IntechOpen

Rice

Germplasm, Genetics and Improvement

Edited by Wengui Yan and Jinsong Bao

RICE - GERMPLASM, GENETICS AND IMPROVEMENT

Edited by **Wengui Yan and Jinsong Bao**

Rice - Germplasm, Genetics and Improvement
http://dx.doi.org/10.5772/51100
Edited by Wengui Yan and Jinsong Bao

Contributors

Jinsong Bao, Sheng Teng, Ying Jie-Zheng, Zhan Xiaodeng, Liyong Cao, Wengui Yan, Aye Aye Khaing, Young-Jin Park, Jiankun Xie, Zhang Fantao, Yulin Jia, Georgia Eizenga

Notice

First published in Croatia, 2014 by INTECH d.o.o.
eBook (PDF) Published by IN TECH d.o.o.
Place and year of publication of eBook (PDF): Rijeka, 2019.
IntechOpen is the global imprint of IN TECH d.o.o.
Printed in Croatia

Legal deposit, Croatia: National and University Library in Zagreb

Additional hard and PDF copies can be obtained from orders@intechopen.com

Rice - Germplasm, Genetics and Improvement
Edited by Wengui Yan and Jinsong Bao

p. cm.

ISBN 978-953-51-1240-2

eBook (PDF) ISBN 978-953-51-4234-8

Meet the editors

Wengui Yan has been a Research Geneticist since 1996 at the USDA-ARS Dale Bumpers National Rice Research Center. Dr. Yan received both B.S. (1981) and M.S. (1984) in Agronomy from Sichuan Agricultural University, China and Ph.D (1992) in Plant Breeding and Genetics from University of Arkansas, USA. His research interests cover phenotypic and genotypic characterization of over 20,000 rice accessions originated from 116 countries in the USDA Plant Germplasm System http://www.ars-grin.gov/npgs/index.html, germplasm identification for various traits essential for resistances to biotic and abiotic stresses, and grain yield, quality and nutritional values, and gene and/or QTL mapping along with molecular marker development for those traits to assist cultivar improvement using genomic technologies. The research program led by him has published 83 peer-reviewed scientific papers, 4 book chapters, 104 proceedings and abstracts for academic conferences and 1 patent in USA, China, Philippines, etc. At present, he is serving as academic editor for PLOS ONE, Plant Omics Journal and The Crop Journal, and reviewer for tens of scientific journals.

Jinsong Bao is a professor of Plant Genetics and Biotechnology at Zhejiang University, China. He received his B.S. (1993) and M.S. (1996) degrees in horticulture and Ph.D. (1999) degree in biophysics from Zhejiang University. His research interests are in molecular genetics of rice quality, more specifically in the areas of starch quality, nutritional quality, genetic mapping, and molecular breeding. He has published more than 60 peer-reviewed research articles and five book chapters in these areas, and has received two professional awards for his achievements in the genetic study and molecular improvement of rice quality from Zhejiang Provincial Government. He has awarded the Young Scientist Research Award from the American Association of Cereal Chemists International, and Zhejiang Young Science and Technology Award from the Zhejiang Association of Science and Technology. He is a member of the editorial board of the Cereal Chemistry, Genes & Genomics, and Open Journal of Genetics.

Contents

Preface

Rice is a staple food for half of the world's population mostly in Asia. Productivity of rice has largely been improved since the Green Revolution in 1960s. Further improvement of rice yield is necessary to keep pace with population growth, which is a challenging task for breeders. This book, Rice - Germplasm, Genetics and Improvement, as its name implies, comprehensively reviews current knowledge in germplasm exploration, genetic basis of complex traits, and molecular breeding strategies in rice. In the germplasm part, we highlight the application of wild rice in rice breeding. In the genetics part, most of the complex traits related with yield, disease, quality have been covered. In the improvement part, Chinese experiences in hybrid rice breeding have been summarized together with many molecular breeding practices scattering in different chapters.

Dr. Jinsong Bao
College of Agriculture and Biotechnology, Zhejiang University,
Hangzhou, China

Dr. Wengui Yan
USDA-ARS Dale Bumpers National Rice Research Center, USA

Chapter 1

Unraveling the Secrets of Rice Wild Species

Ehsan Shakiba and Georgia C. Eizenga

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/58393

1. Introduction

The world is facing a new challenge with global population predicted to plateau at nine billion people by the middle of this century (Godfray et al. 2010). Increasing food production to feed the world's population is an even greater challenge considering that agriculture is experiencing greater competition for land, water and energy, as well as, the effects of substantial climate change and the unintended effects of crop production on the environment. Part of the solution to increasing food production on the same or less cultivated land lies in exploiting the subset of genes lost during the domestication process and subsequent targeted breeding. Currently, these valuable genes are found only in the progenitor species genepool for crop cultivars. Cultivated plants having desirable genes were utilized in intensive breeding projects focused on increasing yield for particular environments and management systems but this process has narrowed the genetic diversity (Rausher 2001). For cultivated plants, this unexploited genetic material includes both landraces and the more exotic wild relatives. Improving our understanding of this tertiary gene pool and exploiting it for crop improvement is paramount to meeting the challenges of feeding the world in this century through the integration of classical genetics and genomics-enabled research paradigms.

The loss of genetic diversity can be more problematic for self-pollinated plant species where the rate of cross pollination is below five percent, thus making it more difficult to reintroduce the lost diversity. In the case of the two major grain crops, rice (*Oryza sativa* L.) and wheat (*Triticum aestivum* L.), both self-pollinated, the re-introduction genetic diversity from the wild is central to the continued success of breeding, given that viruses, fungi, and bacteria, three main causal agents of biotic stress, are constantly evolving to cause the breakdown of the host plant's defense mechanisms (Rausher 2001).

Abiotic stress, including salinity, aluminum toxicity and acid sulfate soils, as well as, temperature and drought, complicate the difficulty of improving crop yields, especially in the face of

global warming (Brooker 2006; Tilman and Lehman 2001), which makes modern cultivars even more vulnerable. Genetic sources of resistance or tolerance offer the most promising mechanism to protect plants against these unfavorable conditions. Often wild species are not included as parental lines in cultivar development because it is relatively difficult to harness desirable genes by genetic recombination and many undesirable genes are introgressed from the wild parent resulting in inferior yield, undesirable plant architecture, and/or poor grain quality (Tanksley and McCouch 1997). Recent studies, however, in rice (McCouch et al. 2007) and tomato, *Lycopersicon esculentum* Mill. (Grandillo and Tanksley 2003), have shown that wild species contain genomic components that could result in genetic gains in terms of agronomic performance.

The rapid advancement in molecular technologies allows for genotyping plants much more quickly and inexpensively than ever before. The availability of high resolution genotypic information creates the opportunity to further explore an expanding number of accessions in a greater depth, and harness this information to enhance the efficiency and accuracy of introgression. These developments create opportunities not previously possible, to identify molecular markers associated with desirable traits in wild species and transfer these traits into elite lines and/or varieties, as well as, to unravel multi-genic traits for crop improvement (Tanksley and McCouch 1997; McCouch et al. 2012).

Our main objective is to summarize efforts over the past 15 years to identify useful novel alleles in the *Oryza* species that were lost during evolution and domestication, genetically dissect the traits encoded by these alleles through chromosome mapping, and incorporate these traits or alleles into an agronomically useful genetic background. To do this we will (a) briefly describe the relationships among the species in the genus *Oryza*, (b) describe the types of populations that have been developed for mapping desirable traits identified in the wild *Oryza* species to a chromosome location, and (c) summarize the quantitative trait locus (QTL) studies focused on mapping the useful traits and novel alleles to specific locations in the genomes of *Oryza* species.

2. Phylogeny of the *Oryza* genus

The *Oryza* genus includes two cultivated species, Asian rice, *O. sativa*, which is grown throughout the tropical and temperate climates of the world, and African rice, *O. glaberrima*, which is found in sub-Saharan Africa along the Niger River. The 22 wild species composing the *Oryza* genus are characterized by eleven different genomes identified as the A-, B-, C-, D-, E-, F-, G-, H-, J-, K-and L-genomes and arranged in the following 10 genome types AA, BB, CC, BBCC, CCDD, EE, FF, GG, HHJJ and KKLL. Four of the wild *Oryza* species are tetraploid and the remaining 18 are diploid, as well as, the two cultivated species (Table 1).

Species[†]	No. of chromo-somes (2n)[†]	Genome[†]	Genome size (Mbp)[‡]	Distribution[†]
Section Oryza				
Oryza sativa complex				
O. sativa L. (ssp. *japonica*, ssp. *indica*)	24	AA	420, 466	Worldwide
O. nivara Sharma et Shastry	24	AA	448	Tropical and subtropical Asia
O. rufipogon Griff.	24	AA	439, 450	Tropical and subtropical Asia, Tropical Australia
O. glaberrima Steud.	24	A^gA^g	354	West Africa
O. barthii A. Chev.[§]	24	A^gA^g	411	Africa
O. glumaepatula Steud.	24	$A^{gp}A^{gp}$	464	South and Central America
O. longistaminata A. Chev. et Roehr.	24	A^lA^l	352	Africa
O. meridionalis Ng	24	A^mA^m	435	Tropical Australia
Oryza officinalis complex				
O. punctata Kotschy ex Steud.	24 48	BB, BBCC	423 (BB)	Africa
O. minuta J.S. Presl. ex C.B. Presl.	48	BBCC	1124	Philippines, Papua New Guinea
O. eichingeri A. Peter	24	CC		South Asia and East Africa
O. officinalis Wall ex Watt	24	CC	653	Tropical and subtropical Asia, Tropical Australia
O. rhizomatis Vaughan	24	CC		Sri Lanka
O. alta Swallen	48	CCDD	1124	South and Central America
O. grandiglumis (Doell) Prod.	48	CCDD		South and Central America
O. latifolia Desv.	48	CCDD		South and Central America
O. australiensis Domin.	24	EE	960	Tropical Australia
Section Brachyantha				
O. brachyantha A. Chev. et Roehr.	24	FF	338	Central Africa

Species†	No. of chromo-somes (2n)†	Genome†	Genome size (Mbp)‡	Distribution†
Section Padia				
Oryza granulata complex				
O. granulata Nees et Am. ex Watt	24	GG	862	South and Southeast Asia
O. meyeriana (Zoll. et (Mor. ex Steud.) Baill.)	24	GG		Southeast Asia
Oryza ridleyi complex				
O. longiglumis Jansen	48	HHJJ		Irian Jaya, Indonesia, Papua New Guinea
O. ridleyi Hook. F.	48	HHJJ	1283	South Asia
Oryza schlechteria complex				
O. coarctata Tateoka	48	KKLL	771	Asian coastal area
O. schlerchteri Pilger	48	KKLL		Papua New Guinea

† Classification for species, genome designation and distribution based on Brar and Singh (2011), Lu et al. (2014), Sanchez et al. (2013) and Vaughan (2003). The superscripts for the A-genome indicate a variation of the type of A-genome.

‡ Genome size based on the following: *O. sativa* subsp. *japonica* (Goff et al. 2002), *O. sativa* subsp. *indica* (Yu et al. 2002) and *Oryza* species (Ammiraju et al. 2010).

§ *O. barthii* is also classified as *O. breviligulata* A. Chev. et Roehr.

Table 1. Taxonomic classification of *Oryza* species including the chromosome number, genome designation, genome size and distribution for each species.

Rice is the only major cereal found in the ancient lineage of the Bambusoideae and is currently placed in the subfamily Erhartoideae. Historically, the grass family, Poaceae, is thought to have evolved about 70-55 mya (million years ago) with the tribes Oryzeae and Pooideae (wheat and oats) diverging about 35 mya [reviewed by Kellogg (2009) and Vaughan et al. (2008)]. The Oryzinae and Zizaninae subtribes diverged about 20-22 mya and the *Oryza* and *Leersia* genera about 14.2 mya. The genus *Oryza* is divided into three sections: *Padia*, *Brachyantha* and *Oryza*. *Padia* includes the forest-dwelling *Oryza*, which are distributed into the *O. granulata* (GG), *O. ridleyi* (HHJJ) and *O. schlechteria* (KKLL) complexes. The *O. granulata* complex is thought to have diverged from the other *Oryza* species about 8 mya. *O. brachyantha* (FF) is the only species in the section *Brachyantha*. This species is widely distributed across Africa, growing in iron-pan rock pools.

Section *Oryza* consists of two species complexes, the *O. officinalis* complex with the B-, C-, D- and E-genomes and the *O. sativa* complex, which includes all the A-genome species. Within

the *O. officinalis* complex, *O. australiensis* (EE) is the most diverged and *O. eichingeri* (CC) appears to be the most basal of the C-genome species.

The species in the *O. sativa* complex prefer full sun, and grow near lakes, rivers and seasonal pools of water. Molecular data suggests that *O. meridionalis* diverged from the other A-genome species about 2 mya. Also, the perennial African species, *O. longistaminata*, diverged from the Asian A-genome species about the same time period, 2-3 mya. The second divergence between the Asian and African A-genome species, *O. barthii* and *O. glaberrima*, occurred 0.6 to 0.7 mya. More recently, possibly about 0.4 mya (or more than 0.2 mya), the *O. rufipogon* clade(s) that eventually diverged into the *O. sativa* subspecies (subsp.) *Japonica* and *Indica*. Later, the *Indica* subspecies differentiated into the *indica* and *aus* subpopulations and the *Japonica* subspecies into the *aromatic* (Group V), *tropical japonica* and *temperate japonica* subpopulations (Garris et al. 2005, Zhao et al. 2011, Huang et al. 2012). Archaeobotanical evidence from spikelet bases and changes in grain size document this domestication process (Fuller et al. 2010). Recently, based on genome sequences of 446 geographically diverse *O. rufipogon* accessions, Huang et al. (2012) further subdivided *O. rufipogon* accessions into three major *O. rufipogon* clades: one closely aligned with *O. sativa* subsp. *japonica*, one aligned with *O. sativa* subsp. *indica*, and the third clade was independent of *O. sativa*. Furthermore, as part of this study, a neighbor-joining tree constructed from sequence differences of 15 representative A-genome accessions suggested within *Indica*, different *O. rufipogon* clades were associated with the *aus* and *indica* subpopulations, whereas the three *Japonica* subpopulations arose from a single *O. rufipogon* clade. This phylogenetic tree also supported the aforementioned genetic distance between *O. meridionalis*, *O. longistaminata*, *O. barthii* and *O. glaberrima*.

Rice, *O. sativa*, the first monocot plant with a reference genome, is the central comparative genomics model for all grasses, and has been compared to all major cereals. To lay the foundation for interrogating the rice wild relatives, 18 bacterial artificial chromosome (BAC) libraries for 16 different *Oryza* species spanning all 10 *Oryza* genome types including the AA-genome species (*O. nivara*, *O. rufipogon*, *O. glaberrima*, *O. barthii*, *O. glumaepatula*, *O. longistaminata*, *O. meridionalis*), *O. punctata* (BB), *O. officinalis* (CC), *O. minuta* (BBCC), *O. alta* (CCDD), *O. australiensis* (EE), *O. brachyantha* (FF), *O. granulata* (GG), *O. ridleyi* (HHJJ) and *O. coarctata* (HHKK), were generated through the *Oryza* Map Alignment Project (OMAP) as summarized by Ammiraju et al. (2010). Subsequently, the International OMAP consortium was formed in 2007 to (a) generate reference sequences and transcriptome data sets of the eight A-genome species and representative species of the other genome types, (b) generate advanced mapping populations for the A-genome species, and (c) identify naturally occurring populations of the wild *Oryza* species for diversity and evolutionary analyses, as well as, conservation (Jacquemin et al. 2013; Sanchez et al. 2013). The species included in the sequencing effort were A-genome species (*O. nivara*, *O. rufipogon*, *O. barthii*, *O. glaberrima*, *O. glumaepatula*, *O. longistaminata*, *O. meridionalis* and both *O. sativa* subsp. *indica* and subsp. *japonica*), *O. punctata* (BB), C-genome species (*O. officinalis*, *O. eichingeri*, *O. rhizomatis*), *O. australiensis* (EE), *O. brachyantha* (FF), *O. granulata* (GG), and the outgroup, *Leersia perrieri*. To date, the sequencing of nine genomes and *L. perrieri* has been completed, in addition to the established reference sequences for *O. sativa* subsp. *japonica* and subsp. *indica* genomes (Wing 2013). Currently, two additional *O. sativa*

subsp. *indica* cultivars are being sequenced representing the *aus* (DJ123) and *indica* (IR64) subpopulations (McCombie 2013).

3. Methods for developing *Oryza* interspecific mapping populations

Traits are classified as either qualitative or quantitative traits. Qualitative traits are controlled by one or a few genes with major effects while quantitative characters are controlled by many genes with minor effects (Poehlman and Sleper 1994). Identification of genes associated with quantitative traits is always more complicated compared to those involving qualitative traits.

Interspecific and intergenomic hybridization, hybridization between species with the same or different genomes, have been used to transfer desirable genes or QTL associated with simple or complex traits from wild species into a cultivated genetic background (Brar and Khush 1997; Dalmacio et al. 1995; Tanksley and McCouch 1997). Nevertheless hybridization success can be hindered by genomic incompatibilities and sterility barriers (Ishii et al. 1994; McCouch et al. 2007; Wang et al. 2005). The utilization of embryo rescue and other methods of producing viable and fertile hybrids combined with robust molecular markers and associated computational and statistical analyses, led to the successful generation of interspecific genetic populations that were used to link desirable traits to molecular markers and subsequent identification of the actual genes controlling the traits of interest (Ali et al. 2010; Chen et al. 2010; Ghesquière et al. 1997; Guo et al. 2013; Lexer and Fay 2005; McCouch et al. 2007). Six types of mapping populations are generated from interspecific crosses between *Oryza* species and *O. sativa* including (a) recombinant inbred line (RIL), (b) advanced backcross (AB), (c) backcross inbred line (BIL), (d) chromosome segment substitution line (CSSL), (e) near isogenic line (NIL) and (f) multi-parent advanced generation inter-cross (MAGIC). A discussion of each of these populations follows and examples are included in the third section describing agronomically important traits attributed to the *Oryza* species donor.

3.1. Recombinant Inbred Line (RIL) population

RIL populations have been the most common type of mapping population used in rice genetics and breeding when both parents are *O. sativa* but a limited number of interspecific populations have been reported. To develop a RIL population, two contrasting cultivars or accessions for the trait(s) to be mapped are crossed together to create an F_1 hybrid. By successive self-pollination starting from the F_1 generation, subsequent generations of segregants are produced (up to F_3), representing multiple rounds of recombination and eventually fixation to homozygosity towards either of the parental alleles (Fig. 1). This derived population is advanced for several generations by the single seed descent (SSD) method, where a single F_3 seed from each F_2 plant is planted to produce the F_4 generation, subsequently a single F_4 seed is selected from each line to produce the F_5 generation with the SSD method usually continuing until F_8 seed are produced. At the F_7, the RILs exhibit genetic homogeneity, such that the genomic contribution of each parent is fixed,

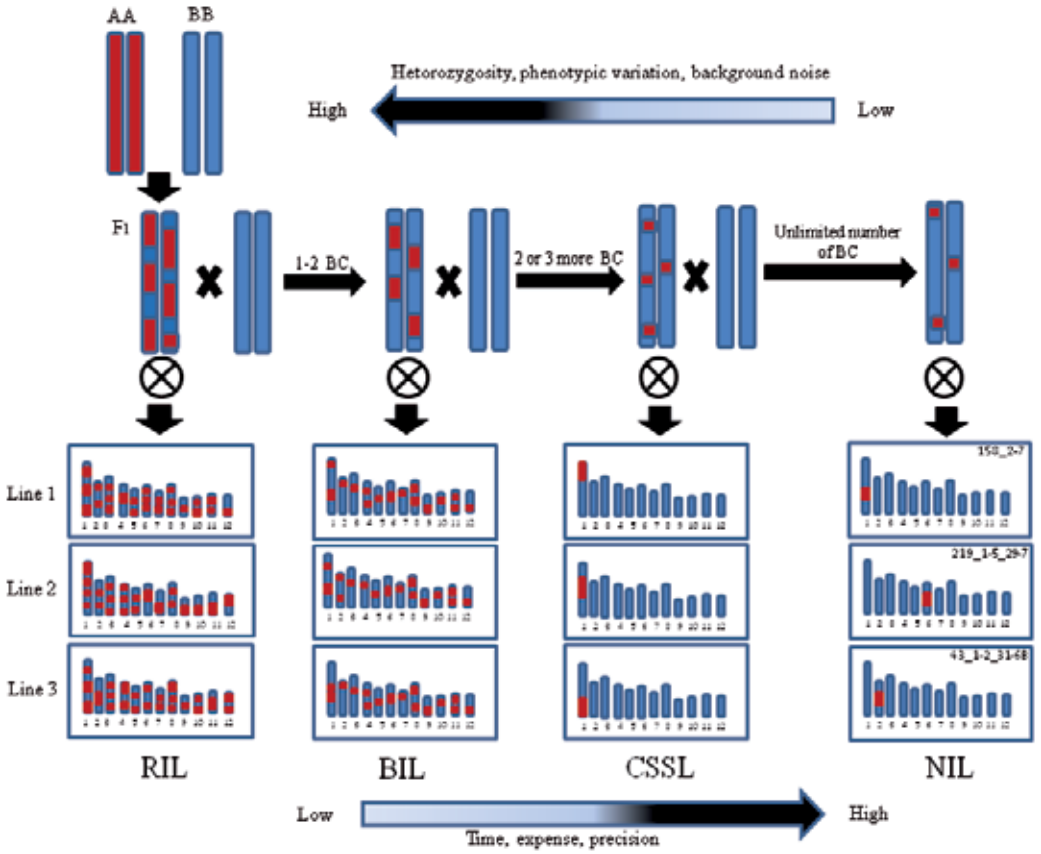


Figure 1. A comparison of the methods for creating primary and advanced bi-parental mapping populations, including recombinant inbred lines (RILs), backcross inbred lines (BILs), chromosome segment substitution lines (CSSLs) and near isogenic lines (NILs) as summarized by Fukuoka et al. (2010). Also shown are the number of backcrosses (BC) required and the genotypes of the lines obtained by each method. Karyotypes of the three CSSLs illustrate how chromosome 1 of the donor can be introgressed into the recurrent parent. The three NIL genotypes are based on the JeffersonNILs, each with a different *O. rufipogon* (IRGC105491) introgression selected for a different yield QTL (Imai et al. 2013).

and together these RILs compose a mapping population. If selections are being made for improved lines with a particular trait(s), this selection often begins in the F_5-F_6 if individual plants can be selected for the trait; otherwise, the selection is postponed to later generations (F_7-F_n) (Nguyen et al. 2003, Poehlman and Sleper 1995). The procedure continues until the superior lines with desirable traits are produced.

The main advantage of the RIL method is that no backcrossing is necessary but when a wild *Oryza* species is a parent, often undesirable traits associated with the wild parent, especially shattering and sterility are problematic, thus it is often necessary to backcross. RIL populations are suitable for identifying major gene(s) or QTL(s), and to detect genetic interactions such as epistasis (Fukuoka et al. 2010). Other advantages are, the individual

RIL may contain more than one introgressed segment in their chromosomes, representing different recombination events and a higher recombination frequency. As a result, fewer progeny lines are required to cover the complete donor genome as compared to other types of bi-parental mapping populations that include a backcross generation. Moreover, epistatic effects can be detected in RILs due to the presence of several introgressed segments in each line (Keurentjes et al. 2007). Because several segments of each parent are present in each individual line composing the population, there is less homogeneity in RIL populations as compared to most other types of populations. This heterogeneity is easy to observe and provides an excellent opportunity for phenotypic evaluation. In summary, the RIL method has proven to be useful when both parents are *O. sativa* but with interspecific and intergenomic crosses, backcrossing is often necessary (Fukuoka et al. 2010).

Commonly used softwares for creating the linkage map from the genotypic (molecular marker) data of the population for QTL analyses include MapMaker-QTL (Lander and Botstein 1989), JoinMap (Van Ooijen 2006) and MapDisto (Lorieux 2012). The possible chromosome location of the QTL for the trait being evaluated is based on the QTL having a significant LOD [logarithm (base 10) of odds] score with the LOD score detecting linkage between the molecular marker and the trait of interest. Several softwares are freely available for conducting the QTL analysis, including MapMaker-QTL (Lander and Botstein 1989), QTLCartographer (Wang et al. 2012), QGene (Joehanes and Nelson 2008), MapDisto (Lorieux 2012) and QTLNetwork (Yang et al. 2008). It is important to confirm that the software being used for QTL analysis can correctly analyze the population type since some cannot be used with BC_2F_2 populations based on differences in fundamental assumptions. Most recent QTL analyses with rice have been performed using either composite interval mapping (CIM) (Zeng 1994) or multiple interval mapping (MIM) (Kao and Zeng 1999) with single point analysis (SPA) (Tanksley et al. 1982), marker regression (Kearsey and Hyne 1994) and interval mapping (IM) (Haley and Knott 1992; Lander and Botstein 1989) being used in earlier analyses.

3.2. Advanced Backcross (AB) population

The advanced backcross (AB)-quantitative trait locus (QTL) analysis is a powerful strategy to map desirable trait(s) discovered in the wild species (Tanksley and Nelson 1996). This method was first applied to QTL mapping in tomato, and subsequently to several other crops, including rice (Grandillo and Tanksley 2003; McCouch et al. 2007). In the process of developing the AB populations used for QTL analysis, plants or lines with unfavorable genes derived from donor parents like sterility and sometimes shattering, are often eliminated from the population after phenotypic and genotypic evaluation. Due to artificial selection in favor of lines with desirable alleles and the genetic background from the recurrent parent, the distribution can be skewed toward the recurrent parent, therefore, after the BC_3 generation, the power of the statistical analysis to detect QTL decreases. Since sequential backcrossing in AB-QTL removes epistatic interactions, the chance of detecting QTLs with epistatic interactions among alleles from the donor parent decreases, while the ability to detect additive QTLs increases (Tanksley and Nelson 1996; Grandillo and Tanksley 2003).

To create an AB mapping population, one parent, usually the wild *Oryza* species, identified as the donor parent, is crossed with the recurrent parent, usually an elite cultivar, which will be crossed with the hybrid parent in subsequent crosses (illustrated in Ali et al. 2010). Often the donor parent is used as a male and the recurrent parent as the female to avoid the cytoplasmic male sterility and because it is usually easier to emasculate the cultivated parent. The F_1 plant(s) is one parent in the second generation and it is crossed with the recurrent parent, which is defined as backcrossing. The resulting first backcross generation (BC_1) may be backcrossed again with the recurrent parent to generate a BC_2 population. If the BC_2 progeny are sterile, it is best to advance the population to the BC_3 generation by crossing the BC_2 plants to the recurrent parent a third time. After the progeny lines are advanced to the BC_2 (or BC_3) generation and allowed to self pollinate, these BC_2F_2 (or BC_3F_2) progeny plants are grown to collect phenotypic and genotypic data for the QTL analysis. After the AB-QTL mapping, the AB population can be advanced by (a) allowing all the progeny lines to self-pollinate and be advanced by SSD for three to four additional generations, thus developing a BIL population or (b) backcrossing the progeny lines additional generations to develop a library of CSSLs or NILs for targeted traits (Fig. 1).

3.3. Backcross Inbred Line (BIL) population

BIL populations are used to introgress desirable traits from the wild *Oryza* species donor into rice with the potential of improving the agronomic performance of elite cultivars and develop mapping populations (Fig. 1). After backcrossing, as described in the aforementioned AB population development, the individual lines, BC_1, BC_2 or BC_3 generation, are self-pollinated for about four generations to the BC_2F_5, as described in the RIL population development. If a specific trait is being selected, the BILs will be screened for that trait and backcrossed as described in the NIL section (Blanco et al. 2003; Fukuoka et al. 2010; Fulton et al. 1997; Bernacchi et al. 1998; Talamè et al. 2004).

The advantages of utilizing BILs are that the method is relatively straightforward and the lines are more homogeneous, having less linkage drag and fewer untargeted segments from the donor parent as compared to RILs. Furthermore, BIL populations can be used to identify major QTLs and single genes, detect QTLs with epistatic or additive effects, as well as, provide an accurate estimation of genotype x environment interactions. It takes more time to develop a BIL population than a RIL population but less time than developing CSSLs and NILs because there are fewer backcrosses to do and less emphasis on targeted segments (Fukuoka et al. 2010; Fulton et al. 1997; Jaquemin et al. 2013). Some disadvantages of this method are the genetic background of the donor parent is higher in the BILs as compared to the CSSLs and NILs, and the lines require more phenotypic evaluation but less genotypic characterization. As a result, mapping in a BIL population is more labor intensive and costly compared to RILs but less costly than NILs and CSSLs. Unfortunately, only limited success has been reported for improving quantitative traits with low heritability and identifying minor QTLs. Also, it is difficult to transfer a relatively large number of genes or QTLs associated with the desirable traits from the wild donor to an elite cultivar using lines selected from a BIL population.

3.4. Chromosome Segment Substitution Line (CSSL) library

A CSSL "library" is a set of near isogenic lines, often ranging from 26 to 80 lines, which cover the entire donor genome when the segments included in each introgression line are in the background of the recurrent parent (Fig. 1; Ali et al. 2010). The concept of CSSL libraries was initially proposed by Eshed and Zamir (1995) as introgression lines and Ghesquière et al. (1997) as contig lines. To develop CSSLs, the initial crossing follows the same scheme as described for AB and BIL populations where the wild, unadapted *Oryza* species is the donor parent and the recurrent parent is usually an elite cultivar. To confirm the entire donor genome is included in the CSSL library, a set of polymorphic markers is often used to assist in selecting lines for each generation, beginning with the BC_1F_1 generation. To develop a CSSL library usually requires backcrossing to the recurrent parent for three to four additional generations (BC_4F_1 or BC_5F_1). The set of polymorphic markers can be used each generation to confirm the targeted segment is present in each line composing the CSSL library as illustrated in Ali et al. (2010). Alternatively, several hundred lines can be backcrossed for 4 to 5 generations and a CSSL library can be selected after genotyping in the BC_4 or BC_5 generation. Once the desired $BC_{4:5}F_1$ lines are selected, the lines are self-pollinated to achieve homozygosity and the lines homozygous for the individual targeted segment are selected from the $BC_{4:5}F_2$ progeny lines. The $BC_{4:5}F_3$ seed is used to establish the CSSL library composed of a set near isogenic lines covering the entire donor genome (Ali et al. 2010; Fukuoka et al. 2010).

A CSSL library has several advantages compared to BILs or an AB mapping population in that it can be used for fine mapping, to identify both major and minor QTLs, and validate genetic interactions. Also, due to the recurrent parent background in CSSLs, linkage drag and its negative effects on the QTL studies are significantly reduced or eliminated. This uniform genetic background enables one to make rapid progress in linkage mapping of targeted QTLs. Lastly, individual CSSLs which carry a specific trait can be used for fine mapping and gene pyramiding (Ali et al. 2010; Fukuoka et al. 2010), as illustrated by the identification of the rice stripe necrosis virus resistance introgression from *O. glaberrima* (Gutiérrez et al. 2010).

The rice universal core genetic map is a set of uniformly distributed polymorphic SSR markers that clearly differentiate *O. sativa* cultivars and wild *Oryza* species accessions, especially within the AA genome (Orjuela et al. 2010). If polymorphic SSR (simple sequence repeat) markers for several different CSSL libraries or other mapping populations are selected from the core map, such that the markers are in approximately the same location, comparisons can be made across several different CSSL libraries or mapping populations. More recently, SNP (single nucleotide polymorphism) markers have been used to genotype the putative lines being selected for the CSSL libraries. For this purpose, several different 384-SNP assays have been used to identify the target donor segment and recurrent parent background (Ali et al. 2010; Tung et al. 2010; McCouch et al. 2010) and most recently a single 6,000 SNP assay is being employed (Zhou et al. 2013; SR McCouch, Cornell University, personal communication).

3.5. Near Isogenic Lines (NILs)

The procedure for developing a set of NILs is similar to CSSLs except the number of backcrosses is unlimited because the focus is on incorporating a single segment with the trait(s) of interest identified in the *Oryza* species donor into the background of the recurrent parent (Fig. 1). With NILs, the focus is on a particular set of lines for the trait(s) of interest, not covering the entire donor genome as with a CSSL library. As with CSSLs, once the targeted segment is introgressed into the recurrent parent background, the pre-NIL lines are allowed to self-pollinate, so that the NILs will be homozygous for the targeted segment. Molecular markers, such as SSRs and SNPs, are used to select for the targeted segment and determine the number of chromosomal segments from the donor parent remaining in the background (Fukuoka et al. 2010).

NILs are often developed to fine map QTLs identified in primary mapping populations, like RIL or BIL, because the QTLs can be mapped precisely as single Mendelian factors (McCouch et al. 2007). Use of NILs, like CSSLs, increases the power to detect small-effect QTL and can overcome or minimize genetic incompatibility, linkage drag, cytoplasmic sterility and epistatic effects, all of which are common obstacles in wide hybridization efforts because the genetic background is more or less uniform. Although developing NILs, like CSSLs, is labor intensive, time consuming, and expensive, NILs are a valuable tool for exploring the genes underlying QTLs because the epistatic effects are removed or minimized making it easier to measure gene expression (Keurentjes et al. 2007). Finally, those NILs with valuable genes introgressed from the wild *Oryza* species donor, can be used as parental lines in breeding programs.

3.6. Multi-parent Advanced Generation Inter-Cross (MAGIC) population

Recently, some efforts have turned to MAGIC populations (Cavanagh et al. 2008; Kover et al. 2009) which can serve the dual purpose of permanent mapping populations for precise QTL mapping, and for direct or indirect use in variety development, especially when the parents used to develop the population are the source of agronomically useful traits (Bandillo et al. 2013). MAGIC populations are developed by systematically crossing several F_1 hybrids involving four to sixteen different parental lines to create a set of double crosses, then systematically crossing the double cross hybrids to create a set of 4-, 8-or 16-way crosses. As the final step, the lines composing the population are advanced four or more generations by single seed descent to obtain a set of advanced intercrossed lines (AILs). Bandillo et al. (2013) reported four different types of MAGIC populations being developed in rice (*O. sativa*) at the International Rice Research Institute (IRRI) which are described as (1) *Indica* MAGIC composed of 1,831 S_8 AILs; (2) MAGIC Plus with 2,214 S_6 AILs; (3) *Japonica* MAGIC with approximately 400 S_6 AILs; and (4) MAGIC Global with 1,402 AILs in the S_5 generation. Currently, a Wild MAGIC population is being developed by a team at IRRI (K. Jena, H. Leung, K. McNally) in collaboration with J. Hibberd (University of Cambridge, U.K.), and I. Mackay (NIAB, Cambridge, U.K.) using multiple accessions of all eight A-genome species (McNally, personnel communication). In most cases, for this population, the initial crosses had *O. sativa* as the female parent, and the goal is to produce 16-way crosses with highly mixed genomes.

4. Useful agronomic traits mapped in *Oryza* species and transferred into cultivated rice

Trait	Donor species	Donor accession	Recurrent parent(s)	Type of mapping popu-lation†	QTL mapping analysis‡	Chromo-some location§	Type of mark-er¶	Reference
Vegetative Growth Stages								
Days to flowering	*O. australiensis*	IRGC100882	IR31917-45-3-2	IL		10	RFLP	Ishii et al. (1994)
Days to heading	*O. glaberrima*		IR64	BC_2F_3	SPA	2, 10	SSR, STS	Bimpong et al. (2011)
	O. glaberrima	IRGC103544	Milyang 23	BC_3F_2	SPA	1, 4, 7, 8	SSR	Suh et al. (2005)
	O. glumaepatula		Taichung 65	BC_4F_2		7	RFLP	Sanchez et al. (2003)
	O. grandiglumis	IRGC101154	Hwaseongbyeo	AB-QTL	SPA	6	SSR	Yoon et al. (2006)
	O. meyeriana	Y73	IR24	RIL	CIM	6, 7, 8, 11	SSR, STS	Chen et al. (2012)
	O. nivara	IRGC100898	Bengal	AB-QTL	MIM	3, 6	SSR	Eizenga et al. (2013)
	O. nivara	IRGC100898	Bengal	AB-QTL	MIM	3, 4, 6, 8	SSR	Eizenga et al. (2013)
	O. nivara	IRGC100195	M-202	AB-QTL	MIM	3, 8	SSR	Eizenga et al. (accepted)
	O. rufipogon	IRGC105491	Jefferson	AB-QTL	SPA, IM, CIM	1, 2, 3, 4, 10	RFLP, SSR	Thomson et al. (2003)
	O. rufipogon	IRGC105491	IR64	AB-QTL	SPA, IM, CIM	2, 7	RFLP, SSR	Septiningsih et al. (2003)
	O. rufipogon	IRGC105491	V20A, V20B	BC_2	ANOVA	6, 12	RFLP	Xiao et al. (1998)
	O. rufipogon	IRGC105491	Hwaseongbyeo	AB-QTL, NIL	SPA, IM, ANOVA	6, 9	SSR	Jin et al. (2009), Xie et al. (2008)
	O. rufipogon	IRGC105491	MR219	AB-QTL	CIM	3	SSR	Wickneswari et al. (2012)
	O. rufipogon	W1944	Hwayeongbyeo	IL	SPA, IM	1	SPA, IM	Yuan et al. (2009)

Trait	Donor species	Donor accession	Recurrent parent(s)	Type of mapping population†	QTL mapping analysis‡	Chromosome location§	Type of marker¶	Reference
Days to maturity	*O. rufipogon*	IRGC105491	IR64	AB-QTL	SPA, IM, CIM	4, 7, 8	RFLP, SSR	Septiningsih et al. (2003)
	O. rufipogon	IRGC105491	V20A, V20B	BC_2	ANOVA	6, 12	RFLP	Xiao et al. (1998)
	O. rufipogon	IRGC105491	MR219	AB-QTL	CIM	4, 6	SSR	Wickneswari et al. (2012)
Seedling height	*O. rufipogon*	W1944	Hwayeongbyeo	RIL	SPA, CIM	1	SSR	Lee et al. (2005)
Culm length	*O. glaberrima*	IRGC103544	Milyang 23	BC_3F_2	SPA	2, 10	SSR	Suh et al. (2005)
	O. grandiglumis	IRGC101154	Hwaseongbyeo	AB-QTL	SPA	1, 4	SSR	Yoon et al. (2006)
	O. minuta	IR71033-121-15	Junambyeo	$F_{2:3}$	SPA, IM	6, 7, 12	SSR, STS	Rahman et al. (2007)
	O. rufipogon	W1944	Hwayeongbyeo	RIL	SPA, CIM	1, 6	SSR	Lee et al. (2005)
	O. rufipogon	IRGC105491	MR219	AB-QTL	CIM	1, 3, 9	SSR	Wickneswari et al. (2012)
	O. rufipogon	W1944	Hwayeongbyeo	IL	SPA, IM,	1, 12	SPA, IM	Yuan et al. (2009)
Plant height	*O. glaberrima*		IR64	BC_2F_3	SPA	1	SSR, STS	Bimpong et al. (2011)
	O. longistaminata		RD23	BC_7F_2	CIM	1	SSR	Chen et al. (2009)
	O. nivara	IRGC100898	Bengal	AB-QTL	MIM	1	SSR	Eizenga et al. (2013)
	O. nivara	IRGC104705	Bengal	AB-QTL	MIM	1, 12	SSR	Eizenga et al. (2013)
	O. rufipogon	IRGC105491	Jefferson	AB-QTL	SPA, IM, CIM	1	RFLP, SSR	Thomson et al. (2003)
	O. rufipogon	IRGC105491	IR64	AB-QTL	SPA, IM, CIM	1, 4, 10, 11	RFLP, SSR	Septiningsih et al. (2003)
	O. rufipogon	IRGC105491	Hwaseongbyeo	AB-QTL, NIL	SPA, IM, ANOVA	7, 9	SSR	Jin et al. (2009), Xie et al. (2008)

Trait	Donor species	Donor accession	Recurrent parent(s)	Type of mapping population†	QTL mapping analysis‡	Chromosome location§	Type of marker¶	Reference
	O. rufipogon	IRGC105491	MR219	AB-QTL	CIM	1, 3, 9	SSR	Wickneswari et al. (2012)
	O. rufipogon	IC22015	IR 58025A	AB-QTL	IM, CIM	1	SSR	Marri et al. (2005)
Plant type (Culm habit or tiller angle)	*O. nivara*	IRGC100898	Bengal	AB-QTL	MIM	9	SSR	Eizenga et al. (2013)
	O. nivara	IRGC104705	Bengal	AB-QTL	MIM	9	SSR	Eizenga et al. (2013)
	O. nivara	IRGC100195	M-202	AB-QTL	MIM	9	SSR	Eizenga et al. (accepted)
Flag leaf length	*O. minuta*	IR71033-121-15	Junambyeo	$F_{2:3}$	SPA, IM	8, 9	SSR, STS	Rahman et al. (2007)
Third node width	*O. rufipogon*	W1944	Hwayeongbyeo	RIL	SPA, CIM	1	SSR	Lee et al. (2005)
Tiller number	*O. glaberrima*		IR64	BC_2F_3	SPA	2, 7	SSR, STS	Bimpong et al. (2011)
	O. glumaepatula	RS-16	BG90-2	BC_2F_2	SPA, IM	4, 5, 7, 8, 11	SSR, STS	Brondani et al. (2002)
	O. glumaepatula	RS-16	Cica8	$BC_2F_{2\text{-}9}$	CIM	7, 11	SSR	Rangel et al. (2013)
	O. minuta	IR71033-121-15	Junambyeo	$F_{2:3}$	SPA, IM	3	SSR, STS	Rahman et al. (2007)
	O. rufipogon	IRGC105491	MR219	AB-QTL	CIM	2, 5, 8	SSR	Wickneswari et al. (2012)
Panicle Development								
Panicle exsertion	*O. rufipogon*	W1944	Hwayeongbyeo	RIL	SPA, CIM	1	SSR	Lee et al. (2005)
Panicle density	*O. rufipogon*	IRGC 105491	Hwaseongbyeo	NIL	ANOVA	9	SSR	Xie et al. (2008)
Panicle number	*O. glaberrima*	IRGC103544	Milyang 23	BC_3F_2	SPA	4	SSR	Suh et al. (2005)
	O. glumaepatula	RS-16	BG90-2	BC_2F_2	SPA, IM	5, 8, 11	SSR, STS	Brondani et al. (2002)
	O. glumaepatula	RS-16	Cica8	$BC_2F_{2\text{-}9}$	CIM	7, 11	SSR	Rangel et al. (2013)

Trait	Donor species	Donor accession	Recurrent parent(s)	Type of mapping population†	QTL mapping analysis‡	Chromosome location§	Type of marker¶	Reference
	O. minuta	IR71033-121-15	Junambyeo	$F_{2:3}$	SPA, IM	4	SSR, STS	Rahman et al. (2007)
	O. nivara	IRGC100195	M-202	AB-QTL	MIM	7	SSR	Eizenga et al. (accepted)
	O. rufipogon	IRGC105491	Jefferson	AB-QTL	SPA, IM, CIM	3, 7	RFLP, SSR	Thomson et al. (2003)
	O. rufipogon	IRGC105491	IR64	AB-QTL	SPA, IM, CIM	2	RFLP, SSR	Septiningsih et al. (2003)
	O. rufipogon	IRGC105491	V20A, V20B	BC_2	ANOVA	1, 2	RFLP	Xiao et al. (1998)
	O. rufipogon	IRGC105491	MR219	AB-QTL	CIM	2, 8	SSR	Wickneswari et al. (2012)
	O. rufipogon	IC22015	IR58025A	AB-QTL	IM, CIM	2	SSR	Marri et al. (2005)
	O. rufipogon	W1944	Hwayeongbyeo	RIL, IL	SPA, IM, CIM	1, 7, 12	SSR	Lee et al. (2004), Yuan et al. (2009)
	O. rufipogon	YJCW	93-11	AB-QTL	SPA, IM, CIM	1, 2, 7, 8, 11	SSR	Fu et al. (2010)
Panicle length	*O. glaberrima*	IRGC103544	Milyang 23	BC_3F_2	SPA	2, 5, 6, 10, 12	SSR	Suh et al. (2005)
	O. meyeriana	Y73	IR24	RIL	CIM	1, 2	SSR, STS	Chen et al. (2012)
	O. minuta	IR71033-121-15	Junambyeo	$F_{2:3}$	SPA, IM	6, 7, 8	SSR, STS	Rahman et al. (2007)
	O. rufipogon	IRGC105491	Hwaseongbyeo	NIL	SPA, IM, ANOVA	9	SSR	Xie et al. (2008)
	O. rufipogon	IRGC105491	IR64	AB-QTL	SPA, IM, CIM	1, 9, 10	RFLP, SSR	Septiningsih et al. (2003)
	O. rufipogon	IRGC105491	Jefferson	AB-QTL	SPA, IM, CIM	1, 2, 4, 9, 12	RFLP, SSR	Thomson et al. (2003)
	O. rufipogon	IRGC105491	V20A, V20B	BC2	ANOVA	1, 2, 4, 8, 9, 12	RFLP	Xiao et al. (1998)
	O. rufipogon	IC22015	IR 58025A	AB-QTL	IM, CIM	2, 5, 9	SSR	Marri et al. (2005)

Trait	Donor species	Donor accession	Recurrent parent(s)	Type of mapping population†	QTL mapping analysis‡	Chromosome location§	Type of marker¶	Reference
	O. rufipogon	W1944	Hwayeongbyeo	RIL	SPA, CIM	1	SSR	Lee et al. (2005)
	O. rufipogon	W1944	Hwayeongbyeo	IL	SPA, IM,	1, 2	SPA, IM	Yuan et al. (2009)
Primary branches per panicle	*O. minuta*	IRGC101144	Hwaseongbyeo	NIL		7	SSR	Balkunde et al. (2013)
	O. rufipogon	W1944	Hwayeongbyeo	RIL	SPA, CIM	1	SSR	Lee et al. (2005)
Secondary branches per panicle	*O. rufipogon*	IRGC105491	Hwaseongbyeo	AB-QTL	SPA, IM	6, 8	SSR	Jin et al. (2009)
	O. rufipogon	W1944	Hwayeongbyeo	RIL, IL	SPA, IM, CIM	1, 2, 9	SSR	Lee et al. (2005), Yuan et al. (2009)
Reproductive Growth Stages								
Pollen (male) sterility	*O. glumaepatula*	IRGC105688	Taichung 65	BC_4F_2		2, 7	RFLP	Sobrizal et al. (2000a, 2000b)
	O. longistaminata		RD23	BC_7F_2	CIM	6	SSR	Win et al. (2009; 2011)
	O. nivara	IRGC105444	Taichung 65	IL-BC_4F_1		4, 8, 12	RFLP, SSR, SNP	Chen et al. (2009)
Hybrid breakdown locus	*O. nivara*	IRGC105444	Koshihikari	BC_4F_3		2	SSR, SNP	Miura et al. (2008)
Panicle fertility	*O. glaberrima*		IR64	BC_2F_3	SPA	2, 10	SSR, STS	Bimpong et al. (2011)
Productive panicle number	*O. rufipogon*	G52-9	Yuexiangzhan	AB-QTL	CIM	2, 3, 7	SSR	Jing et al. (2010)
Spikelets per plant	*O. minuta*	IRGC101144	Hwaseongbyeo	NIL		7	SSR	Balkunde et al. (2013)
	O. rufipogon	G52-9	Yuexiangzhan	AB-QTL	CIM	2	SSR	Jing et al. (2010)
	O. rufipogon	IC 22015	IR 58025A	AB-QTL	IM, CIM	2, 5	SSR	Marri et al. (2005)

Trait	Donor species	Donor accession	Recurrent parent(s)	Type of mapping population†	QTL mapping analysis‡	Chromosome location§	Type of marker¶	Reference
	O. rufipogon	IRGC105491	V20A, V20B	BC_2	ANOVA	1	RFLP	Xiao et al. (1998)
	O. rufipogon	IRGC105491	MR219	AB-QTL	CIM	1	SSR	Wickneswari et al. (2012)
Spikelets per panicle	*O. glaberrima*	IRGC103544	Milyang 23	BC_3F_2	SPA	3	SSR	Suh et al. (2005)
	O. grandiglumis	IRGC101144	Hwaseongbyeo	AB-QTL	SPA	2, 3, 4, 11	SSR	Yoon et al. (2006)
	O. minuta	IR71033-121-15	Junambyeo	$F_{2:3}$	SPA, IM	6	SSR, STS	Rahman et al. (2007)
	O. rufipogon	IRGC105491	Hwaseongbyeo	NIL, AB-QTL	SPA, IM, ANOVA	8, 9	SSR	Xie et al. (2008), Jin et al. (2009)
	O. rufipogon	IRGC105491	Jefferson	AB-QTL	SPA, IM, CIM	2, 3, 9	RFLP, SSR	Thomson et al. (2003)
	O. rufipogon	IRGC105491	V20A, V20B	BC_2	ANOVA	1, 9	RFLP	Xiao et al. (1998)
	O. rufipogon	W1944	Hwayeongbyeo	IL, RIL	SPA, IM, CIM	1	SSR	Yuan et al. (2009), Lee et al. (2005)
	O. rufipogon	YJCW	93-11	AB-QTL	SPA, IM, CIM	3	SSR	Fu et al. (2010)
	O. glaberrima		Milyang 23	BC_2F_5		2	SSR	Kang et al. (2008)
	O. rufipogon	IRGC105491	MR219	AB-QTL	CIM	3	SSR	Wickneswari et al. (2012)
	O. minuta	IRGC101144	Hwaseongbyeo	NIL		7	SSR	Balkunde et al. (2013)
Spikelet fertility	*O. glaberrima*	IRGC103544	Milyang 23	BC_3F_2	SPA	2, 4, 8	SSR	Suh et al. (2005)
	O. longistaminata		RD23	BC_7F_2	CIM	6	SSR	Chen et al. (2009)
	O. minuta	IR71033-121-15	Junambyeo	$F_{2:3}$	SPA, IM	6	SSR, STS	Rahman et al. (2007)

Trait	Donor species	Donor accession	Recurrent parent(s)	Type of mapping population†	QTL mapping analysis‡	Chromosome location§	Type of marker¶	Reference
Shattering	*O. rufipogon*	IRGC105491	Jefferson	AB-QTL	SPA, IM, CIM	8	RFLP, SSR	Thomson et al. (2003)
	O. rufipogon	W1944	Hwayeongbyeo	RIL	SPA, CIM	1, 3, 6	SSR	Lee et al. (2005)
	O. rufipogon	W1944	Hwayeongbyeo	IL, RIL	SPA, IM, CIM	1, 4, 5	SSR	Yuan et al. (2009), Lee et al. (2005)
Grains per panicle	*O. minuta*	IRGC101144	Hwaseongbyeo	NIL		7	SSR	Balkunde et al. (2013)
	O. rufipogon	IRGC105491	Jefferson	AB-QTL	SPA, IM, CIM	2, 3, 8, 9	RFLP, SSR	Thomson et al. (2003)
	O. rufipogon	IRGC105491	V20A, V20B	BC_2	ANOVA	1, 8, 12	RFLP	Xiao et al. (1998)
	O. rufipogon	IRGC105491	Hwaseongbyeo	AB-QTL, NIL	SPA, IM, ANOVA	8, 9	SSR	Jin et al. (2009), Xie et al. (2008)
	O. rufipogon	IC22015	IR 58025A	AB-QTL	IM, CIM	2, 5	SSR	Marri et al. (2005)
	O. rufipogon	G52-9	Yuexiangzhan	AB-QTL	CIM	4, 10, 11	SSR	Jing et al. (2010)
	O. rufipogon	YJCW	93-11	AB-QTL	SPA, IM, CIM	1, 3	SSR	Fu et al. (2010)
Percent seed set	*O. meyeriana*	Y73	IR24	RIL	CIM	8	SSR, STS	Chen et al. (2012)
	O. rufipogon	IRGC105491	MR219	AB-QTL	CIM	3	SSR	Wickneswari et al. (2012)
	O. rufipogon	IRGC105491	V20A, V20B	BC_2	ANOVA	2, 4	RFLP	Xiao et al. (1998)
	O. rufipogon	W1944	Hwayeongbyeo	IL, RIL	SPA, IM, CIM	10	SSR	Lee et al. (2005)
Awn length	*O. minuta*	IRGC101144	Hwayeongbyeo	AB-QTL	SPA, CIM	6, 9	SSR	Linh et al. (2004)
	O. minuta	IR71033-121-15	Junambyeo	$F_{2:3}$	SPA, IM	5, 9	SSR, STS	Rahman et al. (2007)

Trait	Donor species	Donor accession	Recurrent parent(s)	Type of mapping popu-lation†	QTL mapping analysis‡	Chromo-some location§	Type of mark-er¶	Reference
	O. rufipogon	IRGC105491	Hwaseongbyeo	AB-QTL	SPA, IM	8	SSR	Jin et al. (2009)
	O. rufipogon	W1944	Hwayeongbyeo	RIL	SPA, CIM	8, 11	SSR	Lee et al. (2005)
	O. rufipogon	W1944	Hwayeongbyeo	IL	SPA, IM,	1, 8, 11, 12	SPA, IM	Yuan et al. (2009)
Grain (kernel) Traits								
Grain (kernel) length	*O. grandiglumis*	IRGC101154	Hwaseongbyeo	AB-QTL	SPA	11	SSR	Yoon et al. (2006)
	O. minuta	IR71033-121-15	Junambyeo	$F_{2:3}$	SPA, IM	3, 5, 6, 7, 9	SSR, STS	Rahman et al. (2007)
	O. nivara	IRGC100195	M-202	AB-QTL	MIM	1	SSR	Eizenga et al. (accepted)
	O. nivara	IRGC100898, IRGC104705	Bengal	AB-QTL	MIM	1, 9	SSR	Eizenga et al. (2013)
	O. rufipogon	IRGC105491	Hwaseongbyeo	NIL	SPA, IM, ANOVA	8	SSR	Xie et al. (2006)
	O. rufipogon	W1944	Hwayeongbyeo	RIL	SPA, CIM	1, 2, 3, 5, 6	SSR	Lee et al. (2005)
Grain (kernel) width	*O. glaberrima*	IRGC103544	V20A	AB-QTL	SPA, IM, CIM	10, 11	RFLP, SSR	Li et al. (2004)
	O. nivara	IRGC81848	Swarna	BC_2F_2	IM, CIM	3, 6	SSR	Swamy et al. (2012)
	O. rufipogon	IRGC105491	Hwaseongbyeo	NIL	SPA, IM, ANOVA	8	SSR	Xie et al. (2006)
Grain (kernel) length to width ratio	*O. glaberrima*	IRGC103544	Caiapó	BC_3F_1	IM, CIM	1	SSR	Aluko et al. (2004)
	O. glaberrima	IRGC103544	V20A	AB-QTL	SPA, IM, CIM	12	RFLP, SSR	Li et al. (2004)
	O. grandiglumis	IRGC101154	Hwaseongbyeo	AB-QTL	SPA	2, 11	SSR	Yoon et al. (2006)
	O. rufipogon	W1944	Hwayeongbyeo	RIL	SPA, CIM	1, 2, 5	SSR	Lee et al. (2005)
	O. minuta	IR71033-121-15	Junambyeo	$F_{2:3}$	SPA, IM	2, 3, 5	SSR, STS	Rahman et al. (2007)

Trait	Donor species	Donor accession	Recurrent parent(s)	Type of mapping popu-lation†	QTL mapping analysis‡	Chromo-some location§	Type of mark-er¶	Reference
	O. nivara	IRGC100195	M-202	AB-QTL	MIM	1, 5	SSR	Eizenga et al. (accepted)
	O. nivara	IRGC81848	Swarna	BC_2F_2	IM, CIM	12	SSR	Swamy et al. (2012)
Grain thickness	*O. grandiglumis*	IRGC101154	Hwaseongbyeo	AB-QTL	SPA	6, 11	SSR	Yoon et al. (2006)
	O. rufipogon	IRGC105491	Hwaseongbyeo	NIL	SPA, IM, ANOVA	8	SSR	Xie et al. (2006)
	O. rufipogon	W1944	Hwayeongbyeo	RIL	SPA, CIM	1, 12	SSR	Lee et al. (2005)
Pericarp color	*O. rufipogon*	IRGC105491	Ce64, Caiapó, Hwacheong, Jefferson, IR64	AB-QTL	IM, CIM	7	SSR	McCouch et al. (2007)
	O. rufipogon	W1944	Hwayeongbyeo	IL	SPA, IM,	1, 7	SPA, IM	Yuan et al. (2009)
Yield Traits								
Grain weight	*O. glaberrima*	IRGC103544	Milyang 23	BC_3F_2	SPA	2, 3	SSR	Suh et al. (2005)
	O. grandiglumis	IRGC101154	Hwaseongbyeo	AB-QTL	SPA	3, 6, 8, 11	SSR	Yoon et al. (2006)
	O. meyeriana	Y73	IR24	RIL	CIM	3, 9, 11	SSR, STS	Chen et al. (2012)
	O. minuta	IR71033-121 -15	Junambyeo	$F_{2:3}$	SPA, IM	3, 7, 11	SSR, STS	Rahman et al. (2007)
	O. nivara	IRGC100195	M-202	AB-QTL	MIM	10	SSR	Eizenga et al. (accepted)
	O. rufipogon	IRGC105491	Hwaseongbyeo	NIL	SPA, IM, ANOVA	8	SSR	Xie et al. (2006)
	O. rufipogon	IRGC105491	Jefferson	AB-QTL	SPA, IM, CIM	1, 5	RFLP, SSR	Thomson et al. (2003)
	O. rufipogon	IRGC105491	IR64	AB-QTL	SPA, IM, CIM	1, 3	RFLP, SSR	Septiningsih et al. (2003)
	O. rufipogon	IRGC105491	V20A, V20B	BC_2	ANOVA	4, 8, 9, 11, 12	RFLP	Xiao et al. (1998)
	O. rufipogon	IRGC105491	MR219	AB-QTL	CIM	6	SSR	Wickneswari et al. (2012)

Trait	Donor species	Donor accession	Recurrent parent(s)	Type of mapping population†	QTL mapping analysis‡	Chromosome location§	Type of marker¶	Reference
	O. rufipogon	IRGC105491	Hwaseongbyeo	NIL	ANOVA	9	SSR	Xie et al. (2008)
	O. rufipogon	IRGC105491	Hwaseongbyeo	AB-QTL	SPA, IM	8	SSR	Jin et al. (2009)
	O. rufipogon	IRGC105491	Ce64&V20A, Caiapó, Hwacheong, Jefferson, IR64	AB-QTL	IM, CIM	3	SSR	McCouch et al. (2007)
	O. rufipogon	IC22015	IR 58025A	AB-QTL	IM, CIM	2, 9	SSR	Marri et al. (2005)
	O. rufipogon	W1944	Hwayeongbyeo	IL	SPA, IM	1	SPA, IM	Yuan et al. (2009)
	O. rufipogon	YJCW	93-11	AB-QTL	SPA, IM, CIM	1	SSR	Fu et al. (2010)
Brown rice yield	*O. glaberrima*	IRGC103544	V20A	AB-QTL	SPA, IM, CIM	12	RFLP, SSR	Li et al. (2004)
Grain yield per plant	*O. glaberrima*		IR64	BC_2F_3	SPA	2, 6, 8, 9	SSR, STS	Bimpong et al. (2011)
	O. glaberrima	IRGC103544	V20A	AB-QTL	SPA, IM, CIM	12	RFLP, SSR	Li et al. (2004)
	O. rufipogon		MR219	AB-QTL		1	SSR	Bhuiyan et al. (2011)
	O. rufipogon	G52-9	Yuexiangzhan	AB-QTL	CIM	1, 2, 3		Jing et al. (2010)
	O. rufipogon	IC22015	IR 58025A	AB-QTL	IM, CIM	2, 9	SSR	Marri et al. (2005)
	O. rufipogon	IRGC105491	IR64	AB-QTL	SPA, IM, CIM	1	RFLP, SSR	Septiningsih et al. (2003)
	O. rufipogon	IRGC105491	Hwaseongbyeo	NIL	ANOVA	9	SSR	Xie et al. (2008)
	O. rufipogon	IRGC 105491	V20A, V20B	BC_2	ANOVA	1, 2, 8	RFLP	Xiao et al. (1998)
Yield	*O. glaberrima*	IRGC103544	Milyang 23	BC_3F_2	SPA	2, 3, 4, 6, 8	SSR	Suh et al. (2005)
	O. glaberrima	IRGC103544	Milyang 23	BC_2F_5		2	SSR	Kang et al. (2008)

Trait	Donor species	Donor accession	Recurrent parent(s)	Type of mapping population†	QTL mapping analysis‡	Chromosome location§	Type of marker¶	Reference
	O. grandiglumis	IRGC101154	Hwaseongbyeo	AB-QTL	SPA	2	SSR	Yoon et al. (2006)
	O. minuta	IRGC101144	Hwaseongbyeo	NIL		7	SSR	Balkunde et al. (2013)
	O. meyeriana	Y73	IR24	RIL	CIM	6	SSR, STS	Chen et al. (2012)
	O. rufipogon		MR219	AB-QTL		4	SSR	Bhuiyan et al. (2011)
	O. rufipogon	IC22015	IR58025A	AB-QTL	IM, CIM	1, 2, 8	SSR	Marri et al. (2005)
	O. rufipogon	IRGC105491	Jefferson	AB-QTL	SPA, IM, CIM	2, 3, 6, 9	RFLP, SSR	Thomson et al. (2003)
	O. rufipogon	IRGC105491	IR64	AB-QTL	SPA, IM, CIM	1	RFLP, SSR	Septiningsih et al. (2003)
	O. rufipogon	IRGC105491	V20A, V20B	BC_2	ANOVA	1, 2, 8, 12	RFLP	Xiao et al. (1998)
	O. rufipogon	IRGC105491	Hwaseongbyeo	AB-QTL	SPA, IM	8	SSR	Jin et al. (2009)
	O. rufipogon	YJCW	93-11	AB-QTL	SPA, IM, CIM	1	SSR	Fu et al. (2010)
Harvest index	*O. glaberrima*		IR64	BC_2F_3	SPA	2, 7	SSR, STS	Bimpong et al. (2011)
	O. rufipogon	IC22015	IR58025A	AB-QTL	IM, CIM	2	SSR	Marri et al. (2005)

† Abbreviations for mapping population types are: AB-QTL, advanced backcross-quantitative trait locus; IL, inbred line; NIL, near isogenic line; RIL, recombinant inbred line.

‡ Abbreviations for QTL analysis method are: ANOVA, analysis of variance; CIM, composite interval mapping; IM, interval mapping; MIM, multiple interval mapping; SPA, single point analysis.

§ Only the chromosomes where the QTL increase is attributed to the wild parent are listed.

Abbreviations for types of markers are: RAPD, random amplified polymorphic DNA; RFLP, restriction fragment length polymorphism; SNP, single nucleotide polymorphism; SSR, simple sequence repeat; STS, sequence-tagged site.

Table 2. Summary of QTLs for improved yield and yield components attributed to the non-*O. sativa* parent.

4.1. Yield enhancing QTL from exotic *Oryza* genomes

Several plant traits directly or indirectly affect rice grain yield including days to heading and maturity; plant height; panicle length; number of panicles per plant, spikelets per panicle and

grains per panicle; seed set; grain weight; grain size and shape; and shattering (Table 2). The most important yield components in rice are the number of panicles per plant, number of grains per panicle, and grain weight (Chen et al. 2012; Lee et al. 2004; Septiningsih et al. 2003; Thomson et al. 2003). Yield improvement can be achieved as a result of the vast allelic diversity for these traits found in interspecific populations, especially number of grains per panicle which has proven to have the greatest relevance for rice breeding programs (Li et al. 1998; Liu et al. 2008; Tian et al. 2006).

Modern rice varieties are developed after an extensive selection process to improve a few targeted traits related to cultivation and end-use quality but primarily those associated with yield components, such as resistance to shattering, compact growth habit and improved seed germination (Tanksley and McCouch 1997). This prolonged breeding procedure can lead to a reduction in the genetic variability found in modern cultivated rice (Rangel et al. 2008), thus identifying genetic sources for agronomically important traits from wild *Oryza* species and introgressing them into cultivated rice is desirable and necessary. Although wild *Oryza* species are inferior in grain yield, especially when compared to cultivated rice, transgressive segregation resulting from a cross between cultivated rice and a wild *Oryza* species, especially the ancestral species, *O. rufipogon* and *O. nivara*, revealed the presence of favorable alleles from the wild parent that can increase yield in the genetic background of cultivated rice (Brar and Singh 2011). Xiao et al. (1996) developed a genetic population by initially crossing the cytoplasmic male sterile parent, V20A, with *O. rufipogon* (IRGC105491), the donor, as male parent. Subsequently, F_1 plants and later BC_1 plants selected for vigor, plant type, maturity and fertility, were backcrossed to V20B (maintainer line of V20A). A selected subset of 300 BC_2F_1 lines was crossed with Ce64 to create the genotype of the Chinese hybrid rice variety V/64, developed from V20A x Ce64. Xiao et al. (1996) reported that 15% of the testcross families outperformed the recurrent parent, 14% of the yield improvement was related to grains per plant and 56% was related to 1000-grain weight. Subsequently, Xiao et al. (1998) identified 35 QTLs associated with yield improvement, 19 of which, including *yld1.1* and *yld 2.1*, were located on chromosomes 1 and 2, respectively. They also observed no undesirable alleles causing negative effects on yield components, thus the presence of alleles in wild *O. rufipogon* can improve rice yields.

Other AB-QTL populations developed using the same *O. rufipogon* (IRGC105491) donor parent with recurrent parents representing various *O. sativa* subpopulations including *indica* with IR64 (Septiningsih et al. (2003), upland *tropical japonica* with Caiapó (Moncada et al. 2001), irrigated *tropical japonica* with Jefferson (Thomas et al. 2003), and *temperate japonica* with Hwaseongbyeo (Xie et al. 2006 & 2008), revealed enhanced yield and yield components attributed to the *O. rufipogon* donor parent. Selected progeny lines were advanced to BILs or NILs and this yield enhancement was confirmed in field studies with the recurrent parents IR64 (Cheema et al. 2008a), Jefferson (Imai et al. 2013), and Hwaseongbyeo (Jin et al. 2011). Also, an epistatic interaction was noted between the QTLs for grain weight on chromosomes 8 and 9 in the Hwaseongbyeo background (Jin et al. 2011).

Tian et al. (2006) selected an introgression line, SIL040, from the BC_4F_4 lines of *O. rufipogon* (Dongxiang) x Guichao 2, an *indica* rice cultivar. High resolution QTL mapping and analysis

in the SIL040 x Guichao 2 F_2 progeny for yield components revealed *gpa7* (grains per panicle) on the short arm of chromosome 7. This QTL contained five putative genes associated with five panicle traits: panicle length, number of primary and secondary branches per panicle, and number of grains on primary and secondary branches, in the same region. These findings supported the importance of *gpa7* in rice domestication and yield enhancement.

Two AB-QTL populations were developed using the *O. rufipogon* identified as YJCWR (collected from Yuanjiang County, Yunnan Province, P.R. China) as a donor, and TeQing, a popular *indica* cultivar (Tan et al. 2008) and 93-11, a two-line elite *indica* restorer (Fu et al. 2010), as the recurrent parents. Both studies revealed QTL attributed to *O. rufipogon* having a beneficial effect on yield related traits. A CSSL library of 120 lines selected from the TeQing AB-QTL population and evaluated at two locations confirmed a yield advantage associated with *O. rufipogon* alleles for all traits evaluated except 1000-grain weight (Tan et al. 2007).

Similarly, a CSSL library composed of 133 lines selected from an AB-QTL population with an *O. rufipogon* collected in Hainan Province, P.R. China, as the donor, and TeQing as the recurrent parent was used to identify *spd6*, a gene on chromosome 6 responsible for the small panicle and dwarf traits (Shan et al. 2009). This gene, *spd6*, had pleiotropic effects on panicle number per plant, grain size, grain weight, grain number per panicle and plant height, suggesting it may have played a role in the domestication of rice.

To identify the genetic potential of *O. glumaepatula* ($A^{gp}A^{gp}$ genome) as a genetic resource for cultivar improvement, Brondani et al. (2002) developed an AB-QTL population using RS-16, an accession of the Amazonian native rice wild species, *O. glumaepatula*, as the donor parent, and BG90-2, a Latin American *indica* rice, as the recurrent parent. QTL analysis of 96 BC_2F_2 progenies for eleven agronomic traits with *O. glumaepatula* alleles revealed none were positively associated with yield traits. However, several BC_2F_2 lines indicated the presence of introgressed alleles related to yield improvement which were not detected in the QTL analysis. Later, Rangel et al. (2008) evaluated the agronomic performance of 35 BC_2F_8 ILs selected from this population over two years and in multiple locations by measuring grain yield and grain quality traits. The six highest yielding ILs had the highest percentage of recurrent parent genomic background. One of the six ILs, CNAi 9930, was recommended for cultivar release due to its good cooking and milling qualities, and high yield stability. Also, all six ILs contained novel alleles, thus were incorporated as parents in the breeding program for developing high yielding cultivars.

BILs in the $BC_5F_{5:6}$ were derived from *O. grandiglumis* (IRGC101154; CCDD) as the donor parent, and Hwaseongbyeo (Yoon et al. 2006). One BIL, HG101, was backcrossed, and evaluation of the targeted IL, CR1242, revealed the beneficial effect of the *O. grandiglumis* allele on yield and yield components. Further analysis of the QTL, *tgw11*, on chromosome 11 associated with 1000-grain weight showed that a single gene in this QTL controls three grain traits: grain weight, grain width and grain thickness (Oh et al. 2011).

To evaluate the effect of *O. minuta* (IRGC101141) with a BBCC genome on yield components, a single plant, WH79006, was selected from the Hwaseongbyeo x *O. minuta* BC_5F_3 families and selfed (Jin et al. 2004). QTL analysis of Hwaseongbyeo x WH79006 $F_{2:3}$ progeny identified four

QTLs, *sw7* (seed width), *sl11* (seed length), *tsw7* (1000-seed weight) and *lw10* (seed length to width ratio). Similarly, WH29001 was selected from the BC_5F_3 families, selfed and by QTL analysis the co-located QTLs for days to heading, *dth6* and *dth8*, and awn length, *awn6* and *awn8*, were identified on chromosomes 6 and 8, respectively (Linh et al. 2006). Subsequently, a new QTL, *spp7*, for spikelets per panicle, was detected on the long arm of chromosome 7 with the allele attributed to the *O. minuta* parent and validated in the F_3 and F_4 progeny (Linh et al. 2008). Similarly the introgression line IR71033-121-15 was selected from the BC_3 progeny of the same *O. minuta* (IRGC101141) x *indica* cultivar, IR31917. To introgress the *O. minuta* genome into *japonica*, IR71033-121-15 was crossed with Junambyeo, a Korean *japonica* cultivar, and QTL analysis of F_2 progeny identified 14 QTLs associated with agronomic traits reported in previous studies and 22 novel QTLs related to yield components (Rahman et al. 2007).

Awns are an important trait in wild rice species because it protects the seeds from birds and other animals. By contrast, the majority of modern rice cultivars have short awns so that it is easier to harvest the seed. This trait is reported to be controlled by several genes located in different chromosomes, including *An-1* on chromosome 3, *An-2* and *an-5(t)* on chromosome 4, and *An-3* on chromosome 5 (Hu et al. 2011; Nagao and Takahashi 1963; Takamure and Kinoshita 1991). *O. meridionalis* has long awns, ranging in length from 7.8-10.3 cm (Vaughan 1994) and two genes, *An7* and *An8*, associated with the trait, were identified on chromosomes 5 and 4, respectively (Kurakazu et al. 2001). Analysis of *O. meridionalis* x *O. sativa* $BC_4F_{2:6}$ and $BC_4F_{2:8}$ revealed the presence of two dominant genes at different locations on chromosome 1, *An9* and *An10*, and a new allele, *An6-mer* on chromosome 6 (Matsushita et al. 2003a). Another study of an *O. sativa* x *O. glumaepatula* population also identified new alleles, *An7* and *An8* (Matsushita et al. 2003b), thus confirming awn length is controlled by several genes.

A doubled haploid (DH) population was developed from Caiapó (*tropical japonica*, recurrent parent) x *O. glaberrima* (donor parent, IRGC103544, MG12) BC_3F_1 progeny (Aluko et al. 2004). This population was evaluated for agronomic traits including yield and yield components in Colombia and Louisiana, USA (Gutiérrez et al. 2010). Strong segregation distortion was found on chromosomes 3 and 6 indicating the presence of interspecific sterility genes. Evaluation of the phenotypic data revealed transgressive segregation for several traits. A set of 34 CSSLs was selected from Koshihikari, an elite *temperate japonica* rice cultivar (recurrent parent) x *O. glaberrima* (donor parent, IRGC104038) BC_5F_1 progeny, advanced to the F_7 generation, and genotyped with 142 SNP markers (Shim et al. 2010). QTL analysis of phenotypic data from field grown plants revealed 105 putative QTL of which 84 were positive with 64 being related to grain yield components, suggesting the possible use of favorable alleles in *O. glaberrima* for improvement of cultivated rice.

These studies give several examples of QTL or genes for yield and yield components being attributed to the wild donor parent not only the ancestral A-genome species, *O. rufipogon* or *O. nivara*, but also in the more distant tetraploid *O. minuta* with a BBCC genome. These observations confirm that not only single genes and alleles are affecting these traits but there are epistatic interactions and epigenetic interactions, as well as environmental factors affecting many of these traits, resulting in the phenomenon often described as transgressive variation. Currently, six CSSL libraries are under development with three different *O. rufipogon*/*O.*

nivara donor accessions, representing the *Indica*, *Japonica* and independent groups of *O. rufipogon* (Huang et al. 2012), and two recurrent parents, IR64, an *indica* representing the *Indica* subspecies, and Cybonnet, a U.S. *tropical japonica*, representing the *Japonica* subspecies to further explore these interactions resulting in transgressive variation (Tung et al. 2010; SR McCouch, Cornell University and GC Eizenga, personal communication).

4.2. Genes for sterility

Reproductive barriers, such as crossability, hybrid seed inviability, hybrid sterility and hybrid breakdown, have significantly limited the success of interspecific hybridization between *O. sativa* and non-A genome *Oryza* species. Several studies reported the production of F_1 seeds by crossing male sterile lines and *Oryza* species (Huang et al. 2001; Luo et al. 2000). The crossability rate between *O. sativa* and other *Oryza* species vary; however, the rate of crossability between A-genome and non-A genome diploid *Oryza* species is higher than with tetraploid *Oryza* species, none of which has an A-genome (Jena and Khush 1989 & 1990; Yasui and Iwata 1991).

The phenomenon of transmission ratio distortion (TRD) where one allele is transmitted more frequently than the opposite allele in interspecific and intraspecific hybrids has been discovered in a broad range of organisms and is often a reproductive barrier (Koide et al. 2012). Recently, Koide et al. (2012) identified a unique sex-independent TRD (*si*TRD) where one allele is preferentially transmitted through both the male and female parent derived from *O. rufipogon* (W593). This research showed the S_6 allele on chromosome 6 is responsible for the *si*TRD allele and influenced by other unlinked modifiers. The locus, *sa1*, conferring pollen sterility was found in *O. glaberrima* (W025) x T65*wx* progeny (Sano 1990) where T65*wx* is an NIL derived from Taichung 65 (*japonica* rice) x Peiku (*indica* rice) with a Taichung 65 background and the Peiku *waxy* gene on chromosome 6. Other studies identified several pollen sterility loci, *S-1, S3, S18, S19, S20, S21, s25, s27, S29, S29(t)* and *s36*, in populations resulting from interspecific hybridizations between various *O. sativa* cultivars and the *Oryza* species, *O. glumaepatula*, *O. glaberrima* and *O. nivara* (Doi et al. 1998 & 1999; Hu et al. 2006, Sano 1983 & 1986; Taguchi et al. 1999; Win et al. 2009). To overcome such barriers, several methods have been suggested including anther culture, backcrossing, marker-assisted selection (MAS) and asymmetric somatic hybridization, (Fu et al. 2008; Sarla et al. 2005). Also, Deng et al. (2010) demonstrated the fertility in *O. glaberrima* x *O. sativa* crosses could be improved by using an *O. sativa* bridging parent. The bridging parent had the *O. glaberrima* sterility gene, *S1-g* on chromosome 6, introgressed into the particular *O. sativa* cultivar background.

4.3. Grain quality traits

Acceptable rice grain quality is a major goal of rice breeding programs worldwide because it determines the acceptability of cooked rice to the consumer. Grain quality is a combination of several components including milling efficiency, physical appearance, cooking and eating characteristics, and nutritional quality (Aluko et al. 2004; Li et al. 2004). A few interspecific populations were evaluated for grain quality traits (Table 3). These studies showed the *Oryza* parent affects the apparent amylose content, alkali spreading value, protein content, rice bran

percentage, milled rice percentage and seed size. What is desirable for these traits is determined for the most part, by consumer preference and marketing classes. When selecting for these traits, often the grain quality of the recurrent parent is preferred.

Most interesting was the BC_3F_1 progeny of the Caiapó x *O. glaberrima* (IRGC103544, MG12) doubled haploid population (Aluko et al. 2004). For this population, the QTL analysis revealed 27 QTLs associated with rice quality of which seven QTLs including percent rice bran, percent milled rice, alkali spreading value (inversely related to gelatinization temperature), percent protein and grain dimensions (length to width ratio), were traced to alleles originating from the *O. glaberrima* parent.

Trait	Donor species	Donor accession	Recurrent parent(s)	Mapping popu-lation†	QTL mapping analysis‡	Chromo-some location§	Type of mark-er¶	References
Grain Quality								
Apparent amylose content	*O. glaberrima*	IRGC103544	V20A	AB-QTL	SPA, IM, CIM	6, 12	RFLP, SSR	Li et al. (2004)
	O. grandiglumis	IRGC101154	Hwaseong-byeo	AB-QTL	SPA	3, 5, 7	SSR	Yoon et al. (2006)
	O. nivara	IRGC81848	Swarna	BC_2F_2	IM, CIM	2	SSR	Swamy et al. (2012)
	O. nivara	IRGC100195	M-202	AB-QTL	MIM	6	SSR	Eizenga et al (accepted)
	O. rufipogon	IRGC105491	IR64	AB-QTL	IM, CIM	6	RFLP, SSR	Septiningsih et al. (2003b)
Alkali spreading value (ASV) or gel consistency	*O. glaberrima*	IRGC103544	Caiapó	BC_3F_1	IM, CIM	6	SSR	Aluko et al. (2004)
	O. glaberrima	IRGC103544	V20A	AB-QTL	SPA, IM, CIM	12	RFLP, SSR	Li et al. (2004)
	O. nivara	IRGC100195	M-202	AB-QTL	MIM	6	SSR	Eizenga et al. (accepted)
	O. rufipogon	IRGC105491	IR64	AB-QTL	IM, CIM	6	RFLP, SSR	Septiningsih et al. (2003b)
Kernel elongation	*O. glaberrima*	IRGC 103544	V20A	AB-QTL	SPA, IM, CIM	3	RFLP, SSR	Li et al. (2004)
Protein	*O. glaberrima*	IRGC103544	Caiapó	BC_3F_1	IM, CIM	2, 6	SSR	Aluko et al. (2004)
	O. glaberrima	IRGC103544	V20A	AB-QTL	SPA, IM, CIM	8	RFLP, SSR	Li et al. (2004)

Trait	Donor species	Donor accession	Recurrent parent(s)	Mapping popu-lation†	QTL mapping analysis‡	Chromo-some location§	Type of mark-er¶	References
Percent rice bran	*O. glaberrima*	IRGC103544	Caiapó	BC_3F_1	IM, CIM	4, 7	SSR	Aluko et al. (2004)
Percent milled rice	*O. glaberrima*	IRGC103544	Caiapó	BC_3F_1	IM, CIM	5	SSR	Aluko et al. (2004)
	O. nivara	IRGC81848	Swarna	BC_2F_2	IM, CIM	1	SSR	Swamy et al. (2012)
Biotic Stress-Diseases								
Bacterial blight	*O. australiensis*		IR31917-45-3-2	MAAL		12		Multani et al. (1994)
	O. brachyantha O. longistaminata O. officinalis			AIL		5, 6, 8, 11	SSR	Hechanova et al. (2008)
	O. latifolia	IRGC100914	IR31917-45-3-2	AIL	ANOVA	12, others	SSR, SNP, STS, InDel	Angeles-Shim et al. (accepted)
	O. longistaminata	WLO2	BS125	NIL		11	RFLP, RAPD	Ronald et al. (1992)
	O. longistaminata		IR24			11		Khush et al. (1990)
	O. meyeriana	Y73	IR24	RIL	CIM	1, 3, 5, 10, 11	SSR, STS	Chen et al. (2012)
	O. minuta	78-1-5	IR24	F_2- BC_1		6	RAPD, AFLP	Gu et al. (2004)
	O. nivara	IRGC81825	PR114	RIL, BIL, IL	SMA-IM	4	SSR, STS	Cheema et al. (2008)
Blast disease	*O. australiensis*	IRGC100882	Lijiangxintuan-heigu			6	CAPS, SSR, STS	Jeung et al. (2007)
	O. minuta	IRGC101141	IR31917	F_2		6	RAPD	Liu et al. (2002)
	O. nivara	IRGC100898	Bengal	AB-QTL	MIM	8	SSR	Eizenga et al. (2013)
	O. nivara	IRGC104705	Bengal	AB-QTL	MIM	8, 12	SSR	Eizenga et al. (2013)

Trait	Donor species	Donor accession	Recurrent parent(s)	Mapping population†	QTL mapping analysis‡	Chromosome location§	Type of marker¶	References
	O. rufipogon	IRGC104812	Koshihikari	IL		3, 11		Hirabayashi et al. (2010); Sobrizal et al. (1999)
	O. rufipogon	IRGC104814	Koshihikari	IL		3, 5, 6		Hirabayashi et al. (2010)
Sheath blight disease	*O. nivara*	IRGC100898	Bengal	AB-QTL	MIM	6	SSR	Eizenga et al. (2013)
	O. nivara	IRGC104705	Bengal	AB-QTL	MIM	3, 6	SSR	Eizenga et al. (2013)
Stem rot	*O. rufipogon*	IRGC100912 (87-Y-550)	L-201 (long grain-breeding lines)	F_2	SPA	2, 3	AFLP	Ni et al. (2001)
Grassy stunt virus	*O. nivara*	IRGC101508	IR8, IR20, IR22, IR24, IR773A-1-3	F_2				Nuque et al. (1982)
Rice stripe necrosis resistance	*O. glaberrima*	IRGC103544	Caiapó	CSSL	IM, CIM	11	SSR	Gutiérrez et al. (2010)
Biotic Stress-Insects								
Brown planthopper	*O. australiensis*	IRGC100882	IR31917-45-3-2	IL		12	RFLP	Ishii et al. (1994)
	O. australiensis	IR65482-7-216-1-2	Jinbubyeo	F_2, BC_2F_2	ANOVA	12	SSR, STS	Jena et al. (2006)
	O. australiensis		IR31917-45-3-2	MAAL		12		Multani et al. (1994)
	O. eichingeri	IRGC105159	2428	F_2, BC_1F_1		2	RFLP, SSR	Guoqing et al. (2001)
	O. latifolia	B14	Taichung	RIL		4	SSR, RFLP	Yang et al. (2002)
	O. minuta	101141	Junambyeo	F_3		4, 12	SSR, STS	Rahman et al. (2009)
	O. officinalis	IRGC100878, IRGC100896, IRGC101150,	IR31917-45-3, IR25587-109-3	BC_2F_8		4, 10, 12	RFLP	Jena et al. (1992)

Trait	Donor species	Donor accession	Recurrent parent(s)	Mapping popu-lation†	QTL mapping analysis‡	Chromo-some location§	Type of mark-er¶	References
		IRGC101412, IRGC102385						
	O. officinalis	IR54745-2-21-12-1 7-6	IR50			3	RAPD, STS	Renganayaki et al. (2002)
	O. officinalis	B5	1826, 93-11			3, 4	SSR	Li et al. (2006)
	O. officinalis	IRGC100896	IR31917-45-3-2	F_2		11	RAPD	Jena et al. (2002)
	O. officinalis					3	RFLP	Hirabayshi et al. (1998)
	O. officinalis	IR54745-2-21-12-1 7-6	IR50	RIL		3	RAPD	Renganayaki et al. (2002)
	O. officinalis	B5	Minghui 63	F_2		3	RFLP	Huang et al. (2001)
	O. officinalis	B5		RIL		4	AFLP, RFLP, SSR	Yang et al. (2004)
	O. rufipogon	WBO1	Minghui 63	F_2		4, 8	SSR	Hou et al. (2011)
Green rice leafhopper	*O. glaberrima*	IRGC104038	Taichung 65	NIL	IM, CIM	3, 7, 9, 10	SSR	Fujita et al. (2010)
	O. rufipogon	W1962	Taichung 65	NIL, BC_4F_2		8	SSR	Fujita et al. (2006)
White-backed planthopper	*O. officinalis*	B5	Minghui 63	RIL		3, 4	SSR	Tan et al. (2006b)
	O. rufipogon	BILs-DWR/ Dingxiang	XieqingzaoB	BIL	CIM, MIM	2, 5, 9	SSR	Chen et al. (2010)
Abiotic stress								
Aluminum tolerance	*O. rufipogon*	IRGC106424	IR64	RIL	IM	1, 3, 9 (2, 7, 8)	RFLP	Nguyen et al. (2003)
Drought tolerance	*O. rufipogon*		Guichao 2	IL	SMR	2, 12	SSR	Zhang et al. (2006)
	O. rufipogon	W630	Nipponbare	BIL	IM	1, 5	SSR	Thanh et al. (2011)

Trait	Donor species	Donor accession	Recurrent parent(s)	Mapping population†	QTL mapping analysis‡	Chromosome location§	Type of marker¶	References
Heat tolerance	*O. rufipogon*	YJCWR	TeQing	IL	CIM	1	SSR	Lei et al. (2013)
Low temperature tolerance	*O. nivara*	IRGC100195	M-202	AB-QTL	MIM	5	SSR	Eizenga et al. (accepted)
	O. rufipogon	W1943	Guang-lu-ai 4	BC_4F_2	IM, CIM	3, 11	SNP	Koseki et al. (2010)
	O. rufipogon	Dongxiang	Nanjing11	BC_2F_1	CIM	10	SSR	Xia et al. (2010)
	O. rufipogon	W1944	Hwayeong-byeo	RIL	SPA, CIM	2, 5	SSR	Lee et al. (2005)
Salt tolerance	*O. rufipogon*		TeQing	IL	SPA	1, 2, 3, 6, 7, 10	SSR	Tian et al. (2011)
Submergence stress	*O. rufipogon*					9		Li et al. (2011)
Biomass	*O. glaberrima*		IR64	BC_2F_3	SPA	1, 2, 3, 6, 10	SSR, STS	Bimpong et al. (2011)

† Abbreviations for mapping population types are: AB-QTL, advanced backcross-quantitative trait locus; AIL, alien introgression line; BIL, backcrossed inbred line; CSSL, chromosome segment substitution line; IL, inbred line; MAAL, monosomic alien addition line; NIL, near isogenic line; RIL, recombinant inbred line.

‡ Abbreviations for QTL analysis method are: ANOVA, analysis of variance; CIM, composite interval mapping; IM, interval mapping; MIM, multiple interval mapping; SMR, single marker analysis; SPA, single point analysis.

§ Only the chromosomes where the QTL increase is attributed to the wild parent are listed.

Abbreviations for types of markers are: AFLP, amplified fragment length polymorphism; CAPS, cleaved amplified polymorphic sequence, InDel, insertion-deletion polymorphism, RAPD, random amplified polymorphic DNA; RFLP, restriction fragment length polymorphism; SNP, single nucleotide polymorphism; SSR, simple sequence repeat; STS, sequence-tagged site.

Table 3. Summary of QTLs for grain quality, biotic stress tolerance, abiotic stress tolerance and biomass attributed to the non-*O.sativa* parent.

4.4. Resistance to biotic stress

4.4.1. Disease resistance

Pathogenic microorganisms, such as fungi, oomycetes, viruses and bacteria, and pests, such as insect herbivores, significantly reduce rice seed yield and quality. The most destructive rice diseases include bacterial blight caused by *Xanthomonas oryzae* pv. *oryzae* Ishiyama Dye (Cheema et al. 2008), blast caused by the fungus *Magnaporthe oryzae* B. Couch (Couch and Kohn 2002), and sheath blight caused by the soil-borne fungus *Rhizoctonia solani* Kühn (Zhang 2007). The first reported successful introduction of an agronomically important trait from a

wild *Oryza* species was the introgression of grassy stunt virus resistance from the AA-genome species *O. nivara* into the cultivated *O. sativa* genetic background (Khush et al. 1977). The resistance mechanism was subsequently introduced into several other rice cultivars (Sanchez et al. 2013). Since this first introduction, wild *Oryza* accessions have been screened as a potential source of resistance genes to biotic and abiotic stresses, as well as, yield and yield components, as previously discussed. These screening efforts, including successful introduction of stress resistance genes from *Oryza* species were recently summarized by Ali et al. (2010), Brar and Singh (2011) and Sanchez et al. (2013). Table 3 summarizes the efforts to identify the chromosome location of stress resistance genes introduced from the wild *Oryza* species by QTL and fine mapping analyses.

Seed yield losses due to bacterial blight were reported to be up to 75% in India, Indonesia, and the Philippines, and 20 to 30% in Japan (Mew et al. 1993; Nino-Liu et al. 2006). Thus far 31 bacterial blight resistance genes have been reported and six of these were identified in species other than *O. sativa*. These resistance genes were identified in several wild *Oryza* species, including *Xa21* in *O. longistaminata*, *Xa23* in *O. rufipogon*, *Xa27* in *O. minuta* (IRGC101141), *Xa29*(t) in *O. officinalis* (B5), and *Xa30*(t) in *O. nivara* (IRGC81825) (Brar and Singh 2011; Cheema et al. 2008b; Gu et al. 2004; Khush et al. 1990; Tan et al. 2004; Zhang et al. 1998). Most recently, *Xa34*(t) exhibiting broad spectrum resistance, was identified in *O. brachyantha* (IRGC101232) as a single dominant gene after examining the crossed progeny of two resistant introgression lines derived from IR56 (recurrent parent) and *O. brachyantha* (Ram et al. 2010a).

Both bacterial blight and blast resistance were identified in the tetraploid CCDD genome species, *O. minuta* (IRGC101141). To transfer these resistance genes into the background of diploid, cultivated rice, Amante-Bordeos (1992) used embryo rescue and backcrossing to produce interspecific hybrids between the elite *O. sativa* line, IR31917-45-3-2 (recurrent parent) and *O. minuta* (donor parent).

Lastly, the line Y73 was selected for a high level of bacterial blight resistance from the hybrid progeny of an asymmetric somatic hybridization between a resistant *O. meyeriana* and a *O. sativa* subsp. *japonica* cultivar (Yan et al. 2004). Subsequently, Chen et al. (2012) developed a RIL population from Y73 x IR24 (*O. sativa*) and identified five QTLs, two were major QTLs located on chromosomes 1 and 5, and three were minor QTLs on chromosomes 3, 10 and 11. They also mapped 21 additional QTLs related to yield components and yield.

Blast is considered the most destructive fungal disease in irrigated rice. The symptoms include lesions on leaves, stems, peduncles, panicles, seeds and roots (Savary and Willocquet 2000; Khush et al. 2009). To date, 41 blast resistance genes have been reported; however, there are only two genes, *Pi9* and *Pi40(t)*, that have been identified in wild *Oryza* species, *O. minuta* and *O. australiensis*, respectively. *Pi40(t),* which confers broad spectrum of blast resistance, was introgressed into the elite breeding line, IR65482-4-136-2-2 (Liu et al. 2002; Jeung et al. 2007). Qu et al. (2006) cloned the *Pi9* gene via a positional (map-based) cloning technique and found the gene is a member of a group of six resistance-like genes, which encodes a nucleotide-binding site (NBS) and leucine-rich repeats (LRRs). Silué et al. (1992) screened 99 *O. glaberrima* accessions for blast resistance by inoculating with ten *M. oryzae* strains from Cote d'Ivoire. The results revealed that nine accessions were resistant to all *M. oryzae* strains and 32 accessions

were moderately resistant, suggesting these accessions may be the source of novel resistance genes. Eizenga et al. (2009) identified resistance to U.S. blast races in some *Oryza* species but no accession exhibited resistance to all races. Subsequently, two AB-QTL populations with two different resistant *O. nivara* (IRGC100898; IRGC104705) accessions as donor parents x Bengal, a U.S. medium grain *tropical japonica*, as recurrent parent were evaluated for reaction to two U.S. blast races. QTL analysis of these populations mapped resistance to U.S. leaf blast races *IB1* and *IB49* to chromosomes 8 and 12 (Eizenga et al. 2013).

Rice sheath blight, *Rhizoctonia solani* Kühn, was reported for the first time in Japan in 1910 and since then, it has spread to many rice growing areas worldwide (Lee and Rush 1983; Savary et al. 2000). To date, no major resistance gene(s) that confers complete resistance to sheath blight has been discovered, only genes conferring partial resistance (Pinson et al. 2005; Jia et al. 2009). Several *Oryza* species accessions were screened for sheath blight resistance at the International Rice Research Institute (IRRI) in the Philippines with resistance being identified in accessions of *O. minuta* and *O. rufipogon* (Amante et al. 1990), and a resistant *O. officinalis* accession being the donor of sheath blight resistance genes in *O. sativa* introgression lines (Lakshmanan, 1991). Prasad and Eizenga (2008) screened a collection of 73 *Oryza* species accessions using three different methods and identified seven accessions with moderate resistance including three *O. nivara* accessions and one each of *O. barthii*, *O. meridionalis*, *O. nivara*/*O. sativa* hybrid, and *O. officinalis*. Based on these results, Eizenga et al. (2013) developed two AB-QTL populations using two of these *O. nivara* accessions (IRGC100898; IRGC104705) as the donor parents, and Bengal as the recurrent parent for both populations. QTL analysis identified a major QTL, *qShB6*, associated with sheath blight attributed to the wild rice parent. Two other minor QTLs, *qShB1* and *qShB3*, were identified but not attributed to the *O. nivara* parent in all sheath blight tests. A third AB-QTL population with the more distant A-genome species, *O. meridionalis* (IRGC105306), as the donor parent, and Lemont, a U.S. long grain *tropical japonica*, as the recurrent parent, was evaluated for reaction to sheath blight disease and the QTL analysis is currently underway (Eizenga, unpublished data).

Stem rot, a fungal disease caused by *Sclerotium oryzae* Catt., causes yield losses by reduced tillering, more unfilled grains per panicle, chalky grain, lower milling yields and increased lodging (Ou 1985). The germplasm line 87-Y-550 (PI566666) was derived from a cross between the stem rot resistant *O. rufipogon* (IRGC100912) and L-201, a long grain California (USA) *temperate japonica* cultivar (Tseng and Oster 1994). To identify the location of this resistance gene and associated molecular markers, Ni et al. (2001) developed four RIL populations from crosses between 87-Y-550 and four susceptible long grain *O. sativa* breeding lines. Following the bulk segregant analysis method, QTLs revealed an association between the AFLP marker, TAA/GTA167, on chromosome 2 and SSR marker, RM232, on chromosome 3.

African cultivated rice, *O. glaberrima*, is an excellent, potential donor of genes to improve the tolerance of Asian cultivated rice, *O. sativa*, to biotic stresses, including bacterial blight, rice blast, rice stripe necrosis virus (RSNV), nematodes (*Meloidogyne graminicola* Golden and Birchfield) and especially rice yellow mottle virus, RYMV (Djedatin et al. 2011; Gutiérrez et al. 2010; Ndjiondjop and Alber 1999; Plowright et al. 1999; Silue et al. 1992; Thiémélé et al. 2010). A set of 64 CSSLs selected from a Caiapó x *O. glaberrima* (IRGC103544) DH, BC_3F_1 population

was used to identify a major QTL controlling RSNV resistance on chromosome 11 (Gutiérrez et al. 2010). RYMV is one of the most destructive rice viral diseases. Few RYMV resistance genes have been found in *O. sativa* accessions, but 8% of the *O. glaberrima* accessions that were screened exhibited resistant to the virus with three recessive resistance alleles *rymv1-3*, *rymv1-4*, and *rymv1-5* and one dominant resistance allele, *RYMV1*, were identified. Three lines, TOG5681, TOG5672 and TOG7291 initially derived from the wild *Oryza glaberrima* showed resistance to RYMV, blast, and lodging (Futakuchi et al. 2008; Sié et al. 2002; Thiémélé et al. 2010).

4.4.2. Insect resistance

Genetic resistance is an effective method of protecting rice from insect pests in Asia and the Americas (Kiritani 1979; Way 1990) and avoids the possible environmental contamination associated with chemical control (Zhang 2007). The brown planthopper, *Nilaparvata lugens* (Stål), one of the most devastating herbivores of rice in Asia, causes damage by feeding on rice plants and transmitting two viruses, rice ragged stunt virus and rice grassy stunt virus. A total of 26 brown leafhopper resistance genes have been reported, of which 10 genes are recessive and 18 are dominant. Of the 26 genes, 12 genes, *Bph1, bph2, Bph3, bph4, bph5, Bph6, bph7, bph8, Bph9, bph19, Bph25* and *Bph26,* are found in *O. sativa;* two genes, *Bph10* and *Bph18,* are found in *O. australiensis;* seven genes, *Bph11, bph11, bph12, Bph14, Bph15, bph16* and *Bph17,* in *O. officinalis;* one gene, *Bph13*, in *O. eichingeri;* one gene, *Bph17*, in *O. latifolia*; two genes, *Bph20(t)* and *Bph21(t),* in *O. minuta*; one gene, *bph22(t)* in *O. glaberrima*; and three genes, *Bph22(t), Bph23(t)* and *Bph24(t),* in *O. rufipogon* (Deen et al. 2010; Jena 2010; Oryzabase 2014; Ram et al. 2010, Zhang 2007).

Early efforts to evaluate the *Oryza* species accessions as a source of brown planthopper resistance and incorporation of this resistance into *O. sativa* were in a large part due to the efforts at IRRI. Early reports include introgression of resistance from *O. australiensis* through backcrossing into an *O. sativa* background and using RFLP markers to resolve the position of the gene in chromosome 12 (Ishii et al. 1993). Introgression of resistance to three brown planthopper biotypes from five different *O. officinalis* accessions into cultivated *O. sativa* breeding lines resulted in 52 BC_2F_8 ILs (Jena and Khush 1990; Jena et al. 1992). Genotyping of these lines with RFLP markers showed *O. officinalis* introgressions in 11 of the 12 rice chromosomes with markers on chromosomes 4, 10 and 12 appearing to be associated with BPH resistance but not unequivocally. More recently, a single dominant gene, *bph22(t),* was discovered in *O. glaberrima* and transferred into *O. sativa* (Ram et al. 2010).

The white-backed planthopper, *Sogatella furcifera* (Horvath), is another serious insect pest of rice in Asia (Chen et al. 2010). Six major QTLs, *Wbp1, Wbp2, Wbp3, wbh4, Wbp5* and *Wbp6*, have been identified (Angeles et al. 1981; Hernandez and Khush 1981; Oryzabase 2014; Ravindar et al. 1982; Sidhu and Khush 1979; Min et al. 1991; Wu and Khush 1985). *O. officinalis* has been reported as a source of resistance to both white-backed and brown planthoppers. Further study identified two QTLs, *Wph8(t)* and *Bph15* on chromosome 4, with *wph7*(t) and *Bph14* on chromosome 3 (Huang et al. 2001; Oryzabase 2014; Tan et al. 2004). Chen et al. (2010) developed a BIL population derived from *O. sativa* x *O. rufipogon* and found three QTLs from wild *Oryza*

http://dx.doi.org/10.5772/58393

including *qWph2* on the short arm of chromosome 2, *qWph5* on the long arm of chromosome 5, and *qWph9*, which confers high resistance on the long arm of chromosome 9. In addition, these wild alleles reduced the rate of seedling mortality.

Guo et al. (2013) analyzed 131 BC_4F_2 ILs resulting from a cross between *O. minuta* (IRGC101133) x IR24 (*O. sativa*) by using 164 SSR markers, and estimated the average length of introgressed segments to be 14.78 cM. They observed a range of morphological and yield traits, as well as, resistance to bacterial blight, brown planthopper, and white-backed planthopper among the populations.

Rice monosomic alien addition lines (MAALs) contain the complete *O. sativa* chromosome complement ($2n=24_{AA}$) plus an additional, unpaired chromosome from a wild *Oryza* (alien) donor, thus the designation $2n=24_{AA}+1_{alien}$ (Jena 2010). MAALs have been used to identify important genes conferring resistance to biotic stresses, such as bacterial blight, brown planthopper and white-backed planthopper, from *O. australiensis* (EE) and *O. latifolia* (CCDD) into cultivated *O. sativa* (Multani et al. 1994 & 2003). Recently, Angeles-Shim (accepted) evaluated a set of 27 alien introgression lines developed from the *O. sativa* breeding line IR31917-45-3-2 x *O. latifolia* (IRGC100194) MAALs and identified putative QTLs for resistance to four Philippine races of bacterial blight, as well as, yield components and stem strength.

Green leafhopper [*Nephotettix virescens* (Distant)] is an insect found in wetlands including irrigated and rainfed environments. Six resistance loci, *Grh1*, *Grh2*, *Grh3*, *Grh4*, *Grh5* and *Grh6*, and one QTL, *qGRH4*, conferring resistance to green leafhopper have been reported. *Grh5* was identified in the wild rice, *O. rufipogon*, for the first time. *Ghr6* was identified in both cultivated rice and *O. nivara* (Fujita et al. 2003, 2004 & 2010; Fukuta et al. 1998; Saka et al. 1997; Tamura et al. 1999; Yasui and Yoshimura 1999; Yazawa et al. 1998).

The wild *Oryza* species have been used successfully as a source of novel alleles conferring resistance to both rice diseases and insect pests because in many instances these alleles could be transferred to *O. sativa* by backcrossing and screening the progeny. In fact, several of these alleles were successfully transferred even before the advent of molecular markers. With molecular markers, it is now possible to expedite the introduction of these novel alleles because marker-assisted breeding techniques can be used. With the molecular tools currently available, it should be possible to unravel those resistances which are quantitatively inherited like sheath blight.

4.5. Genes for abiotic stress

Abiotic stresses, including drought and flood, high and low temperatures, high salinity, high aluminum and acid sulfate soils, have a negative impact on rice productivity worldwide. Rice, like other plant species, has evolved to adapt to different environmental stresses using different mechanisms and strategies with multiple sensors. When the sensors identify a stress, a signal transduction pathway is invoked, which activates genes conferring the initial response for short term or long term tolerance to the stress (Grennan 2006; Lexer and Fay 2005). Recent studies identified many genes involved in plant tolerance to abiotic stress, which are classified into two groups based on their products. The first group includes genes that protect the cells

via synthesis of chaperones, a group of proteins that help non-covalent folding and unfolding of other proteins in the cell under stress conditions, and enzymes for protecting metabolites and proteins. The second group includes those genes that regulate stress responses acting as transcriptional factors to control stress genes or by producing hormones (Grennan 2006).

4.5.1. Tolerance to drought and heat

Drought reduces grain yield and affects yield stability in many rainfed regions by decreasing the number of tillers per plant, plant height, number of leaves and leaf width; and delaying anthesis and maturity as shown by Ndjiondjop et al. (2010) using 202 BILs derived from WAB56-104 (*O. sativa*) x CG14 (*O. glaberrima*) to identify the effect of drought on rice agronomic traits. Despite the fact that African rice (*O. glaberrima*) has low productivity and grain yield, it is an excellent source of genes associated with drought tolerance (Blum 1998; Hanamaratti et al. 2008; Manneh et al. 2007; Ndjiondjop et al. 2010).

Bocco et al. (2012) evaluated the morphological and agronomical traits of 60 genotypes including 54 BC_3F_6 introgression lines from IR64 (recurrent parent, elite *indica* cultivar) x TOG5681 (*O. glaberrima*), two parents (IR64 and TOG5681) and four NERICA-L cultivars derived from the same parents, for comparison as controls. These genotypes were evaluated in two time periods corresponding to the dry season under irrigated (control) and drought conditions to identify the most drought tolerance introgression lines. Plant height, spikelet fertility, grain yield and leaf area at harvesting were consistently reduced by drought and values for leaf temperature, leaf rolling, leaf tip drying, leaf blast disease, days to flowering and days to maturity were increased under drought conditions. From this evaluation, five BC_3F_6 lines were identified that out yielded the four NERICA-L cultivars described as drought tolerant.

Several accessions of *O. barthii*, *O. meridionalis* and *O. australiensis* were screened for heat and drought tolerance at the University of Arizona, which is located in a desert environment at Tuscon, Arizona, USA (Sanchez et al. 2013). One of the most tolerant *O. meridionalis* accessions was crossed with M-202, a California (USA) medium grain, *temperate japonica* cultivar. From the backcross progeny, two heat-tolerant advanced backcross lines resembling the M-202 parent were selected for variety release as 'Arizona Rice-1' and 'Arizona Rice-2'.

4.5.2. Tolerance to low temperatures

Low temperatures during the rice growing season causes poor germination, slow growth, withering and anther injury (Andaya et al. 2007; Hu et al. 2008). To cope with cold stress, many plant species including rice have developed several physiological and biochemical pathways for surviving and adapting to stress conditions (Ingram and Bartels 1996; Pastori and Foyer 2002; Hu et al. 2008). Rice is predominately grown in tropical and sub-tropical regions; therefore, many cultivars are sensitive to cold temperature especially during the seedling stage. The optimum temperature range for germination and early seedling growth is 25-30^{o}C, and temperatures below 15-17^{o}C during this period delay plant establishment, reduce plant competitive ability against weeds, delay plant maturity, and decrease grain yield. Improving

cold tolerance at this stage is one of the most effective ways to achieve yield stability and genetic tolerance is the most promising strategy (Andaya and Mackill 2003; Fujino et al. 2004; Koseki et al. 2009). Overall, the *Indica* subspecies is more sensitive to cold stress than *Japonica* rice. Several QTLs associated with cold tolerance have been identified, especially in populations derived from crosses between *Japonica* and *Indica* cultivars (Lu et al. 2007; Zhang et al. 2005).

Wild rice species, such as *O. rufipogon*, contain QTLs that can be integrated into cultivated rice to improve cold tolerance (Koseki et al. 2010). Lee et al. (2005) constructed a RIL population consisting of 120 BC_1F_7 lines derived from a cross between the *japonica* cultivar, Hwayeongbyeo and *O. rufipogon* (W1944). The population was genotyped with 124 SSR markers and evaluated for 20 agronomic traits including leaf discoloration which is associated with cold stress. Of the 63 QTLs identified, there were two QTLs for decreased leaf discoloration, in other words, increased cold tolerance, attributed to the *O. rufipogon* parent. These QTLs, *dc2* located on chromosome 2 and *dc5* on chromosome 5, accounted for 11.2% and 11.1% of the phenotypic variation, respectively. The *O. rufipogon* parent also contributed favorable alleles to panicle length, spikelets per panicle and days to heading.

Koseki et al. (2010) analyzed 184 F_2 introgression lines from crosses of Guang-lu-ai 4 (cold sensitive, *indica* cultivar) x W1943 (cold tolerant, *O. rufipogon*) for cold tolerance at the seedling stage (CTSS). Three *Ctss*-QTLs were detected with those on chromosomes 3 (*qCtss 3*) and 11 (*qCtss*11) attributed to the *O. rufipogon* parent, and on chromosome 10 (*qCtss10*) to Guang-lu-ai 4. The major QTL, *qCtss11*, explained 40% of the phenotypic variation and using backcross progenies, it was fine-mapped to a 60kb candidate region defined by markers AK24 and GP0030 with Os11g0615600 and/or Os11g0615900 hypothesized as the causal gene(s) for cold tolerance.

Seedling cold tolerance was measured in the M-202 (medium grain, U.S. *temperate japonica*) x *O. nivara* (IRGC100195) AB-QTL population using a slant board method [Jones and Petersen 1976; Eizenga et al. (accepted)]. In this study, QTLs for increasing coleoptile length and shoot length were identified in the same region on chromosome 5 and attributed to the *O. nivara* parent. QTLs for increased shoot length and root length were found on chromosome 8 and 6, respectively, and attributed to the M-202 parent.

4.5.3. Tolerance to aluminum and acid soils

Aluminum toxicity is another abiotic stress that causes grain yield reduction especially when rice is grown in an acidic soil (IRRI 1978). If the soil pH falls below 5.5, aluminum will more likely separate from the soil colloids and come into a solution phase. Aluminum at toxic levels slows root development, reduces the plant's ability to take up water and nutrients, and decreases plant growth, consequently reducing grain yield and grain quality (Foy 1992). Application of lime to the soil, reduces soil acidity and improves soil fertility but the results have showed limited success in overcoming the effects of aluminum toxicity. Aluminum tolerance is a quantitative trait and varies among rice species. Both additive and dominance effects contribute to the genetic heritability of aluminum tolerance as documented by the

importance of both general combining ability and specific combining ability (Howeler and Cadavid 1976; Wu et al. 1997).

In the past decade, one *O. rufipogon* (IRGC106424) accession found growing in an acid sulfate soil in Vietnam (Sanchez et al. 2013) has proven to be valuable for improving tolerance to both aluminum and acid sulfate soils in cultivated rice. Initially, Nguyen et al. (2003) evaluated 171 F_6 RILs derived from IR64 (*indica*, susceptible) x *O. rufipogon* (IRGC106424, tolerant) for aluminum tolerance. QTL analysis revealed QTLs for root length under stress conditions attributed to the *O. rufipogon* parent in six different chromosomal regions on chromosomes 1, 2, 3, 7, 8 and 9 that individually explained 9.0–24.9% of the phenotypic variation and were controlled by additive effects. The major QTL on chromosome 3, explaining 24.9% of the variation, was found to be conserved across cereal species. During the same time period, the tolerance to acid sulfate soils identified in this *O. rufipogon* accession was introgressed into the IR64 background through breeding efforts. The selected introgression line, IR73678-6-9-B, was released by IRRI as variety AS996 (Sanchez et al. 2013). AS996 is currently grown on 100,000 ha in the Mekong Delta and described as moderately tolerant to acid sulfate soils and tolerant to brown planthopper and blast.

Even though traits associated with abiotic stress are more difficult to evaluate because of environmental effects and interactions between genes, the development of the AS996 variety is an exciting success story. The release of Arizona-1 and Arizona-2 could make significant contributions to improving rice yields in areas where high temperatures routinely lower yield. With the improved molecular techniques for dissecting these traits and the gene functions related to abiotic stress, more significant advances should be made in the near future, especially as the scientific community provides the tools for rice producers to deal with global climate change.

5. Conclusions

The repositories of *Oryza* species accessions found around the world are a storehouse of novel alleles and traits lost during the evolution and domestication of cultivated rice as we know it today. The fact that introgression lines derived from crosses between Asian rice and it's ancestral species, *O. rufipogon* and *O. nivara*, exhibited notable improvement in yield and yield components through the phenomenon known as transgressive variation, was surprising and unexpected. The identification of novel alleles related to biotic stress, especially insect pests like brown planthopper and bacterial leaf blight, and more recently abiotic stresses like acid sulfate soils and drought, underscore the importance of mining these collections. The advent of molecular marker technology and development of mapping populations, especially AB-QTL and CSSL, have made it possible to map many of these alleles to chromosome location and begin to dissect the interactions between various genes. The fact that high quality genome sequences are now available or will soon be available, make it possible to interrogate the wild *Oryza* species accessions at a level that was not possible before. These resources will allow us

to move swiftly beyond the first step of QTL identification to fine mapping traits of interest; introgressing desirable traits into elite breeding lines using markers within the gene, thus decreasing linkage drag; identifying genotype by environment interactions; determining the effect of epistasis (non-allelic genes) on traits of interest; discovering epigenetic effects such as histone modification or DNA methylation; and finally unraveling other genetic phenomenon like gene silencing. In summary, the interspecific and intergenomic mapping populations available or soon to be available, and the increased availability of SNP data, resequencing data and advanced statistical software, create even more opportunities to investigate novel alleles for agronomically important traits discovered in the *Oryza* species and increase our understanding of the mechanisms underlying these traits to deal with the challenges of climate change and feeding nine billion people.

Acknowledgements

The support of National Science Foundation-Plant Genome Project: "The Genetic Basis of Transgressive Variation in Rice" (Award no. 1026555) to Ehsan Shakiba is gratefully acknowledged. Dr. Paul L. Sanchez and Dr. Benildo G. de los Reyes are acknowledged for their critical reading of this manuscript.

Author details

Ehsan Shakiba[2] and Georgia C. Eizenga[1*]

*Address all correspondence to: georgia.eizenga@ars.usda.gov

1 USDA-ARS Dale Bumpers National Rice Research Center, Stuttgart, AR, USA

2 University of Arkansas Rice Research and Extension Center, Stuttgart, AR, USA

References

[1] Ali ML, Sanchez P, Yu SB, Lorieux M, Eizenga GC. Chromosome segment substitution lines: a powerful tool for the introgression of valuable genes from *Oryza* wild species into cultivated rice (*O. sativa*). Rice 2010;3(4):218-34.

[2] Aluko G, Martinez C, Tohme J, Castano C, Bergman C, Oard J. QTL mapping of grain quality traits from the interspecific cross *Oryza sativa* x *O. glaberrima*. Theoretical and Applied Genetics 2004;109(3):630-9.

[3] Amante A, De L, Sitch L, Leung H, Mew T. Sheath blight (ShB) resistance in wild rices. International Rice Research Institute Newsletter 1990;15(3).

[4] Amante-Bordeos A, Sitch L, Nelson R, Dalmacio R, Oliva N, Aswidinnoor H, et al. Transfer of bacterial blight and blast resistance from the tetraploid wild rice *Oryza minuta* to cultivated rice, *Oryza sativa*. Theoretical and Applied Genetics 1992;84(3-4): 345-54.

[5] Ammiraju JS, Song X, Luo M, Sisneros N, Angelova A, Kudrna D, et al. The *Oryza* BAC resource: a genus-wide and genome scale tool for exploring rice genome evolution and leveraging useful genetic diversity from wild relatives. Breeding Science 2010;60(5):536-43.

[6] Andaya V, Tai T. Fine mapping of the *qCTS4* locus associated with seedling cold tolerance in rice (*Oryza sativa* L.). Molecular Breeding 2007;20(4):349-58.

[7] Angeles E, Khush G, Heinrichs E. New genes for resistance to whitebacked planthopper [*Sogatella furcifera*] in rice. Crop Science 1981;21(1):47-50.

[8] Angeles-Shim RB, Vinarao RB, Marathi B, Jena KK. Molecular analysis of *Oryza latifolia* Desv. (CCDD genome)-derived introgression lines and identification of value-added traits for rice (*O. sativa* L.) improvement. Journal of Heredity (provisional acceptance).

[9] Balkunde S, Le HL, Lee HS, Kim DM, Kang JW, Ahn SN. Fine mapping of a QTL for the number of spikelets per panicle by using near-isogenic lines derived from an interspecific cross between *Oryza sativa* and *Oryza minuta*. Plant Breeding 2013;132(1): 70-6.

[10] Bandillo N, Raghavan C, Muyco PA, Sevilla MAL, Lobina IT, Dilla-Ermita CJ, et al. Multi-parent advanced generation inter-cross (MAGIC) populations in rice: progress and potential for genetics research and breeding. Rice 2013;6(1):1-15.

[11] Bernacchi D, Tanksley S, Beck-Bunn T, Uhlig J, Eshed Y, Zamir D, et al. Advanced backcross QTL analysis in tomato. I. Identification of QTLs for traits of agronomic importance from *Lycopersicon hirsutum*. Theoretical and Applied Genetics 1998;97(3): 381-97.

[12] Bhuiyan M, Narimah M, Rahim HA, Abdullah M, Wickneswari R. Transgressive variants for red pericarp grain with high yield potential derived from *Oryza rufipogon* x *Oryza sativa*: field evaluation, screening for blast disease, QTL validation and background marker analysis for agronomic traits. Field Crops Research 2011;121(2):232-9.

[13] Bimpong IK, Serraj R, Chin JH, Ramos J, Mendoza EM, Hernandez JE, et al. Identification of QTLs for drought-related traits in alien introgression lines derived from crosses of rice (*Oryza sativa* cv. IR64) x *O. glaberrima* under lowland moisture stress. Journal of Plant Biology 2011;54(4):237-50.

[14] Blanco A, Gadaleta A, Simeone R. Variation for yield and quality components in durum wheat backcross inbred lines derived from ssp. *dicoccoides*. Bodenkultur 2004;54(3):163-70.

[15] Blum A. Plant breeding for stress environments. BocaRaton, Forida, USA: CRC Press; 1998.

[16] Bocco R, Lorieux M, Seck P, Futakuchi K, Manneh B, Baimey H, et al. Agro-morphological characterization of a population of introgression lines derived from crosses between IR64 (*Oryza sativa Indica*) and TOG5681 (*Oryza glaberrima*) for drought tolerance. Plant Science 2012;183:65-6.

[17] Brar D, Khush G. Alien introgression in rice. Plant Molecular Biology 1997;35(1-2): 35-47.

[18] Brar D, Singh K. *Oryza*. In: Kole C, editor. Wild crop relatives: Genomic and breeding resources: Cereals. Dordrecht London, New York: Springer Heidelberg;2011. p 321-36.

[19] Brar D, Toriyama K, Heong K, Hardy B. Broadening the gene pool of rice through introgression from wild species. Rice is life: Scientific perspectives for the 21st century. Proceedings of the World Rice Research Conference. Tsukuba, Japan, 4-7 November 2004. 2005:157-60.

[20] Brondani C, Rangel P, Brondani R, Ferreira M. QTL mapping and introgression of yield-related traits from *Oryza glumaepatula* to cultivated rice (*Oryza sativa*) using microsatellite markers. Theoretical and Applied Genetics 2002;104(6-7):1192-203.

[21] Brooker RW. Plant–plant interactions and enviromental change. New Phytologist 2006;171(2):271-84.

[22] Cavanagh C, Morell M, Mackay I, Powell W. From mutations to MAGIC: resources for gene discovery, validation and delivery in crop plants. Current Opinion in Plant Biology 2008;11(2):215-21.

[23] Cheema K, Bains N, Mangat G, Das A, Vikal Y, Brar D, et al. Development of high yielding IR64 x *Oryza rufipogon* (Griff.) introgression lines and identification of introgressed alien chromosome segments using SSR markers. Euphytica 2008a;160(3): 401-9.

[24] Cheema KK, Grewal NK, Vikal Y, Sharma R, Lore JS, Das A, et al. A novel bacterial blight resistance gene from *Oryza nivara* mapped to 38kb region on chromosome 4L and transferred to *Oryza sativa* L. Genetical Research 2008b;90(5):397.

[25] Chen J, Huang DR, Wang L, Liu GJ, Zhuang JY. Identification of quantitative trait loci for resistance to whitebacked planthopper, *Sogatella furcifera*, from an interspecific cross *Oryza sativa* x *O. rufipogon*. Breeding Science 2010;60(2):153-9.

[26] Chen L, Zhang W, Cheng X, Yang Y, Yan C, Wang X, et al. Identification of quantitative trait loci for bacterial blight resistance derived from *Oryza meyeriana* and agro-

nomic traits in recombinant inbred lines of *Oryza sativa*. Journal of Phytopathology 2012;160(9):461-8.

[27] Chen Z, Hu F, Xu P, Li J, Deng X, Zhou J, et al. QTL analysis for hybrid sterility and plant height in interspecific populations derived from a wild rice relative, *Oryza longistaminata*. Breeding Science 2009;59(4):441-5.

[28] Cho Y, Suh J, Choi I, Hong H, Baek M, Kang K, et al. QTLs analysis of yield and its related traits in wild rice relative *Oryza rufipogon*. Trends in Crop Research 2003;4:19-29.

[29] Couch B, Kohn L. A multilocus gene genealogy concordant with host preference indicates segregation of a new species, *Magnaporthe oryzae*, from *M. grisea*. Mycologia 2002;94(4):683-93.

[30] Deen R, Ramesh K, Gautam S, Rao Y, Lakshmi V, Viraktamath B, et al. Identification of new gene for BPH resistance introgressed from *O. rufipogon*. Rice Genetics Newsletter 2010;25:70-1.

[31] Doi K, Taguchi A, Yoshimura A. A new locus affecting high F_1 pollen sterility found in backcross progenies between *Japonica* rice and African rice. Rice Genetics Newsletter 1998;15:146-8.

[32] Doi K, Taguchi K, Yoshimura A. RFLP mapping of *S20* and *S21* for F_1 pollen semisterility found in backcross progeny of *Oryza sativa* and *O. glaberrima*. Rice Genetics Newsletter 1999;16:65-8.

[33] Eizenga G, Jia Y, Agrama H, Lee F. Exploring genetic diversity and potential novel disease resistance genes in a collection of rice (*Oryza* spp.) wild relatives. Genetic Resources and Crop Evolution 2009;56(1):65-76.

[34] Eizenga GC, Prasad B, Jackson AK, Jia MH. Identification of rice sheath blight and blast quantitative trait loci in two different *O. sativa*/*O. nivara* advanced backcross populations. Molecular Breeding 2013;31:889–907.

[35] Eizenga G, Neves P, Bryant RJ, Agrama H, Mackill DJ. Evaluation of a M-202 x *Oryza nivara* advanced backcross mapping population for seedling vigor, agronomic traits, yield components, yield and grain quality. Euphytica (provisional acceptance).

[36] Eshed Y, Zamir D. An introgression line population of *Lycopersicon pennellii* in the cultivated tomato enables the identification and fine mapping of yield-associated QTL. Genetics 1995;141(3):1147.

[37] Foy CD. Soil chemical factors limiting plant root growth. Limitations to plant root growth: Springer; 1992. p 97-149.

[38] Fu Q, Tan L, Zhu Z, Ma D, Fu Y, Cai H, et al. Analysis of QTLs for yield-related traits in Yuanjiang common wild rice (*Oryza rufipogon* Griff.). Journal of Genetics and Genomics 2010;37(2):147-57.

[39] Fu X, Lu Y, Liu X, Li J. Progress on transferring elite genes from non-AA genome wild rice into *Oryza sativa* through interspecific hybridization. Rice Science 2008;15(2):79-87.

[40] Fujino K, Sekiguchi H, Sato T, Kiuchi H, Nonoue Y, Takeuchi Y, et al. Mapping of quantitative trait loci controlling low-temperature germinability in rice (*Oryza sativa* L.). Theoretical and Applied Genetics 2004;108(5):794-9.

[41] Fujita D, Doi K, Yoshimura A, Yasui H. Mapping a new resistance gene for green rice leafhopper introgressed from *Oryza rufipogon* Griff. into cultivated rice, *Oryza sativa* L. Rice Genetics Newletter 2003;20:79-80.

[42] Fujita D, Doi K, Yoshimura A, Yasui H. Introgression of a resistance gene for green rice leafhopper from *Oryza nivara* into cultivated rice, *Oryza sativa* L. Rice Genetics Newsletter 2004;21: 64–6.

[43] Fujita D, Doi K, Yoshimura A, Yasui H. Molecular mapping of a novel gene, *Grh5*, conferring resistance to green rice leafhopper (*Nephotettix cincticeps* Uhler) in rice, *Oryza sativa* L. Theoretical and Applied Genetics 2006;113(4):567-73.

[44] Fujita D, Doi K, Yoshimura A, Yasui H. A major QTL for resistance to green rice leafhopper (*Nephotettix cincticeps* Uhler) derived from African rice (*Oryza glaberrima* Steud.). Breeding Science 2010;60(4):336-41.

[45] Fujita D, Yoshimura A, Yasui H. Development of near-isogenic lines and pyramided lines carrying resistance genes to green rice leafhopper (*Nephotettix cincticeps* Uhler) with the Taichung 65 genetic background in rice (*Oryza sativa* L.). Breeding Science 2010;60(1):18-27.

[46] Fukuoka S, Nonoue Y, Yano M. Germplasm enhancement by developing advanced plant materials from diverse rice accessions. Breeding Science 2010;60(5):509-17.

[47] Fukuta Y, Tamura K, Hirae M, Oya S. Genetic analysis of resistance to green rice leafhopper (*Nephotettix cincticeps* Uhler) in rice parental line, Norin-PL6, using RFLP markers. Breeding Science 1998;48:243–9.

[48] Fuller D, Castillo C, Weisskopf A, Kingwell-Banham E, Song J, Sato Y, et al. Consilience of genetics and archaeobotany in the entangled history of rice. Archaeological and Anthropological Sciences 2010:1-17.

[49] Fulton T, Beck-Bunn T, Emmatty D, Eshed Y, Lopez J, Petiard V, et al. QTL analysis of an advanced backcross of *Lycopersicon peruvianum* to the cultivated tomato and comparisons with QTLs found in other wild species. Theoretical and Applied Genetics 1997;95(5-6):881-94.

[50] Futakuchi K, Fofana M, Sié M. Varietal differences in lodging resistance of African rice (*Oryza glaberrima* Steud.). Asian Journal of Plant Sciences 2008;7(6):569-73.

[51] Garris A, Coburn J, Kresovich S, McCouch S, Tai T. Genetic structure and diversity in *Oryza sativa* L. Genetics 2005;169(3):1631-8.

[52] Ghesquière A, Séquier J, Second G, Lorieux M. First steps towards a rational use of African rice, *Oryza glaberrima*, in rice breeding through a 'contig line'concept. Euphytica 1997;96(1):31-9.

[53] Godfray HCJ, Beddington JR, Crute IR, Haddad L, Lawrence D, Muir JF, et al. Food security: the challenge of feeding 9 billion people. Science 2010;327(5967):812-8.

[54] Goff SA, Ricke D, Lan TH, Presting G, Wang R, Dunn M, Glazebrook J, Sessions A, Oeller P, Varma H et al. A draft sequence of the rice genome (*Oryza sativa* L. ssp. *japonica*). Science 2002;296:92–100.

[55] Grandillo S, Tanksley SD. Advanced backcross QTL analysis: Results and perspectives. In: Tuberosa R, Phillips RL, Gale M. (editors) In the Wake of the Double Helix: From the Green Revolution to the Gene Revolution, Proceedings of the International Congress, 27-31 May 2003 Bologna, Italy; 2005.

[56] Grennan AK. Abiotic stress in rice. An "omic" approach. Plant Physiology 2006;140(4):1139-41.

[57] Gu K, Tian D, Yang F, Wu L, Sreekala C, Wang D, et al. High-resolution genetic mapping of *Xa27(t)*, a new bacterial blight resistance gene in rice, *Oryza sativa* L. Theoretical and Applied Genetics 2004;108:800-7.

[58] Guo SB, Wei Y, Li XQ, Liu KQ, Huang FK, Chen CH, et al. Development and identification of introgression lines from cross of *Oryza sativa* and *Oryza minuta*. Rice Science 2013;20(2):95-102.

[59] Gutierrez A, Carabalí S, Giraldo O, Martínez C, Correa F, Prado G, et al. Identification of a *Rice stripe necrosis virus* resistance locus and yield component QTLs using *Oryza sativa* x *O. glaberrima* introgression lines. BMC Plant Biology 2010;10(1):6.

[60] Haley CS, Knott SA. A simple regression method for mapping quantitative trait loci in line crosses using flanking markers. Heredity 1992;69(4):315-24.

[61] Hanamaratti N, Prashanthi S, Salimath P, Hanchinal R, Mohankumar H, Parameshwarappa K, et al. Traditional and races of rice in Karnataka: reservoirs of valuable traits. Current Science 2008;94:242–7.

[62] Hechanova S. Molecular characterization of introgression and putative genes for bacterial blight resistance in wide-cross derivatives of rice (*Oryza sativa*). Food and Agriculture Organization of the United Nations. 2008. http://agris.fao.org/agris-search/search.do?f=2009/PH/PH0909.xml;PH2009000711 (accessed 24 January 2014).

[63] Hernandez JE, Khush GS. Genetics of resistance to white-backed planthopper in some rice (*Oryza sativa* L.) varieties. *Oryza* 1981;18(1):44-50.

[64] Hirabayashi H, Angeles E, Kaji R, Ogawa T, Brar D, Khush G. Identification of brown planthopper resistance gene derived from *O. officinalis* using molecular markers in rice. Breeding Science 1998;48(Suppl 1):82.

[65] Hirabayashi H, Sato H, Nonoue Y, Kuno-Takemoto Y, Takeuchi Y, Kato H, et al. Development of introgression lines derived from *Oryza rufipogon* and *O. glumaepatula* in the genetic background of japonica cultivated rice (*O. sativa* L.) and evaluation of resistance to rice blast. Breeding Science 2010;60(5):604-12.

[66] Hou Ly, Yu P, Xu Q, Yuan XP, Yu HY, Wang YP, et al. Genetic analysis and preliminary mapping of two recessive resistance genes to brown planthopper, *Nilaparvata lugens* Stål in rice. Rice Science 2011;18(3):238-42.

[67] Hu F, Xu P, Deng X, Zhou J, Li J, Tao D. Molecular mapping of a pollen killer gene *S29(t)* in *Oryza glaberrima* and co-linear analysis with *S22* in *O. glumaepatula*. Euphytica 2006;151(3):273-8.

[68] Hu G, Zhang D, Pan H, Li B, Wu J, Zhou X, et al. Fine mapping of the awn gene on chromosome 4 in rice by association and linkage analyses. Chinese Science Bulletin 2011;56(9):835-9.

[69] Hu H, You J, Fang Y, Zhu X, Qi Z, Xiong L. Characterization of transcription factor gene SNAC2 conferring cold and salt tolerance in rice. Plant Molecular Biology 2008;67(1-2):169-81.

[70] Huang X, Kurata N, Wei X, Wang ZX, Wang A, Zhao Q, et al. A map of rice genome variation reveals the origin of cultivated rice. Nature 2012;490(7421):497-501.

[71] Huang Z, He G, Shu L, Li X, Zhang Q. Identification and mapping of two brown planthopper resistance genes in rice. Theoretical and Applied Genetics 2001;102(6-7): 929-34.

[72] Imai I, Kimball JA, Conway B, Yeater KM, McCouch SR, McClung A. Validation of yield-enhancing quantitative trait loci from a low-yielding wild ancestor of rice. Molecular Breeding 2013;32:1-20.

[73] Ingram J, Bartels D. The molecular basis of dehydration tolerance in plants. Annual Review of Plant Biology 1996;47(1):377-403.

[74] Ishii T, Brar D, Multani D, Khush G. Molecular tagging of genes for brown planthopper resistance and earliness introgressed from *Oryza australiensis* into cultivated rice, *O. sativa*. Genome 1994;37(2):217-21.

[75] Jacquemin J, Bhatia D, Singh K, Wing RA. The international *Oryza* map alignment project: development of a genus-wide comparative genomics platform to help solve the 9 billion-people question. Current Opinion in Plant Biology 2013;16:147-56.

[76] Jena KK. The species of the genus *Oryza* and transfer of useful genes from wild species into cultivated rice, *O. sativa*. Breeding Science 2010;60(5):518-23.

[77] Jena K, Jeung J, Lee J, Choi H, Brar D. High-resolution mapping of a new brown planthopper (BPH) resistance gene, *Bph18(t)*, and marker-assisted selection for BPH resistance in rice (*Oryza sativa* L.). Theoretical and Applied Genetics 2006;112(2): 288-97.

[78] Jena K, Khush GS. Monosomic alien addition lines of rice: production, morphology, cytology, and breeding behavior. Genome 1989;32(3):449-55.

[79] Jena KK, Khush G. Introgression of genes from *Oryza officinalis* Well Ex. Watt to cultivated rice, *O. sativa* L. Theoretical and Applied Genetics 1990;80:737-45.

[80] Jena K, Kochert G, Khush G. RFLP analysis of rice (*Oryza sativa* L.) introgression lines. Theoretical and Applied Genetics 1992;84(5-6):608-16.

[81] Jena K, Pasalu I, Rao Y, Varalaxmi Y, Krishnaiah K, Khush G, et al. Molecular tagging of a gene for resistance to brown planthopper in rice (*Oryza sativa* L.). Euphytica 2002;129(1):81-8.

[82] Jeung JU, Kim BR, Cho YC, Han SS, Moon HP, Lee YT, Jena KK. A novel gene, *Pi40(t)*, linked to the DNA markers derived from NBS-LRR motifs confers broad spectrum of blast resistance in rice. Theoretical and Applied Genetics 2007;115(8): 1163-77.

[83] Jia Y, Liu G, Costanzo S, Lee S, Dai Y. Current progress on genetic interactions of rice with rice blast and sheath blight fungi. Frontiers of *Agriculture* in *China* 2009;3(3):231–9.

[84] Jin F, Kwon S, Kang K, Jeong O, Le L, Yoon D, et al. Introgression for grain traits from *Oryza minuta* into rice, *O. sativa*. Rice Genetics Newsletter 2004; 21:15.

[85] Jin FX, Ji SD, Xie XB, Kang JW, Ju HG, Ahn SN. Detection of epistatic interaction of two QTLs, *gw8.1* and *gw9.1*, underlying grain weight using nearly isogenic lines in rice. Breeding Science 2011;61(1):69-75.

[86] Jin FX, Kim DM, Ju HG, Ahn SN. Mapping quantitative trait loci for awnness and yield component traits in isogenic lines derived from an *Oryza sativa*/*O. rufipogon* cross. Journal of Crop Science and Biotechnology 2009;12(1):9-15.

[87] Joehanes R, Nelson JC. QGene 4.0, an extensible Java QTL-analysis platform. Bioinformatics 2008;24(23):2788-9.

[88] Jones DB, Peterson ML. Rice seedling vigor at sub-optimal temperatures. Crop Science 1976;16(1):102-5.

[89] Kang J, Suh J, Kim D, Oh C, Oh J, Ahn S. QTL mapping of agronomic traits in an advanced backcross population from a cross between *Oryza sativa* L. cv. Milyang 23 and *O. glaberrima*. Korean Journal of Breeding Science 2008;40(3):243-9.

[90] Kao CH, Zeng ZB, Teasdale RD. Multiple interval mapping for quantitative trait loci. Genetics 1999;152(3):1203-16.

[91] Kellogg EA. The evolutionary history of Ehrhartoideae, Oryzeae, and *Oryza*. Rice 2009;2(1):1-14.

[92] Keurentjes JJB, Bentsink L, Hanhart CJ, Vries HBD, Koornneef M, Vreugdenhil D, et al. Development of a near-isogenic line population of *Arabidopsis thaliana* and comparison of mapping power with a recombinant inbred line population. Genetics 2007;175(2):891-905.

[93] Khush GS. Disease and insect resistance in rice. Advances in Agronomy 1977;29:265-341.

[94] Khush G, Ling K, Aquino R, Aguiero V. Breeding for resistance to grassy stunt in rice. Plant Breeding Papers; International Congress of the Society for the Advancement of Breeding Researches in Asia & Oceania. 1977.

[95] Khush GS, Bacalangco E, Ogawa T. A new gene for resistance to bacterial blight from *O. longistaminata*. Rice Genetic Newsletter 1990;7:121-2.

[96] Koide Y, Shinya Y, Ikenaga M, Sawamura N, Matsubara K, Onishi K, et al. Complex genetic nature of sex-independent transmission ratio distortion in Asian rice species: the involvement of unlinked modifiers and sex-specific mechanisms. Heredity 2012;108(3):242-7.

[97] Koseki M, Kitazawa N, Yonebayashi S, Maehara Y, Wang ZX, Minobe Y. Identification and fine mapping of a major quantitative trait locus originating from wild rice, controlling cold tolerance at the seedling stage. Molecular Genetics and Genomics 2010;284(1):45-54.

[98] Kover PX, Scarcelli N, Valdar W, Trakalo J, Durrant C, Mott R, et al. A multiparent advanced generation inter-cross to fine-map quantitative traits in *Arabidopsis thaliana*. PLoS Genetics 2009;5(7). DOI: 10.1371/journal.pgen.1000551

[99] Kurakazu T, Sobrizal, Yoshimura A. RFLP mapping of genes for awn on chromosomes 4 and 5 in rice using *Oryza meridionalis* introgression lines. Rice Genetics Newsletter 2001(18):28-30.

[100] Lakshmanan P, Velusamy R. Resistance to sheath blight (ShB) and brown spot (BS) in lines derived from *Oryza officinalis*. International Rice Resesarch Institute Newsletter 1991;16:8.

[101] Lander ES, Botstein D. Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 1989;121(1):185-99.

[102] Lee FN, Rush MC. Rice sheath blight: a major rice disease. Plant Disease 1983;67:829–32.

[103] Lee H, Sasaki K, Higashitani A, Ahn S, Sato T. Mapping and characterization of quantitative trait loci for mesocotyl elongation in rice (*Oryza sativa* L.). Rice 2012;5(13).

[104] Lee SJ, Oh CS, Suh JP, McCouch S, Ahn SN. Identification of QTLs for domestication-related and agronomic traits in an *Oryza sativa* x *O. rufipogon* BC_1F_7 population. Plant Breeding 2005;124(3):209-219.

[105] Lei D, Tan L, Liu F, Chen L, Sun C. Identification of heat-sensitive QTL derived from common wild rice (*Oryza rufipogon*, Griff.). Plant Science 2013;201-202:123-7.

[106] Lexer C, Fay M. Adaptation to environmental stress: a rare or frequent driver of speciation? Journal of Evolutionary Biology 2005;18(4):893-900.

[107] Li C, Zhou A, Sang T. Genetic analysis of rice domestication syndrome with the wild annual species, *Oryza nivara*. New Phytologist 2006;170(1):185-94.

[108] Li J, Thomson M, McCouch SR. Fine mapping of a grain-weight quantitative trait locus in the pericentromeric region of rice chromosome 3. Genetics 2004;168(4):2187-95.

[109] Li J, Xiao J, Grandillo S, Jiang L, Wan Y, Deng Q, et al. QTL detection for rice grain quality traits using an interspecific backcross population derived from cultivated Asian (*O. sativa* L.) and African (*O. glaberrima* S.) rice. Genome 2004;47(4):697-704.

[110] Li JB, Xia MY, Qi HX, He GC, Wan BL, Zha ZP. Marker assisted selection for brown planthopper (*Nilaparvata luge*ns Stål) resistance genes *Bph14* and *Bph15* in rice. Scientia Agricultura Sinica 2006;39(10):2132-7.

[111] Li Z, Pinson SR, Stansel JW, Paterson AH. Genetic dissection of the source-sink relationship affecting fecundity and yield in rice (*Oryza sativa* L.). Molecular Breeding 1998;4(5):419-26.

[112] Li ZX, Septiningsih EM, Quilloy-Mercado SM, McNally KL, Mackill DJ. Identification of *SUB1A* alleles from wild rice *Oryza rufipogon* Griff. Genetic Resources and Crop Evolution 2011;58(8):1237-42.

[113] Linh LH, Hang NT, Jin FX, Kang KH, Lee YT, Kwon SJ, et al. Introgression of a quantitative trait locus for spikelets per panicle from *Oryza minuta* to the *O. sativa* cultivar Hwaseongbyeo. Plant Breeding 2008;127(3):262-7.

[114] Linh LH, Jin FX, Kang KH, Lee YT, Kwon SJ, Ahn SN. Mapping quantitative trait loci for heading date and awn length using an advanced backcross line from a cross between *Oryza sativa* and *O. minuta*. Breeding Science 2006;56(4):341-9.

[115] Liu G, Lu G, Zeng L, Wang GL. Two broad-spectrum blast resistance genes, *Pi9(t)* and *Pi2(t)*, are physically linked on rice chromosome 6. Molecular Genetics and Genomics. 2002;267(4):472-80.

[116] Liu G, Yan H, Fu Q, Qian Q, Zhang Z, Zhai W, et al. Mapping of a new gene for brown planthopper resistance in cultivated rice introgressed from *Oryza eichingeri*. Chinese Science Bulletin 2001;46(17):1459-62.

[117] Liu G, Yang J, Xu H, Hayat Y, Zhu J. Genetic analysis of grain yield conditioned on its component traits in rice (*Oryza sativa* L.). Crop and Pasture Science 2008;59(2): 189-95.

[118] Lorieux M. MapDisto: Fast and efficient computation of genetic linkage maps. Molecular Breeding 2012;30(2):1231-5.

[119] Lu BR, Jackson M, Vaughn D. Wild rice taxonomy. In: Rice knowledge bank, International Rice Research Institute, Philippines. 2014; http://www.knowledgebank.irri.org/extension/index.php/ wild-rice-taxonomy (accessed 23 January 2014).

[120] Luo DG, Zhang SP. Preliminary study on simplified distant cross techniques by using male-sterile lines as maternal parent and *O. gradiglumis* as paternal parent. Seed 2000;3:49–50.

[121] Manneh B, Kiepe P, Sie M, Ndjiondjop M, Drame N, Traore K, et al. Exploiting partnerships in research and development to help African rice farmers cope with climate variability. Journal of SAT Agricultural Research 2007;4(1):1-10.

[122] Marri PR, Sarla N, Reddy LV, Siddiq E. Identification and mapping of yield and yield related QTLs from an Indian accession of *Oryza rufipogon*. BMC Genetics 2005;6(1):33.

[123] Matsushita S, Kurakazu T, Sobrizal, Doi K, Yoshimura A. Mapping genes for awn in rice using *Oryza meridionalis* introgression lines. Rice Genetics Newsletter 2003;20:17.

[124] Matsushita S, Sanchez P, Sobrizal, Doi K, Yoshimura A. Identification of new alleles of awnness genes, *An7* and *An8*, in rice using *Oryza glumaepatula* introgression lines. Rice Genetics Newsletter 2003;20:19-20.

[125] McCombie R. New whole genome de-novo assemblies of three divergent strains of rice document novel gene space in *aus* and *indica* missed by previous reference assemblies. 7th International Rice Genetics Symposium Program and Abstract Book; Manila, Philippines: 5-8 November 2013; p 120.

[126] McCouch SR, McNally KL, Wang W, Hamilton RS. Genomics of gene banks: a case study in rice. American Journal of Botany 2012;99(2):407–23.

[127] McCouch SR, Sweeney M, Jiang H, Thomson M, Septiningsih E, Edwards J, et al. Through the genetic bottleneck: *O. rufipogon* as a source of trait-enhancing alleles for *O. sativa*. Euphytica 2007;154(3):317-39.

[128] McCouch SR, Zhao K, Wright M, Tung CW, Ebana K, Thomson M, et al. Development of genome-wide SNP assays for rice. Breeding Science 2010;60(5):524-35.

[129] Mew T, Alvarez A, Leach J, Swings J. Focus on bacterial blight of rice. Plant Disease 1993;77(1):5-12.

[130] Miura K, Yamamoto E, Morinaka Y, Takashi T, Kitano H, Matsuoka M, et al. The hybrid breakdown 1 (t) locus induces interspecific hybrid breakdown between rice *Ory-*

za sativa cv. Koshihikari and its wild relative *O. nivara*. Breeding Science 2008;58(2): 99-105.

[131] Moncada P, Martinez C, Borrero J, Châtel M, Gauch Jr H, Guimaraes E, et al. Quantitative trait loci for yield and yield components in an *Oryza sativa* x *Oryza rufipogon* BC_2F_2 population evaluated in an upland environment. Theoretical and Applied Genetics 2001;102(1):41-52.

[132] Multani D, Jena K, Brar D, de los Reyes B, Angeles E, Khush G. Development of monosomic alien addition lines and introgression of genes from *Oryza australiensis* Domin. to cultivated rice *O. sativa* L. Theoretical and Applied Genetics 1994;88(1): 102-109.

[133] Multani D, Khush G, de los Reyes B, Brar D. Alien genes introgression and development of monosomic alien addition lines from *Oryza latifolia* Desv. to rice, *Oryza sativa* L. Theoretical and Applied Genetics 2003;107(3):395-405.

[134] Hanamaratti NG, Prashanthi SK, Salimath PM, Hanchinal RR, Mohankumar HD, Parameshwarappa KG, Raikar SD. Traditional land races of rice in Karnataka: reservoirs of valuable traits. Current Science 2008;94:242-7.

[135] Nagao S, Takahashi T. Trial construction of twelve linkage groups in Japanese rice. Genetical studies on rice plant, XXVII Journal of the Faculty of Agriculture, Hokkaido University. 1963:72-130.

[136] Ndjiondjop MN, Manneh B, Cissoko M, Drame NK, Kakai RG, Bocco R, et al. Drought resistance in an interspecific backcross population of rice (*Oryza* spp.) derived from the cross WAB56-104 (*O. sativa*) x CG14 (*O. glaberrima*). Plant Science 2010;179(4):364-73.

[137] Ndjiondjop MN, Manneh B, Cissoko M, Drame NK, Kakai RG, Bocco R, et al. Drought resistance in an interspecific backcross population of rice (*Oryza* spp.) derived from the cross WAB56-104 (*O. sativa*) x CG14 (*O. glaberrima*). Plant Science 2010;179(4):364-73.

[138] Ndjiondjop MN, Albar L, Fargette D, Fauquet C, Ghesquière A. The genetic basis of high resistance to rice yellow mottle virus (RYMV) in cultivars of two cultivated rice species. Plant Disease 1999;83(10):931-35.

[139] Nguyen BD, Brar DS, Bui BC, Nguyen TV, Pham LN, Nguyen HT. Identification and mapping of the QTL for aluminum tolerance introgressed from the new source, *Oryza rufipogon* Griff., into indica rice (*Oryza sativa* L.). Theoretical and Applied Genetics 2003;106(4):583-93.

[140] Ni J, Colowit P, Oster J, Xu K, Mackill D. Molecular markers linked to stem rot resistance in rice. Theoretical and Applied Genetics 2001;102(4):511-6.

[141] Niño-Liu DO, Ronald PC, Bogdanove AJ. *Xanthomonas oryzae* pathovars: model pathogens of a model crop. Molecular Plant Pathology 2006;7(5):303-24.

[142] Nuque F, Aguiero V, Ou S. Inheritance of resistance to grassy stunt virus in rice. Plant Disease 1982;66(1):63-4.

[143] Oh JM, Balkunde S, Yang P, Yoon DB, Ahn SN. Fine mapping of grain weight QTL, *tgw11* using near isogenic lines from a cross between *Oryza sativa* and *O. grandiglumis*. Genes and Genomics 2011;33(3):259-65.

[144] Orjuela J, Garavito A, Bouniol M, Arbelaez JD, Moreno L, Tohme J, et al. A universal core genetic map for rice. Theoretical and Applied Genetics 2010;120(3):563-72.

[145] Oryzabase. List of Gene 2014 (Updated 20 Jan. 2014) http://www.shigen.nig.ac.jp/rice/oryzabaseV4/gene/list (accessed 24 January 2014).

[146] Ou SH. Rice Diseases. Commonwealth Agricultural Bureau, Kew, UK. 1985.

[147] Pastori GM, Foyer CH. Common components, networks, and pathways of cross-tolerance to stress. The central role of "redox" and abscisic acid-mediated controls. Plant Physiology 2002;129(2):460-8.

[148] Pinson SRM, Capdevielle FM, Oard JH. Confirming QTLs and finding additional loci conditioning sheath blight resistance in rice using recombinant inbred lines. Crop Science 2005; 45:503–10

[149] Plowright RA, Nash P, Coyne DL, Jones MP. Resistance to the rice nematodes *Heterodera sacchari*, Meloidogyne graminicola and *M. incognita* in *Oryza glaberrima* and *O. glaberrima* x *O. sativa* interspecific hybrids. Nematology 1999;1(7-8):745-51.

[150] Poehlman JM, Sleper DA. Breeding Field Crops, Fourth edition: Iowa State University Press 1995.

[151] Prasad B, Eizenga GC. Rice sheath blight disease resistance identified in *Oryza* spp. accessions. Plant Disease 2008;92(11):1503-9.

[152] Qu S, Liu G, Zhou B, Bellizzi M, Zeng L, Wang GL, et al. The broad-spectrum blast resistance gene *Pi9* encodes a nucleotide-binding site-leucine-rich repeat protein and is a member of a multigene family in rice. Genetics 2006;172(3):1901-14.

[153] Wing R. 15 million years of *Oryza* evolution as seen through the analysis of 12 high quality genome assemblies. 7th International Rice Genetics Symposium Program and Abstract Book; Manila, Philippines: 5-8 November 2013; p 121-2.

[154] Rahman ML, Jiang W, Chu SH, Qiao Y, Ham TH, Woo MO, Lee J, Khanam MS, Chin JH, Jeung JU, Brar DS, Jena KK, Koh HJ. High-resolution mapping of two rice brown planthopper resistance genes, *Bph20 (t)* and *Bph21 (t)*, originating from *Oryza minuta*. Theoretical and Applied Genetics 2009;119(7):1237-46.

[155] Rahman L, Koh HJ, Khanam MS. QTL analysis for yield related traits using populations derived from an indica-japonica hybrid in rice (*Oryza sativa* L.). Czech Journal of Genetics and Plant Breeding 2008;44(3):93-104.

[156] Ram T, Deen R, Gautam S, Ramesh K, Rao Y, Brar D. Identification of new genes for brown planthopper resistance in rice introgressed from *O. glaberrima* and *O. minuta*. Rice Genetics Newsletter 2010;25:67.

[157] Ram T, Laha G, Gautam S, Deen R, Madhav MS, Brar D, et al. Identification of new gene introgressed from *Oryza brachyantha* with broad-spectrum resistance to bacterial blight of rice in India. Rice Genetics Newsletter. 2010;25:57-8.

[158] Rangel PN, Brondani RPV, Rangel PHN, Brondani C. Agronomic and molecular characterization of introgression lines from the interspecific cross *Oryza sativa* (BG 90-2) x *Oryza glumaepatula* (RS-16). Genetic and Molecular Research 2008;7(1):184-95.

[159] Rangel PN, Vianello RP, Melo ATO, Rangel PHN, Mendonça JA, Brondani C. Yield QTL analysis of *Oryza sativa* x *O. glumaepatula* introgression lines. Pesquisa Agropecuária Brasileira 2013;48(3):280-6.

[160] Rausher MD. Co-evolution and plant resistance to natural enemies. Nature 2001;411(6839):857-64.

[161] Ravinder SS, Khush GS, Heinrichs EA. Genetic analysis of resistance to whitebacked planthopper, *Sogatella furcifera* (Horvath), in some rice varieties. Crop Protection 1982;1:289-97.

[162] Renganayaki K, Fritz AK, Sadasivam S, Pammi S, Harrington SE, McCouch SR, et al. Mapping and progress toward map-based cloning of brown planthopper biotype-4 resistance gene introgressed from *Oryza officinalis* into cultivated rice. Crop Science 2002;42(6):2112-2117.

[163] Ronald PC, Albano B, Tabien R, Abenes L, Wu KS, McCouch S, et al. Genetic and physical analysis of the rice bacterial blight disease resistance locus, *Xa21*. Molecular and General Genetics 1992;236(1):113-20.

[164] Saka N, Toyama T, Tsuji T, Nakamae H, Izawa T. Fine mapping of green ricehopper resistant gene *Grh-3(t)* and screening of *Grh-3(t)* among green ricehopper resistant and green leafhopper resistant cultivars in rice. Breeding Science 1997; 47(1):55.

[165] Sanchez PL, Sobrizal, Ikeda K, Yasui H, Yoshimura A. Identifying late heading genes in rice using *Oryza glumaepatula* introgression lines. In: Advances in Rice Genetics. International Rice Research Institute, Los Baños, Laguna, Philippines. 2003; p 153-4.

[166] Sanchez PL, Wing RA, Brar DS. The wild relative of rice: genomes and genomics. In: Zhang Q, Wing RA, editors. Genetics and Genomics of Rice, Plant Genetics and Genomics: Crops and Models 5. New York: Springer Science: 2013; p 9-25.

[167] Sano Y. A new gene controlling sterility in F_1 hybrids of two cultivated rice species: Its association with photoperiod sensitivity. Journal of Heredity 1983;74(6):435-9.

[168] Sano Y. Sterility barriers between *Oryza sativa* and *O. glaberrima*. In: Rice genetics, Proceedings of the International Rice Genetics Symposium, International Rice Research Institute, Los Baños, Philippines: 27–31 May 1985; p 109–18.

[169] Sano Y. The genic nature of gamete eliminator in rice. Genetics 1990;125(1):183-91.

[170] Sarla N, Swamy BM. *Oryza glaberrima*: a source for the improvement of *Oryza sativa*. Current Science 2005;89(6):955-63.

[171] Savary S, Willocquet L, Elazegui FA, Castilla NP, Teng PS. Rice pest constraints in tropical Asia: quantification of yield losses due to rice pests in a range of production situations. Plant Disease 2000;84(3):357-69.

[172] Septiningsih E, Prasetiyono J, Lubis E, Tai T, Tjubaryat T, Moeljopawiro S, et al. Identification of quantitative trait loci for yield and yield components in an advanced backcross population derived from the *Oryza sativa* variety IR64 and the wild relative *O. rufipogon.* Theoretical and Applied Genetics 2003;107(8):1419-32.

[173] Shan JX, Zhu MZ, Shi M, Gao JP, Lin HX. Fine mapping and candidate gene analysis of *spd6*, responsible for small panicle and dwarfness in wild rice (*Oryza rufipogon* Griff.). Theoretical and Applied Genetics 2009;119(5):827-36.

[174] Shim RA, Ashikari M, Angeles ER, Takashi T. Development and evaluation of *Oryza glaberrima* Steud. chromosome segment substitution lines (CSSLs) in the background of *O. sativa* L. cv. Koshihikari. Breeding Science 2010;60(5):613-9.

[175] Sidhu G, Khush G. Linkage relationships of some genes for disease and insect resistance and semidwarf stature in rice. Euphytica 1979;28(2):233-7.

[176] Sié M, Blaise K, Drissa S, Yonnelle D. Selection of intraspecific (*Oryza sativ*a x *O. sativa*) and inter-specific (*O. sativa* x *O. glaberrima*) lines for their tolerance to blast in Burkina Faso. Institut de l'Environnement et de Recherches Agricoles (INERA) 01 BP 910 Bobo-Dioulasso 1:23-37.

[177] Silué D, Nottéghem JL, Chaume J. Etude de la résistance spécifique d'*Oryza glaberrima* Steud à la pyriculariose du riz (translation Study of the specific resistance of *Oryza glaberrima* Steud to rice blast). Agronomie Tropicale 1992;42:121-9.

[178] Sobrizal, Ikeda K, Sanchez PL, Yasui H, Yoshimura A. Development and evaluation *Oryza glumaepatula* introgression lines in rice, *Oryza sativa* L. Rice Genetics Newsletter 1999;16:107-8.

[179] Sobrizal, Matsuzaki Y, Sanchez PL, Ikeda K, Yoshimura A. Identification of a gene for male gamete abortion in backcross progeny of *Oryza sativa* L. and *Oryza glumaepatula* Steud. Rice Genetics Newsletter 2000a;17:59-61.

[180] Sobrizal, Matsuzaki Y, Sanchez PL, Ikeda K, Yoshimura A. Mapping of F_1 pollen semi-sterility found in backcross progeny of *Oryza sativa* L. and *Oryza glumaepatula* Steud. Rice Genetics Newsletter 2000b;17:61-3.

[181] Suh JP, Ahn SN, Cho YC, Kang KH, Choi IS, Kim YG, Suh HS, Hwang HG. Mapping of QTLs for yield traits using an advanced backcross population from a cross between *Oryza sativa* and *O. glaberrima.* Korean Journal of Breeding Science. 2005;37(4): 214-20.

[182] Swamy BM, Kaladhar K, Rani NS, Prasad G, Viraktamath B, Reddy GA, et al. QTL analysis for grain quality traits in 2 BC_2F_2 populations derived from crosses between *Oryza sativa* cv Swarna and 2 accessions of *O. nivara*. Journal of Heredity 2012;103(3): 442-52.

[183] Takamure I, Kinoshita T. Linkage analysis in chromosomes 3 and 6. Rice Genetics Newsletter 1991;8:98-100.

[184] Talamè V, Sanguineti M, Chiapparino E, Bahri H, Salem M, Forster B, et al. Identification of *Hordeum spontaneum* QTL alleles improving field performance of barley grown under rainfed conditions. Annals of Applied Biology 2004;144(3):309-319.

[185] Tamura K, Fukuta Y., Hirae M, Oya S, Ashikawa I, Yagi.T. Mapping of the *Grh1* locus for green rice leafhopper resistance in rice using RFLP markers. Breeding Science 1999; 49:11–14.

[186] Tamura K, Fukuta Y, Hirae M, Oya S, Ashikawa I., Yagi T. RFLP mapping of a new resistance gene for green rice leafhopper in Kanto PL10. Rice Genetics Newsletter 2004;21: 62–4.

[187] Tan G, Weng Q, Ren X, Huang Z, Zhu L, He G. Two whitebacked planthopper resistance genes in rice share the same loci with those for brown planthopper resistance. Heredity 2004;92(3):212-7.

[188] Tan GX, Ren X, Weng QM, Shi ZY, Zhu LL, He GC. Mapping of a new resistance gene to bacterial blight in rice line introgressed from *Oryza officinalis*. Acta Genetica Sinica 2004;31(7):724-9.

[189] Tan L, Liu F, Xue W, Wang G, Ye S, Zhu Z, et al. Development of *Oryza rufipogon* and *O. sativa* introgression lines and assessment for yield-related quantitative trait loci. Journal of Integrative Plant Biology 2007;49(6):871-84.

[190] Tan L, Zhang P, Liu F, Wang G, Ye S, Zhu Z, et al. Quantitative trait loci underlying domestication-and yield-related traits in an *Oryza sativa* x *Oryza rufipogon* advanced backcross population. Genome 2008;51(9):692-704.

[191] Tan LB, Fu YC, Liu FX, Wang XK, Sun CQ, Zhang PJ. Identification of quantitative trait loci controlling plant height and days to heading from Yuanjiang common wild rice (*Oryza rufipogon* Griff.). Acta Genetica Sinica 2004;31(10):1123-8.

[192] Tanksley S, Grandillo S, Fulton T, Zamir D, Eshed Y, Petiard V, et al. Advanced backcross QTL analysis in a cross between an elite processing line of tomato and its wild relative *L. pimpinellifolium*. Theoretical and Applied Genetics 1996;92(2):213-24.

[193] Tanksley S, Nelson J. Advanced backcross QTL analysis: a method for the simultaneous discovery and transfer of valuable QTLs from unadapted germplasm into elite breeding lines. Theoretical and Applied Genetics 1996;92(2):191-203.

[194] Tanksley SD, McCouch SR. Seed banks and molecular maps: unlocking genetic potential from the wild. Science 1997;277(5329):1063-6.

[195] Tanksley SD, Medina-Filho H, Rick CM. Use of naturally-occurring enzyme variation to detect and map genes controlling quantitative traits in an interspecific backcross of tomato. Heredity 1982;49 (1):11-25.

[196] Thanh PT, Phan PDT, Mori N, Ishikawa R, Ishii T. Development of backcross recombinant inbred lines between *Oryza sativa* Nipponbare and *O. rufipogon* and QTL detection on drought tolerance. Breeding Science 2011;61(1):76-9.

[197] Thiémélé D, Boisnard A, Chéron S, Ghesquière A, Albar L, Aké S, et al. Identification of a second major resistance gene to rice yellow mottle virus, *RYMV2*, in the African cultivated rice species, *O. glaberrima*. Theoretical and Applied Genetics 2010;121(1): 169-79.

[198] Thomson MJ, Tai TH, McClung AM, Lai XH, Hinga ME, Lobos KB, Xu Y, Martinez CP, McCouch SR. Mapping quantitative trait loci for yield, yield components and morphological traits in an advanced backcross population between *Oryza rufipogon* and the *Oryza sativa* cultivar Jefferson. Theoretical and Applied Genetics 2003;107(3): 479-93.

[199] Tian F, Zhu Z, Zhang B, Tan L, Fu Y, Wang X, et al. Fine mapping of a quantitative trait locus for grain number per panicle from wild rice (*Oryza rufipogon* Griff.). Theoretical and Applied Genetics 2006;113(4):619-29.

[200] Tian L, Tan L, Liu F, Cai H, Sun C. Identification of quantitative trait loci associated with salt tolerance at seedling stage from *Oryza rufipogon*. Journal of Genetics and Genomics 2011;38(12):593-601.

[201] Tilman D, Lehman C. Human-caused environmental change: impacts on plant diversity and evolution. Proceedings of the National Academy of Sciences 2001;98(10): 5433-40.

[202] Tseng S, Oster J. Registration of 87-Y-550, a rice germplasm line resistant to stem rot disease. Crop Science 1994;34(1)314.

[203] Tung CW, Zhao K, Wright MH, Ali ML, Jung J, Kimball J, et al. Development of a research platform for dissecting phenotype–genotype associations in rice (*Oryza* spp.). Rice 2010;3(4):205-17.

[204] Van Ooijen J. JoinMap 4. Software for the calculation of genetic linkage maps in experimental populations Kyazma BV, Wageningen, Netherlands: 2006.

[205] Vaughan DA. The wild relatives of rice: a genetic resources handbook: Manila, Philippines: International Rice Research Institute;1994.

[206] Vaughan DA, Ge S, Kaga A, Tomooka N. Phylogeny and biogeography of the genus *Oryza*. Rice Biology in the Genomics Era. Berlin-Heidelberg: Springer; 2008; p 219-34.

[207] Vaughan DA, Morishima H. Biosystematics of the genus *Oryza* In: Smith CW, Dilday RH, editors. Rice: Origin, History, Technology, and Production 6: John Wiley & Sons; 2002; p 27-65.

[208] Wang J, Wan X, Li H, Pfeiffer WH, Crouch J, Wan J. Application of identified QTL-marker associations in rice quality improvement through a design-breeding approach. Theoretical and Applied Genetics 2007;115(1):87-100.

[209] Wang S, Basten CJ, Zeng ZB. Windows QTL Cartographer 2.5. Department of Statistics, North Carolina State University, Raleigh, NC.2012. http://statgen.ncsu.edu/qtlcart/WQTLCart.htm (accessed 24 January 2014).

[210] Wang YM, Dong ZY, Zhang ZJ, Lin XY, Shen Y, Zhou D, et al. Extensive *de novo* genomic variation in rice induced by introgression from wild rice (*Zizania latifolia* Griseb.). Genetics 2005;170(4):1945-56.

[211] Wickneswari R, Bhuiyan M, Lim LS, Thomson MJ, Narimah MK, Abdullah MZ. Identification and validation of quantitative trait loci for agronomic traits in advanced backcross breeding lines derived from *Oryza rufipogon* x *Oryza sativa* cultivar MR219. Plant Molecular Biology Reporter 2012;30(4):929-39.

[212] Win KT, Kubo T, Miyazaki Y, Doi K, Yamagata Y, Yoshimura A. Identification of two loci causing F_1 pollen sterility in inter-and intraspecific crosses of rice. Breeding Science 2009;59(4):411-8.

[213] Win KT, Yamagata Y, Miyazaki Y, Doi K, Yasui H, Yoshimura A. Independent evolution of a new allele of F1 pollen sterility gene *S27* encoding mitochondrial ribosomal protein L27 in *Oryza nivara*. Theoretical and Applied Genetics 2011;122(2):385-94.

[214] Wu P, Zhao B, Yan J, Luo A, Wu Y, Senadihra D. Genetic control of seedling tolerance to aluminum toxicity in rice. Euphytica 1997;97:289-293.

[215] Xia R, Xiao N, Hong Y, Zhang C, Su Y, Zhang X, et al. QTLs mapping for cold tolerance at seedling stage in Dongxiang wild rice (*Oryza rufipogon* Griff.). Scientia Agricultura Sinica 2010;43:443-51.

[216] Xiao J, Grandillo S, Ahn SN, McCouch SR, Tanksley SD, Li J, et al. Genes from wild rice improve yield. Nature (London) 1996;384(6606):223-4.

[217] Xiao J, Li J, Grandillo S, Ahn SN, Yuan L, Tanksley SD, et al. Identification of trait-improving quantitative trait loci alleles from a wild rice relative, *Oryza rufipogon*. Genetics 1998;150(2):899-909.

[218] Xie X, Song MH, Jin F, Ahn SN, Suh JP, Hwang HG, et al. Fine mapping of a grain weight quantitative trait locus on rice chromosome 8 using near-isogenic lines de-

rived from a cross between *Oryza sativa* and *Oryza rufipogon*. Theoretical and Applied Genetics 2006;113(5):885-94.

[219] Yan CQ, Qian KX, Yan QS, Zhang XQ, Xue GP, Huangfu WG, et al. Use of asymmetric somatic hybridization for transfer of the bacterial blight resistance trait from *Oryza meyeriana* L. to *O. sativa* L. ssp. *japonica*. Plant Cell Reports 2004;22(8):569-75.

[220] Yang J, Hu C, Hu H, Yu R, Xia Z, Ye X, et al. QTL Network: mapping and visualizing genetic architecture of complex traits in experimental populations. Bioinformatics 2008;24(5):721-3.

[221] Yang H, Ren X, Weng Q, Zhu L, He G. Molecular mapping and genetic analysis of a rice brown planthopper (*Nilaparvata lugens* Stål) resistance gene. Hereditas 2002;136(1):39-43.

[222] Yang H, You A, Yang Z, Zhang F, He R, Zhu L, He G. High-resolution genetic mapping at the *Bph15* locus for brown planthopper resistance in rice (*Oryza sativa* L.). Theoretical and Applied Genetics 2004;110(1):182-91.

[223] Yasui H, Iwata N. Production of monosomic alien addition lines of *Oryza sativa* having a single *O. punctata* chromosome. In: Rice Genetics II: Proceedings of the 2nd International Rice Genetics Symposium. 14-18 May 1990. International Rice Research Institute;1991; p 147-55.

[224] Yasui H, Yoshimura A. QTL mapping of antibiosis to green leafhopper, *Nephotettix virescens* Distant and green rice leafhopper, *Nephotettix cincticeps* Uhler in rice, *Oryza sativa* L. Rice Genetics Newsletter 1999;16:96-8.

[225] Yazawa S, Yasui H, Yoshimura A, Iwata N. RFLP mapping of genes for resistance to green rice leafhopper (*Nephotettix cincticeps* Uhler) in rice cultivar DV85 using near isogenic lines. Science Bulletin of the Faculty of Agriculture, Kyushu University 1988; 52:169–75.

[226] Yoon DB, Kang KH, Kim HJ, Ju HG, Kwon SJ, Suh JP, et al. Mapping quantitative trait loci for yield components and morphological traits in an advanced backcross population between *Oryza grandiglumis* and the *O. sativa* japonica cultivar Hwaseongbyeo. Theoretical and Applied Genetics 2006;112(6):1052-62.

[227] Yu J, Hu S, Wang J, Wong GKS, Li S, Liu B, et al. A draft sequence of the rice genome (*Oryza sativa* L. ssp. *indica*). Science 2002;296(5565):79-92.

[228] Yuan PR, Kim HJ, Chen QH, Ju HG, Lee SJ, Ji SD, et al. QTL dissection of agronomic and domestication traits using introgression lines carrying wild rice (*Oryza rufipogon* Griff.) segments in cultivated rice (*O. sativa* L.) background. Journal of Crop Science and Biotechnology 2009;12(4):245-52.

[229] Zeng ZB. Precision mapping of quantitative trait loci. Genetics 1994;136:1457-68.

[230] Zhang Q, Lin SC, Zho BY, Wang CL, Yang WC, Zhou YL, Li DY, Chen CB,. Zhu LH. Identification and tagging a new gene for resistance to bacterial blight (*Xanthomonas oryzae* pv. oryzae) from *O. rufipogon*. Rice Genetics Newsletter 1998;15: 138-42.

[231] Zhang Q. Strategies for developing green super rice. Proceedings of the National Academy of Sciences (USA) 2007;104(42):16402-9.

[232] Zhang X, Zhou S, Fu Y, Su Z, Wang X, Sun C. Identification of a drought tolerant introgression line derived from Dongxiang common wild rice (*O. rufipogon* Griff.). Plant Molecular Biology 2006;62(1-2):247-59.

[233] Zhao K, Tung CW, Eizenga GC, Wright MH, Ali ML, Price AH, Norton GJ, Islam MR, Reynolds A, Mezey J, McClung AM, Bustamante CD, McCouch SR. Genome-wide association mapping reveals a rich genetic architecture of complex traits in *Oryza sativa*. Nature Communications 2011;2:467. DOI: 10.1038/ncomms1467

[234] Zhou F, He H, Chen H, Yu H, Lorieux M, He Y. Genomics-based breeding technology.In: Zhang Q, Wing RA, editors. Genetics and Genomics of Rice, Plant Genetics and Genomics: Crops and models 5. New York: Springer; 2013; p 329-48.

Chapter 2

Genes and QTLs Resistant to Biotic and Abiotic Stresses from Wild Rice and Their Applications in Cultivar Improvements

Fantao Zhang and Jiankun Xie

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/56825

1. Introduction

Rice (*Oryza sativa* L.) is one of the most important food crops for mankind because it feeds more than half the world population, especially in developing countries (Maclean et al. 2002). Although rice production in the world has increased markedly in the past decades, it is still insufficient to cope with the ever-increasing global demands (Sasaki et al. 2000). This is not an easy task in view of the fact that the land available for cultivation is decreasing year by year, especially in Asia where 90 percent of the world's rice is produced and consumed (Khush 1997; Fischer et al. 2005). Meanwhile, since rice is grown worldwide and under a wide range of agro-climatic conditions, its productivity is affected by many abiotic and biotic stresses. Major biotic stresses including insect pests, such as brown planthopper (*Nilaparvata lugens* Stål), green rice leafhopper (*Nephotettix cincticeps*), and stem borer (*Chilo suppressalis*); and diseases, such as sheath blight, bacterial leaf blight, tungro virus; and abiotic stresses including salinity, acidity, drought, cold, and iron toxicity severely affect rice production.

During domestication process from wild species to cultivated rice, selecting desirable-agronomic traits to keep achieving high yield allows many genes to be either directly selected or filtered out, resulting in a significant reduction of genetic diversity in rice gene pool (Brar et al. 2003). Sun et al (2001) revealed that the number of alleles in cultivated rice had been reduced by 50-60% compared to wild rice. Thus, it is necessary to broaden the gene pool in rice breeding from diverse sources, especially from wild rice.

In the genus of Oryza, there are two cultivated species and more than 20 wild species. Both of the cultivated species, O. sativa and O. glaberrima, are diploid (2n = 24) and have the AA genome. Wild species have evolved in a wide range of environments over millions of years

(Stebbins 1981). The wild species have either 2n = 24 or 2n = 48 chromosomes, and seven genomes (AA, BB, CC, BBCC, CCDD, EE, and FF) have so far been designated for 17 species (Vaughan 1994; Brar et al. 1997). Common wild rice (Oryza rufipogon Griff.), due to its long-term growth in the wild conditions, possesses numerous advantages such as genetic diversity, excellent agronomic traits, and resistance against various biotic and abiotic stresses, proved to be an important resource for genetic improvement of rice (Song et al. 2005). Dongxiang wild rice (O. rufipogon Griff.) is in the northern most habitats among O. rufipogon populations to be discovered in the world (Chen et al. 2008; Xie et al. 2010; Figure 1), and displays strong tolerance to low temperature (Figure 2). It is for certain that many valuable traits exist in the wild rice species, but the most challenges to us are how to explore the valuable genes from wild rice and effectively transfer them into the cultivated rice for diversifying genetic basis of cultivated rice. Recently, many genes and QTLs have been mined from the wild rice, which functions include disease and insect resistances, abiotic stress tolerances, high yield, and so on. In this chapter, we will summarize current research progresses in mining elite genes and QTLs from wild rice for cultivar improvement in breeding programs.

Figure 1. Dongxiang wild rice (*Oryza rufipogon* Griff.) is a common wild rice located at 28°14′N latitude and 116°30′E longitude in Dongxiang county, Jiangxi province, China, which is considered to be the northernmost region in the world where *O. rufipogon* is found.

http://dx.doi.org/10.5772/56825

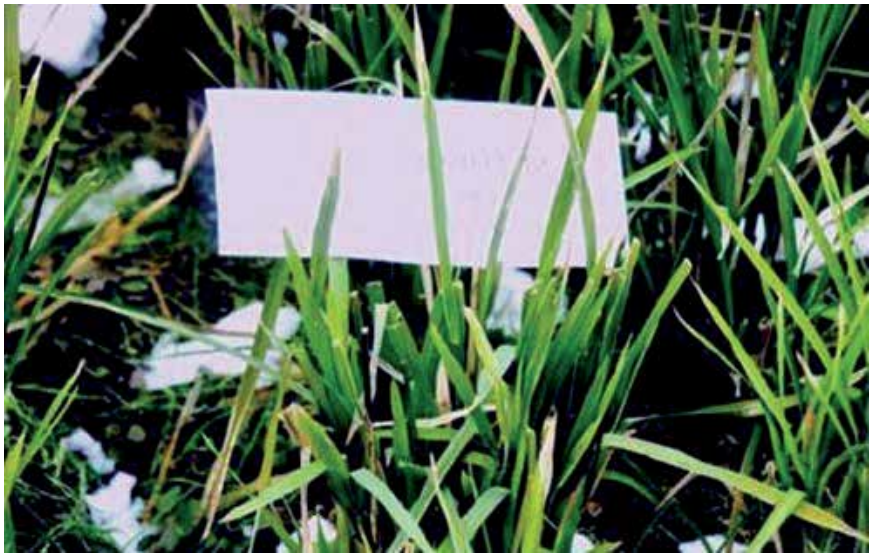


Figure 2. Dongxiang wild rice can survive under freezing conditions.

2. Disease resistance genes and QTLs in wild rice

Rice diseases such as blast, bacterial blight and sheath blight are major obstacles for achieving optimal yields. To complement conventional breeding method, molecular or transgenic method represents an increasingly important approach for genetic improvement of disease resistance and reduction of pesticide usage. During the past two decades, a wide variety of genes and mechanisms involved in rice defense response have been identified and elucidated. However, most of the cloned genes confer high level of race specific resistance in a gene-for-gene manner, and the resistance is effective against one or a few related races or strains of the pathogens. The resistance is effective for only few years because the pathogen race or strain keeps changing for survival in nature. Therefore, there is an urgent need to broaden the rice gene pool from diverse resources, of which the wild rice is an ideal option.

2.1. Rice blast resistance

Rice blast, caused by pathogen *Xanthomonas oryzae* pv. *oryzae*, is considered as a major disease of rice because of its wide distribution and destructiveness under favorable conditions. Rice blast causes lesion symptoms on leaves, stems, peduncles, panicles, seeds, and even roots. The potential threat for cropping failure makes this disease ranked among the most devastating diseases in rice. It is reported that the rice blast disease can lead to lose one million hectares annually in China alone (Savary et al. 2000; Khush et al. 2009). Exploitation of resistance gene resources for rice breeding is one of the most important ways to control the disease.

Oryza minuta J. S. Presl ex C. B. Presl is a tetraploid wild rice, highly resistant to rice blast. By genetic analysis, Amante-Bordeos et al (1992) found that the disease resistance was controlled by a single dominant gene, named *Pi9*. Subsequently, Liu et al (2002) mapped *Pi9* in an approximately 100-kb region on chromosome 6, tightly linked with RFLP markers R2132 and RG6. Finally, this broad-spectrum rice blast resistance gene was cloned using a map-based cloning strategy. It turns out that *Pi9* encodes a nucleotide-binding site-leucine-rich repeat protein, as a member of a multi-gene family in rice (Qu et al. 2006).

Jeung et al (2007) identified a new gene in the introgression line IR65482-4-136-2-2 that has inherited the resistance gene from an EE genome wild *Oryza* species, *O. australiensis* (Acc. 100882). Genetic and molecular analysis localized a major resistance gene, *Pi40*(*t*), on the short arm of chromosome 6, within 70-Kb chromosomal region narrowed by two molecular markers RM3330 and S2539. *Pi40*(*t*) was validated using the most virulent isolates identified in Korea and the Philippines, suggesting a broad resistance spectrum.

Li et al (2009) evaluated blast resistance for 21 progenies from crossing with common wild rice, and obtained three stably resistance progenies. Preliminary analysis showed that the rice blast resistance was controlled by dominant genes. Geng et al (2008) cloned rice blast resistance gene *Pi-ta*$^{+}$ from Jinghong erect type of common wild rice. The *Pi-ta*$^{+}$ coding region shares 99.86% and 98.78% of homologous identity with Japanese waxy cultivar Yashiro-mochi and Yuanjiang type of common wild rice, respectively in the corresponding regions. There are four nucleotides difference in the coding region and six in intron of the cloned *Pi-ta*$^{+}$ gene, compared with *Pi-ta* from Yashiro-mochi. The allele in the Jinghong *Pi-ta*$^{+}$ gene is very rare in nature, because there is an alanine rather than a serine at position 918 within the predicted amino acid sequence of *Pi-ta*$^{+}$. The *Pi-ta*$^{+}$ allele can display disease resistance in response to blast pathogens in rice plant cells.

2.2. Bacterial blight resistance

Bacterial blight is caused by *Xanthomonas oryzae* pv. *oryzae*. Yield losses due to bacterial blight are variable, heavily dependent on the cultivar used and the environment. In Japan, yield losses ranged typically between 20% and 30% after distribution of high-yielding dwarf varieties (Mew et al. 1993). Among tropical climates, yield losses up to 75% were reported in Indonesia, India, and the Philippines (Nino-Liu et al. 2006). It is of great importance to explore elite bacterial blight resistance genes in rice. By now, a total of 35 related genes have been reported, and nine of which were from wild rice, i.e. *Xa21*(*t*), *Xa23*(*t*), *Xa27*(*t*), *Xa29*(*t*), *Xa30*(*t*), *Xa32*(*t*), *xa32*(*t*), *Xa35*(*t*) and *Xa36*(*t*).

In 1977, Dr. S. Devadath found that a strain of *Oryza barthii* from Mali is resistant to all the races of bacterial blight in India. Then, Khush et al (1989) found that this strain is akin to *O. longistaminata*, thus crossed it with IR24, which is susceptible to six races of bacterial blight in Philippines. The F_1 was resistant to the six races, thereby showing that the resistance of *O. longistaminata* was dominant. They designated this gene as *Xa21*(*t*). By developing nearly isogenic lines of *Xa21*(*t*), Ronald et al (1992) mapped locus *Xa21*(*t*) to a region larger than 270 kb on chromosome 11. By positional cloning, Song et al (1995) isolated *Xa21*(*t*). The sequence of the predicted protein, which carries both a leucine-rich repeat motif and a serine-threonine

http://dx.doi.org/10.5772/56825

kinase-like domain, suggests a role in cell surface recognition of a pathogen ligand and subsequent activation of an intracellular defense response. Furthermore, they demonstrated that the transgenic rice plants carrying the cloned *Xa21*(*t*) gene display high levels of resistance to the pathogen.

Xa23(*t*) was first detected from *O. rufipogon* by Zhang (2005), showing resistance to race 6 of bacterial blight in the Philippines. Wang et al (2005) constructed a F_2 population of JG30/CBB23 for molecule mapping of the *Xa23*(*t*) in rice. Based on their previous mapping of *Xa23*(*t*) gene, 12 EST markers from Rice Genome Program (RGP) database were surveyed in the susceptible F_2 plants and two markers, C189 and CP02662, were found to flank *Xa23*(*t*) gene, with genetic distance of 0.8 cM and 1.3 cM, respectively.

Jin et al (2007) identified a rice bacterial blight resistance germplasm (Y238) from the wild rice species *Oryza rufipogon*, and then they transferred the resistance locus into the cultivated rice to breed near isogenic line. By molecular mapping, the gene *Xa30*(*t*) was mapped on the long arm of rice chromosome 11. Linkage analysis revealed that four molecular markers RM1341, V88, C189 and 03STS located on the same side of *Xa30*(*t*), with genetic distances of 11.4 cM, 11.4 cM, 4.4 cM and 2.0 cM to the candidate gene, respectively.

Gu et al (2004) performed disease evaluation to a *Xa27*(*t*) near-isogenic line, IRBB27 with 35 *Xanthomonas oryzae* pv. *oryzae* strains collected from 11 countries. The *Xa27*(*t*) gene conferred a high level of resistance to 27 strains and moderate resistance to three strains. Resistance of the *Xa27*(*t*) gene was developmentally regulated in IRBB27 and showed semi-dominant or a dosage effect in the cv. CO39 genetic background. Molecular mapping located *Xa27*(*t*) within a genetic interval of 0.052 cM, flanked by markers M964 and M1197 and co-segregated with markers M631, M1230, and M449.

Guo et al (2010) transferred a new rice bacterial blight resistance gene *Xa35*(*t*) from the wild rice species *Oryza minuta* (Acc. No. 101133) into *Oryza sativa* L. (IR24). Through genetic analysis and identification of resistance spectrum, *Xa35*(*t*) showed a high level of resistance to PXO61, PXO112 and PXO339, but was susceptible to PXO86 and PXO99 after inoculation with the five strains of *Xanthomonas oryzae* pv. *oryzae*. With SSR marker analysis, the *Xa35*(*t*) locus was mapped to a 1.80 cM region. This locus was co-segregated with marker RM144, and was 0.7 cM from marker RM6293 on one side and 1.1 cM from marker RM7654 on the other side on rice chromosome 11.

Xa29(*t*), which was detected from the wild rice *Oryza officinalis*, has a high resistance to bacterial blight. By molecular mapping, the *Xa29*(*t*) gene was mapped within a 1.3 cM region flanked by RFLP markers C904 and R596 on chromosome 1 (Tan et al. 2004). *Xa32*(*t*), a bacterial blight resistance gene from *Oryzae ustraliensis*, was resistant to *Xanthmonas oryzae* pv. *oryzae* strains P1 (PXO61), P4 (PXO71), P5 (PXO112), P6 (PXO99), P7 (PXO145), P8 (PXO280), P9 (PXO339), and KX085, but susceptible to P2 (PXO86) and P3 (PXO79). *Xa32*(*t*) was mapped within a 2.0 cM interval flanked by two SSR markers RM2064 and RM6293 on the long arm of rice chromosome 11 (Zheng et al. 2009). Miao et al (2010) detected that the rice germplasm C4059 harbored a bacterial blight resistance gene, and designated it tentatively as *Xa36*(*t*). By

analyzing the mapping populations, the gene *Xa36*(*t*) was mapped within a length of 4.5 cM flanked by RM224 and RM2136 on the long arm of rice chromosome 11.

2.3. Others

Bacterial leaf streak (BLS) is caused by *Xanthomonas oryzae* pv. *Oryzicola* in rice. BLS occurs in Asia and West Africa, and yield losses are up to 30 percent. The symptoms of BLS include translucent interveinal streaks extending to orange lesions which may kill the leaf. Yellowish bacterial exudates may be seen. Bacteria may enter through small wounds on the leaf surface, including insect damage. Plants are susceptible at all stages, but infection is most damaging at the tillering stage. BLS is often prevalent in the rainy season. In order to determine if the resistance genes to the BLS disease were from Guangxi wild rice in China, Huang et al (2008) screened 1655 accessions of Guangxi *Oryza rufipogon Griss*, and identified 57 (1.87%) accessions to be resistant. In another screening, 15 (48.4%) out of 31 accessions of *O. officinalis* Wall. ex Watt were resistant.

Sheath blight disease, caused by a soilborne necrotrophic fungus *Rhizoctonia solani* Kühn, is one of the most important diseases in cultivated rice. This disease was first reported in Japan in 1910 and subsequently discovered worldwide (Rush et al. 1992). At present, rice sheath blight widely occurs in most rice-growing areas, including temperate, tropical and subtropical regions in diverse rice production systems (Lee et al. 1983). Sheath blight disease causes approximately 50% yield reduction in test plots of susceptible cultivars (Savary et al. 1996). To identify resistant germplasm to sheath blight disease, Prasad et al (2008) reported seven *Oryza spp*. accessions as moderately resistant, three were *O. nivara* accessions (IRGC104705, IRGC100898, and IRGC104443), *O. barthii* (IRGC100223), *O. meridionalis* (IRGC105306), *O. nivara*/*O. sativa* (IRGC100943), and *O. officinalis* (IRGC105979). Greater effort should be paid to search sheath blight resistant germplasm from wild rice and to transfer the resistant genes into the cultivated rice in the future.

3. Insect resistance genes and QTLs identified in wild rice

Insects are serious constraints to rice production. In Asia alone, yield loss due to insects has been estimated at about 25% (Savary et al. 2000). Insects not only damage the plant by feeding on its tissue, but also are vectors of devastating rice viruses in many cases. All portions of the plant, from panicle to root, are possibly attacked by various insects. And all growth stages of the rice plant, from the seedling to mature stages, are vulnerable. Even after harvest, the grain in store might face the attack from insects (Cramer et al. 1967). Because the resistance sources in cultivated rice are limited, it is important to keep exploring resistant germplasm from wild rice species for cultivar improvements.

Brown planthopper (BPH) is a destructive insect pest to rice in Asian countries where most rice is produced in the world, including China, India, the Philippines, Japan, Korea, Vietnam, etc (Khush 1984). BPH directly damages the plant phloem by using its piercing-sucking mouthparts, resulting in “hopper burn” in the most serious cases. Furthermore, it is also a

http://dx.doi.org/10.5772/56825

vector for rice grassy stunt virus and ragged stunt virus, which may cause further yield losses in many Asian countries (Chelliah et al. 1993). Identification and incorporation of new BPH resistance genes from wild rice into modern cultivars are important breeding strategies to control the damage caused by the BPH.

Ishii et al (1994) found an introgression line from wild species *Oryza australiensis* resistant to three biotypes of BPH, and named the gene *Bph10*(*t*). RFLP analysis resulted in a linkage of the gene *Bph10*(*t*) with RG457 on chromosome 12 at a distance of 3.68 +/- 1.29 cM. A BPH biotype-4 resistance gene *Bph13*(*t*) was identified from *Oryza officinalis* Wall. Using RILs where parents "IR50" (cultivar which is susceptible to BPH Biotype-4) and "IR54745-2-21-12-17-6" (a line with *Oryza officinalis*-derived resistance to BPH biotype-4) are included, *Bph13*(*t*) was located on chromosome 3, linked with a RAPD marker AJ09b with the distance of 1.3 cM (Renganayaki et al. 2002).

Later, Jena et al (2006) identified a major BPH resistance gene *Bph18*(*t*) from an introgression line (IR65482-7-216-1-2) with wild species *Oryza australiensis*. Genetic analysis concluded that *Bph18*(*t*) is a dominant gene located within a 0.843 Mb physical interval flanked by markers R10289S and RM6869 on the long arm of chromosome 12, where three BAC clones are present.,. Subsequently, Jena et al (2010) successful cloned the *Bph18*(*t*) gene. *Bph14* is a BPH resistance gene at seedling and maturity stages. Du et al (2009) cloned *Bph14* gene to encode a coiled-coil, nucleotide-binding, and leucine-rich repeat (CC-NB-LRR) protein. Sequence comparison indicates that *Bph14* carries a unique LRR domain that might function in recognizing the BPH insect invasion and activating the defense response. *Bph14* is predominantly expressed in vascular bundles, the site of BPH feeding. Expression of *Bph14* activates the salicylic acid signaling pathway and induces callose deposition in phloem cells and trypsin inhibitor production after BPH infestation, thus reducing the BPH feeding to yield low growth rate and longevity of BPH insects.

Rahman et al (2009) conducted a genetic analysis of BPH resistance using an F_2 population derived from a cross between an introgression line, IR71033-121-15 from *Oryza minuta* (Accession number 101141) and a susceptible Korean *japonica* variety, Junambyeo. Two major QTLs were identified for BPH resistance. One was mapped to 193.4 kb region located on the short arm of chromosome 4, and the other was mapped to a 194.0 kb region on the long arm of chromosome 12.

4. Abiotic stress resistance genes and QTLs identified in wild rice

Abiotic stresses including high salinity, drought and flood, high and low temperatures are largely limiting productivity of rice crops in large areas of the world. According to Hossain (1996), abiotic stresses affect rice cultivation more than the biotic stresses. Improving the resistance to abiotic stresses will increase agricultural productivity and extend cultivatable areas of rice. There is, therefore, a strong demand for rice cultivars resistant to abiotic stresses.

Based on physiological studies on stress responses, recent progress in plant molecular biology has enabled discovery of many genes involved in stress tolerance. These genes include

functional genes which protect the cell (e.g., enzymes for generating protective metabolites and proteins), and regulatory genes which regulate stress response (e.g., transcription factors and protein kinases). Wild rice is the ancestor of cultivated rice, having been an important gene pool due to its survival ability in wild conditions and suffering from natural selection. Therefore, it is of great significance to study genetic basis of abiotic stress resistance as well as to explore new related genes in wild rice.

4.1. Cold resistance

Cold stress is a common problem for rice cultivation, and is a significant factor affecting global food production since cold stress can cause poor germination, slow growth, withering, and anthers injury on rice plants (Andaya et al. 2007). Annually, about 15 million hectares of rice in the world suffered from cold damage (Zhang et al. 2005). In south Asia, about 7 million hectares cannot be planted timely because of the low temperature stress (Sthapit et al. 1998). Consequently, development of rice cultivars with cold tolerance is recognized as one of the important breeding objectives.

Various methods have been adapted to improve rice resistance to low temperature stress (Bertin et al. 1997; Takesawa et al. 2002). With increasing emphasis on F_1 hybrid rice production in public institutions and private breeding companies, lots of landraces with diversified genetic background continue to decrease, which makes the genetic base of parental materials become more and more narrower. As a result, development of cultivars for strong cold tolerance becomes increasingly difficult using intra-variation. There is thus an urgent need to study the cold-tolerance character and excavate related genes in wild rice to broaden rice gene pool for developing cold tolerance cultivars.

Genetic analysis of cold tolerance at seedling and/or booting stage has resulted in the identification of many QTLs (Lou et al. 2007; Zeng et al. 2009). Zheng et al (2011) constructed chromosome segment substitution line (CSSL) populations using two core accessions of common wild rice (DP15 and DP30) as donor parents and cultivar 9311 as recipient parent. Thus, they identified cold tolerance QTLs effective at the seedling stage. Two donor lines, DP15 and DP30, are different in the number, location and effect of QTLs for cold tolerance. A total of 19 cold tolerance QTLs were detected, and clustered on chromosome 3 and chromosome 8. The survival rates ranged 8 – 74% after cold treatment among the CSSLs. A major QTL *qSCT-3-1* was mapped between SSR markers RM15031 and RM3400, near the centromere of chromosome 3 on the long arm with a distance of 1.8 cM.

Dongxiang wild rice can winter over successful in Wuhan, Hubei province, China, where the lowest temperature can be down to -12C in winter (Liu et al. 2003). In order to transfer cold tolerance gene from Dongxiang wild rice, we have developed introgression lines (ILs) through a backcrossing and single-seed descent program using an elite *indica* restoring cultivar Xieqingzao B (*O. sativa* L.) as recipient and Dongxiang wild rice as donor parent (Jian et al. 2011). Analyzing the introgression lines found that the IL5243 and IL5335 were the best for cold tolerant ability (Chen et al. 2013). Genetic analysis using SSR markers further confirmed that a part of alien DNA has been transferred from the common wild rice into IL5243 and

IL5335. Therefore, IL5243 and IL5335 might be excellent bridging germplasm for breeding programs to improve cultivar tolerance to cold stress.

4.2. Soil salinity resistance

Soil salinity is one of the major agricultural problems affecting crop productivity worldwide (Rozema et al. 2008). Of the cereals, rice is one of the most salt-sensitive crops (Shelden et al. 2013). The effects of salinity on rice have been reported to reduce seed germination (Hakim et al. 2010), decrease growth and survival of seedlings (Lutts et al. 1995), damage the structure of chloroplasts (Yamane et al. 2008), reduce photosynthesis (Moradi et al. 2007) and inhibit seed set and grain yield (Asch et al. 2000). Improving evaluation methodologies to identify genetic sources and excavating responsible genes for improving cultivar salt resistance is of continuing importance in rice. *Oryza coaretata* is an Asian wild rice species, occurring mostly in the coastal areas of India. This species is highly resistant to salt because of survival ability in the coastal environments. *O. coarctata* has some special unicellular hairs (trichomes) on the adaxial surface of leaves. The hairs efficiently maintain a low concentration of toxic salts in the plant tissue (Bal et al. 1986).

4.3. Low-phosphorus resistance

Phosphorus is one of essential nutritive elements for rice growth and development (Abel et al. 2002). The phosphorus content may be too little in the soil to be able to meet the needs of rice growth. It has been estimated that 5.7 billion hectares of land are deficient in phosphorus worldwide. Phosphorus deficiency is considered as one of the greatest limitations in agricultural production (Schachtman et al. 1998; Lynch et al. 2008).

Chen et al (2011) identified the low-phosphorus resistance ability of Dongxiang wild rice at the seedling stage by using the cultivated low-phosphorus sensitive varieties as the control. The results showed that Dongxiang wild rice has strong low-phosphorus resistance ability. And then, they developed BILs by using Dongxiang wild rice as donor parent and the low-phosphorus sensitive variety Xieqingzao B as recurrent parent. By analyzing the morphological indices, they found that the low-phosphorus resistance lines under low-phosphorus stress had higher values of relative leaf age, relative plant height, relative shoot dry mass, and relative soluble content, but low values of relative yellow leaf number and relative malondialdehyde content, suggesting that the low-phosphorus resistance capability of the low-phosphorus resistance lines was mainly attributed to the high phosphorus utilization efficiency of the lines, namely, low-phosphorus resistance lines had stronger capability in synthesizing dry mass with per unit phosphorus uptake (Chen et al. 2011).

4.4. Drought resistance

Because of global climate warming and increasing scarcity of water resource, drought stress and water scarcity have severely impacted the security of rice production (Farooq et al. 2009). At least 23 million hectares of rice area in Asia are estimated to be drought-prone (Pandey et al. 2005). To date, however, the major challenge for research communities is the relatively

limited progress achieved in developing high yielding rice cultivars with drought resistance (Rabello et al. 2008). Therefore, the improvement of drought resistance in newly developed cultivars, for the wide adaptability across rice-growing ecologies, has become a major priority in rice breeding programs. Accordingly, identifying genes from new germplasm resources such as wild rice has become extremely important for drought resistance, which will lay the foundation for utilization of drought resistance gene and genetic improvement of drought resistance (Xie et al. 2004).

Our group has already carried out preliminary experiments for many years on characterization of Dongxiang wild rice for genetic differentiation and conservation, and utilization (Xie et al. 2010). We proved that Dongxiang wild rice has strong drought resistance (Figure 3). Subsequently, Hu et al (2013) constructed BIL population using *Indica* restorer line R974 (*Oryza sativa* L.) and Dongxiang wild rice. Using a mixed inheritance model for both major genes and minor genes, they found that the inheritance of drought-resistance at seedling stage was controlled by two independent genes plus polygenes. Therefore, Dongxiang wild rice could be precious resource for genetic improvement of drought resistance in cultivar development.

Figure 3. Dongxiang wild rice has strong drought resistance.

5. Yield-enhancing QTLs from wild rice

In general, wild rice has smaller seeds and other undesirable traits compared to cultivars, and thus appears not to be appropriate for a donor to enhance yield in cultivars. However, molecular studies have demonstrated that phenotypically poor wild rice contains some genes important for improving cultivar yield (Tanksley et al. 1996). Some wild-QTL alleles

http://dx.doi.org/10.5772/56825

are favorable for some traits, but may be associated with deleterious effects on other traits. The positive QTLs from *O. rufipogon* may be potentially useful for breeding high yield cultivar if the disadvantage linkage drag could be broken through careful selection. In addition, other potentially beneficial QTLs for yield-related traits are often linked to the QTLs conferring negative traits. For example, *gpp1.1* with yield increasing effect is closely linked with a negative QTL to increase plant height because this QTL is closely linked to *sd1* locus (Cho et al. 2003). Brondani et al (2002) detected specific marker regions to strongly associate with multiple yield-related traits including panicle number, spikelets per panicle, seed set percentage, 100-grain weight, grain yield per plant, filled grain number per panicle and grain yield per panicle.

By using a BC_2F_5 population derived from the cross between Zhenshan 97 and a wild rice, Wu et al (2012) identified a QTL region flanked by SSR marker RM481 and RM2 on chromosome 7. This QTL has pleiotropic effects on heading date, spikelets per panicle, and grain yield per plant. The alleles from wild rice have increasing effects on these phenotypic traits contributable to grain yield.

Fu et al (2010) developed an advanced backcross population by using an accession of common wild rice collected from Yuanjiang County, Yunnan Province, China, as the donor and an elite cultivar 9311 as the recurrent parent. From this population, several QTLs originating from *O. rufipogon* display beneficial effects for yield-related traits in the 9311 genetic background. In addition, five QTLs controlling yield and its components are newly identified, and they are potentially novel alleles in Yuanjiang common wild rice. Three regions underling significant QTLs for several yield-related traits are detected on chromosome 1 (RM212-RM5362), 7 (RM125-RM1135) and 12 (RM7003-RM277).

Xiao et al (1998) identified two yield-enhancing QTLs, *yld1.1* and *yld2.1*, from *O. rufipogon* using BC_2 populations. QTLs *yld1.1* and *yld2.1* have been transferred to the elite restorer lines Ce64-7, 9311 and Minghui63 by marker-assisted selection (MAS), and they are confirmed to produce significant yield-enhancing effects in field tests. Xie et al (2006) fine mapped a yield-enhancing QTL cluster using a BC_3F_4 population derived from a cross between the Korean *japonica* cultivar Hwaseongbyeo and *O. rufipogon.* The cluster contained seven QTLs for 1000-grain weight, spikelets per panicle, grains per panicle, panicle length, spikelet density, heading date and plant height. The alleles from the low-yielding *O. rufipogon* parent are beneficial in the Hwaseongbyeo background.

6. Present problems and future directions

As the wild relatives and ancestor of cultivated rice, wild rice carries various characteristics resistant to biotic and abiotic stresses, beneficial agronomic traits, and abundant genetic diversity, which have been lost in the cultivated rice due to breeding activities (Sakai et al. 2010). Thus, it is an extremely important resource for improving important traits in cultivated rice (Xie et al. 2004). However, loss of wild rice genetic diversity was sped up

by increasing deterioration of original habitat. For example, the Dongxiang wild rice was sharply reduced from nine populations in nine isolated areas in 1978 to three in 1995 (Hu et al. 2011). The dramatic reduction makes the unique gene pool endangered. Therefore, it is necessary to accelerate a rational conservation for effective utilization of these survived genetic resources.

Breeders have long recognized the intrinsic value of wild rice for improving the traits of modern cultivars. The most successful examples to utilize wild rice in the history of rice breeding include the use of *Oryza nivara* genes to provide long-lasting resistance to grassy stunt virus (Plucknett et al. 1987), and the use of *O. spontanea* as a source of wild abortive cytoplasmic male sterility, which has made a cornerstone for today's hybrid rice (Li et al. 1988). However, despite these successes, it is still difficult to utilize wild rice for the improvement of quantitatively inherited traits. Great progress of molecular markers and maps makes it possible to identify individual QTL associated with elite traits from wild rice, which will help transfer the valuable QTLs into modern cultivars to improve their qualities (Tanksley et al. 1996).

Nowadays, QTL studies for mining favorable genes from wild rice species are receiving more and more attentions in global rice community. Several studies have successfully identified and introduced the QTL enhancing alleles from wild rice for yield-related traits into high-yielding elite cultivars (He et al. 2006; Deng et al. 2007; Tan et al. 2008). In addition, some QTLs related to rice quality traits were also detected using wild rice introgression lines (Hao et al. 2006; Garcia-Oliveira et al. 2009). Molecular mapping of these good genes will help discover and make full use of the elite resources of wild species to broaden the genetic base of modern cultivars. However, only a few genes have been cloned from wild rice, and the mechanism for those excellent traits from wild rice are far from being clarified. Cloning more genes from wild rice should be emphasized in the future, which will help make full use of these elite resources more effectively.

In summary, as a rare germplasm resource, wild rice is of great significance to our agricultural heritage and biodiversity protection. Research reveals that wild rice not only has many elite genes which have lost in cultivated rice, but also maintains a greater genetic diversity than cultivated rice. We should use the wild rice to broaden genetic diversity of cultivated rice, by which new cultivars could withstand biotic and abiotic stresses. This is of great significance to assure both high yield and quality in rice production.

Author details

Fantao Zhang and Jiankun Xie*

*Address all correspondence to: xiejiankun@yahoo.com

College of Life Sciences, Jiangxi Normal University, China

References

[1] Abel S, Ticconi CA, Delatorre CA (2002) Phosphate sensing in higher plants. Physiol Plant 115:1–8

[2] Amante-Bordeos A, Sitch LA, Nelson R, Damacio RD, Oliva NP, Aswidinnoor H, Leung H (1992) Transfer of bacterial blight and blast resistance from the tetraploid wild rice *Oryza minuta* to cultivated rice, *Oryza sativa*. Theor Appl Genet 84:345–354

[3] Andaya VC, Tai TH (2007) Fine mapping of the *qCTS4* locus associated with seedling cold tolerance in rice (*Oryza sativa* L.). Mol Breeding 20:349–358

[4] Asch F, Dingkuhn M, Dorffling K, Miezan K (2000) Leaf K/Na ratio predicts salinity induced yield loss in irrigated rice. Euphytica 113:109–118

[5] Bal AR, Dutt SK (1986) Mechanism of salt tolerance in wild rice (*Oryza coarctata Roxb*). Plant and soil 92:399–404

[6] Bertin P, Bouharmont J (1997) Use of somaclonal variation and in vitro selection for chilling tolerance improvement in rice. Euphytica 96:135–142

[7] Brar DS, Khush GS (1997) Alien introgression in rice. Plant Mol Biol 35:35–47

[8] Brar DS, Khush GS (2003) Utilization of wild species of genus *Oryza* in rice improvement. In: Monograph of genus *Oryza*, Nanda, J.S. and S.D. Sharma (eds). Science Publishers, Inc., UK., pp:283–310

[9] Brondani C, Rangel PHN, Brondani RPV, Ferreira ME (2002) QTL mapping and introgression of yield-related traits from *Oryza glumaepatula* to cultivated rice (*Oryza sativa*) using microsatellite markers. Theor Appl Genet 104:1192–1203

[10] Chelliah S, Bharathi M (1993) Biotypes of the brown planthopper, *Nilaparvata lugens* (homoptera: Delphacidae) – host inxuenced biology and behavior. Chemical ecology of phytopathogous insects. International Science Publishers, New York:133–148

[11] Chen XR, Chen M, He HH, Zhu CL, Peng XS, He XP, Fu JR, Ouyang LJ (2011) Identification of low-phosphorus-tolerance in Dongxiang wild rice (*Oryza rufipogon* Griff.). Acta Agriculturae Universitatis Jiangxiensis 33:405–411 (Chinese with English abstract)

[12] Chen XR, Chen M, He HH, Zhu CL, Peng XS, He XP, Fu JR, Ouyang LJ (2011) Low phosphorus tolerance and related physiological mechanism of Xieqingzao B // Xieqingzao B / Dongxiang wild rice BC_1F_9 populations. Chinese Journal of Applied Ecology 22:1169–1174 (Chinese with English abstract)

[13] Chen XR, Yang KS, Fu JR, Zhu CL, Peng XS, He P, He HH (2008) Identification and genetic analysis of fertility restoration ability in Dongxiang wild rice (*Oryza rufipogon*). Rice Sci 15:21–28

[14] Chen YL, Luo XD, Zhang FT, Dai LF, Hu BL, Xie JK (2013) Cloning and expression analysis of retrotransposon reverse transcriptase in introgression lines from Dongxiang wild rice. Chinese Bulletin of Botany 48:138–144 (Chinese with English abstract)

[15] Cho YC, Suh JP, Choi IS, Hong HC, Baek MK, Kang KH, Kim YG, Ahn SN, Choi HC, Hwang HG, Moon HP (2003) QTLs analysis of yield and its related traits in wild rice relative *Oryza rufipogon*. Treat of Crop Res 4:19–29

[16] Cramer DA, Barton RA, Shorland FB, Chochanska Z (1967) A comparison of the effects of white clover (*Trifolium repens*) and of perennial ryegrass (*Lolium perenne*) on fat composition and flavour of lamb. J Agric Sci 69:367–373

[17] Deng HB, Deng QY, Chen LY, Yang YS, Liu GM, Zhuang W, Xiong YD (2007) Yield-increasing effect of yield-enhancing QTLs from *O. rufipogon* transferred to 9311, a male parent of medium super hybrid rice. Hybrid Rice 22:49–52 (Chinese with English abstract)

[18] Du B, Zhang WL, Liu BF, Hu J, Wei Z, Shi ZY, He RF, Zhu LL, Chen RZ, Han B, He GC (2009) Identification and characterization of *Bph14*, a gene conferring resistance to brown planthopper in rice. PNAS 106: 22163–22168

[19] Farooq M, Wahid A, Kobayashi N, Fujita D, Basra SMA (2009) Plant drought stress: effects, mechanisms and management. Agron Sustain Dev 29:185–212

[20] Fischer J, Fazey I, Briese R, Lindenmayer DB (2005) Making the matrix matter: challenges in Australian grazing landscapes. Biodivers Conserv 14: 561–78

[21] Fu Q, Zhang PJ, Tan LB, Zhu ZF, Ma D, Fu YC, Zhan XC, Cai HW, Sun CQ (2010) Analysis of QTLs for yield-related traits in Yuanjiang common wild rice (*Oryza rufipogon* Griff.). J Genet Genomics 37:147–157

[22] Garcia-Oliveira AL, Tan LB, Fu YC, Sun CQ (2009) Genetic identification of quantitative trait loci for contents of mineral nutrients in rice grain. J Integr Plant Biol 51:84–92

[23] Geng XS, Yang MZ, Huang XQ, Cheng ZQ, Fu J, Sun T, Li J (2008) Cloning and analyzing of rice blast resistance gene *Pi-ta*$^{+}$ allele from Jinghong erect type of common wild rice (*Oryza rufipogon* Griff) in Yunnan. Hereditas (Beijing) 30(1):109–114 (Chinese with English abstract)

[24] Gu K, Tian D, Yang F, Wu L, Sreekala C, Wang D, Wang GL, Yin Z (2004) High-resolution genetic mapping of *Xa27*(*t*), a new bacterial blight resistance gene in rice, *Oryza sativa* L. Theor Appl Genet 108:800–807

[25] Guo SB, Zhang DP, Lin XH (2010) Identification and mapping of a novel bacterial blight resistance gene *Xa35*(*t*) originated from *oryza minuta*. Sci Agri Sin 43:2611–2618 (Chinese with English abstract)

[26] Hakim MA, Juraimi AS, Begum M, Hanafi MM, Ismail MR, Selamat A (2010) Effect of salt stress on germination and early seedling growth of rice (*Oryza sativa* L.). Afr J Biotechnol 9:1911–1918

[27] Hao W, Jin J, Sun SY, Zhu MZ, Lin HX (2006) Construction of chromosome segment substitution lines carrying overlapping chromosome segments of the whole wild rice genome and identification of quantitative trait loci for rice quality. J Plant Physiol Mol Biol 32:354–362 (Chinese with English abstract)

[28] He GM, Luo XJ, Tian F, Li KG, Su W, Zhu ZF, Qian XY, Fu YC, Wang XK, Sun CQ, Yang JS (2006) Haplotype variation in structure and expression of a gene cluster associated with a quantitative trait locus for improved yield in rice. Genome Res 16: 618–626

[29] Hossain M (1996) Economic prosperity in Asia: Implication for rice research. IRRI. Rice Genetics III. Proceedings of the third international rice genetics symposium. 16–20 Oct., 1995, Manila, Philippines

[30] Hu BL, Fu XQ, Zhang T, Wan Y, Li X, Huang YH, Dai LF, Luo XD, Xie JK (2011) Genetic analysis on characteristics to measure drought resistance using Dongxiang wild rice (*Oryza rufupogon* Griff.) and its derived backcross inbred lines population at seedling stage. Agricultural Sciences in China 10:1653–1664

[31] Hu BL, Yang P, Wan Y, Li X, Luo SY, Luo XD, Xie JK (2013) Comprehensive assessment of drought resistance of BILs population derived from Dongxiang wild rice (*Oryza rufupogon* Griff.) at seedling stage and its genetic analysis. Journal of Plnat Genetic Resources 14:249–256 (Chinese with English abstract)

[32] Huang DH, Cen ZL, Liu C, He WN, Chen YZ, Ma ZF, Yang L, Wei SL, Liu YL, Huang SL, Yang XQ, Li RB (2008) Identification and genetic analysis of resistance to bacterial leaf streak in wild rice. Journal of plant genetic resources 9:11–14 (Chinese with English abstract)

[33] Ishii T, Brar DS, Multani DS, Khush GS (1994) Molecualar tagging of genes for brown lanthopper resistance and earliness introgressed from *Oryza australiennsis* into cultivated rice, *O. sativa*. Genome 37:217–221

[34] Jena KK, Jeung JU, Lee JH, Choi HC, Brar DS (2006) High-resolution mapping of a new brown planthopper (BPH) resistance gene *Bph18*(*t*), and marker-assisted selection for BPH resistance in rice (*Oryza sativa* L.). Theor Appl Genet 112:288–297

[35] Jena KK, Kim SM (2010) Current status of brown planthopper (BPH) resistance and genetics. Rice 3:161–171

[36] Jeung JU, Kim BR, Cho YC, Han SS, Moon HP, Lee YT, Jena KK (2007) A novel gene, *Pi40*(*t*), linked to the DNA markers derived from NBS-LRR motifs confers broad spectrum of blast resistance in rice. Theor Appl Genet 115:1163–1677

[37] Jian SR, Wan Y, Luo XD, Fang J, Chu CC, Xie JK (2011) Genetic analysis of cold tolerance at the seedling stage in Dongxiang wild rice (*Oryza rufipogon*). Chinese Bulletin of Botany 46: 21–27 (Chinese with English abstract)

[38] Jin XW, Wang CL, Yang Q, Jiang QX, Fan YL, Liu GC, Zhao KJ (2007) Breeding of near-isogenic line CBB30 and molecular mapping of *Xa30*(*t*), a new resistance gene to bacterial blight in rice. Sci Agri Sin 40:1094–1100 (Chinese with English abstract)

[39] Khush GS (1984) Breeding rice for resistance to insects. Protection Eco 7:147–165

[40] Khush GS, Mackill DJ, Sidhu GS (1989) Breeding rice for resistance to bacterial blight. In: Bacterial blight of rice. International Rice Research Institute, Manila, Philippines: 207–217

[41] Khush GS (1997) Origin, dispersal, cultivation and variation of rice. Plant Mol Biol 35: 25–34

[42] Khush GS, Jena KK (2009) Current status and future prospects for research on blast resistance in rice (*Oryza sativa* L.). Advances in genetics, genomics and control of rice blast disease. Berlin: Springer:1–10

[43] Lee FN, Rush MC (1983) Rice sheath blight: a major rice disease. Plant Dis 67:829–832

[44] Li SH, Yang QW, Zheng GB, Li H, Lu XL, Han GM (2009) The genetic analysis on resistance to rice blast of rice progeny introduced with wild rice genes. Seed 28:36–40 (Chinese with English abstract)

[45] Li Z, Zhu Y (1988) Rice male sterile cytoplasm and fertility restoration. In: Hybrid Rice. International Rice Research Institute. Manila, Philippines:85–102

[46] Liu FX, Sun CQ, Tan LB, Fu YC, Li DJ, Wang XK (2003) Identification of QTL for cold tolerance at booting and flowering stage in Jiangxi Dongxiang wild rice. Chin Sci Bull 48:1864–1867 (Chinese with English abstract)

[47] Liu G, Lu G, Zeng L, Wang GL (2002) Two broad-spectrum blast resistance genes, *Pi9*(*t*) and *Pi2*(*t*), are physically linked on rice chromosome 6. Mol Genet Genomics 267:472–80

[48] Lou QJ, Chen L, Sun ZX, Xing YZ, Li J, Xu XY, Mei HW, Luo LJ (2007) A major QTL associated with cold tolerance at seedling stage in rice (*Oryza sativa* L.). Euphytica 158: 87–94

[49] Lutts S, Kinet J M, Bouharmont J (1995) Changes in plant response to NaCl during development of rice (*Oryza sativa* L.) varieties differing in salinity resistance. J Exp Bot 46:1843–1852

[50] Lynch JP, Brown KM (2008) Root strategies for phosphorus acquisition. Plant Ecophysiol 7:83–116

[51] Maclean JL, Dawe DC, Hardy B, Hettel GP (eds) (2002) Rice almanac (Third Edition). Philippines, IRRI, WARDA, CIAT and FAO

[52] Mew TM, Alvarez AM, Leach JE, Swings J (1993) Focus on bacterial blight of rice. Plant Dis 77:5–12

[53] Miao LL, Wang CL, Zheng CK, Che JY, Gao Y, Wen YC, Li GQ, Zhao KJ (2010) Molecular mapping of a new gene for resistance to rice bacterial blight. Sci Agri Sin 43: 3051–3058 (Chinese with English abstract)

[54] Moradi F, Ismail AM (2007) Responses of photosynthesis, chlorophyll fluorescence and ROS-scavenging systems to salt stress during seedling and reproductive stages in rice. Annals of Botany 99:1161–1173

[55] Nino-Liu DO, Ronald PC, Bogdanove AJ (2006) *Xanthomonas oryzae* pathovars: model pathogens of a model crop. Mol Plant Pathol 7:303–324

[56] Pandey S, Bhandari H, Sharan R, Naik D, Taunk SK, Sastri ASRAS (2005) Economic costs of drought and rainfed rice farmers' coping mechanisms in eastern India, Final report. International Rice Research Institute, Los Baños, Philippine.

[57] Plucknett DL, Smith NJH, Williams JT, Anishetty NM (1987) Gene banks and the world's food. Princeton University Press, Princeton, New Jersey

[58] Prasad B, Eizenga GC (2008) Rice sheath blight disease resistance identified in *Oryzas pp*. *accessions*. Plant Dis 92:1503–1509

[59] Qu SH, Liu GF, Zhou B, Bellizzi M, Zeng LR, Dai LY, Han B, Wang GL (2006) The broad-spectrum blast resistance gene *Pi9* encodes a nucleotide-binding site-leucine-rich repeat protein and is a member of a multigene family in rice. Genetics 172:1901–1914

[60] Rabello AR, Guimaraes CM, Rangel PH, da Silva FR, Seixas D, de Souza E, Brasileiro AC, Spehar CR, Ferreira ME, Mehta A (2008) Identification of drought-responsive genes in roots of upland rice (*Oryza sativa* L). BMC Genomics 9:485

[61] Rahman ML, Jiang W, Chu SH, Qiao Y, Ham TH, Woo MK, Lee J, Khanam MS, Chin JH, Jeung JU, Brar DS, Jena KK, Koh HJ (2009) High-resolution mapping of two brown planthopper resistance genes, *Bph20*(*t*) and *Bph21*(*t*), originating from *Oryza minuta*. Theor Appl Genet 119:1237–1246

[62] Renganayaki K, Fritz AK, Sadasivam S, Pammi S, Harrington SE, McCouch SR, Kumar SM, Reddy AS (2002) Mapping and progress toward map-based cloning of brown planthopper biotype-4 resistance gene introgressed from *Oryza officinalis* into cultivated rice, *O. sativa*. Crop Science 42:2112–2117

[63] Ronald PC, Albano B, Tabien R, Abenes L, Wu KS, McCouch S, Tanksley SD (1992) Genetic and physical analysis of the rice bacterial blight disease resistance locus, *Xa21*. Mol Gen Genet 236:113–120

[64] Rozema J, Flowers T (2008) Crops for a salinized world. Science 322:1478–1480

[65] Rush MC, Lee FN (1992) Sheath blight. Pages 22–23 in: Compendium of rice diseases. R. K. Webster and P. S. Gunnell, eds. American Phytopathological Society, St. Paul, MN

[66] Sakai H, Itoh T (2010) Massive gene losses in Asian cultivated rice unveiled by comparative genome analysis. BMC Genomics 11:121

[67] Sasaki T, Burr B (2000) International Rice Genome Sequencing Project: The effort to completely sequence the rice genome. Curr Opin Plant Biol 3:138–141

[68] Savary S, Mew TW (1996) Analyzing crop losses due to *Rhizoctonia solani*: Rice sheath blight, a case study. In: *Rhizoctonia* Species: Taxonomy, Molecular Biology, Ecology, Pathology and Disease Control. B. Sneh, S. Jabajihare, S. Neate, and G. Dijst, eds. Kluwer Academic Publisher, Dordrecht, The Netherlands:237–245

[69] Savary S, Willocquet L, Elazegui FA, Castilla N, Teng PS (2000) Rice pest constraints in tropical Asia: quantification of yield losses due to rice pests in a range of production situations. Plant Dis 84:357–369

[70] Schachtman DP, Reid RJ, Ayling SM (1998) Phosphorus uptake by plants: from soil to cell. Plant Physiol 116:447–453

[71] Shelden MC, Roessner U (2013) Advances in functional genomics for investigating salinity stress tolerance mechanisms in cereals. Frontiers in plant science 4:123

[72] Song WY, Wang GL, Chen LL, Kim HS, Pi LY, Holsten T, Gardner J, Wang B, Zhai WX, Zhu LH, Fauquet C, Ronald P (1995) A receptor kinase-like protein encoded by the rice disease resistance gene, *Xa21*. Science 270:1804–1806

[73] Song ZP, Li B, Chen JK, Lu BR (2005) Genetic diversity and conservation of common wild rice (*Oryza rufipogon*) in China. Plant Spec Biol 20:83–92

[74] Stebbins GL (1981) Why are there so many species of flowering plants? Bioscience 31:573–577

[75] Sthapit BR, Witcombe JR (1998) Inheritance of tolerance to chilling stressing rice during germination and plumule greening. Crop Sci 38:660–665

[76] Sun CQ, Wang XK, Li ZC, Yoshimura A, Iwata N (2001) Comparison on the genetic diversity of common wild rice (*Oryza rufipogon* Griff.) and cultivated rice (*O. sativa* L.) using RFLP markers. Theor Appl Genet 102:157–162

[77] Takesawa T, Ito M, Kanzaki H, Kameya N, Nakamura I (2002) Over-expression of ζ glutathione S-transferase in transgenic rice enhances germination and growth at low temperature. Mol Breed 9:93–101

[78] Tan GX, Ren X, Weng QM, Shi ZY, Zhu LL, He GC (2004) Mapping of a new resistance gene to bacterial blight in rice line introgressed from *Oryza officinalis*. Acta Genetica Sinica 31:724–729 (Chinese with English abstract)

[79] Tan LB, Zhang PJ, Liu FX, Wang GJ, Ye S, Zhu ZF, Fu YC, Cai HW, Sun CQ (2008) Quantitative trait loci underlying domestication and yield-related traits in *Oryza rufipogon* × *Oryza sativa* advanced backcross population. Genome 51:692–704

[80] Tanksley SD, Grandillo S, Fulton TM, Zamir D, Eshed Y, Petiard V, Lopez J, Beck-Bunn T (1996) Advanced backcross QTL analysis in a cross between an elite processing line of tomato and its wild relative L. pimpinellifolium. Theor Appl Genet 92:213–224

[81] Tanksley SD, Nelson JC (1996). Advanced backcross QTL analysis: a method for the simultaneous discovery and transfer of valuable QTLs from unadapted germplasm into elite breeding lines. Theor Appl Genet 92:191–203

[82] Vaughan DA (1994) The wild relatives of rice: a genetic resources guide book. International Rice Research Institute, Los Banos, The Philippines

[83] Wang CL, Qi HX, Pan HJ, Li JB, Fan YL, Zhang Q, Zhao KJ (2005) EST-markers flanking the rice bacterial blight resistance gene *Xa23* and their application in marker-assisted selection. Sci Agri Sin 38:1996–2001 (Chinese with English abstract)

[84] Wu B, Han ZM, Li ZX, Xing YZ (2012) Discovery of QTLs increasing yield related traits in common wild rice. Yi Chuan 34:215–22 (Chinese with English abstract)

[85] Xiao JH, Li JM, Grandillo S, Ahn SN, Yuan LP, Tanksley SD, McCouch SR (1998) Identification of trait-improving quantitative trait loci alleles from a wild rice relative, *Oryza rufipogon*. Genetics 150:899–909

[86] Xie JK, Agrama HA, Kong D, Zhuang J, Hu B, Wan Y, Yan W (2010) Genetic diversity associated with conservation of endangered Dongxiang wild rice (*Oryza rufipogon*). Genet Resour Crop Evol 57:597–609

[87] Xie JK, Hu BL, Wan Y, Zhang T, Li X, Liu RL, Huang YH, Dai LF, Luo XD (2010) Comparison of the drought resistance characters at Seedling Stage between Dongxiang Common Wild rice (*Oryza rufipogon* Griff.) and cultivars (*Oryza sativa* L.). Acta Ecologica Sinica 30:1665–1674 (Chinese with English abstract)

[88] Xie JK, Kong XL, Bao JS, Wan Y (2004) Recent advances in molecular mapping and cloning of useful genes from wild rice and their application in breeding. Hereditas (Beijing) 26:115–121 (Chinese with English abstract)

[89] Xie X, Song MH, Jin F, Ahn SN, Suh JP, Hwang HG, McCouch SR (2006) Fine mapping of a grain weight quantitative trait locus on rice chromosome 8 using near-isogenic lines derived from a cross between *Oryza sativa* and *Oryza rufipogon*. Theor Appl Genet 113:885–894

[90] Yamane K, Kawasaki M, Taniguchi M, Miyake H (2008) Correlation between chloroplast ultrastructure and chlorophyll fluorescence characteristics in the leaves of rice (*Oryza sativa* L.) grown under salinity. Plant Prod Sci 11:139–145

[91] Zeng YW, Yang SM, Cui H, Yang XJ, Xu LM, Du J, Pu XY, Li ZC, Cheng ZQ, Huang XQ (2009) QTLs of cold tolerance-related traits at the booting stage for NIL-RILs in rice revealed by SSR. Genes Genom 31:143–154

[92] Zhang Q (2005) Highlights in identification and application of resistance genes to bacterial blight. Chinese J Rice Sci 19:453–459 (Chinese with English abstract)

[93] Zhang ZH, Li S, Wei L, Wei C, Zhu YG (2005) A major QTL conferring cold tolerance at the early seedling stage using recombinant inbred lines of rice (*Oryza sativa* L.). Plant Sci 168:527–534

[94] Zheng CK, Wang CL, Yu YJ, Liang YT, Zhao KJ (2009) Identification and molecular mapping of *Xa32*(*t*), a novel resistance gene for bacterial blight (*Xanthomonas oryzae* pv. *oryzae*) in rice. Acta Agronomica Sin 35:1173−1180 (Chinese with English abstract)

[95] Zheng JX, Ma ZF, Song JD, Liu C, Li YT, Huang DH, Wei SL, Zhang YX, Mi K, Huang JY, Chen M, Meng JR, Li RB, Chen BS (2011) Identification and mapping of QTLs for cold tolerance at the seedling stage in common wild rice (*Oryza rufipogon*). Chinese J Rice Sci 25:52–58 (Chinese with English abstract)

Chapter 3

Rice Germplasm in Korea and Association Mapping

Aye Aye Khaing, Gang Li, Xiao Qiang Wang,
Min Young Yoon, Soon Wook Kwon,
Chang Yong Lee, Beom Seok Park and Yong Jin Park

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/56614

1. Introduction

Rice is a traditional staple food crop in Korea and many other countries. Although the center of rice origin is still unclear, it is believed to be introduced from China to the Korean Peninsula in the early Bronze Age via one of two possible routes—across the West Sea or along the northeastern seashore from China according to Hammer (2005) and Vavilov (1935). Rice germplasm has evolved through several millennia of cultivation and selection by our farming ancestors. An important consequence of the domestication of both plants and animals is a reduction of genetic variability (Hancock, 2004). Maintaining biodiversity is an important worldwide problem and different countries have various policies intended to preserve biodiversity. Because conservation of biodiversity and ecosystems is closely linked to the quality of human life, the preservation and improvement of ecosystems are problematic for agriculture.

Genetic diversity in a crop species is essential for sustained high productivity. Breeding efforts have been devoted to improving grain quality, yield potential, resistance to diseases and insect pests, and environmental stress tolerance. Progress in plant breeding requires a continuous supply of genes or gene-complexes. In this respect, the researcher is often handicapped by the limited availability of germplasm resources. The assembly of large varietal collections, systematic screening for desired traits and subsequent incorporation of the relevant genes into existing cultivars is imperative to meet these needs. The use of landrace varieties has increased in recent years. Wild rice accessions have contributed greatly to rice breeding as a source of resistance genes (*e.g., Xa21, BPH14, BPH15*) (Ronald *et al*., 1992; Song *et al*., 1995; Yang *et al*., 2004; Du *et al*., 2009; Hu *et al*., 2012). Much of the diversity in the rice gene pool is contained in gene banks around the world. Molecular biology has contributed significantly to an increased

understanding of many aspects of plant biology by generating technologies and methods of analysis that provide new approaches or supplement classical methods of analysis. Plant genetic resource scientists and other researchers are increasingly aware of the potential benefits of applying new technologies to germplasm conservation and research.

The integration of genetic data with molecular biotechnology will help breeders produce new rice varieties with the desired traits and make the conservation of rice genetic resources more efficient. Because of newly developed methods for association mapping of genes or QTLs related to desired traits, many genome-wide association analyses have been conducted in rice and resulted in valuable genome-wide association maps to describe the genetic architecture of complex traits. However, further efforts are needed to obtain more genomic information to fill in the gaps of our knowledge and meet the needs and challenges of rice breeding. This chapter will focus on the status of rice germplasm preservation activities, research programs, and outcomes of association mapping in rice in Korea.

2. Research on rice germplasm in Korea

The Ministry of Foreign Affairs and Trade (2009) had outlined eight major environmental issues as current threats to Korea; global warming, desertification, wildlife extinction, rain forest reduction, acid rain, depletion of the ozone layer, marine pollution, and air pollution. The rate of climate change is faster in Korea than the global average, leading to a rapid reduction in national biodiversity. One hundred and ninety families comprising 4000 species of vascular plants and ferns occur in Korea (Lee and Yim, 1978). Approximately 3700 different kinds of flowering plants are estimated to occur naturally (Chung, 1957; Lee, 1980). Four hundred and seven different endemic taxa in six genera are distributed throughout Korea (Lee, 1982). However, some plant species are on the verge of extinction because of pollution and a wide range of developmental activities during the last 20 years in Korea (Ministry of Environment, 1994), highlighting the importance of conservation efforts (Ahn *et al.*, 1994). Conservation programs usually involve activities such as collection, characterization, evaluation, regeneration, documentation, and storage of each germplasm accession.

The National Biodiversity Strategy was implemented in 1997 to integrate and consolidate plans formulated by various ministries and government institutes, including Comprehensive Biological Resources Conservation Plans. The Rural Development Administration (RDA) Gene Bank is one of the institutions responsible for these plans. Rice research programs covering agronomic practices, physiology, post-harvest technology, grain quality evaluation, rice breeding and genetics, and biotechnology, are led by the National Institute of Crop Science (NICS) under the RDA. Other institutions affiliated with NICS carry out rice research programs to target specific problems in various regions of Korea. From 1980 to 1990, rice sheath blight (*Acrocylindirum oryzae*) was the most destructive disease affecting production from damaging approximately 555,000 hectares of rice paddy fields in Korea. Furthermore, rice pests including brown plant hopper, white-backed plant hopper, and small brown plant hopper attacked 586,000 hectares of rice nationwide during the same period (NASTI, 1996). A continuous

cultivation of only five or six cultivars countrywide should be responsible to the extensive losses from the pests.

Rice season normally begins in mid-April and ends in mid-October in Korea. The lowest temperature in both April and October is 13°C. Because of the unprecedented yield loss due to cold damage in 1980 (damage to 80% of total rice hectarage and a yield reduction of 3.9 tons per hectare), cultivation of high-yielding "Tongil-type" rice cultivars declined rapidly, and only high-yielding japonica cultivars have been grown since 1990. In 2010, 20 mid- to late-maturing japonica cultivars were grown on 891,493 hectares, accounting for 92.9% of the total rice production area (Kang and Kim, 2012). Large decrease of temperature also occurred in 1971 and 2003, causing damage to 17% and 20% of total rice hectarage, respectively. Preharvest sprouting may become a serious problem for rice production, as well as for other crops, because of the trend in recent years for frequent and unusually heavy rain at harvest time. Breeders are making efforts to address this problem.

Rice breeders see the development of genetically improved cultivars using modern breeding techniques as an efficient way to reduce the losses in rice production caused by biotic and abiotic stresses. Sequencing the rice genome for genotyping and developing marker-assisted selection (MAS) system have fast-tracked research efforts. In the past, most national programs gave a lower priority to collecting wild relatives of rice than to collecting rice cultivars. Wild rice resources are agronomically unattractive, relatively expensive to conserve, and difficult to use. However, wild rice germplasms are known to contain a broad array of useful genes (Hodgkin, 1991). The benefits for the landrace germplasms to be used in breeding new cultivars in response to climate and environment changes in Korea are resistances to diseases in order to maintain superior qualities suited to consumers' preferences. Plant germplasm resource activities in Korea are performed by The Basic Conservation Programme for Nature and Environment (1994–2003) under the Ministry of Environment (NASTI, 1996).

The RDA Gene Bank conserves 24,673 rice accessions, including Korean landraces and wild types. Many gene banks are having financial difficulty to maintain germplasm collections due to a rapid increase of accession number. These problems may restrict a full exploitation, evaluation, and utilization of these accessions, thus managing such collections presents major challenges (Holden, 1984). The concept of a core collection for resolving these problems has received increased attention over the last few years. Germplasm sampling methods include sequential, stratified, biased (for example, by ecology or country), and random sampling. An understanding of factors underlying the traits being sought will help reduce the time required for identification of useful genes. For very rare traits, such as some associated with resistance to virus infection, searching among wild *Oryza* species and *O. glaberrima* may be most appropriate. Efficient methods for evaluation of germplasm to identify genes for crop improvement will promote the use of conserved germplasm. Frankel and Brown (1984) proposed the concept of a core set of lines to resolve such problems. A desire core set should include the maximum genetic diversity in a crop species including its wild relatives with minimum repetition and provide a manageable set of accessions to gene bank managers, plant breeders, and research scientists. Such a core collection would become the focus of the search for desirable new characteristics, detailed evaluation, and development of new techniques. An initial set of 4406 rice accessions was selected based on ecological types and accession passport information,

including their countries of origin. Using simple sequence repeat (SSR) genotype information, a final core set comprising the 166 conserved accessions currently used by the RDA was generated by a heuristic approach using the PowerCore software developed by Kim *et al*. (2007). Based on this resulted core set, some association mapping studies have been conducted and further researches are still being undertaken.

3. Association mapping in rice

Association mapping analyzes loci in diverse populations and associates them with both one another and with phenotypes. It is a powerful genetic mapping tool for crops and provides high-resolution, broad allele coverage, and cost-effective gene tagging for the evaluation of plant germplasm resources. Genetic mapping of QTL can be performed in two main ways (Ross-Ibarra *et al*., 2007): (1) Linkage-mapping as well as "gene tagging" using experimental populations (also referred to as "biparental" mapping populations) and (2) LD-mapping or "association mapping" using diverse lines from the natural populations or germplasm collections (Abdurakhmonov and Abdukarimov, 2008). LD mapping is based on identification of associations between phenotype and allele frequencies. The advantage of LD mapping for the breeder is that mapping and commercial variety development can be conducted simultaneously (Langridge and Chalmers, 2005). For phenotypes or traits that are governed by multiple genes or QTLs, diverse alleles or advantageous allele combinations should be mined by association mapping followed by gene-tagging efforts using biparental crosses.

The localization of alleles relies on creating a statistical association between markers and QTL alleles and on the efficacy of markers. For markers to be effective, they must be closely linked to the target locus and be able to detect polymorphisms in material likely to be used in a breeding program. Improvements in marker screening techniques have facilitated the tracking of genes (Subudhi *et al*., 2006). Isoenzyme and other protein-based marker systems had in wide long been used before DNA markers became popular (Langridge and Chalmers, 2005). Since then, a variety of DNA-based molecular markers have been developed, including restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNAs (RAPDs) (Williams *et al*., 1990), amplified fragment length polymorphisms (AFLPs) (Vos *et al*., 1995), SSRs (Litt and Luty, 1989), single-strand conformational polymorphisms (SSCPs), cleaved amplified polymorphic sequence (CAPS) markers (Koniecyzn and Asubel, 1993), sequence tagged sites (STSs) (Olson *et al*. 1989), sequence-characterized amplified region (SCAR) markers (Martin *et al*., 1991), and single nucleotide polymorphisms (SNPs) (Brookes, 1999). A total of 2740 SSRs were experimentally confirmed for rice in 2002 or approximately one SSR for every 157 kb (McCouch, 2002). The highly polymorphic nature of many microsatellites is of particular value (Banni *et al*., 2012, Yoon *et al*., 2012, Moe and Park, 2012, Zhao *et al*., 2012a; Khaing *et al*., 2013) for analysis of closely related genotypes or within narrowly adapted gene pools. Thus, the availability of a high-density SSR map is a valuable public resource for interpretation of the functional significance of the rapidly emerging rice genome sequence information.

The next generation of genetic markers is based on SNPs, which provide an attractive tool for QTL mapping studies and marker-assisted selection in plant breeding programs (Mohler and

Schwarz, 2005). SNP discovery is performed primarily *in silico* or using new sequencing approaches (Henry and Edwards, 2009). Large-scale SNP analysis is now possible in plants using a range of platforms. The increasing ease of sequencing and automated genotyping has made association mapping in plants a more attractive option by altering the conventional plant genome mapping method, which involves linkage analysis in a segregating population. This trend is likely to continue as the sequencing of genomes increases. Recently, genome-wide association studies (GWAS) with SNP variants have been conducted using new sequencing platforms (Table 1).

3.1. International rice association-mapping activity

Genome mapping of rice was first attempted using linkage analysis of appearance or phenotype (Nagao and Takahashi, 1963). Nowadays, improvement of the linkage map has been achieved using isozymes (Nakagahra, 1977) and RFLPs and SSRs (McCouch *et al*., 1988; Saito *et al*., 1991; McCouch *et al*., 1991 and Yu *et al*., 1991, Tanksley *et al*., 1991, Causse *et al*., 1994; Kurata *et al*., 1994; Harushima *et al*., 1998; McCouch *et al*., 2002). Relatively few association-mapping studies in rice have been performed. Some rice association-mapping studies using various populations and molecular markers are summarized in Table 1 in which most are conducted using SSR markers.

Whole-genome resequencing is a promising strategy to identify the relationship between sequence variation and normal or mutant phenotypes. High-throughput genome resequencing - if accurate - has the advantage of allowing researchers to identify the specific nucleotides associated with a given phenotype, and allowing the effective detection and analysis of genetic variations important for molecular breeding. An important application of NGS is the resequencing of targeted regions for the identification of mutant alleles, and we believe that mapping by sequencing will become a centerpiece in efforts to discover the genes responsible for QTLs. Generally speaking, the availability of a wide range of low- and high-multiplex single nucleotide polymorphism (SNP) assay methods (sequencing accuracy and depth of coverage relies on the experimental design) makes SNPs an ideal marker option for QTL mapping, association analysis, MAS, and the construction of high-density genetic maps for fine mapping and cloning of agronomically important genes (McCouch *et al*., 2010).

SNP discovery by resequencing whole-genome or subgenome of sample materials is often among the first use of a reference genome sequence. For inbreeding species such as rice, lines to be resequenced are normally purified through 1 or 2 generations of inbreeding (via single seed descent). After a DNA sample is resequenced using NGS technology, SNPs can be identified by comparing the sequenced genome with a reference genome like Nipponbare for japonica rice. For example, using information on the features of the B73, Gore *et al*. (2009) targeted the gene fraction of the maize genome for resequencing in the founder inbred lines of the nested association mapping population. Two data sets comprising 3.3 million SNPs were used to produce a first haplotype map ("HapMap") and to analyze the distribution of recombination and diversity along the maize chromosomes.

A suitable example is the construction of a comprehensive HapMap for rice that was used for the genome-wide associate study of 14 agronomic traits, such as heading date and tillering (Huang *et al*., 2010). The researchers made use of low-coverage (1-fold per rice line) sequence

data across lines, for a combined coverage of ~508-fold, and detected 3.6 million SNPs which can explain ~36% of the phenotypic variance for 14 agronomic traits. This work provided a new approach to low-fold sequence coverage, which can be used to detect not only SNPs but also more complex polymorphisms, and partially overcome the need for deeper sequence coverage (Clark, 2010). Further study was performed with the similar strategy for 950 world-wide rice varieties by Huang et al. (2012) and thirty-two novel loci associated with flowering time and ten grain-related traits were identified. Additionally, 40 cultivated accessions selected from the major groups of rice and 10 from their wild progenitors (*O. rufipogon* and *O. nivara*) were resequenced to >15X raw data coverage (Xu *et al*., 2012). After mapping the sequence read back to an IRGSP reference genome, the authors investigated the genome-wide variation pattern in a comparative analysis. The data revealed examples of structural variation in genomes and included 6.5 million high-quality SNPs after excluding sites with missing data in any accession. Using these population and SNP data, the authors also identified thousands of new rice genes and tracked down those with a significantly lower diversity in cultivated, but not wild rice. These variants represent a valuable resource for those interested in improving rice cultivars.

Preferences in terms of the processing, cooking, and eating qualities of rice differ globally. Plant breeders are attempting to fulfill consumer demand for rice varieties with specific qualities. The major components of rice grain quality include appearance, milling, cooking, eating, and nutritional aspects. The chemical composition of rice grain is important because of its relationship with eating quality of rice. Amylose content is one of the most important traits that determine cooking quality, which is controlled by a major locus waxy (*Wx*) on chromosome 6 (Wang *et al*., 1992; He *et al*., 1999; Tan *et al*., 1999). Genes associated with amylose content, such as starch synthase IIa (*SSIIa*) and *Wx*, are of particular interest. Sano *et al*. (1986) identified two alleles of the *Wx* locus using RFLP markers that correspond to the indica and japonica subspecies. Most grain quality mapping studies have used the *O. sativa* germplasm (He *et al*., 1999; Tan *et al*., 1999, 2000, 2001; Zhou *et al*., 2003). Borba *et al*. (2010) conducted association mapping study on yield traits and grain quality traits including amylose content, and the significant association detected between amylose content and RM190 was in agreement with previous QTL analyses. Zhao *et al.* (2011) identified 44,100 SNP variants across 413 diverse rice accessions collected from 82 countries and observed GWAS for six categories of traits covering morphology related traits; yield-related traits; seed and grain morphology related traits; stress-related phenotypes; cooking, eating and nutritional-quality-related traits; and plant development, represented by flowering time. This study demonstrated that different traits have different genetic architectures.

Olsen and Purugganan (2002) elucidated the origin and evolution of glutinous rice based on the haplotype of the *Wx* gene. By using dCAPS markers, waxy mutations and waxy rice cultivation were shown to have occurred predominantly in the japonica line during the evolution of domestic rice cultivation (Yamanaka *et al*., 2004). Genetic polymorphisms of starch-synthesis genes have been demonstrated to be associated with starch physicochemical properties using molecular markers such as SSRs, SNPs, and STSs. These markers can be extremely useful in marker-assisted breeding (Bao *et al*., 2002; Bao *et al*., 2006a). *SSIIa* was identified as the major gene responsible for determination of gelatinization temperature (GT). Among four SNPs in the *SSIIa* gene, some that cause amino acid substitutions have been

identified. The GC/TT SNP is strongly associated with GT (Bao *et a*l., 2006b; Nakamura *et al*., 2005; Umemoto and Aoki, 2005; Waters *et al*., 2006).

Rice nutritional quality is another important factor for consumer acceptance. In developing countries where rice is the main food, its nutrient content makes a significant contribution to the intake of some essential nutrients. Interest in natural antioxidants in rice is growing due to their role in preventing oxidative stress-related diseases (Aguilar-Garcia *et al*., 2007; Willcox *et al*., 2004; Zhang *et al*., 2013). Rice contains antioxidant compounds such as γ-oryzanols, tocols, and polyphenols, which are associated with a reduced risk of developing chronic diseases such as cardio vascular disease, type 2 diabetes, and some cancers (Liu, 2007; Tan *et al*., 2001; Toyokuni *et al*., 2002). Pigments and flavonoids in colored rice are positively correlated with the antioxidant capacity (Xia *et al*., 2003; Yawadio *et al*., 2007). Association mapping of pigments and flavonoid contents was carried out in brown rice using SSR markers. Significant correlations between phytochemical content and marker loci were found and markers associated with multi-phenotypic traits such as grain color, phenolic content and antioxidant capacity were identified (Shao *et al*., 2011).

The amino acid composition of rice grain is an important characteristic related to nutrient quality. Environmental conditions, potash, and nitrogen dramatically influence the amino acid and protein contents of rice (Wu *et al*., 2004). Few reports of mapping of QTLs for the contents of protein and amino acids in rice grain have been published. Twelve main effect QTLs (M-QTLs) were identified for 10 components of amino acid content in milled rice. Most of the main effect QTLs for amino acid content tended to co-localize within the genome (Lu et al., 2009).

Although many QTL analyses and genetic mapping studies of grain quality have been conducted, association-mapping studies of biotic and abiotic traits in rice are few in number. The genes or QTLs related to these traits are complex. Genetic mapping, including association mapping and linkage mapping, are useful methods of identifying alleles for these traits. As shown in Table 1, most association-mapping studies focused on morphological and agronomic characteristics. Four studies were related to grain and eating quality and only one addressed disease resistance and aluminum tolerance. Biotic and abiotic stress-tolerance traits remain to be explored by association mapping.

Reference	Number of accessions and population type	Number and types of markers used	Traits
Virk *et al*., 1996	200 rice accessions	7 RAPD	Ten morphological traits; culm number, culm length, culm diameter, grain length, grain width, leaf length, leaf width, days to 50% flowering, panicle length and seedling height
Zhang *et al*., 2005	218 inbred lines, worldwide germplasms	60 SSR, 114 RFLPs	Multiple agronomic traits such as plant height, heading date, flag leaf length and width, tiller number, stem diameter, panicle length, grain length and width, grain length/width ratio, grain thickness, 1000-grain weight

Reference	Number of accessions and population type	Number and types of markers used	Traits
Iwata *et al*., 2007	332 rice accessions	179 RFLPs	Size and shape of milled rice grains
Agrama *et al*., 2007	183 rice accessions	123 SSRs	Grain length and width, grain length/width ratio, 100 grain weight, grain thickness
Yan *et al*., 2009	90 accessions	108 SSRs + 1 indel	Single, dual and total stigma exsertions and spikelet characteristics
Wen *et al*., 2009	170 rice accessions	126 SSRs, 6 indels	Heading date, plant height, panicle length
Borba *et al*., 2010	242 inbred lines, worldwide germplasms	86 SSRs	Yield, amylose content, head-milled rice
Huang *et al*., 2010	517 landraces including japonica and indica	~3.6 million SNPs	Fourteen agronomic traits
Iwata *et al*., 2010	332 rice accessions	179 RFLPs	Grain shape variation
Jin *et al*., 2010	416 rice accessions	100 SSRs	Grain color
Ordonez Jr. *et al*., 2010	192 elite rice breeding lines and tropical japonica germplasm base	97 SSRs	Grain quality and flowering time
Zhao *et al*., 2010	395 diverse O. sativa accessions	1,536 SNPs	Amylose content
Famoso *et al*., 2011	373 diverse *O. sativa* accessions	36,901 SNPs	Al tolerance
Hu *et al*., 2011	303 *O. sativa* accessions	24 SSRs	Awness
Zhao *et al*., 2011	413 diverse accessions of *O. sativa*	44,100 SNPs	Thirty-four traits of agronomic characteristics, cooking and eating quality, disease resistance
Bryant *et al*., 2011	174 accessions	156 SSRs	Silica concentration in rice hulls
Li *et al*., 2011	217 accessions	154 SSRs and 1 indel	Yield and yield components among 14 traits
Lou *et al*., 2011	48 accessions	218 markers (SSRs + indels)	Grain metabolites
Shao *et al*., 2011	416 rice accessions including 361 white rice, 50 red rice, and 6 black rice	100 SSRs	Color parameters of brown rice grain, phenolic content, flavonoid content and antioxidant activity
Zhang *et al*., 2011	A core collection consisting of 150 rice varieties	274 SSRs	Six morphological traits: glume hair, phenol reaction, length of 1st-2nd rachis internode, glume color at heading, leaf hair, and grain length/width
Zhou *et al*., 2012	128 japonica rice varieties	152 SSRs	Eleven quantitative traits of agronomic and economic importance

http://dx.doi.org/10.5772/56614

Reference	Number of accessions and population type	Number and types of markers used	Traits
Huang *et al*., 2012	950 worldwide rice cultivars	1,345,417 SNPs	Flowering time and grain yield traits
Jia *et al*., 2012	217 entries	154 SSR markers and 1 indel marker	Sheath blight resistance
Li *et al*., 2012	203 accessions	154 SSRs and 1 indel	Harvest index and related components among 14 traits
Clark *et al*., 2013	233 rice (*Oryza sativa*) accessions	36,901 SNPs	Root growth and development

Table 1. International association-mapping studies of various traits using various markers in rice.

3.2. Association mapping of rice in Korea

To identify useful alleles from a representative core set of rice lines for transferring into elite lines, an allele-mining set of 166 accessions (Zhao et al., 2010) was successfully developed from 4046 rice accessions which were selected from 10368 accessions in the Korea RDA Gene Bank by 39 phenotype traits (Chung and Park, 2009), through a modified heuristic algorithm approach based on 15 SSR markers using the PowerCore software (Kim *et al*., 2007). Chung et al. also employed the PowerCore software of Kim et al. to develop the first preliminary core set by phenotypes. The gene diversity and population structure (Q) were analyzed using PowerMarker 3.25 (Liu and Muse, 2005) and Structure 2.2 (Evanno *et al*., 2005) based on 170 SSR markers. Analysis of these data identified the major substructure groups when the number of populations was set at four (Fig. 1).

Association mapping was conducted on this core set of lines over the past 2 years (as shown in Table 2). Zhao *et al*. (2012b) analyzed 130 accessions from the core set using 170 SSR markers for association analysis of physicochemical traits related to eating quality. Linkage disequilibrium (LD) patterns and distributions are of fundamental importance for genome-wide mapping associations. The mean r^2 value for all intrachromosomal loci pairs was 0.0940. LD between linked markers decreased with distance. Marker–trait associations were investigated using the unified mixed-model approach considering both Q and kinship (*K*). In total, 101 marker–trait associations (P <0.05) were identified using 52 SSR markers covering 12 chromosomes (Fig. 2.). Although direct comparisons of the chromosomal locations of marker–trait associations with previously reported QTLs are difficult because different materials and mapping molecular markers were used, most marker–trait associations were located in regions containing QTLs associated with a given trait. Indeed, some were located in similar or proximal regions related to starch synthesis. The new markers related to eating quality will facilitate the understanding of QTLs and marker-assisted selection (Zhao *et al*., 2012b).

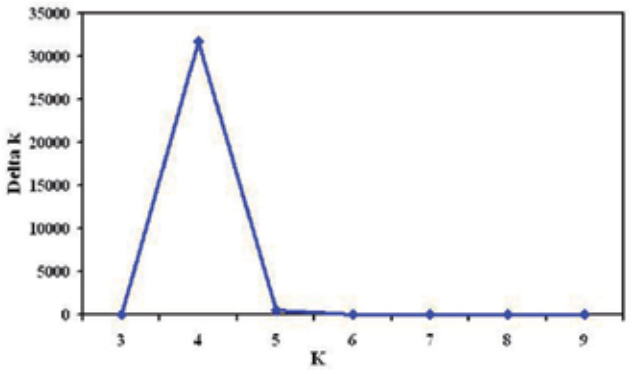


Figure 1. Values of Δ*K*, with its modal value used to detect the true *K* of four groups (*K* = 4). For each *K* value, five independent runs (blue diamonds) were considered and averaged over the replicates (Zhao *et al*., 2012b).

Reference	Numbers of lines and population type	Number and types of markers used	Traits
Zhao *et al*., 2009	84 accessions from land race core set	25 SSRs	16 amino acids
Zhao *et al*., 2012	130 accessions from core set	170 SSRs	Eating quality
Lu *et al*., 2012a	104 accessions from core set	86 SNPs and indels	Amylose content, RVA
Lu *et al*., 2012b	107 accessions from core set	83 SNPs, indels, and SSRs	Amylose content, RVA

Table 2. Rice association-mapping studies for various traits and marker types in Korea.

Association analysis of candidate genes has been used to trace the origin of agronomically important traits. Lu *et al*. (2012a) used the rice core lines for association-mapping to investigate the relationship between sequence variations from parts of 10 SSRGs and the amylose content (AC) and rapid viscosity analysis (RVA) profiles. Eighty-six sequence variations were found in 10 sequenced amplicons including 79 SNPs, six insertion-deletions (indels), and one polymorphic SSR. Among them, 61 variations were exon-based, of which 41 should lead to amino acid changes. The association mapping results showed a sum of four significant associations between three phenotypic indices and three sequence variations. An ADP - glucose pyrophosphorylase small subunit 1 (*OsAGPS1*) SNP (A to G) was significantly associated with increased AC (P <0.001, r^2 = 15.6%) while a 12-bp deletion of an *AGPase* large subunit 4 (OsAGPL4) (Table 3) was significantly related to decreased breakdown viscosity (P <0.001, r^2 = 16.6%) in both general linear model (GLM) and mixed linear model (MLM) (Lu *et al*., 2012a). One SNP with a g/c transversion at the 63rd nucleotide downstream of the *OsBEIIb* gene termination codon on rice chromosome 2 was significantly associated with multiple trait

http://dx.doi.org/10.5772/56614

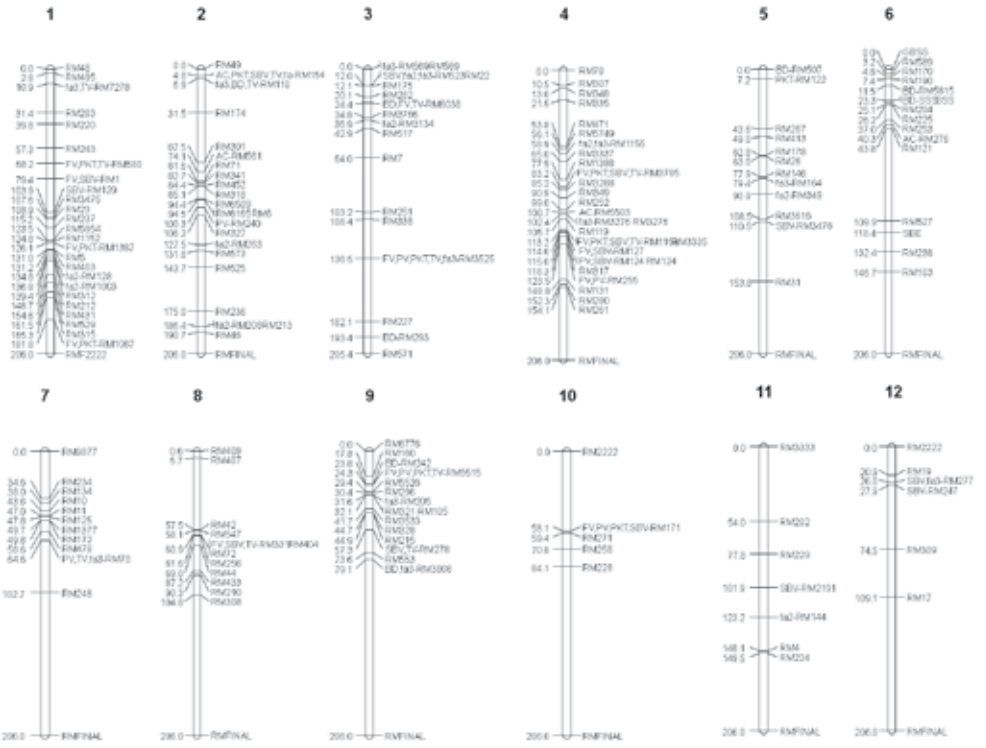


Figure 2. The positions of markers used and marker–trait associations on 12 chromosomes except unmapped markers. Genetic distances are indicated as cM on the left of each map and the corresponding trait-marker names are indicated on the right. AC, amylase content; PKV, peak viscosity; TV, trough viscosity; BD, breakdown viscosity; FV, final viscosity; SBV, setback viscosity; PKT, peak time; fa, degree of polymerization (DP) ≤12; fb_2, 24<DP ≤36; fb_3, DP<36 (Zhao *et al*., 2012b).

indices in both the GLM and MLM, including the final viscosity (P <0.001, r^2 = 23.87%), in both 2009 and 2010, and AC (P <0.01, r^2 = 11.25%) and trough viscosity (P <0.01, r^2 = 20.43) in 2010 (Table 4). This study provided a new perspective on the use of allele mining in breeding strategies based on marker-assisted selection (Lu *et al*., 2012b).

Gene	Location	Nucleotide variations	AAc	Trait	P_GLM	P_MLM	Rsq_Marker
OsAGPL4	1st exon 106–117th	del(TCCGCCGCCGCC)	del(SAAA)	BDV	9·99E−04	2·64E−05	0·1656
OsAGPL4	1st intron 36th	del(cgcgtgcg)	–	BDV	9·99E−04	2·64E−05	0·1656
OsAGPS1	3rd exon 133rd	AAT–>GAT	N–D	AC	9·99E−04	2·03E−04	0·1555
				FV	6·00E−03	9·05E−04	0·1236

AAc, amino acid changes; P_GLM, adjusted *P*-values with 1000 permutations; P_MLM, *P*-values significant in the FDR test; amino acid code: S, serine; A, alanine; N, asparagine; D, aspartic acid; BDV, breakdown viscosity; AC, amylase content; FV, final viscosity (Lu and Park, 2012a).

Table 3. Association between sequence variations and phenotype

Gene	Location	SNP	Trait	Year	P_GLM	P_MLM	Qvalue	R^2
OsBEIIb	3end63rd	g/c	AC	2010	2.10E-03	2.19E-04	9.64E-03	0.1125
			PV	2010	3.10E-03	5.82E-04	2.56E-02	0.1590
			TV	2009	2.00E-04	7.41E-04	1.12E-02	0.1893
				2010	1.00E-04	9.76E-05	3.14E-03	0.2043
			FV	2009	1.00E-04	4.77E-05	1.31E-03	0.2267
				2010	1.00E-04	3.85E-06	1.69E-04	0.2387
OsBE	-224th	c/t	TV	2010	3.60E-03	7.10E-03	3.80E-02	0.1784
OsSSSIIb	-1042nd	t/g	TV	2010	ns	5.90E-03	3.80E-02	0.0986
	-984th	g/t	TV	2010	ns	6.90E-03	3.80E-02	0.0885
	-957th	c/t	TV	2010	ns	6.90E-03	3.80E-02	0.0885
	-782nd	a/g	TV	2010	ns	6.90E-03	3.80E-02	0.0885

P_GLM: adjusted *P*-values with 10,000 permutations in GLM; P_MLM: nominal *P*-values in MLM; Q value: adjusted nominal *P*-value in MLM by false discovery rate; AC: amylose content; PV: peak viscosity; TV: trough viscosity; FV: final viscosity (Lu and Park, 2012b).

Table 4. Associations between sequence variations and eating quality indicators.

Zhao *et al*. (2009) evaluated the contents of 16 amino acids in brown rice by genotyping using 25 SSR markers. A total of 42 marker-trait associations for amino acid content covering three chromosomes (P <0.05) were identified by the MLM model (Fig. 4), which accounted for more than 40% of the total variation (Zhao *et al*., 2009). In our research group, association mapping of rice traits related to cold-stress tolerance during germination, preharvest sprouting resistance, salt tolerance, blast disease resistance, and grain physicochemical properties are undertaken using SSRs and SNP variants on advanced resequencing platforms.

In conclusion, association mapping is a promising approach to overcoming the limitations of conventional linkage mapping in plant breeding. Recent research has demonstrated the significant potential of LD-based association mapping of physicochemical traits and other important agronomic traits in rice accessions using SSR/SNP markers. This type of mapping could be a useful alternative to linkage mapping for the detection of marker–trait associations, and lead to implementation of marker-assisted selection in rice breeding programs.

4. Future directions

4.1. Genomics and GWAS in germplasm research

With the development of next- and third-generation sequencing technologies, the whole genomes of individual rice accessions can now be sequenced with less than $ 1000 (US dollar).

http://dx.doi.org/10.5772/56614

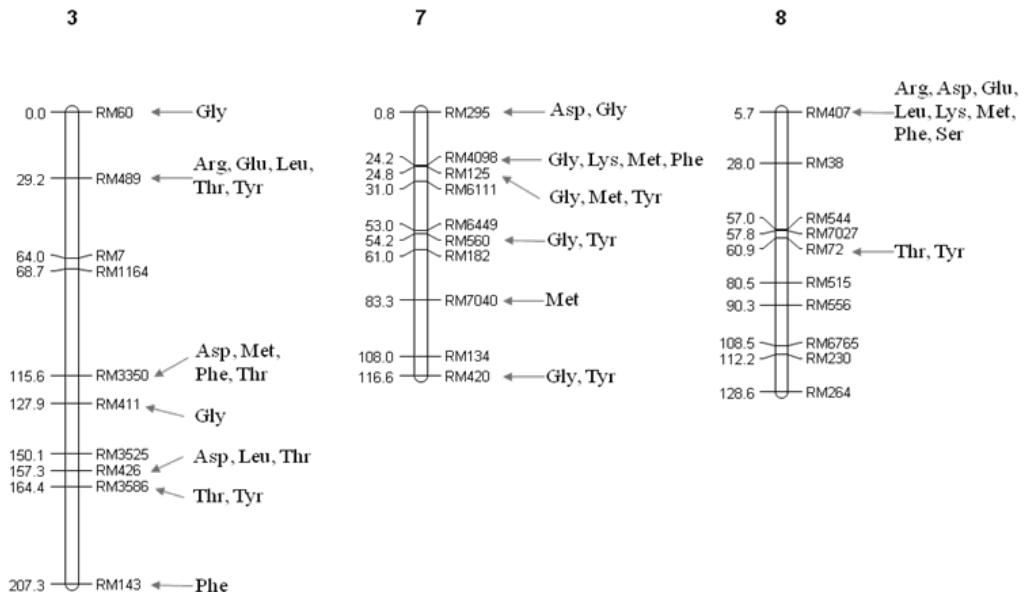


Figure 3. Three regions of putative marker–trait associations on three chromosomes (3, 7, and 8) for amino acid content in brown rice. Genetic distances are indicated in cM on the left of each map and the corresponding marker names are indicated on the right. Ala, alanine; Arg, arginine; Asp, aspartic acid; Glu, glutamine; Gly, glycine; His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; Met, methionine; Phe, phenylalanine; Pro, proline; Ser, serine; Thr, threonine; Tyr, tyrosine; Val, valine (Zhao *et al*., 2009).

Also, new efficient genotyping technologies, such as RADs (Restriction Associated DNAs) (Baird et al., 2008) and GBS (Genotyping-by-Sequencing) allow the generation of genotyping data for up to 40,000 genes at low cost in few days.

Natural alleles and alleles obtained from artificially mutagenized populations provide an important resource for crop breeding. By using all available alleles and detailed phenotypic data from core sets of rice lines, new genes and useful traits can be identified. Molecular tags for useful traits developed using GWASs based on genotypic and phenotypic information can be used to track target traits during segregation of populations in rice breeding (Figure 4).

To identify new alleles from a representative core set of rice lines and transfer them into elite lines, we finally selected 166 from ~25,000 accessions in the RDA Gene Bank. We completed whole-genome resequencing of 84 core accessions with 7x coverage in 2012. We plan to resequence the whole genomes of the remaining 82 core accessions in addition to 84 bred varieties from a validation set in 2013. We are currently undertaking the phenotyping of the core accessions for agronomic traits, and chemical composition for the GWAS analysis with the resequence information. We are also planning to improve the software algorithm for the association analysis to increase the ability to identify alleles from the core set of lines using whole genomic SNP or indel genotype data and phenotypic information. More precise characterization of rice traits that confer resistance to stress from climate change is required to

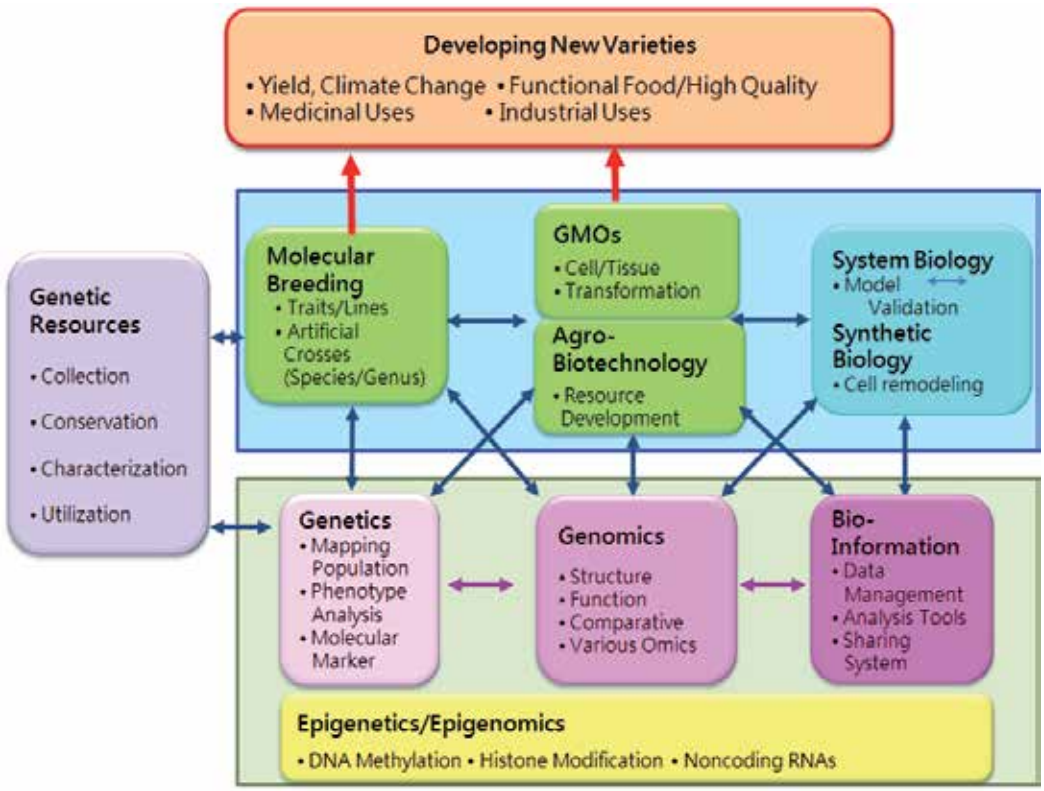


Figure 4. A schematic illustration of inter-disciplinary relationships between genomic research and other fields in the breeding of crop species.

screen useful alleles using GWASs. Using whole-genome genotype information, we are able to develop large numbers of molecular tags across 12 different rice linkage groups based on their contributions to specific phenotypes.

4.2. Strategy for identification of major and minor QTLs for molecular breeding

The core accessions are highly diverse with many traits useful for rice breeding. Upon selection of an accession with a desirable trait, bi-parental mapping populations will be developed using two japonica varieties (Shindongjinbyeo and Junambyeo) and one indica variety (Hanareumbyeo). Major QTLs will be surveyed with an F_8 RIL-segregating population using whole-genome resequencing of 96 samples for first mapping, and then, we can resequence this target region using the expanded 3000 to 5000 samples for fine mapping till the targeted gene can be cloned. We expect that all major QTLs will contribute more than 10% to target traits. To identify minor QTLs that contribute less than 10% to a target trait, BC_4F_1 population will be first developed, and then, selfing will be done till BC_4F_8. The recurrent parent will be an elite line for the purposes of QTL mapping and for transferring target traits into the elite lines. Mapping of minor QTLs will be performed using a BC_4F_8 segregating population (as shown in Fig. 5).

http://dx.doi.org/10.5772/56614

Natural variation results from the expression of different alleles during evolution. As a result of the contributions to farmers over the past ~8000 years, many important traits have been accumulated in the natural germplasm collections currently maintained in seed banks. Whole genome resequencing allows efficient identification of unused alleles from conserved germplasm. We are at present developing a platform for allele mining in rice breeding systems using GWAS approaches and diverse germplasm accessions with the support of the Next-Generation BioGreen 21 Program (No.PJ009099) from Rural Development Administration, Republic of Korea. We believe our effort will facilitate the molecular breeding of rice.

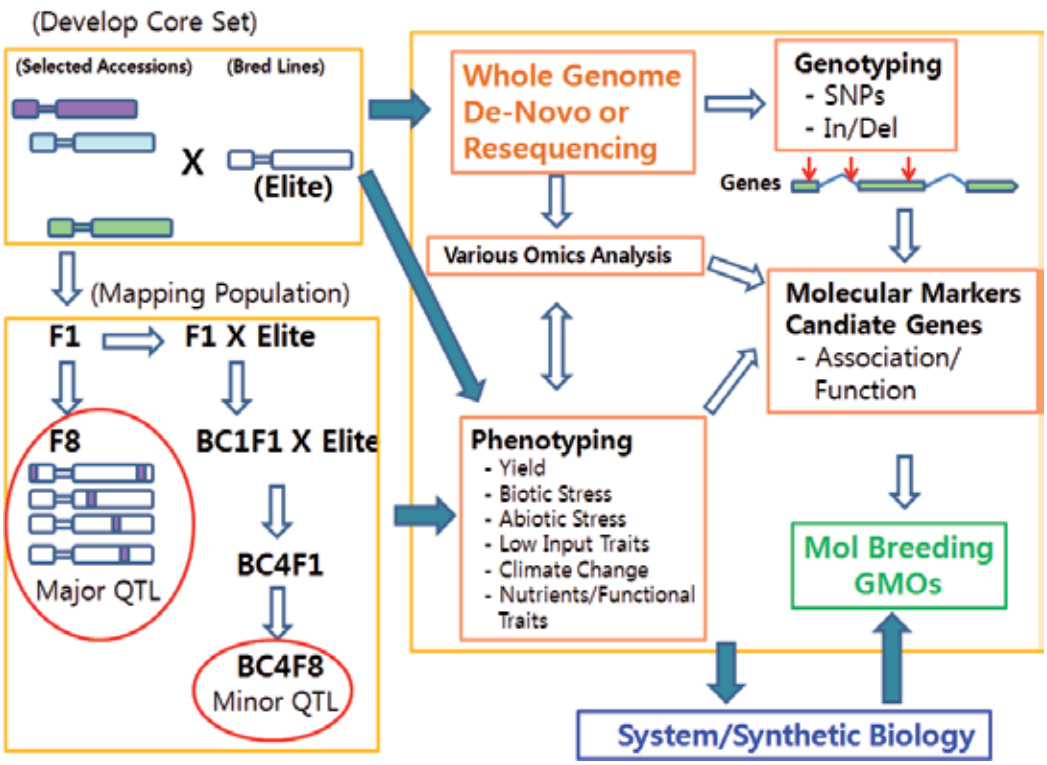


Figure 5. Strategies for identification of major and minor QTLs in rice from selected accessions carrying useful traits through GWAS. The major QTLs will be localized and tagged by molecular markers in the F_8 generation. Minor QTLs will be localized using a BC_4F_8 population.

Author details

Aye Aye Khaing[1], Gang Li[1], Xiao Qiang Wang[1], Min Young Yoon[1], Soon Wook Kwon[2], Chang Yong Lee[3], Beom Seok Park[4] and Yong Jin Park[1,5*]

*Address all correspondence to: yjpark@kongju.ac.kr

1 Department of Plant Resources, College of Industrial Sciences, Kongju National University, Yesan, Republic of Korea

2 Department of Plant Bioscience, College of Natural Resources & Life Science, Pusan National University, Milyang, Republic of Korea

3 Department of Industrial and Systems Engineering, Kongju National University, Kongju, Republic of Korea

4 Department of Agricultural Bio-resources, National Academy of Agricultural Science (NAAS), Rural Development Administration (RDA), Suwon, Republic of Korea

5 Legume Bio-Resource Center of Green Manure (LBRCGM), Kongju National University, Yesan, Republic of Korea

References

[1] Abdurakhmonov IY and Abdukarimov A (2008) Application of association mapping to understanding the genetic diversity of plant germplasm resources. International Journal of Plant Genomics. Vol. 2008, 1–18

[2] Agrama H A, Eizenga GC and Yan W (2007) Association mapping of yield and its components in rice cultivars. Molecular Breeding, Vol. 19, No. 4, pp. 341–356

[3] Aguilar-Garcia C, Gavino G, Baragaño-Mosqueda M, Hevia P, Gavino VC (2007) Correlation of tocopherol, tocotrienol, γ-oryzanol and total polyphenol content in rice bran with different antioxidant capacity assays. Food Chemistry 102(4): 1228–1232

[4] Ahn WS, Kang JH and Yoon MS (1994) Genetic erosion of crop plants in Korea, In Proceedings of Genetic Conservation and Utilization of Biodiversity. Daegu, Korea: pp.13-27

[5] Baird NA, Etter PD, Atwood TS, Currey MC, Shiver AL, Lewis ZA, Selker EU, Cresko WA, and Johnson EA (2008) Rapid SNP discovery and genetic mapping using sequenced RAD markers, Plos One 3:3376-84

[6] Banni K, Moe KT and Park YJ (2012) Assessing genetic diversity, population structure and gene flow in the Korean red bean [*Vigna angularis* (Willd.) Ohwi & Ohashi] using SSR markers. Plant Genetic Resources 10: 74–82

[7] Bao J, Corke H and Sun M (2002) Microsatellites in starch-synthesizing genes in relation to starch physicochemical properties in waxy rice (*Oryza sativa L.*). Theoretical and applied genetics 105(6-7): 898–905

[8] Bao JS, Corke H and Sun M (2006a) Microsatellites, single nucleotide polymorphisms and a sequence tagged site in starch-synthesizing genes in relation to starch physicochemical properties in nonwaxy rice (*Oryza sativa L.*). Theoretical and applied genetics 113(7): 1185–1196

[9] Bao JS, Corke H and Sun M (2006b) Nucleotide diversity in starch synthase IIa and validation of single nucleotide polymorphisms in relation to starch gelatinization temperature and other physicochemical properties in rice (*Oryza sativa L.*). Theoretical and applied genetics 113(7): 1171–1183

[10] Borba TC de O, Brondani RPV, Breseghello F, Coelho ASG, Mendonça JA, Rangel PHN and Brondani C (2010) Association mapping for yield and grain quality traits in rice (*Oryza sativa L.*), Genetics and Molecular Biology, 33(3): 515–524

[11] Brookes AJ (1999) The essence of SNPs. Gene 234:177–186

[12] Bryant R, Proctor A, Hawkridge M, Jackson A, Yeater K, Counce P, Yan W, McClung A and Fjellstrom R (2011) Genetic variation and association mapping of silica concentration in rice hulls using a germplasm collection. Genetica 139:1383–1398

[13] Causse MA, Fulton TM, Cho YG, Ahn SN, Chunwongse J, Wu KS, Xiao JH, Yu ZH, Ronald PC, Harrinton SE, Second G, McCouch SR and Tanksley SD (1994) Saturated molecular map of the rice genome based on an interspecific backcross population. Genetics 138:1251–1274

[14] Chung, TH (1957) Korean flora I & II, Singi Ltd., Seoul.

[15] Chung JW and Park YJ (2009) Genetic diversity and population structure of Korean rice core collection. Korean J Intl Agri 21: 282-288

[16] Clark RM (2010) Genome-wide association studies coming of age in rice. Nat. Genet. 42: 926-927

[17] Clark RT, Famoso AN, Zhao K, Shaff J, Craft EJ, Bustamante CD, McCouch SR, Aneshansley DJ and Kochian LV (2013) High-throughput two-dimensional root system phenotyping platform facilitates genetic analysis of root growth and development. Plant, Cell and Environment 36, 454–466

[18] Du B, Zhang WL, Liu BF, Hu J, Wei Z, Shi AY, He RF, Zhu LL, Chen RZ, Han B and He GC (2009) Identification and characterization of Bph14, a gene conferring resistance to brown planthopper in rice. Proc Natl Acad Sci USA 106: 22163–22168

[19] Evanno G, Regnaut S and Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol Ecol 14: 2611–2620

[20] Famoso AN, Zhao K, Clark RT, Tung C-W, Wright MH, Bustamante C, Kochian LV, McCouch SR (2011) Genetic Architecture of Aluminum Tolerance in Rice (*Oryza sativa*) Determined through Genome-Wide Association Analysis and QTL Mapping. PLoS Genetics 7(8):e1002221

[21] Frankel OH and Brown AHD (1984) Plant genetic resources today: a critical appraisal. In: J. H. W. Holden, and J. T. Williams (eds), Crop Genetic Resources: Conservation and Evaluation, 249–257. Allen & Unwin Ltd, London.

[22] Gore MA, Chia JM, Elshire RJ, Sun Q, Ersoz ES, Hurwitz BL, Peiffer JA, McMullen MD, Grills GS, Ross-Ibarra J, Ware DH and Buckler ES (2009) A first-generation haplotype map of maize. Science 326: 1115-1117

[23] Hancock JF (2004) Plant Evolution and the Origin of Crop Species, Second Edition. CABI Publishing, 875 Massachusetts Avenue, Cambridge, USA. pp. 313

[24] Hammer, K., 2005: Korea's importance in the East Asiatic gene center. Genetic Resources and Crop Evolution 52, 115-116.

[25] Harushima Y, Yano M, Shomura P, Sato M, Shimano T, Kuboki Y, Yamamoto T, Lin SY, Antonio BA, Parco A, Kajiya H, Huang N, Yamamoto K, Nagamura Y, Kurata N, Khush GS and Sasaki T (1998) A high-density rice genetic linkage map with 2275 markers using a single F-2 population. Genetics 148:479–494

[26] He P, Li SG, Qian Q, Ma YQ, Li JZ, Wang WM, Chen Y and Zhu LH (1999) Genetic analysis of rice grain quality. Theor Appl Genet 98:502–508

[27] Henry R and Edwards K (2009) New tools for single nucleotide polymorphism (SNP) discovery and analysis accelerating plant biotechnology. Plant Biotechnology Journal. 7, pp. 311

[28] Hodgkin T (1991) Improving utilization of plant genetic resources through core collections. In Rice germplasm: collecting, preservation, use. IRRI. Rice Germplasm Collecting, Preservation, Use. Proceedings of third international workshop, 10-12 May, 1990. pp. 99–104

[29] Holden JHW (1984) The second 10 years. Pages 277–285 in Crop genetic resources: conservation and evaluation. 27—285. Allen and Unwin, Winchester

[30] Hu G, Zhang D, Pan H, Li B, Wu J, Zhou X, Zhang Q, Zhou L, Yao G, Li J, Li J, Zhang H, Li Z (2011) Fine mapping of the awn gene on chromosome 4 in rice by association and linkage analyses. Chinese Sci Bull 56(9):835-839

[31] Hu J, Li X, Wu CJ, Yang CJ, Hua HX, Gao GJ, Xiao JH and He GC (2012) Pyramiding and evaluation of the brown planthopper resistance genes Bph14 and Bph15 in hybrid rice. Mol Breeding 29: 61–69

[32] Huang X, Wei X, Sang T, Zhao Q, Feng Q, Zhao Y, Li C, Zhu C, Lu T, Zhang Z, Li M, Fan D, Guo Y, Wang A, Wang L, Deng L, Li W, Lu Y, Weng Q, Liu K, Huang T, Zhou T, Jing Y, Li W, Lin Z, Buckler E, Qian Q, Zhang Q, Li J and Han B (2010) Genome-wide studies of 14 agronomic traits in rice landraces. Nature Genetics, Vol. 42, No. 11, 961–967

[33] Huang X, Zhao Y, Wei X, Li C, Wang A, Zhao Q, Li W, Guo Y, Deng L, Zhu C, Fan D, Lu Y, Weng Q, Liu K, Zhou T, Jing Y, Si L, Dong G, Huang T, Lu T, Feng Q, Qian Q, Li J and Han B (2012) Genome-wide association study of flowering time and grain yield traits in a worldwide collection of rice germplasm. Nature Genetics 44:32–42

[34] Iwata H, Ebana K, Uga Y, Hayashi T and Jannink JL (2010) Genome-wide association study of grain shape variation among *Oryza sativa L.* germplasms based on elliptic Fourier analysis. Mol Breeding 25:203–215

[35] Iwata H, Uga Y, Yoshioka Y, Ebana K and Hayashi T (2007) Bayesian association mapping of multiple quantitative trait loci and its application to the analysis of genetic variation among *Oryza sativa L.* germplasms. Theoretical and Applied Genetics, vol. 114, no. 8, pp. 1437–1449

[36] Jia L, Yan W, Zhu C, Agrama HA, Jackson A, Yeater K, Li X, Huang B, Hu B, McClung A and Wu D (2012) Allelic analysis of sheath blight resistance with association mapping in rice. PLoS One 7:3, e32703

[37] Jin L, Lu Y, Xiao P, Sun M, Corke H and Bao J (2010) Genetic diversity and population structure of a diverse set of rice germplasm for association mapping. Theor Appl Genet 121:475–487

[38] Kang KH and Kim YG (2012) Temperate rice in Korea In KK Jena and B Hardly (ed.), Advances in temperate rice research, IRRI, pp. 43-48

[39] Khaing AA, Moe KT, Chung JW, Baek HJ, *et al*. (2013). Genetic diversity and population structure of the selected core set in *Amaranthus* using SSR markers. Plant Breeding Doi:10.1111/pbr.12027

[40] Kim KW, Chung HK, Cho GT, Ma KH, Chandrabalan D, Gwag JG, Kim TS, Cho EG and Park YJ (2007) PowerCore: a program applying the advanced M strategy with a heuristic search for establishing core sets. Bioinformatics 23, 2155–2162

[41] Koniecyzn A and Asubel FM (1993) A procedure for mapping Arabidopsis mutations using co-dominant ecotype-specific PCR-based markers. Plant J 4:403–410

[42] Kurata N, Nagamura Y, Yamamoto K, *et al*. (1994) A 300-kilobase-interval genetic map of rice including 883 expressed sequences. Nat Genet 8:365–372

[43] Langridge P and Chalmers K (2005) The Principle: Identification and Application of Molecular Markers. In H. Lorz and G. Wenzel (Ed.), Biotechnology in Agriculture and Forestry: Molecular Marker Systems in Plant Breeding and Crop Improvement. Springer-Verlag Berlin Heidelberg. pp. 1–23

[44] Lee TB (1980) Illustrated flora of Korea, Hyangmoon Ltd., Suweon.

[45] Lee TB (1982) Classification of endemic taxa in Korea. Seoul Nat'l Univ. Agri. Research 8:71–113

[46] Lee WT and Yim YJ (1978) Studies on the distribution of vascular plants in the Korean peninsula. Korean J. Pl. Tax. 8 (suppl): 1–33

[47] Li X, Yan W, Agrama H, Jia L, Jackson A, Moldenhauer K, Yeater K, McClung A and Wu D (2012) Unraveling the complex trait of harvest index with association mapping in rice (*Oryza sativa L.*). PLoS One 7:1 e29350

[48] Li X, Yan W, Agrama H, Jia L, Shen X, Jackson A, Moldenhauer K, Yeater K, McClung A and Wu D (2011). Mapping QTLs for improving grain yield using the USDA rice mini-core collection. Planta 234:347–361

[49] Litt M and Luty JA (1989) A hypervariable microsatellite revealed by *in vitro* amplification of a dinucleotide repeat within the cardiac muscle actin gene. Am J Hum Genet 44:397–401

[50] Liu RH (2007) Whole grain phytochemicals and health. Journal of Cereal Science 46(3):207-219

[51] Liu K and Muse SV (2005) PowerMarker: an integrated analysis environment for genetic marker analysis. Bioinformatics 21: 2128–2129

[52] Lou Q, Ma C, Wen W, Zhou J, Chen L, Feng F, Xu X, Lu X, Luo L, Mei H and Xu G (2011) Profiling and association mapping of grain metabolites in a subset of the core collection of Chinese rice germplasm (*Oryza sativa L.*). J. Agric. Food Chem., 59, 9257–9264

[53] Lu FH and Park YJ (2012a) Sequence variations in OsAGPase significantly associated with amylose content and viscosity properties in rice (*Oryza sativa L.*). Genet Res (Camb) 94: 179–189

[54] Lu FH and Park YJ (2012b) An SNP downstream of the OsBEIIb gene is significantly associated with amylose content and viscosity properties in rice (*Oryza sativa L.*). Journal of Cereal Science 56: 706–712

[55] Lu K, Li L, Zheng X, Zhang Z, Mou T and Hu Z (2009) Genetic dissection of amino acid content in rice grain. Journal of the Science of Food and Agriculture 89(14): 2377–2382

[56] Martin GB, Williams JGK and Tanksley SD (1991) Rapid identification of markers linked to a *Pseudomonas* resistance gene in tomato by using random primers and near-isogenic lines. Proc Natl Acad Sci USA. 88:2336–2340

[57] McCouch SR, Kochert G, Yu ZH, Wang ZY, Khush GS, Coffman WR and Tanksley SD (1988) Molecular mapping of rice chromosomes. Theor Appl Genet 76:815–829

[58] McCouch SR, Khush GS and Tanksley SD (1991) Tagging genes for disease and insect resistance via linkage to RFLP markers. In: Rice Genetics II. IRRI, Manila, Philippines, pp. 443–449

[59] McCouch SR, Teytelman L, Xu YB, Lobos KB, Clare K, Walton M, Fu BY, Maghirang R, Li ZK, Xing YZ, Zhang QF, Kono I, Yano M, Fjellstrom R, DeClerck G, Schneider D, Cartinhour S, Ware D and Stein L (2002) Development and mapping of 2240 new SSR markers for rice (*Oryza sativa L.*). DNA Res 9:199–207

[60] McCouch SR, Zhao K, Wright M, Tung CW, Ebana K,Thomson M, Reynolds A, Wang D, DeClerck G, Ali ML, McClung A, Eizenga G and Bustamante C (2010) Development of genome-wide SNP assays for rice. Breed. Sci. 60: 524-535

[61] Ministry of Environment (1994) Illustrated Threatened Wild Animals and Plants in Korea. Ministry of Environment Ltd. (Seoul).

[62] Ministry of Foreign Affairs and Trade (2009) Fourth National Report to the Convention on Biological Diversity. Republic of Korea.

[63] Moe KT and Park YJ (2012) Analysis of population structure revealed apparent genetic disturbance in Korea Cymbidium collection. Scientia Horticulturae 134:157–162

[64] Mohler V and Schwarz G (2005) Genotyping Tools in Plant Breeding: From Restriction Fragment Length Polymorphisms to Single Nucleotide Polymorphisms. In H. Lorz and G. Wenzel (Ed.), Biotechnology in Agriculture and Forestry: Molecular Marker Systems in Plant Breeding and Crop Improvement. Springer–Verlag Berlin Heidelberg, pp. 1–23

[65] Nagao S and Takahashi M (1963) Trial construction of twelve linkage groups in Japanese rice. Genetic studies on rice plant. XXVII. J Fac Agri Hokkaido Univ 53:72–130

[66] Nakagahra M (1977) Genetic analysis for esterase isoenzymes in rice cultivars. Jap J Breed 27:141–148

[67] Nakamura Y, Francisco P, Hosaka Y, Sato A, Sawada T, Kubo A and Fujita N (2005) Essential amino acids of starch synthase IIa differentiate amylopectin structure and starch quality between japonica and indica rice varieties. Plant Molecular Biology 58(2): 213–227

[68] NASTI (National Agricultural Science and Technology Institute) (1996) Republic of Korea: Country report to the FAO international technical conference on plant genetic resource. pp.59

[69] Olsen KM and Purugganan MD (2002) Molecular evidence on the origin and evolution of glutinous rice. Genetics 162(2): 941-950

[70] Olson M, Hood L, Cantor C and Botstein D (1989) A common language for physical mapping of the human genome. Science 245(4925): 1434–1435

[71] Ordonez Jr. SA, Silva J and Oard JH (2010) Association mapping of grain quality and flowering time in elite japonica rice germplasm. Journal of Cereal Science 51: 337-343

[72] Ronald PC, Beng A, Rodante T, Abenes L, Wu KS, McCouch SR and Tanksley SD (1992) Genetic and physical analysis of the rice bacterial blight disease resistance locus, Xa21. Mol Gen Genet 236: 113–120

[73] Ross–Ibarra J, Morrell PL and Gaut BS (2007) Plant domestication, a unique opportunity to identify the genetic basis of adaptation. Proceedings of the National Academy of Sciences of the United States of America, vol. 104, supplement 1, pp. 8641–8648

[74] Saito A, Yano M, Kishimoto N, *et al*. (1991) Linkage map of restriction-fragment-length polymorphism loci in rice. Jap J Breed 41:665–670

[75] Sano Y, Katsumata M and Omura K (1986) Genetic studies of speciation in cultivated rice. Inter- and intra-specific differentiation in the waxy gene expression of rice. Euphytica 35:1–9

[76] Shao Y, Jin L, Zhang G, Lu Y, Shen Y and Bao J (2011) Association mapping of grain color, phenolic content, flavonoid content and antioxidant capacity in dehulled rice. Theoretical and applied genetics 122(5):1005–1016

[77] Song WY, Wang GL, Chen LL, Kim HS, Pi LY, Holsten T, Gardner J, Wang B, Zhai WX, Zhu LH, Fauquet C and Ronald P (1995) A receptor kinase-like protein encoded by the rice disease resistance gene *Xa21*. Science 270: 1804–1806

[78] Subudhi P K, Sasaki T and Khush GS (2006) Rice. In C. Kole (Ed.), Genome Mapping and Molecular Breeding in Plants, Volume 1, Springer-Verlag Berlin Heidelberg, pp. 1–78

[79] Tan YF, Li JX, Yu SB, Xing YZ, Xu CG and Zhang Q (1999) The three important traits for cooking and eating quality of rice grains are controlled by a single locus in an elite rice hybrid, Shanyou 63. Theor Appl Genet 99:642–648

[80] Tan YF, Sun M, Xing YZ, Hua JP, Sun XL, Zhang QF and Corke H (2001) Mapping quantitative trait loci for milling quality, protein content and color characteristics of rice using a recombinant inbred line population derived from an elite rice hybrid. Theor Appl Genet 103:1037–1045

[81] Tan YF, Xing YZ, Li JX, Yu SB, Xu CG and Zhang QF (2000) Genetic bases of appearance quality of rice grains in Shanyou 63, an elite rice hybrid. Theor Appl Genet 101:823–829

[82] Tanksley SD, Ahn N, Causse M, Coffman R, Fulton T, McCouch SR, Second G, Tai T, Wang Z, Wu K and Yu Z (1991) RFLP mapping of the rice genome. In: Rice Genetics II. IRRI, Manila, Philippines, pp. 435–442

[83] Toyokuni S, Itani T, Morimitsu Y, Okada K, Ozeki M, Kondo S, Uchida K, Osawa T, Hiai H and Tashiro T (2002) Protective effect of colored rice over white rice on Fen-

ton Reaction-based renal lipid peroxidation in rats. Free Radical Research 36(5): 583–592

[84] Umemoto T and Aoki N (2005) Single-nucleotide polymorphisms in rice starch synthase IIa that alter starch gelatinization and starch association of the enzyme. Functional Plant Biology 32(9): 763–768

[85] Vavilov N.I. 1935. The phytogeographical basis for plant breeding. Theor. Basis Plant Breeding, Moscow (in Russian) 1: 17–75

[86] Virk PS, Ford-Lloyd BV, Jackson MT, Pooni HS, Clemenot TP and Newbury HJ (1996). Predicting quantitative variation within rice germplasm using molecular markers. Heredity 76 296–304

[87] Vos P, Hogers R, Bleeker M, Reijans M, van de Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, and Zabeau M (1995) AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res 23:4407–4414

[88] Wang AY, Yu WP, Juang RH, Huang JW, Sung HY and Su JC (1992) Presence of three rice sucrose synthase genes as revealed by cloning and sequencing of cDNA. Plant Mol Biol 18:1191–1194

[89] Waters DLE, Henry RJ, Reinke RF and Fitzgerald MA (2006) Gelatinization temperature of rice explained by polymorphisms in starch synthase. Plant Biotechnology Journal 4(1):115–122

[90] Wen W, Mei H, Feng F, Yu S, Huang Z, Wu J, Chen L, Xu X and Luo L (2009). Population structure and association mapping on chromosome 7 using a diverse panel of Chinese germplasm of rice (*Oryza sativa L.*). Theoretical and Applied Genetics, Vol. 119, No. 3, 459–470

[91] Willcox JK, Ash SL and Catignani GL (2004) Antioxidants and Prevention of Chronic Disease. Critical Reviews in Food Science and Nutrition 44(4):275-295

[92] Williams JG, Kubelik AR, Livak KJ, Rafalski JA and Tingey SV (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res 18:6531–6535

[93] Wu JG, Shi CH, Zhang XM and Katsura T (2004) Genetic analysis of non-essential amino acid contents in rice (*Oryza sativa L.*) across environments. Hereditas 141(2): 128–134

[94] Xia M, Ling WH, Ma J, Kitts DD and Zawistowski J (2003) Supplementation of diets with the black rice pigment fraction attenuates atherosclerotic plaque formation in apolipoprotein E deficient mice. The Journal of Nutrition 133(3):744–751

[95] Xu X, Liu X, Ge S, Jensen JD, Hu F,12, Li X, DongY, Gutenkunst RN, Fang L, Huang L, Li J, He W, Zhang G, Zheng X, Zhang F, Li Y, Yu C, Kristiansen K, Zhang X, Wang J, Wright M, McCouch S, Nielsen R, Wang J and Wang W (2012) Resequencing 50 ac-

cessions of cultivated and wild rice yields markers for identifying agronomically important genes. Nat. Biotechnol. 30: 105-111

[96] Yamanaka S, Nakamura I, Watanabe K and Sato YI (2004) Identification of SNPs in the waxy gene among glutinous rice cultivars and their evolutionary significance during the domestication process of rice. Theoretical and applied genetics 108(7): 1200–1204

[97] Yan W, Li Y, Agrama H, Luo D, Gao F, Lu X and Ren G (2009) Association mapping of stigma and spikelet characteristics in rice (*Oryza sativa L.*). Mol. Breed., 24: 277–292

[98] Yang HY, You AQ, Yang ZF, Zhang FT, He RF, Zhu LL and He GC (2004) High-resolution genetic mapping at the *Bph15* locus for brown planthopper resistance in rice (*Oryza sativa L.*). Theor Appl Genet 110: 182–191

[99] Yawadio R, Tanimori S and Morita N (2007) Identification of phenolic compounds isolated from pigmented rices and their aldose reductase inhibitory activities. Food Chemistry 101(4): 1616–1625

[100] Yoon MY, Moe KT, Kim DY, Rho RI, Kim S, Kim KT, Won MK, Chung JW, and Park YJ (2012) Genetic diversity and population structure analysis of strawberry (*Fragaria* x *ananassa Duch.*) using SSR markers. Electronic Journal of Biotechnology 15:71–85

[101] Yu Z, Mackill D J, Bonman J M and Tanksley S D (1991) RFLP tagging of blast resistance genes in rice. Pages 451–458 in Rice genetics II. International Rice Research Institute, P.O. Box 933, Manila, Philippines.

[102] Zhang GY, Liu RR, Xu G, Zhang P, Li Y, Tang KX, Liang GH, Liu QQ (2013) Increased α-tocotrienol content in seeds of transgenic rice overexpressing Arabidopsis γ-tocopherol methyltransferase. Transgenic Research 22(1):89–99

[103] Zhang N, Xu Y, Akash M, McCouch S and Oard JH (2005) Identification of candidate markers associated with agronomic traits in rice using discriminant analysis. Theor Appl Genet 110: 721–729

[104] Zhang P, Li J, Li X, Liu X, Zhao X and Lu Y (2011). Collection (*Oryza sativa L.*) investigated with SSR markers. PLoS One 6:1–13

[105] Zhao K, Wright M, Kimball J, Eizenga G, McClung A, Kovach M, Tyagi W, Ali ML, Tung CW, Reynolds A, Bustamante CD and McCouch SR (2010) Genomic Diversity and Introgression in *O. sativa* Reveal the Impact of Domestication and Breeding on the Rice Genome PLoS One 5(5): e10780

[106] Zhao K, Tung CW, Eizenga GC, Wright MH and Ali ML (2011). Genome-wide association mapping reveals a rich genetic architecture of complex traits in *Oryza sativa*. Nature Communications 2:467

[107] Zhao WG, Chung JW, Kwon SW, Lee JH, Ma KH and Park YJ (2012a) Development and use of novel SSR markers for molecular genetic diversity in Italian millet (*Setaria italica L.*). Genes & Genomics 34(1): 51–57

[108] Zhao WG, Lee GA, Kwon SW, Ma KH, Lee MC and Park YJ (2012b) Association analysis of physicochemical traits on eating quality in rice (*Oryza sativa L.*). Euphytica: 10.1007/s10681-012-0820-z

[109] Zhao WG, Cho GT, Ma, KH, Chung JW, Gwag JG and Park YJ (2010) Development of an allele-mining set in rice using a heuristic algorithm and SSR genotype data with least redundancy for the post-genomic era. Molecular breeding 26: 639–651

[110] Zhao WG, Park EJ, Chung JW, Park YJ, Chung IM, Ahn JK and Kim GH (2009) Association analysis of the amino acid contents in rice. J Integr Plant Biol 51: 1126–1137

[111] Zhou PH, TanYF, HeYQ, Xu CG and Zhang Q (2003) Simultaneous improvement for four quality traits of Zhenshan 97, an elite parent of hybrid rice, by molecular marker-assisted selection. Theor Appl Genet 106:326–331

[112] Zhou J, You A, Ma Z, Zhu L and He G (2012) Association analysis of important agronomic traits in japonica rice germplasm. African Journal of Biotechnology Vol. 11(12): 2957–2970

Chapter 4

Association Mapping of Four Important Traits Using the USDA Rice Mini-Core Collection

Wengui Yan, Aaron Jackson, Melissa Jia, Wei Zhou,
Haizheng Xiong and Rolfe Bryant

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/56830

1. Introduction

Classical QTL mapping reveals only a slice of the genetic architecture for a trait because only two alleles that differ between the two parental lines segregate. A comprehensive analysis of genetic architecture requires consideration of a diverse population that represents genetic variation in a species. Association mapping provides an effective method to identify QTL that have effects across a broad spectrum of germplasm (Yu et al. 2006). Many studies have used association mapping for important traits since it was introduced from human genetics (Yu et al. 2006; Kim et al. 2006; Huang et al. 2010; Kang et al. 2008). Genome-wide association scans are expected to be effective when linkage disequilibrium (LD) and marker density are sufficiently high, so that the random markers have a greater chance of being in disequilibrium with QTL across diverse genetic materials (Kim et al. 2006). A substantial number of QTL at close to gene resolution for important traits have been identified by genome-wide association studies (GWAS) in rice (Zhao et al. 2007). Recently, the USDA Rice Mini-Core (URMC) collection was developed and serves as a genetically diversified panel for mining genes of interest (Li et al. 2010). The URMC was derived from 1,794 accessions in the USDA rice core collection using PowerCore software based on 26 phenotypic traits and 70 molecular markers (Agrama et al. 2009). The core collection represents over 18,000 accessions in the USDA global genebank of rice (Yan et al. 2007). The URMC contains 217 accessions originating from 76 countries and covering 14 geographic regions worldwide. The Objective of this review is to analyze the genetic diversity and differentiation of the URMC for genome-wide association mapping of harvest index, grain yield, sheath blight resistance and hull silica concentration.

2. Materials and methods

2.1. Rice association panel

Of 217 accessions in the URMC, 203 belong to *sativa* whereas the remaining belong to other species in *Oryza*, including 8 to *O. glaberrima*, 2 each to *O. nivara* and *rufipogon*, and 1 each to *O. glumaepatula* and *latifolia* (Agrama et al. 2009). Pure seed of these accessions were provided by the Genetic Stock *Oryza* Collection (GSOR) (www.ars.usda.gov/spa/dbnrrc/gsor). In this study, 217 accessions were used for genetic structure and diversity analyses, but only 203 *O. sativa* accessions were used for association mapping analyses because the wild relatives, *O. glaberrima*, *nivara*, *rufipogon*, *glumaepatula* and *latifolia*, contain many rare alleles, and rare alleles are one of the factors that increase the risk of type I errors or spurious associations (Breseghello and Sorrells 2006).

2.2. Location and field experiment

Evaluations were conducted for 14 traits in two field locations, USDA-ARS Dale Bumpers National Rice Research Center near Stuttgart, Arkansas and USDA-ARS Rice Research Unit near Beaumont, Texas during the growing season of 2009. The Stuttgart test site is located at N $34^0$27′44″ and W $91^0$24′59″, representing a temperate climate with a 243 d frost free period and average temperature of 23.9 C during the growing season. The Beaumont test site is located at N $30^0$03′47″ and W $94^0$17′45″, representing a subtropical climate with a 253 d frost free period and an average temperature of 26.1 C during the growing season. The experiments at both locations utilized a randomized complete block design having three replications with nine plants spaced 0.3×0.6 m in each plot. Li et al. (2012) had a detail description of experimental methods and field managements.

2.3. Phenotyping

Data collection followed procedures described by Yan et al. (2005a; 2005b) with modifications. Fourteen characteristics were recorded using the methods described by Li et al. (2010; 2011; 2012), including heading days, plant height, plant weight, tiller per plant, grain yield per plant, harvest index, main panicle length, panicle branches, Grain per panicle, seed set percentage, 1000 grain weight, grains per cm panicle, grains per branch panicle, and grain weight per panicle.

2.4. Genotyping

Bulk tissue from five plants was collected from each accession as described by Brondani et al. (2006) and total genomic DNA was extracted using a rapid alkali extraction procedure (Xin et al. 2003) and a CTAB method as described in Hulbert and Bennetzen (1991). The bulked DNA allowed identification of the origin of heterogeneity, which can result from the presence of heterozygous individuals or from a mix of individuals with different homozygous alleles (Borba et al. 2005). A total of 155 molecular markers covering the entire rice genome, with approximately one marker per 10 cM on average, were used to genotype the URMC accessions.

http://dx.doi.org/10.5772/56830

Among the markers, 149 SSRs were obtained from the Gramene database (http://www.gramene.org/), and five SSRs (AP5652-1, AP5652-2, AL606682-1, con673 and LJSSR1) were identified by Li et al(2011). The remaining marker was an *indel* at the *Rc* locus, named *Rid 12* and is responsible for rice pericarp color (Sweeny et al. 2006). Polymerase chain reaction (PCR) marker amplifications were performed as described in Agrama et al. (2009).

2.5. Statistical analysis marker and phenotype profile

Genetic distance was calculated from 155 molecular markers using Nei's method (Nei and Takezaki 1983). Phylogenetic reconstruction was based on the UPGMA method implemented in *PowerMarker* version 3.25 (Liu and Muse 2005). *PowerMarker* was also used to calculate the average number of alleles, gene diversity, and polymorphism information content (PIC) values. The tree to visualize the phylogenetic distribution of accessions and ancestry groups was constructed using MEGA version 4 (Tamura et al. 2007).

Each of the 14 phenotypic traits was modeled independently with the MIXED procedure in SASv.9.2, where genotype, location and interaction of location with genotype were defined as fixed effects while replication within a location (block effect) was a random effect. Broad-sense heritability was calculated using formula $H^2 = \sigma_g{}^2/(\sigma_g{}^2+\sigma_e{}^2/n)$, where $\sigma_g{}^2$ as the genotypic variance, $\sigma_e{}^2$ as the environmental variance and n as the number of replications (Wang et al. 2007). Spearman rank correlation coefficients between each pair of the 14 traits were calculated using the mean of 9 plants, 3 in each of three replications for an accession, using the CORR procedure in SASv.9.2. Correlation coefficients were graphically displayed based on un-weighted pair-group method using arithmetic average (UPGMA) by *NTSYSpc* software version 2.11V (Rohlf 2000).

2.6. Population structure

The model-based program *STRUCTURE* (Prichard et al. 2000) was used to infer population structure using a burn-in of 100,000, a run length of 100,000, and a model allowing for admixture and correlated allele frequencies. The number of groups (K) was set from 1 to 10, with ten independent runs each. The most probable structure number of (K) was calculated based on Evanno et al. (2005) using an *ad hoc* statistic *D(K)*, assisted with *L(K)*, *L'(K)* and *(L"K)*. The *D(K)* perceives the rate of change in log probability of the data between successive (K) values rather than just the log probability of the data. Determination of mixed ancestry (an accession unable to be clearly assigned to only one group) was based on 60% (Q) as a threshold to consider an individual with its inferred ancestry from one single group. Principal component analysis (PCA), that summarizes the major patterns of variation in a multi-locus data set, was performed with *NTSYSpc* software version 2.11 (Rohlf 2000). Two principal coordinates were used to visualize the dispersion of the mini core accessions graphically. *Fst* indicative of ancestral relationship between genetic groups was calculated using an AMOVA approach in Arlequin V2.000 (Weir 1996; Schneider and Excoffier 1999). The number of private alleles was estimated by Genetic Data Analysis (GDA) program (Lewis and Zaykin 2001).

Fourteen phenotypic characteristics were used to calculate Mahalanobis distance as a measurement of genetic differentiation among the groups (Kouame and Quesenberry 1993). The Mahalanobis distance and Canonical discriminant analysis were performed by the procedures PROC CANDISC of the *SAS* version 9.1 statistical packages. Eventually, the correlation of genetic structure differentiation resulting from the genotypic markers with phenotypic traits was assessed using the Mantel test (Mantel 1967) performed by PowerMarker.

2.7. Model comparisons and association analysis

The flexible mixed model (Yu et al. 2006) was used to control population structure. The methods for model comparisons and association mapping are referred to Li et al. (2012) for harvest index, Li et al. (2011) for grain yield, Jia et al. (2012) for sheath blight resistance and Bryant et al. (2011) for silica concentration in rice hulls.

3. Analysis of genetic structure and genetic diversity

3.1. Profile of DNA markers

Among 217 accessions in the URMC, the average number of alleles per locus was 13.5 ranging from 2 for RM338 to 57 for con673. PIC mean was 0.71 ranging from 0.30 for AP5625-1 to 0.97 for con673 among these markers. Since every accession was analyzed as a bulk of five plants, 54 (42.19%) loci showed heterozygosity and 38 (17.51%) accessions showed heterogeneity for at least one locus. Nei genetic distance (Nei and Takezaki 1983) was estimated for each pair of the 217 rice accessions which ranged from 0.021 to 1.000, with an average 0.752.

In previous studies, the average number of alleles per locus was 5.1 in Cho et al. (2000), 7.8 in Jain et al. (2004), 11.9 in Xu et al. (2004) and 11.8 in Garris et al. (2005). Recently, 13 alleles per locus were reported in the rice population studied by Thomson et al. (2007), 5.5 by Thomson et al. (2009) and 12.4 by Borba et al. (2009). The PIC in the URMC was 0.71, larger than it in the population studied by Cho et al. (2000) (0.56 PIC), Jain et al. (2004) (0.60), Xu et al. (2004) (0.66), Garris et al. (2005) (0.67), Thomson et al. (2007) (0.66), and Thomson et al. (2009) (0.45). The PIC was slightly less in our study than in the population studied by Borba et al. (2009) (0.75). Both the average allele number and PIC values are indicative of genetic diversity or gene richness in a germplasm collection. The higher the genetic diversity is in a collection, the greater the probability is for a gene of interest to be mined from the collection. Greater genetic diversity in the URMC is due to its global originations, multiple *Oryza* species, and the way of sampling with PowerCore software (Kim et al. 2007) based on 26 phenotypic traits and 70 SSR markers in order to capture the most diversity in the core collection (Agrama et al. 2009). Most other rice collections are either for a single country (Thomson et al. 2007), or for certain groups (Jain et al. 2004) and regions in a country (Thomson et al. 2009), or for special interests (Xu et al. 2004; Garris et al. 2005).

http://dx.doi.org/10.5772/56830

3.2. Genetic structure and differentiation derived from DNA markers

UPGMA tree showed that the accessions of *Oryza sativa* were classified to two main branches equivalent to *lowland* and *upland* cultivars, respectively. Ecogeographically, *indica* is primarily known as *lowland* rice and is grown throughout tropical Asia, while *japonica* often referred to as *upland* rice is typically found in temperate East Asia, upland geographic regions of Southeast Asia, and high elevations of South Asia (Garris et al. 2005). The *lowland* branch was further distinguished into two minor groups corresponding to AUS and IND accessions, while the *upland* branched into three groups, TEJ, TRJ and ARO (Fig. 1a). Wild germplasm cluster separates from the two main branches. Eight accessions of *O. glaberrima* stayed together, distinguishable from *O. latifolia*, and *glumaepatula* on one side, and *O.nivara* and *O.sativa* (PI 430909) grouped together on the other side of the tree. Although PI 430909 from Pakistan was classified *O. sativa* in the Germplasm Resources Information Network (GRIN) at www.ars-grin.gov, it exhibited shattering, had a spreading plant type, black hulls with fulllong awns, and small red kernels; all of which are typical characteristics of wild rice. Surprisingly, PI 590422 from Myanmar in 1995 and PI 346371 from Brazil in 1969 were classified as *O. rufipogon* in GRIN, but the former was clustered with *indica* (Q-indica = 0.77) and the latter with an admixture of *aus* and *indica* (Q-aus = 0.59, Q-indica = 0.41). The disagreement of cluster analysis in the study with traditional classification in GRIN is worthy of further attention.

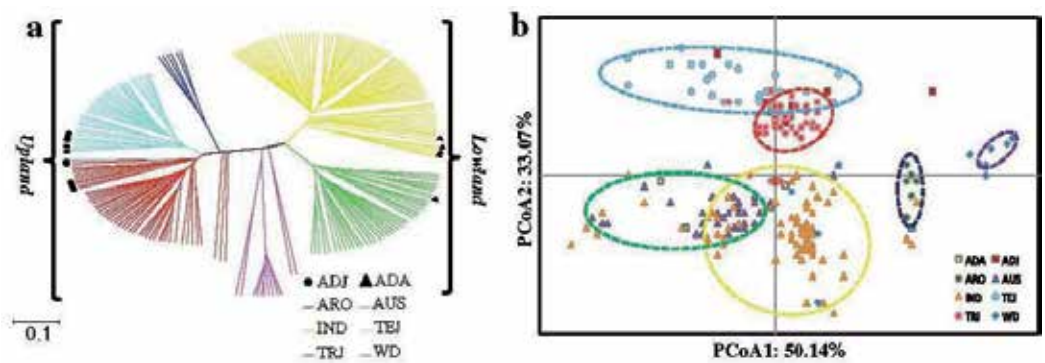


Figure 1. UPGMA tree (a) and principal coordinate analysis for 217 accessions in the USDA rice mini-core collection, both visualizing six main groups, AUS *aus*, IND *indica*, ARO *aromatic*, TEJ *temperate japonica*, TRJ *tropical japonica*, WD *wild rice*, ADA *admix of AUS and IND*, and ADJ *admix of TEJ and TRJ* (Li et al. 2010).

The ancestry of each accession was inferred from the Q value and classified into one of the six groups which corresponded to *aromatic* (ARO), *aus* (AUS), *indica* (IND), *temperate japonica* (TEJ), *tropical japonica* (TRJ) and *wild rice* (WD) based on reference cultivars reported previously by Garris et al. (2005), Agrama and Eizenga (2008), Agrama and Yan (2009). The classification was clear for a single group when the Q value was greater than 60%, otherwise an accession of germplasm was considered admixture with another group. In total, 21 accessions (9.68%) in the URMC had admixed ancestry either between TEJ and TRJ (ADJ) or between AUS and IND (ADA) (Fig. 1a, b).

The first-two axes in PCoA with 83.2% of total variation sufficiently discriminated the six main groups and two admixture groups (Fig. 1b). Each main group was distinguishable from another, but overlaps existed either among *temperate* and *tropic japonica* and their admixtures, or among *indica, aus* and their admixtures. The PCoA visualization and UPGMA tree were in agreement, which demonstrates a correct division of genetic structure in the URMC.

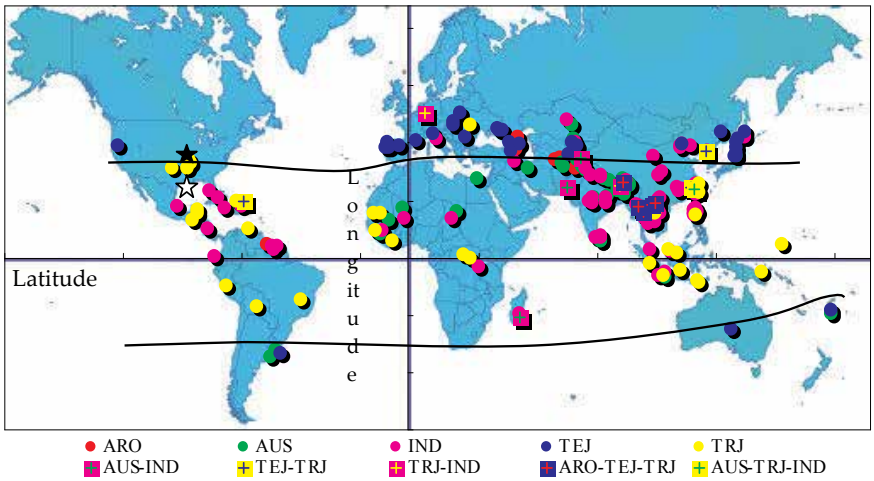


Figure 2. Geographic distribution of each mini-core accession with ancestral classification based on the latitude and longitude of germplasm origination, location of phenotypic evaluation ★: Stuttgart AR, ☆: Beaumont TX (Li et al. 2012).

Each accession with ancestry information was plotted on a world map using its latitude and longitude of geographic origin (Fig. 2). TEJ accessions were mainly distributed between latitudes 30 and 50 degrees north and south of the equator (i.e. temperate zone) while the other four groups scattered between latitude N 30 and S 30 degrees (i.e. tropical and subtropical zone).

In the URMC, the majority of accessions were IND (33%), followed by TRJ and AUS (18% each), TEJ (15%), WD (6%) and ARO with only six accessions (Fig. 3). All the marker loci were polymorphic in IND (Table 1). TRJ had 99% polymorphic loci, followed by WD, AUS, TEJ and ARO. IND had the most alleles per locus, TRJ and AUS the second most, TEJ and WD the third most and ARO had the fewest alleles. The largest number of private alleles per locus (alleles unique in one group and not found in another group) were found in WD (41.89%), followed by IND (23.78%) and AUS (17.66%). TRJ and TEJ had about equal private alleles, and the least was found in ARO. Gene diversity averaged 0.47 among the groups ranging from 0.37 in ARO to 0.52 in both IND and AUS. TRJ and WD had the same diversity (0.50), slightly greater than TEJ (0.43).

Results from the AMOVA showed that 37.92% of total variation was due to differences among groups, 61.21% within groups and 0.88% within individuals. Pair-wise estimates of *Fst* using

http://dx.doi.org/10.5772/56830

the AMOVA approach indicated a high degree of differentiation among the six main model-based groups (Fig. 3a). The mean *Fst* of all group pairs was 0.39 ranging from 0.24 between TRJ and TEJ to 0.48 between ARO and WD (Table 2). All pair-wise *Fst* values for the six groups were significant. The greatest genetic distance (0.990) among the 217 mini-core accessions was observed for PI 590413, an *O. glumaepatula* accession from the WD with 22 IND accessions and three accessions admixed AUS and IND, followed by the distance of 0.981 for PI 590413 with 9 AUS and 28 IND accessions, and the distance of 0.981 for PI 269727, an *O. latifolia* accession from the WD with 4 TEJ accessions. Two IND accessions, PI 202864 and PI 214077 had the shortest distance (0.021).

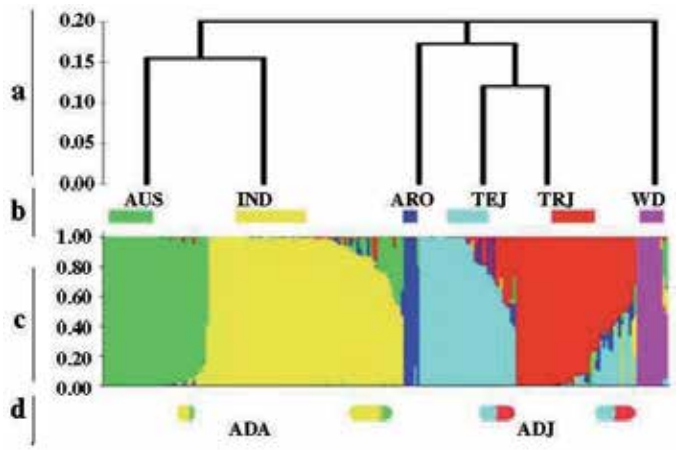


Figure 3. Dendrogram of genetic differentiation based AMOVA (Fst) (a) using DNA markers for six main groups (b), estimated group structure with each individual represented by *a horizontal bar* (c) and two admixture groups (d) (Li et al. 2010).

Group	Accessions*	Polymorphic loci %	Total alleles	Alleles/ locus	Private alleles	Private/total alleles %	Gene diversity
ARO	6	76	297	2.32	12	4.04	0.37
AUS	39	95	708	5.53	125	17.66	0.52
IND	71	100	900	7.03	214	23.78	0.52
TEJ	32	88	570	4.45	80	14.04	0.43
TRJ	40	99	758	5.92	103	13.59	0.50
WD	12	98	530	4.14	222	41.89	0.50

ARO, *aromatic*; AUS: *aus*; IND, *indica*; TEJ, *temperate japonica*; TRJ, *Tropic japonica*; WD, *wild rice*.

* Excluding admixed accessions.

Table 1. Analysis of genetic diversity among structural groups for 217 accessions in the USDA rice mini-core collection genotyped with DNA markers (Li et al. 2010).

3.3. Phenotypic analysis

Statistical analysis using a mixed model demonstrated that the differences due to genotypes and genotype × location interactions were highly significant at the 0.001 level of probability for all of the 14 traits (Table 2). The differences due to location were also significant for all traits except for panicle branches and seed set. Heritability was very high for all 14 traits. Heading had the highest heritability which was close to 100%. Although seed set had the lowest heritability, it was still above 70%. Heritability ranged from 77 to 97% among the other 12 traits. Harvest index had a heritability of 83% at Stuttgart and 90% at Beaumont. Correlation coefficients for each pair of the 14 traits were calculated using Spearman rank in each location for visualizing their relationships using PCA where the first two axes accounted for more than 50% phenotypic variation (Fig. 4a, b). At Stuttgart, 47 out of 91 correlations among the 14 traits were significant (<0.0001) (Fig. 4a), and 40 correlations were significant at Beaumont (Fig. 4b). Thirty four correlations were uniformly significant across two locations and their correlation directions (positive or negative) were also same across two locations.

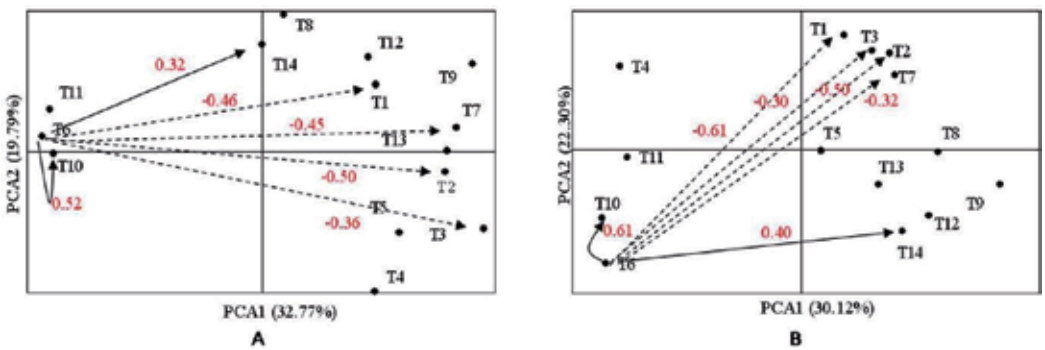


Figure 4. Relationship map constructed by PCA for 14 traits at Stuttgart, Arkansas (A) and Beaumont, TX (B). The distance between traits is inversely proportional to the sizes of the correlation coefficients. *Solid* and *dashed lines* indicate positive and negative correlations, respectively. Trait number corresponds to Table 2, and red numbers show the significant correlation coefficient with Trait 6: Harvest Index (Li et al. 2012).

3.4. Genetic structure and differentiation derived from phenotypic traits

Canonical discriminant analysis of 14 phenotypic traits for the mini-core accessions clearly separated the six plus two admixture model-based genetic groups derived from molecular data (Fig. 5). The first four significant ($P < 0.001$) canonical discriminant functions (CAN) explained 92.02% of the total variance, 54.87 % by the first CAN and 18.08% by the second CAN function, respectively. The accessions in group of AUS, ARO, IND, TEJ, TRJ and WD were clustered into their groups with various overlaps. The *upland* (ARO, TEJ and TRJ) were obviously discriminated from the *lowland* (AUS, IND), and the admixed groups ADA was scattered across AUS and IND while ADJ across TEJ and TRJ.

All 14 traits were significantly different among the eight (six plus two admixtures) model-based genetic groups. However, only three traits, plant weight (biomass), tillers and grain yield

http://dx.doi.org/10.5772/56830

Trait	Location	Mean ± SD	Range	Heritability (%)	Genotype		Location (LOC)		Genotype*LOC	
					F value	Pr>F	F value	Pr>F	F value	Pr>F
1 Heading	Arkansas	99.33±21.31	34.00~181.67	98.08	341.53	0.0000	2634.77	0.0000	12.45	0.0000
(days)	Texas	87.55±22.63	38.00~182.00	98.64						
2 Plant height	Arkansas	109.73±20.20	61.08~153.92	97.11	127.48	0.0000	1676.50	0.0000	45.63	0.0000
(cm)	Texas	124.74±22.45	67.00~178.78	95.73						
3 Plant weight	Arkansas	168.71±79.88	27.83~548.42	86.33	30.87	0.0000	122.48	0.0004	9.94	0.0000
(g/plant)	Texas	219.02±87.70	35.93~558.02	86.83						
4 Tillers/plant	Arkansas	23.95±11.20	6.42~67.75	86.53	35.27	0.0000	818.76	0.0000	7.10	0.0000
	Texas	41.13±15.83	13.00~85.89	87.16						
5 Grain yield	Arkansas	60.02±25.51	8.54~127.27	87.05	29.06	0.0000	98.37	0.0006	8.33	0.0000
(g/plant)	Texas	76.67±30.05	5.64~165.97	84.03						
6 Harvest index	Arkansas	30.44±7.02	3.40~45.06	82.75	35.79	0.0000	2174.76	0.0000	6.10	0.0000
(%)	Texas	38.98±10.51	6.25~60.02	89.98						
7 Panicle length (main)	Arkansas	26.66±3.81	14.21~37.19	89.86	46.56	0.0000	293.26	0.0001	3.68	0.0000
(cm)	Texas	24.75±3.44	16.84~38.40	88.34						
8 Panicle branches (main)	Arkansas	10.97±2.15	5.44~17.78	85.65	29.97	0.0000	31.18	0.0046	2.40	0.0000
	Texas	10.64±2.06	5.56~16.33	81.68						
9 Kernels /panicle (main)	Arkansas	194.97±57.49	68.56~399.00	86.48	29.94	0.0000	367.90	0.0000	4.45	0.0000
	Texas	155.77±45.46	50.00~318.33	86.92						
10 Seed set (main)	Arkansas	78.15±15.23	25.48~96.97	78.39	15.39	0.0000	14.26	0.0191	4.39	0.0000
(%)	Texas	73.55±12.65	35.07~95.29	72.66						
11 1000 Seed weight	Arkansas	25.77±5.07	11.17~44.74	91.79	69.00	0.0000	75.18	0.0005	3.94	0.0000
(g)	Texas	24.41±4.66	12.32~43.86	95.52						
12 Kernels/cm panicle	Arkansas	7.30±1.80	3.25~14.61	84.71	28.72	0.0000	218.17	0.0001	3.60	0.0000
	Texas	6.31±1.63	2.80~12.27	87.02						
13 Kernels/branch panicle	Arkansas	17.88±4.24	11.56~37.10	82.66	19.90	0.0000	353.27	0.0001	4.31	0.0000
	Texas	14.67±2.98	9.61~23.23	77.42						
14 Grain weight/panicle	Arkansas	3.79±1.18	0.68~8.62	82.29	21.86	0.0000	241.69	0.0001	3.94	0.0000
(g)	Texas	2.75±0.95	0.63~6.27	80.72						

Table 2. Statistical analysis of 14 traits generated at Stuttgart, Arkansas and Beaumont, Texas in 2009 among 203 *O. sativa* accessions in the USDA rice mini-core collection (Li et al. 2011).

per plant, had larger variation among groups than within groups. Therefore, they are considered the main discriminatory characters (r^2 >=0.49) in differentiating these genetic groups. The first canonical loading was 0.81 for grain yield and tillers and 0.78 for plant weight. The second canonical loading was dominated by panicle length (0.59), heading days (0.55) and seed weight (0.51).

The most tillers were observed in AUS accessions PI 385697 (93) and 352687 (86), while the lowest were in TRJ PI 584567 (9) and PI 154464 (10). WD had the most tillers (60), followed by AUS (46), ADA (44), AUS (46), IND (38), ARO (27), TEJ (24), ADJ (21) and TRJ (18). The greatest plant weight was 731 g for PI 549215 (IND), and the lowest was 37g for PI 281630 (TEJ). Again, WD had the most plant weight (442g) and TEJ had the lowest (127g). PI 373335 (IND) had the highest grain yield per plant at 175g and PI 389933 (IND) had the lowest at 11g. ADA had the most grain yield per plant (127 g per plant), while TEJ had the lowest (55g).

3.5. Relationship between genetic and phenotypic differentiation

Both the dendrograms based on the Mahalanobis distance (D^2) using the 14 phenotypic traits (Fig. 5) and based on the *Fst* genetic differentiation from AMOVA using DNA markers (Fig. 3a) produced similar results. The two dendrograms differentiated the *lowland* including IND, AUS and their admixtures from the *upland* having TEJ, TRJ, ARO and their admixtures. The WD or non-*sativa* accessions remained independent from the others.

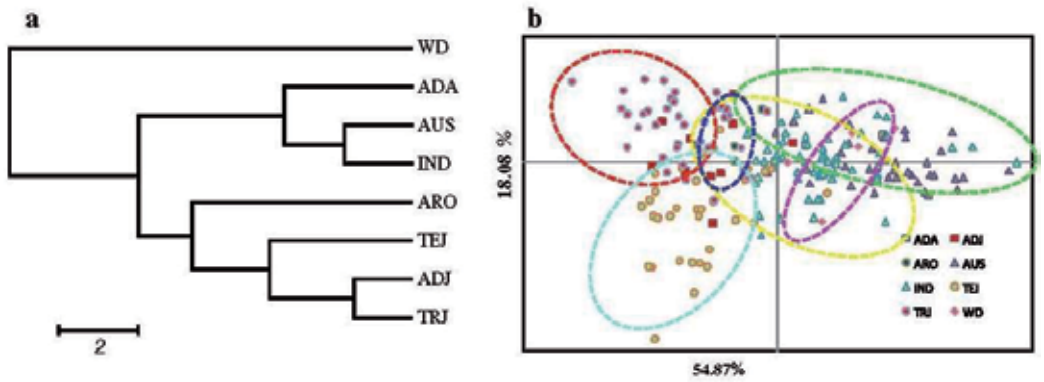


Figure 5. Dendrogram of differentiation based on Mahalanobis distance (left) and Canonical discriminant analysis (CDA) (right) using 14 phenotypic traits among structural groups for 217 accessions in the USDA rice mini-core collection (Li et al. 2010).

Group	ARO	AUS	IND	TEJ	TRJ	WD
ARO	-	0.37	0.41	0.38	0.31	0.48
AUS	12.90	-	0.31	0.44	0.40	0.38
IND	10.96	3.36	-	0.45	0.40	0.38
TEJ	13.08	15.92	9.59	-	0.24	0.46
TRJ	9.37	16.08	9.36	8.03	-	0.41
WD	21.47	14.90	17.10	22.70	22.57	-

All AMOVA-based Fst estimates from 110 permutations were significant (P<0.001), and all Mahalanobis distance (D^2) estimates were significant (P<0.001).

Table 3. Pairwise comparison of Fst values above the diagonal based on DNA markers and Mahalanobis distance (D^2) below the diagonal based on 14 phenotypic traits among structural groups for 217 accessions in the USDA rice mini-core collection (Li et al. 2010).

Analysis developed by Mantel (1967) is widely used to describe the genetic relationship between genotypic and phenotypic measurements (Gaudeul et al. 2000, Gizaw et al. 2007). In our study, genetic distance derived from the DNA markers among the six plus two admixture model-based groups was highly and significantly correlated with the distance derived from 14 phenotypic traits (r = 0.85, P =0.000<0.001). This explains the correspondence of the two dendrograms in Fig. 3a and Fig. 5(left), and similar pattern of D^2 and *Fst* in Table 3.

In rice ancestry, structure and genetic diversity of germplasm collections has been studied using a variety of molecular markers such as SNP (Zhao et al. 2011), SSR (Cho et al. 2000; Jain et al. 2004; Xu et al. 2004; Garris et al. 2005; Thomson et al. 2007; 2009; Borba et al. 2009), RAPD (Mackill 1995) and isozyme (Glaszmann 1987) markers. Phenotypic characteristics are rarely used to analyze genetic diversity or structure in rice germplasm collections. Zeng et al. (2003)

http://dx.doi.org/10.5772/56830

collected samples from each of six genetic groups for a diversity analysis using 31 phenotypic traits, but failed to reveal their genetic differentiations.

However, assessment of genetic diversity and structure using both genotypic and phenotypic characterization and relationship or accuracy between the genotypic and phenotypic assessments has long been attractive to the scientific community. Elias et al. (2001a) reported a significant positive association between genotypic and phenotypic distances (r = 0.204, p = 0.054) using eight SSRs and 14 traits for 38 accessions of cultivated cassava (*Manihot esculenta* Crantz). The association was improved (r = 0.283, $p < 0.01$) in a set of 29 cassava accessions genotyped with AFLP markers and phenotyped for 14 morphological and four agronomic traits (Elias et al. 2001b). A set of 68 sweet sorghum and four grain sorghum (*Sorghum bicolor* L.) accessions were genotyped with 41 SSRs and phenotyped for six traits (Ali et al. 2008). The genotypic analysis classified the 72 accessions in 10 clusters and the phenotypic variation among the clusters was described. Similarly, 15 morpho-physiological traits were used to describe four major groups of 61 tomato (*Solanum lycopersicum* L.) accessions classified by genotypic data of 29 SSRs (Mazzucato et al. 2008). In barley (*Hordeum vulgare* L.), based on five cultivars phenotyped for 18 traits and genotyped with 11 AFLP markers, trait relationships were demonstrated using simple correlation, path analysis and GGE biplot. The cultivars were clustered based on genetic dissimilarity estimated by the AFLP markers (Akash and Kang 2009).

We use both genotypic and phenotypic characterizations to analyze genetic differentiation in a plant germplasm collection. The present study in rice has a much greater association (r = 0.85, P =0.000<0.001) of genetic distance derived from genotypic characterizations with phenotypic characterizations than the previous study in cassava.

4. Association mapping of harvest index and components

Harvest index is a ratio of grain yield to total biomass, which measures farming success in partitioning assimilated photosynthate to harvestable product (Hay 1995; Sinclair 1998). In cereal crops, dramatic improvements of harvest index during domestication have made commercial cultivars dramatically different from their wild ancestors (Gepts 2004). Rice (*Oryza sativa* L.) is one of the most important staple foods (Tyagi et al. 2004), and can be highly productive if high harvest index genotypes are grown with optimal management practices (Raes et al. 2009). Harvest index is one of the most complex traits in rice involving number of panicles per unit area, number of spikelets per panicle, percentage of fully ripened grains, kernel size (Terao et al. 2010) and plant height (Marri et al. 2005). Marri et al. (2005) found that harvest index was negatively correlated with plant height, but positively correlated with grain number per panicle, tiller number per plant, seed set, kernel size and grain yield per plant in rice. Similarly in maize, harvest index is negatively correlated with plant height, and positively correlated with grain yield (Can and Yoshida 1999). In sorghum, harvest index is negatively correlated with forage yield (Mohammad et al. 1993), but positively correlated with growth rate and grain filling rate (Soltani et al. 2001). The correlated traits are interrelated in most

cases, so that increases in one component may lead to either decreases or increases in others. Therefore, scientists aim to identify genes or QTL that increase one aspect of a target trait without affecting others, or improve the target trait indirectly through an improvement of its related traits.

In rice, previous studies on harvest index have identified numerous QTL all using a classic linkage-mapping strategy with two parents. Mao et al. (2003) reported four main QTL on chromosome (Chr) 1, 4, 8 and 11 and an epistatic interaction between two QTL respectively on Chr 1 and Chr 5. Sabouri et al. (1999) identified three QTL each on Chr 2, 3 and 5, and two QTL close to each other on Chr 4. Lanceras et al. (2004) described harvest index QTL on Chr 1 and 3. However, mapping populations developed from different parental combinations and/or experiments conducted in different environments often result in partly or wholly non-overlapping sets of QTL (Hao et al. 2010).

4.1. Traits correlated with harvest index in our study

Six traits were significantly correlated with harvest index and these correlation directions were the same across the two locations. The correlations with harvest index were negative for heading (-0.46 at Stuttgart and -0.61 at Beaumont), plant height (-0.50 and -0.50), plant weight (-0.36 and -0.30), panicle length (-0.45 and -0.32), while positive for seed set (0.52 and 0.61) and grain weight/panicle (0.32 and 0.40) (Fig. 4a, b). In the PCA based on phenotypic traits of 203 mini-core accessions, four traits negatively correlated with harvest index were plotted on opposing axis from harvest index (Fig. 4a, b). Conversely, two traits positively correlated with harvest index were plotted in the same axis relatively close to harvest index.

4.2. Marker-trait associations

At Stuttgart, a total of 36 markers were significantly associated with harvest index traits at the 6.45×10^{-3} level of probability (the Bonferroni corrected significance level). Among 36 markers, seven were associated with harvest index, five with heading, three with plant height, six with plant weight, five with panicle length, nine with seed set and one with grain weight/panicle. Eight trait-marker associations have been reported by previous linkage mappings. Additionally, seven markers were associated with two or more harvest index traits, named "consistent" markers (Pinto et al. 2010). Out of the seven consistent markers, RM600, RM5 and RM302 were co-associated with harvest index and seed set, RM431 with heading and seed set, RM341 with plant height and panicle length, RM471 with heading and plant weight, and RM510 with three traits, plant height, harvest index and seed set.

At Beaumont, we identified 28 markers significantly associated with harvest index's traits. Among 28 markers, two were associated with harvest index, three with heading, nine with plant height, six with plant weight, four with panicle length, three with seed set and one with grain weight/panicle. Similarly with Stuttgart, 11 trait-marker associations have been identified in previous QTL studies. Two consistent markers were RM208 co-associated with harvest index and seed set, and RM55 co-associated with plant height and plant weight.

http://dx.doi.org/10.5772/56830

Associations of RM431 with plant height, Rid12 and RM471 with plant weight and RM24011 with panicle length were found in both locations. The four markers that associated with the same trait across both locations are called "constitutive QTL" markers, while others that associated with a certain trait only at one location are called "adaptive QTL" markers (Mao et al. 2003).

4.3. Allelic effects

The allelic effects of the constitutive markers associated with their traits were estimated with the least square mean (LSMEAN) of phenotypic value and presented in Fig. 6. Meanwhile, an algorithm was employed to generate a letter-based representation of all-pairwise comparisons for allelic effect. For RM431, allele 253bp had a significantly larger effect than all other 6 alleles at Beaumont and than 4 others at Stuttgart to reduce plant height. For RM24011, allele 390bp had the greatest effect on decreasing panicle length while allele 411bp had the largest effect on increasing panicle length at both locations. However, for Rid12, the allelic effects were opposite between two locations. Allele 151bp of Rid12 had a decreasing effect on plant weight at Stuttgart, but an increasing effect at Beaumont instead. The 165 allele of Rid12 had an opposite effect to 151bp on plant weight. For RM471, the allelic effects on plant weight were not consistent from one location to another. The 109bp allele had the largest effect on decreasing plant weight at Stuttgart, but a fairly larger effect on increasing plant weight at Beaumont.

4.4. Genetic dissection of harvest index

Harvest index is an integrative trait including the net effect of all physiological processes during the crop cycle and its phenotypic expression is generally affected by genes responsible for non-target traits, such as heading (Lanceras et al. 2004; Hemamalini et al. 2000), plant height (Lanceras et al. 2004) and panicle architecture (Ando et al. 2008). The magnitude and direction of these gene functions on different phenotypes would bear heavily on the utility of such genes for improvement of these traits. In the current study, the traits like heading, plant height, plant weight and panicle length had a strong negative correlation with harvest index, while seed set and grain weight/panicle were positively correlated with harvest index. These phenotypic correlations were consistently reflected in the identification of molecular markers associated with harvest index and related traits. For example, four consistent markers at Stuttgart, RM600, RM302, RM25, and RM431, were associated with not only harvest index itself, but also for one or more additional traits correlated with harvest index. Another consistent marker, Rid12, associated with both heading and plant weight was close to a reported QTL "*qHID7-1*" responsible for harvest index and the gene "*Ghd7*" which effects grains per panicle, plant height and heading in rice (Hittalmani et al. 2003). At Beaumont, the consistent marker RM55 associated with both plant height and plant weight was adjacent to a QTL "*qHID3-2*" for control of harvest index (Hemamalini et al. 2000). RM431 co-associated with plant height and harvest index in this study has been reported to be closely linked to gene ''*sd1*'' (Xue et al. 2008; Peng et al. 2009). *sd1* is involved in gibberellic acid biosynthesis, decreases plant height and thus increases harvest index. The decreased height confered by *sd1*allows the plant to have a reduced risk of lodging,

be more tolerant to heavy doses of nitrogen fertilizer, and allows for planting increased stand densities. The *sd1* gene has greatlyimproved grain yield and has contributed to the Green Revolution in cereal crops including rice (Fu et al. 2010).

Other markers were associated with the traits correlated with harvest index, but not with harvest index directly in this study. These markers have been reported either nearby or flanking the QTL for harvest index. RM5, which was associated with plant height in the Stuttgart study, was close to a reported QTL for harvest index on Chr 1 (Marri et al. 2005). RM471 associated with plant weight was close to the reported *qHID4-1* and *qHID4-2* for harvest index (Hemamalini et al. 2000). Furthermore, RM257 and RM22559 associated with seed set were co-localized with a known QTL on Chr 9 (Marri et al. 2005), and with *qHID8-1* (Hemamalini et al. 2000) for harvest index, respectively. Similarly, at Beaumont, RM44 associated with plant height was close to *qHID8-1* (Hemamalini et al. 2000), and RM263 associated with heading was adjacent to *hi2.1* (Marri et al. 2005). The chromosomal regions where numerous correlated traits are mapped indicate either pleiotropy of a single gene or tight linkage of multiple genes. Fine-mapping of such chromosomal regions would help discern the actual genetic control of these congruent traits. Development of markers for such traits in specific regions could lead to a highly effective strategy of marker-assisted selection for improving harvest index.

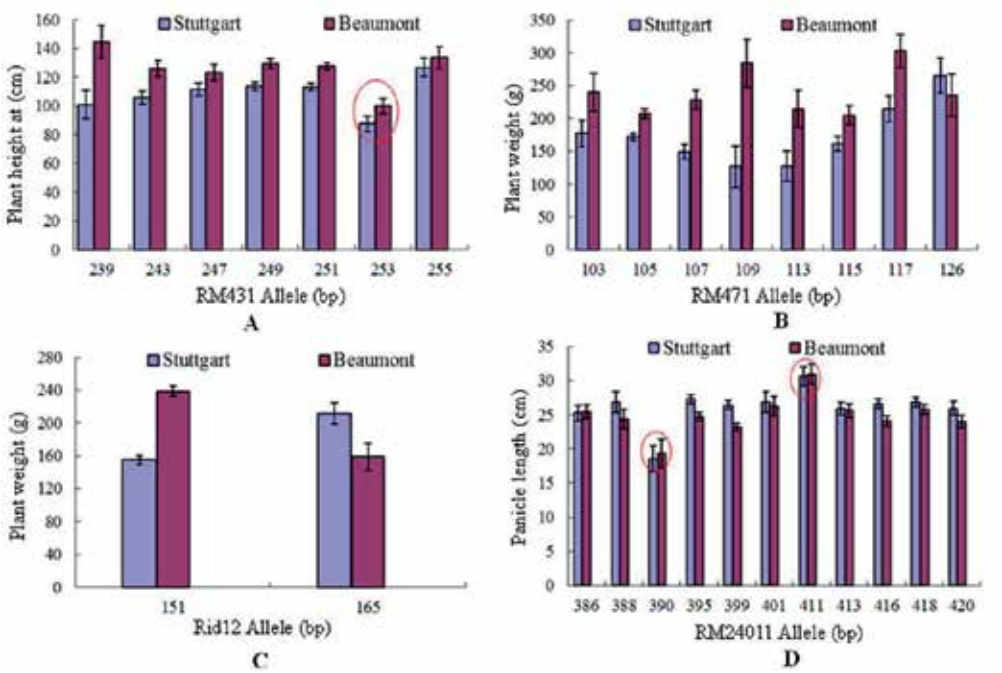


Figure 6. Comparisons of allelic effects of four constitutive marker loci RM431 (a) associated with plant height, RM471 (b) and Rid12 (c) associated with plant weight, RM24011 (d) associated with panicle length constitutively at both Stuttgart, Arkansas and Beaumont, Texas (Li et al. 2012).

http://dx.doi.org/10.5772/56830

5. Association mapping of grain yield and components

Yield is one of the most important and complex traits in crops that does not evolve independently but shows correlations with other traits. Thus, breeders have to consider correlated traits in breeding programs. Yield and its related traits are quantitatively inherited and controlled by many genes with small effects subject to environmental effects (Inostroza et al. 2009; Shi et al. 2009). Many studies have focused on the improvement and inheritance of agronomically important yield-related traits for achieving greater yield (Gravois and McNew 1993; Samonte et al. 1998). Other traits such as biomass, plant architecture, adaptation, and resistance to biotic and abiotic constraints may also indirectly affect yield through yield components or other physical and physiological mechanisms. Hence, estimation of the positions and effects of quantitative trait loci (QTL) for traits related to yield is of central importance for marker-assisted selection for yield improvement. In rice genetics, most QTLs related to yield have been identified through classic linkage mapping approaches (Moncada et al. 2001; Brondani et al. 2002; Thomson et al. 2003; Jiang et al. 2004; Suh et al. 2005). With a few notable exceptions, most of these QTLs have not been successfully validated or consistently used in crop improvement (Bernardo 2008). The classic approaches are too simplistic to effectively model most of the genetic variation for complex traits because they are unable to reflect the genetic realities of these traits (Cooper et al. 2005; Holland 2007).

Trait	Locus	Chr. no.	Position (cM)	Prob F			Annotation
Grain yield	OSR13	3	36.0	0.0046	#		
(4 markers)	RM471	4	45.0	0.0004	#		
	RM247	12	26.7	0.0062			
	RM7003	12	41.0	0.0011	#	*	GY_QTL Marker (Thomson et al. 2003; Fu et al. 2010)
Plant height	RM5	1	98.5	0.0061		*	
(3 markers)	RM431	1	154.6	0.0001	#	*	GY_QTL Marker (Fu et al. 2010)
	RM509	5	59.0	0.0043	#		
Plant weight	RM279	2	14.0	0.0011	#		
(6 markers)	RM471	4	45.0	0.0008	#		
	RM527	6	59.0	0.0048			
	Rid12	7	41.0	0.0006	#	*	GY_QTL Marker (Xue et al. 2008)
	RM224	11	115.0	0.0033	#	*	
	RM7003	12	41.0	5.87×10^{-6}	#		GY_QTL Marker (Thomson et al. 2003; Fu et al. 2010)
Tillers	RM431	1	154.6	0.0033	#		GY_QTL Marker (Fu et al 2010)
(9 markers)	RM279	2	14.0	0.0014	#		

Trait	Locus	Chr. no.	Position (cM)	Prob F		Annotation
	RM341	2	70.0	0.0023	*	
	RM3558	4	69.8	0.0024		
	Rid12	7	41.0	0.0001	#	GY_QTL Marker (Xue et al. 2008)
	RM125	7	41.0	0.0012		GY_QTL Marker (Jiang et al. 2004; Fu et al. 2010; Borba et al. 2010)
	RM484	10	71.4	0.0005		
	RM287	11	68.0	0.0050		GY_QTL Marker (Moncada et al. 2001)
	RM224	11	115.0	0.0002	#	
Panicle length	RM509	5	59.0	0.0036	#	
(5 markers)	RM510	6	11.5	0.0003		
	RM24011	9	27.4	0.0033		
	RM245	9	91.8	0.0040		GY_QTL Marker (Suh et al. 2005)
	RM3739	12	97.0	0.0009		
Kernels/branch	OSR13	3	36.0	0.0016	#	
(3 markers)	RM471	4	45.0	0.0048	#	
□	RM1335	7	106.0	0.0005		

#: The marker was associated with two or more traits

*: The marker was reported to be associated with the corresponding trait previously

GY_QTL Marker: The markers were identified to be linked or close to yield QTL in previous studies

Table 4. Marker loci significantly associated with grain yield and its related traits mapped in the USDA rice mini-core collection (Li et al. 2011)

5.1. Traits correlated with grain yield per plant in our study

The traits significantly correlated with grain yield were plant height (0.43), plant weight (0.81), tillers (0.77), panicle length (0.30) and kernels/branch (0.40). All these traits were clustered into one branch except kernels/branch. This exploratory assessment showed that grain yield and the set of five correlated traits would serve as an appropriate base population for an association mapping application.

5.2. Marker-yield trait associations

Using the selected PCA model, a total of 30 marker loci were identified to have significant marker-trait associations at the 6.45×10^{-3} level of probability (the Bonferroni corrected significance level) for yield and its correlated traits (Table 4). Out of the 30 markers, four were associated with grain yield, three with plant height, six with plant weight, nine with tillers, five with panicle length and three with kernels/branch. Six markers were co-localized with

http://dx.doi.org/10.5772/56830

previous identified QTL (Thomson et al. 2003; Jiang et al. 2004; Xue et al. 2008; Fu et al. 2010; Borba et al. 2010; Moncada et al. 2001) (Table 4).

Most importantly, eight of the 30 markers were synchronously associated with two or more traits (Table 4). RM471 was co-associated with three traits, grain yield, plant weight and kernels/branch. Three markers Rid12, RM224 and RM279 were co-associated with plant weight and tillers. RM431 was co-associated with plant height and tillers; RM509 with plant height and panicle length; RM7003 with grain yield and plant weight; and OSR13 with grain yield and kernels/branch. Three markers, OSR13, RM471 and RM7003 were included for the allelic analysis because they were not only associated with grain yield directly, but also co-associated with other yield correlated traits (Fig. 7). The allelic effect of each loci associated with the traits was estimated with mean of phenotypic value for each allele. For marker locus RM471, allele 126bp had the highest effect on all three traits (93.48 for grain yield, 266.23 for plant weight and 25.36 for kernels/branch), while two other alleles 109bp and 113bp had the lowest effect on grain yield with 48.19 and 49.90, and plant weight with 17.54 and 19.82, respectively (Fig. 7a and b). For OSR13, allele 123bp had a large effect on both grain yield and kernels/branch with 66.37 and 19.91, respectively while allele 115 had the highest effect on kernels/branch and the lowest on grain yield (Fig. 7c). For RM7003, allele 108bp had the highest effect on both traits (66.37 for grain yield and of 228.05 for plant weight) while the allele 106bp had the lowest effect on both traits (43.19 for grain yield and with 154.48 for plant weight) (Fig. 7d).

5.3. Trait-trait and marker-trait associations

Correlation among phenotypic traits is a common phenomenon in biology. Plant breeders need to consider trait correlations for either improving numerous correlated traits simultaneously or reducing undesirable side effects when their goal is only one of the correlated traits (Chen and Lubberstedt 2010). In this study, 34 of 91 pairs (37.36%) of 14 traits were observed to have significant correlation, and five traits were correlated with grain yield among 203 mini-core accessions. The correlations exhibited a complex network among these traits. Numerous researchers have concluded that rice yield is highly dependent on the number of productive tillers or panicles (Sharma and Choubey 1985; Dhanraj and Jagadish 1987), which is recently verified with a high correlation between tillers and yield ($r=0.88$; $p < 0.01$) by Borba et al. (2010). Panicle characters including panicle length, number of primary branches, secondary branches per primary branch, total kernels and seed set in a panicle, are reported to be tightly related to yield performance (Thomson et al. 2003; Ando et al. 2008; Terao et al. 2010). Although seed set and kernel weight per panicle were not directly correlated with yield in this study, they may be correlated in other panels of germplasm or may be indirectly contributable to yield. For example, seed weight per panicle, seed set and 1000 kernel weight are identified to be highly correlated with yield in wild rice (Oryza *rufipogon* Griff.) (Fu et al. 2010). Similarly, seed weight per panicle and seed set have correlations with yield in an advanced backcross population between *Oryza rufipogon* and the *Oryza sativa* cultivar Jefferson (Thomson et al. 2003). These different results could be expected since different materials were used in those studies.

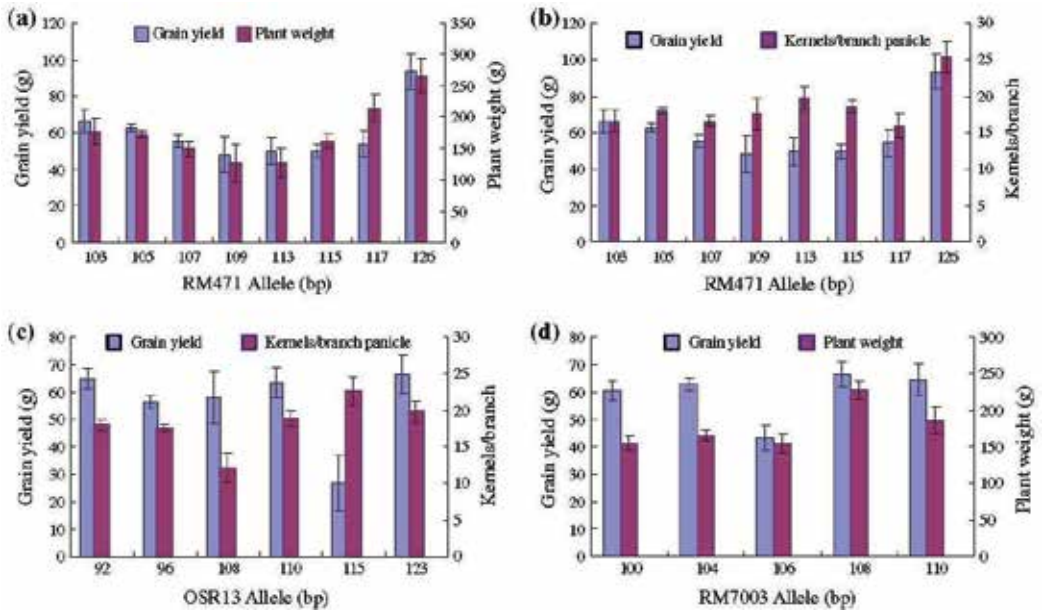


Figure 7. Comparisons of allelic effects of three marker loci on yield traits: RM471 (a, b), OSR13 (c) and RM7003 (d). RM471 co-associated with grain yield, plant weight and kernels/branch panicle; OSR13 co-associated with grain yield and kernels/branch; RM7003 co-associated with grain yield and plant weight; bars are the standard error (Li et al. 2011).

Morphological correlations could be explained by either pleiotropy or linkage disequilibrium. The former describes the impact of a single gene on multiple phenotypic traits. The latter deals with influence of two or more genes on multiple traits, where the genes are physically located so close to each other, that they cannot be practically separated (Chen and Lubberstedt 2010). Co-association of a single gene (or two linked genes) with multiple traits that are phenotypically correlated has occurred in numerous studies. Yan et al. (2009) reported five SSRs that were co-associated with two correlated traits affecting stigma exertion, another five SSRs with two traits correlated to spikelets, and one SSR with three correlated traits to spikelets in rice. Similarly, Terao et al. (2010) identified the gene of *APO1* that increases both the primary rachis branches and grains per panicle in rice. Gene *DEP1* increases both rachis branches and grain yield in rice (Huang et al. 2009). Gene *Ghd7* has major effects on grains per panicle, plant height and heading date in rice (Xue et al. 2008). Further, developmentally related traits (like number of tillers and roots) have been mapped to the same chromosome regions (Hemamalini et al. 2000; Brondani et al. 2002, Li et al. 2006; Thomson et al. 2003, Fu et al. 2010). In this study, eight markers were co-associated with two or more correlated traits and some QTLs related to yield and yield components have been reported to be near these regions. RM7003 co-associated with grain yield and plant weight is reported to flank a major yield QTL (*yld12.1*) (Thomson et al. 2003; Fu et al. 2010). Also, RM7003 is near the QTL *gpp12.1* which influences grains per panicle (Thomson et al. 2003), the QTL *pss12.1* which effects seed set (Fu et al. 2010) and another QTL *qFG12-2* which is involved with filled grain number (Li et al. 2002). Interestingly, five particular

http://dx.doi.org/10.5772/56830

markers were not associated with yield directly in this study, but they were all identified to be the markers flanking grain yield QTL in other studies. RM431, RM340, and RM245 were found to be associated with yield QTLs, *yld1.1* (Fu et al. 2010), *qYI-6-1* and *qYI-9* (Suh et al. 2005), respectively. Rid12 co-associated with tillers and plant weight was found to be very close to *Ghd7* that had major effects on grain yield, plant height and heading date (Xue et al. 2008) in addition to its function for rice pericarp color (Sweeney et al. 2006; Brooks et al. 2008). RM125 associated with tillers was also identified to have a strong association with yield (Borba et al. 2010, Jiang et al. 2004). RM431 co-associated with plant height and tillers in this study has been reported to be closely linked with a QTL "*sd1*" to decrease plant height and increase yield (Peng et al. 1999; Fu et al. 2010). The chromosomal regions where numerous traits are mapped indicate either pleiotropy resulting from a single gene or tight linkage of multiple genes. Fine-mapping of such chromosomal regions would help discern the actual genetic control of these congruent traits. Development of markers for such traits in these regions could lead to a highly effective strategy of marker-assisted selection.

Several genes for grain yield and its related traits have been recently cloned, and each of these genes has a clearly distinct biological function (Li et al. 2003; Ashikari et al. 2005; Fan et al. 2006; Song et al. 2007). Molecular cloning and functional analyses of several genes have shown that these genes are mostly related to the synthesis and regulation of the phytohormone gibberellin (Peng et al. 1999; Ashikari et al. 1999; Spielmeyer et al. 2002; Itoh et al. 2004). For example, a semidwarf QTL "*sd-1"* close to RM431 contains a defective gibberellin 20-oxidase gene responsible for height reduction. The shorter statured plants have a decreases lodging threat and tolerates higher dosags of nitrogen fertilization, thus dramatically increases grain yield. Furthermore, the photoperiod pathway controls flowering time or heading directly, thus affects plant weight and yield indirectly (Xue et al. 2008). Two other genes regulating heading have been identified. One is *Hd6* which encodes a subunit of protein kinase CK2 (Takahashi et al. 2001), and the other is *Ehd1* which encodes a B-type response regulator (Doi et al. 2004). Also, a gene *GHD7* has been identified to simultaneously control yield, plant height and heading in rice (Xue et al. 2008). This gene locates close to Rid12 and encodes a CCT (CO, Co-like and Timing of CAB1) domain protein. These findings demonstrate that genes regulating yield usually share some common pathways for traits that contribute to yield. Regions with either tightly linked QTLs or pleiotropic effects would become QTL hot spots, worth further investigation.

Comparison of the allelic effect among different alleles at the same locus could determine which specific alleles would be most informative for marker assisted selection. For example, allele 126bp of RM471 and 108bp of RM7003 were considered major alleles with a positive effecton increasing yield among all the alleles in the loci (Fig. 7). Howeve, the allele 106bp of RM7003 would be less desirable because it had a negetaive effect which is associated with a decrease of both grain yield and plant weight among accessions containing the allele. Results of the present study demonstrated that genome-wide association mapping in the USDA rice mini-core collection could complement and enhance the information from linkage-based QTL studies, and help increase yield through improvement of these related traits by marker-assisted selection either directly or indirectly.

6. Association mapping of resistance to Sheath Blight disease

Rice sheath blight (ShB), caused by the soil-borne fungal pathogen *Rhizoctonia solani* Kühn, is a major disease of rice that greatly reduces yield and grain quality worldwide (Savary et al. 2006). Due to the high cost of cultural practices and the phytotoxic influence associated with the application of fungicides, the use of ShB resistant cultivars is considered the most economical and environmentally sound strategy in managing this disease. Understandings of genetic control will facilitate cultivar improvement for this disease and secure global food production.

The necrotrophic ShB pathogen has a broad host range and no complete resistance has been identified in either commercial rice cultivars or wild related species (Mew et al. 2004; Eizenga et al. 2002). However, substantial differences in susceptibility to ShB among rice cultivars have been observed under field conditions (Jia et al. 2007). Differential levels of resistance and the associated resistance genes have been studied among rice germplasm accessions (Manosalva et al. 2009). Rice ShB resistance is believed to be controlled by multiple genes or quantitative trait loci (QTLs) (Pinson et al. 2005). Since Li et al. (1995) first identified ShB QTLs using restricted fragment length polymorphism (RFLP) markers under field conditions, over 30 resistant ShB QTLs have been reported using various mapping populations, such as F_2s (Sharma et al. 2009; Che et al. 2003), double haploid (DH) lines (Kunihiro et al. 2002), recombinant inbred lines (RILs) (Liu et al. 2009; Jia et al. 2007; Prasad and Eizenga 2008), near-isogenic introgression lines (NIL) (Loan et al. 2004) and backcross populations (Zuo et al. 2007; Sato et al. 2004). 'Teqing' and 'Jasmine 85' have been repeatedly involved in these studies as the ShB resistant parents. We are the first to map rice ShB QTLs using an association mapping strategy in a global germplasm collection (Jia et al. 2012).

6.1. Phenotypic evaluation of Sheath Blight resistance

The isolate RR0140-1 of *R. solani* was selected from 102 isolates collected state-wide from Arkansas rice fields due to its slow growing phenotype (Wamishe et al. 2007). Field evaluations have showed similar disease reactions between slow growing and fast growing isolates (Wamishe et al. 2007). Further, the RR0140-1 isolate has been adapted by numerous studies (Liu et al. 2009; Jia et al. 2007; Prasad and Eizenga 2008). Pathogen preparation and inoculation are referred to Jia et al. (2007; 2011; 2012).

Plant response to the sheath blight pathogen was measured using the ratio between the height of the pathogen growing up the plant and the height of the leaf collar on the last emerged leaf. Because mature plant height varied from 70 to 202 cm in this collection (Yan et al. 2007), the ratio excluded possible interference of plant height in scoring disease response. Therefore, the smaller the ratio, the greater the resistance was for an entry. Measurements were taken when the ratio reached 1.0 for 75% of the susceptible check plants, Lemont, so that maximum susceptibility was scored as 1.0.

ShB rating data were analyzed using the GLIMMIX procedure in SAS version 9.1.3. The experimental design of randomized incomplete block formed the basis of the statistical model,

http://dx.doi.org/10.5772/56830

where the accession is a fixed effect and block is treated as random effect. The LSMEANS option was used to calculate the least-square means (LSMs) from 18 plant scores in 6 replicates of each entry and the LSMs were used for the association mapping. The statistical differences of the accession to each check (Jasmine 85 and Lemont) were determined by a Dunnett's multiple comparison test, using the diff=control option.

6.2. Phenotypic variation of Sheath Blight severity ratings

The ShB severity ratings among the 217 entries were distributed normally, ranging from 0.256 ± 0.111 to 0.909 ± 0.096 with an average of 0.521 ± 0.008 (Fig. 8). The resistant check Jasmine 85 was rated 0.472 ± 0.021 and susceptible check Lemont was rated 0.946 ± 0.080. Twenty-four entries (11.1 %) were significantly more resistant to ShB than Jasmine 85 at the 5% level of probability while 54 others (24.9%) had similar resistance.

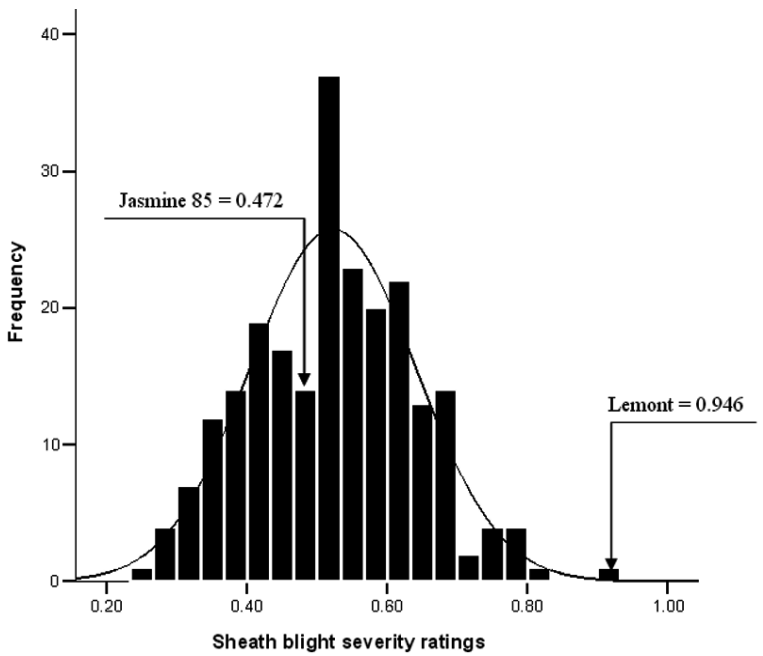


Figure 8. Distribution of sheath blight severity ratings among 217 mini-core accessions averaged over 18 plant scores, 3 in each of 6 replicates using a micro-chamber method with resistant check Jasmine 85 and susceptible Lemont (Jia et al. 2012).

6.3. Marker loci and their alleles associated with Sheath Blight resistance

Ten marker loci were identified to be significantly associated with ShB resistance at the probability level of 5% or lower, three on chromosome (Chr) 11, two on Chr1, and one each on Chr2, 4, 5, 6 and 8 (Table 5). RM237 on Chr1 at 27.1 Mb had the highest significance rating for ShB at the 0.002 level of probability. RM11229 on the long arm of Chr1 explained the most

phenotypic variation (9.5%) with significance at the 0.044 level of probability. RM11229 and 1233 each had six alleles, the most among the 217 mini-core entries, followed by RM341 and 254 (five alleles), RM237, 8217,146 and 408 (four), RM133 (three) and RM7203 (two) (Table 5).

Among the six alleles of RM11229, allele 158 was present in 18 entries that had the lowest average ShB rating (0.414), and thus, it was designated as the 'putative resistant allele' of this marker locus. Accordingly, ten alleles, one each from the ten associated marker loci, were noted as the putative resistant allele in Table 5 because they had the greatest effect to decrease ShB among all the alleles for their respective loci (Table 5). ShB rating was the smallest for putative resistant allele 158 of RM11229 among the ten putative resistant alleles. Of the other five putative resistant alleles, 139 of RM341 (present in 17 entries), 340 of RM146 (28 entries), 88 of RM7203 (120 entries), 169 of RM254 (12 entries) and 177 of RM1233 (35 entries), had lower ShB means ranging 0.447 - 0.470 than the resistant check Jasmine 85 (0.472), suggesting a stronger effect for resistance to ShB than Jasmine 85. The remaining four putative resistant alleles had similar ShB ratings with Jasmine 85, suggesting a similar effect for the level of ShB control.

Marker	Chr.	Position (Mb)	P value	Rsq_Marker [a]	Allele (bp)	Number of Entries	ShB Mean [b]
RM11229	1	22.4	0.044	9.5%	158*	18	0.414
					192	21	0.515
					195	21	0.473
					198	13	0.532
					207	14	0.608
					224	12	0.466
RM237	1	26.8	0.002	6.9%	122	19	0.526
					128*	32	0.473
					130	105	0.515
					132	20	0.635
RM341	2	19.3	0.041	4.1%	135	89	0.558
					138	39	0.545
					139*	17	0.447
					141	15	0.579
					171	39	0.461
RM8217	4	32.7	0.044	3.2%	178	67	0.581
					182	19	0.534
					184	65	0.482
					186*	49	0.476
RM146	5	18.1	0.021	3.8%	330	26	0.539

http://dx.doi.org/10.5772/56830

					332	127	0.512
					340*	28	0.463
					344	28	0.591
RM133	6	0.2	0.043	2.4%	228	89	0.557
					230*	104	0.479
					232	22	0.577
RM408	8	0.1	0.023	4.0%	117	18	0.577
					119*	105	0.478
					125	18	0.498
					127	57	0.591
RM7203	11	1.1	0.033	1.9%	88*	120	0.470
					104	80	0.589
RM254	11	23.8	0.030	5.3%	159	25	0.580
					161	36	0.564
					163	54	0.511
					167	50	0.480
					169*	12	0.463
RM1233	11	26.5	0.036	5.1%	158	97	0.538
					164	12	0.543
					168	15	0.593
					173	12	0.520
					177*	35	0.451
□	□	□	□	□	179	12	0.524
Resistant check 'Jasmine 85'							0.472

[a] Rsq_Marker - total explained phenotypic variation.

[b] The mean of ShB severity rating for the entries with the allele.

Allele*: Putative resistant allele which had the lowest ShB mean at the marker locus.

Table 5. Marker loci significantly associated with sheath blight resistance, their physical locations on chromosomes (Chr), allele size in 217 mini-core entries, number of entries with the allele, and their mean sheath blight (ShB) rating (Jia et al. 2012)

Among the ten putative resistant alleles, allele 88 of RM7203 was the most prevalent and existed in 120 (55%) of 217 entries in the mapping panel, followed by allele 230 of RM133 and 119 of RM408 (48% of the lines), allele 186 of RM8217 (23%), allele 340 of RM146, 128 of RM237 and 177 of RM1233 (13-16%), allele 139 of RM341 and 158 of RM1229 (8%), and allele 169 of RM254 (6%).

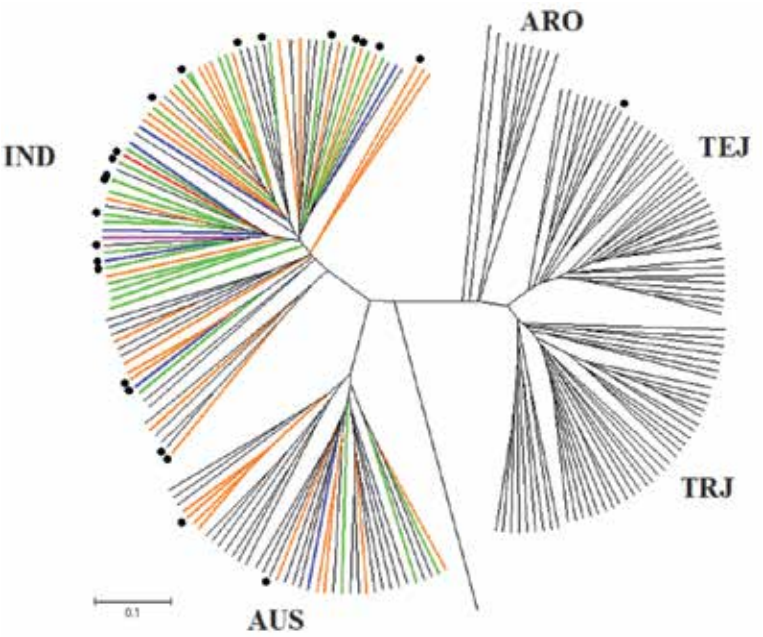


Figure 9. UPGMA tree based on Nei genetic distance for 217 mini-core entries where 24 marked with ● were significantly more resistant to sheath blight than the resistant check 'Jasmine 85'. Presence of 'putative resistant alleles' is distinguished by branch color: Red= eight putative resistant alleles, Pink= seven, Blue= six, Green= five, and Orange = four (Jia et al. 2012).

6.4. Number of putative resistant alleles and Sheath Blight resistance

The number of putative resistant alleles increased along with an increase of sheath blight resistance in an accession of rice germplasm. GSOR 310389 from Korea contained the most putative resistant alleles, eight out of ten, and had a ShB rating of 0.351 which was significantly more resistant than the resistant check Jasmine 85 which contained three putative resistant alleles and had a ShB rating of 0.472. Among seven entries containing six putative resistant alleles with a mean of 0.386 ShB, GSOR 310475 and 311475 were more resistant than Jasmine 85 and had ShB ratings of 0.324 and 0.336, respectively. Among 28 entries having five putative resistant alleles with a mean ShB rating of 0.444, seven were significantly more resistant than Jasmine 85. Seven, out of 35 entries which carried four putative resistant alleles and had a mean ShB of 0.466, were identified to be significantly more resistant than Jasmine 85. The mean ShB ratings for entries containing three, two, one and zero putative resistant alleles were 0.483, 0.535, 0.582 and 0.598, respectively. There was a strong and negative correlation between the ShB severity rating and number of putative resistant alleles (r = -0.535, p<0.0001).

Our mapping results showed that most entries containing a large number of putative resistant alleles were IND (Fig. 9). All entries with six or more putative resistant alleles were IND with only one exception of AUS. Among 28 entries with five putative resistant alleles, 25 were IND and the remaining three were AUS. There were 35 entries with four putative resistant alleles,

http://dx.doi.org/10.5772/56830

nine were AUS, one was admix of TRJ, AUS and IND, and the remaining 25 were IND. Among 35 entries with three putative resistant alleles, 18 were IND, eight AUS, seven TRJ and two admixes of IND. However, among 51 entries without a single putative resistant allele, 26 were TEJ, 18 TRJ, four ARO and two admixes of TRJ-TEJ-ARO, and one IND. Among 72 entries that carried four or more putative resistant alleles, 58 (81%) were IND and 13 AUS (18%) plus admix of TRJ-AUS-IND.

6.5. Putative resistant alleles and ancestry background for Sheath Blight resistance

Jia et al. (2011) reported 52 entries that are significantly more resistant to ShB than Jasmine 85. The resistant entries were identified from 1,794 entries of the USDA rice core collection that has 35% *indica*, 27% *temperate japonica*, 24% *tropical japonica*, 10% *aus* and 4% *aromatic* genotypes (Agrama et al. 2010). Based on the ancestry classification, there are 621 *indica* entries in the core and 45 of them are included in the resistant list, making a resistance frequency of 7.2% for *indica* germplasm. Accordingly, the resistance frequency is 2.8% for *aromatic*, 1.7% for *aus*, and 0.2% each for *temperate japonica* and *tropical japonica*. In a study conducted by Zuo et al. (2007), *japonica* cultivars showed higher sheath blight severity than *indica* cultivars. They describe a general observation that *japonica* rice is more susceptible than *indica* rice. Furthermore, Jasmine 85, Tetep and Teqing, used as parents in many studies on mapping ShB resistance, are all *indica*.

Our study demonstrated that: 1) a majority of the ShB putative resistant alleles existed in *indica* germplasm, 2) most of the resistant entries with a large number of putative resistant alleles were *indica*, conversely 3) only a very small portion of putative resistant alleles existed in *japonica,* and 4) the most susceptible entries with very few or no putative resistant alleles were *japonica* (Fig. 8). Entry GSOR 310389 is an example which had eight out of ten putative resistant alleles, showed a high level of resistance to ShB, and is *indica*. The results from association mapping match well with the phenotypic observation that most resistant genotypes are *indica* and resistant germplasm is rare in *japonica*.

7. Association mapping of silica concentration in rice hulls

Rice (*Oryza sativa* L.) accumulates silicon (Si) in various tissues including hulls. Although Si is not an essential nutrient, it plays an important role in the growth and health of rice plants. Silicic acid is actively taken up by rice roots, which is then translocated in the form of mono-silicic acid (silica gel) through the xylem (Mitani et al. 2005; Ma and Yamaji 2006) to the leaves, stems, hulls and grains of the plant where it converted to silica (SiO_2) (Ma et al. 2007). Often, rice hulls are burned in the mills to produce steam or electricity. However, disposal of the rice hull ash is difficult due to the high silica content (70-95%) (Marshall 2004). Unused hulls and ash are taken to a landfill where they remain for years due to their chemical stability. Another approach for reducing the amount of hulls and ash going to the landfill is to use the silica for value-added products. Rice hulls have been used to produce particle board, poultry bedding, brick making, package cushioning, and absorbents. Due to the high silicon content, rice hulls and ash are good raw materials in the production of silicon-based industrial materials with

high economic value, including silicon carbide, silica, silicon nitride, silicon tetrachloride, pure silicon, and zeolite (Sun and Gong 2001). Since the Si in rice hulls is amorphous, it can be extracted at lower temperatures than Si derived from other conventional sources, thus reducing the cost of Si production (Kalapathy et al. 2002). Understanding the genetic control of Si content in rice will facilitate the development of new varieties with either high or low Si content. Varieties with high silica content in their hulls would be useful for raw material for silica based industrial compounds, while varities with low silica hulls would be more biodegradable and better suited for energy producing purposes (i.e. cleaner energy production at the mills and possible use in bioenergy production).

7.1. Chemical analysis of silica concentration in rice hulls

The rough rice samples from test plots were dehulled with a Satake Rice Machine (Satake Engineering Co., LTD, Ueno Taito-Ku, Tokyo). After drying at 80°C for 2 hr, the hulls (~3g) were stored in 50 ml polypropylene tubes (Cat. # 05-539-5, Fisher Scientific, Houston, TX) at room temp. (22°C) until analyzed. Silica was determined using the molybdenum yellow method described by Saito et al. (2005) and Bryant et al. (2011).

7.2. Variation of silica concentration in the USDA rice mini-core collection

Si content averaged 200 mg g^{-1} and ranged from 118 mg g^{-1} for ACNO 430909, an Admixture of *aus* (AUS), *indica* (IND) and *wild rice* (WD) from the Punjab region of Pakistan, to 249 mg g^{-1} for ACNO 353722, an AUS accession from Assam, India. The non-Admix accession with the lowest Si was ACNO 439683, a TEJ from Eastern Europe, having a Si of 147 mg g^{-1}. Wide variation of Si was seen in all genetic groups. Mean Si of the TRJ (219 mg g^{-1}) and AUS (208 mg g^{-1}) was greater while other groups were less than the overall mean. All the accessions native to Central America region (n = 9), except a TRJ ACNO 2169 from Guatemala, were above the Mini-Core mean value, whereas the Si contents of accessions native to the Mideast (n = 5), Eastern Europe (n = 8), Central Asia (n = 9) and North America (n = 3) were below the Mini-Core mean with a few exceptions. The variation due to genetics (accessions) accounted for 32.4% of the total Si variation in the Mini-Core. The silica content of samples grown in Beaumont, TX (186±1.3 mg g^{-1}) was lower than those grown in Stuttgart, AR (211±1.2 mg g^{-1}), with Location accounting for 19.5% of the silica content variation.

7.3. Marker loci associated with silica concentration

We identified four associated markers in AR, and they were different from the four identified in TX (Table 6). Three out of four AR markers were among seven associated markers mapped in the combined location, whereas none of the TX markers were in. The 19.5% of the total silica content variation due to the difference of AR from TX might be responsible for the mapping results. It is known that the amount of silica present in the soil, the presence of other elements and/or nutrients, the amount of light, and temperature are all factors that affect silica concentrations in the plant (Ma and Takahashi 2002; Ma et al. 2002). RM263 from AR, RM6544 from both AR and Combined location and RM5371 from TX are all within a 1.5 Mb region where additive by additive QTL effects were previously identified by Dai et al. (2005). In summary,

http://dx.doi.org/10.5772/56830

five of the marker-trait associations found in this study are within 1.5 Mb of the reported QTLs for silica concentrations from linkage mapping studies, and one marker-trait association (RM5371 on chromosome 6 at 25.83 Mb) overlaps with a QTL involved in grain arsenic concentration as well as silica concentration (Dai et al. 2005). The present study demonstrates that association mapping of the diverse germplasm in the USDA rice Mini-Core collection is an effective method for identifying new genetic markers and validating previously reported marker regions associated with silica concentration.

Location	Locus	Chr.	Position (Mb)	*P Value*	-$\log_{10}$P	Major allele (bp)	Effect	# of Observed Genotypes
Arkansas	RM23869	9	6.3	2.30E-04	3.64	182	-14.6	37
	RM6544[ab]	11	3.9	3.10E-03	2.51	165	20.2	11
						170	-17.9	47
	RM5953	4	9.4	3.30E-03	2.48	111	15.3	35
	RM263[b]	2	25.9	5.20E-03	2.28	176	-22.8	6
						158	22.0	30
Texas	RM1335[a]	7	28.3	3.70E-04	3.44	173	22.8	7
						163	-22.3	10
						165	21.1	6
						171	-19.1	13
	RM5371[bc]	6	25.8	6.30E-04	3.2	170	-18.6	7
	RM1186	7	9	1.40E-03	2.85	120	-17.5	18
	RM178[a]	5	25.1	4.40E-03	2.36	114	-23.6	72
Combined	RM5953	4	9.4	8.60E-04	3.07	111	14.6	35
	RM23869	9	6.3	2.30E-03	2.64	182	-9.9	37
	RM283[a]	1	4.9	5.10E-03	2.29	150	-19.7	5
	RM6544[ab]	11	3.9	6.80E-03	2.17	165	14.9	11
	RM489	3	4.3	7.90E-03	2.1	269	15.2	25
	RM171	10	19.1	8.40E-03	2.08	328	-18.8	42
	RM484	10	21.1	8.80E-03	2.06	290	-22.4	4

[a] Markers that occur within 1.5 Mb or less of previously identified silica QTL's.

[b] Markers that occur within 1.5 Mb or less of previously identified additive-by-additive QTL regions.

[c] Marker that occur within 1.5 Mb or less of previously identified arsenic QTL's.

Table 6. Marker loci associated with hull silica content at less than 0.01 probability mapped among 174 mini-core accessions genotyped with 164 SSR markers and phenotyped at Stuttgart, Arkansas (AR) and Beaumont, Texas (TX) (Bryant et al. 2011)

Acknowledgements

The authors thank Tiffany Sookaserm, Tony Beaty, Yao Zhou, Biaolin Hu, LaDuska Simpson, Curtis Kerns, Sarah Hendrix, Bill Luebke, Jodie Cammack, Kip Landry, Carl Henry, Jason Bonnette, and Piper Roberts for technical assistance; Xiaobai Li, Limeng Jia, Chengsong Zhu, Robert Fjellstrom and Anna McClung for professional assistance.

Author details

Wengui Yan[1], Aaron Jackson[1], Melissa Jia[1], Wei Zhou[1,2], Haizheng Xiong[1,3] and Rolfe Bryant[1]

1 United States Department of Agriculture, Agricultural Research Service, Dale Bumpers National Rice Research Center, Stuttgart, Arkansas, USA

2 University of Arkansas at Pine Bluff, Pine Bluff, Arkansas, USA

3 Zhejiang University, State Key Lab of Rice Biology, Institute of Nuclear-Agricultural Sciences, Hangzhou, Zhejiang, China

References

[1] Agrama HA, Eizenga GC (2008) Molecular diversity and genome-wide linkage disequilibrium patterns in a worldwide collection of *Oryza sativa* and its wild relatives. Euphytica 160:339–355

[2] Agrama HA, Yan WG, Lee F, Fjellatrom R, Chen MH, et al. (2009) Genetic assessment of a mini-core subset developed from the USDA rice genebank. Crop Sci. 49:1336-1346

[3] Akash WM, Kang SM (2009) Molecular Clustering and Interrelationships among Agronomic Traits of Jordanian Barley Cultivars. J. Crop Impro. 24: 28 – 40

[4] Ali ML, Rajewski JF, Baenziger PS, Gill KS, Eskridge KM (2008) Assessment of genetic diversity and relationship among a collection of US sweet sorghum germplasm by SSR markers. Mol Breed 21:497-509

[5] Ando T, Yamamoto T, Shimizu T, Ma XF, Shomura A, et al. (2008) Genetic dissection and pyramiding of quantitative traits for panicle architecture by using chromosomal segment substitution lines in rice. Theor Appl Genet 116: 881-890

[6] Ashikari M, Sakakibara H, Lin S, Yamamoto T, Takashi T, et al. (2005) Cytokinin oxidase regulates rice grain production. Science 309:741-745

[7] Ashikari M, Wu J, Yano M, Sasaki T, Yoshimura A (1999) Rice gibberellin-insensitive dwarf mutant gene Dwarf 1 encodes the alpha-subunit of GTP-binding protein. Proc Natl Acad Sci USA 96: 10284-10289

[8] Bernardo R (2008) Molecular markers and selection for complex traits in plants: Learning from the last 20 years. Crop Sci 48:1649-1664

[9] Borba TCO, Brondani RPV, Breseghello F, Coelho ASG, Mendonça JA, et al. (2010) Association mapping for yield and grain quality traits in rice (*Oryza sativa* L.). Genet Mol Biol 33:515-524

[10] Borba TCO, Brondani RPV, Rangel PHN, Brondani C (2009) Microsatellite marker–mediated analysis of the EMBRAPA Rice Core Collection genetic diversity. Genetica 137:293–304

[11] Borba TCO, Brondani RPV, Rangel PHN, Brondani C (2005) Evaluation of the number and information content of fluorescent-labeled SSR for rice germplasm characterization. Crop Breed Appl Biotechnol 2: 157-165

[12] Bradbury PJ, Zhang Z, Kroon DE, Casstevens TM, Ramdoss Y, et al. (2007) TASSEL: software for association mapping of complex traits in diverse samples. Bioinformatics 23: 2633-2635

[13] Breseghello F, Sorrells ME (2006) Association analysis as a strategy for improvement of quantitative traits in plants. Crop Sci 46: 1323-1330

[14] Brondani C, Borba TCO, Rangel PHN, Brondani RPV (2006) Determination of traditional varieties of Brazilian rice using microsatellite markers. Genet Mol Biol 29: 676-684

[15] Brondani C, Rangel N, Brondani V, Ferreira E (2002) QTL mapping and introgression of yield-related traits from *Oryza glumaepatula* to cultivated rice (*Oryza sativa*) using microsatellite markers. Theor Appl Genet 104:1192-1203

[16] Brooks SA, Yan W, Jackson AK, Deren CW (2008) A natural mutation in *rc* reverts white-rice-pericarp to red and results in a new, dominant, wild-type allele:*Rc-g*. Theor Appl Genet 117:575-580

[17] Bryant R, Proctor A, Hawkridge M, Jackson A, Yeater K, et al. (2011) Genetic variation and association mapping of silica concentration in rice hulls using a germplasm collection. Genetica 139:1383-1398

[18] Can ND, Yoshida T (1999) Genotypic and phenotypic variances and covariances in early maturing grain sorghum in a double cropping. Pl Prod Sci 2: 67-70

[19] Che KP, Zhan QC, Xing QH, Wang ZP, Jin DM, et al. (2003) Tagging and mapping of rice sheath blight resistant gene. Theor Appl Genet 106: 293-297

[20] Chen Y, Lubberstedt T (2010) Molecular basis of trait correlations. Trends in Plant Sci 15:454-461

[21] Cho YG, Ishii T, Temnykh S, Chen X, Lipovich L, et al. (2000) Diversity of microsatellites derived from genomic libraries and genbank sequences in rice (*Oryza sativa* L.). Theor Appl Genet 100:713–722

[22] Cooper M, Podlich DW, Smith OS (2005) Gene-to-phenotype models and complex trait genetics. Aust J Agric Res 56:895-918

[23] Dai WM, Zhang KQ, Duan BW, Zheng KE, Zhuang JY, et al. (2005) Genetic dissection of different organs in rice. Crop Sci 45:1345-1352

[24] Devlin B, Roeder K (1999) Genomic control for association studies. Biometrics 55: 997-1004

[25] Dhanraj A, Jagadish CA (1987) Studies on character association in the F2 generation of ten selected crosses in rice (*Oryza sativa* L.). Journal of Research-A FAU 15:64-65

[26] Doi K, Izawa T, Fuse T, Yamanouchi U, Kubo T, et al. (2004) *Ehd1*, a B-type response regulator in rice, confers short-day promotion of flowering and controls *FT-like* gene expression independently of *Hd1*. Genes Dev 18:926-936

[27] Eizenga GC, Lee FN, Rutger JN (2002) Screening *Oryza* species plants for rice sheath blight resistance. Plant Dis 86: 808-812

[28] Elias M, Penet L, Vindry P, McKey D, Panaud O, et al. (2001a) Unmanaged sexual reporoduction and the dynamics of genetic diversity of a vegetatively propagated crop plant, cassava (*Manihot esculenta* Crantz), in a traditional farming system. Mol Eco 10:1895-1907

[29] Elias M, McKey D, Panaud O, Anstett MC, Robert T (2001b) Traditional management of cassava morphological and genetic diversity by the Makushi Amerindians (Guyana, South America): perspectives for on-farm conservation of crop genetic resources. Euphytica 120:143-157

[30] Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol Ecol 14: 2611-2620

[31] Falush D, Stephens M, Prithchard JK (2003) Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. Genetics 164: 1567-1587

[32] Fan C, Xing Y, Mao H, Lu T, Han B, Xu C, Li X, Zhang Q (2006) *GS3*, a major QTL for grain length and weight and minor QTL for grain width and thickness in rice, encodes a putative transmembrane protein. Theor Appl Genet 112: 1164-1171

[33] Fu Q, Zhang P, Tan L, Zhu Z, Ma D, et al. (2010) Analysis of QTLs for yield-related traits in Yuanjiang common wild rice (*Oryza rufipogon Griff.*). J Genet Genomics 37: 147-157

[34] Garris AJ, Tai TH, Coburn J, Kresovich S, McCouch SR (2005) Genetic structure and diversity in *Oryza sativa* L. Genetics 169:1631–1638

[35] Gaudeul M, Taberlet P, Till-Bottraud I (2000) Genetic diversity in an endangered alpine plant, Eryngium alpinum L. (Apiaceae), inferred from amplified fragment length polymorphism markers. Molecular Ecol 9: 1625–1637

[36] Gepts P (2004) Crop domestication as a long-term selection experiment. Plant breeding reviews 24: 1-44

[37] Gizaw S, Van Arendonk JAM, Komen H, Windig JJ, Hanotte O (2007) Population structure, genetic variation and morphological diversity in indigenous sheep of Ethiopia. Animal Genetics 38: 621-628

[38] Glaszmann JC (1987) Isozymes and classification of Asian rice varieties. Theor Appl Genet 74: 21–30

[39] Gravois KA, McNew RW (1993) Genetic relationships among and selection for rice yield and yield components. Crop Sci 33:249-252

[40] Hao Z, Li X, Liu X, Xie C, Li M, et al. (2010) Meta-analysis of constitutive and adaptive QTL for drought tolerance in maize. Euphytica 174: 165–177

[41] Hay RKM (1995) Harvest index: a review of its use in plant breeding and crop physiology. Annu Appl Biol 126: 197-216

[42] Hemamalini GS, Shashidhar HE, Hittalmani S (2000) Molecular marker assisted tagging of morphological and physiological traits under two contrasting moisture regimes at peak vegetative stage in rice (*Oryza sativa* L.). Euphytica 112: 69-78

[43] Hittalmani S, Huang N, Courtois B, Venuprasad R, Shashidhar HE, et al. (2003) Identification of QTL for growth- and grain yield-related traits in rice across nine locations of Asia. Theor Appl Genet 107: 679-690

[44] Holland J (2007) Genetic architecture of complex traits in plants. Curr Opin Plant Biol 10:156-161

[45] Huang X, Wei X, Sang T, Zhao Q, Feng Q, et al. (2010) Genome-wide association studies of 14 agronomic traits in rice landraces. Nat Genetics 42: 961-969

[46] Huang X, Qian Q, Liu Z, Sun H, He S, et al. (2009) Natural variation at the *DEP1* locus enhances grain yield in rice. Nat Genet 41:494-497

[47] Hulbert SH, Bennetzen JL (1991) Recombination at the Rp1 locus of maize. Mol Gen Genet 226:377–382

[48] Inostroza L, Pozo AD, Matus I, Castillo D, Hayes P, et al. (2009) Association mapping of plant height, yield, and yield stability in recombinant chromosome substitution lines (RCSLs) using *Hordeum vulgare* subsp. spontaneum as a source of donor alleles in a *Hordeum vulgare* subsp. Vulgare background. Mol Breed 23:365-376

[49] Itoh H, Tatsumi T, Sakamoto T, Otomo K, Toyomasu T, et al. (2004) A rice semi-dwarf gene, *Tan-Ginbozu (D35)*, encodes the gibberellin biosynthesis enzyme, *ent*-kaurene oxidase. Plant Mol Biol 54:533-547

[50] Jain S, Jain RK, McCouch SR (2004) Genetic analysis of Indian aromatic and quality rice (*Oryza sativa* L.) germplasm using panels of fluorescently–labeled microsatellite markers. Theor Appl Genet 109:965–977

[51] Jia LM, Yan WG, Zhu C, Agrama HA, Jackson A, et al. (2012) Allelic analysis of sheath blight resistance with association mapping in rice. PLoS ONE 7(3): e32703. doi:10.1371

[52] Jia L, Yan W, Agrama HA, Yeater K, Li X, et al. (2011) Searching for germplasm resistant to sheath blight from the USDA rice core collection. Crop Sci 51: 1507-1517

[53] Jia Y, Correa-Victoria F, McClung A, Zhu L, Liu G, et al. (2007) Rapid determination of rice cultivar responses to the sheath blight pathogen *Rhizoctonia solani* using a micro-chamber screening method. Plant Dis 91: 485-489

[54] Jiang GH, Xu CG, Li XH, He YQ (2004) Characterization of the genetic basis for yield and its component traits of rice revealed by doubled haploid population. Acta genetica Sinicavol 31:63-72

[55] Kalapathy U, Proctor A, Shultz J (2002) An improved method for production of silica from rice hull ash. Bioresource Technol 85:285-289

[56] Kang HM, Zaitlen NA, Wade CM, Kirby A, Heckerman D, et al. (2008) Efficient control of population structure in model organism association mapping. Genetics 178: 1709-1723

[57] Kim KW, Chung HK, Cho GT, Ma KH, Chandrabalan D, et al. (2007) PowerCore: A program applying the advanced M strategy with a heuristic search for establishing core sets. Bioinformatics 23:2155–2162.

[58] Kim S, Zhao K, Jiang R, Molitor J, Borevitz JO, et al. (2006) Association mapping with single-feature polymorphisms. Genetics 173: 1125-1133

[59] Kouame CN, Quesenberry KH (1993) Cluster analysis of a world collection of red clover germplasm. Genet Res Crop Evo 40:39-47

[60] Kunihiro Y, Qian Q, Sato H, Teng S, Zeng D, et al. (2002) QTL analysis of sheath blight resistance in rice (*Oryza sativa* L.). Chin J Genet 29: 50-55

[61] Lanceras JC, Pantuwan GP, Jongdee B, Toojinda T (2004) Quantitative trait loci associated with drought tolerance at reproductive stage in rice. Plant Physiol 135: 384–399

[62] Li, X., Yan W, Agrama H, Jia L, Jackson AK, et al. 2012. Unraveling the complex trait of harvest index with association mapping in rice (Oryza sativa L.). PLoS One. 7(1): e29350. doi:10.1371/journal.pone.0029350

[63] Li X, Yan W, Agrama H, Jia L, Shen X, et al. (2011) Mapping QTLs for improving grain yield using the USDA rice mini-core collection. Planta 234: 347-361

[64] Li X, Yan W, Agrama H, Hu B, Jia L, et al. (2010) Genotypic and phenotypic characterization of genetic differentiation and diversity in the USDA rice mini-core collection. Genetica 138: 1221-1230

[65] Li C, Zhou A, Sang T (2006) Genetic analysis of rice domestication syndrome with the wild annual species, *Oryza nivara*. New Phytologist 170:185-194

[66] Li X, Qian Q, Fu Z, Wang Y, Xiong G, et al. (2003) Control of tillering in rice. Nature 422:618-621

[67] Li DJ, Sun CQ, Fu YC, Chen L, Zhu ZF, et al. (2002) Identification and mapping of genes for improving yield from Chinese common wild rice (*O. rufipogon* Griff.) using advanced backcross QTL analysis. Chin Sci Bull 18:1533-1537

[68] Li Z, Pinson SRM, Marchetti MA, Stansel JW, Park WD (1995) Characterization of quantitative trait loci (QTLs) in cultivated rice contributing to field resistance to sheath blight (*Rhizoctonia solani*). Theor Appl Genet 91: 382-388

[69] Liu G, Jia Y, Correa-Victoria FJ, Prado GA, Yeater KM, et al. (2009) Mapping quantitative trait loci responsible for resistance to sheath blight in rice. Phytopathology 99: 1078-1084

[70] Liu K, Muse SV (2005) Powermarker: an integrated analysis environment for genetic marker analysis. Bioinformatics 21: 2128-2129

[71] Lewis PO, Zaykin D (2001) Genetic data analysis: computer program for the analysis of allelic data version 1.0. http://lewis.eeb.uconn.edu/lewishome/software.html. Cited 21 Nov 2001

[72] Loan LC, Du PV, Li Z (2004) Molecular dissection of quantitative resistance of sheath blight in rice (*Oryza sativa* L.). Omonrice 12: 1-12

[73] Loiselle BA, Sork VL, Nason J, Graham C (1995) Spatial genetic structure of a tropical understory shrub, *Psychotria officinalis* (Rubiaceae). Am J Bot 82: 1420-1425

[74] Ma JF, Yamaji N (2006) Silicon uptake and accumulation in higher plants. Trends Plant Sci 11: 392-397

[75] Ma JF, Yamaji N, Tamai K, Mitani N (2007) Genotypic difference in silicon uptake and expression of silicon transporter genes in rice. Plant Physiology 145:919-924

[76] Ma JF, Takahashi E (2002) Soil, fertilizer, and plant silicon research in Japan. Elsevier Science

[77] Ma JF, Tamai K, Ichii M, Wu GF (2002) A rice mutant defective in Si uptake. Plant Physiol 130:2111–2117

[78] Mackill DJ (1995) Plant genetic resources: Classifying japonica rice cultivars with RAPD markers. Crop Sci 35: 889-894

[79] Manosalva PM, Davidson RM, Liu B, Zhu X, Hulbert SH, et al. (2009) A germin-like protein gene family functions as a complex quantitative trait locus conferring broad-spectrum disease resistance in rice. Plant Physiol 149: 286-296

[80] Mantel N (1967) The detection of disease clustering and a generalized regression approach. Cancer Res., 27:209–220

[81] Mao B-B, Cai W-j, Zhang Z-h, Hu Z-L, Li P, et al. (2003) Characterization of QTLs for Harvest Index and Source-sink Characters in a DH Population of Rice (*Oryza sativa* L.). Acta Genetica Sinica 30: 1118-1126

[82] Marri PR, Sarla N, Reddy LV, Siddiq EA (2005) Identification and mapping of yield and yield related QTLs from an Indian accession of *Oryza rufipogon*. BMC Genet 13: 33-39

[83] Marshall WE (2004) Utilization of rice hull and rice straw as adsorbents. In: Champagne ET (ed), Rice Chemistry and Technology. American Association of Cereal Chemists, Inc., St. Paul, MN, pp. 611-630

[84] Mazzucato A, Papa R, Bitocchi E, Mosconi P, Nanni L, et al. (2008) Genetic diversity, structure and marker-trait associations in a collection of Italian tomato (Solanum lycopersicum L.) landraces. Theor Appl Genet 116:657-669

[85] Mew TW, Leung H, Savary S, Cruz CMV, Leach JE (2004) Looking ahead in rice disease research and management. Cr Rev Plant Sci 23: 103-127

[86] Mitani N, Ma JF, Iwashita T (2005) Identification of the silicon form in xylem sap of rice (*Oryza sativa* L.). Plant Cell Physiol 46:279-283

[87] Mohammad D, Cox PB, Posler GL, Kirkham MB, Hussain A, et al. (1993) Correlation of characters contributing to grain and forage yields and forage quality in sorghum (*Sorghum bicolor*). Indian J Agric Sci 63: 92–95

[88] Moncada P, Martinez CP, Borrero J, Chatel M, Gauch H, et al. (2001) Quantitative trait loci for yield and yield components in an *Oryza sativa* × *Oryza rufipogon* BC_2F_2 population evaluated in an upland environment. Theor Appl Genet 102:41-52

[89] Nei M, Takezaki N (1983) Estimation of genetic distances and phylogenetic trees from DNA analysis. Proc 5th World Cong Genet Appl Livstock Prod 21: 405–412

[90] Peng J, Richards DE, Hartley NM, Murphy GP, Devos KM, et al. (1999) 'Green revolution' genes encode mutant gibberellin response modulators. Nature 400: 256-261

[91] Pinson SRM, Capdevielle FM, Oard JH (2005) Confirming QTLs and finding additional loci conditioning sheath blight resistance in rice using recombinant inbred lines. Crop Sci 45: 503-510

[92] Pinto RS, Reynolds MP, Mathews KL, McIntyre CL, Olivares-Villegas JJ, et al. (2010) Heat and drought adaptive QTL in a wheat population to minimize confounding agronomic effects. Theor Appl Genet 121: 1001-1021

[93] Prasad B, Eizenga GC (2008) Rice sheath blight disease resistance identified in *Oryza* spp. accessions. Plant Dis 92: 1503-1509

[94] Prichard JK, Stephens M, Rosenberg NA, Donnelly P (2000) Association mapping in structured populations. AM J Hum Genet 67: 170-181

[95] Raes D, Steduto P, Hsiao TC, Fereres E (2009) AquaCrop-The FAO Crop Model to Simulate Yield Response to Water: II. Main Algorithms and Software Description. Agron J 101: 438-447

[96] Rohlf F (2000) FNTSYS-PC numerical taxonomy and multivariate analysis system ver 2.11L. Applied Biostatistics, NY

[97] Saito K, Yamamoto A, Sa T, Saigusa M (2005) Rapid, micro-methods to estimate plant silicon content by dilute hydrofluoric acid extraction and spectrometric molybdenum method: I. Silicon in rice plants and molybdenum yellow method. Soil Sci Plant Nutr 51:29-36

[98] Samonte SOP, Wilson LT, McClung AM (1998) Path analyses of yield and yield-related traits of fifteen diverse rice genotypes. Crop Sci 38:1130-1136

[99] Sato H, Ideta O, Ando I, Kunihiro Y, Hirabayashi H, et al. (2004) Mapping QTLs for sheath blight resistance in the rice line WSS2. Breeding Sci 54: 265-271

[100] Savary S, Teng PS, Willocquet L, Nutter FW (2006) Quantification and modeling of crop losses: a review of purposes. Annu Rev Phytopathol 44: 89-112

[101] Schneider S, Excoffier L (1999) Estimation of demographic parameters from the distribution of pairwise differences when the mutation rates vary among sites: application to human mitochondrial DNA. Genetics 152:1079–1089

[102] Sharma A, McClung AM, Pinson SRM, Kepiro JL, Shank AR, et al. (2009) Genetic mapping of sheath blight resistance QTLs within *tropical japonica* rice cultivars. Crop Sci 49: 256-264

[103] Sharma RS, Choubey SD (1985) Correlation studies in upland rice. Indian Jour Agro 30:87-88

[104] Shi J, Li R, Qiu D, Jiang C, Long Y, Morgan C, Bancroft I, Zhao J, Meng J (2009) Unraveling the complex trait of crop yield with quantitative trait loci mapping in *Brassica napus*. Genetics 182:851-861

[105] Sinclair TR (1998) Historical changes in harvest index and crop nitrogen accumulation. Crop Sci 38: 638-643

[106] Soltani A, Rezai AM, Pour MRK (2001) Genetic variability of some physiological and agronomic traits in grain sorghum (*Sorghum bicolor* L.). J Sci Tech Agric Nat Resources 5: 127–137

[107] Song XJ, Huang W, Shi M, Zhu MZ, Lin HXA (2007) QTL for rice grain width and weight encodes a previously unknown RING-type E3 ubiquitin ligase. Nat Genet 39: 623-630

[108] Spielmeyer W, Ellis MH, Peter M (2002) Semidwarf (*sd-1*), "green revolution" rice, contains a defective gibberellin 20-oxidase gene. Proc Natl Acad Sci USA 99:9043-9048

[109] Suh JP, Ahn SN, Cho YC, Kang KH, Choi IS, et al. (2005) Mapping of QTLs for yield traits using an advanced backcross population from a cross between *Oryza sativa* and *O. glaberrima*. Korean J Breed 37:214-220

[110] Sun L, Gong K (2001) Silicon-based materials from rice husks and their applications. Ind Eng Chem Res 40:5861-5877

[111] Sweeny MT, Thomson MJ, Pfeil BE, McCouch S (2006) Caught red-handed: Rc encodes a basic helix-loop-helix protein conditioning red pericarp in rice. Plant Cell 18: 283-294

[112] Takahashi Y, Shomura A, Sasaki T, Yano M (2001) *Hd6*, a rice quantitative trait locus involved in photoperiod sensitivity, encodes the alpha subunit of protein kinase CK2. Proc Natl Acad Sci USA 98:7922-7927

[113] Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol Bio Evol 24: 1596-1599.

[114] Terao T, Nagata K, Morino K, Hirose T (2010) A gene controlling the number of primary rachis branches also controls the vascular bundle formation and hence is responsible to increase the harvest index and grain yield in rice. Theor Appl Genet 120: 875-893

[115] Thomson MJ, Polato NR, Prasetiyono, Trijatmiko KR, Silitonga TS, et al. (2009) Genetic diversity of isolated populations of Indonesian landraces of rice (Oryza sativa L.) collected in east Kalimantan on the island of Borneo. Rice 2:80-92

[116] Thomson MJ, Septiningsih EM, Suwardjo F, Santoso TJ, Silitonga TS, et al. (2007) Genetic diversity analysis of traditional and improved Indonesian rice (*Oryza sativa* L.) germplasm using microsatellite markers. Theor Appl Genet 114:559–568

[117] Thomson MJ, Tai TH, McClung AM, Lai XH, Hinga ME, et al. (2003) Mapping quantitative trait loci for yield, yield components and morphological traits in an advanced backcross population between *Oryza rufipogon* and the *Oryza sativa* cultivar Jefferson. Theor Appl Genet 107:479-493

[118] Tyagi A, Khurana JP, Khurana P, Raghuvanshi S, Gaur A, et al. (2004) Structural and functional analysis of rice genome. J Genet 83: 79-99

[119] Wamishe YA, Jia Y, Singh P, Cartwright RD (2007) Identification of field isolates of *Rhizoctonia solani* to detect quantitative resistance in rice under greenhouse conditions. Front Agric China 4: 361-367

[120] Wang LQ, Liu WJ, Xu Y, He YQ, Luo LJ, et al. (2007) Genetic basis of 17 traits and viscosity parameters characterizing the eating and cooking quality of rice grain. Theor Appl Genet 115: 463-476

[121] Weir BS (1996) Genetic Data Analysis II: Methods for Discrete Population Genetic Data. Sinauer Assoc., Inc., Sunderland, MA, USA

[122] Xin Z, Velten JP, Oliver MJ, Burke JJ (2003) High throughput DNA extraction method suitable for PCR. Biotechniques 34: 820-826.

[123] Xu YB, Beachell H, McCouch SR (2004) A marker–based approach to broadening the genetic base of rice in the USA. Crop Sci 44:1947–1959

[124] Xue W, Xing Y, Weng X, Zhao Y, Tang W, et al. (2008) Natural variation in *Ghd7* is an important regulator of heading date and yield potential in rice. Nat Genet 143: 1-7

[125] Yan WG, Li Y, Agrama HA, Luo D, Gao F, et al. (2009) Association mapping of stigma and spikelet characteristics in rice (*Oryza sativa* L.). Mol Breed 24:277-292

[126] Yan WG, Rutger JN, Bryant RJ, Bockelman HE, Fjellstrom RG, et al. (2007) Development and evaluation of a core subset of the USDA rice (*Oryza sativa* L.) germplasm collection. Crop Sci 47: 869-878

[127] Yan WG, Rutger JN, Bockelman HE, Tai TH (2005a) Agronomic evaluation and seed stock establishment of the USDA rice core collection. In: Norman RJ et al, editor BR Wells Rice Research Studies 2004 Univ of Arkansas, Agri Exp Sta Res Ser 529: 63-68

[128] Yan WG, Rutger JN, Bockelman HE, Tai TH (2005b) Evaluation of kernel characteristics of the USDA rice core collection. In: Norman RJ et al, editor BR Wells Rice Research Studies 2004 Univ of Arkansas, Agri Exp Sta, Res Ser 529: 69-74

[129] Yu JM, Pressoir G, Briggs WH, Bi IV, Yamasaki M, et al. (2006) A unified mixed-model method for association mapping that accounts for multiple levels of relatedness. Nat Genet 38: 203-208

[130] Zeng Y, Shen S, Li Z, Yang Z, Wang X, et al. (2003) Ecogeographic and genetic diversity based on morphological characters on indigenous rice (Oryza sativa L.) in Yunnan, China. Genet Res Crop Evo 50:567-577

[131] Zhang YM, Mao Y, Xie C, Smith H, Luo L, et al. (2005) Mapping quantitative trait loci using naturally occurring genetic variance among commercial inbred lines of maize (*Zea mays* L.). Genetics 169: 2267-2275

[132] Zhao K, Tung C, Eizenga GC, Wright MH, Ali M, et al. (2011) Genome-wide association mapping reveals rich genetic architecture of complex traits in Oryza sativa. Nature Communications. Available: DOI: 10.1038/ncomms1467.

[133] Zhao K, Aranzana MJ, Kim S, Lister C, Shindo C, et al. (2007) An Arabidopsis example of association mapping in structured samples. PLoS Genet 19: 3(1):e4

[134] Zuo S, Yin Y, Zhang L, Zhang Y, Chen Z, et al. (2007) Breeding value and further mapping of a QTL qSB-11 conferring the rice sheath blight utilized resistance. Chin J Rice Sci 21: 136-142

Chapter 5

Identification and Utilization of Elite Genes from Elite Germplasms for Yield Improvement

Dawei Xue, Qian Qian and Sheng Teng

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/56390

1. Introduction

Rice is a major food crop in the world. Half of the world's population relies on rice as their staple food. Due to continuous growth of human population, the area of arable land decreases every year. Therefore, ensuring adequate grain production has become a challenge for many countries. Rice production has an important role in global food security, poverty alleviation and rural employment. The current rate of increase in mean rice yield per annum is only 0.8%, which falls behind the rate of population growth annually. An annual mean increase in rice production of 1.2% is required between 2001 and 2030 in order to catch up with the growing food demand resulting from increase in population[1,2].

Constrained by lack of water resources and arable land, the area for rice cultivation has decreased in the context of economic development and urbanization. It is evident that rice yield cannot be increased by simply expanding the cultivation area. In light of this, it is important to pay attention to increasing rice yield per unit area. There are two major approaches for achieving this goal: improving cultivation conditions and technology, and breeding rice varieties with higher yield potential. In order to improve the cultivation technique, selecting superior cultivars is essential. Practices in rice science and production have shown that high-yield breeding of rice is essential for yield increase, and a breakthrough is usually made through discovery and effective utilization of specific germplasm (gene). The first leap in rice yield per unit area came from breeding semidwarf rice varieties and their popularization. In the 1940s, with rapid development of the chemical industry, chemical fertilizers were applied extensively in rice production. Tall rice varieties showed very low potential for yield increase due to their low tolerance for fertilizers and easily lodging. Chinese rice breeders first proposed the strategy of dwarfing breeding. In the late 1950s, successful breeding of Taichung Native 1 (TN1), Aijiaonante and Guangchangai rice varieties, which were

high-yielding dwarf rice varieties, marked a new epoch in dwarf rice breeding[80]. Later, the International Rice Research Institute cross-bred the Dijiaowujian rice as a dwarf-gene source with tall Pitai rice. The dwarf hybrid IR8, known as miracle rice, was developed in 1964. Compared with tall rice varieties, dwarf rice varieties have several advantages, including fertilizer tolerance, anti-lodging, erect leaves, more panicles and high harvest index. The yield per unit area was increased over 30% by the dwarf rice varieties. The successful breeding of dwarf rice varieties was just the beginning of the global "green revolution" [3]. Academician Yuan Longping discovered wild-abortive cytoplasmic male sterile line, and was the first to realize three-line combination in 1973 with the establishment of hybrid rice seed production system. The success of three line combination directly resulted in yield per mu exceeding 500 kg in many areas of China. The yield increase was over 15%-20% compared with conventional varieties. The discovery of photoperiod-thermo sensitive genic sterile gene facilitates the transition from three-line system to two-line system, which is especially useful for developing hybrids. Their effective utilization enabled the second leap in rice yield, which is also known as the second "Green Revolution" in rice production history.

The global rice yield of current varieties seems to be at a standstill. Reduction of arable land and global warming are also threatening rice production. For this reason, increasing yield per unit area is very important for boosting total yield. Rice yield per unit area heavily depends on the yield potential of the rice variety. In order to achieve the third leap in yield per unit area, many countries have successively put forward the plan of super rice breeding by adopting the technical route of combining ideal plant type with indica-japonica heterosis utilization [4].

In 1918, Japanese rice breeders had suggested super high-yield breeding of rice via indica-japonica cross. The International Rice Research Institute launched a new plant type breeding program based on Javanica rice in 1989. They proppsed morphological indices for higher yield rate were specified as follows: low tillering (3-4 tillers), no unproductive tiller, large panicle, sturdy stem, dark green, thick and erect leaves, vigorous root systems and high harvest index [5]. In 1996, China started the super rice research program which consisted of two parts: conventional super rice employing the technical route of indica-japonica cross, and super hybrid rice breeding with the combination of plant type improvement, intersubspecific heterosis utilization and distant favorable genes. Chinese rice breeders proposed different ideal plant types based on long-term production practice. These ideal plant types included an erect large spike type in north China, an early-growth deep-root type in south China, a sparse-seeding heavy-panicle type in the upper reach of Yangtze River, a functional-leaf erect type and later-stage functional type in middle and lower reaches of Yangtze River [4]. All ideal plant types of rice share some common features: low tiller number, no ineffective tiller, robust stalk, anti-lodging, large panicle, large grain number per spike, high yield per spike, high biological production, high harvest index and vigorous root systems.

Exploitation of F1 hybrid heterosis for the purpose of reaping economic benefits is referred to as hybrid heterosis exploitation. Research on the mechanism of hybrid heterosis is of great significance for the exploitation of heterosis of hybrids in crop genetic breeding. Pei'ai 64S is a low thermo-sensitive dual-purpose genic male sterile line developed by China National

http://dx.doi.org/10.5772/56390

Hybrid Rice Engineering Research Center, with Nongken 58S as the female parent and Indica Pei'ai 64 as the male parent. Pei'ai 64S was obtained after crossing and backcrossing, as well as multi-generation selection. Because of its stable sterility, seed production security, combination freedom and strong hybrid heterosis, it has been extensively applied in breeding. Pei'ai 64S is China's first indica male sterile line with practical utilization value. Medium maturity indica rice variety 9311 (Yangdao 6) was bred by the Research Institute of Agricultural Sciences in the Lixia River region of Jiangsu Province. It has been extensively applied in rice production for its high quality, high yield, multiple resistance and strong combing ability. Yangdao 6 has served as the male parent for key hybrid varieties including Liangyoupeijiu, Fengliangyou 1, Yangliangyou 6 and Yueyou 938, and it is also the first indica variety used in the sequencing of rice genome framework under the name of 9311.

Effective tiller, grain number per spike and 1000 grain weight are the three major elements used for determining rice yield. These three metrics are also the indicators of hybrid heterosis. Spike number is closely related to rice tillering. Grain number per spike is associated with spike length and grain density. Grain weight depends on grain length, width and thickness. These yield components are considered in ideal plant type. Plant height, tiller, panicle type and grain weight are determined by the interaction between growth, hormone and environment. Generally, these yield-related traits are controlled by quantitative trait loci (QTL) or major genes. At present, some QTL/genes related to yield and heterosis have been cloned and the regulatory mechanism of ideal plant type and hybrid heterosis at the molecular level is being revealed. Knowledge of these mechanisms is especially important in the context of rice breeding.

2. Plant type

The ideal plant type was first proposed in 1968. It is defined as the combination of several traits favorable for photosynthesis, growth and grain yield in one plant type. In such ideal plant type, competition between individuals is reduced and solar energy utilization in the population is maximized, leading to minimal consumption and maximum dry matter accumulation [6]. In a narrow sense, plant type of a crop refers to plant morphology and spatial arrangement of the tillering and leaves. It is also related to other plant traits including plant height, tiller number, tiller angle, panicle type, leaf morphology and leaf angle. In a general sense, plant type may also consist of functional traits related to solar energy utilization of the population, including nitrogen content, photosynthetic efficiency, chlorophyll content, stoma density and extinction coefficient [7].

Apart from rice morphology and spatial arrangement, plant type also covers some functional traits directly related to solar energy utilization of the population. Breeders from the International Rice Research Institute announced a new plant type (NPT) in 1989. It had fewer tillers (5-6 per plant) and no ineffective tiller; large spike, with grain number reaching 150-200 per panicle; plant height of 90-100 cm; anti-lodging robust stalk; thick, erect and dark green leaves; and well-developed root systems [8]. Chinese rice breeders also conducted a series of research

on ideal plant type in different ecological conditions for high-yield breeding and cultivation. Several models of ideal plant type were developed. Prof. Yang Shouren of Shenyang Agricultural University proposed the "short-branch and erect-leaf, large and straight panicle" type for japonica rice in north China [9]. In 1997, Prof. Zhou Kaida of Sichuan Agricultural University proposed "intersubspecific large-spike type", which is adapted to conditions of less wind, high moisture and high temperature in the Sichuan Basin [10]. Similarly, Yuan Longping of China National Hybrid Rice Engineering Research Center proposed "high-canopy and low-spike-layer type", which is also referred to as the "gold hidden in the leaf" type, for the ecological area of middle and lower reaches of the Yangtze River [11]. Huang Yaoxiang of the Research Institute of Guangdong Academy of Agricultural Sciences proposed the "semi-dwarf clustered high-growth super high yield type" for indica rice in south China in 2001 [12]. The China National Rice Research Institute [13], by combining various components of super-high-yield plant type, proposed the "later-stage functional super rice type". In-depth analysis showed that the ideal plant types of China and other countries share the following characteristics: reduced tiller number, fewer ineffective tillers, robust stem, anti-lodging, large spike, large grain number per spike, large grain yield per spike, increased biomass yield, high harvest index and highly developed root system.

Breeding ideal plant types is the goal of super high yield rice breeding in the future. Construction of the ideal plant type in rice has to be done with consideration for traits such as tiller, stem, leaf shape and panicle type. Spike number is used for determining rice yield, and is closely related to tillering. Grain number per spike is associated with spike length and grain density. Grain weight is determined by grain length, width and thickness. These yield components are covered by the factors of the ideal plant type of rice. Plant type, tiller, panicle type and grain weight are determined by interactions between growth hormones and the environment. Successful cloning of QTL/genes related to yield has greatly contributed to our understanding of the regulatory mechanism of ideal plant type at the molecular level.

2.1. Plant height

Plant height is an important component of plant type in rice. Since the end of the 1950s when the first "green revolution" in rice yield was triggered by dwarf breeding, rice grain yield per unit area has increased substantially. This achievement can be attributed to the application of dwarf germplasm, especially semi-dwarf germplasm in breeding. Several studies have shown that dwarfism in many dwarf and semi-dwarf rice varieties is controlled by a recessive major gene and is subject to certain modifier genes [14]. For indica rice, application of the semi-dwarf gene sd-1 is a major contributor to rice yield improvement. The major dwarf cultivars of indica rice are Aijiaonante and Aizizhan. The majority of semi-dwarf indica rice varieties that have been bred are either directly or indirectly derived from the above two cultivars [15]. In 2002, three research groups successively published results of map-based cloning of the sd-1 gene, which supports the first green revolution in molecular level. The sd-1 gene controls plant height in rice. Mutation of sd-1 directly leads to different degrees of dwarfism in rice. The sd-1 protein is involved in the biosynthesis of gibberellin, encoding GA20 oxidase (GA20ox) composed of 389 amino acids. GA20ox is a key enzyme in the gibberellin synthetic pathway,

which catalyzes the conversion of GA53 to GA20. The sd-1 gene is located in chromosome 1 in rice, corresponding to 38381423 - 38384165 map position (5'-3') in Nipponbare. Monna et al. [16] showed that Nipponbare, Sasanishiki and Calrose rice, which are ordinary wild-type rice, contain three exons, with sizes of 558, 318 and 291 bp, and 2 introns with sizes of 105 and 1471 bp. Deo-geo-woo-gen type semi-dwarf varieties IR24, Habataki and Milyang 23 have a 383 bp deletion in the middle of exon 1, covering 278 bp in exon 1 and 2, and 105 bp in the intron. Calrose76 is the outcome of CTC-to-TTC mutation at position 265 in exon 2, which changes a Leu (leucine) residue to Phe (phenyl alanine) residue. Sasaki et al. [17] proposed that the GA20ox-1 gene is independent of sd-1, while the newly discovered GA20-ox-2 is linked to sd-1. Sequences of the sd-1 gene in four cultivars and one wild-type variety were compared. It was found that the length of three exons in SD-1 gene of wild-type variety were 557, 321 and 291 bp respectively while the length of two introns were 103 and 1472 bp. A 383 bp deletion was found in the dwarf Deo-geo-woo-gen variety and its derivative, including 103 bp of intron 1. A GGG-to-GTG mutation at position 94 in Jikkoku rice results in change of glycine to valine. A CTC-to-TTC mutation at position 266 in Calorose 76 rice leads to change of leucine to phenyl alanine. A GAC-to-CAC mutation at position 349 in the dwarf variety Remei leads to a change from aspartic acid to histidine. GA20ox-2 is strongly expressed in the leaves, stem and unbloomed flowers of rice. However, GA20ox-1 is only expressed in unbloomed flowers, which is why the sd-1 gene controls plant height but not the yield. Spielmeyer et al. [18] reported that the 3 sd-1 exons in wild-type variety have lengths of 557, 322 and 291 bp. A 280 bp deletion was found in the GA20ox2 coding region (exon 1 and 2) in the DGWG semi-dwarf indica rice variety. Until now, a total of 5 alleles have been discovered for the sd-1 locus including sd-1 in the wild-type variety, sd-1-d from Deo-geo-woo-gen and its derivative, sd-1-r from Reimei, sd-1-c from Calrose 76 and sd-1-j from Jikkoku. Recently, Asano et al. [77] found that *SD-1* has been subjected to artificial selection in rice evolution and that ancient humans took advantage of functional nucleotide polymorphisms (FNPs) from two SNPs in sd-1 in *japonica* rice.

2.2. Tillering

Rice tillering is an important agronomic character in rice production. Effective tiller number per unit area determines the spike number, which is among the three components of rice yield per unit area, the other two being grain number per spike and 1000 grain weight. Therefore, reducing unproductive tiller is important to improve rice yield. MOC1 was the first gene identified to be related to rice tillering. Li et al. [19] employed map-based cloning to narrow down the location of MOC1 to a 20 kb region in the long arm of Chromosome 6. MOC1 is a member of the GRAS transcription factor family. It is closely related to the LAS gene in Arabidopsis thaliana and the 1s gene in tomato. MOC1 is necessary for initiation of axillary meristem. Loss of MOC1 leads to defects in tiller bud initiation and consequently to complete absence of tillering. Over-expression of MOC1 results in massive tillering in transgenic plants. *OsTB1* and *OSH1* are expressed at lower levels in loss-of-function *MOC1* mutants. Therefore, *MOC1* gene may act as a master regulator in the control of rice tillering. In addition to affecting tillering of rice stem, MOC1 also significantly reduces panicle branches. Cloning of *MOC1* has facilitated our understanding of the regulatory mechanism of rice tillering. However, the

molecular mechanisms regulating *MOC1* remains to be elucidated. Recently, two Chinese research groups found that TAD1/TE directly regulates MOC1, thus revealing an important molecular mechanism of regulation of rice tillers [78,79]. *TAD1*/*TE* encodes a co-activator of the anaphase-promoting complex (APC/C), a multi-subunit E3 ligase. TAD1 interacts with MOC1, forms a complex with OsAPC10, and functions as a co-activator of APC/C to target MOC1 for degradation in a cell cycle-dependent manner. These findings uncovered a new mechanism underlying shoot branching and found novel determinants of plant architecture and grain yield.

2.3. Panicle type

Grain number per spike of rice is an important agronomic character of rice spike that is directly related to rice yield. Increasing the grain number per spike is part of the goal of high yield breeding. At present, most high yield varieties have significantly increased grain number per spike. Some genes, including Gn1, DEP1 and DEP2, that have a major role in influencing grain number per spike, have been successfully cloned.

QTL-Gn1 was cloned by a Japanese research group [20]. Map-based cloning and sequencing showed that QTL-Gn1 encodes cytokinin oxidase (OsCKX2). Using a cross between the japonica rice cultivar Koshihikari and the indica rice cultivar Habataki, they located QTL-Gn1, a major gene controlling grain number, on the short arm of Chromosome 1. This gene accounts for 44% of phenotypic variation in grain number per panicle. NIL-Gn1 heterozygous plants (Gn1/gn1) were used to generate 96 F2 plants, to decompose Gn1 into two loci, Gn1a and Gn1b, which are of equal effect. Gn1a is located in a region less than 2 cM between R3192 and C12072S, and Gn1b is located upstream of Gn1a. NIL-Gn1a heterozygous plants (Gn1a/gn1a) were used to generate 13000 F2 plants and then finely map the Gn1a gene to a 6.3 kb region between 3A28 and 3A20. There is only one open reading frame in this region, and it belongs to the OsCKX2 gene, which is highly homologous to cytokinin oxidase/dehydrogenase. Sequencing analysis showed that compared with Koshihikari, there are deletions of 16 bases and 6 bases in the 5' untranslated region and exon 1, respectively, of OsCKX2 in Habataki. A 3-base substitution in exon 4 causes changes in the protein product was also found in Habataki. These results suggested the loss of function or deletion of OsCKX2 may lead to increase in rice yield. Complementation tests showed that OsCKX2 is the same gene as Gn1a. OsCKX2 is strongly expressed in leaf, stem, inflorescence meristem and flower, but weakly in apical meristem of rice plants; it is not expressed in the root and embryo. The Gn1a locus is an allele of Habataki, which has increased grain number per spike. Loss of this gene leads to significantly increased content of cytokinin in spikes and increased number of spikelets, i.e. grain number, and consequently increased rice yield. Ashikari et al. [21] obtained NIL-Gn1-Sd-1 by crossing and screening using Koshihikari as the genetic background and NIL-Gn1 and NIL-Sd-1, which are Gn1 alleles from Habataki controlling grain number and Sd-1 allele controlling plant height, respectively. This line has 26% higher grain yield and 18% lower plant height compared to Koshihikari.

Spike characters include spike shape, spike-layer uniformity and grain density. High spike-layer uniformity is conducive to ventilation and photopermeability of the lower part of the

http://dx.doi.org/10.5772/56390

plant population, which provides a favorable condition for consistent maturity. Developed primary branches and moderate grain density helps reduce spike length, lower the center of gravity of plants, and ensure the consistency of grain maturity. Panicle type is an important agronomic character, depending on the morphology and number of primary and secondary branches. Panicle type can be erect, semi-erect and curved. It is generally believed that erect panicle type has higher solar energy utilization, and is conducive for CO_2 diffusion. It can also modify the biological environment of the population, adjust inter-plant temperature and reduce moisture. The erect-panicle type has higher accumulation of photosynthestic products, better fertilizer tolerance and anti-lodging properties. Yang Shouren et al. first proposed a super high yield japonica rice type with erect panicles. These new lines, which include Shennong 265 and Shennong 89366 that are bred based on this plant type, feature high yield potential [22] and erect panicle, which are desired traits in rice in north China. Therefore, these lines have attracted increasing attention from breeders in that region.

DEP1 is a key pleiotropic gene isolated from Shennong 265 (super rice variety in north China) controlling rice yield. The DEP1 locus is a major QTL controlling yield-related trait of rice, located between SSR markers RM3770 and RM7424 on Chromosome 9. DEP1 corresponds to the 16410553 - 16414701 map position (5'-3') in Nipponbare, as a qPE9-1 allele [24]. The dominant gene at this locus is caused by an acquired mutation, which results in failure to encode a protein similar to phosphatidylethanolamine-binding protein. This mutant DEP1 promotes cell division, reduces the length of neck-panicle node and increases grain density in a panicle. Moreover, a higher branch number and grain number per panicle results in rice yield increase by 15%-20%. Researchers have found that mutation in DEP1 is widely present in erect and semi-erect-panicle high-yield rice that is grown in the middle and lower reaches of the Yangtze River. Thus, it is evident that the DEP1 gene has played an important role in rice yield increase in China [23]. DEP1 not only increases the yield in rice, but also in other crops, such as wheat and barley. Thus, DEP1 is very important in high-yield molecular breeding and for breeding new high-yielding varieties of crops.

DEP2 is responsible for the trait of erect and dense spike in rice. It is located in Chromosome 7, has 10 exons and encodes a protein with 1365 amino acids and unknown function. Sequence analysis of dep2-1 mutant showed that there is a 31-base deletion in exon 6 and a G/A transversion in intron 2. The deletion of 31 bases leads to shift in the reading frame, while the G/A transversion changes the editing position in intron 2, causing another frame shift. DEP2 mainly influences rachis development, promoting the elongation of primary and secondary branches. Mutation in DEP2 causes cell proliferation disorder in spike differentiation, resulting in the phenotype of erect and dense spikes [25]. It has also been shown that DEP2 is at the same locus as the small and round seed 1 (SRS1) gene [26] and EP2 [27]. SRS1/DEP2 not only regulates spike type in rice, but also its seed size. SRS1/DEP2 is mainly expressed in young tissues, such as young spikes. Mutation in srs1/dep2 leads to erect panicle, and small and round seed.

Recently, dense and erect panicle 3 (DEP3) was identified by map-based cloning. It is located in Chromosome 6. *dep3* is an erect and dense panicle mutant of the japonica rice variety. In the wild-type variety, the panicle begins to droop after flowering, which is accompanied by

changes in panicle length, grain shape and grain number per spike. However, dep3 mutants have smaller vascular bundles and thicker stems, which account for the erect-panicle phenotype. Thus, the erect and dense panicle phenotype in rice is controlled by a single recessive gene dep3. It is predicted to encode a patatin-like phospholipase A2 (PLA2) super family domain-containing protein. The mutant allele of dep3 has a deletion of 408 bp at LOC_Os06g46350, which covers 47 bp after the coding region in exon 3 and 361 bp before the 3' untranslated region [28].

2.4. Ideal plant type

Yield of rice is determined by wide diversity of agronomical traits including tiller number, grain number per spike, grain weight, grain-filling rate, plant type, etc.. It is a complex quantitative trait controlled coordinately by multiple genes and environment. In order to improve the yield potential of rice, concept of new plant type is proposed by rice breeders. New plant type is also called as ideal plant type and its key characteristics include decreased tillering, no ineffective tillering, increased grain number per spike, thick and strong stem and developed root system. Theoretical analysis shows that the yield of rice varieties of ideal plant type could increase by 25% than that of the current variety under the equatorial drought conditions. It is commendable to find the favorable mutant of ideal plant type. Using the japonica line "Shaoniejing" possessing characteristics of ideal plant type, Chinese scientists isolated and cloned the major quantitative trait gene IPA1 (Ideal Plant Architecture 1) which controls the ideal plant type of rice in 2010 [29]. Compared with the conventional cultivar such as indica rice TN1, "Shaoniejing" has less tillering, larger spikes, higher grain number per spike, thicker stem and more developed root system with the characteristics of ideal plant type. Backcrossing was performed between Shaoniejing, TN1 (recurrent parent) and conventional japonica rice Hui7 (recurrent parent). It was found that traits including plant height, tiller number stem thickness and panicle type have a co-segregation relationship. Thus, it is indicated that the phenotypic difference between Shaoniejing and conventional variety might be controlled by the same major gene. Analysis of near isogenic lines in Shaoniejing and Hui7 showed that phenotype of plant with IPA1 locus in heterozygote state (IPA1/ipal) was between homozygous wild type (IPA1/IPA1)and homozygous mutant type (ipal/ipal), indicating that IPA1 is a semidominant gene. Using mapping population of BC2F2 constructed by Shaoniejing and TN1, a major QTL—QTL8 was identified and cloned on chromosome 8 using map-based cloning. Sequence analysis found that the third extron in QTL8 of Shaoniejing had a C to A point mutation, causing amino acid to change from leucine to isoleucine. Through constructing plasmid gIPA1 carrying full-length gene and transforming Nipponbare as a receptor, it was found that transgenic plants had decreased tiller, thick stem, increased branch number and grain number per spike. Contrarily, when IPA1 expression was downregulated in RI 22 (it had the same point mutation as Shaoneijing), it was found that the transgenic plant had increased tillering, decreased plant height and thin stem with significant declining in branch and grain numbers. Sequence analysis revealed that IPA1

http://dx.doi.org/10.5772/56390

encoded the transcription factor OsSPL14 which contained the SBP structural domain. IPA1 was located in the nucleus with the transcriptional activity. Analysis of mRNA *in situ* expression showed that IPA1 had the highest expression in stem tips during vegetative growth period and branch primodium during reproductive growing period. IPA1 contains target site of miR156 and could be regulated by miR156 *in vivo* by means of transcriptional segmentation and translational repression. As point mutation of Shaoniejing occurs at the target site of miR156, the two regulatory channels are influenced, causing simultaneous increase of transcript and protein amount in IPA1. Transgenic study revealed that, although point mutation induced changes in amino acid, it caused no influence on the function of IPA1 protein. Application value of IPA1 gene was explored and ipa1 mutant gene was introduced into rice "Xiushui 11" through backcrossing. Analysis of near isogenic lines of backcross offspring found that strains carrying ipa1 mutant gene had the typical characteristics of ideal plant type and their yield had an increase of over 10% in the field plot experiment compared with their parent strain "Xiushui 11". Therefore, mutation of this gene has induced decreased tillering, thick stem and obvious increase in grain number per spike and 1000 grain weight, and the rice variety had the typical features of ideal plant type. It is a powerful tool for improving the plant type of current rice cultivars and enhancing the rice yield with great application potential in rice breeding[29]. At the same time, this gene was also cloned successfully by the Japanese research group using "Nipponbare" which was widely used in Japan and high-yield rice variety“ST-12”. This allelic gene was introduced into "Nipponbare" whose yield was low with an average production of 2200 grains per plant. Heading number of "Nipponbare" was strengthened after introducing this gene and its yield reached to 3100 grains by about 40% [30]. Using hybrid segregating population of Nipponbare and ST-12, they found gene Gn1a which controlled the grain number per spike on chromosome 1 and WFP gene which controlled the primary branch number on chromosome 8. Through selecting lines with 4 different combinations of Gn1a and WFP genes from BC2F2 population, primary branch number and grain number per spike among different lines were compared. It was found that the pyramiding of Gn1a and WFP from ST-12 could increase the grain number per spike by 40% - 50%, which effectively improved the rice yield [30].

2.5. Summary

Improving yield of rice is an important means to ensure food security in the world today. In order to further strengthen the yield potential of current cultivars to meet people's food demand, super-high-yield breeding based on ideal plant type has become the goal of rice breeders. Studying the control mechanism of ideal plant type to clone the related control gene is of great significance in breeding higher-yield rice variety using genetic engineering. Meanwhile, as model crop of monocotyledon, research of control gene in ideal plant type will contribute to clarifying the molecular mechanism of growth and development of monocotyledon significantly.

3. Hybrid heterosis

The phenomenon of plant heterosis was first described by Shull as the promoting effect of plant development after copulation of gametes of different genotypes[31]. Heterosis of crops was first discovered in tobacco in the middle of the 18th century. Rice heterosis was initially reported by American scientist Jones, who found that some F1 hybrids of rice had increased tillering and higher yield compared with the parents [32]. Later, heterosis in self-pollinated plants was studied and confirmed by more scientists. In the late 1950s, in the context of successful commerical exploitation of corn heterosis in America, rice breeders broaden the exploration channel of heterosis. In 1960s, scientists in India, US, Japan and China began to study the rice heterosis and its application in commercial production successively. For the first time, Xincheng Changyou from Japan achieved three-line combination of japonica rice in 1968. Study on heterosis application of rice began in China when male sterile plant was discovered by Yuan Longping et al. in 1964. Li Bihu in 1970 discovered a wild-type rice with pollen abortion in Nanhong Farm in Yaxian County of Hainan, which was a major breakthrough for the breeding and selection of male sterile rice in China[11]. Three-line combination of hybrid indica rice was achieved successfully in China in 1973. Indica hybrid rice began to receive extensive popularization in China in 1976 and China became the first country in the world to realize the commerical utilization of rice heterosis. Hybrid rice planting resulted in large rice yield increase in China from 1976 to 1995, which was a significant achievement. Cumulative planting area of hybrid rice reached 250 million hm^2 in 1999 with an increased crop production of 370 million tons[11]. Hybrid rice had made great contribution to the food production of China and the world.

3.1. Cytoplasmic male sterility and the fertility-restoring genes

Cytoplasmic male sterility (CMS) refers to the biological phenomena that the male reproductive system of plant cannot develop normally to produce the viable pollen, but the female reproductive system has normal development and vegetative growth. As the major type of hybrid rice combination, cytoplasmic male sterility in rice has attracted more and more attention. Sterile line of rice can be divided into genic male sterility and cytoplasmic male sterility based on sterility mechanism. Genic male sterility can be further divided into dominant male genetic sterility, recessive genic male sterility and environmental sterility. Based on genetic characters of male sterility, cytoplasmic male sterility is classified into sporophyte sterility and gametophyte sterility. Gametophyte sterility mainly consists of Baotai type (BT type), Dian type, Honglian type(HL) and Lide type. Source of sterile cytoplasm in sporophyte is abundant, and wild abortion type (WA), dwarf abortion type (DA), D type, G type, K type, Indonesia paddy type, etc. have large planting area in China (Table 1)[77].

As cytoplasmic male sterility and its fertility restoration are a basis for three-line hybrid rice breeding and production application. Topics on the mechanism of cytoplasmic male sterility and its fertility restoration in rice have attracted attentions of many scientists. Cytoplasmic male sterility is manifested as maternal inheritance and generally related with abnormal open reading frame of mitochondrial genome. In most cases, male sterility could be restored by the

Types	Sources	CMS varieties
Wild abortion type (WA)	Hainan wild rice	Zhenshan 97A, V20A
Honglian type(HL)	Red awl wild rice	Honglian A, YuetaiA, Yuefeng A
Baotai type (BT)	Boro-Taizhong 65	Fengjin A, Hanfeng A
Dwarf abortion type (DA)	Jiangxi dwarf wild rice	Xieqingzao A
K type	West Africa indica varieties	Chaoyang 1A, Gang46A
D type,	Dish D52	D Shan A, D297A, D62A
Indonesia paddy type	Indonesia varieties	Zhong 9A, II-32A
Dian type	Taipei varieties	Liuqianxin A, Ning 67A

Table 1. The CMS types

fertility-restoring gene (Rf) encoded by nucleus [33]. Therefore, CMS/Rf system is the ideal model for studying the interaction between mitochondrial genome and nuclei genome. It has been widely applied in hybrid breeding in order to improve the yield of crop. Various types of CMS have been found in rice and the main applied types in indica hybrid rice include wild abortion type (CMS-WA), Hongling type(CMS-HL), dwarf abortion type (CMS-DA) and so on. The typical representatives are Zhenshan 97A, Congguang 41A and Xieqingzao A. Main applied types in japonica hybrid rice are Baotai type(BT) and Dian type, representatives being Fengjin A and Liuqianxin A[79].

At present, fertility-restoring gene in cytoplasmic male sterility has been positioned and cloned in *Zea mays*, *Petunia hybrida*, *Daucus carota* and other plants [34, 35, 36]. For rice, two research groups in Japan [37, 38] have reported the fine positioning and cloning of fertility-restoring gene Rf-1 for BT type. The results showed that Rf-1 (PRR791) gene also encodes a mitochondial positioning protein which contains PPR. Recently, fertility-restoring gene Rf5 which could restore the cytoplasmic male sterile line of Honglian type was obtained by Hu et al. [39] through map-based cloning and proved to be consistent with Rf1a. Results by Akagi et al. [38] also indicated that a PRR homologous gene (Rf-1b) exists beside this Rf-1 gene (also called as Rf-1a). However, it was supposed that Rf-1b has no restoring function. Study of cytoplasmic male sterility mechanism and functional analysis of fertility-restoring gene in rice CMS-BT was published by Chinese research group in 2006[40]. Results revealed that cytoplasmic male sterile line of BT type contains an abnormal mitochondrial open reading frame—orf79. There is a cotranscription with atp6 gene to encode a cytotoxic peptide. Using transgenic plant, it was proved that the expression of orf79 in rice caused male gametophyte sterility of pollen. A polygene cluster encoding PPR protein was found in Rf-1 loci of chromosome 10. At least 2 members including Rfla (it was reported as PRR791 by the Japanese research group) and Rf1b were proved to have fertility restoring function for BT type.

Studies showed that Rf1 could restore the fertility of cytoplasmic male sterile line of Baotai type. Rf5 could also restore the fertility of cytoplasmic male sterile line of Honglian type. For the near isogenic line with cytoplasmic male sterility, the pollen is fertile if it carries Rf-1 gene,

and otherwise, sterile. Varieties carrying Rf-1 gene such as IR24, IR36 and MTC-18R could correct the cytoplasmic male sterility of BT type but varieties carrying recessive gene rf-1 could not, such as Nipponbare. Rf-1 cDNA has a full length of 2760bp in MTC-10R. It only contains 1 exon which encodes a protein product composed of 791 amino acids. The product contains 16 trigonous pentapeptide repeat sequence motifs and mitochondrial targeting peptides. The near isogenic line MTC-10R(Rf-1/Rf-1)could restore the cytoplasmic male sterility, but the near isogenic line MTC-10A(rf-1/rf-1)with 1bp and 547bp deletion in Rf-1A locus could not restore the cytoplasmic male sterility [38]. With a full length of 3870bp, Rf1b cDNA only has one exon in restoring line Minghui 63. The exon encodes a protein product composed of 506 amino acids containing PPR motif and mitochondrial targeting peptide. The proteins encoded by fertility-restoring allele of 6 restorer lines (male sterile line or maintainer line) are different on 9 amino acids from non-fertility restoring allele of 6 non-restorer lines. The shared difference is that the base A at position 1235 in the fertility-restoring allele Rf1b is replaced by G in the non-fertility restoring allele, causing the changing of asparaginate into serine at position 412 [40]. Fertility-restoring allele Rf1a in the restorer line could encode the complete protein. However, due to frameshift mutation, allele of non-restorer line of japonica rice encodes a truncated protein which only contains 266 amino acids. Protein encoded by allele of non-restorer line of indica rice has a transformation of 55 amino acids [40].

Two open reading frames of Rf-1A and Rf-1B are found in isogenic line MTC-10R which could correct the cytoplasmic male sterility. Due to the presence of Rf-1B, the terminator codon occurs in advance, causing the formation of a short protein with no mitochondrial targeting peptide, Rf-1A is exactly the Rf-1 gene. Rf-1A encodes a protein which contains 16 trigonous pentapeptide repeat (PPR) motifs and is targeted to mitochondrion. Rf-1A is expressed in inflorescence during booting stage and PPR motif with tandem duplication is considered to be capable of having specific binding with RNA and DNA. Therefore, Rf-1A is a fertility-restoring gene through processing the atp6/orf79 transcript from mitochondrial genome in BT type. Cytoplasmic male sterility of BT type could be corrected by rice varieties carrying Rf-1 gene, which has no effect on WA type [38]. In Boro II rice, abnormal mitochondrial open reading frame orf79 has co-transcription with doubled atp6 gene, encoding a cytotoxic peptide. Specific accumulation of this toxic polypeptide causes male sterility of gametophyte. The two related fertility-restoring genes Rf1a and Rf1b are located in the typical Rf-1 locus as members of polygenic cluster, encoding the trigonous pentapeptide repeat protein. RF1A and RF1B are both targeted to the mitochondrion and they prevent the formation of toxic peptide to restore the fertility by restriction and decomposition of B-atp6/orf79 mRNA. For decomposing mRNA, RF1A is epistatic over RF1B. Besides, RF1A could not only degrade B-atp6/orf79 mRNA but also promote the editing of atp6 mRNAs [40].

Rf1a and Rf1b are both fertility restoring-gene with expression in spikes, leaves and roots. Proteins RF1A and RF1B encoded by them are both targeted at mitochondrion. Via restriction, B-atp6/orf79 mRNA is blocked by RF1A to prevent the generation of ORF79 protein to restore the fertility. And fertility is restored by RF1B via degrading B-atp6/orf79 mRNA. When RF1A and RF1B are present simultaneously, RFIA functions with preference, that is, RF1A is epistatic over RF1B in mRNA processing. In addition to the function of dissecting B-atp6/orf79 mRNA,

RF1A could also improve atp6 mRNA editing. It is presumed that the latter is the basic function of RF1A and the former the new function developed during the evolution [40].

Through forming the complex with $GRP_1$62-rich glycine protein, trigonous pentapeptide protein RF5 restores the fertility of cytoplasmic male sterile line of Honglian type [39]. Two non-allelic nuclear restorer genes including Rf5 and Rf6 are involved in the gametophyte fertility restoring model of Honglian type (Rf6 is a new restorer gene locus located in the short arm on chromosome 8). Half of the pollens in F_1 plants carrying either Rf5 or Rf6 are fertile and fertility of 75% pollens is normal in hybrid carrying both Rf5 and Rf6. Seed setting rate of F_1 plants carrying 2 non-allelic genes is higher than that of F1 carrying only 1 restorer gene under adverse environment [41].

3.2. Photoperiod (thermo)-sensitive male sterile

For the first time in 1973, natural nuclear male sterile line Nongken 58S which was mediated by photoperiod and thermal was discovered by Shi Mingsong in a late japonica rice field in Hubei province. The discovery and effective utilization of photoperiod (thermo)-sensitive genic male sterile (PTGMS) line Nongken 58S opened a new chapter in China's hybrid rice research. Because the PTGMS line could be dually used as sterile and maintainer lines, the maintainer line is no longer needed in the two-line hybrid rice cultivation. Under different thermal and photoperoid conditions, the PTGMS line could be used not only as sterile line for hybrid seed production, but also as maintainer line for self reproduction. Thus, process of seed reproduction and breeding are simplified, reducing the production cost of hybrid seeds. Besides, it is not restricted by restoring and maintaining relationship. Therefore, it could strengthen the degree of genetic complexity of breeding parents in the rice hybrid breeding and expand the genetic distance between the 2 parents. So it is favorable for selecting and breeding strong and optical combination with higher heterosis. However, study of fertility transition mechanism of PTGMS line is still weak and could not adapt to the development of application studies on two-line hybrid rice. Especially, the studies on genetic mechanism and regulatory mechanism in photoperiod thermo-sensitive genic male sterile line are not very intensive. Therefore, strengthening the studies on fertility transition mechanism of photoperoid thermo-sensitive genic male sterile line of rice, especially the studies on the genetics and molecular biology, and finding the gene and protein closely related with fertility transition regulation are important. The achievements made in these respect will promote the breeding, selection and mating of photoperiod thermo-sensitive genic male sterile lines and the utilization of heterosis in other crops in future. Photoperiod thermo-sensitive genic male sterile lines show diversity in genetics. This is because sterility is a kind of biological phenomenon related t photoperoid and thermal ecological conditions and expression of sterile gene requires optimal light and temperature conditions. Researchers have already carried out a great number of studies on the sterility inheritance rules of all kinds of photoperiod thermo-sensitive male sterile resources including Nongken 58S, Annong S-1, Hengnong S-1 and 546OS.Some basic inheritance rules have been clarified.

At present, gene pms3 which controls the photoperoid-sensitive male sterility in japonica rice Nongken 58S [42] and gene p/tms12-1 which controls the thermo-sensitive male sterility in

indica rice Peiai 64S [43] already have been cloned. Studies proved that located at the same locus, they are a non-coding RNA. Researchers in Huazhong Agricultural University successfully cloned gene pms3 controlling the photoperoid-sensitive genetic sterile line of rice in 2012. They found that it is a long non-coding RNA that controls the sterility of Nongken 58S; pms3 is the transcript 1 of LOC_12g36030. Studies indicated that it is a RNA molecule associated with male sterility specific to long-time lighting with a length of 1236bp (LDMAR). For normal rice under long-day condition, the expression of this gene could ensure normal pollen development and male fertility. However, for photoperoid-sensitive genic male sterile line of rice, base mutation of pms3 interval causes methylation of promotor interval in this gene with decreased expression. As a result, it could not meet the requirement of pollen development. Thus, this causes the male sterility under long-day condition [42]. Gene p/tms12-1 which controls the thermo-sensitive male sterility was cloned from Peiai 64S, which was the parent of thermo-sensitive genic male sterile line for two-line hybrid indica rice with the largest planting area by researches from South China Agricultural University. This gene is a non-coding RNA gene and its original transcript produces a small RNA after processing at least 2 times. Compared with the normal rice variety, thermo-sensitive male sterile line of rice had a single base mutation in this small RNA. It was further revealed by the studies that Nongken 58S also has the same gene mutation and this single base mutation is the common cause for thermo-sensitive male sterility of indica rice and photoperoid-sensitive male sterility of japonica rice. In normal rice, the expression of wild-type P/TMS12-1 restrains the occurrence of thermo-sensitive or photoperoid-sensitive male sterility. However, for thermo-sensitive and photoperoid-sensitive male sterile line of rice, the expression level of small RNA and its interaction with target gene are influenced by mutation of p/tms12-1, causing male sterility [43]. Successful cloning of pms3(p/tms12-1)gene had a very great significance for accelerating the breeding of two-line male sterile varieties of rice and promoting the research and development of crop heterosis utilization.

3.3. Wide and specific compatibility genes and subspecies heterosis

Making full utilization of heterosis between subspecies of indica and japonica rice is a major and effective means to increase the rice yield per unit area. However, this utilization is restricted by the low fertility of indica-japonica hybrid F1. Asian cultivated rice is divided into 2 subspecies, *indica* and *japonica*. Heterosis of intersubspecific indica-japonica hybrid is far greater than that of intrasubspecific hybrid. However, because reproductive isolation exists widely between subspecies in nature, hybrid fertility of the intersubspecific hybrid declines, which results in low seed setting rate. Breeding of hybrid rice had been limited within the subspecies for a long time because of this restriction because of the difficulty to utilize the stronger intersubspecies heterosis. Later, rice resources which could break the reproductive isolation are discovered by scientists, and known as wide compatibility varieties. Using indica rice varieties IR36 and IR50 and japonica rice varieties Qiuguang and Ribenyou as testers, Japanese scientist IKehashi, et al. [44] performed the hybrid fertility identification for 74 intermediate varieties. Six varieties including Ketan NangKa, Cpslo-17, etc. and hybrid F1 of indica rice and japonica rice all had high seed setting rate. They were believed to have wide compatibility gene (WCG), and named as wide compatibility varieties (WCV). After extensive

http://dx.doi.org/10.5772/56390

testing by Chinese researchers, Balila, Qiuguang, Nantehao and IR36 were officially assigned as the testers for wide compatibility in China in 1989[78]. At present, a great number of wide compatibility lines are selected for hybrid rice breeding through different ways. For example, WA type cytoplasmic male sterile line 02428A, Reyan 1A, Peiai 64S and other wide compatible sterile lines were bred through backcrossing between wide compatible materials and gene-cytoplasmic male sterile lines. Wide compatibility restorer lines including H108, H64, H921, D069, P26, JM-2, Zhong 413, T2070, 9308 were bred with japonica-indica rice cross.

Sterility of indica-japonica hybrid is the key obstacle to taking advantage of hybrid vigor, and its mechanism has for a long time remained as one of the research hotspots for rice breeding and molecular genetics. For the past decades, genetic analysis has already located a host of loci related to the sterility of hybrid rice, but still little is known about the molecular mechanism for the reproductive segregation between the two rice subspecies. In 1984, Japan's rice breeding expert Ikehashi argued that the sterility of indica-japonica hybrid is mainly controlled by the allele at S5 locus on Chromosome 6. S5-n is known as a WCG, and the rice variety containing S5-n gene is a WCV, whose hybrids with indica and japonica show normal fertility [45]. In 2008 after many years of extensive research, Chinese scientists successfully cloned S5 gene and preliminarily illuminated the molecular mechanism for S5 to regulate the sterility of hybrid [46]. The research shows that S5-j is located on Chromosome 6, cDNA having a total length of 2495 bp and containing three exons. It encodes aspartyl protease made up of 472 amino acids and the product contains signal peptides, central domain, N terminal and C terminal. S5 is not expressed in leaves, but in the developing panicle. *In-situ* hybridization shows that S5 is expressed in various organs of ovule, including nucleus, integument, macrospore mother cell and embryo sac. S5 gene regulates seed setting percentage by controlling the sterility of female gamete. Protein s5-i and s5-j of indica and japonica are different on two amino acids. Located in the central domain, both two amino acids may have an effect on the activity or stability of aspartyl protease. Just like Nipponbare and Balilla, in indica rice-japonica hybrid, locus 273 is lLeucine and locus 471 is valine; for indica Nanjing 11, locus 273 is phenylalanine and locus 471 is alanine. At locus 172 bp in the downstream of terminator coden, there is deletion of an A; wide-compatibility variety 02428: deletion appears at 67 pb before ATG and 69 bp after ATG transcription start site, totaling 136bp, resulting in the deletion of 115 amino acids at N terminal of signal peptides and rendering it unable to be located on the cell wall. Therefore, the deletion of large segment on S5 gene of wide-compatibility variety has led to loss of function. Neither the hybrid with indica nor with japonica can affect hybrid fertility. Sequencing of 16 different varieties (including indica and japonica and wide-compatibility variety) has further confirmed the above results. At locus S5-i and S5-j of indica and japonica, indica-japonica differentiation occurs due to the infertility of indica-japonica hybrid, thus creating rich diversity of rice varieties and leading to reproductive segregation. However, the existence of wide-compatibility gene S5-n has provided a bridge for the gene exchange between sub-species of indica-japonica hybrid, maintaining the integrity of rice variety. Wide-compatibility gene S5-n enjoys bright prospect for application in the breed improvement of rice variety, for it can be directly used to develop other wide-compatibility genes and also in breeding wide-compatibility varieties as molecular marker. Effective application of wide-compatibility genes can help

overcome the infertility of the hybrid between indica and japonica rice subspecies so as to improve rice yield by relying on the strong hybrid vigor of indica-japonica sub-species[47].

It's worth noting that the research findings of Aradidopsis indicate that aspartyl protease is mainly involved in the transduction of disease resistant signal and the programmed cell death of regenerative tissues. Although the current research has failed to fully reveal the functional mechanism of S5, they can be sure that S5 has close ties with the emergence and survival of macrospore. According to the analysis of crystalline structure, aspartyl protease has three structural domains, namely, central structural domain, ring structure of N-terminal and ring structure of C-terminal. Sequence alignment and analysis show that the two mutational sites amino acid 273 and 471 in S5 are located in the central domain. However, the problems of the decreasing extent in the activity of aspartyl protease are connected with the fertility of female gamete (embryo sac), and the reason forthe functionally deficient S5-n not to affect the fertility of female gamete (embryo sac) in homozygosity and heterozygosity need further research. Recently, researchers discover a "killer-protector" system encoded by three closely interlocked open reading frames (ORF3, ORF4 and ORF5), which controls the fertility of indica-hybrid hybrid. ORF5 gene plays the "killer" role, assisted by ORF4. Conversely, ORF3, as the protector, has the opposite function. In the forming process of gynospore, the action of ORF5+ ("killer") and ORF4+ ("partner") can cause the stress response of endoplasmic reticulum (ER), while ORF3+ ("protector") blocks the ER stress response in cells and facilitates cells to produce normal gamete. But ORF3- cannot block ER stress response, thus causing programmed cell death and embryo sac abortion to happen in advance [48]. This research has given a relatively complete elaboration of the molecular mechanism of S5, and revealed the molecular mechanism for controlling the fertility of indica-japonica hybrid. It provides reference for studying the sterility of indica-japonica hybrid, molecular mechanism for reproductive segregation and biological evolution. This killer-protector system regulates the sterility of a hybrid from two subspecies. The non-fatal combination of ORF4 and ORF5 allows the indica-japonica hybrid to pass its genes to the next generation, thus overcoming the hybrid sterility and laying the foundation for the development of ideal rice varieties. This finding has vast application potential in improving rice varieties. The relevant information can be directly used to develop other wide-compatibility genes and breed wide-compatibility varieties. It will help fix reproductive segregation, overcome sterility of hybrid between indica-japonica sub-species and make use of hybrid vigor of indica-japonica sub-species to increase rice yield.

Besides wide-compatibility genes, there are also some specific-compatibility genes present in rice. Based on the systematic research on pollen fertility, Zhang Guiquan et al. [49] put forward the theory of specific compatibility genes, holding that the pollen fertility of indica-japonica hybrid is controlled by at least six loci, namely, S-a, s-b, S-c, S-d, S-e and S-f. The pollen sterility of hybrid is mainly determined by the number of heterozygous loci and the differentiation distance of alleles. Heterozygous alleles lead to sterility, while homozygous alleles lead to compatibility. Such gene is called specific compatibility gene. On these loci, indica variety often carries S^i/S^i, while japonica carries S^j/S^j. In their hybrid, the interaction of S^i gene and S^j gene causes the abortion of S^j-carrying male gamete. [50]. Sa gene locus affects the fertility of F_1 hybrid between indica-japonica subspecies and the interaction of indica-japonica alleles leads

http://dx.doi.org/10.5772/56390

to the abortion of male gamete and reduces the seed setting percentage. Using cultivar Taichung 65 and isogenic F_1 sterile line TISL4 as the materials, Zhuang Chuxiong et al (51) employed such technologies as RFLP and RAPD to locate S-a locus on Chromosome 1 and the genetic distance from CDO548 is 6.4 cM.

Further research found that Sa locus is actually made up of two adjacent gene loci SaM and SaF, encoding ubiquitin-like modifier E3 ligase and F-box proteins[52]. Allele SaM^+ encodes an ubiquitin-like modifier E3 ligase made up of 257 amino acids, while a G→T single site mutation at intron 5 in SaM^-, causing premature termination of translation and the end product, is only made of 217 amino acids. SaF encodes a F-box protein composed of 476 amino acids. Compared with SaF^+, a single nucleotide mutation occurs in SaF^-, resulting in phenylalanine for serine substitution at position 287[52]. The haplotype in most indica varieties is SaM^+SaF^+, while SaM^-SaF^- in all japonica varieties. The semi-sterility of indica-japonica hybrid is due to SaF^+'s direct interaction with SaM^- and indirect interaction with SaM^+, which has led to the abortion of pollen that carries SaM^-. Due to the existence of repression domain, SaM^+ does not directly interact with SaF^+, but SaM^+ will inevitably cause male sterility. Male sterility would be impossible if any of SaM^+, SaM^- or SaF^+ is lacking. This "two pairs of alleles/three elements" interaction model has provided a satisfactory explanation for the incompatibility of indica-japonica hybrid [52].

In F_1 plant, combinations of alleles at adjacent positions (SaM^+SaF^+ or SaM^-SaF^-) separate in the haploid microspore. Therefore, only the protein migration between spores can result in the concurrence of SaM^+, SaM^- and SaF^+. It may be impossible for SaM^- to migrate due to the deletion of a domain in its truncated proteins, so SaF^+ and SaM protein need transport from its own microspore to the microspore that carries SaM^- for the interaction to happen. SaF^+SaM^- complex further interacts with SaM^+, leading to male sterility by resulting in killing the microspore that carriers SaM^-. Since the male developmental defect of hybrid occurs in the early period of microspores, the transport of these proteins may occur via the cytoplasmic channel during the tetrad period. The SNPs analysis of SaF and SaM shows that the functional variation on SaF has already existed before the evolution and seperation of most rice varieties. The mutation on SaM occurs in the population of ordinary wild-type rice (Oryza rufipogon) that carries SuM^+SuF^- in south China, thus creating SuM^-SaF haplotype. Through analysis, the authors conclude that their research data agree with the recently presented assumption that indica and japonica originate from different wild rice populations [53]. Some varieties containing SaM^+SaF haplotype have also been found in indica. Since its hybrid with indica or japonica lacks SaM^+ or SaF^+, it is fertile. Therefore, $SaM^+$and SaF can be defined as compatibility locus Sa-n. Sa-n (SaM^+SaF^-), Sa-i (SaM^+SaF^+) and Sa-j (SaM^-SaF^-) are similar to S5 locus, thus forming a three-allele system to control rice hybrid's male sterility and fertility (compatibility). The molecular mechanisms for the sterility of rice hybrid are thus unified.

Considering that indica-japonica hybrid has great application prospect in improving rice variety, the obtaining of relevant information about Sa and S5 genes can facilitate its use as molecular marker in large-scale screening for compatible germplasm of rice varieties. Or people can also use transgenic technology to create new compatibility hybrid lines. The breakthrough in the research of relevant molecular mechanism for the sterility of indica-

japonica rice hybrid has laid a solid foundation for making use of the strong hybrid vigor of inica-japonica subspecies to increase rice yield.

4. Grain shape

Rice's grain shape traits are important agronomic characters directly related to yield, so to reveal the genetic and development mechanism of grain shape and apply it in breeding is an important means to increase the per unit yield of rice. Since grain shape in rice is closely connected with its appearance, processing quality, cooking and edible qualities, grain shape traits affect not only rice yield, but also rice qualities, playing an important role in the forming of yield and quality in rice [54]. The grain shape of the world's rice varieties can be divided into several types: coarse grain, fine grain, short grain, long grain and ultra-large grain. Grain shape traits mainly include grain's length, width, length/width ratio and length/thickness ratio. Many tests show that the inheritance of grain length is controlled by single gene, double gene, polygene and minorgene. Grain width and thickness are mostly in normal distribution, indicating that this trait is controlled by polygene; grain weight is one of the important factors to constitute yield-related trait as well as the integrated indicator of grain length, grain width and grain thickness. It is generally believed that grain weight is controlled by polygene. Therefore, grain's length, width, thickness, length/width ratio and weight belong to quantitative traits controlled by polygene. Meanwhile, there is correlation between different traits [55]. QTL positioning is an important means to analyze the inheritance of quantitative traits. Up to now, the number of already positioned QTL for controlling rice's grain shape has exceeded 200 [56]. The positioning, cloning and functional analysis of the important genes that control rice's yield-related traits can help improve the molecular genetics of rice's yield-related traits and increase per unit yield of rice. Grain size is an important determinant factor of the yield of rice grain as well as the objective trait for crop domestication and artificial breeding. At present, some grain shape-related genes have been cloned by means of map-based cloning, such as GS3, GW2, GW5, GS5 and GW8.

4.1. GS3

GS3 is the first cloned major QTL controlling grain length and weight and also the minor QTL controlling grain width and plumpness. Fan et al [57] used Minghui 63 (large grain) as the recurrent parent in continuous cross breeding and backcrossing with Chuan 7 (small grain) and constructed the near-isogenic lines for positioning GS3. Through analysis of 201 random samples in the offsprings of BC_3F_2, it is found that GS3 has accounted for the 80-90% variation in the grain weight and length of the population. They built advanced backcross population BC_3F_1 and selected recombinants for the target zones. They conducted fine mapping in the Minghui 63-based BC3F2 (GS3-NIL) plant population, and selected single plants that display recessive phenotype for recombinant screen, positioning GS3 within the range of 7.9 kb. Spanning over a length of 956 bp, GS3 cDNA contains 5 exons, encoding a transmembrane protein made up of 232 amino acids. The protein product consists of the following four structural domains: a structural domain for adjusting the size of organs unique to plants (OSR),

http://dx.doi.org/10.5772/56390

a transmembrane domain, the cysteine-rich homologous region of tumor necrosis factor recipient/nerve growth factor receptor (TNFR/NGFR) and von willebrand factor type C at C terminal (VWFC module). OSR domain was previously called PEBP domain. Sequence analysis shows that compared with small grain varieties, the coden IGC encoding cysteine at position 55 in the second exon in large grain varieties mutates to stop coden TGA and causes the advance termination of protein translation (deletion of 178 animo acids). Finally, this results in the deletion of PEBP-like domains and other three domains. Apparently, GS3-encoded protein can negatively regulate grain weight [57]. It is found in the latest database software analysis that GS3 does not belong to the PEBP family. By comparison, they found that the predicted GS3 PEBP is only about 1/3 of the actual PEBP, with 20.3%-28.4% similarity. By comparing to a database sequence, it is shown that the N terminal of GS3 has a highly similar and conserved 66 aa structural domain in most angiosperm, e.g. DEP 1 for controlling panicle type. The author temporarily re-names the domain as OSR [58]. GS3 acts as negative regulator for the size of rice grain and organ. In-situ hybridization shows that GS3 is expressed in young panicles and decreased as the panicles grow. It is also slightly expressed in other tissues like embryo, apical meristem, leaves and stalk, but largely expressed in roots and crowns. Real-time PCR has also proved the above results. Wild-type allele contains four presumed structural domains: OSR domain at N terminal, a transmembrane domain, the TNFR/NGFR family cysteine-rich domain and VWFC at C terminal. It is found that the protein encoded by this gene consists of two confrontational parts and the "gaming" of the two parts at the beginning and end of GS3 protein finally determines the size of grains. The rice varieties without GS3 protein (or the protein is non-functional) is long-grain type (about 10 mm long); the rice varieties containing complete GS3 protein belongs to medium-grain type (about 8mm); the rice varieties containing only ORS belongs to short-grain type (about 6 mm) [58]. The research also found that almost all the excellent indica rice varieties contain complete GS3 protein, and therefore are medium-grain type. The GS3 protein is not functional in long-grain-type indica varieties. Gene transfer and substitution can effectively change the grain shape of rice variety, indicating that GS3 plays a decisive role in the yield and quality of rice and also in the mutation and evolution of grain shape. Homologous gene to GS3 is also found in other species, including corn, barley and soybean, while OSR exits in all these homologous genes, indicating that these genes may also control the seed size of corresponding species. Therefore, this finding will have significant prospect of application. First of all, genetic variation can be directly used in breeding varieties for desired grain size and improving the yield of rice. Secondly, based on the research information about rice, the GS3 homologous genes of other species can be cloned so as to guide the breed improvement of corresponding species. Researchers have already been done on how to apply GS3 gene in rice breeding design. Yang et al [59] used the single-segment substitution line developed from indica variety " Huaxian 74" carrying GS3 gene, to perform pyramiding breeding with single-segment substitution line carrying other favorable genes. Twenty-six homozygous pyramided lines containing GS3 and other favorable genes were obtained in F4. The measurement of grain length confirmed that these pyramided lines have desired long grain length, with much improved appearance qualities. This indicates that using single-segment substitution lines and GS3 can help realize rice breeding design aim to modify the grain length.

4.2. GW2

GW2 is a major gene controlling grain width and weight. Song et al [60] used the offsprings of F2 between WY3 and FAZ1 for preliminary QTL positioning. It is found that the allele coming from WY3 has significantly increased the grain width and weight. Advanced backcross population and the screened recessive single plants were used to position GW2 within the range of 8.2 kb. GW2 in FAZ1 contains eight exons. With a total length of 1634 bp, cDNA encodes the protein composed of 425 amino acids of 47kDa. The deletion of a base on exon 4 causes GW2 allele to terminate the translation in advance, and the product is only composed of 115 amino acids. GW2 encodes a ring-type E3 ubiquitin ligase in the cytoplasm and performs negative regulation on cell division by anchoring the substrate to the proteasome for degradation. The absence of GW2 function renders it impossible to transfer ubiquitin to target protein, making the supposedly degradable substrate hard for specific recognition, activating cell division in spikelet and increasing the width of husk. On the other hand, the grain filling rate is also raised, followed by the growth in the size of endosperm. As a result, the width of the husk, the weight of the grain and yield all increased. The histological analysis of AZ1 and NIL (GW2) indicates that larger rice husk of NIL (GW2) is mainly due to the growth in the number rather than the size of cells. The growth in the endosperm of NIL (GW2) is mainly caused by the growth in the size rather than the number of cells. Compared with FAZ1 (recurrent parents), near isogenic line NIL(GW2) can significantly increase the width and tiller number of grains. GW2 (WY3) allele has significantly increased grain width and 1000 seed weight, thus raising single plant yield. This allele can also increase panicles per plant and prolong the growth period while greatly reducing the seeds per panicle and the length of main panicle, indicating pleiotropism of GW2. Through molecular-marker-assisted selection, researchers transferred the GW2 gene in large-grain varieties to small-grain variety FAZ1 to breed new line. By comparison of the yield of NIL (GW2) and small-grain parent FAZ1, it is found that plant yield of NIL (GW2) increased by 19.7% and plot yield increased by 15.9% over small-grain parent, indicating that this gene is valuable in high-yield breeding. In order to prove whether the growth in grain size and yield in NIL (GW2) can affect rice quality, the rice quality was compared between NIL (GW2) and small-grain parent (FAZ1). It turned out that GW2 large-grains allele had an effect on the appearance of rice grains, while the physical and chemical indicators remained unchanged. It is speculated that the edible and cooking qualities are not much affected. Meanwhile, it is also found that both corns and wheat contain GW2 allele, so the discovery of this gene will greatly advance the research on high-yield breeding of crops [60].

4.3. GW5

Another cloned gene for controlling gain width is GW5, which affects the grain width and weight of rice. The allele coming from Asominori significantly increases grain width and weight [61]. GW5 is preliminarily located between SSR marker RM3328 and RMw513 on the short arm of Chromosome 5 at 2.33 cM and 0.37 cM, respectively. By expanding the population and developing CAPS marker, GW5 was narrowed down to OJ1097-A12 and between CAPS markers Cw5 and Cw6. Within this region, compared with wide-type rice with slender grains,

http://dx.doi.org/10.5772/56390

1212 bp nucleotide is deleted in wide-grain variety. GW5 for controlling grain width is exactly in this deleted sequence [61]. GW5 encodes a nuclear-localised protein made up of 144 amino acids, which contains a nuclear localization signal and a histidine-rich domain. The yeast two-hybrid experiment proved that GW5 interacts with polyubiquitin chain, indicating that GW5 may regulate grain width and weight through ubiquitin proteasome. The lack of GW5 function renders it impossible to transfer ubiquitin to target protein, making the supposedly degradable substrate hard for specific recognition and thus activating cell division in spikelet. As a result, the husk width, grain weight and yield all increase [61].

4.4. GS5

The already cloned GS3, GW2 and GW5 grain shape-related genes are all in negative correlation with grain shape. That is, a high gene expression level corresponds to the decrease in seed size. The cloned GS5 is a positive regulator for grain width, seed setting percentage and thousand seed weight. High GS5 expression level can help accelerate cell cycle and facilitate the transverse cell division of spikelet, thus increasing husk width and speeding up the filling of rice grains and the development of endosperm. This will finally lead to larger and heavier seeds and higher single plant yield [62]. A lot of researches show that besides the difference in grain size, in the two genetic materials with identical genetic background, large-grain materials have higher GS5 expression than small-grain ones. The grain width, thousand seed weight and single plant yield also increase by 8.7%, 7.0% and 7.4% respectively. GS5 is located between RM593 and RM574 on the short arm of Chromosome 5, encoding serine carboxypeptidase. The sequencing of recombinant single plant and the transformation test of GS5 show that GS5's influence on grain size comes from promoter variation. The comparative sequencing of GS5 promoter for 51 rice varieties from different parts of Asia shows that GS5 has three different combinations in nature: GS5 large-grain haplotype, GS5 medium-grain haplotype and GS5 small-grain haplotype. They perfectly correspond to the three grain widths of different varieties: wide, medium and narrow shape. Of the above three types, GS5 small-grain haplotype is wild type, while GS5 large-grain haplotype is the mutant with acquired functions in rice domestication and breeding. The mosaic transformation of promoter further shows that the forming of these mutants relies on the natural variation of GS5 promoter. Therefore, GS5 plays an important role in the artificial domestication and breeding of rice and contributes greatly to the diversity of genes controlling the grain size of rice. These results indicate that the mutation of GS5 gene is related to the grain size of rice. The discovery of this mutation can help boost rice yield and may also help increase the yield of other crops [62].

4.5. GW8

Chinese researchers discover a key functional gene GW8 which can simultaneously affect rice quality and yield. By making use of QTL mapping and advanced backcross population, it is located at the distance of about 7.5 Kb on Chromosome 8 between marker RM502 and PSM711. The sequencing shows that a SBP transcription factor OsSPL16 encoded by this gene can

simultaneously control grain size, grain shape and rice quality [63]. In the Pakistan's Basmati rice including the most delicious varieties in the world, the variation of OsSPL16 promoter is observed, which results in decreased expression. Moreover, the gene overexpression can promote cell division, broaden the grain, improve grain filling rate and increase thousand-grain weight. All these will contribute to rice yield increase. The research also finds that GW8 gene is present in high-yield rice which is grown over large areas in China at present. GW8 gene has been discovered to play an important role in the China's rice yield increase. Later in the experimental fields in Beijing, Guangzhou and Hainan, the researchers discover a variant type of GW8 gene in high-yield rice. The key site mutation not only improves rice quality, but increases grain number per spike. If the new variation site of GW8 gene is introduced into Basmati rice, the yield will increase by 14% with high quality; if it is introduced into China's high-yield rice variety, the rice quality will be remarkably enhanced with unchanged yield. In the meantime, GW8 gene has been used in molecular breeding program, and new variety Huabiao No. 1 containing excellent genes such as GW8 was successfully bred in 2009. Huabiao No. 1 has passed variety certification in Guangdong [63]. Therefore, successful cloning of GW8 gene and expounding of molecular mechanism provide new genes with important application value for high-yield and good-quality molecular breeding of hybrid rice. These achievements help reveal molecular mechanism of product synergy for rice quality.

4.6. Utilization of grains shape genes

Those cloned genes controlling rice grain shape related to yield trait are not only favorable to reveal the complex genetic mechanism of rice yield-related trait, but also provide theoretical and technical basis for molecular marker-assisted selection in rice. Through researches on primary core collection of 170 rice varieties and 10 oversea rice varieties, Fan et al [64] found that C-A single base mutation (SNP) on the second exon of GS3 is highly associated with grain length. On this basis, they developed a functional marker SF28, which can be applied to molecular marker-assisted selection of GS3 gene to improve rice appearance and yield. Song et al [60] also make in-depth researches on yield and quality in rice breeding, and discovered that NIL (*GW2*) increased single plant grain yield by 19.7% compared to FAZ1. However, the grain number per spike decreased by 29.9%, and *GW2* alleles from WY3 had no influence on leaf morphology, grain filling and edible quality of FAZ1.

In the breeding practice, cloning of the important genes controlling rice grain shape gives some revelation to its molecular mechanism, but its application in breeding is still difficult. For example, some gene resources may originate from natural selection before Indica-Japonica differentiation or artificial selection after Indica-Japonica differentiation. The fulfillment of some gene functions requires specific genetic factors under different genetic backgrounds. Quality declining due to enlarging grain size and increasing grain weight is another problem that needs to be solved. Therefore, in addition to making use of single gene, the genetic improvement of important agronomic characters of rice is also necessary. Mining key genes with pleiotropism or gene pyramiding is an effective and quick means to breed super-high-yield rice varieties.

5. Genes with pleiotropic effect to yield related traits

High and stable yield has always been considered as one of the most important objectives in crop research. The genes related to rice yield are the key object of the research on rice breeding and molecular biology. Rice yield per unit area depends on grain number per spike, effective panicle number per plant, thousand-grain weight and seed setting percentage. Meanwhile, plant height and growth period exert huge influences on rice plant morphology and adaptability. The research also finds that many pleiotropic genes are present in rice and involved in regulating multiple growth and development processes, as well as rice vegetative growth and reproduction. Pleiotropic genes are crucial in regulating rice morphogenesis and flower organ development, directly associated with rice yield. Yield and heading date are the basic properties to evaluate practical value of rice. The former reflects income, while the latter decides rice adaption area and season. At present, some pleiotropic genes affecting yield, composition factors and heading date have been cloned.

5.1. Ghd7

Ghd7 is the first reported pleiotropic gene which exerts major influence on rice heading date and yield-related trait [65]. It also can control grain number per spike, plant height and heading date simultaneously. Among the $F_{2:3}$ and recombinant inbred line population constructed by Zhenshan 97 and Minghui 63, *Ghd7* is located between marker R1440 and C1023 on Chromosome 7, and further accurately located on 79kb between RM5436 and RM2256. Through backcrossing, the fragment containing *Ghd7* in Minghui 63 is introduced into Zhenshan 97, and near isogenic line is obtained based on Zhenshan 97. Compared to the recurrent parent Zhenshan 97, NIL heading date is late by 21.2 days, and plant height higher by 33cm, main stem spikelets increased from 130 to 216, and yield per plant also increased by 50%. Through classical map-based cloning, three NIL-F2 macro-populations are adopted to locate Ghd7 to the range of 0.28 cM. In comparison with Zhenshan 97, Minghui 63 allele at the genetic locus delays heading, and enhances plant height, grain number per panicle and yield. In fact, the pleiotropism of Ghd7 region has been generally revealed in the preliminary mapping. Among Zhenshan 97/Minghui 63RIL populations, Minghui 63 allele delays heading, and enhances plant height, grain number per panicle or thousand-grain weight [66]; while in F2:3 population, Minghui 63 allele delays heading, and enhances plant height, grain number per panicle, thousand-grain weight and rice yield [67]. Through map-based cloning, this Minghui 63 *Ghd7* cDNA has a total length of 1013bp, and encodes a nucleoprotein composed of 257 amino acids; the product is CCT (CO, CO-LIKE and TIMING OF CAB1) structural protein, which is similar to CCT domain of Arabidopsis thaliana CO protein, but is obviously different from the latter. Ghd7 protein has no obvious zinc finger, and no homology relation with the Arabidopsis thaliana CO protein. Ghd7 expression is mainly in tender leaves, apical meristem, secondary branch differentiation primordium and phloem of vascular bundle in mature leaves. In view of microscopic structures of plants with Ghd7 expression, cell number obviously increases, so it is supposed that Ghd7 accelerates cell division. More secondary branches are differentiated in the development of immature spike, which become an anatomical base of improving grain number per spike. At the same time, thickening of stems also is in favor of keeping good plant

shape to facilitate stable yield. Ghd7 expression is controlled by photoperiod, and mRNA expression is characterized by day and night rhythm. The expression is inhibited in short days; while in long days, Ghd7 inhibits Hd3a and Ehd1 expression, and this mechanism of flowering control may be unique to rice. As this gene significantly prolong the plant growth period, the panicles have a longer time for development which results in higher grain number and spikes become larger. Moreover, this gene also affects flowering time, plant height and other traits, showing obvious pleiotropic feature. Under the long-day conditions, *Ghd7* over-expression can delay heading, increase plant height and grain number per panicle. The natural mutants with weakened functions can be grown in temperate or even colder regions. Therefore, *Ghd7* plays an important role in yield potential and adaptability on global scale of rice [65]. The subsequent researches discover that single phyA or combination of phyB and phyC can induce accumulation of Ghd7 mRNA, and phyB can independently reduce Ghd7m RNA to a certain extent. Furthermore, phyB and phyA can separately affect the activity of Ghd7 and Hd1 [68]. However, Shibaya et al. [69] indicated genetic interaction between *Hd2* and H*d16* or *Hd2* and *Ghd7*. *Hd2* and the related genetic interaction play an important role in controlling heading date under the long-day conditions.

Researches prove that most of high-yield rice varieties contain *Ghd7* gene. Wild-type *Ghd7* gene can delay heading, and improve plant height and grain number per panicle. Comparative sequencing of *Ghd7* alleles among 19 rice cultivers over Asia found that five genotypes had the proteins encoded by various *Ghd7* alleles. Minghui 63 is the representative of the first genotype, and alleles for this genotype have stronger functions. The varieties with this genotype are mainly distributed in rice production areas in the south of China as well as tropical and sub-tropical region, with longer growth time. Cultivar Nipponbare represents the second genotype, and alleles for this genotype have weakened functions. The varieties with this genotype are mainly distributed in North China and the same latitude regions. Hejiang 19 and Mudanjiang 8 are the representatives of the third genotype, and the alleles totally lose the functions due to terminator mutation. The varieties with this genotype are mainly distributed in Heilongjiang province in the north of China, and rice growth period is adaptable to shorter-summer condition. The fourth genotype is only discovered in Teqing varieties, and this genotype also has stronger functions. The distributed geographical regions are similar to the first one. The last genotype is deficiency of *Ghd7*, and the varieties with this genotype are mainly distributed in China's double cropping rice areas. Based on the above-mentioned researches, it is known that different genotypes of *Ghd7* are associated with rice variety distribution. These genes are not only involved in development regulation, but also are related to plant geographical distribution. The successful cloning of rice *Ghd7* gene greatly deepens the understanding of genetic and molecular basis of complex quantitative traits. This also constitutes a good illustration of pleiotropism rarely discovered in traditional genetics. The isolation of *Ghd7* gene demonstrates that complex quantitative traits such as yield can be improved through biotechnology like qualitative traits. Relevant information of this gene can be directly used in the mining of genes that are significant to improving yield and ecological adaptability to realize genetic improvement. *Ghd7* gene, which has been isolated with confirmed function in yield increase, can greatly shorten the screening time of high-yielding rice variety. This is a key step taken towards improving crop yield in the global range.

5.2. DTH8 (Ghd8)

The effect of *DTH8 (Ghd8)* is similar to that of *Ghd7* in delaying rice heading and improving yield-related traits. Other functions include enhancing plant height, grain number per panicle, total grain number per panicle and yield [70,71]. The pleiotropism is also observed in preliminary mapping. In the Zhenshan 97/HR5 RIL population, HR5 allele delays heading, and improves plant height and total grain number per spike [72]. Through construction of near isogenic lines containing target genes, *Ghd8* is isolated and cloned by virtue of preliminary mapping, comparative sequencing and genetic transformation. It encodes a HAP3 subunit containing CCAAT-box-binding transcription factor [70, 71]. HAP complex consists of three subunits, HAP2/NF-YA/CBF-B, HAP3/NF-YB/CBF-A and HAP5/NF-YC/CBF-C. Moreover, HAP complex can bind to CCAAT sequence in the promoter, and regulate expression of target genes. OsHAP3H is a HAP3 subunit of HAP complex [73]. *DTH8*/*Ghd8*/*LHD1* is proved to be the HAP3H subunit encoding "CCAAT box binding protein" of the transcription factor. It can simultaneously regulate rice yield, plant height and heading date [70, 71, 74]. It is reported that *DTH8* can be expressed in the multiple tissues, down-regulate the transcription of *Ehd1* and *Hd3a* under long-day conditions, and is independent of *Ghd7* and *Hd1.* Under long-day condition, the introduction of *DTH8* allele in Asominori can obviously prolong heading date, and increase plant height and grain number per panicle of *CSSL61 [72]. Ghd8* can delay rice flowering by regulating Ehd1, RFT1 and Hd3a under long-day condition, but promote rice flowering in short-day condition. Also Ghd 8 can up-regulate the expression of MOC1 gene controlling rice tillering and lateral branches to increase tillering number, primary and secondary branch numbers [71]. However, some variations in LHD1 (Ghd8) coding area are related to late panicles. LHD1 can down-regulate the expression of some flowering transcription activators such as Ehd1, Hd3a and RFT1 in long-day condition, but not inhibit these genes in short-day condition. This indicates that LHD1 can delay flowering through inhibiting their expression in long-day condition [74]. By main variation sites and character association analysis of Ghd8 and also cluster analysis of monoploidy of different protein sequences, near-isogenic lines of different allelotypes are constructed to obtain four favorable Ghd8 allelotypes. Among them, Ghd8-9311 and Ghd8-ruf allelotypes can increase yield but not delay flowering, so these alleles are suitable for varieties grown in areas with good sunlight and temperature. Ghd8-MH63 and Ghd8-Nip allelotypes are photostable, so they are applicable to increase yield of varieties in short-day areas.

5.3. APO1

APO1 is also a pleiotropic gene, and can simultaneously affect vegetative growth and reproductive development. During vegetative growth stage, apo1 mutant can promote blade growth and blade number more than wild type. During reproductive growth stage, apo1 mutant can lead to smaller panicle, and smaller primary branch and spikelet numbers than wild type. apo1 mutant transforms flower stamens into lodicules, causing carpel to abnormally stretch and carpellody in glumes. Thus, lodicule number increases and stamen reduces. APO1 encodes an F-box protein, which is mainly expressed in apical meristem and lateral organ primordium. APO1 plays an important role in regulation of meristem destiny, and positively regulates the

primary branch and spikelet numbers. Spikelet meristems of apo1 mutant form early and the formation period of lodicules and carpel is also prolonged. This inferred infer that APO1 participates in time regulation of meristem attributes. APO1 positively regulates the expression of C-class gene related to homoetic transformation, and affects flower organ attributes [75]. In 2010, Japanese scholars made use of chromosomal segment substitution lines (CSSL) to identify a QTL effectively controlling stalk thickness, which is named as SCM2. SCM2 is equivalent to previously reported APO1 gene controlling panicle structure through map-based cloning. The near isogenic lines containing SCM2 have phenotypes of reinforced stalk strength and increased spike number, showing that this gene has pleiotropic effects. Although SCM2 is the functionally acquired mutant of APO1, it has no negative effects associated with overexpression of APO1 mutant as have been previously, including decreasing spike number and abnormal spikelets. SCM2 can prevent yield reduction from lodging due to application of chemical fertilizer in high-plant varieties [76].

6. Perspective

Discovery of semi-dwarf gene sd1 from DGWG and Aizizhan triggers a revolution in global rice production. Cytoplasmic male sterile gene found in wild rice enables the rice heterosis to be fully shown and realized through three line system, followed by another leap in rice yield. The application of excellent germplasm resources and their genes plays a key role in enhancing rice yield. With the development of new technology, germplasm will find more applications. In face of new requirements for current world's production development, the following respects should be considered in the researches on rice yield increase: (1) select and breed high yield, high quality new rice variety with endurable storage and stress tolerance; (2) select and breed new rice variety for special purposes; (3) trans-breed a series of cytoplasmic male sterile lines and restorer lines with stable, high quality and combining fertility; (4) clone and isolate genes controlling important agronomic characters. In general, core collection, backbone parents, excellent medium material and excellent variety are mainly considered as the basis to systematically create and construct saturated mutant library and genotype-phenotype database. Gene cloning, association analysis and gene regulatory network analysis should be adopted to study the molecular mechanism of good character formation, and explore allelism difference and genetic effect of relevant characters such as high yield, high quality and stress resistance of super rice. High-throughput genotyping, favorable gene pyramiding and improvement, conventional and molecular breeding are proper techniques to breed the new lines and variety of super rice with advantages in yield, quality and stress resistance. To breed the above-mentioned varieties, the key is to further develop and utilize new gene resources based on the existing varieties, including gene resources contained in indica and japonica subspecies, *Oryza* species and mutant species. At the same time, we need to improve breeding method and identification technology, including combined adoption of transgene technology, composite hybridization and rice molecular marker-assisted selection. In particular, the conventional breeding method should be combined with biotechnology, which will be the important way to breed super rice of super-high yield.

Author details

Dawei Xue[1], Qian Qian[2] and Sheng Teng[3*]

*Address all correspondence to: steng@sibs.ac.cn

1 College of Life and Environmental Sciences, Hangzhou Normal University, Hangzhou, Zhejiang Province, P.R.China

2 State Key Laboratory of Rice Biology, China National Rice Research Institute, Chinese Academy of Agricultural Sciences, Hangzhou, P.R.China

3 Shanghai Institute of Plant Physiology and Ecology, Shanghai Institute for Biological Sciences, The Chinese Academy of Sciences, Shanghai, P.R.China

References

[1] FAOSTAT. http: //faostat. fao. org/faostat

[2] van Nguyen N, Ferrero A. Meeting the challenges of global rice production. Paddy Water Environment, 2006, 4: 1-9

[3] Chang TT, Zuno C, Marciano-Romena A, et al. Semidwarf in rice germplasm collections and their potentials in rice improvement. Phytibreedon, 1985, 1 (1): 1-9

[4] Xue DW, Qian Q. Genetic basis and resources innovation of super rice Breeding in China. Journal of Shenyang Agricultural University, 2007- 10, 38 (5): 667-675

[5] Khush, G. S. Breaking the yield frontier of rice. Geo. J. 1995, 35, 329-332

[6] Donald C M. The breeding of crop ideotypes. Euphytica, 1968, 17: 385-403

[7] Chen WF, Xu ZJ, Zhang LB. Physiological basis of super rice breeding. Shenyang: Liaoning Science&Technology Press Publishing House, 1995

[8] Khush GS. Green revolution: the way forward. Nat Rev Genet, 2001, 2 (10): 815-822

[9] Yang SR, Zhang BL, Wang JM. The theory and method of ideal plant morphology in rice breeding. Scientia Agricultura Sinica, 1984 (3): 6-12

[10] Zhou KD, Wang XD, Li SG, et al. The study on heavy panicle type of inter-subspectic hybrid rice (*Oryza sativa* L.). Scientia Agricultura Sinica, 1997, 30 (5): 91-93

[11] Yuan LP. Hybrid rice breeding for super high yield. Hybrid Rice, 1997, 12 (6): 1-6

[12] Huang YX. Semi-dwarf, Ecological breeding engineering of Chinese super rice with early growth, deep root, super high yield and quality. Guangdong agricultural sciences, 2001 (3): 2-6

[13] Cheng Shi-hua, Cao Li-yong, Chen Shen-guang et al. Conception of Late-Stage Vigor Super Hybrid Rice and Its Biological Significance. Chinese Journal of Rice Science, 2005, 19: 280-284

[14] Singh V. Mode of inheritance of dwarf stature and allelic relationships among various spontaneous and induced dwarfs of cultivated rice. Theor Appl Genet, 1979, 55: 169-176

[15] Gu MH, Pan XB, Li X. genetic analysis of the pedigrees of the improved cultivars in *Oryza sativa* L. subsp. *hsien* in South China. Scientia Agricultura Sinica, 1986: 41-47

[16] Monna L, Kitazawa N, Yoshino R, Suzuki J, Masuda H, Maehara Y, Tanji M, Sato M, Nasu S, Minobe Y. Positional cloning of rice semidwarfing gene, *sd-1*: rice "green revolution gene" encodes a mutant enzyme involved in gibberellin synthesis. DNA Research, 2002, 9 (1): 11-17

[17] Sasaki A, Ashikari M, Ueguchi-Tanaka M, Itoh H, Nishimura A, Swapan D, Ishiyama K, Saito T, Kobayashi M, Khush G S, Kitano H, Matsuoka M. Green revolution: a mutant gibberellin-synthesis gene in rice. Nature, 2002, 416 (6882): 701-702

[18] Spiemyer W, Ellis M H, Chandler P M. Semidwarf (*sd-1*), "green revolution" rice, contains a defective gibberellin 20-oxidase gene. Proc Natl Acad Sci USA, 2002, 99 (13): 9043-9048

[19] Li X, Qian Q, Fu Z, et al. Control of tillering in rice. Nature, 2003, 422: 618-621

[20] Ashikari M, Sakakibara H, Lin S, et al. Cytokinin oxidase regulates rice grain production. Science, 2005, 309 (5735): 741-745

[21] Ashikari M, Matsuoka M. Identification, isolation and pyramiding of quantitative trait loci for rice breeding. Trends Plant Sci, 2006, 11 (7): 344–350

[22] Chen WF, Xu ZJ, Yang SR. Creation of new plant type and breeding super rice in northern China. Chinese Rice Research News Letter, 2000, 8 (3): 13-l4

[23] Huang X, Qian Q, Liu Z, et al. Natural variation at the *DEP1* locus enhances grain yield in rice. Nat Genet, 2009, 41 (4): 494-497

[24] Zhou Y, Zhu J Y, Li Z Y, et al. Deletion in a Quantitative Trait Gene *qPE9-1* associated with panicle erectness improves plant architecture during rice domestication. Genetics, 2009, 183: 315-324

[25] Li F, Liu W, Tang J, et al. Rice *Dense and Erect Panicle 2* is essential for determining panicle outgrowth and elongation. Cell Res, 2010, 20 (7): 838-849

[26] Abe Y, Mieda K, Ando T, Kono I, Yano M, Kitano H, Iwasaki Y. The Small and Round Seed1 (SRS1/DEP2) gene is involved in the regulation of seed size in rice. Genes & Genetic Systems, 2010, 85 (5): 327-339

[27] Zhu K M, Tang D, Yan C J, et al. *Erect panicle 2* encodes a novel protein that regulates panicle erectness in indica rice［J］.Genetics, 2010, 184 (2): 343-350

[28] Qiao Y, Piao R, Shi J, Lee SI, Jiang W, Kim BK, Lee J, Han L, Ma W, Koh HJ. Fine mapping and candidate gene analysis of dense and erect panicle 3, *DEP3*, which confers high grain yield in rice (*Oryza sativa* L.). Theor Appl Genet. 2011, 122 (7): 1439-1449

[29] Jiao Y, Wang Y, Xue D, Wang J, Yan M, Liu G, Dong G, Zeng D, Lu Z, Zhu X, Qian Q, Li J. Regulation of *OsSPL14* by OsmiR156 defines ideal plant architecture in rice. Nature Genetics, 2010, 42 (6): 541-544

[30] Miura K, Ikeda M, Matsubara A, Song XJ, Ito M, Asano K, Matsuoka M, Kitano H, Ashikari M. *OsSPL14* promotes panicle branching and higher grain productivity in rice. Nat Genet, 2010, 42 (6): 545-549.

[31] Shull, GH. The composition of a field of maize. Am Breed Assoc Rep, 1908, 4: 296-301

[32] Jones, J.W. Hybrid vigor in rice. J AM Soc Agron, 1926, 18: 424-428.

[33] Schnable PS, Wise RP. The molecular basis of cytoplasmic male sterility and fertility restoration. Trens Plant Sci, 1998, 3: 175-180

[34] Cui X, Wise RP, Schnable PS. The *rf2* nuclear restorer gene of male-sterile T-cytoplasm maize，Science，1996，272：1334-1336

[35] Bentolila S，Alfonso A，Hanson M．A pentatricopeptide repeat-containing gene restores fertility to male-sterileplants，Proc Natl Acad Sci，2002，99：10887-1089

[36] Koizuka N，Imai R，Fujimoto H，Hayakawa T，Kllnura Y，Kohno-Murase J，Sakai T，Kawasaki S，Imamura J．Genetic characterization of a pentatricopeptide repeat protein gene，*orf687*，that restores fertility in the cytoplasmic male-sterile Kosena radish，Plant J，2003，34：407-415

[37] Akagi H, Nakamura A, Yokozeki-Misono Y, Inagaki A, Takahashi H, Mori K, Fujimura T. Positional cloning of the rice Rf-1 gene, a restorer of BT-type cytoplasmic male sterility that encodes a mitochondria-targeting PPR protein. Theoretical and Applied Genetics, 2004, 108 (8): 1449-1457

[38] Komori T, Ohta S, Murai N, Takakura Y, Kuraya Y, Suzuki S, Hiei Y, Imaseki H, Nitta N. Map-based cloning of a fertility restorer gene, *Rf-1*, in rice (*Oryza sativa* L.). Plant J. 2004, 37 (3):315-325

[39] Hu J, Wang K, Huang W, Liu G, Gao Y, Wang J, Huang Q, Ji Y, Qin X, Wan L, Zhu R, Li S, Yang D, Zhu Y. The rice pentatricopeptide repeat protein RF5 restores fertility in Hong-Lian cytoplasmic male-sterile lines via a complex with the glycine-rich protein GRP162. The Plant Cell, 2012, 24 (1): 109-122

[40] Wang Z, Zou Y, Li X, Zhang Q, Chen L, Wu H, Su D, Chen Y, Guo J, Luo D, Long Y, Zhong Y, Liu YG. Cytoplasmic male sterility of rice with boro II cytoplasm is caused

by a cytotoxic peptide and is restored by two related PPR motif genes via distinct modes of mRNA silencing. The Plant Cell, 2006, 18 (3): 676-687

[41] Huang W, Hu J, Yu C, Huang Q, Wan L, Wang L, Qin X, Ji Y, Zhu R, Li S, Zhu Y. Two non-allelic nuclear genes restore fertility in a gametophytic pattern and enhance abiotic stress tolerance in the hybrid rice plant. Theoretical and Applied Genetics, 2012, 124 (5): 799-807

[42] Ding J, Lu Q, Ouyang Y, Mao H, Zhang P, Yao J, Xu C, Li X, Xiao J, Zhang Q. A long noncoding RNA regulates photoperiod-sensitive male sterility, an essential component of hybrid rice. Proc Natl Acad Sci USA, 2012, 109 (7): 2654-2659

[43] Zhou H, Liu Q, Li J, Jiang D, Zhou L, Wu P, Lu S, Li F, Zhu L, Liu Z, Chen L, Liu YG, Zhuang C. Photoperiod- and thermo-sensitive genic male sterility in rice are caused by a point mutation in a novel noncoding RNA that produces a small RNA. Cell Research, 2012, 22 (4): 649-660

[44] Ikchashi H, Arxki H. Screening and genetic analysis of wide-compatibility in F1 hybrid of distant crosses in rice (*Oryza. sativa* L). Ibaraki: Technical Bulletin of Tropical Agriculture center, 1987, 1-79.

[45] Ikehashi H, Araki H. Variety screening of compatibility types revealed in F_1 fertility of distant cross in rice, Jpn. J. Breed., 1984, 34 (3): 304-313

[46] Chen J, Ding J, Ouyang Y, Du H, Yang J, Cheng K, Zhao J, Qiu S, Zhang X, Yao J, Liu K, Wang L, Xu C, Li X, Xue Y, Xia M, Ji Q, Lu J, Xu M, Zhang Q. A triallelic system of S5 is a major regulator of the reproductive barrier and compatibility of indica-japonica hybrids in rice. Proc Natl Acad Sci USA, 2008, 105 (32): 11436-11441

[47] Ji Q, Zhang M, Lu J, Wang H, Lin B, Liu Q, Chao Q, Zhang Y, Liu C, Gu M, Xu M. Molecular basis underlying the S5-dependent reproductive isolation and compatibility of indica/japonica rice hybrids. Plant Physiology, 2012, 158 (3): 1319-1328

[48] Yang J, Zhao X, Cheng K, Du H, Ouyang Y, Chen J, Qiu S, Huang J, Jiang Y, Jiang L, Ding J, Wang J, Xu C, Li X, Zhang Q. A killer-protector system regulates both hybrid sterility and segregation distortion in rice. Science, 2012, 337 (6100): 1336-1340

[49] Zhang Guiquan, Lu Yonggen. Genetic Studies of The Hybrid Sterility in Cultivated Rice (*Oryza sativa*)II.A Genic Model for F1 Pollen Sterility. Acta Cenetica Sinica, 1993, 20 (3): 222-228

[50] Zhang Guiquan, Lu Yonggen, Liu Guifu, Yang Jinchang, Zhang Hua. Genetic Studies of the Hybrid Sterility in Cultivated Rice (*Oryza sativa*). Allele Differentiation of F1 Pollen Sterility in Different Types of Varieties. Acta Cenetica Sinica, 1993, 20 (6): 541-551)

[51] Zhuang Chu-Xiong, Zhang Gui-Quan, Mei Man-Tong, Lu Yong-Gen. Molecular Mapping of the *S-a* Locus for F1 Pollen Sterility in Cultivated Rice (*Oryza sativa* L.). Acta Cenetica Sinica, 1999, 26 (3): 213-218

[52] Long Y, Zhao L, Niu B, Su J, Wu H, Chen Y, Zhang Q, Guo J, Zhuang C, Mei M, Xia J, Wang L, Wu H, Liu YG. Hybrid male sterility in rice controlled by interaction between divergent alleles of two adjacent genes. Proc Natl Acad Sci U S A. 2008, 105 (48):18871-18876

[53] Londo J.P., Chiang Y.C., Hung K.H., Chiang T.Y., and Schaal B.A., 2006, Phylogeography of Asian wild rice, *Oryza rufipogon*, reveals multiple independent domestications of cultivated rice, *Oryza sativa*. Proc. Natl. Acad. Sci., USA, 103 (25): 9578-9583

[54] Xu Zheng-Jin, Chen Wen-Fu, Ma Dian-Rong, Lv Ying-Na, Zhou Shu-Qing, Liu Li-Xia. Correlations between Rice Grain Shapes and Main Qualitative Characteristics. Acta Agron Sin, 2004, 30 (09): 894-900.

[55] Shi Chunhai, Shen Zongtan. Inheritance and Improvement of Grain Shape in indica Rice. Chinese Journal of Rice Science, 1995, 9 (1): 27-32

[56] Gao Z, Zhan X, Liang Y, Cheng S, Cao L. Progress on genetics of rice grain shape trait and its related gene mapping and cloning. HEREDITAS (Beijing), 2011, 33 (4): 314-321

[57] Fan CC, Xing YZ, Mao HL, Lu TT, Han B, Xu CG, Li XH, Zhang QF. *GS3*, a major QTL for grain length and weight and minor QTL for grain width and thickness in rice, encodes a putative transmembrane protein. Theor Appl Genet, 2006, 112 (6): 1164–1171

[58] Mao H, Sun S, Yao J, Wang C, Yu S, Xu C, Li X, Zhang Q. Linking differential domain functions of the GS3 protein to natural variation of grain size in rice. Proc Natl Acad Sci USA, 2010, 107 (45): 19579-19584

[59] Yang Tifeng, Zeng Ruizhen, Zhu Haitao, Chen Lan, Zhang Zemin, Ding Xiaohua, Li Wentao, Zhang Guiquan. Effect of Grain Length Gene GS3 in Pyramiding Breeding of Rice. Molecular Plant Breeding, 2010, 8: 59-66

[60] Song XJ, Huang W, Shi M, Zhu MZ, Lin HX. A QTL for rice grain width and weight encodes a previously unknown RING-type E3 ubiquitin ligase. Nat Genet, 2007, 39 (5): 623–630

[61] Weng J, Gu S, Wan X, Gao H, Guo T, Su N, Lei C, Zhang X, Cheng Z, Guo X, Wang J, Jiang L, Zhai H, Wan J. Isolation and initial characterization of GW5, a major QTL associated with rice grain width and weight. Cell Research, 2008, 18 (12): 1199-1209

[62] Li Y, Fan C, Xing Y, Jiang Y, Luo L, Sun L, Shao D, Xu C, Li X, Xiao J, He Y, Zhang Q. Natural variation in GS5 plays an important role in regulating grain size and yield in rice. Nature Genetics, 2011, 43 (12): 1266-1269

[63] Wang S, Wu K, Yuan Q, Liu X, Liu Z, Lin X, Zeng R, Zhu H, Dong G, Qian Q, Zhang G, Fu X. Control of grain size, shape and quality by *OsSPL16* in rice. Nat Genet. 2012, 44 (8): 950-954

[64] Fan CC, Yu SB, Wang CG, Xing YZ. A causal C-A mutation in the second exon of *GS3* highly associated with rice grain length and validated as a functional marker. Theor Appl Genet, 2009, 118 (3): 465–472

[65] Xue W, Xing Y, Weng X, Zhao Y, Tang W, Wang L, Zhou H, Yu S, Xu C, Li X, Zhang Q. Natural variation in Ghd7 is an important regulator of heading date and yield potential in rice. Nature Genetics, 2008, 40 (6): 761-767

[66] GUO Long-biao, LUO Li-jun, XING Yong-zhong et al. Dissection of QTLs in Two Years for Important Agronomic Traits in Rice (*Oryza sativa* L.). Chinese Journal of Rice Science, 2003, 17 (3): 211-218

[67] Yu SB, Li JX, Xu CG, et al. Identification of quantitative trait loci and epistatic interactions for plant height and heading date in rice. Theor Appl Genet, 2002, 104: 619-625

[68] Osugi A, Itoh H, Ikeda-Kawakatsu K, Takano M, Izawa T. Molecular dissection of the roles of phytochrome in photoperiodic flowering in rice. Plant Physiology, 2011, 157 (3): 1128-1137

[69] Shibaya T, Nonoue Y, Ono N, Yamanouchi U, Hori K, Yano M. Genetic interactions involved in the inhibition of heading by heading date QTL, Hd2 in rice under long-day conditions. Theoretical and Applied Genetics, 2011, 123 (7): 1133-1143

[70] Yan WH, Wang P, Chen HX, Zhou HJ, Li QP, Wang CR, Ding ZH, Zhang YS, Yu SB, Xing YZ, Zhang QF. A major QTL, Ghd8, plays pleiotropic roles in regulating grain productivity, plant height, and heading date in rice. Molecular Plant, 2011, 4 (2): 319-330

[71] Wei X, Xu J, Guo H, Jiang L, Chen S, Yu C, Zhou Z, Hu P, Zhai H, Wan J. DTH8 suppresses flowering in rice, influencing plant height and yield potential simultaneously. Plant Physiology, 2010, 153 (4): 1747-1758

[72] Zhang Y, Luo L, Xu C, Zhang Q, Xing Y. Quantitative trait loci for panicle size, heading date and plant height co-segregating in trait-performance derived near-isogenic lines of rice (*Oryza sativa*). Theoretical and Applied Genetics, 2006, 113 (2): 361-368

[73] Thirumurugan T, Ito Y, Kubo T, Serizawa A, Kurata N. Identification, characterization and interaction of HAP family genes in rice. Molecular Genetics and Genomics, 2008, 279 (3): 279-289

[74] Dai X, Ding Y, Tan L, Fu Y, Liu F, Zhu Z, Sun X, Sun X, Gu P, Cai H, Sun C. LHD1, an Allele of DTH8/Ghd8, Controls Late Heading Date in Common Wild Rice (*Oryza rufipogon*). Journal of Integrative Plant Biology, 2012, 54 (10): 790-799

[75] Ikeda K, Ito M, Nagasawa N, Kyozuka J, Nagato Y. Rice *Aberrant Panicle Organization 1*, encoding an F-box protein, regulates meristem fate. The Plant Journal, 2007, 51 (6): 1030-1040

[76] Ookawa T, Hobo T, Yano M, Murata K, Ando T, Miura H, Asano K, Ochiai Y, Ikeda M, Nishitani R, Ebitani T, Ozaki H, Angeles ER, Hirasawa T, Matsuoka M. New ap-

proach for rice improvement using a pleiotropic QTL gene for lodging resistance and yield. Nature Communications, 2010, 1: 132

[77] Zhang JW Xie JK, Li X, et al. Research Advances in Fertility Restoration of Cytoplasmic Male Sterility in Rice. Journal of Anhui Agri Sci 2010, 38 (31): 17391-17394

[78] Gu MH, Pan XB, Chen ZX, et al. A study on compatibility of standard wide compatibility testers of rice in China. Scientia Agricultura Sinica, 1991, 24 (6): 27-32

[79] Li G, Yao F, Zhuang J, et al. Inheritance of Fertility Restoration and Molecular Mapping of Restoring Genes of CMS in Rice. Hybrid Rice, 2006, 21 (3): 1-6

[80] Gu F, Zhai H, Wan J, Zhang H Study on inheritance of dwarf character and its utilization in rice (*Oryza sativa* L.) breeding. Jiangsu Agricultural Sciences, 2003, 19 (1): 48-54

Chapter 6

Functional Characterization of Genes/QTLs for Increasing Rice Yield Potential

Jie-Zheng Ying, Yu-Yu Chen and Hong-Wei Zhang

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/56820

1. Introduction

Rice (*Oryza sativa* L.) as the monocot model plant and an important food crop is cultivated worldwide. Due to the rapid growth of the world's population, rice yield is urgently required to increase to meet world food demand. In the last century, rice yield experienced rapid growth twice in China, which is mainly attributed to the exploitations of *semi-dwarf 1*(*sd1*) gene and heterosis of F_1 hybrid. Before the green revolution, rice varieties were tall and had a low harvest index. Introgression of *sd1* into the varieties significantly reduced the plant height and increased the harvest index, which resulted in a dramatic increase of rice productivity [1]. Heterosis breeding has been widely used to improve rice yield potential. Hybrid rice varieties usually have a yield advantage of 10-20% over the conventional inbred varieties, thus cover more than half of the total rice area in China at present [2, 3]. However, rice yield per unit area has not been much elevated and the arable land for rice cultivation has kept decreasing during the past two decades. New genetic improvement strategies are urgently required to break the bottleneck of yield potential of current varieties, which largely rely on the elucidation and exploitation of genetic and molecular basis for rice yield traits [4].

Rice yield traits are complex agronomic traits governed by multiple genes called as quantitative traits loci (QTLs), which usually show a continuous phenotypic distribution in a segregating population derived from a cross of a pair of inbred lines. Most QTLs for yield traits show small genetic effect and are difficult to be identified. These minor QTLs play a vital role in regulating yield trait and are widely utilized in commercial rice varieties, so that fine-mapping and map-based cloning of these QTLs will be beneficial for breeding. Number of panicles per plant, number of grains per panicle, and grain weight are three component traits which are determined by tiller, panicle and grain development. Dissecting the genetic basis of these traits by QTL mapping can facilitate breeding high yield varieties. However, it is rather

difficult to isolate these QTLs because each contributes little effect to yield traits, and the effect is strongly affected by the environment. In recent years, tremendous progress has been attained and many QTLs for rice yield traits have been isolated and functionally analyzed in detail, which provides new sights into the molecular mechanisms of the formation of rice yield traits. Meanwhile, mutant analysis has also functionally characterized many genes involved in yield traits because of the availability of rice genome and rice mutant collections. These studies greatly strengthen our understanding of regulatory mechanisms of these traits. In this chapter, we summarize the recent progress in the genetic and molecular mechanisms underlying rice yield traits and illustrate a strategy to develop varieties with higher yield potential.

2. Identification and validation of QTLs for rice yield traits

2.1. Identification of QTLs

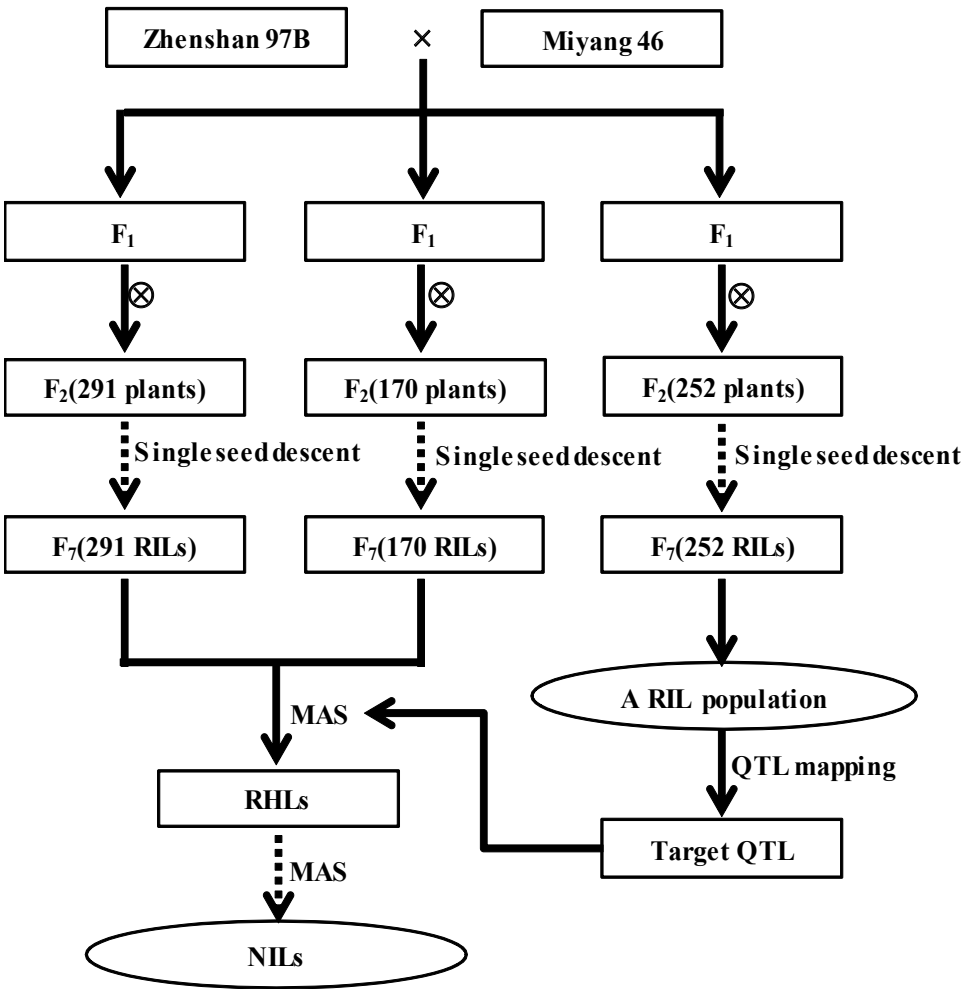
QTL mapping of a target trait is defined as the chromosomal location and genetic characterization of QTLs for the trait through the association between genetic markers and phenotypic variations. To facilitate this mapping, development of mapping population, construction of linkage map and phenotypic evaluation are essential for QTL analysis.

Typically, mapping population includes F_2 plants, doubled haploid lines (DHLs) and recombinant inbred lines (RILs). F_2 population that carries the complete genetic information from the parents can be easily developed, but its phenotypic evaluation cannot be replicated [5]. Due to the inherent homozygosity in the lines, both DHL and RIL populations can be planted repeatedly in different planting seasons and environment conditions as many times as necessary. DHL population is limitedly used in QTL mapping due to the difficulties in the plant regeneration from cultured anthers, especially for *indica* rice varieties [6, 7]. RIL population is widely used in QTL mapping although it is time-consuming and labor-intensive to prepare the population. Many RIL populations have been developed from inter-subspecies crosses [8, 9], intra-subspecies crosses [10-12], or crosses between commercial cultivars and wild rice [13].

Linkage map is composed of many linkage groups according to different chromosomes, which are constructed by genotyping using genome-wide polymorphic markers. DNA based molecular markers, such as restriction fragment length polymorphism (RFLP), simple sequence repeat (SSR), cleaved amplified polymorphic sequence (CAPS) and single nucleotide polymorphism (SNP), are widely applied in the construction of linkage map. Based on the complete genome-wide sequence of rice, it becomes easier to design genome-wide polymorphic markers and construct high density molecular linkage map [14].

Yield trait conditioned by QTLs usually varies continuously in a mapping population. Phenotypic values are difficult to be accurately measured due to environmental influences, especially for F_2 population without replication. The precision of phenotypic data greatly affects the resolution of QTL mapping [15].

Thousands of QTLs for rice yield traits have been detected and are distributed throughout the whole genome while many of them are co-localized (http://www.gramene.org). We use one of our studies to illustrate the general process for the classical characteristics of QTLs (Figure 1). A RIL mapping population is developed from an *indica-indica* cross between Zhenshan 97B and Milyang 46 using single seed descent method. QTL analysis shows that each yield trait is controlled by several QTLs. These QTLs are dispersedly distributed on chromosomes, and function on yield productivity not only by their own effects, but also by within-locus and inter-locus interactions [11].



RIL, recombinant inbred line; MAS, marker-assisted selection; RHL, residual heterozygous line; NIL, near isogenic line; QTL, quantitative trait locus

Figure 1. Flowchart for developing NILs through screening RHLs in a Zhenshan 97B/Miyang 46 RIL population.

2.2. Validation of QTLs

Primary mapping cannot delimit an individual QTL in a precise location, so that further experiments are necessary to validate the biological function of target QTLs individually. Development of near isogenic lines (NILs) is an efficient strategy for QTL validation. NILs contain the segregated target QTL region in a homogeneous genetic background. In general, NILs are produced by consecutive backcrosses with a recurrent parent aided by molecular marker assisted selection (MAS). Only the plants carrying the target QTL in the recurrent parent background will be selected to further develop NILs. Many QTLs controlling rice yield traits have been validated by this classical method. However, successful backcross combining with MAS is laborious and time-consuming. During the process of developing Zhenshan 97B / Miyang 46 RIL population, we form a new method for developing NILs by screening residual heterozygous lines (RHLs). The RHLs should contain a heterozygous chromosomal segment at the target QTL region in a nearly homozygous genetic background [2] (Figure 1). Following MAS in a high density marker linkage map, a series of RHLs with overlapping segregated segments for the target QTL are selected from F_7 RILs. This method has proven to be efficient, and several yield trait QTLs, such as *qGY6* and *qGL7-2*, have been successfully fine-mapped and validated [2, 16].

Introgression lines (ILs) and chromosome segment substitution lines (CSSLs), which are developed by backcrossing repeatedly with the recurrent parent, can also be used for QTL validation, fine-mapping and breeding superior rice varieties[17,18].

3. QTLs/genes for rice yield traits

3.1. QTLs/genes for tillering

Rice tillers are mainly composed of primary, secondary and tertiary tillers, which are shoot branches arising from the unelongated basal internodes. Tillering starts with the appearance of the fourth leaf from the main culm. Usually, the duration of tillering will last about 25-30 days. The number of panicles and yield potential are determined by panicle-bearing tillers, and grain yield are mainly contributed by primary and secondary tillers. Therefore, tiller number is considered a key component in determining rice yield. Some key genetic factors responsible for rice tillering have been molecularly characterized (Figure 2 and Table 1).

Rice tillering undergoes two major processes, the formation and outgrowth of tiller bud. Isolation and characterization of *MONOCULM* (*MOC1*) provide a new sight for the formation of tiller bud. The *moc1* mutant phenotypically exhibits only one main culm without any tillers due to the deficiency to form axillary bud. *MOC1* encodes a member of *GAI*, *RGA* and *SCR* (GRAS) family nuclear proteins to function on the formation of axillary buds [19].

http://dx.doi.org/10.5772/56820

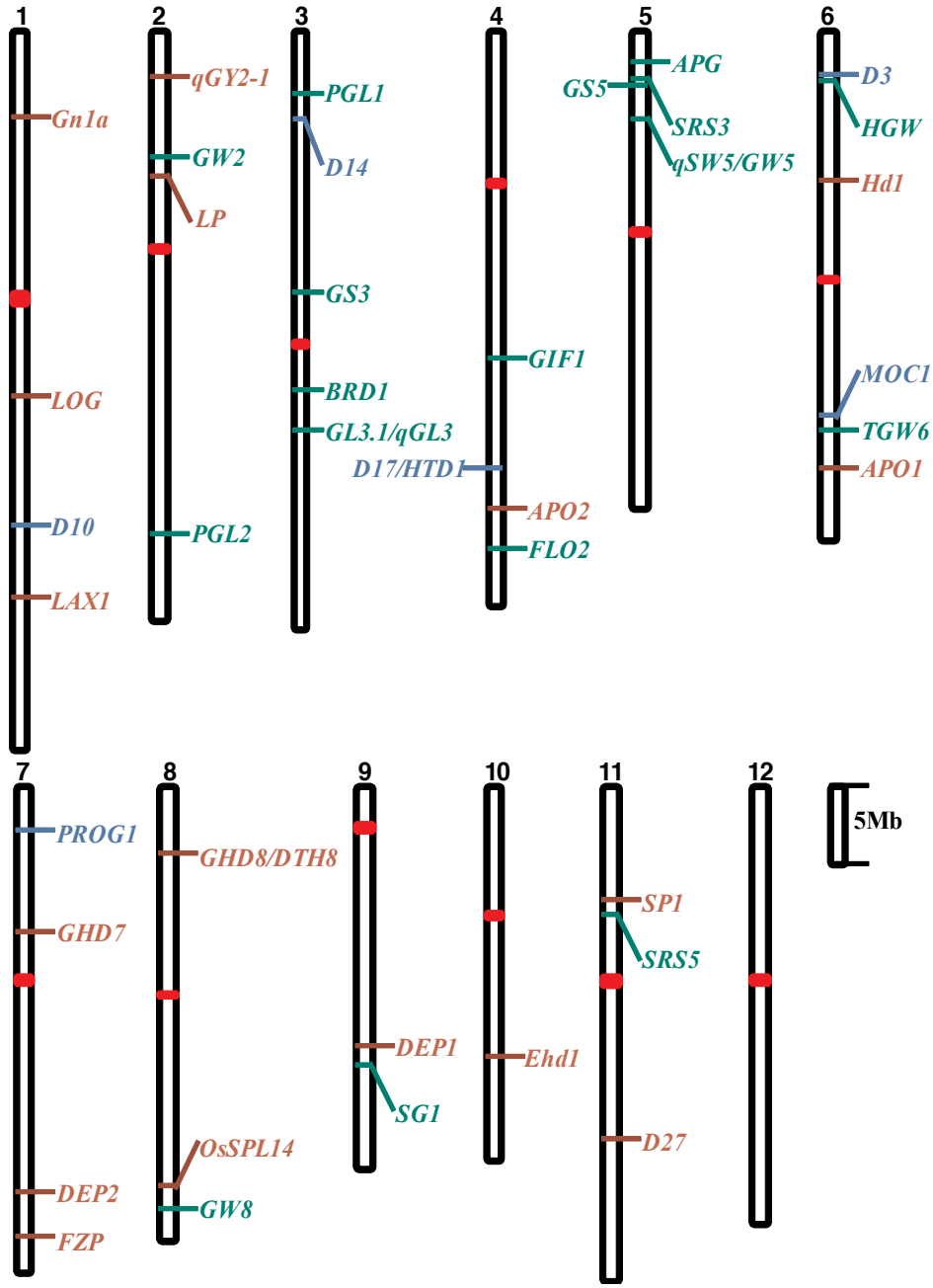


Figure 2. Distribution of the cloned genes/QTLs for rice yield traits in a physical map based on Nipponbare genome sequence; Red ellipses indicate the centromere region according to the data from the International Rice Genome Sequencing Project (http://rgp.dna.affrc.go.jp/IRGSP/); the color horizontal lines represent the locations of each gene/QTL on the chromosome for each trait; blue for number of panicles per plant (NPP); crimson for number of grains per panicle (NGP); green for grain weight (GW).

Traits	Gene/QTL	MSU-ID	Encoded protein	M/QTL	Refs
NPP	*D27*	LOC_Os11g37660	Iron-containing protein	M	[24]
NPP	*D10*	LOC_Os01g54270	Carotenoid cleavage dioxygenase 8	M	[23]
NPP	*D14*	LOC_Os03g10620	Alpha/beta-fold hydrolase superfamily protein	M	[26]
NPP	*D17/HTD1*	LOC_Os04g46470	carotenoid cleavage dioxygenase	M	[22]
NPP	*D3*	LOC_Os06g06050	F-box leucine-trich repeat protein	M	[25]
NPP	*MOC1*	LOC_Os06g40780	GRAS family nuclear protein	M	[19]
NPP, NGP	*OsSPL14*	LOC_Os08g39890	Souamosa promoter binding protein-like 14	QTL	[27, 28]
NPP, NGP	*PROG1*	LOC_Os07g05900	Zinc-finger nuclear transcription factor	QTL	[29, 30]
NPP, NGP	*qGY2-1*	LOC_Os02g05980	Leucine-rich repeat receptor-like kinase	QTL	[31]
NGP	*Gn1a*	LOC_Os01g10110	Cytokinin oxidase/dehydrogenase	QTL	[39]
NGP	*LOG*	LOC_Os01g40630	Cytokinin-activating enzyme	M	[40]
NGP, NPP	*LAX1*	LOC_Os01g61480	A bHLH transcription factor	M	[32]
NGP	*LP*	LOC_Os02g15950	Kelch repeat-containing F-box protein	M	[41]
NGP, NPP	*APO2*	LOC_Os04g51000	Plant-specific transcription factor	M	[35]
NGP	*APO1*	LOC_Os06g45460	F-box protein	M	[34]
NGP, NPP	*DEP2*	LOC_Os07g42410	Plant-specific protein	M	[38]
NGP, NPP	*FZP*	LOC_Os07g47330	Ethylene-responsive element-binding factor	M	[33]
NGP	*SP1*	LOC_Os11g12740	Transporter of the peptide transporter family	M	[36]
NGP	*Hd1*	LOC_Os06g16370	Protein with a zinc finger domain	QTL	[67]
NGP	*Ghd7*	LOC_Os07g15770	CCT domain protein	QTL	[62]
NGP	*Ghd8/DTH8*	LOC_Os08g07740	OsHAP3 subunit of a CCAAT-box binding protein	QTL	[63, 64]
NGP	*EHD1*	LOC_Os10g32600	B-type response regulator	QTL	[65, 66]
NGP	*DEP1*	LOC_Os09g26999	PEBP-like domain protein	QTL	[37]
GW, GS	*TGW6*	LOC_Os06g41850	Iindole-3-acetic acid (IAA)-glucose hydrolase	QTL	[45]
GW, GS	*GW2*	LOC_Os02g14720	RING-type E3 ubiquitin ligase	QTL	[53]
GW, GS	*PGL2*	LOC_Os02g51320	Atypical bHLH protein	Hom*	[49]
GW, GS	*PGL1*	LOC_Os03g07510	Atypical bHLH protein	Hom*	[48]
GW, GS	*GS3*	LOC_Os03g29380	Trans-membrane protein	QTL	[42]
GW, GS	*BRD1*	LOC_Os03g40540	Brassinosteroid-6-oxidase	M	[51]

Traits	Gene/QTL	MSU-ID	Encoded protein	M/QTL	Refs
GW, GS	*GL3.1/qGL3*	LOC_Os03g44500	Phosphatase with Kelch-like repeat domain	QTL	[43, 44]
GW, GF	*GIF1*	LOC_Os04g33740	Cell-wall invertase	M	[58]
GW, GF	*FLO2*	LOC_Os04g55230	Protein with a tetratricopeptide repeat motif	M	[60]
GW, GS	*APG*	LOC_Os05g04740	Typical bHLH protein	Hom*	[48]
GW, GS	*SRS3*	LOC_Os05g06280	Kinesin 13 protein	M	[46]
GW, GS	*GS5*	LOC_Os05g06660	Putative serine carboxypeptidase	QTL	[56]
GW, GS	*qSW5/GW5*	LOC_Os05g09520	Nuclear protein	QTL	[54, 55]
GW, GF	*HGW*	LOC_Os06g06530	ubiquitin-associated domain protein	M	[59]
GW, GS	*GW8*	LOC_Os08g41940	Squamosa promoter-binding protein–like 16	QTL	[57]
GW, GS	*SG1*	LOC_Os09g28520	Novel protein	M	[52]
GW, GS	*SRS5*	LOC_Os11g14220	Alpha-tubulin protein	M	[47]

NPP, number of panicles per plant; NGP, number of grains per panicle; GW, grain weight; GS, grain size; GF, grain filling; M/QTL, mutant/QTL; Hom*, Homolog.

Table 1. Genes/QTLs for rice yield traits are reviewed in this chapter

Phytohormone pathways play a crucial role in controlling the outgrowth of tiller bud from leaf sheath. Plant hormones interact to regulate axillary bud outgrowth. It is well known that auxin maintains shoot apical dominance and inhibit axillary bud outgrowth, whereas cytokinins promote branches development [4]. Strigolactones, as a new kind of terpenoid plant hormones, might act as the downstream of auxin to inhibit axillary bud outgrowth. Several genes involved in the synthesis and signaling pathway of strigolactones are isolated and functionally characterized through analyzing a serious of tillering dwarf mutants [20, 21]. *DWARF17* (*D17*)/ *HIGH-TILLERING DWARF1* (*HTD1*), *DWARF10* (*D10*) and *DWARF27* (*D27*) are involved in the biosynthesis of strgolactones, while *DWARF3* (*D3*) and *DWARF14* (*D14*) act in the signaling pathway in rice [22-26]. Their loss-of-function causes similar phenotype of enhanced shoot branches accompanying with reduced plant height. Although the relationship among phytohormones in regulating axillary bud outgrowth is complex and requires more proof to substantiate, recent advances in the regulatory mechanisms involved in phytohormones help further understand rice tillering.

Tiller number and angle are major determinants of rice plant architecture. New plant type known as ideal plant architecture (IPA) is proposed with reduced tiller number with almost no unproductive tillers to improve cultivar yield potential. A major QTL for IPA encoding SOUAMOSA PROMOTER BINDING PROTEIN-LIKE 14 (OsSPL14) has been cloned. *OsSPL14* is regulated by microRNA OsmiR156, and increasing level of the *OsSPL14* transcript and protein results in an IPA phenotype and higher grain productivity [27, 28]. *PROSTRATE*

GROWTH 1(*PROG1*) controlling wide tiller angle and great number of tillers in wild rice species encodes a zinc-finger nuclear transcription factor and is highly expressed in the axillary meristems. An amino acid substitution caused by a SNP in *PROG1* leads to the transition from prostate growth of the wild rice *O. rufipogon* to erect growth of the domesticated rice *O. sativa* [29, 30]. In addition, *qGY2-1*, a major QTL for grain yield per plant, encodes leucine-rich repeat receptor-like kinase (LRK), and over-express of *LRK1* causes more tillers and greater grain yield than the wild type [31].

3.2. QTLs/genes regulating number of grains per panicle

Number of grains per panicle is an important agronomic trait for grain productivity, which is determined by the panicle formation. During the past two decades, many genes/QTLs controlling panicle development have been characterized (Figure 2 and Table 1). Rice panicle developed from a terminal inflorescence at the top of a stem contains panicle axis, primary and secondary branches, pedicel and spikelets. Pedicels arise from the primary and secondary branches and bear spikelets on the top. Panicles and the bearing spikelets on them directly determine the rice yield.

Inflorescence development determines the formation of rice panicle. Inflorescence meristem generates primary and secondary branches meristems, and subsequent spikelet meristems. Several genes involved in the formation of inflorescence branch and spikelet meristems are identified through mutant analysis. *LAX1* encodes a basic helix-loop-helix (bHLH) transcription factor and is required for the initiation/maintenance of inflorescence branch meristems. The *lax1* mutant produces severely reduced primary and secondary branches and spikelets [32]. *FRIZZY PANICLE* (*FZP*), which encodes an ethylene-responsive element-binding factor (ERF), is responsible for the establishment of floral meristem identity through suppressing the formation of axillary meristems within the spikelet meristem. The *fzp* mutant is deficient in spikelet development and exhibits sequential rounds of branching instead of the formation of florets [33]. *ABERRANT PANICLE ORGNIZATION* (*APO1*), which encodes an F-box protein, functions in preventing the precocious transition from branch meristems to spikelet meristems. The *apo1* loss-of-function mutants produce small panicles with greatly reduced branches and spikelets [34]. In addition, *APO2* interacts with *APO1* to regulate panicle development [35].

Rice panicle size is largely determined by the number and length of primary and secondary branches. *SHORT PANICLE 1* (*SP1*) encodes a putative transporter of the peptide transporter family and participates in the elongation of rice panicle. The mutation of *SP1* causes a short-panicle phenotype due to the defect in the elongation of inflorescence branches in the *sp1* mutant [36]. *OsSPL14* not only controls tillering, but also promotes panicle branching and produces larger panicles with more spikelets [27, 28].

Rice panicle architecture is mainly determined by the arrangement of primary and secondary branches and grain density. Erect panicle is an important agronomic trait closely related to grain yield. *DENSE AND ERECT PANICLE1* (*DEP1*) encodes a phosphatidylethanolamine-binding (PEBP) protein-like domain protein and controls panicle branches, grain density and erect panicle. The gain-of-function mutation in *DEP1* resulted in the phenotype of increased primary and secondary branches and number of grains per panicle, and decreased panicle

length [37]. *DEP2*, which encodes a plant-specific protein and is strongly expressed in young panicles, is responsible for panicle outgrowth and elongation. The *dep2* mutant displays a dense and erect panicle phenotype [38].

Cytokinins regulate number of spikelets per panicle. A major QTL, *GRAIN NUMBER1* (*Gn1a*), which encodes cytokinin oxidase/dehydrogenase (OsCKX2), controls number of grains per panicle. Repression of *OsCKX2* leads to cytokinin accumulation, which finally results in the increase of number of grains per panicle and grain yield [39]. *LONELY GUY* (*LOG*) is responsible for shoot meristem activity and encodes cytokinin-activating enzyme for the conversion from inactive cytokinin nucleotides to the free-base forms. Loss of function of *LOG* results in producing small panicles with reduced panicle branches and grains in the *log* mutant [40]. *LARGER PANICLE* (*LP*) encoding a Kelch repeat-containing F-box protein regulates panicle architecture. Larger panicle with more primary branches and grains is observed in the *LP* loss-of-function mutants. *LP* could regulate panicle architecture by modulating cytokinin level due to the significant down-regulation of *OsCKX2* expression level in the mutants [41]. Furthermore, *DEP1* might control the number of panicle branches through cytokinin pathway because expression level of *OsCKX2* is clearly down-regulated in NIL-dep1 plant [37]. These studies imply that the phytohormone cytokinin plays a vital role in regulating panicle development.

3.3. QTLs/genes controlling grain weight

Rice grain is closely enclosed by a hull which is composed of one palea, lemma, rachilla and two sterile lemmas. A brown rice mainly consists of bran, endosperm and embryo. During the process of grain filling, endosperm cells expand and accumulate a massive amount of nutrients, mainly starch. Rice grain weight is largely determined by the endosperm size. Dozens of genes/QTLs involved in rice grain weight have been isolated and molecularly characterized (Figure 2 and Table 1).

Given that each grain in a rice panicle can be fully filled, grain weight is determined by grain size, which can be measured with grain length, width and thickness. *GS3*, *GL3.1*/*qGL3* and *TGW6*, three major QTLs controlling grain length, are map-based cloned and functionally analyzed [42-45]. *GS3* encodes a putative trans-membrane protein containing four putative domains, a plant-specific organ size regulation (OSR) domain, a trans-membrane domain, a tumor necrosis factor receptor/nerve growth factor receptor (TNFR/NGFR) family cysteine-rich domain and a von Willebrand factor type C (VWFC). Loss-of-function or deletion of plant-specific OSR domain results in long grain phenotype [42]. *GL3.1*/*qGL3* encodes Ser/Thr phosphatase of phosphatase kelch family to regulate grain length and yield. *GL3.1*/*qGL3* directly down-regulates Cyclin-T1;3 to dephosphorylate Cyclin-T1;3 and results in short grain shape [43,44]. *THOUSAND-GRAIN WEIGHT 6* (*TGW6*) controls grain length and weight, which expression is especially high around the endosperm in the pericarp. *TGW6* possesses indole-3-acetic acid (IAA)-glucose hydrolase to decompose IAA-glucose into IAA and glucose, which influences the transition timing from the syncytial to the cellular phase and results in short grain phenotype [45]. *SMALL AND ROUND SEED* (*SRS3*), which encodes a protein of the kinesin 13 subfamily containing a kinesin motor domain and a coiled-coil structure, is strongly expressed in developing organs and regulates rice grain length. The *srs3* mutant

shows shorter cells compared to the wild type, which causes the small and round seed phenotype [46]. *Srs5* encodes alpha-tubulin protein and its mutation produces a semi-dominant mutant exhibiting similar phenotype with the *srs3* mutant [47]. *POSITIVE REGULATOR OF GRAIN LENGTH 1*(*PGL 1*) and *ANTAGONIST OF PGL1* (*APG*) encode an antagonistic pair of bHLH proteins and interact to regulate rice grain length [48]. *PGL 1* and *PGL 2* redundantly suppress the function of *APG* to form elongated grains [49]. In addition, brassinosteroid (BR) pathway affects rice grain size. A series of mutants related to the synthesis and signaling pathway of BR such as *d61*, *brd1*and *short grain1* (*sg1*) display shorter grain phenotype than their wild types [50-52].

Four QTLs conditioning grain width, *GW2*, *qSW5*/*GW5*, *GS5* and *GW8*, have been isolated and characterized. *GW2* encodes a RING-type protein with E3 ubiquitin ligase activity to function in the protein degradation through the ubiquitin-proteasome pathway. *GW2 E3* ligase negatively regulates cell division and the mutant allele of *GW2* promotes spikelet hull cell division to result in an increase of grain width and weight [53]. *GW5* and *qSW5* are the same QTL on chromosome 5 in fact, identified by two research groups separately [54, 55]. *qSW5*/*GW5* encodes a novel nuclear protein, physically interacting with polyubiquitin and acting in the ubiquitin-proteasome pathway to regulate cell division. *qSW5*/*GW5* is also a negative regulator for grain width and its mutant allele causes an increase of grain width [54]. *GS5* encodes a putative serine carboxypeptidase and positively regulates grain width. Over expression of *GS5* promotes cell division and results in increased grain width [56]. *GW8*, synonym with *OsSPL16*, encodes a positive regulator of cell proliferation and conditioning grain width and yield. Enhanced expression level of *GW8* promotes cell division and grain filling, while its loss-of-function forms a slender grain [57].

Grain thickness largely depends on the ability of grain filling. *GRAIN INCOMPLETE FILLING 1*(*GIF1*), which encodes a cell-wall invertase to download sucrose in the ovular and stylar vascular tissues and hydrolyzes sucrose to glucose and fructose for the starch synthesis in the endosperm, is responsible for rice grain-filling and yield. Mutation in the *GIF1* causes slower grain-filling to result in reduced levels of glucose, fructose and sucrose in the *gif1* mutants. Compared to the wild type *GIF1*, the cultivated *GIF1* displays a restricted expression during the filling stage to bring about grain weight increase [58]. Expression level of *GIF1* is substantially low in the *heading and grain weight* (*hgw*) mutant, which delays the heading date and reduces grain weight. *HGW* encodes a novel plant-specific ubiquitin-associated domain protein and acts through *GIF1* to regulate grain width and weight [59]. *FLOURY ENDOSPERM2* (*FLO2*) encodes a protein harboring a tetratricopeptide repeat motif and preferentially expresses in developing seeds. *FLO2* positively regulates the expression of genes involved in production of storage starch and proteins in the endosperm, so mutation of *FLO2* causes significantly smaller grain size phenotype [60].

4. QTLs/genes for rice yield-related traits

Plant height and heading date are two important agronomic traits closely related to rice yield. The Green Revolution has made a tremendous contribution to solve the global food crisis, and

this mark achievement in rice is caused by the application of *sd1* gene. *SD1* encodes an oxidase enzyme involved in the biosynthesis pathway of gibberellin, which is one of the most important determining factors of plant height. Mutation of *SD1* produces semi-dwarf phenotype and significantly increase rice yield [61].

Genes/QTLs controlling heading date usually prolong the duration of panicle differentiation to produce more spikelets per panicle and enhance grain yield potential. *Ghd7* and *Ghd8*/*DTH8* are key genes regulating heading date to enhance grain yield and plant height under long-day conditions. *Ghd7* encodes a CCT domain protein and pleiotropically controls an array of traits such as number of grains per panicle, plant height and heading date. Increased expression level of *Ghd7* under long-day conditions suppresses the expression of *Hd3a*, which results in delaying heading date and prolongs the duration of panicle differentiation [62]. Similarly, *Ghd8*/*DTH8* encodes the OsHAP3 subunit of a CCAAT-box binding protein and simultaneously regulates grain yield, heading date, and plant height. *Ghd8*/*DTH8* can down-regulate the express level of *Early heading date 1*(*Ehd1*) and *Hd3a* under long-day conditions, which leads to delay heading date and produce 50% more grains per plant [63, 64]. *Ehd1* and *Hd1* can regulate panicle development. Increased expression level of *Hd3a* and *RFT1* reduces number of primary branches per panicle in the line combining *Hd1* and *Ehd1* [65, 66]. In addition, *Hd1* increases number of spikelets per panicle and grain yield by suppressing *Hd3a* expression and delaying heading date [67].

5. Future perspectives

As mentioned above, cloning and functional characterization of genes/QTLs have greatly strengthened our understanding in the genetic and molecular mechanisms underlying rice yield traits, which has facilitated the breeding efforts for higher yield potential varieties. Pyramiding of favorable genes/QTLs has become an efficient strategy in rice genetic improvement and is widely adopted by rice breeders. For instance, combination of *Gn1* (*Gn1a*+*Gn1b*) and *sd1* into Koshihikari has simultaneously improved two traits with increased grain numbers per plant by 23% and reduced plant height by 18% as compared to wild type Koshihikari [39]. The NIL (*GW8*/*gs3*) with a pyramiding of *GW8* and *gs3* produces longer and wider grains than the wild type NIL (*gw8*/*GS3*) [57].

Although tremendous progress has been made in the studies of rice yield trait, there is still a long way to clearly elucidate the molecular mechanisms responsible for the formation of rice yield traits. Almost all the rice yield traits including number of panicles per plant, number of grains per panicle and grain weight exhibit comprehensive and continuous variations in the genetic population or among the commercial varieties, typically due to the function of multiple genes called as QTLs. According to the Gramene database, thousands of QTLs conditioning rice yield traits have been detected by QTL mapping and majorities of them are minor QTLs with small genetic effect, which are difficult to be identified through mutant analysis. However, minor QTLs may participate in different molecular pathways to regulate rice yield traits and play a vital role in improving yield potential. During the long domestication process, these

minor QTLs have been selected and combined relying on the breeders' experience to develop cultivated varieties. Therefore, more efforts are necessary to isolate minor QTLs and elucidate the functional mechanisms in the future.

Natural variation exists widely in the genes/QTLs, resulting in many alleles for each gene/QTL. For example, *Ghd7* has at least five alleles including *Ghd7-0*, *Ghd7-0a*, *Ghd7-1*, *Ghd7-2* and *Ghd7-3* which enable rice to be cultivated in different ecotype regions. Till now, it is still rather difficult to combine favorable alleles freely in breeding higher yield potential varieties. Mining the alleles is a key base to combine the alleles. Based on the affordable next-generation sequencing technology, association mapping is a promising strategy to mine favorable alleles using a set of diverse germplasm accessions. On the basis of available and favorable alleles, an efficient breeding strategy has been proposed to exploit rice yield potential, involved in identifying the genes/QTLs for rice yield traits, mining alleles of target genes/QTLs through candidate-gene association mapping, developing functional markers and combining favorable alleles in cultivated varieties (Figure 3).

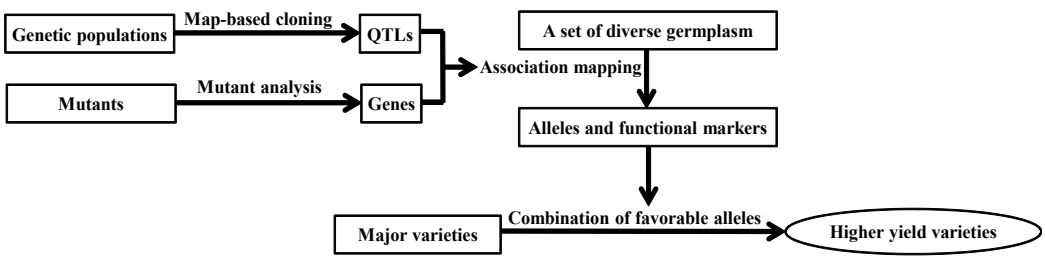


Figure 3. Flowchart for depicting a new strategy to breed higher yield varieties

Acknowledgements

This work was supported by the Chinese High-Yielding Transgenic Program (2013ZX08001004-006), the Chinese 863 Program (2012AA101102), and the Zhejiang Provincial Natural Science Foundation (Y3110394). We are grateful to Dr. Jie-Yun Zhuang at China National Rice Research Institute for valuable discussions and suggestions.

Author details

Jie-Zheng Ying*, Yu-Yu Chen and Hong-Wei Zhang

*Address all correspondence to: yingjiezheng@caas.cn

State Key Laboratory of Rice Biology and Chinese National Center for Rice Improvement, China National Rice Research Institute, Hangzhou, China

References

[1] Khush GS: Green revolution: the way forward. *Nat Rev Genet* 2001, 2:815-822.

[2] Cheng SH, Zhuang JY, Fan YY, Du JH, Cao LY: Progress in research and development on hybrid rice: a super-domesticate in China. *Ann Bot* 2007, 100:959-966.

[3] Jeon JS, Jung KH, Kim HB, Suh JP, Khush GS: Genetic and Molecular Insights into the Enhancement of Rice Yield Potential. *JOURNAL OF PLANT BIOLOGY* 2011, 54:1-9.

[4] Xing Y, Zhang Q: Genetic and molecular bases of rice yield. *Annu Rev Plant Biol* 2010, 61:421-442.

[5] Ying JZ, Gao JP, Shan JX, Zhu MZ, Shi M, Lin HX: Dissecting the genetic basis of extremely large grain shape in rice cultivar 'JZ1560'. *J Genet Genomics* 2012, 39:325-333.

[6] Lin YJ, Zhang Q: Optimising the tissue culture conditions for high efficiency transformation of indica rice. *Plant Cell Rep* 2005, 23:540-547.

[7] Nishimura A, Aichi I, Matsuoka M: A protocol for Agrobacterium-mediated transformation in rice. *Nat Protoc* 2006, 1:2796-2802.

[8] Lee SY, Ahn JH, Cha YS, Yun DW, Lee MC, Ko JC, Lee KS, Eun MY: Mapping of quantitative trait loci for salt tolerance at the seedling stage in rice. *Mol Cells* 2006, 21:192-196.

[9] Andaya VC, Mackill DJ: QTLs conferring cold tolerance at the booting stage of rice using recombinant inbred lines from a japonica x indica cross. *Theor Appl Genet* 2003, 106:1084-1090.

[10] Xing Z, Tan F, Hua P, Sun L, Xu G, Zhang Q: Characterization of the main effects, epistatic effects and their environmental interactions of QTLs on the genetic basis of yield traits in rice. *Theor Appl Genet* 2002, 105:248-257.

[11] Zhuang JY, Fan YY, Rao ZM, Wu JL, Xia YW, Zheng KL: Analysis on additive effects and additive-by-additive epistatic effects of QTLs for yield traits in a recombinant inbred line population of rice. *Theor Appl Genet* 2002, 105:1137-1145.

[12] Wissuwa M, Ismail AM, Yanagihara S: Effects of zinc deficiency on rice growth and genetic factors contributing to tolerance. *Plant Physiol* 2006, 142:731-741.

[13] Onishi K, Horiuchi Y, Ishigoh-Oka N, Takagi K, Ichikawa N, Maruoka M, Sano Y: A QTL cluster for plant architecture and its ecological significance in Asian wild rice. *BREEDING SCIENCE* 2007, 57:7-16.

[14] Project IRGS: The map-based sequence of the rice genome. *Nature* 2005, 436:793-800.

[15] Bai X, Wu B, Xing Y: Yield-related QTLs and their applications in rice genetic improvement. *J Integr Plant Biol* 2012, 54:300-311.

[16] Shao G, Tang S, Luo J, Jiao G, Wei X, Tang A, Wu J, Zhuang J, Hu P: Mapping of qGL7-2, a grain length QTL on chromosome 7 of rice. *J Genet Genomics* 2010, 37:523-531.

[17] Hao W, Zhu MZ, Gao JP, Sun SY, Lin HX: Identification of quantitative trait loci for rice quality in a population of chromosome segment substitution lines. *J Integr Plant Biol* 2009, 51:500-512.

[18] mai I, Kimball J, Conway B, Yeater K, McCouch S, McClung A: Validation of yield-enhancing quantitative trait loci from a low-yielding wild ancestor of rice. *Mol Breeding* 2013:1-20.

[19] Li X, Qian Q, Fu Z, Wang Y, Xiong G, Zeng D, Wang X, Liu X, Teng S, Hiroshi F, et al.: Control of tillering in rice. *Nature* 2003, 422:618-621.

[20] Gomez-Roldan V, Fermas S, Brewer PB, Puech-Pages V, Dun EA, Pillot JP, Letisse F, Matusova R, Danoun S, Portais JC, et al.: Strigolactone inhibition of shoot branching. *Nature* 2008, 455:189-194.

[21] Umehara M, Hanada A, Yoshida S, Akiyama K, Arite T, Takeda-Kamiya N, Magome H, Kamiya Y, Shirasu K, Yoneyama K, et al.: Inhibition of shoot branching by new terpenoid plant hormones. *Nature* 2008, 455:195-200.

[22] Zou J, Zhang S, Zhang W, Li G, Chen Z, Zhai W, Zhao X, Pan X, Xie Q, Zhu L: The rice *HIGH-TILLERING DWARF1* encoding an ortholog of Arabidopsis *MAX3* is required for negative regulation of the outgrowth of axillary buds. *Plant J* 2006, 48:687-698.

[23] Arite T, Iwata H, Ohshima K, Maekawa M, Nakajima M, Kojima M, Sakakibara H, Kyozuka J: *DWARF10*, an *RMS1*/*MAX4*/*DAD1* ortholog, controls lateral bud outgrowth in rice. *Plant J* 2007, 51:1019-1029.

[24] Lin H, Wang R, Qian Q, Yan M, Meng X, Fu Z, Yan C, Jiang B, Su Z, Li J, et al.: DWARF27, an iron-containing protein required for the biosynthesis of strigolactones, regulates rice tiller bud outgrowth. *Plant Cell* 2009, 21:1512-1525.

[25] Ishikawa S, Maekawa M, Arite T, Onishi K, Takamure I, Kyozuka J: Suppression of tiller bud activity in tillering dwarf mutants of rice. *Plant Cell Physiol* 2005, 46:79-86.

[26] Arite T, Umehara M, Ishikawa S, Hanada A, Maekawa M, Yamaguchi S, Kyozuka J: *d14*, a strigolactone-insensitive mutant of rice, shows an accelerated outgrowth of tillers. *Plant Cell Physiol* 2009, 50:1416-1424.

[27] Miura K, Ikeda M, Matsubara A, Song XJ, Ito M, Asano K, Matsuoka M, Kitano H, Ashikari M: *OsSPL14* promotes panicle branching and higher grain productivity in rice. *Nat Genet* 2010, 42:545-549.

[28] Jiao Y, Wang Y, Xue D, Wang J, Yan M, Liu G, Dong G, Zeng D, Lu Z, Zhu X, et al.: Regulation of *OsSPL14* by OsmiR156 defines ideal plant architecture in rice. *Nat Genet* 2010, 42:541-544.

[29] Jin J, Huang W, Gao JP, Yang J, Shi M, Zhu MZ, Luo D, Lin HX: Genetic control of rice plant architecture under domestication. *Nat Genet* 2008, 40:1365-1369.

[30] Tan L, Li X, Liu F, Sun X, Li C, Zhu Z, Fu Y, Cai H, Wang X, Xie D, et al.: Control of a key transition from prostrate to erect growth in rice domestication. *Nat Genet* 2008, 40:1360-1364.

[31] Zha X, Luo X, Qian X, He G, Yang M, Li Y, Yang J: Over-expression of the rice *LRK1* gene improves quantitative yield components. *Plant Biotechnol J* 2009, 7:611-620.

[32] Komatsu M, Maekawa M, Shimamoto K, Kyozuka J: The *LAX1* and *FRIZZY PANICLE 2* genes determine the inflorescence architecture of rice by controlling rachis-branch and spikelet development. *Dev Biol* 2001, 231:364-373.

[33] Komatsu M, Chujo A, Nagato Y, Shimamoto K, Kyozuka J: *FRIZZY PANICLE* is required to prevent the formation of axillary meristems and to establish floral meristem identity in rice spikelets. *Development* 2003, 130:3841-3850.

[34] Ikeda K, Ito M, Nagasawa N, Kyozuka J, Nagato Y: Rice *ABERRANT PANICLE ORGANIZATION 1*, encoding an F-box protein, regulates meristem fate. *Plant J* 2007, 51:1030-1040.

[35] Ikeda-Kawakatsu K, Maekawa M, Izawa T, Itoh J, Nagato Y: *ABERRANT PANICLE ORGANIZATION 2/RFL*, the rice ortholog of Arabidopsis LEAFY, suppresses the transition from inflorescence meristem to floral meristem through interaction with APO1. *Plant J* 2012, 69:168-180.

[36] Li S, Qian Q, Fu Z, Zeng D, Meng X, Kyozuka J, Maekawa M, Zhu X, Zhang J, Li J, et al.: *Short panicle1* encodes a putative PTR family transporter and determines rice panicle size. *Plant J* 2009, 58:592-605.

[37] Huang X, Qian Q, Liu Z, Sun H, He S, Luo D, Xia G, Chu C, Li J, Fu X: Natural variation at the *DEP1* locus enhances grain yield in rice. *Nat Genet* 2009, 41:494-497.

[38] Li F, Liu W, Tang J, Chen J, Tong H, Hu B, Li C, Fang J, Chen M, Chu C: Rice *DENSE AND ERECT PANICLE 2* is essential for determining panicle outgrowth and elongation. *Cell Res* 2010, 20:838-849.

[39] Ashikari M, Sakakibara H, Lin S, Yamamoto T, Takashi T, Nishimura A, Angeles ER, Qian Q, Kitano H, Matsuoka M: Cytokinin oxidase regulates rice grain production. *Science* 2005, 309:741-745.

[40] Kurakawa T, Ueda N, Maekawa M, Kobayashi K, Kojima M, Nagato Y, Sakakibara H, Kyozuka J: Direct control of shoot meristem activity by a cytokinin-activating enzyme. *Nature* 2007, 445:652-655.

[41] Li M, Tang D, Wang K, Wu X, Lu L, Yu H, Gu M, Yan C, Cheng Z: Mutations in the F-box gene *LARGER PANICLE* improve the panicle architecture and enhance the grain yield in rice. *Plant Biotechnol J* 2011, 9:1002-1013.

[42] Mao H, Sun S, Yao J, Wang C, Yu S, Xu C, Li X, Zhang Q: Linking differential domain functions of the GS3 protein to natural variation of grain size in rice. *Proc Natl Acad Sci U S A* 2010, 107:19579-19584.

[43] Zhang X, Wang J, Huang J, Lan H, Wang C, Yin C, Wu Y, Tang H, Qian Q, Li J, et al.: Rare allele of *OsPPKL1* associated with grain length causes extra-large grain and a significant yield increase in rice. *Proc Natl Acad Sci U S A* 2012, 109:21534-21539.

[44] Qi P, Lin YS, Song XJ, Shen JB, Huang W, Shan JX, Zhu MZ, Jiang L, Gao JP, Lin HX: The novel quantitative trait locus *GL3.1* controls rice grain size and yield by regulating Cyclin-T1;3. *Cell Res* 2012, 22:1666-1680.

[45] Ishimaru K, Hirotsu N, Madoka Y, Murakami N, Hara N, Onodera H, Kashiwagi T, Ujiie K, Shimizu B, Onishi A, et al.: Loss of function of the IAA-glucose hydrolase gene *TGW6* enhances rice grain weight and increases yield. *Nat Genet* 2013, 45:707-711.

[46] Kitagawa K, Kurinami S, Oki K, Abe Y, Ando T, Kono I, Yano M, Kitano H, Iwasaki Y: A novel kinesin 13 protein regulating rice seed length. *Plant Cell Physiol* 2010, 51:1315-1329.

[47] Segami S, Kono I, Ando T, Yano M, Kitano H, Miura K, Iwasaki Y: *Small and round seed 5* gene encodes alpha-tubulin regulating seed cell elongation in rice - Springer. *Rice* 2012, 5:4.

[48] Heang D, Sassa H: Antagonistic actions of HLH/bHLH proteins are involved in grain length and weight in rice. *PLoS One* 2012, 7:e31325.

[49] Heang D, Sassa H: An atypical bHLH protein encoded by *POSITIVE REGULATOR OF GRAIN LENGTH 2* is involved in controlling grain length and weight of rice through interaction with a typical bHLH protein APG. *Breed Sci* 2012, 62:133-141.

[50] Yamamuro C, Ihara Y, Wu X, Noguchi T, Fujioka S, Takatsuto S, Ashikari M, Kitano H, Matsuoka M: Loss of function of a rice brassinosteroid insensitive1 homolog prevents internode elongation and bending of the lamina joint. *Plant Cell* 2000, 12:1591-1606.

[51] Mori M, Nomura T, Ooka H, Ishizaka M, Yokota T, Sugimoto K, Okabe K, Kajiwara H, Satoh K, Yamamoto K, et al.: Isolation and characterization of a rice dwarf mutant with a defect in brassinosteroid biosynthesis. *Plant Physiol* 2002, 130:1152-1161.

[52] Nakagawa H, Tanaka A, Tanabata T, Ohtake M, Fujioka S, Nakamura H, Ichikawa H, Mori M: *Short grain1* decreases organ elongation and brassinosteroid response in rice. *Plant Physiol* 2012, 158:1208-1219.

[53] Song XJ, Huang W, Shi M, Zhu MZ, Lin HX: A QTL for rice grain width and weight encodes a previously unknown RING-type E3 ubiquitin ligase. *Nat Genet* 2007, 39:623-630.

[54] Weng J, Gu S, Wan X, Gao H, Guo T, Su N, Lei C, Zhang X, Cheng Z, Guo X, et al.: Isolation and initial characterization of *GW5*, a major QTL associated with rice grain width and weight. *Cell Res* 2008, 18:1199-1209.

[55] Shomura A, Izawa T, Ebana K, Ebitani T, Kanegae H, Konishi S, Yano M: Deletion in a gene associated with grain size increased yields during rice domestication. *Nat Genet* 2008, 40:1023-1028.

[56] Li Y, Fan C, Xing Y, Jiang Y, Luo L, Sun L, Shao D, Xu C, Li X, Xiao J, et al.: Natural variation in *GS5* plays an important role in regulating grain size and yield in rice. *Nat Genet* 2011, 43:1266-1269.

[57] 57. Wang S, Wu K, Yuan Q, Liu X, Liu Z, Lin X, Zeng R, Zhu H, Dong G, Qian Q, et al.: Control of grain size, shape and quality by *OsSPL16* in rice. *Nat Genet* 2012, 44:950-954.

[58] Wang E, Wang J, Zhu X, Hao W, Wang L, Li Q, Zhang L, He W, Lu B, Lin H, et al.: Control of rice grain-filling and yield by a gene with a potential signature of domestication. *Nat Genet* 2008, 40:1370-1374.

[59] Li J, Chu H, Zhang Y, Mou T, Wu C, Zhang Q, Xu J: The rice *HGW* gene encodes a ubiquitin-associated (UBA) domain protein that regulates heading date and grain weight. *PLoS One* 2012, 7:e34231.

[60] She KC, Kusano H, Koizumi K, Yamakawa H, Hakata M, Imamura T, Fukuda M, Naito N, Tsurumaki Y, Yaeshima M, et al.: A novel factor *FLOURY ENDOSPERM2* is involved in regulation of rice grain size and starch quality. *Plant Cell* 2010, 22:3280-3294.

[61] Sasaki A, Ashikari M, Ueguchi-Tanaka M, Itoh H, Nishimura A, Swapan D, Ishiyama K, Saito T, Kobayashi M, Khush GS, et al.: Green revolution: a mutant gibberellin-synthesis gene in rice. *Nature* 2002, 416:701-702.

[62] Xue W, Xing Y, Weng X, Zhao Y, Tang W, Wang L, Zhou H, Yu S, Xu C, Li X, et al.: Natural variation in *Ghd7* is an important regulator of heading date and yield potential in rice. *Nat Genet* 2008, 40:761-767.

[63] Wei X, Xu J, Guo H, Jiang L, Chen S, Yu C, Zhou Z, Hu P, Zhai H, Wan J: *DTH8* suppresses flowering in rice, influencing plant height and yield potential simultaneously. *Plant Physiol* 2010, 153:1747-1758.

[64] Yan WH, Wang P, Chen HX, Zhou HJ, Li QP, Wang CR, Ding ZH, Zhang YS, Yu SB, Xing YZ, et al.: A major QTL, *Ghd8*, plays pleiotropic roles in regulating grain productivity, plant height, and heading date in rice. *Mol Plant* 2011, 4:319-330.

[65] Endo-Higashi N, Izawa T: Flowering time genes *Heading date 1* and *Early heading date 1* together control panicle development in rice. *Plant Cell Physiol* 2011, 52:1083-1094.

[66] Doi K, Izawa T, Fuse T, Yamanouchi U, Kubo T, Shimatani Z, Yano M, Yoshimura A: Ehd1, a B-type response regulator in rice, confers short-day promotion of flowering and controls FT-like gene expression independently of Hd1. *Genes Dev* 2004, 18:926-936.

[67] Zhang ZH, Wang K, Guo L, Zhu YJ, Fan YY, Cheng SH, Zhuang JY: Pleiotropism of the photoperiod-insensitive allele of *Hd1* on heading date, plant height and yield traits in rice. *PLoS One* 2012, 7:e52538.

Chapter 7

Current Advances on Genetic Resistance to Rice Blast Disease

Xueyan Wang, Seonghee Lee, Jichun Wang, Jianbing Ma, Tracy Bianco and Yulin Jia

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/56824

1. Introduction

1.1. The historical and contemporary aspects of rice blast disease

Rice (*Oryza sativa L.*) is one of the most important staple foods that feed more than half of the world's population, with Asia and Africa as the largest consuming regions [1]. Blast disease caused by *Magnaporthe oryzae* (Hebert) Barr is one of the most damaging diseases of rice. This disease was first known as rice fever disease in China as early as 1637 [2]. Blast disease was first reported in the United States in 1876, and has been identified in 85 rice-producing countries or regions worldwide (Figure 1).

Blast severely affects lowland rice in temperate and subtropical areas of Asia, and is highly destructive to upland rice in tropical areas of Asia, Latin America, and Africa [3]. Although blast is considered the most destructive rice disease due to the favorable environmental conditions for disease occurrence and worldwide distribution, little information about annual yield losses are available. Table 1 summarizes reported blast outbreaks with annual yield losses from five countries. In China, 40-50% yield losses were observed under severe rice blast infection; in some cases, 100% yield losses were found in severely infected fields [4]. Yield losses of 5-10%, 8%, and 14% were reported in India from 1960 to 1961, Korea from the mid-1970s, and China from 1980 to 1981, respectively [3]. The highest yield losses were recorded in the Philippines; ranging from 50% to 85% in 1963 [3]. It was estimated that 1.6 billion dollars were lost from 1975-1990 due to blast disease worldwide [5]. The estimated annual loss of rice was enough to feed 60 million people for one year [6] (Table 1).

Figure 1. Worldwide distribution of rice blast disease. Red dots show the countries or regions where blast disease has been reported.

Yield loss (%)	Country	Year
5-10	India	1960-61
50-60	Philippines	1963
70-85	Philippines	1969-70
8	Korea	mid-70s
14	China	1980-81
60	Thailand	1982

Source: http://www.knowledgebank.irri.org/ipm/rice-blast.html

Table 1. Yield losses due to blast.

1.2. The biology of *M. oryzae*

The most common symptoms in commercial rice fields induced by *M. oryzae* can be found on all the above ground parts of the rice plant at all growth stages. Seeds display brown spots after infection, which may have resulted from the infection of the florets as they mature into seeds. Infected roots have also been observed; however, lesions on the sheaths were relatively rare. Infections on young seedlings are initiated when the conidia are deposited on the surface of the leaves. Water is essential for spores to germinate and attach to the leaf surface [7, 8]. Under optimal conditions, spore germination occurs rapidly and the polarized germ tubes are

formed within hours after landing on the leaf [9]. The secondary cycles are initiated by the spores produced by the lesions on the young seedlings, which can be repeated many times through the growing season. Thousands of spores can be produced from a single lesion in 15 days after infection. Typically blast lesions are diamond shaped (Figure 2A). Initial lesions appear dark green or grey with brown borders; while, older lesions are light tan with necrotic borders. Under favorable conditions, lesions can merge together and rapidly enlarge to several centimeters in length, eventually killing the leaf, and ultimately resulting in plant death. On resistant cultivars, lesions induced by *M. oryzae* usually remain small in size (1-2mm) and appear brown to dark brown in color. Disease severity of rice blast and the amount of spores produced on single lesion depends on temperature, field conditions, relative humidity, fertilization levels, and genotype of rice cultivars. In general, moderate temperatures (~24°C), high relative humidity (90-92%), and high moisture with at least a 12-hour period are advantageous for rice blast. The disease severity of the vegetative phase during the growing season highly influences the amount of disease during the reproductive phase. Spores produced at the end of the growing season may result in collar blast and neck blast; neck blast often causes direct crop loss (Figure 2B).

Existence of physiological races of *M. oryzae* complicates the identification of resistance *(R)* genes. Physiological races of *M. oryzae* were first reported by Sasaki in Japan as early as 1922 [10]. From 1950s to 60s differential rice lines resistant to races of *M. oryzae* were identified in Japan, the United States, India, the Philippines, and South Korea. In 1961, 18 physiological races of *M. oryzae* were identified with 12 differential rice varieties in Japan. During that time, an international differential system using 8 rice varieties was established [11]. In China, the identification of *M. oryzae* races was initiated in late 1970s. Seven rice varieties, Tebo, Zhenlong13, Sifeng43, Dongnong363, Kanto51, Hejiang18, and Lijiangxintuanheigu (LTH), and 43 isolates of *M. oryzae* were used. In 1976, Yamada and his colleagues identified 23 races of *M. oryzae* from 2245 isolates with 9 differential rice varieties. Duan et al [12] used Yuyun 1 (with *Pia*), Gaoliangdao (with *Pii*), Kanto51 (with *Pik*), Chugeng1 (with *Pikm*), Dianyu1 × Fook Kam (with *Piz*), Dali782 (with *Pita*), Dan83-3 (with *Pita2*), and Chengbao1 (with*Pizt*) as differential varieties to characterize races of *M. oryzae* in China. These blast *R* genes are described in more details in the part II of this chapter. Near-isogenic lines (NILs) were chosen to better identify races of *M. oryzae* in a gene-for-gene specific manner. The NILs with *indica* high-susceptible variety CO39 background was developed at the International Rice Research Institute (IRRI), the Philippines [13]. In the United States, Marchetti [14] reported that the races IB-54, ID-13, IG-1, and IH-1 of *M. oryzae* were the most common. Most recently, monogenic lines with 24 major blast *R* genes in BC1 of LTH were developed by scientists at IRRI and Japan [15].

Extensive analysis of rice germplasm with physiological races in the past century reveals that complete genetic resistance (vertical resistance) is conferred by major blast *R* genes named as *Piricularia* genes or *Pi*-genes. These genes are often specific in preventing infections by strains of *M. oryzae* that contain the corresponding avirulence genes; whereas, incomplete resistance (slow-blasting components or horizontal resistance, field resistance, or dilatory resistance) is often conditioned by more than one gene on different chromosomal regions. These genes are

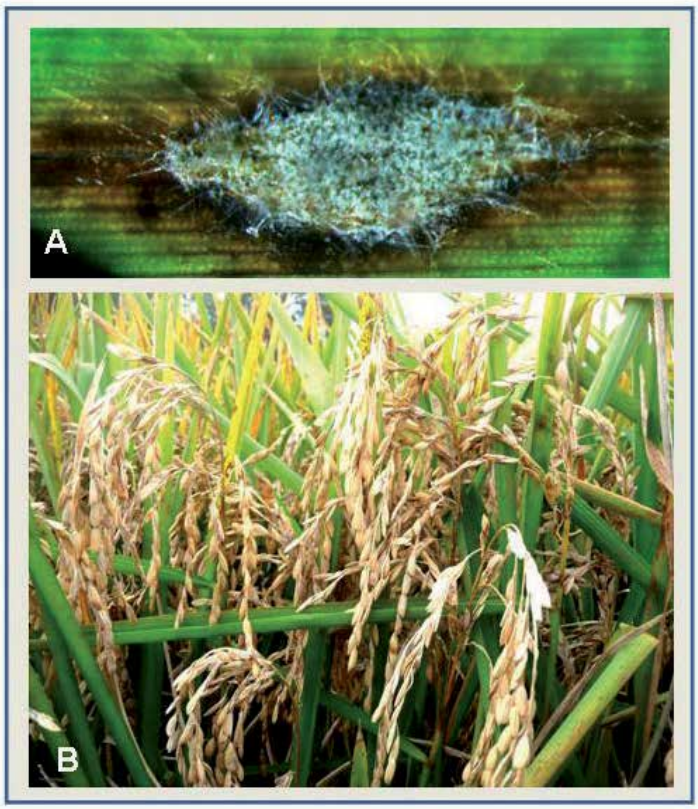


Figure 2. Symptoms of leaf (A) and neck blast diseases (B) in commercial rice fields.

referred to as quantitative resistant loci (QTLs). Resistant germpalsms carrying both major and minor *R* genes and are extremely important genetic resources that rice breeders can use to improve blast resistance in elite rice varieties.

2. Mapped blast *R* genes

Blast *R* genes are predicted to play important roles in the frontier of rice defense responses. During interactions between rice and blast pathogens, products of the *R* gene can specifically recognize the corresponding elicitors of *M. oryzae*. Since the *Pia* gene, indentified in 1967 by Kiyosawa as the first blast *R* gene from the *japonica* variety Aichi Asahi [16], 99 blast *R* genes have been identified; in which 45% were found in *japonica* cultivars, 51% in *indica* cultivars, and the rest 4% in wild rice species (Table 2 to 5). Most deployed *R* genes have often been identified in Asian cultivated rice, specially rice cultivars from Japan and China, with the exception of *Pi9*, *Pi54rh*, *Pi40(t)*, and *Pirf2-1(t)*, which were domesticated from *O. minuta*, *O.*

rhizomatis, *O. australiensis*, and *O. rufipogon*, respectively. All *R* genes have been mapped on all rice chromosomes except for chromosome 3 (Tables 2 to 4; Fig 3). Host genotypes, chromosomal loci, and molecular markers that are tightly linked to blast *R* genes are summarized in Figure 2 and Table 2 (60 major *R* gene) and Table 3 (17 minor *R* gene). Among them, three major *R* gene clusters have been well characeterized: the *Piz* locus on Chromosome 6, the *Pik* locus on Chromosome 11, and the *Pita* locus on Chromosome 12 (Figure 3). More detailed imformation of mapped blast *R* genes can be found at http://www.ricedata.cn/gene/, http://www.shigen.nig.ac.jp/rice/oryzabaseV4/, and http://www.gramene.com.

Chromosome	Major R genes mapped*	Minor R genes mapped*	*R gene cloned**	Total*
1	2	2	3	7
2	7	3	1	11
3	0	0	0	0
4	1	2	1	4
5	2	1	0	3
6	12	1	6	19
7	1	0	0	1
8	4	2	1	7
9	3	0	1	3
10	1	1	0	2
11	12	3	8	23
12	15	2	1	18
Total	60	17	22	99

* refers to number of the genes on the chromosome.

Table 2. Summary of blast *R* (major and minor, mapped and cloned) genes on rice chromosomes.

Chr.	Name of *R* gene	Name of germplasm	Map position (cM) >,#	Markers	Name of pathogenic Strains	Ref.
1	*Pi27(t)**	Q14	28.4-38.8	RM151, RM259	CHL0335, CHL0888, CHL0918	[17]
1	*Pitp(t)*	Tetep	114.1	RM246	IC9	[18]
2	*Pi14(t)*	Maowangu		Amp-1		[19]
2	*Pi16(t)*	Aus373		Amp-1	Hoko1, Ina72, TH67-22, Ai75-61	[20]
2	*PiDa(t)*	Dacca6	10.8-14.4	RM211, RM5529		[21]

Chr.	Name of *R* gene	Name of germplasm	Map position (cM) >#	Markers	Name of pathogenic Strains	Ref.
2	*Pid1(t)*	Digu	87.5-89.9	RM262	ZB13	[22]
2	*Pitq-5*	Teqing	150.5-157.5	RG520, RZ446b	IC-17, IB-49, IE-1, IG-1	[23]
2	*Pig(t)*	Guangchangzhan	142.0-154.1	RM166, RM208	Ken53-33	[24]
2	*Piy(t)*	Yanxian No.1	153.2-154.1	RM3284, RM208	97-27-2, Zhong10-8-14	[25]
4	*Pi39(t)**	Chubu 111	107.4-108.2	RM3743, RM5473		[26]
5	*Pi10(t)*	Tongil	88.5-102.8	RG13	IB46	[27]
5	*Pi23(t)*	Suweon 365	59.3-99.5			[28]
6	*Pi2-1*	Tianjingyeshengdao	Allilic to Pi2/9	AP4791, AP4007	CHL477, CHL473, P06-6, IC-17, 87-4	[29]
6	*Pi2-2*	Jefferson	58.7	RM19817, AP5659-5	HN318-2, CHL438, KJ201, ROR1, PO6-6	[30]
6	*Pi8(t)*	Kasalath	74.6-78.2	Amp-3	Race 447.1	[31]
6	*Pi13(t)**	Maowangu	74.6-78.2	Amp-3		[19]
6	*Pi13(t)**	Kasalath	67.7-68.5	RM2123, RM20155	Ken54-04, 95Mu-29, Ina86-137	[32]
6	*Pi22(t)*	Suweon 365	38.4-41.9		KJ-201	[28]
6	*Pi26(t)**	Gumei 2	51.0-61.6	B10, R674	Ca89	[33]
6	*Pi40(t)*	IR65482-4-136-2-2 *O. australiensis*	54.1-61.6	RM527, RM3330	KJ105, Ca89, PO6-6, M101-1-29-1, M64-1-3-9	[34]
6	*Pi50(t)*	Er-Ba-Zhan	46.8	GDAP51, GDAP16	09-3041a, SC0602, SCRB14, HN0102, W06-18a	[35]
6	*Pigm(t)*	Gumei 4	65.8	C5483, C0428	CH109 (ZC13), CH147 (ZB25), CH131 (ZA1)	[36]
6	*Piz*	Zenith	58.7	z4792, z60510, z5765		[37]
6	*Pitq-1*	Teqing	103.0-124.4	C236, RG653	IC-17, IB-49, IE-1	[23]
7	*Pi17(t)*	DJ123	94.0-104.0	Est9		[38]
8	*Pi42(t)*	Zhe733	58.5	RM72	IE1K	[39]
8	*Pi33(t)*	IR64	45.4	RM72, C483	Guy11	[40]
8	*Pi55(t)*	Yuejingsimiao 2	99.1-102.1	RM1345, RM3452	CHL688	[41]
8	*PiGD-1(t)*	Sanhuangzhan 2	53.7	RG1034	GD RFDW-I	[42]
9	*Pi3(t)*	C104PKT,	31.3-33.0	40N23r	PO6-6	[43]

http://dx.doi.org/10.5772/56824

Chr.	Name of *R* gene	Name of germplasm	Map position (cM) [>#]	Markers	Name of pathogenic Strains	Ref.
9	*Pi15(t)*	GA25	31.3-34.9	CRG3, CRG4	CHL0416 , Hoku 1	[44]
9	*Pi56(t)*	Sanhuangzhan 2	31.3	RM24022	PO6-6	[42]
10	*PiGD-2(t)*	Sanhuangzhan 2		R16, R14B	PO6-6	[42]
11	*Pi18(t)*	Suweon 365	117.9	RZ536	KI-313	[45]
11	*Pi38(t)*	Tadukan	79.1-88.7	RM206, RM21	B157	[46]
11	*Pi44(t)*	Moroberekan	91.4-117.9	AF349	C9240-1	[47]
11	*PiCO39(t)*	CO39	49.1	S2712	6082	[48]
11	*Pilm-2*	Lemont	56.2-117.9	R4, RZ536	IB54, IG1	[23]
11	*Pi7(t)*	Moroberekan	71.4-84.3	RG103, RG16	PO6-6	[49]
11	*Pi47(t)*	Xiangzi 3150	104.2-120.1	RM206, RM224		[50]
11	*Pi43(t)*	Zhe733	109.5	RM1233	IE1K	[39]
11	*Piks*	Bengal, M201	115.1-117.3	RM224, RM1233		[51]
11	*Pikg(t)*	GA20	119.9-120.3			[19]
11	*Piy(t)*	Yunyin	54	RM202	Sichuang-43	[52]
11	*Pizy(t)*	Ziyu44	102.9	RM206	ZB13, ZE1	[53]
12	*Pi19(t)*	Aichi Asahi	50.4-51.5	RM27937, RM1337	CHNO58-3-1, IRBL19-A	[54]
12	*Pita-2*	Tetep, Pi No.4	50.4			‡
12	*Pi6(t)*	Apura	12.2-47.9	RG457, RG869		[55]
12	*Pi62(t)*	Yashiro-mochi	12.2-26.0	RG9, RZ816	4360-R-62	[56]
12	*Pi24(t)**	Zhong 156	51.5	RG241A	92-183 (ZC15)	[57]
12	*Pi12(t)*	Moroberekan	47.9	RG869		[58]
12	*Pi20(t)*	IR24	51.5-51.8	RM1337, RM5364, RM7102	BN111	[58]
12	*PiGD-3*	Sanhuangzhan 2	55.8	RM179	GD RFDW-IV	[42]
12	*Pi51(t)*	Tianjingyeshengdao		RM5364, RM27990	CHL447, RB5, CHL473, PO6-6	[29]
12	*Pi39(t)**	Q15	50.4	RM27933, RM27940	CHL724	[59]
12	*Pi41(t)*	93-11		RM28130	CHL1789, CHL347, CHL688	[60]
12	*Pi157(t)*	Moroberekan	49.5-62.2	RG341, RG9		[61]

Chr.	Name of *R* gene	Name of germplasm	Map position (cM) >#	Markers	Name of pathogenic Strains	Ref.
12	*Pi48(t)*	Xiangzi 3150		RM5364, RM7102		[50]
12	*Pitq-6*	Teqing	47.9	RG869, RZ397	IC-17, IB-49, IE-1, IB-54	[23]
12	*Pih1(t)*	Hongjiaozhan	47.9	RG869, RG81	ZB1	[62]

* This *R* gene shares the same name with another *R* gene.

The map positions were integrated into IRGSP map according to marker information. Detail information can be found on http://rgp.dna.affrc.go.jp/E/IRGSP/index.html.

‡ Information is known, but has not been published.

Table 3. Summary of major blast *R* genes including their resistance specificity, chromosomal location, map position, and tightly linked DNA markers.

Chr.	Name of *R* gene	Donor	Map position (cM) >#	DNA Markers	Avirulent race/isolate	Ref.
1	*Pi24(t)**	Azucena	64.4	K5	CL6	[63]
1	*Pi35(t)*	Hokkai 188	132.0-136.6	RM1216, RM1003		[64]
2	*Pir2-3*	IR64	141.7	RM263, RM250	Race 173	[65]
2	*Pi25(t)**	IR64	157.9	RG520	BR26, CH66, CH72	[63]
2	*Pirf2-1(t)*	*O. rufipogon*	172.3	RM206, RM266	Race 001	[65]
4	*Pikur1*	Kuroka	86.0			[66]
4	*Pikahei1(t)*	Kahei	108.2	RM17496, RM6629		[67]
5	*Pi26(t)**	Azucena	22.5-24.7	RG313	PH68	[63]
6	*Pi27(t)**	IR64	51.9	Est-2	CH66	[63]
8	*Pi11(t)*	Zhaiyeqing8	53.2-84.8	BP127A, RZ617	18-2, ZH7-2, Zhong10-2-4,	[68]
8	*Pi29(t)*	IR64	69	RZ617, RGA-IR86	CL6	[63]
10	*Pi28(t)*	Azucena	114.7	RZ500	PH68	[63]
11	*Pi30(t)*	IR64	59.4-60.4	OpZ11-f, RGA-IR14	CH66, CH72	[63]
11	*Pi34(t)*	Chubu32	79.1-91.4	Z77, Z150-5		[69]
11	*Pif*	St No. 1	119-120			[70]
12	*Pi31(t)*	IR64	44.3	O10-800	PH68, CD69	[63]
12	*Pi32(t)*	IR64	47.5	AF6	BR26	[63]

* This R gene shares the same name with another R gene.

The map positions were integrated into IRGSP map according to marker information. Detail information can be found on http://rgp.dna.affrc.go.jp/E/IRGSP/index.html.

Table 4. Summary of minor blast *R* genes, donors, map position, tightly linked DNA markers, and associated blast races.

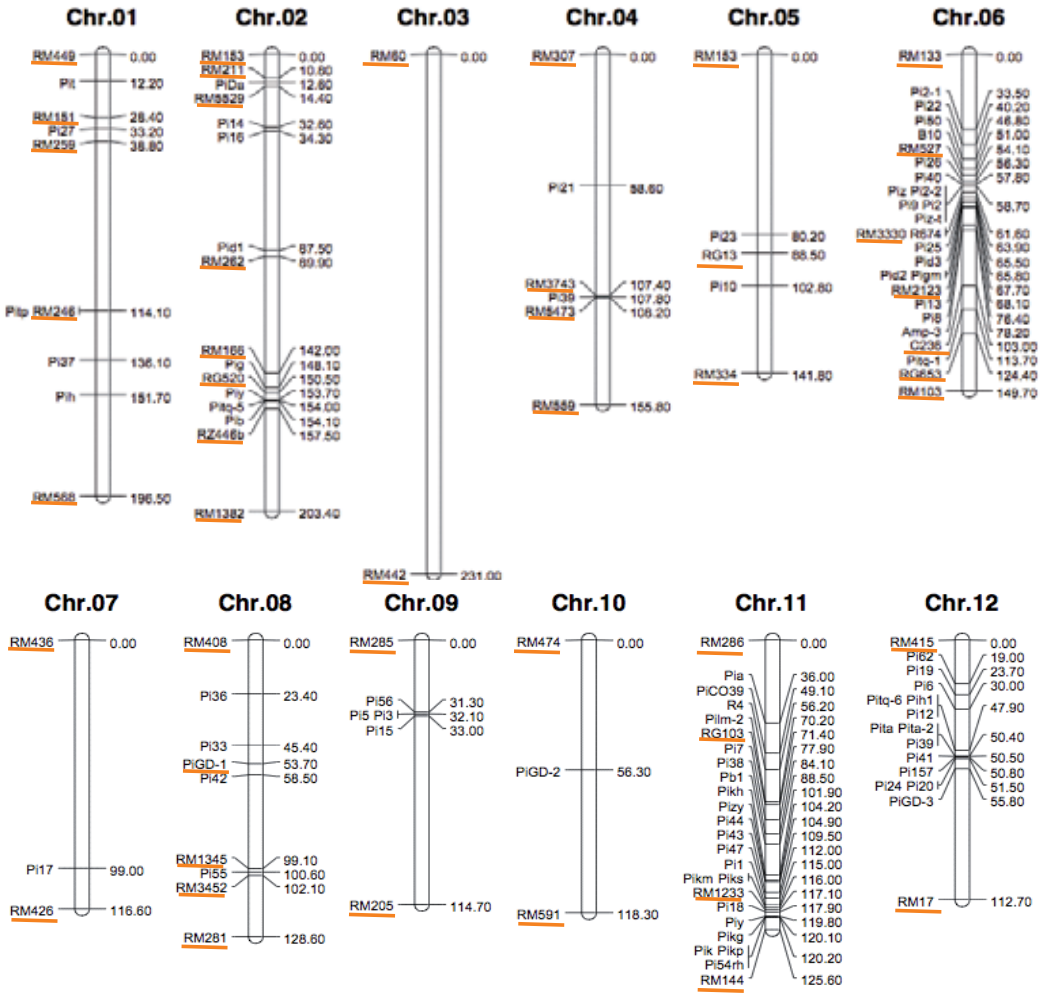


Figure 3. Location of cloned and mapped *R* genes on rice chromosomes. The locations of *R* genes have been integrated into IRGSP map according to marker information, then the map was built using Mapmaker software. Centimorgan was used to measure the map positions showing in the right column of the choromosome. The underlined words indicate either SSR or RFLP markers (see additional resources: http://www.shigen.nig.ac.jp/rice/oryzabaseV4/insd/detail/3554).

Chr.	*R* gene cloned	Donor and cultivar or line carrying the gene	Map position (cM)>#	Markers	Locus structure	Protein type	Subcellular localization	FNPs	Expression	Ref.
1	*Pit*	K59, Tjahaja	12.2	T256	Multiple	CC-NBS-LRR			Repressible	[71]
1	*Pi37*	St. No1	136.1	RM302, RM212	Multiple	NBS-LRR	Cytoplasm	V 239 A, I 247 M	Constitutive	[72]
1	*Pish*	Shin 2, Norin 22	148.7-154.8		Multiple	CC-NBS-LRR			Constitutive	[73]
2	*Pib*	Engkatek, Tohoku IL9, Teqing, Tjinam, BL1	154.1	RM208	Multiple	NBS-LRR			Inducible	[74]
4	*pi21*	Owarihatamochi	58.6	P702D03	Multiple	NBS-LRR	Cytoplasm			[75]
6	*Pid2*	Digu (I)	65.8		Single	Receptor kinase	Membrane	I 441 M	Constitutive	[76]
6	*Pi9*	*O.minuta,* 75-1-127	58.7		Multiple	NBS-LRR			Constitutive	[77]
6	*Pi2*	C101A51	58.7		Multiple	NBS-LRR		R 838 S	Constitutive	[78]
6	*Piz-t*	TKM, Toride 1	58.7	zt56591	Multiple	NBS-LRR		S 839 R	Constitutive	[79]
6	*Pid3*	Digu	65.2-65.8		Single	NBS-LRR		Q 737 Stop		[79]
6	*Pi25**	Gumei 2	63.2-64.6		Multiple	CC-NBS-LRR				[80]
8	*Pi36*	Q61, Kasalath	21.6-25.2	CRG3	Single	NBS-LRR		S 590 D	Constitutive	[81]
9	*Pi5*	Tetep, RIL 260	31.3-33.0	76B14r, 40N23r	Multiple	CC-NBS-LRR	Cytoplasm		Pi5-1 is inducible, Pi5-2 is constitutive	[82]
11	*Pi1*	LAC23, C101LAC	112.1-117.9		Multiple	NBS-LRR				[83]
11	*Pik*	To-To, Kusabue, Kanto 51, K60, Chugoku 31, Shin 2-1, K2, K3, , Minehikari, GA 20	119.9-120.3	k8823, k8824, k3951, k39512	Multiple	CC-NBS-LRR			Constitutive	[84], [85]
11	*Pikm*	Hokushi,Tsuyuake , IRBLkm-Ts	115.1-117.0	k2167, k6441	Multiple	NBS-LRR			Constitutive	[86]
11	*Pikp*	Tetep K60	119.9-120.3	k3957	Multiple	CC-NBS-LRR			Constitutive	[87]
11	*Pikh*	Tetep, K3, Kaybonnet, Lemont, Lebonnet	101.9	RM224	Multiple	NBS-LRR			Inducible	[88]
11	*Pi54rh*	*O. rhizomatis*	119.9-120.3		Multiple	CC-NBS-LRR	Extracellular		Inducible	[89]
11	*Pia*	Aichi Asahi	36.0	Yca72	Multiple	NBS-LRR				[90]
11	*Pb1*	Modan, Tsukinohikari, St NO. 1	85.7-91.4		Single	CC-NBS-LRR			Age-dependent	[91]
12	*Pita*	Tetep, Katy, Teqing	50.4		Single	NBS-LRR	Cytoplasm	A 918 S	Constitutive	[92]

* This R gene shares the same name with another R gene. # The map positions were integrated into IRGSP map according to marker information. Detail information can be found on http://rgp.dna.affrc.go.jp/E/IR.

Table 5. Summary of cloned *R* genes, map position, closely linked DNA markers, and their expression.

http://dx.doi.org/10.5772/56824

3. Structure and function of blast *R* genes

Among the mapped *R* genes (Table 3 and 4), 22 genes including 20 major and 2 minor *R* genes (*Pb1* and *pi21*) have been molecularly characterized (Table 5). Noticeably, *Pid2*, *Pid3*, *Pi36*, *Pb1*, and *Pita* are single copy genes; while others are members of small gene families. A total of eight *R* genes have been identified on chromosome 11, with six at the *Pik* locus; six *R* genes on chromosome 6, four of which are at the *Piz* locus. Most cloned blast *R* genes are adequate in providing complete resistance to strains of *M. oryzae* that contain the corresponding avirulence genes. Interestingly, two different members of each of *Pi5*, *Pik*, *Pikp*, *Pikm*, and *Pia* are required for complete resistance to some avirulent races.

Similar to other plant *R* genes, all cloned blast *R* genes to date encode predicted proteins with centrally located nucleotide binding sites (NBS) and leucine rich repeat (LRR) at the carboxyl terminus (Figure 4), with the exception of *Pid2* and *pi21* encoding a B-lectin kinase protein and a proline containing protein, respectively. Plant NBS-LRR proteins can be divided into two subgroups based on whether they contain a Toll-interleukin receptor (TIR)-like domain (TIR-

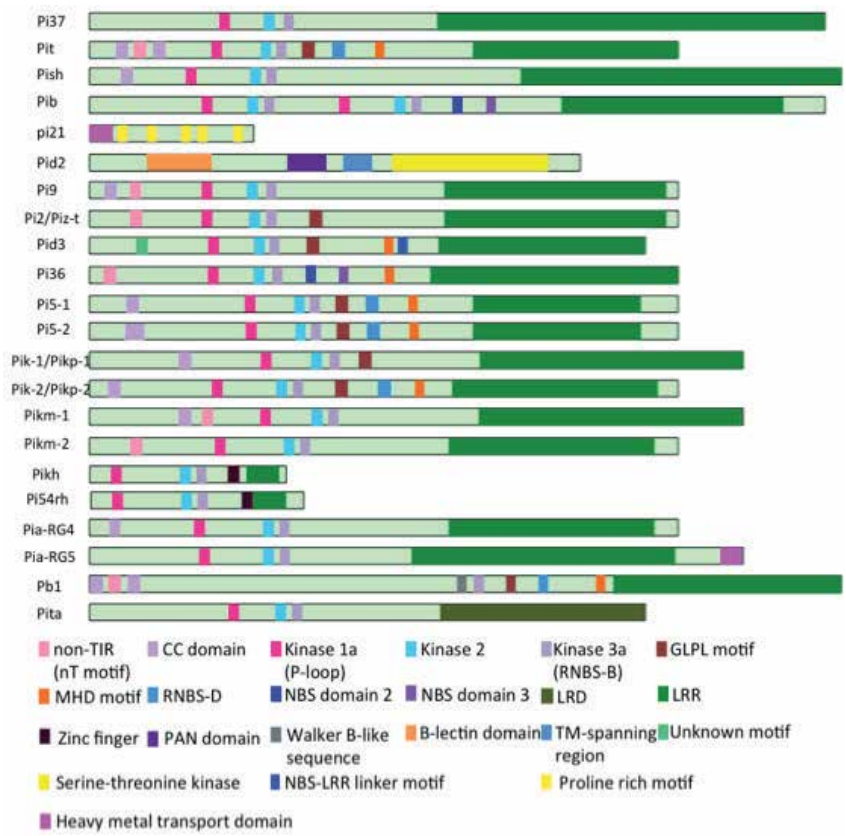


Figure 4. Structure of all cloned *R* genes. The light green bar represents the length of the *R* genes. The highlighted bars represent the different domains of the *R* genes.

NBS-LRR) or a putative coiled-coil (CC) structure (CC-NBS-LRR) in their amino-terminal region. The rice genome has 500 NBS-LRR gene families, and most of them belong to the CC-NBS-LRR family. The NBS domain contains kinase 1a (p-loop), kinase 2 and 3a (RNBS-B) motif, which presumably bind to ATP and trigger downstream signal transduction; whereas, the LRR is predicted to recognize pathogen effectors, either directly or indirectly. Other noticeable protein domains of plant *R* proteins were also summarized in Figure 4.

The observed structural similarities of blast R proteins might imply that their predicted conserved regions are associated with functional roles in triggering resistance to *M. oryzae*. Cloned blast *R* genes can be separated into two clades, I and II (Figure 5). Clade I consists of all NBS-LRR genes and clade II contains both NBS-LRR and non-NBS-LRR gene, such as *Pid2* and *pi21.* Among them, *Pi1-5, Pik-1, Pikp-1*, and *Pikm1-TS* on chromosome 11 share substantial homology to the *Pi9* locus on Chromosome 6; whereas, *Pi1-6, Pik-2, Pikp-2*, and *Pikm2-T* are more similar to *Pid2* and *pi21*, which are not NBS-LRR *R* genes. Homologous sequences of blast *R* genes can be found in the diverse germplasm of cultivated species including domesticated landrace varieties and wild relatives of rice. These observations suggest that genetics of rice immunity is ancient and may have been evolved during speciation and domestication.

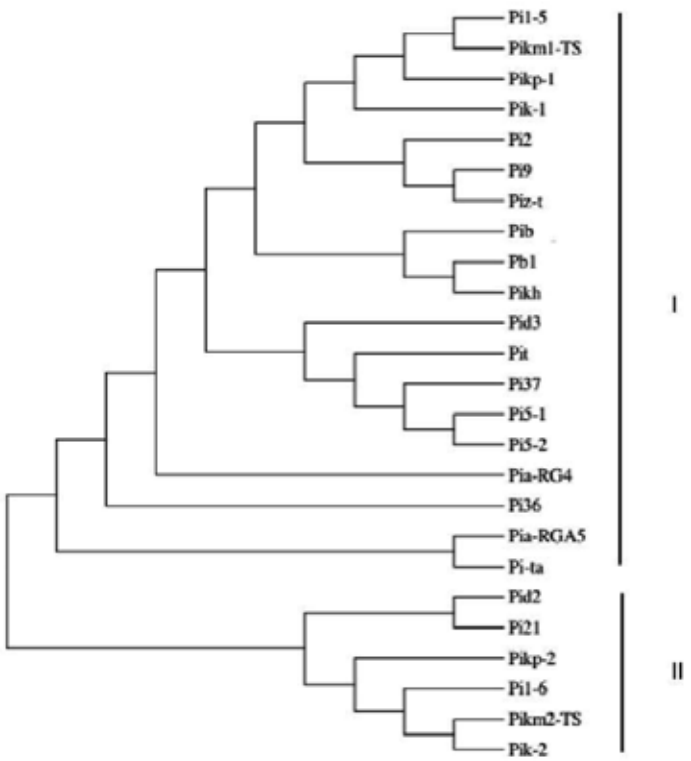


Figure 5. Phylogenetic tree of all cloned blast *R* genes. The tree was constructed using protein sequences by software Mega 5.0 NJ method.

http://dx.doi.org/10.5772/56824

4. *R* gene-mediated signaling transduction pathways

It is now commonly accepted that products of *R* genes in plants can specifically recognize avirulence genes from the pathogen directly or indirectly to initiate innate immunity system responses. Direct recognition of the putative product of the avirulence gene, *AVR-Pita1* by the *Pita* protein was first reported in 2000 [93]. Over a decade later, in 2012, another blast *R* protein, *Pik*, with similar structure to *Pita*, was found to directly recognize the corresponding avirulence gene *AvrPik* [94]. Direct interactions between other blast *R* and avirulence genes have not been reported; suggesting that indirect interactions may be responsible in triggering effective signal transduction pathways. Other plant genes involved in signal transduction have been investigated by the use of *R* proteins as bait in the yeast two-hybrid system (Y2H). While Y2H is a highly effective tool, it is limited in indentifying immediate plant components of *R* proteins. Molecular basis of blast *R* gene-mediated signaling has been a subject of intensive investigation worldwide. Abundant genes that may be involved in *R* gene-mediated signaling have been identified with DNA microarray [95, 96], and most of them were pathogenicity related genes. Genetic analysis using mutagenesis has been another commonly used alternative to identify downstream components. However, most mutants identified, thus far, are lesion mimic mutants [97]. A major effort to identify *Pita* mediated signaling was accomplished by treating 20,000 Katy seeds with *Pita*/*Pita*^{2}/*Pik*^{s} using fast neutrons, ethyl methyl sulfate (EMS), and gamma irradiation [98]. A total of 142 rice seedlings, with altered disease reactions, were identified from independent M2. The susceptibility of M2 individuals was verified in subsequent generations, and 20 of them were confirmed to be derived from Katy using 20 diagnostic single sequence repeat (SSR) markers. Consequently, the *Ptr(t)* gene in rice was identified to be essential for *Pita* mediated signal transudation [99]. Molecular cloning of *Ptr(t)* will shed light on the interaction mechanism of *Pita* and *Ptr(t)*, and subsequent plant genes involved in defense responses.

5. The management of blast disease-marker assisted selection

Blast disease has been effectively managed by a combination of fungicides and *R* genes integrated into diverse cultural practices. These include seed treatment with fungicide; preventive application of fungicide before heading; crop rotation; balanced application of fertilizers with nitrogen, potassium, and phosphate; and maintaining a sufficient water level during tillering and flowering stages. However, the most effective way to manage rice blast is by the utilization of resistant cultivars due to its environmental and economic sustainability. Incorporating major blast *R* genes have been traditionally accomplished by classical breeding methods and can be accelerated by the use of marker assisted selection (MAS) [100]. MAS has become a practical tool in cultivar improvement by selecting important traits at the early growth stages based on DNA markers, thus breeders can screen for resistance without having to maintain pathogen culture. MAS is efficient and consistent in the field and greenhouse [101]. MAS is also reliable in dealing with traits whose phenotype is affected by the environment. To date, 99 blast *R* genes have been mapped with closely linked DNA markers; and some of

them can be used for MAS. DNA markers were also developed from portions of cloned *R* genes, such as *Pi-ta* and *Pi-b*, for their introduction into elite rice cultivars using MAS. Markers for *Pita*, one of the most important *R* genes for blast in the United States, were developed [102]; while, linked markers for 4 blast *R* genes (*Pik*, *Pib*, *Pita2*, and *Pii*) are effective against eight to ten races of *M. oryzae* were identified [103]. Using MAS, *R* genes like *Pi1*, *Pi5*, *Piz-5*, and *Pita* have been established in different rice genotypes [82, 100, 104, 105]

6. Future prospects

Blast disease is a moving target where the fungus can rapidly adapt to the host. The major difficulty in controlling rice blast is the durability of genetic resistance. Rice cultivars containing only a single *R* gene to a specific pathogen race often become susceptible over time due to the emergence of new virulent races. In theory, *R* genes can be found in rice germplasm in different rice production areas. Stacking *R* genes with overlapped resistance spectra can lead to long lasting resistance. Knowledge of genetic identity of contemporary *M. oryzae* is crucial for precise deployment of rice cultivars with different *R* genes [104]. Effective blast management also requires unprecedented international cooperation. IRRI and research institutions worldwide have been coordinating their resources for both genotyping using next generation of DNA sequencing and phenotyping at different geographic locations. The knowledge gained by this massive collaborative effort ought to lead to more effective methods to reduce crop loss due to blast disease worldwide.

Acknowledgements

We thank the Arkansas Rice Research and Promotion Board, and the US National Science Foundation (Plant Research Program no. 0701745), Natural Science Foundation of China (Program no. 31000847), Zhejiang Natural Science Foundation (Program no. Y3100577), and Qianjiang Talents Project supported by Science Technology Department of Zhejiang Province (Program no. 2011R10038) for their partial financial supports. USDA is an equal opportunity provider and employer.

Author details

Xueyan Wang[1], Seonghee Lee[2], Jichun Wang[3], Jianbing Ma[4], Tracy Bianco[5] and Yulin Jia[5*]

*Address all correspondence to: yulin.jia@ars.usda.gov

1 China Jiliang University, Hangzhou, China

2 Noble Foundation, Oklahoma, USA

3 Jilin Academy of Agricultural Sciences, Changchun, China

4 University of Arkansas Rice Research and Extension Center, Stuttgart, Arkansas, USA

5 USDA ARS Dale Bumpers National Rice Research Center, Stuttgart, Arkansas, USA

References

[1] United States Department of Agriculture-Economic Research Service. USDA-ERS: Topics, Crops: rice. http://www.ers.usda.gov/topics/crops/rice/background (Accessed 20 September 2012)

[2] Song Y. Tian Gong Kai Wu. China: 1637.

[3] International Rice Research Institute-Rice Knowledge Bank. IRRI: Crop Health, Disease: Rice Blast. http://www.knowledgebank.irri.org/ipm/rice-blast/symptoms.html

[4] Zhang L, Zhang Q, Cheng Z, Yang H, Zhou Y, Lu C. Resistances of four sets of rice varieties to 19 strains of *Magnaporthe grisea* in Jiangsu area. Jiangsu Journal of Agricultural Science 2010; 26(5) 948-953.

[5] Baker B, Zambryski P, Staskawicz B, Dinesh-Kumar SP. Signaling in plant-microbe interactions. Science 1997; 276(5313) 726-733.

[6] Pennisi E. Armed and dangerous. Science 2010; 327 (5867) 804-805.

[7] Hamer JE, Howard RJ, Chumley FG, Valent B. A mechanism for surface attachment in spores of a plant pathogenic fungus. Science 1988; 239(4837) 288-290.

[8] Bourett TM, Howard RJ. *In vitro* development of penetration structures in the rice blast fungus *Magnaporthe grisea*. Canadian Journal of Botany 1990; 68(2) 329-432.

[9] Talbot NJ. On the Trail of a Cereal Killer: Exploring the biology of *Magnaporthe grisea.* Annual Review of Microbiology 2003; 57(1) 177-202.

[10] Sasaki R. Extensive of strains in rice blast fungus. Journal of plant protection 1992; 9 634-644

[11] Ling KC, Ou SH. Standardization of the international race numbers of *Pyricularia oryzae*. Phytopathology 1969; 59 339-342.

[12] Duan Y, Zhu Y, Chen H, Na X. The blast resistance mechanisms and genetic regularity. Journal of Yunnan Agricultural University 1987; 2(2) 11-16.

[13] Mackill DJ, Bonman JM. Inheritance of blast resistance in near isogenic lines of rice. Phytopathology 1992; 82 (7) 746-749.

[14] Marchetti MA. Race-specific and rate-reducing resistance to rice blast in US rice cultivars. In: Zeigler RS, Leong SA, Teng PS (eds.) Rice blast disease. Wallingford: Cab International; 1994. pp 231-244.

[15] Tsunematsu H, Yanoria MJT, Ebron LA, Hayashi N, Ando I, Kato H, Imbe T, Khush GS. Development of Monogenic Lines of Rice for Rice Blast Resistance. Breeding Science 2000; 50(3) 229-234.

[16] Kiyosawa, S.. Genetic studies on host–pathogen relationship in the rice blast disease. In: Ogura T. (ed.) 1967: rice disease and their control by growing resistant varieties and other measures: proceedings of the Symposium of Agriculture, Forestry and Fisheries Research Council, 1967, Tokyo, Japan.

[17] Zhu M, Wang L, Pan Q. Identification and characterization of a new blast resistance gene located on rice chromosome 1 through linkage and differential analyses. Phytopathology 2004; 94(5) 515-519.

[18] Barman SR, Gowda M, Venu RC, Chattoo BB. Identification of a major blast resistance gene in the rice cultivar 'Tetep'. Plant Breeding 2004; 123(3) 300-302.

[19] Pan Q, Wang L, Ikehashi H, Yamagata H, Tanisaka T. Identification of two new genes conferring resistance to rice blast in the Chinese native cultivar ‘Maowangu’. Plant breeding 1998; 117(1) 27-31.

[20] Pan Q, Wang L, Tanisika T. A new blast resistance gene identified in the Indian native rice cultivar Aus373 through allelism and linkage tests. Plant Pathology Journal 1999; 48(2) 288-293.

[21] Shi B, Zhang J, Zheng Y, Liu Y, Vera Cruz C, Zheng T, Zhao M. Identification of a new resistance gene Pi-Da(t) from Dacca6 against rice blast fungus (Magnaporthe oryzae) in Jin23B background. Molecular Breeding 2012; 30(2)1089-1096.

[22] Chen X, Li S, Xu J, Zhai W, Ling Z, Ma B, Wang Y, Wang W, Cao G, Ma Y, Shang J, Zhao X, Zhou K, Zhu L. Identification of two blast resistance genes in a rice variety, Digu. Journal of Phytopathology 2004; 152(2) 77-85.

[23] Tabien RE, Li Z, Paterson AH, Marchetti MA, Stansel JW, Pinson SRM. Mapping of four major rice blast resistance genes from 'Lemont' and 'Teqing' and evaluation of their combinatorial effect for field resistance. Theoretical and Applied Genetics 2000; 101(8)1215-1225.

[24] Zhou J, Wang J, Xu J, Lei C Ling Z. Identification and mapping of a rice blast resistance gene Pi-g(t) in the cultivar Guangchangzhan. Plant pathology 2004; 53(2) 191-196.

[25] Lei C, Huang D, Li W, Wang J, Liu Z, Wang X, Shi K, Cheng Z, Zhang X, Ling Z, Wan J. Molecular mapping of a blast resistance gene in an indica rice cultivar Yanxian No.1. Rice Genetics Newsletter 2005; 22 76-77.

[26] Terashima T, Fukuoka S, Saka N, Kudo S. Mapping of a blast field resistance gene *Pi39(t)* of elite rice strain Chubu 111. Plant Breeding 2008; 127(5) 485-489.

[27] Naqvi NI, Bonman JM, Makill DJ, Nelson RJ, Chattoo BB. Identification of RAPD markers linked to major blast resistance gene in rice. Molecular Breeding 1995;1(4) 341-348.

[28] Ahn SN, Kim YK, Hong HC, Han SS, Choi HC, McCouch SR, Moon HP. Mapping of genes conferring resistance to Korean isolates of rice blast fungus using DNA markers. Korean Journal of Breeding 1997; 29(4) 416-423.

[29] Wang Y, Wang D, Deng X, Liu J, Sun P, Liu Y, Huang H, Jiang N, Kang H, Ning Y, Wang Z, Xiao Y, Liu X, Liu E, Dai L, Wang G. Molecular mapping of the blast resistance genes *Pi2-1* and *Pi51(t)* in the durably resistant rice 'Tianjingyeshengdao'. Phytopathology 2012; 102(8) 779-786.

[30] Jiang N, Li Z, Wu J, Wang Y, Wu L, Wang S, Wang D, Wen T, Liang Y, Sun P, Liu J, Dai L, Wang Z, Wang C, Luo M, Liu X, Wang G. Molecular mapping of the Pi2/9 allelic gene Pi2-2 conferring broad-spectrum resistance to *Magnaporthe oryzae* in the rice cultivar Jefferson. Rice 2012; 5(29) 1-7.

[31] Pan Q, Wang L, Ikehashi H,Taniska T. Identification of a new blast resistance gene in the *indica* rice cultivar Kasalath using Japanese differential cultivars and isozyme markers. Phytopathology 1996; 86(10) 1071-1076.

[32] Ebitani T, Hayashi N, Omoteno M, Ozaki H, Yano M, Morikawa M, Fukuta Y. Characterization of *Pi13*, a blast resistance gene that maps to chromosome 6 in *indica* rice (*Oryza sativa* L. variety, Kasalath). Breeding Science 2011; 61(3) 251-259.

[33] Wu J, Fan Y, Li D, Zheng K, Leung H, Zhuang J. Genetic control of rice blast resistance in the durably resistant cultivar Gumei 2 against multiple isolates. Theoretical and Applied Genetics 2005; 111(1) 50-56.

[34] Jeung JU, Kim BR, Cho YC, Han SS, Moon HP, Lee YT, Jena KK. A novel gene, *Pi40(t)*, linked to the DNA markers derived from NBS-LRR motifs confers broad spectrum of blast resistance in rice. Theoretical and Applied Genetics 2007; 115 (8) 1163-1177.

[35] Zhu X, Chen S, Yang J, Zhou S, Zeng L, Han J, Su J, Wang L, Pan Q. The identification of *Pi50(t)*, a new member of the rice blast resistance *Pi2*/*Pi9* multigene family. Theoretical and Applied Genetics 2012; 124(7) 1295-1304.

[36] Deng Y, Zhu X, Shen Y, He Z. Genetic characterization and fine mapping of the blast resistance locus *Pigm(t)* tightly linked to *Pi2* and *Pi9* in a broad-spectrum resistant Chinese variety. Theoretical and Applied Genetics 2006; 113(4) 705-713.

[37] Hayashi K, Hashimoto N, Daigen M, Ashikawa I. Development of PCR-based SNP markers for rice blast resistance genes at the *Piz* locus. Theoretical and Applied Genetics 2004; 108(7) 1212-1220.

[38] Pan Q, Tanisaka T, Ikehashi H. Studies on the genetics and breeding of blast resistance in rice VI. Gene analysis for the blast resistance of two Yunnan native cultivars GA20 and GA25. Breeding Science 1996; 46(Suppl 2) 70.

[39] Lee S, Wamishe Y, Jia Y, Liu G, Jia M. Identification of two major resistance genes against race IE-1k of *Magnaporthe oryzae* in the *indica* rice cultivar Zhe733. Molecular Breeding 2009; 24(2) 127-134.

[40] Berruyer R, Adreit H, Milazzo J, Gaillard S, Berger A, Dioh W, Lebrun MH, Tharreau D. Identification and fine mapping of Pi33, the rice resistance gene corresponding to the Magnaporthe grisea avirulence gene ACE1. Theoretical and Applied Genetics 2003; 107(6) 1139-1147.

[41] He X, Liu X, Wang L, Wang L, Lin F, Cheng Y, Chen Z, Pan Q. Identification of the novel recessive gene *pi55(t)* conferring resistance to *Magnaporthe oryzae*. Science China Life Sciences 2012; 55(2) 141-150.

[42] Liu B, Zhang S, Zhu X, Yang Q, Wu S, Mei M, Mauleon R, Leach J, Mew T, Leung H. Candidate defense genes as predictors of quantitative blast resistance in rice. Molecular Plant-Microbe Interactions 2004; 17(10) 1146-1152.

[43] Jeon J S, Chen D, Yi G, Wang G, Ronald PC. Genetic and physical mapping of *Pi5(t)*, a locus associated with broad-spectrum resistance to rice blast. Molecular Genetics and Genomics 2003; 269(2) 280-289.

[44] Lin F, Liu Y, Wang L, Liu X Pan Q. A high-resolution map of the rice blast resistance gene *Pi15* constructed by sequence-ready markers. Plant Breeding 2007; 126(3) 287-290.

[45] Ahn SH, Kim YK, Hong HC, Han SS, Kwon SJ, Choi HC, Moon HP, McCouch SR. Molecular mapping of a new gene for resistance to rice blast (Pyricularia grisea Sacc.). Euphytica 2000; 116(1) 17-22.

[46] Gowda M, Roy-Barman S, Chattoo BB. Molecular mapping of a novel blast resistance gene *Pi38* in rice using SSLP and AFLP markers. Plant Breeding 2006; 125(6) 596-599.

[47] Chen D, dela Viña M, Inukai T, Mackill DJ, Ronald PC, Nelson RJ. Molecular mapping of the blast resistance gene, *Pi44(t)*, in a line derived from a durably resistant rice cultivar. Theoretical and Applied Genetics 1999; 98 1046-1053.

[48] Chauhan RS, Farman ML, Zhang HB, Leong SA. Genetic and physical mapping of a rice blast resistance locus, *Pi-CO39(t)*, that corresponds to the avirulence gene AVR1-CO39 of *Magnaporthe grisea*. Molecular Genetics and Genomics 2002; 267(5) 603-612.

[49] Campbell MA, Chen D, Ronald PC. Development of co-dominant amplified polymorphic sequence markers in rice that flank the *Magnaporthe grisea* resistance gene *Pi7(t)* in recombinant inbred line 29. Phytopathology 2004; 94(3) 302-307

[50] Huang H, Huang L, Feng G, Wang S, Wang Y, Liu J, Jiang N, Yan W, Xu L, Sun P, Li Z, Pan S, Liu X, Xiao Y, Liu E, Dai L, Wang G. Molecular mapping of the new blast

resistance genes *Pi47* and *Pi48* in the durably resistant local rice cultivar Xiangzi 3150. Phytopathology 2011; 101(5) 620-626.

[51] Fjellstrom R, Conaway-Bormans CA, McClung AC, Marchetti MA, Shank A, Park WD. Development of DNA markers suitable for marker assisted selection of three *Pi*-genes conferring resistance to multiple *Pyricularia grisea* pathotypes. Crop Science 2004; 44(5) 1790-1798.

[52] Zhang J, Wang G, Xie H, Ling Z. Genetic analysis and mapping of blast resistance genes in *japonica* rice Yunyin from Yunnan provice. Journal of Agricultural Biotechnology 2003; 11(3) 241-244.

[53] Zhang J, Tan Y, Hong R, Fan J, Luo Q, Zeng Q. Genetic analysis and gene mapping of rice blast resistance in *japonica* variety Ziyu 44. Chinese journal of rice science 2009; 23(1) 31-35.

[54] Koide Y, Telebanco-Yanoria MJ, dela Peña FD, Fukuta Y, Kobayashi N. Characterization of rice blast isolates by the differential system and their application for mapping a resistance gene, *Pi19(t)*. Journal of Phytopathology 2011; 159(2) 85–93.

[55] Causse MA, Fulton TM, Cho YG, Ahn SN, Chunwongse J, Wu K, Xiao J, Yu Z, Ronald PC, Harrington SE, Second G, McCouch SR, Tanksley SD. Saturated molecular map of the rice genome based on an interspecific backcross population. Genetics 1994; 138(4)1251-1274.

[56] Wu KS, Martinez C, Lentini Z, Tohme J, Chumley FG, Scolnik PA, Valent B. Cloning a blast resistance gene by chromosome walking. In: Khush GS. (ed.) IRRI1996: map-based gene cloning: proceedings of the Third International Rice Genetic Symposium, IRRI, 1996, Manila, Philippines.

[57] Zhuang J, Ma W, Wu J, Chai R, Lu J, Fan Y, Jin M, Leung H, Zheng K. Mapping of leaf and neck blast resistance genes with resistance gene analog, RAPD and RFLP in rice. Euphytica 2002; 128(3) 363-370

[58] Inukai T, Zeigler RS, Sarkarung S, Bronson M, Dung LV, Kinoshita T, Nelson RJ. Development of pre-isogenic lines for rice blast-resistance by marker-aided selection from a recombinant inbred population. Theoretical and Applied Genetics 1996; 93(4) 560-567.

[59] Liu X, Yang Q, Lin F, Hua L, Wang C, Wang L, Pan Q. Identification and fine mapping of *Pi39(t)*, a major gene conferring the broad-spectrum resistance to Magnaporthe oryzae. Molecular Genetics and Genomics 2007; 278(4) 403-410.

[60] Yang Q, Lin F, Wang L, Pan Q. Identification and mapping of *Pi41*, a major gene conferring resistance to rice blast in the Oryza sativa subsp. indica reference cultivar, 93-11. Theoretical and Applied Genetics 2009; 118(6) 1027-1034.

[61] Naqvi NI, Chattoo BB. Development of a sequence characterized amplified region (SCAR) based indirect selection method for a dominant blast-resistance gene in rice. Genome 1996; 39(1) 26-30.

[62] Zheng K, Qiang H, Zhuang J, Lu J, Lin H. Mapping of rice resistance genes to blast using DNA marker. Acta Phytopathologica Sinica 1995; 25(4) 307-313.

[63] Sallaud C, Lorieux M, Roumen E, Tharreau D, Berruyer R, Svestasrani P, Garsmeur O, Ghesquiere A, Notteghem JL. Identification of five new blast resistance genes in the highly blast-resistant rice variety IR64 using a QTL mapping strategy. Theoretical and Applied Genetics 2003; 106(5) 794-803.

[64] Nguyen TT, Koizumi S, La TN, Zenbayashi KS, Ashizawa T, Yasuda N, Imazaki I, Miyasaka A. *Pi35(t)*, a new gene conferring partial resistance to leaf blast in the rice cultivar Hokkai 188. Theoretical and Applied Genetics 2006; 113(4) 697-704.

[65] Utami DW, Moeljopawiro S, Aswidinnoor H, Setiawan A, Hanarida I. Blast resistance genes in wild rice *Oryza rufipogon* and rice cultivar IR64. Indonesian Journal of Agriculture 2008; 1(2) 71-76

[66] Goto I. Genetic studies on resistance of rice plant to blast fungus (VII). Blast resistance genes of Kuroka. Annals of Phytopathological Society of Japan 1988; 54 460-465.

[67] Xu X, Chen H, Fujimura T, Kawasaki S. Fine mapping of a strong QTL of field resistance against rice blast, Pikahei-1(t), from upland rice Kahei, utilizing a novel resistance evaluation system in the greenhouse. Theoretical and Applied Genetics 2008; 117(6) 997-1008.

[68] Wang G, Mackill D, Bonman J, McCouch SR, Champoux MC, Nelson RJ. RFLP mapping of genes conferring complete and partial resistance to blast in a durably resistance rice cultivar. Genetics 1994; 136(4) 1421-1434.

[69] Zenbayashi-Sawata K, Fukuoka S, Katagiri S, Fujisawa M, Matsumoto T, Ashizawa T, Koizumi S. Genetic and physical mapping of the partial resistance gene, *pi34*, to blast in rice. Phytopathology 2007; 97(5) 598-602.

[70] Toriyama K. Breeding for resistance to major rice disease in Japan. In: IRRI (ed.) Rice Breeding. Manila: IRRI; 1972. p253-281.

[71] Hayashi K, Yoshida H. Refunctionalization of the ancient rice blast disease resistance gene *Pit* by the recruitment of a retrotransposon as a promoter. The Plant Journal 2009; 57(3) 413-425.

[72] Lin F, Chen S, Que Z, Wang L, Liu X, Pan Q. The blast resistance gene *Pi37* encodes a nucleotide binding site leucine-rich repeat protein and is a member of a resistance gene cluster on rice chromosome 1. Genetics 2007; 177(3) 1871-1880.

[73] Takahashi A, Hayashi N, Miyao A, Hirochika H. Unique features of the rice blast resistance *Pish* locus revealed by large scale retrotransposon-tagging. BMC Plant Biology 2010; 10175.

[74] Wang Z, Yano M, Yamanouchi U, Iwamoto M, Monna L, Hayasaka H, Katayose Y, Sasaki T. The *Pib* gene for rice blast resistance belongs to the nucleotide binding and leucine-rich repeat class of plant disease resistance genes. The Plant Journal 1999; 19(1) 55-64.

[75] Fukuoka S, Saka N, Koga H, Ono K, Shimizu T, Ebana K, Hayashi N, Takahashi A, Hirochika H, Okuno K,Yano M. Loss of function of a proline-containing protein confers durable disease resistance in rice. Science 2009; 325(5943) 998-1001.

[76] Chen X, Shang J, Chen D, Lei C, Zou Y, Zhai W, Liu G, Xu J, Ling Z, Cao G, Ma B, Wang Y, Zhao X, Li S, Zhu L. A B-lectin receptor kinase gene conferring rice blast resistance. The Plant Journal 2006; 46(5) 794-804.

[77] Qu S, Liu G, Zhou B, Bellizzi M, Zeng L, Dai L, Han B, Wang G. The broad-spectrum blast resistance gene *Pi9* encodes a nucleotide-binding site-leucine-rich repeat protein and is a member of a multigene family in rice. Genetics 2006; 172(3) 1901-1914.

[78] Zhou B, Qu S, Liu G, Dolan M, Sakai H, Lu G, Bellizzi M, Wang G. The eight amino-acid differences within three leucine-rich repeats between *Pi2* and *Piz-t* resistance proteins determine the resistance specificity to *Magnaporthe grisea*. Molecular Plant-Microbe Interactions 2006; 19(11) 1216-1228.

[79] Shang J, Tao Y, Chen X, Zou Y, Lei C, Wang J, Li X, Zhao X, Zhang M, Lu Z, Xu J, Cheng Z, Wan J, Zhu L. Identification of a new rice blast resistance gene, *Pid3*, by genomewide comparison of paired nucleotide-binding site--leucine-rich repeat genes and their pseudogene alleles between the two sequenced rice genomes. Genetics 2009; 182(4) 1303-1311.

[80] Chen J, Shi Y, Liu W, Chai R, Fu Y, Zhuang J, Wu J. A *Pid3* allele from rice cultivar Gumei2 confers resistance to *Magnaporthe oryzae*. Journal of Genetics and Genomics 2011; 38(5) 209-216.

[81] Liu X, Lin F, Wang L, Pan Q. The in silico map-based cloning of *Pi36*, a rice coiled-coil nucleotide-binding site leucine-rich repeat gene that confers race-specific resistance to the blast fungus. Genetics 2007; 176(4) 2541-2549.

[82] Lee SK, Song MY, Seo YS, Kim HK, Ko S, Cao PJ, Suh JP, Yi G, Roh JH, Lee S, An G, Hahn TR, Wang GL, Ronald P, Jeon JS. Rice *Pi5*-mediated resistance to *Magnaporthe oryzae* requires the presence of two coiled-coil-nucleotide-binding-leucine-rich repeat genes. Genetics 2009; 181(4) 1627-1638.

[83] Hua L, Wu J, Chen C, Wu W, He X, Lin F, Wang L, Ashikawa I, Matsumoto T, Wang L, Pan Q. The isolation of *Pi1*, an allele at the *Pik* locus which confers broad spectrum resistance to rice blast. Theoretical and Applied Genetics 2012; 125(5) 1047-1055.

[84] Zhai C, Lin F, Dong Z, He X, Yuan B, Zeng X, Wang L, Pan Q. The isolation and characterization of *Pik*, a rice blast resistance gene which emerged after rice domestication. New Phytologist 2011;189(1) 321-334.

[85] Ashikawa I, Hayashi N, Abe F, Wu J,Matsumoto T. Characterization of the rice blast resistance gene *Pik* cloned from Kanto51. Molecular Breeding 2012; 30(1) 485-494.

[86] Ashikawa I, Hayashi N, Yamane H, Kanamori H, Wu J, Matsumoto T, Ono K, Yano M. Two adjacent nucleotide-binding site-leucine-rich repeat class genes are required to confer *Pikm*-specific rice blast resistance. Genetics 2008; 180(4) 2267-2276.

[87] Yuan B, Zhai C, Wang W, Zeng X, Xu X, Hu H, Lin F, Wang L, Pan Q. The *Pik-p* resistance to *Magnaporthe oryzae* in rice is mediated by a pair of closely linked CC-NBS-LRR genes. Theoretical and Applied Genetics 2011; 122(5) 1017-1028.

[88] Sharma TR, Madhav MS, Singh BK, Shanker P, Jana TK, Dalal V, Pandit A, Singh A, Gaikwad K, Upreti HC, Singh NK. High-resolution mapping, cloning and molecular characterization of the *Pi-k(h)* gene of rice, which confers resistance to *Magnaporthe grisea*. Molecular Genetics and Genomics 2005; 274(6) 569-578.

[89] Das A, Soubam D, Singh PK, Thakur S, Singh NK,Sharma TR. A novel blast resistance gene, *Pi54rh* cloned from wild species of rice, *Oryza rhizomatis* confers broad spectrum resistance to *Magnaporthe oryzae*. Functional and Integrative Genomics 2012; 12(2) 215-228.

[90] Okuyama Y, Kanzaki H, Abe A, Yoshida K, Tamiru M, Saitoh H, Fujibe T, Matsumura H, Shenton M, Galam DC, Undan J, Ito A, Sone T, Terauchi R. A multifaceted genomics approach allows the isolation of the rice *Pia*-blast resistance gene consisting of two adjacent NBS-LRR protein genes. The Plant Jounal 2011; 66(3) 467-479.

[91] Hayashi N, Inoue H, Kato T, Funao T, Shirota M, Shimizu T, Kanamori H, Yamane H, Hayano-Saito Y, Matsumoto T, Yano M, Takatsuji H. Durable panicle blast-resistance gene *Pb1* encodes an atypical CC-NBS-LRR protein and was generated by acquiring a promoter through local genome duplication. The Plant Journal 2010; 64(3) 498-510.

[92] Bryan GT, Wu KS, Farrall L, Jia Y, Hershey HP, McAdams SA, Faulk KN, Donaldson GK, Tarchini R, Valent B. tA single amino acid difference distinguishes resistant and susceptible alleles of the rice blast resistance gene *Pi-ta*. The Plant Cell 2000; 12(11) 2033-2046.

[93] Jia Y, McAdams SA, Bryan GT, Hershey HP, Valent B. Direct interaction of resistance gene and avirulence gene products confers rice blast resistance. EMBO Journal 2000; 19(15): 4004-4014.

[94] Kanzaki H, Yoshida K, Saitoh H, Fujisaki K, Hirabuchi A, Alaux L, Fournier E, Tharreau D, Terauchi R. Arms race co-evolution of *Magnaporthe oryzae AVR-Pik* and rice *Pik* genes driven by their physical interactions. The Plant Journal 2012; 72(6) 894-907.

[95] Vergne E, Ballini E, Marques S, Sidi Mammar B, Droc G, Gaillard S, Bourot S, DeRose R, Tharreau D, Notteghem JL, Lebrun MH, Morel JB. Early and specific gene expression triggered by rice resistance gene *Pi33* in response to infection by *ACE1* avirulent blast fungus. New Phytologist 2007; 174(1) 159-171.

[96] Gupta SK, Rai AK, Kanwar SS, Chand D, Singh NK, Sharma TR. The single functional blast resistance gene *Pi54* activates a complex defence mechanism in rice. Journal of Experimental Botany 2012; 63(2) 757-772.

[97] Jia Y. Registration of lesion mimic mutant of Katy rice. Crop Science 2005; 45(4) 1675.

[98] Jia Y, Xie J, Rutger JN. Development and characterization of Katy deletion mutant populations. Plant Mutation Reports 2006; 1(1) 43-47.

[99] Jia Y, Martin R. Identification of a new locus, *Ptr(t)*, required for rice blast resistance gene Pi-ta-mediated resistance. Molecular Plant-Microbe Interactions 2008; 21(4) 396-403.

[100] Hittalmani S, Parco A, Mew TV, Zeigler RS, Huang N. Fine mapping and DNA marker-assisted pyramiding of the three major genes for blast resistance in rice. Theoretical and Applied Genetics 2000; 100(7) 1121–1128.

[101] Moose SP, Mumm RH. Molecular plant breeding as the foundation for 21st century crop improvement. Plant Physiology 2008; 147(3) 969-977.

[102] Jia, Y, Wang, Z, Singh P. Development of dominant rice blast resistance *Pi-ta* gene markers. Crop Science 2002; 42(3) 2145-2149.

[103] Jia, Y. Understanding the molecular mechanisms of rice blast resistance using rice mutants. In: Shu Q. (ed.) Induced Plant Mutations in the Genomics Era. Rome: FAO; 2009. p 375-378.

[104] Liu S, Li X, Wang C, Li X, He Y. Improvement of resistance to rice blast in Zhenshan 97 by molecular markeraided selection. Acta Botanica Sinica 2003; 45(11) 1346-1350.

[105] Narayanan NN, Baisakh N, Vera Cruz CM, Gnanamanickam SS, Datta K, Datta SK. Molecular breeding for the development of blast and bacterial blight resistance in rice cv. IR50. Crop Science 2002; 42(6) 2072-2079.

[106] Xing J, Jia Y, Correll JC, Lee FN, Cartwright R, Cao M, Yuan L. Analysis of genetic and molecular identity among field isolates of the rice blast fungus with an international differential system, Rep-PCR, and DNA sequencing. Plant diease 2013; 97(4) 491-495.

Chapter 8

Rice Straighthead Disease – Prevention, Germplasm, Gene Mapping and DNA Markers for Breeding

Wengui Yan, Karen Moldenhauer, Wei Zhou,
Haizheng Xiong and Bihu Huang

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/56829

1. Introduction

Straighthead is a physiological disorder of rice that results in sterile florets with distorted lemma and palea, and in extreme cases, the panicles or heads do not form at all (Atkins, 1974). As a result, heads remain upright at maturity due to lack of grain development: hence, the name 'straighthead'. The diseased panicles may not emerge from the flag leaf sheath when the disease is severe. Either the lemma or palea or both may be lacking, even if they are present they are distorted and crescent-shaped, particularly in long grain cultivars, forming a characteristic symptom of straighthead called 'parrot beak' (Rasamivelona et al., 1995). Other symptoms include unusually vigorous dark green leaves in mature plants and strikingly abnormal root systems with large, shallow roots with few branches and root hairs (Atkins, 1974; Bollich et al., 1989).

Figure 1. Straighthead symptoms in rice field of the United States (US) (left and middle) and Argentina (right).

Straighthead can cause a complete loss of grain yield in rice when sever (Fig. 1). In a study conducted by Wilson et al. (2001), grain yield reduction due to straighthead was up to 94% for a popular cultivar Cocodrie (Table 1). Yan et al. (2005) concluded that US cultivar Cocodore, Mars, Kaybonnet and Bengal were highly susceptible to straighthead, indicated by a yield reduction from 80% for Bengal to 96% for Mars in a study conducted in 1999 and 2000 (Fig. 2). Similarly, in a study conducted in 2001, Cocodrie and Mars suffered a yield reduction of 97% and 95%, respectively from straighthead (Table 2). Cocodrie, Cypress and Wells were grown on 73% of rice hectares in the southern US in 2001 (RTWG, 2002). The susceptibility of these widely grown cultivars to straighthead represents a potentially serious threat to southern US rice production, especially for Arkansas where about 50% of the US rice is produced (Wilson et al., 2010a). Therefore, the prevention of straighthead is not only an important target in the DD50 Computerized Rice Management Program http://dd50.uaex.edu/dd50Logon.asp (Slaton, 2001), but also is reminded to rice growers each year when the time of its prevention is getting close by Cooperative Extension Agents http://www.uaex.edu (Wilson et al., 2010b; 2010c).

Cultivar	Continuous flood	Drain and dry	10 days delay flood	20 days delay flood	Yield loss %*
Bengal	1210	5695	3629	4435	79
Cocodrie	353	6048	1361	1865	94
Cypress	3427	6250	6602	6300	45
Drew	4032	6905	5292	6451	42
Jefferson	5695	6854	6653	6048	17
Madison	3478	6149	4536	4990	43
Priscilla	5594	7510	7358	5443	26
Wells	5695	7913	6250	7459	28
LSD 0.05	2923 for comparing water managements within a cultivar				
LSD 0.05	1663 for comparing cultivars within a water management				

*Yield loss (%) for each cultivar was calculated by: [(Drain and dry yield – Continuous flood yield) / Drain and dry yield] x 100.

Table 1. Grain yield (kg/ha) of rice cultivars affected by straighthead disease under different water managements at the Rice Research and Extension Center, University of Arkansas near Stuttgart during 1999 (Wilson et al., 2001).

http://dx.doi.org/10.5772/56829

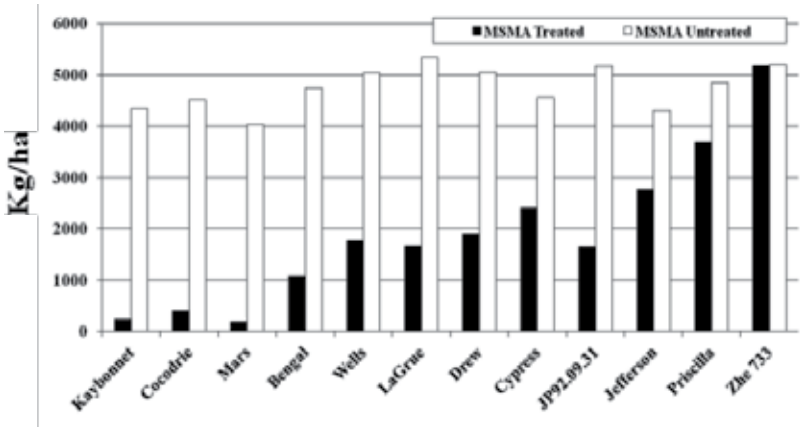


Figure 2. Comparison of grain yield between straighthead affected (MSMA Treated) and un-affected (MSMA Untreated) cultivars at the Dale Bumpers National Rice Research Center near Stuttgart, Arkansas in 1999 and 2000, where straighthead was induced by soil incorporation of 6.7 kg of monosodium methanearsonate (MSMA) per hectare (Yan et al., 2005).

2. Global threat from straighthead and its causal factors

Straighthead was first reported to dramatically affect grain yield in the US by Hewitt (1912). In the early 1900s, Collier (1912) estimated that approximately 20% of the US rice acreage suffered significant yield reductions by 12 to 15% due to straighthead. Afterwards, straighthead researches were published in Japan (Iwamoto, 1969), Portugal (called 'branca') (Cunha and Baptista, 1958), Australia (Dunn et al., 2006), Thailand (Weerapat, 1979), and Argentina (Yan et al., 2010a) (Fig. 1).

No pathogen has been identified to be associated with straighthead, so it is regarded as a physiological disease. The occurrence and severity of straighthead have been associated with soil organic matter (Editor's Note, 1946), low pH and low free iron (Baba and Harada, 1954), thiol compounds (Iwamoto, 1969), sandy to silt loam soil textures (Rasamivelona et al., 1995; Slaton et al., 2000), continuous flooding (Wilson et al., 2001), high soil As (Gilmour and Wells, 1980), N fertilization (Dilday et al., 1984; Dunn et al., 2006), and soil Cu availability (Ricardo and Cunha, 1968). A recent work suggested possible roles of magnesium but not As in naturally-occurring straighthead by chemical analyses of rice plant (node, internode, stem, leaf and root) and seed (brown and milled seed and hull) (Belefant-Miller and Beaty, 2007). Soil aeration is believed to speed the decay of soil organic matter (Editor's Note, 1946) and help oxidize arsenic (As) into arsenate, which is biologically inactive (Marin et al., 1992). Arsenic is toxic to many plant species including snap bean (*Phaseolus vulgaris* L.) (Sachs and Michael, 1971), soybean (*Glycine max* L.), potato (*Solanumtuberosum*L.), cotton (*Gossypiumhirsutum* L.), and rice (Baker et al., 1976).

In a straighthead study conducted by Yan et al. (2008) using resistant and susceptible cultivars in 2004 and 2005, minerals in flag leaves of heading panicles were measured because the

susceptible cultivars could not produce seeds and direct measurement on seeds is not feasible. Straighthead was correlated negatively with grain yield (r=-0.89), plant height (r=-0.60) and flag leaf contents of Ca (r=-0.51), Mn (r=-0.31) and S (r=-0.26) and positively with days to head (r=0.63). Leaf Ca was associated positively with grain yield (r=0.60), leaf Mn (r=0.81), Fe (r=0.42), S (r=0.40) and Cu (r=0.38) and negatively with days to 50% heading (r=-0.64). The increased Mn in the flag leaves was associated with the increased leaf Ca (r=0.81), Fe (r=0.49), Cu (r=0.48), S (r=0.40) and As (r=0.29), but with the decreased days to 50% head (r=-0.56). Flag leaf S concentration was correlated positively with plant height (r=0.37), grain yield (r=0.35) and leaf P (r=0.59), K (r=0.49) and Mn (r=0.40) and negatively with days to head (r=-0.64) and leaf Na (r=-0.41) and Zn (r=-0.41). Leaf As concentration was correlated with the leaf Cu (r=0.65), Na (r=0.58), Fe (r=0.51) and Mn (r=0.29), but negatively with leaf K (r=-0.49) and B (r=-0.42). However, the exact causal factors of naturally occurring straighthead are still unknown.

3. Methods for straighthead evaluation and prevention

3.1. Evaluation methods for straighthead

Because the symptoms of As injury are similar to straighthead of rice, incorporation of As in a form of monosodium methanearsonate (MSMA) has become the common and only practice for evaluating rice susceptibility to straighthead in research and breeding programs up to present (Horton et al., 1983; Frans et al., 1988; Wilson et al., 2001; Dunn et al., 2006; Pan et al., 2012).

A special field has been designated for straighthead research and breeding with MSMA amendment for more than 20 years (Somenahally et al., 2011) at the University of Arkansas, Division of Agriculture, Rice Research and Extension Center (RREC) jointly located with the United States Department of Agriculture (USDA), Agricultural Research Service (ARS), Dale Bumpers National Rice Research Center near Stuttgart, Arkansas. Usually, the MSMA as a solution in a spray volume of 85 L ha^{-1} at a rate of 6.7 kg MSMA ha^{-1} is directly applied to the soil surface with a calibrate CO_2-backpack sprayer and incorporated into the soil before planting the seeds (Yan et al., 2008).

At maturity of growth stage R9 (Counce et al., 2000), straighthead is visually rated in the center of a plot based on floret fertility or sterility and panicle emergence from the flag leaf sheath. The rating scale ranged from 1 to 9, 1 = no apparent sterility (more than 80% grains developed) and 100% of the panicles completely emerged; 2 = 71 to 80% of the grains developed and 96 to 100% of the panicles completely emerged; 3 = 61 to 70% of the grains developed and 91 to 95% of the panicles completely emerged; 4 = 41 to 60% of the grains developed and 85 to 90% of the panicles completely emerged; 5 = 21 to 40% of the grains developed and 75 to 80% of the panicles completely emerged (at this stage distorted and parrot-beak grains initially appear); 6 = 11 to 20% of the grains developed and 65 to 70% of the panicles completely emerged; 7 = 0 to 10% of the grains developed and most of the panicles emerged but remained totally erect; 8 = no grains developed and 0 to 10% of the panicles emerged from the flag leaf sheath but erect; and 9 = short stunted plants with no panicle emergence. Indicated by Table 2, at rate 1

straighthead, cultivars have either no numerical reduction of yield or slightly numerical reductions which are far from statistical significance ($p>0.60$). The yield reduction is not statistically significant at the rate 4 or below, but highly significant ($p<0.0001$) at the rate 7 with a reduction of 95% or above.

			MSMA untreated§					MSMA treated#			Yield differences due to SH††	
	PI†	Subspecies‡	Yield	Heading	Height	Lodging‡‡	SH rate¶	Yield	Heading	Height		
	-		kg ha^{-1}	d	cm			kg ha^{-1}	d	cm	kg ha^{-1}	$P > \|t\|$
					Very early group 1							
Luhoogzao	615199	I	6405	68	92	7	1	7337	70	89	931	0.35
Aijiaonante	614994	I	5826	68	94	4	1	6149	70	90	323	0.72
Zhong 86-44	615202	I	7057	67	97	8	1	7191	69	100	134	0.90
Zhenshan 97	614966	I	6588	69	96	3	1	6467	71	90	−121	0.89
Zao 402	615218	I	6387	68	99	8	1	5929	70	94	−458	0.65
Xiangzaoxian No1	614981	I	9010	68	97	2	1	8550	71	93	−460	0.61
Zanuo No1	614988	I	6927	65	116	5	2	6397	68	110	−523	0.57
Dian No. 01	614991	I	6584	83	128	2	3	5484	87	125	−1100	0.22
Tie 90-1	614999	I	8744	63	89	7	1	7641	66	93	−1102	0.27
LSD 0.05			2375	2	6	3	1	1267	2	8		
CV			27	2	4	48	24	18	2	6		
					Early group 2							
Shufeng 109	615014	I	4782	83	121	6	3	5896	88	119	1115	0.27
Danwanbao 24	615214	I	4889	83	99	1	2	5716	84	84	827	0.35
LSD 0.05			1999	2	6	2	1	1428	3			
CV			21	1	4	59	15	43	2			
					Intermediate group 3							
Gui 99	614958	I	5954	90	122	1	2	5947	93	104	−7	0.99
Shufeng 117	615017	I	7167	89	127	1	2	6256	93	127	−911	0.31
LSD 0.05			2256	1	5	2	1	1414	2	7		
CV			23	1	3	66	14	44	2	5		
					Late group 4							
Jing 185-7	615205	J	6679	88	94	2	3	7072	89	92	393	0.66
Jinnuo No6	614990	I	6383	94	128	3	3	6865	96	117	482	0.59
Sheng 10	615001	I	6935	91	119	1	2	6826	94	110	−108	0.90
Sheng 12	615003	I	6004	91	133	1	3	4901	93	123	−1103	0.22
CDR 22	615008	I	6428	99	113	5	3	5311	102	113	−1117	0.25
Shufeng 121	615015	I	7024	90	122	2	3	5827	93	111	−1197	0.18
LSD 0.05			1514	1	6	2	1	1225	8	10		
CV			16	1	4	57	12	32	6	7		
Zhe 733 (CK)	629016	I	6829	64	99	3	1	6842	67	94	13	0.97
Cocodrie (CK)		J	7409	79	87	1	8	202	89	83	−7207	<0.0001
Priscilla (CK)		J	6350	80	90	1	4	5292	86	86	−1058	0.25
Mars (CK)		J	7006	81	107	1	7	353	88	102	−6653	<0.0001

† PI: Plant Introduction number in the U.S. germplasm system.

‡ Subspecies: I = *indica* and J = *japonica.*

§ No MSMA (monosodium methanearsonate) was applied as check conditions.

Stralghthead (SH) raling 1-9: 1 as normal and 9 as the worst SH.

Soil was treated with 6.7 kg MSMA ha^{-1} to induce straighthead.

†† Yield d ifference = Treated yield - Untreated yield, and *P* is probability of *t* test for the difference.

‡‡ Lodging 1-9 scale: 1 as no plants lodged and 9 as over 80% plants lodged.

Table 2. Nineteen Chinese rice germplasm accessions had no significant yield reductions from straighthead induced by MSMA (monosodium methanearsonate) at 6.7 kg hn^{-1} in 2001 (Yan et al., 2005).

The soil to induce straighthead by application of MSMA for research purposes was studied by Yan et al. (2008) (Table 3). In the straighthead evaluation soil amended by MSMA, pH and Mehlich-3 extractable P, Ca, Mg, Fe, Zn and As concentrations are significantly lower, while S, Mn and As are higher than those in the native soil where MSMA has never been applied. However, soil electronic conductivity, organic matter and K, Na and Cu concentrations are not affected by the amendment of MSMA. Decreased soil pH resulted from the MSMA is

significantly associated with decreased Ca (r=0.92), Mg (r=0.78), and P (r=0.41), but increased As (r=-0.87), S (r=-0.73), and Mn (r=-0.59) concentrations in the soil.

	pH	EC†	P	K	Ca	Mg	S	Na	Fe	Mn	Zn	Cu	As	SOM‡
		Umhos cm^{-1}	mg kg^{-1}											g kg^{-1}
No MSMA	5.9a§	188a	31a	180a	1053a	185a	9b	66a	312a	178b	0.9a	1.1a	5.9c	21a
Before MSMA	5.3b	196a	19b	182a	795b	154b	16a	70a	283b	211a	0.8b	1.2a	16.0b	22a
After MSMA	5.3b	192a	14b	164a	759b	146b	17a	74a	301ab	211a	0.8b	1.2a	19.5a	24a
CV,%	4	20	37	17	6	9	13	23	11	7	21	7	13	12

† EC, soil electrical conductivity.

‡ SOM, soil organic matter.

§ Means in each column with the same letter are not significantly different at the 0.05 probability level

Table 3. Soil properties and minerals for samples collected from the straighthead designated field before (Before MSMA) and after (After MSMA) the application of 6.7 kg MSMA ha^{-1} in comparison with native soil sample which never receives MSMA application (No MSMA) in 2004 and 2005. (Before MSMA soil received MSMA application previously for straighthead studies) (Yan et al., 2008).

3.2. Prevention methods in rice production

The sporadic nature of straighthead and the lack of a specific and definite causal factor have made straighthead difficult to be prevented. Since 1950s, rice researchers had tried to prevent straighthead using chemical application. Evatt and Atkins (1957) applied Feralum, a mixture of ferric and aluminum sulfates to soil for controlling straighthead. In Portugal, Cu deficiency was found to be associated with straighthead (Karim and Vlamis, 1962), and application of copper sulfate to the soil when seedlings were transplanted was reported to prevent or greatly reduce straighthead (Cunha and Baptista, 1958). Ricardo and Cunha (1968) studied copper sulfate as a supplier of Cu for straighthead control since soil organic matter may bind Cu and reduce its availability for uptake by plants. However, chemical prevention never reaches applicable scale because an effective chemical has never been developed, so the control effects are not stable.

A water management practice that is called 'Draining and Drying' was developed by farmers in the early 1900s (Atkins et al., 1957; Slaton, 2001), and is currently used as the only recommended method to prevent straighthead in rice through DD50 Computerized Program and agricultural extension system in the USA (Wilson et al., 2010b; 2010c). Rice fields are drained about 2 weeks after a permanent flood, dried thoroughly until cracks appear in the soil and rice leaves begin to curl and exhibit yellowing as drought stress symptoms, and then re-flooded for the remainder of season. The drying must be completed about 10 to 14 days before the internode elongation starts (Wells and Gilmour, 1977), and the best timing could be predicted by the online DD50 Program http://dd50.uaex.edu/dd50Logon.asp. Fields that favor straighthead are permanent, which means each time when rice is planted, straighthead will develop at some level to cause yield losses if the flood is not drained for the soil to be aerated at

http://dx.doi.org/10.5772/56829

appropriate time (Wilson et al., 2010c). Soil aeration is believed to speed the decay of soil organic matter (Editor's Note, 1946) and help oxidize arsenic (As) into arsenate, which is biologically inactive (Marin et al., 1992). Therefore, once straighthead occurs in a field, growers will keep using the Draining and Drying method permanently because of unaffordable consequences.

Table 1 shows cultivar variation on yield recovery of the Draining and Drying from the traditional-continuous flood. Long grain type cultivar Cocodrie and medium Bengal are high recovery cultivars with about 80% of the recovered yield. Cypress, Drew and Madison are the intermediate recovery cultivars with more than 40% of the yield to be recovered by the Draining and Drying. Jefferson, Priscilla and Wells are the low recovery cultivars because they display certain resistance to straighthead.

Currently, the Draining and Drying method is applied to more than one third of the rice acreage in Arkansas as a preventative measure (Wilson, per. Comm.). Using Arkansas rice harvested area of 723,000 hectares in 2010, K.B. Watkins, agricultural economics professor in the University of Arkansas, Rice Research and Extension Center, made the following estimates: $ 9.21/ha for additional labor cost to open levee gates for the draining, $ 20.93/ha for power cost to water the dried fields afterwards, and $ 56.77/ha for additional application of fungicide to control blast since blast disease is known to be more severe in fields or parts of fields in which the water in paddies falls below recommended levels (TeBeest et al., 2007). As a result, straighthead prevention added either $ 7.264 million for the draining and reflooding only or $ 20.945 million for the draining, reflooding and blast control to rice growers in Arkansas. Furthermore, an additional 308.4 m^3 of water are required to re-flood each hectare after drying, which resulted in an extra 74.324 million m^3 of water utilized for straighthead prevention in Arkansas in 2010. Wasting water is becoming a public concern because Lonoke, Prairie, Arkansas, and Jefferson counties with 150,317 hectares of rice in 2010 have been designated as having critical levels of groundwater (Riley, pers. comm.). Thus, preserving the natural resource of water is important for the long term economic viability of these counties. Therefore, the Draining and Drying method for straighthead prevention is costly for rice growers and wasteful of natural resources, and results in drought-related yield loss.

3.3. Resistant germplasm for straighthead breeding

Varietal resistance is regarded as the most efficient, economical, and environmentally friendly strategy for straighthead prevention (Wilson et al., 2001; Yan et al., 2005; Dunn et al., 2006). The earliest attempt at breeding for straighthead resistance in the USA started in 1950s (Atkins et al., 1957), but little progress had been made because the inheritance of straighthead resistance had not been well understood because of limited resistant germplasm until 2002 (Yan et al., 2002).

In 2001, 124 accessions of germplasm including 109 *indica*and 15 *japonica* cultivars introduced from China were evaluated for straighthead resistance, and 19 showed resistance to straighthead (Table 2) (Yan et al., 2005). Seven had increases of grain yield from 134 to 1115 kg ha^{-1} under the influence of straighthead, and the other 12 had reductions from 7 to 1197 kg ha^{-1}, but all the increases and decreases due to straighthead were not significant. Their straighthead

Core No.	PI	Name	Country	Region	SH03	SH04	PCAx	PCAy
46	11009	GPNO 254	United States	North America	3.7	4.0	0.031	0.069
314	350300	Plovdiv	Bulgaria	Eastern Europe	2.7	3.5	−0.632	−0.1
385	388243	Ponta Rubra	Portugal	Western Europe	2.0	3.3	−0.647	−0.026
488	400345	U.V.S. Unblatuzi	Africa	Africa	3.0	1.8	−0.039	−0.315
671	439687	Linia 84 Icar	Romania	Eastern Europe	4.0	1.8	−0.492	0.006
700	505386	IR 31779-112-1-2-2-3	Philippines	South Pacific	2.7	1.5	−0.036	−0.34
746	596815	376	Cambodia	Southeast Asia	3.7	3.0	−0.166	−0.329
748	596827	IR-44595	Nepal	Subcontinent	3.0	1.5	−0.059	−0.321
980	281758	Cesariot	France	Western Europe	3.3	4.0	−0.519	−0.172
997	291539	Lusitano	Portugal	Western Europe	4.0	4.3	−0.546	0.016
1159	400072	L-IV-34	Romania	Eastern Europe	3.7	3.0	−0.513	−0.006
1178	401458	29 LU 1	China	China	4.0	3.5	−0.041	−0.44
1198	403546	WC 6570	Spain	Western Europe	3.0	4.5	−0.292	−0.083
1344	458488	IR 9209-26-2	Philippines	South Pacific	1.7	1.8	−0.07	−0.335
1347	464599	IR 19759-21-3-3-2	Philippines	South Pacific	3.0	3.2	−0.072	−0.368
1353	494757	Hunan early dwarf No.3	China	China	3.7	3.0	−0.003	−0.48
1356	503036	Chao Lang 1 Hao	China	China	2.3	3.5	−0.072	−0.366
1395	584644	Spalcik	Russian Federation	Eastern Europe	3.3	2.8	−0.78	−0.1
1397	584650	Avangard	Uzbekistan	Central Asia	3.0	2.7	−0.709	−0.098
1405	584678	Huri 282	Colombia	South America	3.0	3.3	−0.189	−0.49
1417	596902	CNTLR80076-44-1-1-1	Thailand	Southeast Asia	2.7	4.0	−0.08	−0.295
1443	614958	GUI 99	China	China	2.3	2.5	−0.221	−0.513
1447	614962	Xiangzhaoxian No.15	China	China	1.3	2.4	−0.073	−0.288
1450	614966	Zhenshan 97	China	China	1.0	1.8	−0.06	−0.441
1460	614979	Wunong No. 2	China	China	2.3	3.2	−0.002	−0.357
1467	614990	Jinnuo No.6	China	China	1.3	1.5	−0.073	−0.423
1470	614994	Aijiaonante	China	China	3.0	1.8	−0.063	−0.484
1475	614999	TIE 90-1	China	China	2.7	1.8	−0.1	−0.457
1491	615192	You-I B	China	China	1.3	2.3	−0.084	−0.355
1497	615198	Chunjiangzao No.1	China	China	3.7	2.5	−0.749	−0.072
1498	615199	Luhongzao	China	China	1.3	1.0	−0.028	−0.374
1499	615200	Zhong 156	China	China	2.3	1.5	−0.003	−0.41
1501	615202	Zhong 86-44	China	China	2.0	1.3	−0.066	−0.332
1502	615203	Zhongyouzao No. 5	China	China	1.0	1.5	−0.083	−0.525
1504	615206	Minkezao No. 22	China	China	1.7	1.7	−0.081	−0.382
1507	615210	Shangyu 394	China	China	2.0	1.7	−0.733	−0.009
1510	615214	Danwanbao 24	China	China	2.0	1.5	−0.106	−0.317
1513	615218	Zao 402	China	China	2.0	1.5	−0.185	−0.384
1514	615219	Chaoyang No. 1	China	China	2.0	1.3	−0.065	−0.414
Jing185_7	615205	Jing185_7	China	China	1.0	1.5	−0.172	−0.261
Shufeng109	615014	Shufeng109	China	China	1.0	1.5	−0.038	−0.412
Zhe733	634573	Zhe 733	China	China	2.3	1.9	−0.017	−0.343

Table 4. USDA core collection number, plant introduction (PI), cultivar name and country of origin, average rate of straighthead in 2003 (SH03) and 2004 (SH04) for resistant accessions rated 4 or less on a 1-9 scale and their positions in principal component analysis (PCA) (Agrama and Yan, 2010).

ratings ranged from 1 to 3 while susceptible check Cocodrie and Mars were rated 8 and 7, respectively.

All the resistant cultivars are *indica*. In terms of the cultivar 'Jing 185-7', ('Jing' means *japonica* in Chinese), a study has indicated that Jing185-7 is an *indica*(Agrama and Yan, 2010). Nine accessions of the resistant germplasm are in the very early group having 63 - 69 days to heading except Dian No. 01, two in the early group having 83 days to heading, two in the intermediate group having 89 - 90 days to heading, and all six in the late group having 90 or more days to heading except Jing 185-7. Preliminary observation of days to heading had incorrectly classified Dian No. 01 in the very early group. Plant heights vary from 89 cm for Tie 90-1 in the very early group to 133 cm for Sheng 12 in the late group. Two accessions, Zanuo No1 and Jinnuo No6, are waxy endosperm type containing no amylose, and the other seventeen non-waxy accessions have amyloses ranging from 14.8% for Shufeng 121 to 27.0% for Shufeng 109 in their endosperms. Aijiaonante is the first semi-dwarf cultivar bred in 1956 in China (Qian and Liu, 1993), and Zhenshan 97 is a popular maintainer line of hybrid rice in China (Virmani, 1994).

http://dx.doi.org/10.5772/56829

In 2002, 1002 accessions selected from 1794 accessions of the USDA Rice Core Collection (Yan et al., 2007; 2010b; Agrama et al., 2010) were evaluated for straighthead resistance in Arkansas (Agrama and Yan, 2010). These selections have proper maturities ranged from 48-110 days and plant heights ranged from 65-150 cm because the maturity and height largely affect the assessment of panicle fertility, which is essential for straighthead infestation. Those rated 4 or less in the 2003 straighthead evaluation were verified in larger plots and more replications in 2004. In total, 42 accessions (4.2%) displayed resistance (Table 4).

The 42 resistant cultivars originate from 15 countries in ten geographic regions worldwide, with the most (24 or 57%) from China, are classified into 5 clusters (Fig. 3) (Agrama and Yan, 2010). Cluster K1 includes three references, indicating none of the resistant cultivars belong to *Deep water*, *Australian* and *Aromatic* type. K2 includes 13 *indica* cultivars referenced by Zhe733, all from China except entry 488 from an unknown country in Africa. Referenced by IR64, K3 consists of another group of 12 *indica* cultivars originated from six countries of five regions: China, South America, South Pacific, Southeast Asia and the Subcontinent. Four Chinese cultivars, entry 1467, 1475, 1502 and Shufeng109, are positioned between K2 and K3. K4 has two *Tropical Japonica* references only and K5 contains 11 *Temperate Japonica* cultivars originating from seven countries of four regions: Centeral Asia, China, and Eastern and Western Europe. Two cultivars are positioned between K4 and K5: entry 46 (GPNO 254) developed in Louisiana, U.S.A. and entered in the germplasm collection in 1977; and entry 1198 (WC 6570) developed in Spain and entered the collection in 1975.

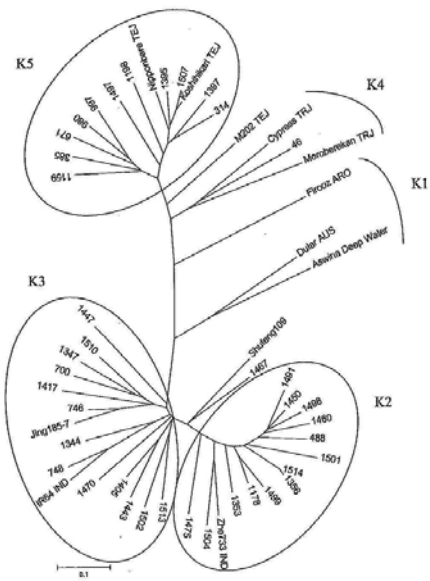


Figure 3. Unrooted neighbor-joining tree based on C.S. Chord (Cavalli-Sforza and Edwards, 1967) for 42 accessions resistant to straighthead rated 4 or less in a 1-9 scale and derived from the USDA rice core collection (Core entry number used in the chart) and reference cultivars (McNally et al. 2006) (AUS-Australia, ARO-Aromatic, IND-Indica, TRJ-Tropical Japonica, TEJ-Temperate Japonica) genotyped with 72 molecular markers (Agrama and Yan, 2010).

4. Gene mapping and development of DNA markers for breeding

4.1. Association mapping of quantitative trait loci (QTL) for straighthead

Because of the sporadic nature of straighthead and its unidentified causes, molecular marker assisted selection is essential for improvement of resistance in breeding programs. To take advantage of recent advances in gene-mapping technology, we executed a genome-wide association mapping study to identify genetic markers associated with straighthead using 547 accessions of germplasm from the USDA rice core collection and 75 simple sequence repeat (SSR) markers covering the entire rice genome (Agrama and Yan, 2009). A mixed-model approach combining the principal component assignments with kinship estimates proved to be particularly promising for association mapping. The extent of linkage disequilibrium was described among the markers. Seven marker loci are highly-significantly associated with straighthead at a significance level of 0.0001 = 4.0 value of $-\log_{10}q$ (Fig. 4).

The SSR markers RM263, RM105 and RM277 on chromosomes (chr) 2, 9 and 12, respectively, show very strong association with straighthead ($p< 9.83x10^{-8}$, $q< 1.31x10^{-6}$). Four other loci, RM490, RM413, RM116 and RM224 are highly associated with the disorder ($p< 0.0001$). Three alleles, each of marker RM490 (87 bp), RM413 (105 bp) and RM277 (122 bp), and two alleles (182 bp and 183 bp) of RM263 show significantly low straighthead rates of resistance. Only three accessions (core entry 748, 1344 and 1402) carrying allele 105 bp of RM413 have the lowest straighthead rate with the average of 3.9. Nine accessions with the allele 122 at RM277 on chr 12 (57.2 cM) have a significantly low straighthead rate (4.1). The rates of 15 accessions with allele 182 at RM263 (chr 2) are lower (4.6), on average, than the accessions with other alleles. Moderate straighthead rates are associated with alleles 87 bp at RM490 (23 accessions), 183 bp at RM263 (15 accessions) and 137 bp at RM105 (59 accessions).

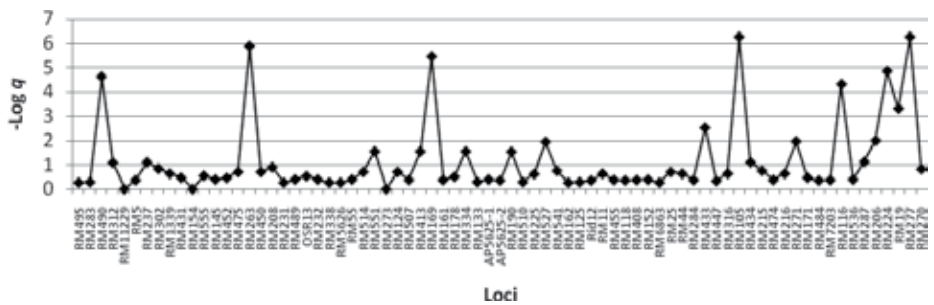


Figure 4. Marker loci significantly associated with straighthead disease with a value of - Log q = 4 which indicates a correlation probability 0.0001 among 547 accessions of germplasm in the USDA rice core collection, which were phenotyped in Arkansas and genotyped with 75 genome-wide SSR markers (Agrama and Yan, 2009).

4.2. Identification of a major QTL for straighthead resistance

We mapped the QTLs for straighthead using two recombined inbred line (RIL) F9 populations, one with 170 lines genotyped with 136 SSRs and another with 91 lines genotyped with 159

http://dx.doi.org/10.5772/56829

SSRs (Pan et al., 2012). These lines were evaluated for straighthead in both 2008 and 2009 with three replicates per year.

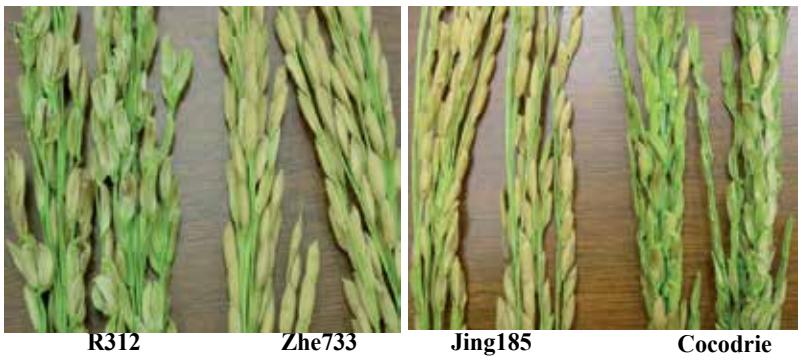


Figure 5. Straighthead phenotypes in parents of mapping populations,resistant parents Zhe733 and Jing185 with fully developed panicles while susceptible parents R312 and Cocodrie with severely distorted spikelets (Pan et al., 2012).

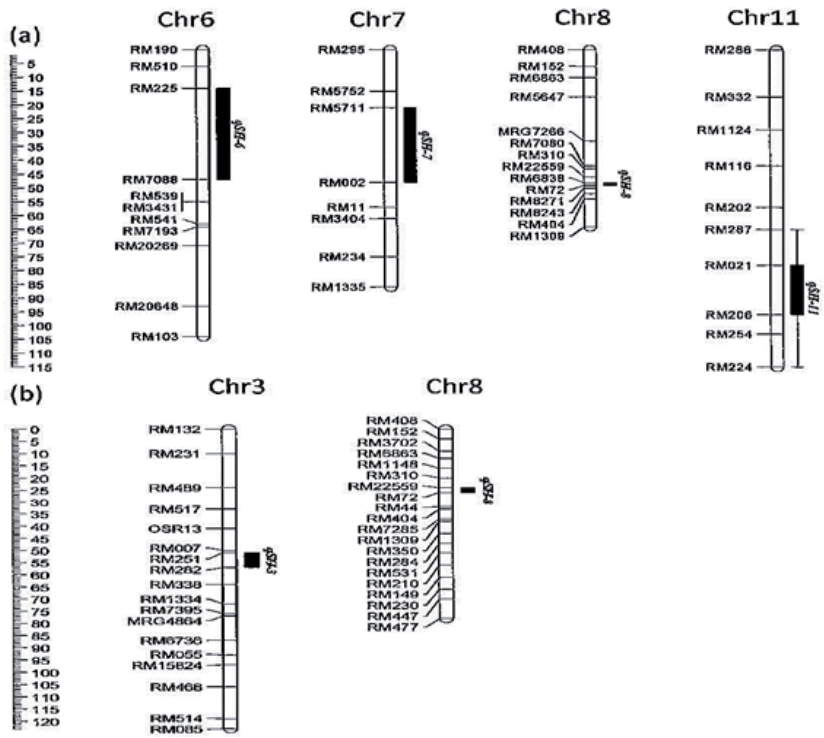


Figure 6. Four QTLs for straighthead resistance are identified from RIL F9 population of Zhe733/R312 (a) and two QTLs from RIL F9 population of Cocodrie/Jing185, are marked by black bar (Pan et al., 2012).

Four QTLs were identified to be associated with straighthead resistance in the Zhe733/R312 population on chr6, 7, 8 and 11 (Fig. 6a). The QTL on chr8 had the largest LOD (23.0), highest additive effect (-2.1) and smallest marker interval (1.0 cM) between RM6838 and RM72, and explained the most total variation (46%) for straighthead among the identified QTLs. From the Cocodrie/Jing185 population, two QTLs were identified (Fig. 6b), one on chr3 (LOD=3.8), and another on chr.8 (LOD= 27.0). The chr.8 QTL is within a 1.9 cM interval between RM22559 and RM 72, has a -2.1 additive effect, and explained 67% of total variation. RM72 at 6.76 Mb is the most distal marker of the chr8 QTL identified in both populations. RM6838 in Zhe733/R312 and RM22559 in Cocodrie/Jing185 are physically located very close to each other at 5.85 Mb and 5.70 Mb, respectively. The overlapping intervals on chr.8 identified in both populations indicate the presence of a major QTL at this location, designated as *qSH-8* (Fig. 5a for Zhe733/R312 and 5d for Cocodrie/Jing185).

4.3. Fine mapping of *qSH-8*, a Major QTL for straighthead resistance

Within the putative region of *qSH-8*, four recombinants (RIL12, 112, 174, and 306) are identified in Zhe733/R312 and four recombinants (RIL418, 423, 480, and 533) are identified in Cocodrie/Jing185 population for fine mapping according to the substitution strategy described by Paterson et al. (1990). Using an additional 16 SSR markers derived from the Gramene database http://www.gramene.org/, and 9 InDel markers designed from the MSU rice genome browser http://rice.plantbiology.msu.edu/cgi-bin/gbrowse/rice/to compare the sequence of Nipponbare with 93-11 in the targeted region,qSH-8 is fine mapped in a 290 kb interval between RM22573 and InDel 27 in the Zhe733/R312 population, and a 690 kb region between InDel 11 and RM22613 in the Cocodrie/Jing185 population (Fig. 7).

Three markers, SSR AP3858-1, InDel 11 and InDel 5 are in the 290 kb interval, and should co-segregate with *qSH-8* to predict either resistance or susceptibility of a rice line to straighthead. Both RIL 12 and 306 in the Zhe733/R312 population have the R312 genotype at AP3858-1, InDel 11 and InDel 5 loci, which matched up with the R312 phenotype, susceptible to straighthead with high ratings (8.7±0.5 for RIL 12 and 6.8±1.3 for RIL 306). Conversely, both RIL 112 and 174 have the Zhe733 resistant genotype at these loci, and have low straighthead ratings (1.6±0.9 for RIL 112 and 1.3±0.5 for RIL 174) as well. These results prove the hypothesis that co-segregation exists between *qSH-8* genotype and straighthead phenotype.

4.4. Marker development for marker-assisted breeding of straighthead resistance

We have tested 72 accessions of global germplasm for a match between straighthead phenotype and *qSH-8* genotype indicated by the markers AP3858-1 and InDel 11. The 72 accessions originated from 28 countries, and a large portion of them (22 accessions) were from China, followed by the Philippines and the USA. Forty of the tested accessions are resistant to straighthead with ratings of 4 or less, and the remaining 32 are susceptible with straighthead ratings of 6 or more based on previous studies by Yan et al. (2002; 2005) and Agrama and Yan (2009; 2010). For InDel 11, 30 accessions have either no alleles of or alleles different from parental Zhe733, R312, Cocodrie and Jing185. The remaining 42 have the parental alleles for InDel 11, where 32 genotypes have a good match with the expected phenotype (Table 5). For

http://dx.doi.org/10.5772/56829

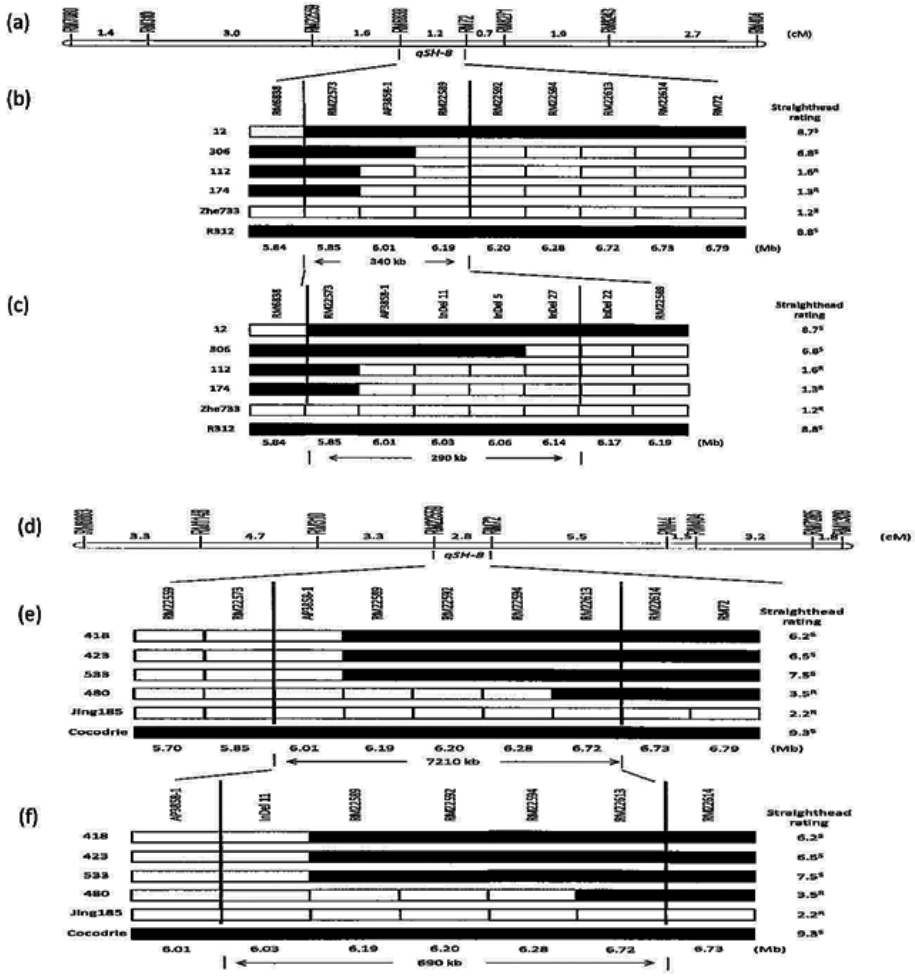


Figure 7. Fine mapping of *qSH-8* using Zhe733/R312 (a-c) and Cocodrie/Jing185 (d-f) F_9 RIL populations. (a) *qSH-8* regionof 1.2 cM between RM6838 and RM72, (b) a 340 kb region between RM22573 and RM22589, (c) a 290 kb region between RM22573 and InDel 27; (d) *qSH-8* regionof 2.8 cM between RM22559 and RM72, (e) a 710 kb region between AP3858-1 and RM22613 and (f) a 690 kb region between InDel 11 and RM22613 (Pan et al., 2012).

marker AP3858-1, 38 accessions do not have the parental alleles, and 25 out of the remaining 34 accessions have a good match between the genotype and phenotype. Because InDel 5 is monomorphic in the Cocodrie/Jing185 population, it is not desirable for screening the global germplasm collection. χ^2 test indicates a high association of InDel 11 with straighthead (P=0.0014), with 76.2% of the genotypes matching the phenotypes among those global accessions (Table 6). Similarly, AP3858-1 is highly associated with straighthead (P=0.0004) with a match of 73.5%. In the Zhe733/R312 population, all three markers (InDel 5, InDel 11, and AP3858-1) are verified by χ^2 test at the P<0.0001 level of significance for all where AP3858-1 has a slightly higher ratio of co-segregation (80.0%) than InDel 11 (79.6%) and InDel 5 (78.5%). InDel 5 is not polymorphic in the Cocodrie/Jing185 population, and the remaining two markers

are verified at the $P<0.0001$ level of significance for both. InDel 11 has slightly higher ratio of co-segregation (85.1%) than AP3858-1 (81.2%) in the Cocodrie/Jing185 population.

ACNO***	ACP	Name	Country of Origin	Allele Size	Genotype	Straighthead rating******
629016****	PI	Zhe733*	China	151	a*****	1.2±0.5
615205	PI	Jing185*	China	151	a	2.2±0.5
606331	PI	Cocodrie**	United States	145	b	9.3±0.5
614959	PI	R312**	China	148	b	8.3±0.5
502680	PI	Catibos	Philippines	145	b	8.7±0.5
12505	Clor	PR 433	Puerto Rico	145	b	8.7±0.5
242804	PI	Mojito Colorado	Bolivia	145	b	9.0±0.0
505386	PI	IR 31779-112-1-2-2-3	Philippines	145/151	h	2.7±0.9
596815	PI	376	Cambodia	145/151	h	3.7±2.1
596827	PI	IR-44595	Nepal	151	a	3.0±0.9
281758	PI	Cesariot	France	145/151	h	3.3±0.5
291608	PI	WC 4443	Bolivia	145	b	8.7±0.5
325909	PI	IR 237-20-1	Philippines	148	b	8.7±0.5
331504	PI	IR 547-54-1-2	Philippines	148	b	8.7±0.5
369804	PI	Blakka Tere Thelma	Suriname	145	b	8.7±0.5
392086	PI	CHONTALPA 437	Mexico	148	b	8.7±0.5
392883	PI	Five Months	Guyana	145	b	8.7±0.5
413734	PI	YR 44	Australia	145	b	8.7±0.5
458488	PI	IR 9209-26-2	Philippines	151	a	1.7±0.9
459028	PI	B 541B-PN-58-5-3-1	Indonesia	148	b	8.7±0.5
464599	PI	IR 19759-21-3-3-2	Philippines	151	a	3.0±0.8
584688	PI	CT9901-1-7-M	Colombia	145	b	9.0±0.0
608418	PI	IR 54055-142-2-1-2-3	Philippines	148	b	8.7±0.5
614958	PI	Gui 99	China	151	a	2.3±0.5
615199	PI	Luhongzao	China	151	a	1.3±0.5
615219	PI	Chaoyang No.1	China	151	a	2.0±0.8
568890	PI	Adair	United States	145	b	6.5±0.6
643127	PI	Banks	United States	145	b	6.0±0.8
PVP		CL 161	United States	145	b	6.8±1.0
634572	PI	KBNT Ipa1-1	United States	145	b	7.3±0.5
551950	PI	Mars	United States	145	b	8.0±0.0
636725	PI	Medark	United States	145	b	6.3±1.3
615014	PI	Shufeng 109	China	151	a	1.5±0.6
548630	PI	Wells	United States	145	b	6.0±0.0
614981	PI	Xiangzaoxian No.1	China	151	a	1.3±0.5
614966	PI	Zhenshan 97	China	151	a	1.0±0.0

* Zhe733 and Jingl85 as the straighthead resistant parents for the RIL populations while

** Cocodrie and R312 as the susceptible parents.

*** Core collection accessions with PI No. and C1or No, PVP as Plant Variety Protection.

***** A total of 42 accessions display parental allele screened by In Del 11. The 32 accessions listed above are those have genotype matched with phenotype, but there are other I 0 accessions which genotypes do not match with phenotypes.

**** 'a' as resistant, 'b' as susceptible, and 'h' as heterozygote genotype but still considered as resistant because straighthead is a dominant trait.

****** Straighthead rating using a 1-9 scale, with 4 or below being resistant and 6 or above being susceptible.

Table 5. Association of marker InDel 11 genotype with straighthead phenotype in a global germplasm collection (Pan et al., 2012).

http://dx.doi.org/10.5772/56829

Population	Marker name	Resistant lines		Susceptible lines		No. of total accessions used for verification	Percent match between phenotype and genotype	χ^2	P Value
		No of resistant genotype	No of susceptible genotype	No of resistant genotype	No of susceptible genotype				
Global germplasm collection*	AP3858-1	7	9	0	18	34**	73.5%	18.25	0.0004
	InDel 11	12	8	2	20	42**	76.2%	15.53	0.0014
Zhe733/R312 RIL F9 population*	AP3858-1	59	9	22	65	155	80.0%	58.02	<0.0001
	InDel 11	60	9	23	65	157	79.6%	49.33	<0.0001
	InDel 5	58	8	24	59	149	78.5%	52.64	<0.0001
Cocodrie/Jing185 RIL F9 population*	AP3858-1	24	0	13	32	69	81.2%	32.02	<0.0001
	InDel 11	25	0	11	38	74	85.1%	39.88	<0.0001

*The accessions or RILs selected for marker verification were either the resistance with straighthead rating 4 or below or the susceptibility with rating 6 or above in global germplasm collection and two F9 populations.

**A total of 34 accessions were selected for verification of AP3 858-1 because remaining 3 8 had either no alleles of or different from parental Zhe733, R312, Cocodrie and Jingl85, and for the same reason, 42 accessions were applied for verification of lnDell 11.

Table 6. Association analysis between marker genotypes and straighthead phenotype (Pan et al., 2012).

4.5. Bridge germplasm for cultivar development

Since the susceptible parent Cocodrie is a widely grown cultivar in the USA (Linscombe et al., 2000), it will be important to improve Cocodrie for straighthead resistance. Among 162 SSRs used for mapping and fine mapping in Cocodrie/Jing185 population, 101 are monomorphic between parent Cocodrie and resistant line RIL506 which is resistant with straighthead rating 2.3. Thus, the genetic similarity between Cocodrie and RIL506 is 62%. In other word, 62% of marker loci are same between Cocodrie and RIL506 in the whole genome. Four other resistant RIL lines 404, 407, 479 and 480 have a genetic similarity of more than 50% with Cocodrie. These resistant lines can be used for improving straighthead resistance in long grain *tropical japonica* cultivars like Cocodrie in the southern US. However, the susceptible R312 is not a commercial cultivar in the USA, so the improvement of straighthead resistance for R312 is not important in the USA.

Acknowledgements

The authors thank everybody who has directly and indirectly contributed to this project, and every institution that has directly and indirectly supported this project.

Author details

Wengui Yan[1], Karen Moldenhauer[2], Wei Zhou[1,2,3], Haizheng Xiong[1,2,4] and Bihu Huang[3]

1 United States Department of Agriculture, Agricultural Research Service (USDA-ARS), Dale Bumpers National Rice Research Center, USA

2 University of Arkansas, Division of Agriculture, Rice Research and Extension Center, USA

3 University of Arkansas at Pine Bluff, USA

4 Zhejiang University, State Key Lab of Rice Biology, Institute of Nuclear-Agricultural Sciences, China

References

[1] Agrama, H. A, & Yan, W. G. (2009). Association mapping of straighthead disorder induced by arsenic in Oryza sativa. Plant Breeding , 128, 551-558.

[2] Agrama, H. A, & Yan, W. G. (2010). Genetic diversity and relatedness of rice cultivars resistant to straighthead disorder. Plant Breeding , 129, 304-312.

[3] Agrama, H. A, Yan, W. G, Jia, M, Fjellstrom, R, & Mcclung, A. M. (2010). Genetic structure associated with diversity and geographic distribution in the USDA rice world collection. Natural Science , 2, 247-291.

[4] Atkins, J. G, Jr, H. M, & Beachell, L. E. Crane.(1957). Testing and breeding rice varieties for resistance to straighthead. International Rice Commission Newsletter. , 7(2), 12-14.

[5] Atkins, J. G. (1974). Rice diseases of the Americas- a review of literature. USDA Agric. Handbook , 448(448), 58-63.

[6] Baba, I, & Harada, T. (1954). Akiochi, Akagare and straighthead. *In*: Physiological diseases of rice plant in Japan. Ministry of Agriculture and Forestry, Japanese Government. Tokyo.

[7] Baker, R. S, Barrentine, W. L, Bowman, D. H, Hawthorne, W. L, & Pettiet, J. V. (1976). Crop response and arsenic uptake following soil incorporation of MSMA. Weed Sci. , 24, 322-326.

[8] Belefant-miller, H. B, & Beaty, T. (2007). Distribution of arsenic and other minerals in rice plants affected by natural straighthead. Agron. J. , 99, 1675-1681.

[9] Bollich, P. K, Linscombe, S. D, Mckenzie, K. S, Leonards, W. J, Jr, S. M, & Rawls, D. M. Walker. (1989). Evaluating commercial rice varieties and experimental lines for straighthead. Louisiana Agri. , 33(2), 4-5.

[10] Cavalli-sforza, L. L, & Edwards, A. W. F. (1967). Phylogenetic Analysis: Models and estimation procedures. American J. of Human Genetics, 19, 233-257.

[11] Collier, J. S. (1912). Rice blight.Illinois Agric. Exp. Sta. Circular 156., 19.

[12] Counce, P. A, Keisling, T. C, & Mitchell, A. J. objective, and adaptive system for expressing rice development. Crop Sci. , 40, 436-443.

[13] Cunha, J. M. A, & Baptista, J. E. (1958). Estudoda branca do arroz. I. Combated a doenca. Agronomia lusit. , 20, 17-64.

[14] Dilday, R. H, Wells, B. R, Klerk, R. A, Kuenzel, K. A, & Johnson, T. H. (1984). Induced straighthead: Interaction of genotype, arsenic, and nitrogen fertility. Proc. 20th Rice Tech. Working Group., 34.

[15] Dunn, B. W, Batten, G. D, Dunn, T. S, Subasinghe, R, & Williams, R. L. (2006). Nitrogen fertilizer alleviates the disorder straighthead in Australian rice. Australian J. Exp. Agri. , 46, 1077-1083.

[16] Editor's Note(1946). Symptoms of straighthead and its control. The Rice Journal. , 49(7), 20-36.

[17] Evatt, N. S, & Atkins, J. G. (1957). Chemical control of straighthead in rice. The Rice Journal. , 60(5), 26-37.

[18] Frans, R, Horton, D, & Burdette, L. (1988). Influence of MSMA on straighthead, arsenic uptake and growth response in rice. Univ. of Arkansas, Agri. Exp. Sta., Res. Ser. , 302, 1-12.

[19] Gilmour, J. T, & Wells, B. R. (1980). Residual effects of MSMA on sterility in rice cultivars. Agro. J., , 72, 1066-1067.

[20] Hewitt, J. L. (1912). Rice blight. Univ. of Ark. Agric. Exp. Stn. Bull:110.

[21] Horton, D. K, Frans, R. E, & Cothren, T. (1983). MSMA-induced straighthead in rice (*Oryza sativa*) and effect upon metabolism and yield. Weed Sci., , 31, 648-651.

[22] Iwamoto, R. (1969). Straighthead of rice plants effected by functional abnormality of thiol-compound metabolism. Memoirs of the Tokoyo University of Agri. XIII: , 63-80.

[23] Karim, A. Q. M. B, & Vlamis, J. (1962). Micronutrient deficiency symptoms of rice grown in nutrient culture solutions. Plant and Soil , 16, 347-360.

[24] Linscombe, S. D, Jodari, F, Bollich, P. K, Groth, D. E, White, L. M, Chu, Q. R, Dunand, R. T, & Sanders, D. E. (2000). Registration of'Cocodrie' Rice. Crop Sci 40: 294.

[25] Marin, A. R, Masscheleyn, P. H, & Patrick, W. H. Jr. (1992). The influence of chemical form and concentration of arsenic on rice growth and tissue arsenic concentration. Plant and Soil , 139, 175-183.

[26] Mcnally, K. L, Bruskiewich, R, Mackill, D, Buell, C. R, Leach, J. E, & Leung, H. (2006). Sequencing multiple and diverse rice cultivars, connecting whole-genome variation with phenotypes. Plant Physiology , 141, 26-31.

[27] Pan, X, Zhang, Q, Yan, W. G, Jia, M, Jackson, A, Li, X, Jia, L, Huang, B, Xu, P, Correa-victoria, F, & Li, S. (2012). Development of genetic markers linked to straighthead resistance through fine mapping in rice (*Oryza sativa* L.). PLOS ONE

[28] Paterson, A. H, Deverna, J. W, Lanini, B, & Tanksley, S. D. (1990). Fine Mapping of Quantitative Trait Loci Using Selected Overlapping Recombinant Chromosomes, in an Interspecies Cross of Tomato. Genetics , 124(3), 735-742.

[29] Qian, Y. W, & Liu, J. Z. (1993). Rice germplasm resources in Guangdong Province. In: C.S. Ying (ed.). Rice germplasm resources in China.China Agricultural Science and Technology Press, , 267.

[30] Rasamivelona, A, Gravois, K. A, & Dilday, R. H. (1995). Heritability and genotype X environment interactions for straighthead in rice. Crop Sci. , 35, 1365-1368.

[31] Ricardo, C. P. P, & Cunha, J. M. A. E. (1968). Study of branca: a physiological disease of rice II. Relation between the soil redox potential and the disease: action of cupper sulfate. AgronomiaLusit , 29(1), 57-97.

[32] RTWG (Rice Technical Working Group)(2002). Rice variety acreage tables. Proc. 29th Conf. Little Rock, AR. Feb. 24-27, 2002., 18-31.

[33] Sachs, R. M, & Michael, J. L. (1971). Comparative phytotoxicity among four arsenical herbicides. Weed Sci. , 19, 558-564.

[34] Slaton, N. A, Jr, C. E, Wilson, S, Ntamatungiro, R. J, & Norman, D. L. Boothe. (2000). Evaluation of new varieties to straighthead susceptibility. *In:* R.J. Norman and C.A. Beyrouty, (eds). B.R. Wells Rice Research Studies 1999.Univ. of Arkansas, Agri. Exp. Sta., Res. Ser. 476., 313-317.

[35] Slaton, N. A. (2001). Rice production handbook. Univ. of Arkansas, Agri. Exp. Sta., MPM-1-01RV., 192-10.

[36] Slaton, N. A, Wilson, C. E, Jr, R. J, & Norman, R. E. DeLong. (2006). Correlation of soil pH and Mehlich-3 phosphorus with postflood rice phosphorus concentrations in Arkansas. Commun. Soil Sci. Plant Anal. , 37, 2819-2831.

[37] Somenahally, A, Hollister, E. B, Yan, W. G, Gentry, T. J, & Loeppert, R. H. (2011). Water management impacts on arsenic speciation and iron-reducing bacteria in contrasting rice-rhizosphere compartments. Envir. Sci.& Tec. , 45, 8328-8335.

[38] TeBeestD.O., C. Guerber, and M. Ditmore. (2007). Rice blast. The Plant Health Instructor.DOI:PHI-I-2007-0313-07

[39] Virmani, S. S. (1994). Heterosis and hybrid rice breeding. Monograph on Theo. & App. Gen. 22. International Rice Research Institute.

[40] Weerapat, P. (1979). Straighthead disease of rice suspected in southern Thailand. IRRN , 4(3), 6-7.

[41] Wells, B. R, & Gilmour, J. T. (1977). Sterility in rice cultivars as influenced by MSMA rate and water management. Agron.J. , 69, 451-454.

[42] Wilson, C. E, Jr, N. A, Slaton, D. L, Frizzell, D. L, Boothe, S, & Ntamatungiro, R. J. Norman. (2001). Tolerance of new rice cultivars to straighthead. *In:* R.J. Norman and J.-F. Meullenet, (eds). B.R. Wells Rice Research Studies 2000. Univ. of Arkansas, Agri. Exp. Sta., Res. Ser. , 485, 428-436.

[43] Wilson, C. E, Jr, S. K, Runsick, R, & Mazzanti, . Trends in Arkansas rice production. In Norman RJ, Moldenhauer KAK (eds) BR Wells Rice Research Studies 2009 AR AgricExpSta: UA, AR, , 11-21.

[44] Wilson, C. E, Jr, R, Cartwright, G, Lorenz, S, & Stiles, . My rice field is no yeilding well, what happened? In'Arkansas rice on Sep 8', UA CES http://arkansasrice.blogspot.com/2010_09_01_archive.html.

[45] Wilson, C. E, Jr, R, Cartwright, B, & Stiles, . Managing for straighthead starts early. In'Arkansas rice on May 11', UA CES http://arkansasrice.blogspot.com/2010/05/managing-for-straighthead-starts-early.html.

[46] Yan, W. G, Rutger, J. N, Moldenhauer, K. A. K, & Gibbons, J. W. (2002). Straighthead-resistant germplasm introduced from China. In Norman RJ, Meullenet JF (eds) BR Wells Rice Research Studies 2001 AR. Agric. Exp. Sta.: UA, AR , 359-368.

[47] Yan, W. G, Dilday, R. B, Tai, T. H, Gibbons, J. W, Mcnew, R. W, & Rutger, J. N. (2005). Differential response of rice germplasm to straighthead induced by arsenic. Crop Sci. , 45, 1223-1228.

[48] Yan, W. G, Rutger, J. N, Bryant, R. J, Bockelman, H. E, Fjellstrom, R. G, Chen, M. H, Tai, T. H, & Mcclung, A. M. (2007). Development and evaluation of a core subset of the USDA rice (*Oryza sativa* L.) germplasm collection. Crop Sci. , 47, 869-878.

[49] Yan, W. G, Agrama, H. A, Slaton, N. A, & Gibbons, J. W. (2008). Soil and Plant Minerals Associated with Rice Straighthead Disorder Induced by Arsenic. Agron J. , 100, 1655-1661.

[50] Yan, W. G, Correa-victoria, F. F, Marin, A, Marassi, J, Li, X, & Re, J. (2010a). Comparative study on induced straighthead in the U.S. with natural straighthead in Argentina.Proc 33rd Rice Technical Working Group conference Feb: , 22-25.

[51] Yan, W. G, Agrama, H, Jia, M, Fjellstrom, R, & Mcclung, A. M. (2010b). Geographic description of genetic diversity and relationships in the USDA rice world collection. Crop Sci. , 50, 2406-2417.

Chapter 9

Genes and QTLs for Rice Grain Quality Improvement

Jinsong Bao

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/56621

1. Introduction

As a major cereal crop, rice (*Oryza sativa* L.) is crucial to food security for at least half the world population. New varieties with high yield potential, good quality and high resistance to biotic and abiotic stresses are needed in order to meet the demand for more food arising from the rapid human population growth and concurrent decrease in arable land. Improvement of rice quality has now become a foremost consideration for rice buyers and breeding programs.

Quality is defined as "the totality of features and characteristics of a product or service that bears its ability to satisfy stated or implied needs" (International Standard Organization (ISO) 8402 1986). Features are identified properties of a product which can be related to the quality characteristics. Grain quality of rice is the totality of features and characteristics of rice or rice product that meets the demand of end-user. The concept of grain quality covers many features ranging from physical to biochemical properties, and includes milling efficiency, grain shape and appearance, cooking easiness, eating palatability, and nutrition. Thus, rice grain quality generally includes four classes, i.e. milling quality, appearance quality, cooking and eating quality, and nutritional quality (Figure 1). Many countries have set up their own protocols to assess the respective quality. International organizations such as ISO, Association of Analytical Communities International (AOAC), and American Association of Cereal Chemists International (AACCI) have set up methods to evaluate some quality parameters, for example, apparent amylose content (AAC). Rice is consumed mainly as milled, so eating quality mentioned in this article generally relates to the cooked milled rice. However, due to the impact of the western life style, whole grain rice or brown rice becomes popular worldwide, so that the nutritional quality has expanded to the nutrients of brown rice.

Grain quality and its assessment are not only important to consumers, end-users, processors, but also to rice breeders who are engaged in creating rice varieties haboring new features such as high quality, high yield potential, highly resistant to abiotic or biotic stresses. It is necessary

for rice breeders to understand how the quality traits are inherited from their parents. Genetic studies have revealed many genes and quantitative trait loci (QTL) for grain quality, though the grain quality traits are complex. Some major genes have been cloned, and their functions in a specific pathway, such as starch, protein, lipid, and flavonoids biosynthesis, have been characterized. Some QTLs have been finely mapped for further map-based cloning and functional characterization. The known genes or QTLs have been successfully applied in breeding programs for marker-assisted selection (MAS) to improve the breeding and selection efficiencies.

This chapter highlights the genes and QTLs available for grain quality of rice, summarizing how many QTLs and genes have been mapped or characterized, and how many could be used in marker assisted selection (MAS), which could help breeders to in-deep understand the genetics of grain quality of rice and apply the knowledge in their breeding practices.

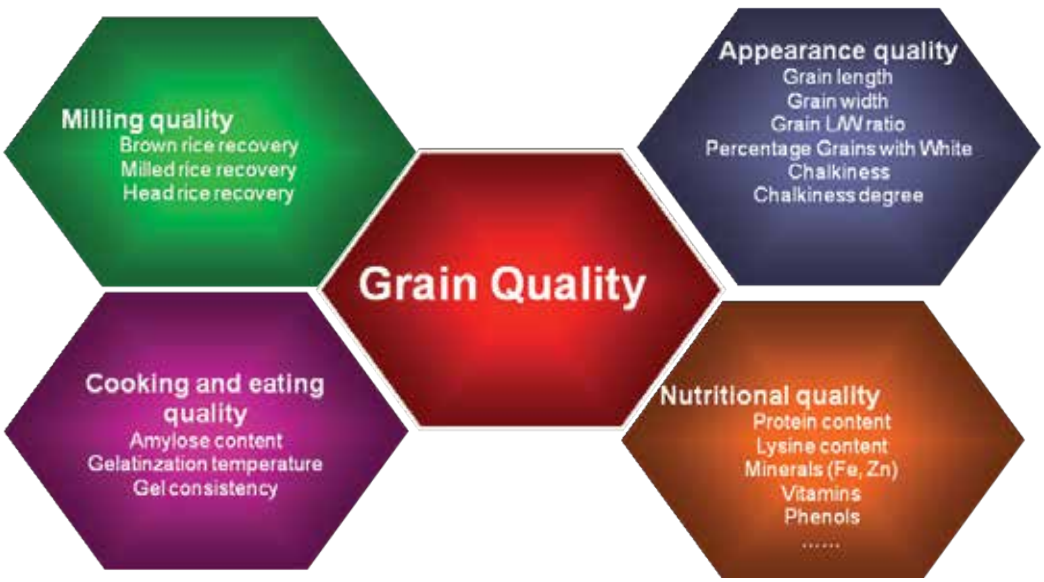


Figure 1. Four facets of grain quality

2. Four facets of grain quality

2.1. Milling quality

Milling quality determines the final yield and the broken kernel rate of the milled rice, which is of concern for consumers and farmers. Three main parameters, brown rice recovery (the percentage of brown rice to rough rice), milled rice recovery (the percentage of milled rice to rough rice), and head rice recovery (the percentage of head rice to rough rice) are used to evaluate the quality and efficiency of the milling process. Brown rice is the de-hulled rice with

the palea and lemma removed. Brown rice itself is a type of whole grain that could be used for cooking and eating. Removing all of the bran which consists of the aleurone and pericarp, and germ or embryo from brown rice results in white (or milled) rice. Some milled grains are broken during milling, head rice is a standard term for the whole milled grain. In calculation of head rice recovery, kernels longer than or equal to 3/4 full length of a kernel were considered as whole grains. Among all three parameters to determine the milling quality, head rice recovery is the main factor determining rice market value and one of the most important criteria of milled rice.

2.2. Appearance quality

Appearance is one of the crucial properties of rice grain affecting its market acceptability. After milling, the appearance of the grain is associated with size, shape (long vs. round), chalkiness, and translucency. Grain length, width, thickness are used to describe the physical dimensions of rice kernels, while the grain shape is expressed as the ratio of length to width. Grain appearance is also largely determined the clarity, the vitreousness, and the translucency of the endosperm, which is specifically required by most segments of the rice industry. According to the location of the chalkiness in the endosperm, it could be classified into three groups, white belly (chalkiness on the dorsal side of the grain), white back (chalkiness on the ventral side) and white core (chalkiness in the center). Generally, the great the chalkiness, the lower the market acceptability. Percentage of chalky grain is the proportion of grains having a chalky spot on (or in) the endosperm. Chalkiness is measured visually with scales for 0 for none, 1 for small (<10%), 5 for medium (10-20%) and 9 for large (>20 % of the area). Grain transparency may be measured using a light permeation instrument or with an image analyzer, with which the size and shape may be measured simultaneously.

2.3. Cooking and eating quality

Cooking and eating quality determines the easiness of cooking, as well as the firmness and stickiness of the cooked rice. Rice cooking and eating quality is highly related to some easily measurable physicochemical properties: apparent amylose content (AAC), gel consistency, gelatinization temperature (GT) and pasting viscosity. All these parameters are related to the properties of starch that makes up 90% of milled rice. Starch consists of two kinds of molecules, the linear and helical amylose and the branched amylopectin. Amylose content is measured with a simplified procedure using I_2-KI solution. Due to the binding ability of long chain of amylopectin with I_2, the amylose content measured with I_2-KI solution is also termed as apparent amylose content (AAC). The AAC of milled rice may be classified as waxy (1-2%), very low (5-12%), low (12-20%), intermediate (20-25%) and high (>25%). Gelatinization is the disruption of molecular orders within the starch granule manifested in irreversible changes in properties such as granular swelling, native crystallite melting, loss of birefringence, and starch solublization. The gelatinization temperature determines the time and energy input required for cooking. Gel consistency was developed as a parameter to index the tendency of cooked rice to harden on cooling, and is normally classified as hard, medium, and soft. Pasting viscosity is another useful parameter to differentiate rice with similar AAC, and is popularly

measured by a Rapid Visco-Analyser (RVA) developed by Newport Scientific Pty Ltd., Australia. RVA records the viscosity continuously as the temperature is increased, held constant for a time, and then decreased.

The above mentioned are objective parameters for the cooking and eating quality. However, eating quality is quite subjective and thus is difficult to define as it depends on consumer preferences. Sensory quality of cooked rice could be evaluated by a trained sensory panel (Champagne et al. 2010). Four steps are used to evaluate the cooked rice texture (Table 1). In addition to texture, the flavor (aromatics, taste, mouthfeel) of cooked rice can also be evaluated by the sensory panel.

Phases/attributes	Definition
Phase I	Place 6–7 grains of rice in mouth behind front teeth. Press tongue over surface and evaluate.
Initial starchy coating	Amount of paste-like thickness perceived on the product before mixing with saliva (3 passes).
Slickness	Maximum ease of passing tongue over the rice surface when saliva starts to mix with sample.
Roughness	Amount of irregularities in the surface of the product.
Stickiness to lips	Degree to which kernels adhere to lips.
Stickiness between grains	Degree to which the kernels adhere to each other.
Phase II	Place 1/2 teaspoon of rice in mouth. Evaluate before or at first bite.
Springiness	Degree grains return to original shape after partial compression.
Cohesiveness	Degree to which the grains deform rather than crumble, crack, or break when biting with molars.
Hardness	Force required to bite through the sample with the molars.
Phase III	Evaluate during chew.
Cohesiveness of mass	Maximum degree to which the sample holds together in a mass while chewing.
Chewiness	Amount of work to chew the sample.
Uniformity of bite	Evenness of force throughout bites to chew.
Moisture absorption	Amount of saliva absorbed by sample during chewing.
Phase IV	Evaluate after swallow.
Residual loose particles	Amount of loose particles in mouth.
Toothpack	Amount of product adhering in/on the teeth.

[1]Adapted from Champagne et al. (2010).

Table 1. Sensory descriptive texture attributes and their definitions used to evaluate cooked rice texture[1]

http://dx.doi.org/10.5772/56621

2.4. Nutritional quality

As one of the most important staple food in the world, nutritional quality is closely related to human health, and thus is highly valued by consumers. Protein is the second most abundant constituent of milled rice, following starch. Lysine is the first limiting essential amino acid in rice based on the human requirements. Protein and lysine content are two important parameters determining nutritional value of rice. With social development, diverse people eating rice as staple food may require rice with distinct nutritional quality. For those in the underdeveloped region where micronutrient deficiency (Vitamins and minerals, such as iron and zinc) is apparent, genetics study for and biofortification of micronutrients by breeding are necessary to improve the nutritional quality of rice. For those with improved living standards, consuming of brown rice as one kind of whole grains becomes popular to combat chronic diseases, such as diabetes. Whole grain rice (brown rice) provides more minerals, vitamins, dietary fibers, and phenolics to human health than milled rice (Bao 2012a).

3. Genes and QTLs for grain quality

3.1. Milling quality

Milling quality is assessed by brown rice recovery, milled rice recovery and head rice recovery, which is one kind of complex quantitative trait whose genetic control is poorly understood. Up to date, no major gene has been genetically identified and functionally characterized. However, many studies have been carried out to search quantitative trait locus (QTL) for the milling quality (Table 2). These researches improve our understanding of the genetic control of milling quality, and provide molecular markers that are useful in breeding for improvement of milling quality in rice.

Population	Property[1]	No. of QTLs	Chromosome distribution[2]	PVE[3]	Reference
Brown rice recovery					
Zhenshan 97/Minghui 63	I/I, RIL	1	5	10	Tan et al. 2001
Nipponbare/Kasalath	J/I, BIL	5	3,4,9,10,11	7.5-17.9	Li et al. 2004a
Asominori /IR24	J/I, RIL	2	9, 10	7.2, 21.3	Dong et al. 2004
Caiapo/ *O. glaberrima*	I/W, DH	3	1, 7, 8	2.8-4.9	Aluko et al. 2004
Zhenshan 97/WYJ-2	I/J, DH	1	12	13.6	Jiang et al. 2005
Teqing/Lemont	I/J, IL	3	5,6,7	5-12.4	Zheng et al. 2007
Chuan7/Nanyangzhan	I/J, RIL	2	1,3	1.9, 3.2	Lou et al. 2009
L204/01Y110	J/J, RIL	3	1, 4, 6	6-11	Nelson et al. 2012
Milled rice recovery					

Population	Property[1]	No. of QTLs	Chromosome distribution[2]	PVE[3]	Reference
Zhenshan 97/Minghui 63	I/I, RIL	2	3, 5	4.8, 7.0	Tan et al. 2001
Nipponbare/Kasalath	J/I, BIL	4	4,9,10,11	7.6-19.9	Li et al. 2004a
Asominori /IR24	J/I, RIL	2	11, 12	7.7, 12.2	Dong et al. 2004
Caiapo/ *O. glaberrima*	I/W, DH	2	5,7	5.3, 6.1	Aluko et al. 2004
Teqing/Lemont	I/J, IL	5	1,2,5,6,7	11.5-30.7	Zheng et al. 2007
Chuan7/Nanyangzhan	I/J, RIL	1	3	6.7	Lou et al. 2009
L204/01Y110	J/J, RIL	3	1, 4, 9	6-9	Nelson et al. 2012
Head rice recovery					
Zhenshan 97/Minghui 63	I/I, RIL	1	3	10.1	Tan et al. 2001
IR64/*O. rufipogon*	I/W, BC2F2	3	1,2,5	5.2-5.5	Septiningsih et al. 2003
Nipponbare/Kasalath	J/I, BIL	3	3, 6,7	9.7-12.2	Li et al. 2004a
Asominori /IR24	J/I, RIL	3	1, 3, 5	8,7-22.1	Dong et al. 2004
Caiapo/ *O. glaberrima*	I/W, DH	5	1,3,6,8,11	7.6-54.1	Aluko et al. 2004
Zhenshan 97/WYJ-2	I/J, DH	2	3, 8	10.1, 16	Jiang et al. 2005
Teqing/Lemont	I/J, IL	3	1,5,6	5.8-5.9	Zheng et al. 2007
Chuan7/Nanyangzhan	I/J, RIL	1	3	29.7	Lou et al. 2009
Cypress/RT0034	J/I, RIL	2	6,9	12, 16	Nelson et al. 2011
Cypress/ LaGrue	J/J, RIL	4	1,5,9,10	8, 12	Nelson et al. 2011
L204/01Y110	J/J, RIL	7	6, 6, 8, 9, 9, 10, 11	3-8	Nelson et al. 2012

1: BC=backcross; BIL=backcross inbred line; DH=doubled haploid; I=*indica* subspecies; J=*japonica* subspecies; RIL=recombinant inbred line; W=wild rice. IL: introgression lines.

2: The value in this column indicates the number of chromosome; the two or three same values in the same line indicate two or three QTLs in the same chromosome.

3: Percentage of total variation explained by a single QTL (%).

Table 2. Summary of main-effect QTLs for milling quality traits mapped on rice genome

3.1.1. Brown rice recovery

A total of 20 QTLs have been identified in eight studies, covering all chromosomes except chromosome 2 (Table 2). A major QTL at the interval between markers RM42 and C734b on chromosome 5 is also responsible for grain width (Tan et al. (2001). A QTL on chromosome 3 likely shares the same genomic region for grain length (Lou et al. 2009). These results indicate that brown rice rate relates to the grain shape and size of rice kernel. Five QTLs were detected in the study of Li et al. (2004a), of which three were expressed in two years, indicating that there are QTL-by-environment interactions effects.

3.1.2. Milled rice recovery

A total of 19 QTLs have been identified in seven studies, covering all chromosomes except chromosome 8 (Table 2). There are no strong or reproducible QTLs for the milled rice recovery. Three independent studies detected QTL for the milled rice recovery on chromosome 5 (Tan et al. 2001; Aluko et al. 2004; Zheng et al. 2007), but there are actually not at the same region. Li et al. (2004a) reported that two of four QTLs were detected in two years, indicating that the QTL-by-environment interactions effects exist.

3.1.3. Head rice recovery

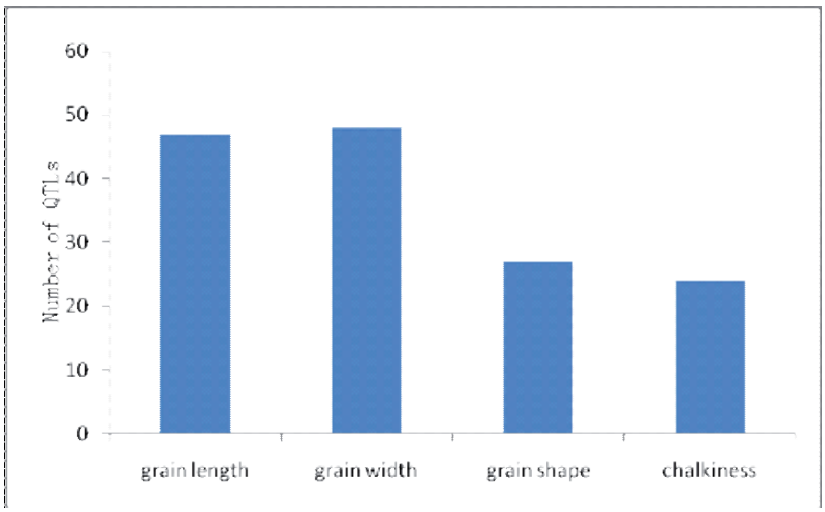
Up to date, a total of 34 QTLs locating at all the chromosomes have been reported in ten studies with the number of QTLs varied from 1 to 7 in different studies. A major QTL located on chromosome 3 is also a major QTL for grain length (Tan et al. 2001), suggesting that genetic relationship exists between grain size or shape and the percentage of head rice. Other studies frequently identified the QTL at chromosome 3 (Li et al. 2004a; Dong et al. 2004; Aluko et al. 2004; Jiang et al. 2005; Lou et al. 2009), proving that there might be a major gene for head rice. In addition, QTLs on chromosome 1, 5 and 6 are also detected by at least three independent studies. Li et al. (2004) detected three QTLs for head rice, but all of them were detected only in a specific year, suggesting that the head rice is largely affected by the environment. However, Nelson et al. (2011) showed that more variance of head rice yield was explained by main-effect QTL than QTL × environment effect in the Cypress/RT0034 RIL population, whereas the main-effect QTLs contributed a little less to genetic variation than those of QTL × environment effect in the Cypress/ LaGrue RIL population. There is a clear coincidence of QTLs for head rice recovery with early-heading QTLs in the hotter growing location, hinting an environmental effect (Nelson et al. 2011). Note that some genetic populations were derived from cultivated rice and wild rice (Septiningsih et al. 2003; Aluko et al. 2004), but all milling-yield-increasing effects came from the cultivated parent.

3.2. Appearance quality

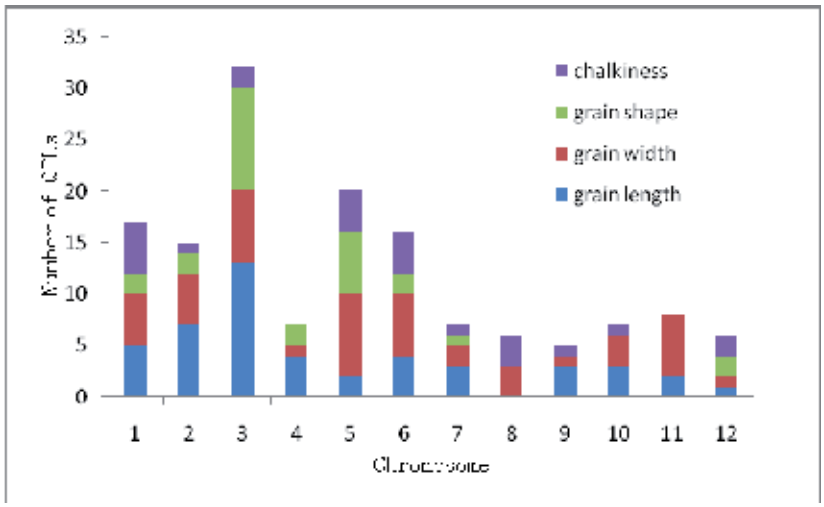
3.2.1. Grain size and shape

Grain shape is not only key determinant of grain quality but also of grain yield potential. A long, slender grain of rice is generally preferred by consumers in Southern China, the USA, and South and Southeast Asian countries, whereas consumers in Japan, Korea, and Northern China prefer short or round grain of rice (Huang et al. 2013).

Grain length, grain width, length-to-width (grain shape) are the most stable properties of the variety, so they are highly heritable. Genetically, a lot of QTLs have been identified for grain length, grain width, and grain shape (Figure 2a). The chromosome 3 harbors more QTLs than others (Figure 2b). Some are major genes that have been map-based cloned with their function characterized (Table 3), some are finely mapped (Table 4), while many are with minor effects and are waiting for further characterization. The finely mapped QTLs provide potential markers for molecular breeding to modify grain shape while use of functional markers derived



(A)



(B)

Figure 2. Number (A) and distribution (B) of QTLs for appearance quality (data from Gramene: http://www.gramene.org/).

from the cloned genes would lead to precise phenotype in breeding. QTL mapping studies also suggest that many QTLs exhibit pleiotropic effects; they control not only grain length, but also grain width, grain shape or grain yield (Bai et al. 2010; Fan et al. 2006; Guo et al. 2009; Li et al. 2011; Song et al. 2007; Wang et al. 2012). Functional characterization of the cloned genes provides evidence underlying the pleiotropic effects (Fan et al. 2006; Li et al. 2011; Song et al. 2007; Wang et al. 2012). A good review of Huang et al. (2013) has summarized the current progress in the genetic base of grain shape of rice.

Gene	Trait	Chromosome	Encoded protein	Reference
GS3	Grain length	3	Member protein with multiple domains	Fan et al.2006
GL3	Grain length	3	protein phosphatase with Kelch-like repeat domain (OsPPKL1)	Zhang et al. 2012; Hu et al. 2012 Qi et al. 2012
GW2	Grain width	2	Ring-type E3 ubiquitin ligase	Song et al. 2007
GW5/qGW5	Grain width	5	Arginine-rich protein of 144 amino acids	Weng et al.2008; Shomura et al. 2008
GS5	Grain width	5	Serine carboxypeptidase	Li et al. 2011
GW8/SPL16	Grain width	8	SQUAMOSA promoter-binding protein-like 16	Wang et al. 2012
ms-h /UGPase1	grain chalkiness, genic male sterility	9	UDP-glucose pyrophosphorylase 1	Woo et al. (2008)
GIF1	grain chalkiness, grain incomplete filling	4	Cell wall invertase	Wang et al. 2008a

Table 3. The cloned genes for grain appearance quality (grain shape and endosperm chalkiness)

3.2.1.1. Grain length

A total of 47 QTLs for grain length have been detected in different populations. Among them, the Chromosome 3 harbors more QTLs than other chromosomes (Figure 2). Up to date, two QTLs have been map-based cloned (Table 3), and seven QTLs have been finely mapped (Table 4).

GRAIN SIZE 3 (GS3) is the first QTL that has been map-based cloned for grain length. It was detected in the RIL population derived from Minghui 63 and Chuan 7, displaying a major role for grain length and weight and a minor role for grain width and thickness and functioning as a negative regulator for grain size (Fan et al. 2006; 2009). The GS3 protein contains an organ size regulation (OSR) domain in the N terminus, a transmembrane domain, a tumor necrosis factor receptor/nerve growth factor receptor (TNFR/NGFR)-like domain, and a von Willebrand factor type C (VWFC) domain in the C terminus. The OSR domain functions as a negative regulator of grain length and deletion mutants of this domain result in the formation of long-grain rice. The C-terminal TNFR/NGFR and VWFC domains act as positive regulators of grain length and loss-of-function mutations of these domains lead to the development of very short grain (Mao et al. 2010; Takano-Kai et al. 2009).

GRAIN LENGTH 3 (qGL3) is a major grain length QTL recently identified in three mapping populations (Zhang et al. 2012; Hu et al. 2012; Qi et al. 2012). qGL3 encodes a putative protein phosphatase with Kelch-like repeat domain (OsPPKL1). A rare allele, i.e a single nucleotide substitution (C→A) leads to a long grain phenotype by an aspartate-to-glutamate transition in

a conserved AVLDT motif of the second Kelch domain in OsPPKL1 (Hu et al. 2012; Zhang et al. 2012; Qi et al. 2012). Genetic analysis of a near-isogenic line (NIL) for qGL3-1 revealed that the allele qGL3-1 from CW23 has an additive or partly dominant effect, and is suitable for use in molecular marker-assisted selection (Hu et al. 2012). A new variety containing the new allele shows increased grain yield, which indicates that GL3 is a powerful tool for breeding high-yield crops (Qi et al. 2012).

3.2.1.2. Grain width

A total of 48 QTLs for grain width have been detected in different populations with more QTLs on chromosome 3 and 5 (Figure 2). Up to date, four QTLs have been map-based cloned (Table 3).

GRAIN WIDTH 2 (GW2) is a major QTL for rice grain width and weight, which was initially detected from a cross between a large-grain japonica rice variety (WY3) and a small-grain *indica* rice variety (Fengaizhan-1). GW2 encodes a RING-type E3 ubiquitin ligase (Song et al. 2007). WY3 has a 1-bp deletion resulting in the introduction of a premature stop codon in its exon 4, causing the large-grain phenotype. GW2 negatively regulates cell division by targeting its substrates to proteasomes for regulated proteolysis; loss of GW2 function results in an increase in cell number in the spikelet hull and acceleration of the grain-milk filling rate, thus enhancing grain width, weight, and yield.

GRAIN WIDTH 5 (GW5) is a major QTL for seed width on chromosome 5 (qSW5) (Wan et al. 2008; Weng et al. 2008; Shomura et al. 2008). A survey of GW5/qSW5 polymorphisms in various rice landraces has revealed that deletions in this gene may have played an important role in the selection of increased grain size from artificial and natural crossings during rice domestication (Shomura et al. 2008). The GW5/qSW5 gene encodes a nuclear protein of 144 amino acids with an arginine-rich domain. Because GW5/qSW5 physically interacts with polyubiquitin, it is likely to act as a regulator in the ubiquitin–proteasome pathway and regulates cell division of the outer glume of the rice spikelet (Wan et al. 2008; Weng et al. 2008; Shomura et al. 2008).

GRAIN SIZE ON CHROMOSOME 5 (GS5) is a major QTL affecting grain width, grain filling, and grain weight (Li et al. 2011). It encodes a serine carboxypeptidase and functions as a positive regulator of grain size. Analysis of genomic DNA sequences and promoter swaps in transgenic plants reveals that nucleotide changes in three segments of the GS5 promoter seem to be responsible for the variations in grain width (Li et al. 2011).

GRAIN WIDTH 8 (GW8) is a major QTL affecting grain width and grain yield from the cross between HXJ74 and Basmati385 (Wang et al. 2012), which encodes SQUAMOSA promoter-binding protein-like 16, referred to OsSPL16, belonging to the protein family of SBP domain-containing transcription factors. Six polymorphisms in the DNA sequence of OsSPL16 exist in the parents HXJ74 and Basmati385. Among them, a 10-bp deletion in the promoter region has been shown to be responsible for the slender grain trait of Basmati385 (Wang et al. 2012).

GS3, GL3, GW2, and GW5/qSW5 are negative regulators of grain size, but GS5 and GW8 are positive regulators of cell proliferation. Other genes associated with grain shape including the SMALL AND ROUND SEED (SRS) loci have been well reviewed in Huang et al. (2013).

Functional markers developed from these major genes and finely mapped QTL resources allow breeders to efficiently manipulate grain size and shape (Tables 3 and 4).

Trait	QTL	Chromosome	Marker interval	Distance[a]	Reference
GL	qGL-3a	3	RMw357–RMw353	87.5kb	Wan et al. 2006
GL	qGL4b	4	RM5586–RM3524	3Mb	Kato et al. 2011
GL/GW	qGL7	7	RID711–RM6389	258kb	Bai et al. 2010
GL	GS7	7	Indel3–Indel5	4.8kb	Shao et al. 2012
GL/GW/GS	qSS7	7	GL293–GL285	23kb	Qiu et al. 2012
GL	qGRL1	1	RM431–CHR1.1	108kb	Singh et al. 2012a
GL	LGS1	2	RM13838–RM13840	0.2cM	Huang et al. 2013
PGWC	qPGWC-8	8	Indel 8G-7-Indel 8G-9	142kb	Guo et al. 2011
PGWC	qPGWC-7	7	Indel14-Indel3 (RM21938)	44kb	Zhou et al. 2009

GL: grain length; GW: grain wideness; GS: grain shape and PGWC: percentage of grains with chalkiness

Table 4. Fine mapped QTLs associated with appearance quality of rice

3.2.2. Grain chalkiness

Chalkiness is a major concern in rice breeding because it is one of the key factors in determining quality and price. The chalky endosperm consists of loosely packed, round and large compound starch granules while the translucent endosperm comprises tightly packed, polyhedral and small single starch granules. The chalky grains show significantly different physicochemical, morphological, thermal, cooking and textural properties from translucent grains. Percentage of grains with chalkiness (PGWC) is one of the main indices of rice-determining appearance quality, which is easily determined.

Many factors contribute to the formation of chalkiness in the rice grain. Environmentally, rice grown at the higher temperature contains more chalky grains. Genetically, defect in genes affecting starch biosynthesis, starch granule structure, and grain filling may lead to endosperm chalkiness. These genes include *starch branching enzyme IIb* (*BEIIb*), *branching enzyme I* (*BEI*), *starch synthase IIIa* (SSIIIa), *floury* and *sugary* genes, etc. It should be noted that many of the genes characterized show pleiotropic effects on other traits in addition to chalkiness.

A rice genic male-sterility gene *ms-h* is recessive and has a pleiotropic effect on the chalky endosperm (Woo et al. 2008). Fine mapping and nucleotide sequencing analysis reveal a single nucleotide substitution at the 3′-splice junction of the 14th intron of the UDP-glucose pyrophosphorylase 1 (*UGPase1*) gene, which causes the expression of two mature transcripts with abnormal sizes caused by the aberrant splicing. Overexpression of UGPase1 in *ms-h* mutant plants restored male fertility and the transformants produced T1 seeds that segregated into normal and chalky endosperms (Woo et al. 2008).

The grain incomplete filling 1 (gif1) mutant defects in grain-filling capacity, but its grains are with more chalkiness as a result of loosely packed starch granules. A frameshift mutation caused by a 1-bp nucleotide deletion in GIF1 results in premature termination of its open reading frame. GIF1 encodes a cell-wall invertase required for carbon partitioning during early grain filling (Wang et al. 2008a).

Two white-core genes have been characterized with knockout mutants. A floury endosperm-4 (flo4) rice mutant with a floury-white endosperm but a normal outer portion was generated by T-DNA insertion into the fifth intron of the OsPPDKB gene encoding pyruvate orthophosphate dikinase (PPDK) (Kang et al. 2005). Other two additional alleles, flo4-2 and flo4-3 also showed the same white-core endosperm phenotype. OsPPDKB was mainly expressed in the endosperm, aleurone, and scutellum of the developing kernel, suggesting that cytosolic PPDK functions in rice to modulate carbon metabolism during grain filling. Ryoo et al. (2007) characterized another white-core floury endosperm mutant (flo5) caused by T-DNA insertion into the SSIIIa.

A floury mutant, flo(a), exhibits floury characteristics in the innermost endosperm, while the outer layer of the endosperm appeared normal (Qiao et al. 2010). The *FLO(a)* gene was isolated via a map-based cloning approach and predicted to encode the tetratricopeptide repeat domain containing protein, OsTPR. Three mutant alleles contain a nucleotide substitution that generated one stop codon or one splice site, respectively, which presumably disrupts the interaction of the functionally conserved TPR motifs (Qiao et al. 2010). The OsTPR motifs may play a significant role in rice starch biosynthetic pathways, which causes the formation of chalkiness. Yang et al. (2012) identified a mutant 'Jiangtangdao 1' which had chalky endosperm with resistant starch content up to 11.67%. The putative gene *starch branching enzymne 3* on chromosome 2 was finely mapped and a cleaved amplified polymorphic sequence (CAPS) marker for marker assisted selection was developed (Yang et al. 2012).

For the naturally occurring chalkiness, earlier studies (Li et al. 2004; Tan et al. 2000; Wan et al. 2005) identified 24 QTLs from three crosses among Asian cultivars (Figure 2). Recently, Wan's group in China (Guo et al. 2011; Liu et al. 2010; Wan et al. 2005; Zheng et al. 2012; Zhou et al. 2009) and others (Yamakawa et al. 2008; Liu et al. 2012) have identified many more QTLs for grain chalkiness. Among them, two QTLs have been finely mapped (Table 4).

qPGWC-8 is a major QTL for the percentage of grains with white chalkiness in the interval G1149-R727 on chromosome 8 which was identified using a chromosome segment substitution line (CSSL). Guo et al. (2011) narrowed down the location of this QTL to a 142 kb region between Indel markers 8G-7 and 8G-9. qPGWC-8 accounted for 50.9% of the difference in PGWC between the parents.

qPGWC-7 is a QTL for the percentage of grain with chalkiness (PGWC) on 7 which was identified using a set of chromosome segment substitution lines, made from a cross between PA64s and 9311. Segregation analysis of the F_2 population from the cross between C-51 (a CSSL harboring qPGWC-7 and having a chalky endosperm) and 9311 showed PGWC is a semi-dominant trait, controlled by a single nuclear gene. Fine mapping of qPGWC-7 with a large

F_2 population constructed from the cross C51 × 9311 delimitated it to a 44-kb DNA fragment, containing thirteen predicted genes (Zhou et al. 2009).

The markers tightly linked to qPGWC-8 and qPGWC-7 facilitate cloning of the gene underlying the QTLs and is of value for marker-assisted selection for endosperm texture. However, it is still far away from clear understanding the mechanism of formation of the grain chalkiness. First, the QTLs mapping results show low coherence in different genetic populations, suggesting many minor QTLs affecting chalkiness exist in different rice germplasm that we do not know. Second, in addition to the major genes or QTLs we have known, how their interactions with each other, and with the major genes for amylose and protein synthesis (Liu et al. 2010; Zheng et al. 2012) that may affect chalkiness are unknown. Third, effect of environment on the formation of chalkiness is well known, but how its effect on the gene expression that leads to the formation of chalkiness is largely unknown.

3.3. Eating and cooking quality

Great progresses have been made in the understanding of the genetic basis of cooking and eating quality (Bao 2012b; Chen et al. 2012). Starch properties play important role in determining the cooking and eating quality, which is highly associated with starch biosynthesis related genes. Starch biosynthesis pathways and genes or enzymes participating in have been well clarified (Figure 3). Amylose is synthesized mainly by GBSSI, and the amylopectin synthesis process is governed by a combination of multiple isoforms of SS, BE, and DBE to produce a uniform number of chains per amylopectin cluster. *Wx* encoding GBSSI is mainly responsible for the natural variation of amylose content, gel consistency and RVA pasting viscosity, while the SSIIa is mainly for gelatinization temperature, thermal properties, and amylopectin structure (Bao 2012b).

3.3.1. Apparent amylose content, gel consistency and RVA pasting viscosity

Wx locus on chromosome 6 is a major QTL for amylose content, gel consistency and RVA pasting viscosity (He et al. 1999; Bao et al. 2000; Bao 2012; Wan et al. 2004; Fan et al. 2005; Septiningsih et al. 2003; Aluko et al. 2004; Lapitan et al. 2009; Lanceras et al. 2000; Tan et al. 1999). Map-based cloning of the qGC-6, a locus for gel consistency, indicates that *Wx* is the major gene controlling it (Su et al. 2011). Five functional markers in the *Wx* gene, a (CT)n microsatellite (or simple sequence repeat, Ayres et al. 1997; Bligh et al. 1995;), a 23bp insertion/deletion sequence (Inukai et al. 2000; Wanchana et al. 2003; Teng et al. 2012)., and three single nucleotide polymorphism (SNP) markers (Bligh et al. 1998; Cai et al. 1998; Hirano et al. 1998; Isshiki et al. 1998; Larkin and Park 2003) are well characterized, with different alleles differing in AAC (Ayres et al. 1997; Bligh et al. 1995; Chen et al. 2008a; Inukai et al. 2000; Larkin and Park 2003), and RVA pasting viscosity (Bao et al. 2006a; Chen et al. 2008b; Larkin et al. 2003; Larkin and Park 2003). Among them, the (CT)n microsatellite in the *Wx* gene located 55 bp upstream of the putative 5′-leader intron splice site has many alleles with n ranging from 8 to 22 in diverse rice germplasm (Ayres et al. 1997; Bergman et al. 2001; Bao et al. 2006a; Chen et al. 2008a; Bao et al. 2002a; Han et al. 2004). Another locus, the G/T single nucleotide polymorphism (SNP) at the putative leader intron 5′ splice site, and a G to T mutation at this site reduces

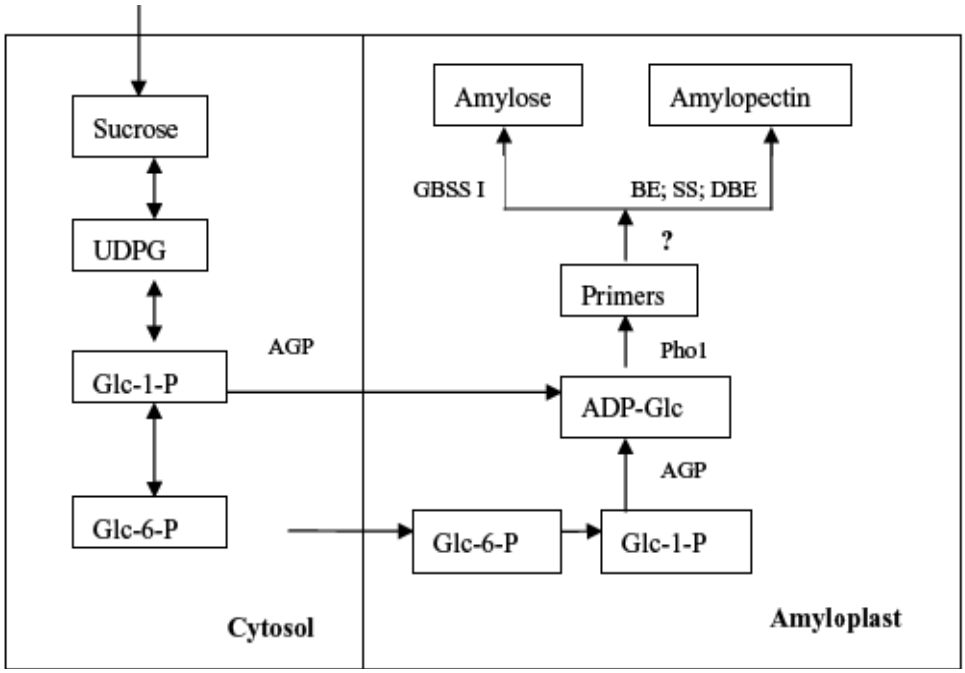


Figure 3. Starch biosynthesis pathway in rice endosperm (modified from Jeon et al. 2010). Starch biosynthesis consists of two distinct phases: the glucan initiation process and the starch amplification process. The plastidial starch phosphorylase (Pho1) extends the chains of the initial priming sites such as free chains of malto-oligosaccharides in the presence of Glc-1-P. The subsequent mechanisms underlying the glucan initiation process remain to be established. Branched dextrins are putatively processed by the coordinated activities of SS, BE, and/or DBE to produce the prototype of an amylopectin cluster structure, which further develops into amylopectin to establish the basic structure. AGPase, ADP glucose pyrophosphorylase; BE, starch branching enzyme; DBE, starch debranching enzyme; GBSSI, granule-bound starch synthase; Pho1, plastidial starch phosphorylase; SS, soluble starch synthase; DBE includes isoamylase (ISA) and pullulanase (PUL).

the efficiency of *Wx* pre-mRNA processing and thus results in the lower level of spliced mature mRNA, Wx protein, and AAC (Wang et al. 1995; Bligh et al. 1998; Cai et al. 1998; Hirano et al. 1998; Isshiki et al. 1998). Waxy, low amylose, and some intermediate amylose rice have the T SNP allele, while some intermediate and high amylose rices have the G allele (Ayres et al. 1997; Bligh et al. 1998; Cai et al. 1998; Isshiki et al. 1998). The G/T SNP explained 80% (Aryes et al. 1997) to 90 % (Bao et al. 2006a) of the total observed variation in AAC in the nonwaxy rice accessions.

Rice with similarly high AAC still differs in cooking and eating quality due to potential effect of amylopectin structure and other factors. Gel consistency and RVA pasting viscosity are effective to differentiate rice with high AAC. Genetic studies show that the exon 10 SNP of *Wx* is responsible for the genetic basis for the gel consistency, the proportion of amylose bound to amylopectin, the proportion of amylose able to leach, gel hardness (Tran et al. 2011) and RVA pasting viscosity (Traore et al. 2011). Tran et al. (2011) indicated that the rice with SNP allele C at exon 10 produces soft, viscous gels, has a soft texture when cooked, but with high

http://dx.doi.org/10.5772/56621

retrogradation, and the rice with SNP allele T gives a short, firm gel, and has a firm texture when freshly cooked with little change in texture over storage. In a cross between two varieties having similar high AAC, but with different paste viscosity properties, Traore et al. (2011) indicated that the exon 10 SNP marker is associated with most RVA pasting measurements and the proportion of soluble to insoluble apparent amylose.

3.3.2. Gelatinization temperature, thermal properties

SSIIa locus on chromosome 6 is a major QTL for gelatinization temperature and amylopectin structure (Aluko et al. 2004; He et al., 1999; Bao et al., 2004; Fan et al., 2005; Wang et al. 2007; Tian et al. 2005; Lapitan et al. 2009; Umemoto et al. 2002). Map-based cloning of the alkali degenerate locus gives evidence that the gene encoding SSIIa is the major gene responsible for gelatinization temperature (Gao et al. 2003). Nakamura et al. (2005) revealed that the function of SSIIa is to elongate the short A and B1 chains with degree of polymerization (DP) < 10 to form long B1 chains of amylopectin. Genetic engineering by introduction of *indica* active *SSIIa* gene into *japonica* rice increases GT and gives longer amylopectin side chain length (Nakamura et al. 2005; Gao et al. 2011).

Four functional SNPs in the SSIIa gene have been revealed (Umemoto et al. 2004; Umomoto and Aoki 2005; Nakamura et al. 2005; Bao et al. 2006b; Waters et al. 2006). The first one is at 264 bp in Exon 1 of AY423717, where a change from G to C results in change of glutamate to aspartate. The second site is at 3799 bp, where glycine encoded by GGC is replaced by serine encoded by AGC. The third site is at 4198 bp, where valine encoded by GTG is replaced by methionine encoded by ATG. The fourth site is at 4330 bp, glycine-leucine encoded by GGGCTC is replaced by glycine-phenylalanine encoded by GGTTTC. *SSIIa* gene fragments shuffling experiments by Nakamura et al. (2005) show that only the third and fourth SNPs are functional, and the third SNP (G/A) is crucial for SSIIa activity, the enzyme is inactive when it is A SNP (coding for methionine) no matter which SNP at 4229/4330 bp (GC/TT) is present. The GC/TT is most common and is strongly associated with GT (Waters et al 2006; Bao et al. 2006b). This GC/TT polymorphism alone can differentiate rice with high or intermediate GT (possessing the GC allele) from those with low GT (possessing the TT allele), explaining 62.4 % of the total variation in pasting temperature (Bao et al. 2006b). Few rice accessions with GC allele have low GT phenotype, which can be explained by their carrying the A SNP allele in the third SNP (Umemoto et al. 2004; Waters et al. 2006; Lu et al. 2010). However, it should be mentioned that the A allele of third SNP (G/A) is quite rare in natural populations. The frequency of A at is 1 in 30 rices (Bao et al. 2006b), 9 in 180 rices (Chen et al. 2003), 127 in 1543 rices (Cuevas et al. 2010), 5 in 65 rices (Umemoto et al. 2004), and 13 in 73 rices (Waters et al. 2005). It should also be noted that genetic control of intermediate GT rice starch remains unknown. Intermediate GT rice is characterized by more chains of DP24-35, which may be synthesized by other enzymes (Cuevas et al. 2010).

3.3.3. Contributions of other starch biosynthesis related genes

Cooking and eating quality is a complex trait which is not only determined by the *Wx* and *SSIIa* genes, but also other genetic factors, such as other starch biosynthesis related genes. Three

evidences show the effect of other genes in determining the cooking and eating qualities. First, In a population derived from two parents having similar intermediate AAC, QTLs rather than *Wx* locus are associated with the RVA pasting viscosities, and two of which might be located close to the *starch branching enzyme 1* (*SBE 1*) and *SBE3* loci (Bao et al. 2002b). Second, in an association mapping with all the starch biosynthesizing genes, additional five genes (AGPlar, PUL, SSI, SSIIa, and SSIII-2) with minor effects were detected when the effect of *Wx* gene was eliminated. Again, with the model controlling for SSIIa, a further search identified *Wx*, *SBE3*, *ISA*, and *SSIV-2* as minor genes that affect GT additively (Tian et al. 2009). Third, what factors will determine the cooking and eating quality of waxy rice is complex, because the GBSS is not active in waxy rice. It is expected that genes other than *Wx* are to control the genetic basis of pasting and thermal properties of waxy rice. Comparing starch physicochemical properties among different microsatellite groups in *starch branching enzyme 1* (*SBE1*) and soluble starch synthase 1 (SSS), waxy rices with the *SBE*-A allele have higher peak viscosity (PV), hot paste viscosity (HPV) and cold paste viscosity (CPV) than those with other alleles, and those with the *SSS*-B allele have higher HPV and CPV than other alleles (Bao et al. 2002a). Han et al. (2004) indicated that nucleotide polymorphisms in both *SBE1* and *SBE3* loci account for 70% of the observed variation in HPV and CPV, and for 40% of the observed variation in PV. Yan et al. (2011) conducted association analysis for pasting viscosity parameters of waxy rice using starch synthesis-related gene markers, showing that 10 gene markers were involved in controlling the pasting viscosity parameters. Among these, the *pullulanase* gene plays an important role in control of PV, HPV, CPV, breakdown viscosity, peak time, and pasting temperature (PT) in glutinous rice.

To date, there are many markers resources derived from starch biosynthesis related genes available for molecular breeding for the purpose of improving the cooking and eating quality (Tian et al. 2010; Bao et al. 2006b; Jin et al. 2010; He et al. 2006; Yan et al. 2011).

3.3.4 Other traits related to cooking and eating quality

In addition to the amylose content, gelatinization temperature, gel consistency and pasting viscosity, other parameters, such as water absorption, volume expansion and cooked rice elongation have been set up to evaluate the cooking characteristics of rice (Bao et al. 2009).

Ahn et al. (1993) identified a QTL on chromosome 8 for cooked rice elongation. Rani (2011) found that a functional marker targeting an SNP in the GS3 is associated with kernel elongation. Tian et al. (2005) detected 3, 2, and 2 QTLs for water absorption, volume expansion and cooked rice elongation, respectively in a DH population. While no QTL on chromosome 3 and 8 was detected, one common QTL for all the traits is at the *Wx* locus on chromosome 6, suggesting that the *Wx* gene plays a major role in determining these cooking characteristics in addition to other cooking and eating quality traits (Tian et al. 2005).

The aroma of cooked rice contributes to consumer sensory acceptance of rice. The aromatic compound 2- acetyl-1-pyrroline (2-AP) reportedly is the primary component of the popcorn-like smell of aromatic rice. Fragrance (fgr) is a recessive trait that is controlled by a major gene on chromosome 8 (Lorieux et al. 1996; Jin et al. 2003). Bradbury et al. (2005a; 2005b) reported that the *badh2* gene could most likely be the *fgr* gene since it has an 8-bp deletion and three

SNPs in its exon 7 compared to the functional *Badh2* gene which encodes putative betaine aldehyde dehydrogenase 2 (BADH2), and developed molecular markers for fragrance genotyping. Shi et al., (2008) found a novel null *badh2* allele (*badh2-E2*), which has a sequence identical to that of the *Badh2* allele in exon 7, but with a 7-bp deletion in exon 2. By map-based cloning strategy, Chen et al. (2008c) confirmed that the full-length BADH2 protein encoded by *Badh2* renders rice nonfragrant by inhibiting biosynthesis of 2-acetyl-1-pyrroline (2AP), a potent flavor component in rice fragrance. Functional markers derived from fgr are sufficient to carry out molecular marker assisted breeding to improve the sensory quality of rice (Shi et al. 2008; Chen et al. 2008c; Jin et al. 2010). So far as we are aware, there is no genetic report on the other sensory characteristics of rice.

3.4. Nutritional quality

Few molecular genetics studies have been conducted for nutritional quality (Table 5), but many molecular breeding activities through transgenic engineering to improve nutritional quality of rice have been reported (see 4.4).

3.4.1. Protein and amino acid content

There are nice reports about QTL mapping for protein content (Table 5). A total of 43 QTLs have been identified covering all 12 chromosomes. Chromosomes 1, 2 and 7 harbor more QTLs than other chromosomes. In addition to the total protein content, Zhang et al. (2008) detected 2, 4, 3 and 4 QTLs for protein fractions, albumin, globulin, prolamin and glutelin, respectively. The QTLs affecting contents of different protein fractions may locate at the same chromosomal region.

Wang et al. (2008c) identified 18 chromosomal regions for 19 individual amino acids, one of which at the bottom of chromosome 1 is a relatively strong QTL cluster, consisting of up to 19 individual QTL. A wide coincidence was found between the QTL and the loci involved in amino acid metabolism pathways, including N assimilation and transfer, and amino acid or protein biosynthesis (Wang et al. 2008c). Hu et al. (2009) identified a total of 12 QTLs for individual amino acid content and total amino acid content on chromosomes 1, 4, 6, 7 and 11. A QTL cluster on chromosome 1 was associated with the content of eight amino acids. The results are useful for candidate gene identification and marker-assisted breeding targeting the development of improved rice amino acid composition for human nutrition.

3.4.2. Fat content

Fat content affects eating quality and nutritional values, and storage stability of rice as well. Apparently, 48 QTLs for fat content have been reported. Chromosome 1, 3 and 6 harbor more QTLs than other chromosomes (Table 5). Liu et al (2009) reported 14 QTLs for crude fat content in brown rice distributing on chromosomes 1, 3, and 5-9. One of which is a major QTL, qCFC5, locating on chromosome 5, which have been detected simultaneously among three populations. Shen et al. (2012) characterized two stably expressed QTLs on chromosome 7, and they were detected in all three environments and were further confirmed by additional lines across

Population	Property[1]	No. of QTLs	Chromosome distribution[2]	PVE[3]	Reference
Protein content					
Koshihikari/Kasalath	J/I, BIC	7	2,2,3,4,7,7,10	4.5-15.8	Zheng et al. 2012
Asominori/IR24	J/I, RIL	3	1,3,8	8.5-13.9	Zheng et al. 2011
Zhenshan 97B/Delong 208.	I/I, RIL	6	1,2,4,7,8,9	4-25.9	Zhong et al. 2011
Asominori/IR24	J/I, CSSL	8	1,1,2,3,6,8,8,11	3-54	Liu et al. 2011
Xieqingzao B/Milyang 46	I/I, RIL	5	3,4,5,6,10	4-19	Yu et al. 2009
Asominori/IR24	J/I, RIL	3	2, 7, 12	11-14	Zhang et al. 2008
Gui 630/02428	I/J, DH	5	1,4,5,6,7,	7-35	Hu et al. 2004
Zhenshan 97B/Minghui 63	I/I, RIL	2	6,7	6, 13	Tan et al. 2001
Caiapo/ *O. glaberrima*	I/W, DH	4	1,2,6,11	3.3-5.8	Aluko et al. 2004
Fat content					
Fengaizhan-1/JZ1560	I/J, F2; F2:3	5	1,3,7,8,10	7.7-13.9	Ying et al. 2012
Samgang/Nagdong	J/I, DH and RIL	8	1,1,3,5,6,6,7,9	6-19	Qin et al. 2010
Sasanishiki/Habataki	J/I, BC	7	1, 2, 3, 6 10, 11,12	5-21	Shen et al. 2010
Xieqingzao B/Milyang 46	I/I, RIL	4	3,5,6,8	7-13	Yu et al. 2009
Zhenshan 97B/Wuyujing 2	I/J, DH and BC1F1	10	1,1,3,3,5,6,7,7,8,9	3.8-21.3	Liu et al. 2009
Asominori/IR24	J/I, RIL	11	1,1,2,3,3,4,4,5,6,6,11	7-14	Wang et al. 2008b
Gui 630/02428	I/J, DH	3	1,2,5	7.7-25.5	Hu et al. 2004

1: BC=backcross; BIL=backcross inbred line; DH=doubled haploid; I=*indica* subspecies; J=*japonica* subspecies; RIL=recombinant inbred line; W=wild rice. IL: introgression lines.

2: The value in this column indicates chromosome number, the two or three same values in the same line indicate two or three QTLs in the same chromosome.

3: Percentage of total variation explained by a single QTL (%).

Table 5. QTLs for protein content and fat content in the rice grain

six environments. The stably expressed QTLs and major QTLs are suitable candidates for the improvement of FC via marker assisted breeding. Dynamic expression of QTLs for fat content during grain filling was detected by Wang et al. (2008b). Eleven unconditional QTL and 10 conditional QTL for FC were identified with more QTL expressed in the early developmental stages. The results suggested that accumulation of fat was governed by time-dependent gene expression. Ying et al. (2012) identified QTLs for fatty acid composition, and 29 associated QTLs were identified throughout the rice genome, except chromosomes 9 and 10. Nine rice

http://dx.doi.org/10.5772/56621

orthologs of *Arabidopsis* genes encoding key enzymes in lipid metabolism co-localized with 11 mapped QTLs. A strong QTL for oleic (18:1) and linoleic (18:2) acid is associated with a gene encoding acyl–CoA:diacylglycerol acyltransferase, while another one for palmitic acid (16:0) is possibly associated with the acyl–ACP thioesterase gene.

3.4.3. Minerals

Stangoulis et al. (2007) mapped the QTLs for inorganic phosphorus (P), total P, Fe, Zn, Cu and Mn concentrations. Norton et al. (2010) mapped 41 QTLs for the concentration of 17 elements in rice grain. Du et al. (2013) identified 23 and 9 QTLs for Ca, Fe, K, Mg, Mn, P, and Zn contents in brown rice in two environments of China, Lingshui of Hainan and Hangzhou of Zhejiang, respectively. Only 2 QTLs for Mg accumulation have been detected in both environments, indicating that mineral accumulation QTLs in rice grains are largely environment-dependent. Garcia-Oliveira et al. (2009) identified 31 putative QTLs for Fe, Zn, Mn, Cu, Ca, Mg, P and K contents with introgression lines derived from a cross between an elite *indica* cultivar Teqing and the wild rice (*Oryza rufipogon*). It was found that wild rice contributed favorable alleles for most of the QTLs (26 QTLs), and chromosomes 1, 9 and 12 exhibited 14 QTLs (45%) for these traits.

Phytic acid (myo-inositol 1,2,3,4,5,6-hexakisphosphate) in rice grain may form complexes with mineral ions, such as Fe, Zn and Ca, leading to be low bioavailability of minerals to humans. A set of low phytic acid rice mutant lines with the aim of increasing the bioavailability of the minerals of rice (Liu et al. 2007) have been isolated. Functional markers have been developed from some mutants (Zhao et al. 2008; Tan et al. 2013), and candidate genes such as multi-drug resistance-associated protein ABC transporter gene 5 (Xu et al. 2009) have been revealed. These mutant and markers tagged for the mutation may help develop new rice with increased mineral bioavailability.

3.4.4. Phenolics

Jin et al. (2009) found via linkage mapping that phenolic content, flavonoid content, and antioxidant capacity were individually controlled by three QTLs. Only one QTL on chromosome 2 was shared by phenolic content and flavonoid content. Shao et al. (2011) identified QTLs for these traits via association mapping using a diverse set of rice germplasm including red rice and black rice. Four, six and six QTLs were found associated with phenolic content, flavonoid content, and antioxidant capacity, respectively. Among them, four QTLs for phenolic content were also shared for other two traits. *Ra* (i.e. *Prp-b* for purple pericarp) and *Rc* (brown pericarp and seed coat) were main-effect loci for rice grain color and nutritional quality traits. Association mapping for the traits of the 361 white or non-pigmented rice accessions (i.e. excluding the red and black rice) revealed marker (RM346) is associated with phenolic content.

Pigmented rice accumulates anthocyanins (black rice) and proanthocyanidin (red rice), which are benefit to human health. Genetically, the pericarp color of red rice was controlled by two complementary genes, *Rc* (brown pericarp) on chromosome 7 and *Rd* (red pericarp) on

chromosome 1. When present together, these loci produce red seed color. *Rc* in the absence of *Rd* produces brown seeds, whereas *Rd* alone has no phenotype (Sweeney et al. 2006; Furukawa et al. 2007). A natural mutation in *rc* has reverted brown pericarp to red pericarp and resulted in a new, dominant, wild-type allele, *Rc-g* (Brooks et al. 2008). The color of dark purple pericarp was also controlled by two complementary genes, *Pb* and *Pp*, located on chromosome 4 and 1, respectively (Wang et al 2009). Wang and Shu (2007) mapped *Pb* gene and suggested that this gene may be *Ra* gene. Markers for these genes may be useful for pigmented rice breeding, especially useful if new rice expects to accumulate both anthocyanins and proanthocyanidin.

4. Molecular breeding

Molecular breeding is the application of molecular biology tools in plant breeding, which is generally include marker assisted selection (MAS) and genetic engineering (genetic transformation) in addition to QTL mapping or gene discovery. Both of MAS and genetic engineering have been applied in grain quality improvement in rice. MAS has been successfully applied for cooking and eating quality improvement because of available of the excellent markers, while the genetic engineering has been widely used to improve nutritional quality of rice.

4.1. Marker assisted selection

QTLs underlying natural occurring variation in grain quality have been widely explored, however, only few of them have been applied in current rice breeding programs. To the best of our knowledge, most of reports in terms of improving grain quality simply mean to improve the eating and cooking quality. The most useful genes are *Wx*, *SSIIa*, and *fragrance* (Table 6). Functional markers developed from GS3 are also available for grain length improvement (Wang et al. 2011). There are two strategies to conduct MAS in the breeding program. One is to improve the grain quality for the rice with high yield potential or high resistance to abiotic or biotic stresses, but with low quality. This is referred to foreground selection, which means that selection of a marker for grain quality trait by MAS denotes a trait obtained. Foreground selection is particularly useful for traits that need laborious or time-consuming phenotypic screening procedures, such as grain quality traits. The other is to improve the yield potential and high resistance for good quality rice, such as basmati or jasmine rice. This is referred to background selection. The markers for grain quality are used as background selection, which is to avoid the loss of good quality traits during introduction of the other traits.

4.1.1. Wx, fgr and SSIIa

Low quality of hybrid rice in China is mainly owing to its poor quality maintainer line. One of good ways is to improve the quality of maintainer line by MAS. Some important maintainer line, such as Zhenshan 97B (Zhou et al. 2003; Liu et al. 2006), Longtefu B (Liu et al. 2006), and II32B (Jin et al. 2010), G46B (Gao et al. 2009) have been the target of transferring the *Wx* allele conferring lower amylose content. The new hybrid rice derived from the improved maintainer line and restorer line is expected to have better quality

because the restorer line generally has good quality. Furthermore, MAS with *Wx* gene marker for quality improvement of the conventional rice has been reported (Yi et al. 2009; Jantaboon et al. 2011; Jairin et al. 2009).

Consumers generally prefer fragrant rice to non-fragrant rice. Functional markers for *fgr* have been developed and successively used to transfer this gene from fragrance rice to the target non-fragrance rice (Yi et al. 2009; Jin et al. 2010; Salgotra et al. 2012; Jantaboon et al. 2011).

SSIIa is responsible for the variation of gelatinization temperature; the functional markers for SSIIa have been developed and used in MAS to improve the cooking quality (Jin et al. 2010; Jantaboon et al. 2011; Lu et al. 2010).).

Gene marker	Forward primer (5'→3')	Reverse primer (5'→3')	Reference
Wx	484: ctttgtctatctcaagacac	485: ttgcagatgttcttcctgatg	Aryes et al. (1997)
SSIIa	NF1 :cgaggcgcagcacaacag F22: caaggagagctggagggggc	NR1: ggccgtgcagatcttaaccat R21: acatgccgcgcacctggaaa	Bao et al. (2006b) and Jin et al. (2010)
fgr	1F : ggagcttgctgatgtgtgtaaa 2F : cctctgcttctgcctctgat	1R : ggaaacaaaccttaaccatag 2R : gattgcgcggaggtacttg	Jin et al. (2010) Shi et al. (2008)

[1]Adapted from Jin et al. (2010)

Table 6. Useful PCR markers for MAS to improve cooking and eating quality of rice[1]

4.1.2. Combining grain quality with other traits

Breeding is working for not only one trait, but all the traits for the formation of a new variety. In addition to grain quality traits, yield and other agronomic or resistance traits are also very important. For those rice cultivars already have good quality, the objective of MAS is to combine the important quality traits with other traits. There are special cases for basmati and jasmine rices which have premium grain quality, and have been widely accepted by consumers worldwide. MAS has been carried out to introduce bacterial blight resistance (Pandey et al. 2013; Win et al. 2012), blast resistance (Singh et al. 2012), brown planthopper resistance (Jairin et al. 2009), submergence tolerance (Jantaboon et al. 2011) and plant stature (Pandey et al. 2013) genes into the basmati or jasmine rices.

4.2. Transgenic engineering

The advantage to conduct MAS is that abundant molecular markers are available for rice and many traits have been tagged with molecular markers. However, the disadvantage is that MAS is only effective when the target traits exist in rice germplasm, and becomes void when the traits of interest are not present in the rice germplasm. In this case, transgenic engineering is useful, which could introduce the new traits into rice by transferring the target gene from other species. Expression of exotic gene in rice could produce the target trait. Transgenic engineering

has some successful examples to introduce new nutrient traits into rice grain, such as vitamine a (Va), that confers rice high nutritional and increased benefit to human health.

4.2.1. Resistant starch

Consumption of resistant starch enriched foods is associated with decrease in the postprandial glycaemic and insulinaemic responses, accompanied by the production of fermentation-related gases in the large bowel. A high-amylose transgenic rice line modified by antisense RNA inhibition of starch branching enzymes has a 8.05% of resistant starch content, which was shown to decrease the postprandial glycaemic and insulinaemic responses and promoted fermentation-related production of H_2 in the large bowel of young and healthy adults who consumed the resistant starch-enriched rice meal (Li et al 2009).

4.2.2. Protein

Expression of a gene encoding a precursor polypeptide of sesame 2S albumin, a sulfur-rich seed storage protein in transgenic rice plants results in the improvement of the nutritive value of rice; the crude protein content in rice grains was increased by 0.64-3.54%, and the methionine and cysteine contents of these transgenic rice grains were respectively elevated by 29-76% and 31-75% compared with those of wild-type rice grains (Lee et al. 2003). Over-expression of aspartate aminotransferase genes in rice results in altered nitrogen metabolism and increased amino acid content and protein contents in seeds (Zhou et al. 2009).

4.2.3. Va

Vitamin A deficiency has been linked to night blindness, corneal scarring and permanent blindness. Vitamin A deficiency increases infant mortality rates and the incidence and severity of infectious diseases. Carotenoids, a precursor of Vitamin A, is an important lipid-soluble antioxidants in photosynthetic tissues, which are known to be completely absent in rice endosperm. The entire β-carotene biosynthetic pathway in rice endosperm has been introduced into rice by transformation of plant phytoene synthase, *Erwinia uredovora* carotene desaturase, and lycopene β-cyclase genes via *Agrobacterium*-mediated transformation. The transgenic rice, Golden Rice 1, can accumulate a maximal level of 1.6 μg/g total carotene in the endosperm. Insertion of the phytoene synthetase gene from maize and the carotene desaturase gene from *Erwinia uredovora* into rice resulted in the greatest accumulation of total carotenoids and β-carotene. Golden Rice 2 contains as much as 37 μg total carotenoids per gram of dry weight of grain, of which 31 μg/g is β–carotene (Paine et al. 2005).

4.2.4. Folate

Folates are B vitamins (vitamin B9). Humans cannot synthesize folates and have to absorb them from the diet, with plants usually being the main dietary sources. Folates play roles in the prevention of neural tube defects and in reducing the risk of cardiovascular disease, colon cancer, and neuropsychiatric disorders. In the United States, folic acid is added to refined cereals and grain products; these products are major contributors to total folate intake. Rice is

http://dx.doi.org/10.5772/56621

a poor source of folates (vitamin B9). Overexpressing two *Arabidopsis thaliana* genes of the pterin and para-aminobenzoate branches of the folate biosynthetic pathway, Storozhenko (2007) obtained transgenic rice with a maximal folate content enhancement as high as 100 times above wild type, with 100 g of polished raw grains containing up to four times the adult daily folate requirement.

4.2.5. Minerals (Fe)

Iron deficiency is the most widespread micronutrient deficiency world-wide that afflicts an estimated 30% of the world population, especially where vegetable-based diets are the primary food source. Expression of the soybean *ferritin* gene (Goto et al. 1999) or pea *ferritin* gene (Ye et al. 2007) in rice produced seeds with greater Fe contents. Especially, Vasconcelos et al. (2003) showed that expression of the soybean *ferritin* gene under the control of the glutelin promoter in rice has proven to be effective in enhancing grain nutritional levels, not only in brown grains but also in polished grains. Expression of a thermotolerant phytase gene from *Aspergillus fumigatus* in rice endosperm is expected to decrease the phytic acid and increase iron bioavailability (Lucca et al. 2001).

4.2.6. Flavonoids

Flavonoids are lacking in the endosperm of rice. Expression of maize C1 and R-S regulatory genes driven by an endosperm specific promoter of a rice prolamin gene in rice grain resulted in dark brown pericarp of the C1/R-S homozygous lines, and the major flavonoids, dihydroquercetin (taxifolin), dihydroisorhamnetin (3′-*O*-methyl taxifolin) and 3′-*O*-methyl quercetin were identified in the rice grain (Shin et al. 2006). These rice lines have the potential to be developed further as a novel variety that can produce various flavonoids in its endosperm.

4.2.7. Serotonin

Serotonin derivatives such as *p*-coumaroylserotonin and feruloylserotonin, a family of plant polyphenol compounds, play roles in an array of biological activities including antioxidative activity, but neither their production nor identification has been reported in crop plants. Transgenic rice expressing the pepper hydroxycinnamoyl-CoA:serotonin N-(hydroxycinnamoyl) transferase gene produced on average 274 ng/g seed weight which was nine-fold higher than wild-type (30 ng/g seed weight) (Kang et al. 2005). Chemical treatments such as trans-cinnamic acid and tyramine increased the serotonin derivatives contents by two- to three fold in both wild-type and transgenic rice. The transgenic rice had higher radical scavenging activities than that of wild-type, suggesting that neutraceutical serotonin derivative could be enriched by transgenic engineering (Kang et al. 2005).

4.2.8. Coenzyme Q

Coenzyme Q (CoQ), also called ubiquinone, is an electron transfer molecule in the respiratory chain. CoQ is also a lipid-soluble antioxidant. Most cereal crops produce mainly CoQ9, which has nine isoprene units, whereas humans produce mainly CoQ10, with 10 isoprene units.

CoQ10 is a very popular food supplement. Takahashi et al. (2009) produced CoQ10-enriched rice plants by introduction of the gene for decaprenyl diphosphate synthase. In CoQ10-enriched rice plants, seed CoQ10 content per weight was increased to up to 10 times that of wild-type rice, but its level is still insufficient for practical use. Combination of the transgene with giant embryo mutant lines produced giant embryo line-type CoQ10-enriched rice with seed CoQ10 content per weight increased to up to 1.4-1.8 times. It was found that CoQ was preferentially accumulated in bran and germ of rice seed.

5. Future directions

Great progress has been achieved in our understanding of the genetic and molecular basis of grain quality of rice. This is especially true for grain appearance and grain shape, since they are not only linked with grain quality, but also with grain yield, a more important trait. Cooking and eating quality has a strong relation with starch biosynthesis pathway which has been well understood. Markers derived from the starch biosynthesis related genes have been widely applied in MAS. However, there are four major problem areas that challenge researchers working on molecular genetics of grain quality.

5.1. Functional genes for milling quality and chalkiness

Genetic understanding of milling quality is quite poor since only limited numbers of QTLs have been detected, and no QTL has been finely mapped or cloned. To make in-deep research into the area of milling quality, (1) rapid and accurate analytical tools are needed to measure the trait; (2) finely dissection of QTLs with large effect should be carried out; (3) because no mutants for milling quality have been reported, the mutants such as those induced by T-DNA insertion may provide a good start to characterize the genes responsible for milling quality. For grain chalkiness, two finely mapped QTLs await further characterization, and transcriptome for chalkiness formation during seed development have been described (Yamakawa et al. 2007; Liu et al. 2010). It looks optimism to see more progress from this area.

5.2. Molecular genetics studies for nutritional quality

Nutrition quality of rice will be a new area for further research because people keep increasingly concern about the health benefit of the food they eat. Nutrition quality covers a wide range of traits, for example, protein, amino acids, fat and phenolics. In this area, naturally occurring variation for protein, amino acids, fat and fatty acid compositions have been under exploration, but only few genes have been characterized. Formation of each nutrient in rice grain requires a complex pathway in which many genes or enzymes are involved. Current advances in protein and fatty acid biosynthesis in other crops and *Arabidopsis* may help understand the pathways in rice.

Phenolics are expected to be an important field because they are proven to benefit human health in many ways (Shao and Bao 2012). Genes for red pericarp formation, *Rc* (brown pericarp) on chromosome 7 and *Rd* (red pericarp) on chromosome 1 have been under-

stood, but their roles in regulating the flavonoids biosynthesis are unknown. The genes for dark purple pericarp formation, *Pb* and *Pp*, wait for finely mapping and functional characterization. In this field, MAS could be conducted to breed rice accumulating not only anthocyanins (a characteristic of black rice) and proanthocyanidin (a characteristic of red rice). Genetic transformation could be conducted to breed rice with accumulation of the anthocyanins or proanthocyanidin in the endosperm, since these phytochemicals accumulate only in the bran layer (Shao and Bao 2012).

5.3. Cooking and eating quality of brown rice

As concerns about nutritional quality rise, consumption of brown rice will become popular in the near future. Cooking and eating quality of brown rice will be another issue. The knowledge we have established for milled rice may not be applicable to the brown rice. Needless to say the genetic control of the cooking and eating quality of brown rice, what parameters to assess these qualities should be firstly considered. How to make brown rice appeal to consumers through suitable cooking methods should also be considered as well. At last, the question is how to improve the cooking and eating quality of brown rice.

5.4. MAS with more genes/QTLs together

Targeting more traits with more markers, such as *Wx*, *SSIIa*, and *fragrance* (Jin et al. 2010), is increasingly needed in the breeding programs. MAS for quality and yield and resistance traits should be considered together in the future. Strategies for more effective selection should be developed when many markers are used at the same time. *In silica* molecular breeding is coming into the era, with which alleles of different markers are designed in the computer; the phenotypes of new rice could also be designed and displayed in the computer.

6. Conclusion

Grain quality of rice as a whole is a complex trait that is comprised of appearance quality, milling quality, eating and cooking quality, and nutritional quality etc. Researches on the genetic control of the quality traits have made a great progress, especially for the appearance quality, cooking and eating quality. More genetic studies are needed for milling quality and nutritional quality.

The progress on the molecular genetics on grain quality has allowed MAS to be conducted more efficiently. However, only MAS for cooking and eating quality and genetic engineering for nutritional quality have made some achievements. More molecular breeding practices are needed for improvement of grain quality.

With social development and improvement of living standards, cooking and eating quality of brown rice will be a new theme that deserves greater attention from researches. Studies including cooking methods, parameters for cooking and eating, genetics, and molecular breeding are among the top priorities.

Acknowledgements

This work was financially supported by Zhejiang Provincial Natural Science Foundation (LZ13C0001) and Special Fund for Agro-scientific Research in the Public Interest (201103007) from the Ministry of Agriculture, China.

Author details

Jinsong Bao*

Address all correspondence to: jsbao@zju.edu.cn

Institute of Nuclear Agricultural Sciences, College of Agriculture and Biotechnology, Zhejiang University, Hangzhou, China

References

[1] Ahn SN, Bollich CN, McClung AM, Tanksley SD (1993) RFLP analysis of genomic regions associated with cooked-kernel elongation in rice. Theor Appl Genet 87: 27-32.

[2] Aluko G, Martinez C, Tohme J, Castano C, Bergman C, Oard JH (2004) QTL mapping of grain quality traits from the interspecific cross *Oryza sativa* × *O. glaberrima.* Theor Appl Genet 109:630–639.

[3] Ayres NM, McClung AM, Larkin PD, Bligh HFJ, Jones CA, Park WD (1997) Microsatellites and a single-nucleotide polymorphism differentiate apparent amylose classes in an expanded pedigree of US rice germ plasm. Theor Appl Genet 94: 773-781.

[4] Bai X, Luo L, Yan W, Kovi MR, Zhan W, Xing Y (2010) Genetic dissection of rice grain shape using a recombinant inbred line population derived from two contrasting parents and fine mapping a pleiotropic quantitative trait locus qGL7. BMC Genet 11: 16.

[5] Bao JS, Zheng XW, Xia YW, He P, Shu QY, Lu X, Chen Y, Zhu LH (2000) QTL mapping for the paste viscosity characteristics in rice (*Oryza sativa* L.). Theor Appl Genet 100: 280-284.

[6] Bao JS, Corke H, Sun M (2002a) Microsatellites in starch-synthesizing genes in relation to starch physicochemical properties in waxy rice (*Oryza sativa* L.). Theor Appl Genet 105: 898-905.

[7] Bao JS, Wu YR, Hu B, Wu P, Cui HR, Shu QY (2002b) QTL for rice grain quality based on a DH population derived from parents with similar apparent amylose content. Euphytica 128: 317-324.

[8] Bao JS, Sun M, Zhu LH, Corke H (2004) Analysis of quantitative trait loci for some some starch properties in rice (*Oryza sativa* L.): thermal properties, gel texture, swelling volume. J Cereal Sci 39: 379-385.

[9] Bao JS, Corke H, Sun M (2006a) Microsatellites, single nucleotide polymorphisms and a sequence tagged site in starch-synthesizing genes in relation to starch physicochemical properties in nonwaxy rice (*Oryza sativa* L.). Theor Appl Genet 113: 1185-1196.

[10] Bao JS, Corke H, Sun M (2006b) Nucleotide diversity in starch synthase IIa and validation of single nucleotide polymorphisms in relation to starch gelatinization temperature and other physicochemical properties in rice (*Oryza sativa* L.). Theor Appl Genet 113: 1171-1183.

[11] Bao JS, Robert Fjellstrom, Christine Bergman (2009) Rice end-use quality, in: Rice improvement in the genomics era. Swapan K Datta (ed.), The CRC Press, US. pp269-305.

[12] Bao JS (2012a) Nutraceuticals properties and health benefits of rice. In: Cereals and Pulses: Nutraceutical Properties and Health Benefits. Yu LL, Tsao R, Shahidi F (eds). Wiley-Blackwell, John Wiley & Sons, Inc. 37-64.

[13] Bao JS (2012b) Towards understanding of the genetic and molecular basis of eating and cooking quality of rice. Cereal Foods World 57: 148-156

[14] Bergman CJ, Delgado JT, McClung AM, Fjellstrom RG (2001) An improved method for using a satellite in rice waxy gene to determine amylose class. Cereal Chem 78: 257-260.

[15] Bligh HFJ, Larkin PD, Roach PS, Jones CA, Fu HY, Park WD (1998) Use of alternate splice sites in granule-bound starch synthase mRNA from low-amylose rice varieties. Plant Mol Biol 38: 407-415.

[16] Bligh HFJ, Till RI, Jones CA (1995) A microsatellite sequence closely linked to the waxy gene of *Oryza sativa*. Euphytica 86: 83-85.

[17] Bradbury LMT, Fitzgerald TL, Henry RJ, Jin QS, Waters DLE (2005a) The gene for fragrance in rice. Plant Biotechnol J 3: 363-370.

[18] Bradbury LMT, Henry RJ, Jin QS, Reinke RF, Waters DLE (2005b) A perfect marker for fragrance genotyping in rice. Mol Breed 16: 279-283.

[19] Brooks SA, Yan WG., Jackson AK, Deren CW (2008) A natural mutation in *rc* reverts white-rice-pericarp to red and results in a new, dominant, wild-type allele, *Rc-g*. Theor Appl Genet 117:575-580.

[20] Cai XL, Wang ZY, Xing Y, Zhang JL, Hong MM (1998) Aberrant splicing of intron 1 leads to the heterogeneous 5′ UTR and decreased expression of waxy gene in rice cultivars of intermediate amylose content. Plant J 14: 459-465.

[21] Champagne ET, Bett-Garber KL, Fitzgerald MA, Grimm CC, Lea J, Ohtsubo K, Jongdee S, Xie L, Bassinello PZ, Resurreccion A, Ahmad R, Habibi F, Reinke R (2010) Important sensory properties differentiating premium rice varieties. Rice 3: 270-281.

[22] Chen MH, Bergman CJ, Fjellstrom RG (2003) *SSSIIa* locus genetic variation associated with alkali spreading value in international rice germplasm. In Proc. Plant Animal Genomes Conf XI: 314.

[23] Chen MH, Bergman C, Pinson S, Fjellstrom R (2008a) Waxy gene haplotypes: Association with apparent amylose content and the effect by the environment in an international rice germplasm collection. J Cereal Sci 47:536-545.

[24] Chen MH, Bergman CJ, Pinson SRM, Fjellstrom RG (2008b) Waxy gene haplotypes: Associations with pasting properties in an international rice germplasm collection. J Cereal Sci 48: 781-788.

[25] Chen S, Yang Y, Shi W, Ji Q, He F, Zhang Z, Cheng Z, Liu X, Xu M (2008c) Badh2, encoding betaine aldehyde dehydrogenase, inhibits the biosynthesis of 2-acetyl-1-pyrroline, a major component in rice fragrance. Plant Cell 20: 1850-1861.

[26] Chen Y, Wang M, Ouwerkerk PBF (2012) Molecular and environmental factors determining grain quality in rice. Food and Energy Security 2012: 1-22.

[27] Cuevas RP, Daygon VD, Corpuz HM, Nora L, Reinke RF, Waters DLE, Fitzgerald MA (2010) Melting the secrets of gelatinization temperature in rice. Funct Plant Biol 37(5): 439-447.

[28] Dong Y, Tsuzuki E, Lin D, Kamiunten H, Terao H, Matsuo M, Cheng S (2004) Molecular genetic mapping of quantitative trait loci for milling quality in rice (*Oryza sativa* L.) J Cereal Sci 40: 109-114.

[29] Du J, Zeng D, Wang B, Qian Q, Zheng S, Ling HQ (2013) Environmental effects on mineral accumulation in rice grains and identification of ecological specific QTLs. Environ Geochem Health, 35: 161-170.

[30] Fan CC, Yu XQ, Xing YZ, Xu CG, Luo LJ, Zhang QF (2005) The main effects, epistatic effects and environmental interactions of QTLs on the cooking and eating quality of rice in a doubled-haploid line population. Theor Appl Genet 110: 1445-1452.

[31] Fan C, Xing Y, Mao H, Lu T, Han B, Xu C, Li X, Zhang Q (2006) GS3, a major QTL for grain length and weight and minor QTL for grain width and thickness in rice, encodes a putative transmembrane protein. Theor Appl Genet 112: 1164-1171.

[32] Fan C, Yu S, Wang C, Xing Y (2009) A causal C-A mutation in the second exon of GS3 highly associated with rice grain length and validated as a functional marker. Theor Appl Genet 118: 465-472.

[33] Furukawa T, Maekawa M, Oki T, Sudal I, Iida S, Shimada H, Takamure I, Kadowaki KI (2007) The Rc and Rd genes are involved in proanthocyanidin synthesis in rice pericarp. Plant J 49: 91-102.

[34] Gao FY, Lu XJ, Wang WM, Sun SS, Li ZH, Li HJ, Ren GJ (2009) Trait-specific improvement of a cytoplasmic male-sterile line using molecular marker-assisted selection in rice. Crop Sci 49: 99-106.

[35] Gao ZY, Zeng DL, Cui X, Zhou YH, Yan M, Huang D, Li JY, Qian Q (2003) Map-based cloning of the ALK gene, which controls the GT of rice. Sci China C Life Sci 46: 661-668.

[36] Gao Z, Zeng D, Cheng F, Tian Z, Guo L, Su Y, Yan M, Jiang H, Dong G, Huang Y, Han B, Li J, Qian Q (2011) ALK, the key gene for gelatinization temperature, is a modifier gene for gel consistency in rice. J Integr Plant Biol 53: 756-765.

[37] Garcia-Oliveira AL, Tan L, Fu Y, Sun C (2009) Genetic identification of quantitative trait loci for contents of mineral nutrients in rice grain. J Integr Plant Biol 51: 84-92.

[38] Goto F, Yoshihara T, Shigemoto N, Toki S, Takaiwa F (1999) Iron fortification of rice seed by the soybean ferritin gene. Nat Biotechnol 17: 282-286.

[39] Guo L, Ma L, Jiang H, Zeng D, Hu J, Wu L, Gao Z, Zhang G, Qian Q (2009) Genetic analysis and fine mapping of two genes for grain shape and weight in rice. J Integr Plant Biol 51: 45-51.

[40] Guo T, Liu X, Wan X, Weng J, Liu S, Liu X, Chen M, Li J, Su N, Wu F, Cheng Z, Guo X, Lei C, Wang J, Jiang L, Wan J (2011) Identification of a stable quantitative trait locus for percentage grains with white chalkiness in rice (*Oryza sativa*). J Integr Plant Biol 53: 598-607.

[41] Han YP, Xu ML, Liu XY, Yan CJ, Korban SS, Chen XL, Gu MH (2004) Genes coding for starch branching enzymes are major contributors to starch viscosity characteristics in waxy rice (*Oryza sativa* L.). Plant Sci 166: 357-364.

[42] He P, Li SG, Qian Q, Ma YQ, Li JZ, Wang WM, Chen Y, Zhu L (1999). Genetic analysis of rice grain quality. Theor Appl Genet 98: 502-508.

[43] He Y, Han Y, Jiang L, Xu C, Lu J, Xu M (2006) Functional analysis of starch-synthesis genes in determining rice eating and cooking qualities. Mol Breed 18: 277-290.

[44] Hirano HY, Eiguchi M, Sano Y (1998) A single base change altered the regulation of the Waxy gene at the posttranscriptional level during the domestication of rice. Mol Biol Evol 15: 978-987.

[45] Hu ZL, Li P, Zhou MQ, Zhang ZH, Wang LX, Zhu LH, Zhu YG (2004) Mapping of quantitative trait loci (QTLs) for rice protein and fat content using doubled haploid lines. Euphytica 135: 47-54.

[46] Hu KY, Li LZ, Zheng XF, Zhang ZH, Mou TM, Hu ZL (2009) Genetic dissection of amino acid content in rice grain. J Sci Food Agric 89: 2377-2382

[47] Hu Z, He H, Zhang S, Sun F, Xin X, Wang W, Qian X, Yang J, Luo X (2012) A kelch motif-containing serine/threonine protein phosphatase determines the large grain QTL trait in rice. (2012) J Integr Plant Biol 54: 979-990.

[48] Huang R, Jiang L, Zheng J, Wang T, Wang H, Huang Y, Hong Z (2013) Genetic bases of rice grain shape: so many genes, so little known. Trend Plant Sci 18:218-226.

[49] Huang X, Qian Q, Liu Z, Sun H, He S, Luo D, Xia G, Chu C, Li J, Fu X (2009) Natural variation at the DEP1 locus enhances grain yield in rice. Nat Genet 41: 494-497.

[50] Inukai T, Sako A, Hirano HY, Sano Y (2000) Analysis of intragenic recombination at wx in rice: correlation between the molecular and genetic maps within the locus. Genome 43: 589-596.

[51] Isshiki M, Morino K, Nakajima M, Okagaki RO, Wessler SR, Izawa T, Shimamoto K (1998) A naturally occurring functional allele of the waxy locus has a GT to TT mutation at the 5′ splice site of the first intron. Plant J 15: 133-138.

[52] Jagadish SVK, Muthurajan R, Oane R, Wheeler TR, Heuer S, Bennett J, Craufurd PQ (2010) Physiological and proteomic approaches to address heat tolerance during anthesis in rice (*Oryza sativa* L.). J Exp Bot 61: 143-156.

[53] Jairin J, Teangdeerith S, Leelagud P, Kothcharerk J, Sansen K, Yi M, Vanavichit A, Toojinda T (2009) Development of rice introgression lines with brown planthopper resistance and KDML105 grain quality characteristics through marker-assisted selection. Field Crop Res 110: 263-271.

[54] Jantaboon J, Siangliw M, Im-mark S, Jamboonsri W, Vanavichit A, Toojinda T (2011) Ideotype breeding for submergence tolerance and cooking quality by marker-assisted selection in rice. Field Crop Res 123: 206-213.

[55] Jeon JS, Ryoo N, Hahn TR, Walia H, Nakamura Y (2010) Starch biosynthesis in cereal endosperm. Plant Physiol Biochem 48: 383-392.

[56] Jiang GH, Hong XY, Xu CG, Li XH, He YQ (2005) Identification of quantitative trait loci for grain appearance and milling quality using a doubled-haploid rice population. J Integr Plant Biol 47: 1391-1403.

[57] Jin L, Xiao P, Lu Y, Shao YF, Shen Y, Bao JS (2009) Quantitative trait loci for brown rice color, phenolics and, flavonoid contents and antioxidant capacity in rice grain. Cereal Chem 86(6): 609-615.

[58] Jin L, Lu Y, Shao YF, Zhang G, Xiao P, Shen SQ, Corke H, Bao JS (2010) Molecular marker assisted selection for improvement of the eating, cooking and sensory quality of rice (*Oryza sativa* L.). J Cereal Sci 51: 159-164.

[59] Jin QS, Waters D, Cordeiro GM, Henry RJ, Reinke RF (2003) A single nucleotide polymorphism (SNP) marker linked to the fragrance gene in rice (*Oryza sativa* L.). Plant Sci 165: 359–364.

[60] Kang HG, Park S, Matsuoka M, An G (2005) White-core endosperm floury endosperm-4 in rice is generated by knockout mutations in the C4-type pyruvate orthophosphate dikinase gene (OsPPDKB). Plant J 42: 901-911.

[61] Kang K, Jang SM, Kang S, Back K (2005) Enhanced neutraceutical serotonin derivatives of rice seed by hydroxycinnamoyl-CoA:serotonin N-(hydroxycinnamoyl) transferase. Plant Sci 168: 783-788.

[62] Kato T, Segami S, Toriyama M, Kono I, Ando T, Yano M, Kitano H, Miura K, Iwasaki Y (2011) Detection of QTLs for grain length from large grain rice (*Oryza sativa* L.). Breeding Sci 61: 269-274.

[63] Lanceras JC, Huang ZL, Naivikul O, Vanavichit A, Ruanjaichon V, Tragoonrung S (2000) Mapping of genes for cooking and eating qualities in Thai jasmine rice (KDML105). DNA Res. 7: 93-101.

[64] Lapitan VC, Redona ED, Abe T, Brar DS (2009) Mapping of quantitative trait loci using a double-haploid population from the cross of indica and japonica cultivars of rice. Crop Sci 49: 1620-1628.

[65] Larkin PD, Park WD (2003) Association of waxy gene single nucleotide polymorphisms with starch characteristics in rice (*Oryza sativa* L.). Mol Breed 12: 335-339.

[66] Larkin PD, Park WD (1999) Transcript accumulation and utilization of alternate and non-consensus splice sites in rice granule-bound starch synthase are temperature-sensitive and controlled by a single-nucleotide polymorphism. Plant Mol Biol 40: 719-727.

[67] Larkin PD, McClung AM, Ayres NM, Park WD (2003) The effect of the Waxy locus (Granule bound starch synthase) on pasting curve characteristics in specialty rice (*Oryza sativa* L.). Euphytica 131: 243-253.

[68] Lee TTT, Leu WM, Yang HH, Chen BCM, Tzen JTC (2006) Sesame oleosin and prepro-2S albumin expressed as a fusion polypeptide in transgenic rice were split, processed and separately assembled into oil bodies and protein bodies. J Cereal Sci 44: 333-341.

[69] Li J, Xiao J, Grandillo S, Jiang L, Wang Y, Deng Q, Yuan L, McCouch SR (2004). QTL detection for rice grain quality traits using an interspecific backcross population derived from cultivated Asian (*O. sativa* L.) and African (*O. glaberrima* S.) rice. Genome 47: 697-704.

[70] Li M, Piao JH, Tian Y, Li WD, Li KJ, Yang XG (2009) Postprandial glycaemic and insulinaemic responses to GM-resistant starch-enriched rice and the production of fermentation-related H2 in healthy Chinese adults. Brit J Nutr 103: 1-6.

[71] Li Y, Fan C, Xing Y, Jiang Y, Luo L, Sun L, Shao D, Xu C, Li X, Xiao J, He Y, Zhang Q (2011) Natural variation in GS5 plays an important role in regulating grain size and yield in rice. Nat Genet 43: 1266-1269.

[72] Li ZF, Wan JM, Xia JF, Zhai HQ, Ikehashi H (2004) Identification of quantitative trait loci underlying milling quality of rice (*Oryza sativa*) grains. Plant Breeding 123: 229-234.

[73] Liu QL, Xu XH, Ren XL, Fu HW, Wu DX, Shu QY (2007) Generation and characterization of low phytic acid germplasm in rice (*Oryza sativa* L.) Theor Appl Genet 114: 803-814.

[74] Liu QQ, Li QF, Cai XL, Wang HM, Tang SZ, Yu HX, Wang ZY, Gu MH (2006) Molecualr marker-assisted selection for improved cooking and eating quality of two elite parents of hybrid rice. Crop Sci 46: 2354-2360.

[75] Liu W, Zeng J, Jiang G, He Y (2009) QTLs identification of crude fat content in brown rice and its genetic basis analysis using DH and two backcross populations. Euphytica 169: 197-205.

[76] Liu X, Guo T, Wan X, Wang H, Zhu M, Li A, Su N, Shen Y, Mao B, Zhai H, Mao L, Wan J (2010) Transcriptome analysis of grain-filling caryopses reveals involvement of multiple regulatory pathways in chalky grain formation in rice. BMC Genomics 11: 730.

[77] Liu X, Wan X, Ma X, Wan J (2011) Dissecting the genetic basis for the effect of rice chalkiness, amylose content, protein content, and rapid viscosity analyzer profile characteristics on the eating quality of cooked rice using the chromosome segment substitution line population across eight environments. Genome 54: 64-80.

[78] Liu X, Wang Y, Wang SW (2012) QTL analysis of percentage of grains with chalkiness in Japonica rice (*Oryza sativa*). Genet Mol Res 11: 717-724.

[79] Lorieux M, Petrov M, Huang N, Guiderdoni E, Ghesquiere A (1996) Aroma in rice: genetic analysis of a quantitative trait. Theor Appl Genet 93: 1145–1151.

[80] Lou J, Chen L, Yue G, Lou Q, Mei H, Xiong L, Luo L (2009) QTL mapping of grain quality traits in rice. J Cereal Sci 50:145–151.

[81] Lu Y, Xiao P, Shao YF, Zhang G, Thanyasiriwat T, Bao JS (2010) Development of new markers to genotype the functional SNPs of SSIIa, a gene responsible for gelatinization temperature of rice starch. J Cereal Sci 52: 438-443

[82] Lucca P, Hurrell R, Potrykus I (2011) Genetic engineering approaches to improve the bioavailability and the level of iron in rice grains. Theor Appl Genet 102: 392-397.

[83] Mao H, Sun S, Yao J, Wang C, Yu S, Xu C, Li X, Zhang Q (2010) Linking differential domain functions of the GS3 protein to natural variation of grain size in rice. Proc Natl Acad Sci USA 107: 19579-19584.

[84] Nakamura Y, Francisco PB, Hosaka Y, Sato A, Sawada T, Kubo A, Fujita N (2005) Essential amino acid of starch synthase IIa differentiate amylopectin structure and starch quality between japonica and indica rice varieties. Plant Mol Biol 58: 213-227.

[85] Nelson JC, Jodari F, Roughton AI, Mckenzie KM, Mcclung AM, Fjellstrom RG, Scheffler BE (2012) QTL mapping for milling quality in elite western U.S. rice germplasm. Crop Sci 52: 242-252.

[86] Nelson JC, Mcclung AM, Fjellstrom RG, Moldenhauer KAK, Boza E, Jodari F, Oard JH, Linscombe S, Scheffler BE, Yeater KM (2011) Mapping QTL main and interaction influences on milling quality in elite US rice germplasm Theor Appl Genet 122: 291-309.

[87] Norton GJ, Deacon CM, Xiong L, Huang S, Meharg AA, Price AH (2010) Genetic mapping of the rice ionome in leaves and grain: Identification of QTLs for 17 elements including arsenic, cadmium, iron and selenium. Plant Soil 329: 139-153.

[88] Paine JA, Shipton CA, Chaggar S, Howells RM, Kennedy MJ, Vernon G, Wright SY, Hinchliffe E, Adams JL, Silverstone AL, Drake R (2005) Improving the nutritional value of golden rice through increased pro-vitamin A content. Nat Biotechnol 23: 482-487.

[89] Pandey MK, Rani NS, Sundaram RM, Laha GS, Madhav MS, Rao KS, Sudharshan I, Hari Y, Varaprasad GS, Rao LVS, Suneetha K, Sivaranjani AKP, Viraktamath BC (2013) Improvement of two traditional Basmati rice varieties for bacterial blight resistance and plant stature through morphological and marker-assisted selection. Mol Breed 31: 239-246.

[90] Qi P, Lin YS, Song XJ, Shen JB, Huang W, Shan JX, Zhu MZ, Jiang L, Gao JP, Lin HX (2012) The novel quantitative trait locus GL3.1 controls rice grain size and yield by regulating Cyclin-T1;3. Cell Res 22: 1666-1680.

[91] Qiao Y, Lee SI, Piao R, Jiang W, Ham TH, Chin JH, Piao Z, Han L, Kang SY, Koh HJ (2010) Fine mapping and candidate gene analysis of the floury endosperm gene, FLO(a), in rice. Mol Cells 29: 167-174.

[92] Qin Y, Kim SM, Sohn JK (2009) Genetic analysis and qtl mapping for grain chalkiness characteristics of brown rice (*Oryza sativa* L.). Genes Genom 31: 155-164.

[93] Qin Y, Kim SM, Zhao X, Lee HS, Jia B, Kim KM, Eun MY, Sohn JK (2010) QTL detection and MAS selection efficiency for lipid content in brown rice (*Oryza sativa* L). Genes Genom 32: 506-512.

[94] Qiu X, Gong R, Tan Y, Yu S (2012) Mapping and characterization of the major quantitative trait locus qSS7 associated with increased length and decreased width of rice seeds. Theor Appl Genet 125: 1717-1726.

[95] Rani NS, Sivaranjani AKP, Madhav MS, Sundaram RM, Prasad GSV, Srikanth S, Pandey M, Suneetha K, Sudharshan I (2011) Identification of molecular markers for cooking quality traits of rice. Indian J Genet Plang Breed 71: 129-138

[96] Ryoo N, Yu C, Park CS, Baik MY, Park IM, Cho MH, Bhoo SH, An G, Hahn TR, Jeon JS (2007) Knockout of a starch synthase gene OsSSIIIa/Flo5 causes white-core floury endosperm in rice (*Oryza sativa* L.). Plant Cell Rep 26: 1083-1095.

[97] Salgotra RK, Gupta BB, Millwood RJ, Balasubramaniam M, Stewart Jr CN (2012) Introgression of bacterial leaf blight resistance and aroma genes using functional marker-assisted selection in rice (*Oryza sativa* L.). Euphytica 187: 313-323.

[98] Septiningsih EM, Trijatmiko KR, Moeljopawiro S, McCouch SR (2003) Identification of quantitative trait loci for grain quality in an advanced backcross population derived from the Oryza sativa variety IR64 and the wild relative O. rufipogon. Theor Appl Genet 107: 1433–1441.

[99] Shao G, Wei X, Chen M, Tang S, Luo J, Jiao G, Xie L, Hu P (2012) Allelic variation for a candidate gene for GS7, responsible for grain shape in rice. Theor Appl Genet 125: 1303-1312.

[100] Shao YF, Jin L, Zhang G, Lu Y, Shen Y, Bao JS (2011) Association mapping of brown rice color, phenolic content, flavonoid content and antioxidant capacity in rice grain. Theor Appl Genet 122: 1005-1016.

[101] Shao YF, Bao JS (2012) Anthocyanins in Rices: Biosynthesis and health benefits. In: Anthocyanins: Structure, Biosynthesis and Health Benefits. Noboru Motohashi (ed.). Nova Science Publishers, Inc. 93-110.

[102] Shen Y, Liu L, Jiang L, Zhang Y, Liu X, Zhai H, Wan J (2010) Identification of quantitative trait loci affecting grain fat content in rice (*Oryza sativa* L.). Cereal Chem 87: 118-124.

[103] Shen Y, Zhang W, Liu X, Chen L, Liu S, Zheng L, Li J, Chen Y, Wu T, Yu Y, Zhong Z, Jiang L, Wan J (2012) Identification of two stably expressed QTLs for fat content in rice (*Oryza sativa*). Genome 55: 585-590.

[104] Shi WW, Yang Y, Chen SH, Xu ML (2008) Discovery of a new fragrance allele and the development of functional markers for the breeding of fragrant rice varieties. Mol Breed 22: 185-192.

[105] Shin YM, Park HJ, Yim SD, Baek NI, Lee CH, An G, Woo YM (2006) Transgenic rice lines expressing maize C1 and R-S regulatory genes produce various flavonoids in the endosperm. Plant Biotechnol J 4: 305-315.

[106] Shomura A, Izawa T, Ebana K, Ebitani T, Kanegae H, Konishi S, Yan, M (2008) Deletion in a gene associated with grain size increased yields during rice domestication. Nat Genet 40: 1023-1028.

[107] Singh R, Singh AK, Sharma TR, Singh A, Singh NK (2012) Fine mapping of grain length QTLs on chromosomes 1 and 7 in Basmati rice (*Oryza sativa* L.). J Plant Biochem Biotechnol 21: 157-166.

[108] Singh VK, Singh A, Singh SP, Ellur RK, Choudhary V, Sarkel S, Singh D, Krishnan SG, Nagarajan M, Vinod KK, Singh UD, Rathore R, Prashanthi SK, Agrawal PK, Bhatt JC, Mohapatra T, Prabhu KV, Singh AK (2012) Incorporation of blast resistance into "PRR78", an elite Basmati rice restorer line, through marker assisted backcross breeding. Field Crop Res 128: 8-16.

[109] Song XJ, Huang W, Shi M, Zhu MZ, Lin HX (2007) A QTL for rice grain width and weight encodes a previously unknown RING-type E3 ubiquitin ligase. Nat Genet 39: 623-630.

[110] Stangoulis JCR, Huynh BL, Welch RM, Choi EY, Graham RD (2007) Quantitative trait loci for phytate in rice grain and their relationship with grain micronutrient content. Euphytica 154: 289-294.

[111] Storozhenko S, De Brouwer V, Volckaert M, Navarrete O, Blancquaert D, Zhang GF, Lambert W, Van Der Straeten D (2007) Folate fortification of rice by metabolic engineering. Nat Biotechnol 25: 1277-1279.

[112] Su Y, Rao Y, Hu S, Yang Y, Gao Z, Zhang G, Liu J, Hu J, Yan M, Dong G, Zhu L, Guo L, Qian Q, Zeng D (2011) Map-based cloning proves qGC-6, a major QTL for gel consistency of japonica/indica cross, responds by waxy in rice (*Oryza sativa* L.). Theor Appl Genet 123: 859-867.

[113] Sweeney MT, Thomson MJ, Pfeil BE, McCouch S (2006) Caught red-handed: Rc encodes a basic helix-loop-helix protein conditioning red pericarp in rice. Plant Cell 18: 283-294.

[114] Takahashi S, Ohtani T, Iida S, Sunohara Y, Matsushita K, Maeda H, Tanetani Y, Kawai K, Kawamukai M, Kadowaki KI (2009) Development of CoQ10-enriched rice from giant embryo lines. Breeding Sci 59: 321-326.

[115] Takano-Kai N, Jiang H, Kubo T, Sweeney M, Matsumoto T, Kanamori H, Padhukasahasram B, Bustamante C, Yoshimura A, Doi K, McCouch S (2009) Evolutionary history of GS3, a gene conferring grain length in rice. Genet 182: 1323–1334.

[116] Tan YF, Li JX, Yu SB, Xing YZ, Xu CG, Zhang Q (1999) The three important traits for cooking and eating quality of rice grains are controlled by a single locus in an elite rice hybrid, Shanyou 63. Theor Appl Genet 99: 642-648.

[117] Tan YF, Xing YZ, Li JX, Yu SB, Xu CG, Zhang Q (2000). Genetic bases of appearance quality of rice grains in Shanyou 63, an elite rice hybrid. Theor Appl Genet 101: 823-829.

[118] Tan YF, Sun M, Xing YZ, Hua JP, Sun XL, Zhang QF, Corke H (2001) Mapping quantitative trait loci for milling quality, protein content and color characteristics of rice using a recombinant inbred line population derived from an elite rice hybrid. Theor Appl Genet 103: 1037-1045.

[119] Tan YY, Fu HW, Zhao HJ, Lu S, Fu JJ, Li YF, Cui HR, Shu QY (2013) Functional molecular markers and high-resolution melting curve analysis of low phytic acid mutations for marker-assisted selection in rice. Mol Breed 31: 517-528.

[120] Teng B, Zeng R, Wang Y, Liu Z, Zhang Z, Zhu H, Ding X, Li W, Zhang G (2012) Detection of allelic variation at the Wx locus with single-segment substitution lines in rice (*Oryza sativa* L.). Mol Breed 30: 583-595.

[121] Tian R, Jiang GH, Shen LH, Wang LQ, He YQ (2005) Mapping quantitative trait loci underlying the cooking and eating quality of rice using a DH population. Mol Breed 15: 117-124.

[122] Tian Z, Qian Q, Liu Q, Yan M, Liu X, Yan C, Liu G, Gao Z, Tang S, Zeng D, Wang Y, Yu J, Gu M, Li J (2009) Allelic diversities in rice starch biosynthesis lead to a diverse array of rice eating and cooking qualities. Proc Natl Acad Sci USA 106: 21760-21765.

[123] Tian ZX, Yan CJ, Qian Q, Yan S, Xie HL, Wang F, Xu JF, Liu GF, Wang YH, Liu QQ, Tang SZ, Li JY, Gu MH (2010) Development of Gene-tagged Molecular Markers for Starch Synthesis-related Genes in Rice. Chinese Sci Bull 55: 3768-3777.

[124] Tran NA, Daygon VD, Resurreccion AP, Cuevas RP, Corpuz HM, Fitzgerald MA (2011) A single nucleotide polymorphism in the Waxy gene explains a significant component of gel consistency. Theor Appl Genet 123: 519-525.

[125] Traore K, McClung AM, Chen MH, Fjellstrom R (2011) Inheritance of flour paste viscosity is associated with a rice Waxy gene exon 10 SNP marker. J Cereal Sci 53: 37-44.

[126] Umemoto T, Yano M, Satoh H, Shomura A, Nakamura Y (2002) Mapping of a gene responsible for the difference in amylopectin structure between japonica-type and indica-type rice varieties. Theor Appl Genet 104: 1-8.

[127] Umemoto T, Aoki N, Lin HX, Nakamura Y, Inouchi N, Sato Y, Yano M, Hirabayashi H, Maruyama S (2004) Natural variation in rice starch synthase IIa affects enzyme and starch properties. Funct Plant Biol 31: 671-684.

[128] Umemoto T, Aoki N (2005) Single-nucleotide polymorphisms in rice starch synthase IIa that alter starch gelatinization and starch association of the enzyme. Funct Plant Biol 32: 763-768.

[129] Vasconcelos M, Datta K, Oliva N, Khalekuzzaman M, Torrizo L, Krishnan S, Oliveira M, Goto F, Datta SK (2003) Enhanced iron and zinc accumulation in transgenic rice with the ferritin gene. Plant Sci 164: 371-378.

[130] Wan X, Weng J, Zhai H, Wang J, Lei C, Liu X, Guo T, Jiang L, Su N, Wan J (2008) Quantitative trait loci (QTL) analysis for rice grain width and fine mapping of an identified QTL allele gw-5 in a recombination hotspot region on chromosome 5. Genet 179: 2239-2252.

[131] Wan XY, Wan JM, Su CC, Wang CM, Shen WB, Li JM, Wang HL, Jiang L, Liu SJ, Chen LM, Yasui H, Yoshimura A (2004) QTL detection for eating quality of cooked rice in a population of chromosome segment substitution lines. Theor Appl Genet 110: 71-79.

[132] Wan XY, Wan JM, Weng JF, Jiang L, Bi JC, Wang CM, Zhai HQ (2005) Stability of QTLs for rice grain dimension and endosperm chalkiness characteristics across eight environments. Theor Appl Genet 110: 1334-1346.

[133] Wan XY, Wan JM, Jiang L, Wang JK, Zhai HQ, Weng JF, Wang HL, Lei CL, Wang JL, Zhang X, Cheng ZJ, Guo XP (2006) QTL analysis for rice grain length and fine mapping of an identified QTL with stable and major effects. Theor Appl Genet 112: 1258-1270.

[134] Wanchana S, Toojinda T, Tragoonrung S, Vanavichit A (2003) Duplicated coding sequence in the waxy allele of tropical glutinous rice (*Oryza sativa* L.). Plant Sci 165:1193-1199.

[135] Wang, C., Chen, S., Yu, S. Functional markers developed from multiple loci in GS3 for fine marker-assisted selection of grain length in rice (2011) Theoretical and Applied Genetics, 122 (5), pp. 905-913.

[136] Wang CX, Shu QY (2007) Fine mapping and candidate gene analysis of purple pericarp gene Pb in rice (*Oryza sativa* L.). Chinese Sci Bull 52: 3097-3104.

[137] Wang E, Wang J, Zhu X, Hao W, Wang L, Li Q, Zhang L, He W, Lu B, Lin H, Ma H, Zhang G, He Z (2008a) Control of rice grain-filling and yield by a gene with a potential signature of domestication. Nat Genet 40: 1370-1374.

[138] Wang HL, Zhang WW, Liu LL, Shen YY, Wang JK, Jiang L, Zhai HQ, Wan JM (2008b) Dynamic QTL analysis on rice fat content and fat index using recombinant inbred lines. Cereal Chem 85: 769-775.

[139] Wang L, Zhong M, Li X, Yuan D, Xu Y, Liu H, He Y, Luo L, Zhang Q (2008c) The QTL controlling amino acid content in grains of rice (*Oryza sativa*) are co-localized with the regions involved in the amino acid metabolism pathway. Mol Breed 21: 127-137.

[140] Wang LQ, Liu WJ, Xu Y, He YQ, Luo LJ, Xing YZ, Xu CG, Zhang QF (2007) Genetic basis of 17 traits and viscosity parameters characterizing the eating and cooking quality of rice grain. Theor Appl Genet 115: 463-476.

[141] Wang S, Wu K, Yuan Q, Liu X, Liu Z, Lin X, Zeng R, Zhu H, Dong G, Qian Q, Zhang G, Fu X (2012) Control of grain size, shape and quality by OsSPL16 in rice. Nat Genet 44: 950-954.

[142] Wang XG, Ji ZJ, Cai J, Ma LY, Li XM, Yang CD (2009) Construction of near isogenic lines for pericarp color and evaluation on their near isogenicity in rice. Chin J Rice Sci 23: 135-140.

[143] Waters DE, Henry RJ, Reinke RF, Fitzgerald MA (2006) Gelatinization temperature of rice explained by polymorphisms in starch synthase. Plant Biotechnol J 4: 115-122.

[144] Weng J, Gu S, Wan X, Gao H, Guo T, Su N, Lei C, Zhang X, Cheng Z, Guo X, Wang J, Jiang L, Zhai H, Wan J (2008) Isolation and initial characterization of GW5, a major QTL associated with rice grain width and weight. Cell Res 18: 1199-1209.

[145] Win KM, Korinsak S, Jantaboon J, Siangliw M, Lanceras-Siangliw J, Sirithunya P, Vanavichit A, Pantuwan G, Jongdee B, Sidhiwong N, Toojinda T (2012) Breeding the Thai jasmine rice variety KDML105 for non-age-related broad-spectrum resistance to bacterial blight disease based on combined marker-assisted and phenotypic selection. Field Crop Res 137: 186-194.

[146] Woo MO, Ham TH, Ji HS, Choi MS, Jiang W, Chu SH, Piao R, Chin JH, Kim JA, Park BS, Seo HS, Jwa NS, McCouch S, Koh HJ (2008) Inactivation of the UGPase1 gene causes genic male sterility and endosperm chalkiness in rice (*Oryza sativa* L.). Plant J 54: 190-204.

[147] Xu XH, Zhao HJ, Liu QL, Frank T, Engel KH, An G, Shu QY (2009) Mutations of the multi-drug resistance-associated protein ABC transporter gene 5 result in reduction of phytic acid in rice seeds. Theor Appl Genet 119: 75-83.

[148] Yamakawa H, Ebitani T, Terao T (2008) Comparison between locations of QTLs for grain chalkiness and genes responsive to high temperature during grain filling on the rice chromosome map. Breeding Sci 58: 337-343.

[149] Yamakawa H, Hirose T, Kuroda M, Yamaguchi T (2007) Comprehensive expression profiling of rice grain filling-related genes under high temperature using DNA microarray. Plant Physiol 144: 258-277.

[150] Yan CJ, Tian ZX, Fang YW, Yang YC, Li J, Zeng SY, Gu SL, Xu CW, Tang SZ, Gu MH (2011) Genetic analysis of starch paste viscosity parameters in glutinous rice (*Oryza sativa* L.). Theor Appl Genet 122: 63-76.

[151] Yang R, Sun C, Bai J, Luo Z, Shi B, Zhang J, Yan W, Piao Z (2012) A putative gene *sbe3-rs* for resistance starch mutated from *SBE3* for starch branching enzyme in rice (*Oryza sative* L.). PLoS One 7: e43026.

[152] Ye X, Al-Babili S, Klöti A, Zhang J, Lucca P, Beyer P. Potroykus I. (2000) Engineering the provitamin A (β-carotene) biosynthetic pathway into (carotenoid-free) rice endosperm. Sci 287:303-305.

[153] Ye HX, Li M, Guo, ZJ, Shu, QY, Xu XH, Bao JS, Shen SQ (2008) Evaluation and application of two high-iron transgenic rice lines expressing a pea ferritin gene. Rice Sci 15: 51-56.

[154] Yi M, New KT, Vanavichit A, Chai-arree W, Toojinda T (2009) Marker assisted backcross breeding to improve cooking quality traits in Myanmar rice cultivar Manawthukha. Field Crop Res 113: 178-186.

[155] Ying JZ, Shan JX, Gao JP, Zhu MZ, Shi M, Lin HX (2012) Identification of quantitative trait loci for lipid metabolism in rice seeds. Mol Plant 5: 865-875.

[156] Yu YH, Li G, Fan YY, Zhang KQ, Min J, Zhu ZW, Zhuang JY (2009) Genetic relationship between grain yield and the contents of protein and fat in a recombinant inbred population of rice. J Cereal Sci 50: 121-125.

[157] Zhang W, Bi J, Chen L, Zheng L, Ji S, Xia Y, Xie K, Zhao Z, Wang Y, Liu L, Jiang L, Wan J (2008) QTL mapping for crude protein and protein fraction contents in rice (*Oryza sativa* L.). J Cereal Sci 48: 539-547.

[158] Zhang X, Wang J, Huang J, Lan H, Wang C, Yin C, Wu Y, Tang H, Qian Q, Li J, Zhang H (2012) Rare allele of OsPPKL1 associated with grain length causes extra-large grain and a significant yield increase in rice. Proc Natl Acad Sci USA 109: 21534-21539.

[159] Zhao HJ, Liu QL, Ren XL, Wu DX, Shu QY (2008) Gene identification and allele-specific marker development for two allelic low phytic acid mutations in rice (*Oryza sativa* L.). Mol Breed 22: 603-612.

[160] Zheng L, Zhang W, Chen X, Ma J, Chen W, Zhao Z, Zhai H, Wan J (2011) Dynamic QTL analysis of rice protein content and protein index using recombinant inbred lines. J Plant Biol 54: 321-328.

[161] Zheng L, Zhang W, Liu S, Chen L, Liu X, Chen X, Ma J, Chen W, Zhao Z, Jiang L, Wan J (2012) Genetic relationship between grain chalkiness, protein content, and paste viscosity properties in a backcross inbred population of rice. J Cereal Sci 56: 153-160.

[162] Zheng TQ, Xu JL, Li ZK, Zhai HQ, Wan JM (2007) Genomic regions associated with milling quality and grain shape identified in a set of random introgression lines of rice (*Oryza sativa* L.) Plant Breeding 126: 158-163.

[163] Zhong M, Wang LQ, Yuan DJ, Luo LJ, Xu CG, He YQ (2011) Identification of QTL affecting protein and amino acid contents in rice. Rice Sci 18: 187-195.

[164] Zhou L, Chen L, Jiang L, Zhang W, Liu L, Liu X, Zhao Z, Liu S, Zhang L, Wang J, Wan J (2009) Fine mapping of the grain chalkiness QTL qPGWC-7 in rice (*Oryza sativa* L.). Theor Appl Genet 118: 581-590.

[165] Zhou PH, Tan YF, He YQ, Xu CG, Zhang Q (2003) Simultaneous improvement for four quality traits of Zhenshan 97, an elite parent of hybrid rice, by molecular marker-assisted selection. Theor Appl Genet 106: 326-331.

[166] Zhou Y, Cai H, Xiao J, Li X, Zhang Q, Lian X (2009) Over-expression of aspartate aminotransferase genes in rice resulted in altered nitrogen metabolism and increased amino acid content in seeds. Theor Appl Genet 118: 1381-1390.

Chapter 10

Chinese Experiences in Breeding Three-Line, Two-Line and Super Hybrid Rice

Liyong Cao and Xiaodeng Zhan

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/56821

1. Introduction

Because rice is a staple food for over half of the world's population, it is estimated that the world rice production must be annually increased by approximately 1% to meet the growing demand for food, resulting from population growth and economic development (Rosegrant et al., 1995). Rice is one of the main food crops in China with the second largest planting area, most total yield and highest per unit yield (Table 1), it feeds more than 60% of the population and contributing nearly 40% of total calorie intake in China (Cheng and Li, 2007). China is the largest producer and consumer of rice, and also a pioneer in the utilization of hybrid rice technology in the world. Hybrid rice has resulted in a substantial increase of food production in China over the past 40 years. China average rice yield has risen from 1.89 tons per ha (t/ha) in 1949 to 6.71 t/ha in 2012, which created the highest historical record (http://futures.xinhua08.com/a/20121018/1042507.shtml). Hybrid rice has played an important role for total grain production to consecutively increase for nine years in China (http://www.aqzyzx.com/system/2012/10/31/006110920.shtml).

Crop	Planting Area (10^4 ha)	Total output (m t)	Yield (kg/ha)
Corn	33541.67	19.28	5741.7
Rice	30057.04	20.10	6680.7
Wheat	24270.00	11.74	4832.4

Source: http://datacenter.cngrain.com/IndexProduce.aspx?Flag=1&IsHome=0&Tld=74&Str=PP

Table 1. Planting area, total production and yield of three main food crops in China in 2011

Variety improvement plays a leading role in increasing of grain yield characterized as two quantum leaps in rice (Chen et al., 2002). The first one was brought about by the development of semi-dwarf varieties in the late 1950s in China and early 1960s at the International Rice Research Institute (IRRI). In 1956, a dwarf mutant was found in *indica* variety Nantehao in Guangdong Province, China. Since then, Huang et al. (2001) had initiated dwarf rice breeding in Guangdong and southern China, and released semi-dwarf *indica* rice varieties Guangluai 4, Guichao 2, Teqin, etc. subsequently. The semi-dwarf varieties displayed a yield potential up to 7.5 t/ha, which is 20%–30% higher than the traditional tall varieties, owing to the improved tolerance to lodging for standing higher rates of fertilizer. In 1966, IR8 as the first semi-dwarf variety from IRRI was released to tropical irrigated lowlands (Peng et al., 1994; 2008). The second leap arose from commercial use of hybrid rice in 1976 in China (Yuan et al., 1994). Compared with inbred rice, hybrid rice can increase grain yield by approximately 20%. These two major breakthroughs have brought China's average rice yield up to a new level in the mid-1970s and mid-1980s, respectively. Thereafter, with popularization of hybrid rice due to improved hybrid seed production methods, rice yield was further elevated to 6.0 t/ha in early 1990s in China, which was the world level, then. However, the yield ceiling witnessed in various crop species has also been encountered in the rice production in China since 1990. Considering that the annual per capita rice consumption is 150 kg and rice cropping area maintains at 31.57 million hectares in China, it is estimated that rice total production and yield per unit must be increased by 35% and 32%, respectively in 2030 (Cheng et al., 2005). This estimation implies a great challenge to rice community, and the 3rd leap of rice yield is definitely needed for the challenge.

In response to the challenge, Chinese Ministry of Agriculture (CMOA) organized China National Super Hybrid Rice Symposium at Shenyang in 1996, where rice scientists from all over China united to design a national proposal to breed super hybrid rice and develop cultivation methodologies to realize yield potential of the super hybrid. In 2000, because of the leadership of CMOA and the leading role of China National Rice Research Institute, China Super Rice Cooperative Research Group released super hybrid rice cultivars and reached the phase◎target of 10.5 t/ha. In 2005, the phase II target of 12 t/ha was accomplished, and cultivation of super hybrid rice cultivars developed in the phase I was dramatically extended to a large area nationwide. Till 2012, the grain production has consecutively increased for nine years, and nationally produced grain of more than 500 million tons has maintained for five consecutive years in China. The 500 million tons set a new record of grain production in China, which is the planning level of grain production in 2020. The abundant harvest will play an important role in maintaining economy to develop steadily in China.

Meanwhile, steady rice production in China has to keep dealing with decreasing growth area along with an increasing population, biotic and abiotic stresses, extensive use of chemical fertilizers, and water shortage. Therefore, it seems at present that the most effective and economic way is to develop and extend super inbred rice and hybrid rice cultivars with wide adaptation and super high yielding potential, which is also an alternative solution to China's future food security problem and an important way to maintain social stability (Chen et al., 2007).

http://dx.doi.org/10.5772/56821

2. Genetic mechanism of rice heterosis

Heterosis, or hybrid vigor, refers to the phenomenon that progeny of diverse inbred varieties is superior over both parents on yield, panicle size, number of spikelets per panicle, number of productive tillers, stress tolerance etc. This phenomenon to be a powerful force in the evolution of plants has been exploited extensively in crop production. Successful development of hybrid maize in 1930 gave great impetus to breeders of other crops including rice to utilize the principle of hybrid production by exploiting heterosis. In fact, the exploitation of heterosis has been the greatest practical achievement of the science of genetics and plant breeding (Alam et al., 2004). The impact of this phenomenon can be judged by the fact that rice dramatically varies on the number of grains per square meter among 1) wild ancestors with only a few hundreds, 2) improved inbred varieties with about 40, 000, and 3) rice hybrids with about 52, 000 (Mir, 2002). Rice heterosis was first reported by Jones (1926) who observed that some F_1 hybrids had more culms and greater yield than their parents. Between 1962 and 1967, a number of suggestions came from different places of the world for commercial exploitation of heterosis to become a major component of rice improvement programs at national and international level. For example, rice breeders from Japan, China, United States, India, the former Soviet Union and Philippines started their projects to utilize rice heterosis. However, progress had not been sound because of the difficulty for rice to be a strictly self-pollinated crop unlike corn, which made out crossing absolutely essential for hybrid seed production extremely difficult.

2.1. Genetic hypotheses for crop heterosis

Classic quantitative genetic explanations for heterosis center on two concepts, dominance and over dominance (Crow, 1952). With advances on genetic study of quantitative traits and high density molecular linkage maps, many research groups prefer epistasis as a major genetic basis of heterosis (Wright, 1968; Hallauer and Miranda, 1988).

"Dominance" originally means that heterosis is resulted from action and interaction of favorable dominant genes brought together in an F_1 hybrid from two parents. This hypothesis assumes that genes that are favorable for vigor and growth are dominant, and the genes contributed by another parent result in more favorable combination of dominant genes in the F_1 than either parent. For instance, we have a combination of five dominant genes ABCDE favorable for yields, patent one (P1) has the genotype AAbbCCDDee (possessing three dominant genes ACD) and parent two (P2) has the genotype aaBBccddEE (possessing two dominant genes BE); the F_1 hybrid derived from the two parents will have five dominant genes as shown below (Fig. 1).

The F_1 hybrid therefore would exhibit higher yield than either of the parents because each parenthas only a part of five dominant genes. According to this hypothesis, inbreeding depression occurs when unfavorable recessive genes hidden in the heterozygous conditions in the F_1 generation become homozygous in subsequent generations with inbreeding. Crossing unrelated homozygous lines obscures the deleterious recessives and restores vigor.

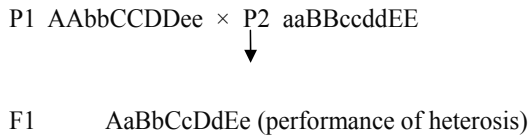


Figure 1. Illustration of dominance hypothesis

The second historical explanation for heterosis is "over dominance, " which refers to allelic interactions in the hybrid, such that the heterozygous class performs better than either homozygous class (Fig. 2). Thus, an individual such as the F_1 hybrid with the greatest number of heterozygous alleles will be mostly vigorous compared to two parents. Because these two explanations for heterosis were developed under the conditions with non-additive effects and supposed all the genes have the same influences to different traits, they have limitations and can't explain the heterosis in molecular level. Therefore, they are of diminished utility for describing the molecular parameters that accompany heterosis.

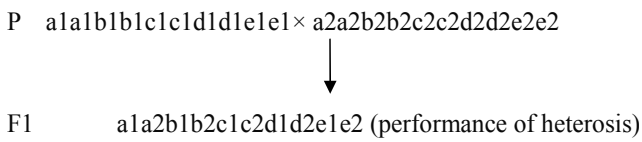


Figure 2. Illustration of over-dominance hypothesis

The hypothesis of over dominance advocating that the hybrids exhibit superiority to the better parent has been agreed by increasing number of studies. However, this hypothesis completely denies the function of dominant genes in heterosis. It is well known that heterosis doesn't perfectly comply with heterozygosity of alleles. For instance, some rice hybrids do not perform better than their homozygous parents at some specific traits.

The hypothesis of epistasis regards heterosis to be genetically controlled by many genes because a complex character such as yield includes many components. Heterozygosity with gene interaction is the primary genetic basis for explanation of heterosis because the hybrid is heterozygous across all genetic loci that different between the parents. Thus, the degree of heterosis depends on which loci are heterozygous and how within locus alleles and inter-locus alleles interact with each other. Interaction of within locus alleles results in dominance, partial dominance, or over dominance, with a theoretical range of dominance degree from zero (no dominance) to larger than 1 (over dominance). Interaction of inter-locus alleles results in epistasis. Genetic mapping results have indicated that most QTLs involve in heterosis and other quantitative traits have a dominance effect. Epistasis has been found more frequently and proven to be a common phenomenon in the genetic control of quantitative traits including heterosis (Yu et al., 1997; Luo et al., 2001; Hua et al., 2003). Study of Yu et al. (1997) provided strong evidence for two-loci and multi-loci interactions (epistasis) especially for traits such as grain yield, which are complex in nature. They found that heterosis is not controlled by a single

http://dx.doi.org/10.5772/56821

locus, even the locus behaves in dominant or overdominant patten, linkage and epistasis has a major role. Thus, the effects of dominance, over dominance and epistasis of various forms are not mutually exclusive in the genetic basis of heterosis, as opposed to what was previously debated in favor of different hypothesis. All of these components have a role to play depending on the genetic architecture of the population (Hua et al., 2003), i. e. single-locus heterotic effects (caused by partial, full-and over-dominance), all three forms of digenic interactions (AA/AD/DA and DD) and probably multi-locus interactions.

Thus, these results may help reconcile the century long debate on the role of dominance, over-dominance and epistasis as genetic basis of heterosis. Two different types of allelic interaction, both within-locus and inter-locus, should play an important role in the genetic control of heterosis. A full understanding of heterosis has to wait for breakthrough achievements on cloning and functional analysis of all genes related to heterosis. This process would be very similar to the understanding of disease resistance genes with aid of standard check variety.

2.2. Molecular basis of heterosis

Since heterosis is a phenomenon of superior growth, development, differentiation, and maturation caused by the interaction of genes, metabolism and environment, a simple explanation of heterosis solely based on the nuclear genome heterozygosity appears untenable. Several distinct lines of evidence from biochemical, physiological, ultrastructural and restriction endonuclease DNA fragment analyses in a variety of organisms are available to show that all three genetic sources of nuclear genome, mitochondrial genome and chloroplast genome, in staed just one of them, are at work during the manifestation of heterosis.

Some molecular studies support the over dominance hypothesis (Stuber et al., 1992, Yu et al., 1997, Li et al., 2001), but another supports the dominance hypothesis (Xiao et al 1995). Yu et al. (1997) reported over dominance at several main-effect quantitative trait loci (QTLs) and a stronger additive epistasis affecting grain yield and its components in F_3 progenies from Shan You 63, the most widely grown hybrid in China. Furthermore, Li et al. (2001) concluded that most QTLs associated with inbreeding depression and heterosis in rice appeared to be involved in epistasis, and almost 90% of the QTLs contributing to heterosis appeared to be over dominant. Zhang et al. (2001) assessed the relationship between gene expression and heterosis by assaying the patterns of different genes expression in hybrids related their parents using a diallel cross. The analysis revealed that differentially expressing fragments occurred in only one parent of the cross were positively correlated with heterosis, but the fragments detected only in F_1 generation not in the respective parents were negatively correlated with heterosis. Using a total of 384 fragments recovered from gels which hybridized with the mRNAs from seedling and flag-leaf tissues, Zhang et al. (2000) detected an overall elevated level of gene expression in the hybrid compared with the parents, where several fragments showed a higher expression in the high-heterotic hybrid than in the low-heterotic hybrids. Studying the molecular mechanism of differential gene expression between Chinese super-hybrid rice cultivars and their parental lines concluded that many genes were up-regulated in the super-hybrid, whereas other genes were down-regulated (Zhang et al, 2006). These findings pointed out a role of enhanced photosynthesis in the heterosis of the super-hybrid combinations. Using

different display techniques for a set of diallel cross involved eight elite hybrid rice parents, Xiong et al. (1998) studied the relationship between banding patterns of differentially displayed gene expressions and the level of heterosis, and showed that dominant type of differential gene expression in flag leaf tissue failed to be correlated with heterosis on yield traits, while differential inhibition of gene expression in the hybrids appeared to be significantly correlated with heterosis. Huang et al. (2006) analyzed gene expression profiles of an elite rice hybrid with the parents at three stages of young panicle development, a cDNA microarray consisting of 9198 expressed sequence tags (ESTs) was used for the objective to reveal gene expression patterns that may be associated with heterosis in yield. The results showed that the biochemical and physiological activities took place in the hybrid relatively rather than in the parents. Identification of genes showing expression polymorphisms among different genotypes and heterotic expression in the hybrid may provide new avenues for exploring the biological mechanisms underlying heterosis.

Nonetheless, a lack of a clear understanding of the genetic or molecular basis of heterosis has not prevented plant breeders from exploiting this phenomenon to raise crop yields.

3. Methods and strategies in hybrid rice breeding

3.1. The methods in hybrid breeding

Prof. Yuan proposed the breeding strategy to Chinese scientists for developing hybrid rice in the following phases. Three approaches are for breeding methodology, 1) three-line method or CMS (cytoplasmic male sterility) system, 2) two-line method or PTGMS (photo/temperature sensitive genic male sterility) system and 3) one-line method or apomixis system. Three ways are for increasing the degree of heterosis, 1) inter-varietal hybrids, 2) inter-sub-specific hybrids and 3) inter-specific or intergeneric hybrids (distant hybrids).

Indica and *japonica* (both tropical and temperate) are two main subspecies of *Oryza sativa* in Asia. *Javanica* is genetically between *indica* and *japonica*. Among them, *indica* and *temperate japonica* subspecies have the most apparent difference in morphological and agronomic traits. Many studies indicate that the degree of heterosis in different kinds of hybrid rice varieties has the following general trend: *indica/japonica>indica/javanica>japonica/javanica>indica/indica>japonica/japonica* (Yuan, 1996). The first three kinds are inter-subspecific hybrid, and the last two are inter-varietal. Currently, hybrid rice technology mainly uses inter-varietal heterosis, *indica* × *indica* and *japonica* × *japonica*. The high-yielding inter-subspecific hybrids yield 15% to 20% more than the best inbred varieties when they are grown under similar conditions. But now, it has been quite difficult to make the genetic difference between parents great enough for the inter-subspecific hybrids, so that their yields have almost reached a ceiling. In *japonica/javanica* hybrids, there are a few fertility problems till present. Therefore, using heterosis between *japonica/japonica* and *indica/japonica* would be an effective approach for increasing rice yields. *Indica/japonica* hybrids posses the highest yield potential in both sink and source. Their theoretical yield can be 30% more

http://dx.doi.org/10.5772/56821

than the best existing inter-varietal hybrid varieties. But inter-subspecific heterosis has not been commercially utilized because of high spikelet sterility and long growth duration.

The discovery of wide compatibility (WC) genes provides a possibility to resolve the problems of seed setting and growth duration in the inter-subspecific hybrids. IRRI, China, and India are making a great effort to develop inter-subspecific hybrids. Recently, Chinese scientists have developed super high-yielding rice hybrids from crosses involving *indica*/*japonica* derivative parents. Most of the inter-specific crosses in cultivated species are within either *O. sativa* or *O. glaberrima*, and their hybrids are heterotic but not so useful in terms of yield and plant stature. Most inter-specific hybrids from wide hybridization have elevation of genetic variability and bring in desirable genes for resistance to several biotic and abiotic stresses, such as the progeny of *O. sativa* × *O. longistaminata*, *O. sativa* × *O. rufipogon*, and *O. sativa* × *O. perennis*.

3.2. The principles for parental selection

High-yield, good quality and multiple resistances are the eternal targets in rice breeding. Matching proper parents together is the key and basis to breed excellent hybrid combinations. Selection of excellent hybrid combinations must base on heterosis with the following specific considerations.

3.2.1. Selection of parents with big variation in genetic basis

Hereditary diversity is the basis of heterosis, and within a certain range, the genetic diversity decides heterosis. Because rice varieties are different in kinship, geographical origin or ecotype, the heterosis is produced by genetic diversity between both parents. Widely commercialized hybrid rice combinations usually have widely different parents in ecological types and geographic origins, such as the parents for Shanyou63, ShanYou10 and XieYou46. Furthermore, the parents may differ in *indica-japonica* affinity.

3.2.2. Screening the parents with good traits

Presently, hybrid rice varieties in commercial production have complementary good traits from their parents. Thus, hybrid rice combinations are comprehensively better than the parents, such as Shanyou63, Shanyou10, Liangyou Peijiu, Zhongzhe You 1 and so on. The hybrid combinations gather many good characteristics of parents such as disease-resistance, late mid-maturity, strong tillering ability and high grain weight. Studies on heredity law of essential traits in hybrid rice showed that some traits of hybrids have certain relationships with the average of parents, such as number of grains per panicle, filled grain number per panicle, grain weight, efficient panicles per unit area, growth period and plant height. These traits have highly significant correlations between a hybrid and the average of its parents. According to this relationship, we may choose the combinations with excellent parents to breed new restorer or maintainer lines.

3.2.3. Selection of parents with good combining ability

General combining ability (GCA) refers to the average performance of an offspring from one parent that is crossed with many other parents. GCA is determined by the number of favorable genes and the size of gene function in parents, and often influenced by additive effects. Specific combining ability (SCA) refers to the performance of a unique offspring deviation from a specific pair of parents. SCA is mainly controlled by non-additive effects. Therefore, not only the yield of parents is necessary for, but also the combining ability of parents is very important for hybrid rice breeding.

3.3. Strategies for hybrid rice breeding

In addition to the breeding principles described above, the following strategies are also very important in hybrid rice breeding to result in hybrid cultivars with high yield, good quality and disease resistance.

3.3.1. Amplification of genetic diversity in parents

Through pedigree analysis, most rice cultivars originate from few parents which genetic diversity is small. The main reason for hybrid rice yield to stand still since 1980s maybe relate to short in genetic diversity in China. Since the discovery of semi-dwarfism in *indica*, genetic diversity among cultivars has become increasingly narrow, so that strong hybrid vigour has hardly been achieved by combining *indicas* cultivars. *Japonica* materials are full developed and utilized in temperate zone, resulting in a narrow genetic diversity among cultivars. The narrow diversity makes it very hard to achieve hybrid preponderance in inter varietal crosses. Tropic *japonica* (*Javanica*) and *nuda* types of rice (*japonica*) are not widely utilized in breeding because they are limited in special regions with some particular traits. Recently breeders have paid much attention to wide compatibility germplasm derived from tropic *japonica* and *nuda* rice. After Yuan (1987) proposed the strategy to utilize heterosis between *indica-japonica*, breeders have made a great effort to amplify parents' genetic diversity and breed super high-yield *indica-japonica* hybrid through effective utilization of wide compatibility. *Indica-japonica* combinations from direct crosses hardly have super high-yield because of sterility. Introgressing *japonica* genes into *indica* restorer lines in the south and introgressing indica genes into *japonica* in the north are proven to be effective for super high-yield hybrid rice breeding in China. Taking this strategy, three new *indica* restorer lines, Zhong413, R9308 and T2070, are bred by China National Rice Research Institute. They all contain about 25% of *japonica* components, and their hybrid combinations have super high-yield.

3.3.2. Increasing the biological outputs

Ideotype is an ideal plant model which is expected to yield the most for a specific environment. Hybrid rice ideotype (HRI) is a best parental combination to yield the most grains with good quality in a certain ecological environment. HRI does not only contain desire morphological characteristics but also have resistances to biotic and abiotic stresses for the environment. Otherwise, the most grains with good quality are not achievable. For instance, Taoyuan in

http://dx.doi.org/10.5772/56821

Yongsheng County, Yunnan Province, China is a perfect ecological environment for rice, where the yield of indica hybrid rice Shanyou63 can be up to 15.27 t/ha with a harvest index up to 0.54 in small field trial. However, under normal conditions in most areas of China, it is not possible for Shanyou63 to yield 15.27 t/ha. This difference may imply the importance of plant biomass for grain yield. Increasing plant biomass with maintaining or improving harvest index may lead to raise grain yield directly. It is well known that high biological-outputs need abundance of sunshine and high levels of nitrogen nutrition. In addition to high plant biomass, high grain yield needs a series of good characteristics such as tough stems, erect leaves and rational operation of photo synthetic products. Otherwise, the population will lodge to flat and the leaves shade each other, favorable for pest and disease infestations. At the end, rice yields will decline instead of increasing.

Increasing the biological outputs is the key to further improve yield potential under a certain harvest index condition for high-yielding dwarf rice. It is no doubt to properly raise plant height beneficial for increasing biological products, but we must enhance the capacity of lodging resistance at the same time and ensure all set grains harvestable. In current high-yielding rice fields, when the number of spikelets per square meter is about 40, 000 or slightly less, 50, 000 to 60, 000 and 60, 000 to 70, 000, the corresponding grain yield could be 7.5 t/ha, 11.25 t/ha and 15 t/ha, respectively. To a specific rice variety, the panicle number per unit and grains per panicle varied upon to ecological conditions, thus, we must improve leaf position and leaf quality to increase the number of spikelets per unit and the total output. Meanwhile, strong root activity, no immature stems and leaves, long time of photosynthesis, rough vascular bundles in stems and spikes are all very important for grain production.

3.3.3. Improvement of leaf posture and quality

Plant growing, tillering and expanding ability of tillers at early stage, stand upright, and enough green leaves at later stage are all extremely important for rice to use solar energy highly efficiently. Thickness of leaves and maximum leaf nitrogen content should be exploited under intensive cultivation conditions because their advantages for improving photosynthetic capacity. Besides maintaining the photosynthetic ability of leaves, maintaining the flag leaves upright is also very important. Studies have indicated that the thicker the leaves are, the more mesophyll cells per unit area and the larger intercellular spaces there are (Zhu et al., 2001; Fu et al, 2012). Therefore, it is also very important to select thicker leaves in hybrid breeding because they are beneficial for diffusing carbon dioxide and maintaining high level of chlorophyll and nitrogen content inside the leaves.

Curling degree of leaf is another important index for leaf posture in super-rice breeding due to its benefit for photopermeability by increasing the under surface. Zhu et al. (2001) studied the relationships between curl degree and photopermeability, and classified the leaf curl degrees into high, intermediate and low with 44°-47°, 15°-16° and 10°-11°, respectively. Testing the photosynthesis rate between under and upper surface of leaves resulted in 1.19-1.32 in the high, 0.90-1.02 in the intermediate and 0.82-0.85 in the low curl degree hybrid combinations, respectively. Compared with the low curl degree combinations, the high and intermediate curl

degree combinations had smaller leaf angle, higher leaf straightness and lower extinction efficient. So, we should select the lines with high or middle leaf curl degree in breeding.

3.3.4. Increasing the ability of root system

Long time ago, researchers were attracted by the influence of root system on rice associated to grain yield. Nagai proposed the concept of root type for the first time in 1957. Ling et al (1989) studied the close relationship between the direction of root stretching and leaf angle, and proposed that cultivating root type was favorable for ideo-type as well as rice high-yield cultivation. Root vigour, especial during the filling stage, guarantees a super high-yield of rice, undoubtedly. A radical reason for bad grain plumpness in hybrid rice is root senescence. However, improving root system properties has not been embodied in rice breeding plan till now, so we should do further and more research in root vigour at various aspects such as methodology study for root characteristic, root physiological characteristic, relationship with ground part, diversity among breeding materials and genetic utilization to construct ideal root type.

4. Three-line system hybrid rice

4.1. Identification and utilization of cytoplasm male sterility

The role of cytoplasm on causing male sterility of rice was first reported in 1954 (Sampath and Mohanty 1954). In 1965, Sasahara and Katsuo studied cytoplasmic differences among rice varieties and developed, for the first time, a male sterile line by transferring the nuclear genotype of rice cultivar Fujisaka 5. However, this cytoplasm male sterility (CMS) line could not be used for breeding rice hybrids because of its instability, poor plant type and photoperiod sensitivity. In 1964, Yuan Long Ping put forward the idea to utilize the heterosis in rice and initiated the research on hybrid rice in China for the first time. In November 1970, a pollen abortive wild rice plant (shortly called wild abortive, i.e., WA) was discovered among the plants of common wild rice (*Oryza rufipogon Griff*. L.) at Nanhong Farm of Ya County, Hainan Island which is the south most province of China. After the discovery of WA, a nationwide cooperative program was immediately established to extensively testcross with the WA and screen for its maintainers and restorers. Soon in 1972, the first group of CMS lines such as Erjiunan 1A, Zhenshan 97A and V20A were developed all using WA as the donor of male sterile genes and all using successive backcrossing method. In 1973, the first group of restorer lines such as Taiyin 1, IR24 and IR661 were screened out through direct test crossing method. In 1974, the hybrids with strong heterosis such as Nanyou 2 and Nanyou 3 were released. In another word, the discovery of WA resulted in the subsequent and successful breakthrough in hybrid rice development, so that the three-line hybrid rice system was established. Therefore, China became the first country to commercialize hybrid rice for food production in the world.

Three-line hybrid system includes the CMS line (A), maintainer line (B) and restorer line (R) for a commercial production of rice hybrids. The A line cannot produce viable pollen due to

http://dx.doi.org/10.5772/56821

the interaction between cytoplasmic and nuclear genes, so called cytoplasmic male sterile, which anthers are pale or white and shriveled. The A line is used as a female parent for hybrid seed production, so it is commonly called the CMS line and the seed parent as well. Because the CMS line is male sterile, it cannot be self-reproduced and has to have a maintainer. The B line is the maintainer line, which morphology is highly similar to its corresponding CMS line except its reproductive function. The B line has viable pollen grains and normal seed setting, so can pollinate the A line and the F_1 plants from this pollination are male sterile, again. In this way, the male sterility of the A line is maintained, and the A line is reproduced for further use or commercial use in a large scale. Similarly, the R line has viable pollen grains and normal seed setting and can pollinate the A line. Differently from the pollination with B line, the F_1 plants from the pollination with R line are highly fertile, or the male sterility of the A line is restored into fertility by R line in their progeny. Therefore, the R line also called the pollen parent, male parent, and/or restoring line.

4.2. Diversification of CMS sources

Chinese rice breeders designated various CMS sources arbitrarily without following any systematic nomenclature. Principally, the CMS sources are designated according to the cultivar name from which the male sterile cytoplasme is derived. In some cases, different symbols are assigned by different researchers for the same material. For example, Shinjyo designated the male sterile cytoplasm of Chinsurah Boro II as [CMS-boro] or [CMS-bo], but the Chinese workers designated it as BT (B for Chinsurah Boro ◎ and T for Taichung 65 which is the nuclear donor cultivar). The first series of released WA-type CMS lines include Zhenshan 97A, V20A, Erjiu Ai 4A, Erjiu Nan 1A and V41A (Mao, 1993). In order to diversify the genetic background of hybrid rice, other CMS types besides WA-type are developed for three-line hybrid rice varieties in China, including Dwarf Abortive (DA) type, Gambiaka and Dissi (G and D) type, Indonesla 6 (ID) type, K type and Hong Lian (HL) type. DA-type CMS lines are derived from the male sterile dwarf wild rice, including Xieqingzao A. G and D type CMS lines are developed from geographically distant crosses, where West Africa *indica* cultivars Gambiaka Kokoum and Dissi D52/37 are crossed with Chinese *indica* cultivar Aijonante, respectively to yield G46A and D62A, major representative of G and D CMS lines (Li 1997). ID type CMS lines are derived from an abortive plant in an Indonesia rice cultivar Indonesia 6. Zhong 9 A and II-32A are two representative CMS lines of ID type. K type CMS lines are derived from the cross of K52 and Luhongzao 1 with representative K qing A and K 17A. HL Type CMS lines are derived from the cross between red awn wild rice (*O.sativa spontanea* L.) and an indica rice cultivar Liantangzao with representative Yuetai A (Zeng et al, 2000). Virmani and Wan (1988) listed some of the CMS sources identified in and outside China, where the CMS sources are designated in principle according to the cultivar name from which the male sterile cytoplasme is derived, as well.

Outside China, IRRI used CMS sources from V20A, Kaliya 1, ARC and Gambiaka to develop CMS lines of IR58025A, IR68275A, 68281A, IR68273A, IR68888A, IR68891A and IR68893A. Also, IRRI developed CMS lines with male sterile cytoplasme sources of *Oryza perennis* (e.g.

IR66707A) and *O. rufipogon* (e.g. OMS1) (Virmani, 1996). Therefore, the genetic backgrund among three-line hybrid rice varieties are greatly broadened or diversified.

4.3. Genetic model of CMS line

As a self-pollinating crop, rice must use an effective male sterility system to develop and produce F_1 hybrid cultivars. The male sterility in CMS system is controlled by an interaction of cytoplasmic and nuclear genes. The presence of homozygous recessive nuclear genes for fertility restoration combining with cytoplasmic genes for sterility makes a plant male sterile. The cytoplasmic genes for sterility exist in mitochondrial DNA. The nucleo-cytoplasmic inter-reaction hypothesis explains genetics of three-line hybrids. In this hypothesis, a CMS or A line has sterility cytoplasm but no dominant restorer genes in nucleus, so sterile. A maintainer or B line also has no dominant restorer genes in nucleus, but has fertile cytoplasm, so fertile.

When B line pollinates A line, the progeny is male sterile because it has a complete sterility cytoplasm of A line with half nucleus from A line and another half nucleus from B line, and both A and B nuclei have no dominant restorer genes. A restoring or R line has dominant restorer genes in the nucleus. Regardless of sterile or fertile cytoplasm in R line, the progeny from crossing A with R line becomes fertile solely because of dominant restorer genes in the nucleus of R line. Accordingly, genetic constitutions can be expressed as *S* (sterile cytoplasm) with *rfrf* (sterile nucleus) in CMS line, *N (*fertile cytoplasm) wit *rfrf* in maintainer line, S/N with *RfRf* (fertile nucleus) in restorer line and S with *Rfrf* in hybrid rice. When the CMS line is crossed with corresponding maintainer, the sterility is maintained and seeds of the CMS line are multiplied. When the CMS line is crossed with the restorer line, the fertility is restored in F_1 generation, namely commercial hybrid seed production.

Zhang (1981) made the following conclusions on male sterility and cytoplasmic regulation of gene reaction in rice:

1. The occurrence of male sterility depends on "affinity" between the cytoplasm and nucleus. The greater the genetic distance between cytoplasm donor and nucleus donor cultivars is, the easier for their offsping to be male sterile and to breed male sterile line. If we assume that the evolutionary order of cultivated rice is wild, *indica* and *japonica,* the genetic distance between wild and *japonica* should be greater than it between wild and *indica*. Then, the cytoplasm of wild rice has less "affinity" with *japonica* than it with *indica*. However, the definition of "affinity" in genetic terms remains unexplained.

2. The cytoplasm and nucleus jointly decide pollen abortion. Pollen abortion is observed from uninucleate stage before first pollen mitosis, until binucleate stage just before anthesis. The earlier the abortion stage is, the more morphologically discernible pollen sterility there is.

3. A genotype can function as either a maintainer to one MS cytoplasm or a restorer to another MS cytoplasm, depending upon the ability of either complete sterility for

http://dx.doi.org/10.5772/56821

maintaining or normal fertility for restoring. Therefore, the cytoplasmic differences between two male-sterile lines derived from two CMS sources can be ascertained through the reaction of maintainer and restorer by crossing with a set of cultivars.

4.4. Achievements on three-line hybrid rice

In order to reduce the potential threats from diseases due to MS cytoplasme uniformity, a variety of cytoplasmic male sterile sources have been utilized by Chinese rice breeders and a number of three-line hybrid CMS lines have been bred. WA CMS source used to be overwhelming for a long time. Other cytoplasm sources named Wild and D type, ID type etc. are gradually increasing lately in commercial extension of hybrid rice. For example, the monopoly of WA CMS source hybrids was broken by D-type CMS source hybrids with heavy panicles that are successully bred in Sichuan on commercial scale.

In recent years, great progresses have been made by Chinese rice breeders to improve grain quality and out-crossing rate of male sterile lines. ID type CMS line Zhong 9A (http://www.ricedata.cn/variety/varis/601141.htm), developed by China National Rice Research Institute, combines high quality and high outcrossing rate up to 80%. The grain quality of Yixiang 1A cultivated by Yibin Institute of Agricultural Sciences in Sichuan province (Jiang et al, 2008), and Yuefeng A cultivated by Guangdong Academy of Agricultural Sciences has reached international standards of first level high quality rice (Li, 2001). The breeding success of these CMS lines improved the quality of hybrid rice, especially for the significant improvement on milled rice rate, chalkiness and amylose content.

Rice statistics shows that three-line hybrids are still dominant in rice production in china (Table 2). From 2009 to 2011, the planting areas of top ten three-line hybrids ranged from 110, 700 ha for Jin you 207 in 2011 to 260, 000 ha for Yue you 9113 in 2009. Many three-line hybrids are elite, such as Yue you 9113, Gang you 188, Q you 6, Gang you 725, Tian you 998 and Zhongzhe you 1. Among them, Yue you 9113 is outstanding with the most total planting areas of 724, 7000 ha in the three years because it has good characteristics of high yield and premium quality, resistance to diseases and suitable maturity. Because three-line hybrid combinations have very significant yield increasing ability, it has proven that hybrid rice has a yield advantage of more than 20% over conventional rice. In recent years, hybrid rice covers about 15.5 million ha annually, accounts for 50% of the total rice area, and produces 60% of the total rice produced in China. From 1976 to 2011, the accumulated planting area of hybrid rice is 500 million ha, from which 500 million tons of paddy rice has increased over conventional rice. Up to now, three-line hybrids include Indica, Japonica and Indica/Japonica types with different maturities. Thus, hybrid rice production distributes to the entire China, from Hainan in the south to Liaoning in the north, and from Shanghai in the east to Yunnan in the west. Hybrid rice shows not only a high-yielding ability but also a wide adaptability. Chinese demonstration has also encouraged IRRI and National rice improvement programs of countries like India, Vietnam, Philippines, USA, Bangladesh and Indonesia to start hybrid rice breeding programs for utilization of heterosis.

Rank	2009		2010		2011	
	Variety name	Area	Variety name	Area	Variety name	Area
1	Yueyou 9113	26.00	Zhongzhe you 1	24.53	Yue you 9113	22.80
2	Tian you 998	25.53	Gangyou 188	23.87	Gang you 188	22.60
3	Gang you 188	24.73	Yueyou 9113	23.67	Q you 6	17.00
4	Jin you 207	21.93	Q you 6	22.80	Gang you 725	14.40
5	Jin you 402	20.33	Gangyou 725	17.93	Tian you 998	13.47
6	Q you 6	19.27	Jin you 207	14.73	Zhongzhe you 1	12.80
7	Zhongzhe you 1	17.33	Tian you 998	14.33	Wu you 308	12.67
8	Gangyou 725	16.60	Ganxin 688	12.80	Tianyou huazhan	12.60
9	Il you 838	15.53	Jin you 402	12.47	Fengyuan you 299	11.73
10	Jin you 463	11.53	Il you 838	12.13	Jin you 207	11.07

Table 2. Planting areas of top 10 three-line hybrid varieties during 2009~2011 (10^4 ha)

5. Breeding and application of two-line hybrid rice

5.1. Photoperiod Sensitive and Thermo-sensitive Genetic Male Sterility

In 1973, Chinese scientist Shi Mingsun discovered a natural male sterile plant in the field of Nongken 58, a *japonican* late maturing variety, at Shahu Farm of Mianyang County, Hubei Province, China. After eight years of in-house study for confirmation, he announced his discovery as a dual-purpose rice line Nongken 58S in 1981, and proposed a new strategy to utilize heterosis in rice, namely two-line system based on his research results (Shi, 1981). Further studies indicate that the critical stage for fertility transformation must be before the 1st or 2nd of September in Wuhan 30-31 N. When Nongken 58S heads during August 5th ~ 1st or 2nd of September, it is male-sterile (99.5-100%). However, its pollen sterility is reduced to 20% and seed setting rate varies between 10-40% when Nongken 58S heads after 1st or 2nd of September. This sterility-fertility change regulated by heading is true in many other regions. Pollen sterility during the sterile stage in summer is stable, but the fertility in autumn is unstable and varies over locations and years. The original sterile plant Nongken 58S becomes the first dual-purpose line in rice and possesses the characteristics of fertility alteration, i.e., completely sterile under long day period and high temperature conditions, and partially fertile under short day period and low temperature conditions.

Japanese rice scientists (Maruyama et a1., 1991a) reported thermo-sensitive genetic male sterility (TGMS) as a mutant from Japanese rice cultivar Remei treated by 20 kr gamma rays for the first time. This male-sterile mutant, designated as H89-1, sets no seeds under 31/24℃, some seeds under 28/21℃ and full seeds under 25/15℃. Pollen sterility in this mutant does not change along with change of day length period (viz.15, 13.5, 12 h). Behavior of this TGMS

http://dx.doi.org/10.5772/56821

mutant is confirmed at IRRI. Like PGMS, TGMS can also be employed to develop rice hybrids rather than three lines. While PGMS can be used in the countries having large territory with striking differences in latitude, TGMS can be used in the area close to the equator where low temperature areas are on top of the hills. Thus, TGMS can be utilized in tropical and subtropical areas.

5.2. Breeding methods for two-line hybrid rice

Two-line hybrid rice research originates in China and successfully reached to production scale in 1995. The male sterile lines in which sterility expression is controlled by temperature are called thermo-sensitive male sterile (TGMS) lines and those in which expression is controlled by day-length period are called photoperiod-sensitive male sterile (PGMS) lines. The PGMS trait has been transferred to several *Indica* and *Japonica* rice cultivars in China by backcrossing. Rice hybrids developed by this male sterility system are being evaluated in multi-location trials in China. Two-line hybrid rice has similar level of heterosis with three-line hybrid rice, but different in technique process. Unlike three-line hybrids, the male parent of two-line hybrid is not restricted by restorer genes, so we can use not only the good restorer lines with high combining ability as the male parent, but also the good conventional varieties without restorer genes as male parent. The non-restriction of restorer genes brings about greater opportunity to breed elite hybrids.

The developed PTGMS lines such as PA64S, GZ63S, Zhun S, etc. have many advantages for hybrid combinations, such as larger freedom for crossing, higher yielding, better quality and resistance to diseases than CMS lines. Commonly, the yield of improved two-line hybrid rice combinations is higher than it of three-line hybrids as checks. Meanwhile, the techniques of seed production and cultivation for two-line hybrids have been sophisticated enough for production application. Breeding of elite restorer lines is the key for matching heterotic combinations. The following three ways are usually used in breeding restorers and new combinations for two-line hybrids.

5.2.1. Testing and screening strong combinations using conventional rice cultivars

China has a very long history for rice cultivation. Thousands of cultivars have been used for production and all these cultivars can be used as a restorer for testing new two-line combinations. Especially for some conventional cultivars bred in recent years, they have many advantages such as high-yield, good resistance and excellent quality, thus are easy to be used in two-line hybrid breeding. At present, two-line hybrid rice combinations applied in large production areas are mostly bred using conventional cultivars. For example, Teqing, Shagnqing11, Yuhong, and 9311 (Yangdao6) are the male parent for elite two-line hybrids Liangyouteqing, Peizashanqing, Peiliangyouyuhong and Liangyoupeijiu, respectively.

5.2.2. Testing and screening strong combinations using cytoplasmic male sterile restorers

Three-line hybrid rice breeding technologies in China are regarded the first class in the world. For the pasted four decades, a large number of restorer lines with strong general combining

ability, good resistance to diseases and high quality have been bred. All these restorer lines can be used as male parent for testing two-line hybrid rice combinations. For example, many two-line hybrid rice combinations which are widely used in production such as Peiliangyou 288, 70 You 9 (Wandao20), Liangyou 2163 and Fuliangyou 63 are configured by three-line restorer R288, Wanhui9, Minghui63, respectively.

5.2.3. Breed new restorers from crossing

Although two-line hybrid rice is superior to three-line hybrid rice in quality traits, resistance and yielding, we also need to expand genetic differences of parents, make crosses and breed new two-line restorers, and overcome the shortcomings of parents using complementary effects. For example, researchers in Yahua Seed Industry in Hunan Academy of Agricultural Sciences have bred a restorer line ZR02 which combines good quality, stable growth period, strong resistance and good out-crossing ability together. We can use this restorer line to cross with other lines to improve the traits such as poor quality, late maturity and poor out-crossing ability. Using this restorer line, a new combination Zhuliangyou 02 is bred as a double-crop early maturing hybrid rice with stable and high yield and good quality. As a result, Zhuliangyou 02 has good prospects in Yangtze River.

5.3. Advantages of two-line hybrids

In the two-line system, only two lines are involved in hybrid rice seed production. One is the male sterile line in which male sterility is genetically controlled by recessive genes, which expression is influenced by environment (temperature, photoperiod, or both). Another is male parent or pollinator line that can be any inbred variety with dominant gene (s) for male sterile locus. There are no constraints for the restoration-maintenance relationship because the male sterility of PGMS and TGMS is controlled by only one or two pairs of recessive genes. There is no need for special R genes to restore fertility, so the choice of parents in developing heterotic hybrids is greatly broadened. Developing hybrid rice varieties with these systems has the following advantages over the classical three-line or CMS system:

1. **Maintainer lines are not needed.** The PGMS lines (under long day-length) and the TGMS lines (under high temperature) show complete pollen sterility and can thus be used for hybrid seed production. Under short day-length or low temperate conditions, they show almost normal fertility and can multiply themselves by selfing. Therefore, in the PGMS/TGMS system, no maintainer line is needed in seed multiplication of male sterile lines, thus the cost to produce hybrid seed is cut down because of the simplified production procedure.

2. **The parental choice for developing heterotic hybrids is greatly broadened.** Studies showed that more than 97% of tested varieties (within subspecies) could restore fertility of MS lines, indicating no need to identify restoring abilities. Thus, our choice of parents in developing heterotic hybrids is broadened in comparison with the CMS system. In addition, PGMS and TGMS genes can be easily transferred to almost any rice lines with desirable characteristics.

http://dx.doi.org/10.5772/56821

3. **Negative effects from the sterile cytoplasm are avoided.** Therefore, the vulnerability to destructive diseases or insects due to uniform resource of male sterile cytoplasme may be eliminated.

Apparently, it is easier to develop rice hybrids that possess higher yield, earlier maturity, better grain quality and improved pest resistance by two-line system than by three-line system. The research findings and production experiences have also proven that two-line hybrid rice out-yields three-line hybrid rice by 5%-10%. Furthermore, it is promising to develop elite hybrids for the early cropping rice with both high yield and early maturity, and to develop heterotic *japonica* hybrids by two-line method, which would very likely break the deadlock of stagnant yield and area in hybrid rice. For example, Xiangliangyou 68, an early-cropping two-line hybrid rice combination with high yield, fine grain quality and early maturity, was successfully released to commercial production in 1998. It shows a very promising prospect in overcoming the great difficult long existing in developing high-yield, good-quality and early-maturity hybrid rice in China.

However, it should be pointed out that two-line hybrid rice also faces the risk of seed purity in case of lower temperature occurred in thermo-sensitive stage of TGMS lines in hybrid rice seed production. Because the fertility alteration of TGMS lines is conditioned by temperature, even in hot season like summer, low temperature may occur in rainy days and last for a few days. Therefore, to guarantee the seed purity in two-line hybrid seed production is essentially important for developing practical TGMS lines with their Critical Sterility Inducing Temperature (CSIT) low enough, generally 23 C for temperate zones and 24 C for subtropical zone.

5.4. Achievements in two-line hybrid rice breeding

Because the PTGMS lines can be used to produce hybrid seeds in the sterile period and to multiply themselves in the fertile period, a nationwide research was organized to study the mechanism of PTGMS and its application after the discovery of Nongken 58S. Soon after, many *japonica* and *indica* PTGMS lines have been released using male sterile genes in the original Nongken 58S. Furthermore, some other germplasms with fertility alteration such as Annong S-1, 5460 S and Hengnong S-1 are also identified. Up to now, tens of practical PTGMS lines in rice which possess the characteristics of low CSIT to secure hybrid seed production have been technically identified and approved. At present, the PTGMS lines used in rice production mainly derive from either PGMS Nongken 58S or TGMS Annong S. More attention should be paid to the following areas in order to improve screening and utilizing efficiency of photo-thermo sensitive male sterility (Lu and Zou, 2000).

1. Seed production safety: Because the fertility of photo-thermo sensitive male sterile lines is regulated by light and temperature, safely producing hybrid seed in the target region must be taken into account during the selection of sterile lines. The window for sterility to stably occur must be more than 30 days. The stable length of sterility period mainly depends on the critical temperature and critical photo-period length for fertility transition in the selected PTGMS lines. In South China, we generally select temperature-sensitive sterile lines because inter-annual light changes are minor and inter-annual temperature

changes are major, where the suitable critical temperature for fertility transition is 24-25°C in this region. In Central China, PGMS, TGMS and P-TGMS are all applicable, but the suitable critical temperature of fertility transition should be 23°C ~ 24°C, and the day length of critical light should be around 13.5 h. We should select the sterile lines with relatively stronger photo-sensitivity but weaker temperature-sensitivity for fertility transition in North and Northeast rice region, because there are longer day length and relatively lower temperature there.

2. Easy to multiply: Because the TGMS lines are fertile when the temperature is below the critical temperature, photo-thermo sensitive male sterile lines must have strong cold tolerance, which makes them survive from the cold water irrigation to have high grain output in multiplication.

3. High combining ability: A successful hybrid with high vigor is directly determined by the combining ability level of sterile lines. Combining ability (CA) includes specific CA (SCA) and general CA (GCA), the former is for crossing with a specific parent and the latter is for crossing with many parents. The GCA is usually highly related with its comprehensive characters, advantages and disadvantages, so the sterile lines with high GCA have high chances to produce high yielding combinations.

According to above principles, the breeders have bred many elite two-line hybrid varieties with good quality, high yield and good resistance, such as Fengliangyou Xiang1, Zhunliangyou 527, Yangliangyou 6, Liangyou 288, Zhuliangyou 02, Zhuliangyou 120, et al. After more than twenty years for nationwide collaborative studies, important progress has been made in two-line hybrid rice in both theoretical mechanism and practical application, which has resulted in a yield advantage of 10 percent over three-line hybrids. Along with improvements of techniques on daily bases, two-line hybrid rice is becoming more and more popular in large-scale application. The area planted to two-line hybrid rice increases year after year from 4, 300 ha in 1991 to 704, 400 ha in 1999 in China. In 2000, the growing area of two-line hybrid rice in China reached 1.5 million ha, and total yield reached 109.3 million tons with average yield of 7287kg/ha which was 4.16% more than that of three-line hybrid rice. Currently, the main combinations in commercial production are Liangyou Peijiu, PZS7, Peiliangyou 288, Xiangliangyou 288 and Fengliangyou 1. In 2002, a two-line hybrid rice Liangyou Peijiu took up the first place of planting areas from Shanyou 63, a three-line hybrid rice that had maintained the leading place for more than ten year in China.

In recent years, more and more two-line hybrid rice varieties are released. In 2011, six and 51 two-line hybrid combinations were released from national and provincial institutions, respectively (Fig. 3). The planting area of two-line hybrid rice reached 2.7 million ha, about 9.0% of total rice cultivation area and 18.6% of hybrid rice planting area. In 2010, the top three hybrid varieties with the most planting area were all two-line combinations. The cultivation area of two-line hybrid rice will further expend with the progress of research on seed production.

http://dx.doi.org/10.5772/56821

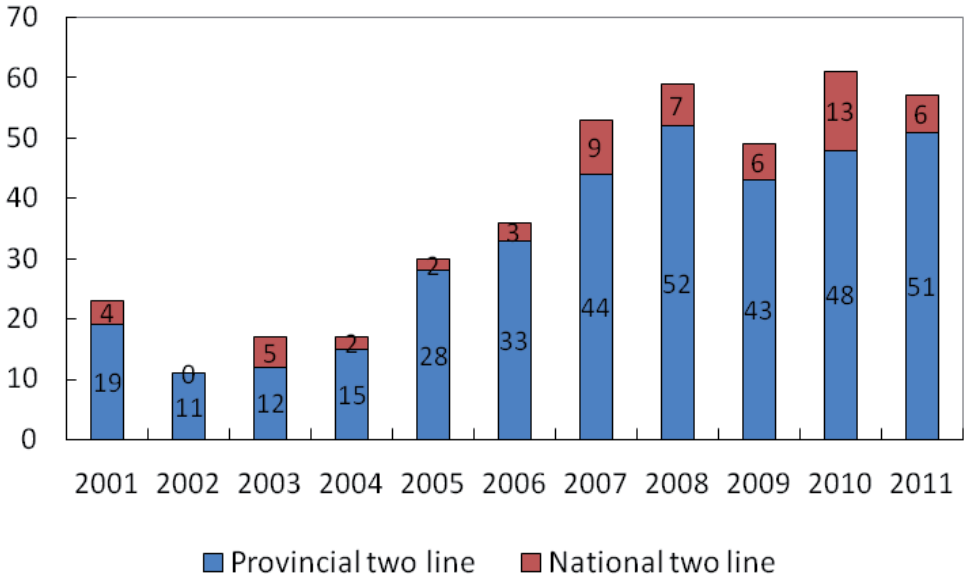


Figure 3. Two-line hybrid rice varieties released in China (2001-2011).

6. Super hybrid rice breeding

In order to achieve another leap of rice yield and secure food supply in China, after summarizing the experiences and lessons at home and abroad, Chinese scientists put forward a national program to breed super rice in 1996. A primary goal of this program is to combine ideal plant type with heterosis of *indica/japonica*, and improve rice yield, quality and resistance. Through joint research, a series of new super rice varieties have been approved for release from national and provincial institutions. The super rice varieties have demonstrated the yield of 12 t/ha in a scale of 100 mu or 6.7 ha model trial. From 1998 to 2004, the accumulative demonstration and extension areas of super hybrid rice have 10 million ha. Practices show that developing super hybrid rice is a necessary choice to increase rice yield, stabilize total production of rice, improve the efficiency of the rice planting, and ensure food security in China.

6.1. Model of super hybrid rice

Backgrounds are confirmed to be unique based on the results of RFLP variations among *indica* hybrid varieties and their parents. The yield ceiling has remained in hybrid rice for nearly 10 years because of insufficient genetic diversity. Optimal combination means that the hybrid rice combination has a reasonable genetic difference between its parents, such as 1) lowland rice with upland rice varieties, 2) geographically different varieties, 3) ecologically different varieties, 4) dominantly different varieties, and 5) *indica* and *japonica* subspecies (Chen et al.,

2007). However, we can only exploit part of the heterosis between *indica* and *japonica* subspecies, but not the heterosis between typical *indica* and *japonica* rice or excessive *indica* and *japonica* ingredients. Cheng et al. (2007) indicated that when *indica* or indicalinous cytoplasmic male sterile (CMS) lines are crossed with restorer lines having different *indica* and *japonica* genetic backgrounds, the hybrids from indicalinous or japonicalinous restorer lines (*indica-japonica* differentiation index 11-15) have the highest yield. Therefore, breeding high yielding hybrids by crossing *indica* with *japonica* with aid of wide compatibility gene has been paid great attentions. In the *indica* rice growing regions, breeders strategically introgress *japonica* consanguinity into *indica* rice, and in the *japonica* rice growing regions, introgress *indica* consanguinity into *japonica* rice, instead. So far, a set of indicalinous or japonicalinous germplasms for super rice breeding have been intentionally developed. Some of such germplasms have been successfully used in breeding of super inbred and hybrid rice (Table 3). For instance, Shennong 89366 is one of the core parents for IRRI to develop new plant type super rice. ed by Shennong 89366 is bred by Shenyang Agricultural University, China and has served as a donor for short sturdy stems and long-big panicles (Chen et al., 2003). R9308, an indica restorer line from a cross of C57//No. 300/IR26, has been successfully used in the breeding program for super hybrid rice by the China National Rice Research Institute (CNRRI). Xieyou 9308 (Xieqingzao A/R9308), a hybrid rice combination with super high yielding, multi-resistance to diseases and good grain quality, was registered in Zhejiang Province, China in 1999. It is estimated that there are 25% *japonica* and 75% *indica* genetic components in R9308. The hybrid Xieyou 9308 has super high yielding potential with harmonious plant type (Cheng et al., 2005). Another example is Liangyou peijiu, a two-line super *indica* hybrid developed by the Jiangsu Academy of Agricultural Sciences collaboratively with the Hunan Hybrid Rice Research Center, China (Lu et al., 2000). Its female parent is Pei'ai 64S, a thermo-sensitive male sterile line with tropic japonica in its pedigree.

Combination name	Parental cross	Pedigree of major parent
Xieyou 9308	Xieqingzao A/R9308	R9308: C57 (*j*) //No.300 (*j*) /IR26 (*i*)
Liangyou Peijiu	Pei'ai 64 S/R9311	Pei'ai 64S: Nongken 58S (*j*) /Peiai 64 (*i*) // Peiai 64 (*i*) /
II you 602	II-32A/Luhui 602	Luhui602: 02428 (*j*) /Gui 630 (*i*) //IR24 (*i*)
II you 7954	II-32A/Zhehui 7954	Zhehui 7954: R9516/M105 R9516: Peiai'64S/Teqing M105: Miyang 46 (*i*) /Lunhui 422 (*m*)
Guodao 1	Zhong 9A/R8006	R8006: IRBB60//T2070/Duoxi 1 T2070: WL1312 (*j*) /Lunhui 422 (*m*) /Minghui 63 (*i*)
Guodao 3	Zhong 8A/R8006	
Guodao 6	Neixiang 2A/R8006	
Liaoyou 5218	Liao 5216A/C418	C418: Lunhui422 (*m*) /Miyang 23 (*i*)

Table 3. Some super rice hybrids derived from gene introgression of *indica* (*i*), *japonica* (*j*) and medium (m) type

http://dx.doi.org/10.5772/56821

6.2. Strategies to breed super hybrid rice

6.2.1. Construction of harmonious plant type based on substantial biomass production

Evolutionary change of rice variety in China indicates that increasing yield through dwarfing is due to the increase of harvest index, whereas through hybrid rice is due to the increase of biological production or biomass. In the experiment under the special ecological conditions at Yongsheng county, Yunnan Province, China, the plot grain yield, biological yield and harvest index of an *indica* hybrid Shanyou 63 were 15.27 t/ha, 28.29 t/ha and 0.54, respectively. Its harvest index under normal ecological conditions is almost the same (about 0.5) (Yang et al., 2006). We think that the key to further increase grain yield is the increase of biomass with a stable harvest index. Undoubtedly, proper increase of plant height is beneficial for increasing biomass, but the lodging resistance should be increased as well. Currently, leaf area index (LAI) in some high yielding varieties is 8-10, which seems to be the maximum. In order to increase the spikelet number and filled grain number per panicle, an indirect strategy is to properly raise plant height, ameliorate leaf stature and leaf quality, and strengthen root system vigor. It is known that the erect and slight rolling uppermost three leaves favor the full utilization of light energy after heading, by promoting CO_2 diffusion, increasing photosynthetic rate on the back face of leaves, accelerating the increase in biological yield, mitigating the conflict between panicle number and size, and improving the lodging resistance of rice plants (Cheng et al., 2007). Now, a set of super hybrids with slight rolling leaves have been developed and used in production. They generally have more than 12 t/ha of yield potential in combination with erect and late senescent leaves and lodging resistant culms.

6.2.2. Utilization of intersubspecific heterosis

It is known that the heterosis of inter-subspecific hybrids is much stronger than that of inter-varietal hybrids. Therefore, utilization of inter-subspecific hybrids is the most feasible approach for realizing super high yield. At present efforts have been focused on using Pei'ai 64S as a major female parent in the selection of super high-yielding combinations. Because Pei'ai 64S is an intermediate type between *indica* and *japonica*, it has a very wide compatibility. To exploit the heterosis of inter-subspecific hybrids and improve the efficiency of super high-yielding hybrid breeding, the emphasis is on the development of various widely compatible lines, especially those that have a broad spectrum of compatibility, including restorer lines and male sterile lines of *indica* type, *japonica* type and the intermediate type with different growth durations. This emphasis will create abundant parental lines for breeding various super high-yielding hybrids to well adapt different ecological environments in China.

6.2.3. Improvement of important agronomic traits by molecular breeding techniques

Genetic engineering techniques, such as anther culture, marker-assisted selection and gene transformation, offer reliable opportunities to accelerate breeding progress, increase selection efficiency and overcome genetic barriers to transfer genes across species. These techniques have played important roles in breeding super hybrid rice as well. For example, dramatic progress has been made in the development of transgenic rice plants with a high level of

resistance to insects (stem borer, brown planthopper), diseases (tungro virus, rice yellow stunt virus, blast, bacterial blight), herbicide (glufosate), and abiotic stresses (salinity and drought), as well as better nutritional value (e.g. glutelin, vitamin A) and higher yield. Some transgenic rice plants have already been subjected to evaluation under field conditions. In 1995, based on molecular analysis and field experiments carried out as part of a cooperative research program with Cornell University, China National Hybrid Rice Research and Development Center (CNHRRDC) identified two favourable quantitative trait loci (QTLs) (yld1 and yld2) from wild rice (*O. rufipogon* L.). Each of the QTL genes contributed a yield advantage of 18% over the high-yielding hybrid V64 (one of the most elite hybrids in China, with a yield potential of 80 kg/ha per day). By means of molecular marker-assisted backcrossing and selection, the development of near-isogenic lines carrying these two QTL genes is under way (Xiao et al., 1996). If biotechnology can be used to transfer apomixis to rice from grass species, hybrid rice production will be revolutionized and reach even higher levels.

6.3. Achievements of super rice breeding in China

In recent years, super rice breeding and extension for heterosis demonstration in China have made outstanding progress. On the bases to actively use conventional hybrid rice technology for new variety breeding, more and more attention is paid to strengthen the breeding technology innovation by combining molecular breeding technology with the conventional breeding technology. Bacterial blight broad-spectrum resistance gene *Xa21* has been introgressed into restorer lines by marker-assisted selection technology to develop restorer line R8006 with disease-resistance, good quality and high combining ability. As a result, R8006 has produced a series of successful combinations such as Guodao 1, Guodao 3, II you 8006 and Guodao 6 in China National Rice Research Institute. Among them, Guodao 6 has tall and erect plant type as obvious high-yield characteristics, so that the record in southern area was broken by it. In 2004, Guodao 6 had the average yield of 12.08 t/ha in a 100 mu or 6.7 ha model trial. It was released by National Crop Variety Approval Committee in 2006. Similarly, bacterial blight broad-spectrum resistance gene *Xa4* and *Xa21* are introgressed into restorer lines by marker-assisted selection technology in Rice Research Institute of Sichuan Agricultural University. This introgression has resulted in developing restorer line Shuhui527 with disease-resistance and high combining ability, from which a series of combinations such as superior two-line hybrid rice combination Zhunliangyou 527 and three-line hybrid rice Dyou 527, Gangyou 527, Xieyou 527, Guyou 527 have been bred. Some of the 527 hybrids reached record of high yield frequently. A comprehensive review paper "Super Hybrid Rice Molecular Breeding Research" published on China Rice Science has been downloaded more than 10, 000 times from China rice information network during last two years.

Over last 16 years, super rice research in China has gained significant advances in the aspects of breeding methodology, creation of breeding materials and selection and promotion of elite rice varieties. New pathway is proposed to utilize inter-subspecies heterosis between *indica* and *japonica* and harmonious plant type construction. Under the guidance of breeding methods, the super rice breeding program has been successively conducted and a series of new super rice varieties have been commercially released, such as the three-line super hybrid

http://dx.doi.org/10.5772/56821

rice combinations Xieyou 9308, II youming 86, II youhang 1, II you 162, D you 527, Zhong 9 you 8012 and II you 602; the two-line super hybrid rice combinations Liangyoupeijiu, Fengliangyou 1, Xinliangyou 6 and Zhunliangyou 527; and super inbred rice varieties Jijing 88, Shennong 265 and Shennong 606. Hitherto, a total of 101 new inbred rice varieties or hybrid rice combinations have been identified and nominated as super rice by the Chinese Ministry of Agriculture (CMOA), about half of which are the three-line hybrid rice combinations from South China. The demonstration and promotion of super rice have resulted in an increase of rice yield. According to the CMOA statistics, the accumulative planting area of super rice has increased to 23.85 million hectares (Fig. 4). The average yield of super rice is over 9 t/ha, 0.75 t/ha higher than that of traditional rice varieties. Totally, super rice yield has increased by 17.7 million tons since 1998. These super rice varieties cover rice regions in the Yangzte River Valley, South China and Northeast of China. In 2011, the yield of super hybrid rice Y Liangyou 2 was up to 13.9 t/ha in 6.6 ha demonstration area in Hunan province, which passed, for the first time, the yield target of the third phase in National Super RiceProgram.

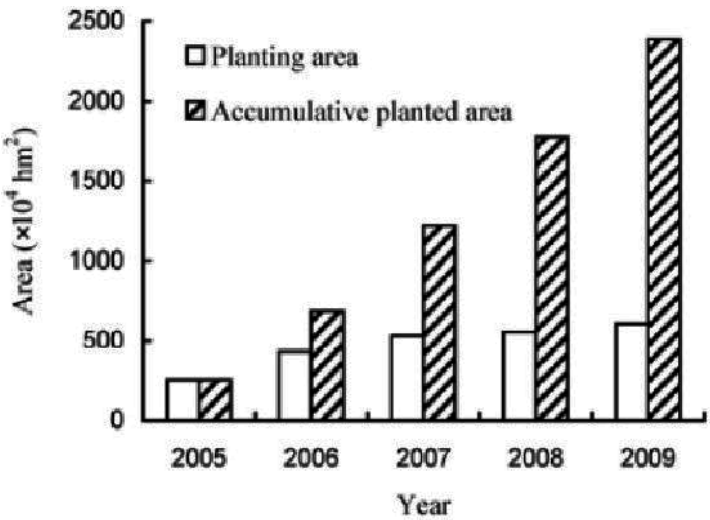


Figure 4. Planting area of super hybrid rice from 2005 to 2009 (the Ministry of Agriculture, P. R. China)

6.4. Outstanding elite hybrid rice varieties in China

Guodao 6 was released in 2007, which elite traits include high yielding and good quality with a yield potential of 12.5 t/ha and planting area of 95, 000 ha in 2010 (Table 4). Y Liangyou 1 was released in 2006, which elite traits include high yielding, good quality, and wide adaptability with a yield potential of 12.50 t/ha and planting area of 353, 000 ha in 2010. Xin Liangyou 6 was released in 2005, which elite traits include good quality and high yielding with a yield potential of 12.5 t/ha and planting area of 271, 000 ha in 2010. Zhongzheyou 1 was released in 2004, which elite traits include high yielding, good quality, and ideotype with a yield potential of 12.3 t/ha and planting area of 245, 000 ha in 2010.

Aariety name	Year of release	Elite traits	Yield potential (t/ha)	2010 planting area (ha)
Guodao 6	2007	high yielding, good quality	12.50	95, 000
Y liangyou 1	2006	high yielding, good quality, wide adaptability	12.50	353, 000
Xin liangyou 6	2005	good quality, high yielding	12.50	271, 000
Zhongzhe you 1	2004	high yielding, good quality, ideotype	12.30	245, 000

Table 4. Outstanding elite hybrid rice varieties.

6.5. Future directions for super rice breeding

Although great achievements have been resulted from past 13 years in super rice breeding and the yields of some hybrids have approached to the designed target, Chinese scientists are consistently making their efforts to further increase grain yield of rice, regardless of more difficulties and more constraints than even before.

6.6. Strengthen the exploitation and utilization of favorable genes

The core of super rice breeding is an effective use of germplasms and favorable genes. Because the genetic diversity in rice variety gene pool is limited, we must extensively utilize exogenous genes for variety improvements. We can exploit valuable genes from not only the cultivated rice, but also wild rice species, and even other crops to increase yield, quality and resistance to diseases and insects, and tolerance to adverse circumstances. The molecular marker-assisted selection techniques should be effectively used for transformation of high-yielding genes and other important agronomic trait genes from various sources into current variety genetic background. Especially for some complicated traits that lack in rice such as high photosynthetic efficiency gene in maize, stem borer resistant gene (*Bt*) and herbicide-resistant gene in microorganism, the transgenic technology is the best choice to improve rice in the future.

6.7. Strengthen the evaluation on root system (including physiological traits)

In recent years of super hybrid rice breeding practices, a conflict of large panicle with premature senescence becomes more and more troublesome to rice breeders and scientists. The large panicle needs longer time for the gains to fully fill and the premature senescence closes the sink before the completion of filling process. Therefore, studying root system to delay senescence of rice plant has become a hot subject in rice community (Wu and Cheng, 2005). Rice root system is not only a vital organ to stand the plant, absorb water and minerals, but also an important place where bioactive substances (hormones, amino acids, etc.) are synthesized. Root senescence at the late developmental stage directly affects the life span of functional leaves, grain filling and root vigor, especially during the grain-filling period. Obviously, the root vigor is the guarantee for high-yielding of super rice. So far, the genetic research on the root-related morphological traits such as root length, thickness, number, dry weight, density,

http://dx.doi.org/10.5772/56821

volume, penetration depth, and root/shoot ratio, as well as other physiological traits such as absorption ability to N, P and K, and root vigor, etc. has achieved great progress (Wu, 2006). However, these studies are still at preliminary stage, and further systematical study is required to propose the key indicator and the appraisal methods for rice breeding, and to explore the gene regulation of the root system and the relationship with the plant organs above ground.

6.8. Strengthen the seed production with high security and efficiency

As we know, super hybrid rice could not be commercially and successfully utilized if hybrid seed production costs too much, seed yield is too low and seed purity is not high enough. In the future, we should strengthen the research on the characteristics of flowering time, stigma exsertion and out-crossing rate of the female parents for super hybrid rice, and establish a new system for super high-yielding seed reproduction. The efficiency of seed production will bring seed price down. The security of seed production will not only yield good quantity and quality of hybrid seed, but also reduce production risk. Therefore, improving seed production will promote the rapid and stable extension of super hybrid rice.

6.9. Strengthen the combination of super rice with suitable cultivation management

China plays a leading role in global rice production, and the key from Chinese experience is the integration of superior varieties with suitable cultivation management. Research and promotion of the cultivation technology have played an important role in the two breakthroughs of rice yield in China. Besides high yielding, super rice should have high grain quality, and high efficiency to utilize resources with low environmental pollution. The superiority of varieties and suitability of cultivation practices jointly determine the production scale for a super hybrid rice to be promoted in commercialization.

7. Challenges and prospects

7.1. Challenges

Although tremendous achievements have been made in hybrid rice breeding, there also are some constraints and challenges in its development. To sum up, the major problems are as follows.

7.1.1. Planting area has not been at a standstill for years

In 1991, the acreage of hybrid rice reached its peak at 17.6 million ha, but after that the acreage decreased and remained at about 14.2 million ha in 2011. The main reasons are considered to be the cease and even decrease in the acreage of double cropping early hybrid rice and japonica hybrid rice. Recently, only 20% of early cropping rice area in South China are covered by hybrid rice, while over 90% of late cropping rice area are under hybrid rice in the same region. The availability of early cropping hybrid rice varieties is very limited to growers because it is very

difficult to integrate short growth duration and acceptable grain quality into elite high yielding combinations.

7.1.2. Grain quality of hybrid rice needs improving

With the increase of living standards for rice consumers in China, grain quality of rice is required to be improved. In comparison with conventional rice, hybrid rice usually has poorer grain quality measured mainly by the traits of head rice recovery and chalkiness. How to develop rice hybrids with both high yield and good grain quality is still a challenge for breeders.

7.1.3. Limited sources of male sterile cytoplasm to develop better CMS lines

Currently, more than 75% of the CMS lines used in commercial production belong to WA types. This dominant cytoplasm creates a great uniformity of WA cytoplasm, and genetic uniformity has been responsible for an epidemic of a destructive pest. Therefore, more efforts should be paid to diversify male sterile cytoplasms.

7.2. Prospcects

Conventional breeding has played an essential role in rice cultivar innovation for decades. Large-area application of three-line hybrid rice has showed that hybrid rice technology brings rice yield up to its potential level of physiological yield. With advanced root system and heterosis of seedling and nutrition in early stage, the application of hybrid rice to not only irrigated areas, but also low-lying fields, rain-fed fields and upland fields should be equally important. To commercialize the hybrid rice worldwide, studies on mechanical operation of hybrid seed production and male sterile line regeneration will also be an important subject in hybrid rice research.

In the past dozen years, we have made great progress in rice genome researches, such as establishing a dense molecular linkage map, locating a large amount of major and minor genes underlying important traits including resistance to bacterial blight and rice blast, plant height, reproductive period and tillers, and fully sequencing both *indica* and *japonica* DNA and subsequent functional genomic studies. With research advancement on rice genome, molecular breeding technology has become a new breeding technology to screen and breed new cultivars according to both phenotype and genotype, thus has been applied to rice breeding. Marker-assisted selection (MAS), quantitative trait locus (QTL) analysis and genetic transformation techniques are the most useful tools for rice molecular breeding, and have been used to identify new germplasms and elite rice cultivars. Chinese rice geneticists and breeders have made great progress in identifying QTLs responsible for important agronomic traits such as grain yield and quality, growth and development, disease and pest resistance and abiotic tolerance (Wang et al., 2005). MAS is a method to use molecular markers closely linked to a target gene as a molecular tag, so that the target gene can be quickly identified from breeding populations in the lab. In China, MAS is widely used to pyramid functional genes into popular

http://dx.doi.org/10.5772/56821

hybrid rice cultivars to improve important agronomic traits of hybrid rice, such as resistance and grain quality.

In summary, hybrid rice has made a great contribution to safeguarding the food supply in China and is still a major source of elite rice cultivars. However, hybrid rice production is rather time-consuming and the limited available genetic resources leave little room for the continued improvement of rice. With the completion of rice genome sequence, scientists are better equipped to unravel rice gene functions on a genome-wide scale, providing breeders with abundant genetic resources for continued generation of elite rice varieties to maintain a sustainable food supply in China. We expect that the successful implementation of a combinatorial approach using hybrid rice technology will play a crucial role in our effort to improve rice cultivars in China. The immediate goal is to breed varieties with a further improved yield potential, enhanced stress resistance and good grain quality by using molecular and genomic information to break the rice yield plateau in the future.

Author details

Liyong Cao* and Xiaodeng Zhan

*Address all correspondence to: caolycgf@mail.hz.zj.cn

China National Center for Rice Improvement, China National Rice Research Institute, Zhejiang,, China

References

[1] Alam M F, Khan M M R, Nuruzzaman M, Parvez S, Swaraz A M, Alam I, Ahsan N (2004) Genetic basis of heterosis and inbreeding depression in rice (*Oryza sativa* L.). Journal of Zhejiang University 5 (4): 406-411.

[2] Chen LY, Xiao YH, Tang WB, Lei DY (2007) Practices and prospects of super hybrid rice breeding. Rice Sci 14 (2):71–77.

[3] Chen WF, Xu ZJ, Zhang LB (2003) Physiological Basis of Super-High-Yield Rice Breeding. Shenyang: Liaoning Science and Technology Press, Pp156–162.

[4] Chen WF, Xu ZJ, Zhang LZ, Zhang WB, Yang SL (2002) Advances and prospects of rice breeding for super high yield. Eng Sci 4 (1): 31–35.

[5] Cheng SH, Cao LY, Chen SG, Zhu DF, Wang X, Min SK, Zhai HQ (2005) Conception of late-stage vigor super hybrid rice and its biological significance. Chin J Rice Sci 19 (3): 280–284.

[6] Cheng SH, Cao LY, Zhuang JY, Chen SG, Zhan XD, Fan YY, Zhu DF, Min SK (2007) Super hybrid rice breeding in China: Achievements and prospects. J Integ Plant Biol 49 (6): 805–810.

[7] Cheng SH, Cao LY, Zhuang JY, Wu WM (2009) Discussion on germplasm and gene utilization in breeding of super rice. Chin J Rice Sci, 23 (3): 223–228.

[8] Cheng SH and Li J (2007) Modern China Rice, published by jindun press in Beijing, p1-2.

[9] Crow J F (1952) Dominance and overdominance. In: Heterosis. Ed. J. W. Gowen. Iowa State College Press, Ames. Pp 282-297.

[10] Fu J, Chen L, Huang ZH, Wang ZQ, Yang JC (2012) Relationship of leaf photosynthetic characteristics and root physiological traits with grain yield in super rice. Acta Agron Sinica 38 (7): 1264-1276.

[11] Hallauer AR, Miranda J B (1988) Quantitative genetics in Maize Breeding. Iowa State College Press, Ames. P468.

[12] Hua J P, Xing YZ, Wu W, Xu CG, Sun X L, Yu S B, Zhang Q (2003) Single locus heterotic effects and dominance by dominance interaction can adequately explain the genetic basis of heterosis in an elite rice hybrid. Proceedings of the National Academy of Sciences of the United States of America, 100: 2574-2579.

[13] Huang YX (2001) Rice ideotype breeding of Guangdong Academy of Agricultural Sciences in retrospect. Guangdong Agric Sci (3): 2–6.

[14] Huang Y, Zhang LD, Zhang JW, Yuan DJ, Xu CG, Li XH, Zhou DX, Wang SP, Zhang QF (2006) Heterosis and polymorphisms of gene expression in an elite rice hybrid as revealed by a microarray analysis of 9198 unique ESTs. Plant Mol Biol 62:579–591.

[15] Jones JW (1926) Hybrid vigor in rice. Journal of the American Society of Agronomy 18: 423-428.

[16] Jiang QS, Lin G, Zhao DM, Li YW, He B, Wang F (2008) Characteriscs and application of Yixiang 1A, a good quality CMS line with fragrant. China Rice, 14 (2):35-37.

[17] Li SF (1997) Breeding, utilization and genetic study on G-type and D-type hybrid rice. Hybrid Rice (Special Issue for G-Type and D-Type Hybrid Rice): 1-25.

[18] Li CG (2001) A chalkiless indica CMS Yuefeng A developed in Guangdong. Hybrid Rice, 1:54.

[19] Li Z, Luo LJ, Mei H, Wang DL, Shu QY, Tabein R, Zhong D, Stansel JW, Khush GS Paterson AH (2001) Genetic basis of inbreeding depression and heterosis in rice. Genetics. 158: 1737-1753.

[20] Lin QH, Lu WP, Cai JZ, Cao XZ (1989) The relationship between root and leaf angle in rice plant. Acta Agron Sinica 15 (2):123-131.

http://dx.doi.org/10.5772/56821

[21] Lu CG, Zou JS (2000) Breeding and utilization of two-line intersubspecific hybrid rice Liangyoupeijiu. Hybrid Rice 15 (2): 4–5.

[22] Luo LJ, Li Z, Mei H, Shu QY, Tabein R, Zhong D, Ying CS, Stansel JW, Khush GS, Paterson AH (2001) Overdominant epistatic loci are the primary genetic basis of inbreeding depression and heterosis in rice. II. Grain yield components. Genetics 158 (4): 1755-1771.

[23] Mao CX (1993) Hybrid rice production in China-New successes, challenges and strategies. Paper presented at the FAO Regional Expert Consultation on Hybrid Seed Production, Development and Security of Major Cereal Crops, 9-12 November, Bangkok, Thailand.

[24] Mir G. N. Development of commercial hybrids for hills-problems, present status and future scope. In: Recent advances in rice production technology in hills, SKUAST-K. 2002, pp. 107-111.

[25] Peng SB, Khush GS, Cassman KG (1994) Evolution of new plant ideotype for increased yield potential. In: Cassman K G. Breaking the Yield Barrier. Manila: IRRI, Pp 5–20.

[26] Peng SB, Khush GS, Virk P, Tang QY, Zou YB (2008) Progress in ideotype breeding to increase rice yield potential. Field Crops Res 108: 32–38.

[27] Rosegrant MW, Sombilla MA, Perez N (1995) Global food projections to 2020: Implications for investment. In: Food, Agriculture and the Environment Discussion Paper No. 5.Washington, DC: IFPRI.

[28] Shi MS (1981) Report on breeding and application of two-line system in later japonica. Hubei Agricultural Sciences, 7:1-3.

[29] Stuber CW, Lincoln SE, Wolf DW, Helenjarisand T, Lander E S (1992) Identification of factors contributing to heterosis in a hybrid from two elite maize inbred lines using molecular markers. Genetics 132: 823-839.

[30] Virmani SS (1996) Hybrid rice. Adv Agron 47: 377-462.

[31] Wang YH, Xue YB, Li JY (2005) Towards molecular breeding and improvement of rice in China. Trend Plant Sci 10 (12):610-614

[32] Wright S (1968) Evolution and genetics of population. University of Chicago Press, Chicago.

[33] Wu WM, Cheng SH (2005) Significance and prospects of breeding for root system in rice (*Oryza sativa* L.). Chin J Rice Sci 19 (2): 174–180.

[34] Wu WM (2006) Genetics and QTL mapping of root traits in rice plant (*Oryza sativa* L.). [Ph.D Dissertation]. Beijing: Chinese Academy of Agricultural Sciences.

[35] Xiao J, Li J, Yuan L, Tanksley SD (1995) Dominance is the major genetic basis of heterosis in rice as revealed by QTL analysis and molecular markers. Genetics 140: 745-754.

[36] Xiong LZ, Yang GP, Xu CG, Zhang QF, Saghai Maroof MA (1998) Relationships of differential gene expression in leaves with heterosis and heterozygosity in a rice diallel cross. Mol Breed 4: 129–136.

[37] Yang SH, Cheng BY, Wu JL, Shen WF, Cheng SH (2006) Review and prospects on rice breeding and extension in China. Rice Sci 13: 1–8.

[38] Yu SB, Li K, Xu CG, Tan YF, Gao YJ, Li XH, Zhang Q, Maroof MA (1997) Importance of epistasis as genetic basis of heterosis in an elite rice hybrid. Proceedings of the National Academy of Sciences of the United States of America 94: 9226-9231.

[39] Yuan LP, Yang Z, Yang J (1994) Hybrid rice in China. In: Virmani S S. Hybrid Rice Technology: New Developments and Future Prospects. Los Banos, Philippines: International Rice Research Institute, Pp143–147.

[40] Yuan LP (1987) Strategic assumption of hybrid rice breeding. Hybrid Rice (1): 1–3.

[41] Yuan LP (1996) Breeding strategies for development of intersubspecific hybrid rice. Hybrid Rice (2):2-3.

[42] Zeng QC, Zhou KD, Zhu Z, Luo Q (2000) Current status in the use of hybrid rice heterosis in China. Chinese J Rice Sci, 14 (4):243-246.

[43] Zhang HQ, Zhou YH (2006) Meiotic pairing behaviour reveals differences in genomic constitution between Hystrix patula and other species of the genus Hystrix Moench (Poaceae, Triticeae). Pl Syst Evol 258: 129–136.

[44] Zhu DF, Lin XQ, Cao WX (2001) Comparison of leaf photosynthetic characteristics among rice hybrids with different leaf rolling index. Acta Agron Sinica 27 (3): 329-333.

[45] Zhang TB (1981) New understanding to male sterility breeding theory. Hubei Agricultural Sciences, 3:8-11.

Edited by Wengui Yan and Jinsong Bao

Rice is a staple food for half of the worlds population mostly in Asia. Productivity of rice has largely been improved since the Green Revolution in 1960s. Further improvement of rice yield is necessary to keep pace with population growth, which is a challenging task for breeders. This book, Rice - Germplasm, Genetics and Improvement, as its name implies, comprehensively reviews current knowledge in germplasm exploration, genetic basis of complex traits, and molecular breeding strategies in rice. In the germplasm part, we highlight the application of wild rice in rice breeding. In the genetics part, most of the complex traits related with yield, disease, quality have been covered. In the improvement part, Chinese experiences in hybrid rice breeding have been summarized together with many molecular breeding practices scattering in different chapters.

ISBN 978-953-51-4234-8

Photo by Modify260 / iStock

IntechOpen