IntechOpen

Global Wheat Production

Edited by Shah Fahad,
Abdul Basir and Muhammad Adnan

GLOBAL WHEAT PRODUCTION

Edited by **Shah Fahad, Abdul Basir** and **Muhammad Adnan**

Global Wheat Production
http://dx.doi.org/10.5772/intechopen.72559
Edited by Shah Fahad, Abdul Basir and Muhammad Adnan

Contributors

Gautam Dadhich, Parul Patel, Mukhtar Ahmed, Sehrish Khan, Shakeel Ahmad, Muhammad Irfan, Ghulam Shabir, Muhammad Aslam, Saule Kenzhebayeva, Gulina Doktyrbay, Fatma Sarsu, Nargul Omirbekova, Alfia Abekova, Dauren Tashenev, WenZhu Yang, Yizhao Shen, Shaima Moustafa, Haifa Alhaithloul, Hani Abdelzaher, Gisela Montero, Marcos A. Coronado, Conrado García, Héctor Campbell, Daniela Montes Nuñez, Ricardo Torres, Laura Pérez, José León, José Ayala, Muhammad Javid Iqbal, Inna Lapochkina, Olga Baranova, Nail Gainullin, Michael Kuzmich, Svetlana Polyakova, Petr Polityko, Ramin Mamedov, Sergey Voronov, Arritokieta Ortuzar-Iragorri, Valentina V. Kolpakova, Irina Gaivoronskaya, Nikolay D. Lukin, Szilvia Veres, Gabrijel Ondrasek, László Zsombik, Guigan Fang, Kuizhong Shen, Thierry Aussenac, Larbi Rhazi

© The Editor(s) and the Author(s) 2018
The rights of the editor(s) and the author(s) have been asserted in accordance with the Copyright, Designs and Patents Act 1988. All rights to the book as a whole are reserved by INTECHOPEN LIMITED. The book as a whole (compilation) cannot be reproduced, distributed or used for commercial or non-commercial purposes without INTECHOPEN LIMITED's written permission. Enquiries concerning the use of the book should be directed to INTECHOPEN LIMITED rights and permissions department (permissions@intechopen.com).
Violations are liable to prosecution under the governing Copyright Law.

Individual chapters of this publication are distributed under the terms of the Creative Commons Attribution 3.0 Unported License which permits commercial use, distribution and reproduction of the individual chapters, provided the original author(s) and source publication are appropriately acknowledged. If so indicated, certain images may not be included under the Creative Commons license. In such cases users will need to obtain permission from the license holder to reproduce the material. More details and guidelines concerning content reuse and adaptation can be foundat http://www.intechopen.com/copyright-policy.html.

Notice

Statements and opinions expressed in the chapters are these of the individual contributors and not necessarily those of the editors or publisher. No responsibility is accepted for the accuracy of information contained in the published chapters. The publisher assumes no responsibility for any damage or injury to persons or property arising out of the use of any materials, instructions, methods or ideas contained in the book.

First published in London, United Kingdom, 2018 by IntechOpen
eBook (PDF) Published by IntechOpen, 2019
IntechOpen is the global imprint of INTECHOPEN LIMITED, registered in England and Wales, registration number: 11086078, The Shard, 25th floor, 32 London Bridge Street
London, SE19SG – United Kingdom
Printed in Croatia

British Library Cataloguing-in-Publication Data
A catalogue record for this book is available from the British Library

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

Global Wheat Production
Edited by Shah Fahad, Abdul Basir and Muhammad Adnan

p. cm.

Print ISBN 978-1-78923-336-0

Online ISBN 978-1-78923-337-7

eBook (PDF) ISBN 978-1-83881-703-9

We are IntechOpen, the world's leading publisher of Open Access books

Built by scientists, for scientists

3,650+
Open access books available

114,000+
International authors and editors

118M+
Downloads

Our authors are among the

151
Countries delivered to

Top 1%
most cited scientists

12.2%
Contributors from top 500 universities

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Meet the editors

Dr. Shah Fahad is currently working as an assistant professor in Agriculture Department, University of Swabi, Khyber Pakhtunkhwa, Pakistan. He studied in Pakistan at Khyber Pakhtunkhwa Agricultural University and Quaid-I-Azam University, Islamabad, where he successfully completed two degrees: a BSC (Hons) in Agronomy and an MPhil in Plant Physiology. As a scholar, he continued for another degree, in graduate studies at Huazhong Agricultural University Wuhan, China, pursuing PhD in Agronomy, which was achieved with honors in 2015. Mr. Shah Fahad did his postdoctorate at Huazhong Agricultural University in 2017. He is a contributor to many international journals with focuses on global warming and their influences on rice crop attributes in his articles. He is a member of the Editorial Board and a critic of ten international journals.

Dr. Abdul Basir completed his PhD degree from Pak-USA in 2015 and is currently working as an associate professor at the Department of Agronomy, University of Swabi (www.uoswabi.edu.pk). He is teaching courses at graduate and postgraduate levels and performs student counseling. His research interests are residue management under diverse tilling and no tilling system for improving soil fertility, soil biology, C sequestration, greenhouse gas emission, and crop productivity across diverse topography. Biochar, organic, and inorganic nitrogen sources impact on crop growth and yield. Advance biostatistics, crop modeling, remote sensing, and their application in the field of agriculture are other sources of his interest. He has more than 14 years of research and outreach experience. He is an author and coauthor of more than 40 peer-reviewed journal articles and 7 conference proceedings and abstracts. He developed a wheat variety "Shahkar-CCRI-2013" for rain-fed areas of the country. He has been awarded many international fellowships such as IRSIP and NFP.

Mr. Muhammad Adnan is a lecturer in the Department of Agriculture at the University of Swabi (UOS), Pakistan. Mr. Adnan is currently obtaining his PhD degree (Soil Fertility and Microbiology) from the Department of Soil and Environmental Sciences (SES), University of Agriculture, Peshawar, Pakistan, and Department of Soil, Plant, and Microbial Sciences, Michigan State University, USA. He has received his MSc and BSc (Hons) degrees in Soil and Environmental Sciences, from the Department of SES, University of Agriculture, Peshawar, Pakistan.

Mr. Adnan's main research interests are soil microbiology and plant nutrition including fertilizer use efficiency, N losses, management of legume N2 fixation for increasing cereal production, and management of organic wastes for sustainable agriculture production. He has published over 73 peer-reviewed articles and has received over 2.4 million PKR in research funding as a Co-PI.

Mr. Muhammad Adnan is the recipient of three Gold medals [one in BSc and two in MSc (Hons)], President of Pakistan award [BSc (Hons)], Indigenous PhD scholarship, and IRSIP grant for Michigan State University, USA, in his educational carrier. He was also awarded Best University Researcher award for the year 2015 by UOS.

Contents

Preface

In the coming years, the agricultural sector will face a challenge to feed an increasingly growing human population while simultaneously facing the need to avoid additional deforestation and land degradation. This challenge requires the sustainable intensification of underperforming agricultural systems that can cope with climate change. With a current production of ~ 700 Mt, wheat is the third largest crop globally and an essential source of calories in human diets. Wheat will remain a crucial component of human nutrition, and increasing its production is therefore an important requirement for food security. Global fertilizer and pesticide use has increased significantly during the last decades leading to an increase in wheat yields in many countries. Yet, approximately 70% more wheat could still be produced if the cropland already under cultivation met its current climatic potential, which can mainly be achieved through improved fertilization and irrigation. A large geographic variation in wheat yields across similar climates points to sizeable yield gaps in many nations and indicates a regionally variable flexibility to increase wheat production. Climate change will alter growing conditions and thus impact future wheat production and management opportunities for sustainable intensification. Wheat is expected to be especially sensitive to rising temperature since it has been among the crops most affected in an already changing climate. Warming is likely to reduce wheat yields due to a shorter grain filling period caused by a more rapid development. On the other hand, increased growth rates during winter or a shift of the grain filling period into a wetter part of the season may result in rising wheat yields in some regions. In addition, precipitation is expected to become an important driver for crop production in many regions, such as in South and West Asia.

The purpose of the book *Global Wheat Production* is an attempt to present a comprehensive picture of the importance of wheat production globally. The book is designed to cater to the needs of researchers, technologists, policy makers, and undergraduates and postgraduate students studying sustainable crop production and crop protection. Libraries in all universities and research establishments where agricultural and agronomical sciences are studied and taught should have multiple copies of this valuable book on their shelves. The book comprises 12 chapters. We are thankful to all authors who contributed their valuable chapters to this book. We are also extremely grateful to **Ms. Romina Skomersic** (Publishing Process Manager) of InTech for helping us to publish the book in an excellent form in the shortest possible time. We owe our sincere thanks and irreparable gratitude to our families whose consistent encouragement and love have been a tremendous impetus for the completion of this book.

Dr. Shah Fahad, Dr. Abdul Basir and Mr. Muhammad Adnan
Agriculture Department
The University of Swabi
Swabi Kyhber Paktunkhwa, Pakistan

Chapter 1

Effect of Phosphorus on Root Signaling of Wheat under Different Water Regimes

Mukhtar Ahmed, Sehrish Khan, Muhammad Irfan, Muhammad Aqeel Aslam, Ghulam Shabbir and Shakeel Ahmad

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.75806

Abstract

Phosphorus (P) is one of the most vital nutrient needed for crop production. Phosphorus plays an important role in root growth and builds resistance against abiotic stresses. In the current study two wheat cultivars (phosphorus responsive) were planted to study the treatment effects in polythene bags. The treatments were 5 different levels of P (P_0 = 0.2 g/bag, P_{60} = 0.4 g/bag, P_{80} = 0.53 g/bag, P_{100} = 0.66 g/bag and P_{120} = 0.8 g/bag) and three water regimes. The data regarding root length, shoot length, root-shoot ratio and yield parameters were collected and analyzed. Among both the genotypes, NARC-2009 performed well compared to Sehar-06. The highest dry matter and yield were obtained under P_{100} compared to other treatments. With the increased phosphorus root and shoot length increased linearly up-to P_{100} while afterward it starts decreasing. The results lead to conclusion that optimum dose of phosphorus could be used to increase root growth and establishment under water stress.

Keywords: phosphorus, abiotic stresses, dry matter, root growth and root establishment

1. Introduction

Root signaling is the response of the plant roots on different stimuli like soil structure, soil nutrients, different chemicals and stress conditions. Root apical meristems are the major sites for different types of activities in response to changes related to roots. Root growth defines the extent to which plant explores soil for water and mineral nutrients. Root systems of individual crop plants may encounter large variations in mechanical impedance to root penetration [1]. Root architecture is a highly plastic and environmentally responsive trait that enables

IntechOpen

© 2018 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

plants to counteract nutrient scarcities with different forging strategies [2]. Root-specific traits such as root system architecture, sensing of edaphic stress and root-to-shoot communication can be exploited to improve resource capture (water and nutrients) and plant development under water-limited conditions [3].

The uptake of nutrients depends upon both the supply of available nutrients in the rooting media and the root system [4]. The ability of plants to respond appropriately to nutrient availability is of fundamental importance for their adaptation to the environment. Nutrients such as nitrate, phosphate, sulfate and iron act as signals that can be perceived. These signals trigger molecular mechanisms that modify cell division and cell differentiation processes within the root and have a profound impact on root system architecture. Important developmental processes, such as root-hair formation, primary root growth and lateral root formation, are particularly sensitive to changes in the internal and external concentration of nutrients [5]. There is no doubt that differences occur in response to mineral nutrition both among species and cultivars, that is, genotypes belonging to the same species.

Phosphorus plays a vital role in crop production and is involved in energy transfer in plants. Carbon dioxide fixation by plants is not possible without phosphorus. Many plant physiological functions such as utilization of sugars, starch, photosynthesis, energy storage and transfer are dependent on phosphorus. It is also a constituent of cell nucleus and is essential for cell division and development of meristematic tissues [6]. Phosphorus has been reported to increase the strength of cereal straw, resist abiotic stresses, stimulate root development, promote flowering, fruit production, and formation of seed and hasten maturity of the crops [7]. Phosphorus utilization efficiency can be improved by mixing it with farm yard manure to increase the yield of wheat. Farm yard manure mixed with single superphosphate in 1:2 ratio increases phosphorus efficiency significantly [8]. It would be advantageous if we select, screen or improve plants for higher capacity to adapt to mineral stresses. This approach is beneficial in developing countries like Pakistan where capital input resources are limited. Farmers in these countries require nutrient efficient crop cultivars which perform better or do something better than other cultivars when given a considerable amount of mineral nutrient.

Cereals are facing acute problem of drought and temperature stress [9]. Low water availability is the major environmental factor which limits crop productivity. Root is the place where plants first encounter drought stress, it is likely that roots may be able to sense and respond to stress condition. Drought stress is the most common adverse environmental condition that can seriously reduce crop productivity [10, 11]. The mechanism of drought tolerance and breeding for drought-resistant crop plants has been major goal of plant biologists and crop breeders. Significant progress has been made in understanding root growth under drought stress. However, there has been no genetically defined drought-adaptive response in root development. But inhibition of lateral root development is a typical adaptive response of roots to drought stress. Despite the lack of understanding of drought tolerance mechanisms, physiological and molecular biological studies have documented several plant responses to drought stress [12].

Lack of sufficient water is the most important factor affecting world agriculture. Thus, increasing the efficiency of water and nutrient use is essential in order to improve yield whilst minimizing damage to the environment [13–18]. Plant depends upon the capacity of roots to obtain water and nutrients from the soil. The root respiration, carbohydrates allocation (root: shoot

ratio) and grain yield are closely related to soil water status. Reductions in root respiration and root biomass under severe soil drying can improve drought tolerant wheat growth and physiological activity during soil drying and improve grain yield, and hence should be advantageous over a drought sensitive cultivar in arid regions. Therefore objectives of the study were (i) to examine the effect of phosphorus on root signaling of wheat and (ii) to determine the effect of water stress on root signaling. The hypothesis, therefore made, was that there is a significant relationship present between wheat roots and P and also between root and drought stress.

2. Root signaling

2.1. Phosphorus and root signaling

Among all essential plant nutrients, phosphorus (P) is the second most abundantly required nutrient element after nitrogen and is an important constituent of many structural components of the plants [19, 20]. In agricultural ecosystems, it determines the soil quality with respect to its production capacity [21]. Being scarce and non-renewable natural resource [22] which is under the threat of rapid depletion as a result of intensive mining across the world more emphasis is being given to increase P use efficiency in soil for successful and sustainable crop production. A field experiment was conducted over 2 years to study the ameliorating effects of P on wheat yield, root cation exchange capacity (CEC) and on different doses of P. Phosphorus was applied as single superphosphate. The application of P increased the root CEC of wheat up to bloom stage only whereas nutrient concentration, uptake and grain and straw yield were found to increase up to maturity [23]. The capacity of plant roots to increase their carboxylate exudation at low plant phosphorus (P) status is an adaptation to acquire sufficient P at low soil P availability. Root mass ratio decreased with increasing P supply for *Triticum aestivum* L. [24]. An experiment was set up to make a critical assessment of the role of organic P in soil solution in the nutrition of wheat plants under sterile conditions. Phosphorus supply had a positive effect on dry matter and P concentration of the plants. Acid phosphatase secretion by plant roots was 5–11 times higher in organic P treatments than in the inorganic P treatments. It was hypothesized that plants secrete phosphatases in response to the presence of organic P in soil solution and organic P might be responsible for the increase in P influx to wheat plants [25].

Root-soil contact is an important factor for uptake of a less mobile soil nutrient such as phosphorus (P) by crop plants. Root hairs can substantially increase root-soil contact. Identification of crop cultivars with more and longer root hairs can, therefore, be useful for increasing P uptake in low input agriculture. The variation in root hair parameters of the cultivars was related to quantity of P depleted from rhizosphere. These results showed that the variation in root hairs of cereal cultivars can be considerable and it can play a significant role in P acquisition, especially in low-P soils [26]. A field trial was conducted to investigate main morphological and physiological changes of different wheat landraces to low-P stress at the stage of seedling. P-deficiency significantly decreased root volume, total leaf area, and plant dry weight, but greatly increased density of root hairs and root top ratio. In addition, P-deficiency induced the significant enhancement of phosphorus utilization efficiency and the amount of

proline, malondialdehyde, acid phosphatase, peroxidase and superoxide dismutase (SOD), but the significant reduction of P uptake and soluble protein content. The results based on the correlation analysis showed that the economic yield of wheat landraces had relationships with their morphological and physiological characteristics under P-deficiency [27].

2.2. Drought and root signaling

Effect of drought on the growth and yield of wheat were investigated. Drought during grain filling further decreased yields. Plots with a lower plant density demonstrated a smaller decrease in yield due to drought. There was a significant positive linear relationship between the number of shoots per plant and nodal root axes per plant. There appeared to be a difference between cultivars in root system architecture, and in their response to drought, but these differences were not reflected in grain yield [28]. Drought-induced loss in crop yield probably exceeds losses from all other causes, since both the severity and duration of the stress are critical. Drought stress reduces leaf size, stem extension and root proliferation, disturbs plant water relations and reduces water-use efficiency. Plants display a variety of physiological and biochemical responses at cellular and whole-organism levels towards prevailing drought stress, thus making it a complex phenomenon. Plants display a range of mechanisms to withstand drought stress. The major mechanisms include curtailed water loss by increased diffusive resistance, enhanced water uptake with prolific and deep root systems and its efficient use, and smaller and succulent leaves to reduce the transpirational loss. At molecular levels several drought-responsive genes and transcription factors have been identified, such as the dehydration-responsive element-binding gene, aquaporin, late embryogenesis abundant proteins and dehydrins. Plant growth substances such as salicylic acid, auxins, gibberellins, cytokinin and abscisic acid modulate the plant responses towards drought. Polyamines, citrulline and several enzymes act as antioxidants and reduce the adverse effects of water deficit [29]. The possibility of reducing the proliferation of roots to increase yields at higher seeding rates and conserving the soil water at different growing stages in water-limited environments was studied. In the severe drought towards the end of the growing season, grain yield decreased as the seeding rate increased, but under the more favorable conditions the reverse was true. Averaged over the seeding rates, grain yield was significantly increased; grain yield and yield components were higher and root pruning at spring-growth stage recorded the highest water use efficiency [30]. The leaf net photosynthetic rate and stomatal conductance were significantly decreased under drought. The leaf transpiration rate was decreased by drought. The intercellular CO_2 concentration was increased under drought, while it was decreased most of the time from midday to the afternoon. The leaf stomatal limitation was increased under drought [31].

Root length, root dry weight and seedling dry weight are the major traits to select for studying tolerant genotypes under water stress conditions [32]. It is reported that drought affect the plant water status during ear formation and flowering stage. Water availability mostly affects growth of leaves, roots, photosynthesis and dry mater accumulation [33].

2.3. Phosphorus × drought and root signaling

Phosphorous availability is correlated with moisture conditions of the soil, because higher water content in soil due to frequent irrigation generally leads to a better mobility and

availability of P [34], which also improves the P conversion in the internal of plant [35], by enhancing root-shoot ratio and root elongation releasing of organic acids or protons [36] and phosphatases [37]. The absorbed P by plant to produce more biomass is another adaptive mechanism to P deficiency in soil, thus low-P also limits the yield and quality of wheat [38], because P can effect on photosynthesis, photo-assimilate transportation and stunt growth of plant [39–41]. Further, the coupling effect of water and chemical fertilizers on different crops or varieties have been reported by many studies, they revealed that water and nutrient uptake were two physiological processes that interacted with each other [42–45]. Therefore, soil water content and P fertilizer, and meanwhile, their interaction plays great key role for crop growth [46], and suitable irrigation and fertilization is the main method to increase production. The effects of drought stress on the phosphorus (P), uptake dynamics throughout the growth cycle were studied. Drought stress induced sharp decreases in total P uptake at different developmental stages and, in particular, detrimentally affected the nutrient uptake capability of roots. The results suggested that plants differ in their ability to maintain nutrient uptake under drought stress, and it is highly dependent on the intensity and duration of drought stress and the developmental stage. The decrease in total P uptake caused by both moderate stress and severe stress was accompanied by reduction in biomass production in drought-stressed tissues. The biomass allocation patterns in response to drought stress fluctuated strong mostly because of competitive changes in the shoot and roots at different stages, thus the root: shoot ratio increased at some stages and decreased at other stages. Severe stress induced a dramatic reduction in the harvest index, whereas moderate stress slightly decreased harvest index. Thus, water limitation caused lower P uptake and harvest index [47].

The water content and nutrient in soil are two main determinant factors to crop yield and quality, managements of which in field are of great importance to maintain sustainable high yield. The objective of the study was to measure the uptake, forms, and use efficiency of phosphorus in wheat under irrigation. The results indicated that P fertilizer combined with irrigation not only improved the activity of phosphatase in soil, but also increased P accumulation in wheat, similar results was found in the grain of wheat, the content of total P increased significantly. The interaction between P and irrigation also significantly affected on the P accumulation, grain total P, grain phospholipids P, and P production efficiency [48].

3. Materials and method

Two experiments were carried out to study root signaling in response to different water regimes and level of phosphorus. First one was about screening of wheat genotypes for drought tolerance conducted in the laboratory. The sowing apparatus used was Petri dishes (9 cm diameter) in which 9 different varieties of wheat were sown under different level of PEG (polyethylene glycol) to induce stress. From these 9 varieties two varieties which gave better results under drought conditions were selected. These two varieties were further sown in the second experiment which was conducted in a polythene bags (2.5 feet long, 10 cm diameter). In the second experiment eight treatments were applied which were replicated thrice. The detail of both the experiments is given as under.

3.1. Experiment # 1

Lab experiment was conducted at PMAS-Arid Agriculture University Rawalpindi. Nine wheat varieties were selected namely, Sehar-06, Wafaq-2001, Freed-06, Dhurabi, NARC-09, NARC-11, Lasani-08, Bars-09 and Punjab-11. Thirty seeds were randomly selected from each variety and were sterilized with ethanol solution. PEG6000 solution was prepared at three different concentrations viz; 12.5 g/250 ml (−0.50 bars) ($PEG_{-0.50}$), 25 g/250 ml (−1.48 bars) ($PEG_{-1.48}$) and 37.5 g/250 ml (−2.95 bars) ($PEG_{-2.95}$). The sterilized seeds of the above mentioned nine varieties were placed in the Petri dishes on the filter papers soaked with the above mentioned solutions of PEG. The sowing was done on 23rd October 2013. The effect of PEG on germination and seedling vigor traits of wheat varieties were studied to check which variety performed well under higher concentrations of PEG producing higher degree of drought.

3.1.1. Germination and seedling vigor traits (10–20 days)

Germination percentage was taken 10 days after sowing. Total number of seeds sown and the number of seeds germinated were counted and germination percentage was calculated. Fresh roots (of one plant per petri dish) were taken and were individually weighed on a weighing balance to get root fresh weight. After taking the fresh weight, the roots were oven dried for 24 h at 65°C. After 24 h they were weighed on a weighing balance for measurement of root dry weight. Length of individual roots was measured with the help of a foot ruler. The roots of the plants were removed and the above root portion, that is, shoot were weight on a weighing balance for the measurement of shoot fresh weight. After taking fresh weight, the shoots were oven dried for 24 h at 65°C and after that they were weighed on a weighing balance for shoot dry weight. Root and shoot lengths were separately measured with the help of a foot ruler and then the ratio was taken.

3.2. Experiment # 2

From experiment # 1 two varieties (NARC-09 and Sahar-06) were selected which performed well under higher PEG concentrations showing their adaptation under drought conditions. These two varieties were then sown for further study. Equal quantity of sand (72 kg) and soil (72 kg) were mixed and filled in polythene bags. Phosphorus was applied to the soil prior to sowing. Ten seeds of selected genotypes were sown in each bag. Measured amount of water was added in treatments involving drought study while in phosphorus treatments water was applied before sowing. The experimental area was covered with polythene sheet to hinder the supply of water to the water treatments due to rain. The treatments includes; T1 = at field capacity (control), T2 = 10% below field capacity, T3 = 20% below field capacity, T4 = 0.2 g/bag (30 kg/ha), T5 = 0.4 g/bag (@ 60 kg/ha), T6 = 0.53 g/bag (@ 80 kg/ha), T7 = 0.66 g/bag (@ 100 kg/ha) and T8 = 0.8 g/bag (@ 120 kg/ha). Phosphorus was applied in the form of P_2O_5. Number of replications were three, therefore, the total number of treatments were 48. The experimental design used was completely randomized (CRD).

3.2.1. Crop parameters and statistical analysis

Root length was taken at three leaf, anthesis and maturity with the help of a foot ruler. Roots of the plant were separated from the shoot and also any soil, if present, was removed. Afterwards the samples were weighed on a weighing balance. Root-shoot ratio was calculated by first measuring the root length and then the shoot length and then the ratio was calculated. Root fresh weight was measured by weighing the root samples on a weighing balance. Fresh root samples were oven dried for 24 h and weighed afterwards on a weighing balance. Root fresh weight and root dry weight are separately measured and then the ratio was calculated. Number of spikelets per spike was calculated of three spikes and then average was taken. Numbers of seeds of three spikes were counted and then its average was taken to get number of seeds per spike. Spikes were collected from the plants and were weighed on a weighing balance to get spike weight. 100 grains were separated on the seed counting tray and weight of those 100 grains were calculated on a weighing balance. The data obtained was statistically analyzed. Analysis of variance (ANOVA) were used to determine means and LSD at 5% level of significance was determined to compare means.

4. Result and discussion

4.1. Experiment # 1 (screening analysis)

Experiment 1 was conducted for screening analysis to select best wheat genotypes. Highest germination percentage was recorded for T2 (87.78%) followed by T1 (87.03) while lowest was at T4 (80.1%) (**Table 1**). There was 20% difference among T2 and T4. In the meanwhile, all the genotypes behaved differently for germination percentage. Maximum germination (96.67%) was recorded for genotype NARC-2009 while minimum germination percentage (76.50%) was recorded for genotype Dhurabi. There was 8% difference among genotype NARC-2009 and Dhurabi for germination percentage. The interactive effects were statistically significant at 1% P level. Maximum germination percentage was recorded for NARC-2009 (100%) at T1 and T2 while minimum germination percentage was recorded for genotype BARS-09 (66.67%) under T1. The treatments depicted significant effect on root fresh weight of different genotypes. All the genotypes varied considerably for root fresh weight (RFW) (**Table 2**). Maximum root fresh weight was recorded for genotype NARC-2009 (0.12 g) while minimum root fresh weight was recorded for genotype Lasani-08 (0.09 g). There was 24% difference among NARC-2009 and Lasani-08 for root fresh weight. Similarly, all the treatments differed potentially for root fresh weight. Maximum root fresh weight was recorded for T1 (0.11 g) while minimum root fresh weight was observed under T3 (0.08 g). In the same way the interactive effects for root fresh weight was potentially significant at 1% P level. Maximum root fresh weight was recoded for genotype NARC-2009 under T1 (0.14 g) followed by genotype Sehar-06 under T2 while minimum root fresh weight (0.06 g) was recorded for genotype Lasani-08 under T4. Results depicted significant variation for root length for different treatments on wheat

Genotypes	Control	$PEG_{-0.50}$	$PEG_{-1.48}$	$PEG_{-2.95}$	Mean
Dhurabi	73.33e–g	76.67d–g	86.67a–e	73.33e–g	77.5CD
Fareed-06	80c–g	86.67a–e	83.33b–f	86.67a–e	84.17BC
NARC-11	96.67ab	93.33a–c	90a–d	73.33e–g	88.33B
Sehar-06	90a–d	80c–g	93.33a–c	93.33a–c	89.17B
Punjab-11	90a–d	93.33a–c	86.67a–e	80c–g	87.5B
Wafaq-2001	96.67ab	93.33a–c	83.33b–f	80c–g	88.33B
NARC-2009	100a	100a	93.33a–c	93.33a–c	96.67A
BARS-09	66.67g	76.67d–g	86.67a–e	76.67d–g	76.67D
Lasani-08	90a–d	90a–d	66.67g	70fg	79.17CD
Mean	87.04A	87.78A	85.56A	80.74B	
LSD for G	6.7819				
LSD for T	4.5213				
LSD for G × T	13.564				

Table 1. Germination percentage for nine wheat genotypes under four treatments (T1 = control, T2 = $PEG_{-0.50}$, T3 = ($PEG_{-1.48}$) and T4 = ($PEG_{-2.95}$)).

genotypes at three leaf stage. All the treatments differed significantly for root length at three leaf stage (Z-13) for wheat crop (**Table 3**). Maximum root length recorded for T2 (10.9 cm)

Genotypes	Control	$PEG_{-0.50}$	$PEG_{-1.48}$	$PEG_{-2.95}$	Mean
Dhurabi	0.08i–n	0.10g–l	0.010e–k	0.10d–j	0.09BC
Fareed-06	0.12b–d	0.09h–m	0.08k–o	0.10b–h	0.09B
NARC-11	0.10b–h	0.10c–i	0.07l–p	0.07m–p	0.08BC
Sehar-06	0.12a–c	0.11b–d	0.10b–g	0.12ab	0.11A
Punjab-11	0.12b–d	0.09g–m	0.07n–p	0.07m–p	0.09C
Wafaq-2001	0.11b–e	0.10d–j	0.07n–p	0.06op	0.08C
NARC-2009	0.14a	0.14a	0.09f–l	0.12ab	0.12A
BARS-09	0.10b–h	0.10b–h	0.08j–m	0.09f–l	0.09B
Lasani-08	0.11b–f	0.10b–h	0.07l–p	0.06p	0.09C
Mean	0.11A	0.10B	0.08D	0.09C	
LSD for G	0.00904		0.242374		
LSD for T	0.006027		0.250676		
LSD for G × T	0.0181		0.569639		

Table 2. Root length for 9 wheat genotypes under 4 treatments (T1 = control, T2 = $PEG_{-0.50}$, T3 = ($PEG_{-1.48}$) and T4 = ($PEG_{-2.95}$)).

Genotypes	Control	$PEG_{-0.50}$	$PEG_{-1.48}$	$PEG_{-2.95}$	Mean
Dhurabi	6.56p–r	14.20ab	8.73l–p	6.52qr	9.00EF
Fareed-06	10.97e–k	10.53f–l	11.27d–j	12.07b–i	11.21C
NARC-11	7.07n–q	8.83k–o	9.23j–n	12.5a–f	9.43DE
Sehar-06	14.54a	10.41f–l	13.29a–d	11.25d–j	12.38B
Punjab-11	12.55a–g	12.42a–i	10.39g–l	6.70o–r	10.52CD
Wafaq-2001	11.67d–i	8.95k–n	8.73l–p	7.93m–q	9.32EF
NARC-2009	12.83a–e	14.24ab	14.54a	14.04a–c	13.92A
BARS-09	9.2j–n	7.45n–q	9.9i–m	10.35h–l	9.23EF
Lasani-08	10.23h–l	11.94c–i	6.42qr	4.51r	8.28F
Mean	10.62A	10.99A	10.28AB		9.55B
LSD for G	1.0972				
LSD for T	0.7315				
LSD for G × T	2.1944				

Table 3. Root fresh weight for 9 wheat genotypes under 4 treatments (T1 = control, T2 = $PEG_{-0.50}$, T3 = ($PEG_{-1.48}$) and T4 = ($PEG_{-2.95}$)).

whereas, minimum root length recorded for T4 (9.6 cm) at three leaf stage. Meanwhile, wheat genotypes differed significantly for root length. Genotype NARC-2009 obtained maximum root length (13.9 cm) at three leaf stage however, genotype Lasani-08 obtained minimum root length (8.3 cm). The interactive effect G x T was highly significant at 1% P level. Maximum root length was recorded for Sehar-06 under T1 (14.5 cm) followed by NARC-2009 under T4 (14.0 cm) whereas, minimum root length was recorded for Lasani-08 under T4 (4.5 cm).

Results illustrated significant difference for shoot length for different treatments on wheat genotypes at three leaf stage. All the treatments differed potentially for shoot length (**Table 4**). Maximum shoot length was recorded for T1 (10.8 cm) while minimum shoot length was recorded for T3 (7.9 cm). In the same way all the wheat genotypes varied considerably for shoot length. Highest shoot length was observed for NARC-2009 (11.7 cm) followed by Sehar-06 (11.1 cm) whereas, lowest shoot length was observed for genotype Lasani-08 (8.4 cm). There was 29% difference among genotypes for shoot length. In the meanwhile, the interactive effect was highly significant for shoot length. Highest shoot length was recorded under T1 for NARC-2009 (13.0 cm) while lowest shoot length was recorded under T4 for Lasani-08 (5.6 cm). There was 56% difference among genotypes under different treatments.

All the treatments varied considerably for shoot fresh weight (**Table 5**). Maximum shoot fresh weight was recorded for T1 (0.21 g) while minimum weight was recorded for T4 (0.15 g). There was 27% difference among different treatments. Highest shoot fresh weight was gained by genotype NARC-2009 (0.23 g) while lowest shoot fresh weight gained by genotype Dhurabi (0.15 g). There was 35% difference among genotypes for shoot fresh weight. The interactive effect was significantly different under all the treatments. Highest shoot fresh weight was

Genotypes	Control	$PEG_{-0.50}$	$PEG_{-1.48}$	$PEG_{-2.95}$	Mean
Dhurabi	8.2j–o	8.7h–m	9.13f–l	9.63e–k	8.92BCD
Fareed-06	11.07b–e	8.53i–n	7.57l–p	9.9c–j	9.27BC
NARC-11	9.9c–j	9.8d–j	7.33m–q	6.9n–q	8.48CD
Sehar-06	11.44a–d	11b–e	10.30b–g	11.59a–c	11.08A
Punjab-11	11.04b–e	8.62h–n	6.65o–q	6.90n–q	8.30D
Wafaq-2001	10.83b–f	9.56e–k	6.62o–q	6.02pq	8.26D
NARC-2009	13.04a	13.039a	8.92g–m	11.64ab	11.66A
BARS-09	11.23b–e	10.03b–i	8.02k–o	9.03g–m	9.58B
Lasani-08	10.53b–g	9.93b–i	7.32m–q	5.62q	8.35D
Mean	10.81A	9.91B	7.98D	8.58C	
LSD for G	0.8636				
LSD for T	0.5757				
LSD for G × T	1.7272				

Table 4. Shoot length for 9 wheat genotypes under 4 treatments (T1 = control, T2 = $PEG_{-0.50}$, T3 = ($PEG_{-1.48}$) and T4 = ($PEG_{-2.95}$)).

accumulated for genotype NARC-2009 under T1 and T2 (0.26 g) while minimum was accumulated for Lasani-08 under T4 (0.11 g).

Genotypes	Control	$PEG_{-0.50}$	$PEG_{-1.48}$	$PEG_{-2.95}$	Mean
Dhurabi	0.16i–m	0.17g–l	0.15k–o	0.12no	0.15D
Fareed-06	0.19b–i	0.17g–l	0.15k–n	0.12no	0.15D
NARC-11	0.17h–m	0.19c–j	0.15k–o	0.14l–o	0.16D
Sehar-06	0.23a–c	0.22b–d	0.20b–g	0.19b–h	0.21B
Punjab-11	0.22b–d	0.17g–l	0.13m–o	0.14l–o	0.16D
Wafaq-2001	0.21b–e	0.19d–j	0.13m–o	0.12no	0.16D
NARC-2009	0.26a	0.26a	0.18f–k	0.23ab	0.23A
BARS-09	0.12b–h	0.19b–h	0.16j–m	0.18e–k	0.18C
Lasani-08	0.21b–f	0.19b–h	0.15k–o	0.11o	0.17D
Mean	0.21A	0.20A	0.15B	0.15B	
LSD for G	0.0179				
LSD for T	0.012				
LSD for G × T	0.0359				

Table 5. Shoot fresh weight for 9 wheat genotypes under 4 treatments (T1 = control, T2 = $PEG_{-0.50}$, T3 = ($PEG_{-1.48}$) and T4 = ($PEG_{-2.95}$)).

Genotypes	Control	$PEG_{-0.50}$	$PEG_{-1.48}$	$PEG_{-2.95}$	Mean
Dhurabi	0.06h–l	0.07d–j	0.06j–m	0.04lm	0.06C
Fareed-06	0.09d–i	0.07e–j	0.07h–l	0.04lm	0.07C
NARC-11	0.07g–k	0.08c–g	0.06j–m	0.05k–m	0.07C
Sehar-06	0.10ab	0.09bc	0.09b–d	0.09b–e	0.09A
Punjab-11	0.09b–f	0.07e–j	0.06k–m	0.06l–m	0.07BC
Wafaq-2001	0.08c–f	0.08c–h	0.06k–m	0.05lm	0.07BC
NARC-2009	0.12a	0.12a	0.08d–h	0.10ab	0.10A
BARS-09	0.08d–h	0.09c–f	0.07g–k	0.07f–k	0.08B
Lasani-08	0.08c–g	0.09c–f	0.06i–m	0.04m	0.07BC
Mean	0.09A	0.08A	0.07B	0.06B	
LSD for G	0.008949				
LSD for T	0.005966				
LSD for G × T	0.0179				

Table 6. Shoot dry weight for 9 wheat genotypes under 4 treatments (T1 = control, T2 = $PEG_{-0.50}$, T3 = ($PEG_{-1.48}$) and T4 = ($PEG_{-2.95}$)).

The treatments varied statistically for shoot dry weight (**Table 6**). Highest shoot dry weight was observed under T1 (0.05 g) while minimum shoot dry weight recorded for T3 (0.04 g). In the same way, genotypes varied potentially for shoot dry weight. The highest shoot dry weight was recorded for genotype NARC-2009 (0.06 g) while, lowest shoot dry weight was recorded for genotype Lasani-08 (0.04 g). There was 23% difference among genotypes for shoot dry weight. In the meanwhile, the interactive effects differed considerably for shoot dry weight under all the treatments. Maximum shoot dry weight was accumulated by NARC-2009 under T1 (0.06 g) whereas, minimum shoot dry weight was accumulated by Lasani-08 under T4 (0.02 g).

The results depicted that there was great difference among treatments and genotypes for root dry weight (**Table 7**). Maximum root dry weight was accumulated for T4 (0.05 g) while minimum root dry weight was recorded for T3 (0.04 g). Similarly, all the genotypes varied potentially for root dry weight. Highest root dry weight was accumulated by genotype NARC-2009 (0.06 g) fallowed by Sehar-06 (0.52 g) while lowest by Lasani-08 (0.04 g). In the same way, the interactive effect for T × G was highly significant. Maximum root dry weight was obtained by NARC-2009 under T4 (0.06 g) while minimum root dry weight was obtained by Lasani-08 under T4 (0.23 g). Maximum root to shoot ratio for fresh weight calculated for T1 (1.08) while minimum was calculated for T3 (0.81) (**Table 8**). In the same way all the genotypes differed significantly for root to shoot ratio. Highest root to shoot ratio was calculated for Dhurabi (1.10) whereas, lowest was calculated for Punjab-11 (0.81). Meanwhile, the interactive effects were highly significant at 1% P level. Highest root to shoot ratio was calculated for NARC-11 under T1 (1.57) while lowest for Wafaq-2001 under T4 (0.76). On the basis of screening results two genotypes were selected for experiment II. Genotypes NARC-2009 and Sehar-06 performed better under treatment 4 so these two genotypes were selected.

Genotypes	Control	$PEG_{-0.50}$	$PEG_{-1.48}$	$PEG_{-2.95}$	Mean
Dhurabi	0.04e–o	0.04f–k	0.04f–k	0.04f–k	0.04B
Fareed-06	0.05c–g	0.04f–m	0.03i–p	0.04f–k	0.04B
NARC-11	0.04f–j	0.04e–h	0.032k–p	0.03n–p	0.04B
Sehar-06	0.06a–d	0.05b–e	0.05e,f	0.06a–c	0.05A
Punjab-11	0.05c–g	0.04f–l	0.03l–p	0.03m–p	0.04B
Wafaq-2001	0.05d–g	0.04e–i	0.03m–p	0.03op	0.04B
NARC-2009	0.06a	0.06ab	0.04e–i	0.06a–c	0.06A
BARS-09	0.04e–i	0.05e–g	0.04h–n	0.04g–n	0.04B
Lasani-08	0.04e–h	0.05e–h	0.03j–p	0.02p	0.04B
Mean	0.05A	0.05A	0.04B	0.04B	
LSD for G	0.00488				
LSD for T	0.003254				
LSD for G × T	0.009761				

Table 7. Root dry weight for 9 wheat genotypes under 4 treatments (T1 = control, T2 = $PEG_{-0.50}$, T3 = ($PEG_{-1.48}$) and T4 = ($PEG_{-2.95}$)).

4.2. Polythene bags results

4.2.1. Shoot length

NARC-2009 exhibited maximum shoot length (6.28) at three leaf stage than Sehar-06 (5.28) (**Table 9**). All the treatments showed significant difference for shoot length at three leaf stage. Highest shoot length was recorded for T7 (8.1 cm) followed by T6 and T8 while lowest shoot length shoot length was recorded for T1 (3.47). In the same way, the interactive effect was also found significant. Maximum shoot length was recorded for NARC-2009 under T7 (8.8 cm) followed by NARC-2009 under T8 (8.54 cm) and Sehar-06 under T7 (8.40 cm) whereas, minimum shoot length was recorded for Sehar-06 under T1 (3.1 cm). NARC-2009 exhibited higher shoot length (63.75 cm) than Sehar-06 (54.37 cm). Meanwhile, all the treatments exhibited significant difference for shoot length at anthesis stage (**Table 9**). Highest shoot length was recorded for T7 (69.00 cm) followed by T8 (64.45), T5 (59.88 cm) and T6 (59.11 cm) while lowest shoot length was recorded by T1 (47.61 cm). In the same way, the interactive effects were varied potentially. Highest shoot length was recorded for NARC-2009 (71.64 cm) under T7 while lowest shoot length was recorded for Sehar-06 under T1 (42.88 cm). Maximum shoot length calculated for NARC-2009 (63.75 cm) while minimum shoot length was calculated for Sehar-06 (54.37 cm) (**Table 9**). In the meanwhile, all the treatments differed significantly for shoot length at maturity stage. Highest shoot length was calculated for T7 (75.21 cm) followed by T8, T5, T4 and T6 while, lowest was calculated for T1 (51.89 cm). Meanwhile, the interactive effects were highly significant at 1% P level. Highest shoot length was calculated for NARC-2009 under T7 (78.08 cm) while lowest for Sehar-06 under T1 (46.74). Crop growth and development is

Genotypes	Control	$PEG_{-0.50}$	$PEG_{-1.48}$	$PEG_{-2.95}$	Mean
Dhurabi	1.26b–d	0.62mn	1.05d–h	1.49ab	1.10A
Fareed-06	1.01d–j	0.84g–m	0.67k–n	0.83g–m	0.84C
NARC-11	1.57a	1.13c–f	0.83g–m	0.55n	1.02AB
Sehar-06	0.79i–n	1.08d–g	0.78i–n	1.03d–i	0.92BC
Punjab-11	0.88f–l	0.69k–n	0.64l–n	1.03d–i	0.81C
Wafaq-2001	0.93e–k	1.07d–h	0.76j–n	0.76j–n	0.88C
NARC-2009	1.02d–j	0.92e–k	0.62l–n	0.83g–m	0.85C
BARS-09	1.22cd	1.35a–c	0.81h–m	0.88g–m	1.06A
Lasani-08	1.03d–i	0.83g–m	1.14c–e	1.24b–d	1.06A
Mean	1.08A	0.95B	0.81C	0.96B	
LSD for G	0.1294				
LSD for T	0.0863				
LSD for G × T	0.2588				

Table 8. Shoot to root ratio for 9 wheat genotypes under 4 treatments (T1 = control, T2 = $PEG_{-0.50}$, T3 = ($PEG_{-1.48}$) and T4 = ($PEG_{-2.95}$)).

primarily dependent upon biotic and abiotic environment prevailing in the vicinity of plants. Roots are the main source of nutrients supply to the plant nutrients to the plant. Our results were in line with earlier work who was of the point of view that phosphorus has been reported to increase the strength of cereal straw, stimulate root development, promote flowering, fruit production, and formation of seed and hasten maturity of the crops [13]. Due to increased availability of P leaf area, green pigments also increased and hence the shoot length increased finally. Our results were supported by Zhang et al. [49] who reported that deficiency of P reduced photosynthetic efficiency in wheat due to reduction in leaf area expansion.

4.2.2. Root length

Both the genotypes varied considerably for root length (**Table 10**) at three leaf stage. Maximum root length was calculated for NARC-2009 (4.3 cm) whereas, minimum root length (3.6 cm) calculated for Sehar-06. There was 14% difference among both the genotypes. Similarly, there was a major difference among all the treatments. Maximum root length (4.6 cm) calculated for treatment T7, minimum root length (3.2 cm) calculated for treatment T1 followed by T2. There was 31% difference among maximum and minimum treatments for root length. The interactions were significant at 1% P level for root length. Highest root length was recorded for NARC-2009 under T7 (4.8 cm) while lowest root length recorded for Sehar-06 (2.9 cm). Wheat genotypes varied considerably for root length at anthesis stage (**Table 10**). Genotype NARC-2009 accumulated highest root length (43.0 cm) whereas, genotype Sehar-06 accumulated lowest root length (35.3 cm). The percentage difference among both genotypes for number of seeds per spike was 18%. In the meanwhile, all the treatments varied noticeably for

Treatments	Three leaf		Mean	Anthesis		Mean	Maturity		Mean
	G1	G2		G1	G2		G1	G2	
T1	3.17d	3.7733d	3.4717D	42.883d	52.337cd	47.61C	46.742d	57.046cd	51.894C
T2	4.2267cd	5.03cd	4.6283CD	53.093b–d	55.72a–d	54.407BC	57.87b–d	60.734a–d	59.302BC
T3	5.2833b–d	6.2867bc	5.785BC	53.093b–d	59.7a–c	56.397BC	57.87b–d	65.072a–c	61.471BC
T4	4.2267cd	5.03cd	4.6283CD	53.093b–d	66.663a–c	59.878AB	57.87b–d	72.664a–c	65.267AB
T5	5.2833b–d	6.2867bc	5.785BC	54.627b–d	68.653ab	61.64AB	59.54b–d	74.833ab	67.186AB
T6	6.34bc	7.5433ab	6.9517AB	51.563cd	66.663a–c	59.113AB	56.201cd	72.664a–c	64.432AB
T7	7.3967ab	8.8a	8.0983A	66.367a–c	71.64a	69.003A	72.338a–c	78.086a	75.212A
T8	6.34bc	7.5433ab	6.9417AB	60.24a–c	68.653ab	64.447AB	65.661a–c	74.833ab	70.247AB
Mean	5.2833B	6.2867A		54.37B	63.754A		59.262B	69.492A	
LSD for G	0.8206			5.6734			6.1844		
LSD For T	1.6412			11.347			12.369		
LSD for G × T	2.3211			16.047			17.492		

Table 9. Shoot length for both genotypes at three leaf, anthesis and maturity.

Treatments	Three leaf		Mean	Anthesis		Mean	Maturity		Mean
	G1	G2		G1	G2		G1	G2	
T1	2.5d	3.49cd	3.17C	35.1b	35.1b	35.1B	34.2b	34.2b	34.2B
T2	3.54b–d	3.71a–d	3.63BC	38.2b	39.3b	38.8B	37.2b	38.2b	37.7B
T3	3.54b–d	3.97a–c	3.75BC	40.3b	63.0a	51.7A	39.2b	61.3a	50.2A
T4	3.54b–d	4.44a–c	3.99AB	31b	32.0b	31.5B	30.1b	31.1b	30.6B
T5	3.64b–d	4.57ab	4.11AB	29.9b	25.8b	27.9B	29.1b	25.1b	27.1B
T6	3.44cd	4.44a–c	3.94AB	37.2b	41.3b	39.3B	36.2b	40.2b	38.2B
T7	4.42a–c	4.77a	4.60A	40.3b	69.2a	54.8A	39.2b	67.3a	53.2A
T8	4.02a–c	4.57ab	4.29AB	29.9b	38.2b	34.1B	29.1b	37.2b	33.1B
Mean	3.63B	4.25A		35.3B	43.0A		34.3B	41.8A	
LSD for G	0.3783			5.8271			5.6636		
LSD For T	0.7565			11.654			11.327		
LSD for G × T	1.0699			16.482			16.019		

Table 10. Root length for both genotypes at three leaf, anthesis and maturity.

root length at anthesis stage. Maximum root length was recorded for T7 (54.8 cm) followed by T3 (51.7 cm) whereas, minimum root length was recorded for T1 (35.13 cm) at anthesis stage. There was 49% difference between T7 and T1 for root length. Similarly, the interactive effects were highly variable at anthesis stage. Highest root length (69.2 cm) was recorded for NARC-2009 under T7 while lowest root length (29.9 cm) was recorded for Sehar-06 under T7. Both the wheat genotypes varied considerably for root length at maturity stage (**Table 10**). NARC-2009 accumulated maximum root length (41.8 cm) while Sehar-06 accumulated minimum root length (34.3 cm). There was 18% difference among both the genotypes for root length accumulation. In the same way all the treatments varied significantly for root length. Highest root length was recorded was recorded for T7 (53.2 cm) followed by T3 (50.2 cm) and lowest root length was recorded for T5 (27.1 cm). There was 49% difference between T7 and T5. Similarly, the interactive effects were also significantly different at 1% P level for root length accumulation at maturity stage. Highest root length was recorded for NARC-2009 under T7 (67.3 cm) while lowest for Sehar-06 under T8 (29.1 cm). In the stress environment the length of roots increased to ensure proper supply of nutrients to the plant body. Phosphorus application enhanced root length to ensure better nutrient supply to the plant body. Our results were in accordance with Fahad and Bano [13] who stated that nutrient enhanced the crop stress tolerance hence help in root elongation. It would be advantageous if we select, screen or improve plants for higher capacity to adapt to mineral stresses. This approach is beneficial in developing countries like Pakistan where capital input resources are limited. Farmers in these countries require nutrient efficient crop cultivars which perform better or do something better than other cultivars when given a considerable amount of mineral nutrient.

4.2.3. Shoot dry weight

Both the genotypes differed considerably for shoot dry weight accumulation (**Table 11**). The results depicted that maximum shoot dry weight was accumulated by NARC-2009 (0.21 g) while minimum shoot fresh weight was accumulated by Sehar-06 (0.14 g) at three leaf stage. There was 32% variation among both the genotypes for shoot dry weight accumulation at three leaf stage. On the other hand, all the treatments exhibited significant difference for shoot dry weight at three leaf stage. Highest shoot dry weight was recorded for T7 (0.25 g) while lowest shoot dry weight was recorded by T4 (0.11 g). There was 57% difference among higher and lower treatments. In the same way, the interactive effects varied potentially. Highest shoot dry weight recorded for NARC-2009 (0.29 g) under T7 while lowest shoot dry weight was recorded for Sehar-06 under T4 (0.08 g). There was 61% difference among maximum and minimum shoot dry weights. Genotype NARC-2009 and Sehar-06 did not varied potentially for shoot dry weight at anthesis stage (**Table 11**). Whereas, all the treatments varied noticeably for shoot dry weight at anthesis stage. Maximum shoot dry was recorded for T7 (1.59 g) followed by other treatments except T4 which accumulated minimum shoot dry weight (1.28 g) at anthesis stage. There was 19% difference between T7 and T4 for shoot dry weight at anthesis. Similarly, the interactive effects were highly variable at anthesis stage. Highest shoot dry weight (1.66 g) was recorded for NARC-2009 under T7 while lowest shoot dry weight (1.23 g) was recorded for Sehar-06 under T4. There was 25% difference among highest and lowest shoot dry weight under all the treatments for both the genotypes. Both the genotypes did not differ considerably for shoot dry weight accumulation at maturity stage (**Table 11**). On the other hand, all the treatments exhibited significant difference for shoot dry weight at maturity stage. Maximum shoot dry weight was accumulated for T7 (2.55 g) while minimum shoot dry weight was recorded by T4 (2.06 g). There was 19% difference among higher and lower

Treatments	Three leaf		Mean	Anthesis		Mean	Maturity		Mean
	G1	G2		G1	G2		G1	G2	
T1	0.08c	0.13bc	0.11C	1.30ab	1.42ab	1.36AB	2.09ab	2.28ab	2.18AB
T2	0.11c	0.17a–c	0.14BC	1.44ab	1.57ab	1.50AB	2.30ab	2.51ab	2.40AB
T3	0.14bc	0.21a–c	0.18A–C	1.3ab	1.42ab	1.36AB	2.08ab	2.27ab	2.18AB
T4	0.11c	0.17a–c	0.14BC	1.23b	1.34ab	1.29B	1.97b	2.15ab	2.06B
T5	0.14bc	0.21a–c	0.18A–C	1.28b	1.40ab	1.34AB	2.06b	2.25ab	2.15AB
T6	0.17a–c	0.25ab	0.21AB	1.31ab	1.43ab	1.37AB	2.10ab	2.29ab	2.19AB
T7	0.20a–c	0.29a	0.25A	1.52ab	1.66a	1.59A	2.44ab	2.66a	2.55A
T8	0.17a–c	0.25ab	0.21AB	1.44ab	1.57ab	1.50AB	2.30ab	2.51ab	2.40AB
Mean	0.14B	0.21A		1.35NS	1.48		2.16NS	2.37	
LSD for G	0.0477			0.1321			0.2118		
LSD For T	0.0954			0.2642			0.4236		
LSD for G × T	0.1349			0.3736			0.5991		

Table 11. Shoot dry weight for both genotypes at three leaf, anthesis and maturity.

treatments. In the same way, the interactive effects varied considerably. Highest shoot dry weight was recorded for NARC-2009 (2.66 g) under T7 followed by all other treatments except Sehar-06 under T4 (1.97 g) fallowed by Sehar-06 under T5 (1.97 g). There was 26% difference among maximum and minimum shoot dry weights. Root signaling influence directly above ground biomass production. With the application of phosphorus roots were able to penetrate deep in the soil to provide better nutrients to the above ground parts. Our results were in accordance to Dewal and Pareek, [50] who stated that dry matter production increased by the addition of phosphorus. Similar results were also reported by Swarup and Yaduvanshi, [51] who concluded that fertilization of crop with phosphatic compounds resulted in enhanced dry matter accumulation.

4.2.4. Root dry weight

Balanced plant nutrition encourages above and below ground plant growth development. Both the genotypes differed considerably for root dry weight at three leaf stage (**Table 12**). Genotype NARC-2009 accumulated maximum root dry weight (0.16 g) while Sehar-06 accumulated minimum root dry weight (0.11 g). There was 31% difference among genotypes for root dry weight at three leaf stage. All the treatments were statistically varied for root dry weight at three leaf stage. The highest root dry weight was recorded for treatment T7 (0.195 g) while, lowest root dry weight was recorded for T1 (0.08 g). In the same way, the interactive effects differed considerably for root dry weight under all the treatments for both genotypes at three leaf stage. Maximum root dry weight was accumulated by NARC-2009 under T7 (0.23 g) whereas, minimum root dry weight was accumulated by Sehar-06 under T1 (0.07 g). Both the genotypes did not varied considerably for root dry weight at anthesis stage (**Table 12**). On the other hand, all the treatments varied noticeably for root dry weight at anthesis stage. Maximum root dry was

Treatments	Three leaf		Mean	Anthesis		Mean	Maturity		Mean
	G1	G2		G1	G2		G1	G2	
T1	0.07c	0.09bc	0.08C	1.2ab	1.31ab	1.26AB	1.53ab	1.66ab	1.59AB
T2	0.09bc	0.13a–c	0.11BC	1.32ab	1.45ab	1.39AB	1.68ab	1.83ab	1.76AB
T3	0.11bc	0.16a–c	0.14ABC	1.2ab	1.31ab	1.25AB	1.52ab	1.66ab	1.59AB
T4	0.09bc	0.13a–c	0.11BC	1.14b	1.24ab	1.19B	1.44b	1.57ab	1.51B
T5	0.11bc	0.16a–c	0.14ABC	1.19b	1.29ab	1.24AB	1.50b	1.64ab	1.57AB
T6	0.13a–c	0.19ab	0.17AB	1.21ab	1.32ab	1.27AB	1.54ab	1.68ab	1.61AB
T7	0.16a–c	0.23a	0.19A	1.41ab	1.53a	1.47A	1.79ab	1.95a	1.87A
T8	0.13a–c	0.19ab	0.16AB	1.32ab	1.45ab	1.39AB	1.68ab	1.83ab	1.76AB
Mean	0.12B	0.16A		1.25NS	1.37		1.58NS	1.73	
LSD for G	0.0368			0.122			0.1547		
LSD For T	0.0736			0.2439			0.3095		
LSD for G × T	0.1041			0.3449			0.4376		

Table 12. Root dry weight for both genotypes at three leaf, anthesis and maturity.

recorded for T7 (1.47 g) fallowed by other treatments except T4 which accumulated minimum root dry weight (1.18 g) at anthesis stage. There was 24% difference between T7 and T4 for root dry weight at anthesis. Similarly, the interactive effects were highly variable at anthesis stage for root dry weight at anthesis stage. Highest root dry weight (1.53 g) was recorded for NARC-2009 under T7 while lowest shoot dry weight (1.14 g) was recorded for Sehar-06 under T4. There was 25% difference among highest and lowest root dry weight under all the treatments for both the genotypes. Both the genotypes were not varied potentially for root dry weight at maturity (**Table 12**). In the meanwhile, all the treatments differed significantly for root dry weight at maturity stage. Highest root dry weight was calculated for T7 (1.86 g cm) followed by all other treatments while, lowest was calculated for T4 (1.51 g). There was 19% variation among highest and lowest treatments for root dry weight. Meanwhile, the interactive effects were highly significant at 1% P level for root dry weight. Highest root dry weight was recorded for NARC-2009 under T7 (1.94 g) while lowest for Sehar-06 under T4 (1.44 g).

4.2.5. Root-shoot ratio

Both the genotypes were non-significant for root to shoot ratio at three leaf stage (**Table 13**). Whereas, all the treatments varied noticeably for root to shoot ratio at three leaf stage. Maximum root to shoot ratio recorded for T1 (0.92) while minimum root to shoot ratio calculated for T6 (0.57) at three leaf stage. There was 38% difference between T1 and T6 for root to shoot ratio at three leaf stage. Similarly, the interactive effects were highly variable at three leaf stage for root to shoot ratio at three leaf stage. Highest root to shoot ratio (0.94) was recorded for NARC-2009 under T1 followed by Sehar-06 under T1 (0.90) while lowest root to shoot ratio (0.54 g)

Treatments	RSRT		Mean	RSRA		Mean	RSRM		Mean
	G1	G2		G1	G2		G1	G2	
T1	0.90a	0.95a	0.93A	0.82b	0.66c–e	0.74BC	0.73b	0.59c–e	0.66BC
T2	0.84ab	0.76bc	0.79BC	0.72b–d	0.69cd	0.71BC	0.64b–d	0.63b–d	0.63BC
T3	0.67c–f	0.65c–g	0.66DE	0.76bc	1.05a	0.91A	0.67bc	0.93a	0.81A
T4	0.84ab	0.90a	0.87AB	0.58e–h	0.48hi	0.53D	0.52e–g	0.42gh	0.47D
T5	0.69c–e	0.75b–d	0.72CD	0.55f–h	0.37i	0.46D	0.49fg	0.33h	0.41D
T6	0.54g	0.60e–g	0.57F	0.72b–d	0.61d–f	0.67C	0.64b–d	0.55d–f	0.59C
T7	0.59e–g	0.56fg	0.58EF	0.64d–g	0.96a	0.78B	0.54d–f	0.85a	0.69B
T8	0.63d–g	0.62e–g	0.63EF	0.49gh	0.55e–h	0.52D	0.44fg	0.49e–g	0.47D
Mean	0.71NS	0.72		0.66NS	0.67		0.59NS	0.6	
LSD for G	0.0426			0.041			0.0368		
LSD For T	0.0852			0.082			0.0737		
LSD for G × T	0.1204			0.116			0.1042		

Table 13. Root-shoot ratio for both genotypes at three leaf, anthesis and maturity.

was recorded for Sehar-06 under T6. There was 42% difference among highest and lowest root dry weight under all the treatments for both the genotypes. Both the wheat genotypes did not varied considerably for root to shoot ratio at anthesis stage (**Table 13**). In the meanwhile, all the treatments varied significantly for root to shoot ratio at anthesis stage. Highest root to shoot ratio was recorded for T3 (0.90) and lowest root to shoot ratio recorded for T5 (0.46). There was 48% difference between T3 and T5. Similarly, the interactive effects were also significantly different at 1% P level for root to shoot ratio at anthesis stage. Highest root to shoot ratio was recorded for NARC-2009 under T3 (1.05) followed by NARC-2009 under T7 (0.96) while lowest for NARC-2209 under T5 (0.37). There was 48% difference among highest and lowest root dry weight under all the treatments for both the genotypes at anthesis stage.

Both the genotypes were not different for root to shoot ratio at maturity stage (**Table 13**). In the meanwhile, all the treatments differed significantly for root to shoot ratio at maturity stage. Highest root to shoot ratio was calculated for T3 (0.81) followed by all other treatments while, lowest was calculated for T5 (0.41). There was 49% variation among highest and lowest treatments for root to shoot ratio at maturity stage. Meanwhile, the interactive effects were highly significant at 1% P level for root to shoot ratio. Highest root to shoot ratio was recorded for NARC-2009 under T3 (0.93) fallowed by NARC-2009 under T7 (0.85) while lowest for NARC-2209 under T5 (0.33). There was 64% difference among highest and lowest root dry weight under all the treatments for both the genotypes at maturity stage. Root architecture is a highly plastic and environmentally responsive trait that enables plants to counteract nutrient scarcities with different forging strategies [7]. Root-specific traits such as root system architecture, sensing of edaphic stress and root-to-shoot communication can be exploited to improve resource capture (water and nutrients) and plant development under resource-limited conditions [8].

Treatments\genotypes	Sehar-06	NARC-2009	Mean
T1	8.4ab	9.1ab	8.7AB
T2	9.2ab	10.0ab	9.6AB
T3	8.3ab	9.1ab	8.7AB
T4	7.9b	8.6ab	8.2B
T5	8.2b	8.9ab	8.6AB
T6	8.4ab	9.2ab	8.8AB
T7	9.8ab	10.7a	10.2A
T8	9.2ab	10.0ab	9.6AB
Mean	8.7B	9.5A	
LSD for G	0.7473		
LSD for T	1.6947		
LSD for G × T	2.3966		

Table 14. Spike length for both genotypes at maturity.

4.2.6. Spike length

Spike length of the both wheat genotypes differed considerably due to their genetic characteristics (**Table 14**). The results illustrated that the higher spike length was recorded for the NARC-2009 (9.5 cm) against Sehar-06 (8.7 cm). The difference between both genotypes was 8%. While discussing about treatments, maximum spike length was recorded for T7 (10.2 cm), while the minimum spike length was noticed under T4 (8.2 cm). There was 14% difference among highest and lowest treatments. Similarly, the interactive effect was significant. Highest spike length was recorded for NARC-2009 under T7 (10.7 cm) while lowest spike length was observed for Sehar-06 under T4 (7.89 cm). There was 21% variation for spike length among highest and lowest interactions. Root signaling played a vital role in the development of the good source-sink relationship. Maximum spike length is produced as translocation of more photo-assimilates takes place efficiently from source to sink. Balanced application of P fertilizers and their availability might be another reason of spike length increment. Our findings were in accordance with Dewal and Pareek [50] and Memon [52] who reported increment in spike length with the addition of P fertilizers. Our results were also confirmed by the findings of Hussain [53] who reported increase in spike length due to P addition.

4.2.7. Spikelets per spike

Spikelets per spike of the both wheat genotypes varied noticeably due to their genetic characteristics (**Table 15**). The results depicted that the higher spikelets per spike was observed for the NARC-2009 (2.7) against Sehar-06 (2.5). The difference between both genotypes was 8%. As regards to treatments, maximum spike length was recorded for T7 (2.9), while the minimum spike length (2.4) was noticed under T4. There was 15% difference among highest and lowest treatments. Similarly, the interactive effect was significant. Highest spike length was recorded for NARC-2009 under T7 (3.1) while lowest spikelets per spike were observed for Sehar-06 under T4 (2.3). There was 22% variation for spikelets per spike among highest and lowest interactions. The variation in number of spikelets per spike might be due to balanced

Treatments/genotypes	Sehar-06	NARC-2009	Mean
T1	2.4ab	2.6ab	2.5AB
T2	2.6ab	2.9ab	2.8AB
T3	2.4ab	2.6ab	2.5AB
T4	2.3b	2.ab	2.4B
T5	2.4b	2.6ab	2.5AB
T6	2.4ab	2.6ab	2.5AB
T7	2.8ab	3.1a	2.9A
T8	2.6ab	2.9ab	2.8AB
Mean	2.5B	2.7A	
LSD for G	0.2234		
LSD for T	0.4867		
LSD for G × T	0.6883		

Table 15. Spikelets per spike for both genotypes at maturity.

application of P fertilizers and enhanced availability as well as uptake of phosphorus through root signaling by plants. Another reason might be spike length which consumes available nutrient resources as well as temperature in more proficient way and accumulated photo-assimilates efficiently. As P application and availability support growth and developmental process in plants through root signaling such as photosynthesis, energy storage, transfer, cell division as well as cell elongation so it also promotes spikelets initiation and finally increases number of spikelets per spike. Similar results were reported by Memon [52] who observed a significant increase in number of spikelets per spike by the application of P fertilizers through enhanced root signaling.

4.2.8. Number of grains per spike

Both the genotypes varied potentially for number of grains per spike (**Table 16**). NARC-2009 exhibited maximum number of grains per spike (23.56) than Sehar-06 (21.45). There was 9% difference among both the genotypes for number of grains per spike. All the treatments showed significant difference for number of grains per spike. Highest number of grains per spike was recorded for T7 (26.99) while lowest number of grains per spike was recorded for T4 (20.07). In the same way, the interactive effect was also found significant. Maximum number of grains per spike was recorded for NARC-2009 under T7 (28.21) whereas, minimum number of grains per spike was recorded for Sehar-06 under T4 (19.21). Root signaling played a vital role in enhancing number of grains per spike. By applying phosphorus root signaling enhanced in the crop. Sufficient availability and the uptake of P facilitate the crop to grow more rapidly and it also enables the crop to capture more solar radiations and consequently more number of grains per spike produced. Insufficiency of P undersized the growth of stem as well as whole plant. However, the addition of P encourages the plant growth which results in increase in number of spikelets per spike due to better root signaling. With the increase in number of spikelets per spike, number of grains per spike also increased. The results of present study were in line with Ali et al. [54] and Dewal and Pareek [50] who observed the reduction in number of grains per

Treatments/genotypes	Sehar-06	NARC-2009	Mean
T1	20.32cd	21.81b–d	21.07BC
T2	22.41a–d	24.42a–d	23.42A–C
T3	20.27cd	22.09a–d	21.19BC
T4	19.21d	20.93b–d	20.07C
T5	20.06cd	21.87b–d	20.97BC
T6	20.85b–d	22.33a–d	21.59BC
T7	25.79a–c	28.21a	26.99A
T8	22.66a–d	26.84ab	24.75AB
Mean	21.45B	23.56A	
LSD for G	2.1023		
LSD for T	4.4173		
LSD for G × T	6.267		

Table 16. Number of grains per spike for both genotypes at maturity.

spike with the reduction in quantity of P applied. Similar results were reported by Poulsen et al. [55] who suggested that P fertilization maximizes number of grains per spike in wheat crop.

4.2.9. Spike weight

Spike weight of the both wheat genotypes differed considerably due to their genetic characteristics (**Table 17**). The results depicted that the higher spike weight was recorded for the NARC-2009 (0.52 g) against Sehar-06 (0.46 g). The difference between both genotypes was 11%. Similarly, all the treatments differed potentially for spike weight. Maximum spike weight was recorded for T7 (0.55 g) followed by T8 (0.53 g), while the minimum spike weight was noticed under T1 (0.43). There was 21% difference among highest and lowest treatments. Similarly, the interactive effect was significant for spike weight. Highest spike weight was recorded for NARC-2009 under T7 (0.57 g) while lowest spike weight was observed for Sehar-06 under T1 (0.41 g). There was 28% variation for spike weight among highest and lowest interactions. Increased spike weight might be due to the adequate accessibility and uptake of P by crop plants. In stressed environment phosphorus played role to enhance root signaling. Due to uptake of P in adequate amount maximum numbers of fertile tillers were produced and the spike length, number of spikelet per spike and grains per spike also increased due to photosynthesis, energy storage, transfer, cell division as well as cell elongation so ultimately it results in increase in grain yield. The findings of current study corroborate the conclusions of Al-Karaki and Al-Omoush, [56], and Mehdi et al. [57] who reported that application of P increases spike weight which ultimately enhanced grain yield. Our results were not in accordance with Somayeh and Bahram [58], who reported enhanced spike weight by addition of phosphorus.

4.2.10. Hundred grain weight

Wheat genotypes due to their genetic behavior differed considerably for hundred grain weight at (**Table 18**). Highest hundred grain weight (4.18 g) calculated for genotype NARC-2009 whereas, lowest hundred grain weight (3.71 g) calculated for genotype Sehar-06. Both the genotypes differed 11% for hundred grain weight. All the treatments varied significantly for hundred grain weight. Highest hundred grain weight (4.38 g) recorded for treatment T7

Treatments/genotypes	Sehar-06	NARC-2009	Mean
T1	0.41i	0.47e–h	0.43E
T2	0.43hi	0.49d–f	0.45DE
T3	0.44g–i	0.49de	0.46DE
T4	0.45f–i	0.51cd	0.48CD
T5	0.47e–g	0.53b–d	0.49BC
T6	0.49de	0.56a–c	0.52AB
T7	0.52b–d	0.57a	0.55A
T8	0.50de	0.56ab	0.53A
Mean	0.46B	0.528A	
LSD for G	0.0149		
LSD for T	0.0298		
LSD for G × T	0.0421		

Table 17. Spike weight for both genotypes at maturity.

Treatments	Sehar-06	NARC-2009	Mean
T1	3.27i	3.74e–h	3.51E
T2	3.41hi	3.91d–f	3.67DE
T3	3.48g–i	3.99de	3.74DE
T4	3.59f–i	4.11cd	3.85CD
T5	3.77e–g	4.22b–d	3.99BC
T6	3.96de	4.40a–c	4.19AB
T7	4.17bcd	4.58a	4.38A
T8	4.01de	4.48ab	4.25A
Mean	3.7125	4.1829	
LSD for G	0.1193		
LSD for T	0.2386		
LSD for G × T	0.3376		

Table 18. Hundred grain weight for both genotypes at maturity.

fallowed by T8 (4.25 g) whereas, lowest hundred grain weight (3.51 g) observed for treatment T1. There was 19% difference among T7 and T1 for hundred grain weight. Similarly, there was significant difference among all the interactive effects at 1% P level. Maximum hundred grain weight observed for genotype NARC-2009 (4.58 g) under T7 whereas, minimum hundred grain weight recorded for Sehar-06 under T1 (3.27 g). There was 28% difference among maximum and minimum interactive effects for hundred grain weight. Grain weight is directly a measure of final productivity of the field crop. Greater the grain weight greater will the economical yield. Phosphorus applications in the stressed environment enhanced root signaling which ultimately enhanced grain weight. The reason of increased hundred grain weight might be due to provision of available phosphates to the plants in sufficient amount. Availability of P encourages root development and stimulates growth at seedling stage, so it promotes the quick establishment of seedling. It also accelerates leaf development and promotes faster growth of shoots and roots. As addition of phosphorus encourages normal growth of plant, ultimately it increased hundred grain weight. Similar results were found by Dewal and Pareek [50] and Memon [52] who observed considerable increase in grain weight in wheat by the addition of phosphorus.

5. Conclusion

Root architecture is a highly plastic and environmentally responsive trait that enables plants to counteract nutrient scarcities with different forging strategies. Root-specific traits such as root system architecture, sensing of edaphic stress and root-shoot communication can be exploited to improve resource capture (water and nutrients) and plant development under resource-limited conditions. The ability of plants to respond appropriately to nutrient availability is of fundamental importance for their adaptation to the environment. These signals trigger molecular mechanisms that modify cell division and cell differentiation processes within the root and have a profound impact on root system architecture. Important developmental processes, such as root-hair formation, primary root growth and lateral root formation, are particularly sensitive to changes in the internal and external concentration

of nutrients. Phosphorus (P) is one of the most vital nutrients needed for wheat production. Phosphorus plays an important role in root growth and builds resistance against abiotic stresses. It functions as one of the major players in process of photosynthesis, nutrient transport, and energy transfer. Drought stress reduces leaf size, stem elongation, root proliferation, as well as, disturbs plant water relations and reduces water use efficiency in plants. The present study conducted in laboratory as well as in polythene bags. In first experiment (screening test), nine wheat genotypes sown in petri dishes using four treatments (control, $PEG_{-0.50}$, $PEG_{-1.48}$ and $PEG_{-2.95}$) as a medium of growth. The data regarding germination percentage, root fresh weight, root dry weight, root length, shoot fresh weight, shoot dry weight and root shoot ratio recorded from experiment one and analyzed statistically. On the basis of stress tolerance two wheat genotypes were selected for next experiment. Genotypes NARC-2009 and Sehar-06 accumulated maximum germination percentage, root length, shoot length, root fresh weight, shoot fresh weight, shoot dry weight and root shoot ratio. So these two genotypes were selected for further experimentation. In experiment # 2 the effect of different treatments of phosphorus and water stress on root signaling checked. The treatments were 5 different levels of P including (P_{30} = 0.26 g/bag, P_{60} = 0.4 g/bag, P_{80} = 0.53 g/bag, P_{100} = 0.66 g/bag and P_{120} = 0.8 g/bag) and three different water levels designated as WFC, W10% < FC and W20% < FC. The data regarding root length, root weight, root-shoot ratio, root length, root fresh weight, root dry weight, root fresh weight: root dry weight, root hair density, root depth and yield and yield parameters collected and analyzed. Among both the genotypes, NARC-2009 performed well compared to Sehar-06. While discussing treatments higher dry matter and yield and yield parameters were recorded under T7 (P_{100}). With the increasing rate phosphorus root and shoot length was increasing linearly up-to P100 then it was declining. So under pot conditions where nutrients are limiting factor higher rate of phosphorus is essential to boost the productivity of the crop through better action of root signaling. Root signaling played important role in the growth and development of wheat crop. Under stressed conditions plant height, root and shoot length, root and shoot fresh and dry weight, yield and yield parameters decreased but in the presence of phosphorus all these parameters increased.

Author details

Mukhtar Ahmed[1]*, Sehrish Khan[1], Muhammad Irfan[1], Muhammad Aqeel Aslam[1], Ghulam Shabbir[2] and Shakeel Ahmad[3]

*Address all correspondence to: ahmadmukhtar@uaar.edu.pk

1 Department of Agronomy, PMAS, Arid Agriculture University Rawalpindi, Pakistan

2 Department Plant Breeding and Genetics, PMAS, Arid Agriculture University Rawalpindi, Pakistan

3 Department of Agronomy, Bahauddin Zakariya University, Multan, Pakistan

References

[1] Bingham IJ, Bengough AG. Morphological plasticity of wheat and barley roots in response to spatial variation in soil strength. Plant and Soil. 2003;**250**:273-282

[2] Kellermeier F, Armengaud P, Seditas TJ, Danku J, Salt DE, Amtmann A. Analysis of the root system architecture of Arabidopsis provides a quantitative readout of crosstalk between nutritional signals. Plant Cell. 2014;**26**(4):1480-1496

[3] Fahad S, Hussain S, Bano A, Saud S, Hassan S, Shan D, Khan FA, Khan F, Chen Y, Wu C, Tabassum MA, Chun MX, Afzal M, Jan A, Jan MT, Huang J. Potential role of phytohormones and plant growth-promoting rhizobacteria in abiotic stresses: Consequences for changing environment. Environmental Science and Pollution Research. 2014;**22**(7): 4907-4921

[4] Wang Y, Jensen LS, Magid J. Low-P solution culture can be used for screening root growth vigor in soil for high nutrient uptake of spring wheat varieties. Acta Agriculturae Scandinavica Section B Soil and Plant Science. 2018;**68**(2):130-138

[5] Fahad S, Chen Y, Saud S, Wang K, Xiong D, Chen C, Wu C, Shah F, Nie L, Huang J. Ultraviolet radiation effect on photosynthetic pigments, biochemical attributes, antioxidant enzyme activity and hormonal contents of wheat. Journal of Food, Agriculture and Environment. 2013;**11**(3-4):1635-1641

[6] Magalhaes JV, de Sousa SM, Guimaraes CT, Kochian LV. Chapter 7—The role of root morphology and architecture in phosphorus acquisition: Physiological, genetic, and molecular basis A2—Hossain, Mohammad Anwar. In: Kamiya T et al., editor. Plant Macronutrient Use Efficiency. Academic Press; 2017. pp. 123-147

[7] Fahad S, Hussain S, Matloob A, Khan FA, Khaliq A, Saud S, Hassan S, Shan D, Khan F, Ullah N, Faiq M, Khan MR, Tareen AK, Khan A, Ullah A, Ullah N, Huang J. Phytohormones and plant responses to salinity stress: A review. Plant Growth Regulation. 2015a;**75**(2):391-404

[8] Ademba JS, Kwach JK, Esilaba AO, Ngari SM. The effects of phosphate fertilizers and manure on maize yields in south western Kenya. East African Agricultural and Forestry Journal. 2015;**81**(1):1-11

[9] Fahad S, Bajwa AA, Nazir U, Anjum SA, Farooq A, Zohaib A, Sadia S, Nasim W, Adkins S, Saud S, Ihsan MZ, Alharby H, Wu C, Wang D, Huang J. Crop production under drought and heat stress: Plant responses and management options. Frontiers in Plant Science. 2017;**8**:1147

[10] Fahad S, Hussain S, Saud S, Hassan S, Ihsan Z, Shah AN, Wu C, Yousaf M, Nasim W, Alharby H, Alghabari F, Huang J. Exogenously applied plant growth regulators enhance the morphophysiological growth and yield of rice under high temperature. Frontiers in Plant Science. 2010;**7**:1250

[11] Fahad S, Hussain S, Saud S, Hassan S, Chauhan BS, Khan F, et al. Responses of rapid viscoanalyzer profile and other rice grain qualities to exogenously applied plant growth regulators under high day and high night temperatures. PLoS One. 2016a;**11**(7):e0159590. DOI: 10.1371/journal.pone.0159590

[12] Luan S. Signaling drought in guard cells. Plant, Cell & Environment. 2002;**25**:229-237

[13] Fahad S, Bano A. Effect of salicylic acid on physiological and biochemical characterization of maize grown in saline area. Pakistan Journal of Botany. 2012;**44**:1433-1438

[14] Fahad S, Hussain S, Saud S, Tanveer M, Bajwa AA, Hassan S, Shah AN, Ullah A, Wu C, Khan FA, Shah F, Ullah S, Chen Y, Huang J. A biochar application protects rice pollen from high-temperature stress. Plant Physiology and Biochemistry. 2015, 2015b;**96**:281-287

[15] Fahad S, Nie L, Chen Y, Wu C, Xiong D, Saud S, Hongyan L, Cui K, Huang J. Crop plant hormones and environmental stress. Sustainable Agriculture Reviews. 2015c;**15**:371-400

[16] Fahad S, Hussain S, Saud S, Hassan S, Chauhan BS, Khan F, et al. Responses of rapid viscoanalyzer profile and other rice grain qualities to exogenously applied plant growth regulators under high day and high night temperatures. PLoS ONE. 2016b;**11**(7):e0159590

[17] Fahad S, Hussain S, Saud S, Khan F, Hassan SA Jr, Nasim W, Arif M, Wang F, Huang J. Exogenously applied plant growth regulators affect heat-stressed rice pollens. Journal of Agronomy and Crop Science. 2016c;**202**:139-150

[18] Fahad S, Hussain S, Saud S, Hassan S, Tanveer M, Ihsan MZ, Shah AN, Ullah A, Nasrullah, Khan F, Ullah S, Alharby H, Nasim W, Wu C, Huang J. A combined application of biochar and phosphorus alleviates heat-induced adversities on physiological, agronomical and quality attributes of rice. Plant Physiology and Biochemistry. 2016d;**103**:191-198

[19] Havlin JL, Beaton JD, Tisdale SL, Nelson WL. Soil Fertility and Fertilizers. 7th ed. USA: Pearson Education Limited; 2005. ISBN: 0-13-027824-6

[20] Brady NC, Weil RR. The Nature and Properties of Soils. New Jersey USA: Pearson Prentice Hal Inc.; 2008. ISBN: 978-0-13-227938-3

[21] Wang Y, Zhang X, Huang C. Spatial variability of soil total nitrogen and soil total phosphorus under different land uses in a small watershed on the Loess Plateau, China. Geoderma. 2009;**150**(1-2):141-149. DOI: 10.1016/j.geoderma.2009.01.021

[22] Jasinski SM. Phosphate Rock, Statistics and Information. US Geological Survey; 2006

[23] Sharma KL, Neelaveni K, Katyal JC, Srinivasa Raju A, Srinivas K, Kusuma Grace J, Madhavi M. Effect of combined use of organic and inorganic sources of nutrients on sunflower yield, soil fertility, and overall soil quality in rainfed alfisol. Communications in Soil Science and Plant Analysis. 2008;**39**:1791-1831. DOI: 10.1080/00103620802073784

[24] Pearse SJ, Veneklaas EJ, Cawthray GR, Bolland MDA, Lambers H. Carboxylate release of wheat, canola and 11 grain legume species as affected by phosphorus status. Plant and Soil. 2006;**288**(1-2):127-139. DOI: 10.1007/s11104-006-9099-y

[25] Tarafdar JC, Claassen N. Organic phosphorus utilization by wheat plants understerile conditions. Biology and Fertility of Soils. 2003;**39**(1):25-29

[26] Zhang C, Simpson RJ, Kim CM, Warthmann N, Delhaize E, Dolan L, et al. Do longer root hairs improve phosphorus uptake? Testing the hypothesis with transgenic *Brachypodium distachyon* lines overexpressing endogenous RSL genes. New Phytologist. 2018;**217**(4):1654-1666

[27] Meyari S, Nouri F, Sasani S, Najafian G, Aghayari F. Correlation and path analysis of grain yield and its components of some bread wheat (*Triticum aestivem* L.) under normal and source restriction conditions. International Journal of Farming and Allied Sciences. 2013;**2**(23):1065-1069

[28] Dickin E, Wright D. The effects of winter waterlogging and summer drought on the growth and yield of winter wheat (*Triticum aestivum* L.). European Journal of Agronomy. 2008;**28**:234-244

[29] Farooq M, Hussain M, Wahid A, Siddique KHM. Drought stress in plants: An overview. Chapter 1. In: Aroca R, editor. Plant Responses to Drought Stress from Morphological to Molecular Features. 2012. pp. 1-33

[30] Fang Y, Xu B, Turner NC, Li FM. Grain yield, dry matter accumulation and remobilization, and root respiration in winter wheat as affected by seeding rate and root pruning. European Journal of Agronomy. 2010;**33**(4):257-266

[31] Gong H, Chen K. The regulatory role of silicon on water relations, photosynthetic gas exchange, and carboxylation activities of wheat leaves in field drought conditions. Acta Physiologiae Plantarum. 2012;**34**:1589-1594

[32] Dhanda SS, Sethi GS, Behl RK. Indices of drought tolerance in wheat genotypes at early stages of plant growth. Journal of Agronomy and Crop Science. 2004;**1**(9):6-8

[33] Katerji N, van Hoorn JW, Hamdy A, Mastrorilli M. Comparison of corn yield response to plant water stress caused by salinity and by drought. Agricultural Water Management. 2004;**65**:95-101

[34] Yang LJ, Zhang YL, Li FS, Lemcoff J. Soil phosphorus distribution as affected by irrigation methods in plastic film house. Pedosphere. 2011;**21**(6):712-718

[35] Smith FW. Plant responses to nutritional stress. In: Hawkesford MJ, Buchner P, editors. Molecular Analysis of Plant Adaptation to the Environment. Dordrecht: Kluwer Academic Publishers; 2001. pp. 249-269

[36] Hinsinger P, Plassard C, Tang C, Jaillard B. Origins of root-induced pH changes in the rhizosphere and their responses to environmental constraints: A review. Plant and Soil. 2003;**248**:43-59

[37] Herzig S, Neumann J. Effects of serine/threonine protein phosphatases on ion channels in excitable membranes. Physiological Reviews. 2001;**1**:173-210

[38] Lutcher LK, Schillinger WF, Wuest SB, Christensen NW, Wysocki DJ. Phosphorus fertilization of late-planted winter wheat into no-till fallow. Agronomy Journal. 2010;**2**(3): 868-874

[39] Smith SE, Smith FA, Jakobsen I. Mycorrhizal fungi can dominate phosphate supply to plants irrespective of growth responses. Plant Physiology. 2003;**133**:16-20

[40] Adrian A, Burner DM, Brauer KD. Soil phosphorus and water effects on growth, nutrient and carbohydrate concentrations, 13C, and nodulation of mimosa (*Albizia julibrissin* Durz.) on a highly weathered soil. Agroforest System. 2009;**76**:317-325

[41] Abbate PE, Cogliatti DH, Andrade FH. Relationship between yield, growth and spike weight in wheat under phosphorus deficiency and shading. The Journal of Agricultural Science. 2010;**148**:83-93

[42] Gorny AG, Garczynski S. Genotypic and nutritiondependent variation in water use efficiency and photosynthetic activity of leaves in winter wheat (*Triticum aestivum* L.). Journal of Applied Genetics. 2002;**43**:145-160

[43] Sardans J, Roda F, Penuelas J. Effects of water and a nutrient pulse supply on Rosmarinus of ficinalis growth, nutrient content and flowering in the field. Journal of Experimental Botany. 2005;**53**:1-11

[44] Zhang HW, Huang Y, Ye XS, Xu FS. Evaluation of phosphorus efficiency in rapeseed (*Brassica napus* L.) recombinant inbred lines at seedling stage. Acta Agronomica Sinica. 2008;**34**:2152-2159

[45] Hua YG, Chen QB, Lin ZM, Luo W. Coupling effects of water and chemical fertilizers on *Hevea brasiliensis* latex yield. Chinese Journal of Applied Ecology. 2008;**19**:1211-1216 (in Chinese)

[46] Bandyopadhyay KK, Misra AK, Ghosh PK, Hati KM, Mandal KG, Moahnty M. Effect of irrigation and nitrogen application methods on input use efficiency of wheat under limited water supply in a vertisol of Central India. Irrigation Science. 2010;**28**:285-299

[47] Tong C, Sui F, Bai L, Sun N, Ge T. Effects of drought stress on phosphorus and potassium uptake dynamics in summer maize (*Zea mays*) throughout the growth cycle. Journal of Plant Physiology. 2012;**34**(6):2179-2186

[48] Yu W, Chi S, Ning T, Tian S, Li Z. Coupling effects of irrigation and phosphorus fertilizer applications on phosphorus uptake and use efficiency of winter wheat. Journal of Integrative Agriculture. 2013;**12**(2):263-272

[49] Zhang W, Chen XX, Liu YM, Liu DY, Du YF, Chen XP, Zou CQ. The role of phosphorus supply in maximizing the leaf area, photosynthetic rate, coordinated to grain yield of summer maize. Field Crops Research. 2018;**19**:113-119

[50] Dewal GS, Pareek RG. Effect of phosphorus, sulphur and zinc on growth, yield and nutrient uptake of wheat (*Triticum aestivum* L.). Indian Journal of Agronomy. 2004;**49**:160-162

[51] Swarup A, Yaduvanshi NPS. Effect of integrated nutrient management on soil properties and yield of rice in alkali soil. Journal of the Indian Society of Soil Science. 2000;**48**:279-282

[52] Memon KS. Soil and fertilizer phosphorus. In: Bashir E, Bantel R, editors. Soil Science. Islamabad, Pakistan: National Book Foundation; 2005. pp. 291-316

[53] Hussain F. Soil fertility monitoring and management in wheat rice system. Ann. Report LRRI. Islamabad: NARC; 2004. https://www.hindawi.com/journals/ija/2014/801626/cta/

[54] Ali MS, Sutradhar A, Edano ML, Edwards JT, Girma K. Response of winter wheat grain yield and phosphorus uptake to foliar phosphite fertilization. International Journal of Agronomy. 2014:1-9

[55] Poulsen KH, Nagy R, Gao LL, Smith SE, Bucher M, Smith FA, Jakobsen I. Physiological and molecular evidence for pi uptake via the symbiotic pathway in a reduced mycorrhizal colonization mutant in tomato associated with a compatible fungus. The New Phytologist. 2005;**168**:445-453

[56] Al-Karaki GN, Al-Omoush M. Wheat response to phosphogypsum and mycorrhizal fungi in alkaline soil. Journal of Plant Nutrition. 2002;**25**(4):873-883

[57] Mehdi SM, Abid M, Sarfraz M, Hafeez M, Hafeez F. Wheat response to applied phosphorus in light textured soil. Journal of Biological Sciences. 2007;**7**:1535-1538

[58] Somayeh AS, Bahram M. Effect of phosphorus fertilization and seed biofertilization on harvest index and phosphorus use efficiency of wheat cultivars. Journal of Food, Agriculture and Environment. 2011;**9**(2):388-391

Chapter 2

Wheat Sensitivity to Nitrogen Supply under Different Climatic Conditions

Veres Szilvia, Ondrasek Gabrijel and Zsombik László

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.76154

Abstract

With the projection of the Earth's population reaching eight billion in coming years and nine billion by 2050 which means increasing demand for food. Wheat (*Triticum aestivum* L.) is the main important and strategic cereal crop for feeding the majority of world's populations. Scientific forecasts predict that wheat production in the future will be affected by climate change and will decrease on the global level. To reduce these risks, the impact of climate change mitigation strategies and management systems for crop adaptation to climate change conditions should be considered. Demand for increases in food production will have to occur on less available arable land, which can only be accomplished by intensifying production. Chemical fertilisers are responsible for 40–60% of the world's food production. Because nonlegume plants generally require 20–50 g of nitrogen to produce 1 kg dry biomass, the natural supply of soil nitrogen usually restricts plants yield in most agricultural cropping system. The goal of ecological intensification is to increase yield per unit of land, intensify production, while meeting acceptable standards of environmental quality. This chapter discusses some aspects of connection between nitrogen supply and different abiotic conditions.

Keywords: wheat, nitrogen, drought, salinisation, climate

1. Introduction

The global wheat consumption has escalated at a faster rate than all other cereals. This growth is accounted for by the increase in developing countries, mainly in China and India, and based on the future projection, the growth of wheat consumption will continue [1]. In these two countries, the use of production inputs, primarily nitrogen fertiliser and irrigation water, has risen dramatically as well. Wheat is an important staple crop, providing 20% of all calories

IntechOpen

© 2018 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. CC BY

consumed by people worldwide. It is the leading source of non-animal protein in human food and also makes a significant contribution to animal feed. Increasing global demand for wheat is also based on the ability to make several food products and the increasing consumption of these with industrialisation. In particular, the properties of the gluten protein fraction allow the processing of wheat to produce bread, other baked goods, noodles and pasta, and a range of functional ingredients [2].

Beside the food demand sustainable nutrient supply and climatic effect on plant productivity are two crucial topics of agricultural development. Applying adequate amount of nutrients based on genotype requirements is hard under potential conditions, especially under different abiotic loads. Nitrogen (N) is an important nutrient, which determines the amount of yield and throughout the proteins the quality as well. The increased crop productivity has been associated with a 20-fold increase in the global use of nitrogen fertiliser during the 50 years [3], and this is expected to increase by threefold by the year 2050 [4]. Inadequate application of N—deficiency and excess—can cause environmental and ecological problems. Climatic factors can improve and deteriorate crop nutrient use efficiency and yield. Drought occurs in all climatic regions and drought-induced crop yield reduction is among the greatest losses in agriculture. About 32% of wheat production areas in developing countries experience serious drought stress in different growth stages [5]. Lobell et al. [6] published that climate trends were large enough in some countries to offset a significant portion of the increases in average yields that arose from technology, fertilisation, and other improving factors. High and low temperature [7–9], irrigation [10–12], salinisation [13, 14], agrotechnology [15–17], and other nutrients [18] also have an effect on N use of wheat. These effects are depending on the adaptation and acclimatisation strategies of different wheat genotypes, the current climatic conditions and its combinations and biotic effects as well [19]. To know more about and improve nitrogen use efficiency of wheat means a way towards the sustainability. Wheat being the basic food plant and the global demand for qualitative perfect food is increasing we have no other alternatives, than step forward to smart-wheat, which will be able to survive unfavourable conditions.

2. Nitrogen requirement and NUE

Nitrogen is one of the nutrients plants need in high quantity [20], as it is a core constituent of a plant's nucleic acid, proteins, enzymes, and cell wall and pigment system [21]. The availability of nitrogen for plants is complex, and depending on different processes in connection with nitrogen cycle in the environment (**Figure 1**). Through the different way of nutrition supply and agrotechnology processes, the agriculture has a main impact on global and local nitrogen cycle. Plants also can have an effect on your own nitrogen supply by connecting different bacteria or releasing different extracts, like nitrification inhibitors. Biological nitrification inhibition (BNI) is the natural ability of certain plant species to release nitrification inhibitors from their roots that suppress nitrifier activity, thus reducing soil nitrification and N_2O emission (**Figure 1**). Among the tropical pasture grasses, the BNI function is the strongest in *Brachiaria* sp. [22].

http://dx.doi.org/10.5772/intechopen.76154

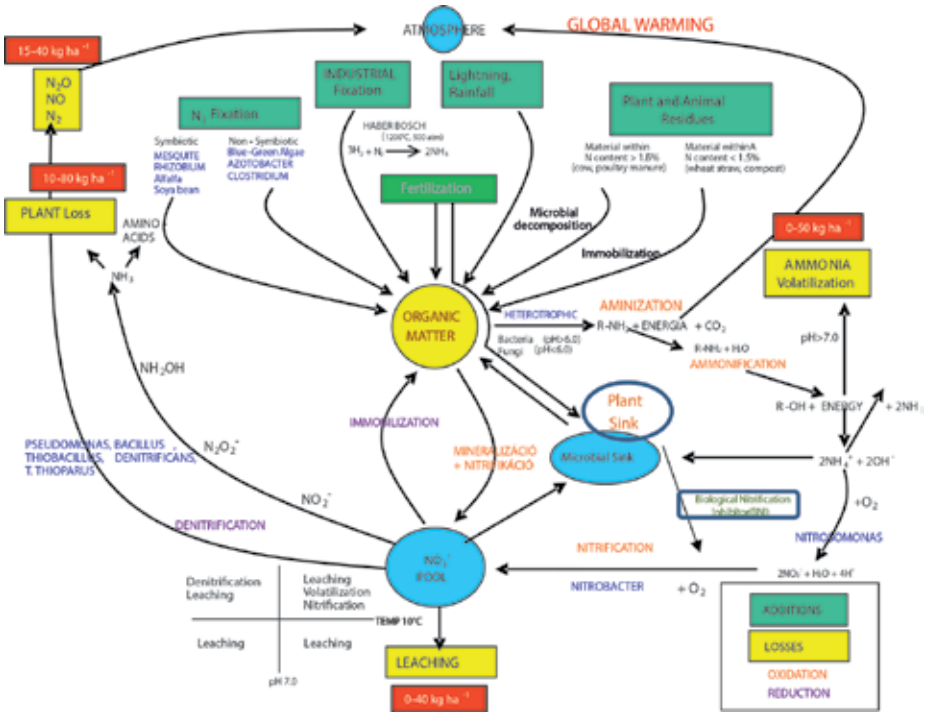


Figure 1. Nitrogen cycle (adapted according to LaRuffa et al. [23]).

Nitrogen availability and using capacity are crucial in plant life. The chlorophyll content of wheat leaves and leaf N is closely related as the photosynthetic machinery accounts for more than half of the N in a leaf [24]. Nitrogen influences carbohydrate source size by leaf growth and leaf area duration and also the photosynthetic rate per unit leaf area and thereby source activity. The availability of N is of agricultural concern because plant metabolism is differently affected by excess, optimal and deficient levels [25]. The concept of nitrogen-use efficiency (NUE) has been widely used to characterise plant responses to different levels of N availability. Moll et al. [26] defined the most use of NUE, at least among breeders, which computes the grain dry mass divided by the total N available to a plant. It is divided into two components: NUE = NUpE × NUtE, where NUpE is the N-uptake efficiency calculated as the total amount of N in above-ground plant at harvest divided by the available N in soil, and NUtE is the utilisation efficiency calculated as the grain dry mass divided by the total amount of N in above-ground plant at harvest. Based on several authors, establishment N remobilisation efficiency (NRE) is also a main component of NUE [27]. The NRE—the proportion of N in the crop or crop component at anthesis which is not present in the crop or crop component at harvest—is the ability of plants to translocate the N after anthesis from the shoot to the grains. Nitrogen is the most limiting nutrient for the production of wheat [28]. Cultivars with higher NRE tend to accelerate the senescence process and increase N levels in grains [29]. It is widely understood that N accumulated before anthesis provides the major source of grain N. In wheat, around 50–95% of the grain N at harvest comes from the remobilisation of N stored in shoots and roots before anthesis [30–32]. In wheat between anthesis and maturity, the leaves had a higher

NRE than the stem and the roots [33]. About 70–80% of nitrogen, which is needed for grain development in cereals, is gained from vegetative organs before flowering stage [34]. Nitrogen use efficiency (NUE) plays a fundamental role in sustainable grain production [35, 36]. Based on several physiological parameters of doubled-haploid mapping wheat populations can lead to identification of specific loci that might be useful in marker-assisted breeding for increased N-use efficiency [35, 37, 38].

3. Temperature influence on nitrogen nutrition

Wheat growth can be impaired by heat stress at any developmental stage, and modelling scenarios predict even warmer temperatures in the future [39]. Production of wheat is affected markedly by high temperature [40, 41]. Elevated temperature alters uptake and allocation of N, thus intensifying N deficiency in plants [42]. Wheat shows enormous diversity in canopy architecture, and it has long been proposed that optimised light distribution could improve radiation use efficiency as well as light interception [43]. In heat tolerance, the activity of enzymes has crucial role. Rubisco's affinity for CO_2 decreases with temperatures [44]. Therefore, increasing affinity would simultaneously improve adaptation to warmer conditions, the proof of concept coming from C_4 species, in which it is achieved by concentrating CO_2 [45]. High temperature not only degrades Rubisco but also accelerates its inactivation by addition of inhibitory sugars to its active site. Moreover, Rubisco has a relatively low turnover number as compared with the other Calvin cycle enzymes. Activity of Rubisco is mainly regulated by a catalytic chaperone—Rubisco activase—which catalyses removal of inhibitory sugars from its active site, switching the enzyme to active mode [46]. Among cereals wheat's Rubisco has one of the best CO_2 affinities. Models where wheat's substrate specificity factor of Rubisco is replaced from *L. gibertii* predicted increases of 12% in net assimilation [47]. Combined stress of high temperature and low nitrogen affected both the abundance and mode of regulation of Rubisco, which catalyses CO_2 fixation and is one of the primary determinants of photosynthetic rate [48].

4. Effect of drought on wheat nutrition

According to the most recent assessment report of the Inter-governmental Panel on Climate Change, published in 2014, levels of anthropogenic emissions of greenhouse gases are now at their highest in history [49]. Agricultural production and its effect on land use are major sources of these emissions by sharing methane and nitrous oxide gases. Greenhouse gases causing air temperatures increase, thus more moisture evaporates from land and water bodies. Warmer temperatures also increase evaporation and evapotranspiration in plants, soils, and on other hand, they will also escalate the water stress frequency and intensity with a rise from 1 to 30% in acute drought land area by 2100 [50].

Under dry conditions in the field, 75–100% of the grain yield could be attributed to stored assimilates, compared with 37–39% under high-rainfall conditions. Drought stress severely

influenced plant water status by reducing the water potential and the relative water content in wheat [51]. Optimal nutrition levels have also alleviated drought stress damage by sustaining metabolic activities under reduced tissue water potential [52]. Nitrogen supply also has a crucial role in combating drought [53]. Efficiency of nitrogen supply declined with increasing of drought stress [54]. Morgan [55], Arun et al. [56] and Binghua et al. [57] who showed that with an application of nitrogen, plants show positive influence in terms of growth and development under drought stress. Although Li et al. [58] mentioned that different grass species under drought stress did not modify physiological functions under varying N application. Water limitation reduces diffusive conductivity which in turn affects other physiological process such as energy and N metabolism. It is concluded that N uptake and its diffusion depend on environmental condition especially to water supply as also indicated by Abreau et al. [59]. Under water deficiency, roots are unable to get optimal amounts of nitrogen from soil, which has general negative effects on plant metabolisms [60]. The main effect of water restriction is certainly a reduction in N demand due to the marked sensitivity of leaf area expansion [61]. Fewer results have about light reaction affected by genotypic and nitrogen supply variations, mainly under stress conditions. By measuring the yield of chlorophyll fluorescence (Chl-fl), information about changes in the efficiency of photochemistry and heat dissipation can be obtained [62]. Under extreme drought stress when the stomatal resistance just around 0.1 mol H_2O m^{-2} s^{-1}, poor performance of photosystem II (F_v/F_m) and downregulated activities of CO_2 assimilating enzymes such as Rubisco become the dominant limitations to reduced photosynthesis [63]. The optimal photochemical activity (F_v/F_m) values were sensitive for the investigated two environmental factors, and genotype differences were established in tolerance [64]. Chl-fl parameter's sensitivity for detecting nitrogen deficiency is different, but some of them are really applicable for describing nitrogen lack [65]. Previous drought stress studies have reported that photosynthetic rate of the leaf under drought stress is closely related to the leaf chlorophyll contents, N concentrations and stay-green characteristics of the leaf, which in turn increases the grain yield by increasing the photosynthetic process [66]. Palta et al. [67] and Hosenlou et al. [54] reported induction of N remobilisation under drought stress. Application of the high amounts of N under drought resulted to the lowest NUE [68]. Critical, sufficient concentration of nitrogen in leaf is 15–40 mg g^{-1} DM [69]. Based on Pepó [16] and Zsombik and Seres [70] results, the dry weight production was mainly influenced by environmental factors and modified by fertilisers and genotypes. Water deprivation means higher strain than nitrogen luck with genotype difference based on dry weight value [65]. Plant responses to drought stress vary at different growth stages of the crop [71]. In wheat, tillering capacity of the crop is a major constituent of the final grain yield [72], but has been reported to be highly vulnerable to drought stress [73].

5. Salinisation and impact to wheat production

Salinisation or increased concentration of dissolved cations/anions in soil solution and/or water resources (e.g. capillary rising of saline groundwater, salinised waters used for irrigation) [74] across the (agro)ecosystems is the principal cause of most widespread abiotic constraint to glycophytes (i.e. the majority of cultivated crops, including wheat) known as salt

stress. Salt stress encompasses wide range of physiological dysfunctions as a consequence of primary salinity effects, that is, osmotic and ionic disorder. Primary salinity effects, depending on the salinity level/duration, crop/genotype type, development stage, and so on, very often cause different secondary salinity-induced effects such as reduced cell expansion and assimilate production (i.e. growth and yield reduction), production of reactive oxygen metabolites and even plant mortality [75]. The general salinity effects are quite visible and assume reduce biomass growth (shoot/root height and weight, leaf area) and changes in root and shoots colour (e.g. presence of leaf tip burns, scorching/firing of leaves) [76]. The extent to which growth and yield will be reduced under salt stress mostly depends on the salinity level and plant (crop) species (**Figure 2**).

Electrical conductivity (EC) is commonly used as an expression of the total dissolved salt concentration in an aqueous sample (e.g. water, soil solution) and usually express soil salinity level based on measured EC of saturated soil paste extracts (EC_e) (e.g. Rhoades et al. [77]). Therefore, ECe threshold level (i.e. ECt), as a numeric value at which crop growth and yield start to decline (more or less intensive under certain slope) can be very useful for categorisation of plants from salt tolerant (halophytes) to salt-sensitive (glycophytes) (**Figure 2**). In general, wheat is categorised as moderately tolerant to soil salinity (e.g. a threshold EC of 6.0 dS/m) [78] although existing significant differences among genotypes that it is difficult to make a categorical statement [79]. The relative effects of salt stress on wheat vegetative

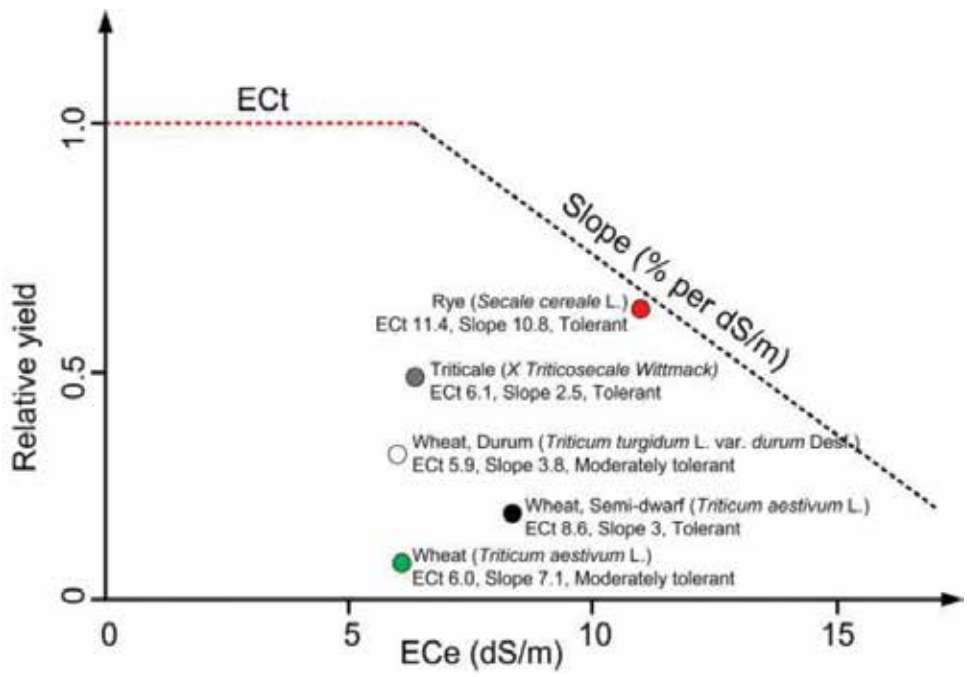


Figure 2. Simplified presence of salt tolerance in some cereals (adapted according to Maas [81]). ECt represents the threshold in soil EC that is expected to cause the initial significant reduction in the maximum expected yield, whereas the slope is the percentage of yield expected to be reduced for each soil salinity unit above the ECt [82].

growth parameters and grain yield can vary significantly among genotypes (**Figure 2**) and with the developmental stage at which salt stress occurs [75] as well under specific environmental conditions given that the interaction of crop (genotype) and environment is not completely understood but is likely to be significant [80].

5.1. Salinisation and wider (agro)ecological impact

Environmental salinisation process represents an increasing environmental issue especially in intensive agroecosystems such as (fert)irrigated areas [83] but also in less intensive rain feed (semi)arid regions [84]. Salt-affected areas are often overlapping with numerous other physical, chemical and/or biological pedosphere constrains such as sandy soils with low water retention capacity, non-structured/dispersed (waterlogged) soils, organically depleted soils with diminished microbial activity/diversity and excessive alkalinity, specific ionic (Al, B) toxicity, and many other [84, 85] (**Figure 2**). Saline or alkaline (sodic) soils due to increased concentration of particular slats (Na^+, Cl^-, Ca^{2+}, Mg^{2+} etc) and ionic interrelations (e.g. Na^+/Ca^{2+}; Na^+/Mg^{2+}) can be recognised and visually by crystallised (precipitated) salts on the soil surface (forming a brighter salt forms on the soil surface; **Figure 3a**) or at later (developed) stages by topsoil crusting, as a consequence of dispersed clay minerals and soil aggregates (**Figure 3b**).

A constitute of structurally dispersed soils (e.g. clay particles, minerals, organics) undergo leaching through the soil profile, accumulating and blocking deeper macro/micro pores, especially in textured-heavier soil layers, and finally causing waterlogging (e.g. Burrow et al. [86]. Thus, salt-affected soils (profiles) depleted in adsorption matrices (organic matter and clay content notably) might be more prone to mobility and transfer of certain pollutants (e.g. toxic trace elements) on the soil/crop/groundwater routes, although certain genotypic differences have to be considered.

For instance, it was shown that raised soil solution salinity can significantly impact mobility of toxic Cd in the rhizosphere and enhance its uptake and root/shoot accumulation in different wheat cultivars [87, 88]. Also, sublimating the results of studies conducted by Norvell et al. [89], Khoshgoftarmanesh et al. [88] and Ozkutlu et al. [90] their outcomes suggest that

Figure 3. Topsoil (a) crystallisation of soluble salts (dotted brighter areas) in a wheat paddock, depleted with soil organic matter, and (b) crusting in an adjacent saline plot with disturbed soil structure (Esperance area, Western Australia).

durum *vs*. bread wheat genotypes could be more effective, not only in Cd root extraction, but also in Cd root to shoot (leaf/grain) translocation and deposition under excessive Cl salinity. Such genotypic differences should be considered also in wheat breeding programs related to salt resistance (next section).

5.2. Sustainable management practices and perspectives for wheat cropping under salinised conditions

Some of the widely used and most promising perspectives and strategies against soil salinity are listed in **Table 1**, and some of them are explained in the next section in more detail. Beside excessive ECe in saline soils, it is of great importance and interrelated concentrations of particular ions (notably portion of exchangeable Na^+; ESP) as well as soil pH reaction. According to some of chemical parameters, salt-affected soils generally can be categorised as: saline (ECe > 4 dS/m, ESP < 15 and pH <8.5), saline-sodic (ECe > 4 dS/m, ESP > 15 and pH <8.5) and sodic/alkaline (ECe >4 dS/m, ESP >15 and pH >8.5), and usually require specific strategy for reclamation often with low benefit/cost ratio for crops (Ondrasek et al. [85] and references therein). Sustainable agricultural management in saline/sodic conditions usually is combination of certain preventive actions (aiming to control salinity/alkalinity level) and/or remediate of saline/alkaline areas. For instance, saline soils might be easier for reclamation than sodic soils because the former often requires only salt leaching while the latter requires addition and certain Ca-/Mg-based soil amendments (e.g. gypsum) to replace excess ESP in addition to leaching (e.g. [91]).

Perspective	Description
Species/varieties selection	Cropping of more salt resistant wheat varieties (genotypes), although genotypic differences related to efficiency of mineral uptake and accumulation (e.g. trace elements) should be considered (explained above)
Amelioration of soil water management	Implementation of subsurface drainage system may be useful approach for: (i) prevention of salt accumulation in sub/surface horizons as a consequence of seasonal sea water intrusion and/or capillary rising and/or (ii) salt leaching from the surface soil layers (e.g. [109]). Implementation of irrigation can decline vegetative growth of wheat cropped on salt-affected soils but without evident yield reductions (e.g. [75])
Soil amelioration by microbes	It was shown that exploitation of certain microbial populations can be a promising alternative to alleviate crops stress under excessive root zone salinity [96]. Thus for instance, inoculation of wheat seeds prior sowing by salt-tolerant microbe colonies might be beneficial strategy for wheat cropping in salt-affected environment (e.g. [97])
Application of inorganic amendments	Addition of natural or synthetic Ca-/Mg-/Zn based sources can ameliorate soil salinity/sodicity [110] and related pedosphere constrains (e.g. Zn-deficiency; see above)
Conservation and increasing of soil organic matter	Over conservation land management (e.g. reduced/minimal/no tillage) is possible to preserve and/or enhance soil–plant water relations, soil organic matter content and rhizosphere biodiversity across the saline paddocks (e.g. [85])
Genetic improvement	Genetic improvement of wheat genomes for salt-tolerance has a great potential of acquiring some halophytic traits such as Na^+ and/or Cl^- exclusion by crossing cultivars of *Triticum aestivum* L. with genetically related (non)halophytes (e.g. [80])

Table 1. Some perspectives for improving wheat cropping in salt-affected agroecosystems.

Application of (in)organic soil amendments, such as mineral/organic fertilisers, lime, gypsum phospho-gypsum, and so on to salt-affected pedosphere has multi-beneficial impact [75]. Introduction of Ca-/Mg-enriched amendments enhances to maintain soil micro-aggregate structure in the soil profile, and consequently improves physical pedovariables such as improved flocculation, reduced spontaneous dispersion (air-dry aggregates) and dispersion of remoulded aggregates, increased hydraulic conductivity and soil aeration [92]. Furthermore, it was confirmed that soil salinity/alkalinity is frequently associated with microelement Zn deficiency, and that under such conditions, application of certain inorganic Zn-based fertilisers is able to improve salt tolerance but also and nutritional value of wheat. Namely, ~40% of the soils used for wheat production in Iran are Zn-deficient [93] and comparing to some other widely cropped cereals, wheat genotypes are especially very sensitive to Zn deficiency which markedly reduce wheat grain yield [94]. However, one of the biggest issues with soil amendments (Ca-/Mg-/Zn-based) application and their beneficial impact to crops in saline conditions is often lacking of their dissolution (i.e. phytoavailability of specific element/substance) due to (semi)arid conditions and/or not implemented irrigation practice.

Another promising strategy to enhance wheat salt tolerance might be introduction of salt more tolerant root-associated microbes that enhance plant growth under excessive salinity. Namely, it was widely discussed how spatial rhizosphere adaptation of plants is also driven by genetic differentiation in closely associated microbe populations such as: (i) arbuscular mycorrhizal fungi (whose hyphal networks ramify throughout the soil and within the plant cells) then (ii) ectomycorrhizal fungi (over a fungal layer around the root system and root intercellular spaces) and (iii) root-associated plant growth-promoting rhizobacteria (see reviews by Rodriguez and Redman [95]; Dodd and Perez-Alfocea [96]). Alleviation of salt stress on yield and mineral nutrition (e.g. increased K/Na ratio) exploiting the arbuscular mycorrhizal fungi was confirmed in certain wheat varieties under field saline conditions [97]. For instance, the mycorrhizal colonisation enhanced grain wheat yield up to >31% in Kavir (spring cultivar), up to >32% in Roshan (spring and semi-early maturing cultivar) and even up to >38% in Tabasi (mutated salt tolerant line) [97]. Furthermore, Sadeghi et al. [76] applying the isolate of *Streptomyces* in cultivated soil with wheat (cul. Chamran) observed: (i) increased the growth/development and shoot concentration of N, P, Fe and Mn in both saline and non-saline conditions and (ii) significant increases in germination rate, percentage and uniformity, shoot length and dry weight of salt-exposed plant (*vs*. non saline control). Also, studying the effect of inoculation of the five halotolerant bacterial strains in alleviation of NaCl-induced stress (80–320 mM) in wheat (var. HD 2733) Ramadoss et al. [98] observed an increase in root elongation (by >90%) and root dry weight (by >17%) in comparison with control (uninoculated) plants. Such beneficial effects of salt-tolerant microbes to (wheat) crops exposed to salinity are explained by improved plant water relations (e.g. due to enhanced accumulation of specific osmolytes), then regulating plant homeostasis and improved phytonutrients (e.g. N, P, K, Zn, Cu, Mn, Fe) uptake as well by enhanced germination rate [96, 97, 99].

Breeding programs to salt tolerance (as relatively long-term approach) are expecting that might have crucial role in (wheat) cropping under saline conditions in the near future (see down). Relatively little work has been done on breeding programs of wheat cultivars for saline conditions [80] given on polygenic character of salt tolerance, but continuous progress

is evident. Namely, hexaploid bread wheat (*Triticum aestivum* L.) has one of the most complex (ABD) genomes (e.g. six copies of each chromosome, numerous of near-identical sequences scattered throughout, overall haploid size of >15 billion bases) [100], thus making wheat highly challenging for genome sequencing and detection of salt-tolerant genes and quantitative trait loci. Also, the huge amount of repetitive sequences poses a big challenge for sequencing the wheat genome [101]. For instance, first assembly of the wheat genome from 2012 was represented by only ~33% (5.42 billion bases) [102], another assembly from 2014 by ~66% (10.2 billion bases) [103] whereas assemblies from 2017 were extended to 78% (12.7 billion bases) [104] and recent assembly was almost completed with >15.3 billion bases [100]. Hence, the genomic complexity and its uncomplete assembly makes the wheat crop additionally extremely difficult for improvement to salt tolerance over conventional (e.g. traditional breeding) and/or modern genetic (e.g. molecular and transgenic breeding) approaches.

Genetic improvement of wheat for salt-tolerance has also a great potential of acquiring some halophytic traits (genes) such as Na^+/Cl^- exclusion and/or compartmentation by crossing wheat genotypes with genetically related halophytic plant species (e.g. *Lophopyrum elongatum*) [105]. In wheat salt resistance is associated with low rates of the root-to-shoot transport of Na^+ with high selectivity for K^+ over Na^+ [106]. Bread wheat genotypes have a low rate of Na^+ accumulation and enhanced K^+/Na^+ discrimination which is controlled by a locus (Kna1) on chromosome 4D [107]. Contrary, durum wheat (tetraploid, AB genomes) have higher rates of Na^+ accumulation and weaker K+/Na + discrimination [80] and is consequently less salt resistant *vs*. bread wheat (**Figure 2**). It was confirmed that salt−/draught-tolerant genes and quantitative trait loci identified in *T. dicoccoides* and *H. spontaneum* have great potential in wheat improvement also [108]. Finally, improvement in salt resistance of modern wheat genotypes will be generated from introducing new gene(s) by (i) crossing with new donor germplasm or (ii) transformation with single genes, and after the progeny has to be back-crossed into adapted cultivars before the donor genes are ready for cultivation [80].

6. Conclusion

Sustainable plant production has three main goals: environmental health, economic profitability, and social and economic equity. It needs to achieve higher and higher amount of quality food by using less stock and energy under actual environmental conditions. Nitrogen is one of the most important nutrients for plants because of the yield quality and quantity as well. Applying adequate amount of nutrients based on genotype requirements is hard under potential conditions, especially under different abiotic loads. About 70% of all fertilisers are used on wheat and rice. Wheat is an important staple crop and crucial source of non-animal protein in human food and also makes a significant contribution to animal feed. The problem is that the utilisation efficiency of nitrogenous fertilisers under field conditions is relatively low, thus the production may become dangerous for the environment, economically inadequate and can result in poor quality. Finding of 'smart' wheat genotypes with high NUE does not mean a solution for the problem of being sustainable. Several environmental conditions have effect on NUE and/or the components of NUE, thus we need more knowledge to locate the final answer our global challenge.

Acknowledgements

Thank you for the support of 'Kiváló malomipari paraméterekkel rendelkező adaptív őszi búza vonalak előállítása' (AGR_PIAC_13-1-2013-0002) grant and the EFOP-3.6.3- VEKOP-16-2017-00008 project. The project is co-financed by the European Union and the European Social Fund.

Conflict of interest

We have no conflict of interest declare.

Author details

Veres Szilvia[1]*, Ondrasek Gabrijel[2] and Zsombik László[3]

*Address all correspondence to: szveres@agr.unideb.hu

1 Faculty of Agricultural and Food Sciences and Environmental Management, Department of Agriculture Botany, Crop Physiology and Biotechnology, Institute of Crop Sciences, University of Debrecen, Debrecen, Hungary

2 Faculty of Agriculture, Department of Soil Amelioration, University of Zagreb, Zagreb, Croatia

3 Institutes for Agricultural Research and Educational Farm, Research Institute of Nyíregyháza, University of Debrecen, Nyíregyháza, Hungary

References

[1] Kearney J. Food consumption trends and drivers. Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences. 2010;**365**(1554):2793-2807. DOI: 10.1098/rstb.2010.0149

[2] Shewry PR, Hey SJ. The contribution of wheat to human diet and health. Food and Energy Security. 2015;**4**(3):178-202. DOI: 10.1002/fes3.64

[3] Glass ADM. Nitrogen use efficiency of crop plants, physiological constraints upon nitrogen absorption. Critical Reviews in Plant Sciences. 2003;**22**:453-470. DOI: 10.1080/07352680390243512

[4] Good AG, Shrawat AK, Muench DG. Can less yield more? Is reducing nutrient input into the environment compatible with maintaining crop production? Trends in Plant Science. 2004;**9**:597-605. DOI: 10.1016/j.tplants.2004.10.008

[5] Ginckel MV, Calhoun DS, Gebeyehu G, Miranda A, Tian-you C, Lara RP, Trethowan RM, Sayre K, Crossa J, Rajaram S. Plant traits related too yield of wheat in early. Iate, or continuous drought conditions. Euphytica. 1998;**100**:109-121

[6] Lobell DB, Schlenker W, Costa-Roberts J. Climate trends and global crop production since 1980. Science. 2011;**333**(6042):616-620

[7] Fahad S, Hussain S, Saud S, Hassan S, Ihsan Z, Shah AN, Wu C, Yousaf M, Nasim W, Alharby H, Alghabari F, Huang J. Exogenously applied plant growth regulators enhance the morphophysiological growth and yield of rice under high temperature. Frontiers in Plant Science. 2016;**7**:1250. DOI: 10.3389/fpls.2016.01250

[8] Peerzada YY, Elsayed FAA, Mohd N, Ambreen A, Abeer H, Abdulaziz AA, Altaf A. Responsive proteins in wheat cultivars with contrasting nitrogen efficiencies under the combined stress of high temperature and low nitrogen. Genes. 2017;**8**(12):356. DOI: 10.3390/genes8120356

[9] Tahir ISA, Nakata N. Remobilization of nitrogen and carbohydrate from stems of bread wheat in response to heat stress during grain filling. Journal of Agronomy and Crop Science. 2005;**191**:106-115

[10] Bandyopadhyay KK, Misra ĆAK, Ghosh ĆPK, Hati ĆKM, Mandal ĆKG, Moahnty ĆM. Effect of irrigation and nitrogen application methods on input use efficiency of wheat under limited water supply in a vertisol of Central India. Irrigation Science. 2008;**28**(4). DOI: 10.1007/s00271-009-0190-z

[11] Majeed A, Muhmood A, Niaz A, Javid S, Ahmad ZA, Shah SSH, Shah AH. Bed planting of wheat (*Triticum aestivum* L.) improves nitrogen use efficiency and grain yield compared to flat planting. The Crop Journal. 2015;**3**:118-124 Available from: http://creativecommons.org/licenses/by-nc-nd/4.0/

[12] Pepó P. Role of fertilization and water supply in wheat production. In: Hidvegi SZ, Gyuricza CS, editors. Hungarian Academy of Sciences. Budapest: Akaprint; 2004. pp. 275-279

[13] Abdul-Kadir SM, Paulsen GM. Effect of salinity on nitrogen metabolism in wheat. Journal of Plant Nutrition. 2008;**5**(9):1141-1151. DOI: 10.1080/01904168209363047

[14] Soliman SM, Shalabi HG, Campbell WF. Interaction of salinity, nitrogen, and phosphorus fertilization on wheat. Journal of Plant Nutrition. 1994;**17**(7):1163-1173. DOI: 10.1080/01904169409364796

[15] Árendás T, Berzsenyi Z, Láng L, Bedő Z. A minőségi búza termesztésének néhány agrotechnikai szempontja a martonvásári kutatási eredmények tükrében. In: Pepó P, editor. Búzavertikum aktuális kérdései. Szaktanácsadási Füzetek 2. Debrecen: Hungarian Academy of Sciences; 2006. pp. 73-83

[16] Pepó P. Efficiency of fertilization in sustainable wheat production. Acta Agraria Debreceniensis. 2002;**1**:1-7

[17] Pepó P, Zsombik L, Vad A, Berényi S, Dóka L. Agroecological and management factors with impact on the yield and yield stability of maize (*Zea mays* L.) in different crop rotation. Analele Universitatii Oradea of Facultatea de Pretectia Mediului. 2007;**13**:181-187

[18] Duan YH, Shi XJ, LI SL, Sun XF, He XH. Nitrogen use efficiency as affected by phosphorus and potassium in long-term rice and wheat experiments. Journal of Integrative Agriculture. 2014;**13**(3):588-596. DOI: 10.1016/S2095-3119(13)60716-9

[19] Lehoczky É, Kismányoky A, Kismányoky T. Study on the weediness of winter-wheat in a long-term fertilization field experiment. Communications in Agricultural and Applied Biological Sciences. 2006;**71**(3a):793-796

[20] Epstein E, Bloom AJ. Mineral Nutrition of Plants: Principles and Perspectives. Sunderland: Sinauer Associates, Inc.; 2005. p. 400

[21] Krapp A. Plant nitrogen assimilation and its regulation: A complex puzzle with missing pieces. Current Opinion in Plant Biology. 2015;**25**:115-122. DOI: 10.1016/j.pbi.2015.05.010

[22] Subbaro GV, Rao IM, Nakahara K, Sahrawat KL, Ando Y, Kawashima T. Potential for biological nitrification inhibition to reduce nitrification and N_2O emissions in pasture crop-livestock systems. Animal. 2013;**7**(2):322-332. DOI: 10.1017/S1751731113000761

[23] LaRuffa J, Thomason W, Taylor S, Lees H. Soil-Plant Nutrient cycling and Environmental Quality. Department of Plant and Soil Sciences, Oklahoma State University. Available from: http://slideplayer.com/slide/9941674/

[24] Evans JR. Nitrogen and photosynthesis in the flag leaf of wheat (*Triticum aestivum* L.). Plant Physiology. 1983;**72**:297-302. DOI: 10.1104/pp.72.2.297

[25] Iqbal N, Umar S, Khan NA. Nitrogen availability regulates proline and ethylene production and alleviates salinity stress in mustard (*Brassica juncea*). Journal of Plant Physiology. 2015;**178**:84-91. DOI: 10.1016/j.jplph.2015.02.006

[26] Moll RH, Kamprath EJ, Jackson WA. Analysis and interpretation of factors which contribute to efficiency of nitrogen utilisation. Agronomy. 1982;**74**:562-564. DOI: 10.2134/agronj1982.00021962007400030037x

[27] Le Gouis J, Beghin D, Heumez E, Pluchard P. Genetic differences for nitrogen uptake and nitrogen utilization efficiencies in winter wheat. European Journal of Agronomy. 2000;**12**:163-173. DOI: http://dx.doi.org/10.1016/S1161-0301(00)00045-9

[28] Pan J, Zhu Y, Jiang D, Dai T, Li Y, Cao W. Modeling plant nitrogen uptake and grain nitrogen accumulation in wheat. Field Crops Research. 2006;**97**:322-336. DOI: 10.1016/j.fcr.2005.11.006

[29] Gaju O, Allard V, Martre P, Le Gouis J, Moreau D, Bogard M, Hubbart S, Foulkes MJ. Nitrogen partitioning and remobilization in relation to leaf senescence, grain yield and grain nitrogen concentration in wheat cultivars. Field Crops Research. 2014;**155**:213-223. DOI: 10.1016/j.fcr.2013.09.003

[30] Palta JA, Fillery IRP. Post-anthesis remobilisation and losses of nitrogen in wheat in relation to applied nitrogen. Plant and Soil. 1993;**155**:179-181

[31] Kichey T, Hirel B, Heumez E, Dubois F, Le Gouis J. In winter wheat (*Triticum aestivum* L.), post-anthesis nitrogen uptake and remobilisation to the grain correlate with agronomic traits and nitrogen physiological markers. Field Crops Research. 2007;**102**:22-32. DOI: 10.1016/j.fcr.2007.01.002

[32] Pask AJD, Sylvester-Bradley R, Jameison PD, Foulkes MJ. Quantifying how winter wheat crops accumulate and use nitrogen reserves during growth. Field Crops Research. 2012;**126**:104-118. DOI: 10.1016/j.fcr.2011.09.021

[33] Zhen-Yuan S, Han BW, Liu SL, Wang HF, Gao RF. Absorption and redistribution of nitrogen during grain-filling period of wheat and their regulation by 6-benzylaminopurine. Acta Phytophysiol Sinica. 1996;**22**:258-264

[34] Mainard SD, Jeuffroy MH. Partitioning of dry matter and N to the spike throughout the spike growth period in wheat crops subjected to N deficiency. Field Crops Research. 2001;**70**:153-165

[35] Malik AI, Rengel Z. Physiology of nitrogen-use efficiency. In: Rengel Z, editor. Improving Water and Nutrient-Use Efficiency in Food Production Systems. UK: Wiley-Blackwell Inc.; 2013. pp. 105-121. DOI: 10.1002/9781118517994.ch7

[36] Asplund L, Bergkvist G, Weih M. Proof of concept: Nitrogen use efficiency of contrasting spring wheat varieties grown in greenhouse and field. Plant and Soil. 2014;**374**:829-842. DOI: 10.1007/s11104-013-1895-6

[37] Malik AI, Veres S, Rengel Z. Differential nitrogen-use efficiency in wheat parents of doubled-haploid mapping populations. Plant and Soil. 2016;**408**:311-325. DOI: 10.1007/s11104-016-2943-9

[38] Veres S, Malik AI, Rengel Z. Differential nitrogen supply causes large variability in photosynthetic traits in wheat germplasm. Crop & Pasture Science. 2017;**68**(8):703-712. DOI: 10.1071/CP17126

[39] Easterling DR, Apps M. Assessing the consequences of climate change for food and forest resources: A view from the IPCC. Climatic Change. 2005;**70**:165-189

[40] Gourdji SM, Sibley AM, Lobell DB. Global crop exposure to critical high temperatures in the reproductive period, historical trends and future projections. Environmental Research Letters. 2013;**8**:24-41. DOI: 10.1088/1748-9326/8/2/024041

[41] Koehler AK, Challinor AJ, Hawkins E, Asseng S. Influences of increasing temperature on Indian wheat, quantifying limits to predictability. Environmental Research Letters. 2013;**8**:034016. DOI: 10.1088/1748-9326/8/3/034016

[42] Tjoelker MG, Reich PB, Oleksyn J. Changes in leaf nitrogen and carbohydrates underlie temperature and CO_2 acclimation of dark respiration in five boreal tree species. Plant, Cell & Environment. 1999;**22**:767-778. DOI: 10.1046/j.1365-3040.1999.00435.x

[43] Murchie EH, Pinto M, Horton P. Agriculture and the new challenges for photosynthesis research. The New Phytologist. 2009;**181**:532-552. DOI: 10.1111/j.1469-8137.2008.02705.x

[44] Jordan DB, Ogren WL. The CO_2/O_2 specificity of ribulose 1,5-bisphosphate carboxylase/oxygenase: Dependence on ribulosebisphosphate concentration, pH and temperature. Planta. 1984;**161**:308-331. DOI: 10.1007/BF00398720

[45] Sage RF. Variation in the k(cat) of rubisco in C(3) and C(4) plants and some implications for photosynthetic performance at high and low temperature. Journal of Experimental Botany. 2002;**53**:609-620

[46] Portis AR. Rubisco activase – Rubisco's catalytic chaperone. Photosynthesis Research. 2003;**75**:11-27. DOI: 10.1023/A:1022458108678

[47] Parry MAJ, Andralojc P, Khan S, Lea P, Keys AJ. Rubisco activity: Effects of drought stress. Annals of Botany. 2002;**89**:833-839. DOI: 10.1093/aob/mcf103

[48] Perdomo JA, Capó-Bauçà S, Carmo-Silva E, Galmés J. Rubisco and rubisco activase play an important role in the biochemical limitations of photosynthesis in rice, wheat, and maize under high temperature and water deficit. Frontiers in Plant Science. 2017;**8**(490). DOI: 10.3389/fpls.2017.00490

[49] Porter JR, Xie L, Challinor AJ, Cochrane K, Howden SM, Iqbal MM, Lobell DB, Travasso MI. Food security and food production systems. In: IPCC. 2014. Climate Change 2014: Impacts, adaptation, and vulnerability. Part A: Global and sectoral aspects. Contribution of Working Group II to the Fifth Assessment Report,of the Intergovernmental Panel on Climate Change. Cambridge/New York: Cambridge University Press; 2014. pp. 485-533

[50] Fischlin A, Midgley GF, Price JT, Leemans R, Gopal B, Turley C. Ecosystems, their properties, goods, and services. Climate change 2007: Impacts, adaptation and vulnerability. In: Parry ML, Canziani OF, Palutikof JP, Hanson CE, editors. Proceedings of the Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge: Cambridge University Press; 2007. pp. 211-272

[51] Siddique MRB, Hamid A, Islam MS. Drought stress effects on water relations of wheat. Botanical Bulletin Academia Sinica. 2001;**41**:35-39

[52] Zhang LX, Li SX, Zhang H, Liang ZS. Nitrogen rates and drought stress effects on production, lipid peroxidation and antioxidative enzyme activities in two maize (*Zea mays* L.) genotypes. Journal of Agronomy and Crop Science. 2007;**193**:387-397. DOI: 10.1111/j.1439-037X.2007.00276.x

[53] Marschner H. editor: Mineral Nutrition of Higher Plants. 3rd ed. London: Academic Press; 2011. 672 p. ISBN: 978-0-12-384905-2

[54] Hoseinlou SH, Ebadi A, Ghaffari M, Mostafaei E. Nitrogen use efficiency under water deficit condition in spring barley. International Journal of Agronomy and Plant Production. 2015;**4**:3681-3687

[55] Morgan JA. The effects of N nutrition on the water relations and gas exchange characteristics of wheat (*Triticum aestivum* L.). Plant Physiology. 1986;**80**:52-58. DOI: 10.1104/pp.80.1.52

[56] Arun T, Upadhyaya SD, Upadhyay A, Preeti SN. Responses of moisture stress on growth, yield and quality of isabgol (*Plantago ovata* Forsk). Journal of Agricultural Technology. 2012;**8**:563-570

[57] Binghua L, Liang C, Mingjun L, Dong L, Yangjun Z, Fengwang M. Interactive effects of water and nitrogen supply on growth, biomass partitioning, and water-use efficiency of young apple trees. African Journal of Agricultural Research. 2012;**7**:978-985. DOI: 10.5897/ajar11.1212

[58] Li P, Chen J, Wu P. Agronomic characteristics and grain yield of 30spring wheat genotypes under drought stress and nonstress conditions. Agronomy Journal. 2011;**103**:1619-1628. DOI: 10.2134/agronj2011.0013

[59] Abreau JPD, Folres I, De-Abrea FMG, Medeira MV. Nitrogen uptake in relation to water availability in wheat. Plant and Soil. 1993;**154**(1):89-96

[60] Waraich EA, Ahmad R, Saifullah U, Ashraf MY, Ehsanullah L. Role of mineral nutrition in alleviation of drought stress in plants. Australian Journal of Crop Science. 2011;**5**:764-777

[61] Gonzales-Dugo V, Durand JL, Gastal F. Water deficit and nitrogen nutrition of crops. A review. Agronomy for Sustainable Development. 2010;**30**:529

[62] Maxwell K, Johnson GN. Chlorophyll fluorescence — A practical guide. Journal of Experimental Botany. 2000;**51**:659-668. DOI: 10.1093/jexbot/51.345.659

[63] Grassi G, Magnani F. Stomatal, mesophyll conductance and biochemical limitations to photosynthesis as affected by drought and leaf ontogeny in ash and oak trees. Plant, Cell & Environment. 2005;**28**:834-849. DOI: 10.1111/j.1365-3040.2005.01333.x

[64] Sz V, Simkó A, Kiss L, Zsombik L. Wheat genotypes under reduced nitrogen supply: Changes in chlorophyll fluorescence parameters. Columella. 2017;**4**(1 Suppl):53-58. DOI: 10.18380/SZIE.COLUM.2017.4.1.suppl

[65] Veres S, Marko P, Makleit P, Kiss L, Gáspár S, Frommer D, Rengel Z. Physiological detection of water and nitrogen deprivation. Annals series on agriculture. Silviculture and Veterinary Medicine Sciences. 2017;**6**(1):152-158

[66] Park JH, Lee BW. Photosynthetic characteristics of rice cultivars with depending on leaf senescence during grain filling. Journal of Crop Science. 2003;**48**:216-223

[67] Palta JA, Kobata T, Turners NC, Fillery IR. Remobilization of carbon and nitrogen in wheat as influenced by post-anthesis water deficits. Crop Science. 1994;**334**:118-124

[68] Gouarda G, Padovan S, Delougu G. Grain yield, nitrogen use efficiency and baking quality of old and modern Italian bread - wheat cultivars grown at different N levels. European Journal of Agronomy. 2004;**21**:181-192

[69] White PJ, Brown PH. Plant nutrition for sustainable development and global health. Annals of Botany. 2010;**105**(7):1073-1080

[70] Zsombik L, Seres E. The effect of genotype and nitrogen supply on the agronomical parameters of winter wheat (*Triticum aestivum* L.). In: LOTEX 2017, International Conference on Long-Term Field Experiments. 27-28. September; Nyíregyháza: Research Institute of Nyíregyháza; 2017

[71] Shi J, Yasuor H, Yermiyahu U, Zuo Q, Ben-Gal A. Dynamic responses of wheat to drought and nitrogen stresses during re-watering cycles. Agricultural Water Management. 2014;**146**:163-172. DOI: 10.1016/j.agwat.2014.08.006

[72] Ata-Ul-Karim ST, Nadeem A, Ehsan U. Improvement in wheat crop growth and grain yield under different planting techniques in Faisalabad zone. Journal of Environmental Agricultural Sci. 2015;**5**:71-79

[73] Blum A, Ramaiah S, Kanemasu ET, Paulsen GM. Wheat recovery from drought stress at the tillering stage of development. Field Crops Research. 1990;**24**:67-85. DOI: 10.1016/0378-4290(90)90022-4

[74] Muyen Z, Moore GA, Wrigley RJ. Soil salinity and sodicity effects of wastewater irrigation in South East Australia. Agricultural Water Management. 2011;**99**(1):33-41

[75] Eynard A, Lal R, Wiebe K. Crop response in salt-affected soils. Journal of Sustainable Agriculture. 2005;**27**(1):5-50

[76] Sadeghi A, Karimi E, Dahaji PA, Javid MG, Dalvand Y, Askari H. Plant growth promoting activity of an auxin and siderophore producing isolate of Streptomyces under saline soil conditions. World Journal of Microbiology and Biotechnology. 2012;**28**:1503-1509

[77] Rhoades JD, Chanduvi F, Lesch S. Soil salinity assessment-methods and interpretation of electrical conductivity measurements. FAO Irrigation and Drainage Paper 57. Italy, Rome: FAO-UN; 1999. 165 p

[78] Francois LE, Maas EV. Crop response and management on salt-affected soils. In: Pessarakli M, editor. Handbook of Plant and Crop Stress. New York: Marcel Dekker Press Inc.; 1999. pp. 169-201

[79] Munns R, Husain S, Rivelli AR, James RA, Condon AG, Lindsay MP, Lagudah ES, Schachtman D, Hare RA. Avenues for increasing salt tolerance of crops, and the role of physiologicallybased selection traits. Plant and Soil. 2002;**247**:93-105

[80] Munns R, James RA, Lauchli A. Approaches to increasing the salt tolerance of wheat and other cereals. Journal of Experimental Botany. 2006;**57**:1025-1043

[81] Maas EV. Salt tolerance of plants. Applied Agricultural Research. 1986;**1**:12-26

[82] Shannon MC, Grieve CM. Tolerance of vegetable crops to salinity. Scientia Horticulturae. 1999:78.5-78.7838

[83] Metternicht GI, Zinck JA. Remote sensing of soil salinity: Potentials and constraints. Remote Sensing of Environment. 2003;**85**:1-20

[84] Shrivastava P, Kumar R. Soil salinity: A serious environmental issue and plant growth promoting bacteria as one of the tools for its alleviation. Saudi Journal of Biological Sciences. 2015;**22**:123-131

[85] Ondrasek G, Rengel Z, Veres S. Soil salinisation and salt stress in crop production. In: Shanker AK, Venkateswarlu B, editors. biotic Stress in Plants: Mechanisms and Adaptations. Rijeka: InTechOpen; 2011. pp. 171-190

[86] Burrow DP, Surapaneni A, Rogers ME, Olsson KA. Groundwater use in forage production: The effect of saline–sodic irrigation and subsequent leaching on soil sodicity. Australian Journal of Experimental Agriculture. 2002;**42**:237-247

[87] Khoshgoftar AH, Shariatmadari H, Karimian N, Khajehpour MR. Responses of wheat genotypes to zinc fertilization under saline soil conditions. Journal of Plant Nutrition. 2006;**29**:1543-1556

[88] Khoshgoftarmanesh AH, Shariatmadari H, Karimian N, Kalbasi M, van der Zee SEATM. Cadmium and zinc in saline soil solutions and their concentrations in wheat. Soil Science Society of America Journal. 2006;**70**(2):582-589

[89] Norvell WA, Wu J, Hopkins DG, Welch RM. Association of cadmium in durum wheat grain with soil chloride and chelate extractable soil cadmium. Soil Science Society of America Journal. 2000;**64**:2162-2168

[90] Ozkutlu F, Ozturk L, Erdem H, McLaughlin MJ, Cakmak I. Leaf-applied sodium chloride promotes cadmium accumulation in durum wheat grain. Plant and Soil. 2007;**290**:323-331

[91] Tanji KK. Salinity in the soil environment. In: Läuchli A, Lütthe U, editors. Salinity: Environments-Plants-Molecules. Dordrecht: Kluwer; 2002. pp. 21-52

[92] Wheaton AD, McKenzie BM, Tisdall JM. Management of a sodic soil for wine grape production. Australian Journal of Experimental Agriculture. 2002;**42**:333-339

[93] Balali MR, Malakouti MJ, Mashayekhi H, Khademi Z. The effect of micronutrient on the yield increase of wheat in ten provinces of Iran. Journal of Soil and Water. 1999;**12**(8):111-119

[94] Broadley MR, White PJ, Hammond JP, Zelko I, Lux A. Zinc in plants. New Phytologist. 2007

[95] Rodriguez R, Redman R. More than 400 million years of evolution and some plants still can't make it on their own: Plant stress tolerance via fungal symbiosis. Journal of Experimental Botany. 2008;**59**:1109-1114

[96] Dodd IC, Perez-Alfocea F. Microbial amelioration of crop salinity stress. Journal of Experimental Botany. 2012;**63**(9):3415-3428

[97] Daei G, Ardekani MR, Rejali F, Teimuri S, Miransari M. Alleviation of salinity stress on wheat yield, yield components, and nutrient uptake using arbuscular mycorrhizal fungi under field conditions. Journal of Plant Physiology. 2009;**166**:617-625

[98] Ramadoss D, Lakkineni VK, Bose P, Ali S, Annapurna K. Mitigation of salt stress in wheat seedlings by halotolerant bacteria isolated from saline habitats. Springer Plus. 2013;**2**(6):1-7

[99] Nadeem SM, Zaheer ZA, Naveed M, Nawaz S. Mitigation of salinity-induced negative impact on the growth and yield of wheat by plant growth-promoting rhizobacteria in naturally saline conditions. Annales de Microbiologie. 2013;**63**(1):225-232

[100] Zimin AV, Puiu D, Hall R, Kingan S, Clavijo BJ, Salzberg SL. The first near-complete assembly of the hexaploid bread wheat genome, *Triticum aestivum*. GigaScience. 2017;**6**:1-7

[101] Li W, Zhang P, Fellers JP, Friebe B, Gill BS. Sequence composition, organization, and evolution of the core Triticeae genome. The Plant Journal. 2004;**40**(4):500-511

[102] Brenchley R, Spannagl M, Pfeifer M. Analysis of the bread wheat genome using whole-genome shotgun sequencing. Nature. 2012;**491**(7426):705-710

[103] International Wheat Genome Sequencing Consortium (IWGSC). A chromosome-based draft sequence of the hexaploid bread wheat (*Triticum aestivum*) genome. Science. 2014;**345**(6194):1251788

[104] Clavijo BJ, Venturini L, Schudoma C. An improved assembly and annotation of the allohexaploid wheat genome identifies complete families of agronomic genes and pro vides genomic evidence for chromosomal translocations. Genome Research. 2017;**27**(5):885-896

[105] Omielan JA, Epstein E, Dvorak J. Salt tolerance and ionic relation of wheat as affected by individual chromosomes of salt-tolerant *Lophopyrum elongatum*. Genome. 1991;**34**(6):961-974

[106] Gorham J, Wyn Jones RG, Bristol A. Partial characterization of the trait for enhanced K^+-Na^+ discrimination in the D genome of wheat. Planta. 1990;**180**:590-597

[107] Dubcovsky J, Santa Maria G, Epstein E, Luo MC, Dvorak J. Mapping of the K^+/Na^+ discrimination locus Kna1 in wheat. Theoretical and Applied Genetics. 1996;**2**:448-454

[108] Nevo E, Chen G. Drought and salt tolerances in wild relatives for wheat and barley improvement. Plant, Cell and Environment. 2010;**33**:670-685

[109] Ondrasek G, Rengel Z, Petosic D, Filipovic V. Land & water management strategies for the improvement of crop production. In: Ahmad P, Rasool S, editors. Emerging Technologies and Management of Crop Stress Tolerance. Vol. 2. A Sustainable Approach. London: Elesvier; 2014. pp. 291-310

[110] Dang YP, Dalal RC, Routley R, Schwenke GD, Daniells I. Subsoil constraints to grain production in the cropping soils of the north-eastern region of Australia. Australian Journal of Experimental Agriculture. 2006;**46**:19-35

Chapter 3

Role of Osmolytes and Antioxidant Enzymes for Drought Tolerance in Wheat

Muhammad Javid Iqbal

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.75926

Abstract

Plants are vital to life as their presence maintains ecosystem on this living globe. Environmental stresses trigger multiple responses initiated by plant cells to save plant life, from altered gene expression up to changes in cellular metabolism to regulate plant growth rates, which lead to better crop yield. The production of different osmoprotectants like proline, glycine-betaine (GB), trehalose, and antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase has shown a promising role to keep away cells from immediate cellular damage. Root-to-shoot ratio was enhanced in the drought-affected genotypes, while osmolytes and antioxidant enzymes take up the role to overcome drought situation in wheat germplasm. PEG-induced protocol was used to find out the production of osmolytes (proline, glycine-betaine, and trehalose) and antioxidant enzymes (SOD, CAT, and APX) biochemically. The levels of antioxidant enzymes and osmolytes were enhanced significantly in all germplasms indicating the defensive measures of plant cells in drought situation. DNA fingerprinting results have shown that the different wheat germplasms have an association with the levels of osmoprotectants and antioxidant enzymes during drought stress.

Keywords: drought tolerance, PEG 8000, osmolytes, antioxidant enzymes, wheat germplasm

1. Introduction

Global wheat production in the key production areas is being threatened by recurrent drought situation which is predicted to increase with climate change. Drought-tolerant wheat varieties are the ultimate solution of safeguarding the crop against adverse effects of drought [1]. Plants are frequently exposed to environmental stresses both due to some natural cause and

IntechOpen

© 2018 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. CC BY

rough agricultural practices. Various types of both biotic and abiotic stresses may result in limited plant productivity. Plant stresses like oxidative, chemical toxicity, drought and salinity, extreme temperatures along with the attack of insects, pests, and plant pathogens result in significant crop losses which are a serious threat to agriculture [2].

Drought is significantly damaging the plant that further limits the crop productivity, and most countries are facing this big disaster. Plant productivity is greatly inclined due to stressful conditions that affect almost every aspect of plant growth. All plants develop a unique pattern of biochemical and molecular mechanisms to manage odd situations linked with stress tolerance. Environmental stresses like drought, high salinity, and low temperature initiate gene expression that raise osmolytes and antioxidant enzyme levels in plant cells to tolerate stress responses.

Due to ever-increasing population around the globe, food crop productivity is highly enviable, and it is the need of the hour to expand measures for maximum produce. Wheat occupies an important place as staple food and the yield improvement of the wheat germplasm under different stresses and agro-climatic situations [3] have an essential task for researchers to deal within the present scenario. Human food comprises many valuable produce like rice, pulses, and meats but wheat is among the most important for human consumption worldwide. A part of the total wheat crop production is also used as a feed for livestock. *Triticum aestivum* is common bread wheat, but the other two species of wheat are of commercial importance as *T. durum* is pasta product wheat and *T. compactum* is pastry flour wheat. About 35% of the human population consumes wheat as food, covering 29% of caloric intake. Wheat shares the largest cereal market due to its global production at more than 651. 4 million metric tons per annum [4]. The high nutritive value (>10% protein, 2.4% lipids, and 79% carbohydrates) of wheat is based largely on its ingredients and the versatility of its use in the production of a wide range of food products [5]. The global climate change is facing adverse effects, while some other areas that have adopted the effects of climate change have shown benefits to crop system. Wheat is grown in the regions where rainfall ranges 30–113 cm [6]. Plant productivity is hampered by environmental stresses [7], and water shortage definitely limits plant growth and productivity even more than any other environmental factor [8]. Elevated levels of stress hormone viz. ABA has been associated with water stress tolerance in crop plants. Heat regulates stomatal conductance and water loss under desiccation that results in the accumulation of osmolytes like proline, mannitol, glycine-betaine, and soluble sugars like trehalose which lower the osmotic potential of the cell sap and thus prevent the movement of water out of the cell [9].

Pakistan has faced recently a big problem of wheat shortage due to drought situation and had to import wheat to fulfill the need of the country. Our globe is affected by drought: about 45% of the land area mostly of Africa (Ethiopia) and its surroundings, most of the Mediterranean, Mexico, Australia, and some parts of Middle East, India and Sindh province area of Pakistan. Irrigated land is only 15% of total cultivated land which yields twice as much as rain-fed land and producing one-third of the world's food [10]. Plant stress also plays a major role in determining the distribution of plant species due to soil texture and climate limitations. To maintain a gradient of water flow into the plant, the soil water potential is much important but its reduction may lead to increased soil solutes that make it increasingly difficult to establish osmotic pressure. The resulting osmotic stress leads to stomatal closure in some plant species [11] and a reduced rate of photosynthesis [12].

http://dx.doi.org/10.5772/intechopen.75926

2. Plant stresses

Environmental stresses are the main cause of limited crop production in the world. The land is affected by mineral stress about 20%, by drought stress about 26%, and 15% by freezing stress [13]. Environmental stresses are of two types: biotic stresses and abiotic stresses. Biotic stresses include infection and competition by other organisms. Abiotic stresses include light, temperature, water (drought), excess (flooding), radiation, and salinity stress. The capacity of plants to cope with unfavorable environments is known as stress resistance. Plant adaptations to tolerate stress depend upon genetically modified resistance genes that improve resistance as a result of prior exposure of a plant to stress. The mechanisms of drought resistance may fluctuate with climate change and soil conditions. Leaf expansion is restricted by water stress as one of the earliest responses occurring when decreases in turgor resulting from water deficit reduce or eliminate the driving force for cell and leaf expansion. Leaf abscission mechanisms start due to water stress and root extension into deeper, wetter soil, and stomatal closure as a response of water deficiency. Water deficit leads to the gene expression involved in acclimation and adaptation to the stress. The sensing and activation of signal transduction cascades mediating these changes in gene expression involve both an ABA-dependent pathway and ABA-independent pathways [9]. Anjum et al. [14] have studied the drought-induced changes in growth, osmolytes accumulation, and antioxidant metabolisms of maize hybrids. Drought stress in crop production system is much more precarious than any abiotic stress due to climatic changes. According to them, physio-biochemical regulation of plants under drought stress can be used as markers for drought tolerance in selection and breeding purposes. The maize growth and yield responses were highly related to ROS production, osmolytes accumulation, and activation of antioxidative defense system under drought situation.

3. Water-use efficiency

The water-limited productivity of plants depends on the total amount of water available and on the water-use efficiency of the plant. Any plant capable of acquiring more water or that has a higher water-use efficiency will resist drought better. When water shortage develops slowly, it is sufficient to permit changes in developmental process as water stress has more than a few adverse effects on plant growth. In this situation, compatible solutes like proline, glycine-betaine, and trehalose are produced to counter the unfavorable cellular conditions. Osmotic adjustment (OA) is a net increase in these solute contents per cell, and it develops slowly in response to tissue dehydration and maintains turgor and osmotic pressure of effected plant species. Osmotic potential fluctuation by the soil solution creates the stress in plants by water ultimately leading to plant death as a result of growth arrest and molecular damage. Osmotic adjustment in plant cells helps to maintain plant water balance to carry on regular life processes [9].

Most plants take up CO_2 from atmosphere while limiting water loss. The cuticle covers exposed plant surfaces as an effective barrier to water loss that protects the plant from desiccation. These plants cannot prevent outward diffusion of water without excluding CO_2 from the leaf. The concentration gradient of CO_2 uptake is much smaller than the concentration gradient that drives water loss. If water reservoir is higher than usual, this triggers regulation

of stomatal apertures at day time and remained close at night. There is no photosynthesis in the night, so no demand for CO_2 inside the leaf; therefore, stomatal apertures are kept small, preventing unnecessary water loss. When water supply is abundant on a sunny morning, the solar radiation incident on the leaf favors a high photosynthetic activity, the demand for CO_2 inside the leaf is large, and the stomatal pores are wide open to claim more CO_2. Through transpiration, water loss is substantial under these conditions, but since the H_2O is plentiful, it is beneficial for the plant to trade water, the product of photosynthesis, which is essential for growth and reproduction. Mild water deficits also affect the development of the root system. Root-to-shoot biomass ratio appears to be governed by a functional balance between water uptake by the root and photosynthesis by the shoot. A shoot will grow with maximum water uptake by the roots and becomes limiting to promote growth until their demand for photosynthate from the shoot equals the supply. This functional balance is shifted if the water supply decreases. On the other hand, when soil water is less abundant, the stomata will open less or even remain closed on a sunny morning. Thus, plants avoid dehydration by keeping its stomata closed in dry conditions [9].

Many organisms accumulate intracellular low-molecular-weight compounds due to water deficit to maintain equal water potential with the external conditions. Osmotic adjustment is contributed by many compounds which besides providing protection to macromolecules such as enzymes, proteins, electrolytes and temperature. Plant cells generally accumulate the inorganic ions mostly present in the soil environment, but in high concentration these become harmful to cellular integrity [15]. The organisms usually accumulate specific types of organic molecules called as compatible solutes required for maintaining the cytoplasm osmotically balanced. The main function of formed osmolytes is to maintain osmotic balance within the cell, and even their high concentrations may not impair the normal physiological function of the cell. As plant life savers, organic osmolytes facilitate osmotic adjustment normally to maintain cellular milieu.

3.1. Root-shoot ratio

Crops of tomorrow are expected to grow under huge levels of atmospheric CO_2. Basic crop growth parameters will be affected and major among those is carbon allocation. The ratio of root to shoot is dependent upon the separation of photosynthate which might be influenced by environmental stimuli. The upper layer of the soil gets dry without water, but the root growth started more to qualify moist zones under the soil. Deeper root growth into wet soil can be considered a second line of defense against drought. Better root growth into moist soil zones during stress requires osmolytes indirectly to maintain osmotic potential in order. During water shortage, the root growth is less prominent in reproductive plants as compared to vegetative plants. Therefore, the plants are more sensitive to water stress during reproduction period.

According to Iqbal et al. [16], the water deficit situation in wheat germplasm has shown detrimental developmental processes which effects plant growth ultimately. To counter this situation, compatible solutes like proline, glycine-betaine, and trehalose protect cellular milieu from dehydration. An increase in root growth in different plants under drought stress was also shown by Tahir et al. [17] and Jaleel et al. [18]. Plants with a higher proportion of roots can

compete more effectively for soil nutrients, while those with a higher proportion of shoots can collect more light energy and perform function accordingly. Root length is a better measure than the surface area of the absorbing ability of roots. Water moves slowly in soil so that a small root is almost as effective as a larger one in absorbing water and nutrients. According to Fahad et al. [19], the large proportions of shoot production are characteristic of vegetation in early succession phases, while high proportions of root production are characteristic of climax vegetation phases. Except for injury to the roots, a reduction in the root-shoot ratio is almost always in response to more favorable growing conditions. An increase in the root-shoot ratio would indicate that a plant was probably growing under less favorable conditions.

4. Drought stress

Drought is the lack of inadequate moisture level in soil, leading toward water stress which adversely affects crop productivity. Indeed, it is hypothesized that differences in drought and salt tolerance arise because of changes in the regulation of a basic set of drought and salt tolerance genes. Attempts to improve the drought tolerance of crops by conventional breeding programs have shown very limited success because of the complexity of the trait. Drought tolerance is a complex process genetically and physiologically [20]. The components of drought resistance in plants include both avoidance and tolerance to water stress and desiccation. Early maturity mechanism helps drought resistance in wheat before the period of drought, deeper root system to efficiently utilize the available moisture, and prolonged closing of stomata during drought stress to decrease water loss. The development of wheat cultivars for drought stress tolerance commonly has narrow leaves and lower shoot/root ratios and may have a low yield potential than varieties developed for irrigated areas. Jaleel et al. [21] has reviewed the drought stress as a changed physiological situation caused by the trend to disturb equilibrium. The damage in physical and chemical change shaped the stresses in plants exposed to drought, oxidative stress, low and high temperature, salt, flooding, and heavy metal toxicity. Drought stress tolerance is observed in most of the plants but its extent varies from species to species and even within species. Water deficit and salt stresses are global issues to ensure the survival of agricultural crops and sustainable food production. A ramified root system is established during drought tolerance and high biomass production primarily due to its ability to extract more water from soil and its transport to above ground parts for photosynthesis. When the beginning of stress is in rapid state or the plant has reached its full leaf area before initiation of stress while on the other side, protective mechanisms started in the plant against immediate desiccation. Under these sub-normal conditions, stomata closure reduces evaporation from the leaf surface area. At this stage, the stomatal closure is considered to be an important line of defense against drought. Uptake and loss of water in guard cells change their turgor and modulate stomatal opening and closing. The guard cells are located in the leaf epidermis which can drop turgor pressure as a result of a direct water loss by evaporation to the atmosphere. Hydropassive closure of stomata is due to the decrease in turgor that operates in air of low humidity and when direct water loss from the guard cells is too rapid to from adjacent epidermal cells. Secondly, hydroactive closure mechanism closes the stomata when the whole leaf or the roots are dehydrated and depends on metabolic processes in the

guard cells. The reduced solute contents in the guard cells results in water loss and decreased turgor for closing stomata. The hydraulic mechanism of hydroactive closure is a reversal of the mechanism of stomatal opening. The loss of solutes from guard cells can be activated by a decreased water content of the leaf where abscisic acid (ABA) plays an important role in this process. Abscisic acid is synthesized continuously at a low rate in mesophyll cells and tends to accumulate in the chloroplasts. When the mesophyll becomes mildly dehydrated, firstly the ABA stored in the chloroplasts is released to the apoplast (the cell wall space) of the mesophyll cell [22]. The pH gradients redistribute ABA molecule within the leaf, making it possible for the transpiration stream to carry some of the ABA to the guard cells. Secondly, leaf apoplast is saturated with ABA synthesized at a higher rate, and this higher ABA concentration appears to enhance or prolong the initial closing effect of the stored ABA, leading to the mechanism of ABA-induced stomatal closure. Leaf dehydration can vary widely both within and across species due to stomatal responses. The drought tolerant species like cowpea (*Vigna unguiculata*) and cassava (*Manihot esculenta*) are more responsive to stomatal conductance and leaf water potential may remain nearly constant during drought due to less transpiration activity. Chemical signals from the root system may affect the stomatal responses to water stress. The stomatal conductance is more closely related to soil water status than to leaf water status because the average root system is directly affected by soil water status. In fact, dehydrating only part of the root system may cause stomatal closure even if the well-watered portion of the root system still delivers ample water to the shoots.

4.1. Osmotic adjustment

Water plays a crucial role in plants' life as approximately 500 g of water is absorbed by the roots for every gram of organic matter made by plant. Imbalance in water flow can cause water shortage that lead to malfunctioning of major cellular processes. The balancing of water uptake and loss is a crucial challenge for photosynthetic plants to utilize CO_2 from atmosphere, and by doing so, plant exposes to water loss and the next threat of dehydration. A main difference between plant and animal cells that affects almost all aspects of their relation with water is the existence in plants of the cell wall. The internal hydrostatic turgor pressure is a result of their normal water balance inside the cell wall. Turgor pressure is essential for many physiological processes including cell enlargement, gases exchange in the leaves, transport in the phloem, and various transport processes across membrane. Turgor pressure also contributes to the rigidity and mechanical stability of non-lignified plant tissues.

Water is essential to land plants to avoid lethal desiccation by water loss to the atmosphere. The large surface area of leaves, their high radiant-energy gain, and their need to have an open pathway for CO_2 uptake may aggravate water loss. Water conservation and the need for CO_2 assimilation are a constant situation in plants for survival. Water makes up most of the mass of the plant cells, as each cell contains large water-filled vacuole whereas water typically constitutes 80–95% of the mass of the growing plant tissues. Seeds with a water content of 5–15% are among the driest of plant tissues that also absorb a considerable amount of water before germination. Plants continuously absorb and lose water during transpiration means and dissipate heat because the escaped water molecules have higher than average energy, breaking the bonds holding them in a liquid form. The transport of water bulk flow from the soil through

the plant body to the atmosphere includes diffusion and osmosis. The plant water can be considered incessant hydraulic system connecting through water in the soil with the water vapors in the atmosphere. Guard cells regulate transpiration through the control of stomatal pore size to meet the photosynthetic demand for CO_2 uptake while limiting water loss to the atmosphere. Large negative pressures (or tensions) in the apoplastic water is generated by water evaporation from the cell walls of the leaf mesophyll cells. Xylem conduits hold these negative pressures, but when transpiration is high, negative pressures in the xylem water may cause cavitations (embolisms) in the xylem that can block water transport and lead to severe water deficits in the leaf. Water deficit plants adapt responses that modify the physiology and development of plants. Water move through soils by bulk flow driven by a pressure gradient, and plants absorb water from soil through roots. The rate of water flow in soils depends upon the factors like pressure gradient through soil and hydraulic conductivity of the soil. As the water content of the soil decreases, the hydraulic conductivity decreases drastically. In very dry soil, water potential may fall below the permanent wilting point. At this point, the water potential of the soil is so low that plant cannot regain turgor pressure which means that the water potential is less than or equal to the osmotic potential of the plant. Different plant species behave differently in soil, and the permanent wilting point is clearly not a unique property of the soil. Water uptake decreases when roots are subjected to low temperature or anaerobic conditions, or treated with respiratory inhibitors like cyanide. The anaerobic roots transport less water to the shoots which suffer net water loss and begin to wilt [15].

4.2. Polyethylene glycol application

Polyethylene glycol (PEG) is a polymer used to modify the osmotic potential of nutrient solutions cultures to induce plant water deficit in experimental protocols. PEG 8000 (18%) is used as an osmoticum to induce drought in wheat genotypes after 1 week of plantation. Water stress greatly suppresses cell expansion and cell growth due to low turgor pressure. Osmotic regulation can enable the maintenance of cellular turgor for plant survival. The reduction in plant height was associated with a decline in the cell enlargement and more leaf senescence. The plants grown in nutrient culture containing PEG suffered from hypoxia, and such system should be oxygenated for running drought-stress experiments [16].

5. Osmolytes

In plants, there are effective mechanisms of osmotic adjustment based on the synthesis of osmolytes which are low-molecular-weight compatible solutes. Osmolytes are frequently used by cells to accommodate osmotic pressure within the effected cells to avoid cellular injury due to oxidation phenomenon. They are highly soluble organic molecules that are synthesized in many organisms in response to different environmental conditions leading to osmotic stress [7]. They accumulate in the cytosol without interfering with the cellular metabolism even at high concentrations. Osmolytes have additional functions during the stress response and act as osmoprotectants by directly stabilizing protein and membrane structures under dehydration conditions. They have a diverse chemical nature, and apart

from contributing to maintain osmotic balance, they do protect cell against oxidative stress as scavengers of "reactive oxygen species" (ROS) [23, 24].

The osmoregulators such as protein, sugars, amino acids, and compounds of quaternary ammonium play a vital role in adjusting the osmotic pressure and stabilizing of plant cells and tissues [25]. Drought stress causes osmotic stress in plants which causes a reduction in growth, imbalance ion transport, and a decrease in transpiration rate and an increase in membrane permeability. Such effects result in less water-absorbing capacity of crop plants, and different plant species and genotypes within a species respond differently to adverse environmental conditions. In order to counteract unfavorable environmental conditions, plants accumulate different types of organic and inorganic solutes in cytosol to decrease osmotic potential by which they can maintain cell turgor.

Plant cells lose water and decrease turgor pressure under water-stress conditions. There is an increase in different plant hormones in case of water stress like abscisic acid, which has important roles in the tolerance of plants to drought, high salinity, and cold. Abiotic stresses, which cause depletion of cellular water, are responsible for the greatest agricultural losses. Upon exposure to these prevalent stresses, the accumulation of osmoprotectants is in sufficient quantity to facilitate osmotic adjustment. The increase in cellular osmolarity due to these compatible solutes is accompanied by the influx of water into the cells, providing the turgor necessary for cell expansion [7]. Water deficit develops slowly enough to allow changes in developmental processes as water stress has several adverse effects on plant growth. In this situation, compatible solutes like proline, glycine-betaine, and trehalose produce to counter the unfavorable cellular conditions. The osmotic potential fluctuation of soil solution creating a water stress in plants ultimately leads to plant death due to growth arrest and molecular damage. Osmotic adjustment of cells helps to maintain plant water balance to establish internal milieu [9].

5.1. Proline

Proline is the most extensively studied osmolyte because of its great importance in stress tolerance [26]. The exogenous application of proline can increase its endogenous levels in plant tissues subjected to water-stress conditions which help maintain osmotic adjustment in plant tissues. It may be a good source of minimizing the adverse effects of water stress on plants, and triggering their growth also depends upon the type of plant species and its concentration [27].

The production of proline is widely present in higher plants and normally accumulates in large quantities in response to environmental stresses [28]. For osmotic adjustment, proline contributes to stabilizing subcellular structures (e.g., membranes and proteins), scavenging free radicals, and buffering cellular redox potential under stress conditions. A rapid breakdown of proline upon relief of stress may provide sufficient reducing agents that support mitochondrial oxidative phosphorylation and generation of ATP for recovery from stress and repairing of stress-induced damages [29]. Iqbal et al. [30] have reported that the accumulation of proline in drought-tolerant and drought-sensitive cultivars has revealed the significance of this osmolyte. The role of proline in induced PEG experiment gave evidence that the higher levels of proline are due to the emergent need of stressed plant. This osmolyte is able to control the osmotic regulation of the cellular environment because of its high water solubility and

its accumulation in the leaves of many halophytic higher plants grown in saline environment. Proline protects membranes against adverse effects of high concentration of inorganic ions and temperature extremes. It is also functional as a protein-compatible hydrotope and as a hydroxyl radical scavenger [31].

5.2. Proline biosynthesis

Proline biosynthesis in plants is initiated with the ATP-dependent phosphorylation of the carboxy group of glutamate by glutamyl kinase (GK).The resulting glutamyl phosphate (GP) is reduced to glutamic semi-aldehyde (GSA) by GSA dehydrogenase and glutamyl kinase which forms obligatory enzyme complex [32]. The accumulation of proline under stress in many plant species has been correlated with stress tolerance, and its concentration has been shown to be generally higher in stress-tolerant than in stress-sensitive plants. In wheat, an assessment of the effects of drought stress on proline accumulation in a drought-tolerant and a drought-sensitive cultivar revealed that the rate of proline accumulation and utilization was significantly higher in the drought-tolerant cultivar [33]. Furthermore, in *B. juncea* plants grown under stress conditions, activities of proline biosynthetic enzymes P5CR and ornithine-aminotransferase (OAT) increased mainly intolerant lines though the activity of proline-degrading enzyme "proline oxidase" decreased in all lines.

5.3. Trehalose

Trehalose is a vital soluble sugar osmolyte frequently used by cells to accommodate osmotic pressure within the effected cells to avoid cellular injury due to oxidation phenomenon. According to recent research, sugar-signaling mechanism plays a vital role in accelerating the photosynthetic performance of plants to its maximum rate in association with trehalose metabolism. These positive effects of trehalose on gas exchange parameters are due to its role in osmoregulation which may affect the stomatal opening. It can be concluded that improvement in growth in wheat cultivars under water-stressed condition with trehalose application may have been due to the role of trehalose in osmotic adjustment. Different plant species respond differently on exogenous application of trehalose and proline. The plant development may be hampered by the external application of these compounds resulting in growth inhibition or yield reduction. The beneficial applications of these osmolytes on crop stress tolerance must carefully be determined for appropriate plant developmental stages.

In plants, trehalose increased the biomass production in shoots and roots in all wheat cultivars under water-stressed conditions as an osmoprotectant under adverse environmental conditions. Exogenous applications of trehalose and proline to plants during or after stress exposure and the increase in the internal levels of these compounds generally enhance plant growth and final crop yield under stress conditions [30].

5.4. Glycine-betaine

Among the many quaternary ammonium compounds known in plants, glycine-betaine (GB) occurs most abundantly in response to dehydration stress. GB is abundant mainly in chloroplast where it plays a vital role in adjustment and protection of thylakoid membrane, thereby

maintaining a photosynthetic efficiency. In higher plants, GB is synthesized in chloroplast from serine via ethanolamine, choline, and betaine aldehyde [34]. The accumulation of two valuable osmolytes like glycine-betaine and proline in different plant species in response to environmental stresses such as drought, salinity, extreme temperatures, UV radiations, and some heavy metals. The role of these compounds has positive effects on enzymes and membrane integrity along with adaptive ways for osmotic adjustment in plants grown under stress conditions.

Cellular responses to stress include changes in the cell cycle and cell division, changes in the endomembrane system and vacuolization of cells, and changes in cell wall architecture, all leading to enhanced stress tolerance of cells. Plants alter metabolism in various ways to accommodate environmental stresses at a biochemical level by producing osmoregulatory compounds such as proline and glycine-betaine. The molecular events linking the perception of a stress signal with the genomic responses leading to tolerance have been intensively investigated in recent years. Certain plants accumulate significant amounts of glycine-betaine [35] in response to high salinity, cold, and drought stress. This quaternary amine has protective functions for macro-components of plant cells such as protein complexes and membranes under stress. GB is known to accumulate in response to stress in many crop plants, including sugar beet (*Beta vulgaris*), spinach (*Spinacia oleracea*), barley (*Hordeum vulgare*), wheat (*T. aestivum*), and sorghum (*Sorghum bicolor*). In these species, tolerant genotypes normally accumulate more GB than sensitive genotypes in response to stress. The relationship between GB accumulation and stress tolerance is species or even genotype specific [36]. The increased biosynthesis of GB from choline in stress-sensitive plants is capable of synthesizing this protective solute as drought-stress management.

All plant species are not equally capable of natural production or accumulation of osmolytes in response to stress. Tolerance to abiotic stresses is very complex at the whole plant and cellular levels. The complexity of interactions between stress factors and various molecular, biochemical, and physiological phenomena affects plant growth and development eventually [24]. Exogenous application of proline as pre-sowing seed treatments significantly affected the shoot and root K^+, Ca^{2+}, P, and N contents of root while this effect of proline on shoot N contents was inconsistent [37]. Similarly, Cuin et al. [38] reported that compatible solutes such as glycine-betaine, proline, and trehalose have explanatory effects on K+ efflux in Arabidopsis under stressed condition.

6. Antioxidant enzymes

The enzymatic and non-enzymatic mechanisms are available for scavenging of reactive oxygen species (ROS) in plants. The biochemical adaptive function of osmoprotectants to scavenge these harmful ROS by-products of hyperosmotic and ionic stresses causes membrane dysfunction and cell death ultimately. These active oxygen species are superoxide (O_2−), hydrogen peroxide (H_2O_2), hydroxyl radical (√OH), and singlet oxygen ($1O_2$) which is produced through oxidation phenomenon. Many plants have the ability to eliminate superoxide with the help of superoxide dismutase (SOD), which catalyzes the superoxide into H_2O_2 and O_2. Thylakoid membrane has potential enzyme ascorbate peroxidase (APX) to eliminate hydrogen peroxide to save cell membrane from severe damage.

6.1. Superoxide dismutase

SOD concentrations typically increase with the degree of stress conditions as the compartmentalization of different forms of SOD throughout the plant makes them counteract stress very effectively. There are three classes of SOD metallic coenzymes that exist in plants that act to control increased levels of oxidative stress. SOD's role as a free radical scavenger is established, and those genotypes have higher levels indicating a higher level of stress tolerance in wheat. The availability of different forms of SOD in plants show a maximum stress tolerance in affected crops, giving protection to the plant. The trends observed in the present research might be due to the reasons discussed earlier.

6.2. Catalase

Catalase is a common enzyme found in nearly all living organisms exposed to oxygen and catalyzes the decomposition of H_2O_2 to water and oxygen. The highest turnover by catalase molecule could convert millions of molecules of H_2O_2 to water and oxygen in each second. Hydrogen peroxide is a toxic by-product of many regular metabolic processes. It must be quickly converted into less toxic substances to prevent most cellular damage and tissue injuries.

6.3. Ascorbate peroxidase

Ascorbate peroxidases (APXs) are the enzymes that detoxify hydrogen peroxide using ascorbate as a substrate. It is frequently used by cells to rapidly catalyze the decomposition of hydrogen peroxide into less reactive gaseous oxygen and water molecules. Higher plants produce active oxygen species during metabolic processes including mitochondrial, chloroplastic, and plasma membrane-linked electron transport system. Due to biotic and abiotic stress, conditions can give rise to excess concentration of these active oxygen species resulting in oxidative damage at a cellular level. At this stage, the antioxidant enzymes have to function to interrupt the cascades of uncontrolled oxidation in each organelle. Ascorbate peroxidase (APX) exists as isoenzymes that play an important role in the metabolism of H_2O_2 in higher plants. APX activities generally increase along with activities of other antioxidant enzymes like catalase, SOD, and GSH reductase in response to various environmental stress factors regulating the components of ROS-scavenging systems. APX has been identified in many higher plants and comprises a family of isoenzymes with different characteristics.

Photosynthetic organisms including higher plants and eukaryotic algae have developed AOS-scavenging systems, including APX isoenzymes. AOS-scavenging system also established in prokaryotic cyanobacteria has an H_2O_2 tolerance system of the Calvin cycle and an H_2O_2 diffusion system. The distinct regulatory mechanisms are expressed by APX isoenzymes in response to various environmental stresses or cell conditions and play a cooperative role to protect each organelle and minimize tissue injury. The action of antioxidant systems under drought has been investigated by many authors in several crops, such as spinach, pea, sorghum and sunflower, and wheat [39]. Richard et al. [40] have studied the responses to abiotic stresses and activities of superoxide dismutase, catalase, and peroxidase, as well as malondialdehyde (MDA) contents and solute potentials in seedlings of seven wheat (*Triticum*) species (nine genotypes representing three ploidy levels: hexaploid,

tetraploid, and diploid) subjected to water stress for 4, 8, and 12 days by withholding water. In most species, the activities of superoxide dismutase and catalase showed an increase in the early phase of drought and then a decrease with further increase in water stress.

The enzymatic activities partly recovered and malondialdehyde contents decreased with re-watering. Under drought situation, hexaploid wheat had higher peroxidase activities and MDA contents than tetraploid and diploid wheat. The solute potentials and the activity of SOD and CAT were similar among three groups. Conventional breeding techniques have been unsuccessful in transferring the drought tolerance trait to the target species [2]. The basic biotechnology tools can be employed to manage stress tolerance, hence improving yield stability. Different genetic markers were identified as linked to different traits of interest to determine polymorphism among a variety of wheat genotypes. Richard et al. [40] have studied the responses of growth and primary metabolism of water-stressed barley roots to rehydration.

The assessment of the quantity of variety detected with microsatellite exposes additional polymorphism among different genotypes. The individuality and the value of microsatellites started their multi-allelic nature, co-dominant transmission, wide genome treatment, and requirement for a small amount of starting DNA. Genetic diversity among adapted cultivars or elite-breeding materials has a considerable impact on the improvement of crop plants. Molecular markers can determine genetic diversity from pedigree analysis or morphological traits, and they can offer the best estimate of genetic diversity since they are independent of the perplexing effects of environmental factors. Several molecular markers like RAPD and SSRs are available to assess the variability and diversity at a molecular level [41].

7. Conclusion

Drought stress causes osmotic stress in plants which causes reduction in growth, imbalance ion transport, and a decrease in transpiration rate and an increase in membrane permeability. Such effects result in less water-absorbing capacity of crop plants, and different plant species and genotypes within a species respond differently to adverse environmental conditions. In order to counteract unfavorable environmental conditions, plants accumulate different types of organic and inorganic solutes in cytosol to decrease osmotic potential by which they can maintain cell turgor. PEG induced severe stress in the selected wheat germplasm, and root-to-shoot ratio was enhanced in the drought-affected germplasm. The levels of antioxidant enzymes were enhanced significantly in all genotypes while glycine-betaine, proline, and trehalose have shown association and positive effects during drought stress. The safety and survival of the plants depends on the coordination of these vital osmoprotectants with antioxidant enzymes.

Author details

Muhammad Javid Iqbal

Address all correspondence to: imjavid@gmail.com

Department of Pharmacy, CPMSATS Institute of Information Technology, Lahore, Pakistan

References

[1] Li P, Chen J, Wu P. Agronomic characteristics and grain yield of 30 spring wheat genotypes under drought stress and non-stress conditions. Agronomy Journal. 2011; **103**(6):1619-1628

[2] Jamil A, Anwer F, Ashraf M. Plants tolerance to biotic and abiotic stresses through modern genetic engineering techniques. In: Crops: Growth, Quality and Biotechnology. Helsinki, Finland: WFL Publishers; 2005. pp. 1276-1299

[3] Zhu Y, Cao W, Dai T, Jiang D. A dynamic knowledge model for wheat target yield design and variety selection. The Journal of Applied Ecology. 2004;**15**(2):231-236

[4] Food and Agriculture Organization. Annual wheat report. Internet source: www.fao.org/worldfoodsituation

[5] Tarek EB. Trehalose metabolism in wheat and identification of trehalose metabolizing enzymes under abiotic stress. (PhD thesis); 2003

[6] Fahad S, Chen Y, Saud S, Wang K, Xiong D, Chen C, Wu C, Shah F, Nie L, Huang J. Ultraviolet radiation effect on photosynthetic pigments, biochemical attributes, antioxidant enzyme activity and hormonal contents of wheat. The Journal of Food, Agriculture & Environment. 2013;**11**(3&4):1635-1641

[7] Nezhadahmadi A, Prodhan ZH, Faruq G. Drought tolerance in wheat. The Scientific-World Journal. 2013:1-12

[8] Bary EA, Seres JB, Weretilnyk E. Responses to abiotic stresses in biochemistry and molecular biology of plants. American Society of Plant Physiologists. 2000:1158-1203

[9] Taiz L, Zeiger E. Plant Physiology. 3rd ed. India: Panama Publishing Co.; 2003. pp. 591-620

[10] Munns R. Comparative physiology of salt and water stress. Plant, Cell and Environment. 2002;**25**:239-250

[11] Lawlor DW. Genetic engineering to improve plant performance under drought: Physiological evaluation of achievements, limitations and possibilities. Journal of Experimental Botany. 2013;**64**(1):83-108

[12] Chaves MM, Pereira JS, Morroco J, Rodrigues ML, Recardo CPP, Osorio ML, Carvalho I, Faria T, Pinhiero C. How plants cope with water stress in the field: Photosynthesis and growth. Annals of Botany. 2002;**89**:907-916

[13] Blum A, Zhang J, Nguyen HJ. Consistent differences among wheat cultivars in osmotic adjustment and their relationship to plant production. Field Crops Research. 1999; **64**:287-291

[14] Anjum SA, Ashraf U, Tanveer M, Khan I, Hussain S, Shazad B, Zohaib A, Abbas F, Saleem MF, Ali I, Wang LC. Drought induced changes in growth, osmolyte accumulation and antioxidant metabolism of three maize hybrids. Frontiers in Plant Science. 2017;**8**(69):1-12

[15] Bartels D. Drought and desiccation induced modulation of gene expression in plants. Journal of Plant Cell Environment. 2002;**25**:141-115

[16] Iqbal MJ, Rahman M, Ashraf M, Sheikh MA, Jamil A. Trehalose expression in hexaploid wheat (Triticum aestivum L.) germplasm under drought stress. Pakistan Journal of life Social Sciences. 2012;**10**(2):106-110

[17] Tahir MHN, Imran M, Hussain MK. Evaluation of sunflower (*Helianthus annuus* L.) inbred lines for drought tolerance. International Journal of Agriculture and Biology. 2002;**3**:398-400

[18] Jaleel CA, Gopi R, Sankar B, Gomathinayagam M, Panneerselvam R. Differential responses in water use efficiency in two varieties of *Catharanthus roseus* under drought stress. Comptes Rendus Biologies. 2008;**331**:42-47

[19] Fahad S, Hussain S, Saud S, Khan F, Hassan S, Jr A, Nasim W, Arif M, Wang F, Huang J (2016b). Exogenously applied plant growth regulators affect heat-stressed rice pollens. Journal of Agronomy and Crop Science. 202:139-150

[20] Flowers TJ. Improving crop salt tolerance. Journal of Experimental Botany. 2004;**55**: 307-319

[21] Jaleel CA, Ragupathi G, Paramasivam M, Panneerselvam R. Soil salinity alter the morphology in *Catharanthus roseus* and its effects on endogenous mineral constituents. Eurasia Journal of Biosciences. 2007;**2**:18-25

[22] Sauter A, Davies WJ, Hurtung W. The long distance abscisic acid signal in the droughted plant: The fate of hormone on its way from root to shoot. The Journal of Experimental Botany. 2001;**52**(363):1991-1997

[23] Serrano R, Culiañz-Maciá FA, Moreno V. Genetic engineering of salt and drought tolerance with yeast regulatory genes. Journal of Science Horticulture. 1999;**78**:261-269

[24] Zhu JK. Salt and drought stress signal transduction in plants. Annual Review of Plant Biology. 2002;**53**:247-273

[25] Hasegawa PM, Bressan RA, Zhu JK, Bohnert HJ. Plant cellular and molecular responses to high salinity. Annual Review of Plant Physiology and Plant Molecular Biology. 2000;**51**:463-499

[26] Kamran M, Shahbaz M, Ashraf M, Akram NA. Alleviation of drought-induced adverse effects in spring wheat (*Triticum aestivum* L.) using proline as a pre-sowing seed treatment. Journal of Botany. 2009;**41**:621-632

[27] Ali G, Srivastava PS, Iqbal M. Proline accumulation, protein pattern and photosynthesis in regenerants grown under NaCl stress. Biologia Plantarum. 2007;**42**:89-95S

[28] Kishore PBK, Hong Z, Miao GH, CAA H, DPS V. Overexpression of 1-pyrroline-5-carboxylate synthase increases proline production and confers osmotolerence in transgenic plants. Plant Physiology. 2007;**108**:842-847

[29] Hare PD, Cress WA. Metabolic implications of stress induced proline accumulation during stress. Plant Growth Regulations. 1997;**21**:79-102

[30] Iqbal MJ, Maqsood Y, Abdin ZU, Manzoor A, Hassan M, Jamil A. SSR markers associated with Proline in drought tolerant wheat germplasm. Applied Biochemistry and Biotechnology. 2016;**178**:1042-1052

[31] Fahad S, Nie L, Chen Y, Wu C, Xiong D, Saud S, Hongyan L, Cui K, Huang J. Crop plant hormones and environmental stress. Sustainable Agriculture Reviews. 2015b;**15**:371-400

[32] Nayyar H. Variation in osmoregulation in differentially drought sensitive wheat genotype involves calcium. Journal of Biologia Plantarum. 2004;**47**:541-547

[33] Mansour MMF. Nitrogen containing compounds and adaption of plants to salinity stress. Biologia Plantarum. 2000;**43**:491-500

[34] Mohanty A, Kathuria H, Ferjani A, Sakamoto A, Mohanty P, Murata N, Tyagi AK. Transgenics of an elite indica rice variety *Pusa Basmati* 1harbouring the *cod A* gene is highly tolerant to salt stress. Theoretical and Applied Genetics. 2002;**106**:51-57

[35] Yang WJ, Rich PJ, Axtell JD, Wood KV, Bonham CC, Ejeta G, Mickelbart MV, Rhodes D. Genotypic variation for glycine betaine in sorghum. Crop Science. 2003;**43**:162-169

[36] Ashraf M, Foolad M. Roles of glycine betaine and proline in improving plant abiotic stress resistance. Journal of Environment and Experimental Botany. 2007;**59**:206-216

[37] Farooq M, Hussain M, Siddique KHM. Drought stress in wheat during flowering and grain-filling periods. Critical Reviews in Plant Sciences. 2014;**33**(4):331-349

[38] Cuin TA, Zhou M, Parsons D, Shabala S. Genetic behaviour of physiological traits conferring cytosolic K^+/Na^+ homeostasis in wheat. Plant Biology. 2012;**14**:438-446

[39] Sgherri CLM, Maffei M, Navari-Izzo F. Antioxidative enzymes in wheat subjected to increasing water deficit and rewatering. Journal of Plant Physiology. 2000;**157**(3):273-279

[40] Richard CS, Timlin D, Bailey B. Responses of growth and primary metabolism of water-stressed barley roots to rehydration. Journal of Plant Physiology. 2012;**169**:686-695

[41] Palombi MA, Damiano C. Comparison between RAPD and SSR molecular markers in detecting genetic variation in kiwifruit (*Actinidia deliciosa* A. Chev). Plant Cell Report. 2002;**20**:1061-1066

Chapter 4

Wheat Straw Open Burning: Emissions and Impact on Climate Change

Gisela Montero, Marcos A. Coronado,
Conrado García, Héctor E. Campbell,
Daniela G. Montes, Ricardo Torres, Laura Pérez,
José A. León and José R. Ayala

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.76031

Abstract

The state of Baja California, Mexico, is the second national wheat producer. Mexicali, the capital of Baja California, is the primary wheat producer, and it represents the most significant crop in the valley, with 90,609 ha of a cultivated surface by 2015; it leads to a wheat production of 585,334 t and a generation of 661,446 t of wheat straw as agricultural residue. The 15% of this waste has various uses. The 85% of wheat straw is open burnt *in situ* to prepare the farmland for the next agricultural cycle. Through the development of an emissions and energy model on iThink®, the emissions of 6,185 t of PM, 35,983 t of CO, and 1,125 t of CH_4 considering a headfire burning or 3,373 t of PM, 30,360 t of CO, and 731 t of CH_4 by backfire burning were estimated. Also, the wheat straw wasted energy was estimated at 8.15 PJ by 2015, with a lower heating value of 14.50 MJ/kg determined experimentally. The results highlight that for each hectare of harvested wheat, 6.205 t of wheat straw are generated and burnt. It represents the emission of pollutants and 89,972.50 MJ of wasted energy.

Keywords: wheat straw, climate change, open burning, emissions, energy

1. Introduction

Agriculture is the oldest economic sector in the world, and it is more reliant on fertile soils and stable climate than any other type of trade [1]. Nowadays, wheat is one of the key cereals cultivated in the world, with an annual production of 733 million tons by 2015 [2]. In the

IntechOpen

© 2018 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. CC BY

same year, the harvested surface dedicated to wheat production was 819,928 ha in Mexico [3]. The wheat varieties *Triticum aestivum* and *T. durum* are the most common. In the fall–winter season, 90% of wheat production is obtained, and the remaining 10% in the spring–summer season. The harvest season is performed predominantly in May and June [4].

The Mexicali Valley is one of the most important agricultural areas of the northwest of Mexico, and it has one of the most extensive surfaces dedicated to wheat production nationally. This valley is located on the state of Baja California and shares the atmospheric basin with the Imperial Valley, USA (**Figure 1**). Its principal crop is wheat, with an average productivity of 6.46 t/ha, of the *T. aestivum* variety. Apart from the favorable climate conditions for this crop in the region, the use of improved varieties of a high productive potential and the experience of the producer in the application of the technological innovations for its management had been determinants to achieve this level of production [5]. After harvesting, it is necessary to dispose large amounts of straw generated as agricultural waste, with a rate of 7.3 t/ha [6]. Usually, 85% of this waste is burnt *in situ* in the open air with the objective of preparing the fields for double-cropping or the next agricultural cycle, and the remaining percentage has various applications [7].

Figure 1. Geographic location of Baja California.

Some wheat producers of the Mexicali Valley that conduct this practice argue that the burning represents a traditional practice and that the incineration of agricultural waste is necessary since it eliminates perennial weeds, diseases, and pests (**Figure 2**). Other producers ensure that for burning wheat straw, the use of machinery is not a requirement, saving money in machinery, diesel, and the tractor's operator and that it gives more time with the purpose of preparing the fields for the next cycle. However, contrary to the producers' assumptions, the burning calcine nitrogen, phosphorus, and the soil organic matter, as well as generating additional costs and a drop in yield and, in consequence, shrinkages on the utility in obtaining less volumes by wheat hectare between cycle and cycle [8], has been demonstrated.

The *in situ* burning of wheat straw implies the emissions of large quantities of PM, CO, and CH_4 that impact the environment, causing deterioration in the air quality of the Valley, including the city of Mexicali, as well as respiratory diseases for the population (**Figure 3**). In this sense, it is relevant to mention that Mexicali is one of the cities of Mexico with a higher level of morbidity from acute respiratory infections.

The emissions caused by the open burning of wheat straw affect the climate. Consequently, it has an impact on crop growth and yields are negatively affected by suboptimal water supply and abnormal temperatures due to physical damages, physiological disruptions, and biochemical changes [9, 10]. The use of conditional promoters driving gene expression at specific developmental stages, in response to specific environmental cues, will make possible the generation of transgenic crops able to grow under various abiotic stresses with minimal yield losses [11].

Also, when the wheat straw is open burnt, the energy contained in the same is wasted. The wheat straw could be valorized and reconverted into biofuels or directly used in electric generation.

The utilization of bioenergy has significant environmental, and also economic, benefits because the biomass waste is valorized as biofuel. The use of wheat straw as raw material for any productive process presents diverse factors that must be considered. Among those factors

Figure 2. Open burning of the wheat straw in Mexicali Valley, Mexico.

Figure 3. Open burning of the wheat straw near the rural population of the Mexicali Valley, Mexico.

are the low density of biomass, handling and high transportation cost, an attractive heating value, and the physicochemical characterization [12].

In this chapter, the emissions caused by the headfire or backfire burning of wheat straw *T. aestivum* in Baja California, Mexico, for the period 1987–2015, were estimated through the development of a model on the iThink® dynamic simulator [13]. Also, the energy emitted by wheat straw burning was calculated considering its significant heating value of 14.50 MJ/kg determined experimentally [14], and it was included in this model.

2. Materials and methods

The emissions and energy associated with the agricultural burnings depend on many parameters; for that, those supported by current and reliable information were selected. The settings used to feed the model are the following:

1. Historical series of the wheat harvested surface,
2. Wheat straw generation index,
3. Wheat straw lower heating value,
4. PM, CO, and CH_4 emission factors by agricultural burning technique.

2.1. Historical series of the wheat-harvested surface

Wheat straw is a waste generated in large quantities during wheat harvesting. To estimate its generation in the Mexicali Valley, information on the annual wheat harvested surface on the 1987–2015 period was used and is presented in **Table 1** [15, 16].

2.2. Wheat straw generation index and lower heating value

To estimate the quantity of wheat straw generated by agricultural cycle, a generation index of 7.3 t/ha was considered [6].

Year	Wheat-harvested surface (ha)	Year	Wheat-harvested surface (ha)
1987	53,098	2002	74,394
1988	50,572	2003	85,320
1989	48,374	2004	80,555
1990	60,366	2005	75,989
1991	79,683	2006	79,946
1992	79,683	2007	81,958
1993	80,018	2008	88,937
1994	69,658	2009	87,724
1995	53,159	2010	87,321
1996	67,224	2011	74,260
1997	54,913	2012	72,153
1998	50,636	2013	83,015
1999	74,273	2014	81,681
2000	68,033	2015	90,609
2001	64,926		

Table 1. Historical series of the wheat-harvested surface, 1987–2015.

The lower heating value of the wheat straw was considered as 14.50 MJ/kg, which was experimentally determined. The tests were realized with the *T. aestivum* wheat variety from Baja California, Mexico [14].

2.3. PM, CO, and CH_4 emission factors by agricultural burning technique

To estimate the PM, CO, and CH_4 emissions, generated by wheat straw burnt *in situ* in the open air, the factors reported by the EPA AP-42 [17], enlisted in **Table 2**, were used. Such report clusters the emission factors according to the incineration technique used by the farmers. It is important to note that in the Mexicali Valley case, both techniques are used by producers, for which the calculations were made considering the two of them. The incinerating techniques according to the EPA are described as follows:

- Headfire: Burning technique where the fire advances in the wind direction;
- Backfire: Burning technique in which the fire advances to the opposite direction of the wind.

2.4. Parameters used in the emissions and energy model and sequence

Figure 4 displays the sequence and relationships between the parameters used in the emissions and energy model.

Type of burning	Emissions factors (kg/t)		
	PM	CO	CH_4
Headfire	11	64	2
Backfire	6	54	1.3

Table 2. Emissions factors.

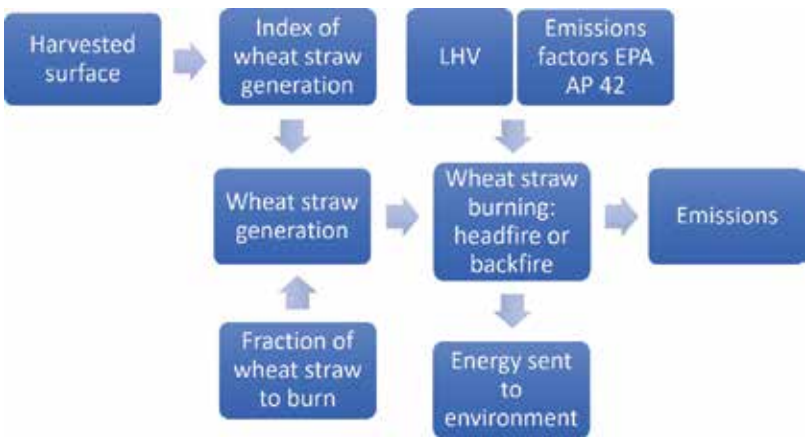


Figure 4. Parameters used in the emissions and energy model.

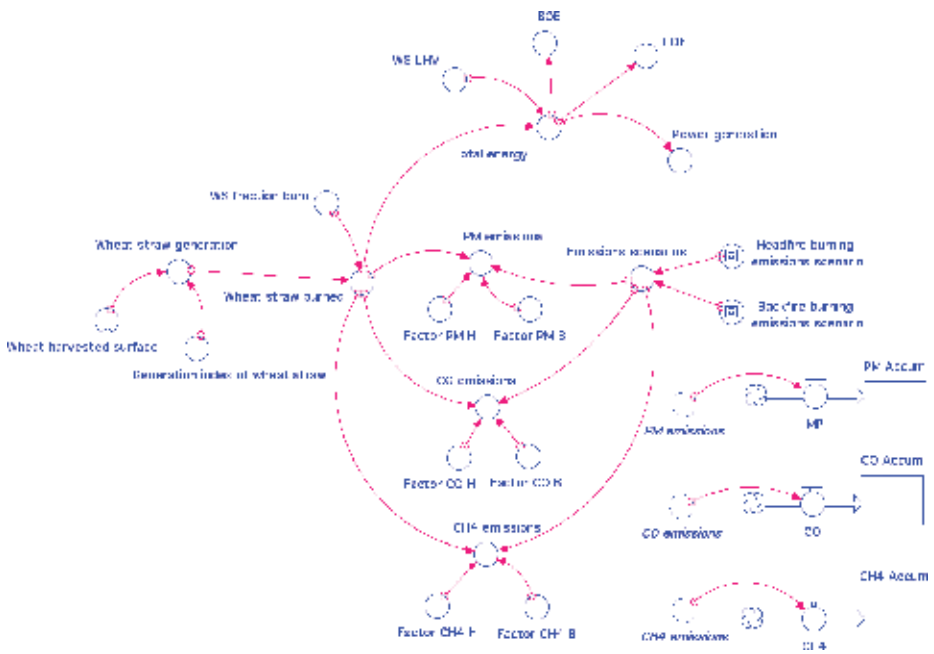


Figure 5. Emissions and energy model developed in iThink®.

2.5. Emissions and energy model

Based on the selected parameters and with the purpose of facilitating the analysis of the emissions associated with wheat straw burning during the 1987–2015 period, a dynamic model was developed on iThink®, whose simplified version is illustrated in **Figure 5**. The development of the model allows to establish and observe practically and graphically the interrelations of the different variables used to estimate the emissions corresponding to wheat straw burning and the quantity of energy generated during the combustion of the agricultural waste under study and associated emissions.

3. Discussion and results

The simulation results indicate that for headfire burning, the annual emissions (PM, CO, and CH_4) increased from 25,370 t (1987) to 43,292 t (2015). While for backfire, the emissions went from 20,197 t (1987) to 34,465 t (2015), which represents an increase of 71%.

Figures 6 and **7** illustrate the accumulated emissions of the period under study. In the headfire burning, 141,951 t of PM, 825,899 t of CO, and 25,809 t of CH_4 are generated. In the backfire burning, the emissions are 77,428 t of PM, 696,853 t of CO, and 16,776 t of CH_4.

The decrease of emission in backfire burning is due to a more significant interaction generated between the wheat straw and the oxygen present in the air because the incineration occurs against the wind which promotes the slow burning of wheat straw and better combustion.

The energy sent to the environment by wheat straw incineration in the 1987–2015 period was estimated at 188.81 PJ, which represents the 2.29% of the primary energy production of Mexico by 2015 [18]. During the analyzed period, there was an increase in the energy sent to the environment that varied from 4.78 PJ in 1987 to 8.15 PJ in 2015. **Figure 8** displays the behavior of the accumulated values of the energy sent to the environment in 1987–2015.

The annual average of discarded energy in the 1987–2015 period was of 6.51 PJ, which represents the 1.81% of the biomass energy in Mexico, 2015 [18]. However, the use of this wasted energy presents some challenges and opportunities that must be taken into consideration, which implies evaluating the technical and economic feasibility of any process.

Figure 9 displays the matter and energy balance corresponding to one wheat hectare harvested in the Mexicali Valley, where the index of wheat production by hectare is of 6.46 t and the generation of wheat straw is 7.3 t. The 15% of wheat straw generated has many applications such as incorporation in agricultural soil, cattle food, construction material elaboration, among others. The 85% of wheat straw, that is to say, 6.205 tons, is openly burnt *in situ*, which represents 89,972.50 MJ of energy sent to the environment and causes pollutant emissions. In the headfire burning, 477.78 kg of contaminants, composed of 68.26 kg PM, 397.12 kg CO, and 12.41 kg CH_4, are generated. In the case of backfire burning, 390.37 kg of contaminants, composed of 37.23 kg PM, 335.07 kg CO, and 8.07 de CH_4, are generated.

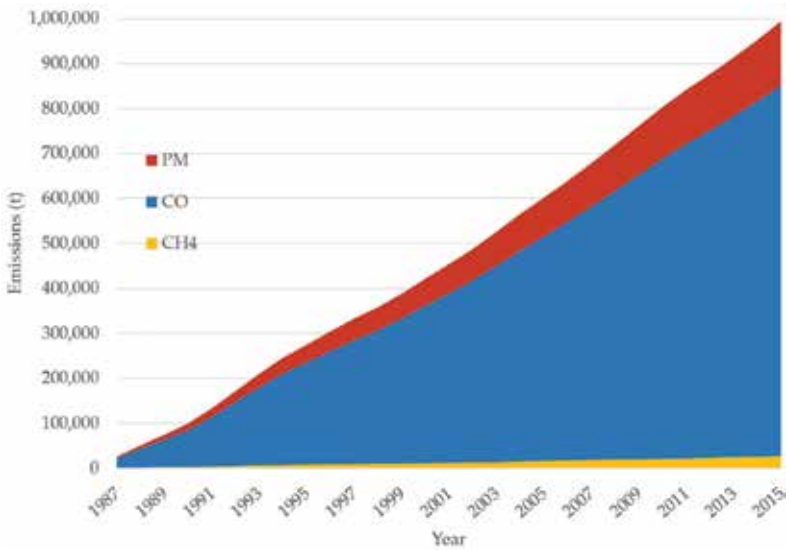


Figure 6. Accumulated emissions by headfire burning.

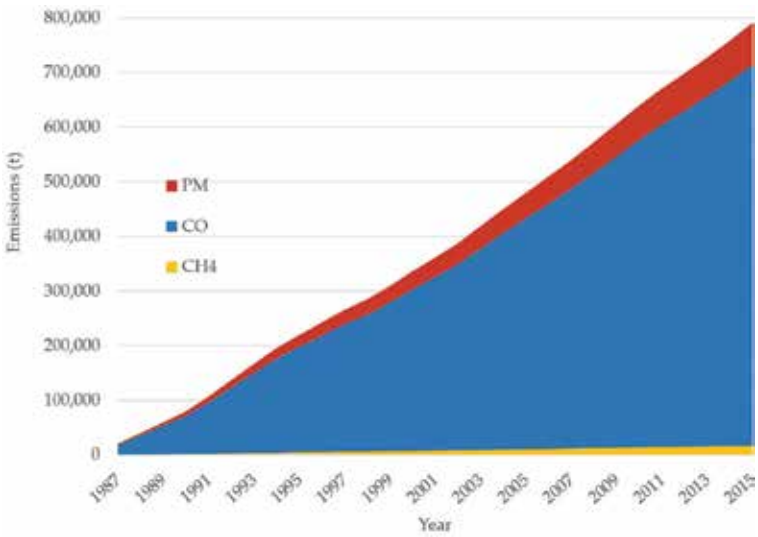


Figure 7. Accumulated emissions by backfire burning.

The balance of energy and matter indicates that for each ton of harvested wheat in the Mexicali Valley, 1,130.03 kg of wheat straw are generated, of which 169.50 kg are used in diverse applications and 960.53 kg are burnt in open air. The incineration of this waste implies that 13,927.63 MJ are wasted without any use, as well as pollutant emissions. In the headfire burning, 73.96 kg of pollutants, composed of 10.57 kg PM, 61.47 kg CO, and 1.92 kg $CH_{4,}$ are generated. As for the backfire burning, 58.88 kg of contaminants, composed of 5.76 kg PM, 51.87 kg CO, and 1.25 kg CH_{4}, are generated.

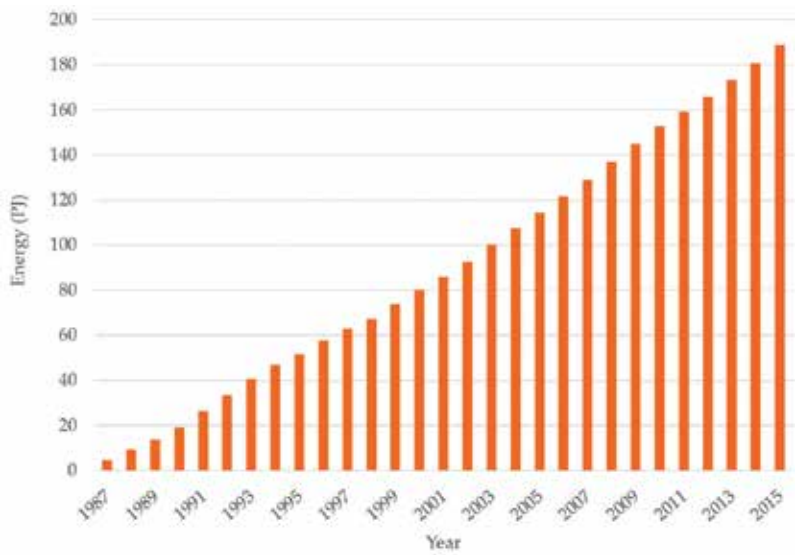


Figure 8. Energy sent to the environment.

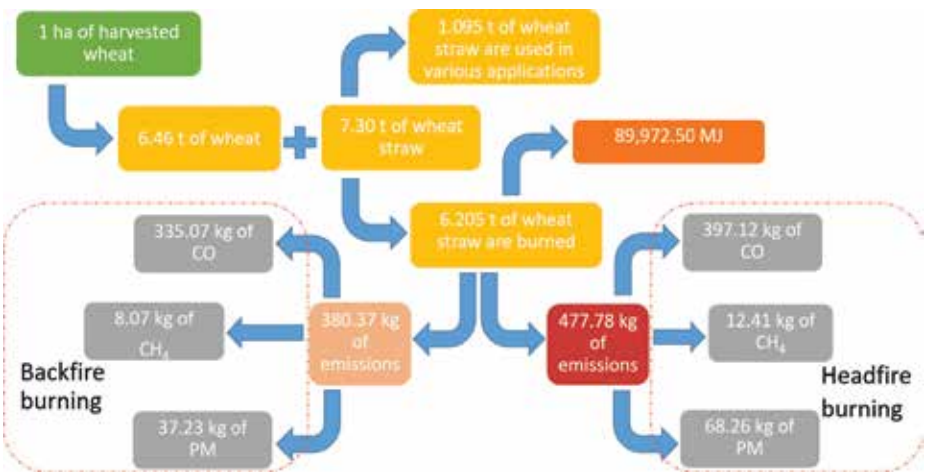


Figure 9. Material and energy balance of one harvested hectare of wheat.

4. Conclusions

Wheat cultivation is an intensive activity of great importance for the economic development of Baja California, Mexico. It also means the generation of vast amounts of wheat straw that is burnt *in situ* and emits large quantities of PM, CO, and CH_4 annually, contaminants that affect the air quality of Mexicali and its valley.

Since 1987 until 2015, the sown surface of wheat has incremented in the Mexicali Valley, resulting in an increase in the polluting emissions and the wasted energy.

Also, the total available energy estimated, draw from wheat straw incineration, for the 1987–2015 period is 188.81 PJ, which represents a high energy potential that can be exploited in productive processes.

Through the development of the model on iThink®, the emissions and the wasted energy, as a result of wheat straw burning, were estimated in the period under study. It demonstrates the severity of the problem and justifies the necessity of promoting sustainable alternatives for the disposal of wheat straw, with a lower environmental impact, among the farmers of the region.

According to the model of headfire burning, the results of the simulation indicate that the annual emission increased from 25,370 t (1987) to 43,292 t (2015), while for the backfire burning from 20,197 t (1987) to 34,465 t (2015), which represents a rise of 71%.

The balance of matter and energy results, developed in the current work, for 1 hectare of wheat harvested in the Mexicali Valley shows that 6.46 t of wheat are produced and 7.3 t of straw are generated; 6.205 t are burnt *in situ* in open fire, which generates 89,972.50 MJ and 477.78 kg of contaminants by the headfire burning and 380.37 kg of pollutants through backfire burning.

Acknowledgements

The authors thank the Engineering Institute of Universidad Autónoma de Baja California, for the facilities to develop this project, and PFCE 2017 for the financial support for the publication of this chapter.

Author details

Gisela Montero*, Marcos A. Coronado, Conrado García, Héctor E. Campbell, Daniela G. Montes, Ricardo Torres, Laura Pérez, José A. León and José R. Ayala

*Address all correspondence to: gmontero@uabc.edu.mx

Universidad Autónoma de Baja California, Institute of Engineering, Mexicali, México

References

[1] Fahad S, Nie L, Chen Y, Wu C, Xiong D, Saud S, Hongyan L, Cui K, Huang J. Crop plant hormones and environmental stress. In: Lichtfouse E, editor. Sustainable Agriculture Reviews. 1st ed. Cham: Springer; 2015. pp. 371-400. DOI: https://doi.org/10.1007/978-3-319-09132-7_10

[2] FAO. Cereal Supply and Demand Brief [Internet]. 2016. Available from: http://www.fao.org/ worldfoodsituation/csdb/en/ [Accessed: Jan 15, 2018]

[3] Agrifood and Fisheries Information Service. Statistical Yearbook of Agricultural Production [Internet]. 2015. Available from: https://datos.gob.mx/busca/dataset/estadistica-de-la-produccion-agricola/resource/2a3fea18-00a4-461f-abf8-15bda6b131e3?inner_span=True [Accessed: Jan 21, 2018]

[4] Current situation and overview of wheat production in Mexico 1990-2000 [Internet]. 2005. Available from: http://www.campomexicano.gob.mx/portal_siap/Integracion/Estadistica-Derivada/ComercioExterior/Estudios/Perspectivas/Trigo90-00.pdf9 [Accessed: Feb 1, 2018]

[5] SAGARPA. Plan rector del sistema producto trigo, Baja California [Internet]. 2011. Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación, México. Available from: http://www.sagarpa.gob.mx/agricultura/Publicaciones/ SistemaProducto [Accessed: Dec 12, 2017]

[6] SENER, BID, GTZ. Potenciales y viabilidad del uso de bioetanol y biodiesel para el transporte en México. Secretaría de Energía, Banco Interamericano de Desarrollo, Cooperación Técnica Alemana. [Internet]. 2006. Available from: http://www.sener.gob.mx/res/169/Biocombustibles_en_Mexixo_Estudio_Completo.pdf. México, D.F. 600 p. [Accessed: Dec 20, 2017]

[7] Quintero M, Moncada A. Contaminación y control de las quemas agrícolas en Imperial, California, y Mexicali, Baja California. Región y Sociedad. 2008;**20**:3-24. DOI: 10.22198/rys.2008.43.a494

[8] SFA. Estudio sobre la utilización de la paja de trigo [Internet]. 2010. Available from: http://www.oeidrus-bc.gob.mx/oeidrus_bca/ [Accessed: Nov 29, 2017]

[9] Fahad S, Bajwa A, Nazir U, Anjum S, Farooq A, Zohaib A, Sadia S, Nasim W, Adkins S, Saud S, Ihsan M, Alharby H, Wu C, Wang D, Huang J. Crop production under drought and heat stress: Plant responses and management options. Frontiers in Plant Science. 2017;**8**:1-16. DOI: 10.3389/fpls.2017.01147

[10] Amanullah FS, Anwar S, Baloch S, Saud S, Alharby H, Alghabari F, Rice IM. Crop responses to global warming: An overview. In: Amanullah FS, editor. Rice – Technology and Production. 1st ed. Croatia: InTech; 2017. pp. 1-10. DOI: 10.5772/68035 Available from: https://www.intechopen.com/books/rice-technology-and-production/rice-crop-responses-to-global-warming-an-overview

[11] Fahad S, Hussain S, Matloob A, Khan F, Khaliq A, Saud S, Hassan S, Shan D, Khan F, Ullah N, Faiq M, Khan M, Tareen A, Khan A, Ullah A, Ullah N, Huang J. Phytohormones and plant responses to salinity stress: A review. Plant Growth Regulation. 2015;**75**:391-404. DOI: https://doi.org/10.1007/s10725-014-0013-y

[12] Montero G, García C, Coronado M, Toscano L, Stoytcheva M, Torres R, Vázquez A, Montes D. SWOT analysis applied to wheat straw utilization as a biofuel in Mexico. In: Jacob-Lopes, editor. Frontiers in Bioenergy and Biofuels. 1st ed. Croatia: InTech; 2017. pp. 483-493. DOI: 10.5772/65496 Available from: https://www.intechopen.com/books/frontiers-in-bioenergy-and-biofuels/swot-analysis-applied-to-wheat-straw-utilization-as-a-biofuel-in-mexico

[13] iThink. Iseesystems [Internet]. 2018. Available from: https://www.iseesystems.com/ [Accessed: Feb 1, 2018]

[14] Montero G, Coronado M, Torres R, Jaramillo B, García C, Stoytcheva M, Vázquez A, León J, Lambert A, Valenzuela E. Higher heating value determination of wheat straw from Baja California, Mexico. Energy. 2016;**109**:612-619. DOI: 10.1016/j.energy.2016.05.011

[15] SAGARPA. Serie histórica de producción de trigo grano, sistema producto trigo [Internet]. 2011. Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación, México. Available from: http://www.oeidrus-bc.gob.mx/sispro/trigobc/ [Accessed: Nov 10, 2017]

[16] OEIDRUS. State Information Office for the Sustainable Rural Development of Baja California (Oficina Estatal de Información para el Desarrollo Rural Sustentable de Baja California) [Internet]. 2016. Available from: http://201.140.167.37/series/ [Accessed: Jan 22, 2018]

[17] EPA. Compilation of air pollutant emission factors. Volume I, Chapter 2: Solid Waste Disposal, Open Burning [Internet]. 1995. Environmental Protection Agency. EPA AP 42, 5a ed. Available from: https://www3.epa.gov/ttnchie1/ap42/ch02/index.html [Accessed: Feb 4, 2018]

[18] SENER. Balance Nacional de Energía 2015. [Internet]. 2016. Available from: https://www.gob.mx/cms/uploads/attachment/file/248570/Balance_Nacional_de_Energ_a_2015__2_.pdf [Accessed: Jan 29, 2018]

Chapter 5

Nitrogen Losses: Gaseous and Leached Nitrogen Balance

Arritokieta Ortuzar-Iragorri, Ander Castellón,
Gerardo Besga, Ana Aizpurua,
Teresa Fuertes-Mendizabal and Jose M. Estavillo

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.75801

Abstract

Nitrogen is the element with the greatest influence on plant production and on protein content in the case of grain crops. Nevertheless, nitrogen over-fertilization produces environmental problems such as water pollution and global warming, which has led to the declaration of vulnerable zones to nitrate pollution in the European Union and to the adhesion of many countries to the Kyoto protocol. In the case of wheat there is a demand for producing quality grain, which is primed with a bonus price. Under these both economical and environmental circumstances, arose the need for a rational system of nitrogen fertilization which enabled the optimization of nitrogen use under the specific edaphoclimatic of Northern Spain. In order to cope with this objective a net of nitrogen fertilization assays was established by means of which a series of fertilization strategies together with some associated diagnosis tools were evaluated. Thus, N losses occurring both by nitrate leaching and by N_2O emissions to the atmosphere were quantified, as well as plant N extractions regarding the different nitrogen fertilizer treatments applied.

Keywords: gaseous N losses, leached N

1. Introduction

Nitrogen (N) plays a key role in the growth and development of wheat; thus, wheat's growth and quality might be modified through N fertilization [48]. However, cultures use N inefficiently, and, in general, 50% of the applied N is not used by plants [12, 41]. Therefore, N losses take place, both gaseous and leached, which cause economic and environmental costs.

IntechOpen

© 2018 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Within the economic costs, that caused due to diminished N use efficiency stands out. Among the environmental costs, the contribution of some N-based gaseous compounds that play a role in the greenhouse effect [10], acid rain [23, 24] and the contamination and eutrophication of waters due to nitrate leaching further than the root zone [1, 17] are notable. In this sense, in Álava, Arrate et al. [6] describe a series of changes in the management of arable land in the years 1967–1997 (wetland drainage, application of large quantities of fertilizers, phytosanitary products etc.) that progressively increased the concentration of N compounds in subsurface waters. Due to such reasons and by application of the European Directive 88/778/EEC related to water for human consumption [13], the zone related to the Eastern sector of the quaternary aquifer of Vitoria was designated as vulnerable to nitrate pollution in the year 1999 [14]. This zone comprises 38% of the area where wheat is grown in Alava, 9500 ha approximately. In this zone, the N fertilization is as much as 140 kg N ha^{-1} depending on the previous culture and soil richness; N fertilization is not allowed at a distance closer than 3 m of any water course.

Nitrous oxide (N_2O) is not a very reactive gas; it persists in the atmosphere for as much as 150 years [47]. This gas adsorbs electromagnetic radiation in various wavelengths in the infrared region between 7.7 and 17 μm [35] and its greenhouse effect per mass unit is some 300 times larger than that of CO_2 [36]. In this sense, it is estimated that in the last 100 years, N_2O has contributed approximately 5% to the warming up of the planet [42, 43]. The origin of 90% of the N_2O emissions is anthropogenic, and agriculture is its main source [22]. N_2O in soils can be produced both due to nitrification and denitrification (**Figure 1**). Nitrification is a microbial aerobic process in which ammonium first oxidizes to nitrite and then to nitrate. In this ammonium to nitrate oxidation process, N_2O can be released into the atmosphere [46]. On the other hand, denitrification is a microbial anaerobic process in which organic carbon is used as the energy source and the nitrate as the last electron acceptor so that it reduces to the last nitrogenous gaseous compounds N_2O and N_2. The nitrification and denitrification processes

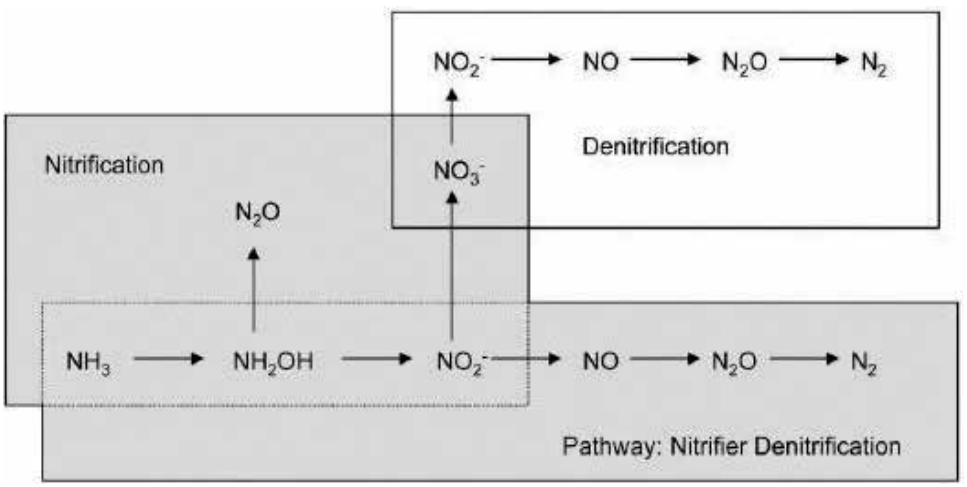


Figure 1. Transformations of mineral nitrogen in the soil [46].

http://dx.doi.org/10.5772/intechopen.75801

can occur simultaneously in the soil since the aerobic and anaerobic conditions can simultaneously take place at the same soil aggregate [26].

The N balance allows the knowledge of the N evolution at the soil–plant system in a settled period of time. It also enables the knowledge of the sources of N other than fertilization, and the rate of transfer between the different components of N, the main mechanisms of N loss, and what amount of N is not likely to be recovered [30]. Therefore, determining the balance of N in a culture system helps explain certain parameters that determine the dose of N needed by the cereal [28, 49], optimizing the nitrogenous nutrition of the plant and reducing the danger of contamination.

The goal of this work was to ascertain the quantity of N lost by leaching or by being released to the atmosphere and to study the factors that have an effect on such losses. Also, the N balance in the soil–plant system was to be determined.

2. Materials and methods

2.1. Assay establishment

A nitrogen fertilization experiment was carried out in Gauna, Álava (average annual rainfall of 779 mm and average annual temperature of 11.5°C) from November 2001 to February 2004 in three consecutive seasons. The assay was conducted in the Western Sector of the quaternary aquifer of Vitoria, adjacent to the area vulnerable to the contamination of nitrates of agricultural origin. The trial was organized in random blocks with four repetitions in which each elementary plot covered an area of 50 m^2. The soil on which the trial was established was classified as Aquertic Eutrudept [40] and was planted with wheat. Some of the soil properties are shown in **Table 1**. Data regarding when sowing, the first, second, and third N broadcastings, and the harvest took place are shown in **Table 2**. Before sowing, 90 kg ha^{-1} of P_2O_5 ha^{-1} and 90 kg ha^{-1} of K_2O ha^{-1} were applied as 0-14-14. Nitrogen doses of 0, 140, and 220 kg N ha^{-1}

Depth (cm)	0–30	30–60
Sand (%)	45.18	48.25
Silt (%)	27.14	39.21
Clay (%)	27.67	12.49
pH	7.98	8.13
Organic matter (%)	2.12	1.52
Phosphorous (P) (mg kg^{-1})	43.30	32.53
Potassium (K) (mg kg^{-1})	135.00	93.00
Carbonates (%)	11.90	22.00

Table 1. Soil properties of the experiment in Gauna (Álava).

Sowing	First broadcast	Second broadcast	Third broadcast	Harvest	Tilling
2001-11-30	2002-03-04	2002-04-08	2002-05-13	2002-07-24	2002-11-17
2002-10-29	2003-01-20	2003-03-24	2003-05-12	2003-07-08	
2003-11-20	2004-03-16	2004-04-15	2004-05-20	2004-07-28	

Table 2. Data for sowing, first, second, and third N broadcasts, harvest and tilling.

were applied as ammonium nitrate (33.51% N g/g) in two or three broadcasts as described in **Table 3**. A control treatment in which no N was applied was included. The treatment in which 140 kg N ha^{-1} were broadcast was applied in two or three amendments (**Table 3**) to observe the splitting effect and to evaluate a low fertilization strategy in three broadcasts suggested as a possible N fertilization management in vulnerable zones.

2.2. Mineral nitrogen (Nmin)

The first Nmin measures, (ammonic and nitric N), took place at start of tillering and at the end of winter 2001. They were determined from a mixture of eight samples throughout the trial taken at the depths 0–30 and 30–60 cm. Subsequently, all Nmin values were determined from a mixture of two samples per treatment, block and depth. These samples were taken before sowing, at the end of winter, at Z20, and also after harvest in all years and all treatments except at the end of winter of year 2003, when only samples of the control treatment were taken.

The soil samples were shredded manually. Stones, roots, and any other type of plant material were discarded. Then, the humidity of the samples was gravimetrically ascertained.

200 mL of 1 M KCl were added to 100 g of soil and the mixture was stirred for 30 min. After filtering, the nitrogen of nitric origin in the filtrate was analyzed by segmented flow injection [3, 4]. The calculation of Nmin per hectare was corrected according to the content of coarse elements of the soil (**Table 4**).

Total dose (kg N ha^{-1})	Treatment	Broadcasts (kg N ha^{-1})		
		Start of tillering (Z20)*	Start of jointing (Z30)	Flag leaf (Z37)
0	0	0	0	0
140	40 + 100	40	100	0
140	40 + 60 + 40	40	60	40
220	80 + 140	80	140	0

*Z20, Z30 and Z37 correspond to Zadoks' scale [50].

Table 3. Dose and broadcasts of the N fertilization treatments.

Depth (cm)	Soil apparent density (g cm^{-3})	Coarse elements (% g/g)	Stone free apparent density (g cm^{-3})
0–20	1.57	20.8	1.4
20–40	1.59	21.5	1.4
40–60	1.93	42.6	1.6

Table 4. Soil apparent density (g cm^{-3}) and coarse elements (% g/g) at 0–20, 20–40, and 40–60 cm depths.

2.3. Leached nitrate

Two ceramic cups were inserted per block and treatment. They were buried at a depth of 60 cm (**Figure 2**). As a test, in the year 2003, a hole in the soil was performed after harvest. It was observed that wheat roots reached a depth of 60 cm as a maximum; so the depth at which the ceramic cups were inserted was considered appropriate and it can be considered that the gathered nitrogen was not profitable for plants. Liquid samples were collected from ceramic cups when it had rained 20–40 mm or every fortnight. The first liquid sample after every insertion was extracted but then discarded. The sampling data occurred between December 20, 2002 and September 18, 2003 and between January 9 and September 27, both data in 2004. At both periods, the ceramic cups were removed at harvest and then inserted again. After every sampling session, a vacuum of approximately 50 kPa was performed with a manual pump. Afterwards, the nitrate concentration in the sampled water was analyzed through segmented flow injection [3, 4].

The water balance was determined in layers 0–20, 20–40, and 40–60 cm depth following Campbell's [11] simplified waterfall method. In this method, it is considered that waters fills

Figure 2. Ceramic capsule in the soil for leached liquid sampling.

up every layer of the soil before it flows to the following layer (Eqs. (1)–(3)). Every time a sample was taken, the humidity of each of the three layers was measured so that the variation of the water reservoir was measured. For every layer, the humidity was measured gravimetrically as well as with an IMKO TDR (time domain reflectrometry) so as to calibrate the later. However, no relation between both measures was observed; thus, only the gravimetrical measures were considered afterward.

The water balance in the 0–20 cm deep layer was calculated as specified in Eq. (1):

$$D_{20} = \mathrm{Pr} - \mathrm{ETc} \pm \mathrm{VR}_{20} \quad (1)$$

where D_{20} is drainage (mm) below 20 cm, Pr stands for rain, ETc is the culture's evapotranspiration (mm) determined according to FAO methodology [2] and VR_{20} (mm) is the variation of the water reservoir in the 0–20 cm layer.

Drainage below 40 and 60 cm was, respectively, ascertained as described in Eqs. (2) and (3):

$$D_{40} = D_{20} \pm \mathrm{VR}_{40} \quad (2)$$

where VR_{40} is the water reservoir variation in the 20–40 cm (mm) layer and D_{40} is drainage beyond 40 cm (mm).

$$D_{60} = D_{40} \pm \mathrm{VR}_{60} \quad (3)$$

where VR_{60} is the water reservoir variation in the 40–60 cm (mm) layer and D_{60} is drainage beyond 60 cm (mm).

Finally, the N mass drained during the sampling period was calculated with Eq. (4).

$$N_i = D_{60} \cdot [\mathrm{N}]_i \cdot 10^{-2} \quad (4)$$

where N_i is the N mass of nitric origin drained per period and treatment (kg N ha^{-1}), D_{60} is the water loss due to deep percolation in the period between sampling days (L m^{-2}) and [N] is the nitric nitrogen concentration in the leachate sampled at the end of the period i (mg L^{-1}).

Finally, the values obtained per day in Eq. (4) for each treatment and sampling day in the period of time comprehended between the days the capsules were inserted and when they were removed were summed up so as to assess the N mass leached per hectare in that period.

A piezometer was installed next to the assay so as to detect the moments when the level of water was beyond 60 cm. This occurred on the days February 5 and May 7, 2003 and March 11, and April 1 and 30, 2004.

2.4. Gaseous losses

2.4.1. N_2O emissions

To assess the fertilizer application effect on N_2O emissions (N_2Oem), in the year 2002, those emissions were measured after the N fertilizer applications, from March 4 to May 17. During

http://dx.doi.org/10.5772/intechopen.75801

Figure 3. Chamber in the soil.

this period of time, samples were taken every 2 days in treatments 0, 40 + 100, 40 + 60 + 40, and 80 + 140. In the year 2003, more exhaustive measures were taken so as to assess not only the differences in the emissions after N applications but also to study the N_2O emissions of the field after harvest and laboring and to determine the effect of the temperature and humidity on emissions. Therefore, that year, samples were taken every 2 days after N broadcasting and every fortnight from January 20, 2003 to February 4, 2004 in treatments 0, 40 + 100, and 80 + 140. Measurement frequency intensified around laboring time.

To perform such measurements, 3 L polyvinyl chloride (PVC) hermetic chambers with a rubber septum were inserted in the soil in every block at a depth of 2 cm (**Figure 3**). Before their placement, four 10 mL samples from the atmosphere of the essay were taken at a height of 2 m. After 45 min, four 10 mL samples of the air of each chamber were taken and kept in Vacutainer® blood sampling tubes. Then, the N_2O in the samples was analyzed with a gas chromatograph (Unicam 8925) equipped with an electron capture detector (ECD). When the described measurements were performed in the field, the temperatures of the air at a height of 2 m and that of the soil at a depth of 10 cm were also measured. In 2003, the humidity of the first 30 cm soil layer was also gravimetrically measured at 4 points of the essay.

With the soil humidity measure, the percentage of soil water-filled pore space was assessed using Eq. (5).

$$WFPS = H\,\rho_{SFS}/(1-\rho_{SFS}/2.65) \tag{5}$$

where WFPS stands for the water-filled pore space (% ml/ml), H, the water percentage in dry soil for the 0–30 cm soil layer (% g/g), and ρ_{SFS}: stone free soil apparent density (g cm^{-3}).

Soil apparent density is the quotient between the weight of the soil solid particles and the in situ total volume of the soil. The in situ volume of the soil was assessed through the excavation method [9] with the adaptation of using polyurethane resin (Wolf, [45]) as the average value obtained at four holes dug in the essay at depths 0–20, 20–40, and 40–60 cm. Due to the

fact that the soil was quite stony, the apparent density required a correction which was executed considering the percentage of gross elements (those that did not pass through a 2 mm sieve) (**Table 4**) and their density, which is acknowledged as 2.65 g cm^{-3} [37].

2.4.2. N_2O production and total denitrification at the arable layer

From January 23 to September 18, 2003, the N_2O production rate (N_2Oprod) was measured in the arable layer at the same treatments and times described for N_2Oem. To achieve this, two 30 cm long and 2.65 cm diameter wide cylindrical samples were taken at the plots of the studied treatments. These two samples were transferred to a hermetic 2 L pot with a rubber septum (**Figure 4**). Then, the pots of the different plots were inserted in a hole next to the studied plot and were covered with the soil of the same hole and kept to incubate for 24 hours. In such a manner, the actual soil temperature and its changes could be mimicked. After 24 hours, 10 mL samples of the atmosphere of each pot were stocked in Vacutainer® tubes for their ulterior N_2O determination by gas chromatography (Unicam 8925). The same process was followed for other pots to which 100 mL of acetylene (C_2H_2) (Air Liquide, SA) were injected making the atmosphere of the pot rich in acetylene by 5% (**Figure 4**). C_2H_2 blocks N_2O reduction to N_2 in the denitrification process and the ammonium oxidation in the nitrification process [33]. Therefore, the N_2O produced in the incubation with an atmosphere of 5% C_2H_2 comprises the joint production rate of $N_2O + N_2$ due to denitrification (Ndeni) [8] (Knowles, [25]).

The cumulated N loss was calculated by the integration of the diary rates of N_2Oem, N_2Oprod, and Ndeni over time.

2.5. Plant N extraction

To determine the N extraction of wheat in the aerial part at harvest, a 0.25 m^2 area in each plot was randomly chosen and the plants in that area were cut to the ground. Grain and straw were separated and dried in an oven at 70°C for 48 h at least to determine biomass. Then, the samples

Figure 4. Incubation pot before it was buried and bag and syringe with acetylene.

http://dx.doi.org/10.5772/intechopen.75801

were ground and sieved through 0.5 and 1 mm sieves respectively. The total N was determined both for straw and grain by Kjeldahl procedure [5] with a Kjeltec Auto sampler System 1035 (Tecator). The N absorption by the aerial part of the plant (Nab) was determined as the sum of the products of the N concentration at grain and straw times their respective biomasses.

The yielded grain quantity datum, needed for the previous calculation was determined by harvesting the central 1.5 m wide aisle of each plot. Yield was referred to a humidity of 120 g kg^{-1}.

2.6. N balance

The N balances are based on the application of the mass conservation principle to the cultures. Thus, the variation of N stocked in a system equals the difference among the inputs and outputs to the system [31]. Often, the N balance is inferred considering the inputs and outputs in relation to the main source, the soil Nmin. This is considered available to plants in spite of the fact that it can also be consumed by microorganisms, dissipate as gas, or leach through the soil [34]. The balance was assessed as described in Eq. (6) [29]:

$$\mathrm{NminS} + \mathrm{Min} + \mathrm{F} = \mathrm{Nab} + \mathrm{Nabr} + \mathrm{Nlix} + \mathrm{N_2Oem} + \mathrm{NminAH} + \mathrm{Nc} \quad (6)$$

where, NminS: Nmin quantity in soil before seeding (kg N ha^{-1}), Min: soil N fraction in the soil due to the conversion of organic nitrogen to ammonium (kg N ha^{-1}) (It is calculated from the adjustment of the balance for the control treatment, i.e., 0 (kg N ha^{-1})), F: fertilizer N applied (kg N ha^{-1}), Nab: N absorbed by the aerial part of the plant (kg N ha^{-1}), Nabr: N absorbed by the roots of the plant (kg N ha^{-1}) (It was estimated that this was 25% of Nab [38]), Nl: leached N (kg N ha^{-1}), N_2Oem: N emitted as N_2O (kg N ha^{-1}), NminAH: Nmin in soil after harvest (kg N ha^{-1}), Nc: Not accounted for.

Thus, a positive Nc means that: (i) the inputs have been overestimated due to an error in its calculation or/and experimental error, (ii) the outputs have been underestimated for the same reasons as in (i), (iii) there are other N outputs that have not been considered, and (iv) a combination of all or some of the situations described here occurs.

N_2Oem was considered in the balance instead of N_2Oprod or Ndeni since these last two refer to gases produced in the arable layer and thus may not exit the system.

2.7. N use efficiency

For the assessment of N use efficiencies, the following parameters were defined (Huggins and Pan, 1993):

Efficiency in the use of the fertilizer (NUE): difference between Nab of the fertilized treatment and the non-fertilized, divided by the quantity of fertilizer applied.

Harvest index (HI): quotient between the N in the grain and the N extracted by the aerial part of the culture (dimensionless).

2.8. Statistics

PROC GLM [39] procedure was used to carry out the variance analysis and then determine the differences between averages with the Duncan procedure.

3. Results and discussion

3.1. Mineral N

Through the Nmin analysis, it was observed that about the 80–95% of the Nmineral (Nmin) analyzed in the floor of the assay was of nitric origin (**Table 5**). The non-fertilized treatment had the least nitric N percentages at every moment and depth in comparison to the percentages in the fertilized treatments. The nitric N percentage was constant or increased with depth, (i.e., from 0–30 to 30–60 cm) except in the moments after harvest in years 2003 and 2004, when the percentage of nitric N was somewhat inferior in the 30–60 cm layer due to the deep and recent extraction of the culture until harvest, close to the moment "after harvest" referred to in this work.

3.2. Leached nitrate

In **Tables 6** and 7, the nitric N leached in the periods comprehended between the beginning of the sampling period until harvest and between the beginning of the sampling period until it finished are, respectively, shown. Statistically significant differences were only observed between treatment 80 + 140 and the rest of treatments at the period of time comprehended between the beginning of the campaign and harvest in year 2003 and all the sampling period in year 2004. Although Webster et al. [44] reported some 19 kg ha^{-1} of N leached for the non-fertilized treatment, a similar figure to that reported in this work in year 2004 (**Table 7**), the 32 kg N ha^{-1} obtained for treatment 80 + 140 is minor to the 50 kg N ha^{-1} quantities reported by the same authors for treatments with similar dosages, presumably due to variables such as different kinds of soils, etc. By the deduction of nitric **N** leached at the non-fertilized treatment, the nitric N quantities leached in years 2003 and 2004 were assessed in the other treatments. Until harvest, those quantities ranged between the 4 and the 6% of the applied N and, when all the sampling periods were considered, they comprehended N quantities that ranged between the 8 and the 14% of the N fertilized.

Concurring with the results obtained for treatment 80 + 140, in a study performed in Denmark, Kjellerup and Kofoed [24] observed that N fertilizer dosages around 200 kg N ha^{-1} significantly raised the leached nitrate in drained waters for they exceeded the adsorbing capacity of the crop; the assessed losses were approximately 40 kg N ha^{-1}. In the neighbor community of Navarra, Arregui [7] also described the augmenting N leaching trend for dosages larger than 110 or 120 kg N ha^{-1}.

Approximately 50% of the leached N-NO_3^- in all the samples in 2004, leached in the period comprehended between the moments after harvest and the end of sampling in September for all treatments (**Table 7**).

http://dx.doi.org/10.5772/intechopen.75801

Moment	Depth (mm)	Treatment			
		0	40 + 100	40 + 60 + 40	80 + 140
		$N\text{-}NO_3^-$/Nmin (%)			
2002					
Before harvest	0–30	82			
	30–60	96			
End of winter	0–30	78			
	30–60	85			
After harvest	0–30	75	89	94	81
	30–60	83	89	94	84
2003					
Before harvest	0–30	82	86	79	88
	30–60	90	89	89	85
End of winter	0–30	67			
	30–60	43			
After harvest	0–30	92	90	86	92
	30–60	89	89	84	87
2004					
Before harvest	0–30	79	84	86	83
	30–60	79	83	83	88
End of winter	0–30	77	87	83	89
	30–60	79	83	82	91
After harvest	0–30	88	88	94	93
	30–60	80	90	85	84

Table 5. Nitric origin N percentage in relation to Nmin in the soil at different depths, moments, and treatments.

In **Tables 6** and 7, the average nitric N in the total quantity of drained water is also shown. It was calculated as the product of the drained total N (kg ha^{-1}) throughout all the drainage period and the drained water (mm) in the same period.

Thus, it can be observed that the maximum average $N\text{-}NO_3^-$ concentration in the sampling period was 7 ppm N in the year 2003 and 14 ppm in the year 2004 for treatment 80 + 140, which was the treatment that regards average $N\text{-}NO_3^-$, statistically differentiated from the rest in years 2003 and 2004 except for treatment 40 + 60 + 40 in year 2004 when all the sampling period was considered. The average concentrations in treatments 40 + 60 + 40 and 80 + 140 in year 2004 when all the periods were considered were larger than the allowed 11.3 mg L^{-1} $N\text{-}NO_3^-$ limit.

Treatment	$N\text{-}NO_3^-$ (kg ha^{-1})	Drainage (mm)	$[N\text{-}NO_3^-]$ average (mg L^{-1})
0	14 b	350	4 b
40 + 100	14 b		4 ab
40 + 60 + 40	12 b		3 b
80 + 140	23 a		7:00 AM

Statistically different values ($\alpha \leq 0.05$) are identified with different letters.

Table 6. $N\text{-}NO_3^-$ leached from January to harvest in year 2003.

3.2.1. Gaseous losses

Through the integration in time of the daily emission and production rates, losses due to N_2O emitted to atmosphere and $N_2O + N_2$ due to denitrification in 2002 and 2003 were assessed (**Table 8**). In spite of the fact that N fertilization has a key role in N_2O emissions (Bouwman, 1996), no statistical differences were observed between fertilizing treatments for the emission or the N_2O cumulated production, as described by Menéndez [32] in an essay carried out in Mediterranean climatic conditions. However, when the N_2Oem quantity corresponding to the non-fertilized treatment is deducted from the other treatments, the N_2O emitted quantities range from 1.8 to 2.9% of the N broadcast as a fertilizer, superior numbers to those cited by the IPCC [20] when estimating the winter house effect caused by agriculture [21].

In 2003, in spite of the fact that applied N dosage had no effect on the N_2O emissions to the atmosphere or on its production in the arable layer, it did affect the $N_2O + N_2$ production due to total denitrification—it was larger for the treatment with the largest N dose, i.e., 80 + 140. As mentioned before, $N_2O + N_2$ peaks due to denitrification were only observed until April 2; thus, these largest losses due to denitrification are imputable to the denitrification peaks after fertilization. Due to the same reason, the cumulated values of $N_2O + N_2$ rates due to denitrification until harvest and after it are similar.

Treatment	Period					
	2004 until harvest*			2004 all the sampling period**		
	$N\text{-}NO_3^-$ (kg ha^{-1})	Drainage (mm)	$[N\text{-}NO_3^-]$ average (mg L^{-1})	$N\text{-}NO_3^-$ (kg ha^{-1})	Drainage (mm)	$[N\text{-}NO_3^-]$ average (mg L^{-1})
0	19 a	371	5 a	38 b	487	8 b
40 + 100	20 a		5 a	41 b		8 b
40 + 60 + 40	31 a		8 a	62 ab		13 ab
80 + 140	32 a		9 a	70 a		14 a

Statistically different values ($\alpha \leq 0.05$) are identified with different letters.*from January 9, 2004 to July 1, 2004.
**from January 9, 2004 to September 27, 2004.

Table 7. $N\text{-}NO_3^-$ leached from January until harvest in all the sampling period of year 2004.

Treatment	2002*	2003 until harvest**			2003 in all the sampling period***
	N_2Oem	N_2Oem	Ndeni	Nprod	N_2Oem
0	2 a	5 a	4 b	7 a	10 a
40 + 100	2 a	4 a	5 b	10 a	9 a
40 + 60 + 40	6 a				
80 + 140	5 a	8 a	9a	11 a	14 a

In the year 2003, N_2Oem accumulation, from the N_2O production rates (N_2Oprod) and $N_2O + N_2$ due to denitrification (Ndeni) from the beginning of the sampling period until harvest and until the end of the sampling period is shown; statistically different values are marked with different letters.*From March 5 until May 17, 2002.

**From January 21 to July 4, 2003.

***From 2003, January 21 to 2004, February 4.

Table 8. Cumulated N_2O emissions (N_2Oem) in all the sampling period in year 2002.

Although Hussain et al. [19] report in their review, that the emission of greenhouse gases is frequently favored by tilling, such a fact was not observed in this work for N_2O, Ndeni, or Nprod.

3.3. Nitrogen balance

Nitrogen balances for the different campaigns are shown in **Tables 9–11**. The yield and thus the extraction of the non-fertilized treatment in year 2002 was larger than in the latter two campaigns, due to the fact that in the first year, the field preceding the essay was thoroughly

Dosage (kg N ha^{-1})	0	140	140	220
Splitting	0	40 + 100	40 + 60 + 40	80 + 140
Nmin initial (NminS)	40±3	40±3	40 ±3	40 ±3
N mineralized (MIN)	120±11	120 ±11	120 ±11	120 ±11
N fertilized (F)	0	140	140	220
NTOTAL INPUTS	160±11	300 ±11	300 ±11	380 ±11
N absorbed by the aerial part (Nab)	113±6	177 ±15	185 ±15	188 ±24
N absorbed by the roots (Nabr)	28 ±2	42 ±3	44 ±3	44 ±5
N gas (N_2O em)	2 ±0	2 ±0	6 ±3	5 ±2
Nmin after harvest (NminAH)	17 ±5	33 ±1	45 ±14	17 ±3
NTOTAL OUTPUTS	160 ±8	254 ±15	280 ±21	254 ±25
N not computed	0 ±14	46±19	19 ±24	126 ±27
Fertilizer use efficiency (NUE)		0.49 ±0.12	0.60 ±0.14	0.35 ±0.12
N harvest index	0.84 ±0.08	0.90±0.04	0.81 ±0.05	0.87 ±0.06

Table 9. N balance (kg N ha^{-1}) during the 2002 campaign; average values ± standard errors are reported.

Dosage (kg N ha^{-1})	0	140	140	220
Splitting	0	40 + 100	40 + 60 + 40	80 + 140
Nmin initial (NminS)	25 ± 5	24 ± 1	25 ± 3	40 ± 10
N mineralized (MIN)	123 ± 12	123 ± 12	123 ± 12	123 ± 11
N fertilized (F)	0	140	140	220
N TOTAL INPUTS	147 ± 13	287 ± 12	288 ± 13	383 ± 15
N absorbed by the aerial part (Nab)	53 ± 6	139 ± 21	145 ± 25	180 ± 22
N absorbed by the roots (Nabr)	13 ± 1	35 ± 5	36 ± 6	45 ± 5
N leached (Nlix)	14 ± 3	14 ± 2	12 ± 2	23 ± 3
N gas (N_2O em)	5 ± 4	4 ± 1		8 ± 3
Nmin after harvest (NminAH)	50 ± 4	37 ± 4	30 ± 3	40 ± 1
N TOTAL OUTPUTS 160 ±	135 ± 8	229 ± 21	224 ± 25	296 ± 22
N not computed	12 ± 16	58 ± 24	65 ± 28	86 ± 27
Fertilizer use efficiency (NUE)		0.76 ± 0.22	0.82 ± 0.21	0.72 ± 0.11
N harvest index (HI)	0.76 ± 0.77	0.77 ± 0.03	0.71 ± 0.08	0.65 ± 0.04

The average values ± standard error are indicated.

Table 10. N balance (kg N ha^{-1}) during campaign 2003.

fertilized while in the following two campaigns, wheat was again preceded by wheat and it was not fertilized. The extraction by the aerial part at the other treatments ranged between 134 and 188 kg N ha^{-1}. Mineralization rate was between 93 and 123 kg N ha^{-1} at the three campaigns.

It can be observed that the non-accounted N augmented with the N fertilizer dose, as it happened with the leached N quantities and the N emitted as N_2O. In general, such an effect was also observed for balances performed in Navarra and Castilla-La Mancha [34]. As Estavillo et al. [15] reported, these facts suggest an N mineralization rate different to that of the non-fertilized treatment and dependent on the broadcast N fertilizer dose. In this sense, Kuzyakova and Stahr [27] observed there was an effect on the mineralization pattern after the fertilizer application and Webster et al. [44] imputed the increasing Nc to short periods of immobilization that increased with the increasing quantity of available Nmin. Another possibility is that the increase of Nc might be due to some other kind of undetermined losses in the study such as those derived from ammonia leaching, which is presumed not to be very large since the ammonia quantity regarding nitrate is around 10% of the Nmin (**Table 5**). The losses corresponding to ammonia volatilization were not accounted for, but in posterior studies in the zone, they have proved to be dismissible (personal communication).

NUE (nitrogen use efficiency) ranged between 0.35 and 0.60 in the year 2002 and between 0.62 and 0.82 in the years 2003 and 2004 (**Tables 9–11**). This occurred due to the major extraction of the non-fertilized treatment at the first campaign as compared to the following two, since in the first campaign, the essay was preceded by a fertilized crop, while in the following two campaigns, the

Dosage (kg N ha^{-1})	0	140	140	220
Splitting	0	40 + 100	40 + 60 + 40	80 + 140
Nmin initial (NminS)	28 ± 2	35 ±2	36 ±7	36 ± 3
N mineralized (MIN)	93 ± 16	93 ± 16	93 ± 16	93 ± 16
N fertilized (F)	0	140	140	220
N TOTAL INPUTS	121 ± 16	268 ± 16	269 ± 17	349 ± 16
N absorbed by the aerial part (Nab)	56 ± 14	134 ± 7	145 ± 10	165 ± 5
N absorbed by the roots (Nabr)	14 ± 3	33 ± 2	36 ± 2	41 ± 1
N leached (Nlix)	19 ± 2	20 ± 2	31 ± 2	32 ± 2
Nmin after harvest (Nmin AH)	32 ± 1	33 ± 1	42 ± 8	53 ± 2
N TOTAL OUTPUTS	121 ± 15	220 ± 8	254 ± 13	291 ± 7
N not computed	0 ± 22	48 ± 18	15 ± 22	58 ± 18
Fertilizer use efficiency (NUE)		0.69 ± 0.18	0.79 ± 0.2	0.62 ± 0.1
Harvest index	0.77 ± 0.26	0.73 ± 0.03	0.87 ±0.04	0.74 ± 0.07

Table 11. N balance (kg N ha^{-1}) during campaign 2004; average values ± standard error.

non-fertilized treatment received no N fertilization. NUE values that range between 0.3 and 0.6 are frequent at the experiments carried out by the ITGA in Navarra [23].

It was observed that the NUE decreased as the fertilizer dose increased, as observed by other authors (Huggins and Pan, [18]). Fischer et al. [16] stated that depending on the quantity and the distribution of N, HI (harvest index) in wheat ranged from 0.64 to 0.85. Similarly, HI comprehended values between 0.65 and 0.87 in our experiment, the most frequent value of HI was about 0.70–0.80 (**Tables 9–11**).

4. Conclusions

1. The average Nmin values in the soils in years 2002, 2003, and 2004 ranged between 12 and 53 kg N ha^{-1}.
2. Nitrate leaching after harvest in the year 2004 accounted for 50% of the total N leached at every treatment. Thus, it was concluded that a significant mineralization took place after harvest that, together with the absence of crop in summer, causes a Nmin accumulation in summer and its ulterior leaching with the rain in autumn.
3. With treatment 80 + 140, more nitrate was leached than with treatments 0 and 40 + 100; losses up to 70 kg N ha^{-1} were assessed in all the sampling periods in 2004. In this very treatment and treatment 40 + 60 + 40, the 11.3 mg L^{-1} $N\text{-}NO_3^-$ limit was exceeded, calculated as the average concentration in drained water in the sampling period. By the subtraction of the nitric N quantity leached for the non-fertilized treatment, it was assessed that the

nitric N quantities leached until years 2003 and 2004 ranged between 4 and 6% of the N broadcast. If the sampling period after harvest in year 2004 was also considered, the losses ranged between 8 and 14% of the N broadcast.

4. N-N_2O quantities emitted to the atmosphere in all the sampling period in 2003 ranged from 10 kg N ha^{-1} in the non-fertilized treatment to 14 kg N ha^{-1} for treatment 80 + 140. By the subtraction of the N_2O emitted by the non-fertilized treatment from that emitted by the rest of the treatments, it was assessed that the emitted N_2O quantities ranged between 1.8 and 2.8% of the N broadcast as fertilizer in the sampling periods in years 2002 and 2003.
5. No effect was observed due to tilling either on the emitted N_2O or on Ndeni or on Nprod.
6. Regarding the N balance, it was concluded that the N inputs to the soil–plant system were beyond the outputs. In fact, the N acquainted for as not computed N in the N balance, increased with the raising dosage of fertilizer, as the leached N quantities and the N_2O emitted quantities did. This fact suggests an N immobilization rate in the fertilized treatments superior to that at the non-fertilized treatments and dependent on the fertilized N dosage.
7. The efficiency in the fertilizer use varied from 0.35 to 0.82 and it was less in year 2002 than in the next 2 years. This was due to a major N extraction at the non-fertilized treatment in the first campaign as compared to the following 2 years, for at the first campaign, the assay was preceded by a fertilized crop, while in the following 2 years, the non-fertilized treatment was not fertilized. The most frequent value for harvest index was around 0.70–0.80.

Acknowledgements

This work was financially supported by the Basque Government, project IT932-16.

Author details

Arritokieta Ortuzar-Iragorri[1]*, Ander Castellón[2], Gerardo Besga[2], Ana Aizpurua[2], Teresa Fuertes-Mendizabal[3] and Jose M. Estavillo[3]

*Address all correspondence to: arritxu.ortuzar@ehu.eus

1 Department of Didactics of Mathematics and Experimental Sciences (UPV/EHU), Teaching Faculty of Bilbao, Leioa, Spain

2 NEIKER-Tecnalia, Derio, Spain

3 Department of Plant Biology and Ecology, University of the Basque Country (UPV/EHU), Bilbao, Spain

References

[1] Addiscot TM, Whtmore AP, Powlson DS. Farming, Fertilizers and the Nitrate Problem. Wallinford, UK: CAB International; 1991

[2] Allen RG, Pereira LS, Raes D, Smith M. Crop Evapotranspiration—Guidelines for Computing Crop Water Requirements—FAO Irrigation and Drainage Paper 56. Water Resources, Development and Management Service; 1998

[3] Alpkem, Nitrate + nitrite nitrogen (A353, S-170). In: Alpkem Corp editor. RFA Methodology. Clackomas, OR, Alpkem Corp; 1986. p. 1-10

[4] Alpkem. Ammonia nitrogen (A303, S-020). In: Alpkem Corp editor. RFA Methodology, Clackomas, OR, Alpkem Corp; 1987: p. 1-7

[5] Association of Official Analytical Chemists International (AOAC). Plants. In: Cunniff P, editor. Official Methods of AOAC International. 16th ed. Gaithersburg, Maryland, USA: AOAC International; 1999. p. 24127

[6] Arrate I, Sanchez-Perez JM, Antigüedad I, Vallecillo MA, Iribar V, Ruiz M. Groundwater pollution in quaternary aquifer of Vitoria–Gasteiz (Basque Country, Spain). Environmental Geology. 1997;**30**:257-265

[7] Arregui LM. Estrategiasparamejorar la eficienciadelnitrógeno en sistemascerealistas de secano en climamediterráneohúmedo [tesis doctoral]. Pamplona: Universidad Pública de Navarra; 2006

[8] Aulakh MS, Doran JW, Moisier AR. Soil denitrification—Significance, measurement and effects of management. Advances in Soil Sciences. 1992;**18**:1-57

[9] Blake GR, Hartge KH. Bulk density. In: Klute A editor. Methods of Soil Analysis. Part 1. Physical and Mineralogical Methods. Madison WI: ASA-SSSA; 1986. p. 363-375

[10] Bouwman AF. Soils and the Greenhouse Effect. New York: Wiley; 1990

[11] Campbell GS. Soil Physics with BASIC: Transport Models for Soil-Plant Systems. Amsterdam: Elsevier; 1985

[12] Dobermann A, Casssman KG. Environmental Dimensions of Fertilizer Nitrogen: What Can Be Done to Increase Nitrogen Use Efficiency and Ensure Global Food Security? In: Moisier. 2004. pp. 261-278

[13] EEC. Council Directive 88/778/EEC of 15 July 1980 relating to the quality of water intended for human consumption. OJL. 1980;**229**:11

[14] Gobierno Vasco-EuskoJaurlaritza (GV-EJ). Decreto 390/1998 por el que se dictannormaspara la declaración de ZonasVulnerables a la contaminación de lasaguaspor los nitratosprocedentes de la actividadagraria and se aprueba el Código de BuenasPrácticasAgrarias de la ComunidadAutónoma del País Vasco. BOPV. 1999;**18**:1448-1474

[15] Estavillo JM, Rodriguez M, Lacuesta M, González-Murua C. Effect of cattle slurry and mineral N fertilizer applications on various components of the nitrogen balane of mown grassland. Plant and Soil. 1997;**188**:49-58

[16] Fisc;her RA, Howe GN, Ibrahim Z. Irrigated spring wheat and timing and amount of nitrogen fertilizer. I. Grain yield and protein content. Field Crops Research. 1993;**33**:37-56

[17] Follet RF, Keeney DR, Cruse RM. Managing Nitrogen for Groundwater Quality and Farm Profitability. Madison: Soil Science Society of America; 1991

[18] Huggins DR, Pan WL. Nitrogen efficiency component analysis: An evaluation of cropping systems differences in productivity. Agronomy Journal. 1993;**85**:898-905

[19] Hussain S, Peng S, Fahad S, Khaliq A, Huang J, Cui K, Nie L. Rice management interventions to mitigate greenhouse gas emissions: A review. Environmental Science and Pollution Research. 2015;**22**:3342-3360. DOI: 10.1007/s11356-014-3760-4

[20] Intergovernmental Panel on Climate Change (IPCC). Climate change. Scientific and technical analysis of impacts, adaptions and mitigation. Contribution of Working Group II to the Second Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge: Cambridge University Press; 1995

[21] Irañeta I, Armesto AP, Segura A, Lafarga A, Arregui LM, Merina M, Baroja M, y Quemada M. Herramientas de ayuda a la decisión para el manejo correcto de fertilizantes nitrogenados en cereales de invierno. Navarra Agraria. 2002;**133**:6-16

[22] Isermann K. Agriculture's share in the emission of trace gases affecting the climate and some cause-oriented proposals for sufficiently reducing this share. Environmental Pollution. 1994;**83**:95-111

[23] Kennedy IR. Acid Soil and Acid Rain. Taunton: Research Studies Press Ltd.; 1992

[24] Kjellerup V, Kofoed A. Nitrogen fertilization in relation to leaching of plant nutrients from soil. Lysimeter experiments with 15N. Tidsskrift for Planteavl. 1983;**87**:1-22

[25] Knowles R. Denitrification. In: Paul EA, y Ladd J, editors. New York: Soil Biochemistry, Marcel Dekker; Vol. 5. 1994. pp. 323-369

[26] Kuenen JG, Robertson LA. Combined nitrification-denitrification processes. FEMS Microbiology Review. 1994;**15**:109-117

[27] Kuzyakova IF, Stahr K. Studying the dynamics of the net N mineralization rate in a catena on loess deposits using time series analysis. Soil Science. 2006;**39**:397-402

[28] Lasa B, Irañeta J, Armesto AP, Lafarga A. Balances de nitrógeno en cereal de invierno en la ComunidadForal de Navarra. In: Quemada M, editor. Balance de nitrógeno en sistemas de cultivo de cereal de invierno and de maíz en variasregionesespañolas. Monografías INIA: Serie Agrícola 22. Madrid: INIA; 2006. pp. 48-58

[29] Legg JO, Meisinger JJ. Soil nitrogen budgets. In: Stevenson FJ, editor. Nitrogen in Agricultural Soils. Madison, WI: American Society of Agronomy; 1982. pp. 1-42

[30] Mary B. Concluding remarks: N hazards to crops and environment. In: Lemaire G editor. Diagnosis of the Nitrogen Status in Crops. Alemania: Springer-Verlag, Berlin; 1997. p. 229-236

[31] Meisinger JJ, Randall GW. Estimating nitrogen budgets for soil-crop systems. In: Follet RF et al, editors. Managing Nitrogen for Groundwater Quality and Farm Profitability. Madison, WI: SSSA; 1991. p. 85-124

[32] Menéndez S. Evaluating gaseous emissions from agricultural soils under different management strategies [tesis doctoral]. Leioa: UPV-EHU; 2006

[33] Okereke GU. Prevalence of nitrous oxide reducing capacity in denitrifiers from a variety of habitats. Plant and Soil. 1984;**81**:421-428

[34] Quemada M, Lloveras J. Conclusiones. In: Quemada M, editor. Balances de nitrógeno en sistemas de cultivo de cereal de invierno and de maíz en variasregionesespañolas. Monografías INIA: Serieagrícola. n° 22. Madrid: INIA; 2006. pp. 134-137

[35] Ramathan V, Ciccerone RJ, Singh HB, Kiehl JT. Trace gas trends and their potential role in climatic change. Journal of Geophysical Research. 1985;**90**:554-5566

[36] Rodhe H. A comparison of the contribution of various gases to the greenhouse effect. Science. 1990;**248**:1217-1219

[37] Rowell DL. Soil Science: Methods and Applications. Essex, England: Longman Scientific & Technical; 1994

[38] Rroço E, Mengel K. Nitrogen losses from entire plants of spring wheat (*Triticum aestivum*) from tillering to maturation. European Journal of Agronomy. 2000;**13**:101-110

[39] SAS Institute. SAS Version 8. Cary, NC: SAS Institute; 1998

[40] Soil Survey Staff. Keys to Soil Taxonomy. Washington, USA: United States Department of Agriculture, Natural Resources Conservation Service; 2006

[41] Tilman D, Cassman KG, Matson PA, Naylor RL, Polasky S. Agricultural sustainability and intensive production practices. Nature. 2002;**418**:671-677

[42] Walkey A. An examination of methods for determining organic carbon and nitrogen in soils. The Journal of Agricultural Science. 1935;**25**:598-609

[43] Watson RT, Zinyowera MC, Moss RH, Dokken DJ. Climate Change 1995, Impacts, Adaptations and Mitigation of Climate Change: Scientific-Technical Analyses. USA: Intergovernmental Panel on Climate Change, Cambridge University Press; 1996

[44] Webster CP, Shepherd MA, Goulding KWT, Lord E. Comparisons of methods for measuring the leaching of mineral nitrogen from arable land. European Journal of Soil Science. 1993;**44**:49-62

[45] Wolf B. Diagnostic techniques for improving crop production. Inc. New York: Food Products Press, The Haworth Press; 1996

[46] Wrage N, Velthof GL, van Beusichem ML, Oenema O. Role of nitrifier denitrification in the production of nitrous oxide. Soil Biology and Biochemistry. 2001;**33**:1723-1732

[47] Yamulki S, Goulding KWT, Webster CP, Harrison R. Studies on NO and N_2O fluxes from a wheat field. Atmospheric Environment. 1995;**29**:1627-1635

[48] Yousaf M, Li J, Lu J, Ren T, Cong R, Fahad S, Li X. Effects of fertilization on crop production and nutrient-supplying capacity under rice-oilseed rape rotation system. Scientific Reports. 2017;**7**:1270. DOI: 10.1038/s41598-017-01412-0

[49] Yousaf M, Li X, Zhang Z, Ren T, Cong R, Ata-Ul-Karim ST, Fahad S, Shah AN, Lu J. Nitrogen fertilizer management for enhancing crop productivity and nitrogen use efficiency in a rice-oilseed rape rotation system in China. Frontiers in Plant Science. 2016;**7**:1496. DOI: 10.3389/fpls.2016.01496

[50] Zadoks JC, Chang TT, Konzak CF. A decimal code for the growth stages of cereals. Weed Research. 1974;**4**:415-421

Chapter 6

Challenges to Safe Wheat Storage

Shaima Mohamed Nabil Moustafa,
Haifa Abdulaziz S. Alhaithloul and
Hani Mohamed Awad Abdelzaher

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.75585

Abstract

There are enormous challenges facing wheat storage, which is the most important crop in existence. Wheat is one of the most famous and important plants in human history. There is no country in the world that does not give up wheat yields. Countries of the world vary and differ in their production and consumption of that important plant. Since ancient times, humans have stored wheat grain in special places. Storage areas were developed until the current silos were reached. With large quantities of wheat stored in silos, there are many challenges to the healthy environment of storage. One of the most important challenges facing quality of wheat stored in silos is the spread of conidia and spores of many dangerous fungi on wheat grains. One of studies conducted by the authors proved presence of some of notorious fungi on and inside wheat mass stored in the silo under study. *Aspergillus flavus*, *A. niger*, *Circinella umbellata*, *Gliocladium* sp., *Penicillium frequentans*, *P. islandicum*, and *Ulocladium atrum* were isolated from wheat samples. All seven isolated fungi demonstrated their ability to analyze human red blood cells with different strengths. These results are consistent with previous studies that confirm the seriousness of presence of these fungi on the health of dealers and exposers especially with bad storage and humidity.

Keywords: fungi, humidity, silos, storage, wheat

1. Introduction

Wheat is one of the most important crops in the world. It is cultivated on an area of about 2 million square kilometers. Its ease of adaptation to the soil has spread to several areas of the world. Wheat is the mainstay of nutrition for many countries in the world. Based on the latest report

IntechOpen

© 2018 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. CC BY

of the Food and Agriculture Organization of the United Nations (FAO), that the global wheat trade in 2017 reached 754.8 million tons; Russia's exports of wheat amounted to 30 million tons, America 26.5 million tons, Europe 25 million tons, Canada 21 million tons, Australia 20 million tons, while exports of Ukraine 15 million tons, Kazakhstan 8.5 million tons, Argentina 8 million tons, the largest countries exporting wheat worldwide (www.fao.org).

Fortunately, global wheat prices have fallen, driven by an increase in global production, which has been affected by good weather and the availability of water to grow in the EU, India, Pakistan, China, and the United States, which was accompanied by a rise in global exports (www.fao.org).

Forecasts indicate that the use of wheat in 2017–2018 will reach 740 million tons. More than 43% of the production is consumed by only six countries: China, India, Russian Federation, the United States of America, and Pakistan. This is because of the population of these countries of nearly three billion people, or half the world's population (www.fao.org).

As many countries in the world do not produce sufficient quantities of wheat for their uses and therefore tend to import this strategic commodity. These wheat-importing countries store large quantities of these grains in storage places called silos. Since ancient times, wheat grains have been stored in huge shipments. Earliest discovery of grain stores dates back to the year 9500 BC [1], and these stores in the settlements of the Neolithic period before the pottery "A" were located in the Jordan Valley, where the first stores were located in places among other buildings. But at the beginning of the 8500 BC, they were moved inside the houses, but with the period of 7500 BC, they were stored in rooms dedicated to it [2]. The area of the first wheat stores was 3 × 3 m from the outside, and had suspended layers in order to protect grain from rodents and insects and provide ventilation [3]. These stores are then located in Mahjara, which is placed in the valley of Sindh since 6000 BC. The ancient Egyptians used to store wheat grains in years of prosperity for use in drought ones. Because Egypt's climate is very drought, Egyptians have been able to store grain in silos without a significant loss of quality. The grain silo, as it is called, is an ideal way to store grains in all lands of the East since time immemorial. In Turkey and Iran, moorings used to buy wheat or barley, which is relatively cheap, and stored it in closed and hidden places in the face of famine. In Malta, relatively large quantities of wheat were stored in hundreds of silos dug into rocks. The silos can store up to 60–80 tons, by taking proper precautions and keeping it in good condition for 4 years or more. By the end of the nineteenth century, stores specifically designed for grain conservation began to spread in Great Britain, but North America was the home for the major stores, called grain levers. There were large-scale climatic obstacles to grain storage in Great Britain on those difficulties significantly. In order to keep the grain in good condition, it should be kept as far away as possible from moisture and heat because new grains tend to release moisture when brought to the store. In this case, microorganisms (mainly fungi) are more active and can heat the grains. If the grain continues to be heated, its quality will be affected. Therefore, effective treatment is to place grains on the ground in the form of non-thick layers, and to keep the place well ventilated. Hence, grain can be configured to store in silos.

In Great Britain, small wheat stores were built on mushroom-shaped logs called stone pillars. It was built on a wooden frame and usually has stone roofs. The large ones resemble the open ceiling from the front, but the upper part is closed. The first floor is usually accessed by a stone

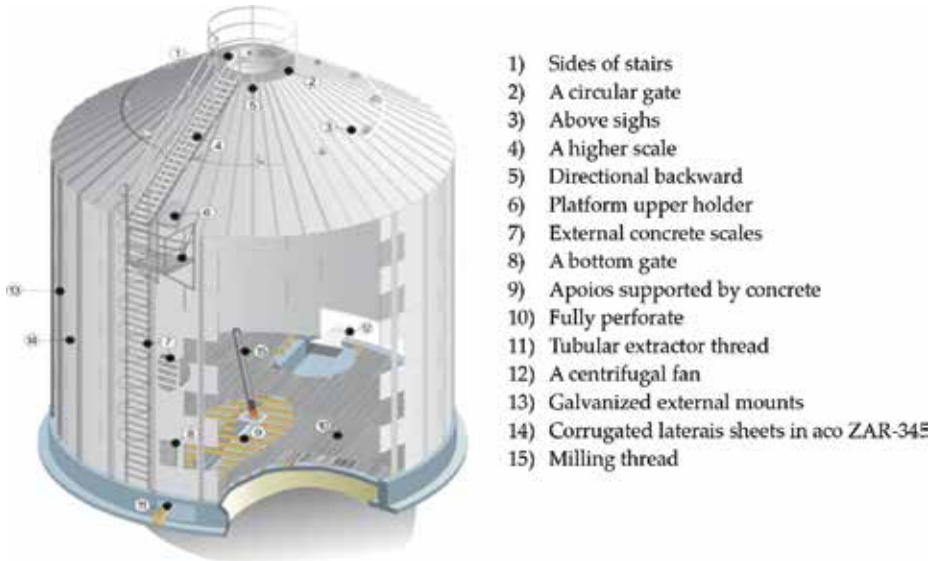


Figure 1. Internal structure of a steel silo as in [3].

staircase on the outer wall. With tremendous advances in engineering technology in the construction of silos, a new generation of forms and possibilities of silos has begun. High-density indoor silos are found in farms, mills, and harbors and can reach a height of 30 m. Largest silos are located in North America at major shipping centers such as Chicago, Kansas city, and Missouri in the United States, and Thunder Bay, Ontario, and Canada. Many cylindrical silos are equipped with a perforated metal floor that allows the air to pass through to keep grain free from moisture. Cylindrical silos are often adjacent, with high mechanical devices nearby.

Generally, there are three types of silos, the first type is made of wood, the second type is made of concrete, and the third type is made of metal (steel and galvanized). Wheat is as barley, oat, rye, and so on, used to store suitably both in concrete and metal silos [3] (**Figure 1**).

Large silos have many appliances including dryers, detergents, workpieces, cranes, and conveyors. Dryers leave grain free from moisture. The air is heated in the dehydrator and directed over the grains and then used air that has not been heated to cool the grains. The use of detergent ensures grain cleanliness. The absorption of dust, husk, or straw is obtained and screening and vibration leads to the elimination of grains that are not of the appropriate size and density. However, some machines use photovoltaic cells to isolate rotten grains.

Cranes and conveyors are used to transport grains. Vertical grain movement is obtained by cranes. Among the most used cranes is a crane with a large conveyor belt and a bundle of buckets. Wheat grains in the bucket are transported to high altitude and poured into storage baskets. Tankers move the grains horizontally across the silo, called a grain lift. It is a silage storage place as well.

Large quantities of wheat are delivered to the silos. Of course, the huge quantities received by silos are coming directly from farms without any transactions. Wheat is then loaded with all the residues of process of flail, agricultural soil, and contaminants of harvesting tools during the

separation of grains from the ears. It is perhaps of standard importance to prepare wheat grains for the flour process to explain how grinding of wheat grains is done by the following procedures. The grain of wheat consists of three main parts: grain coats, embryos, and endosperm. The purpose of the milling process is to separate as much of the endosperm content as possible from the wrappers. The ratio of flour produced to the percentage of grain used in production is known as extraction. The weight of flour produced from 100 g of grain, and depends on this percentage on several factors, the most important; extraction method used, type of mills used, nature, and specific weight of wheat grains. In general, extraction rate is between 70 and 72% in excellent white flour, and this percentage is 90–95% in brown flour. What is left behind from milling, so-called bran, is used to feed animals. Wheat milling is done by the following subsequent serial steps [4]:

1.1. Receiving

Grain coming from fields or silos is received in the mill after sampling and examined to ensure that it meets specifications set by the mill. Grains are received and emptied in conical tanks, each of which can reach 10 tons. Of which is covered with a fixed metal mesh for impurities when unloading grain. The crane and aspirator pull the grain out of the tanks for delivery to the cleaning equipment.

1.2. Cleaning

The cleaning equipment consists of two main units: dry-cleaning unit known as black cleaning unit and the wet-cleaning unit known as the white cleaning unit.

1.2.1. Black cleaning

This unit consists of the following equipment:

A. Compound separation device: It is composed of three wire screens installed and portable on a metal frame suspended by pulley rods spring to generate vibration movement. Sieves are arranged on top of each other so that they have wide holes at the top followed by center holes and small holes. As a result, impurities can be eliminated according to their size. The device is fitted with a fan mounted at the top of the frame to generate a stream of air that helps to breakdown light impurities.

B. Separation device according to the specific weight: This device consists of a metal box revolving inside the fan and a strong aspirator and sieve mounted 12% slope from the horizontal level, working on the suction of grain and the deposition of heavy impurities such as stones and pieces of glass.

C. Magnetic separation device: It is a death pass inside the grain, and is equipped with magnetic plates electric work to attract pieces of iron nails and clumps which are collected in a special drawer.

D. Vibrating machine for the separation of impurities: It is a composite of a serrated cylinder from the inside to be grooves or round pockets to settle round impurities similar to the diameter of the grain and different in terms of form, where they gather in special ditches.

1.3. White cleaning

This unit consists of a peeler and stalker.

A. The peeler consists of a cylinder with rough internal surfaces or coated with a precision metal bed that scrapes centrifugal grains. Shells are separated and pulled by an air stream generated by an electric fan installed at the top of the peeler.

B. The asset consists of a rectangular basin with a nozzle to feed it with grains, as well as an appropriate water source that can be controlled as needed, and ends with a drainage basin topped by an appropriate filter. Inside cylinder with two helical separators working in opposite directions that wash the grains and push them forward and the light impurities on the surface of the water. With the rush of grain forward, it passes over a vibrator strainer that has been removed from the water and then to a rotary roller dryer to complete removing water droplets from the grain. The question now arises: Is the process of drying wheat grains sufficient to remove all moisture from the grain mass? Can wheat grains be washed before entering the silos? What is the cost for this? What are possible risks of increasing humidity and possibility of conditions for infection of fungal spores and conidia?

Perhaps, it is possible in this book to put forward some ideas as follows:

- Presence of units within silos for washing and drying of grains by successive processes of passage of warm air currents, and the source of energy units of solar cells installed one way or another on sides of the silo exposed to sunlight.
- Putting desiccant materials that absorb air moisture inside silos such as sodium chloride and silica gel.
- Exposure of wheat mass to ultraviolet rays for superficial sterilization with constant flipping, taking into account the non-exposure of direct workers to those rays.
- Air fumigation of silos with volatile oils that have the potential to sterilize the air.

Despite all precautions taken in modern silos, many studies have shown that fungi are flourishing on wheat grains and in the air of these reservoirs [5]. Several research findings have confirmed the presence of harmful and toxic fungi in many silos, not only on stored grains but also on wheat flour derived from those silos. Wheat grains are harvested in agricultural fields so they are subjected to contamination with soil particles as well as germs adhered on wheat plant itself. For these reasons, the mass of wheat stored in silos contains large quantities of dust packed with fungal spores. It is worth mentioning that any defect in the system of grain conservation inside silos is followed by the growth of fungal spores among wheat grains, and these molds may be not visible to the eye, which ultimately leads to the arrival of consumers.

The problem seems more complicated if wheat is stored in poor conditions, due to the ability of wheat to imbibe the air humidity of the silos. A study conducted in Zimbabwe indicated that the storage of red wheat in many silos led to a decline in the commercial level of this commodity and an increase in the level of fungal toxins [6].

In one of the literatures of the previous research, presence of fungi was tested in 34 samples collected from 3 silos. Results of this experiment proved that presence of fungi produced aflatoxins in majority of tested samples [5]. It is worth mentioning that fungi represent the main factors of starchy grain contamination (mycotoxigenic). Therefore, it has been found logical to review a study conducted by the authors on presence of fungi that have serious precedents as causes of diseases of respiratory system in humans. We will discuss the danger of these fungi to people dealing with wheat grains from the beginning of harvest until the entry into silos.

This part of the book will present results of a study conducted in one of the giant silos in Sakaka city, Al-Jouf region, Saudi Arabia, in the autumn of 2015. Our results will be discussed with results of previous studies in some countries in order to highlight some of the challenges facing safe storage of wheat grains inside silos.

2. Materials and methods a research on the presence of fungi in wheat stored in a silo in Sakaka city, Saudi Arabia

Twenty samples of wheat grains stored in the large silo in Sakaka were tested for the presence of fungi (part of this work has been reported elsewhere [7]). This was done by placing a known quantity of wheat in a bottle of sterile water next to the sampling area, taking care to ensure that one source of fungus (wheat grains) reaches the collection container. When returning to the laboratory, fungi were isolated by placing 5 ml of water mashed with fungal spores, coming from grain surfaces, in a 9-cm Petri dish and then adding 15 ml of rose-Bengal Potato Dextrose Agar (PDA) medium, all in isolation cabinet under aseptic conditions. Dishes were closed and sealed with parafilm to ensure full closure and placed upside down in plastic bags (previously sterilized by radiation) and incubated at 28°C, and then followed up until appearance of fungal colonies. After emergence of fungal colonies, each colony was purified on its own, to obtain pure single fungal isolate. Pure fungal samples were subjected to ophthalmic and microscopic examinations with imaging and arranged of figurative plates [8, 9]. Risk of isolated fungi has been tested on human health. Hemolytic ability of the isolated fungi to human red blood cells was tested following the method of [10, 11]. Fungal spore suspension (in 0.9% NaCl) was used. Washed (using 0.9% NaCl) 100 μl of blood, plus 900 μl of spore suspension was incubated, sodium chloride solution, which represents a negative control sample, and distilled water (positive control sample), under aseptic conditions, for comparison at 28°C, for 24 h, in the dark. Reaction mixtures were separated with the aid of a centrifuge and absorbance of supernatant was measured using UV-Vis spectrophotometer (spectro uv-2505) at 540 nm to calculate percentage of hemolysis of red blood cells following formula of the equation:

$$\%\text{Hemolytic activity} = \text{absorbance of sample–absorbance of saline/absorbance of dist.water} \times 100.$$

3. Results and discussion

Fungi of *Aspergillus flavus*, *A. niger*, *Circinella umbellata*, *Gliocladium* sp., *Penicillium frequentans*, *P. islandicum*, and *Ulocladium atrum* were isolated from wheat samples. **Table 1** shows prevalence of each fungus.

OR = Occurrence remarks; H = 60% -100.0%, M = 33 - 59.0%, L = 20–32%, and R = 7–19%.

Genera and species	Wheat grains		
	NCl	OR	TC (%)
Aspergillus	145	20H	38.3
A. flavus	73	20H	19.3
A. niger	72	20H	19.0
Circinella umbellata	28	5 L	7.4
Gliocladium sp.	8	4 L	2.1
Penicillium	138	18H	36.5
P. frequentans	70	18H	18.5
P. islandicum	68	17H	18.0
Ulocladium atrum	59	3 L	15.7
Gross total counts	378		
No. of genera	5		
No. of species	7		

Table 1. Gross counts of fungal genera and species derived from 20 samples of wheat grains collected from the main silo, Sakaka, Al-Jouf, Saudi Arabia by germs came from soaked grains in sterilized H_2O, number of cases of isolation (NCl; out of 20 cases), occurrence remarks (OR), percentage of total counts (TC%) on PDA agar at 28°C.

4. Fungal identification

It is worth mentioning that all fungi isolated from wheat surfaces stored in the silo, produces huge amounts of spores and conidia. For example, *Aspergillus flavus* is a fungus of a bad reputation which produces the most dangerous toxin called aflatoxin (AFs). This instinct fungus is famous for corruption and damage of many seeds, grains, and nuts [12]. *A. flavus* produces large quantities of conidia bearing on biseriate sterigmata. *A. niger* produces large quantities of conidia that are carried on two-row stregmata; these conidia are black, in long chains. Conidia of *A. niger* cause respiratory problem in people exposed to inhalation of such germs, such as people working in poultry farms, and so on. *Circinella umbellate* is a fungus belonging to zygomycotina that has huge amounts of spores in many sporangia (**Figures 2–8**). Previous

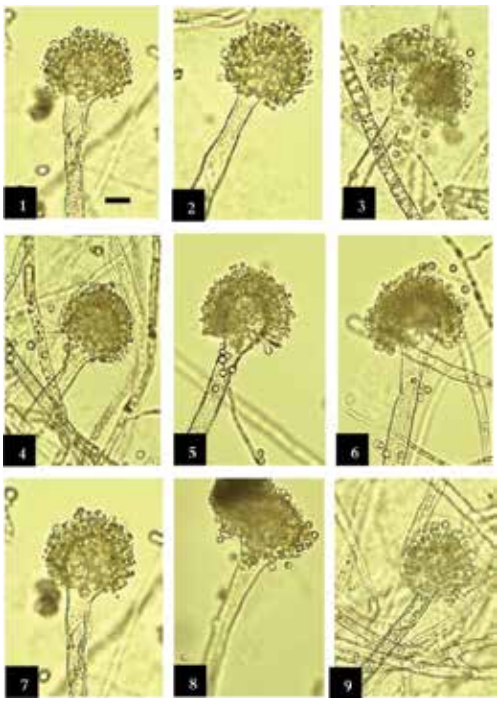


Figure 2. Hyphal growth of *Aspergillus flavus* on PDA at 28°C. Photos 1–9 were shot using ordinary compound microscope. Non-septate conidiophores and foremost radiate heads with mono- and biseriate sterigmata carried on conical-shaped vesicles. Bar 10 μm in the photo 1 is the same for rest of the photos.

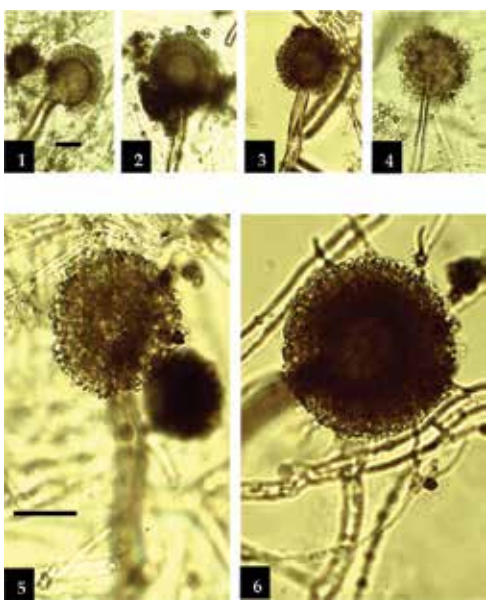


Figure 3. Hyphal growth of *Aspergillus niger* PDA at 28°C. Photos 1–6 taken by ordinary compound microscope. Non-septate conidiophores and radiate heads with biseriate sterigmata. Bar 10 μm in the photo (1) is the same for photos 2–4, and in photo 5 is the same as photo 6.

http://dx.doi.org/10.5772/intechopen.75585

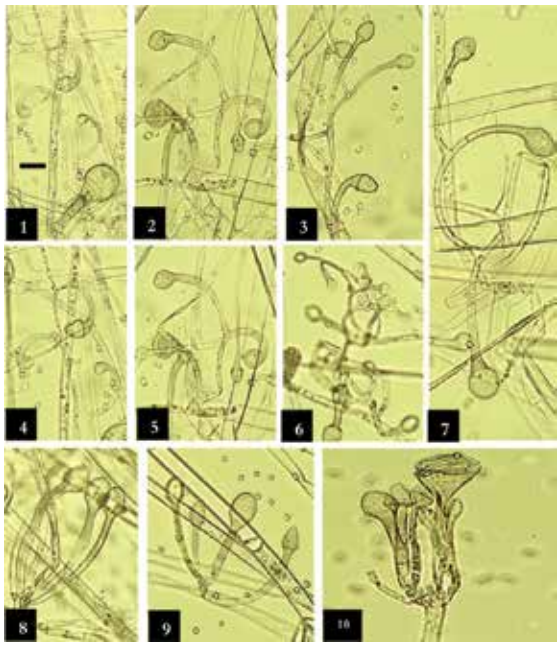


Figure 4. Hyphal growth of *Circinella umbellate* on PDA at 28°C. Photos 1–10 are taken by ordinary compound microscope. 1–7 branched conidiophores with curved side branches cut off by sporangia. 8–10 gatherings tent form sporangiophores. Bar 10 μm in Photo 1 is the same for rest of the photos.

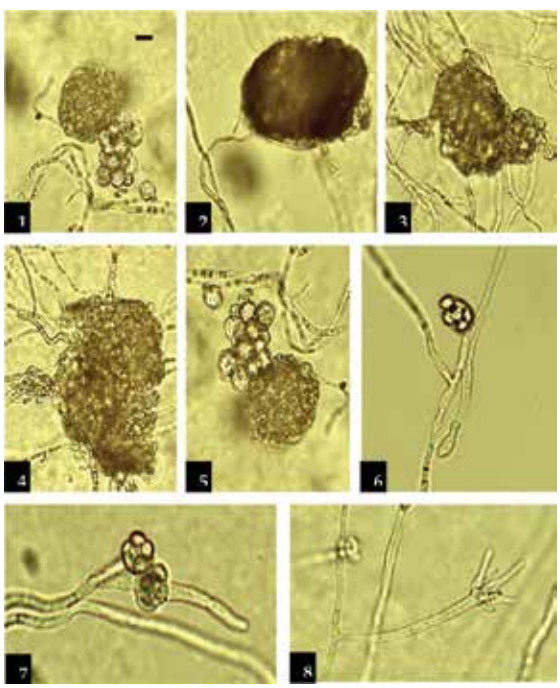


Figure 5. Hyphal growth of *Gliocladium* sp. on PDA at 28°C. Photos 1–8 shot by ordinary compound microscope. 1–7 gelatinous grouping of conidia. 8 A conidiophore loads a distinguishing sterigmata peculiar of this fungus. Bar 10 μm in Photo 1 is the same for the rest of the photos.

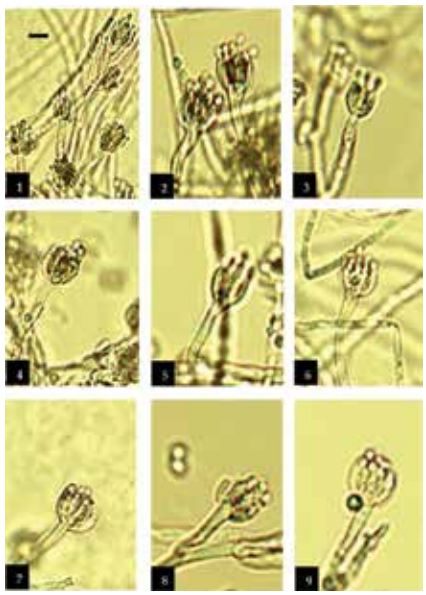


Figure 6. Hyphal growth of *Penicillium frequentans* on PDA at 28°C. Photos 1–9 shot by ordinary compound microscope. 1–9 septate conidiophores carried monoseriate sterigmata. Bar 10 μm in Photo 1 is the same for the rest of the photos.

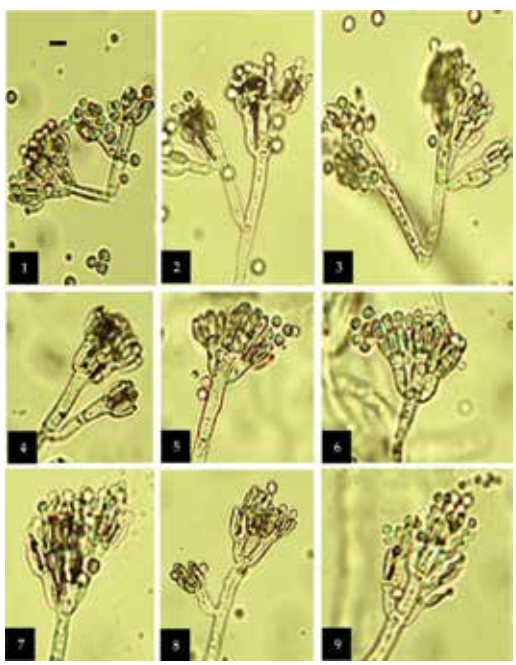


Figure 7. Hyphal growth of *Penicillium islandicum* on PDA at 28°C. Photos 1–9 shot by ordinary compound microscope. 1–9 septate conidiophores carried biseriate sterigmata and symmetrical. Bar 10 μm in the Photo 1 is the same for of the rest of the photos.

http://dx.doi.org/10.5772/intechopen.75585

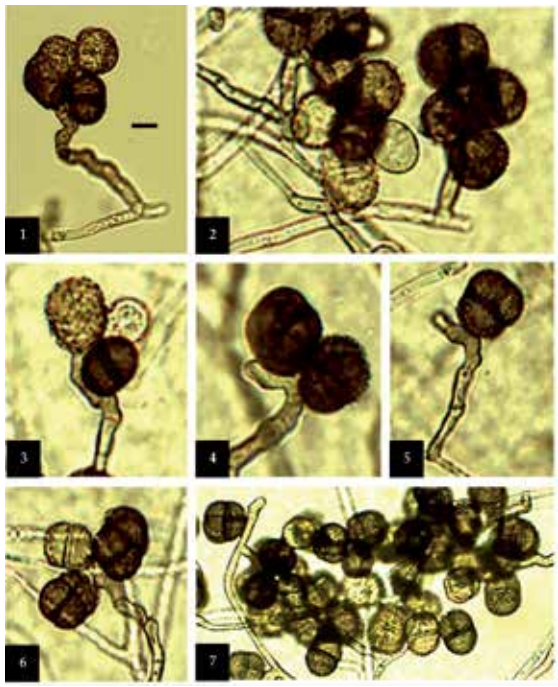


Figure 8. Hyphal growth of *Ulocladium atrum* on PDA at 28°C. Photos 1–7 shot by ordinary compound microscope. 1–7 septate conidiophores carried singularly broad conidia with rough wall has transverse septa (1–3 septa) and longitudinal septum (one or more), comparatively strict at the base and wide at the top. Bar 10 μm in Photo 1 is the same for rest of the photos.

studies suggest that this fungus can cause chest allergic diseases and asthma-like symptoms for people with low immunity who are subject to inhaling its spores [13].

A previous study has shown that *Penicillium frequentans* and *P. islandicum* produced a type of fungal toxin called aflatoxin (AFs) [14]. They further caused pulmonary disease, hypersensitivity, allergy (alveoli), a kind of emphysema [15]. As the two fungi produces chains of conidia, which are easy to spread through dusted air with the mass of wheat stored in the silos, therefore, these toxins can cause risk of spread of diseases for dealers within the silos.

While literature treated *Gliocladium* as a class of "useful fungi," modern research raveled the contribution of it in the production of a toxin named Gliotoxin [16].

Ulocladium atrum is not far from the problems caused by the fungi mentioned above. It was mentioned in some previous studies for its ability to cause allergy in chest and respiratory system in humans [17].

By reviewing the previous narration (above), we can say that the isolated fungi of this study are present in the form of conidia and spores, lying on the surface of wheat grains and between folds of its coats.

There are two types of risks because of the existence of conidia and spores on wheat surfaces. The first risk is the exposure of dealers with large quantities of conidia and spores

inside the silos. The second danger is the possibility of growth of these conidia and spores, with the availability of moisture, to be innate growths producing very dangerous toxins to humans. In order to prove the first risk in a measurable experimental way, an experiment was conducted to determine the damage that could occur as a result of invasion of conidia and spores of the isolated organisms into human lungs and then into the blood via alveoli in one way or another.

5. An experiment showing the effect of inhaling spores and conidia of the isolated fungi on public health of exposed individuals

Biological effect of spores and conidia of *Aspergillus flavus*, *A. niger*, *Circinella umbellate*, *Gliocladium* sp., *Penicillium frequentans*, *P. islandicum*, and *Ulocladium atrum* on the decomposition of red blood cells in humans.

Results of the study showed the ability of each of the tested fungi to analyze the red blood cells in a human blood sample. There was a disparity in the effect of that on the severity of decomposition (hemolytic activity). *P. frequentans* performed highest response to disruption of the human red blood cells (63%), followed by *Gliocladium* sp. (51%), and *A. niger* (50%), respectively. Conidia and spore suspension of each of *Ulocladium atrum*, *Circinella umbellata*, *Aspergillus flavus*, and *Penicillium islandicum* donated sponses of 23, 22, 20, and 19%, respectively (**Figures 9** and **10**).

Despite all evidences from previous studies that confirm the seriousness of isolated fungi on the health of dealers and exposers, we have tested the ability of these fungi on hemolysis

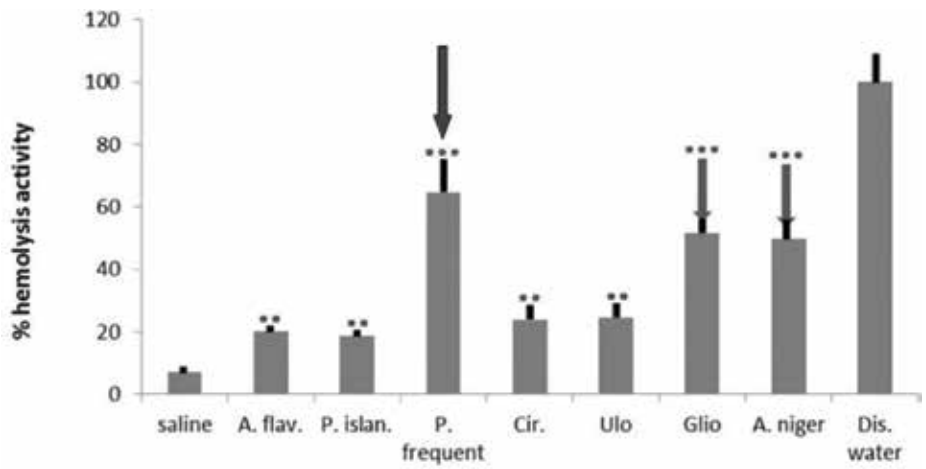


Figure 9. Influence of conidia and spore suspension of *Aspergillus flavus*, *A. niger*, *Circinella umbellate*, *Gliocladium* sp., *Penicillium frequentans*, *P. islandicum*, and *Ulocladium atrum* on breakdown of the human red blood cells. Bars above columns symbolize standard error of average data from three replicates and reveal differences between averages of samples related to control. Significant values against control represent: ** = highly significant at $p < 0.01$, *** = very significant at $p < 0.001$.

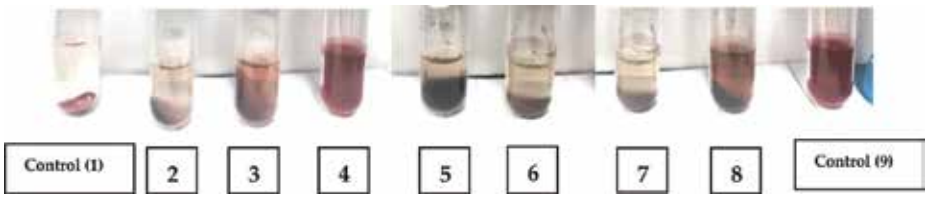


Figure 10. Influence of conidial and fungal spores suspension of *Circinella umbellata* (sample No. 2), *Gliocladium* sp. (sample No. 3), *Penicillium frequentans* (sample No. 4), *Ulocladium atrum* (sample No. 5), *Penicillium islandicum* (sample No. 6), *Aspergillus flavus* (sample No. 7), and *Aspergillus niger* (sample No. 8) on hydrolysis of human red blood cells. Sample 1, is negative control sample [human blood + saline solution (0.9% NaCl)], and Sample 9, is the positive control sample [human blood + distilled water].

of human red blood cells. From our results, *Penicillium frequentans*, *Gliocladium* sp., and *Aspergillus niger* caused significant damage and decomposition of red blood cells at high rates compared to other fungi, while rest of tested fungi also caused damage, but less than the above which highlights the risk of exposure to conidia and spores of those fungi. Our findings here correlate with previous studies on how bad these fungi are, although no data are available on the effect of conidia and spores of some fungi on the decomposition of human red blood cells. All this confirms without any doubt the seriousness of the intense exposure to conidia and spores of fungi and that the huge quantities of wheat stored in silos is dangerous sources for dealers and exposers. We recommend using nasal and oral masks for people working in silos and exposed to dust carrying fungal conidia and spores generated by the movement of wheat grains.

From another dimension, these conidia and spores of fungi are sources of severe contamination to wheat stored in silos. In the case of moisture, wheat becomes an ideal environment for growth, reproduction, and growth of fungi, which may grow inside the wheat mass, producing dangerous fungi that produce toxins.

6. Conclusion

With the steady increase in human numbers and high living and nutrition requirements, it is imperative to increase the production of important cereal crops for large segments of the population. Wheat is a very important component of human needs throughout the world. Therefore, since ancient times people have been interested in working on storing this important and vital commodity to get it in time of need. Granaries and silos were established and they continued to develop until they reached the current structural and architectural design. Nowadays, nearly every country in the world has several silos spread throughout its land to cover the continuing needs of cereals. It should be noted that many countries in the world have a much higher request for wheat than their production. This leads to the import of this important commodity from places of production surplus from the need of producers. Since its harvest, wheat has been subjected to successive steps of transport and conservation, which makes it vulnerable to pollution and damage. As wheat crop is subjected to the sifting process, which removes grain from the harvest residues and soil granules, this factor will be ignored.

Large quantities of wheat during shipping process through giant vessels are exposed to many risks. High humidity of transport containers overseas increases chances of wetting wheat grains and thus chances of increasing the contamination and growth of fungi in wheat. Sometimes conidia and spores of fungi germinate within the mass of wheat grains and producing innate fungi which may not be seen by the naked eye and generating very dangerous toxins. Often, when shipments of wheat arrive via transport to silos, they are loaded with many elements of danger.

In one of the studies conducted by the authors on the presence of harmful fungi in the mass of wheat inside a silo, *Aspergillus flavus*, *A. niger*, *Circinella umbellata*, *Gliocladium* sp., *Penicillium frequentans*, *P. islandicum*, and *Ulocladium atrum* were isolated. Since these fungi have precedents to cause some diseases, an experiment has been conducted to prove this. The test of the ability of the isolated fungi to analyze human red blood cells has been shown to have a high coefficient of effect.

In theory, based on the scientific data and results of the previous studies, we proposed to provide a healthy environment within the silos, which is summarized as follows:

1. Washing wheat grain at the before entering for storage in the silos and then drying with constant exposure and flipping to warm air currents from the sources of solar energy.
2. Exposing air silos to ultra violet light during periods of non-working workers.
3. Use of aerosols and volatile oils to help sterilize air silos.
4. Put moisture absorbent materials such as calcium chloride and silica gel, to ensure dry storage environment.

Author details

Shaima Mohamed Nabil Moustafa[1,2]*, Haifa Abdulaziz S. Alhaithloul[1] and Hani Mohamed Awad Abdelzaher[1,2]

*Address all correspondence to: shymaa.nabil@ju.edu.sa

1 Department of Biology, College of Science, Jouf University, Sakaka, Saudi Arabia

2 Department of Botany and Microbiology, Faculty of Science, Minia University, El-Minia City, Egypt

References

[1] Kuijt I, Finlayson B. In: Bar-Yosef O, editor. Evidence for food storage and predomestication granaries 11,000 years ago in the Jordan Valley. Cambridge, MA: Harvard University; 2008. Approved May 15, 2009; Received for Review December 16

[2] Kuijt I. Demography and storage systems during the Southern Levantine Neolithic demographic transition. In: Bocquet-Appel JP, Bar-Yosef O, editors. The Neolithic Demographic Transition and Its Consequences. New York: Springer; 2008. pp. 287-313

[3] Pekmez H. Cereal storage techniques: A review. Journal of Agricultural Science and Technology B. 2016;**6**:67-71

[4] Mousaily HA. Food Grains (Production–Storage–Manufacturing of Their Products). Dar Alaa Eldeen; 2006. ISBN: 13978-9933-18-193-2

[5] Joshaghani H, Namjoo M, Rostami M, Kohsar F, Niknejad F. Mycoflora of fungal contamination in wheat storage (silos) in Golestan Province, North of Iran. Jundishapur Journal of Microbiology. 2013;**6**(4):e6334

[6] Mhiko TA. Determination of the causes and the effects of storage conditions on the quality of silo stored wheat (*Triticum aestivum*) in Zimbabwe. Natural Products and Bioprospecting. 2012;**2**:21-28

[7] Moustafa SMN, Abdelzaher HMA. Occurrence of hemolytic fungi mounted on wheat grains in the main silo of Sakaka, Saudi Arabia. Journal of Pure and Applied Microbiology. 2016;**10**(3):1817-1824

[8] Leslie J, Summerll BA. *Fusarium* Laboratory Manual. Blackwell Publishing; 2006

[9] Domsch KH, Gams W, Anderson TH. Compendium of Soil Fungi. London/New York: Academic Press; 2007

[10] Tasca T, Adecarrli G. Hemolytic activity of fresh isolates and clones of *Trichomonas gallinae*. Parasitol. Día. Santiago. 1999;**23**:3-4

[11] Mukherjee A, Rajasekaran C. In-vitro hemolytic activity of *Allium stracheyi* Baker. Journal of Pharmacy Research. 2010;**3**(5):1160-1162

[12] Nawar LS. Prevention and control of fungi contaminated stored Pistachio nuts imported to Saudi Arabia. Saudi Journal of Biological Sciences. 2008;**15**(1):105-112

[13] Joseph F. Dail and Hammar's Pulmonary Pathology (Volume I, Nonneoplastic Lung Disease). 3rd ed. Springer Science+Business Media, LLC; 2008. ISBN: 978-0-387-72139-2

[14] Caloni F, Cortinovis C. Toxicological effects of aflatoxins in horses. Veterinary Journal. 2011;**188**:270-273. DOI: 10.1016/j.tvjl.2010.06.002

[15] Pitt JI, Basílico JC, Abarca ML, López C. Mycotoxins and toxigenic fungi. Medical Mycology. 2000;**38**(Suppl. 1):41-46

[16] Puri A, Ahmad A, Panda BP. Development of an HPTLC-based diagnostic method for invasive aspergillosis. Biomedical Chromatography. 2010;**24**:887-892. DOI: 10.1002/bmc.1382

[17] Sharpe RA, Cocq KL, Nikolaou V, Osborne NJ, Thornton CR. Identifying risk factors for exposure to culturable allergenic moulds in energy efficient homes by using highly specific monoclonal antibodies. Environmental Research. 2016;**144**(Pt. A):32-42

Chapter 7

Quality Assessment of Feed Wheat in Ruminant Diets

Wenzhu Yang and Yizhao Shen

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.75588

Abstract

With adverse growing and harvesting conditions as well as the fluctuation of grain pricing, there have increased supplies of feed wheat used as livestock feed. However, the majority of wheat has been used as feed for poultry and swine, and ruminant producers have been reluctant to use large quantities of wheat because feeding wheat increases the risk of rumen acidosis due to rapid wheat starch digestion in the rumen. To avoid this problem, animal producers often believe that they must limit the amount of wheat in the diet to 50% or less. This chapter summarizes some research findings published in peer reviewed and extension articles on the use of feed wheat in ruminant diets. Substantial variation in physical and chemical composition exists among wheat samples, which are mainly influenced by type of wheat, variety and environmental conditions. Feed values of wheat are largely influenced by its physical properties and nutrient content; however, grain processing as well as its interaction with the physical characteristics is a critical consideration to optimize wheat utilization in ruminant diets. Wheat grain can be fed to animals at higher than typically used in the current livestock industry if proper bunk management and processing are employed.

Keywords: wheat grain, kernel hardness, nutrient content, starch digestion, ruminants, rumen acidosis, digestibility, grain quality

1. Introduction

Wheat is not used traditionally as a feed grain because its milling properties make it desirable for use in breads, pastas and noodles; the milling of wheat produces flour for human use and appreciable quantities of by-products for animal feeds. However, recently, there have been significantly increased supplies of feed wheat used as livestock feed, in particularly in ruminant feeding. The livestock industry is interested in increasing wheat portion in animal

IntechOpen

© 2018 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

rations because of competitively priced wheat compared with other feed grains. Wheat is high in starch and protein, and low in fiber [1]. Similar to other cereal grains, feed wheat is primarily used a source of energy in the form of carbohydrates. Available energy expressed as either digestible energy or metabolizable energy per unit of dry matter is similar to corn, but higher than other major grains [2]. The majority of wheat has traditionally been used as feed for poultry and swine because the use of large quantities of feed wheat in ruminant rations has a number of concerns and problems. The primary problem appears that wheat starch is highly fermentable and its rate of digestion in the rumen is greater than that of corn and barley grains that increase the risk of rumen acidosis in animals fed high-grain diet [1, 3]. Furthermore, the physical characteristics and nutrient content of wheat can vary considerably due to different types of wheat: soft, hard and durum, and the growing conditions [4]. Therefore, nutrient contents of wheat, variety and growing conditions need to be considered when quality of feed wheat is assessed. In addition, kernel processing is another key factor affecting wheat quality. Whole wheat kernels are poorly digested due to the resistance of the seed coat to attack by rumen microbial or host enzymes. Therefore, wheat grains require processing to break seed coat. The digestibility of wheat can be increased from about 60% if fed whole to over 90% if properly processed. However, attention to processing is crucial for best results when feeding wheat since over processing can result in digestive upset and several factors (kernel hardness and uniformity, and processing methods) can significantly affect the processing results. Finally, level of deoxynivalenol in wheat grain may affect feed value. The deoxynivalenol, commonly referred to as vomitoxin, is a mycotoxin that may be produced in wheat and barley grain infected by Fusarium head blight or scab [5]. Ruminants are generally considered to have more tolerance to Fusarium toxins such as deoxynivalenol than poultry and swine because of the detoxifying potential of rumen microbes. However, little information is available on the effects of deoxynivalenol level in wheat on performance and health of ruminant animals. The levels of deoxynivalenol vary with type of wheat and the tolerance to deoxynivalenol also differs depending on type of animals (dairy versus beef cattle) or production levels [5]. The present review will be focusing on assessing physical and chemical characteristics of wheat, kernel processing and levels of deoxynivalenol related to feeding value for ruminant animals based on the published results.

2. Physical and chemical characteristics of feed wheat

Wheat quality is a complex term, and it depends upon the end-use. For feed wheat, the quality should be associated primarily to energy level and protein content as well as its digestibility in the digestive tract of animals. The feed wheat quality can be assessed with its nutrient content such as content of starch or protein, and physical characteristics like thousand kernel weight, test weight, and kernel hardness which are easily measured and commonly used by commercial feedlots and feed mills to assess the quality of wheat as animal feed. Wheat cultivars can be classified by planting season (Winter and Spring), hardness of the grain (soft and hard), and color (red and white). Winter wheats are winter hardy, so they are planted in the fall. In the spring they resume maturation and are harvested early in the summer. Spring wheats are planted in the spring and harvested late in

the summer. Soft wheat varieties have starchy kernels and less gluten which mill easier than the hard wheats, whereas hard wheats have higher protein and gluten levels than the soft wheat with the hardest wheat is durum.

2.1. Physical characteristics

Test weight, also referred to as volume weight or bulk density, is one of the criteria used to assess the quality and grade of cereal grains. Test weight is a measure of density and it is measured volume of grain expressed as kilograms per hectoliter (kg/hL; **Table 1**). The test weight is easily measured and commonly used by feed mills to assess the quality (energy value) of grain as animal feed. Heavy kernel typically has larger plumper kernels with greater starch and lower fiber concentrations than light weight grain, but there is no consistent relationship between test weight of grain and animal growth performance [6]. The test weight is influenced by genetics, agronomic management and environment conditions. Each kernel is composed of the bran (seed coat), the germ, and the endosperm. The endosperm is primarily comprised of carbohydrates (starch) with protein woven among the starch granules. Starch weighs more than protein, thus the tighter the starch molecules are woven within a kernel, the greater the test weight. The test weight in itself is not a good indicator of feeding value to ruminants. Generally, cattle fed wheat with test weights greater than 70 kg/hL will have similar feed efficiency. However, usually only wheat with low test weight (<70 kg/hL) will be used as feed, and its energy values will be lower and feed efficiency is poorer. Wheat with low test weight may be more difficult to properly process compared to wheat with higher test weight. In general, kernel size is more variable with low test weight grains, making processing more difficult. When the roller is set properly for larger kernels, many small kernels will pass through the rollers unprocessed. When set properly for smaller kernels, many larger kernels will be processed too finely.

Kernel hardness, defined as the resistance of the kernel to fracture, is a critical factor affecting grain processing and product quality for feed wheat. The wheat industry has applied this trait for decades to differentiate grain quality and market classes. Hard wheat kernels require more force to fracture than do soft wheat grains, which is caused by differences in the endosperm starch-protein matrix [7]. Kernel hardness can be measured as a hardness index using a Single Kernel Characterization System [8], milling energy using a Comparamill [9] or by particle size analysis [10]. The particle size analysis is the measurement of particle size after feed processing, and is a commonly used method to evaluate end-use quality [11]. Soft wheat fractures easily with small particle size and limited starch damage, while hard wheat produces larger particles with increased starch damage. Kernel hardness is closely related to

Item	Mean	STD	Min	Max
1000 Kernel weight, g	36.4	2.9	32.7	39.1
Test weight, kg/hL	81.6	2.4	78.1	84.3
Kernel hardness	18.4	12.3	0	35.5

Table 1. Variation with variety of physical characteristics of wheat; kernel hardness was estimated by particle size index, lower number indicates harder kernel.

the wheat process affecting the starch damage, particle size and process quality. The grain hardness is therefore one of the important distinguishing factors in the wheat evaluation for grain quality and plays an important role with regard to the suitability of processing. Kernel hardness is measured on a scale from 0 to 35 with durum variety being the hardest (0) and soft white spring the softest (35) (**Table 1**). The increased kernel hardness is generally associated with a decrease in the rate of starch digestion, likely because the protein protects starch granules from microbial digestion. Kernel hardness could be a particularly important property as hard kernels may be more susceptible to shattering and generating the fine particles that are often associated with rumen acidosis [12] and bloat in ruminants fed high-grain diet [13]. It has been found that ruminant performance is significantly influenced by particle size of the feed consumed, and a negative relationship between feed particle size and rumen dry matter digestibility of grains was reported [14].

2.2. Kernel structure and chemical composition

The major structures of the wheat kernel include the pericarp (seed coat), the endosperm and the germ. The pericarp covers and protects the endosperm, which is composed of starch granules embedded in a protein matrix (about 80% of dry weight). Starch granules in the endosperm vary in size, shape and molecular structure depending on the variety and the environmental conditions of cultivation. All starches are made up of two types of glucose polymers: amylose and amylopectin. Amylose is a linear polymer with α-(1,4) glycosidic linkages and amylopectin is a branched polymer with both α-(1,4) and α-(1,6) linkages. Cereal starches are typically composed of approximately 25% amylose and 75% amylopectin. Starch digestibility in the digestive tract of animals can vary with relative proportion of amylose and amylopectin starch since the digestion of amylose is slower due to lesser accessibility of digestive enzyme compared with amylopectin.

Corn and barley are two mostly used feed grains worldwide in livestock animal rations. Wheat is in general higher in protein (15.4%) than corn (9.7%) and barley (12.9%) and has starch content intermediate (70%) between corn (76%) and barley (58%) as well as lower in fiber than barley (**Table 2**). As a result, wheat has a total digestible nutrient and net energy for gain content that is comparable to corn but higher than that of barley grain [2]. However, owing to the number of different types of wheat, soft, hard and durum, the nutrient content of wheat can vary considerably. For example, on a dry matter basis, the starch content of wheat can range from 62 to 75%, protein from 9 to 19% and neutral detergent fiber from 10 to 18% (**Table 3**). This variation was most pronounced in protein content which presumably reflects the interaction between proteins and starch granules in the endosperm of wheat [15]. The high protein content of wheat may offer advantages in meeting the protein requirements of growing animals, whereas the low fiber content may contribute to its increased propensity to cause rumen acidosis. Because it exist substantial variation in the chemical composition of wheat, there is an interest in developing predictive tools to relate chemical composition to nutritional quality and animal performance [16]. Seifried et al. [17] observed negative correlation of protein content of wheat with ruminal protein degradability ($r = -0.51$; $P < 0.05$) and negative or positive correlation with some amino acid content. Furthermore, new developments and research in near infrared reflectance spectroscopy may allow accurate and rapid assessment of feed quality characteristics related to utilization and animal performance [18].

Item	Wheat	Barley	Corn	Oats
Organic matter	98.0	97.8	98.5	97.7
Crude protein	15.4	12.9	9.7	12.8
Neutral detergent fiber	13.3	20.5	9.3	24.0
Acid detergent fiber	3.2	6.8	3.3	16.5
Starch	70.3	58.3	75.7	58.1

Table 2. Nutrient contents (% of dry matter) of cereal grains.

Item	Mean	STD	Min	Max
Organic matter, %	98.5	0.15	97.1	98.7
Crude protein, %	12.6	2.9	9.3	19.1
Neutral detergent fiber, %	13.3	1.8	8.6	17.9
Acid detergent fiber, %	3.6	0.3	3.3	5.2
Ether extract, %	1.7	0.2	1.2	3.2
Starch, %	69.1	4.7	62.5	75.6

Table 3. Variation with variety of chemical and physical characteristics of wheat.

3. Biological characteristics

Biological characteristics are here referred to as their digestion characteristics in the digestive tract, especially in the rumen. The quality of feed wheat is not only depending on its physical and chemical characteristics, but also depending on its biological characteristics, i.e., its rate of digestion in the rumen and potential digestibility in the total digestive tract. Among cereal grains, wheat has the most rapid rate of starch digestion in the rumen (**Figure 1**), with a rate that is almost twice that of barley and almost four times that of corn, if the grains are processed similarly. Rapid starch digestion in the rumen increases the production rate of fermentation acids, primarily the volatile fatty acids and if these accumulate, subclinical or clinical ruminal acidosis can occur. However, as with other cereal grains, whole wheat kernels are poorly digested owing to the resistance of the seed coat to attack by rumen microorganisms. Low fiber levels and a rapid rate of starch digestion make wheat more difficult to feed than most other cereal grains. The digestion rates of wheat starch vary with both inherent of kernel nature and the kernel processing including processing method used and extent of processing.

The rates of wheat digestion in the rumen are commonly measured either using in vitro or in situ technique [19]. In vitro methodologies that simulate animal digestive tract conditions become vital in developing feed additive products and screening large number of feed samples at the same time. Batch culture is the one most commonly used in vitro techniques in evaluating grain digestion in the rumen [20]. The grains that are tested in batch culture need to be ground or rolled and incubated in fermentation media containing buffer and

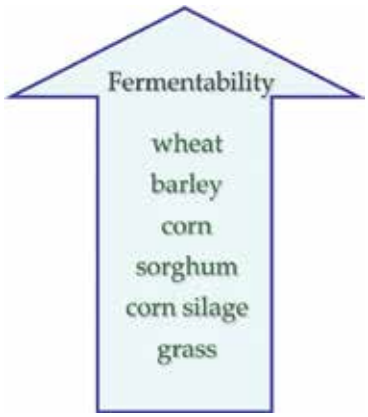


Figure 1. Rumen fermentability of feeds that are commonly fed to ruminants, the highest is wheat grain and lowest is straw.

rumen inoculum at 39°C under anaerobic condition over a period of up to 48 h of incubation. The data that are generated can be used to determine the kinetic parameters of fermentation including the rate and extent of the grain digestion. The in situ techniques have been extensively used for measuring rumen digestion of feeds as well. The dynamic interactions within the rumen are difficult to simulate in vitro, and thus the in situ techniques study digestion within the rumen itself and reduce the need for ruminal simulation. Current nutrition models need for quantitative information on rates and extents of feed digestion in the rumen. The in situ rumen digestion kinetics of grains are measured by filling processed grain in bags and incubated in the rumen via rumen cannula for a period of series times, thus rumen cannulated animals are required.

The rates of wheat dry matter or starch digestion in the rumen varied with type of wheat. McAllister and Sultana [19] measured in situ rumen digestion kinetics of three different wheats (i.e., soft, hard and very hard) with same degree of processing (**Table 4**). These authors found that the rates of dry matter digestion were lowest for durum (4.1%/h) and highest for soft wheat. The digestion rates of wheat in the rumen appeared to be associated with kernel hardness because the kernel hardness can reflect to relative affinity between protein and starch in the endosperm [15]. It suggests that the nature of endosperm protein may influence rumen digestion of wheat. However, Lanzas et al. [21] reported the variation in the fermentation kinetics among wheat samples from various sources, did not attribute to any chemical parameters. Whereas, the study by Lanzas et al. [21] focused mainly on the impact of kernel processing that may confound with chemical effects on the fermentation dynamics of wheat. In fact, it was reported that protein content of wheat was most highly correlated with the rate of wheat digestion ($r^2 = -0.77$) in the study by McAllister and Sultana [19]. It suggests protein characteristics as a factor that influences the digestive properties of wheat in the rumen. This phenomenon could be explained by the nature of wheat protein. Wheat grain has two major proteins, puroindolines A and B that are associated with the fibrillin protein complex on the surface of wheat starch granules which may have a central role in determining the digestion rate of wheat starch [22].

	Kinetics parameters			
Item	**a**	**b**	**c**	**ED, %**
Dry matter				
Durum	0.123a	0.756	0.041	43.0b
Red spring	0.121a	0.736	0.044	43.2b
Soft red winter	0.097b	0.720	0.062	46.3a
Protein				
Durum	0.081a	0.857	0.035	39.7
Red spring	0.027b	0.837	0.051	41.2
Soft red winter	0.008c	0.868	0.053	41.5
Starch				
Durum	0.173b	0.783	0.039b	48.2b
Red spring	0.243a	0.738	0.043b	55.1a
Soft red winter	0.104c	0.744	0.060a	47.6b

Kinetics of nutrient digestions were estimated using the model: $y = a + b(1 - e^{-c(t-lag)})$, a = soluble fraction; b = slowly digestible fraction; c = fractional digestion rate constant at which b is digested; lag = lag time (h), and t = time of incubation (h). Effective degradability (ED) = a + bc/(c + k), where k is the ruminal flow rate assuming 0.06/h. a,b,c means within a column and within a nutrient, with different letters differ ($P \leq 0.05$).

Table 4. Variation with variety of in situ rumen digestion kinetics of wheat.

Rumen digestion kinetics of wheat grain also varies with wheat genotypes. Seifried et al. [17] measured in situ rumen digestion kinetics of over 20 wheat samples varying with genotypes and found considerable variation in digestion kinetics of dry matter, protein and starch among wheat genotypes. The digestion kinetics parameters include soluble fraction which is immediately digestible, potential digestible fraction and rate of digestion. The soluble fraction ranged from 21 to 40% for dry matter, 11 to 22% for protein and 25 to 49% for starch; the potential digestible fraction varied between 53 to 71% for dry matter, 51 to 74% for starch and 75 to 89% for protein; and the digestion rate ranged from 29 to 54%/h for dry matter, from 18 to 27%/h for protein and from 38 to 99%/h for starch [17]. The differences in digestion kinetics among genotypes were explained by the variation in the endosperm characteristics [22]. Therefore, these authors concluded that selection of wheat grains with slower digested wheat can be used to shift starch digestion from the rumen to the small intestine. The potential to shift more starch digestion from the rumen to the small intestine by developing lower ruminal digested wheat will be beneficial to reduce risks of rumen acidosis and improve energy efficiency, in particularly for ruminants fed high-grain rations. It is known better energy efficiency with starch digested in the small intestine than in the rumen [23, 24]. However, although improving wheat as a feed grain by selection of slower rate of digestion in the rumen is a wise consideration for wheat breeder, it may be challenging as all types of wheat (soft, hard and durum) examined in the study of McAllister and Sultana [19] exhibited rapid digestion rates than that of corn.

4. Processing wheat

Processing of cereal grains either by grinding, rolling, tempering (i.e., addition of water prior to rolling), steam-rolling (i.e., exposure to steam prior to rolling) or steam flaking (i.e., longer duration of exposure and higher grain temperature) breaks down barriers such as the hull, pericarp and protein matrix and allows microbes access to the starch harbored within endosperm. Furthermore, these processes reduce the particle size of the grain, increasing the surface area available for microbial attachment that these actions increase the rate and extent of starch digestion [25]. Although wheat has the most rapid rate of starch digestion in the rumen, whole wheat kernels are poorly digested in the rumen, and thus need to be properly processed prior to being fed to animals. In fact, excessive processing of wheat results in fine particle sizes that can cause digestive upsets (rumen acidosis, bloat) that in themselves reduce the profitability of animal production. Conversely, under processing of wheat can result in whole kernels in the diet which are not digested by rumen microorganisms contributing to a loss of valuable starch in the manure.

4.1. Definition of degree of processing

Maintaining an optimum degree of processing that maximizes the utilization of wheat grain, and while ensuring animal health is challenging and critical to the livestock industry. The quality of the processed wheat and its particle size can be affected by kernel uniformity, test weight, kernel plumpness, and wheat variety. Kernel uniformity is a major concern for the efficiency of rolling as grain kernels vary in size and shape, making it impossible to achieve optimal processing for all kernels with a single roller setting.

There is no standardized measurement that has been established to assess the degree of grain processing [4]. Coarse, medium and fine are descriptors commonly used in research reports, but these terms are relative, and specific only to the treatments within a given study [26]. As a consequence, medium-processed grain referred to in one study may actually be equivalent to coarsely processed grain in another study. The need for a quantitative measurement of grain processing is evident. In the feed industry, the degree of grain processing has been described using a processing index, which refers to the volume weight (g/L) of grain after processing expressed as the percentage of its volume weight before processing [26]. This index reflects the fact that the more extensively wheat is processed, the finer the particle will be, hence, the lower the volume weight will be, and consequently, the lower the processing index. However, this processing index is influenced by the processing method used. The values generated with dry-rolling can differ substantially, depending on the hardness of wheat kernels, whereas, temper- or steam-rolling make fractured particles that are more likely to adhere together contributing to reduced fines.

4.2. Grinding versus dry-rolling processing

The rate and extent of dry matter digestion varies among wheat sources and with the extent of processing, but seldom have both of these properties been studied in the same experiment. A batch culture study was conducted to assess the effects of wheat grain source and processing

method on dry matter and protein digestibility. Eight wheat samples collected from various sources were either ground through 2-mm sieve or dry-rolled to have processing index of 80%, and incubated for 24 h in batch culture. Dry matter digestibility ranged from 60 to 68% and from 28 to 38%, respectively, for ground and rolled wheat (**Figure 2**). The digestibility of protein varied from 52 to 62% or from 25 to 48%, respectively, for ground and rolled wheat. There was no interaction between wheat source and degree of processing. As expected, the digestibility of dry matter and protein was greater ($P < 0.01$) for ground wheat (64 and 56%) than for rolled wheat (34 and 38%) after 24 h of incubation. In vitro digestibility of dry matter and protein linearly ($P < 0.01$) increased with increasing incubation time and consistently higher ($P < 0.01$) with ground than rolled wheat, whereas no interaction between processing and incubation time was noticed (**Figure 3**). These results showed evident impact of processing method on the extent of wheat digestion in the rumen. The study also demonstrated the variation in the digestive value of commercially available wheat grain and emphasized the need to have an accurate and rapid means of quality assessment at the point of sale.

4.3. Micronization processing

Micronization is a dry-heat process that generates infrared electromagnetic short waves to heat the feedstuff to approximately 110–115°C. It has been used to process grains to increase their utilization [27]. Rapid internal heating is accompanied by a rise in water vapor pressure that the feedstuff is cooked from the inside out and the kernel expands to the point of eversion. This process has been widely used to process grains for livestock consumption [28]. Wang et al. [29] reported that the micronization reduced the in situ dry matter digestibility of both full-fat canola seed and flaxseed. McAllister and Sultana [19] compared three different wheats varying with kernel hardness (i.e., soft, hard and durum) and found that in situ digestibility of dry matter, crude protein and starch were reduced by micronization processing in all three types of wheat. However, the reductions were greater with soft than

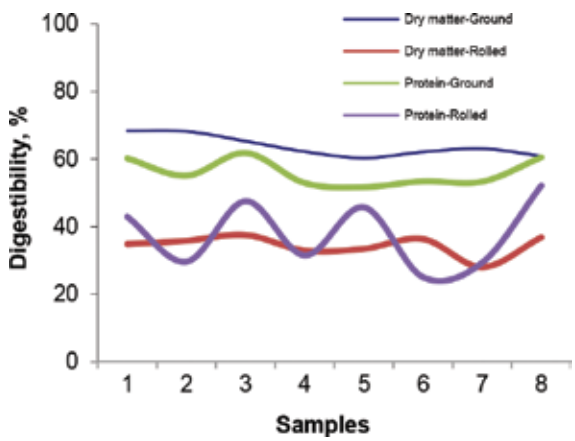


Figure 2. Effects of processing and wheat source on in vitro rumen dry matter and protein digestibility. For dry matter digestibility, SEM = 2.9%, processing ($P < 0.01$), wheat ($P < 0.20$) and process x wheat ($P < 0.45$). For crude protein digestibility, SEM = 4.7%, processing ($P < 0.01$), wheat ($P < 0.01$) and process x wheat ($P < 0.19$).

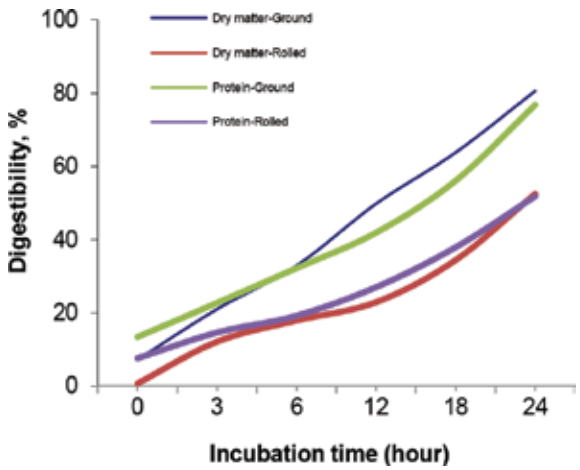


Figure 3. Effects of processing and incubation time on in vitro rumen dry matter and protein digestibility. For dry matter digestibility, SEM = 1.1%, processing ($P < 0.01$), incubation time ($P < 0.01$) and process x incubation time ($P < 0.68$). For crude protein digestibility, SEM = 2.3%, processing ($P < 0.01$), wheat ($P < 0.01$) and process x wheat ($P < 0.79$).

hard wheat varieties. These authors suggested that the micronization altered the properties of the endosperm in soft wheat that may be more closely resembles that of the harder wheat. The micronization may also change wheat starch that may be related to the nature of the endosperm with alterations of proteins within the fibrillin complex [30]. The reduction of rate and extent of wheat starch digestion using micronization method may provide an effective processing technique to modulate the rate of acid production during the fermentation of wheat in the rumen, thus reduce the severity of rumen acidosis.

4.4. Impact of degree of processing on the feed value of wheat

Recently, a series of experiments using beef cattle were conducted in our laboratory at the Lethbridge Research and Development Centre to determine the maximum level of wheat grain that could be included in finishing cattle rations, the effects of degree of grain processing on wheat utilization and comparison the feeding value between hard versus soft wheat. A study was conducted to compare inclusion of 90% wheat grain processed to processing index of either 75 or 85% on the growth performance of finishing beef cattle [31]. Compared to steers fed dry-rolled wheat with a processing index of 75%, steers fed wheat with a processing index of 85% ate 0.4 kg per day more feed. However, this difference in feed intake did not alter the daily gain or final live weight of steers. As a result, the feed efficiency, expressed as daily per unit of feed consumption, of steers fed wheat with a processing index of 85% was lower than for steers fed wheat with a processing index of 75%. Carcass traits had a trend to be different with higher back fat thickness but lower rib eye area and lower quality grade (% Canada AAA) for steers fed wheat with a processing index of 85% than with a processing index of 75%. The steers fed wheat with a processing index of 85% also had more numbers of liver abscesses. Therefore, a high processing index (85%, i.e., coarsely processed) increased feed consumption but reduced feed efficiency and adversely impacted carcass quality, including saleable meat

yield, back fat thickness, and rib eye area. The greater feed intake but lower feed efficiency for steers fed with coarsely processed wheat may be resulted from an increased an amount of unprocessed whole kernel, in particularly when the uniformity of kernel size is poor and rollers are set to roll the large kernels [32]. The unprocessed wheat kernel is often poorly digested in the digestive tract of cattle because of the seed coat protection from microbial and host enzyme access and its faster passage through the digestive tract as well. Consequently, the digestibility of coarsely processed wheat would be lower and thus animals need to increase feed intake to meet their nutrient requirement. These results appeared to contrast to the general recommendation that wheat should be coarsely processed with processing index of 80 to 85%. The increased feed intake by steers fed wheat with 85% of processing index would contribute to the adverse effects on feed efficiency and risks of liver abscesses. The optimum processing index may depend on starch content of the wheat as well [16].

4.5. Level of wheat in the diet

Wheat grain is generally recommended to be fed to ruminants in combination with more fibrous or slowly fermented feed grains and limited to 40 or 50% of the diet (dry matter basis) because of its rapidly fermentable starch in the rumen. A study using rumen cannulated beef heifers was conducted to compare inclusion level of wheat relative to barley grain in finishing beef cattle rations on measuring rumen pH and fermentation, and digestibility if nutrients in the total digestive tract [33]. In this study wheat was substituted for barley grain at 0, 30, 60, or 90% of the diet dry matter with the remainder of the diet composed of 6% barley silage and 4% vitamin and mineral supplement. All grains were dry-rolled to a processing index of 80%. Increasing wheat level from 30, 60 to 90% in the diet linearly increased the duration of time that rumen pH was under 5.8, but ruminal pH below 5.5 and 5.2 were not affected. These results indicated that subclinical rumen acidosis was not exacerbated with increase of wheat grain up to 90%. Rumen acidosis includes acute acidosis and subacute acidosis (also called subclinical rumen acidosis). The acute acidosis is characterized by sustained low pH (<5.2) without recovery unless intervention is used [12]. The subacute rumen acidosis occurs in repeated bouts where pH is <5.6 for >3 h per day [34], but unlike the situation for acute acidosis, the pH recovers between bouts. The subacute rumen acidosis is a common metabolic disorder in animals fed high-grain diet with rapid fermentation of feed in the rumen and subsequent accumulation of volatile fatty acids (acetate, propionate, butyrate), whereas acute acidosis is caused by accumulation of lactic acid in the rumen and much less happen. Feed intake, animal performance and feed efficiency are adversely impacted when animals suffer from subacute rumen acidosis [12]. However, there was no effect of feeding increasing levels of wheat on rumen fermentation and nutrient digestibility, which suggest that the levels of wheat included in finishing diets of beef cattle could be higher than typically used in the feedlot industry if proper bunk management and processing are employed.

4.6. Impact of wheat type on feed value

Grain hardness is a trait that has been used for decades by the wheat industry to differentiate quality and market classes, and it is characterized as the resistance of the kernel to fracture [35].

The differences in kernel hardness are the result of differences in affinity of starch and protein within endosperm, higher affinity decreases both the rate and extent of starch digestion in the rumen [36]. Although the endosperm within different wheat types differs in hardness, all wheat types are digested rapidly in the rumen. As a result, the information on the rate and extent to which hardness influences the site and extent of starch digestion in wheat is scarce. Soft wheat generally exhibits a faster rate of digestion than hard wheat in the rumen [3, 19]. However, this relationship is also dependent on the processing method used or the degree of wheat processing [31]. The hard wheat kernel may be more susceptible to shattering and generating the fine particles that are readily fermentable in the rumen [37]. Swan et al. [22] reported that starch granules from soft wheat appeared even more resistant to rumen digestion than the starch granules from hard wheat because of greater damage to the surface of starch granules in hard wheat after cracking using a mill. Recently, we conducted a study using rumen cannulated beef heifers fed either soft or hard wheat-based rations [3], there were no differences in the rumen pH and rumen acid concentrations between beef heifers fed soft or hard wheat. The lack of differences between soft and hard wheats can be explained by the fact that wheat grain was processed coarsely (i.e., processing index >80%) to avoid digestive upsets. Similarly, a feedlot study using beef steers that were fed soft or hard wheat with the similar wheat processing as did in the study by Yang et al. [3], did not show the differences in feed intake (averaged 11.3 kg dry matter/day), daily weight gain (1.79 kg), feed efficiency (160 g weight gain/kg dry matter intake), and net energy for growth [38]. It concluded that soft and hard wheat exhibited the similar feed value for feeding feedlot beef cattle if the ration is formulated with the same energy level and wheat is processed at the same degree of processing.

5. Deoxynivalenol content in wheat

Deoxynivalenol, commonly referred to as vomitoxin, is a mycotoxin that may be produced in wheat and barley grain infected by Fusarium head blight or scab [39]. The fusarium head blight may infect grain heads when wet weather occurs during the flowering and grain filling stages of plant development. Although the occurrence of fusarium head blight is not necessary to mean that deoxynivalenol is present, a high level of scabby kernels in the harvested grain means deoxynivalenol will likely be present. Levels of deoxynivalenol do not necessarily correlate with levels of physical damage in grain. The impact of deoxynivalenol in feed grains on animal performance and health vary with type of livestock animals. The evident production losses were observed in non-ruminant animals, in particularly swine when vomitoxin-infested grains were fed [40, 41]. Research conducted with vomitoxin-infested barley indicates no apparent problems when fed to growing and finishing cattle or gestating or lactating beef cows [42]. It appears that cattle can tolerate high levels of vomitoxin (21 mg deoxynivalenol/kg wheat) without impacts on performance or health of the cattle [43]. However, exercise caution with wheat or any grain that has gone out of condition or has mold damage. The possibility exists that molds and toxins will impact feeding value through reduced feed acceptance, intake and performance, as well as higher incidence of morbidity, the possibility of abortion in pregnant cattle and, in some cases, even death. Young calves, gestating cows and animals under nutritional stress are most vulnerable [43].

The deoxynivalenol can be measured using several laboratory procedures. The most common method used by the Federal Grain Inspection Service and most grain handling and processing facilities is the immunological-antibody method called Enzyme Linked-Immunosorbent Assay (ELISA) because it is relatively fast and cheap. The gas chromatography-electron capture (GC-EC) analytical method is quantitative and used to calibrate ELISA test kits.

The inability to feed wheat with high levels of deoxynivalenol to be fed to swine and poultry contributes to the lower price of wheat, but the impacts of deoxynivalenol on the feed value of wheat for beef cattle are largely unknown. According to [5], the level of deoxynivalenol in North American wheat ranges from 0.3 to 1.0 mg/kg, however the level of deoxynivalenol measured in specific lots can reach levels of up to 20 mg/kg. The highest deoxynivalenol levels are also usually associated with soft rather than hard wheat [5]. The maximum tolerated deoxynivalenol level by Canadian Food Inspection Agent in diets for swine, young calves, and lactating dairy animals is 1 mg/kg, and 5 mg/kg in diets for cattle and poultry. Ruminants are considered quite resistant to Fusarium toxins such as deoxynivalenol because of the detoxifying potential of rumen microbes. Previous studies have shown that the epoxide group-bearing parent toxin deoxynivalenol is metabolized to de-epoxy-deoxynivalenol [44]. However, little is known about the effects of Fusarium toxins (i.e., deoxynivalenol, fumonisins, trichothecenes, zearalenone) or their metabolites on the activity of rumen microbes and the consequent effect on feed efficiency in ruminant animals.

6. Sprouted, frosted and drought-damaged wheat

Wheat can be priced competitively with other feed grains because of damage from disease, drought, or sprouting. Wet conditions during fall harvesting can cause widespread sprout damage to the grain crop. Physical and chemical characteristics could be different between sprouted grains and non-sprouted grains such as lower text weight and starch content but higher crude protein due to the concentration effect that occurs when starch is expended during the germination process. However, it has been reported that animal performance is similar when consuming sprout-damaged grain versus non-sprouted wheat grain. Rule et al. [45] reported no differences in growth performance or carcass characteristics when comparing sprouted wheat with non-sprouted wheat in finishing rations containing 77% wheat-based concentrate. Reed et al. [46] concluded that sprouted wheat is palatable, digestible sources of nutrients that can be used in beef cattle diets. These authors further indicated that the sprouted wheat should be processed similar to non-sprouted wheat for optimal utilization by the animal. Growth performance and feed efficiency were improved for steers fed diets containing rolled sprouted wheat compared with whole sprouted wheat [46].

Little data is available regarding the feeding value of frosted wheat. However, research conducted in Western Canada with frosted wheat indicates no difference in feeding value of frosted grain, compared with non-frosted grain when it was fed in feedlot rations. Drought-damaged wheat generally has smaller kernels and is lower in starch content than wheat grown without drought stress. Nitrate toxicity should not be a concern with wheat grain. Wheat does not transfer nitrate into the seed during drought stress.

7. Conclusion

Feed values of wheat used in ruminant animal rations can vary substantially, depending on types of wheat, physical, chemical and biological characteristics, kernel processing, level of wheat in rations, kernel uniformity, kernel damage, contamination of deoxynivalenol, etc. Although wheat grain is high in starch and protein, its rapid starch fermentation in the rumen is a great concern on developing rumen acidosis when high proportion of wheat is included in the ruminant diets. Kernel processing including the selection of processing method and manipulation of degree of processing is critical to optimize the wheat utilization in ruminant diets. Type of wheat (soft and hard) and physical characteristics (kernel hardness, kernel uniformity) could interact with quality of processing, thus impact on feed values of wheat. This information will be useful to wheat breeder to develop suitable variety to improve feed value when wheat that fails quality grade for milling is used as livestock feed. Although the limited information is available, the adverse impact on animal growth performance and health is not apparent for feeding cattle with deoxynivalenol contaminated wheat, sprouted wheat or damaged kernels due to frosting and drought stress.

Author details

Wenzhu Yang[1]* and Yizhao Shen[2]

*Address all correspondence to: wenzhu.yang@agr.gc.ca

1 Agriculture and Agri-Food Canada, Lethbridge Research and Development Centre, Lethbridge, Alberta, Canada

2 Laboratory of Metabolic Manipulation of Herbivorous Animal Nutrition, College of Animal Science and Technology, Yangzhou University, Yangzhou, Jiangsu, P.R. China

References

[1] Herrera-Saldana RE, Huber JT, Poore MH. Dry matter, crude protein, and starch degradability of five cereal grains. Journal of Dairy Science. 1990;**73**:2386-2393. DOI: 10.3168/jds.S0022-0302(90)78922-9

[2] National Academies of Sciences, Engineering, and Medicine (NASEM). Nutrient Requirements of Beef Cattle. 8th ed. Washington: The National Academies Press; 2016. 494 p

[3] Yang WZ, Xu L, Zhao YL, Chen LY, McAllister TA. Impact of hard vs. soft wheat and monensin level on rumen acidosis in feedlot heifers. Journal of Animal Science. 2014; **92**:5088-5098. DOI: 10.2527/jas.2014-8092

[4] McAllister TA, Yang WZ, Oba M. Matching processing with grain size. In: Proceedings of the Western Nutrition Conference; Edmonton, Alberta. September 14-15, 2011. pp. 149-163

[5] Bianchini A, Horsley R, Jack MM, Kobielush B, Ryu D, Tittlemier S, Wilson WW, Abbas HK, Abel S, Harrison G, Miller JD, Shier WT, Weaver G. DON occurrence in grains: A North American perspectives. Cereal Food World. 2015;**60**:32-56. DOI: 10.1094/CFW-60-1-0032

[6] Hunt CW. Factors affecting the feeding quality of barley for ruminants. Animal Feed Science and Technology. 1996;**46**:41-51. DOI: 10.1016/S0377-8401(96)01004-8

[7] Chandra R, Singh SP, Gupta K. Damping studies in fiber-reinforced composites–a review. Composite Structures. 1999;**46**:41-51. DOI: 10.1016/S0263-8223(99)00041-0

[8] Martin CR, Rousser R, Brabec DL. Development of a single kernel wheat characterization system. Transactions of the ASAE. 1993;**36**:1399-1404. DOI: 10.13031/2013.28477

[9] Allison MJ, Cowe IA, Borzucki R, Bruce F, McHale R. Milling energy of barley. Journal of the Institute of Brewing. 1979;**85**:262-264. DOI: 10.1002/j.2050-0416.1979.tb03919.x

[10] American Assoc. of Cereal Chemists (AOAC). Approved Methods of the American Association of Cereal Chemists. St. Paul: American Assoc. of Cereal Chemists; 1983. Method 39-70A

[11] Morris CF, Rose SP. Wheat. In: Henry RJ, Kettlewell PS, editors. Cereal Grain Quality. London: Chapman & Hall; 1996. pp. 3-54. DOI: 10.1007/978-94-009-1513-8_1

[12] Owens FN, Secrist DS, Hill WJ, Gill DR. Acidosis in cattle: A review. Journal of Animal Science. 1998;**76**:275-286. DOI: 10.2527/1998.761275x

[13] Cheng KJ, McAllister TA, Popp JD, Hristov AN, Mir Z, Shin HT. A review of bloat in feedlot cattle. Journal of Animal Science. 1998;**76**:299-308. DOI: 10.2527/1998.761299x

[14] Galyean ML, Wagner DG, Owens FN. Dry matter and starch disappearance of corn and sorghum as influenced by particle size and processing. Journal of Dairy Science. 1981;**64**:1804-1812. DOI: 10.3168/jds.S0022-0302(81)82769-5

[15] Barlow KK, Buttrose MS, Simmonds DH, Vesk M. The nature of the starch-protein in wheat endosperm. Cereal Chemistry. 1973;**50**:443-454

[16] Koenig KM, Beauchemin KA, Yang WZ. Processing feed grains: Factors affecting the effectiveness of grain processing for beef and dairy cattle production. In: Western Nutrition Conference; Saskatoon. September 24-26, 2013; pp. 62-73

[17] Seifried N, Steingass H, Hoffmann N, Rodehutscord M. In situ starch and crude protein degradation in the rumen and in vitro gas production kinetics of wheat genotypes. Journal of Animal Physiology and Animal Nutrition. 2017;**101**:779-790. DOI: 10.1111/jpn.12529

[18] Jancewicz LJ, Swift ML, Penner GB, Beauchemin KA, Koenig KM, Chibisa GE, He ML, McKinnon JJ, Yang WZ, McAllister TA. Development of NIRS calibrations to estimate fecal composition and nutrient digestibility in beef cattle. Canadian Journal of Animal Science. 2017;**97**:51-64. DOI: 10.1139/cjas-2016-0107

[19] McAllister TA, Sultana H. Effects of micronization on the in situ and in vitro digestion of cereal grains. Asian-Australasian Journal of Animal Sciences. 2011;**24**:929-939. DOI: 10.5713/ajas.2011.10387

[20] Yang WZ, Oba M, Swift ML, McAllister TA. Short communication: Variation in response to processing, in vitro gas production and fermentation of western Canadian feed barley. Canadian Journal of Animal Science. 2014;**94**:725-729. DOI: 10.1139/CJAS-2014-053

[21] Lanzas C, Fox DG, Pell AN. Digestion kinetics of dried cereal grains. Animal Feed Science and Technology. 2007;**136**:265-280. DOI: 10.1016/j.anifeedsci.2006.09.004

[22] Swan CG, Bowman JG, Martin JM, Giroux MJ. Increased puroindoline levels slow ruminal digestion of wheat (*Triticum aestivum* L.) starch by cattle. Journal of Animal Science. 2006;**84**:641-650. DOI: 10.2527/2006.843641x

[23] McLeod KR, Baldwin RL, Harmon DL, Richards CJ, Rumpler WV. Influence of ruminal and postruminal starch infusion on energy balance in growing steers. In: Chwalibog A, Jakobsen K, editors. Energy Metabolism in Animals. Wageningen, Wageningen Pers; 2001. pp. 385-388

[24] Huntington GB, Harmon DL, Richards DJ. Sites, rates, and limits of starch digestion and glucose metabolism in growing cattle. Journal of Animal Science. 2006;**84**:E14-E24. DOI: 10.2527/2006.8413_supplE14x

[25] McAllister TA, Bae HD, Jones GA, Cheng KJ. Microbial attachment and feed digestion in the rumen. Journal of Animal Science. 1994;**72**:3004-3018. DOI: 10.2527/1994.72113004x

[26] Yang WZ, Beauchemin KA, Rode LM. Effects of barley grain processing on extent of digestion and milk production of lactating cows. Journal of Dairy Science. 2000;**83**:554-568. DOI: 10.3168/jds.S0022-0302(00)74915-0

[27] Mercier C. Effects of various U.S. grain processes on the alteration and in vitro digestibility of starch granule. Feedstuffs. 1971;**43**:33-47

[28] Zollitsch W, Wetscherek W, Lettner F. Use of differently processed full fat soybeans in a diet for pig fattening. Animal Feed Science and Technology. 1993;**41**:237-246. DOI: 10.1016/0377-8401(93)90016-D

[29] Wang Y, McAllister TA, Zobell DR, Pickard MD, Road LM, Mir Z, Cheng KJ. The effect of micronization of full-fat canola seed on digestion in the rumen and total tract of dairy cows. Canadian Journal of Animal Science. 1997;**77**:431-440. DOI: 10.4141/A96-113

[30] White GA, Doucet FJ, Hill SE, Wiseman J. Physicochemical changes to starch granules during micronisation and extrusion processing of wheat, and their implications for starch digestibility in the newly weaned piglet. Animal. 2008;**2**:1312-1323. DOI: 10.1017/S1751731108002553

[31] Moya D, He ML, Jin L, Wang Y, Penner GB, Schwartzkopf-Genswein KS, McAllister TA. Effect of grain type and processing index on growth performance, carcass quality, feeding behavior, and stress response of feedlot steers. Journal of Animal Science. 2015;**93**:3091-3100. DOI: 10.2527/jas.2014-8680

[32] Zhao YL, Yan SM, He ZX, Anele UY, Swift ML, McAllister TA, Yang WZ. Effects of volume weight, processing method and processing index of barley grain on in situ digestibility of dry matter and starch in beef heifers. Animal Feed Science and Technology. 2015;**199**:93-103. DOI: 10.1016/j.anifeedsci.2014.11.005

[33] He ML, Long J, Wang Y, Penner G, McAllister TA. Effect of replacing barley with wheat grain in finishing feedlot diets on nutrient digestibility, rumen fermentation, bacterial communities and plasma metabolites in beef steers. Livestock Science. 2015;**176**:104-110. DOI: 10.1016/j.livsci.2015.03.024

[34] Plaizier JC, Krause DO, Gozho GN, McBride BW. Subacute ruminal acidosis in dairy cows: The physiological causes, incidence and consequences. The Veterinary Journal. 2008;**176**:21-31. DOI: 10.1016/j.tvjl.2007.12.016

[35] Anjum FM, Walker CE. Review on the significance of starch and protein to wheat kernel hardness. Journal of the Science of Food and Agriculture. 1991;**56**:1-13. DOI: 10.1002/jsfa.2740560102

[36] McAllister TA, Rode LM, Major DJ, Cheng KJ, Buchanan-Smith JG. Effect of ruminal microbial colonization on cereal, grain digestion. Canadian Journal of Animal Science. 1990;**70**:571-579. DOI: 10.4141/cjas90-069

[37] Varner LW, Woods W. Influence of wheat variety upon in vitro and in vivo lactate levels. Journal of Animal Science. 1975;**41**:900-905. DOI: 10.2527/jas1975.413900x

[38] Xu L, He ML, Liang RF, McAllister TA, Yang WZ. Effects of grain source and monensin level on growth performance, carcass traits and fatty acid profile in feedlot beef steers. Animal Feed Science and Technology. 2014;**198**:141-150. DOI: 10.1016/j.anifeedsci.2014.10.015

[39] Streit E, Naehrer K, Rodrigues I, Schatzmay G. Mycotoxin occurrence in feed and feed raw materials worldwide: Long-term analysis with special focus on Europe and Asia. Journal of the Science of Food and Agriculture. 2013;**93**:2892-2899. DOI: 10.1002/jsfa.6225

[40] Dıaz-Llano G, Smith TK. Effects of feeding grains naturally contaminated with Fusarium mycotoxins with and without a polymeric glucomannan mycotoxin adsorbent on reproductive performance and serum chemistry of pregnant gilts. Journal of Animal Science. 2006;**84**:2361-2366. DOI: 10.2527/jas.2005-699

[41] Frobose HL, Stephenson EW, Tokach MD, DeRouchey JM, Woodworth JC, Dritz SS, Goodband RD. Effects of potential detoxifying agents on growth performance and deoxynivalenol (DON) urinary balance characteristics of nursery pigs fed DON-contaminated wheat. Journal of Animal Science. 2017;**95**:327-337. DOI: 10.2527/jas2016.0664

[42] Ingalls JR. Influence of deoxynivalenol on feed consumption by dairy cows. Animal Feed Science and Technology. 1996;**60**:297-300. DOI: 10.1016/0377-8401(96)00984-4 60

[43] Aakre D, Flaskerud G, Hellevang K, Lardy G, McMullen M, Ransom J, Sorenson B, Swenson A. DON (Vomitoxin) in Wheat: Basic Questions and Answers. North Dakota State University; Fargo, North Dakota. September, 2005

[44] Swanson SP, Nicoletti J, Rood HD, Buck WB, Cote LM, Yoshizawa T. Metabolism of three trichothecene mycotoxins, T-2 toxin, diacetoxyscirpenol and deoxynivalenol, by bovine rumen microorganisms. Journal of Chromatography B: Biomedical Sciences and Applications. 1987;**414**:335-342. DOI: 10.1016/0378-4347(87)80058-0

[45] Rule DC, Preston RL, Koes RM, McReynolds WE. Feeding value of sprouted wheat (*Triticum aestivum*) for beef cattle finishing diets. Animal Feed Science and Technology. 1986;**15**:113-121. DOI: 10.1016/0377-8401(86)90018-0

[46] Reed JJ, Bauer ML, Loe ER, Caton JS, Lardy GP. Effects of processing on feeding value of sprouted barley and sprouted durum wheat in growing and finishing diets for beef cattle. The Professional Animal Scientist. 2005;**21**:7-12. DOI: 10.15232/S1080-7446(15)31159-1

Chapter 8

Storage Proteins Accumulation and Aggregation in Developing Wheat Grains

Aussenac Thierry and Rhazi Larbi

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.75182

Abstract

The aggregative properties of wheat grain prolamins are largely responsible for the technological functionalities of the flours and doughs. The ability of wheat prolamins to form a plastic three-dimensional network during the mixing depends to a large extent on their ability to interact. These aggregative properties, which can be evaluated by measuring their molecular weight distribution, are dependent on the polymorphism of the protein subunits present but also on the environmental conditions that are applied during grain development. Much progress has been made in the last 30 years at a genetic level to better understand and/or to favour the interaction properties of the storage proteins. However, these improvements can be strongly limited by environmental conditions. Any modification of the redox status of the grain cells in response to an oxidative stress can lead to a decrease in the degree of association of the prolamins by limiting the protein-protein interactions during the grain desiccation. Considering the current and projected environmental impacts (i.e. climate change with increasing heat stress), it is essential to better understand these phenomena to implement new breeding strategies for a sustainable quality.

Keywords: wheat, storage proteins, aggregation, breadmaking quality

1. Introduction

During the last 60 years, in the field of cereal chemistry, the scientific community has been working to determine in an ever more precise way, the nature of the constituents responsible for the acquisition of technological properties (i.e. breadmaking properties for common wheat doughs and/or pasta properties in the case of durum wheat). Particular emphasis has been placed on those whose (quantitative and/or qualitative) variations account for observed and measured changes in processing ability.

IntechOpen

© 2018 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. CC BY

As early as the 1950s, thanks to very good recombination experiments with flour constituents, Finney [1] confirms that the baking capacity is essentially conferred by gluten. Gluten, which can be defined as a viscoelastic protein complex formed after hydration and the addition of flour, consists of a heterogeneous mixture of prolamins (i.e. gliadins and glutenins) associated with covalent (S-S) and non-covalent bonds (hydrogen, hydrophobic and ionic). The specific role of certain protein fractions (monomer to polymeric proteins ratio) in the different properties of wheat doughs was also highlighted.

During the period 1970–1990, it became clear that the variation of the baking capacity of a flour is based on the ability of its storage proteins (i.e. prolamins) to form, during mixing, a three-dimensional plastic structure (**Figure 1A** and **B**). This remarkable structure creates a cohesive and viscoelastic network, insoluble in water, ensuring the retention of carbon dioxide, ethanol and aromas, during the fermentation of the dough and unlike other cereals for which these properties are non-existent (**Figure 2**). Thus, a common wheat is all the more breadmaking that its storage proteins have a strong tendency to aggregate into a three-dimensional viscoelastic network during mixing. Thus, gluten is considered a transient network whose mechanical properties depend on the density of the junction zones between the elements that compose it [2].

Since the 1990s, thanks to the integration of many complementary scientific approaches (i.e. molecular biology, biochemistry, analytical chemistry, rheology, etc.), a clearer vision of the transformation processes and the role of the main protein constituents within them have begun to take shape [3]. Thus, attention has been focused on the (polymeric) glutenin fraction because a strong relationship has been established between breadmaking properties, such as mixing time, extensibility and loaf volume and the molecular weight distribution (MWD) of the polymeric protein components [4].

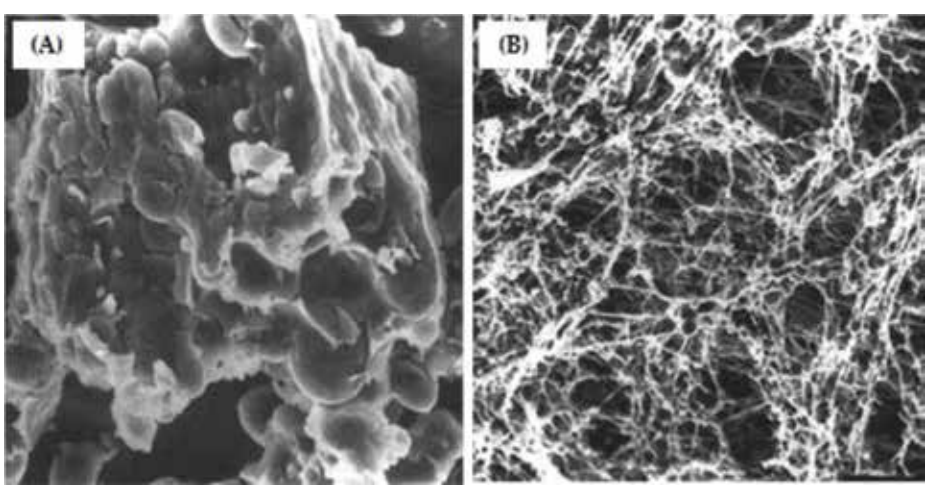


Figure 1. Scanning electron micrographs of durum wheat (A) flour and (B) dough particles (From Hoseney and Rogers [3]).

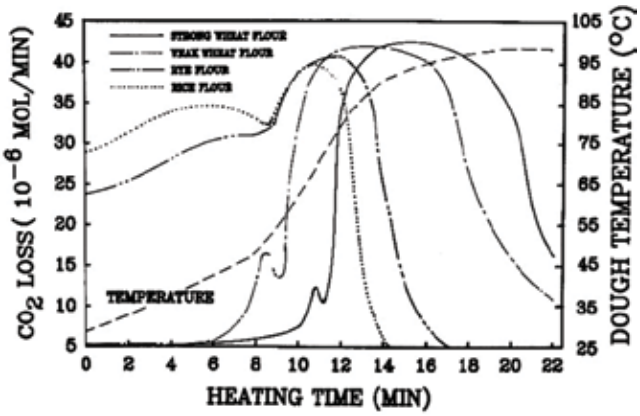


Figure 2. Loss of carbon dioxide and increase in temperature in relation to the heating time for different doughs of different cereal grains (From Hoseney and Rogers [3]).

To the extent that any changes (genetically and/or environmentally controlled) in the molecular size and/or aggregation status of these polymeric proteins can potentially result in very significant changes in the technological properties of the products concerned, it is important to understand how they are synthesized and accumulated in grains of wheat during their development. This knowledge is essential if we are to manipulate wheat quality in the future for traditional or new end users.

This chapter reviews the definition of the molecular weight distribution of wheat storage proteins, their changes during grain development and the impacts of environmental factors.

2. Molecular weight distribution (MWD) of wheat storage proteins

2.1. Classification and polymorphism of wheat grain proteins

Like all grain seeds, wheat grain contains a large number of proteins classified as structural proteins, functional proteins and reserve proteins. They are unequally distributed within the different cell of the grain. A natural gradient of distribution can be highlighted. As a result, the starch to protein ratio significantly increases from the peripheral to the central regions of the grain. Given the relative weight of these different cells, 70–80% (w/w) of the proteins are in the albumen.

The classification system for cereal proteins is mainly based on Osborne's historical work, in 1907 [5], based on their differences in solubility later used in sequential extractions (**Table 1**). As a result, four major protein fractions have been defined: albumins (soluble in water), globulins (soluble in dilute salt solutions), gliadins (soluble in diluted alcohols, 70% ethanol) and finally, glutenins (residual proteins, partially soluble in diluted acids and bases). Other authors have enriched these classifications based on structural and/or functional properties [6, 7].Within the large family of the storage proteins (prolamins), two main classes can be

Osborne [5] classification			**Shewry et al. [6] classification**		**Singh and Shepherd [7] classification**	
Protein fraction	Solubility	MW (kDa)	Composition	Structure	Gene localization*	Function
Albumins and globulins	Water neutral salts	5–90				Structural and functional proteins
Gliadins	Diluted alcohols	25–75		Monomers		Storage proteins (prolamins)
- ω			Poor in S		*Gli-1*(1A,1B,1D)S	
- α			Reach in S		*Gli-2*(6A,6B,6D)S	
- β					*Gli-2*(6A,6B,6D)S	
- γ					*Gli-1*(1A,1B,1D)S	
Glutenins	Acids, bases, reductants, detergents	100 to several millions	Reach in S	Polymers		
- LMW					*Glu-3*(1A,1B,1D)S	
- HMW					*Glu-1*(1A,1B,1D)L	

**Allelic blocks*, wheat homologous chromosomes (noted 1–6), wheat genomes (A, B and D) and chromosome position: (S) short arm, (L) long arm.

Table 1. Classification of wheat grain proteins.

differentiated due, to their degree of aggregation/polymerization. Thus, on the one hand, gliadins (soluble monomeric proteins in aqueous alcohols), which represent approximately 30–40% (w/w) of flour proteins and on the other hand, glutenins representing, 40–45% (w/w) of the total flour proteins. The latter are polymeric and aggregated proteins, forming a much more complex material than the gliadins.

Gliadins correspond to a mixture of monomeric proteins of molecular weight between 25 and 75 kDa and are characterized by their richness in glutamine and proline. They represent 45% (w/w) of the total prolamins. There are four classes based on their electrophoretic behaviour (i.e. increasing mobility in acid medium): α/β, γ and ω-gliadins (which, respectively, represent 44–60%, 30–46% and 6–20% of total gliadins) [8].

Glutenins, for their part, represent 40–50% (w/w) of total proteins; they are rich in proline and glutamic acid and their content in basic amino acids is higher than that of gliadins. They constitute a much more complex material formed of an assembly of polypeptide chains, commonly called subunits, linked together mainly by intermolecular disulphide bridges. These subunits have been grouped into two different subgroups: low molecular weight subunits (LMW-GS) and high molecular weight subunits (HMW-GS).

LMW-GS account for an average of two-thirds of total glutenins. They are very polymorphic and have molar masses between 30 and 50 kDa. Given their similarity to some gliadins, these have sometimes been difficult to quantify. HMW-GS, as their name indicates, have higher molecular weights ranging from 95 to 130 kDa. According to their SDS-PAGE migration, they fall into two groups: HMW-GS*y* (67–74 kDa) and HMW-GS*x* (83–88 kDa).

Gliadins have a large genetic polymorphism, it has been possible to detect between 20 and 40 different constituents for a wheat variety [9]. Within a class of gliadins, it is possible to find several sub-families depending on the composition and richness of certain amino acids (the ω1 and ω5 gliadins differ in basic amino acids, glutamine and proline, than that γ1, γ2 and γ3 differ in their richness in tyrosine, lysine and methionine). Thus, the polymorphism of gliadins is very important that it serves as a basis for the varietal identification of wheat [10].

The polymorphism of low molecular weight glutenic subunits (LMW-GS) is less important than that of gliadins. For a given variety, there are 7–6 LMW-GS. But 40 different LMW-GS were found in 222 varieties of soft wheat [11]. Finally, high molecular weight glutenic subunits (HMW-GS) are the prolamins that have the lowest polymorphism. The association of two genes at each Glu-A1, Glu-B1 and Glu-D1 locus was noted. The *x*-type genes express subunits of masses greater than those encoded by *y*-type genes in SDS-PAGE [12]. However, some *y*-type HMW-GS (notably subunit 12) have been shown to have important immunochemical similarities with α/β and γ-gliadins [13]. In all cases, recombination between these genes is very rare. The different wheat varieties contain between 3 and 5 HMW-GS. Indeed, 1Ay genes are never expressed, and 1By and 1Ax genes are only expressed in some varieties [14].

The primary structure of the storage proteins is well understood. They comprise three distinct domains (**Figure 3**): a central domain made up of repeated sequences and two domains formed of non-repeated sequences at the ends (i.e. C- and N-terminal). The understanding of these sequences has made it possible to locate particularly important cysteine residues because of their ability to form disulphide bonds (intra and/or intermolecular). α-, β- and γ-gliadins are provided with cysteines at their C-terminal domains; these all being involved in the formation of intramolecular disulphide bridges. HMW-GS have unpaired cysteine in their C-terminal domain and several others in their N-terminal domain; LMW-GS carrying seven C-terminal cysteines and one N-terminal cysteine. Thanks to these unpaired cysteines, unlike gliadins, HMW-GS and LMW-GS are able to form intermolecular disulphide bridges. Some authors report a globular type structure for the N- and C-terminal and a spiral structure for the repetitive domain (**Figure 3**).

2.2. Gliadin to glutenin ratio

Generally, it is accepted that the functional properties of gluten proteins are related to their ability to form a network during technological processes [17, 18]. However, gliadins and glutenins do not have the same effect on the rheological properties of doughs or glutens. Consequently, gliadins explain the viscous nature, while glutenins determine elasticity. In fact, the small quantity of cysteine residues in these storage proteins makes it possible to establish an important structural and functional distribution between gliadins and glutenins (**Figure 4**).For the former, all cysteine residues are involved in the establishment of intramolecular disulphide bridges while for both high and low molecular weight glutenins, a number of cysteines not involved in intramolecular bonds are therefore available to establish intermolecular links with other subunits. Glutenins are therefore likely to constitute polymers with a real consistency, thanks to the formation of intermolecular disulphide bridges, while gliadins remain in the monomeric state. The latter may, however, be aggregated by weak bonds

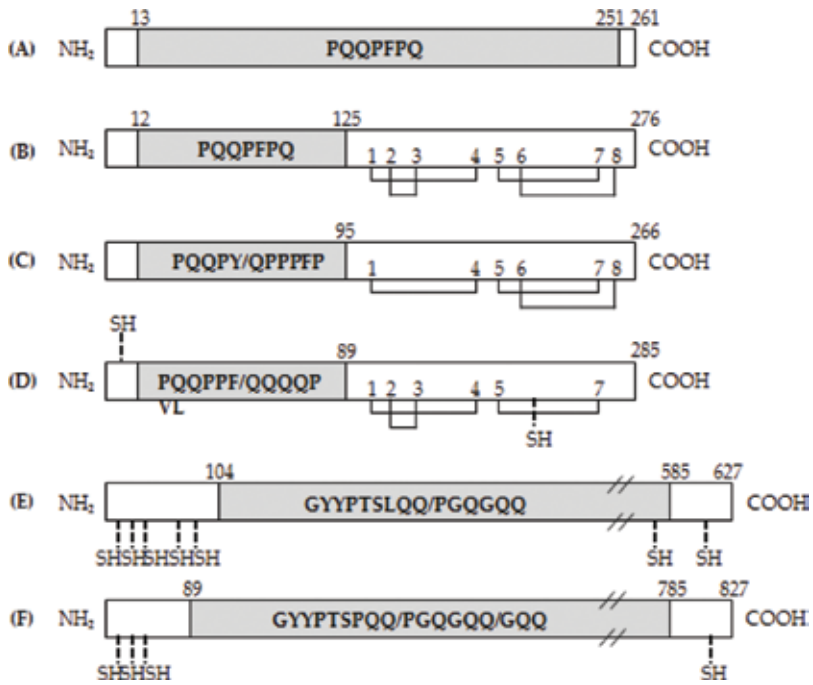


Figure 3. Schematic structures of typical primary structures of (A) ω-giadin, (B) α-gliadin, (C) γ-gliadin, (D) LMW-GS, (E) HMW-GS*y* and (F) HMW-GS*x* [15, 16]. Repetitive sequences are shaded and disulphide bonds between conserved cysteine residues (1–8) in the γ-gliadin are shown as lines. SH denotes the positions of cysteine residues in the HMW prolamins. Single letter abbreviations for amino acids: F = phenylalanine; G = glycine; L = leucine; P = proline; Q = glutamine; S = serine; V = valine and Y = tyrosine.

(hydrogen and hydrophobic). The viscoelasticity of gluten depends on its state of polymerization and the interactions between polymers [2].

A large number of conventional fractionation and reconstitution tests have been conducted based on the differential physical properties observed in purified gliadins and glutenins. The aim of these studies was to link variations in molecular weight distribution (i.e. monomer to polymer ratio) with the rheological characteristics of the glutens obtained. In the majority of cases, the results obtained during these different reconstitution studies have demonstrated that the rheological properties of the restructured flours generated are strongly influenced by the ratio of these two protein fractions [20, 21]. With a constant amount of prolamins, the strength of the reconstituted flour, measured at the time of the dough making with a mixograph (i.e. peak time value mix (MPT)), is related to the proportion of polymeric proteins.

The development of the original analytical approaches (i.e. high performance liquid chromatography of size exclusion, SEC-HPLC) during the 1980s confirmed the vast majority of these hypothesis, which were essentially based on results obtained from differential solubility protocols (i.e. gliadins vs. glutenins). Thus, many authors [22–29] have confirmed the existence of a significant relationship between the relative amount of glutenin aggregates and the baking quality of many everyday wheat genotypes.

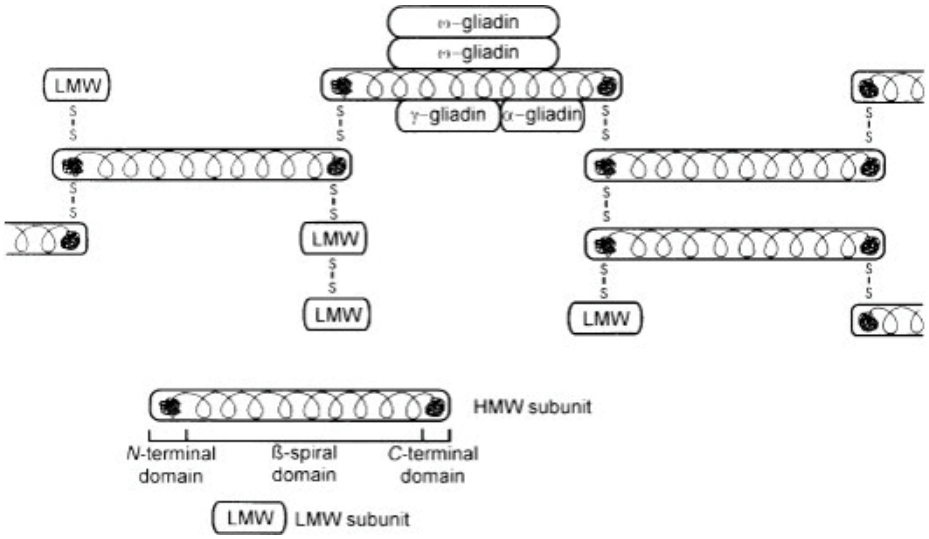


Figure 4. A structural model for wheat gluten in which the HMW subunits provide a disulphide-bonded backbone which interacts with other gluten proteins through disulphide bonds (LMW subunits) and non-covalent interactions (gliadins) (From Shewry et al. [19]).

The molecular weight distribution (MWD) of prolamins is becoming recognized as the main determinant of physical dough properties [30, 31]. However, in theory, the MWD can be altered from one sample of wheat (or one cultivar) to another by changes in the relative proportions of monomeric proteins and polymeric proteins (gliadins to glutenins ratio) but also by changes in the size distribution of polymeric proteins [32].

2.3. Size distribution of polymeric proteins

Chen and Bushuk [33] revealed that part of the glutenin is soluble in acetic acid thus making the distinction between an insoluble and a soluble fraction. The importance of this distinction became clear when Orth and Bushuk [34] demonstrated a positive correlation between the amount of acetic acid insoluble glutenin and bread loaf volume. From then on, insoluble glutenin became widely recognized as the key protein fraction that can explain differences in dough strength and breadmaking quality [35]. The use of detergents (SDS) and organic solvents (propanol) [36] allowed an even better separation and led to the conclusion that insolubility was due to size and a very high degree of polymerization. Other groups developed methodology with propanol to further separate soluble protein parts from the insoluble glutenin. Currently, two main methods are in use to quantify and characterize this fraction. The first corresponds to the so-called unextractable polymeric protein (UPP) method using propanol and during which unextractable polymeric protein (UPP) fraction is obtained. Upon sonication, this fraction becomes soluble in SDS [28, 29] and can be analysed using size exclusion chromatography [27, 37]. The other method is the SDS method as advanced by Graveland et al. [38] resulting in the SDS-insoluble gel protein fraction. This fraction was renamed glutenin macro polymer (GMP) to reflect its highly aggregated nature [39, 40]. Moonen et al. [41]

found that the SDS-insoluble glutenin-gel protein fraction highly correlated with SDS sedimentation values and loaf volume. Weegels et al. [40, 42] studied this fraction in great detail and presented firm evidence that GMP quantity correlates to bread loaf volume.

In addition to these classical approaches (UPP and/or GMP), new analytical protocols have been developed since the early 2000s to separate and more accurately characterize the molecular size distribution of the polymeric proteins. Flow field-flow fractionation (FFFF) [43–46], which is a new separation technique without any stationary phase, and which is therefore not hampered by a steric exclusion limit [47–49], has been used successfully to separate a number HMW fractions [50–52]. Furthermore, the MALLS technique which is one of the most effective means of determining molecular weight, size and conformation of glutenin polymers without reference to standards [48, 53–56] has been applied in combination with the A-FFFF method to accurately measure size and conformation of wheat glutenins [57] (**Figure 5**).

The glutenin association level (i.e. the size distribution) is strongly correlated with the HMW-GS/LMW-GS [58–60] ratio and the nature of the HMW-GS present (especially HMW-GS pair 5 + 10 vs. HMW-GS pair 2 + 12 coded by Glu-*D1*). As demonstrated by the different experimental approaches carried out in recent years [61–64], the different glutenin subunits (i.e. HMW-GS, LMW-GS and HMW-GS *x* and *y* type) are unequally distributed within polymers. These results demonstrate the existence of a highly ordered structures in which some subunits play a predominant role, notably because of their difference in functionality (i.e. number and especially position of cysteine residues capable of forming intermolecular bonds) [65] (**Figure 6**).

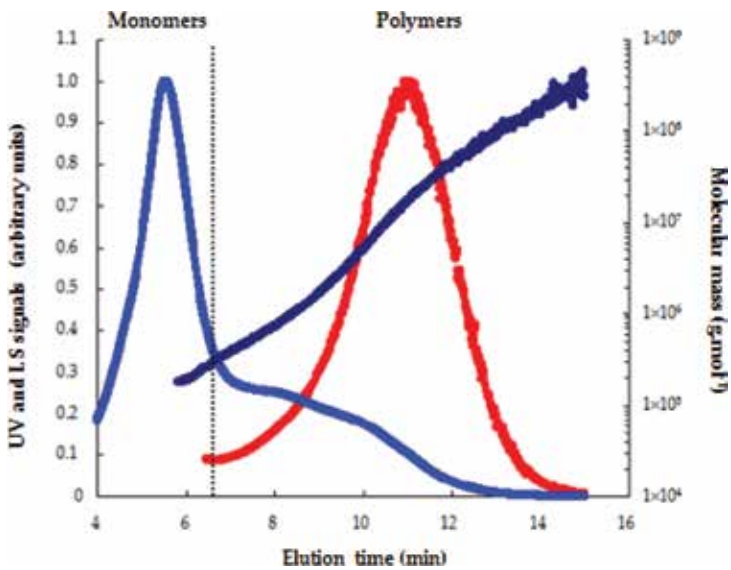


Figure 5. Asymmetrical flow field-flow fractionation (A4F) profiles of total solubilized storage proteins of a common French wheat cultivar (Soissons). UV (blue line), light scattering at 90° (red line) and molecular weight in relation to elution time (dark line) (from Lemelin et al. [57]).

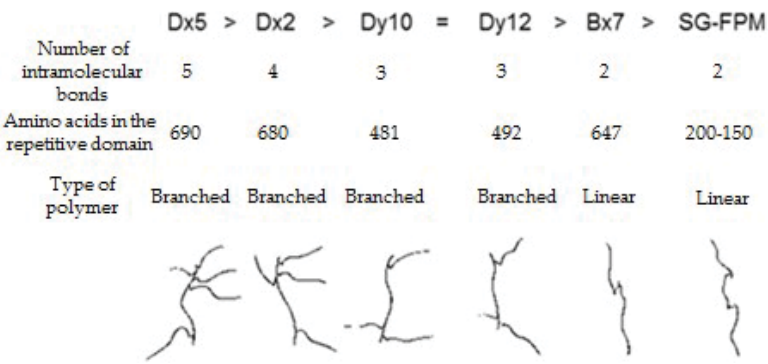


Figure 6. Hierarchical arrangement of HMW-GS and LMW-GS in relation to their intrinsic contribution to polymer formation (from Kasarda [65]).

3. Accumulation of prolamins in developing wheat grains

3.1. Endosperm development

The development of the wheat endosperm which has been well described at the microscopic level, as reviewed by Bechtel et al. [66], can be quite easily characterized by the study of the temporal variations of several quantitative components of it such as the accumulation of the total dry matter, the water content of the grain and the accumulation of total protein and starch [67] (**Figure 7**).

The accumulation of total dry matter in the grain provides a good insight into the functioning of different accumulation metabolisms (i.e. nitrogen translocation and post-flowering photosynthesis) [68]. Thus, after an initial lag phase (up to 10–15 days after anthesis (DAA)), it is easy to observe a phase of linear accumulation of this dry matter; wheat grains reaching a maximum dry weight from 40 DAA.

During this linear phase, the observed phenomena depend on two main variables: the duration (D) and the speed or flux of assimilates towards the grain (V), so that the weight of a grain (P) is given by the relation $P = V \times D$ [69]. D can be expressed in days or in the sum of average daily temperatures (i.e. degree-days (DD)). The filling speed is the limiting factor in the development of the weight of a grain. This speed is mainly by the number of grains per m^2. Finally, under natural conditions, the duration D cannot compensate for the weight loss produced by any reduction in the rate of accumulation. The amount of water per grain that gradually increases to about 20 DAA remains relatively constant up to ≈ 35 DAA (i.e. "water plateau" phase) before decreasing at harvest time.

The higher the rate of water accumulation in the grain, the greater the height of the "water plateau" and the higher the weight of the grain at maturity [70]. Based on changes in the amount of water and total dry matter per grain after anthesis, three particular phases of grain development can be estimated: the cell division phase, the cell enlargement phase (i.e. grain

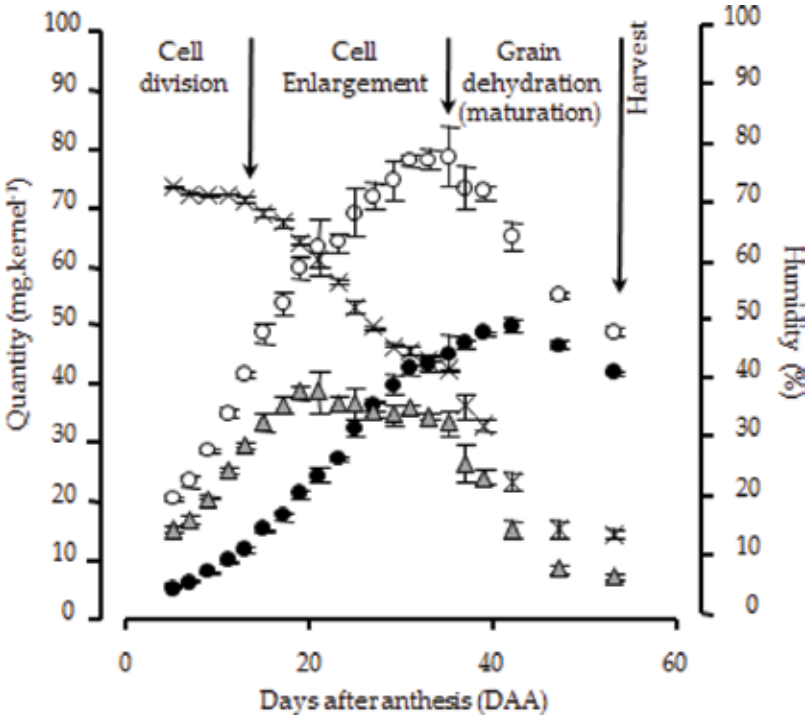


Figure 7. Grain filling period for a common wheat cultivar (*Soissons*). Evolution of (●) dry matter per kernel, (O) fresh matter per kernel, (△) water quantity per kernel and (X) grain humidity. The vertical lines represent the standard deviation ($n = 3$) (from Carceller and Aussenac [67]).

filling phase) and the grain desiccation phase (the beginning of this phase corresponding to the acquisition of physiological maturity) [71] during which protein bodies disappear to form the protein matrix [72, 73].

Since 1970s, a great deal of work has been done to evaluate the effects of the environment on grain development. Thus, the effects of several environmental variables (i.e. light, temperature, water availability and nutrient availability), taken individually or in combination, have been studied [74–85]. In general, temperature and water availability strongly affect the filling rate (V) and the duration of grain filling (D), although some differences in behaviour may exist between wheat genotypes. Consequently, differences in thermal regimes and/or water regimes cause profound changes in the accumulation of the total dry matter (P) by affecting indifferently and without compensating the speed and duration of filling [86].

3.2. Accumulation of Storage Proteins

The accumulation of different protein fractions (albumins-globulins, gliadins and glutenins) is progressive from flowering until the acquisition of the physiological maturity of the grains (≈ 35–40 DAA). However, even if the time of initiation of the biosynthesis of the different proteins of the grain is not significantly different (5–7 DAA) [87], their rate of accumulation

varies considerably, suggesting a phenomenon of differential regulation of this biosynthesis (**Figure 8A** and **B**).

Thus, a certain accumulation asynchrony in the protein fractions of the grain can be highlighted. The albumins-globulins accumulate most rapidly in the grain, followed by the monomeric prolamins and finally the polymeric prolamins. As many researchers have shown [88–93], the accumulation of albumins-globulins is maintained only during the cell division phase, contrary to that of prolamins. This confirms the functional and/or structural role of these specific proteins.

While the ratio between polymeric proteins and monomeric proteins is stable during the first stages of grain development (i.e. cell division and cell enlargement), this ratio increases significantly during the grain desiccation phase (i.e. after 35 DAA) (**Figure 8C**). A number of results in the literature are quite contradictory [90, 94, 95]. In our opinion, and in accordance with the

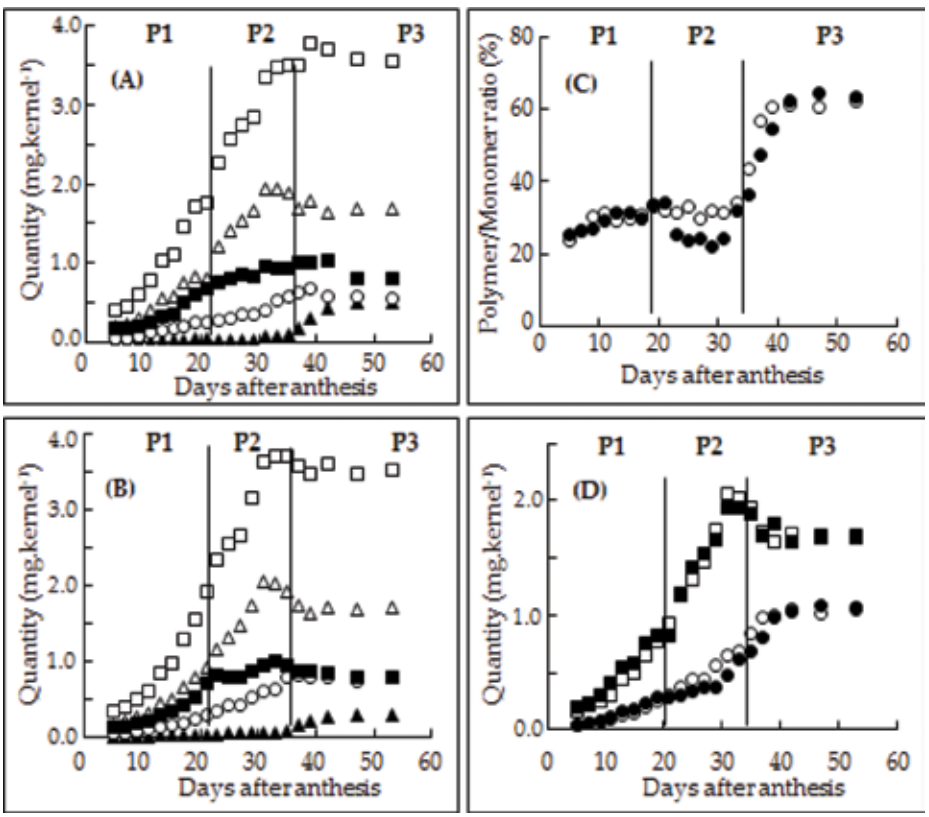


Figure 8. Evolution of the quantity of the different protein fractions (mg.kernel^{-1}) for two common French wheat cultivars (A) Soissons and (B) Thésée, as a function of the days after anthesis. (▲) SDS-insoluble polymers; (O) SDS-soluble polymers; (■) albumins and globulins; (△) monomers and (□) total proteins. Evolution of (C) the polymer/monomer ratio (%) and (D) the quantity of monomers and total polymers (mg.kernel^{-1}) as a function of days after anthesis. (O,●) total polymers of Thésée and Soissons, respectively; (□,■) Monomers of Thésée and Soissons, respectively. Stages: (P1) cell division; (P2) cell enlargement and (P3) grain desiccation and maturation (from Carceller and Aussenac [67]).

remarks of Stone and Nicolas [92], most of these differences can be explained by the fact that the methods of extraction and analysis of the polymeric proteins retained are extremely varied from one research group to another; it is therefore certain that all the researchers did not take into account the same protein entities in the calculation of the polymers/monomers ratio.

The accumulation of SDS-soluble polymers that starts very early in the grain (from 7 DAA), is very slow and continues up to the beginning of the drying phase of the grain. The accumulation of SDS-insoluble polymers (i.e. UPP) is, in turn, really visible only when the grain begins to lose its water balance (i.e. end of the "water plateau") [67, 92, 96] (**Figure 9B**).

These elements must be compared with the observations of researchers such as Woodman and Engledow who, as early as the 1920s, noted the increase in the ability of proteins to form a coherent mass, gluten, in relation to the beginning of the grain desiccation [97]. The accumulation of the protein polymers in the broad sense coincides perfectly with the accumulation of the different glutenin subunits (LMW-GS and HMW-GS) in the grain [91, 98]; the HMW-GS/LMW-GS ratio being an important parameter for differentiating wheat genotypes from each other. For example, in the framework of our own research [67, 99], we have been able to demonstrate that at harvest time, the association state of polymeric proteins (i.e. SDS-insoluble polymers/total polymers ratio) is strongly correlated with the HMW-GS/LMW-GS ratio. Thus, at maturity, with the same total polymer amount (**Figure 9A**), the wheat genotype Soissons, which is characterized by a HMW-GS/LMW-GS ratio twice that of the wheat genotype Thesée, has a SDS-insoluble polymer/total polymer ratio twice as large that of Thésée (**Figure 9B**).

3.3. Unextractable polymeric protein (UPP) accumulation

The formation and accumulation of polymeric protein fractions characterized by high levels of aggregation (indifferently qualified in the literature of SDS-insoluble polymeric proteins, unextratable polymeric proteins (UPP) and glutenin macro polymers (GMP)) have been the focus

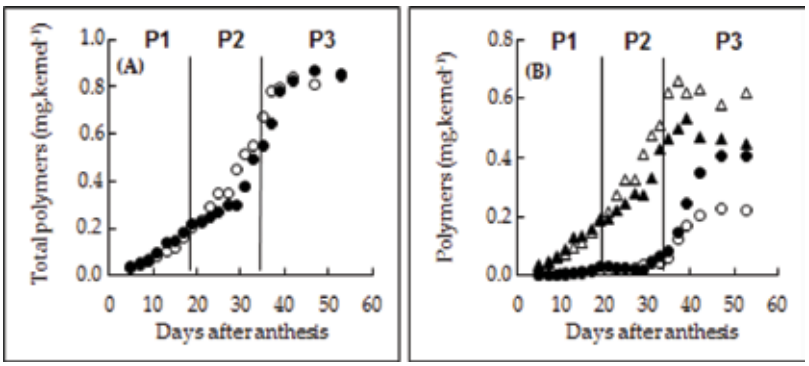


Figure 9. Accumulation of total polymers, SDS-soluble and SDS-insoluble polymers as a function of the days after anthesis. (A) Total polymers (mg.kernel^{-1}) for (●) Soissons and (O) Thésée. (B) Open symbols are for Thésée and closed symbols are for Soissons. (△) SDS-soluble polymers and (O) SDS-insoluble polymers. Stages: (P1) cell division; (P2) cell enlargement and (P3) grain desiccation and maturation (from Carceller and Aussenac [67]).

of attention during the last 15 years because these fractions became widely recognized as the key protein fraction that can explain differences in dough strength and breadmaking quality.

According to the various physiological observations carried out since the early 2000s [67, 93, 100–102], it appears that the UPP accumulation phase coincides very strongly with the grain desiccation phase (**Figure 9B**), whatever the culture conditions applied (i.e. light, temperature, water availability and nutrient availability). Thus, 95–100% (w/w) of the UPPs present in the grain at harvesting accumulates during the grain desiccation phase. Finally, several experiments of artificial dehydration of wheat grains have confirmed the strong relationship between grain water loss and UPP accumulation [93, 102].

Although today the mechanisms responsible for the formation of UPPs are still the subject of discussions and/or hypotheses, many observations seem to confirm that the strengthening of the aggregation character in these polymeric proteins during grain desiccation results from the reinforcement of intermolecular interactions (mainly covalent interactions) between the different glutenin subunits (HMW-GS and LMW-GS) [103, 104]. This phenomenon has led to a very significant increase in the different molecular dimensions (Mw and Rg) of the glutenin polymers [103].

Studying the function of free glutenin sulfhydryl (SH) and disulphide (SS) groups in glutenins of developing wheat for UPP formation, we showed that the major wheat glutenin subunits residing in the protein bodies undergo redox change during the development and the maturation of the grain [103] (**Figure 10**). Indeed, during the cell division and grain filling, glutenin

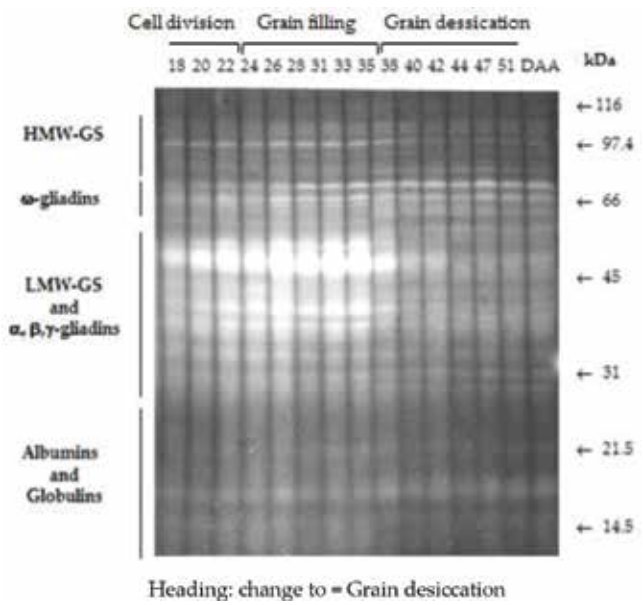


Figure 10. Change in sulfhydryl status of wheat proteins during grain development and maturation. MBBr-derivatized (fluorescence photography) storage proteins of a common French wheat cultivar (Soissons). Days after anthesis (DAA) (from Rhazi et al. [103]).

subunits and particularly LMW-GS have a large amount of free SH groups and become oxidized during grain desiccation which coincided with the accumulation of UPP. Moreover, monobromobimane (mBBr) derivatized of free glutenin SH groups before the artificial grain desiccation totally inhibits the UPP deposition [104].

In our hypothesis which is very close to the model proposed by Hamer and van Vliet [105] for the gluten structure termed "hyper aggregation" model, the grain desiccation promotes the aggregation of polymers already present (i.e. SDS-soluble polymers or level I aggregates in the "hyper aggregation" model) by facilitating specifically the formation of interchain hydrogen bonding between the repeat regions of glutenin subunits [106–108], which can bring glutenin free accessible SH groups into close proximity to form additional intermolecular disulphide bridges.

4. Impacts of environmental factors on MWD of prolamins

The multiple agronomic studies which were done during the last 25 years indicate that environmental conditions affect the amount, composition and polymerization of the gluten proteins [109–119]. Furthermore, the impact of environmental components on the molecular weight distribution of the prolamins is significantly greater than that of genetic components (i.e. $\sigma^2_E/\sigma^2_R > \sigma^2_G/\sigma^2_R$) (**Table 2**) [120–122]. This is why, in a context of profound environmental changes [123], it is very important to better understand the mechanisms responsible for these effects in order to better anticipate them.

The availability of nutrients (nitrogen and/or sulphur availability) and the temperature (thermal regime) are the two main environmental factors responsible for these protein changes.

Parameters	Maximum value	Minimum value	Mean value	σ^2_G/σ^2_R	σ^2_E/σ^2_R
Total proteins[1]	8.700	15.100	11.187	11.411^{***}	271.577^{***}
Total polymers[1]	2.785	5.755	4.016	11.104^{***}	187.462^{***}
Polymer/monomer	0.321	0.700	0.561	14.845^{***}	72.611^{***}
Polymer M_n[2]	0.730×10^6	9.609×10^6	0.972×10^6	1.068^{NS}	4.383^{***}
Polymer M_w[2]	1.142×10^6	22.970×10^6	7.640×10^6	3.370^{***}	38.974^{***}

[1]Quantity in mg/100 g DM.

[2]M_n = Molecular weight number-average and M_w = Molecular weight-average ($g.mol^{-1}$).

$^{***}F$-test significance at 0.1% level of probability; σ^2_G/σ^2_R = Genetic variance/Residual variance ratio and σ^2_E/σ^2_R = Environmental variance/Residual variance ratio.

NS: Not significant.

Table 2. Genetic (G) and environmental (E) influence on molecular weight distribution of storage proteins determined by analysis of variance (***F***-test) for 130 common French wheat genotypes cultivated in three different locations for 2005 and 2006 (from Aussenac et al. unpublished data).

High nitrogen availability translates into high protein contents in the grain and flour but also by changes in protein composition. With increasing protein content, gliadins tend to increase at a greater rate than other proteins. This can lead to MWD alterations which results from decreases in the polymeric-to-monomeric protein ratio and/or increases in the HMW-GS to LMW-GS ratio [113, 124, 125].

When sulphur fertilization is limited, the molecular distribution of glutenins is strongly affected insofar as this limitation results in a significant modification of the HMW-GS/LMW-GS ratio [109, 126]. The increase in the HMW-GS/LMW-GS ratio which is linked to the fact that the high molecular weight glutenin subunits are much less affected by a sulphur limitation because they are poorer in corresponding amino acids, therefore results in an increase in the average molecular weight of the polymers. Finally, sulphur deficiency is accentuated by higher nitrogen levels [127].

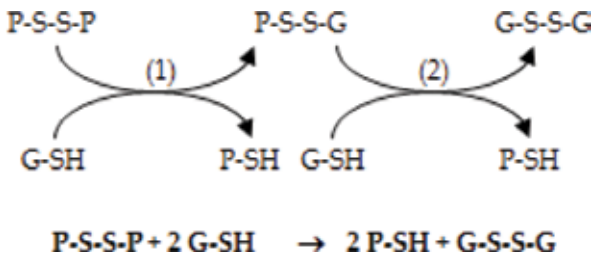
Temperature (i.e. daily mean temperature, temperature regime and temperature application stage) can induce very large changes in the association state of polymers during grain filling [110, 128–130]. Thus, in the great majority of the work carried out in recent years, various researchers have shown that the increase in temperature and/or the sudden change in the thermal regime during grain filling could lead to a significant decrease in the association status of prolamins resulting in a decrease of MWD (or solubility) of glutenins [131–133].

In the majority of the work to which we have just referred to above, the effects observed are most often attributed to modifications in the synthesis activities of the different storage proteins (i.e. gliadins vs. glutenins and/or HMW-GS vs. LMW-GS) resulting from modulation of the expression of storage protein genes [85]. Today, it seems that other phenomena could also be reasonably involved. These phenomena could be based, in particular, on important variations in the cellular redox status in response to environmental stimuli (i.e. environmental stress).

It has long been established that desiccation of plant tissues causes the appearance of free radicals. Although this phenomenon is a very general mechanism, a large number of observations have been made from seeds of various species [134–137]. In the majority of these studies, the presence of free radicals has been correlated with viability losses [138]. Among these implemented detoxification mechanisms, the ascorbate/glutathione cycle (i.e. trapping of H_2O_2 generated) is one of the most efficient. This essential cycle in chlorophyll tissues [139, 140] has also been studied in seeds [141, 142].

At a cellular level, thiols are the first compounds affected by oxidative stress in general because of the high sensitivity to the oxidation of sulfhydryl (SH) groups. The predominant non-protein thiol in most plant species is glutathione (GSH). This tripeptide ensures the maintenance of the redox status at a cellular level but also the storage and transport of the reduced sulphur necessary for the synthesis of proteins [143–145]. The first compound resulting from the oxidation of glutathione is its dimer (GSSG) which is produced in vivo largely thanks to SH/SS exchanges with proteins (noted P) [146]. The reactions below illustrate these exchanges.

The GSSG dimer is normally reduced in GSH by glutathione reductase (GR) activity. Thus, under normal conditions, glutathione is very much present at a cellular level in its reduced



form (i.e. high GSH/GSSH ratio) which has the effect both for maintaining the SH status of proteins (to maintain enzymatic activities [147]) and continue to trap H_2O_2.

Under the influence of oxidative stress, the redox status of glutathione will be modified; GSSG dimer will accumulate due to either an increase in GSH oxidation and/or a decrease in GSSG reduction activity (i.e. decrease of GSH/GSSH ratio). Such changes in the SH/SS status have already been widely observed in response to oxidative stress, especially during seed desiccation [148]. Glutathione which is able to bind to protein thiols is considered a "protective" element of these protein compounds since it prevents the formation of intramolecular disulphide (S-S) bridges during the desiccation phenomena [149]. In this way, GSH contributes both to limit the protein denaturation phenomena and to modulate enzymatic activity [150]. In contrast to desiccation, the imbibition phenomenon preceding germination causes the reduction of the disulphide bonds (SS) of a large number of compounds such as, GSSG [151, 152], protein-SSG conjugates [153], α-amylases [154] or the storage proteins [155, 156]. A synthesis of the presumed role of glutathione can be postulated, referring in particular to the hypothesis formulated by Kranner and Grill [150] (**Figure 11**).

Glutathione may occur endogenously in wheat flour in the free reduced glutathione (GSH) and free oxidized glutathione disulphide (GSSG) forms as well as in the form of protein-glutathione mixed disulphides (PSSG) [146–159]. Moreover, approximately 85% of PSSG in mature wheat grains are represented by polymeric proteins (PP) conjugated to glutathione

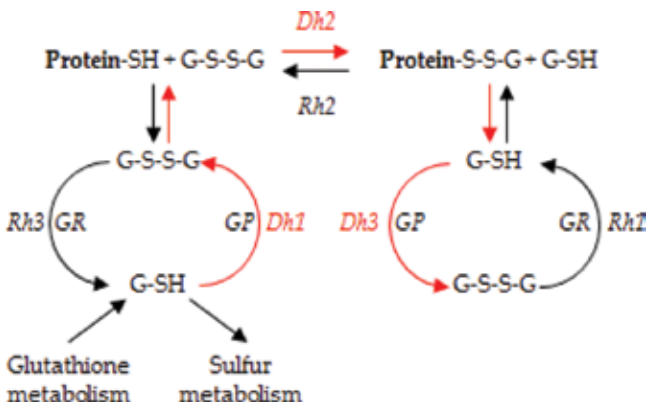


Figure 11. SH/SS interchange during dehydration/rehydration phenomenon. (*Dh*) dehydration step, (*Rh*) rehydration step, (*GP*) glutathione peroxidase, (*GR*) glutathione reductase (from Kranner and Grill [150]).

(PPSSG) [159]. Even if glutathione is able to bind to the storage proteins during grain filling, the formation of PSSG is not however correlated with the accumulation of the storage proteins in the grain but coincide rather with the grain desiccation during which the major wheat storage proteins residing in protein bodies undergo redox change (i.e. become oxidized) and UPP are accumulated [103, 159, 160].

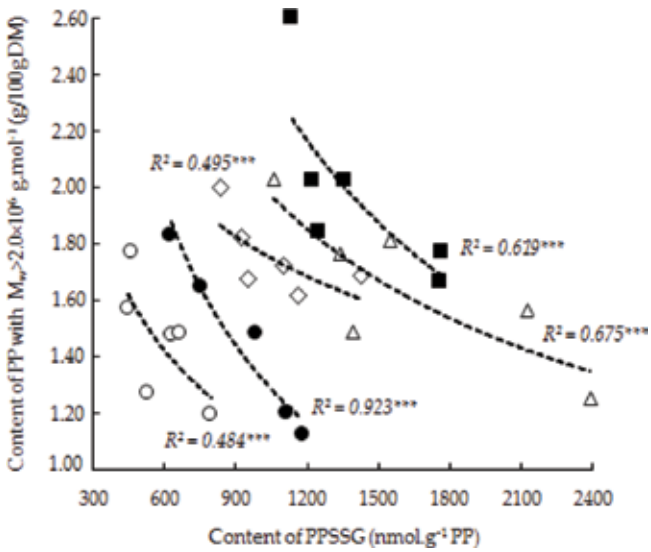


Figure 12. The relationship between the content of high aggregated polymeric proteins (PP with $M_w > 2.0\times10^6$ g.mol^{-1}) and the content of polymeric proteins conjugated to glutathione (PPSSG) for five different common French wheat cultivars (harvest 2005 and 2006 in three locations) (from Aussenac et al. Unpublished data).

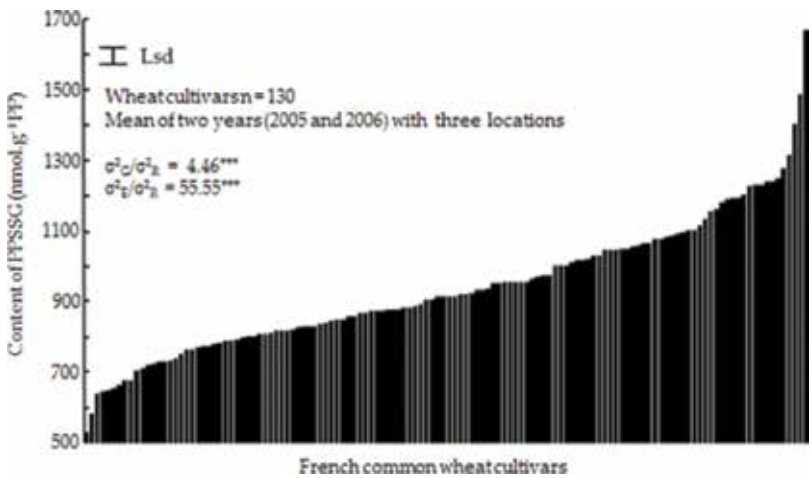


Figure 13. Variation of the content of polymeric proteins conjugated to glutathione (PPSSG) for a significant set of common French wheat cultivars (harvest 2005 and 2006 in three locations). σ^2_G/σ^2_R = genetic variance/Residual variance ratio and σ^2_E/σ^2_R = environmental variance/residual variance ratio (from Aussenac et al. Unpublished data).

Low molecular weight endogenous thiols such as glutathione, which mainly act as "protein protectors" [149] through the formation of PSSG during tissue desiccation, are responsible in wheat grains during its desiccation to a significant reduction of the MWD of the polymeric proteins by the formation of PPSSG (**Figure 12**). This action is all the more important because it is very targeted because GSH was bound almost exclusively to those cysteine residues that have been proposed to form intermolecular disulphide bonds (in particular, cysteines Cb* and Cx, which are responsible for the aggregative nature of LMW-GS) as Köhler et al. [161] has been able to demonstrate it by using ^{35}Slabelled GSH.

Consequently, it is now clear that glutathione conjugation with polymeric proteins during the grain development resulting in drastic changes of the cellular redox status (largely due to environmental factors - **Figure 13**) plays a crucial role in controlling the MWD of the polymeric proteins which has been shown to be important in determining baking performance.

5. Conclusions

Since the 1990s, there has been a broad consensus within the scientific community that the value of using of a wheat flour depends mainly on the quality of the assembly of its prolamins (glutenins in particular) which are themselves largely under the control of protein polymorphism (the nature and relative abundance of LMW-GS and HMW-GS) and the conditions of development and maturation of the grains from which it is made. Although much progress has been made in the field of characterization of polymeric structures, in particular through the implementation of new analytical approaches (A-4F/MALLS), the fact remains that significant work needs to be done to better understand the structure of its protein assemblies of technological interest (UPP or GMP).

This chapter demonstrates that to achieve these objectives, it is essential to better understand the mechanisms that govern the formation of these polymers and/or protein aggregates in wheat grains during the final stages of their development which are subject to changing environmental conditions (i.e. rising temperatures). In this context, the important role of cellular redox status is addressed by highlighting the significant effects of particular free thiols such as glutathione on the state of association of glutenins. These compounds, of which one of the main functions is to limit the deleterious effects of oxidative stress on protein structures by combining with them, will at the same time reduce the inter-prolamin interactions in the grain thus limiting their technological functionalities. The current improved understanding of these cellular mechanisms will undoubtedly open up new avenues for exploring redox strategies for wheat improvement required for a sustainable quality.

Acknowledgements

The authors would like to thank the FSOV (French Support Fund for Plant Breeding) and the Conseil Régional HdF (Regional Council of Hauts de France) for supporting part of our research.

Author details

Aussenac Thierry* and Rhazi Larbi

Address all correspondence to: thierry.aussenac@unilasalle.fr

Transformations and Agro-Resources (UP 2018.C103), Institut Polytechnique UniLaSalle, Beauvais, France

References

[1] Finney KF, Barmore MA. Loaf volume and protein content of hard winter and spring wheats. Cereal Chemistry. 1948;**25**:291-312

[2] Cornec M, Popineau Y, Lefebvre J. Characterization of gluten subfractions by SE-HPLC and dynamic rheological analysis in shear. Journal of Cereal Science. 1994;**19**:131-139. DOI: 10.1006/jcrs.1994.1018

[3] Hoseney RC, Rogers DE. The formation and properties of wheat flour doughs. Critical Reviews in Food Science and Nutrition. 1990;**29**:73-93. DOI: 10.1080/10408399009527517

[4] Gupta RB, Batey IL, MacRitchie F. Relationships between protein composition and functional properties of wheat flour. Cereal Chemistry. 1992;**69**:125-131

[5] Osborne TB. The proteins of the wheat kernel. Carnegie Institution of Washington D.C. 1907; Pub. N° 84: 119 pp

[6] Shewry PR, Tatham AS, Forde J, Kreis M, Miflin BJ. The classification of wheat gluten proteins; a reassessment. Journal of Cereal Science. 1986;**4**:97-106. DOI: 10.1016/S0733-5210(86)80012-1

[7] Singh NK, Shepherd KW. Linkage mapping of genes controlling endosperm storage proteins in wheat. I – Genes on the short arms of group 1 chromosomes. Theoretical and Applied Genetics. 1988;**75**:628-641. DOI: 10.1007/BF00289132

[8] Wieser H, Seilmeier W, Belitz HD. Quantitative determination of gliadin subgroups from different wheat cultivars. Journal of Cereal Science. 1994;**19**:149-155. DOI: 10.1006/jcrs.1994.1020

[9] Brown JWS, Flavell RB. Fractionation of wheat gliadin and glutenin subunits by two dimensional electrophoresis and the role of group 6 and group 2 chromosomes in gliadin synthesis. Theoretical and Applied Genetics. 1981;**59**:349-359. DOI: 10.1007/BF00276448

[10] Autran JC, Bourdet A. L'identification des variétés de blé : établissement d'un tableau général de détermination fondé sur le diagramme électrophorétique des gliadines du grain. Annales de l'Amélioration des Plantes. 1975;**25**:277-301

[11] Gupta RB, Shepherd KW. Two step one dimensional SDS-PAGE analysis of LMG subunits of glutenin. I - Variation and genetic control of the subunits in hexaploid wheats. Theoretical and Applied Genetics. 1990;**80**:65-74. DOI: 10.1007/BF00224017

[12] Payne PI, Holt LM, Law CN. Structural and genetical studies on the high-molecular-weight subunits of wheat glutenin. Part I: Allelic variation in subunit amongst varieties of wheat (*Triticum aestivum*). Theoretical and Applied Genetics. 1981;**60**:229-236. DOI: 10.1007/BF02342544

[13] Curioni A, Dal Belin Peruffo A, Pressi G, Pogna NE. Immunological distinction between x-type and y-type high molecular weight glutenin subunits. Cereal Chemistry. 1991;**68**:200-204

[14] Payne PI, Lawrence GJ. Catalogue of alleles for the complex gene loci, Glu-A1, Glu, B1, and Glu-D1 which code for high-molecular-weight subunits of glutenin in hexaploid wheat. Cereal Research Communications. 1983;**11**:29-34

[15] Shewry PR, Tatham A, Halford NG. The prolamins of the Triticeae. In: Shewry P, Casey R, editors. Seed Proteins. Dordrecht, The Netherlands: Kluwer Academic Publishers; 1999. p. 35-78. DOI: 10.1007/978-94-011-4431-5

[16] Shewry PR, Halford NG. Cereal seed storage proteins: Structures, properties and role in grain utilization. Journal of Experimental Botany. 2002;**53**:947-958. DOI: 10.1093/jexbot/53.370.947

[17] MacRitchie F. Baking quality of wheat flour. Advanced in Food Research. 1984;**29**:201-277. DOI: 10.1016/S0065-2628(08)60058-0

[18] MacRitchie F, Ducros DL, Wrigley CW. Flour polypeptide related to wheat quality. In: Pomeranz Y, editor. Advances in Cereal Science and Technology. St Paul, USA: American Association of Cereal Chemists; 1990. Vol. X. p. 79-145. DOI: 10.1002/food.19910350434

[19] Shewry PR, Popineau Y, Lafiandra D, Belton P. Wheat glutenin subunits and dough elasticity: Findings of the EUROWHEAT project. Trends in Food Science and Technology. 2001;**11**:433-441. DOI: 10.1016/S0924-2244(01)00035-8

[20] Lee JW, MacRitchie F. The effect of gluten protein fractions on dough properties. Cereal Chemistry. 1971;**48**:620-625

[21] MacRitchie F. Studies of gluten proteins from wheat flours. Cereal Foods World. 1980;**25**:382-385

[22] Bietz JA. Analysis of wheat gluten proteins by high-performance liquid-chromatography. Part I. Baker's Digest. 1984;**58**:15-17, 20-21, 32

[23] Bietz JA. High performance liquid chromatography of cereal proteins. In: Pomeranz Y, editor. Advances in Cereal Science and Technology. Vol. Vol. VIII. St-Paul: AACC; 1986. pp. 105-170

[24] Huebner FR, Bietz JA. Detection of quality differences among wheats by high performance liquid chromatography. Journal of Chromatography. 1985;**327**:333-342. DOI: 10.1016/S0021-9673(01)81662-9

[25] Huebner FR, Wall JS. Fractionation and quantitative differences of glutenin from wheat varieties varying in baking quality. Cereal Chemistry. 1976;**53**:258-260

[26] Orsi F, Pallagi E, Bekes F, Lasztity L. Investigation of wheat proteins by high performance gel chromatography. In: Proceedings of the 3rd Int. Workshop Gluten Proteins, Budapest. Singapore: World Scientific; 1987. pp. 141-160

[27] Dachkevitch T, Autran JC. Prediction of baking quality of bread wheats in breeding programs by size-exclusion high-performance liquid chromatography. Cereal Chemistry. 1989;**66**:448-456

[28] Singh NK, Donovan R, Batey IL, MacRitchie F. Use of sonication and size exclusion high-performance liquid chromatography in the study of wheat flour proteins. I. Dissolution of total proteins in the absence of reducing agents. Cereal Chemistry. 1990;**67**:150-161

[29] Singh NK, Donovan R, MacRitchie F. Use of sonication and size exclusion high-performance liquid chromatography in the study of wheat flour proteins. II. Relative quantity of glutenin as a measure of breadmaking quality. Cereal Chemistry. 1990;**67**:161-170

[30] Weegels PL, Hamer RJ, Schofield JD. Critical review. Functional properties of wheat glutenin. Journal of Cereal Science. 1996;**23**:1-18. DOI: 10.1006/jcrs.1996.0001

[31] Southan M, MacRitchie F. Molecular weight distribution of wheat proteins. Cereal Chemistry. 1999;**76**:827-836. DOI: 10.1094/CCHEM.1999.76.6.827

[32] MacRitchie F, Lafiandra D. Structure-function relationships of wheat proteins. In: Damodaran S, Paraf A, editors. Food Proteins and Their Applications. New York: Marcel Dekker; 1997. pp. 293-323

[33] Chen CH, Bushuk W. Nature of proteins in Triticale and its parental species I. Solubility characteristics and amino-acid composition of endosperm proteins. Canadian Journal of Plant Science. 1970;**50**:9-14. DOI: 10.4141/cjps70-002

[34] Orth RA, Bushuk W. A comparative study of proteins of wheats of diverse baking quality. Cereal Chemistry. 1972;**49**:268-275

[35] Khan K, Bushuk W. Glutenin: Structure and functionality in breadmaking. Baker's Digest. 1978; April:14-20

[36] Graveland A. Extraction of wheat proteins with sodium dodecyl sulphate. Annales de Technologie Agricole. 1980;**29**:113-123

[37] Gupta RB, Khan K, MacRitchie F. Biochemical basis of flour properties in bread wheats. I. Effects of variation in the quantity and size distribution of polymeric protein. Journal of Cereal Science. 1993;**18**:23-41. DOI: 10.1006/jcrs.1993.1031

[38] Graveland A, Bosveld P, Lichtendonk WJ, Moonen JHE. Extraction and fractionation of wheat flour proteins. Journal of the Science of Food and Agriculture. 1982;**33**:1117-1128. DOI: 10.1002/jsfa.2740331109

[39] Weegels PL, Flissebaalje T, Hamer R. Factors affecting the extractability of the glutenin macropolymer. Cereal Chemistry. 1994;**71**:308-309

[40] Weegels PL, Van de Pijpekamp AM, Graveland A, Hamer RJ, Schofield JD. Depolymerisation and repolymerisation of wheat gluten during dough processing I. Relationships between GMP content and quality parameters. Journal of Cereal Science. 1996;**23**:103-111. DOI: 10.1006/jcrs.1996.0010

[41] Moonen JHE, Scheepstra A, Graveland A. Use of the SDS-sedimentation test and SDSpolyacrylamide gel electrophoresis for screening breeder's samples of wheat for bread-making quality. Euphytica. 1986;**31**:677-690. DOI: 10.1007/BF00039206

[42] Weegels PL, Hamer RJ, Schofield JD. Depolymerisation and re-polymerisation of wheat glutenin during dough processing. II. Changes in composition. Journal of Cereal Science. 1997;**25**:155-163. DOI: 10.1006/jcrs.1996.0082

[43] Giddings JC. Field-flow fractionation: Analysis of macromolecular, colloids, and particulate materials. Science. 1993;**260**:1456-1465. DOI: 10.1126/science.8502990

[44] Giddings JC, Benincasa MA, Liu MK, Li P. Separation of water soluble synthetic and biological macromolecules by flow field-flow fractionation. Journal of Liquid Chromatography. 1992;**15**:1729-1747. DOI: 10.1080/10826079208018323

[45] Wahlund KG, Giddings JC. Properties of an asymmetrical flow field-flow fractionation channel having one permeable wall. Analytical Chemistry. 1987;**59**:1332-1339. DOI: 10.1021/ac00136a016

[46] Litzén A, Walter JK, Krischollek H, Wahlund KG. Separation and quantification of monoclonal antibody aggregates by asymmetrical flow field-flow fractionation and comparison to gel permeation chromatography. Analytical Biochemistry. 1993;**212**:469-480. DOI: 10.1006/abio.1993.1356

[47] Bean SR, Lookhart GL. Factors influencing the characterization of gluten proteins by size-exclusion chromatography and multiangle laser light scattering (SEC-MALLS). Cereal Chemistry. 2001;**78**:608-618. DOI: 10.1094/CCHEM.2001.78.5.608

[48] Carceller JL, Aussenac T. Size characterisation of glutenin polymers by HPSEC-MALLS. Journal of Cereal Science. 2001;**33**:131-142. DOI: 10.1006/jcrs.2000.0356

[49] Giddings JC, Yang FJ, Myers MN. Flow field-flow fractionation as a methodology for protein separation and characterization. Analytical Biochemistry. 1977;**81**:395-407. DOI: 10.1016/0003-2697(77)90710-2

[50] Stevenson SG, Preston KR. Flow field-flow fractionation of wheat proteins. Journal of Cereal Science. 1996;**23**:121-131. DOI: 10.1006/jcrs.1996.0012

[51] Wahlund KG, Gustavsson M, MacRitchie F, Nylander T, Wannerberger L. Size characterisation of wheat proteins, particularly glutenin, by asymmetrical flow field-flow fractionation. Journal of Cereal Science. 1996;**23**:113-119. DOI: 10.1006/jcrs.1996.0011

[52] Ueno T, Stevenson SG, Preston KR, Nightingale MJ, Marchylo BM. Simplified dilute acetic acid-based extraction procedure for fractionation and analysis of wheat flour protein by size exclusion HPLC and flow field-flow fractionation. Cereal Chemistry. 2002;**79**:155-161. DOI: 10.1094/CCHEM.2002.79.1.155

[53] Wyatt PJ. Combined differential light scattering with various liquid chromatography techniques. In: Harding SE, Sattelle DB, Bloomfield VA, editors. Laser Light Scattering in Biochemistry. Wiltshire, UK: Royal Society of Chemistry; 1992. pp. 35-58

[54] Egorov TA, Odintsova TI, Shewry PR, Tatham AS. Characterisation of high *M*r wheat glutenin polymers by agarose gel electrophoresis and dynamic light scattering. FEBS Letters. 1998;**434**:215-217. DOI: 10.1016/S0014-5793(98)00983-1

[55] Stevenson SG, You S, Isydorczyk MS, Preston KR. Characterization of polymeric wheat proteins by flow field-flow fractionation/MALLS. Journal of Liquid Chromatography and Related Technololgy. 2003;**26**:2771-2781. DOI: 10.1081/JLC-120025044

[56] Arfvidsson C, Wahlund KG, Eliasson AC. Direct molecular weight determination in the evaluation of dissolution methods for unreduced glutenin. Journal of Cereal Science. 2004;**39**:1-8. DOI: 10.1016/S0733-5210(03)00038-9

[57] Lemelin E, Aussenac T, Violleau F, Salvo L, Lein V. Impact of cultivar and environment on size characteristics of wheat proteins using asymmetrical flow field-flow fractionation and multi-angle laser light scattering. Cereal Chemistry. 2005;**82**:28-33. DOI: 10.1094/CC-82-0028

[58] Gupta RB, Paul JG, Cornish GB, Palmer GA, Bekes F, Rathjen AJ. Allelic variation at glutenin subunit and gliadin loci, *Glu-1*, *Glu-3*, and *Gli-1*, of common wheats. I. Its additive and interaction effects on dough properties. Journal of Cereal Science. 1994;**19**:9-17. DOI: 10.1006/jcrs.1994.1004

[59] Gupta RB, Popineau Y, Lefebre J, Cornec M, MacRitchie F. Biochemical basis of flour properties in bread wheats. II. Changes in polymeric protein formation and dough/gluten properties associated with the loss of low Mr or high Mr glutenin subunits. Journal of Cereal Science. 1995;**21**:103-116. DOI: 10.1016/0733-5210(95)90026-8

[60] Larroque OR, Gianibelli MC, Batey IL, MacRitchie F. Electrophoretic characterisation of fractions collected from gluten protein extracts subjected to size-exclusion high-performance liquid chromatography. Electrophoresis. 1997;**18**:1064-1067. DOI: 10.1002/elps.1150180706

[61] Lindsay MP, Skerritt JH. Examination of the structure of the glutenin macropolymer in wheat flour and doughs by stepwise reduction. Journal of Agricultural and Food Chemistry. 1998;**46**:3447-3457. DOI: 10.1021/jf980315m

[62] Skerritt JH, Hac L, Bekes F. Depolymerisation of the glutenin macropolymer during dough mixing. I. Changes in levels, molecular weight distribution, and overall composition. Cereal Chemistry. 1999;**76**:395-401. DOI: 10.1094/CCHEM.1999.76.3.395

[63] Aussenac T, Carceller JL, Kleiber D. Changes in SDS solubility of glutenin polymers during dough mixing and resting. Cereal Chemistry. 2001;**78**:39-45. DOI: 10.1094/CCHEM.2001.78.1.39

[64] Veraverbeke WS, Courtin CM, Verbruggen IM, Delcour JA. Factors governing levels and composition of the sodium dodecyl sulphate unextractable gutenin polymers during straight dough breadmaking. Journal of Cereal Science. 1999;**29**:129-138. DOI: 10.1006/jcrs.1998.0232

[65] Kasarda DD. Glutenin polymers: The in vitro to in vivo transition. Cereal Foods World. 1999;**44**:566-571

[66] Bechtel D, Abecassis J, Shewry PR, Evers T. The development, structure and mechanical properties of the wheat grain. In: Khan K, Shewry PR, editors. Wheat: Chemistry and Technology. fourth ed. St Paul, MN, USA: AACC; 2009. pp. 51-95

[67] Carceller JL, Aussenac T. Accumulation and changes in molecular size distribution of polymeric proteins in developing grains of hexaploid wheats: Role of the dessication phase. Australian Journal of Plant Physiology. 1999;**26**:301-310. DOI: 10.1071/PP99010

[68] Jenner CF, Ugalde TD, Aspinall D. The physiology of starch and protein deposition in the endosperm of wheat. Australian Journal of Plant Physiology. 1991;**18**:211-226. DOI: 10.1071/PP9910211

[69] Triboï E. Modèle d'élaboration du poids du grain chez le blé tendre (*Triticum aestivum* em. Thell.). Agronomie. 1990;**10**:191-200

[70] Dale EH, Houstley TL. Sucrose synthase activity in developing wheat endosperms differing in maximum weight. Plant Physiology. 1986;**82**:7-10. DOI: 10.1104/pp.82.1.7

[71] Wang YP, Gifford RM. A model of wheat grain growth and its applications to different temperature and carbon dioxide levels. Australian Journal of Plant Physiology. 1995;**22**:843-855. DOI: 10.1071/PP9950843

[72] Bechtel DB, Gaines RL, Pomeranz Y. Protein secretion in wheat endosperm—formation of the matrix protein. Cereal Chemistry. 1982;**59**:336-343

[73] Tosi P. Trafficking and deposition of prolamins in wheat. Journal of Cereal Science. 2012;**56**:81-90. DOI: 10.1016/j.jcs.2012.02.004

[74] Brooks A, Jenner CF, Aspinall D. Effects of water deficit on endosperm starch granules and on grain physiology of wheat and barley. Australian Journal of Plant Physiology. 1982;**9**:423-436. DOI: 10.1071/PP9820423

[75] Nicolas ME, Gleadow RM, Dalling MJ. Effects of drought and high temperature on grain growth in wheat. Australian Journal of Plant Physiology. 1984;**11**:553-566. DOI: 10.1071/PP9840553

[76] Morris CF, Paulsen GM. Development of hard winter wheat after anthesis as affected by nitrogen nutrition. Crop Science. 1985;**25**:1010. DOI: 10.2135/cropsci1985.0011183X002500060026x

[77] Wardlaw IF, Dawson IA, Munibi P, Fewster R. The tolerance of wheat to high temperatures during reproductive growth. I. Survey procedures and general response patterns. Australian Journal of Agricultural Research. 1989;**40**:1-13. DOI: 10.1071/AR9890001

[78] Tashiro T, Wardlaw IF. The response to high temperature shock and humidity changes prior to and during the early stages of grain development in wheat. Australian Journal of Plant Physiology. 1990;**17**:551-561. DOI: 10.1071/PP9900551

[79] Randall PJ, Moss HJ. Some effects of temperature regime during grain filling on wheat quality. Australian Journal of Agricultural Research. 1990;**41**:603-617. DOI: 10.1071/AR9900603

[80] Kobata T, Palta JA, Turner NC. Rate of development of postanthesis water deficits and grain filling of spring wheat. Crop Science. 1992;**32**:1238-1242. DOI: 10.2135/cropsci1992.0011183X003200050035x

[81] Gibson LR, Paulsen GM. Yield components of wheat grown under high temperature stress during reproductive growth. Crop Science. 1999;**39**:1841-1846. DOI: 10.2135/cropsci1999.3961841x

[82] Daniel C, Triboi E. Effects of temperature and nitrogen nutrition on the grain composition of winter wheat: Effects on gliadin content and composition. Journal of Cereal Science. 2000;**32**:45-56. DOI: 10.1006/jcrs.2000.0313

[83] Yang J, Zhang J, Zhu Q, Wang L. Remobilization of carbon reserves is improved by controlled soil-drying during grain filling of wheat. Crop Science. 2000;**40**:1645-1655. DOI: 10.2135/cropsci2000.4061645x

[84] Guedira M, McCluskey PJ, MacRitchie F, Paulsen GM. Composition and quality of wheat grown under different shoot and root temperatures during maturation. Cereal Chemistry. 2002;**79**:397-403. DOI: 10.1094/CCHEM.2002.79.3.397

[85] Altenbach SB, DuPont FM, Kothari KM, Chan R, Johnson EL, Lieu D. Temperature, water and fertilizer influence the timing of key events during grain development in a US spring wheat. Journal of Cereal Science. 2003;**37**:9-20. DOI: 10.1006/jcrs.2002.0483

[86] DuPont FM, Altenbach SB. Molecular and biochemical impacts of environmental factors on wheat grain development and protein synthesis. Journal of Cereal Science. 2003;**38**:133-146. DOI: 10.1016/S0733-5210(03)00030-4

[87] Tosi P, Parker M, Gritsch CS, Carzaniga R, Martin B, Shewry PR. Trafficking of storage proteins in developing grain of wheat. Journal of Experimental Botany. 2009;**60**:979-991. DOI: 10.1093/jxb/ern346

[88] Jennings AC, Morton RK. Changes in carbohydrate, protein and non-protein nitrogenous compounds of developing wheat grains. Australian Journal of Biological Sciences. 1963;**16**:318-331. DOI: 10.1071/BI9630318

[89] Flint D, Ayers GS, Ries SK. Synthesis of endosperm proteins in wheat seed during maturation. Plant Physiology. 1975;**56**:381-384. DOI: 10.1104/pp.56.3.381

[90] Stenram U, Heneen WK, Oleredd R. The effect of nitrogen fertilizers on protein accumulation in wheat (Triticum aestivum L.). Swedish Journal of Agricultural Research. 1990;**20**:105-114

[91] Gupta RB, Masci S, Lafiandra D, Bariana HS, MacRitchie F. Accumulation of protein subunits and their polymers in developing grains of hexaploid wheats. Journal of Experimental Botany. 1996;**47**:1377-1385. DOI: 10.1093/jxb/47.9.1377

[92] Stone PJ, Nicolas ME. Varietal differences in mature protein composition of wheat resulted from different rates of polymer accumulation during grain filling. Australian Journal of Plant Physiology. 1996;**23**:727-737. DOI: 10.1071/PP9960727

[93] Shewry PR, Underwood C, Wan YF, Lovegrove A, Bhandari D, Toole G, Mills ENC, Denyer K, Mitchell RAC. Storage product synthesis and accumulation in developing grains of wheat. Journal of Cereal Science. 2009;**50**:106-112. DOI: 10.1016/j.jcs.2009.03.009

[94] Kaczkowski J, Kos S, Moskal M. Protein functional composition in developing wheat grains. Die Nahrung. 1986;**30**:437-439

[95] Huebner FR, Kaczkowski J, Bietz JA. Quantitative variations of wheat proteins form grain at different stages of maturity and from different spike locations. Cereal Chemistry. 1990;**67**:464-470

[96] Bénétrix F, Kaan F, Autran JC. Changes in protein complexes of durum wheat in developing seed. Crop Science. 1994;**34**:462-468. DOI: 10.2135/cropsci1994.0011183X003400020029x

[97] Woodman HE, Engledow FJ. A chemical study of the development of the wheat grains. The Journal of Agricultural Science. 1924;**14**:563-586

[98] Seilmeier W, Wieser H, Belitz HD. Weizen während der Reifung. Analyse der gliadine und glutenine mittels RP-HPLC. Zeitschrift für Lebensmittel-Untersuchung und -Forschung. 1990;**191**:99-103

[99] Carceller JL, Aussenac T. SDS-insoluble glutenin polymer formation in developing grains of hexaploid wheat: The role of the ratio of high to low molecular weight glutenin subunits and drying rate during ripening. Australian Journal of Plant Physiology. 2001;**28**:193-201. DOI: 10.1071/PP00002

[100] Ferreira MS, Samson MF, Bonicel J, Morel MH. Relationship between endosperm cells redox homeostasis and glutenin polymers assembly in developing durum wheat grain. Plant Physiology and Biochemistry. 2012;**61**:36-45. DOI: 10.1016/j.plaphy.2012.08.015

[101] Ferrise R, Bindi M, Martre P. Grain filling duration and glutenin polymerization under variablenitrogen supply and environmental conditions for durum wheat. Field Crops Research. 2015;**171**:23-31. DOI: 10.1016/j.jcs.2016.12.003

[102] Koga S, Böcker U, Wieser H, Koehler P, Uhlen AK, Moldestad A. Polymerisation of gluten proteins in developing wheat grain as affected by desiccation. Journal of Cereal Science. 2017;**73**:122-129. DOI: 10.1016/j.jcs.2016.12.003

[103] Rhazi L, Cazalis R, Aussenac T. Sulfhydryl-disulfide changes in storage proteins of developing wheat grain: Influence on the SDS-unextractable glutenin polymer formation. Journal of Cereal Science. 2003;**38**:3-13. DOI: 10.1016/S0733-5210(03)00019-5

[104] Gobin P, Ng PKW, Buchanan BB, Kobrehel K. Sulfhydryl-disulfide changes in proteins of developing wheat grain. Plant Physiology and Biochemistry. 1997;**35**:777-783

[105] Hamer R, van Vliet T. Understanding the structure and properties of gluten and overview. In: Shewry PR, Tatham AS, editors. Wheat Gluten. Cambridge: Royal Society of Chemistry; 2000. pp. 125-131

[106] Belton PS, Colquhoun IJ, Grant A, Wellner N, Field JM, Shewry PR, Tatham AS. FTIR and NMR studies of high Mr subunit of glutenin. International Journal of Biological Macromolecules. 1995;**17**:74-80. DOI: 10.1016/0141-8130(95)93520-8

[107] Belton PS. On the elasticity of wheat gluten. Journal of Cereal Science. 1999;**29**:103-107. DOI: 10.1006/jcrs.1998.0227

[108] Chen X, Schofield JD. Determination of protein-glutathion mixed disulfides in wheat flour. Journal of Agricultural and Food Chemistry. 1995;**43**:2362-2368. DOI: 10.1021/jf00057a009

[109] Wrigley CW, DL dC, Fullington JG, Kasarda DD. Changes in polypeptide composition and grain quality due to sulfur deficiency in wheat. Journal of Cereal Science. 1984;**2**:15-24. DOI: 10.1016/S0733-5210(84)80003-X

[110] Ciaffi M, Tozzi L, Borghi B, Corbellini M, Lafiandra D. Effect of heat shock during grain filling on the gluten protein composition of bread wheat. Journal of Cereal Science. 1996;**24**:91-100. DOI: 10.1006/jcrs.1996.0042

[111] Graybosch RA, Peterson CJ, Baenziger PS, Shelton DR. Environmental modification of hard red winter wheat flour protein composition. Journal of Cereal Science. 1995;**22**:45-51. DOI: 10.1016/S0733-5210(05)80006-2

[112] Borghi B, Corbellini M, Ciaffi M, Lafiandra D, De Stefanis E, Sgrulleta S, Boggini G, Di Fonzo N. Effects of heat shock during grain filling on grain quality of bread and durum wheats. Australian Journal of Agricultural Research. 1995;**46**:1365-1380. DOI: 10.1071/AR9951365

[113] Wieser H, Seilmeier W. The influence of nitrogen fertilization on quantities and proportions of different protein types in wheat flour. Journal of the Science of Food and Agriculture. 1998;**76**:49-55. DOI: 10.1002/(SICI)1097-0010(199801)76:1<49::AID-JSFA950>3.0.CO;2-2

[114] Panozzo JF, Eagles HA. Cultivar and environment effects on quality characters in wheat. II. Protein. Australian Journal of Agricultural Research. 2000;**51**:629-636. DOI: 10.1071/AR99137

[115] Luo C, Branlard G, Griffin WB, McNeil DL. The effect of nitrogen and sulphur fertilization and their interaction with genotype on wheat glutenins and quality parameters. Journal of Cereal Science. 2000;**31**:185-194. DOI: 10.1006/jcrs.1999.0298

[116] Johansson E, Prieto-Linde ML, Jonsson JO. Effects of wheat cultivar and nitrogen application on storage protein composition and breadmaking quality. Cereal Chemistry. 2001;**78**:19-25. DOI: 10.1094/CCHEM.2001.78.1.19

[117] Vaughan B, Westfall DG, Barbarick KA. Nitrogen rate and timing effects on winter wheat grain yield, grain protein, and economics. Journal of Production Agriculture. 1990;**3**:324-328. DOI: 10.2134/jpa1990.0324

[118] Uhlen AK, Dieseth JA, Koga S, Böcker U, Hoel B, Anderson JA, Moldestad A. Variation in gluten quality parameters of spring wheat varieties of different origin grown in contrasting environments. Journal of Cereal Science. 2015;**62**:110-116. DOI: 10.1016/j.jcs.2015.01.004

[119] Johansson E, Malik AH, Hussain A, Rasheed F, Newson WR, Plivelic T, Hedenqvist MS, Gällstedt M, Kuktaite R. Wheat gluten polymer structures: The impact of genotype, environment, and processing on their functionality in various applications. Cereal Chemistry. 2013;**90**:367-376. DOI: 10.1094/CCHEM-08-12-0105-F

[120] Zhu J, Kahn K, Huang S, O'Brien L. Allelic variation at Glu-D1 locus for high molecular weight (HMW) glutenin subunits: Quantification by multistacking SDS–PAGE of wheat grown under nitrogen fertilization. Cereal Chemistry. 1999;**76**:915-919. DOI: 10.1094/CCHEM.1999.76.6.915

[121] Finlay GJ, Bullock PR, Sapirstein HD, Naeem HA, Hussain A, Angadi SV, DePauw RM. Genotypic and environmental variation in grain, flour, dough and bread-making characteristics of western Canadian spring wheat. Canadian Journal of Plant Science. 2007;**87**:679-690. DOI: 10.4141/P06-150

[122] Gouache D, Le Bris X, Bogarda M, Deudonc O, Pagé C, Gate P. Evaluating agronomic adaptation options to increasing heat stress under climate change during wheat grain filling in France. European Journal of Agronomy. 2012;**39**:62-70. DOI: 10.1016/j.eja.2012.01.009

[123] Jia YQ, Fabre JL, Aussenac T. Effects of growing location on response of protein polymerization to increased nitrogen fertilization for the common wheat cultivar Soissons: Relationship with some aspects of the breadmaking quality. Cereal Chemistry. 1996;**73**:526-532

[124] MacRitchie F, Gupta RB. Functionality-composition relationships of wheat flour as a result of variation in sulfur availability. Australian Journal of Agricultural Research. 1993;**44**:1767-1774. DOI: 10.1071/AR9931767

[125] Wooding AR, Martin RJ, MacRitchie F. Effect of sulfur nitrogen treatments on work input requirements for dough mixing in a second season. In: Proceedings of the 44th Australian Cereal Chemistry Conf. RACI. Australia, Melbourne; 1994. pp. 219-222

[126] Blumenthal CS, Barlow EWR, Wrigley CW. Growth environment and wheat quality. Journal of Cereal Science. 1993;**18**:3-21. DOI: 10.1006/jcrs.1993.1030

[127] Bernardin JE, Witt SC, Milenic J. Effect of heat stress on the pattern of protein synthesis in wheat endosperm. In: Proceeding of the 44th Australian Cereal Chemistry Conference RACI. Melbourne; 1994. pp. 37-41

[128] Stone P, Nicolas ME. Wheat cultivars vary widely in their responses of grain yield and quality to short periods of post-anthesis heat-stress. Functional Plant Biology. 1994;**21**:887-900. DOI: 10.1071/PP9940887

[129] Stone P, Nicolas ME. Effect of timing of heat stress during grain filling on two varieties differing in heat tolerance. II. Fractional protein accumulation. Australian Journal of Plant Physiology. 1996;**23**:739-749. DOI: 10.1071/PP9960739

[130] Johansson E, Nilsson H, Mazhar H, Skerritt J, MacRitchie F, Svensson G. Seasonal effects on storage proteins and gluten strength in four Swedish wheat cultivars. Journal of the Science of Food and Agriculture. 2002;**82**:1305-1311. DOI: 10.1002/jsfa.1185

[131] Quartacci MF, Navari-Izzo F. Water stress and free radical mediated changes in sunflower seedlings. Journal of Plant Physiology. 1992;**139**:621-625. DOI: 10.1016/S0176-1617(11)80381-0

[132] Simontacchi M, Puntarulo S. Oxygen radical generation by isolated microsomes from soybean seedlings. Plant Physiology. 1992;**100**:1263-1268. DOI: 10.1104/pp.100.3.1263

[133] Leprince O, Deltour R, Thorpe PC, Atherton NM, Hendry GAF. The role of free radicals and radical processing systems in loss of dessication tolerance in germinating maize (Zea mays L.). New Phytologist. 1990;**116**:573-580. DOI: 10.1111/j.1469-8137.1990.tb00541.x

[134] Leprince O, Atherton NM, Deltour R, Hendry GAF. The involvement of respiration in free radical processesduring loss of dessication tolerance in germinationg Zea mays L. An electron paramagnetic resonance study. Plant Physiology. 1994;**104**:1333-1339. DOI: 10.1104/pp.104.4.1333

[135] Hendry GAF. Oxygen, free radical processes and seed longevity. Seed Science Research. 1993;**3**:141-153. DOI: 10.1017/S0960258500001720

[136] Foyer CH, Halliwell B. The presence of gluthatione and glutathione reductase in chloroplasts: A proposed role in ascorbic acid metabolism. Planta. 1976;**133**:21-25. DOI: 10.1007/BF00386001

[137] Foyer CH, Lelandais M, Kunert K. Photooxydative stress in plants. Physiologia Plantarum. 1994;**92**:696-717. DOI: 10.1111/j.1399-3054.1994.tb03042.x

[138] Klapheck S, Zimmer I, Cosse H. Scavenging of hydrogen peroxide in the endosperm of Ricinus communis by ascorbate peroxydase. Plant and Cell Physiology. 1990;**31**:1005-1013. DOI: 10.1093/oxfordjournals.pcp.a077996

[139] Cakmak I, Marschner H. Activities of hydrogen peroxide-scavenging enzymes in germinating wheat seeds. Journal of Experimental Botany. 1993;**44**:127-132. DOI: 10.1093/jxb/44.1.127

[140] Renneberg H. Glutathione metabolism and possible biological roles in higher plants. Phytochemistry. 1982;**21**:2771-2781. DOI: 10.1016/0031-9422(80)85045-X

[141] Alsher RG. Biosynthesis and antioxydant function of glutathione in plants. Physiologia Plantarum. 1989;**77**:457-464. DOI: 10.1111/j.1399-3054.1989.tb05667.x

[142] Smith IK, Polle A, Renneberg H. Glutathione. In: Alsher RG, Cumming J, editors. Stress Responses in Plants. New York: Wiley-Liss Inc; 1990. pp. 201-215

[143] Jocelyn PC. Biochemistry of the SH group. London: Academic Press; 1972. pp. 63-136

[144] Gilbert HF, McLean V, McLean M. Molecular and cellular aspects of thiol-disulfide exchange. Advances in Enzymology and Related Areas of Molecular Biology. 1990;**63**:169-172. DOI: 10.1002/SERIES2011

[145] Dhindsa RS. Glutathione redox status and protein synthesis during drought and subsequent rehydration. Plant Physiology. 1987;**83**:816-919. DOI: 10.1104/pp.83.4.816

[146] De Gara L, Pinto M, Moliterni V, D'Egidio M. Redox regulation and storage processes during maturation in kernels of *Triticum durum*. Journal of Experimental Botany. 2003;**54**:249-258. DOI: 10.1093/jxb/erg021

[147] Kunert KJ, Foyer C. Thiol/disulfide exchange in pants. In: Dekok LJ, Stulen I, Rennenberg H, Brunold C, Rauser WE, editors. Sulfur Nutrition and Assimilation in Higher Plants. The Hague: SPB Academic Publishing; 1993. pp. 139-151

[148] Kranner I, Grill D. Significance of thiol-disulfide exchange in resting stages of plant development. Botanica Acta: Journal of the German Botanical Society. 1996;**109**:8-14. DOI: 10.1111/j.1438-8677.1996.tb00864.x

[149] Fahey RC, Di Stefano DL, Meier GP, Bryan RN. Role of hydration state and thiol-disulfide status in the control of thermal stability and protein synthesis in wheat embryo. Plant Physiology. 1980;**65**:1062-1066. DOI: 10.1104/pp.65.6.1062

[150] Kranner I, Grill D. Content of low-molecular-weight thiols during the imbibition of pea seeds. Physiologia Plantarum. 1993;**88**:557-562. DOI: 10.1111/j.1399-3054.1993.tb01371.x

[151] Butt AD, Ohlrogge JB. Acyl carrier protein is conjugated to glutathione in spinach seed. Plant Physiology. 1991;**96**:937-942. DOI: 10.1104/pp.96.3.937

[152] Kobrehel K, Yee BC, Buchanan BB. Role of the NADP/thioredoxin system in the reduction of α-amylase and trypsin inhibitor proteins. The Journal of Biological Chemistry. 1991;**266**:16135-16140

[153] Kobrehel K, Wong JH, Balogh A, Kiss F, Yee BC, Buchanan BB. Specific reduction of wheat storage proteins by thioredoxin. Plant Physiology. 1992;**99**:919-924. DOI: 10.1104/pp.99.3.919

[154] Gerna D, Roach T, Stöggl W, Wagner J, Vaccino P, Limonta M, Kranner I. Changes in low-molecular-weight thiol-disulphide redox couples are part of bread wheat seed

germination and early seedling growth. Free Radical Research. 2017;**51**:568-581. DOI: 10.1080/10715762.2017.1338344

[155] Sarwin R, Walther C, Laskawy G, Butz B, Grosch W. Determination of free reduced and total glutathione in wheat flours by a radioisotope dilution assay. Zeitschrift für Lebensmittel-Untersuchung und -Forschung. 1992;**195**:27-32

[156] Rhazi L, Cazalis R, Lemelin E, Aussenac T. Changes in the glutathione thiol–disulfide status during wheat grain development. Plant Physiology and Biochemistry. 2003;**41**:895-902. DOI: 10.1016/S0981-9428(03)00134-7

[157] Köhler P, Hüttner S, Wieser H. Binding sites of glutathione in gluten proteins. In: Wrigley CW, editor. Gluten 96. North Melbourne, Australia: Royal Australian Chemical Institute; 1996. pp. 137-140

[158] Luque A, Hegedus S. Science and Engineering. 2nd ed. Chichester: Wiley; 2011. p. 1132. DOI: 10.1002/9780470974704

[159] Luque A, Hegedus S, editors. Handbook of Photovoltaic Science and Engineering. 2nd ed. Chichester: Wiley; 2011. p. 1132. DOI: 10.1002/978047974704

[160] Ceccaroli B, Lohne O. Solar grade silicon feedstock. In: Luque A, Hegedus S, editors. Handbook of Photovoltaic Science and Engineering. 2nd ed. Chichester: Wiley; 2011. pp. 169-217. DOI: 10.1002/978047974704.ch5

[161] Kajihara A, Harakawa T. Model of photovoltaic cell circuits under partial shading. In: Proceedings of the IEEE International Conference on Industrial Technology (ICIT '05); 14-17 December 2005. Hong Kong. New York: IEEE; 2006. pp. 866-870

Chapter 9

Mutant Resources of Spring Wheat to Improve Grain Quality and Morphology

Saule Kenzhebayeva, Gulina Doktyrbay,
Fatma Sarsu, Nargul Omirbekova,
Alfia Abekova and Dauren Tashenev

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.75706

Abstract

The objective of this study was to broaden genetic variation of spring common wheat, evaluate and identify among M_5 mutant lines those with high-yield and improved grain quality characteristics. New lines were generated by initial treatment of variety of Eritrospermum-35 adapted to conditions of Kazakhstan by irradiation with 100-Gy and 200-Gy doses from a ^{60}Co source. Yield-associated traits including grain number and weight per main spike, grain weight (GW) per plant, and the thousand-kernel weight; grain size and grain shape variations; as well as quality characteristics such as grain protein content (GPC), iron, and zinc concentrations were evaluated. Mutant lines with high iron and zinc concentrations and high protein content were identified as those which have 1.6–3.4 and 1.4–2.9 times more as well as 3.7–16.9% more higher data of target concentrations than parental variety had, respectively. Several mutant lines showed significant increase in both grain iron and zinc concentrations. The positive correlation of grain quality parameters with grain area, length, and width suggest that they are related to each other. Wheat grain can be biofortified with micronutrients without negative impact on crop productivity using new mutant lines. Mutation breeding can significantly contribute to human health malnutrition and improve nutrition quality diet.

Keywords: biofortification, high productivity, new mutation resources, spring wheat, micronutrients

IntechOpen

© 2018 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. CC BY

1. Introduction

Bread wheat (*Triticum aestivum* L.) is a main crop with global importance for food safety and one of the major cereal source of nutrients for both humans and animals. This balance of consumption of required nutrient for human metabolic needs generally resulted in serious metabolic violations leading to sickness, poor health, suppressing of children development, and high economic expenses for society [1]. It is necessary for agricultural systems to ensure proper products, which will balance quantity of nutrients to support healthy life. However, in many developing countries, agriculture does not meet these requirements [2].

Presently, over three billion people suffer due to micronutrient malnutrition and the numbers are increasing [3]. There are many human diseases associated with nutritional deficiency, and around two-thirds of all children's deaths are related to malnutrition [4, 5].

Improvement of many agronomic traits including grain quality requires their genetic variation, which should be separable from non-genetic impacts. Over the years, the main focus of wheat breeding programs was the replacement of traditional varieties with modern high-yield ones that led to reduction of its genetic diversity mainly by end-use quality characteristics and nutrition quality (FAO Document Repository, 2015). The key desired traits for breeding were high yield and disease resistance. It is also known that the genetic variability of major crops currently have systematically decreased due to the repeated utilization of the local adapted genotypes in breeding processes which leads to the decreasing of the wide genetic recombination which may posse the novel traits [6].

Crops' genetic variability and therefore crop improvement could be powerfully generated through mutation breeding. Over the past 80 years, this approach has been applied for development of new mutant varieties of both seeds and vegetatively propagated crops [7–9]. According to the FAO/IAEA Mutant Variety Database, in 2014, there were 3220 mutant plant varieties of 214 plant species all round the world [10] (http://mvgs.iaea.org/). Application of mutagenesis in crop breeding has two main steps: the selection of individual mutants with improved traits after the mutagenic treatment and utilization of selected plants or lines in breeding programs [7].

One of the greatest contributions into crop yield is grain weight trait [11] which is directly related to two important morphometric characteristics such as grain size and grain shape. Grain size and grain shape are essential breeding traits because they are phenotypically the most stable of the yield components. They also greatly influence the grinding process, yield of flour, which is the main source for human consumption with a lot of other end-use utilizations, and starch damage [12]. Larger grains have strong contribution to higher grain weight in addition to increase in seedling vigor and production of dry matter of the raw material in the field [12, 13]. Grain size can be generally characterized by grain width (GW) and grain area (GA), however grain shape also determines along the grain's main growth axis. Grain shape is measured by grain length (GL), grain width (GW), vertical perimeter, sphericity, and determination along the horizontal axis [12, 14]. It is very substantial to improve the wheat grain morphometry according to the demand of grain market and processing industries by various breeding approaches. Therefore, the estimation of genetic diversity of the

traits related to grain morphometry will be very important for feather genetic improvement of wheat varieties according to their end-use quality demand.

The grain protein content (GPC) is an economically valuable trait which plays one of the key roles in the determination of the wheat grain nutrition quality and has strong impact on the bread-making and end-use quality [15]. Despite the great importance of GPC, advancement in wheat breeding for high GPC is quite poor. One of the explanations of this situation is low variations by GPC among commercial cultivars; in addition, breeding for high GPC is very difficult and time consuming due to the trait complicated genetic control. The GPC is routinely screened in wheat breeding programs and selected the accessions with high protein content being for bread making and accessions with low protein content being for feed and other directions of industrial utilizations. One more limitation in wheat breeding programs for high GPC is the strong negative correlation between GPC and grain yield [16–18]. Nevertheless, it was shown that in common and spelt wheat populations, there are lines with simultaneous high GPC and yield components [19].

The evidence of correlation between high GPC and grain morphometry such as grain size and grain shape has not been pointed out. The wild tetraploid wheat (*Triticum turgidum L. var. dicoccoides*) has gene for GPC as a promising source for wheat improvement [20], which is located on chromosome 6B [21]. The high protein content gene from *T. dicoccoides* has been transferred into hexaploid wheat [22]. However, the exclusive milling and baking properties of bread wheat are not found among the diploid and tetraploid wheats. Mainly because of the fact that only the hexaploid wheats have the subgenome D chromosomes derived from diploid *T. tauschii* which determine the quality parameters of bread making of common wheat as widely suggested by wheat scientists [23]. In order to improve the modern wheat cultivars by GPC characteristics without reductions in yield, it is very important to develop wheat genotypes with more high N-use efficiency which involves improved N-uptake and/or N-remobilization [16]. The significant variations in GPC was related to post-anthesis N uptake independently of anthesis date and total N at anthesis [24, 25]. One of the last results on bread wheat highlights the correlation with GPC of post-flowering N uptake occurring early during grain development [26].

Iron and zinc deficiency is a widespread food-related health problem and affects over half of the world population [27, 28]. Wheat is the cheapest and primary source, which supplies the bulk of nutrients for the human diet. If compared to cultivated wheat species, the wild species appear to be a rich genetic resource for high Fe and Zn concentrations [29]. Meanwhile, despite cultivated wheat had mainly lower Fe and Zn concentrations that it was indicated for wild species, more their screening is required in order to find out elite germplasm with high Fe and Zn concentrations already possessing good agronomic productivity [30]. Wheat grain has low content micronutrients. For this reason, there is need for genetic enhancement with more of this nutrient being one of the most cost-effective and powerful method of diminishing global micronutrients malnutrition [31].

Biofortification of cereal grains is one of the most economic effective ways for solving the global micronutrients malnutrition issue. Biofortification has multiplicative advantages [31], and it is considered to be a promising and cost-effective approach for decreasing malnutrition and human health improvement [32].

The aims of present studies were: (1) to generate M_5 mutant lines of spring common wheat in genetic background of cv. Eritrospermum-35; (2) to evaluate variability in components of productivity, including grain number and weight per main spike (GNS and GWS), grain weight per plant (GWP) and 1000-grain weight (TKW), variability in grain morphometry (size and shape), and quality parameters, namely GPC, grain Fe (GIC), and grain Zn (GZnC) concentrations in parent and M_5 mutant lines from generation developed by irradiation treatment of seeds with 100 Gy and 200 Gy and identify those that have high-yield characteristics and improved grain quality traits; and (3) to estimate relationship between two sets of data, including grain quality and agronomic performance parameters.

2. Materials and methods

2.1. Plant material and application of induced mutagenesis

Grains of spring bread wheat variety cv. Eritrospermum-35 (*Triticum aestivum* L.) were irradiated by doses of 100 Gy and 200 Gy from a Co^{60} source at the Kazakh Nuclear Centre. Grains were planted immediately after irradiation in order to obtain M_1 plants. The M_1 generation was grown in the experimental field of the Kazakh Institute of Agricultural and Farming near Almaty according to the standard agricultural practice. Single spikes were harvested from each plant in order to develop the M_2 generation. Selection of the best lines from M_1 to M_5 was carried out based on individual plants. The plants of M_3 and M_4 generations were planted in randomized blocks in three replications. The best lines were tested with their parent variety in order to select advanced mutants. The selection criteria for these lines were GWS and GWP, which were applied in the M_3 and M_4 generations (2011 and 2012) and based on the values for the parent cv. Eritrospermum-35 grown under the same trial conditions. In 2011 and 2012, the parent line had a mean of GWS of 0.79 ± 0.24 g and 0.80 ± 0.26 g, a mean of GWP of 2.06 ± 06 g, and 1.41 ± 0.43 g yield values, respectively. The threshold criteria for selection in the M_3 and M_4 generation were GWS >1.1 g and GWP > 2.2 g for mutant lines. Grains of the best mutants were individually selected in each generation. After harvesting the M_5 plants, 15 lines from the original 100-Gy radiation dose were selected. These lines were numbered as follows: 105(1), 108(1), 113(1), 113(5), 118(1), 118(2), 118(3), 135(1), 136(1), 138(6), 140(2), 140(3), 140(4), 232(1), and 242(2)). Another 15 lines were selected from the 200-Gy radiation dose, which were numbered: 144(1), 144(2), 149(2), 150(7), 152(1), 152(4), 152(5), 152(6), 152(7), 152(8), 153(4), 153(5), 153(6), 153(7) and 153(8).

Samples of grains of each mutant line and the parent were tested. Grain samples from each mutant line, together with the parent Eritrosperumum-35, were planted in a field trial for further evaluation. Each line was grown in three replicate three-row plots, 2 m long, 1.20 m wide with 20 cm between rows, and 30 seeds planted per row. The trial was managed according to the local recommendations for agronomic practices [33]. The following productivity parameters, GWP, GNS, GWS, and TGW calculated as the mean weight of three sets of 100 grains per line were estimated.

2.2. Grain morphometric analysis

Morphometric analysis was performed with the WinRHIZO and image analysis system ((version 1.38 2007, Reagent Instruments Inc., Canada) for GL, GW, and GA on 50–60 grains per line and the GL:GW ratio was also calculated.

2.3. Estimation of grain protein content, iron and zinc concentrations

Grain protein content was estimated with near infrared reflectance (NIR) spectroscopy of whole grains (ZX50 Portable Grain Analyzer, USA) using respective calibration software provided by Zeltex. Three repetitions were studied using 25 grains per line.

The measurements of grain iron and zinc concentrations were described in detail [33]. Briefly, grain samples (100-Gy- and 200-Gy-dosed M_5 mutant lines and cv. Eritrosperumum-35) were washed with sodium dodecyl sulfate (0.1%) with the following several times washings in deionized water, dried, ground with a mixer mill (Retsch MM400 GmbH) and digested (0.2 g) with a mixture of nitric acid (65%, analytical grade) and hydrogen peroxide (30%) (5:1, v/v) using digestion automat K-438 and scrubber K-415 model triplescrub (BÜCHI Labortechnik AG CH-9230 Flawil 1/Switzerland). The sample was diluted to 20 mL with twice-distilled water. The Fe and Zn concentrations were analyzed using flame atomic absorption spectroscopy (Analytic Jena NovAA350, Germany). Estimation of mineral nutrients were checked against the certified reference values from the State standard samples LLC "HromLab," Zn 7837–2000, Fe 7835–2000 diluted by 0.3% HNO3. Three extracts and analysis repetitions were performed.

2.4. Statistical analysis

All data were evaluated in R 3.0.2 (R Core Development Team 2013). The simultaneous tests of general linear hypotheses, Dunnett Contrasts, were used for multiple comparisons of the means. Summarized data are reported as a mean values ± standard deviations. Correlation coefficients between productivity components and grain-quality parameters and p values were calculated using the GenStat software (10th edition). A p-value ≤0.05 was considered statistically significant.

3. Results and discussion

In the current study, GPC showed considerable variation from 12.60 to 14.73% with a mean of 13.62 ± 0.60% (**Figure 1**). It identified 11 advanced M_5 mutant lines (37.7%), of which 5 lines were from 100-Gy gamma-irradiated lines, which demonstrated significant differences from the parent up to 5.7–11.0% GPC higher.

The screening of mutant lines (n = 90) for Fe concentration (GIC) showed great variations in this grain quality parameter (**Figure 2**). The GIC varied from 13.49 to 65.53 mg/kg with a mean

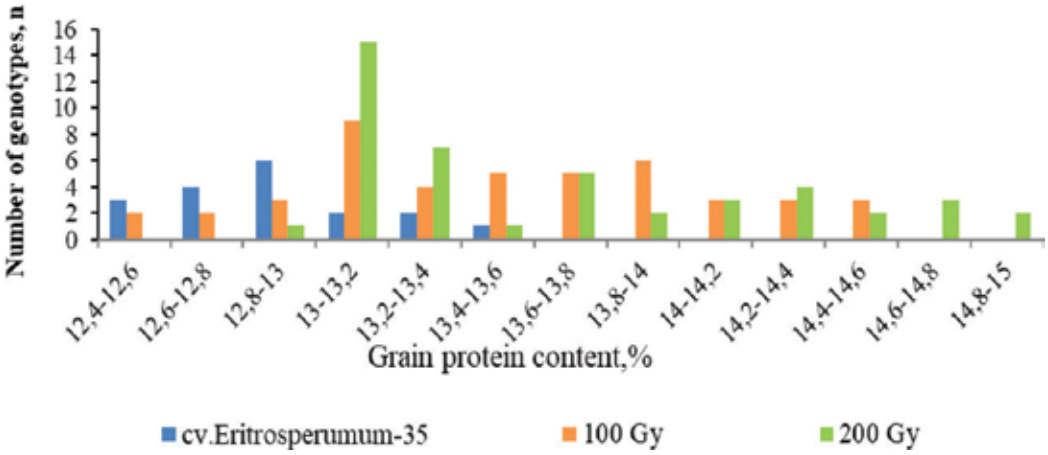


Figure 1. Frequency distribution for range of GPC in 100-Gy- and 200-Gy-dosed M_5 wheat mutant lines and parent cv. Eritrospermum-35.

of 44.95 ± 13.95 mg/kg. The 16 M_5 lines (53%) had significantly enhanced GIC with regard to cv. Eritrospermum-35 such that it exceeds the parent by 1.3 to 1.9 times. The highest values of GIC were revealed in 200-Gy mutant germplasm.

Considerable increase in GIC of mutant lines if compared to parent is the useful tool for further crop improvement.

The ranges of GZnC in mutant lines (n=90) were more higher comparing with that of GIC, from 25.97 to 106.23 mg/kg with a mean of 65.73±26.39 mg/kg (**Figure 3**).

Therefore, identification of genetically determined high GIC and GZnC in mutant germplasm and afterward development of iron and zinc biofortified varieties is a very important and

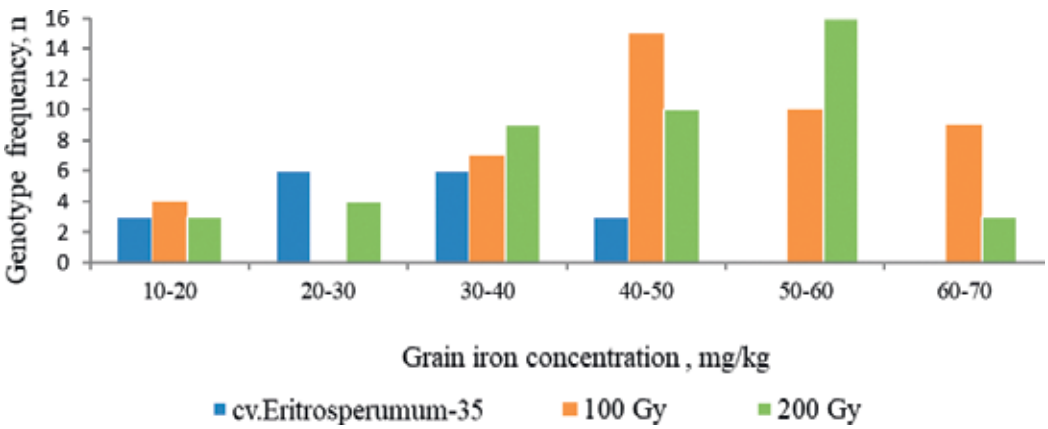


Figure 2. Frequency distribution for wheat grain Fe concentration (GIC) in 100- and 200-Gy-dosed M_5 wheat mutant lines and parent cv. Eritrospermum-35.

promising approach. Varieties and lines with high GIC and GZnC can be used to reduce the human nutrition deficiencies in iron, zinc, and other micronutrients [34, 35].

The key basis of crops improvement is the range of their genetic variability. These ranges of values define the genetic variability that exists in the pooled parent and gamma-irradiated M5 lines under one set of environmental conditions. We revealed that 19 M5 mutant lines (63%) of which 14 lines were from 100 Gy treatment had significantly higher GZnC by 1.24-3.62 times than that of the parent variety. The great variation in GIC and GZnC among wheat mutant lines suggests that it is possible to identify and develop cultivars with high metal concentration. These grain metal accumulations can occur without adversely affecting plant biochemical and physiological functions and they indicate the potential to induce mutations in genes involved in mineral homeostasis processes. The advantage of wild emmer over cultivated wheat for higher grain nutrient concentrations has been previously consistently demonstrated [28]. However, because of sexual incompatibility between the crop and its wild relative, it may require embryo rescue or use tissue culture to recover fertile embryos.

The results of comparison of mutant lines with significantly enhanced grain Fe and Zn concentrations pointed out that eight lines of 100- and 200-Gy-gamma-irradiated M_5 (generation 26.7%, the same number for each) had concomitant increase in both GIC and GZnC (**Figures 4** and **5**). Identification of genetically determined high GIC and GZnC in mutant germplasm and afterward development of Fe and Zn biofortified varieties is very important and promising approach. Resources with high GIC and GZnC can be used to reduce the human nutrition deficiencies in Fe, Zn, and other micronutrients [34, 35].

The effect of gamma irradiation on averages of GIC and GZnC of 100- and 200-Gy-dosed M_5 mutant wheat lines indicated that irradiation treatment of 100-Gy induced greater variations

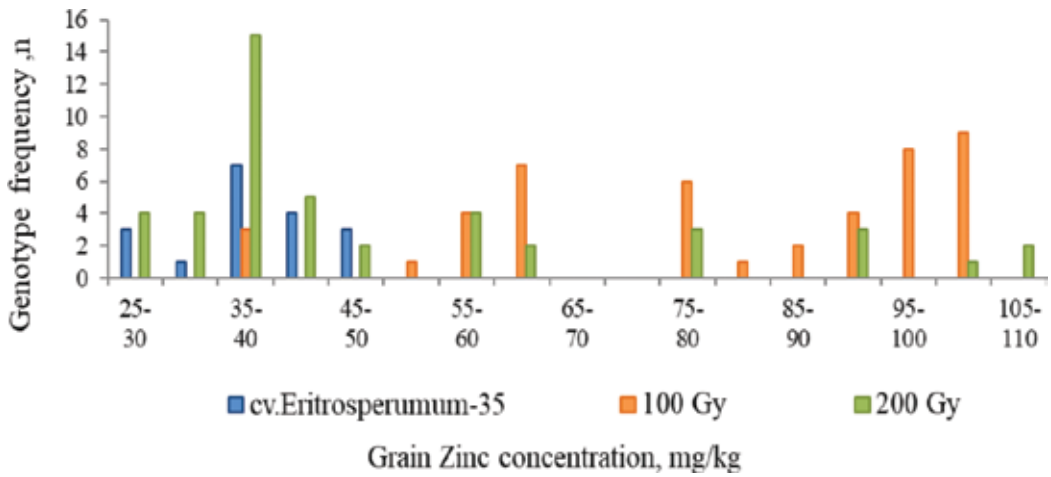


Figure 3. Frequency distribution for wheat grain Zn concentration (GZnC) in 100-and 200-Gy-dosed M_5 mutant lines and parent cv. Eritrospermum-35.

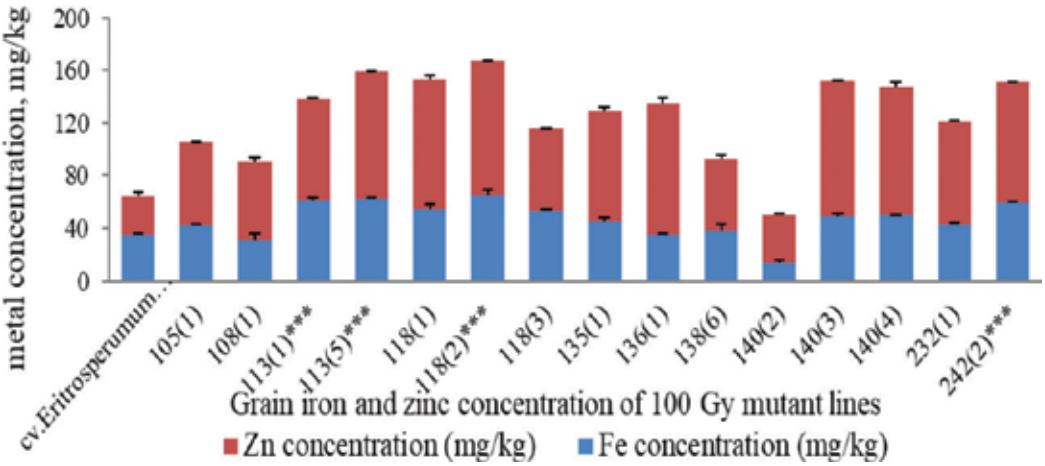


Figure 4. Comparison of 100 Gy-dosed M_5 wheat mutant lines and parent (cv. Eritrospermum-35) with simultaneous enhancement of grain Fe and Zn concentrations.

for GZnC in comparison to GIC, and there is significant difference in GZnC between the dose of irradiation (**Figure 6**).

Spring wheat mutant lines generated by irradiation treatments were characterized by grain morphometric parameters, namely grain area (GA), grain length (GL), grain width (GW), and the grain length to width ratio (GL:GW ratio) with comparison to the parent. The GA of 100- and 200-Gy-treated mutant lines from 16.74 to 23.46 mm^2 and from 19.65 to 23.31 mm^2 with means of 19.68 ± 2.31 mm^2 (n = 45) and 21.27 ± 1.28 mm^2 (n = 45), respectively (**Figure 7A**). Among 20 genotypes (67%), mostly 200-Gy-treated lines had significantly higher GA than that of the parent on intervals between 10.95% and 34.4%.

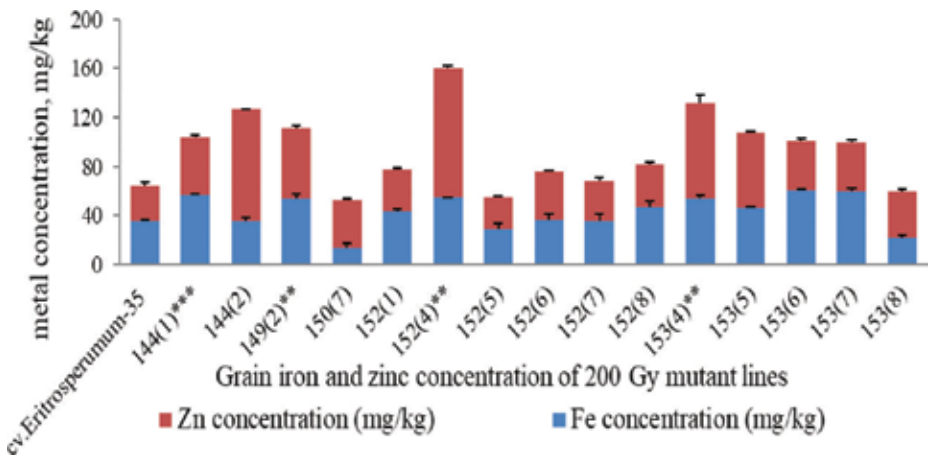


Figure 5. Comparison of 200 Gy-dosed M_5 wheat mutant lines and parent (cv. Eritrospermum-35) with significantly enhanced grain Fe and Zn concentrations.

http://dx.doi.org/10.5772/intechopen.75706

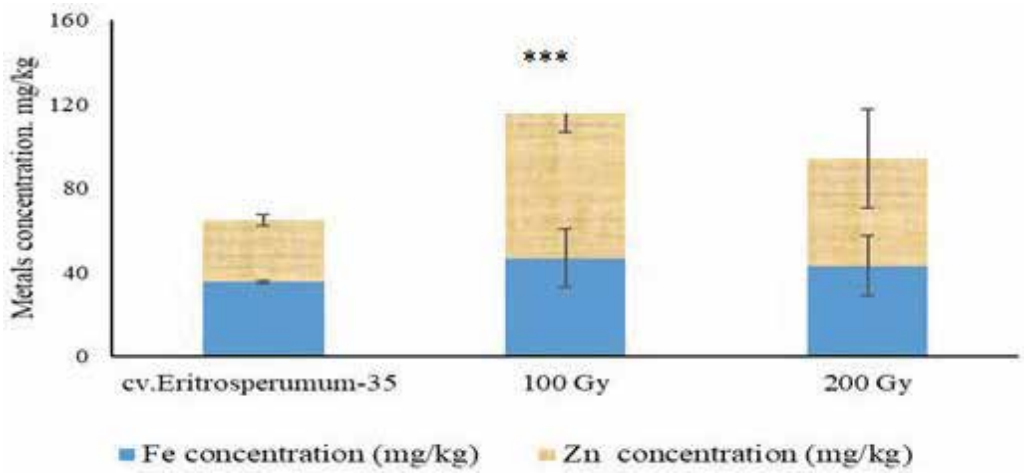


Figure 6. The effect of gamma irradiation on averages of grain Fe and Zn concentrations of 100 Gy and 200-Gy-dosed M_5 and the parent cv. Eritrospermum-35.

Grain length (GL) of 100- and 200-Gy-dependent mutant lines varied from 6.81 to 9.37 mm with a mean of 8.68 ± 0.21 mm (n = 90) (**Figure 7B**). The majority, 21 M_5 lines (77.0%), showed significantly longer grains by intervals of 12.09% and 28.0%.

The GW data of 100- and 200-Gy-dosed mutant lines ranged from 3.24 to 4.77 mm with 4.06 ± 0.26 mm and from 3.65 to 4.79 mm (n = 225) with a mean of 4.43 ± 0.27, respectively. With the exception of four 100-Gy-dependent radiation mutant lines (26.67%), most of them had significantly lower GW than the parent cv. Eritrospermum-35. Opposite, in 200-Gy-dosed lines, 12 lines (80.0%) showed significant wider grains by 12.9–13.5% than the parent.

The means of GW, GL, GL/GW ratio, and GA of M_5 mutant lines generated by gamma irradiation with 100- and 200-Gy doses and the parent cv. Eritrospermum-35 are shown in **Figure 8**.

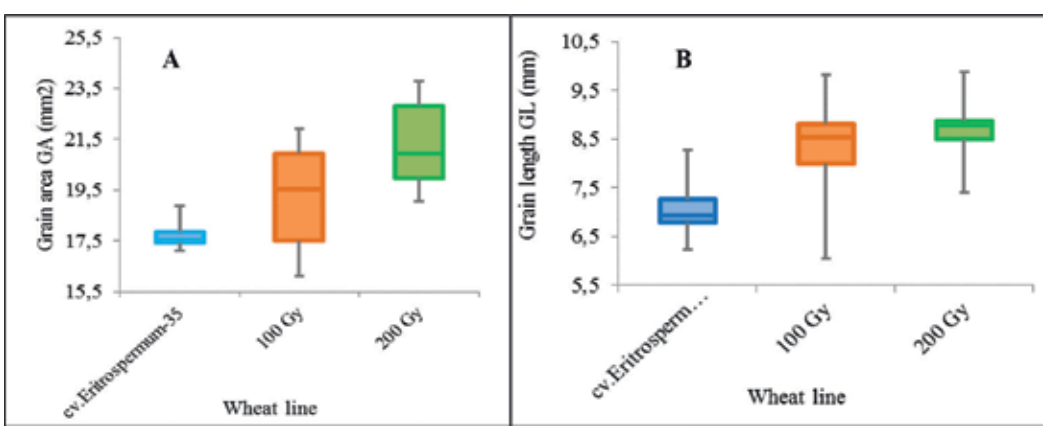


Figure 7. Phenotypic screening grain area (GA) (A) and grain length (GL) (B) of 100- and 200-Gy-dosed M_5 lines and the parent cv. Eritrospermum-35.

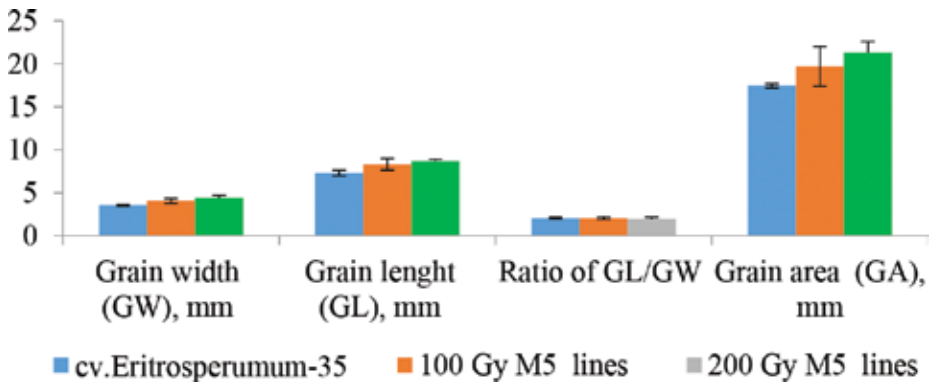


Figure 8. Phenotypic variation in grain morphometric parameters (GW, GL, ratio of GL/GW, and GA) of M_5 mutant lines generated by irradiation with 100- and 200-Gy doses and the parent cv. Eritrospermum-35. Means of GL, GW, GL/GW, and GA with standard error bars.

These results indicate that among grain morphometric parameters of mutation resources, phenotypic variation in GA and to a lesser degree GL were the most variable phenotypic traits. Variations in GW and the GL:GW ratio were moderate and less variable. Moreover, most of the longer and wider grains were found in lines developed by 200-Gy dose treatment. We did not find out the dose-dependent pattern for all grain morphometric parameters. Meanwhile, in our previous study with mutant germplasm generated on the base of cv. Almaken, we revealed that GW is a dose-dependent pattern and the 200-Gy gamma irradiation induced significant higher variation in this shape-characterizing parameter [33].

The generated by 100 Gy and 200 Gy treatments on the base of the cv. Eritrospermum-35 mutant lines were evaluated on the following yield-associated parameters: grain weight per plant (GWP), grain number per main spike (GNS), and grain weight per main spike (GWS) (**Table 1**). Among these productivity components, TGW of mutant lines showed the highest number of genotypes with significantly greater means in comparison to the parent (total 17 lines) and followed by a GWP trait (total 14 lines). The TGW in the 100- and 200-Gy-treated germplasm varied from 27.64 to 58.54 g with mean of 46.98 ± 8.27 (n = 225) and from 33.58 to 57.18 g with mean of 46.98 ± 8.27 (n = 225), respectively.

Another productivity element such as GWP in mutant lines was the most variable trait (**Table 1**). Its range was from 2.34 to 6.53 g in the 100-Gy-dosed lines with 8 genotypes (53.3%), having significant higher GWP in comparison to the parent. The variability in GWP of 200-Gy-dosed germplasm was from 1.84 to 5.37 g with mean of 3.75 ± 1.18 (n = 225). The 14 lines (47.0%) were identified as those which had significant higher GWP by 1.86–3.36 times than that of the parent.

The GNS means were 46.98 ± 8.27 and 48.69 ± 5.95 (n = 225) in the 100- and 200-Gy-dosed mutant lines with their ranges of 30.33–56.67 and of 38.58–58.67, respectively (**Table 1**). When compared to parent and other mutant lines, 11 lines of 100- and 200-Gy-generated lines were identified as those having significant high GNS. Concerning the GWS trait, the differences were not significant between each of M_5 lines and the parent cv. Eritrospermum-35.

Genotypes	Grain number per main spike	Grain weight per main spike (g)	Grain weight per plant (g)	1000 Grain weight (g)
cv. Eritrospermum-35	30.33 ± 8.21	1.55 ± 0.52	1.94 ± 0.07	34.12 ± 1.17
100-Gy-dosed M_5 mutant lines				
105(1)	38.33 ± 7.51	2.26 ± 1.25	2.34 ± 0.71	41.86 ± 1.02
108(1)	37.33 ± 5.13	1.69 ± 0.27	2.42 ± 0.51	42.17 ± 2.05
113(1)	43.67 ± 7.37	2.01 ± 0.34	2.72 ± 0.56	39.63 ± 0.78
113(5)	43.00 ± 4.36	2.22 ± 0.33	3.28 ± 1.39	45.34 ± 0.82
118(1)	40.33 ± 8.01	1.42 ± 0.53	2.70 ± 0.27	39.32 ± 1.74
118(2)	44.67 ± 1.16	2.20 ± 0.07	2.85 ± 0.34	49.97 ± 0.84**
118(3)	46.33 ± 6.25*	2.70 ± 0.47	3.61 ± 0.72*	49.88 ± 0.58**
135(1)	56.33 ± 4.47***	2.64 ± 0.45	6.53 ± 0.52***	58.54 ± 1.49***
136(1)	42.33 ± 6.11	2.53 ± 0.12	6.13 ± 0.18***	53.54 ± 2.27***
138(6)	45.66 ± 5.06	2.05 ± 0.49	6.37 ± 0.63***	50.11 ± 2.83***
140(2)	56.67 ± 3.61***	2.77 ± 0.67	4.01 ± 0.73**	57.23 ± 1.78***
140(3)	40.00 ± 2.03	1.91 ± 0.16	3.13 ± 1.04	56.94 ± 2.16***
140(4)	42.33 ± 4.93	1.88 ± 0.17	3.68 ± 0.32*	48.51 ± 2.38***
232(1)	30.33 ± 5.51	1.46 ± 0.38	2.48 ± 0.46	27.64 ± 1.56
242(2)	47.67 ± 7.02**	2.27 ± 0.49	2.61 ± 0.23	43.98 ± 2.32
200-Gy-dosed M_5 mutant lines				
144(1)	44.33 ± 3.79	2.22 ± 0.21	4.32 ± 2.28***	49.67 ± 1.89**
144(2)	41.10 ± 3.52	1.80 ± 0.03	1.84 ± 0.41	33.58 ± 2.49
149(2)	55.34 ± 2.31***	2.63 ± 0.04	5.37 ± 0.64***	57.18 ± 1.23***
150(7)	52.34 ± 3.65***	2.29 ± 0.38	3.01 ± 0.23	49.89 ± 1.24**
152(1)	47.33 ± 6.66**	2.37 ± 0.35	3.75 ± 0.42*	46.21 ± 2.75*
152(3)	43.33 ± 1.53	2.12 ± 0.53	2.59 ± 0.96	39.85 ± 2.16
152(4)	42.00 ± 3.01	1.88 ± 0.30	2.48 ± 0.60	46.86 ± 2.33*
152(5)	50.43 ± 4.04**	2.43 ± 0.12	4.28 ± 0.96***	51.77 ± 1.39***
152(6)	42.67 ± 4.62	2.22 ± 0.16	3.07 ± 0.21	49.18 ± 2.42
152(7)	41.33 ± 1.53	1.92 ± 0.04	2.24 ± 0.65	45.59 ± 1.88
152(8)	38.00 ± 4.58	1.73 ± 0.23	3.14 ± 0.47	43.23 ± 1.68
153(4)	39.67 ± 2.52	1.83 ± 0.07	3.22 ± 0.23	45.87 ± 1.42
153(5)	58.67 ± 4.02***	2.87 ± 0.58	4.43 ± 0.87***	53.77 ± 1.86***
153(6)	51.75 ± 3.42**	2.48 ± 0.78	5.97 ± 1.37***	54.56 ± 1.38***
153(7)	44.30 ± 3.69	2.07 ± 0.32	5.03 ± 0.74***	54.17 ± 2.36***
153(8)	51.00 ± 3.84**	2.21 ± 0.49	4.16 ± 1.16***	49.79 ± 1.86

*, **, and *** denote significance at 0.05, 0.01, and 0.001 probability levels, respectively. The lines are significantly different from parent line. Grain number and weight per main spike, grain weight per plant are a means of 15 randomly selected spikes/plants.

Table 1. Comparing yield-associated traits of advanced spring wheat M_5 mutant lines developed using 100 Gy and 200 Gy and the parent cv. Eritrospermum-35.

Comparing yield-associated traits of Eritrospermum-35 spring wheat M_5 mutant lines generated by treatments of 100 Gy and 200 Gy showed that 8 lines numbered by 118(3), 135(1), 140(2), 149(2), 152(1), 152(5), 153(5), 153(6) and accounting for 26.7% of the total number of mutated genotypes had simultaneous significant higher GNS, GWP, and TGW than the parent (**Table 1**). In our previous study with mutant germplasm developed on cv. Almaken, we were able to generate only three lines which had high GNS, GWP, and TGW [33]. This number is less than the Eritrospermum-35 mutant lines.

Thus, the radiation doses of 100 Gy and 200 Gy had generated mutations with respect to components of productivity such as GWS, GWP, and TGW in comparison to the parent, with the greatest variation being for GWP and TGW. High GWP and TGW of these mutant populations indicate that genetic background of variety selected for irradiation treatment is of great importance to generate broad and valuable variability in productivity traits (**Table 1**).

One of the important outputs revealed in the present study was the correlation between concentrations of grain nutrients, grain characteristics, and plant productivity. Parent variety showed a significant correlation of TGW with GNS and grain morphometric parameter of GW with yield-associated components such as TGW and GWS (**Table 2**). There was no relation between grain quality characteristics (GPC, GIC, and GZnC) and plant productivity and grain morphometry.

In 100-Gy-dosed M_5 mutant lines, there was significant correlation of productivity components such as GNS with GWS, GNS with GWP (**Table 2**). Both grain metals, namely GZnC and GIC, were related to each other indicating that metal accumulation may be controlled by the same loci. These results which describe positive correlation between grain Zn and Fe concentrations are similar to those which were observed in domesticated wheat and synthetic hexaploids [31]. This fact suggested that the alleles for Zn and Fe deposition co-segregate or are pleiotropic effect and therefore grain metal concentrations possibly improve metals accumulation simultaneously. It was also found that another nutritional value parameter such as GPC was significantly correlated with one of the grain morphometric parameter such as GW.

This finding suggests that this grain quality character may be genetically linked to grain morphometry. In our studies reported for Almaken mutant lines such kind of relation was revealed for GA, GL, and GW at 200-Gy gamma irradiation treatment [33]. Concerning correlations between parameters characterizing grain size and shape, we determined that there was a high relation of GA with GL and GW for 200-Gy-gamma irradiated population.

Similar to 100-Gy-dosed M_5 mutant germplasm, GNS was significantly related to GWS with high r^2 mean as well as grain metals, GZnC and GIC, were correlated to each other in 200-Gy-dosed M_5 mutant lines (**Table 2**). The interesting fact, which was only revealed for these lines, is that GIC except GZnC significantly related to GPC. Thus, in mutant populations, both metals, GIC and GZnC, are associated with each other. For parameters characterizing grain morphometry, we revealed that there were high correlations of GW with GA and GL.

	GWS (g)	GWP (g)	TGW (g)	GPC (%)	GIC (mg/kg)	GZnC (mg/g)	GL (mm)	GW (mm)	GA (mm²)
cv. Eritrosperumum-35									
GNS	0.003	0.115	0.171*	0.056	0.001	0.012	0.011	0.006	0.008
GWS		0.089	0.021	0.007	0.000	0.000	0.078	0.142*	0.004
GWP			0.155	0.005	0.000	0.007	0.120	0.042	0.096
TGW				0.011	0.003	0.065	0.008	0.163**	0.000
GPC					0.021	0.008	0.004	0.001	0.000
GIC						0.000	0.001	0.014	0.008
GZnC							0.030	0.045	0.008
GL								0.115	0.017
GW								—	0.008
GNS	*0.470***	*0.176***	*0.051*	*0.093*	*0.000*	*0.001*	*0.003*	*0.043*	*0.036*
GWS)		*0.099*	*0.002*	*0.038*	*0.000*	*0.017*	*0.019*	*0.073*	*0.110*
GWP			*0.030*	*0.098*	*0.083*	*0.002*	*0.062*	*0.071*	*0.087*
TGW				*0.050*	*0.033*	*0.044*	*0.010*	*0.046*	*0.079*
GPC					*0.025*	*0.031*	*0.063*	*0.107**	*0.020*
GIC						*0.417****	*0.000*	*0.006*	*0.064*
GZnC							*0.000*	*0.025*	*0.038*
GL								*0.211**	*0.048*
GW									*0.181**
GNS	0.870***	0.070	0.018	0.048	0.014	0.038	0.011	0.074	0.016
GWS		0.071	0.064	0.011	0.011	0.050	0.088	0.028	0.020
GWP			0.004	0.043	0.072	0.122	0.021	0.006	0.002
TGW				0.059	0.034	0.000	0.018	0.006	0.015
GPC					0.251*	0.036	0.019	0.002	0.144
GIC						0.224*	0.035	0.004	0.000
GZnC							0.045	0.000	0.044
GL								0.400***	0.451***
GW									0.503***

*, **, and *** denote significance at 0.05, 0.01, and 0.001 probability levels, respectively.

Table 2. R2 correlation between yield-associated traits (TWG, GNS, GWS, and GWP) grain protein content and micro-elements concentrations, and grain morphometric parameters (GA, GL, GW, and GL:GW ratio) in cv. Eritrospermum-35 parent and 100 Gy-(orange) and 200-Gy-(gray) M_5 mutant lines.

4. Conclusions

This study aimed to develop M_5 mutant lines of spring wheat by 100-and 200-Gy gamma irradiation treatment and identify genetic variability in grain nutritional value including grain protein content, grain Fe and Zn concentrations, and yield-associated traits such as grain number and weight per main spike, grain weight per plant, and 1000-grain weight and grain morphometric parameters, namely grain area, length, and width and M_5 mutant lines in comparison to the cv. Eritrospermum-35 parent. We identified mutant lines with high Fe and Zn concentrations and grain protein content as those which have 1.6–3.4 and 1.4–2.9 times more as well as 3.7–16.9% than target parameters of parent variety, respectively. Several mutant lines showed significantly simultaneous increase of metals concentrations. The positive correlation of grain protein content with grain width suggests that they are genetically related. This relation could be a good candidate for evaluating genotypes for improvement of grain protein content. Wheat grain can be biofortified by micronutrients without negative impact on crop productivity using new mutant lines. Mutation breeding can significantly contribute to human health malnutrition and improve nutrition quality diet.

Acknowledgements

The authors thank the Ministry of Education and Sciences of the Republic of Kazakhstan for funding the project 074/GF "The creation and study of mutant genotypes of wheat for identifying valuable breeding forms and new alleles of genes controlling key adaptive properties" and AP05131881 "Development of integrated approaches for bifortification, high bioavailability of the most important micronutrients of spring wheat and health." The authors wish to acknowledge the technical financial support of the International Atomic Energy Agency (IAEA, Austria) for National TC project KAZ/5003, "Increasing Micronutrient Content and Bioavailability in Wheat Germplasm by Means of an Integrated Approach."

Conflict of interest

The authors have declared that no competing interests exist.

Author details

Saule Kenzhebayeva[1]*, Gulina Doktyrbay[1], Fatma Sarsu[2], Nargul Omirbekova[1], Alfia Abekova[3] and Dauren Tashenev[1]

*Address all correspondence to: kenzhebaevas@mail.ru

1 Kazakh National University the Named after al-Farabi, Almaty, Kazakhstan

2 The Plant Breeding and Genetics Section, Joint FAO/IAEA Division, International Atomic Energy Agency, Vienna, Austria

3 Kazakh Institute of Agricultural and Breeding, Almaty, Kazakhstan

References

[1] Graham R, Senadhira D, Beebe S, Iglesias C, Monasterio I. Breeding for micronutrient density in edible portions of staple food crops: Conventional approaches. Field Crops Research. 1999;**60**:57-80. DOI: 10.1016/S0378-4290(98)00133-6

[2] Borrill P, Connorton JM, Balk J, Miller AJ, Sanders D, Uauy C. Biofortification of wheat grain with iron and zinc: Integrating novel genomic resources and knowledge from model crops. Frontiers in Plant Science. 2014;**5**:1-8. DOI: 10.3389/fpls.2014.00053

[3] Welch RM, Graham RD. Breeding for micro-nutrients in staple food crops from a human nutrition perspective. Journal of Experimental Botany. 2004;**55**:353-364. DOI: 10.1093/jxb/erh064

[4] FAO. The Food and Agriculture Organisation of the United Nations. (FAOSTAT.URL). 2017. http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567#ancor

[5] Muthayya S, Rah JH, Sugimoto JD, Roos FF, Kraemer K, Black RE. The global hidden hunger indices and maps: An advocacy tool for action. PLoS One. 2013;**8**:e67860. DOI: doi.org/10.1371/journal.pone.0067860

[6] Reif JC, Zhang P, Dreisigacher S, Warburton ML, Ginkel M, Hoisington D, Bohn M, Melchinger AE. Wheat genetic diversity trends during domestication and breeding. Theoretical and Applied Genetics. 2005;**110**:859-864. DOI: 10.1007/s00122-004-1881-8

[7] Parry MAJ, Madgwick PJ, Bayon C, Tearall K, Hernandez-Lopez A, Baudo M e a. Mutation discovery for crop improvement. Journal of Experimental Botany. 2009;**60**:2817-2825. DOI: 10.1093/jxb/erp189

[8] Tomlekova NB, Kozgar MI, Wani MR. Mutagenesis: Exploring Genetic Diversity of Crops. Wageningen Academic Publishers; 2014. pp. 253-264

[9] Pathirana R. Plant mutation breeding in agriculture. CAB reviews Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources. 2011;**6**(032):1-20. DOI: 10.1079/PAVSNNR20116032

[10] http://mvgs.iaea.org/

[11] Huang Y, Kong Z, Wu X, Cheng R, Yu D, et al. Characterization of three wheat grain weight QTLs that differentially affect kernel dimensions. Theoretical and Applied Genetics. 2015;**128**(12):2437-2445. DOI: 10.1007/s00122-015-2598-6

[12] Gegas VC, Nazari A, Griffiths S, Simmonds J, Fish L, Orford S, Sayers L, et al. A genetic framework for grain size and shape variation in wheat. The Plant Cell. 2010;**22**:1046-1056. DOI: 10.1105/tpc.110.074153

[13] Botwright TL, Condon AG, Rebetzke GJ, Richards RA. Field evaluation of early vigour for genetic improvement of grain yield in wheat. Australian Journal of Agricultural Research. 2002;**53**:1137-1145. DOI: 10.1071/AR02007

[14] Okamoto YT, Kajimura TM, Ikeda TS. Evidence from principal component analysis for improvement of grain shape- and spikelet morphology-related traits after hexaploid wheat speciation. Genes & Genetic Systems. 2012;**87**:299-310. DOI: 10.1266/ggs.87.299

[15] Balyan HS, Gupta PK, Kumar S, Dhariwal R, Jaiswal V, et al. Genetic improvement of grain protein content and other health-related constituents of wheat grain. Plant Breeding. 2013;**132**:446-457

[16] Brevis JC, Dubcovsky J. Effect of chromosome region including the *Gpc-B1* locus on wheat protein and protein yield. Crop Science. 2010;**50**:93-104. DOI: 10.2135/CROPSCI2009.02.0057.

[17] Kang MS, Banga SS. Global agriculture and climate change. Journal of Crop Improvement. 2013;**27**:667-692. DOI: 10.1080/15427528.2013.845051

[18] Monaghan JM, Snape JW, Chojecki AJS, Kettlewell PS. The use of grain protein deviation for identifying wheat cultivars with high grain protein concentration and yield. Euphytica. 2001;**122**:309-317

[19] Zanetti S, Winzeler M, Feuille C, Keller B, Messmer M. Genetic analysis of bread-making quality in wheat and spelt. Plant Breeding. 2001;**120**:13-19

[20] Avivi L. Utilization of Triticum dicoccoides for the improvement of grain protein quantity and quality in cultivated wheat. lsraeli-Italian Joint Meet. Genet. Breed. Crop Plants, Rom Mon. Genet. Agr. 1978:27-38

[21] Joppa LP, Cantrell RG. Chromosomal location of genes for grain protein content of wild tetraploid wheat. Crop Science. 1990;**30**(5):1059-1064

[22] Khan IA, Procunier JD, Humphreys DG, Tranquilli G, Schlatter AR, Marcucci-Poltri S, Frohberg R, Dubcovsky J. Development of PCR–based markers for a high grain protein content gene from *Triticum turgidum ssp. dicoccoides* transferred to bread wheat. Crop Science. 2000;**40**:518-524

[23] Belderok B, Mesdag J, Donner DA. Bread Making Quality of Wheat: A Century of Breeding in Europe. Dordrecht, the Netherlands: Kluwer Academic Publisher; 2000. pp. 30-31

[24] Bogard M, Allard V, Brancourt-Hulmel M, Heumez E, Machet J-M, Jeuffroy M-H, Gate P, Martre1 P, Le Goui J. Deviation from the grain protein concentration–grain yield negative relationship is highly correlated to post-anthesis N uptake in winter wheat. Journal of Experimental Botany. 2010;**61**(15):4303-4312. DOI: 10.1093/jxb/erq238

[25] Taulemesse F, Le Gouis J, Gouache D, Gibon Y, Allard V. Bread wheat (*Triticum aestivum* L.) grain protein concentration is related early to post-flowering nitrate uptake under putative control of plant satiety level. PLoS One. 2016;**11**(2):e0149668. DOI: 10.1371/journal.pone.0149668

[26] Campos-Bowers MH, Wittenmyer BF. Biofortification in China: Policy and practice. Health Research Policy and Systems. 2007;**5**:1-7. DOI: 10.1186/1478-4505-5-10

[27] Borrill P, Connorton JM, Balk J, Miller AJ, Sanders D, Uauy C. Biofortification of wheat grain with iron and zinc: Integrating novel genomic resources and knowledge from model crops. Frontiers in Plant Science. 2014;**5**:1-8. DOI: 10.3389/fpls.2014.00053

[28] Chatzav M, Peleg Z, Ozturk L, Yazici A, Fahima T, et al. Genetic diversity for grain nutrients in wild emmer wheat: Potential for wheat improvement. Annals of Botany. 2010;**105**:1211-1220. DOI: 10.1093/aob/mcq024

[29] Graham R, Senadhira D, Beebe S, Iglesias C, Monasterio I. Breeding for micronutrient density in edible portions of staple food crops: Conventional approaches. Field Crops Research. 1999;**60**:57-80. DOI: 10.1016/S0378-4290(98)00133-6

[30] Velu GI, Ortiz-Monasterio I, Cakmak Y, Hao, Singh RP. Bio fortification strategies to increase grain zinc and iron concentrations in wheat. Journal of Cereal Science. 2013;**59**:365-372

[31] Welch RM, Graham RD. Breeding for micronutrients in staple food crops from a human nutrition perspective. Journal of Experimental Botany. 2004;**55**:353-364. DOI: 10.1093/jxb/erh064

[32] Kenzhebayeva SS, Doktyrbay G, Capstaff NM, Sarsu F, Omirbekova NZ, Eilam T, Tashenev DK, Miller AJ. Searching a spring wheat mutation resource for correlations between yield, grain size, and quality parameters. Journal of Crop Improvement. 2017;**31**:208-228. DOI: 10.1080/15427528.2016.1276990

[33] Bouis HE, Welch RM. Biofortification - a sustainable agricultural strategy for reducing micronutrient malnutrition in the global south. Crop Science. 2010;**50**:S-20-S-32. DOI: 10.2135/cropsci 2009.09.0531

[34] Miller DD, Welch RH. Food system strategies for preventing micronutrient. Food Policy. 2013;**42**:115-128. DOI: doi.org/10.1016/j.foodpol.2013.06.008

[35] Zhao FJ, Su YH, Dunham SJ, Rakszegi M, Bedo Z, Mcgrath SP, Shewry PR. Variation in mineral micronutrient concentrations in grain of wheat lines of diverse origin. Journal of Cereal Science. 2009;**49**:290-295. DOI: 10.1016/j.jcs.2008.11.007

Chapter 10

Genetic Improvement of Bread Wheat for Stem Rust Resistance in the Central Federal Region of Russia: Results and Prospects

Inna Lapochkina, Olga Baranova, Nail Gainullin, Michael Kuzmich, Svetlana Polyakova, Petr Polityko, Ramin Mamedov and Sergey Voronov

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.75379

Abstract

Advanced breeding lines of spring and winter wheat with several effective resistance genes to stem rust, including its aggressive race Ug99, were developed for the first time for the non-Chernozem zone of Russia. Modern wheat varieties cultivated in this region have high productivity and grain quality. However, they are susceptible to fungal diseases and therefore are cultivated using frequent fungicides treatments. The introgression wheat lines with multiple alien translocations ("Arsenal" collection) have been developed in the Moscow Scientific Research Institute of Agriculture "Nemchinovka" by using gamma irradiation of pollen of wild wheat relatives (*Aegilops speltoides, Ae. triuncialis, Triticum kiharae, Secale cereale*). Initial material with several effective Sr resistance genes for wheat breeding was developed using donors from the "Arsenal" and the VIR collections. The created initial material can compete with modern varieties, as it has resistance to leaf rust and powdery mildew, high productivity and numerous other advantages. On this basis, a new direction in breeding of spring and winter wheat is developed for this region, that is, creation of wheat cultivars with resistance to fungal diseases. This allows to reduce the fungicide load during cultivation with the goal of producing ecologically clean grain for healthy diet.

Keywords: bread wheat, stem rust, donors, Sr genes, grain quality

IntechOpen

© 2018 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

1. Introduction

1.1. Geographical position of the Central Federal District of Russia, achievements in the selection of wheat and the directions of its improvement

The Central Federal District of Russia (CFDR) is an area of more than 650,000 km^2, which includes 18 oblasts with the capital city of Moscow (**Figure 1**). The CFDR lies within the Atlantic-continental climatic region of the north temperate zone. It is characterized by not too cold winter and warm, but not excessively hot summer. The lowest temperatures are observed in January: on the average from −8 to −12°C. Summer temperature ranges from 18 to 20°C. The average duration of the frost-free period is 125–140 days, and the sum of the effective temperatures is 1800–2300, which allows to successfully cultivate most of the cereals, potatoes, vegetables, fodder grasses and flax in the CFDR. The average annual precipitation is 450–600 mm [1] (http://studopedya.ru/2-68711.html). This economic region includes about 16,000,000 ha of arable land, in which winter and spring wheat are the leading crops. The area under these crops is 3,600,000 and 620,000 ha, respectively. Traditionally, until the late 1960s of the twentieth century, rye was grown in this region, which is less demanding for the fertility of sod-podzolic soils. However, rye gave way to wheat due to the efforts of breeders. Breeders P. Lukyanenko (Bezostaya 1, Karlik 1), V. Remeslo (Mironovskaya 808), G. Lapchenko (PPG-1, PPG-186), E. Varenitsa (Zarya), E. Nettevich (Moskovskaya 35, Priokskaya, Lada) and B. Sandukhadze (Inna, Galina, Moskovskaya 39, Nemchinovskaya 17) made a great contribution to the creation of wheat cultivars.

Modern cultivars of bread wheat, derived in the Moscow Scientific Research Institute of Agriculture "Nemchinovka" (Moscow Sc. Res. Inst. of Agr. "Nemchinovka"), are characterized by high winter hardiness, productivity and grain quality. They are cultivated according to intensive technologies with the application of mineral fertilizers up to 150 kg N, 120 kg P_2O_5, 150 kg K_2O, against the background of manure and annual grasses. Seed treatment before sowing, threefold

Figure 1. The Central Federal District on the map of Russia (a) and its composition (b): 1—Belgorod Oblast, 2—Bryansk Oblast, 3—Vladimir Oblast, 4—Voronezh Oblast, 5—Ivanovo Oblast, 6—Kaluga Oblast, 7—Kostroma Oblast, 8—Kursk Oblast, 9—Lipetsk Oblast, 10—Moscow, 11—Moscow Oblast, 12—Oryol Oblast, 13—Ryazan Oblast, 14—Smolensk Oblast, 15—Tambov Oblast, 16—Tver Oblast, 17—Tula Oblast and 18—Yaroslavl Oblast.

http://dx.doi.org/10.5772/intechopen.75379

application of fungicides, herbicides, insecticides and growth regulators for the season ensure the yield of spring wheat up to 7 tons/ha and winter wheat up to 10 tons/ha. However, these cultivars are susceptible to the majority of fungal diseases common in this zone (powdery mildew, leaf rust, stem rust and *Septoria* leaf spot). To date, only one cultivar of winter wheat Nemchinovskaya 24 with two resistance genes *Lr9* and *Lr46* has genetic protection against leaf rust. Therefore, the development of cultivars with increased immunity to fungal diseases is actual for the CFDR. Extensive transport and economic relations in the globalization of the world do not exclude the importation of quarantine diseases into our territory, for example, the aggressive race of stem rust Ug99. The worsening of the phytopathological situation requires increased research in this area, especially in recent years, when the cases of crop damage caused by stem rust occur more frequently (2010, 2013, 2016 and 2017). The aim of this study was to identify the sources and donors of resistance to stem rust, including the race Ug99, from the collection of VIR and "Arsenal" collection, and to create on their basis, the initial material of spring and winter wheat with durable resistance to stem rust.

2. The development of the initial material of spring and winter wheat with several Sr genes resistant to *Puccinia graminis* f. sp. *tritici*

2.1. The modern phytopathological stem rust situation in CFDR and possible threats

The situation in the CFDR reflects the general trend observed in the populations of *P. graminis* in all areas of pathogen distribution; the fungus actively evolves. Differences concern only the speed and genes of the pathogen virulence, depending on the geographical location. In the case of Ug99 (TTKSK), the process is very fast (in 18 years, 13 biotypes of the fungus appeared); on the other hand, in the territory of CFDR, the change of the dominant races took place for 57 years. The phytopathological situation is complicated by the proximity of the CFDR to European countries, where aggressive pathogens of *P. graminis* have been identified recently. Six races (TKTTF, TKKTF, TKPTF, TKKTP, PKPTF and MMMTF) were retrieved from 48 isolates, obtained from the *P. graminis* population in 2013 in Germany [2]. The detection of the TKKTP race causes concern because of its virulence to the *Sr24*, *SrTmp* and *Sr1RSAmigo* genes, although it has been determined that none of these races belongs to the race group TTKSK (Ug99), and the German isolates of the TKTTF race are phenotypically different from the TKTTF race that caused plant disease epidemic in Ethiopia in 2013/2014. It is known that 55% of North American and international cultivars and selection lines resistant to the race TTKSK (Ug99) are susceptible to the TKKTP race [2]. On the Italian island of Sicily, a new race of stem rust, the TTTTF, hit several thousand hectares of durum wheat in 2016, leading to the largest outbreak of stem rust in Europe in recent decades. TTTTF is a newly identified race of stem rust that can soon spread over long distances along the Mediterranean basin and the Adriatic coast [3] (http://www.fao.org/news/story/en/item/469467/icode/).

The analysis of the racial composition of *R. graminis* f. sp. *tritici* in CFDR was held annually since 1960. During this time, significant changes occurred in the composition of the dominant races. In the 1960s–1970s, the population of stem rust included physiological races 21, 17 and

34 according to Stakman's nomenclature [4]. Races 11 and 14 were detected regularly, but were not widely distributed. In the 1960s–1970s, only the resistance genes *Sr7b* and *Sr9g* were completely ineffective. Virulence to the genes *Sr5, Sr21, Sr9e, Sr11, Sr6, Sr8a, Sr36, Sr9b, Sr30* and *Sr17* was low or absent [5]. In subsequent years, the fungal pathotypes, virulent to the resistance genes *Sr5, Sr21, Sr6, Sr8a* and *Sr17*, appeared. Races of the pathogen MKCT, MKCK, MKBK, MKBS, MKBT, RKCT and RKBS dominated in CFDR in 2004 [6]. During this period, the *Sr9e, Sr11, Sr36, Sr9b* and *Sr30* genes were effective. Over the past decade, the structure of the population on the basis of virulence has changed toward the predominance of several aggressive virulent races, including races that are virulent to genes *Sr5, Sr21, Sr9e, Sr7b, Sr6, Sr8a, Sr9g, Sr36, Sr30, Sr9a, Sr9d, Sr10* and *SrTmp.* Among samples from the European part of the Russian Federation, the races of stem rust MKBT and MRLT in 2002 and TKNT, TKST, TTNT in 2005 dominated [7]. The race composition of *P. graminis* f. sp. *tritici* populations in the CFDR in the period 2000–2009 is presented in the work of Skolotneva et al. [8]. They analyzed 387 isolates of the fungus using the North American set of differentiators. Samples were obtained from cereals (wheat and barley), wild herbs and barberry. As a result of the study, 45 races of *P. graminis* f. sp. *tritici* were identified. The predominant races TKNT and TKNTF were isolated. The Ug99 race and its derivatives were not found in the Russian Federation.

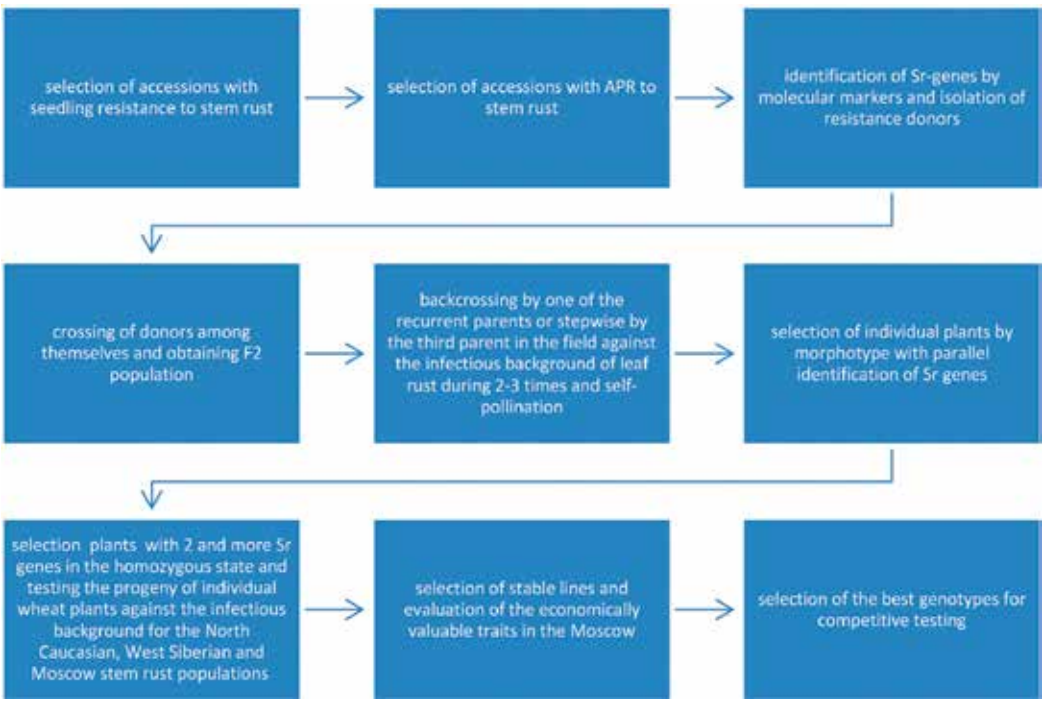
According to the data obtained in 2013 [9], during assessing the collection of lines with known Sr genes, the effective genes of resistance to stem rust in CFDR were the genes *Sr2*, *Sr9e*, *Sr13, Sr25, Sr26, Sr31, Sr32, Sr36, Sr44, SrWld* and the combination of genes *Sr17 + Sr13*, *Sr31 + Sr38*. According to the data of Skolotneva et al. [8], the resistance genes *Sr9e* and *Sr36* in 2009 were ineffective in the Central region of Russia. Differences in the data are probably due to a change in the composition of the pathogen population. Thus, in the same work of Skolotneva, a change in the percentage of fungal isolates virulent to the *Sr17* gene was noted from 92.5% in 2000 to 0% in 2008, and the genes of *Sr31* and *Sr24* remained effective against all local races of stem rust. Observations of the pathogen development in the period 2013–2017, conducted in the All-Russian Research Institute of Phytopathology, showed the annual development of stem rust. The development of the disease on the susceptible genotype of Khakasskaya in 2017 reached 100% [10].

2.2. Development of bread wheat lines with several resistance Sr genes

The process of creating the initial material of bread wheat with several resistance genes passed in several stages. *First stage:* Isolation of resistance sources in the seedling stage; evaluation of the spring bread wheat line against the background of Ug99 natural infection in Ethiopia. *Second stage:* Identification of resistance Sr genes using specific molecular markers and isolation of resistance donors. *Third stage:* Selection of pairs for crossing and hybridization of donors among themselves, obtaining of the segregative population of hybrids F_2. *Fourth stage:* Performing backcrossing by one of the recurrent parents or stepwise hybridization of individual plants by the third parent in the field against the infectious background of leaf rust; subsequent self-pollination or repeated backcrossing with test of progeny against infectious background of leaf rust. At this stage, work with spring and winter plants was carried out in parallel at different plots and with different planting times. *Fifth stage:* Selection of individual

plants by morphotype with parallel identification of Sr genes. *Sixth stage:* Testing the progeny of individual spring wheat plants against the infectious background for the North Caucasian and West Siberian populations of stem rust, and plants of winter wheat for the North Caucasian population of stem rust (infectious background) and natural epidemic development of stem rust in the Moscow Oblast. *Seventh stage:* Evaluation of the economically valuable traits of selected stable lines in the Moscow Oblast conditions in comparison with standard cultivars, selection of the best genotypes for competitive testing.

Schematically, the process of breeding lines with several effective Sr genes can be represented in the following sections.



2.2.1. First stage

The identification of the resistance sources to the race Ug99 of stem rust was started by an employee of FSBSI VIZR Anna Anisimova together with the scientists from Minnesota University (USA) in 2010. At the seedling stage, 386 accessions of bread wheat from the collection of VIR and the "Arsenal" collection from Moscow Sc. Res. Inst. of Agr. "Nemchinovka" were evaluated, six accessions of winter wheat and one accession of spring wheat with resistance to this disease were selected (type of reaction during pathogen penetration from 0 to 2) [11]. It is the selection line GT 96/90 (hereinafter referred to as line 96) from Bulgaria with genetic material of the species *T. timopheevii*, a cultivar of winter wheat Donskaya polukarlikovaya

(hereinafter referred to as D) in the pedigree of which *Aegilops tauschii* was present (accessions from the collection of VIR). From the collection, "Arsenal" lines with translocations from *Ae. speltoides* were selected: 9/00w (*2n = 42*), disomic addition lines of *Ae. speltoides:* 19/95w and 141/97w (*2n = 44*); wheat-*Ae. speltoides*-rye line 119/4-06rw (*2n = 42*), hereinafter referred to as line 119. The only stable accession of spring wheat 113/00i-4 (*2n = 42*) (in the text accession 113), obtained from crossing the spring cultivar Rodina with irradiated pollen of the species *Ae. triuncialis* [12] and then crossed with the line with the genetic material of *T. kiharae*, showed immunity to the natural infection of stem rust race Ug99 in Ethiopia at the stage of an adult plant [13].

2.2.2. Second stage

Identification of resistance Sr genes was carried out using molecular markers both to effective genes against the Ug99 race (*Sr2, Sr22, Sr24, Sr25, Sr26, Sr32, Sr35, Sr36, Sr39, Sr40, Sr44, Sr47*) and to ineffective ones: *Sr9a, Sr15, Sr17, Sr19* and *Sr31*, but providing resistance to local populations of the pathogen. The list of molecular markers is given in **Table 1**. For each primer, the most optimal PCR conditions were selected; the conditions given in the original studies are taken as basis. Separation of amplification products was performed by electrophoresis in 2% agarose and 8% polyacrylamide gels stained with ethidium bromide at a voltage of 100 V for

Sr gene	Chromosome	Marker	References
Sr2	3B	Xgwm533	[14]
Sr9a	2BL	Xgwm47	[15, 16]
Sr15	7AL	STS638	[17]
Sr17	7BL	Wpt5343	[18]
Sr19	2BS	Wpt9402	[18, 19]
Sr22	7AL	Xbarc121, cfa2123	[20, 21]
Sr24/Lr24	3DL/1BS	Sr24#12, Sr24#50	[22]
Sr25/Lr19	7DL	Gb	[23]
Sr26	6AL	Sr26#43	[22]
Sr31	1R/1B	Scm9	[24]
Sr32	2AS,2B	Xbarc55, Xstm773	[19, 25, 26]
Sr35	3AL	Xcfa2170, BE485004	[27]
Sr36	2BS	Xwmc477, Xstm773-2	[28]
Sr39	2BS	Sr39#22	[29]
Sr40	2BS	Xgwm344	[30]
Sr44	7DS	Wpt2565	[18]
Sr47	T2BL-2SL·2SS	Xgwm501	[31]

Table 1. Specific primers used to identify Sr genes.

3 h in 0.5× TBE buffer. As markers of molecular weights, 50 bp, 100 bp and 1 kb GeneRulerTM DNA Ladder from "Fementas" were used. The results of gene identification in new sources are given in **Table 2** [32].

We explain a wide range of identified genes in donors from the "Arsenal" collection by multiple alien translocations of the genetic material of species *Aegilops speltoides, Ae. triuncialis, Triticum kiharae, Secale cereale*, arising during the irradiation of pollen, and in the selection line GT 96/90 by the presence of translocations from the species *T. timopheevii*. The use of such donors, even in paired crosses, can lead to the creation of plant forms with an unusual combination of resistance Sr genes due to the recombination of genes in meiosis. However, since we were faced with the task of obtaining the initial material for the selection process, we had to take into account not only the level of donors ploidy but also the presence of economically valuable traits. It should be noted that despite the positive identification of the *Sr22* gene in the wheat-*Aegilops* lines (9/00w, 141/97w and 119/4-06rw) using the *Xbarc121* and *Xcfa2123* markers, the absence in the pedigree of these lines of the genetic material *T. monococcum* leaves doubt in the presence of this gene.

2.2.3. Third stage

We rejected the use of disomic addition lines with chromosomes of *Aegilops speltoides* in the selection of pairs for crossing, since the supplemented alien chromosome with which we bind resistance was rarely conjugated to wheat chromosomes and was lost in the process of division in meiosis. The remaining donors had an euploid number of chromosomes, but were different according to the morphophysiological and agronomic characteristics (terms of ear formation, height, susceptibility to powdery mildew). The D cultivar and the GT 96/90 line had a very short stem (60–70 cm), early ear formation (late May to early June) and were affected by powdery mildew to a high degree (severity 30–50%), remaining resistant to leaf rust. For donors from the "Arsenal" collection, on the contrary, later ear formation, long stem, but the high resistance to powdery mildew and leaf rust were characteristic. Parent pairs for crossing were selected with alternative development of traits (short stem, early ear formation,

Line, cultivar	Identified Sr genes	Severity, %		Height, cm	Grain weight per ear, g	1000 grains weight, g
		Powdery mildew	Leaf rust			
9/00w	*Sr22, Sr32, Sr44, Sr15*	0–5	0	70–80	1.5	40.0
141/97w	*Sr22, Sr44*	0–10	0	90–110	1.4	36.0
113/00i-4	*Sr2, Sr36, Sr39, Sr40, Sr44, Sr47, Sr15*	0–1	0	90–110	1.4	41.0
119/4-06rw	*Sr22, Sr32, Sr44, Sr9a, Sr17, Sr19*	0–1	$10/_1$	80–100	1.5	42.0
GT 96/90	*Sr24,Sr36, Sr40, Sr47, Sr15, Sr17, Sr31*	30	0	60–70	1.5	42.0
Donskaya polukarlikovaya	*Sr32, Sr44, Sr9a, Sr17, Sr19*	50	$5/_2$	60–70	1.3	40.0

Table 2. Results of the identification of Sr genes in resistance donors to stem rust [32] and their economically useful traits.

susceptibility to powdery mildew) x (long stem, later ear formation, resistance to powdery mildew). The first crossings were conducted in 2010 in the greenhouse. The following pairs of direct crossing and backcrossing were successful: (GT 96/90 × 113/00i-4), (119/96rw × GT 96/90), (113/00i-4 × 119/96rw). In the conditions of the greenhouse, the D cultivar was found to be the earliest ripening, and it was not possible to hybridize with it because of the mismatch of the flowering periods. Later, this cultivar was used in stepwise hybridization. F_1 plants were also grown in the greenhouse. The fact of the segregation of future F_2 populations into winter and spring genotypes from the crossing of winter lines with the spring line 113/00i-4 was taken into account when planning crossings. Crossing with the productive wheat-*Ae. speltoides* line 145/05i (grain weight from ear is 1.9 g, weight of 1000 grains is 49.0 g), which had group resistance to powdery mildew and leaf rust, but was susceptible to Ug99, was additionally planned in order to shift the formative process toward the isolation of productive spring forms of plants.

2.2.4. Fourth stage

Beginning with F_2, the work with spring and winter forms of plants was carried out against the infectious background of leaf rust at different seeding times. Half of the seeds were sown in February in the heated plot after snow melting. After the emergence of shoots, the heating of the soil was switched off, and the plants passed vernalization at natural low temperatures and natural snow cover. In this case, spring plants perished, and winter plants formed the ear. The second half of the seeds were sown in the field in spring. Under these conditions, spring plants formed the ear, and winter crops remained in the tillering phase. Backcrossing of plants resistant to leaf rust, beginning with F_2, was conducted by recurrent spring parent or line 145/05i (when working with spring genotypes) and winter recurrent parent or D cultivar (when creating winter wheat lines). The infectious background of leaf rust was created using all races characteristic for the Moscow Oblast. For further hybridization, only plants resistant to leaf rust were selected. The second backcrossing or self-pollination was carried out under the conditions of a greenhouse, the progeny was sown on the appropriate soil background, and the process of backcrossing on the infectious background of leaf rust was repeated again. Then self-pollination of plants was carried out. The scheme of the selection process for obtaining spring and winter lines with several resistance Sr genes is shown in **Figure 2**.

2.2.5. Fifth stage

In the progeny of self-pollinated plants, which were sown as lines of different generations $BC1F_3$, $BC2F_2$, $BC3F_2$, F_4, F_5, individual plants were selected by morphotype with parallel identification of Sr genes by PCR analysis. During the selection, attention was drawn to the habitus of the plant (bush form, the number of productive shoots), the location of the leaves, the shape of the ear, the presence of marker morphological features (the presence of awns and anthocyanin on different parts of the plant such as stem, ear, anther), the date of ear formation and the degree of severity of affection by powdery mildew and leaf rust were taken into account. Preference was given to plants with group resistance to diseases, with optimal plant height (80–110 cm), early ripening and an ear with 19–21 developed spikelets, that is, the

http://dx.doi.org/10.5772/intechopen.75379

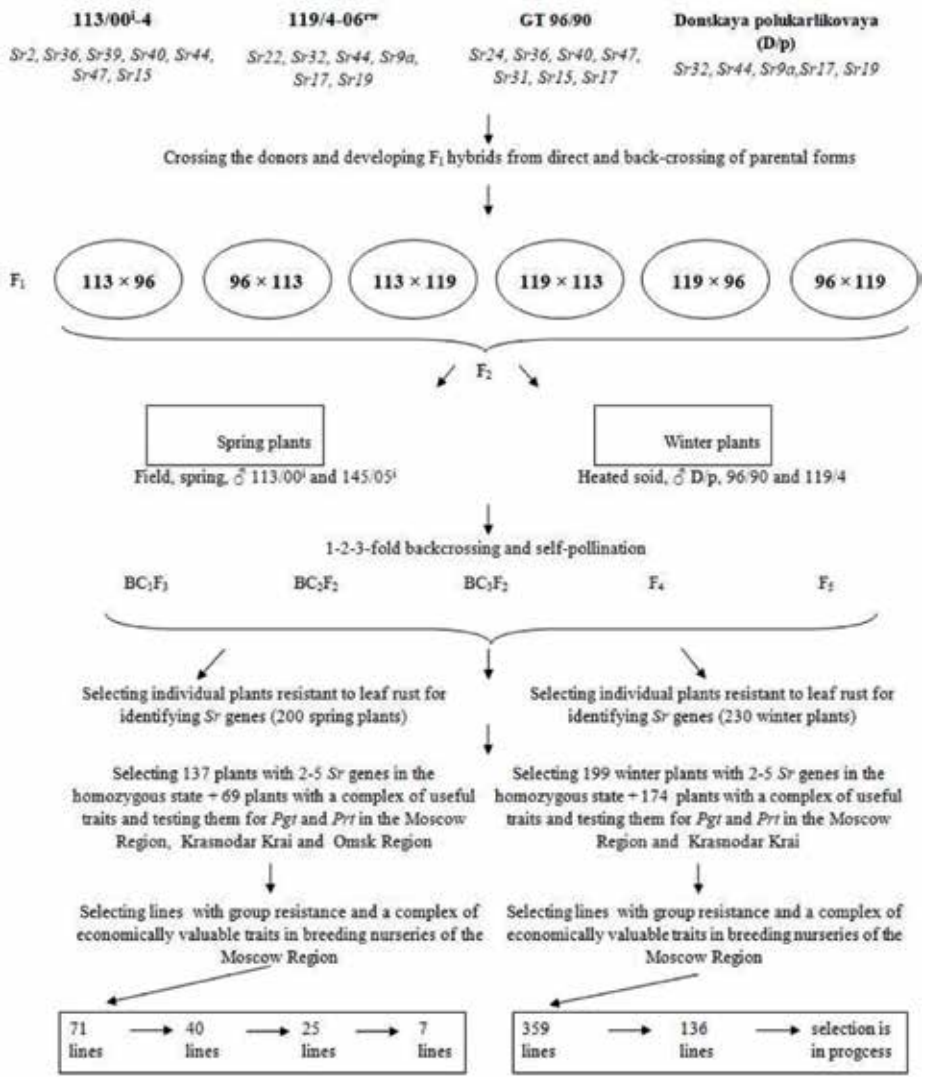


Figure 2. Scheme of development of spring and winter wheat lines with several resistance Sr genes.

selection of individual plants was not accidental, but aimed to combining economically valuable traits. In such plants, a piece of leaf was taken to isolate DNA and to identify Sr genes. In total 200 spring plants and more than 200 winter plants were selected for PCR analysis. The spectrums of identified effective Sr genes in spring and winter plants differed.

In spring plants, the genes *Sr2, Sr44, Sr36* and *Sr40* were found most often in the homozygous state (71, 89, 78 and 26%, respectively, of the number of plants tested). The *Sr22* gene, which was originally identified in the winter donor 119/4-06rw using two markers Xbark 121 and cfa 2123, was detected in the progeny of selected spring plants at a frequency of 20%, when the donor was used for backcrossing and the resulting progeny was self-pollinated. The *Sr39* and

Sr47 genes were rare, with a frequency of 4.4 and 1.4%, respectively. After PCR analysis, 137 individual plants with several Sr genes in the homozygous state were selected from 200 spring plants, namely: with two resistance genes—54 plants, with three—64 plants, with four—15 plants and with five genes—4 plants.

In individual winter plants, selected from the hybrid population represented by the families F3, BC1F2, BC1F3, BC2F3, BC3F2 of different origin, eight genes were identified, which form a row: *Sr2* > *Sr44* > *Sr32* > *Sr36* > *Sr22* > *Sr31* > *Sr47* > *Sr39* and *Sr40* by the frequency of occurrence in progeny. The combination spectrum of the identified genes in winter wheat plants differed from the spectrum of genes identified in spring wheat lines. This is connected with the orientation of backcrossings conducted in winter and spring wheat. The combination of Sr genes compound in the genotypes of winter wheat is more diverse. The plants with the combination of the *Sr22, Sr32* and *Sr44* genes in the homozygous state were most often encountered. Plants with a unique combination of genes characteristic only of winter plants have been found: *Sr2 + Sr22, Sr2 + Sr32, Sr2 + Sr36, Sr36 + Sr44, Sr36 + Sr47, Sr32 + Sr44, Sr22 + Sr44, Sr31 + Sr36, Sr31 + Sr47, Sr31 + Sr44, Sr22 + Sr44 + Sr47, Sr22 + Sr31 + Sr32, Sr22 + Sr31 + Sr44, Sr22 + Sr36 + Sr44, Sr32 + Sr44 + Sr47, Sr31 + Sr36 + Sr47, Sr36 + Sr39 + Sr47, Sr2 + Sr22 + Sr36 + Sr44,Sr2 + Sr31 + Sr36 + Sr44, Sr22 + Sr32 + Sr40 + Sr44, Sr22 + Sr31 + Sr36 + Sr44, Sr2 + Sr22 + Sr32 + Sr44, Sr2 + Sr22 + Sr32 + Sr40 + Sr44.* Specific features in transmission of some resistance genes are noted. In particular, no plants with the *Sr24* gene were detected. The second feature is associated with the *Sr2* gene (the gene was originally identified only in spring wheat 113/00i-4). The *Sr2* gene was in a heterozygous state in more than 70% of winter plants in which it was identified (**Figure 3**).

The presence of *Sr32, Sr39, Sr40, Sr44* genes, which are poorly studied in relation to other *Pgt* races and rarely used in selection programs, with the resistance *Sr2* gene of an adult plant showing "slow rusting" effect, gives particular value to the selected winter plants. However, the presence of the recessive *Sr2* gene of resistance in the heterozygous state in most winter wheat plants will require additional efforts to transfer it to a homozygous state. In particular, we have planned experiments on the production of digaploid lines using androgenesis method. Individual plants with the identified genotype of resistance to stem rust differed greatly in height (75–145 cm), ear productivity (1.0–2.7 g), weight of 1000 grains (36–60 g) and morphological features. For further testing in infectious nurseries of stem and leaf rust, 373 individual winter wheat plants were selected: 199 plants with the identified Sr genes and 174 plants selected for a set of other economically valuable traits. From the populations of spring

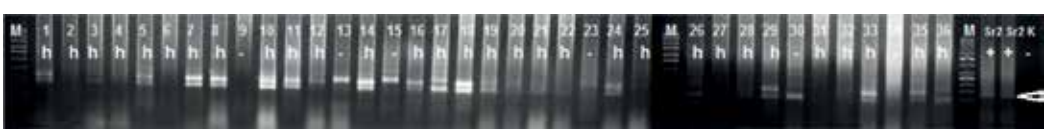


Figure 3. Identification of the *Sr2* gene using the molecular marker *Xgwm533* in winter plants 1–36: M—molecular weight marker of 50 bp "Fermentas", *Sr2*—positive control Pavon76, K—negative control Saratovskaya 29 cultivar. The arrow indicates a diagnostic fragment with a molecular weight of 120 bp. The amplification products were separated in 2% agarose gel. "+"—presence of the diagnostic fragment; "–"—absence of a diagnostic fragment; h—heterozygote.

wheat, only 198 spring plants were selected for further testing: 129 plants with identified Sr genes and 69 plants with a set of valuable traits.

2.2.6. Sixth stage

2.2.6.1. Spring wheat

Progeny testing of individual spring wheat plants was carried out against the infectious background for the North Caucasian and West Siberian populations of stem rust and leaf rust, and against the natural background of the disease course in the Moscow Oblast. It should be clarified that in the south of Russia (Krasnodar Krai), most of the known resistance genes are ineffective against the causative agent of stem rust. Genes *Sr1, Sr5, Sr6, Sr9a, Sr9e, Sr13, Sr24, Sr27, Sr31, Sr32, Sr35, Sr36* remain effective [33]. One hundred and fifty-eight lines of spring wheat (or 81% of the number of studied lines) showed high resistance to infection (0R) by the North Caucasian population of stem rust, and 160 lines were resistant to leaf rust.

Testing of the same set of spring lines in Western Siberia (Omsk), which were sown with a special late spring sowing (late crops are more affected than those sown in the optimal time) led to the death of some lines, but from the 167 surviving lines, 111 lines (66.5%) with resistance to stem rust were selected. In the year of testing (2015), strong epidemic of stem rust was observed in the region. Under these conditions, only a small group of genes, according to the observations of the researchers, was effective *(Sr2, Sr9e, Sr11, Sr12, Sr13, Sr19, Sr24, Sr25, Sr26, Sr27, Sr30, Sr31, Sr35 Sr37),* but none of these genes provided full protection against the disease. The severity of lines with known resistance genes varied from 5 to 30% in comparison with 50–60% severity of cultivars without effective genes [34]. Selected in such harsh conditions, stable lines with group resistance to stem and leaf rust are valuable initial material for the selection of spring wheat in this region. Structural analysis performed in comparison with the standard cultivar Omskaya 37 allowed to select 20 lines with the least decrease in productivity in the unfavorable dry conditions of Western Siberia. In 2016, these lines were involved in crosses with the best adapted varieties cultivated in this region (Shamanin, personal communication).

In the Moscow Oblast, in 2015, no development of stem and leaf rust was observed even on the highly susceptible line Khakasskaya because of unfavorable weather conditions for the development of these pathogens (low air humidity, lack of dew, strong wind). However, in the Moscow suburbs, the spring lines were evaluated for resistance to powdery mildew. After that, the results of lines estimates at three geographic locations were combined, and genotypes that showed resistance simultaneously to leaf and stem rust in Krasnodar and Omsk and resistance to powdery mildew in the Moscow Oblast (71 genotypes) were selected. In 2016, under the conditions of epidemic development of stem rust in the Moscow Oblast, after negative selection for resistance to diseases, the timing of the ear formation, height and the presence of segregation by morphological features, 40 genotypes were left for further tests. After the statistical evaluation of the productivity elements (yield of grain from 0.3 m^2, productivity of the ear, weight of 1000 grains), 25 best genotypes with a set of economically valuable traits were selected (see Stage 7).

2.2.6.2. Winter wheat

The progeny of winter wheat plants was tested in two geographical locations: the Moscow Oblast against the natural but epidemic course of stem rust in 2016 and Krasnodar for the North Caucasian population of stem rust against the infectious background. In the Moscow Oblast in 2016, favorable conditions for the epidemic development of stem rust arose on wheat crops. The focus of the disease arose on winter wheat in the phase of milk ripeness of grain and then switched to spring wheat. The disease affected the standard winter wheat cultivar Moskovskaya 39 by 40% with the type of response to infection 3–4, and allowed a clear differentiation of the genotypes among the sown source material on the basis of resistance, and also the evaluation of the spring wheat lines collection with known resistance Sr genes for the effectiveness of individual genes in the Moscow Oblast.

During assessing the collection of lines with known Sr genes in 2016, it was found that compared to 2013, the spectrum of effective resistance genes to this disease narrowed, which indicates possible mutational processes in the fungus population or various sources of plant disease epidemic. If in 2013, the following genes: *Sr2, Sr9e, Sr13, Sr22, Sr25, Sr26, Sr28kt, Sr30, Sr31, Sr32, Sr36, Sr44, SrWld* and combinations *Sr13 + Sr17* and *Sr31 + Sr38* were effective, then in 2016, only lines with the following Sr genes showed high resistance (severity 0) or resistance (up to 1% severity with the reaction type of 1 point): *Sr28kt, Sr30, Sr31, Sr32* and *SrWld*, and lines with the *Sr9e, Sr17, Sr25, Sr26, Sr33* and *Sr40* genes showed moderate resistance (from 5 to 20% severity with the reaction type of 2 points).

The evaluation of the created winter wheat lines for fungal diseases showed high resistance of most genotypes to local populations of leaf rust and stem rust and to powdery mildew. Only 14 out of 373 sown lines (about 4% of the genotypes) were susceptible to the *P. graminis* of the Moscow population and segregating along this trait. Even more lines (98.7%) were resistant to *P. triticina.* In the test material, there were 147 lines resistant to powdery mildew with severity up to 10% (**Table 3**). One hundred and thirty-six lines with group resistance to the three diseases were selected.

The evaluation of 367 winter wheat lines in the Krasnodar Krai made it possible to isolate 146 immune lines (severity 0) and 22 resistant lines (up to 5% severity, reaction type of 1, 2 points), that is, 46% of the genotypes which showed resistance to the North Caucasian population of stem rust. By comparing the results obtained in the Moscow Oblast and in the Krasnodar Krai, 50 genotypes that showed stability in both geographically remote points were selected.

Pathogen	Total number of lines, pcs.	Immune and resistant lines allocated, pcs.	Susceptible and segregating lines allocated, pcs.
Pucinia triticina	373	368	5
Pucinia graminis	373	359	14
Blumeria graminis	373	147	226

Table 3. The results of estimations of winter wheat lines for fungal diseases against the natural background of leaf, stem rust and powdery mildew development in the Moscow Oblast (2016).

http://dx.doi.org/10.5772/intechopen.75379

2.2.7. Seventh stage

Evaluation of the economically valuable traits of selected stable lines in the Moscow Oblast conditions in comparison with standard cultivars, selection of the best genotypes for competitive testing.

2.2.7.1. Spring wheat

During the selection of spring genotypes, we were guided by such characteristics as the earlier (43–46 days) or simultaneous ear formation with the standard spring cultivar Lada, the optimum height of the plant (up to 110 cm), the grain mass from the ear (1.6–2.6 g) and weight of 1000 grains (45–50 g). During the selection of winter lines, the wintering of the lines was taken into account, and we also oriented toward the listed characteristics and compared them to the standard winter cultivar Moskovskaya 39. The reliability of the differences in the indices (the productivity of the ear, the mass of 1000 grains, the height) was estimated upon the results of a single-factor disperse analysis using the "Agros" statistical analysis algorithms [35]. Protein and gluten content in the grain was determined on an infrared analyzer SpectraStar 2400 in the productive lines with large grain. The content of gluten in the flour was analyzed on Glutamatik Perten device, and the quality of gluten on an IGD-3 M (the measuring instrument of gluten deformation). Other indicators of flour quality (strength and dilution) were determined on alveograph and farinograph. The main physiological trait of the selected lines is the group resistance to fungal diseases (leaf and stem rust, powdery mildew) and the presence of several identified genes of resistance to stem rust that must provide durable resistance to the *Pgt* population in the Central Federal District of the Russian Federation and on the territory of Western Siberia. The distinctive morphological sign of the majority of the lines is the presence of anthocyanin on the pericarp of the grain, which causes the grain to acquire a different degree of coloration (from dark red to dark purple). As a rule, lines with purple grain have the manifestation of anthocyanins on other organs too (stems, ears, anthers). As stated earlier, the 25 best genotypes with a set of economically valuable traits were selected among the spring progeny, which were estimated in 2017 in the control nursery for resistance to diseases, grain harvest from the plot, grain nature and its quality. The control nursery was laid in triple replication in the conditions of the Moscow Oblast (area of the registration plot 1.5 m^2). All tested lines of spring wheat confirmed their high resistance to stem and leaf rust, but none of the lines exceeded the standard cultivar Lada by the yield of grain from the plot. Only three accessions (11-17, 21-17 and 23-17) out of 25 produced a crop that is not inferior to this standard. The second standard cultivar Zlata was strongly affected by stem and leaf rust (up to 70%) and formed a yield significantly lower than Lada and some tested lines (**Table 4**). Some of the selected lines, when compared with standard cultivars, look attractive in terms of the number of days before ear formation, which was reduced by 1–2 days and height (lines 1-17, 12-17, 23-17), group resistance to diseases (lines 1-17, 4-17, 7-17, 8-17, 9-17, 12-17, 15-17, 16-17, 20-17, 23-17), the grain size (lines 12-17, 21-17, 23-17) and grain nature (lines 11-17, 15-17).

According to the results of complex assessments, seven genotypes were selected for the evaluation of grain quality (**Table 5**) and ecological testing in CFDR (Moscow Oblast, Vladimir Oblast, Tula Oblast). After the results of the environmental test, which is planned in 2018, the best prototype of the cultivar will be sent to the State Test and determination of the cultivation regions.

Line, cv	*Sr* genes	Days to heading	Height, cm	Disease severities in Moscow Region, %			Yield, g/1.5 m^2	Nature of the grain, g/l	1000 grain mass, g
				Pgt	*Pt*	*Bgt*			
1-17	*2,36,40h,44*	41	119	0	0	0	780	774	41.2
2-17	*2,36,40,44*	43	128	0	0	15	794	778	47.4
4-17	*2,36,40h*	45	132	0	0	10	735	758	47.8
7-17	*2,32,40,44*	43	140	0	0	1	664	732	40.4
8-17	*2,36,40h,44*	45	147	0	$10/_1$	10	750	782	45.0
9-17	*2,36,40h,44*	42	128	0	0	7	574	764	45.3
11-17	—	43	142	0	0	15	866	794	44.2
12-17	*2,36,40,44*	42	120	0	$1/_1$	5	630	758	48.0
15-17	—	42	135	0	0	10	790	790	46.0
16-17	—	44	123	0	0	10	650	778	47.5
20-17	*2,36,39,40,47,44*	44	123	0	0	0	661	758	41.4
21-17	*2,36,40h,44*	43	120	0	0	15	814	752	50.5
23-17	—	42	120	0	0	3	925	754	49.0
Zlata (St)	—	43	125	70	70	15	625	782	41.0
Lada (St)	—	44	131	40	40	15	1012	792	44.0
LSD < *p* 0.05			6	—	—	—	198	6	4.4

Table 4. Variety of spring wheat lines from the control nursery for some qualitative and quantitative traits (2017).

The analysis of grain and flour samples in 2017 is presented in **Table 5**. Grain has a good nature, corresponding to the first class and the high weight of 1000 grains (see **Table 4**). Almost all the lines have an increased grain hardness, according to the protein content in the grain, they correspond to the first class (>14.5%), and to the gluten content in grain to the second class (> 28%). This allows us to attribute them to a group of strong wheat and use them in mill grist to improve lower quality grain. The high content of gluten in flour characterizes it as a premium product. However, the quality of gluten is characterized as satisfactorily weak in the indications of the measuring instrument of gluten deformation (third group). Only one sample (8-17) for gluten quality corresponds to second group. The strength of the flour (245), determined on the alveograph, allows it to be attributed to a good filler group, and according to the dilution factor of the dough (80), the flour is at the standard level and corresponds to valuable wheat. The results of the baking test show that the volume yield of tin bread of this line exceeds the standard, and the color and porosity of the crumb are not inferior to it. Due to the high content of protein and gluten in the flour of other lines, one should also consider their other purpose, for example, making flour confectionery products, where satisfactorily weak gluten is required (GDI > 85).

According to the data available in the literature, grain cereals with anthocyanin coloration have increased antioxidant activity [36]. However, during obtaining the premium flour, the

Line	Color of grain*	Grain hardness, %	protein in grain, %	Gluten in flour, %	GDI, units the scale of the instrument	Alveograph, W	Dough dilution, units the scale of the instrument	Volume yield of tin bread cm^3	Crumb color	Crumb porosity
4-17	3	74	15.0	37.3	97	145	130	660	2.8	3.0
7-17	2	80	13.6	29.7	98	130	110	610	3.0	3.0
8-17	3	63	14.4	32.3	87	245	80	970	4.3	4.5
9-17	4	72	15.5	33.7	97	150	100	790	2.8	3.3
12-17	3	57	16.0	36.3	97	93	150	640	2.8	2.5
16-17	3	55	15.1	40.1	102	93	175	460	2.8	2.0
17-17	4	54	15.5	34.6	105	71	125	580	2.0	1.5
St cv. Lada	2	46	13.7	29.3	71	306	80	950	4.5	4.8

*1—light red, 2—red, 3—dark red, 4—purple.

Table 5. Indicators for the quality of grain, gluten and test baking of bread in spring wheat lines with different intensity of grain coloring (harvest of 2017).

colored shells of the grain go into the bran. An attempt was made to use whole-wheat flour of bread wheat purple grain lines with a high content of antioxidants (up to 70 mg/100 g) in confectionery technologies. Whole-grain flour had an increased water holding capacity, far exceeding control. But according to their technological properties, the samples were inferior to the standard—the dough formed worse, crumbled, less amenable to laminating. The best technological properties, closest to the standard, were shown by the samples of lines 8-17, 9-17 and 17-17. Sample 17-17 was distinguished by the presence of large bran, which prevented the formation of the dough. The baked sugar cookies were better on the indicators of swelling in water (up to 78%) and specific volume (up to 0.76 g/cm^3) compared to the standard (58% and 0.62 g/cm^3, respectively). The structure of the cookies from all the samples was more crumbly and fragile than that of the standard, and the organoleptical properties (taste, smell, appearance, cross-sectional texture) were at the standard level. Using flour from whole grains in industrial conditions will allow to obtain pastry with a high yield of products suitable for healthy eating.

2.2.7.2. Winter wheat

From the 373 winter wheat lines created during the experiment, 137 were selected for further testing in breeding nurseries of the Moscow Oblast. This group also included 49 stable genotypes, which were selected during the study in the Krasnodar Krai. **Table 6** shows the diversity of the best winter wheat lines by the identified resistance Sr genes and some economically valuable traits in comparison with the standard cv. Moskovskaya 39 in the Moscow Oblast.

Lines	Pedigree	genes	Days to heading	Height, cm	Grain per ear, g	1000 grain mass, g
1-16	(113/119)/D/D	*Sr2h***,*22,44,47*	257	90	2.1	50.0
149-16	(113/119)/D/D/D	*Sr2h,22,32,44*	262	95	2.2	48.0
198-16	(113/119)	*Sr31,36*	268	130	1.9	52.0
167-16	(113/119)/D/D	*Sr2h,22,36*	260	83	1.7	51.0
165-16	(96/113)/ D/96	*Sr2h,36,44*	263	97	2.0	47.0
30-16	(96/113)/ D/D/D	*Sr2h,31,47*	260	86	2.0	48.0
16-16	(96/113)/ D/D	*Sr32h,36 h,22*	259	106	1.7	46.0
43-16	(113/96)/ D/D/D	*Sr2h,22,32,40*	259	78	1.5	48.0
45-16	(113/96)/ D/D/D	*Sr22,32,44*	259	87	2.0	54.0
48-16	(113/96)/ D/96	*Sr2h,32 h,36 h*	260	93	2.4	51.0
54-16	(113/96)/ D/D	*Sr22,31,32 h,36 h*	259	99	1.9	49.0
85-16	(113/96)/96/96	*Sr2h,36,47*	262	99	2.0	45.0
86-16	(113/96)/96/96	*Sr2h,36*	263	98	2.7	49.0
99-16	(113/96)/119/96	*Sr2h,36,39,47*	264	97	1.9	54.0
326-16	(113/96)/119	*Sr2,32*	264	135	2.1	44.0
103-16	[(119/96) × (113 × 96)]/96	*Sr22,36*	264	95	1.8	46.0
128-16	(119/96)/ D/ D	*Sr2h,22,32*	261	91	1.9	61.0
138-16	(119/96)/ D/ D	*Sr2h,22,32*	259	98	2.2	57.0
129-16	(119/96)/ D/ D	*Sr22,32,44*	261	84	2.1	63.0
124-16	(119/96)/ D/ 96	*Sr2h,22,31,32*	262	115	2.3	61.0
131-16	(119/96)/D/96/96	*Sr2h,31,36,47*	263	90	1.8	49.0
St cv. Moskovskaya 39			265	115	1.9	49.0
LSD < p 0.05				25	0.50	$F_{actual} < F\ t_{theoretical}$

***h*—heterozygous state of gene.

Table 6. Some economically valuable traits of the winter wheat lines with identified genotype of resistance to *Pgt*.

Among the winter genotypes, it was possible to select the lines that formed ear 2–8 days earlier than the standard and had a shorter stem than the standard cv. Moskovskaya 39. Both attributes are of selective importance for the Central Federal District of the Russian Federation, and breeders tend to create early ripening short-stemmed analogues of productive cultivars. This is due to the climatic conditions of the CFDR: abundant rainfall with the wind during the ripening of cereals lead to lodging of cereals and crop losses. Thick stiff short stem provides resistance to lodging. Most of the created lines form a large grain with the mass of 46–60 g and the productive ear at the standard level. Several lines (86-16, 48-16) were selected, which are superior to the standard cultivar according to the productivity of the ear (grain mass from ear of 2.7 and 2.4 g, respectively). Preliminary evaluation of lines by grain quality (protein and gluten content in grain) on an infrared analyzer showed an increased value of these parameters in comparison with the Moskovskaya 39 cultivar, which is a quality standard in the non-Chernozem zone of the Russian Federation. The fluctuation of the protein content in the grain

from the isolated lines was in the range of 15.2–20.2%, and the gluten content was 29.7–41.5% (cv. Moskovskaya 39 had 17.6% of protein and 31.4% of gluten in the grain). An additional assessment of the gluten content in flour, carried out on the Glutomatik device, confirmed such a high gluten content in the selected lines (37–61.3%), but the quality of gluten of most lines corresponded to the third class (GDI unit of the instrument 92–114). Such gluten is characterized as satisfactorily weak. Flour with such indicators is used in the confectionery industry for baking biscuits and cookies.

Selected winter wheat lines will have to undergo tests at the control nursery in the Moscow Oblast, and then environmental testing at three geographical locations, before they receive the status of the prototype of a new cultivar.

3. Conclusion

During the period 2010–2017, the initial material of spring and winter wheat was developed in the Moscow Scientific Research Institute of Agriculture "Nemchinovka," which differs fundamentally from the varieties of wheat that have been obtained to date. Namely, for the first time, prototypes of cultivars with group resistance to the most widespread fungal diseases in the Central Federal District of Russia (leaf and stem rust and powdery mildew) were developed. Resistance to stem rust is determined by the presence of 2–4 effective resistance genes not only to the European but to the North Caucasian and West Siberian *Pgt* pathogen populations. Taking into account the presence of the APR gene *Sr2* with other effective genes *Sr22, Sr32, Sr39, Sr40, Sr44* and *Sr47*, lines can also have a selection value for regions where the rust race Ug99 is common. The genetic diversity of lines, as far as the spectrum of resistance genes is concerned, differs from that obtained earlier in the world practice. The possibility of creating such genotypes in a short time is explained by the presence of original resistance donors having in their genealogy an alien genetic material of species relatives (*Aegilops speltoides, Ae. triuncialis, Triticum kiharae, Secale cereale, T. timopheevii, Ae. tauschii*) and the presence of several effective Sr genes in donors identified using specific molecular markers. The advantage of the used donors was the presence of other selection valuable traits such as resistance to leaf rust and powdery mildew, early ripeness and the presence of a short stem. As a result of simple, stepped and backcross crossings with subsequent self-pollination, hybrid populations were obtained from which the individual plants were initially selected, and then on their basis, lines were obtained that were tested for resistance to stem rust at three geographical locations: Moscow, Krasnodar and Omsk. According to the results of testing, the progeny in breeding nurseries of the Moscow Oblast and the results of genotypes resistance to stem rust, the lines of spring and winter wheat are selected in three geographical locations, which form the crop at the level of standard cultivars without the use of chemical protection agents during cultivation. This technology allows to get environmentally friendly products for a healthy diet. In fact, these are new prototypes of spring and bread wheat cultivars for the Central Federal District of Russia, which can be used as donors of resistance to stem rust while improving wheat in other regions. These lines have some morphophysiological features such as the presence of anthocyanin on the stem, anthers and grain. The presence of anthocyanins gives the grain an increased

content of antioxidants and increased resistance to unfavorable environmental factors, according to the literature. Technological evaluation of the grain from the created lines of spring and winter wheat showed an increased content of protein and gluten in flour, which allows them to be classified as a group of strong wheat and used in mill grist to improve the lower quality grain in baking. However, the quality of gluten in new lines is characterized as satisfactorily weak. An attempt to define a different direction for the use of such grains in the food industry, taking into account grain coloring by anthocyanins, namely, in the production of flour confectionery products (sugar cookies) has been undertaken. Product from whole-wheat flour exceeded the standard baking on the basis of the features of swelling in water, volume, crumbliness and fragility, but in organoleptical indicators was not inferior to the standard. It is concluded that the use of whole-grain flour with increased antioxidant activity for baking confectionery products determines the use of this grain for healthy food (not only because of the lack of residual chemical protection agents not used in cultivating such varieties, but also due to the presence of anthocyanins in the grain and its antioxidant properties). Taking into account the conducted researches, a new direction in selection for the Central Federal District of Russia is defined: development of spring and winter wheat varieties with group resistance to fungus diseases and with grain suitable for healthy nutrition.

Acknowledgements

This study was partially supported by the Russian Foundation for Basic Research in 2013–2015.

Conflict of interest

The authors declare that they have no conflict of interest.

Author details

Inna Lapochkina[1]*, Olga Baranova[2], Nail Gainullin[1], Michael Kuzmich[1], Svetlana Polyakova[3], Petr Polityko[1], Ramin Mamedov[1] and Sergey Voronov[1]

*Address all correspondence to: inna-lapochkina@yandex.ru

1 Moscow Scientific Research Institute of Agriculture “Nemchinovka”, Novoivanovsky, Russia

2 All-Russian Institute of Plant Protection, St. Petersburg, Russia

3 All-Russian Scientific Research Institute of Confectionery Industry – Branch of V.M. Gorbatov Federal Research Center for Food Systems, Moscow, Russia

References

[1] Central Federal District Economic Natural. Available from: http://studopedya.ru/2-68711.html

[2] Olivera P, Newcomb M, Flath K, Sommerfeldt N, et al. Characterization of *Puccinia graminis* f. sp. *tritici* isolates derived from an unusual wheat stem rust outbreak in Germany in 2013. British Society for Plant Pathology. 2017;**66**(8):1258-1266. DOI: 10.1111/ppa.12674

[3] Spread of Damaging Wheat Rust Continues: New Races Found in Europe, Africa, Central Asia. Available from: http://www.fao.org/news/story/en/item/469467/icode/

[4] Stakman EC, Levine MN, Loegering WQ. Identification of Physiologic Races of *Puccinia graminis var. tritici*. Washington DC, USA: USDA-ARS; 1962. E617

[5] Volkova VT. The population structure of stem rust pathogen *Puccinia graminis* pers. in the middle and low volga. [PhD thesis] Moscow, Russia: Moscow Lomonosov State University; 1978

[6] Lekomtseva SN, Volkova VT, Zaitseva LG, Chaika MN. Pathotypes of wheat stem rust pathogen *Puccinia graminis* f. sp. *tritici* from various host plants in 1996–2000. Mycology and Phytopathology. 2004;**33**:68-74 (in Russian)

[7] Lekomtseva SN, Skolotneva ES, Volkova VT, Zaitseva LG. Assessment of the phenotypes diversity of the causative agent of wheat stem rust *Pucinia graminis* f. sp. *tritici pers*. In: The Second All-Russian Conference. Modern Problems of Plant Immunity to Harmful Organisms. St. Petersburg, September 29–October 2, 2008; St. Petersburg: Innovation Center for Plant Protection; 2008. pp. 63-65 (in Russian)

[8] Skolotneva ES, Lekomtseva SN, Kosman E. The wheat stem rust pathogen in the Central Region of the Russian Federation. Plant Pathology. 2013;**62**:1003-1010. DOI: 10.1111/ppa.12019

[9] Lapochkina IF, Baranova OA, Shamanin VP, Volkova GV, et al. The development of the initial material of spring common wheat for breeding for resistance to stem rust (*Puccinia graminis* Pers. f. sp. *tritici*) including the Ug99 race in Russia. Russian Journal of Genetics: Applied Research. 2017;**7**(3):308-317. DOI: 10.1134/S207905971703008X

[10] Kiseleva MI, Kolomiets TM, Scolotneva ES, Vetrova MA. Developing stem rust population on winter and spring wheat in Moscow Region in 2014–2017. In: Procceedings of the International Conference "Epidemics of Plant Diseases: Monitoring, Prognosis, Control"; November 13–17 2017; Bolshie Vyazemy, Moscow Region, Russia. 2017. pp. 65-73

[11] Anisimova AV, Steffenson B, Mitrofanova OP, Lapochkina IF, Afanasenko OS. Resistance of assortment of wheat and Aegilops specimens from the collection of VIR to stem rust race Ug99 (TTKSK). In: Tekhnologii sozdaniya i ispol'zovaniya sortov i gibridov s gruppovoi i kompleksnoi ustoichivost'yu k vrednym organizmam v zashchite rastenii (Technologies of Development and Use of Cultivars and Hybrids with Group and Complex Resistance to Pests in Plant Protection, St. Petersburg). 2010. pp. 153-158. (In Russian)

[12] Lapochkina IF. Cytogenetic and morphological features of common wheat hybrids obtained with the use of irradiated pollen of *Aegilops triuncialis* L. Rusian Journal of Genetics. 1998;**34**(9):1064-1068

[13] Gainullin N, Lapochkina I, Iordanskaya I, Baranova O, Anisimova A. Development of initial material of spring and winter wheat on the basis of new sources of resistance to leaf and stem rust. In: International Plant Breeding Congress. 10–14 November 2013; Antalya, Turkey. 2013. p. 191

[14] Hayden MJ, Kuchel H, Chalmers KJ. Sequence tagged microsatellites for the *Xgwm533* locus provide new diagnostic markers to select for the presence of stem rust resistance gene *Sr2* in bread wheat (*Triticum aestivum* L.). Theoretical and Applied Genetics. 2004; **109**:1641-1647. DOI: 10.1007/s00122-004-1787-5

[15] Tsilo J, Yue J, Anderson J. Microsatellite markers linked with stem rust resistance allele *Sr9a* in wheat. Crop Science. 2007;**47**:2013-2020. DOI: 10.2135/cropsci2007.02.0087

[16] Röder MS, Korzun V, Wendehake K, Plaschke J, Tixier M-H, Leroy P, Ganal MW. A microsatellite map of wheat. Genetics. 1998;**149**:2007-2023

[17] Neu CH, Stein N, Keller B. Genetic mapping of the *Lr20-Pm1* resistance locus reveals suppressed recombinations on chromosome arm 7AL in hexaploid wheat. Genome. 2002; **45**:737-744. DOI: 10.1139/G02-040

[18] Crossa J, Burgueño J, Dreisigacker S, Vargas M, Herrera-Foessel SA, Lillemo M, Singh RP, Trethowan R, Warburton M, Franco J, Reynolds M, Crouch JH, Ortiz R. Association analysis of historical bread wheat germplasm using additive genetic covariance of relatives and population structure. Genetics. 2007;**177**:1889-1913. DOI: 10.1534/genetics.107.078659

[19] Yu LX, Abate Z, Anderson JA, Bansal UK, Bariana HS, Bhavani S, Dubcovsky J, Lagudah ES, Liu SX, Sambasivam PK, Singh RP, Sorrells ME. Developing and Optimizing Markers for Stem Rust Resistance in Wheat. In: McIntosh RA, BGRI Technical Workshop, editors. Borlaug Global Rust Initiative, Cd. Obregón, Sonora, Mexico. 2009. pp. 117-130

[20] Khan RR, Bariana HS, Dholakia BB, Naik SV, Lagu MD, Rathjen AJ, Bhavani S, Gupta VS. Molecular mapping of stem and leaf rust resistance in wheat. Theoretical and Applied Genetics. 2005;**111**:846-850. DOI: 10.1007/s00122-005-0005-4

[21] Yu LX, Liu S, Anderson JA, Singh RP, Jin Y, Dubcovsky J, Brown-Guidera G, Bhavani S, Morgounov A, He Z, Huerta-Espino J, Sorrells ME. Haplotype diversity of stem rust resistance loci in uncharacterized wheat lines. Molecular Breeding. 2010;**26**:667-680. DOI: 10.1007/ s11032-010-9403-7

[22] Mago R, Bariana HS, Dundas IS, Spielmeyer W, Lawrence GJ, Pryor AJ, Ellis JG. Development of PCR markers for the selection on wheat stem rust resistance genes *Sr24* and *Sr26* in diverse wheat germplasm. Theoretical and Applied Genetics. 2005;**111**:496-504. DOI: 10.1007/ s00122-005-2039-z

[23] Ayala-Navarrete L, Bariana HS, Singh RP, Gibson JM, Mechanicos AA, Larkin PJ. Trigenomic chromosomes by recombination of *Thinopyrum intermedium* and *Th. ponticum*

translocations in wheat. Theoretical and Applied Genetics. 2007;**116**:63-75. DOI: 10.1007/s00122-007-0647-5

[24] Weng Y, Azhaguvel P, Devkota RN, Rudd JC. PCR-based markers for detection of different sources of 1AL.1RS and 1BL.1RS wheat–rye translocations in wheat background. Plant Breeding. 2007;**126**:482-486. DOI: 10.1111/j.1439-0523.2007.01331.x

[25] Dundas IS, Anugrahwati DR, Verlin DC, Park RF, Bariana HS, Mago R, Islam AKMR. New sources of rust resistance from alien species: Meliorating linked defects and discovery. Australian Journal of Agricultural Research. 2007;**58**:545-549

[26] Somers DJ, Isaac P, Edwards K. A high-density microsatellite consensus map for bread wheat (*Triticum aestivum* L.). Theoretical and Applied Genetics. 2004;**109**:1105-1114. DOI: 10.1007/s00122-004-1740-7

[27] Zhang W, Olson E, Saintenac C, Rouse M, Abate Z, Jin Y, Akhunov E, Pumphrey M, Dubcovsky J. Genetic maps of stem rust resistance gene *Sr35* in diploid and hexaploid wheat. Crop Science. 2010;**50**:2464-2474. DOI: 10.2135/cropsci2010.04.0202

[28] Tsilo TJ, Jin Y, Anderson JA. Diagnostic microsatellite markers for detection of stem rustresistance gene *Sr36* in diverse genetic backgrounds of wheat. Crop Science. 2008;**48**: 253-261. DOI: 10.2135/cropsci2007.04.0204

[29] Mago R, Zhang P, Bariana HS, Verlin DC, Bansal UK, Ellis JG, Dundas IS. Development of wheat lines carrying stem rust resistance gene *Sr39* with reduced Aegilops speltoides chromatin and simple PCR markers for marker assisted selection. Theoretical and Applied Genetics. 2009;**119**:1441-1450. DOI: 10.1007/s00122-009-1146-7

[30] Wu S, Pumphrey M, Bai G. Molecular mapping of stem-rust-resistance gene *Sr40* in wheat. Crop Science. 2009;**49**:1681-1686. DOI: 10.2135/ cropsci2008.11.0666

[31] Faris JD, Xu SS, Cai X, Friesen TL, Jin Y. Molecular and cytogenetic characterization of a durum wheat-*Aegilops speltoides* chromosome translocation conferring resistance to stem rust. Chromosome Research. 2008;**16**:1097-1105. DOI: 10.1007/s10577-008-1261-3

[32] Baranova OA, Lapochkina IF, Anisimova AV, Gajnullin NR, Iordanskaya IV, Makarova IY. Identification of Sr genes in new common wheat sources of resistance to stem rust race Ug99 using molecular markers. Russian Journal of Genetics: Applied Research. 2016;**6**(3): 344-350. DOI: 10.1134/S20790559716030011

[33] Sinyak EV, Volkova GV, Nadykta VD. Characteristics of *Puccinia graminis* f. *tritici* population by virulence in the North Caucasus Region of Russia. Russian Agricultural Sciences. 2014;**40**:32-34

[34] Shamanin V, Salina E, Wanyera R, Zelenskiy Y, Olivera P, Morgunov A. Genetic diversity of spring wheat from Kazakhstan and Russia for resistance to stem rust Ug99. Euphytica 2016;**212**:287-296. DOI: 10.1007/s10681-016-1769-0

[35] Martynov SP. Statistical and Biometrics–Genetic Analysis in Plan Breeding and Selection. Software Package AGROS, ver. 2.09. Tver. 1999 (in Russian)

[36] Yashin A, Yashin Y, Fedina P, Chernousova N. Determination of natural antioxidants in food cereals and legumes. Analytics. 2012;**2**:32-36 (in Russian)

Chapter 11

Interrelation of Functional Properties of Protein Products from Wheat with the Composition and Physicochemical Characteristics of Their Proteins

Valentina V. Kolpakova, Nikolay D. Lukin and
Irina S. Gaivoronskaya

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.75803

Abstract

The results of studies of the correlation relationship between the functional properties of dry wheat gluten, protein concentrates from wheat bran and their granulometric fractions with the features of the chemical composition, and the physicochemical properties of their proteins are presented. Granulometric fractions of bran were obtained from grinding process systems with different particle sizes. The correlation interrelation between the functional properties of protein products (solubility, water binding capacity, fat-binding capacity, and foaming capacity) with a mass fraction of components, fractional amino acid composition, the number of thiol metabolism groups (–S-S– and –SH) and the aggregation capacity of whole gluten proteins, its fractions (gliadin, glutenin) and products from wheat bran. The obtained information is expedient for using when modifying the properties of wheat protein products with the purpose of expanding the directions of their use.

Keywords: protein products from wheat, chemical composition, functional properties, physicochemical characteristics of proteins, interrelation

1. Introduction

Cereals crops on a global scale are the largest (or most spread) sources of proteins. Among them, wheat occupies an important place, the world production of which has increased from 450 million tons in 1981–750 million tons at present. Wheat is the only type of grain crop

IntechOpen

© 2018 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. CC BY

from which spare proteins in the dry wheat gluten (DWG) form were extracted industrially, intended as a protein ingredient to improve the baking properties of flour and meat substitution in sausage products. In time of processing of wheat grains on DWG forms bran, which is additionally a source of valuable food protein. Therefore, this chapter is devoted to the results of a study of the physicochemical properties of DWG proteins and protein concentrates from wheat bran for the purpose of applying the information obtained for practical purposes to improve and regulate the functional properties of protein ingredients in the development of food formulas.

Functional properties of protein products are understood as physicochemical indicators that determine the behavior of proteins in the production of food products, providing the necessary structure, and consumer properties [1]. The indicators characterize the parameters of products, some of which are substituted or supplemented with protein in the technological processes of food production. The functional properties of protein products are evaluated both in numerical values and in profiles of dependencies on various technological factors (temperature, pH, processing time, etc.) [2–5]. This approach to the evaluation of properties is reflected in the term "techno-functional," which includes the features of the reactivity of proteins in the technological processes of production and storage of food systems. Functional properties for concrete food systems are usually evaluated on model recipes, and then compared with the properties of traditional or known protein products. The presence of hydrophilic and hydrophobic groups in one chain ensures the interaction of proteins with water, lipids, carbohydrates, other compounds and leads to the formation of stable emulsions, foams, gels, and so on. In solutions proteins can perform a dispersing and suspending roles, it's able to cling to solid particles and by that forming cementing structures. The presence of polar and nonpolar, charged and uncharged groups in one polymer chain allows proteins to interact with different types of compounds and, thereby, influence the quality of food products.

The most important functional properties of protein products are hydration, fat-binding capacity, foam ability, stability of emulsions, foam stability (FS), gel-forming ability, adhesion, rheological properties (viscosity, elasticity), ability to spin and texturing [1, 6, 7]. The values of the functional properties of protein products always determine the directions of their use in the production of food products as technological or nutritional ingredients, but not always these properties satisfy the requirements of the consumer; therefore, in the chemistry of dietary protein, there is a direction devoted to regulating the quality indices of vegetable protein products by various modification processes [8–12].

It is known that the functional properties of protein products depend on the chemical nature of the raw materials (wheat, rye, soybean, etc.), methods of isolation, processing, and technological regimes of food production (pH, temperature, recipe, etc.) [13–14]. When analyzing the nature of vegetable proteins, food recipes' designers limit themselves, as a rule, to a statement of facts showing how a particular kind of raw material affects the functional properties but does not study the molecular basis that conditions these properties. In the practice of using protein products, at best, technological factors affecting their functional properties (temperature, pH, electrolytes, etc.) are taken into account, whereas the characteristics of the chemical,

biochemical composition, and physicochemical properties of the polypeptides themselves are practically not are considered. Despite the fact that, for example, dry wheat gluten (DWG) is widely used in the production of bread as an improver or filler [15–19], the areas of its use can be expanded by modifying the functional properties.

The choice of DWG is conditioned not only by the fact that wheat is one of the traditional cultures of many peoples of the world for bread production but also because the increasing volumes of its cultivation are aimed at producers to use it in technologies and other types of food products. In addition, the amount of secondary products of wheat processing in the form of bran is also increasing. Taking into account the functional properties on the basis of DWG, we have developed special mixtures for the production of oil cakes and protein-containing biscuits [20], based on the gel-forming and foaming ability—marshmallows with the replacement of egg protein on DWG [21], based on enzymatically hydrolyzed DWG-bread with increased content protein from amaranth (20–25%) for diabetics (unpublished data). However, the processes of modifying the functional properties of protein products from wheat, the prophylactic and dietary properties of products from them, can be more effective if one has more information about the structural features and properties of their proteins, as it is known for proteins from other cultures [22–24] that additional studies are needed on the characteristics of the composition and properties of protein products from wheat, are the following facts. Thus, it is known that soluble proteins have a greater set of functional properties than poorly soluble proteins. They have little change in viscosity, gelatinization, but they have a high ability to stabilize suspensions, emulsions, and foams. However, there are proteins that do not fall under these patterns. So DWG proteins, despite their low solubility in water (1–3%), form structured gels that withstand heating, freezing, and drying. Therefore, they are used to prepare protein fibers as a binding agent in the production of film membranes, meat analogs, and non-food products [25, 26].

Another example is protein flour made from wheat bran. Having relatively low solubility values (10–20%), it has a high fat-emulsifying ability (FEA) and foaming capacity (FC): 72–97% and 74–100%, respectively [2, 3]. It is possible to increase the solubility of proteins to 25–100% by heating to 40–90°C, changing the ionic strength of the system or pH [3], but it is difficult to predict the final result of solubility control, as well as other functional properties, because it will often have a "one-time" nature and do not provide, as a rule, a stable forecasting of the quality of finished products. Hence, in order to predict stable results of the modification of the quality of protein products, the purpose of the present study was to study the composition and physicochemical properties of DWG proteins and products from wheat bran and to establish a correlation relationship between the results and the basic functional properties of the ingredients.

2. Materials and methods

2.1. Materials

As protein products, two samples of dry wheat gluten were used from LLC "BM" (Kazakhstan) and "Royal Ingredients Group BV" (the Netherlands), as well as concentrates from wheat

bran and their fractions obtained according to the technology developed by us [27]. To study the amino acid composition of proteins, three samples of wheat gluten were used, which were manually washed from the flour of a typical "strong" sort of grain Saratov 29 (spring), typically "weak"—Akmolinka 1 (spring) and typically medium Gorkovskaya 52 (winter). The crude gluten was dried on a lyophilic plant, it was regenerated by washing in tap water for 15 min and the deformation index on the IDG-1 instrument was determined. Regenerated gluten in the first grade of grain was characterized as slightly elongated, "strong," with an indicator of the device of 58 units, in the second—tensile, "normal" with an indicator of 70 equipment units, the third—as very extensible and "weak" with an indicator of 100 equipment units.

Protein concentrates from wheat bran were obtained from various systems of the technological process of JSC "Moscow Combine of Bread Products," the quality of grain and bran was in accordance with the requirements of standards. The bran was combined, sieved through a sieve of different diameters, and granulometric fractions with a particle size of more than 1000, 670, 195, and less than 195 μm were obtained.

To compare the results of the relationship between functional properties and physicochemical parameters for proteins from wheat and protein products from another type of raw material, soy concentrate, soy isolate Supro 760 from "Soloe" Supro (USA), soy isolate ArdexF ADM (USA), concentrates from amaranth and grain of rye, obtained by our methods [28, 29].

2.2. Determination of chemical composition

Indicators of the chemical composition of protein products were determined by the methods of state standards of the Russian Federation and generally accepted methods. The mass fraction of moisture was determined in accordance with GOST 13586.5-85; ash content—GOST 10847-74; mass fraction of fat—according to the method of Soxhlet in the apparatus of the firm "Buchi"—GOST 29033-91, the mass fraction of protein—in the automated Kjeldahl system of the firm "Buchi"—GOST 10846-91, fiber—according to Gennesberg and Shtoman—GOST 31675-2012. Carbohydrates were calculated as the difference between 100% and the sum of the mass fraction of protein, fat, ash, and fiber.

2.3. Determination of the amino acid composition of proteins

A liquid chromatograph from Hitachi (Japan) was used in a mode with a sulfonated styrene-divinylbenzene copolymer and a step gradient of sodium citrate buffer solutions with increasing pH and molarity. The data were processed in an online system "MultiChrome 1.52" for Windows 98. A sample of 3–5 mg sample was placed in a glass ampoule, 300 μl of a mixture of concentrated hydrochloric acid and trifluoroacetic acid (2, 1) with 0.1% 2-mercaptoethanol was added. The sample was frozen in liquid nitrogen, evacuated and hydrolyzed at 155°C for 1 h. The hydrolyzable mixture was evaporated on a rotary evaporator (Centrivap Concentrator Labconco, USA). To the residue, 0.1N HCl was added and centrifuged for 5 min at 800 g on a Microfuge 22R centrifuge (Beckman-Coulter, USA).

http://dx.doi.org/10.5772/intechopen.75803

2.4. Determination of the fractional composition of proteins

1 g of the protein product, weighed to within 0.001 g, was placed in a centrifuge tube, 10 cm^3 of a 0.5 mol/dm^3 NaCl solution was added, shaken for 1 h and centrifuged for 15 min at 8000 g. The centrifugate was drained, 10 cm^3 of cold distilled water was added to the precipitates, thoroughly mixed, and centrifuged again. The combined centrifuges were taken as albumins and globulins. To extract the gliadin proteins, 20 cm^3 of 70% ethanol was added to the precipitates, shaken at 180–200 rpm for 1 h and left overnight at room temperature. The next day the sample was shaken for 30 min and centrifuged at 8000 g for 15 min. The centrifugate (gliadin) was drained, 20 cm^3 of 0.1 mol/dm^3 acetic acid was added to the precipitates and again shaken for 1 h. The suspension was centrifuged under the same conditions. The extraction procedure was repeated one more time. The combined solutions of proteins soluble in acetic acid were considered to be soluble glutenin. To isolate insoluble glutenin to the precipitates, 20 cm^3 of AUC included 0.1 N acidic acid, 6M urea, and cetyl three methyl ammonium bromide solvent (pH 4.1) were added [24]; the tubes were shaken for 1 h and centrifuged. The extraction operation was repeated once more, after which the centrifuges were combined and the protein content of Kjeldahl was determined therein. The protein precipitate was designated as an insoluble protein. The amount of each fraction was expressed as the percentage of soluble and insoluble protein from the total amount of protein in the sample.

2.5. Determination of the functional properties of protein products

Functional properties of DWG samples, protein products from wheat bran, amaranth, rye, and soybean were determined by the methods described in [30].

2.6. The content of thiol exchange groups

The content of disulfide bonds and sulfhydryl groups in protein preparations from wheat bran was analyzed by the Ellman method in Bogdanov's modification [31].

2.7. Determination of the constant of the final stage of protein aggregation

To determine the aggregating properties of proteins, a sample of the product 1.0 g with an accuracy of ±0.001g was suspended in 10 cm^3 of a 0.05 mole/ dm^3 solution of CH_3COOH for 1 h on a mechanical shaker. The solution was then centrifuged for 15 min at 3000 g, the centrifugate was filtered and the Lowry protein was determined in the filtrate. The solution was diluted with 0.05 mol/dm^3 acetic acid to a concentration of 0.02% protein. To 1.3 cm^3 of the protein solution, 1.3 cm^3 of 0.2 mol/dm^3 phosphate buffer containing 2 mol/dm^3 NaCl (pH 5.6) was added to the spectrophotometer cuvette. Then, after 10 min at a wavelength of 350 nm, the optical density (turbidity) of the solution was measured. The constant of the final stage of aggregation (τ_{10}/C) was calculated as the ratio of turbidity (τ) to protein concentration (C) [32].

Analyses were carried out in 3–5 replicates, the results were represented as arithmetic means. To determine the confidence interval of the average arithmetic result, the Student's test was

used at the significance level $p = 0.05$. The statistical processing of the results was carried out with the programs Statistica 6.0 and Mathematica 5.2.

3. Results and discussion

3.1. Dependence of the functional properties of protein products on the chemical composition

To study the relationship between the chemical composition and the functional properties of protein products from wheat, the mass fraction of the main components was determined. It was established that all the products, depending on their belonging to one or another group, contained different amounts of protein, carbohydrates, fat, ash, and fiber (**Table 1**).

The high correlation between the mass fraction of components and the functional properties of protein products from wheat (**Table 2**) was not found, which is confirmed by the calculation of the correlation coefficients (r) for variant pairs for different pairs of indices (**Table 3**). Correlation dependence at the mean level is established only for the mass fraction of protein with FEA, water-binding capacity (WBC), and FC protein products ($r = 0.51 - 0.60$). Correlation coefficients for the mass fraction of the remaining parameters of the chemical composition with fat-binding ability (FBA), foam stability (FS), and protein solubility (PS) are relatively low.

The correlation relationship between the chemical composition of other vegetable protein products (**Table 1**) and their functional properties (**Table 2**) was established at a high level ($r = 0.75 - 0.79$) for the mass fraction of protein with FEA and FC and a relatively low for other indicators ($r = <0.5$).

3.1.1. Effect of amino acid composition on the functional properties of protein products

The interrelation of functional properties with the amino acid composition of proteins was studied on three gluten samples of different quality (strong, good, and weak), three of its fractions (gliadin, soluble, insoluble glutenin), and three protein concentrates from unconventional grains: amaranth, wheat bran, and rye. The results of determining the amino acid composition of gluten and its fractions are given in **Table 4**, and for other protein products in **Table 5**. Based on the data obtained, the sums of polar (lysine, arginine, aspartic, glutamic acid) and nonpolar amino acids (glycine, phenylalanine, alanine, leucine, methionine, isoleucine, valine, and proline), on the ratio of which the surface properties of proteins will depend, hence their functional properties: solubility, foaming, the ability to bind and emulsify fat, and so on (**Tables 4** and **5**). Coefficients of pair correlation, reflecting the interrelation between the amino acid composition and functional properties, are shown in **Table 6**. It is shown that for gluten of different quality (weak, good, and strong), a high positive correlation between the sum of polar amino acids and FBA is detected, high negative with solubility. A high, directly proportional relationship was found for FEA and the sum of nonpolar amino acids ($r = 0.86$).

Protein products	Humidity, %	Mass fraction, % on dry substance				
		Protein	Fat	Carbohydrates	Insoluble fibers	Ash
Concentrates from wheat:						
DWG (Kazakhstan)	4.0 ± 004	75.0 ± 0.9	1.0 ± 0.04	22.0 ± 0.6	1.0 ± 0.06	1.0 ± 0.12
DWG (The Netherlands)	5.0 ± 0.10	74.0 ± 0.7	1.0 ± 0.08	23.0 ± 1.0	1.0 ± 0.08	1.0 ± 0.17
Concentrate from wheat bran	5.3 ± 0,05	77.9 ± 2,1	1.3 ± 0.06	14.7 ± 1.0	2.8 ± 0.08	3.3 ± 0.05
Concentrates from wheat bran fractions, N of sieve, d, mcm:						
1,0 > 1000 (descent)	4.1 ± 0.02	72.6 ± 0.21	4.0 ± 0.04	12.8 ± 0.15	4.5 ± 0.03	6.1 ± 0.02
067 670 (descent)	4.7 ± 0.31	69.4 ± 0.32	3.8 ± 0.06	17.4 ± 0.04	3.8 ± 0.01	5.6 ± 0.03
38 195 (descent)	5.1 ± 0.22	72.5 ± 0.51	2.2 ± 0.07	17.3 ± 0.12	3.2 ± 0.06	4.8 ± 0.02
38 <195 (pass)	5.6 ± 0.30	75.7 ± 0.14	1.4 ± 0.02	19.0 ± 0.08	2.1 ± 0.02	1.8 ± 0.05
Protein products from other types of raw materials:						
Soy flour (Belgium)	9.0 ± 0.15	43.0 ± 0.5	14.0 ± 0.7	34.0 ± 0.50	5.0 ± 1.00	4.0 ± 1.00
Soy concentrate (Russia)	4.0 ± 0.13	61.0 ± 1.2	5.0 ± 1.0	26.0 ± 0.25	4.0 ± 0.09	4.0 ± 0.09
Soy Isolate Supro 760	4.0 ± 0.20	92.0 ± 1.5	0.5 ± 0.02	1.5 ± 0.80	3.0 ± 0.08	3.0 ± 0.05
Soy isolate Ardex F	4.0 ± 0.40	91.0 ± 1.1	0.5 ± 0.01	2.0 ± 0.40	3,0 ± 0,05	3.5 ± 0.08
Amaranth concentrate	8.0 ± 0.20	71.6 ± 1.4	1.0 ± 0.08	20.0 ± 1.1	3.0 ± 0.20	4.4 ± 0.10
Concentrate from rye grains	6.2 ± 0.13	75.5 ± 2.0	1.3 ± 0.04	17.8 ± 0.09	2.6 ± 0.05	2.8 ± 0.08

Table 1. Chemical composition of protein products from plant material.

For the gliadin fraction, the regularities in the relationship between the sum of polar amino acids and FBA and solubility are similar to those of the whole gluten complex, and additional regularities were revealed for the sum of nonpolar amino acids: the more they were contained in the gliadin, the higher the FC, FS, and FEA gluten (r = 0.70 ± 0, 99). For the sum of nonpolar amino acids in the soluble fraction of glutenin, a high negative correlation with FBA was found, a high positive with solubility and FA. The more the amount of polar amino acids in the fraction was, the lower the values of the FBA, FEA, and FA but higher solubility.

Protein products	PS, % *	WBC, g/g	FBA, g/g	FEA, %	SE, %	FC, %	FS, %
Concentrates from wheat:							
DWG (Kazakhstan)	1.2 ± 0.50	2.39 ± 1.00	2.32 ± 0.25	64 ± 1.6	92 ± 2.5	220 ± 2.8	65 ± 2.0
DWG (The Netherlands)	1.1 ± 0.15	2.27 ± 0.90	1.24 ± 0.50	50 ± 2.0	70 ± 1.6	182 ± 1.9	59 ± 2.0
Concentrate from wheat bran	16.0 ± 0.9	2.90 ± 0.08	4.20 ± 0.5	56 ± 1.3	80 ± 1.2	85 ± 1.3	70 ± 2.0
Concentrates from wheat bran fractions, N of sieve, d, mcm:							
1.0 > 1000 (descent)	13.7 ± 0.6	1.1 ± 0.10	2.4 ± 0.09	95 ± 1.2	90 ± 2.2	132 ± 3.2	75 ± 1.8
067 670 (descent)	21.5 ± 1.1	1.0 ± 0.09	2.4 ± 0.07	89 ± 1.1	85 ± 1.3	130 ± 2.2	74 ± 2.1
38 195 (descent)	26.9 ± 1.6	0.7 ± 0.05	2.8 ± 0.04	72 ± 1.5	79 ± 1.4	129 ± 1.2	65 ± 1.2
38 <195 (pass)	9.8 ± 0.6	0.5 ± 0.06	4.9 ± 0.07	61 ± 2.0	66 ± 2.7	107 ± 1.2	63 ± 1.7
Protein products from other types of raw material:							
Soy flour (Belgium)	45.1 ± 2.0	1.60 ± 0.80	1.20 ± 0.08	49 ± 1.2	47 ± 1.0	80 ± 2.1	60 ± 1.7
Soy concentrate (Russia)	75.3 ± 1.3	7.40 ± 0.35	2.20 ± 0.50	61 ± 0.9	48 ± 0.2	50 ± 2.0	68 ± 1.5
Soy Isolate Supro 760	72.6 ± 1.5	7.90 ± 1.00	1.80 ± 0.05	55 ± 0.5	55 ± 1.2	110 ± 2.5	57 ± 1.2
Soy isolate Ardex F	78.5 ± 2.0	6.00 ± 1.50	1.20 ± 0.08	48 ± 0.9	45 ± 1.0	95 ± 2.0	55 ± 1.2
Amaranth concentrate	46.0 ± 1.0	2.60 ± 0.05	2.60 ± 0.80	57 ± 1.20	54 ± 1.0	200 ± 2.0	65 ± 1.5
Concentrate from rye grains	18.2 ± 1.0	1.80 ± 0.50	2.20 ± 0.90	87 ± 1.5	88 ± 1.1	100 ± 1.5	40 ± 1.6

*PS—protein solubility; WBC—water-binding capacity; FBA—fat-binding ability; FEA—fat-emulsifying ability; SE—stability of the emulsion; FC—foaming capacity; FS—foam stability.

Table 2. Functional properties of protein products.

It was found that the functional properties of gluten are also interrelated with the characteristics of the amino acid composition of insoluble glutenin: the more nonpolar amino acids were FEA. The number of polar amino acids is also directly proportional to the FC values but is inversely proportional to WBC and FBA.

Functional properties	Fraction of total mass, % by dry matter			
	Protein	Carbohydrates	Fat	Insoluble fibers
PS, %	0.44	0.15	0.30	−0.11
WBC, g/g	0.55	0.22	0.13	0.27
FBA, g/g	−0.22	0.41	0.14	0.42
FEA, %	0.60	0.20	0.36	0.32
FC, %	0.51	0.25	0.40	0.18
FS, %	0.23	0.37	0.24	0.28

Table 3. Correlation coefficients (***r***) between functional properties and chemical composition of protein products.

The results of the dependence of the functional properties of the whole complex of dry wheat gluten on the characteristics of the amino acid composition of its proteins were confirmed by data obtained for protein concentrates from amaranth, rye, and wheat bran. It is also shown that the values of Foaming Capacity and Foam Stability are directly proportional to the content of the polar (r = 0.93) and nonpolar (r = 0.68 and 0.85) amino acids, and the values of FEA are inversely proportional to the sum of nonpolar amino acids (r = −0.79).

3.1.2. Effect of the fractional composition of proteins on the functional properties of protein products

The effect of the fractional composition of proteins on the functional properties of protein products was studied using protein products from wheat bran and their granulometric fractions as an example. Protein products obtained from bran fractions with different particle sizes differed both in their functional properties (**Table 7**) and in the fractional composition of their proteins (**Figure 1**). The highest amount of albumins and globulins (44%) had protein products obtained from the bran fraction with a particle size of 195–670 μm, the lowest (32%) protein products from the fraction with a particle size <195 μm.

The highest amount of gluten proteins (gliadin, glutenin) was observed for products isolated from the fraction with a size of <195 μm, the smallest for products from the cut fraction with a particle size> 1000 μm. A large amount of insoluble glutenin (37%) differed products from a large bran fraction (> 1000 μm), a smaller (12%) from a granulometric fraction with a particle size <195 μm. Mathematical processing of the data showed that protein solubility was directly proportional to the sum of albumins and globulins (r = 0.90), FBA—from the amount of both gluten fractions and their sums (r = 0.78–0.90) and the FEA–of the amount of high molecular weight (MM) glutenin and insoluble proteins (r = 0.73–0.78) (**Figure 1**). A high inverse relationship was found for the solubility of wheat bran concentrate proteins on the amount of gliadin and the average negative correlation (r = −0.51–0.69) for WBA on the amount of gliadin and sum insoluble proteins.

Amino acid	Strong DWG				Good DWG				Weak DWG			
	1	2	3	4	1	2	3	4	1	2	3	4
Lysine	2.24	0.70	1.17	2.63	1.57	0.60	1.27	1.74	1.51	0.79	1.21	1.20
Histidine	2.27	1.81	1.47	2.24	1.99	1.70	1.60	1.76	2.04	1.49	1.20	1.67
Arginine	3.62	2.87	2.85	3.96	3.76	2.55	3.62	4.24	3.43	2.51	3.10	3.44
Aspardic acid	3.75	2.84	2.69	4.09	3.17	3.12	2.62	3.89	3.32	2.95	1.99	4.44
Threonine	2.37	2.20	2.73	5.13	5.16	2.24	2.58	3.95	2.55	2.49	2.96	4.40
Serine	4.87	4.12	3.33	4.25	3.05	4.64	4.61	5.23	4.93	3.97	6.08	8.12
Gluetamic acid	40.16	50.86	49.56	33.15	43.59	50.53	44.36	31.88	43.66	50.96	42.06	30.47
Proline	18.19	18.09	17.40	12.08	17.16	20.95	15.44	10.73	19.12	18.73	14.62	8.47
Glycine	3.96	1.89	3.43	5.32	3.66	1.69	3.51	4.37	4.16	1.63	3.55	5.90
Alanine	3.34	2.50	2.53	2.71	3.34	2.27	2.05	2.90	2.89	2.25	1.97	3.19
Valine	4.58	5.15	4.53	3.96	5.08	5.11	3.69	4.56	4.52	4.74	3.58	3.79
Methionine	2.13	1.64	1.78	1.79	1.73	1.48	1.21	1.33	1.91	0.95	1.21	1.59
Cysteine (1/2)	5.43	5.10	5.74	5.10	3.25	2.72	3.91	2.81	1.91	2.84	4.23	2.60
Isoleucine	4.80	5.10	3.65	2.95	4.49	4.84	3.42	3.33	4.42	4.42	3.32	3.26
Leucine	7.84	8.44	7.34	6.82	8.08	7.83	6.34	7.33	7.99	7.15	5.92	6.37
Tyrosine	3.88	3.01	3.53	3.90	3.65	2.87	3.55	3.45	3.48	2.88	3.48	4.62
Fenylalanine	6.76	6.61	5.97	4.06	7.48	7.07	5.25	5.49	7.05	6.68	5.20	2.83
The sum of polar amino acids	49.77	57.27	56.27	43.83	52.09	56.80	50.60	41.75	51.92	57.21	44.05	39.55
The sum of nonpolar amino acids	52.20	49.42	46.63	39.69	51.02	51.27	40.91	40.04	52.06	46.55	39.93	35.4

Note: 1–gluten; 2–gliadin; 3–soluble glutenin; 4–insoluble glutenin.

Table 4. Amino acid composition of wheat gluten of different quality and its fractions, g/100 g of protein.

3.1.3. Effect of the number of thiol exchange groups and the coefficient of the final stage of protein aggregation on the functional properties of protein products

It is known that the covalent (disulfide) and non-covalent (hydrogen, ionic) bonds and hydrophobic interactions play an important role in the structure of vegetable proteins. To identify the participation of these types of interactions in the formation of the functional properties of products from wheat bran, the content of sulfhydryl groups, disulfide bonds, and the aggregating capacity of proteins were determined (**Figure 2**). It was taken into account that the aggregation of proteins in the presence of sodium chloride molecules is carried out with the participation of hydrophobic interactions. It is established that the values

Amino acid	Protein concentrate from:		
	Amaranth	Wheat bran	Rye grains
Lysine	5.43 ± 0.50	6.90 ± 0.40	2.55 ± 0.30
Histidine	3.11 ± 0.25	4.86 ± 0.15	1.58 ± 0.09
Arginine	10.57 ± 1.0	6.21 ± 0.60	3.11 ± 0.10
Aspardic acid	9.51 ± 1.0	8.56 ± 0.50	5.43 ± 1.00
Threonine	3.85 ± 0.25	4.62 ± 0.80	2.37 ± 0.55
Serine	5.40 ± 0.50	6.09 ± 0.80	2.49 ± 0.30
Gluetamic acid	17.88 ± 0.90	14.40 ± 1.1	23.78 ± 1.20
Proline	4.64 ± 0.15	3.77 ± 0.55	9.62 ± 1.00
Glycine	5.57 ± 0.50	6.99 ± 0.90	3.51 ± 0.60
Alanine	4.36 ± 0.20	5.92 ± 0.70	4.07 ± 1.10
Valine	5.17 ± 0.20	5.11 ± 0.50	1.47 ± 0.20
Methionine	4.02 ± 0.3	2.84 ± 0.15	2.13 ± 0.25
Cysteine (1/2)	1.25 ± 0.15	5.10 ± 06	5.43 ± 0.32
Isoleucine	4.63 ± 0.9	3.34 ± 0.62	1.82 ± 0.15
Leucine	7.57 ± 1.05	9.35 ± 1.1	5.60 ± 0.35
Tyrosine	4.61 ± 0.10	5.19 ± 1.0	2.32 ± 0.10
Fenylalanine	10.57 ± 1.5	5.83 ± 1.0	3.96 ± 0.06
The sum of polar amino acids	43.39 ± 1.8	36.07 ± 0.89	34.87 ± 1.12
The sum of nonpolar amino acids	46.53 ± 1.3	43.15 ± 0.67	32.18 ± 1.11

Table 5. Amino acid composition of protein concentrates, g/100 g of protein.

of FC and FEA protein concentrate are interrelated with the content of -SH-groups and -S-S-bonds: the smaller the -S-S bonds and more -SH-groups, the FC and FEA are higher, r = 0.896 and 0.732, respectively.

For FBA, the inverse relationship was observed, the more -S-S bonds in proteins, the indicator, on the contrary, was higher (r = 0.755). For solubility and WBC, there was no significant correlation with the indices of thiol metabolism.

FEA and SE of protein products from wheat bran were positively correlated with the coefficient of the final stage of aggregation of τ10/C proteins, as well as with the number of -SH-groups. Therefore, one can indirectly conclude that the stronger the property of the surface hydrophobicity of proteins, the ability to emulsify fat and the stability of the emulsion in foods is higher. These results are consistent with the number of non-polar amino acids in gluten and wheat gliad, which included amino acids with hydrophobic radicals (**Table 6**).

The sum of amino acids	Functional properties					
	PS, %	WBC, g/g	FBA, g/g	FEA, %	FC, %	FS, %
Wheat gluten and its fractions:						
	Wheat gluten					
Polar	−0. 96	0.54	0.95	−0.78	−0.66	0.22
Nonpolar	0.98	−0.36	−0.76	0.86	−0.11	−0.36
	Gliadin					
Polar	−0.97	−−0.20	0.78	−0. 84	0.21	0.21
Nonpolar	−0.21	−0.42	−0.30	0.70	0.79	0.99
	Soluble glutenin					
Polar	0.62	−0.67	−0.85	−0.62	−0.67	0.54
Nonpolar	0.88	0.62	−0.92	−0.39	0.80	0.14
	Insoluble glutenin					
Polar	0.24	−0.98	−0.86	−0.61	0.97	−0.49
Nonpolar	0.10	0.57	−0.52	−0.74	0.95	0.90
Protein concentrates from wheat bran						
Polar	0.15	0.54	−0.17	−0.37	0.93	0.44
Nonpolar	0.06	0.65	0.10	−0.79	0.68	0.85

Table 6. Coefficients of correlation between functional properties protein products and the sum of amino acids.

Raw material	PS, %	FEA, %	SE, %	FBA, g/g	FC, %
Total insoluble fibers	16.0 ± 0.3	89	87	3.6	119
> 1000	13.7 ± 0.2	95	90	2.4	132
670–1000	21.5 ± 0.3	89	85	2.4	130
195–670	26.9 ± 0.5	72	79	2.8	129
< 195	9.30 ± 0.1	61	66	4.9	107

Table 7. Functional properties of protein preparations.

The functional properties of DWG depend on the molecular weight (MM) of the individual electrophoretic components obtained in PAGE. Using the example of native and modified DWG obtained by the limited proteolysis method, we showed that single-chain polypeptides with low (<40 kDa) and medium (40-60 kDa) MM were included in the composition of DWG

http://dx.doi.org/10.5772/intechopen.75803

with increased FC and solubility [30]. In multi-stranded polypeptides, DWG, there were more single-chain peptides with low MW (12–16 kDa) and fewer with medium (27–39 kDa) and high (69–108 kDa) MM.

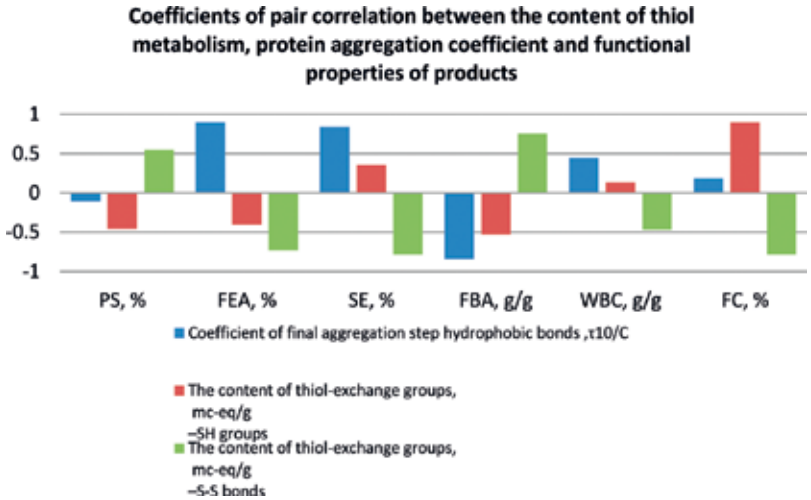


Figure 1. Coefficients of pair correlation between functional properties products and fractional composition of proteins.

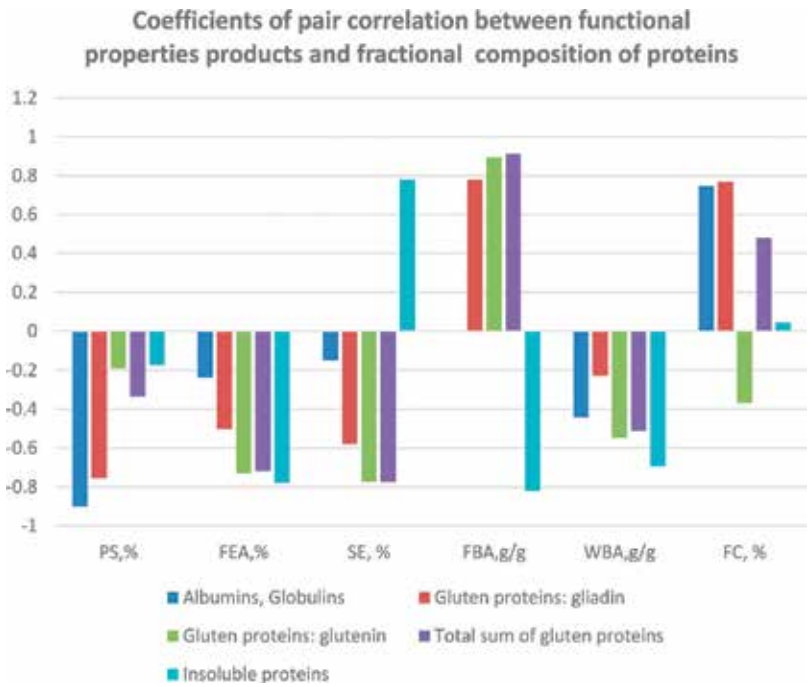


Figure 2. Coefficients of pair correlation between the content of thiol metabolism, protein aggregation coefficient, and functional properties of products.

The revealed regularities of interrelation of functional properties with the component composition were intended for the use of DWG in the production of confectionery products.

4. Conclusion

The results of studies of the chemical composition, physicochemical characteristics of proteins, and functional properties of dry wheat gluten, its components, protein concentrates from wheat bran, and their granulometric fractions have shown that it is advisable to regulate the quality indices of protein products with the aim of improving them and taking into account the revealed regularities. A high correlation positive dependence was established for the solubility of wheat gluten proteins, protein concentrates from wheat bran and their fractions with the amount of albumins and globulins, the sum of nonpolar amino acids (gluten, gliadin, soluble glutenin), and a negative correlation with gliadin gluten. With the indices of thiol metabolism, the relationship between solubility and WBA is not revealed.

For WBC of protein products, the reverse dependence on the sum of the polar amino acids of both fractions of glutenin is typical; for FBA, it is a direct relationship with the sum of gluten proteins and polar amino acids in gliadin and whole gluten and the inverse relationship was observed for the sum of nonpolar amino acids in the alcohol-soluble fraction. The lower the protein aggregation coefficient, hence, the less degree of hydrophobic interactions, less than -SH-groups, but more -S-S-bonds in proteins, the higher the FBA.

FEA positively correlated with the amount of glutenin and insoluble residue in proteins from wheat bran and the sum of nonpolar amino acids in gluten, gliadin. A negative relationship is established for the sum of polar amino acids, as whole gluten, and all its fractions. The higher the degree of hydrophobic interactions in protein products and the less disulfide bonds in them, the ability to emulsify fat and stabilize the emulsion is higher.

The average correlation dependence was revealed for FC and the mass fraction of protein for all types of protein products studied. The FC of gluten proteins positively correlated with the sum of nonpolar amino acids of gliadin, soluble, insoluble glutenin, and polar amino acids of insoluble glutenin. The sum of two kinds of amino acids also positively influenced the FC of other protein products. The higher the mass fraction of albumins, globulins, and gliadin in gluten, the more FC products are. As for FEA protein products from wheat bran, it was found that the higher the content of SH groups and the lower the number of S-S bonds in protein products, the more FC protein products are higher.

Consequently, the main functional properties of the protein products studied from wheat are interrelated with the protein mass fraction, the features of the fractional, amino acid composition of proteins, the number of covalent disulfide bonds, sulfhydryl groups, and non-covalent (hydrophobic) interactions. Thus, in order to predict the high and stable functional properties of protein products from wheat for production or their modification, it is advisable to take into account the patterns of interrelation of these properties with the chemical composition and the physicochemical properties of their proteins.

Conflict of interest

The authors declare no conflict of interest.

Abbreviations

DWG	dry wheat gluten
PS	protein solubility
FBA	fat-binding ability
FC	foaming capacity
WBC	water-binding capacity
ST	stability of emulsion
FS	foam stability
–S-S–	disulfide bonds
–SH	sulfhydryl groups
MM	molecular masses

Author details

Valentina V. Kolpakova*, Nikolay D. Lukin and Irina S. Gaivoronskaya

*Address all correspondence to: val-kolpakova@rambler.ru

All-Russian Scientific Research Institute of Starch Products–A Branch of the V.M. Gorbatov Federal Research Center for Food Systems of Russian Academy of Sciences, Kraskovo, Russia

References

[1] Tolstoguzov VB. New Forms of Protein Food. M: Agropromizdat. 1987;303

[2] Kolpakova VV, Volkova AE, Nechaev AP. Protein from wheat bran. Functional properties of protein flour: Emulsifying and foaming properties. Izvestiya Vuzov. Food Technology. 1995;**1-2**:34-37

[3] Kolpakova VV, Nechaev AP. Protein from wheat bran. Functional properties of protein flour: Solubility and water binding ability. Izvestiya Vuzov. Food Technology. 1995;**1-2**:31-33

[4] Dorglamud S, Suwannaporn P, Huang T-C, Tester F. Physicochemical properties of protease-treated rice flour. Starch/Stärke. 2013;**65**:613-620. DOI:10.1002/star.201500103

[5] Akintayo ET, Oshodi AA, Esuoso KO. Effects of NaCl, ionic strength and pH on the foaming and gelation of pigeon pea (Cajanus Cajan) protein concentrates. Food Chemistry. 1999;**66**: 51-56. DOI: 10.1016/S0308-8146(98)00155-1

[6] Söderberg J. Functional properties of legume proteins compared to egg proteins and their potential as egg replacers in vegan food. Second cycle, A2E. Uppsala: SLU, Dept. of Food Science. 2013. Available from: https://stud.epsilon.slu.se

[7] Vegetable Protein: New Perspectives/ed. HER. Braudo. Moscow: Pishchepromizdat. 2000;180

[8] Schwenke KD. Structural modification as an instrument for the functionalization of proteins of oilseed and leguminous seeds. In: Braudo EE, editor. Vegetable Protein: New Perspectives/ed. HER. Braudo. Moscow: Pishchepromizdat; 2000. pp. 40-60

[9] Kolpakova VV, Chumikina LV, Vasil'ev AA, Arabova LI, Topunov AF. Wheat gluten proteolysis by enzyme preparations of directional action. International Journal of Agronomy and Agricultural Research (IJAAR). 2014;**5**(2):72-86. ISSN: 2223-7054 (print) 2225-3610 (Online). Available from: http//www.innspub.net

[10] Wang J, Zhao M, Yang X, Jiang Y. Improvement on functional properties of wheat gluten by enzymatic hydrolysis and ultrafiltration. Journal of Cereal Science. 2006;**44**:93-100. DOI: 10.1016/j.jcs.2006.04.002

[11] Zhang H, Claver JP, Li Q, Zhu K, Peng W, Zhou H, Structural modification of wheat gluten by dry heat – enhanced enzymatic hydrolysis. Food Technology and Biotechnology. 2012;**50**:53-58

[12] Popineau Y, Huchet B, Lare B, Berot S. Foaming and emulsifying properties of fractions of gluten peptides obtained by limited enzymatic hydrolysis and ultrafiltration. Journal of Cereal Science. 2002;**35**:327-335. DOI: 10.1006/jcrs.2001.0437

[13] Kolpakova VV, Vanin SV. Influence of hydrocolloids of polysaccharide nature on the foaming properties of protein products. Storage and Processing of Agricultural Raw Materials. 2008;**1**:57-59

[14] Vanin SV, Kolpakova VV. Functional properties of dry wheat gluten of different quality. Izvestiya Vuzov. Food Technology. 2007;**1**:21-24

[15] Toufeili I, Ismail B, Shadarevian S, Baalbaki R, Khatkar BS, Bell AE, Schofield JD. The role of gluten proteins in the baking of Arabic bread. Journal of Cereal Science. 1999;**30**:255-265. DOI: 10.1006/jcrs.1999.0286

[16] Kolpakova V, Yudina T, Vanin S, Lomakin A. Dry wheat gluten is an effective flour improver with short-glutinous gluten. Bread Products. 2007;**2**:50-52

[17] Lazaridou A, Duta D, Papageorgiou M, Belc N, Biliaderis CG. Effects of hydrocolloids on dough rheology and bread quality parameters in gluten free formulations. Journal of Food Engineering. 2007:1033-1047. DOI: 10.1016/j.jfoodeng.2006.03.032

[18] Noorfarahzilah M, Lee JS, Sharifudin MS, Mohd FA, Hasmadi M. Applications of composite flour in development of food products. International Food Research J. 2014;**21**(6):2061-2074

[19] Esteller MS, Pitombo RNM, Lannes SCS. Effect of freeze-dried gluten addition on texture of hamburger buns. Journal of Cereal Science. 2005;**41**:19-21. DOI: 10.1016/j.jcs.2004.08.013

[20] Kolpakova VV, Vanin SV. Method of Biscuit Production. 2008. The Patent of the Russian Federation No 2316968, IPC A 21 D 13/08

[21] Studennikova OU, Kolpakova VV. Method of Zephyr Production. The patent of the Russian Federation No 2432771 IPC A23G3/00

[22] Kinsella JE. Functional properties of proteins: possible relationships between structure and function in foams. Food Chemistry. 1981;**7**:273-288. DOI: 10.1016/0308-8146(81)90033-9

[23] Nakai S. Structure-function relationships of food proteins: with an emphasis on the importance of protein hydrophobicity. Journal of Agricultural and Food Chemistry. 1983;**31**:676-683

[24] Danilenko AN, Dianova VT, Braudo EE, Henning T, Mothes R, Schwenke KD. A novel approach to the evaluation of hydrophobocoty of food proteins. Nahrung. 1998;**44**:179-182

[25] Day L, Augustin MA, Batey IL, Wrigley CW. Wheat-gluten uses and industry needs. Trends in Food Science &Technology. 2006;**17**:82-90. DOI:10.1016/j.tifs.2005.10.003

[26] Guilbert S, Gontard N. Agro-polymers for edible and bodegradable films: Review of agricultural polymeric materials, physical and mechanical characteristics. Innovations in Food Packaging A volume in Food Science and Technology. 2005;**1**:263-276. DOI: 10.1016/B978-012311632-1/50048-6

[27] Kolpakova VV, Zaitseva LV, Osipov EA. Protein from wheat bran: increase in yield and functional properties. Storage and Processing of Agricultural Raw Materials. 2007;**2**: 23-25

[28] Abramov IA, Eliseeva NE, Kolpakova VV, Piskun TI. Amaranth: chemical composition, biochemical properties, processing methods. Storage and processing of agricultural raw materials. 2011;**6**:46-47

[29] Kolpakova VV, Bakhitov TA, Chelnokova EYa, Zaitseva LV. Resource-saving ways of processing grain of rye. Food Technology Izvestiya Vuzov. 2008;**5-6**:49-51

[30] Kolpakova VV, Chumikina LV, Arabova LI, Lukin DN, Topunov AF, Titov EI. Functional technological properties and electrophoretic composition of modified wheat gluten. Foods and Raw Materials. 2016;48-57. DOI: 10.21179/2308-4057-2016-2-48-57

[31] Bogdanov VP, Vukolova GN, Gruzdev LG Vakar AB. Determination of disulfide bonds and sulfhydryl groups in wheat storage proteins biochemistry and microbiology. 1980;**16**(1):127-138

[32] Arakawa T, Yonesawa D. Compositional difference of wheat flour glutens in relation to ther aggregation behaviours. Agricultural and Biological Chemistry. 1975;**39**(11):2123-2128

Chapter 12

Wheat Straw Pulping for Paper and Paperboard Production

Guigan Fang and Kuizhong Shen

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.77274

Abstract

This chapter covers relative topics on wheat straw applied as fibrous raw materials for pulping and papermaking industry, i.e., chemical components and anatomy of wheat straw, pulping process and pulping properties, paper and paperboard products from wheat straw pulps, as well as environmental protection issues. Wheat straw is a kind of annual vascular-bundled herbal arthrophyte containing cellulose fibers that are acceptable for pulping and papermaking. The chemical components and anatomy of wheat straw were discussed; practically, soda or soda-AQ chemical pulping processes are common techniques often applied for chemical pulping from wheat straw, conventional and advanced bleaching sequences were introduced, and especially ECF and TCF bleaching techniques have been successfully applied in China's paper mills. Cooking and black liquor extraction equipment and facilities are explained; chemi-mechanical pulping properties of wheat straw, corrugated medium, and linerboard products from wheat straw CMP pulps are evaluated; and chemical recovery from chemical cooking black liquor and effluent treatment processes are discussed. In this chapter, not only laboratory research results but also some commercial operation experiences are shared. These information and knowledge described in this chapter will help readers to have a good understanding about wheat straw pulping and papermaking; they are useful for pulping and papermaking engineer as reference for design and operation management of wheat straw pulping lines.

Keywords: wheat straw, chemical components, fiber anatomy, chemical pulping, chemi-mechanical pulping, writing and printing paper, linerboard, corrugated medium, effluent characteristics

IntechOpen

© 2018 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

1. Preface

Wheat cultivation area is the world's largest agricultural lands, wheat being the most prolific and most widely distributed food crops. At present, the global sum of wheat plant area is of more than 2.2 million ha, with the annual wheat grain output of 730 million tons, accounting for one-third of the world's total food production [1]. Wheat straw is a good fiber material, produced annually in huge quantities worldwide in a much shorter growing cycle than wood. The utilization of no-woody fiber materials to produce cellulosic pulps is the most economically justified solution fitting with the EU's environmental directives, which aim to reduce the consumption of wood fiber in paper and board products and replace it with other plant biomasses [2]. From the production point of view, the world's largest wheat production country is China, followed by India, the United States, and Russia, and these four countries produce 45% of the total world wheat production. Therefore, there are more than 650 million tons of wheat straws available annually [3, 4]. How to utilize these lignocellulosic biomass resources efficiently has been becoming a very urgent issue worldwide [3]. Practically, some countries start to produce electricity by using biomass fuel boilers, and some parts of the world feed straws back to agricultural land by cutting, but most of those resources have not been properly utilized. There are some countries making paper and paperboard products from wheat straw, such as China, Spain, and so on.

The term "pulping," in technical processes or methods for production of fibers from cellulosic raw materials, might be classified mainly as chemical pulping, chemi-mechanical pulping, and mechanical pulping processes. The principle of pulping is to separate cellular fibers from plant tissues by chemical cooking to remove lignin or mechanical separation combined with chemical softening, etc.

2. Fiber anatomic structure, chemical composition and pulping properties of wheat straw

Wheat is a kind of annual vascular-bundled herbal arthrophyte (**Figures 1** and **2**) [5], containing spike head, straw stalk (nodes and internodes), and root parts. Countering its mass percentage of each part, there are proximate ranges of internodes (68% w/w), leaves-sheaths (20% w/w), leaves-blades (6% w/w), nodes and fines (4% w/w), and grain and debris (2% w/w). Practically, only internode parts are acceptable for pulping [6]. The physical and optical properties of the stubble pulp were better than those of whole wheat straw pulp [7]. Therefore, at a commercial pulping process line, about 25–30% w/w loss of biomass, unsuitable parts for pulping, should be separated during wheat straw chip preparation (**Figure 3**), for purposes of chemical saving and pulp quality improvement as well as silica content decrements of black liquor from cooking [6, 8].

Papermaking is the process to form a fiber mat by a mesh net from fiber suspension stock through draining water. Fibers are the key element of pulp furnishes for papermaking. With contrast to wood fibers, wheat straw fibers are characterized as shorter in average fiber length and narrow in width, and there are more non-fiber cell contents. After proper pulping, cleaning, and screening, pulps from wheat straw can be used for manufacturing various paper product grades, such as linerboard, corrugated medium, writing and printing papers, etc.

http://dx.doi.org/10.5772/intechopen.77274

Figure 1. Baled wheat straw.

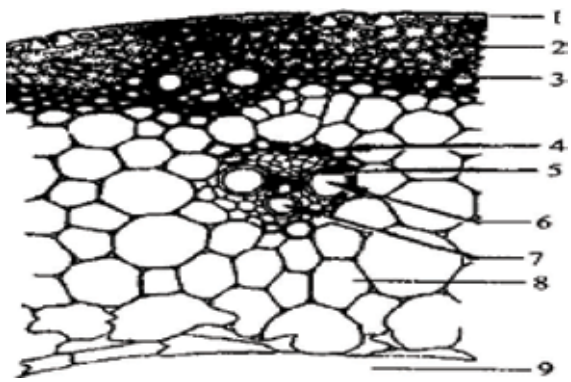


Figure 2. Cross section of wheat straw [5]. (1) Outer epidermal cells, (2) fibrous tissue band, (3) vessel, (4) bundle sheath, (5) vascular bundle, (6) xylem, (7) phloem, (8) parenchyma cell, (9) internal epidermal membrane.

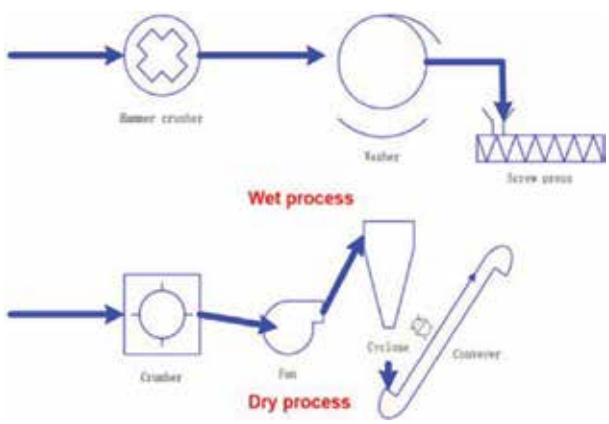


Figure 3. Two types of pretreatment processes for wheat straw chip preparation [6, 8].

In some case, it also can be used in cigarette paper furnish with less than 30% proportion to adjust paper's air permeability. Fiber morphology data of wheat straw are listed in **Table 1**.

With contrast to wood materials, there are less lignin and more hemicellulose contents in wheat straw [9] and extremely high content of extractives by 1% caustic soda solution, but its biological organizations are bulky and loosened, which is helpful for pulping process. In fact, there were a series of pulping processes, successfully employed by wheat straw pulping practices, such as soda pulping (soda), kraft pulping (KP), and neutral or alkaline sulfite pulping (NS or AS) for production of brown pulps and bleached pulps. Chemical compositions of one kind of wheat straw from Hebei province, China, are illustrated in **Table 2** [10].

Zhan et al. [11] analyzed chemical components of differential parts from wheat straw, and results show significant differences on chemical compositions from each part, listed in **Table 3**. Internodes of wheat straw contain higher hemicellulose which is the key element of pulps.

Species	Fiber length (mm)		Width (um)		Rate of L/W	Wall thickness (um)	Lumen diameter (um)	2 W/D	Non-fiber cell content (%)
	Avg.	Range	Avg.	Range					
Wheat straw	1.32	1.03–1.60	12.9	9.3–15.7	102	5.2	2.5	4.16	37.9
Birch (*Betula alba*)	1.21	1.01–1.47	18.7	14.7–22.0	65	—	—	—	26.7-
Pinus massoniana	3.61	2.33–5.06	50.0	36.3–65.7	72	3.8E) 8.7(L)	33.1 (E) 16.6 (L)	0.23 (E) 1.05 (L)	1.5

Table 1. Fiber morphology datum of wheat straw and wood [10].

Sample	Ash	Extractives				Pentosanes	Lignin	Nitrate-EtOH cellulose
		Cold water	Hot water	Benzene-EtOH	1%NaOH			
Wheat straw (Hebei)	10.65	5.36	23.15	—	44.56	25.56	22.34	40.40

Table 2. Chemical composition of wheat straw (%, wt/wt) [10].

	Internodes	Nodes	Leaves
Klason lignin (%)	19.66	19.98	14.18
Holocellulose (%)	70.27	67.97	60.95
Ash (%)	6.27	8.69	12.06

Table 3. Chemical compositions of wheat straw parts (% wt/wt) [30].

http://dx.doi.org/10.5772/intechopen.77274

3. Chemical pulping and pulp bleaching

Soda and soda-anthraquinone (AQ) cooking, as sulfur-free processes, are more suitable in producing pulp fibers from some no-woody raw materials [12]. Most common operated chemical pulping processes are soda or soda-AQ cooking, using caustic soda or caustic soda-AQ solution as cooking liquor to dissolve and remove lignin from wheat straw. It was proven that AQ can improve selectivity of delignification by caustic soda to prevent carbohydrates from hydrolysis [12, 13].

Since chemical pulping process was employed for wheat straw pulping, some disadvantages occurred during commercial practices, i.e., higher steam consumption, shortage of heat recovery, changeable in pulp qualities, lower pulp yield, poor pulp drainability, high viscosity of black liquor, and so on, which caused some challenges as high production cost, limited application of pulps, difficulties on chemical recovery, etc. Some advanced techniques have been improved and invented in the past decades [14], summarized as follows: (1) the wheat straw chip preparation processes combined with dry method and wet method were developed to remove nonfibrous components selectively and efficiently; (2) rapid cooking process at lower temperature combined with mechanical fiber dissociation to enhance uniform cooking effects and improve pulp qualities; and (3) elemental chlorine-free and total chlorine-free bleaching processes invented to eliminate generation of AOX and other bio-toxicities from pulp bleaching operations.

The first installation of continuous cooking system with an annual capacity of 100,000 metric tons of wheat straw pulps was operated at Quanlin Paper Group, Shandong, China. This successful practice scales up an individual pulp production line capacity from non-wood materials in the world and improves significantly technical specifications of wheat straw pulping. The detailed technical specifications are shown in **Table 4** [8].

A new type of pulp washer series, i.e., serialized ZXV-type pulp washers, has been innovated by Wenrui Machinery Co. Ltd., Shandong, China. The new pulp washers, with the maximum filtration area of each drum being up to 120 m^2, and with a conical chamber structure designed and plane distributing valves being applied to improve water flow turnover and keep higher vacuum degree in sucking chambers, resulted in a good pulp cleanness and high extraction rate of black liquor; on the other hand, dispersing press, agitating device applied to improve washing efficiency [3]. The proposed acceptable washing-screening process is the sequence with press extraction—replacement washing—closure screening; it has been proven by many commercial operations that the new concept of the combined countercurrent washing sequence improves black liquor extraction rate significantly, resulting a remarkable water-saving effect. For instance, the black liquor extraction rate reaches up to 94.6%. The water consumption can be reduced to 40m^3/t pulp or less in Xinya Paper Group, contrasting to normal water consumption of more than 100 m^3/t pulp by traditional process [4].

The bleaching techniques had experienced a long-term development in China's wheat straw pulping practices. Early, the single-stage low consistency bleaching with hypochlorite was commonly applied in many non-wood pulping lines, and a three-stage bleaching sequence

of CEH (chlorination—alkali extraction—hypochlorite) was not applied until the 1980s of the twentieth century [14]. All of the above bleaching sequences contain elemental chlorine chemicals with generation of AOX and other organic toxicities in these bleaching effluents. Due to poor bleaching selectivity upon residue lignin, there were a large amount of carbohydrates degraded while bleaching, resulting a large water consumption and high chemical dosage, to produce bleached pulps with low brightness and weak physic strength as well as poor drainability of water. Following more and more strict regulations to effluent discharge, elemental chlorine chemicals were forbidden to be used for pulp bleaching, and the bleaching techniques were developed toward more environmentally friendly processes, such as ECF (elemental chlorine-free) and TCF (totally chlorine-free) bleaching sequences. The first mid-consistency and shortened TCF bleaching sequence in the world, namely, OQPo (oxygen delignification—chelating metal ions—peroxide bleaching assisted with oxygen) sequence, was successfully commercially operated with the capacity of 150 t/d by Xianhe Co., Henan, China (**Figure 4**) in 2008 [15]. By this bleaching sequence, bleached wheat straw pulps (BWSPs) with brightness of more than 80%ISO, pulp viscosity of 653 ml/g and breaking length of more than 7000 m (**Tables 5** and **6**) and less amount bleaching effluent generated about 30 m^3/t pulps, decreased by more than 60% of a traditional CEH bleaching process for wheat straw pulps [15]. Later, a bleached wheat straw pulp production line by TCF sequence with an annual capacity of 200 t/d was installed in Baiyuan Paper Co., Henan, China. Also, enzymes have been considered by many pulp scientists to assist bleaching operation with a decrement of chlorine-contained bleaching chemicals, providing an optional measure for bleached pulp quality improvement and chlorine-contained bleaching chemical dosage decrement toward an environmentally friendly clean production concept.

Items	Technical specification
1. Biomass lose rate at wheat straw chip preparation stage (% w/w)	23–28
2. Caustic soda dosage in cooking on raw materials saved (% w/w)	2.5
3. Bleached pulp yield increased on raw materials (% w/w)	5.0
4. Pulp brightness after oxygen delignification (% ISO)	50–55
5. Tensile strength of the unbleached wheat straw pulp (N.m/g)	58.8
6. Black liquor properties at the exit of the black liquor extraction stage:	
(i) Extraction efficiency (%)	>90
(ii) Viscosity of black liquor (cp) (100°C, 50% solid consistency)	<500
7. Black liquor properties at the exit of normal vacuum evaporation system (%w/w)	60–62
8. Mid-stage water discharge	
(i) Water discharge amount (m^3/t o.d. pulp)	<50
(ii) COD of treated effluent (mg/L)	<60

Table 4. Advanced technical specifications of wheat straw pulping in Quanlin paper group [15].

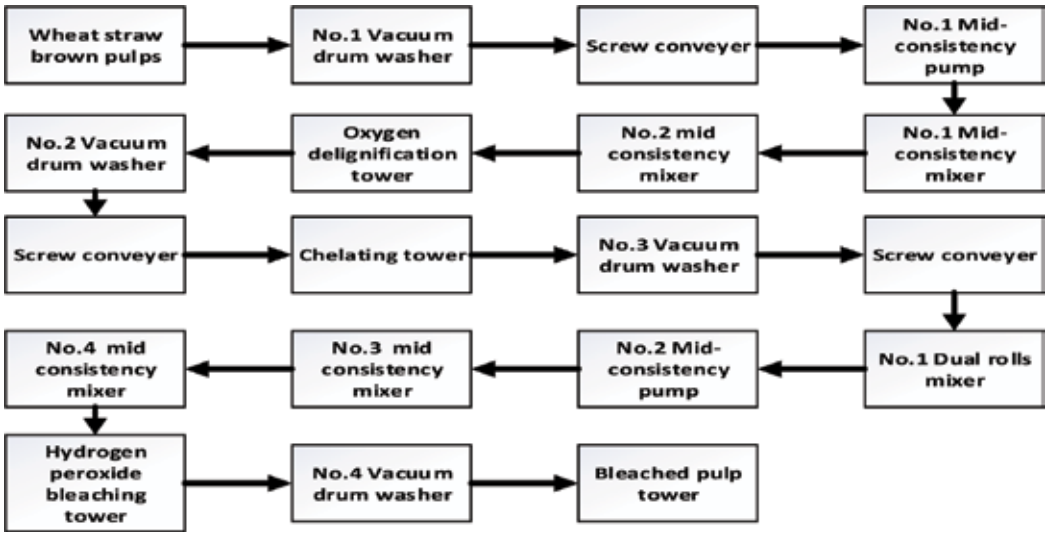


Figure 4. A short OQPo bleaching sequence for production of bleached wheat straw pulps [15].

Grammage (g/m^2)	Beating degree (°SR)	Tensile (N.m/g)	Breaking length (km)	Tear (mN. m^2/g)	Burst (kPa. m^2/g)	Fold endurance times (1.5 kgf tension)
58.3	**44.3**	**71.23**	**7.16**	**4.83**	**4.68**	**24**

Table 5. Physical strength properties of bleached wheat straw pulps by an OQPo sequence [15].

Wheat straw pulps	Brightness (%ISO)	Viscosity (mL/g)
Screened brown pulp	34.7	965
Pulp after oxygen stage	48.0	850
Pulp after Po stage	81.6	653

Table 6. Viscosity and brightness of wheat straw pulps from different stages [15].

4. Paper products from wheat straw pulps

As previous discussed, bleached wheat straw pulps can be characterized with good strength properties, reasonable brightness, good printability, etc., which can be used to manufacture a wide range of paper and paperboard grades, such as light-weighted printing paper [16], letterpress printing paper, typing paper, writing paper, aluminum foil base paper, cylinder polished paper, tissue and sanitary papers, white board, coated ivory board, and almost all grades of paper and paperboard grades [17–22]. Practically, to improve pulp stock wet-web

Pulps	Ratio (%)	Beating degree (°SR)	Wet weight (g)
Bleached wheat straw pulp	70–50	48–53	>2.2
Bleached softwood pulp	30–50	48–65	5–8

Table 7. Portion of furnish for bible paper [18].

Specific properties		unit	Bible paper product	
			Produced product	GB 1913–1989 (Grade A)
Grammage		g/m^2	40	40 ± 1.5
Density		g/cm^3	0.80	≥0.8
Fold endurance CD		Times	5	≥5
Breaking length		m	3000	≥2500
Smoothness	Average of T-B surfaces	s	102	≥100
	Variation	%	23	≤25
Brightness		%	80.0	≥79.0
Opacity		%	87.0	≥85.0
Dust content	0.3–0.5 mm^2	Number/m^2	40	≤50
	>1.5 mm^2		no	Not allowed
Moisture content		%	6.0	6.0 ± 1.0
Ash		%	19	—

Table 8. Quality specification to bible paper products [18].

run ability in paper machine, wheat straw pulp furnishes must be mixed with some portion of bleached wood pulps or bamboo pulps occasionally [18, 20].

Some paper mills produce Bible paper products from bleached wheat straw pulps, the formula of pulp furnishes is given in **Table 7**, and the quality specifications of Bible paper are illustrated in **Table 8** [18].

5. Chemi-mechanical pulping process and CMP pulp properties

The chemi-mechanical pulping process, combined with multiple stages as chemical pretreatment, fiber mechanical separation, and enzymatic hydrolysis modification, has many significant advantages contrasting to chemical pulping process, i.e., higher pulp yield, lower pollution load, and easily treated effluent. In fact, chemi-mechanical pulping process has been developed rapidly and widely applied for wood pulping. In the early 1990s of the

twentieth century, China's wood pulp production from fast-grown woodchips by promotion of chemi-mechanical pulping technologies, such as CTMP (chemi-thermo-mechanical pulping), APMP (alkaline peroxide mechanical pulping), P-RC APMP (preconditioning-refiner chemical APMP), and CTMP-c (chemi-thermo-mechanical pulping with enhanced chemical impregnation) processes, has increased rapidly [23].

Chemi-mechanical pulping from wheat straw studies lasted for more than 30 years in China. In the early 1990s of the twentieth century, Liu and Fang [24] investigated chemi-mechanical pulping properties from wheat straw using caustic soda impregnation, characteristics of effluent, and proper treatment process. It was proven that wheat straw can be used to produce chemi-mechanical pulps using small amount of caustic soda with advantages as high pulp yield, good physical strength, and proper biodegradable effluents [25–27]. A-grade corrugated medium was produced from this kind of wheat straw CMP pulps (**Table 9**), and also A-grade linerboard from WSCMP mixed with 15–30% kraft wood pulps was prepared [17, 24].

Zhang et al. [28] also investigated wheat straw chemi-mechanical pulping properties using two-stage caustic soda impregnation followed with two-stage atmospheric refining, while a total dosage of caustic soda 3–5% was applied at the impregnation stage, resulting pulp yield of 73.1% w/w–78.9% w/w, pulp tensile strength of 19.49–41.27 N.m/g, the specific refining energy consumption from 827 to 336 kWh/t pulp (**Table 10**), and COD load in effluent of 270.5–446.0 kg/t o.d. pulp (**Table 11**); the relationships between pulp yield versus caustic soda dosage and COD load versus pulp yield are illustrated in **Figures 5** and **6**.

Specification	**GB1302**	**Corrugated medium from WSCMP (g/m²)**	
		140	**180**
Grammage (g/m²)	140 ± 2.0	140.3	180.6
Density (g/cm³)	≥0.5	0.56	0.61
Breaking length (m)	≥4.30	4.94	4.71
Ring crush (N.m/g)	≥	14.61	16.94

Table 9. Quality specifications of corrugated medium from wheat straw CMP pulps [17].

Pulp sample	**Caustic soda dosage (%)**	**Pulp yield (%)**	**Specific refining energy (kWh/t pulp)**	**Bulk (cm³/g)**	**Tensile (N.m/g)**	**Burst (kPa. m²/g)**	**Tear (mN. m²/g)**	**Breaking length (m)**
CTMP-1	3.0	78.9	827	3.23	19.5	1.04	4.23	2460
CTMP-2	4.0	75.5	555	2.66	32.6	1.60	4.56	3330
CTMP-3	5.0	73.1	336	2.35	41.3	2.31	4.08	4210

Table 10. CTMP pulping properties of wheat straw [28].

Effluent	Caustic dosage (%)	Color times	pH number	Pollutant load (kg/t)						
				TOC	SS	TS	COD	BOD5	TN	TP
CTMP-1	3.0	5166	9.1	150	47.6	188.3	271	181	6.04	1.90
CTMP-2	4.0	5244	9.6	173	78.5	277.1	369	200	6.75	1.92
CTMP-3	5.0	6127	10.1	219	124.3	315.9	446	228	8.08	1.93

Table 11. Characteristics of effluent from wheat straw CTMP pulping [28].

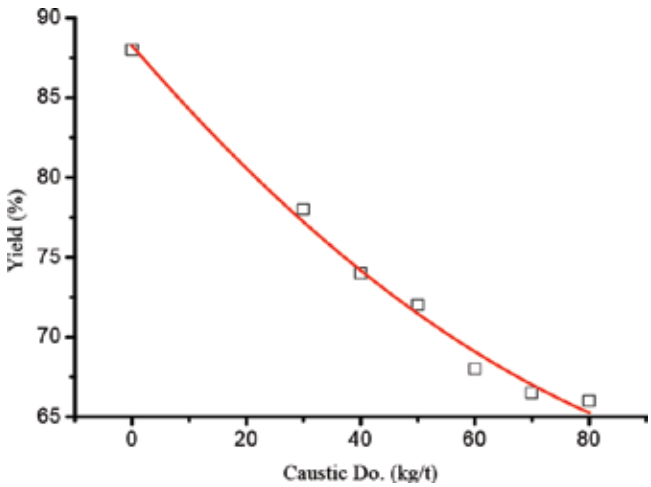


Figure 5. Wheat straw CTMP pulp yield decreases with increment of caustic soda dosage [24, 28].

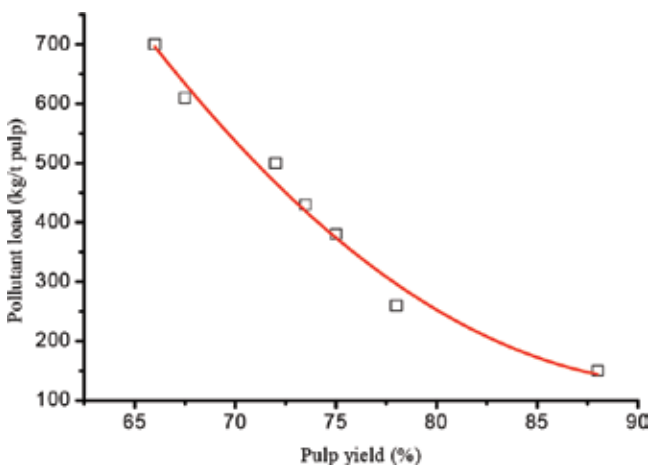


Figure 6. Pollutant load of effluent decreases with increment of pulp yield [24, 28].

6. Effluent treatment

It is clear that pollution load from pulping process can be decreased by increasing pulp yield. Liu and Fang [24] summarized a relationship between COD load (y) and pulp yield (x) of wheat straw pulping, i.e., $y = 4929.58426–104.0789x + 0.5883x^2$, $r^2 = 0.910$. It was proven that effluent from wheat straw chemi-mechanical pulping has a good biodegradability contrasting with that from OCC (old corrugated container) pulping. Effluent from wheat straw CTMP pulping has been characterized and illustrated in **Table 12** [24].

The proposed flow diagram for installation of effluent treatment plant is illustrated in **Figure 7** [24].

Since less amount of chemicals is applied for wheat straw impregnation, the contaminant of such pulping effluent consists of low polymerized or monomolecular carbohydrate compounds, which are biodegradable. With consideration of effluent characteristics from each stage and quality requirements of process water, it is possible to reuse the used water in a short circuit after suitable individual specific treatment. If chemi-mechanical pulping line is going to be installed in recycled fiber pulping and papermaking mill, the integrated effluent from wheat straw CTMP or CMP line will contribute to improve biodegradability for recycled fiber pulping effluent, carefully considering the water balance, and it is possible to produce wheat straw chemi-mechanical pulps without consumption of any fresh water [17]. Therefore, the total volume of discharge effluent will not be increased. It means that the original effluent treatment can be operated with improvement of efficiency [17, 25, 27].

Parameters	**Wheat straw CTMP effluent**		
	Screw press after pre-steaming	**Pulp washing**	**Integrated effluent**
Effluent discharge (m^3/BDT)	0.32	12.05	12.37
pH	8.3	8.5	8.1
COD concentration (mg/L)	13,580	17,280	17,185
COD load (kg/BDT)	4.35	208.19	212.54
BOD concentration (mg/L)	—	—	7888
BOD load (kg/BDT)	—	—	97.57
Ratio of BOD_5/COD	—	—	0.459
SS (g/L)	3.7	11.81	11.60
TS (g/L)	7.7	19.04	19.32
Ash content in TS (%)	26.7	35.20	34.06
TN (mg/L)	110.0	146.0	145.0
TP (mg/L)	99.0	57.0	58.0

Table 12. Effluent characteristics from wheat straw CTMP pulping [24].

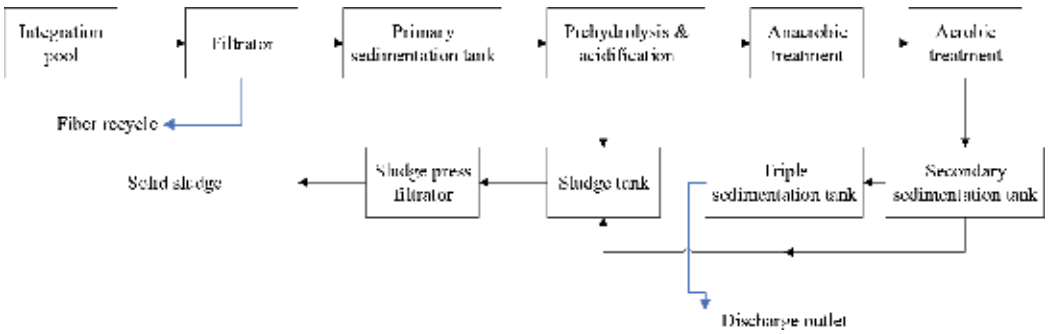


Figure 7. Diagram of an effluent treatment plan [24].

7. Desilication and chemical recovery of black liquor

7.1. Desilication in wheat straw cooking

Two different kappa number pulps of wheat straw soda-AQ cooking were studied followed by O_2 cooking for elucidating the effects of alkali charge, temperature and oxygen pressure on the black liquor desilication, pulp yield, and pulp delignification. The results showed that the soda-AQ cooking conditions had an influence on the properties of the pulp and the black liquor in the O_2 cooking stage. And, there were proper alkali charge, temperature and pressure ranges for the desilication, the pulp yield, and the black liquor viscosity in the O_2 cooking system at given other conditions. The results also showed that silica content in the O_2 cooking pulp did not always decrease with the rise of system pH and silica content of black liquor did not always increase with the rise of system pH. The O_2 cooking in soda-AQ/O_2 two-stage cooking cannot only successfully desilicate from the black liquor but also delignify effectively itself. Hence, the soda-AQ/O_2 cooking process could be an environmentally friendly potential pulping process [29].

Desilication kinetics of the O_2 stage in the wheat straw soda-AQ/O_2 two-stage cooking were investigated. In addition, the pulp yield, alkali concentration, and O_2 pressure in the O_2 cooking were studied. The results showed that desilication reaction from the O_2 cooking black liquor could be divided into two phases, a rapid initial desilication phase followed by a slow final desilication phase. They both follow pseudo-first-order reaction toward the silica concentration in black liquor with the reaction energy 64 and 79 kJ/mols, respectively. The differential state expressions of two phases were deduced [8, 29].

Studies also indicated that under the experiment conditions, the pulp yield increased by 0–3.5% and the O_2 pressure and alkali concentration in the system decreased with the extending time at given temperature in the O_2 cooking process.

The successful experiences for chemical recovery in China's wheat straw chemical pulping mills indicate the following: (1) to enhance the loss of wheat straw preparation is helpful to decrease the silica content and viscosity of black liquor; (2) rapid cooking at lower temperature

can prevent degradation of cellulose and provide wheat straw chemical pulps with good physic strength and drainability; and (3) high-efficient extraction washer for black liquor is helpful to increase the initial concentration of black liquor, resulting steam savings during thickening black liquor and also decrement of fresh water consumption in pulp washing stage [4, 8].

For instance, Quanlin Paper Group has successfully operated its chemical recovery system for wheat straw chemical pulping black liquor, with an alkali recovery rate of more than 85%, and its mid-stage water can be treated economically to meet an extremely strict effluent discharge standard (COD concentration is less than 60 mg/L). There are many new techniques applied in its wheat straw pulping line, such as application of flying hammer chipper to separate more non-fiber components and to improve uniform cooking effects resulting in short cooking time and lower chemical dosage, decrement of black liquor viscosity from 1000cp to less than 500cp (solid content (55%) and temperature (100°C)); a vertical replacement cooking digester with large ration of chips to water was installed, leading to significant chemical dosage decrement and initial concentration of black liquor increment to save evaporation cost and to obtain more thickened black liquor which is a benefit for chemical recovery boiler [3, 4].

7.2. Application of straw industrial lignin

Although being a highly abundant aromatic feedstock, lignin is still largely regarded simply as a source for heat and power for the biorefining or pulping process that liberates the lignin. The lack of established processes that add value to the lignin component can be largely attributed to its chemical recalcitrance and structural complexity. Adding to this complexity, the lignin structure is highly dependent on both the feedstock and lignin isolation process [30]. However, a part of lignin could be converted into value-added products including bio-based aromatic chemicals, as well as building blocks for materials [31]. The soda lignin has more value than kraft lignin from wheat straw in manufacturing lignin-derivative compounds in terms of the industrial lignin structure and the lignin recovery cost [30, 32]. Lignosulfonates and their modified products have always been traditionally and extensively employed as a class of thinning agent for drilling fluids. SFP lignin is a kind of lignosulfonate in the waste liquor from the pulping of wheat straw with Na_2SO_3, HCHO, and AQ [33, 34]. Experimental results showed that SFP lignin exerts three effects on drilling fluid dilution, foaming, and flocculation. Therefore, SFP lignin and its modified products can be used as a thinning and foaming agent for drilling fluids, as well as a flocculating agent for treating waste drilling fluids [34, 35].

8. Conclusion

Wheat is a kind of annual vascular-bundled herbal arthrophyte with only internodes of about 68%w/w of the whole stalk being acceptable for pulping and the remainder of 20–30%, i.e., leaves-sheaths, leaves-blade, nodes, grain, debris, etc., being removed for chemical saving and pulp quality improvement as well as silica content decrements of black liquor from cooking at a commercial pulping process line. There are a series of pulping processes, such as

the soda, soda-AQ, KP, NS and AS process, etc., successfully employed by wheat straw pulping practices for production of brown pulps or bleached pulps, among which the soda-AQ (anthraquinone) process is most commonly applied in producing the bleached pulps from wheat straw, for AQ to be used in improving selectivity of delignification.

The first installation of continuous cooking system with an annual capacity of 100,000 metric tons of wheat straw pulps was successfully operated in China, scaling up an individual pulp production line capacity from non-wood materials in the world, with the tensile strength of wheat straw pulp up to 6000 m, the mid-water discharge amount of less than $50m^3$/t o.d. pulp, COD less than 60 mg/L of treated effluent, and the black liquor extraction rate up to 94.6%.

The first mid-consistency and shortened TCF bleaching sequence in the world, namely, OQPo (oxygen delignification—chelating metal ions—peroxide bleaching assisted with oxygen) sequence, was successfully commercially operated in China in 2008, with the brightness of bleached wheat straw pulps (BWSPs) of more than 80%ISO, the pulp viscosity of 653 ml/g, breaking length of more than 7000 m, less amount bleaching effluent generated about 30 m^3/t pulps, and decrease by more than 60% of a traditional CEH bleaching process. The BWSPs can be used to manufacture a wide range of paper and paperboard grades as the full or part furnishes, such as light-weighted printing paper, letterpress printing paper, typing paper, writing paper, aluminum foil base paper, cylinder polished paper, tissue and sanitary papers, white board, coated ivory board, and almost all grades of paper and paperboard grades.

Chemi-mechanical pulps, meeting the requirement of manufacturing A-grade corrugated medium, A-grade linerboard, etc., can be produced from wheat straw using a small amount of caustic soda with advantages as high pulp yield (73.1–78.9%), good physical strength (a tensile strength of 19.49–41.27 N.m/g), and proper biodegradable effluents. A relationship between COD load (y) and pulp yield (x) of wheat straw pulping, i.e., $y = 4929.58426 – 104.0789x + 0.5883x^2$, $r^2 = 0.910$, is summarized, and it is proven that effluent from wheat straw chemi-mechanical pulping has a good biodegradability contrasting with that from OCC (old corrugated container) pulping.

The O_2 cooking in soda-AQ/O_2 two-stage cooking is benefited for the desilicate of black liquor from wheat straw chemical pulping line. The successful experiences for chemical recovery in China's wheat straw chemical pulping mills indicate the following: to enhance the loss of wheat straw preparation being helpful to decrease the silica content and viscosity of black liquor, rapid cooking at lower temperature preventing the degradation of cellulose and providing wheat straw chemical pulps with good physic strength and drainability, and high efficient extraction washer for black liquor added to increase the initial concentration of black liquor and to result in steam savings during thickening black liquor and also decrement of fresh water consumption in pulp washing stage. SFP lignin, a kind of lignosulfonate in the waste liquor from the pulping of wheat straw with Na_2SO_3, HCHO, and AQ and its modified products, can be used as a thinning and foaming agent for drilling fluids, as well as a flocculating agent for treating waste drilling fluids.

Acknowledgements

This work was funded the Fundamental Research Funds for the Central Non-profit Research Institution of CAF (CAFYBB2017ZX003–08) and the Le'an Scholarship Program, Shandong province, China.

Author details

Guigan Fang* and Kuizhong Shen

*Address all correspondence to: fangguigan@icifp.cn

Institute of Chemical Industry of Forestry Products (CAF), Key Lab of Forest Chemical Engineering (SFA), National Engineering Lab for Biomass Chemical Utilization, Key Lab of Biomass Energy and Material of Jiangsu, Nanjing, PR China

References

[1] Lu YJ, Li X, Jiang N. The development and trend analysis of the world wheat industry in 2017. China Market. 2017;(26):63-65. DOI: 10.13939/j.cnki.zgsc.2017.26.063

[2] Danielewicz D, Surma-S'lusarska B. Properties and fiber characterization of bleached hemp, birch and pine pulps: A comparison. Cellulose. 2017;**24**:5173-5186

[3] Li ZZ. Overview of recent development of non-wood fibers pulping in China. China Pulp & Paper. 2011;**30**(11):55-63

[4] Lin WY. Review on progress of non-wood fibers pulping and chemical recovery in China. China Pulp & Paper Industry. 2005;**26**(5):22-26

[5] Wu YM. Chemistry of Plant Fiber. 2nd ed. Beijing: China Light Industry Press; 1991. p. 54

[6] Li ZZ. Non-wood fibers pulping theories and techniques. China Pulp & Paper Industry. 2007;**26**(12):71-74

[7] Tutuş A, Çiçekler M. Evaluation of common wheat stubbles (Triticum aestivum L.) for pulp and paper production. Drvna Industrija. 2016;**67**(3):271-279. DOI: 10.5552/drind.2016.1603

[8] Song MX, Li ZZ, Chen ST. Principals for non-wood fibers pulping–A successful wheat straw pulping practice in Quanlin paper group. China Pulp & Paper. 2009;**28**(2):69-72

[9] Plazonić I, Barbarić-Mikočević Ž, Antonović A. Chemical composition of straw as an alternative material to wood raw material in fiber isolation. Drvna Industrija. 2016; **67**(2):119-125. DOI: 10.5552/drind.2016.1446

[10] Yang SH. Chemistry of Plant Fiber. 3rd ed. Beijing: China Light Industry Press; 2011. pp. 19-44

[11] Zhan DT, Liu XB, Li ZZ. Fiber morphology and chemical compositions of different parts of wheat straw. China Pulp & Paper. 1990;**9**(4):16-21

[12] Saeed HAM, Liu Y, Lucia LA, Chen HL. Suitable approach using agricultural residues for pulp and paper manufacturing. Nordic Pulp & Paper Research Journal. 2017;**32**(4): 674-682. DOI: 10.3183/NPPRJ-2017-32-04-p674-682

[13] Zhan HY. The Principle and Engineering of Pulping. 3rd ed. Beijing: China Light Industry Press; 2011. pp. 74-77

[14] Zan HY, Fu SY, Li HL. Technical progress of non-wood fibers pulping in China- bamboo and wheat straw. China Pulp & Paper Industry. 2011;**32**(9):12-16

[15] Li J, Li K, Wu HM, Li WW, Zeng J, Xu J, Chen KF. Commercial operation of wheat straw pulping bleaching by a OQPo sequence. China Pulp & Paper. 2009;**28**(10):79-81

[16] Zhang Q, Peng JJ, Chen LH, Shen ZH. Manufacture of low density printing paper using wheat straw chemical pulp. China Pulp & Paper. 2017;**36**(8):30-34

[17] Fang GG, Liu MS, Shen KZ, Deng YJ, Li YR, Chen TA. Pollution controllable non-wood fibers pulping techniques—High yield pulping. In: Proceedings of Materials Science and Engineering Technology; Beijing: The Third Annual Youth Academy of China Association for Science and Technology; 1998. pp. 276-278

[18] Shen KZ, Fang GG, Liu MS, Li P. Technology of producing bible paper with a furnish of wheat-straw bleached chemical pulp and Bleached SKP. In: Proceedings of the Ninth Annual Academic Conference of China Paper Society; Nanjing; 1999. pp. 873-876

[19] Zhao SZ, Li YL, Ying SY, Ye JL. A new application technology of chemicals to decrease wet-deformation and to increase surface strength of wheat straw-base paper. World Pulp and Paper. 2001;**20**(4):16-22

[20] Huang SR. The produce high-grade cultural paper from the mixture of bleached wheat straw pulp and bleached bamboo pulp. In: Proceedings of the Eleventh Annual Academic Conference of China Paper Society, Transactions of China Pulp and Paper; 18(z) Rizhao; 2003. pp. 195-197

[21] Wang FM, Wang DF. Synthetic surface sizing agent to size offset paper made by wheat straw pulp. Paper and Paper Making. 2003;(Z1):15-16

[22] Xu HY, Zhou GW, Qi GW, Huang KP. Micro-particle retention and drainage system for producing offset using wheat straw pulp. In: Proceedings of the Fourteenth Seminar on the Development and Application Technology of Papermaking Chemicals; Shanghai: China Paper Chemical Industry Association; 2007. pp. 219-231

[23] Shen KZ, Fang GG. State-of-the-art bleaching technology of mechanical pulps. In: Proceedings of the Sixth Annual Academic Conference of China Paper Society. Transactions of China Pulp and Paper, 29(z), Beijing; 2014. pp. 13-20

[24] Liu GL, Fang GG. Promotion and development of non-wood fibers high yield pulping-a successful solution to pollution control for non-wood fibers pulping mills. Sichuan Paper. 1997;**26**(1):30-32

[25] Li HB, Fang GG, et al. High brightness bleaching of wheat straw CMP pulps. China Paper Industry. 2008;**29**(24):44-47

[26] Li HB, Fang GG, et al. Enforced chemical impregnation measures for wheat straw CMP pulping. Chemistry and Industry of Forest Products. 2009;**29**(2):81-84

[27] Wang Q, Chen JC, et al. Factors screening for wheat straw CMP pulping. China Pulp & Paper Industry. 2009;**30**(6):48-51

[28] Zhang HL, Fang GG, et al. Characterization of effluent and pollution load from a two-stage impregnation wheat straw CMP pulping process. Biomass Chemistry and Engineering. 2016;**50**(5):39-44

[29] Cao YF, Yang YQ, Zhang XL, Li ZZ. Properties of black liquor of two-stage alkali-oxygen pulping of wheat straw. Transactions of China Pulp and Paper. 2002;**17**(1):64-67

[30] Constant S, Wienk HLJ, Frissen AE, Peinder P, Boelens R, Es DS, Grisel RJH, Weckhuysen BM, Huijgen WJJ, Gosselink RJA, Bruijnincx PCA. New insights into the structure and composition of technical lignins: A comparative characterization study. Green Chemistry. 2016;**18**:2651-2665. DOI: 10.1039/c5gc03043a

[31] Santos JI, Fillat U, Martin-Sampedro R, Eugenio ME, Negro MJ, Sallesteros I, Rodriguez A, Ibarra D. Evaluation of lignins from side-streams generated in an olive tree pruning-based biorefinery: Bioethanol production and alkaline pulping. International Journal of Biological Macromolecules. 2017;**105**(1):238-251. DOI: 10.1016/j.ijbiomac.2017.07.030

[32] Kumar H, Alén R, Sahoo G. Characterization of hardwood soda-AQ lignins precipitated from black liquor through selective acidification. BioResources. 2016;**11**(4):9869-9879

[33] Cao JW. Separation and modification of lignin from wheat straw pollution-free decomposition solution [master's dissertations]. Shaanxi University of Science and Technology; 2011. pp. 1-88

[34] Liu QX. Study on comprehensive utilization of lignin in alkaline pulping black liquor [doctoral dissertations]. Tianjin College of Light Industry; 2001. pp. 1-110

[35] Zhou Q. Catalytic sulfonation of wheat straw alkali lignin and its application in petroleum recovery [doctoral dissertations]. South China University of Technology; 1998. pp. 1-132

Edited by Shah Fahad, Abdul Basir and Muhammad Adnan

Global wheat consumption in the 2016/2017 season is forecasted to reach a record high 736m tonnes, showing a growth of 25% in the last 15 years. This raises the question which outlets the wheat is going into, what the growth of these outlets is, which regions or countries have grown the most, and where do we see future potential. Strong competition of other feed grains like corn is expected to slow the growth of wheat used for feed in the next years, and in the future, companies involved in the grain supply chain and feeding industry will need to be flexible enough to continue to meet this fast-changing demand for feed grains. For feed producers, this means they need to be able to access supplies of different grains from different origins to allow for the cheapest composition of their feed, while grain suppliers need to be able to continuously best engage with global trading opportunities to originate grains in various regions and move them to demand regions as cost-effectively as possible.

Published in London, UK
© 2018 IntechOpen
© yul38885 / iStock

IntechOpen

ISBN 978-1-83881-703-9