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# Wheat Improvement, Management and Utilization

*Edited by Ruth Wanyera and James Owuoche*

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Edited by **Ruth Wanyera** and **James Owuoche**

**Wheat Improvement, Management and Utilization**
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# Meet the editors

Ruth Wanyera is a principal research scientist and works as a plant pathologist at Kenya Agricultural and Livestock Research Organization, Njoro, since 1981. She is the head of the Department of Plant Pathology, a national wheat coordinator, and a graduate from the Ukrainian Agricultural Academy, Russia. She has extensive research experience in wheat rust diseases, including phenotyping, surveys, and surveillance. She is an outstanding ABCF alumnus and an African Women in Agricultural Research and Development fellow. She has contributed to the release of wheat varieties with adult plant resistance to the wheat stem rust race Ug99 and its variants. She is a member of the American Phytopathological Society and Crop Science Society of America and founder member of Kenya Professional Association of Women in Agriculture and Development. She has written and published several technical reports and journal papers and contributed to book chapters. She has coordinated research projects funded by international, regional, and national bodies. She has won a number of awards including the 2015 Borlaug Global Rust Initiative Gene Stewardship Award.

James Owuoche is a wheat breeder with vast experience in wheat, barley, and triticale. Dr. Owuoche holds BSc in Agriculture (Nairobi), MSc in Wheat Breeding (University of Alberta), and PhD from Kansas State University (US). He has previously worked at the Wheat Research Center (current KALRO) as a breeder and is interested in developing wheat and small grain cereals resistant to biotic and abiotic stresses with suitable traits for baking and industrial end-use qualities using both conventional and molecular breeding. Currently, Dr. Owuoche is a lecturer at Egerton University, Kenya and a reviewer with reputable international journals, and has supervised 12 MSc students and 5 PhD students and published over 20 papers in refereed journals. During this period of his career, he has contributed to the development and release of several wheat and sorghum varieties for baking and industrial use in Kenya.

# Contents

# Preface

Wheat is the second most produced cereal grain after maize worldwide and a source of multiple nutrients and dietary fiber. The wheat book shares knowledge on some of the key topics on wheat improvement; aspects of production, quality, and utilization; biotic and abiotic stresses; and good crop husbandry that contributes to improved varieties. The safety of the products being consumed was also considered in this book. The relevant sections in this book provide readers with current knowledge on wheat improvement that contributes to high-quality products and wheat improvement, production, quality, utilization, and safety. Exploitation of relevant innovations specified in this book will improve wheat production worldwide and benefit scientists and consumers. The contribution of knowledge to the publication of this book by authors from various countries is highly acknowledged.

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Section 1

# Wheat Genetics and Improvement

Chapter 1

# Wheat in Kenya: Past and Twenty-First Century Breeding

Godwin Macharia and Bernice Ngina

Additional information is available at the end of the chapter

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**Abstract**

Plant breeders aim to improve crop varieties to benefit humankind. Since wheat was introduced in Kenya, numerous varieties have been released and cultivated to varying extents. Past genetic gains have been fragile due to various environmental challenges-mostly rust diseases, and unfavorable socio-economic national policy for the crop. The role and the contribution of wheat breeding to the success of the crop in Kenya for over a century is reviewed. It is considered that systematic exploitation of local and introduced genetic diversity has contributed to release of varieties with superior genetics over time, enhancing productivity from 1 ton/ha in the 1920's to approximately 3 tons/ha recently. Consistent rise in demand to about 1 million metric tons suggests that the national wheat breeding research program must be remodeled to leverage modern tools and best practices; to reconsider its target range of breeding environments in the wake of climate change; to entrench its engagement with the international wheat research programs; and to promote a culture of continuous mentorship. Here, cases are highlighted where the national program has moved in such positive directions to address the varietal needs of a crop that has fully integrated in the economy and the diets of many Kenyans.

**Keywords:** Kenya wheat, rust, breeding cycle, phenotyping, genomics

## 1. Introduction

Traditionally, wheat (*Triticum* spp.) is considered one of the several founder crops domesticated in the "Fertile Crescent" [1] and significantly contributed to "Neolithic Revolution" [2]. This is initially attributed to the cultivation of diploid (genome $A^m$, $2n = 14$) einkorn wheat (*Triticum monococcum*) and tetraploid (genomes BBAA, $2n = 28$) emmer wheat (*Triticum turgidum* spp. *dicoccoides*) around 10 millennia [3] Tanno and Willcox 2006 which triggered the evolution of

human societies and the hallmark transition from hunting and gathering of food to agrarian lifestyles [4]. It is estimated that bread wheat (*Triticum aestivum* spp. *aestivum* L.), an allohexaploid (genomes AABBDD, 2*n* = 42) hybrid of emmer wheat with goat grass (*Aegilops tauschii*, genome DD) [5], accounts for over 95% of all cultivated wheat. Instructively, since its emergence approximately 8000 years ago, this species is not only deemed among the most important cereal crops in global production that also includes rice (*Oryza sativa*) and maize (*Zea mays*) but also in its ecological range of cultivation, cultivar diversity, and the extent to which it has become inseparable to the cultures and religions of diverse societies worldwide [3].

In Kenya, wheat was introduced in the early twentieth century, while wheat breeding research through introduction, hybridization, and selection has been underway in the country [6] for over a century. Past achievements have led to the development of cultivars highly adaptable to the Kenya highlands with most commercial production practiced at altitudes above 1500 m. Diseases, especially rusts, have reduced wheat productivity in Kenya ever since the crop was first grown commercially in 1906 [7–11]. Devastating historical and current epidemics (**Figure 1**) including the highly virulent race *Ug99* of wheat stem rust (*Puccinia graminis* Pers. f. sp. *tritici* Eriks) [12] and other related races [13] have reduced Kenya and regional countries to perennial net wheat grain importers. This is in the backdrop of increased consumption needs, estimated at more than 150% of local production [14].

**Figure 1.** Stem rust disease has been a key deterrent to Kenya's wheat productivity for nearly a century. A devastating epidemic of the disease caused major losses in fields planted to variety Robin in 2014.

### 1.1. Wheat growing conditions and a reflection of the origin and objectives of the national breeding program

Considering that a vast majority of Kenya's wheat production is accomplished in the medium-to-high-altitude zones, the uniqueness of the growing conditions and hence breeding objectives is encapsulated in Sir Rowland Biffen's 1926 address to a farmer's gathering following a tour of the Kenya wheat fields. As at that time, just as is today, Sir Biffen reflected that the growing

conditions were characterized by a continuous growing period, where the crop is practically grown at any time of the year such that it is not uncommon to find within the same vicinity a field being prepared for sowing, while an adjacent one has a crop at tillering, booting, or even grain-filling growth stages [15]. Kenya unfortunately sits at the epicenter of wheat rust diseases where devastating epidemics of particularly stem and stripe rusts driven by rapid evolution of new races have been recurrent over the last century [16]. In his address, Biffen posed: "…I have never yet seen wheat so badly attacked by rusts as I have in this country. I have been impressed by the variety of the rust attacks and it soon became quite clear that the incidence of the rust on wheat was going to determine whether Kenya is ever to be a producing country…."

Dixon [6] traces the origin of bread wheat in Kenya initially to introductions of Australian germplasm at the beginning of the twentieth century followed by a gradual succession with a few Egyptian, Italian, and Canadian founder lines decades later. Moreover, development of breeding populations and variety release in 1930–1950 was largely based on crosses within that core diversity with relatively limited additions from contemporary international programs. But for a short stint during the late 1980s through early 1990s, during which period the national program devoted substantial resources to breeding for drought tolerance [17, 18] and insect pests [19], an overarching objective throughout the history of wheat in Kenya has been that for rust resistance.

Today the goal of the breeding program is to design cultivars that are high yielding, widely adapted, and resistant/tolerant to prevailing biotic and abiotic stresses, particularly rust diseases, drought, and Russian wheat aphid. Moreover, the breeding effort as a priority releases cultivars that are of good end user quality.

### 1.2. Remodeling the future of wheat breeding in Kenya

Through breeding efforts and better management practices, grain yield of wheat in Kenya has increased (**Figure 2**) from an average of 1.0 ton/ha during the 1920s to 3 tons/ha during the 2010s [20]. Yet, the demand for wheat grain through the last century has risen from an average of about 0.02 million metric tons in 1920 to about 1.0 million metric ton in 2014—a 50-fold increase.

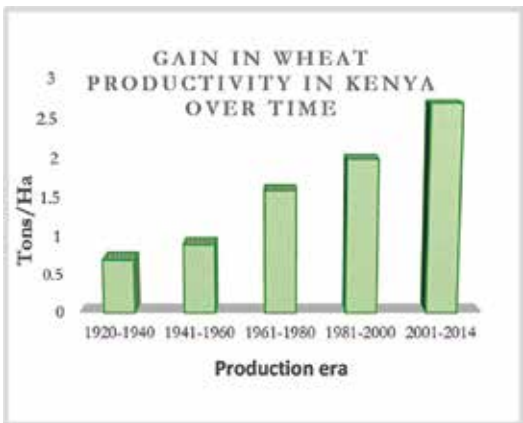


**Figure 2.** Significant gain in Kenya's wheat productivity since the earlier years of the crop is evident. Faster gain through a remodeled breeding scheme would be instrumental in achieving self-sufficiency.

Demand for bread and related food products in the country is expected to increase even further owing to changing diets that favor wheat-based diets over traditional food sources and generally due to increased human populations. Generally, crop researchers and more so breeders agree that improvement and selecting for high yields and yield stability as well as maintaining resistance to insect pests and disease pathogens are objective and priority traits for any crop [21], including wheat in Kenya; it behooves to consider that how breeding is implemented, and what goals are achieved, is a function of biological feasibility, consumer demand, and production economics [22]. Twenty-first century wheat breeding in Kenya must audit (e.g., [23]; **Table 1**) and systematically exploit the genetic diversity within its reach vis-à-vis the target growing environments, reevaluate what specific trait growers value most alongside traditional target traits, and importantly consider designing cultivars that are responsive to current and future management practices including zero tillage and irrigated environments.

| | **No. of marker** | **No. of alleles** | **Mean** | | |
|---|---|---|---|---|---|
| | | | **MAF** | **PIC** | $H_e$ |
| **Chromosome** | | | | | |
| 1A | 510 | 1015 | 0.29 | 0.30 | 0.38 |
| 1B | 358 | 716 | 0.27 | 0.29 | 0.36 |
| 1D | 91 | 182 | 0.33 | 0.30 | 0.29 |
| 2A | 325 | 650 | 0.31 | 0.30 | 0.39 |
| 2B | 625 | 1250 | 0.28 | 0.30 | 0.37 |
| 2D | 98 | 196 | 0.22 | 0.25 | 0.31 |
| 3A | 390 | 780 | 0.26 | 0.28 | 0.35 |
| 3B | 402 | 800 | 0.29 | 0.29 | 0.37 |
| 3D | 33 | 66 | 0.25 | 0.27 | 0.34 |
| 4A | 360 | 720 | 0.27 | 0.29 | 0.36 |
| 4B | 159 | 318 | 0.27 | 0.29 | 0.37 |
| 4D | 28 | 56 | 0.13 | 0.18 | 0.21 |
| 5A | 408 | 812 | 0.28 | 0.30 | 0.38 |
| 5B | 511 | 1017 | 0.30 | 0.30 | 0.38 |
| 5D | 73 | 146 | 0.20 | 0.24 | 0.29 |
| 6A | 417 | 834 | 0.26 | 0.28 | 0.36 |
| 6B | 403 | 802 | 0.30 | 0.31 | 0.39 |
| 6D | 52 | 104 | 0.27 | 0.27 | 0.34 |
| 7A | 397 | 794 | 0.27 | 0.29 | 0.36 |
| 7B | 279 | 558 | 0.31 | 0.30 | 0.39 |
| 7D | 43 | 86 | 0.28 | 0.27 | 0.35 |

| | No. of marker | No. of alleles | Mean | | |
|---|---|---|---|---|---|
| | | | MAF | PIC | $H_e$ |
| **Genome** | | | | | |
| A | 2807 | 5605 | 0.28 | 0.29 | 0.37 |
| B | 2737 | 5461 | 0.29 | 0.30 | 0.38 |
| D | 418 | 836 | 0.24 | 0.26 | 0.32 |

**Table 1.** Number of markers and alleles, minor allele frequency (MAF), polymorphism information content (PIC) and expected heterozygosity ($H_e$) averaged across 5962 mapped SNP loci in an East African enriched set of 297 wheat lines.

Like any typical breeding program and for its success, the implementation of wheat improvement must be approached as both an art and a science. Conceptually current and future wheat breeders must be guided by a range of both subjective and objective judgments in the design and implementation of the program and in deciding which parents to cross, which selection methods to use, which progenies to keep, and which cultivars to release [24]. The latter implies that the Kenyan wheat program might in future explore development of hybrid wheat besides pure lines. That consistently the breeding program must maintain a sufficient return on investment in people, money, and time and generate benefits in the most efficient way. Routine self-audit on what genetic gain is made per unit time and cost and in every cycle of breeding would be a healthy practice moving into the future. The latter consideration of a routine self-audit would provide a major paradigm shift from the current situation where in general no empirical assessment of genetic progress is purposely done in the program.

### 1.3. The need for enhanced collaborations

From a wider perspective, research collaborations between African scientists and foreign agencies have been known to yield important results [25] in addressing a myriad of agricultural problems in the continent. The success of the Kenyan wheat breeding program can significantly be attributed to close networks that have been created with the wheat community globally. These collaborations that extend back to the beginning of the twentieth century revolve around sharing germplasm and information as well as in training (**Figure 3**). For instance, beginning mid-1950s, there was a major shift in the national program in which event breeders reasoned that continued under performance and attack of wheat crops by rust diseases was partly due to low genetic diversity of cultivated material. During this decade, breeders at the national program devoted systematic effort to introduce a new gene pool comprising of cultivars identified from the International Spring Wheat Nursery initiated in 1950 by B. B. Bayles and R. A. Rodenhiser of United States Department of Agriculture-Agricultural Research Services (USDA-ARS) as well as screening nurseries emanating from Food and Agriculture Organization [6].

Beginning mid-1960s, the national program increasingly utilized germplasm developed at CIMMYT culminating in the release of many superior cultivars that were not only shorter in height but were resistant to stem rust and had a significant yield advantage [23]. The collaboration with CIMMYT has today gone full cycle. The shuttle breeding program (**Figure 4**) in which crosses made at CIMMYT are tested for stem rust in the global rust phenotyping platform at

Njoro-Kenya, for stripe rust resistance, leaf rust resistance, and heat tolerance at Toluca, El Batan, and Ciudad Obregon, respectively, is a case study of how future collaborations should be modeled.

**Figure 3.** Breeding effort must address training for future breeders. In this image, students participate in selection of rust-resistant plants at the KALRO-Njoro rust phenotyping facility.

An example at the regional level that might generate significant gain and progress for the wheat breeding program and hence the crop's success is envisioned in the recent dialogue about an "opened seed space." The rationale is to align seed laws as well as harmonize seed trade regulations across countries in the COMESA region. The outcome is that superior cultivars released under similar growing conditions in the pertinent countries will not necessarily be subjected to lengthy testing in Kenya, and benefits in their adoption and use should accrue immediately. At the national level, expedient production and distribution of seed of released cultivars need to be strengthened through both private-public and public-public partnerships. Neighboring countries could also be co-opted in varietal maintenance and initial seed increases so that each country need not maintain every variety it uses [26].

### 1.4. Breeding wheat for nontraditional environments

Wheat breeding in Kenya will continue to play a key role in the coordinated need and effort for increased food production. In the background of current yield trends, predicted population growth, and pressure on the environment, traits relating to yield stability and sustainability should be a major focus of plant breeding efforts [27]. These traits include durable disease resistance, abiotic stress tolerance, and nutrient and water-use efficiency [28–30]. Designing and developing cultivars that are adaptable to marginal lands, conservation agriculture, irrigated conditions, etc., is likely to be a key driver of the future of wheat breeding in Kenya. In this context, consideration needs to be prioritized for cultivars that are resilient to climate change, well aware that this phenomenon negatively impacts economies largely based on rain-fed agriculture [23], the traditional source for Kenya's wheat. Rigorous and inclusive wheat research that also involves multifaceted technological approaches in various frontiers beyond conventional breeding is paramount.

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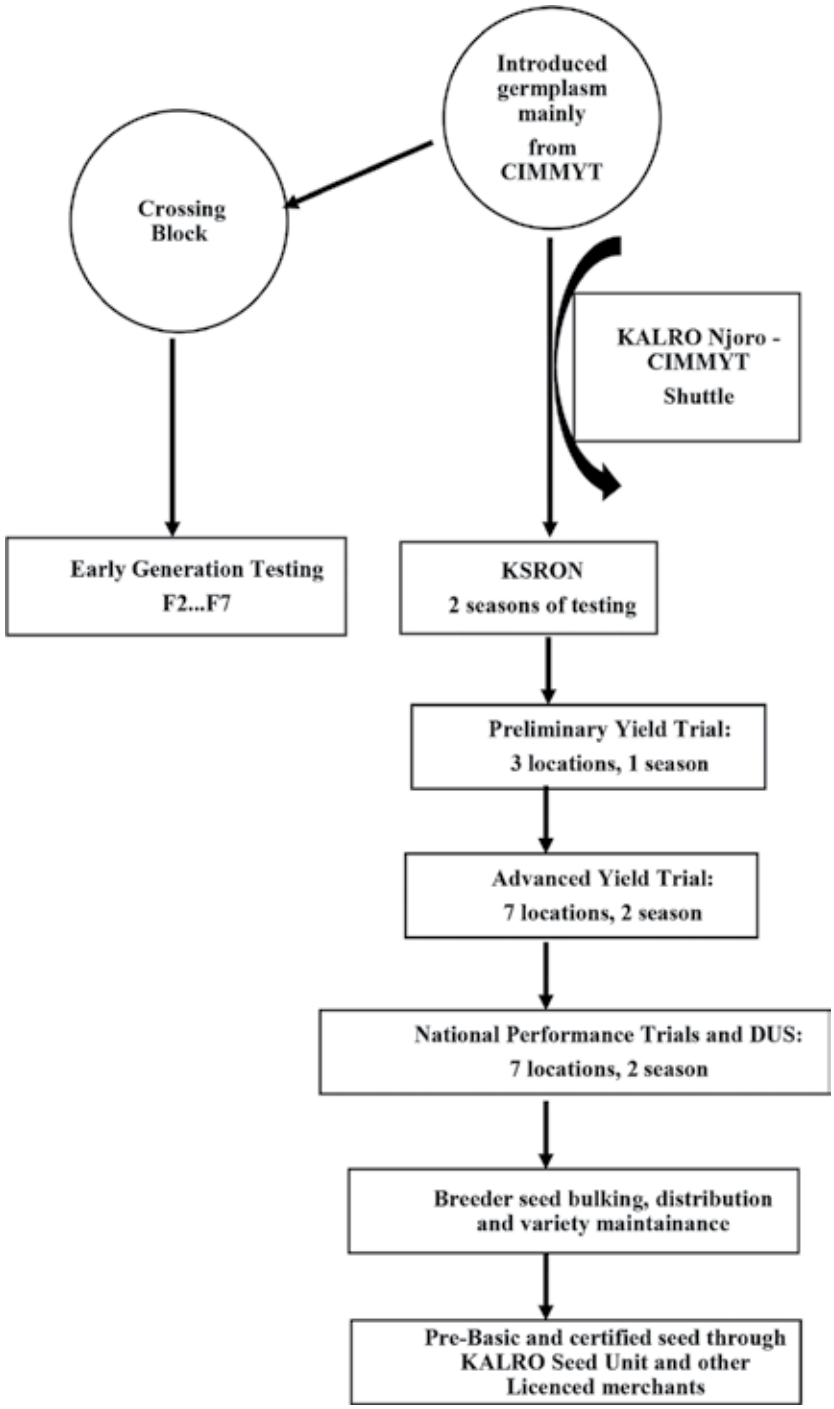


**Figure 4.** Kenya wheat breeding scheme has lately incorporated systematic germplasm sharing between KALRO-Njoro and CIMMYT through a shuttling program. Benefits from breeding effort can also be fast tracked through incorporation of biotechnology.

### 1.5. Leveraging modern breeding tools and best practices

Modern plant breeding increasingly utilizes innovations that promise greater efficiencies over current breeding methods. A key approach has been the utilization of biotechnology in many breeding programs globally. While the Kenya breeding program prides success in releasing cultivars through conventional selection methods, DNA-based marker-assisted selection (MAS) still largely underutilized might expedite development of desired cultivars if well implemented. MAS is applicable in four main areas that wheat breeders in Kenya often encounter: for efficient detection and selection of a small number of traits that are difficult to manage via phenotype and usually characterized with low penetrance and/or complex inheritance, for the retention of recessive alleles in backcrossing pedigrees, for the pyramiding of disease-resistance genes, and for aiding in the choice of parents in crossing, to ensure minimal levels of duplication [31]. However, just as this author posits, wheat breeding will continue to be mostly characterized by selection in the breeding plots, rather than detection in the microtiter plots per se.

Since selection in the breeding plots has traditionally been based on phenotyping, success in the future for the Kenya wheat breeding program must be inbuilt on robust phenotyping platforms. The objective of modern phenotyping is to increase the accuracy, precision, and throughput of phenotypic estimation at all levels of biological organization while reducing costs and minimizing labor through automation, remote sensing, improved data integration, and experimental design [32]. Hence robust phenotyping assays for the nation program with the objective of reducing inefficiencies in development and release of superior cultivars would immensely benefit from investments in infrastructure and human capacities in biometrics in plant breeding.

The bigger picture is in utilizing methodologies that combine accurate phenotyping and sufficient genotyping in modeling gene discovery and introgression in breeding populations. Recently, the national program collaborating with other researchers has implemented both biparental and association mapping works for rust resistance genes (e.g., [23, 33, 34]). Such effort will contribute to faster cultivar development.

## 2. Conclusion

The wheat breeding program in Kenya has become of age. Ever since the first crosses were made in the 1910s leading to the release of cultivar *Equator* in 1920 and subsequently over 130 other cultivars (**Table 2**), measurably genetic gain has been made. However, recent trends both in the country's production and consumption landscapes necessitate that for wheat to continue to play its rightful roles as a food crop for an increasing Kenyan population, breeding efforts must not only be enhanced but such should be systematic and guided by international best practices for it to create novelty and stimulate industry. Lastly, there is optimism that a large potential for enhancing wheat productivity through breeding, and of course management avails for Kenya especially if synergies among local, regional, and international collaborations are enriched.

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| Variety name/code | Breeder | Year of release | Variety name/ code | Breeder[a] | Year of release | Variety name/ code | Breeder | Year of release |
|---|---|---|---|---|---|---|---|---|
| 1061.K.1 | NBS | *Unknown* | Kenya8 | NBS | *Unknown* | Lenana | NBS | 1963 |
| 1061.K.4 | NBS | *Unknown* | KenyaB-256-G | NBS | *Unknown* | Menco | NBS | 1963 |
| 1200.M | NBS | *Unknown* | Kenya cheetah | NBS | *Unknown* | Fanfare | NBS | 1964 |
| 291J.1.I.1 | NBS | *Unknown* | KenyaFL.1.158 | NBS | *Unknown* | Fury | NBS | 1964 |
| BF236C1L | NBS | *Unknown* | Equator | NBS | 1920 | Gem | NBS | 1964 |
| EgyptianNa95 | NBS | *Unknown* | Kenya Governor | NBS | 1925 | Kenya Hunter | NBS | 1964 |
| FLIKenya9 | NBS | *Unknown* | Kenya | NBS | 1929 | Kenya Plume | NBS | 1965 |
| H441 | NBS | *Unknown* | Kenya Standard | NBS | 1930 | Bailey | NBS | 1966 |
| K-360-H | NBS | *Unknown* | Kenya Plowman | NBS | 1950 | Bonny | NBS | 1966 |
| Kenya 291 J.1.I.1 | NBS | *Unknown* | 338AA1A2 | NBS | 1951 | Bounty | NBS | 1966 |
| Kenya-117A | NBS | *Unknown* | Kenya-184-P | NBS | 1951 | Brewster | NBS | 1966 |
| Kenya117C | NBS | *Unknown* | Kenya Farmer | NBS | 1954 | Kenya civet | NBS | 1966 |
| Kenya-122 | NBS | *Unknown* | Kenya-362-B-1A | NBS | 1956 | Kenya Grange | NBS | 1966 |
| Kenya-131 | NBS | *Unknown* | 321BT11B1 | NBS | 1960 | Kenya Jay | NBS | 1966 |
| Kenya155 | NBS | *Unknown* | Africa Mayo | NBS | 1960 | Kenya Kudu | NBS | 1966 |
| Kenya-294-B-2A-3 | NBS | *Unknown* | Equator1 | NBS | 1960 | Kenya Leopard | NBS | 1966 |
| Kenya-318.O.3B.2 | NBS | *Unknown* | Kentana Yaqui | NBS | 1960 | Goblet | NBS | 1967 |
| Kenya-318-AJ-4A-1 | NBS | *Unknown* | Kenya-5 | NBS | 1960 | Mentor | NBS | 1967 |
| Kenya-358-AC | NBS | *Unknown* | Kenya-1 | NBS | 1961 | Beacon-Ken | NBS | 1968 |
| Kenya501 | NBS | *Unknown* | Kenya Mamba | NBS | 1962 | 1010AM2 (L) | NBS | 1969 |
| Kenya-58 | NBS | *Unknown* | Catcher | NBS | 1963 | 1010F3SEL.13C | NBS | 1969 |
| Kenya-6297-2 | NBS | *Unknown* | Fronthatch | NBS | 1963 | 1010F3SEL.4 | NBS | 1969 |
| Kenya6820 | NBS | *Unknown* | Gabrino | NBS | 1963 | 1010F3SEL.7 | NBS | 1969 |
| Kenya7 | NBS | *Unknown* | Kenya page | NBS | 1963 | 1012B.1 (L) | NBS | 1969 |
| 1016.P.2 | NBS | 1969 | Kenya Kanga | NBS | 1977 | Njoro BW1 | KARI | 2001 |
| 1016P.1 | NBS | 1969 | Kenya Kifaru | NBS | 1977 | Njoro BWII | KARI | 2001 |
| 1076.D.7 | NBS | 1969 | Kenya Ngiri | NBS | 1979 | KS-Simba | KSC | 2007 |
| 688F4SEL3 | NBS | 1969 | Kenya Nyangumi | NBS | 1979 | KS-Chui | KSC | 2008 |
| 690F4SEL.D.1 | NBS | 1969 | Kenya Paa | KARI | 1981 | Kenya Ibis | KARI | 2008 |

| Variety name/code | Breeder | Year of release | Variety name/code | Breeder[a] | Year of release | Variety name/code | Breeder | Year of release |
|---|---|---|---|---|---|---|---|---|
| Kenya Sungura | NBS | 1969 | Kenya Kanga | NBS | 1977 | Robin | KARI | 2011 |
| Kenya Swara | NBS | 1972 | Kenya Kifaru | NBS | 1977 | Eagle10 | KARI | 2011 |
| Kenya Nyati | NBS | 1973 | Kenya Ngiri | NBS | 1979 | Kenya Hawk 12 | KARI | 2012 |
| Kenya Mbweha | NBS | 1974 | Kenya Nyangumi | NBS | 1979 | Kenya Tai | KARI | 2012 |
| Kenya Nungu | NBS | 1975 | Kenya Zabadi | NBS | 1979 | Kenya SunBird | KARI | 2012 |
| Kenya Nyoka | NBS | 1975 | Kenya Paa | KARI | 1981 | Kenya Wren | KARI | 2012 |
| Kenya Paka | NBS | 1975 | Kenya Popo | KARI | 1982 | Kenya Korongo | KARI | 2012 |
| Kenya Tembo | KARI | 1975 | Kenya Nyumbu | KARI | 1982 | Kenya Kingbird | KARI | 2012 |
| Kenya Kongoni | KARI | 1975 | Kenya Tumbili | KARI | 1984 | KS-Kanga | KSC | 2013 |
| Kenya Fahari | KARI | 1977 | Kwale | KARI | 1987 | KS Nyota | KSC | 2013 |
| Kenya Kanga | NBS | 1977 | Mbuni | KARI | 1987 | Eldo Baraka | UoE | 2014 |
| Kenya Kifaru | NBS | 1977 | Kenya Chiriku | KARI | 1989 | Eldo Mavuno | UoE | 2014 |
| Kenya Ngiri | NBS | 1979 | Pasa | KARI | 1989 | Kenya Hornbill | KALRO | 2016 |
| Kenya Nyangumi | NBS | 1979 | Duma | KARI | 1998 | Kenya Peacock | KALRO | 2016 |
| Kenya Zabadi | NBS | 1979 | Mbega | KARI | 1998 | Kenya Songbird | KALRO | 2016 |
| Kenya Paa | KARI | 1981 | Chozi | KARI | 1998 | Kenya Pelican | KALRO | 2016 |
| Kenya Popo | KARI | 1982 | Ngamia | KARI | 1998 | Kenya Falcon | KALRO | 2016 |
| Kenya Nyumbu | KARI | 1982 | Kenya Heroe | KARI | 1999 | Kenya Deer | KALRO | 2016 |
| Kenya Tumbili | KARI | 1984 | Kenya Yombi | KARI | 1999 | Kenya Weaverbird | KALRO | 2016 |
| Kwale | KARI | 1987 | KSMwamba | KSC | 2001 | | | |

[a]Breeder refers to institution under which the variety was developed and is maintained: NBS, national breeding station. Now defunct; KARI, Kenya agricultural research institute. Now defunct; KALRO, Kenya agricultural and livestock research organization; KSC, Kenya seed company; and UoE, University of Eldoret.

**Table 2.** List of bread wheat varieties for commercialization in Kenya over a century of crop improvement.

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Chapter 2

# Past, Present and Future Molecular Approaches to Improve Yield in Wheat

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Additional information is available at the end of the chapter

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**Abstract**

This chapter addresses the development and use of molecular markers for yield enhancement in wheat. Since their key goal for breeding is to maximize yield, extensive efforts have been made toward the improvement of yield. Agronomic traits related to yield, yield-related, disease resistance, and abiotic stresses are considered to be quantitative traits (QTLs), also known as complex traits, because they are controlled by numerous genes and are affected by environmental factors. Researchers have been studying such traits in the past decades for the development of molecular markers which can be used in various wheat breeding studies mainly involving restriction fragment length polymorphism (RFLP), simple sequence repeat (SSR), single nucleotide polymorphism (SNP), random amplified polymorphic DNA (RAPD), and amplified fragment length polymorphism (AFLP). Furthermore, the advent of next-generation sequencing (NGS) has accelerated the discovery of agronomically important genes. All of the technologies have enabled great advances for increasing the productivity of wheat. Here, the past history of first-generation sequencing, present status of second-generation sequencing, and future potential of translational genomics linked to the yield will be discussed.

**Keywords:** molecular markers, yield, quantitative traits (QTLs), next-generation sequencing (NGS)

## 1. Introduction

As the world's population is projected to reach approximately 9 billion by 2050, grain production of major staple crops needs to double to meet global food needs [1]. Together with rice and maize, wheat is one of the major staple crops widely grown in many countries, providing one-fifth of the calories and the protein for the world's population. In addition,

bioethanol is made primarily from wheat in Europe. Wheat starch is a major component for the production of bread, porridge, cakes, biscuits, and cereals which is a highly versatile crop for the human diet. In 2013, wheat was the third most produced cereal crop (713 million tons), after maize (1016 million tons) and rice (545 million tons). There are two distinct types of wheat, spring wheat and winter wheat, cultivated in many countries based on growing seasons, of which spring wheat is planted in most countries except in the United States and Northern Europe where the predominant crop is winter wheat. The global consumption of wheat has increased at a much faster rate than all other crops, because of the scale-up cultivation in developing countries, particularly in China [2]. Currently, out of the total cultivation area of more than 217 million hectares, the European Union countries has the largest area, followed by China, India, Russia, United States, and Canada [3]. Therefore, there has been an extensive effort over the past decades to increase wheat production through the application of molecular techniques which are powerful tools for enhancing effectiveness in breeding.

The most common or bread wheat species, *Triticum aestivum*, is an annual grass cultivated in temperate zones worldwide that belongs to the genus *Triticum* of the tribe *Triticeae*, and the family Poaceae. The most economically important cereals in the Poaceae family are maize, wheat, rice, barley, and millet. In the genus *Triticum*, there are approximately 25 species including wild and domesticated consisting of a series of diploid, tetraploid, and hexaploid forms, including the diploid einkorn wheat, *Triticum monococcum* (AA genome), the diploid wild wheat, *Triticum urartu* (AA genome), the allotetraploid emmer wheat, *Triticum turgidum* var. *durum* (AABB genome), the allohexaploid common wheat, *T. aestivum* L. (AABBDD genome), and the autoallohexploid *Triticum zhukovskyi* (AAAAGG genome). Of these, *T. aestivum* has 42 chromosome pairs that are derived from two rounds of polyploidization events. Its genome size is approximately 17 Gb, composed of A, B, and D genomes created from the hybridization of three different species. The first hybridization between *T. urartu* (2n = 2x = 14) and a B genome species that has not yet been identified occurred 0.20–1.3 million years ago (MYA) to form the tetraploid *Triticum dicoccoides* (2n = 4x = 48) [4, 5]. The B genome is still unclear and may be extinct, but cytological evidence suggests that the S genome of *Aegilops speltoides* is a closely related species or an ancestral progenitor to the B genome of wheat [6, 7]. The second hybridization event resulted in the complete form of the hexaploid genome *T. aestivum* (2n = 6x = 42), which occurred between the domesticated *Triticum dicoccum* or *T. durum*, a wild goatgrass, and *Aegilops Tauschii* about 8000–10,000 years ago [8–10]. Like most allopolyploid plants, wheat also has the diploid-like chromosome pairing behavior during meiosis, preventing multivalent formation created by multiple homologous or homoeologous chromosomes [11].

In wheat breeding, a strong emphasis has been put toward the improvement of grain yield as it the most important goal in wheat breeding. There have been concerns about the stagnation or decline of the staple crops in some parts of the world. It has been detected that 37% of wheat areas have experienced the yield stagnation [12]. If the breeder develops an improved wheat variety, having a superior of trait, but produces low yields, producers unlikely will grow it because the yield is necessary for economic feasibility. The grain yield is a complex character with low heritability which is influenced not only by genes but also by the effects of the environment. In wheat, it has been documented that the higher yield is inversely related to

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the protein content and can also delay maturity. Furthermore, abiotic stress factors including drought, salinity, extreme temperatures, and acidity contribute the most to yield loss, ranging between 60 and 82% [13]. As a consequence, extensive efforts have been made to identify the QTLs associated with the yield and its related traits which can be deployed by breeders through marker-assisted selection (MAS). The first report of the genome-wide assessment of molecular marker-based map in the nuclear genome of wheat began in the 1989 with the use of restriction fragment length polymorphism (RFLP) [14]. Subsequent analyses have been performed for construction of genetic maps to improve the efficiency of conventional breeding based on amplified-fragment-length polymorphism (AFLP), single-nucleotide polymorphism (SNP), diversity arrays technology (DArT), simple sequence repeat (SSR) or microsatellite, random DNA marker (RDM), gene targeted marker (GTM), and functional marker (FM). Based on these markers, there are 180 genetic maps extrapolated in wheat, most of which are developed by SSR markers. For example, molecular markers such as SSR and DArT have been used to detect QTLs for fusarium head blight (FHB) resistance, which can further be implemented in breeding studies [14]. Since 2007, a number of research studies have been taken to identify QTLs related to yield based on different mapping populations such as kernel length, kernel width, spike length, spike number, the grain number of spike, sterile spikelet number per spike, fertile spikelet number per spike, and thousand kernel weight [15–22]. However, the development of molecular markers and their applications in breeding have been relatively difficult in wheat because of its three closely related subgenomes and a large genome size consisted of high amounts (80%) of repetitive sequences. In 2012, the availability of wheat whole genome sequences has provided a framework for understanding of polyploidization, and domestication by comparing its sequences with ancestral and progenitor genomes, enabling us to understand the genetic diversity of wheat, which may help accelerate breeding programs [11]. Up to now, there are a total of 217,907 loci and 273,739 transcripts identified, of which 104,091 have been assigned as coding genes and 10,156 as long ncRNAs, according to Ensembl Plants (www.plants.ensembl.org). The chapter addresses molecular areas of research for yield improvement in wheat, focusing on finding QTLs for traits that affect yield. There are three objectives of this chapter as follows: (1) to explore the use of major molecular markers that have been used to identify yield and its-related QTLs in the past; (2) the current progress of molecular markers for linkage map construction; (3) to assess genomic studies of wheat; and (4) to discuss the potential of translational genomics in wheat using well-studied grasses such as rice and barley.

## 2. First-generation sequencing resources for yield improvement in wheat

### 2.1. Restriction fragment length polymorphism (RFLP) markers

Earlier molecular studies in wheat have shown that the RFLP markers are the common tools used as the oldest method of molecular markers for the construction of genetic maps. RFLP are typically inherited as simple Mendelian codominant markers, and are not influence by the environment, which could serve as highly heritable genetic markers for the study of inheritance of a trait. Chao et al. created the first linkage maps of the homoeologous group

7 chromosomes on the wheat genome using 18 cDNA clones across six mapping populations from ten varieties [14]. They mapped 31 RFLP loci on chromosomes 7A, 7B, and 7D. In 1991, detailed linkage maps were constructed with a total size of 1800 cm consisting of 197 RFLP loci [23]. Ma et al. [24] and Anderson et al. [25] identified RFLP markers associated with two Hessian fly resistance genes from *Triticum taushii*, and preharvest sprouting genes from two recombinant inbred populations of white wheat (*T. aestivum* L. em. Thell.). Based on comparative mapping among cereal species such as wheat, rye, and barley, the development of detailed RFLP maps of the homoeologous group-2 chromosomes has established and showed that they had collinear relationships, indicating the high degree of conservation in gene orders [26]. The RFLP markers flanking the resistance to cereal cyst nematode were identified to be *Xglk605* and *Xcdo588*, which were mapped at 7.3 and 8.4 cm from the *Cre* locus [27]. Later, RFLP-based genetic maps belonging to different homoeologous groups were generated including homoeologous group 1 [28], group 2 [29], group 3 [30], and group 5 [31], and group 6 [32]. During this period, the powdery mildew genes were detected by RFLP probes. Ma et al. [33] reported that the powdery mildew resistant gene *Pm2* was located at 3.5 cm away from the RFLP marker *BCD1871*, and both *Pm1* and *Pm4a* showed cosegregation of markers. In addition, the *Pm1a* and *Pm2* were positioned on the RFLP maps at 3 cm away from *Xwhs178-7A*, at 2.7 cm away from *Whs296*, respectively [34]. To increase the frequency of polymorphism detection in wheat, a non-intervarietal cross (W7984 X Opata85) developed at the International Maize and Wheat Improvement Center (CIMMYT) was used creating a dense map which consisted of about 1000 RFLP loci on group 1 [28], group 2 [29], group 3 [30], and group 6 [35]. Other disease-resistant genes of *Lr9* responsible for leaf rust and *Sr22* responsible for stem rust were characterized and mapped using RFLP markers [36, 37]. Furthermore, the feasibility of the 37 RFLP probes on group 5 chromosomes from Thatcher near isogenic lines (NILs) for leaf rust resistance gene *Lr1* was conducted by Feuillet et al., of which three were investigated to be linked the gene [38]. Galiba et al. analyzed the cross between a frost-sensitive, vernalization-insensitive substitution line, *Triticum spelta* 5A and a frost-tolerant, vernalization-sensitive line, Cheynne 5A and showed cosegregation with *Vrn1* and *Xpsr426* RFLP marker [39]. There was also a linkage detected between *Vrn1* and *Xwg644* in accordance to Korzun et al. [40]. The mapping of a single locus controlling the aluminum tolerance gene *Alt2* was completed on the Chinese Spring chromosome arm 4DL derived from homoeologous recombination between *T. aestivum* cv. Chinese Spring chromosome 4D and *Triticum turgidum* cv. Cappelli chromosome 4B.

Starting from 1998, QTLs for yield and yield-related traits were investigated. The dwarfing genes, *Rht-B1* and *Rht-D1*, associated with plant height in wheat were firstly mapped on the short arms of chromosomes 4BS (*Xfba1-4B*) and 4DS (*Xfba211-4D*) [41]. A year later, thirteen RFLP probes and one morphological marker locus, *Eps*, were used to develop a genetic linkage map and identified that an RFLP marker *Xcdo549* on the short arm of chromosome 3A was associated with plant height, kernel number spike-1, and 1000-kernel weight [42]. Araki et al [43] reported one QTL for yield, *Qyld.ocs-4A.1*, and other yield-related traits of spikelet number ear, *QSpn.ocs-4A.1*, and grain weight/ear *QGwe.ocs.-4A.1*, on chromosome 4A which were detected by the *Xbcd1738* marker. As wheat lodging can result in yield losses, nine

QTLs for lodging resistance were detected with the genetic distance between the flanking RFLP markers, of which seven coincided with QTLs for morphological traits [44]. Since the year 2000, there have been many studies published for loci associated with grain yield, heading date, disease, and spike morphology. Kato et al. conducted mapping of QTLs for grain yield and its components on chromosome 5A and confirmed that the grain-yield QTLs were closely linked to QTLs for yield components [45]. They found RFLP markers associated with grain yield, tiller number/plant, ear grain weight, 50-grain weight, and spikelet number/ear based on a homozygous population of single-chromosome recombinant lines. Genetic mapping of QTLs conferring resistance to stripe (yellow) rust and powdery mildew was performed by Singh et al. [46] and Tao et al. [47]. The resistance gene, *Yr28*, derived from *Ae. tauschii* and the adult-plant resistance (APR) gene *Yr18* were mapped on chromosome arm 4DS and 7DS, respectively. They found that *Yr18* was closely correlated with leaf-rust gene Lr34. In addition, the RFLP *xbcd135* and *xbcd266* loci mapped at a genetic distance of 1.6 and 4.8 cm were identified to be closely linked to *Pm6*, a gene conferring resistance to the powdery mildew. Later, two of the powdery resistant genes, *Pm26* and *Pm29*, were mapped on the RFLP linkage map [48, 49]. Several RFLP markers within six major QTL regions have been identified to be tightly linked traits related to compactness such as spike length and number of spikelets [50]. Based on a set of 114 recombinant lines (RILs) of the 'International Triticeae Mapping Initiative' mapping population, morphological, agronomical, and disease resistance traits have been studied. Börner et al. [51] mapped 211 QTLs distributed over 20 chromosomes, of which they detected 64 major QTLs with a LOD score of >3.0 conferring glume color, leaf erectness, peduncle length, ear emergence time, flowering time, plant height, ear length, winter hardiness, grain-filling, grain number, thousand-grain-weight, fusarium resistance, powdery mildew resistance, and leaf rust resistance. The mapping of an earliness *per se* gene in wheat was carried out by Bullrich et al. [52]. This gene was located between the RFLP *Xcdo393* and *Xwg241* on the log arm of chromosome 1A, which showed a large effect on heading date with a phenotypic variance of 0.47. Despite RFLP markers are highly reproducible and used as the primary way for most genetic work in wheat, it has been difficult to use RFLP markers due to the low levels of polymorphism detected in wheat.

### 2.2. Random amplified polymorphic DNA (RAPD) markers

The applications of RAPD markers have been beneficial to improve breeding programs in wheat because they are simple and fast PCR based, require no prior knowledge of target DNA sequence, and are analyzed either by the presence or absence of an amplicon via agarose gel electrophoresis. In wheat, RAPD has been used since 1990 [53]. Devos and Gale confirmed that a degree of polymorphism detected by six RAPD primers was comparable with RFLP markers [54]. They identified four RAPD markers with bread wheat cultivar 'Chinese Spring.' The application of both bulk segregant analysis (BSA) and RAPD has started in 1994 by Eastwood et al. [55]. Using BSA on DNA enriched for low-copy sequences by RAPD markers, the *Cre3* gene resistance to cereal cyst nematode (CCN) in *Triticum tauschii*, was mapped on the long arm of chromosome 2D (Ccn-D2) [55]. More efforts have been made to distinguish the wheat varieties resistant to cadmium stress [56], powdery mildew [57], and

common bunt [58]. The RAPD marker *OPC20* was closely correlated with a gene controlling cadmium uptake in western Canadian durum wheat (*T. turgidum* L. var. *durum*) [56]. In addition, a RAPD marker *OPH17-1900* located on the chromosome arm 6VS, was detected that could be used for the detection of a powdery mildew resistance gene, *Pm21*, in breeding [57]. For common bunt, also known as stinking smut and covered smut, the polymorphic RAPD marker, *BW553*, was identified between resistant and susceptible NILs [58]. Furthermore, identification of RAPD markers linked to the *Yr15* gene controlling stripe rust resistance was conducted using 340 RAPD primers, six of which were detected to be polymorphic [59]. The *OPB13* RAPD marker was the only one that produced polymorphism in 123 $F_2$ individuals and showed that it was linked to *Yr15* through screening a series of NILs each consisted of a different gene for Hessian fly resistance using 1600 random 10-mer primers. Another RAPD marker, *OPE-13*, showed the complete cosegregation with the H21 Hessian fly resistance gene in wheat [60]. Dweikat et al. developed RAPD markers linked to 11 different Hessian fly resistance loci that could be used for determining the presence or absence of specific Hessian fly resistance genes [61]. More RAPD markers including *OPX061050*, *OPAG04950*, and *OPAI14600*, was observed to be linked to the new powdery mildew resistance *Pm25* gene, where the linkage distance between them was 12.8, 17.2, and 21.6 cm, respectively [62]. The application of the BSA approach on DNA enriched for low-copy sequences was used by Eastwood et al. [55] and William et al. [63], generating an increased level of polymorphism and in repeatability. Hu et al. [64] identified two RAPD markers, *UBC320420* and *UBC638550*, that cosegregated with *Pm1a* and one RAPD marker, *OPF12650*, tightly linked to the resistance gene. In these studies, it has been observed that RAPD markers also have been difficult to use in wheat like RFLP markers due to the very low level of polymorphism. Furthermore, the reproducibility of this RAPD bands have been found to be questionable. Despite this, continuous efforts have been made to develop RAPD genetic markers for the discrimination of species/cultivars from each other [65, 66].

### 2.3. Amplified fragment length polymorphism (AFLP) markers

For assessment of large numbers of polymorphic loci, the AFLP technology has been implemented as a powerful tool because of its advantage of having good levels of reproducibility, insensitivity, fast, and no need of sequence information required for primer design [67]. In 1995, a novel PCR-based assay for plant DNA fingerprinting using AFLP markers has resulted in high levels of DNA polymorphism [68]. In fact, the AFLP technique has observed to be more efficient and less expensive and less labor intensive compared to the RFLP technique in wheat [69]. Earlier AFLP-based marker studies have been found to be informative in assessment of genetic diversity in wheat varieties started in 1998 [70, 71]. Regarding the investigation of traits associated with yield, Goodwin et al. [72] and Hartl et al. [73] initiated the AFLP technique to develop an AFLP marker associated with resistance to Septoria tritici blotch and powdery mildew, respectively. Hartl et al. identified several AFLP markers closely linked to the *Pm1c* and *Pm4a* [73]. Out of 92 AFLP primer combinations, 31 polymorphic fragments detected between the resistance and susceptible lines, of which eight were found to be the most reliable polymorphic markers. One of the AFLP markers, *18M2*, was detected as being highly specific for the *Pm1c* gene, while the 4aM1 AFLP marker was identified at 3.5 cm

from *Pm4a*. A couple of years later, an AFLP analysis was conducted using NILs of the strip rust resistance gene *Yr10* and designed AFLP primers that can be useful in detecting the *Yr10* gene [74]. Cao et al. analyzed the 119 individuals of H9020-17-5 x Mingxian169 $F_2$ population to detect AFLP markers linked to the strip rust resistance gene *YrHua* [75]. They found the two markers, *PM14(301)* and *PM42(249)*, of which *PM14(301)* was converted to PCR marker that could be a useful tool for MAS. In 2006, nine AFLP markers that showed polymorphism between the Argentinian wheat cultivar Sinvalocho MA and the rust leaf susceptible cultivar Gamma 6 were used to construct a linkage map of the *Lr3* gene for leaf rust resistance on chromosome 6BL of wheat [76]. The development of AFLP markers was carried out by Li et al. [77]. They detected seven markers linked to *Lr19* resistance to wheat leaf rust using *Mse I* and *Pst I* based on Thatcher, 23 NILs and $F_2$ generation of TcLr19 x Thatcher. Dhillon et al. detected putative AFLP markers linked to leaf rust resistance genes *Lr9, Lr19*, and *KLM4-3B* using NILs of wheat [78]. More likely, the AFLP technique has been approached for developing polymorphic markers underlying a trait.

### 2.4. Simple sequence repeat (SSR) and intersimple sequence repeat (ISSR) markers

Among all available markers, SSR (or microsatellite) markers have become the best suited tool in plant breeding programs, because they are practical, convenient, easy to use, and inexpensive. Moreover, SSRs with tandem repeats of a motif of <6 bp are the most polymorphic, codominant, easy for scoring banding patterns, and have wide genomic distribution, high reproducibility, and a multiallelic nature [79]. SSR analysis has been conducted in most of the QTL studies for mapping various traits. Up to 2015, there are more than 4000 SSR markers that have been developed and used for the construction of wheat genetic maps [80]. The high level of variability and Mendelian inheritance of SSR DNA markers have been first reported by Devos et al. [81] and Röder et al. [82]. Moreover, Röder et al. [83] and Stephenson et al. [84] placed SSR loci onto the genetic map, providing a starting point for developing a saturated map of the wheat genome. For example, SSR markers have been implemented for tagging and mapping important yield-related genes such as the dwarfing genes *Rh8* [85], *Rht12*, and the vernalization gene *Vrn1* [40], and a gene for preharvest sprouting tolerance [86]. SSR primers also have been used to detect some of the wheat resistance genes. Peng et al. found nine microsatellite loci found to be linked to *YrH52* with recombination frequencies of 0.2–0.35, and LOD scores of 3.56–54.22 [87]. The identification chromosomes with QTLs underlying 1000 grain weight (GW) has conducted by Varshney et al. [88]. They found the SSR marker Xwmc333 as being linked to GW and the major QTL for GW (*QGw1.ccsu-1A*) with a $R^2$ value of 0.1509. Moreover, the SSR markers of *Xgwm210, Xgwm296*, and *Xgwm455*, were detected to be polymorphic and linked to the leaf rust resistance gene *Lr39*, of which *Xgwm210* was the closest marker mapped 10.7 cm from *Lr39* [89]. Ammiraju et al. [90] found four intersimple sequence repeat (ISSR) markers, *UBC8181000, UBC842600, UBC8431200*, and *UBC814750*, controlling seed size in wheat, which can be measured indirectly by 1000-kernel weight (TKW). These markers showed a signification association with gene effects of 84.662.92, and 2.61%, contributing a total of 31% of the phenotypic variance in seed size. The following year, Zhou et al. [91] identified six SSR markers linked to the major QTL for scab resistance, which were *Xgwm389, Xgwm533, Xgwm493, Xbarc75, Xbarc88*, and

*Xbarc147* spanning a region of approximately 20 cm on chromosome 3BS. Additional SSR markers have been reported for strip rust resistance genes, including the *Xgwm501* marker linked to *Yr5* [92], and the *Xpsp3000* marker linked to *Yr10* [93]. A microsatellite linkage map of the powdery mildew resistance gene *Pm5e* on chromosome 7B has constructed with 20 microsatellite loci, consisting of two codominant markers *Xgwm783* and *Wgwm1267* located close to *Pm5e* with a linkage distance of 11.0 cm and 6.6 cm, respectively [94]. The detection of QTL linked to FHB resistance has found that two microsatellite loci, *Xgwm533* and *Xgwm*, were significantly associated with QTL for FHB [95]. In 2004, a SSR-based consensus map has been completed by Somers et al. [96] that has been widely used a reference. Furthermore, five QTLs, *QYld.crc-1A*, *QYld.crc-2D*, *QYld.crc-3B*, *QYld.crc-5A.1*, and *QYld.crc-5A.2*, controlling grain yield have been identified on chromosomes 1A, 2D, 3B, and 5A [97]. Other QTLs associated with 1000 grain weight, spikes meter$^{-2}$, seed number spike$^{-1}$, average seed weight spike$^{-1}$, harvest index, days to heading, days to maturity, and grain filling time have also been detected in the Superb/BW278 mapping population. Liu et al. [98] performed association mapping by genotyping wheat germplasm accession from China using SSR markers and EST-SSR markers, detecting 10 SSR markers on chromosome 4A associated with plant height, spike length, spikelets per spike, spikelet density, grains per spike and thousand-kernel weight. Even though studies involving RFLP, RAPD, and AFLP markers have only been used for identification and mapping of QTLs and genes, they are less likely to be applied into breeding programs because the application of these markers is likely to be less efficient for MAS [99].

## 3. Second-generation sequencing resources for yield improvement in wheat

Researchers have focused on discovering SNPs to be used as genetic markers, because they have many advantages. SNPs act as codominant, single-locus, biallelic markers offering a lower error rate, and higher accuracy than SSR markers. However, the nature of polyploidy and having similar sequences among the A, B, and D genomes makes it difficult to identify SNPs [100]. Therefore, progress in SNP detection has been limited, especially related to yield and its related traits. With the advancement in next-generation sequencing (NGS), sequencing has become increasingly popular due to the rapid development of NGS technologies, including SOLiD/Ion Torrent PGM from Life Science, Genome Analyzer/HiSeq 2000/MiSeq from Illumina, and 454 FLX Titanium/GS Junior by Roche [101]. These technologies have led to the implementation of high-density SNP genotyping in wheat [99, 102].

An important milestone in wheat genomic research was accomplished in 2012 with the completion of *de novo* sequencing of bread wheat, the variety Chinese Spring (CS42), facilitating advances in genomic research into the genus *Triticum* and providing insights into the polyploidization and domestication of wheat. Brenchley et al. sequenced the wheat genome using Roche 454 pyro-sequencing technology (GS FLX Titanium and GS FLX+ Platforms [11]. To identify the three component genomes (A, B, and D) of hexaploid wheat, the following technologies have been applied: Illumina sequence assemblies of *Triticum monococcum* (related

to the A-genome donor), *Ae. speltoides* complementary DNA assemblies, and 454 sequences from *Ae. tauschii* (the D-genome donor). Sequence analysis revealed that the A, B, and D genomes have estimated to consist of approximately 28,000, 38,000, and 36,000 genes, respectively. As a consequence, they identified the number of genes in wheat to be between 94,000 and 96,000. Like *G. hirsutum* [103] and soybean [104], wheat also has experienced a recent whole genome duplication (WGD) at 0.5 MYA and about 3.5 MYA. Brenchley et al. also examined gene conservation between wheat and its most closely related species *Brachypodium distachyon* and detected a high degree of conservation between the two species [11]. A large set of SNPs (132,000 SNPs) in A, B, and D genes will enhance future studies aimed at identifying QTLs and discovering associations of traits.

Since the completion of the draft sequence of wheat, extensive efforts have put into the identification of various molecular markers influencing yield to increase MAS efficiency. Based on the genotype by sequencing (GBS) approach, the linkage map of wheat comprised of markers including 538 GBS Bin, 258 AFLPs, 175 SSRs, and an EST has been constructed in 2014 [105]. They identified five QTL regions linked to thylakoid membrane damage (TMD), SPAD chlorophyll content (SCC), and plasma membrane damage (PMD), known as indicatives of high temperature tolerance, on chromosomes 6A, 7A, 1B, 2B and 1D and also detected some of the SSR markers associated with these traits such as the SSR marker *Xbarc121* and *Xbarc49* for all three traits and *gwm18* and *Xbarc113* for SCC. More SNP and SSR markers have been investigated using the 9 K Infinium iSelect Beadchips [106]. The SNP distribution between cultivars and landraces has provided impacts on our understanding of crop improvement on the structure of genetic diversity and insight into signatures of selection. Liu et al. reported six SNPs from two genes, *wsnp_CAP11_c209_198467* and *wsnp_JD_c4438_5568170*, showed significant association with soil-borne wheat mosaic virus (SBWMV) resistant which can be used in MAS to improve SBWMV resistance in wheat breeding [107]. Using the high-density Illumina iSelect 90K SNP assay, a linkage map spanning 3609.4 cm was constructed based on 5636 polymorphic SNP markers, with an average length of 171.9 cm per chromosome and marker density of 0.64 cm [108]. Association of agronomic traits with 1,366 SNP markers in durum wheat has been performed by Hu et al. [109]. By genotyping 150 accessions of durum wheat germplasm based on the Illumina Bead Array platform and Golden Gate Assay, a large amount of SNP markers were detected associated with key yield-related traits including plant height, number of effective spikes, length of main spike, number of spikelets per plant, panicle neck length of main spike, grain number per plant, grain weight per plant, and 1000-grain weight. These SNP markers have enhanced the previous QTL analyses and can be utilized for MAS to improve yield in wheat.

## 4. Future directions: translational genomics

The wheat genome is very complex as it is a polyploid species consisted of three diploid progenitor genomes. Recent advances in genome sequencing technologies have accelerated efforts to complete genomes of many crop species, which has opened the door for discovery and knowledge of the genetic basis of a number of important agronomic traits, with the final

| Species | | Arabidopsis | Barley | | Rice | | Wheat | | Maize | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Trait** | | **Locus** | **Locus** | **Identity** | **Locus** | **Identity** | **Locus** | **Identity** | **Locus** | **Identity** |
| Flowering | Early flowering | At1g28380 | KR706151 | 42.43 | XM_015770121 | 36.86 | KX161741.1 | 44.78 | EU241899.1 | 41.78 |
| | | At5g44040 | HM133570 | 43.85 | XM_015777033 | 37.49 | KJ711537 | 43.1 | KP202720.1 | 42.53 |
| | Late flowering | At1g01580 | EU331897.1 | 44.13 | XM_015787003 | 38.04 | KJ711539.1 | 43.16 | | |
| | | At1g04400 | AB476614.1 | 43.88 | XM_015756636 | 36.72 | | | | |
| Growth | Dwarf/small | At5g19530 | AY750996.1 | 42 | AB630963.1 | 43.05 | AY747606.1 | 43.1 | | |
| | | At1g02730 | EU331690.1 | 43.14 | AY747605.1 | 43.1 | KR816810.1 | 42.96 | | |
| | | At4g10180 | KT247893.1 | 42.47 | | | KT750252.1 | 42.82 | | |
| | Growth defective | At4g01690 | | | | | AY244509.2 | 42.97 | | |
| | | At1g02910 | | | | | JF965395.1 | 42.79 | | |
| | | At1g65260 | | | | | | | | |
| Stems | Waxy | At1g09560 | AK366020.1 | 58.91 | FJ487950.1 | 58.98 | KU376264.1 | 58.75 | | |
| | | At1g02205 | AK360068.1 | 58.91 | FJ487949.1 | 58.86 | KU376267.1 | 58.98 | | |
| | Fasciation | At1g64670 | AJ567377.2 | 60.29 | FJ501983.1 | 59.64 | EU981913.1 | 59.82 | | |
| | | | AK354338.1 | 57.95 | EU981914.2 | 60.39 | | | | |
| | | | AK356998.1 | 59.25 | | | | | | |
| | Short petiole | At4g10180 | AK360706.1 | 36.39 | AY585350.1 | 54.03 | M11336.1 | 55.81 | | |
| | | At3g03860 | AK353983.1 | 37.12 | GQ389628.1 | 58.62 | DQ457416.2 | 54.2 | | |
| | Twisted petiole | At4g27060 | | | EF190873.1 | 58.4 | EU189093.1 | 54.23 | | |
| Leaves | Abnormal shape | At3g16830 | | | JX828333.1 | 58.57 | AY831792.1 | 56.52 | | |
| | | At1g61940 | | | | | JX878122.1 | 58.41 | | |

| Species | | Arabidopsis | Barley | Rice | Wheat | Maize |
|---|---|---|---|---|---|---|
| | Rough surface | At5g04660 | | | | |
| | | At5g11060 | | | | |
| | | At1g32640 | | | | |
| Flowers | Carpel | At4g32551 | AK368072.1 | CT831121.1 | AY887064.1 | NM_001112060.1 |
| | | At5g11320 | AK253078.1 | AK100856.1 | BT008997.1 | AY898650.1 |
| | Stamen | At5g48390 | AB085818.1 | | AK334664.1 | DQ343238.1 |
| | | At1g63990 | | | | |
| | | At1g05160 | | | | |
| | Petal | At4g32551 | | | | |
| | | At1g55320 | | | | |
| | Sepal | At2g20860 | | | | |
| Fruits | Short | At2g02000 | EU333863.1 | AP014958.1 | KP749902.1 | FJ573211.1 |
| | | At1g69180 | AK250398.1 | | KP749901.1 | NM_001151022.1 |
| | | At1g68560 | | | | EU968771.1 |
| | Abnormal shape | At4g05200 | | | | |
| | | At5g04660 | | | | |
| | | At1g74720 | | | | |

**Table 1.** Comparative analysis of yield-related genes in Arabidopsis, wheat, barley, and maize.


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aim of crop improvement and production. Moreover, the completion of sequences has enabled assessment of translational genomics which is an effective way for researchers and breeders to transfer knowledge of genetic and genomic information among related species, such as rice and wheat [110]. The translational genomics tool is known as 'model to crop' translation that can be contributed to the implementation of genetic and genomics in crop species [111]. There are three well-characterized model grass species rice [112, 113], *Brachypodium* [114], and barley [115], which can be used to accelerate the application of translational genomics among the Poaceae family. The large amount of insufficient knowledge such as biological processes that are controlled by genes can be filled from well-studied species through translational biology approach [110]. In translational genomics, comparative genomics studies can take advantage from available genomes and can provide information on the extrapolation of knowledge of gene functions among species [110]. Genome sequences are currently available for wheat, but information QTL mapping as compared to other model grass species have been less reported. Comparative analysis based on the candidate gene approach (CGA) is known as the most powerful tool for exchange information among species. We conducted a brief comparative analysis of yield-related genes in Arabidopsis, barley, rice, wheat, and maize. The 37 Arabidopsis genes controlling flowering, growth, stems, leaves, flowers, and fruits, have been used to investigate homologous sequences in the barley, rice, wheat, and maize genomes using BLAST [116]. Using the detected homologs among the grass species, we found a number of homologous sequences with the sequence identity values ranging between 36.39 and 60.39 based on MUSCLE v3.51 [117] (**Table 1**).

The release of genomic sequence of wheat [11], barley [115], and maize [118], provides a new opportunity for translational genomics. Since comparative genomics focuses on comparing genomes among plant species looking for similarities and differences of DNA sequence, protein sequence, and gene orders, information from well-studied and analyzed species can be applied for less studied crops to improve a specific target trait, which can be implemented in crop breeding and improvement. For example, genome-wide comparative analysis of flowering-related genes in Arabidopsis, wheat, and barley has revealed that there are 900 and 275 putative orthologs in wheat and barley, respectively [119]. In addition, they showed many orthologous genes having similar expression profiles in different tissues of wheat and barley based on their *in silico* expression analyses. Such a work will help researchers to investigate candidate genes controlling the time of flowering in rice and barley which can be incorporated into molecular breeding for early flowering in wheat and barley in short-season cropping region [119].

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Chapter 3

# Association Mapping of Root Traits for Drought Tolerance in Bread Wheat

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Additional information is available at the end of the chapter

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**Abstract**

Bread wheat (*Triticum aestivum* L., 2n = 6x = 42, AABBDD) is one of the most important crops, making staple food for more than 40 countries and over 35% of the global population. Drought stress is among the major constraints to wheat production as it affects plant growth, gene expression and yield potential of the crop. Development of elite wheat cultivars with the ability to grow and reproduce in water-limited soils seems to be the most enduring solution of addressing drought stress. A total of 100 lines including well-adapted wheat cultivars were evaluated for important root traits and complemented with 102 PCR-based markers aiming to understand their genetic structure and to identify molecular markers that are closely associated to quantitative trait loci (QTLs) of important root traits. Alleles per locus are counted and polymorphic information content (PIC) values are calculated. Population structure of these lines was analyzed with general linear model (GLM) and mixed linear model (MLM) approaches for identification of QTLs associated with important root traits. The results indicated the presence of two novel QTLs on the homoeologous group 2 and group 5 of wheat that may be related to drought stress resistance. Our results may facilitate the development of agronomically desirable drought stress-resistant wheat germplasm.

**Keywords:** bread wheat, genetic variation, drought tolerance, association mapping, QTL

## 1. Introduction

Wheat (*Triticum* spp.) is one of the most important and widely cultivated crops with the annual yield of 694 million metric tons. More than 40 countries and over 35% of the world population use wheat as their staple food [1, 2]. Wheat is cultivated on larger area than other

cereals and modified to different climatic conditions [3, 4]. Bread wheat (2n = 6x = 42) and durum wheat (4x = 28) are the two common cultivated species around the world. Bread wheat supplies about 95% wheat globally, while durum and other wheats (emmer (4x = 28), einkorn (2x = 14) and spelt (6x = 42)) provide only 5% of the world wheat [2, 5]. Human population is increasing rapidly and is estimated to reach 9.4 billion by 2050. Therefore, food production will require a greater yield from the present cropland without horizontal expansion [6]. Population growth, environmental pollution and utilization of croplands for other purposes may reduce the croplands by 10–20% [7]. To meet the growing demand of global food shortage of 2050, total food production must increase by 50% at least to meet out demands of 2050. Among the crop plants, wheat is an economic and rich source of energy and proteins and supplies one fifth of all human calories for the world population [8]. Plant breeders are always trying to find wheat germplasm having desirable traits such as tolerance to diseases and other abiotic stresses [7, 9]. There is no doubt that cereals such as wheat, rice and maize are the world's leading food crops for all humans and are the principal resources that have led to the emergence of human civilization.

## 2. Global wheat production

Wheat is one of the most important cereal and staple food crop around the world. It ranks first due to its area and production and contributes more calories to the world's human diet than any other crop. On the other hand, wheat also maintains its first rank among major cereals due to its higher protein and gluten contents [8–10]. In 1986–1987, the wheat production across the world, which was 521 million metric tons, was increased to approximately 572 million metric tons in 2005–2006 from an area of 220 million hectares [11] and 694 million metric tons in 2011–2012. In 2011, the European Union (137 million tons) was top ranking in wheat production countries followed by China (118 million tons) and the United States of America (54 million tons). Further, Canada, Australia, India, Pakistan and Argentina contribute about 79% of the total wheat production. The world trade market was very feasible for wheat in 2011, and 129 million tons of wheat was traded in the world market [12].

## 3. Drought stress

Drought is defined as water deficiency in the root zone of crops that result to decrease in yield during the plant life cycle [13]. The capability of a plant to grow and reproduce in water-limited area is referred to as drought tolerance. Drought stress is changeable in its intensity, length and effectiveness, and crop plants are required not only be able to survive, but their ability to produce a harvestable yield under drought stress is of practical importance [14]. Drought tolerance is a quantitative trait, influenced by complex phenotype and genetic interactions. Understanding the genetic basis of drought tolerance in crop plants is a prerequisite for developing superior genotypes. High temperatures, radiation, water and nutrient deficiencies are commonly encountered under normal growing conditions also pose somewhat similar challenges. Further, certain soil properties such as composition and structure can also affect the balance of these different stresses; see, *for example*, [15, 16].

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Drought is the main environmental problem that causes high negative effect on cereal crops particularly wheat. During drought conditions, plants show a wide range of behaviors varying from great sensitivity to high tolerance [17]. Seasonal cyclic drought has great involvement in reduction of wheat, barley and other cereal yields [18]. Drought stress greatly affects plant growth, gene expression, distribution, yield and quality of crop in arid and semiarid areas around the world [19]. About 60% of crop production around the world is from arid and semiarid regions. The rate of rainfall critically fluctuates in these areas. In developing countries 37% of wheat is commonly grown in drought susceptible areas [20]. The major constraint to wheat production around the world is inadequate supply of water. Within the United States of America alone, about 67% of crop losses over the last 50 years have been due to drought. The 2012 drought in the United States of America was the worst in the last 60 years, and more frequent occurrences of water shortages are expected due to climate projections and increasing competition for water among urban, industrial and agricultural demand.

The plants' reaction to drought stress depends on plant growth (development), stress period and plant genetics [21, 22]. Drought can also influence morphophysiological features of plant such as growth, anatomy, morphology, physiology (stomatal closure, low photosynthesis, transpiration rate), biochemistry and ultimately productivity [23, 24]. Yield is the basic criteria for cultivation of crop varieties under drought conditions. Therefore, it is a great challenge for crop breeders to produce cultivars having good potential of survival in stressed (drought, salinity, cool) environment [14, 15, 25]. Breeding for drought tolerance is further complicated by the fact that several types of abiotic stress can challenge crop plants simultaneously. Further, given the complexity of drought tolerance, marker-assisted selection has not contributed significantly to cultivar improvement, and breeding for dry environments has relied on direct phenotypic selection. However, recent technological advances and the great potential in wheat to ensure sustainable food production have driven research programmes to improve this crop genetically despite the size and complexity of the genome. Nonetheless, drought tolerance breeding may be effective if the marker-assisted selection-based molecular linkage maps for crop species are available [15, 26].

### 3.1. Drought stress in Pakistan

Diverse climatic and soil conditions are available for wheat growing in Pakistan. About one third of the total land area comes under rain-fed regions where rainfall is unusual [27]. Drought and salinity are very common around the world and are among the most serious problems to the agriculture in Pakistan [28]. Arid and semiarid regions of the world are badly affected by water stress, and as result crop production is reduced. Irrigated areas sometimes face drought conditions due to inadequate supply of water and canal closures [23, 29]. Drought-tolerant varieties are those, where grain yield is least affected by drought stress, or drought-tolerant crops are those that take up maximum amount of water and lose minimum of water during dry conditions [1–5, 30]. To ensure high crop production in rain-fed areas, different aspects of agriculture like holding precipitation, reducing evapotranspiration and sowing of drought tolerant varieties are important. Wheat varieties cultivated in rain-fed areas of Pakistan are usually low yielding as well as pests and diseases that are susceptible but are well adapted and flourish in dry conditions. Still, the need to increase yield to meet the demands of growing population to ensure food security requires well-integrated efforts. Although global water scarcity may be an abstract concept to many and a reality for others.

But with no confusion, it is the result of myriad environmental, political, economic and social issues. The current global climatic conditions are to hit Pakistan, and therefore, the search for diverse and drought-tolerant sources of crop plants is of paramount significance to feed its ever-growing population. Marker-assisted selection is a cry of the day for yield improvement in drought stress areas of Pakistan. Thus, the use of molecular markers for tagging of drought resistance genes is needed [14, 15, 31].

## 4. Materials and methods

During the current study, 100 wheat lines (**Table 1**) including well-adapted wheat cultivars were evaluated for important root traits. A total of 102 PCR-based markers were applied aiming to understand their genetic structure and to identify molecular markers that are closely associated to quantitative trait loci (QTLs) of important root traits (**Table 2**). Plant germination, DNA extraction and PCR profiling followed previously published standard procedures [32]. Further, population structure of these lines was analyzed with general linear model (GLM) and mixed linear model (MLM) approaches using TASSEL software with their default setting for identification of QTLs associated with important root traits.

| S. no | Genotypes | S. no | Genotypes |
|---|---|---|---|
| 1 | Sonalika | 2 | Shalimar 88 |
| 3 | Merco 2007 | 4 | Khyber 83 |
| 5 | Manther | 6 | Chenab 70 |
| 7 | Lr-230 | 8 | Soghat 90 |
| 9 | Ksk | 10 | Pari -73 |
| 11 | Maxi pak | 12 | Chakwal 86 |
| 13 | Indus 79 | 14 | Wadanak 98 |
| 15 | Bakhtawar 94 | 16 | Nori -70 |
| 17 | Wadanak 85 | 18 | ZA-77 |
| 19 | Abdaghar 97 | 20 | Kaghan 93 |
| 21 | Margalla 99 | 22 | Dawar 96 |
| 23 | Uqab 2000 | 24 | Suliman 96 |
| 25 | Raskoh | 26 | AS-2002 |
| 27 | Haider 2002 | 28 | LYP-73 |
| 29 | Local white | 30 | Noshera 96 |
| 31 | MH-97 | 32 | Sindh 81 |
| 33 | Zarlashta 90 | 34 | Fakhri sarhad |
| 35 | Punjab-76 | 36 | 10737 |

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| S. no | Genotypes | S. no | Genotypes |
|---|---|---|---|
| 37 | Faisalabad 85 | 38 | 10776 |
| 39 | Barani 70 | 40 | 10748 |
| 41 | Rawal 87 | 42 | 10724 |
| 43 | NIAB 83 | 44 | 10792 |
| 45 | GA 2002 | 46 | Pirsabak 2008 |
| 47 | Chenab 79 | 48 | Punjab-96 |
| 49 | Saleem 2000 | 50 | Mumal-2002 |
| 51 | Zamindar-80 | 52 | SA-42 |
| 53 | Iqbal-2000 | 54 | Marwat-01 |
| 55 | SH-2003 | 56 | Barani-83 |
| 57 | Anmol-91 | 58 | Potohar-93 |
| 59 | LU-26 | 60 | Kohinoor-83 |
| 61 | Chenab-96 | 62 | Potohar-70 |
| 63 | Faisalabad-83 | 64 | Pak-81 |
| 65 | Zarghoon-79 | 66 | Pirsabak-85 |
| 67 | C-228 | 68 | C-273 |
| 69 | Shahkar-95 | 70 | Tandojam-83 |
| 71 | Punjab-88 | 72 | Dirk |
| 73 | 10793 | 74 | Bahalwapur-79 |
| 75 | Punjab-81 | 76 | Lasani-08 |
| 77 | C-591 | 78 | Sussi |
| 79 | Sutlag-86 | 80 | Khyber-79 |
| 81 | C-250 | 82 | FPD-08 |
| 83 | Blue silver | 84 | Sandal |
| 85 | RWP-94 | 86 | Kiran |
| 87 | Sariab-92 | 88 | Wardak-85 |
| 89 | Wafaq-2008 | 90 | Meraj-08 |
| 91 | 10742 | 92 | C-518 |
| 93 | 010724-YR | 94 | potohar-90 |
| 95 | AUP 5000 | 96 | Mehran-89 |
| 97 | WL-711 | 98 | Janbaz |
| 99 | SA-75 | 100 | AUP-4008 |

**Table 1.** List of wheat lines and cultivars used in the current study.

| Marker | Marker | Marker | Marker | Marker | Marker |
|---|---|---|---|---|---|
| *Cfd 15* | *Xbarc 154* | *Xwmc 232* | *Xgwm 372* | *Xwmc 177* | *Xgwm 293* |
| *Cfd 18* | *Xbarc 158* | *Xwmc 233* | *Xgwm 389* | *Xwmc 181* | *Xgwm 299* |
| *Xwmc 24* | *Xbarc 159* | *Xwmc 235* | *Xgwm 443* | *Xwmc 182* | *Xgwm 302* |
| *Xwmc 25* | *Xbarc 163* | *Xwmc 398* | *Xgwm 471* | *Xwmc 216* | *Xgwm 325* |
| *Xwmc 27* | *Xbarc 164* | *Xwmc 420* | *Xgwm 469* | *Xwmc 219* | *Xgwm 359* |
| *Xwmc 43* | *Xbarc 165* | *Xwmc 606* | *Xgwm 484* | | |
| *Xwmc 51* | *Xbarc 167* | *Xwmc 718* | *Xgwm 544* | | |
| *Xwmc 52* | *Xbarc 172* | *Xwmc 749* | *Xgwm 608* | | |
| *Xwmc 94* | *Xbarc 173* | *Xwmc 798* | *Xgwm 609* | | |
| *Xwmc 97* | *Xbarc 175* | *Xbarc 42* | *Xgwm 642* | | |
| *Xwmc 104* | *Xbarc 264* | *Xbarc 45* | *xgwm 908* | | |
| *Xwmc 147* | *Xgwm 4* | *Xbarc 76* | *Xgdm 3* | | |
| *Xwmc 149* | *Xgwm 10* | *Xbarc 101* | *Xgdm 5* | | |
| *Xwmc 153* | *Xgwm 33* | *Xbarc 127* | *Xgdm 6* | | |
| *Xwmc 154* | *Xgwm 37* | *Xbarc 128* | *Xgdm 19* | | |
| *Xwmc 157* | *Xgwm 55* | *Xbarc 134* | *Xgdm 28* | | |
| *Xwmc 161* | *Xgwm 60* | *Xbarc 137* | *Xgdm 33* | | |
| *Xwmc 163* | *Xgwm 71* | *Xbarc 140* | *Xgdm 46* | | |
| *Xwmc 166* | *Xgwm 99* | *Xbarc 141* | *Xgdm 114* | | |
| *Xwmc 167* | *Xgwm 111* | *Xbarc 144* | *VRN AF* | | |
| *Xwmc 168* | *Xgwm 136* | *Xbarc 147* | *VRN B1 R3* | | |
| *Xwmc 169* | *Xgwm 194* | *Xbarc 148* | *PpD1 R1* | | |
| *Xwmc 175* | *Xgwm 261* | *Xbarc 149* | *PpD 1 R2* | | |

**Table 2.** List of PCR primers/molecular markers used in the current study.

## 5. Root trait analysis and its significance to drought

To understand the performance of wheat crop under drought conditions, it is necessary to have a sound knowledge about root traits. Root traits vary from species to species on the base of water availability, growth, physiology and architecture [33]. Root surface area and root length in wheat crop play an important role in water uptake. A well-organized root system is necessary for efficient water uptake. In crops, fibrous root system consists of two types as seminal and nodal roots [34]. Well-developed root system could play positive role in water deficit (drought) areas. Root morphological traits greatly affect water and nutrient uptake. Herbaceous plants with fine roots, smaller diameter and greater root length are better adapted to dry conditions [35]. Root traits greatly influence the resource uptake and sustaining crop yield under drought stress conditions. For maximum grain yield in wheat, active and well-developed root system is necessary [36, 37].

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## 6. Association mapping between root traits and SSR markers

In the present study, association mapping was applied for identification of association between root traits and SSR markers. Marker-trait association (MTA) based on polymorphism found in SSR markers applied on diverse wheat genotypes. Two different models were used for identification of QTLs associated with root traits as GLM (general linear model) and MLM (mixed linear model). GLM requires no kinship, and only Q matrix was used to determine association between markers and mean of phenotypic traits. The level of significance of P value was measured at $p \leq 0.01$ in both GLM and MLM models. The QTLs having LOD values above 2.5 were considered for both GLM and MLM.

A sum of 102 molecular markers were used in the present study. Most of the markers showed high level of polymorphism. A total of 271 polymorphic alleles were generated. The alleles per locus ranged from 1 to 3 and an average of 2.63 per locus. Polymorphic information content (PIC) values of the markers were also calculated in the range of 0.03–0.59. Initially, in order to investigate the genetic diversity of the material, 100 wheat genotypes were grouped into different cluster populations (**Figure 1**). Population structure may lead to spurious association between marker and traits [38]. Therefore, a model-based approach was used for association mapping. Both the general linear model (GLM) and mixed linear model (MLM) were applied. The association analysis also concluded that hundreds of genotypes having different genetic backgrounds were classified into 13 distinct groups, viz., G1, G2, G3, G4, G5, G6, G7, G8, G9, G10, G11, G12 and G13.

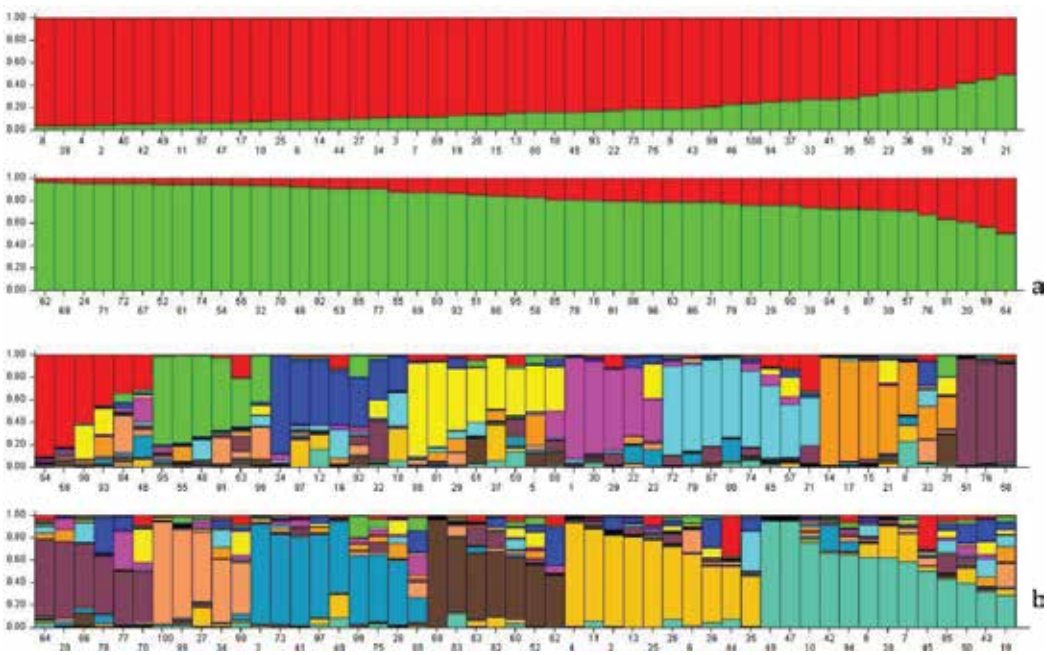


**Figure 1.** Population structure analysis of wheat genotypes based on SSR markers. (a) Graphical bar plot at k = 2 presenting two subgroups (G1 and G2). (b) Graphical bar plot at k = 13 presenting 13 subgroups (G1–G13). The X-axis shows accession numbers, and Y-axis shows subgroup membership.

### 6.1. Total root length

Total root length per unit ground area (La) is often considered to be directly related to the amount and rate of water uptake. Total root length (TRL) is associated with drought tolerance in wheat because it marks the spreading of roots in the soil and affects the resource uptake [39]. The genotype Pirsabak-85 ranked high on the base of TRL and R:S and considered to be the best for drought tolerance by extracting water stored in the deep soil layers. Further, in GLM model the SSR marker *Xgdm 5* on chromosome 2 was significantly associated with total root length, but no association of marker with TRL was found in MLM. The phenotypic variance (r2) was 0.10. The p value was recorded as 0.0016, and LOD is 2.78 as shown in **Figure 2**. The present research revealed that GLM model confirmed MTA for TRL was found to be located on chromosome 2D and the results are in accordance with previous results where MTA for TRL was reported on chromosome 2 at 3.4 cM [40].

For root fresh weight, the GLM model identified MTA associated with RFW, located on chromosome 5B. The marker *Xwmc 235* attributed to trace the QTL on specific chromosome for RFW. The phenotypic variance (r2) was found as 0.10, and LOD was 3.56 as shown in **Figure 3**. The previous report of Ayman, A.D., M. A.M. Atia, H.A. H. Ebtissam, A.H. Hashem and S.A. Sami. 2013. A multidisciplinary approach for dissecting QTL controlling high yield and drought tolerance-related traits in durum wheat. *Int. J. Agri. Sci. Res*.3: 99-116 confirmed that four QTLs are associated with RFW located on 2B, 5B, 6A and 6B chromosomes. Our results which did not localize other QTLs due to lesser number of markers have been used. Similarly, for root dry weight, the *PpD1* marker revealed marker trait association (MTA) for RDW in GLM model only. The MTA was found to be located on chromosome 2A having r2 0.41 and LOD of 2.7 as in **Figure 4**. These results were partly in agreement with results of [41] where the authors found that 5 QTLs for RDW were grouped in chromosomes 2A and 7A.

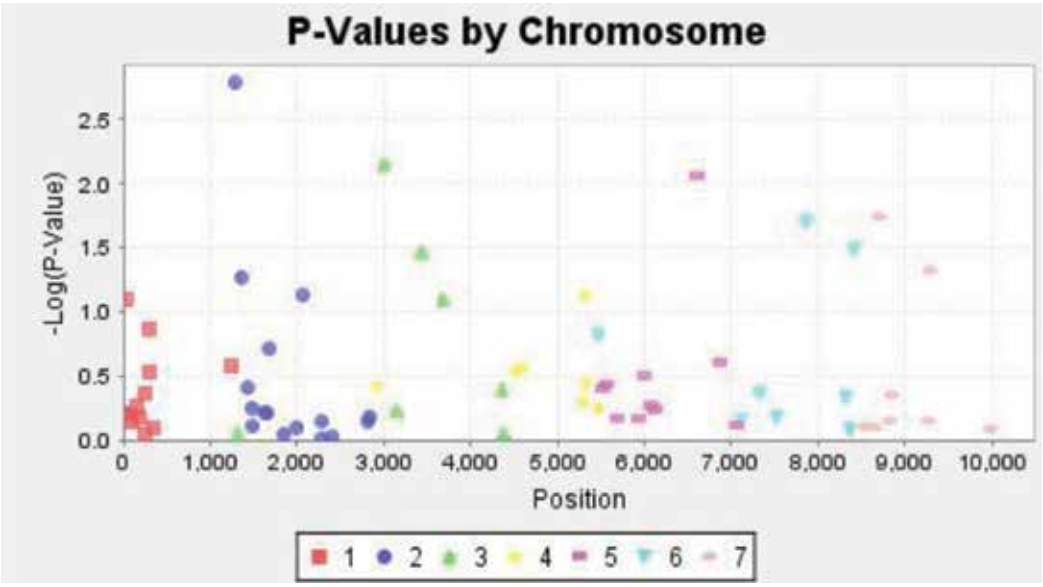


**Figure 2.** QTL identified for TRL on the basis of LOD in GLM.

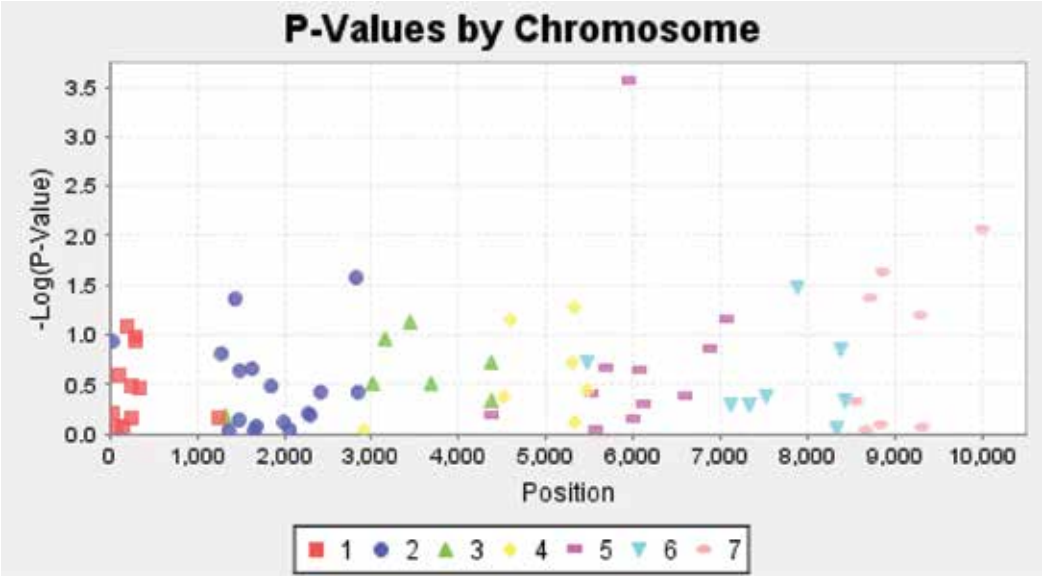


**Figure 3.** QTL identified for RFW on the basis of LOD in GLM.

### 6.2. Maximum root length

The maximum root length (MRL) evolved to capture deeper water from the soil under drought stress [42]. The Abdaghar-97 genotype recorded the maximum root length (MRL) to capture deep soil moisture in dry areas. Two MTAs were identified for MRL located on chromosomes 2A and 5B. MTA of chromosome 2A was marked by *Xgwm 10* having LOD (2.68) and that of 5B was

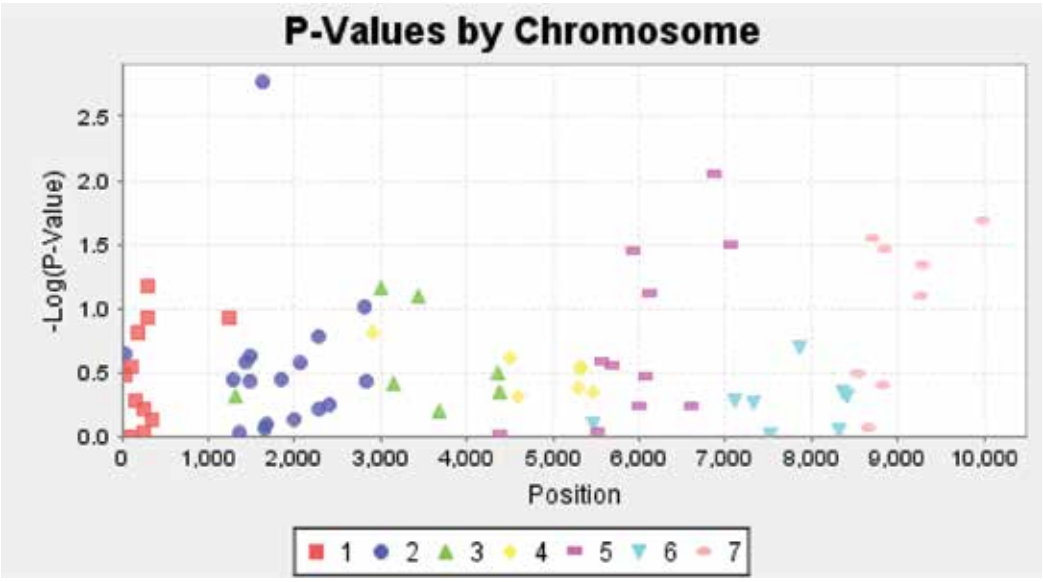


**Figure 4.** QTL identified for RDW on the basis of LOD in GLM.

attributed by *Xwmc 149* having LOD of 2.86 as in **Figure 5a** and **b**. So far only one QTL for maximum root length located on chromosome 4B has been reported [14]. Similarly, QTL was identified for MRL located on chromosome 5 at 158.5 cM [43]. Therefore, the MTA identified on 2A chromosome in the present study was not reported before and considered to be novel QTL for MRL.

### 6.3. Number of nodal roots

The bulk of roots would increase with the increase in number of tillers. Nitrogen uptake is affected by length and number of nodal roots [44]. The uptake of nutrients is 2–6 times

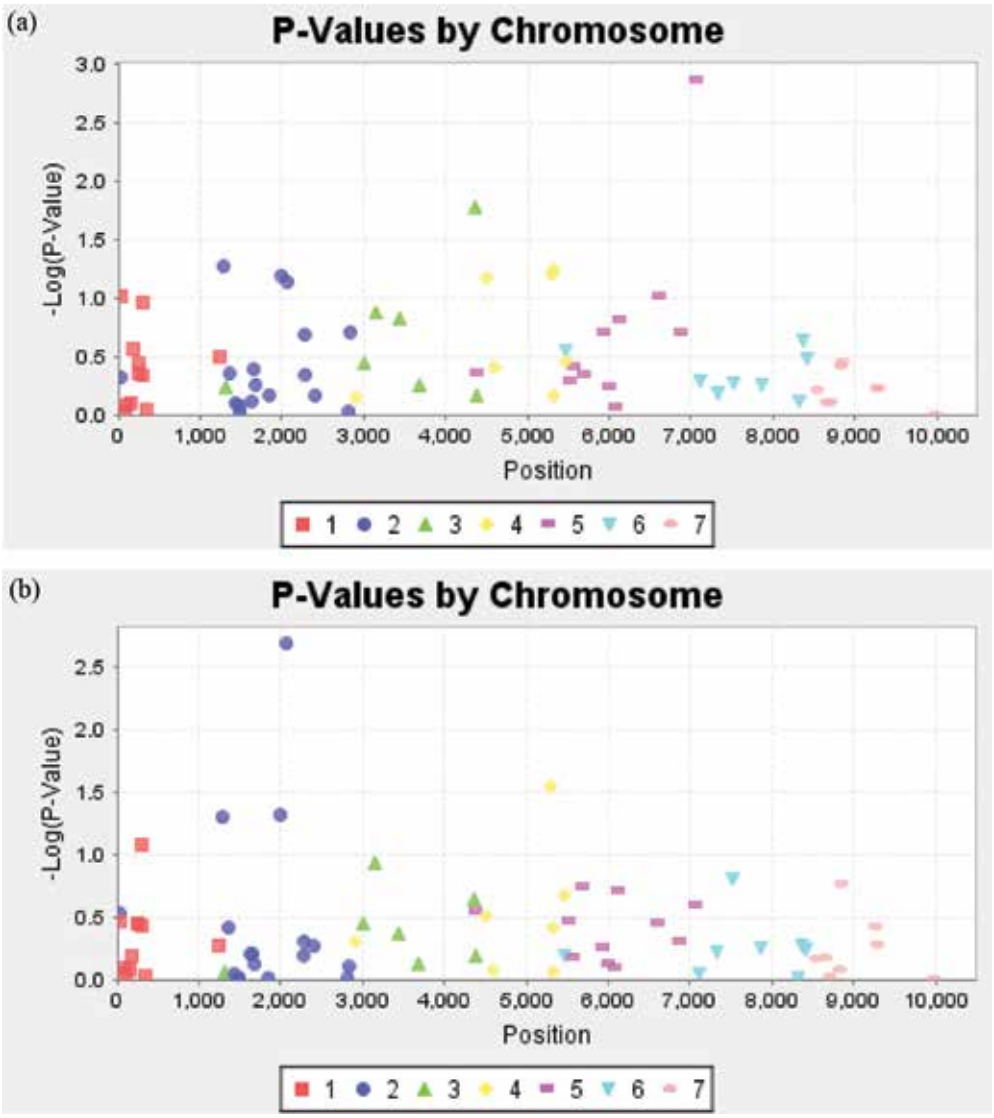


**Figure 5.** QTL identified for MRL on the basis of (a) GLM; (b) LOD in MLM.

more for nodal roots than seminal roots, and thus growing such genotypes in rain-fed areas would be desirable [45]. The results of the present study found Meraj-08 with high number of nodal roots and would be better for nitrogen and water uptake in rain-fed areas. As for as the number of nodal roots MTAs was concerned, the MTA for number of nodal roots located on chromosome 2B. SSR marker *Xwmc 175* recognized the MTA for NNR on chromosome 2B as shown in **Figure 6**. MTA for NNR was found at LOD 2.5, p value 0.00306, while the (r2) 0.17. Our results were accordance with result of [46] where the same QTL is reported on chromosome 2B. Two MTAs (QTLs) were found associated with root angle in GLM model. The MTAs were found to be located on chromosomes 7B and 6D. The MTA located on chromosome 7B recognized by *Xgwm 302* and that of 6D was identified by *Xwmc 749* as in **Figure 7**. The results are consistent with previous results where QTL for RA was located on chromosome 7B at 86cM, and reported four QTLs for RA was located on chromosome 2A, 3D, 6A and 6D [47, 48].

### 6.4. Root density

Root density (RDT) increases the efficiency of the root system and is considered to be the most important trait for uptake of phosphorus in wheat [42]. The genotype Soghat-90 ranked first on the base of RDT and is considered to be good for phosphorus uptake. Further, root density has been reported to be positively correlated with total root length, root diameter and water use efficiency [49]. Two MTAs were identified for root density (RDT) in both GLM and MLM models located on chromosomes 2B and 5B. The MTA for chromosome 2B was attributed by *Xwmc 175* and 5B by *Xwmc 235* having LOD of 3.28 and 2.5 as in

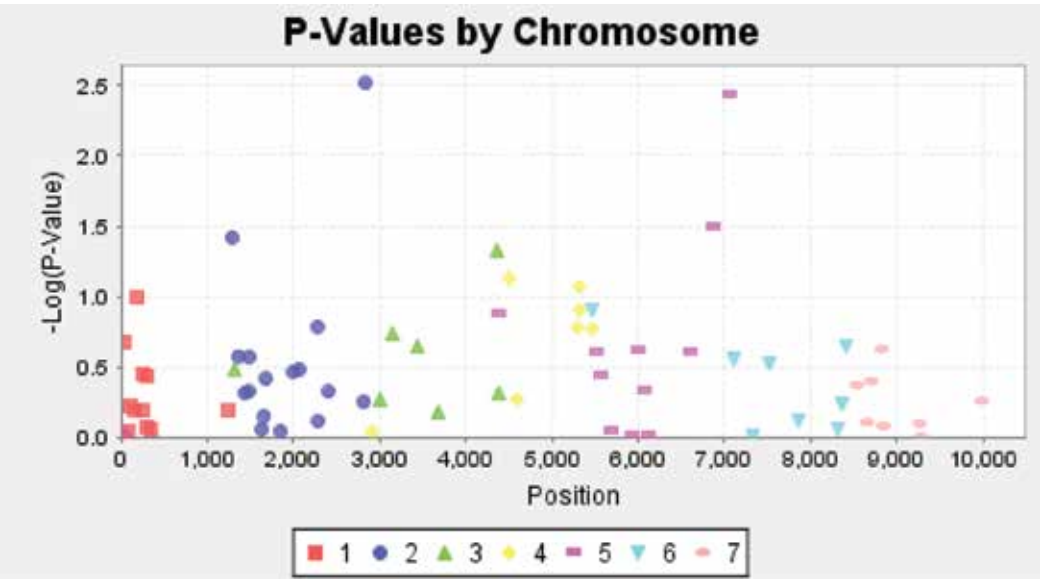


**Figure 6.** QTL identified for NNR on the basis of LOD in GLM.

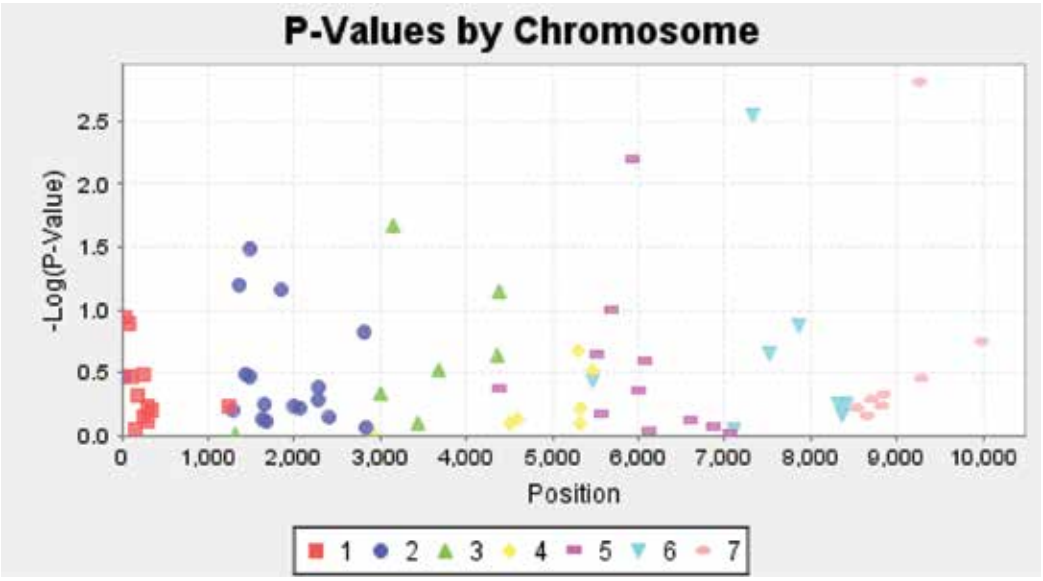


**Figure 7.** QTL identified for RA on the basis of LOD in GLM.

**Figure 8a** and **b**. The results of the present study are in accordance with the earlier reports. Previously QTL for RDT has been reported on chromosome 2B at 158.5 cM and 5B at 47 cM [46, 50]. The number of seminal roots may result in better adaptation to drought conditions in wheat. Further, the number of seminal roots was negatively correlated with water use efficiency [51, 52]. The strong root system will reduce the WUE and hence will reduce biomass production. Therefore, it is needed to improve the root system function rather than a strong root growth for wheat survival in drought conditions. In the present study, the genotype Marwat-01 is recorded the highest NSR and is suggested to be good in more water uptake in rain-fed areas.

### 6.5. Root diameter

The high root diameter (RD) is associated with drought tolerance in wheat. The genotypes showing the highest RD are supported for drought stress tolerance due to large xylem vessels with increased resource uptake and are well organized in searching deep soil layers to extract water [53]. Further, total root length, maximum root length and root density increase or decrease extremely with a small change in root diameter and decrease in root diameter would increase crop yield under drought. Significant reduction in root diameter, total root length and root density under drought conditions has been previously documented [37, 54]. Two MTAs were identified for RD, each in GLM and MLM. Both MTAs were located in chromosome 5B, attributed by *Xwmc 233* having LOD 3.1 and 3.3 as in **Figure 9**. Our results were consistent with earlier reports, where QTLs for RD on chromosome 5B at 4.5 cM have been mentioned [55].

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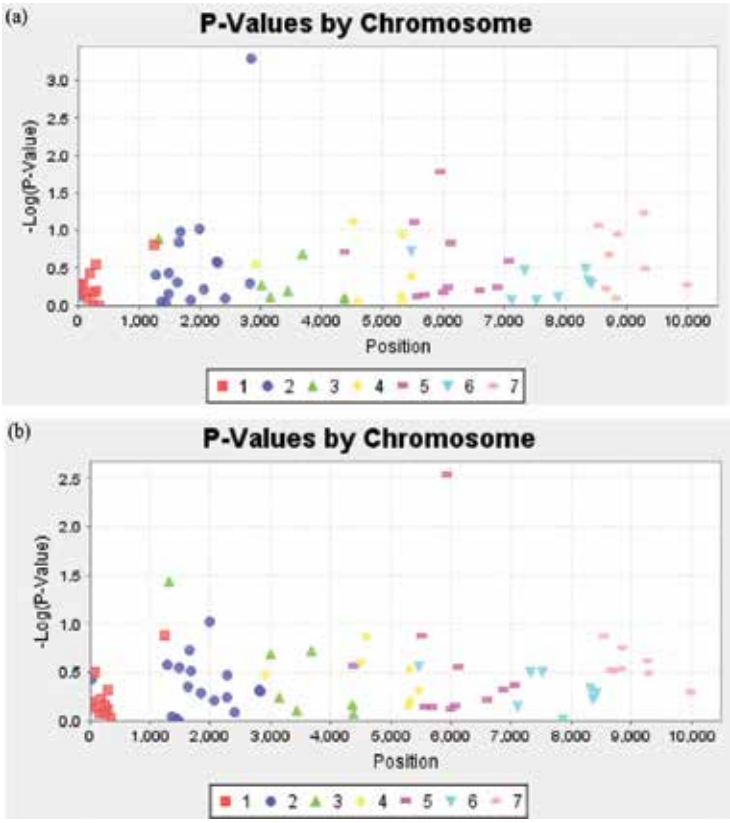


**Figure 8.** QTL identified for RDT on the basis of LOD in (a) GLM; (b) MLM.

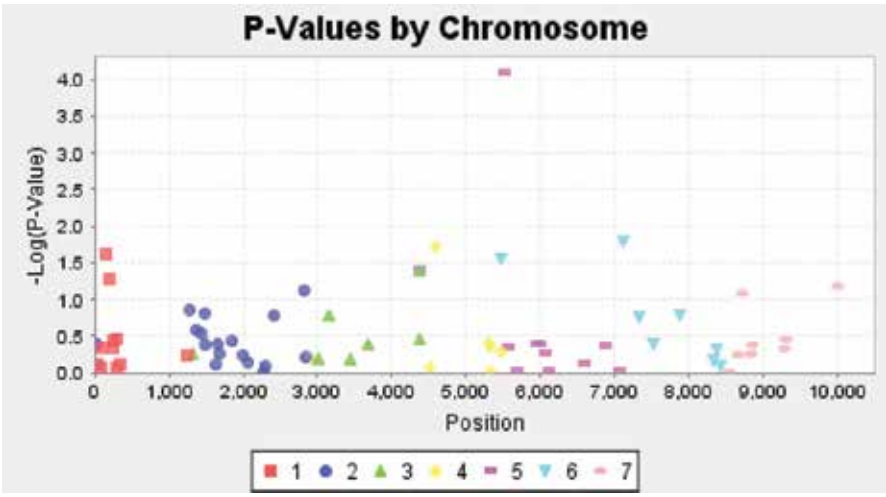


**Figure 9.** QTL identified for RD on the basis of LOD in GLM.

## 7. Conclusion

Among the abiotic stresses that limit wheat crop productivity, drought stress alone is by all means one of the most devastating factors. In the past, breeding efforts to improve drought tolerance response have been hindered primarily by its quantitative nature as well as our poor understanding of the physiological basis of yield in water-deficient conditions [16]. So far, most QTLs for drought tolerance in wheat have been identified through yield and yield component measurements under water-limited conditions. No doubt, yield is the most desirable trait to breeders; still, it is very difficult to relate water use efficiency and identify potential target regions for positional cloning [15]. Only few studies have associated QTLs with specific components of drought response. Although the development of gene-based molecular markers and genome sequencing should accelerate positional cloning, the genomic regions associated with individual QTL are still very large and are usually unsuitable for breeding programme [51–55]. From an application point of view, it is imperative to select genotypes that are able to optimize water use efficiency while maximizing yield in response to drought. Improving the competence of root systems to extract water from the soil is highly desirable, and any extra water extracted during grain filling definitely remarkably increases the yield in wheat. Thus, identification of markers or genes associated with root growth and architecture would be particularly useful for breeding programmes to improve root traits by molecular marker-assisted selection.

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Chapter 4

# Potential of Wide Crosses to Improve the Resistance to Vomitoxin Accumulation in Wheat Following Infection by Fusarium Head Blight

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Additional information is available at the end of the chapter

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**Abstract**

Deoxynivalenol (DON) levels were determined in landraces of rye from Brazil, in a collection of triticales and a series of triticale amphiploids. Two of three rye landraces showed a resistant reaction to DON. Seven triticale accessions of the 371 score showed lower levels of incidence, severity and DON content. A total of eight Tritordeum (*Triticum durum* × *Hordeum chilense amphiploids*) were scored and showed lower DON levels. Stable lines with lower Fusarium head blight (FHB) and DON levels were selected in progenies from crosses of wheat to preselected accessions of *Triticum monococcum and Aegilops speltoides*. Both selections compared favourably to the check cultivars in term of agronomic traits indicating minimal linkage drag. One stable resistant line with lower DON levels was isolated in the F7 generation of progenies from crosses to *Tritium timopheevii*. Lower DON levels were observed in field trials of advanced generation progeny from crosses of wheat to *Aegilops cylindrica* and *Triticum miguschovae*. The findings indicate that the alien species accessions or segregating populations from the inter-specific or inter-generic hybridization can provide material with variability for DON content.

**Keywords:** deoxynivalenol (DON), interspecific/intergeneric, hybrids, segregating, populations

## 1. Introduction

Fusarium head blight (FHB) is a ubiquitous fungal disease of wheat, barley, oats, rye and ear rot of maize. Deoxynivalenol (DON) is a secondary metabolite of Fusarium head blight. DON content renders the harvested grain unsuitable for food or feed. It can cause malfunction of respiratory, immune and even reproduction systems. It has been estimated that for

each 1 ppm increase in DON content in harvested grain, feed consumption in swine decreases by 7.5% [1, 2]. Additional costs are incurred in lowering the DON level of threshed grain. In North America, the US-FDA has set tolerance limits for DON of 1 ppm in processed grain [1, 2], whereas Health Canada has set regulations of 2 ppm in uncleaned soft wheat for use in non-staple foods and 1 ppm in uncleaned soft wheat for use in baby foods [3, 4].

In an epidemic year such as 1966 in Southwest Ontario in Canada, samples of winter wheat taken directly from farmer's combine showed a range in DON content of 1.1–13.9 ppm [5]. These findings point to the fact that genetic resistance must be put into the wheat crop to reduce the DON content.

In terms of breeding for resistance to FHB, earlier efforts were focussed on accumulating genes that reduced the symptoms of Type I and Type II resistance. Bai [5] was among the first to consider the inheritance of two other traits, Fusarium damaged kernels (FDK) and DON content. These two factors are receiving additional attention lately.

The correlation between FDK and DON is in the order of 0.81 [6] but they are much lower for incidence/severity and DON content, indicating that FDK and DON deserve additional attention as measures of FHB resistance.

Somers et al. [7, 33] were the first to suggest that DON accumulation was controlled by independent quantitative trait loci (QTL). These QTL were located on chromosome 5A, on 2D (coincident with a plant height QTL) and on chromosome 3BS (coincident with a QTL for Type I resistance). In addition, a number of minor QTL that were not specifically mapped were revealed in that study and shown in **Figure 1** of that publication.

**Figure 1.** Resistance to Fusarium head blight in an accession of *Triticum monococcum*. Disease symptoms developed at 21 days after artificial inoculation.

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This was followed by similar reports [8, 9, 10]. In the latter study, a major QTL for DON content was mapped on chromosome 2AS that was independent of FHB severity. The cultivar CJ9306 was the source of several QTL for resistance to DON accumulation [11]. Two new QTLs were reported, in that study QFhs.nau-2DL and QFhs.nau-1AS, whereas two others, QFhs.ndsu-3BS and QFhs.nau-SAS, were validated in that study.

This overview will discuss the variability for DON content in alien relatives of wheat and in progenies obtained from wide crosses with wheat.

## 2. Materials and methods

At the start of the project, large numbers of accession of alien wheat relatives were acquired from numerous gene banks. In addition, cytogenetic stocks and inter-specific and inter-generic hybrids were screened for resistance. Some of the numbers of accessions acquired for screening included 200 accessions of *Triticum monococcum* and 370 accessions of triticales plus lower numbers of other species and hybrids.

The initial screening invariably consisted of inoculation with point inoculation in greenhouses or corn spawn in field plots, followed by scoring of symptoms. In the initial screen, the obvious susceptible lines were discarded. Screening on promising lines was repeated. Evaluation of DON content on ground seeds harvested from inoculated plots was carried out on lines that showed minimal scab symptoms.

The lines showing lowest scab symptoms and lower DON content were then crossed to wheat. In most cases, this involved the application of growth hormones following pollination, then rescuing of hybrid embryos and culturing on artificial media. In most cases, backcrossing to a recurrent parent was necessary to restore full fertility. Screening of progenies from wide crosses was carried out by selecting resistant segregates with minimal symptoms following inoculation. DON contents were determined on lines with minimal symptoms. In some cases, DON contents were determined directly on alien species or cytogenetic stocks following inoculation.

For point inoculation—Type I resistance—plants were grown in controlled environments at day/night temperatures of 20/15°C and 16 hours photoperiods supplied by a combination of florescent and incandescent lamps. Spikes at the 50% flowering stage were point inoculated by injecting 10 μl of a 50,000 spores/ml suspension into a central floret on the spikes. Inoculated plants were retained in a unit maintained at 25°C for 48 hours and 95% RH, then moved to a normal growth cabinet. Symptoms were read at 21 days after inoculation. Symptom scores were expressed as % infected florets. Other symptoms such as blackened rachis were also recorded [12].

Type II resistance was usually evaluated in field plots in the epiphytotic nursery. Where seed quantities were adequate the plots consisted of two 1-m rows spaced at 6 inches apart and ideally replicated three times. At the boot leaf stages, corn spawn consisting of inoculated corn and barley seed was spread between the rows at the rate of 80 g/m$^2$. Applications

were repeated 1 week later. An irrigation system was activated twice a day to maintain a high relative humidity to enhance sporulation of the inoculum. Flowering dates of each plot were recorded, defined as the stage of 50% anthesis. At 21 days after the flowering date, disease incidence and severity was estimated visually for each plot and recorded. FHB indices were calculated from these readings. The plots were hand harvested at physiological maturity.

Threshing was done with a small plot thresher adjusted to retain the shrunken Fusarium damaged kernels (FDK). Two 1-g aliquots were removed from each sample and ground in a Wiley mill. To ensure homogeneity of the aliquots, the seed was put through a seed divider.

DON contents were estimated by an ELISA test using established methods [13]. Don contents of plots were expressed as parts per million. The check cultivars in field plots were Roblin as the susceptible check and Sumai3 as resistant. Other checks were selected as those that were parents of the various populations.

## 3. Results

### 3.1. Triticum monococcum

Excellent reviews have been written listing the variability for resistance to FHB in alien species [14, 15–18, 34]. *T. monococcum* was not listed in those reviews. *T. monococcum* was one of the species screened for FHB resistance in our studies. We started by screening 200 accessions of *T. monococcum* that were obtained from M. Trottet of INRA. After repeated screening, line 10-1 was identified as having a fair level of FHB resistance (**Figure 1**) [19, 20, 21]. Line 10-1 was crossed to the spring wheat cultivar AC Domain. After repeated backcrossing and screening, line M321 was selected. The values for percent infected florets following point inoculation were 8% compared to 4% for the resistant check Sumai3 and 32% for Roblin the susceptible check. The DON content of M321 was 5.5 ppm compared to Sumai3 at 2.1 and Roblin at 17.2 (**Table 1**). M321 was crossed to AC Domain and a doubled haploid mapping population of 80 lines was produced by the maize pollination method [22]. A QTL for FHB resistance was located in chromosome 5A, linked to the marker Xwme705 [18].

The agronomic characteristics of line M321 are shown in **Table 1**. Line M321 compares favourably with check cultivars in terms of agronomic traits such as plant height, yield, thousand kernel weight (TKW), protein content and even flour yield. The grain yield of this line is reasonable compared to AC Barrie, a check cultivar. The data in **Table 1** indicate that there is minimal linking drag in M321. The lowered DON content relative to the checks could be a useful attribute for improvement of disease resistance of wheat.

### 3.2. Aegilops speltoides

FHB resistance was also sought in *Aegilops speltoides*. In this case, 50 accessions were screened and line S184 selected [19, 23]. It has previously been shown that different accessions of *Ae. speltoides* can lead to different levels of meiotic chromosome pairing in F1 hybrids with wheat. The hybrid

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| Genotypes | Yield (kg/ha) | TSTWT (kg/hl) | HT (cm) | Protein (%) | Flour yield (%) | DON (ppm) |
|---|---|---|---|---|---|---|
| Sumai3 | 2895 | – | 88.5 | 13 | – | 2.1 |
| M 321 | 3272 | 79.3 | 76 | 13.9 | 57.5 | 5.5 |
| S 184 | 3246 | 80.3 | 86 | 13.3 | 67.2 | 3.4 |
| AC Barrie | 3304 | 80.5 | 79 | 13.7 | 66.8 | 6.5 |
| Roblin | – | – | – | – | – | 17.2 |

**Table 1.** Agronomic characteristics and DON content of FHB resistant lines introgressed into wheat from *T. monococcum* (M321) and *Ae. speltoides* (S184).

between AC Domain and the resistant speltoides accession showed an average of 3–4 bivalents at meiosis, i.e. the accession that we chose produced a high level of chromosome pairing in the F1 hybrid. The level of recombination between wheat chromosomes and those of *Ae. speltoides* would then be relatively high. Despite this, three backcrosses were required to restore fertility in the progeny. The agronomic characteristics of line S184 are shown in **Table 1**.

For most agronomic traits, such as plot yield, plant height protein content and even flour yield, the values for S184 compared favourably with the check cultivars (**Table 1**) [35]. Perhaps the most important attribute of this line is the lowered DON content. The DON content as shown in **Table 1** is 3.4 ppm compared to Sumai3 at 2.1, AC Barrie at 6.5 and Roblin at 17.2.

### 3.3. Triticum timopheevii

A resistant accession of *T. timopheevii* (AAGG genome) was crossed to the wheat cultivar Crocus which has all three crossability genes. The F1 was backcrossed to Crocus [24]. A population of 1500 $BC_1F_2$ plants was established and 535 BC1F7 lines were developed in the greenhouse using single seed descent. One hundred lines were selected based on full plant fertility and good agronomic traits and evaluated for their FHB reaction in the field. The line TC67 was selected based on its enhanced FHB resistance (**Figure 2**) and good agronomic traits. To map the resistance trait, a mapping population was established by crossing TC67 to the moderately susceptible cultivar AC Brio. An F7 population of 230 RIL was established by SSD and evaluated for a number of FHB-selected traits in field and greenhouse plantings.

As shown in **Table 2**, the DON content of TC67 and Brio was 1.3 and 3.0 ppm, respectively. The population mean for DON content was 2.2 with a range of 1.0–5.1 ppm. The QTL for this trait was mapped to chromosome 5A [25].

### 3.4. Aegilops cylindrica

*Aegilops cylindrica* is a tetraploid with the CCDD genome constitution. An accession collected in the wild by Alexander Rybalka of the Plant Breeding and Genetics Research Institute at Odessa Ukraine showed resistance to FHB. It was crossed to a local cultivar and a FHB resistant, stable derivative Cyl-1 was selected in the progeny. In our tests that line gave DON ratings intermediate between Sumai3 and Roblin. The DON content of Cyl-1 was 4.5 ppm


**Figure 2.** Resistance to Fusarium head blight expressed in TC67 an introgression from *T. timopheevii*. Disease symptoms expressed at 21 days after inoculation. Roblin is the susceptible check.

| Trait | Parents | | Population mean | Population range | Heritability |
|---|---|---|---|---|---|
| | TC 67 | Brio | | | |
| Disease spread within the spike (%) | 5.1 | 35.1 | 35.2 | 5.1–99.2 | 0.89 |
| Disease incidence (%) | 18.0 | 42.6 | 36.4 | 12.4–65.4 | 0.60 |
| Disease severity (%) | 41.3 | 41.8 | 50.8 | 25.5–76.7 | 0.47 |
| FDK (%) | 2.4 | 6.3 | 7.4 | 1.7–22.3 | 0.67 |
| DON content (ppm) | 1.3 | 3.0 | 2.2 | 1.0–5.1 | 0.69 |

**Table 2.** FHB scores and DON content, means, ranges and heritability in a mapping population derived from TC67, a derivative from *Triticum timopheevii* and wheat cultivar Brio.

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compared to Sumai3 and Roblin at 3.0 and 10.0, respectively. Cyl-1 was crossed to North America cultivars AC Superb, AC Barrie and Alsen as shown in **Table 3**.

The populations were advanced to F4, F6 and F7. Progenies were grown in field plots and DON contents determined. The distribution of DON levels was similar for the three populations. Although the DON levels in the checks Sumai3 and Roblin were at expected levels, the levels in the populations were unusually low and will need to be repeated.

Continued selection for a combination of improved agronomic traits and lower DON content resulted in line Odessa129-2 with a DON content of 9.6 ppm compared to Sumai3 and AC Superb at 3.9 and 47.2, respectively.

### 3.5. Triticum miguschovae

*Triticum miguschovae* is an amphiploid between *T. timopheevii* (AAGG genome) and *T. tauschii* (DD genome) [26, 27].

The spikes of the amphiploid display many alien species traits as shown in **Figure 3**. Following point inoculation with a 50,000 spores/ml, suspension of *Fusarium graminearum* spores, the symptoms did not spread beyond the inoculated floret (**Figure 3**). A similar display of symptoms was observed in $BC_2$ progeny following backcrossing to AC Superb (**Figure 4**). AC Superb has no FHB resistance so the observed resistance must be contributed by the alien parent.

The progenies of $BC_2$ plants were advanced to F5 with selections made on point inoculation symptoms at each generation.

A total of 35 F5 lines were grown in the epiphytotic nursery in single row plots and only one replicate. The DON content of the 35 lines ranged from 0.6 to 11.3 ppm (**Table 4**). Ten of the best F7 lines grown in the field gave a range of DON values of 3.5–8.2 ppm. The mean DON

| Derivatives | Generation | DON levels (ppm) | | | | No. of lines |
|---|---|---|---|---|---|---|
| | | <1 ppm | 1–2 ppm | 2–5 ppm | > 5 ppm | |
| *Cyl-1/AC Superb | F7 | 11 | 8 | 10 | 4 | 33 |
| Cyl-1/AC Barrie | F6 | – | 4 | 14 | 9 | 27 |
| Cyl-1/Alsen | F4 | 7 | 6 | 6 | 4 | 23 |
| Checks | | | | | | |
| Sumai3 | | | 1.2 | | | |
| Roblin | | | | | 11 | |
| Strongfield | | | | | 17.6 | |

*Note*: *Cyl-1 FHB-resistant accession of *Aegilops cylindrical*.

**Table 3.** DON content of FHB-resistant lines derived from progenies of *Aegilops cylindrical* crossed to wheat.

**Figure 3.** Resistance to Fusarium head blight expressed on spike of *Triticum miguschoae* (AGD) (R) at 21 days after inoculation. Roblin (L) is the susceptible check.

**Figure 4.** Symptoms on $BC_2$ spike of hybrid between Superb and *T. miguschovae* at 21 days after point inoculation.

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| Generation | No. of lines | DON content (range) |
|---|---|---|
| F5 | 35 | 0.6–11.3 |
| F7 | 10 | 3.5–8.2 |
| **Checks** | | |
| Sumai3 | | 2.7 |
| Fukuhokomuji | | 13.5 |
| AC Superb | | 17.8 |

**Table 4.** FHB symptoms and DON content (ppm) in progenies from intercrosses of bread wheat cultivar AC Domain with *Triticum miguschovae*.

levels of the checks for the field tests were Sumai3 at 2.7 ppm, Fukuho at 13.5 ppm and AC Superb at 17.8 ppm.

Continued selection for a combination of improved agronomic traits and lower DON content resulted in the line MSB55 that had a DON content of 10.8 ppm compared to Sumai3 at 3.9 and AC Superb at 47.2.

### 3.6. Tritordeum

Resistance to FHB in durum wheat is very poor and variability for this trait in the tetraploid gene pool is very limited [28]. After screening some accessions of *Hordeum chilense* and detecting some variability for reaction to FHB, we crossed the better accessions to the durum cultivar Ma (which in our experience had better crossability than other durum cultivars). A chromosome preparation of the amphiploid is shown in **Figure 5**. Seven of the tritordeum amphiploids were evaluated for DON content and the results are shown in **Table 5**. Compared to Medora, a susceptible check, all seven amphiploids showed improved levels of DON content. Some of the values shown in **Table 5** are unrealistically low and should be

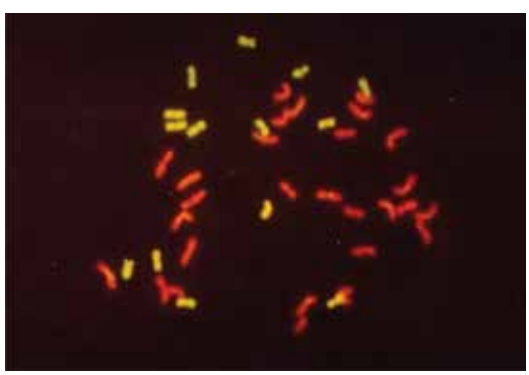

**Figure 5.** GISH pattern on a chromosome preparation from a Tritordeum (*Hordeum chilense* × *Triticum durum* amphiploid) showing 14 *Hordeum chilense* chromosomes (light color) and 28 durum chromosomes.

| Strain | DON content | |
|---|---|---|
| | Mean + SD | Range |
| HT-8 | 0.32 + 0.05 | 0.27–0.37 |
| HT-10 | 1.24 + 1.52 | 0–2.76 |
| HT-18 | 2.52 + 3.36 | 0–5.88 |
| HT-31 | 2.31 + 0.71 | 1.60–3.02 |
| HT-47 | 1.62 + 1.92 | 0.26–3.54 |
| HT-166 | 6.83 + 6.01 | 6.82–12.84 |
| HG-174 | 1.83 + 2.01 | 0.54–3.75 |
| Medora | 8.83 + 2.01 | 6.66–10.54 |

**Table 5.** DON content (ppm) of amphiploids between *Triticum turgidum* (AABB) and *Hordeum chilense* (HH).

re-evaluated; however, this appears to be a potential source of lower DON levels. A variety of FHB responses are shown in **Figure 6**, following the point inoculation of four tritordeum amphiploids plus a Roblin check.

The amphiploids show normal meiotic behaviour, are stable and perfectly fertile with perfect transmission of all chromosomes. There is no meiotic pairing between *Hordeum chilense* chromosomes and those of wheat. In order to induce pairing between homoeologous chromosomes, the amphiploid was crossed to the Capelli *Ph* mutant then backcrossed once to place the mutant in a homozygous recessive condition. The progeny resulting from the *Ph* mutant treatment were further backcrossed and advanced to the $BC_3F_4$ generation. Selection for reduced DON content and desirable agronomic traits was practiced during this procedure. Seventeen $BC_3F_4$

**Figure 6.** FHB symptoms on spikes of Tritordeum lines at 21 days after inoculation.

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lines were evaluated in the epiphytotic nursery, and results are shown in **Table 6**. The DON content in these lines ranged from 3.3 to 27.7 ppm compared to 19.1 for Strongfield the recurrent parent. Derivatives from this process appeared to have lower symptoms.

### 3.7. Triticale

Triticale, a wheat-rye amphiploid is used primarily as a feed grain worldwide, but has never reached its true potential. For a feed grain, DON content is a highly significant component. It has been shown that for each ppm of DON, feed consumption by monogastric animals decreases by 7.5%. Triticale was considered to be a major carbohydrate for bio-fuel production because of its high yields of biomass and tolerance to poor soils. Some consideration has been given for triticale to be used for ethanol production. However, it has been shown that the DON content in distiller's grains can be three lines as high as in the original grain. Therefore to fully realize the potential of triticale, its DON content must be reduced.

In general, triticale strains are notorious for poor FHB resistance. To put a wider perspective on this problem, we started by acquiring 371 strains of triticale from Plant Gene Resources of Canada (PGRC) to begin FHB testing. The testing was done in an epiphytotic nursery to evaluate Type II resistance. Visual rating of incidence and severity was done on the field plots and aliquots of seed ground for DON analysis. For the majority of the strains tested, the incidence and severity values exceeded 50%. Seven of the best strains were selected and shown in **Table 7**.

As shown in **Table 7**, the DON values of the seven strains ranged from 2.1 to 7.7 ppm, compared to Sumai3 at 1.2. The DON values in 2007 were low overall in that year. They were somewhat higher in 2008, ranging from 3.2 to 9.0.

AC Ultima was used a check triticale cultivar. It was a recently licensed cultivar in Canada and superior for most agronomic traits. Its DON content was 17.5 in 2007 and 16.0 in 2008.

The triticale strain TMP16315 was selected for further study. It was tested at numerous locations across Canada and proved to be stable in its reaction to FHB. Its pedigree is undefined, but believed to originate from a Polish gene pool.

A study was initiated to identify the QTL combining the FHB resistance/lower DON levels. Line TMP16315 was crossed to AC Ultima. A mapping population of 150 DH lines using microspore culture (Francois Eudes pc) method was produced from the F1 hybrid. The

| Generation | No. of lines | DON content (range) |
|---|---|---|
| $BC_3F_4$ | 17 | 3.3–27.7 |
| **Checks** | | |
| Strongfield | | 14.1 |
| AC Superb | | 16.3 |
| Roblin | | 17.2 |

**Table 6.** DON content (ppm) in progenies of intercrosses between *Tritordeum* (ABH) and *Capelli Ph* mutant followed by backcrosses to durum cultivar AC Strongfield.

| Line | 2007 | | | 2008 | | |
|---|---|---|---|---|---|---|
| | Incidence (%) | Severity (%) | DON (ppm) | Incidence (%) | Severity (%) | DON (ppm) |
| PI 355949 | 10.0 | 10.0 | 3.6 | 17.5 | 10.0 | 5.2 |
| PI 428748 | 10.0 | 10.0 | 3.2 | 10.0 | 5.0 | 4.4 |
| PI 428754 | 10.0 | 10.0 | 2.1 | 7.5 | 7.5 | 5.4 |
| PI 428814 | 30.0 | 20.0 | 7.7 | 20.0 | 15.0 | 9.0 |
| PI 428846 | 15.0 | 10.0 | 2.4 | 15.0 | 15.0 | 3.8 |
| CN 42948 | 20.0 | 20.0 | 5.0 | 10.0 | 10.0 | 4.5 |
| TMP 16315 | 15.0 | 15.0 | 4.1 | 20.0 | 10.0 | 5.2 |
| Sumai3 | 5.0 | 5.0 | 1.2 | 5.0 | 5.0 | 3.2 |
| AC Ultima | 85.0 | 50.0 | 17.5 | 45.0 | 45.0 | 16.0 |

**Table 7.** FHB symptoms and DON content of seven best resistant accessions of triticale and the checks Sumai3 and AC Ultima in the field nursery in 2007 and 2008.

mapping population in three replicates was grown at three locations in eastern Canada and data collected on incidence severity, FDK and DON content. The QTL for the various FHB related traits will be determined from these data.

### 3.8. Rye

In screening of numerous accessions of rye from numerous sources, we were not able to find any lines with even minor improvements in FHB resistance. There were reports of Brazilian land races of rye with improved levels of FHB resistance [29]. The lines were evaluated for resistance by plating on media containing from $10^{-3}$ to $10^{-6}$ M levels of DON [32] to evaluate their levels of tolerance to DON. Lines that showed no variable effects on a medium containing $10^{4}$–$10^{-3}$ M DON were considered to be resistant, whereas lines showing retarded growth on media containing $10^{-5}$–$10^{-6}$ M levels of DON were considered to be susceptible. As shown in **Table 8**, the landraces from Poula Frontin were susceptible to DON, whereas landraces from Lagoon Vermellia and Sao Paulo gave a resistant reaction. A number of the resistant lines were used as pollen parents on wheat cultivars Encruzilhada, Maringa, Max and NyuBay to produce octoploid amphiploids (as shown in **Table 9**).

| Accession | DON* | Reaction to DON |
|---|---|---|
| Rye from Poula Frontin, Parana | $10^{-5}$ | S |
| Rye from Lagoon Vermellia, Rio di Sul | $10^{-4}$ | R |
| White rye, Sao Paulo | $10^{-3}$ | R |

*Note*: *DON levels in culture media (ppm).

**Table 8.** Tolerance of Brazilian rye landraces to deoxynivalenol (DON) following plating on DON-containing media.

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| Hybrid combination | No. tested | DON* | Reaction to DON |
|---|---|---|---|
| Encruzilhada X 14A | 1 | $10^{-5}$ | S |
| Maringa X 26A | 3 | $10^{-5}$ | S |
| Max X 2C | 1 | $10^{-4}$ | R |
| | | $10^{-6}$–$10^{-5}$ | $10^{-3}$–$10^{-4}$ |
| | | S | R |
| NyuBay X Rye lines | 13 | 8 | 5 |

*Note*: *DON levels in culture media (ppm).

**Table 9.** Reaction of octoploid triticale strains to deoxynivalenol (DON) following plating on DON-containing media.

One amphiploid combination with wheat cultivar Max gave a resistant reaction and five amphiploids with NyuBay were also resistant by growing in a medium with a $10^{-3}$ level of DON [12].

## 4. Discussion

Reviews have been written showing the variability for FHB resistance in alien species [14, 20]. Less information is available on variation for DON content in alien species [18].

This review has shown that there are ranges for DON values in progenies obtained from several combinations of inter-specific/inter-generic hybrids. Although some of the data represent analyses from single years, there are indications of the potential of lowering the DON content by means of wide crosses.

In all cases, the screening of alien species parents was initially conducted by point or spray inoculation. The progenies in most cases were screened by several methods. Perhaps a more concerted effort needs to be employed to initially screen wild species for DON content.

Of various inoculation methods evaluated and methods of disease evaluation scored, including incidence, severity and FDK, it was found that DON evaluation gave the most reliable estimates of FHB resistance [14]. Considering that reducing DON content is the most important aspect of FHB resistance, DON evaluations should receive higher priority in future studies. Transgressive segregation for DON content was obtained in breeding populations of wheat and rye [30]. It was suggested that selection for lower DON content could be initiated as early as F3.

Transgressive segregation for DON content was observed in populations described in this paper, especially in progenies of crosses to *T. timopheevii* derivatives.

It has been shown by numerous studies beginning with Somers et al. [7] that DON content in wheat is controlled by distinctive QTL. That study also showed that minor QTL for DON content were present in the mapping population derived from Wuhan and NyuBay. These observations indicate that the potential exists for employing a combination of marker-assisted

selection plus a high selection pressure in an epiphytotic nursery to increase the overall resistance to DON accumulations as has been done for visual symptoms QTL [31].

Detailed screening of alien species collections for DON content should be done to the same extent as screening for visual symptoms of FHB resistance. Preliminary results shown in this paper indicated that such an approach would be warranted, to be followed by mapping of additional QTL. Such QTL would very likely be unique and would add to the toolbox of resources available for breeding for reduced DON content.

In order to effectively transfer FHB resistance from alien species to wheat, sufficiently large populations need to be grown. It has been showed in numerous studies that sufficient number of major and minor QTL need to be transferred to obtain effective resistance.

In conclusion, these studies have shown considerable variability for DON content can be obtained from species relatives alien to wheat. A focussed approach would be required to tag the various QTL and systematically integrate them into bread wheat. This is anticipated to be an incremental process. The end products would be crop cultivars that would be resistant to the head scab phase of FHB with the added benefit of lower DON accumulation, making them more suitable for feed and food.

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Chapter 5

# Impact of Growth Habit and Architecture Genes on Adaptation and Performance of Bread Wheat

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**Abstract**

In bread wheat (*Triticum aestivum* L.), flowering time and plant stature are important phenological and agronomic traits for adaptation, yield potential, and yield stability. Timely flowering is critical for production, and the flowering window has to be late enough to avoid early season frosts but early enough to avoid late season stresses such as heat and terminal drought. Flowering time is controlled mainly by vernalization, photoperiod response, and earliness *per se* genes, which can be exploited to fine-tune growth and tailor flowering time for the production of desirable wheat cultivars. Tailoring flowering time could help reduce preharvest sprouting problems by escaping high temperatures and late season rainfall, which promote preharvest sprouting, hence yield loss. Concisely summarizing available information on flowering time and identifying research gaps could provide direction for future research. This chapter, therefore, discusses: (i) the progress made in discovering genes involved and the impact of their extensive allelic variation on flowering time, (ii) the potential benefits of tailoring wheat's flowering time to improve yield, and (iii) the benefits of introgressing genes for other complimentary traits, such as semidwarf and preharvest sprouting resistance on advanced lines to achieve higher yield, thus, sustainable food security.

**Keywords:** earliness *per se*, flowering time, photoperiod response, preharvest sprouting, semidwarf, vernalization, yield

## 1. Introduction

The performance of a wheat cultivar, which is normally measured by its adaptability and yield potential under target environments, is dependent on genetic and environmental factors as well as the interaction between these factors. Timely flowering, that is the switch

from the vegetative phase to the reproductive phase, and the duration of the life cycle fine-tune a cultivar to the targeted environment [1, 2]. Flowering is essential for reproductive success and occurs when conditions are favorable to maximize pollination, seed development, seed dispersal, and subsequent germination [1]. Flowering success is demonstrated by the ability of the plant to efficiently use a range of available resources including water, nutrients, temperature, day length, radiant energy, and relevant endogenous signals to maximize its potential yield and to escape stressful conditions during growth and development [1, 3]. Consequently, there is a need to better understand the genetic control of flowering time in wheat. Understanding the genetic control of the components of the life cycle, although complex, will enable plant breeders to exploit associated genes, thus fine-tune the growth and development of the crop to fulfill the demands of a specific environment and to increase yield [1, 4]. Discovering genes that control flowering time in wheat have been one of the key research goals for decades [1] and is increasingly gaining importance due to the impact of projected climate change [5]. As a result, many loci influencing flowering time has been successfully mapped and their effects determined [1, 4].

The duration of the life cycle of bread wheat is controlled by numerous genes, including those associated with seed germination, vegetative growth, flowering time, seed maturation, and seed dispersal [6]. These processes form the foundation of the reproductive strategy of flowering plants. The interaction between these genes and the environment defines the ultimate phenotype [7, 8]. Flowering time is an important component of the life cycle with a very wide and complex genetic control. Three groups of genes with major influence on flowering time of wheat include vernalization response genes, photoperiod response genes, and genes controlling the developmental rate (earliness *per se* (*eps*)) when vernalization and photoperiod response requirements have been met [1, 9, 10]. With the exception of *eps* genes, the environment plays a role in the expression of vernalization and photoperiod response genes and thus, to their contribution towards flowering time and growth of wheat [1, 11, 12]. Reviewing currently available genetic and genomic resources for flowering time and the progress made so far toward introgressing known genes in elite germplasm is vital to guide future research. This chapter, therefore, discusses the progress made in discovering genes involved and the impact of their extensive allelic variation on flowering time. Additionally, the potential benefits of tailoring the flowering time of wheat to improve yield in the wheat production industry are also discussed. Furthermore, the chapter discusses the benefits of introgressing genes for other complimentary traits such as semidwarf and preharvest sprouting resistance on promising or advanced wheat breeding lines.

## 2. The process of flowering in bread wheat

The process of flowering involves multiple interactions between major genes (vernalization, photoperiod response, and *eps* genes) and endogenous factors such as the developmental stage and floral gene activities acting together to promote flower initiation [13]. Crucial to the process of floral initiation is the establishment and maintenance of meristems. A specified

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class of vernalization response genes called *Vrn-1* series (*Vrn-A1*, *Vrn-B1*, and *Vrn-D1*) is responsible for this task in wheat [14–16]. The process consists of pools of undifferentiated cells that could either give rise to lateral organs such as leaves, auxiliary shoots (including flowers), and internode tissue, or that could serve as a continuing supply of new meristem cells. As a result, the type of cells produced and their ultimate developmental fate as part of vegetative or reproductive structures determine whether flowering occurs [13]. To initiate flowering, the flowering response or signal must be transferred through florigen to apices and induces meristem identity genes involved in the initiation of flowering following the accumulation of a light signal (photoperiod response) on the leaves [17–19]. This process is mediated by both vernalization and photoperiod response genes [18, 20]. Future research should identify the meristem identity genes controlling floral transition and inflorescence development in wheat and other cereal crops [21].

## 3. The influence of vernalization genes on the flowering time of bread wheat

Bread wheat is generally classified as spring or winter types according to its response to low temperatures during the vegetative phase [22–24]. Exposure to low temperatures (0–10°C) for several weeks (usually 6–8 weeks) is necessary for the development of tillers and the induction of flowering in winter wheat, whereas tillering and flowering of spring wheat occur regardless of temperature [22, 25]. The flowering models of the temperate cereals indicate that before vernalization, *Vrn-3* series is repressed by *Vrn-2* and long exposure to low temperatures is necessary for the upregulation of *Vrn-1* series and downregulation of *Vrn-2* in the leaves. Failure of these processes will delay the flowering process [26–28]. As spring approaches, the *Vrn-3* levels are upregulated (a process mediated by photoperiod genes) and signals are sent from the leaves to shoot apices to increase the *Vrn-1* transcription above threshold levels for the induction of flowering [10, 28]. Winter wheat types are considered to be ancestral to spring wheat types [29] and the winter alleles of *Vrn-A1* genes are considered to be ancestral to the spring alleles. The insensitivity of spring wheat to vernalization is due to mutational loss of the repressor binding site in the regulatory region of one or more *Vrn-1* genes [30] and is responsible for the early flowering ability of spring wheat [31]. Spring wheat varieties have been bred to adapt to diverse agroclimatic conditions attributing to their much shorter flowering time as compared to winter wheat [4, 32].

Substitution line analyses have identified four major series of genes controlling the length of the vernalization period in bread wheat (**Table 1**). According to Yan et al [17], the *Vrn-B3* gene (7BS) was identified as a flowering time (FT)-like gene, as have its homologous *Vrn-A3* and *Vrn-D3* on 7AS and 7DS, respectively [19]. Among the identified major genes controlling vernalization response, the *Vrn-1* series (*Vrn-A1*, *Vrn-B1*, *Vrn-D1*) is the predominant one in reducing vernalization requirement [31, 33]. However, even with fulfilled vernalization requirement, photoperiod sensitive bread wheat cannot flower until a critical day length has been reached [5].

| Major gene series | Gene(s) comprised | Gene location | Reference |
|---|---|---|---|
| *Vrn-1* | *Vrn-A1*, *Vrn-B1* and *Vrn-D1* | Long arms of chromosomes 5A, 5B and 5D, respectively | [31, 34] |
| *Vrn-2* | Not specified | chromosomes 4B, 4D and 5A | [35] |
| *Vrn-3* | *Vrn-A3*, *Vrn-B3* and *Vrn-D3* | Short arms of chromosomes 7A, 7B and 7D, respectively | [36] |
| *Vrn-4* | *Vrn-D4* | Chromosome 5D | [37] |

**Table 1.** Vernalization genes/class of genes identified in bread wheat to date.

## 4. The influence of photoperiod response genes on the flowering time of bread wheat

Photoperiod is the day length and number of long days that a wheat cultivar must reach (a threshold) for floral initiation [38]. The duration of exposure to light can be categorized into three groups namely, short-day (SD, 11-14 h), long-day (LD, 18 h), and day-neutral (DN) or facultative [39]. The winter wheat and spring wheat varieties can be photoperiod-sensitive or photoperiod-insensitive. Photoperiod-insensitive varieties are early flowering both under SD and LD conditions, in contrast to the photoperiod-sensitive varieties that require exposure to LD for weeks before they can initiate flowering [38, 40]. Several genes controlling photoperiod response have been successfully identified in wheat (**Table 2**). The *Ppd-1* genes (*Ppd-A1*, *Ppd-B1*, and *Ppd-D1*) induce flowering time irrespective of the day length in contrast to the *Ppd-B2* gene reported on the short arm of chromosome 7B, which accelerates flowering time only under LD conditions [41, 42, 43]. The potency of the insensitivity of the photoperiod response genes has been ranked in the order:

*Ppd-D1* > *Ppd-B1* > *Ppd-A1* [38, 44].

| Gene | Gene location | Reference |
|---|---|---|
| *Ppd-D1* (formerly *Ppd1*) | Chromosome 2D | [27, 38, 40, 44, 45, 49] |
| *Ppd-B1* (formerly *Ppd2*) | Chromosome 2B | [27, 38, 40, 44, 45, 49] |
| *Ppd-A1* (formerly *Ppd3*) | Chromosome 2A | [27, 38, 40, 44, 45, 49] |
| *Ppd-B2* | Short arm of chromosome 7B | [50] |

**Table 2.** Photoperiod response genes identified in bread wheat to date.

Photoperiod insensitivity is beneficial for crops grown in short-growing seasons with high summer temperatures in order to avoid heat stress during grain-filling stages [44, 45, 46]. Earlier flowering conferred by the *Ppd-D1a* insensitive allele has broadened the adaptation of cultivars over a range of environments and increased yield potential in improved cultivars, in southern Europe, Asian, Mediterranean, and North African regions [11, 47, 48]. However, the desirability of this allele depends on the target environment. For example, in the northern parts of Europe, which do not experience late season stress, the *Ppd-D1a* allele is not selected

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for due to the shortened vegetative phase associated with this allele, which results in considerably lower yield potential in these environments [5].

## 5. The interaction of *Vrn* and *Ppd* alleles and its impact on flowering time of bread wheat

Various studies have been conducted to study the interaction between *Vrn-1* and *Ppd-1* active alleles and the impact of their different combinations on the flowering time of bread wheat. Cultivars containing different combinations of *Vrn-1* and *Ppd-1* alleles will respond differently under different environmental conditions and thus, display different heading dates or flowering times [3, 9, 23, 51, 52]. It was discovered that *Vrn-A1* genotypes (either single or in combination with other *Vrn* alleles) are the earliest in flowering followed by *Vrn-B1* and then *Vrn-D1* genotypes [53]. In fully vernalized winter wheat, *Ppd-D1a* allele advanced flowering time by up to 24 days [54, 55]. However, in the presence of an active allele of *Vrn-1*, the flowering time of wheat was reduced by at least 30 days [54, 56]. It was reported that wheat genotypes with all three dominant alleles of *Vrn-1* genes (*Vrn-A1, Vrn-B1*, and *Vrn-D1*) head quite early compared to mono- or di-dominant gene combinations [28]. Similar information was also reported by various authors [5, 9, 57–59]. From the results of the above-mentioned studies, it was shown which combinations of alleles perform better than others. In agreement with Zhang et al. [53], it was demonstrated in the other studies mentioned above that *Vrn-B1* and/or *Vrn-D1* alleles are less effective in advancing flowering time as compared to the *Vrn-A1* allele. The *Vrn-1* genotypes are reported to be marginally early in flowering time in the following order:

*Vrn-A1 Vrn-B1 Vrn-D1* > *Vrn-A1 Vrn-B1*, *Vrn-A1 VrnD1* or *Vrn-A1* > *Vrn-B1* or *Vrn-D1*.

An epistatic interaction between the *Vrn-A1* and *Vrn-D1* active alleles was demonstrated in a study [9]. The same study confirmed an additive/complementary interaction for flowering time between the photoperiod-insensitive *Ppd-D1a* allele and the *Vrn-1* active alleles [57, 60]. Moreover, it was noted that although genotypes carrying *Vrn-1* and *Ppd-D1a* alleles are early flowering under both SD and LD conditions, the flowering time is delayed by low temperatures under SD conditions. Overall, it is concluded that *Ppd-1* and *Vrn-1* genes participate in a similar pathway to control flowering time [10]. This implies that even though vernalization and photoperiod responses are independent processes, fulfillment of both requirements is necessary for early flowering of bread wheat. Flowering will be delayed if these processes did not occur [28] and the extent of this delay will depend on the *Ppd* gene present in the variety, as well as the environmental conditions [43].

## 6. The influence of earliness *per se* (*eps*) genes on the flowering time of bread wheat

A third class of genes controlling flowering time of wheat is the earliness *per se* (*eps*) genes. *Eps* genes affect phenological development of wheat when all photoperiod and vernaliza-

tion requirements have been satisfied [1, 11]. However, genes of all three classes (*Vrn*, *Ppd*, and *eps*) exert pleiotropic effects on other aspects of plant growth and development [1]. Whereas the major *Vrn* and *Ppd* genes govern the gross adaptation to environments, the *eps* genes have been shown to largely fine-tune the flowering time of wheat varieties for their regional adaptations [1, 61–63]. Sufficient information is now available on the effect of *eps* genes in determining flowering time of wheat. Genetic analyses show that these loci have been mapped only as QTL effects rather than major genes because of their relatively small effect [1, 6]. This makes it difficult to undertake a comparative analysis of *eps* effects with confidence. Nevertheless, comparative genetic studies indicate that most wheat chromosomes harbor *eps* genes [1, 11, 64]. Worland [11] reported the likelihood of the existence of these genes on chromosome groups 2, 3, 4, 6, and 7. It was suggested in the same study that these genes fine-tune flowering time probably by determining the amount and rate at which vegetative and floral primordia are produced. A detailed mapping in bread wheat has detected *eps* loci on chromosomes of homologous group 2 and on the short arm of chromosome 3A [65, 66]. A locus on chromosome 2B is orthologous with the *eps2* gene in barley (*Hordeum vulgare*) [6, 11, 64], whereas the one on chromosome 3A is orthologous with the *Eps*-3*Am* gene in einkorn wheat (*Triticum monococcum*) [67]. The locus on chromosome 3A has been reported to also have significant effects on plant height, thousand kernel weight, and number of grains per plant [66]. However, no *eps* genes have been cloned as yet in bread wheat [12, 68] as compared to barley [6, 61, 69] and einkorn wheat [67]. More than 90 QTL for heading date, with most of them believed to play a role in fine-tuning flowering time, have been reported to be spread over almost the entire wheat genome [62, 70]. Recently, Zikhali et al. [12] validated the presence of an *eps* effect on 1DL in hexaploid wheat. Some qualities of *eps* genes, such as high heritability and their independency on the environment, display a platform for this class of genes to be efficiently used in breeding programs to modify the flowering time of wheat by advancing/shortening its life cycle [61]. With further studies, it will be possible to fine-tune flowering time to regional climatic variations using these loci (including those of *Vrn* and *Ppd*) once their primary and pleiotropic effects have been identified.

## 7. The influence of height-reducing genes on the flowering time of bread wheat

Among the most important growth habit parameters influencing adaptation and yield potential of bread wheat to various environments is plant height. The most common genes for reduced height (*Rht*, also called semidwarf) in wheat have been mapped on *Rht-B1* and *Rht-D1* loci on chromosomes 4B and 4D, respectively [71]. Another potentially valuable height reducing gene, designated *Rht*8, has been mapped on chromosome 2D of bread wheat [72, 73]. The alleles of the two genes, *Rht-B1b* and *Rht-D1b*, inhibit cell elongation due to insensitivity to the growth hormone gibberellic acid in contrast to the *Rht*8 gene. The primary mechanism of height reduction caused by these alleles (*Rht-B1b* and *Rht-D1b*) is a reduction in the rate of stem development and dry matter accumulation in vegetative tissue, leading to increased

partitioning of water and nutrients to the spike [74]. Consequently, more fertile florets and more seeds per spike are produced.

*Rht*8 genotypes were reported to compare very well with *Rht-B1b* and *Rht-D1b* genotypes in hot and dry environments (i.e. short growing season) [73]. This was evident in a study conducted by Lanning et al. [71] under terminal drought stress in Montana and Washington. In the study, the *Rht*8 semidwarf lines appeared to have superior seed characteristics (significantly higher kernel weight and grain protein content) relative to the *Rht-B1b* and *Rht-D1b* lines which even had reduced grain protein content relative to the wild-type. However, other studies reported higher yield potential associated with *Rht-B1b* and *Rht-D1b* genotypes under high input growing conditions (i.e., irrigated) as compared to *Rht*8 and standard height genotypes [75, 76]. The performance of semidwarf wheat lines was evaluated relative to standard height lines using a recombinant inbred line (RIL) population grown in both rain-fed and irrigated conditions in Montana [77]. Semidwarf lines containing *Rht-D1b* were discovered to have superior yield as compared to standard height lines. Moreover, McNeal et al. [78] observed that semidwarf wheat lines containing either *Rht-B1b* or *Rht-D1b* outyielded tall lines in Montana, except in very low yield potential environments where tall lines were superior. From these results, it can be concluded that when opting for high yield potential under normal or high input growing conditions, *Rht-B1b* and *Rht-D1b* genotypes are the best, but when planting in hot and dry (low yielding) environments, *Rht*8 genotypes should be selected for. The success of the *Rht* genes has resulted in their wide deployment in wheat breeding programs globally [79].

A moderate but significant correlation between flowering time and plant height has been reported in bread wheat [5, 80–82]. Shorter genotypes tend to flower earlier than the taller ones [5]. This effect was proposed to be mainly due to the *Rht-B1b* allele, suggesting a possible effect of the *Rht-B1* gene on heading date in wheat. Similar results were also reported by Wilhelm et al. [80], confirming the significant effect of *Rht-B1* on flowering time and suggested a possibility of genes controlling plant height to also affect flowering time. However, other studies report that earliness is often associated with reduced height and potentially reduced resource capture, therefore, reduced yield [42, 83]. This suggested negative correlation between earliness and yield remains a challenge in wheat breeding programs, posing a need to modify flowering time to suit local climatic conditions while maintaining or even increasing yield potential. The biggest challenge is to incorporate all or as many of the favorable and/or agricultural important traits as possible in one cultivar [84, 85].

## 8. The potential benefits of tailoring flowering time of wheat in the wheat production industry

Flowering time is a complex trait that is responsible for wide adaptation of wheat (and other cereal crops) to different environments [4, 21, 86]. This trait could be modified or tailored to local climatic conditions to achieve desired characteristics such as improved yield [87, 88].

Similar studies have been conducted successfully whereby high temperatures and drought stress during anthesis and grain filling were avoided through tailoring flowering time of wheat to local climatic conditions [42, 89].

The potential advantage of tailoring flowering time can be used to escape environmental conditions that lead to yield loss, such as high temperatures or conditions that lead to poor wheat quality, such as rain during harvest time. This could contribute to reducing the worldwide physiological phenomenon of preharvest sprouting (PHS). Preharvest sprouting, which is the germination of seed grains in the mother ear before harvest due to humid conditions, is prevalent in wheat-growing regions experiencing high rainfall during the period of grain maturity and ripening [90]. This results in significant losses in the wheat production industry such as the downgrading of premium milling quality wheat to feed quality [91]. Resistance to PHS is a highly desirable trait sought by plant breeders globally [92, 93]. In addition to breeding for resistance of this trait, tailoring flowering time for the production of early flowering cultivars, which will escape conditions favorable to PHS, could help reduce the problem.

## 9. The role of diagnostic molecular markers in the detection of allelic variation among the major growth habit genes influencing the flowering time of bread wheat

The fact that current wheat germplasm has not been characterized fully in terms of important agronomic traits limits the use of wheat germplasm to a certain extent. Identifying the alleles of these genes and estimating the effects of their combination on growth, heading date, and ultimately grain yield will enhance the selection of cultivars with wide adaptability to a set of environments [57]. This knowledge can help accelerate the introgression of adaptability and yield-contributing genes by predicting the best combinations for enhanced yield potential and adaptation [28]. Moreover, the identification of alleles of growth habit genes subsequently leads to the development of a series of molecular markers (allele-specific DNA markers) for improved identification of these alleles in future [46, 86, 94].

The development of allele-specific DNA markers has allowed for efficient detection of extensive allelic variation existing among genes controlling flowering time in bread wheat [35, 46, 95]. Through these markers, it has been revealed that the allelic variation at the *Vrn-A1* locus (*Vrn-A1a, Vrn-A1b*, and *Vrn-A1c*) results from mutations within the promoter sequence [26] and/or deletions within the first intron of this gene [26, 95]. For the *Vrn-B1* and *Vrn-D1* loci, their allelic variation is determined only by deletions within the first intron sequence of the gene [95]. Diagnostic markers are available to differentiate among these forms and consequently, significant progress in understanding the molecular basis of vernalization has been made in wheat and barley species [15, 16, 94, 96].

For photoperiod response genes, photoperiod insensitivity is induced by indels in the 5′ upstream region of pseudoresponse regulator (PPR) genes, which do not exist in photoperiod-sensitive varieties [41, 46, 49]. For instance, a 2 kb deletion in the *Ppd-D1* promoter region of chromosome

http://dx.doi.org/10.5772/67116

2D results in a semi-dominant, photoperiod-insensitive allele (*Ppd-D1a*). The semidominant *Ppd-D1a* mutation has been identified as the major source of earliness in wheat varieties globally. This most potent allele upregulates the expression of the *Vrn-3* gene, which is a homologue of the *flowering locus T* (*FT*) of *A. thaliana* [9], under both SD and LD conditions, therefore confers early flowering in wheat [13, 43].

The *Ppd-D1* gene is said to exist in several forms. Six haplotypes (alleles) of this gene have been identified [40], four of which were common in bread wheat. The same study provided molecular markers to distinguish among these alleles and elucidated that they have different levels of expression. Haplotype I, which is equivalent to *Ppd-D1a* in Beales et al [46] and Eagles et al [57], had the highest level of expression, and it was suggested that Haplotype II is a progenitor of the others and probably photoperiod-sensitive. To further their work, Guo et al. [40] developed a method which identified allelic variation within a locus that Eagles et al. [57] and Fischer [97] labeled as *Ppd-D1b*, eventually dividing that single classification into three alleles.

The alleles of *Ppd-D1* were also identified by Cane et al. [98] using allele-specific molecular markers. Lines carrying *Ppd-D1a* were identified with a large deletion in the promoter region by the method described in references [46] and [60]. Lines with a deletion in exon 7 were classified as *Ppd-D1d* carriers. Lines harboring *Ppd-D1c* alleles manifested a *mariner*-like transposable element in intron 1 and lines not characterized as either *Ppd-D1a*, *Ppd-D1c*, or *Ppd-D1d* were designated as *Ppd-D1b*. Frequent alleles of *Ppd-D1*, *Vrn-A1*, *Vrn-B1*, and *Vrn-D1* genes can be accurately identified using current molecular techniques. Inaccurate classification of alleles could reduce the accuracy of estimation of their effects on flowering time [57, 98].

Besides the *Ppd-D1* gene, two more loci namely, *Ppd-A1* and *Ppd-B1*, have been identified and shown to have effects as strong as that of *Ppd-D1* in accelerating flowering time in bread wheat [20, 41, 42, 99]. In a study to investigate the effect of *Ppd-B1a* allele on flowering time in wheat, it was shown that *Ppd-B1* displays copy number variation (CNV) [20]. Wheat genotypes with only one copy allele are photoperiod-sensitive whereas an increased copy number (2–4 copies) results in a day-neutral, early flowering phenotype. A complementary study [100] confirmed that wheat genotypes with the three-copy allele (termed *Ppd-B1a*) and the four-copy allele (termed *Ppd-B1c*) exhibit reduced days to heading as compared to the one-copy allele (termed *Ppd-B1b*) whereas the two-copy allele (termed *Ppd-B1d*) displays increased days to heading (late flowering). These results indicate that the CNV at the *Ppd-B1* locus contributes in fine-tuning the adaptation of wheat to local climatic conditions, in addition to the major effect of *Ppd-D1*.

## 10. Concluding remarks and future breeding perspectives

The three classes of genes (*Vrn*, *Ppd*, and *eps*) play a vital role in the adaptation and protective mechanisms to ensure successful reproduction of wheat in diverse environments around the world. It has been revealed that a combination of *Vrn*-1 (especially *Vrn-A*1) and *Ppd-D1a*

results in genotypes that are early flowering under both SD and LD conditions, but flowering time is delayed under SD conditions. Moreover, a significant correlation between flowering time and plant height has been reported suggesting the possibility of genes regulating flowering time to also regulate height [5, 80]. Therefore, semidwarf genotypes are said to flower earlier (and may give higher yield) as compared to taller or normal ones depending on the environment and the genotype by environment interactions.

In the view of the current and projected climate change, which will include extreme hot and dry conditions, selecting for *Rht*8 genotypes could be beneficial relative to the *Rht-B1b* and *Rht-D1b* genotypes, which only perform well under high input conditions. In contrast, *Rht*8 genotypes have been shown to perform well and give higher yields under hot and dry environments. Climate change necessitates that the genetic structure of current breeding programs be shaped accordingly. Therefore, breeding for wheat cultivars with flexible response in different environments and that exhibit superior performance under extreme conditions, such as hot and dry environments, should become a priority. Photoperiod insensitivity is usually an advantage in most regions [100]. Therefore, selecting for the trait and incorporating genes for other complementary traits, such as preharvest sprouting resistance into the advanced lines, could be an added advantage in addition to significant yield improvement. Selecting for favorable alleles in targeted environments will contribute to yield improvement in the wheat production industry. This will help to meet the ever-increasing demand, which will mean sustainable food security.

Selection of favorable alleles could increase the level of variation and/or introduce novel sources of resistance to diseases and unfavorable weather conditions into breeding populations [9, 26, 95, 101]. This allows the transfer of genotypes between regions with different climatic conditions but still maintains their level of agronomic performance [5]. The *Ppd-D1a* allele has been selected for by plant breeders in different countries for several decades to enhance yield in certain climatic conditions [10, 46, 100]. Selecting for favorable alleles also allows the development of allele-specific DNA markers for efficient detection of extensive allelic variation among genes controlling traits of agronomic importance [35, 46, 95]. As a result, the genetic components of flowering time and other traits of agronomic importance are now better understood and some of the associated genes are isolated and cloned in wheat and closely related species [15–17, 46, 101]. The information provided in this chapter will therefore be helpful in the current and future breeding programs when breeding for adaptation and improved yield in wheat.

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Section 2

# Wheat Ecology and Physiology

Chapter 6

# Inoculation with *Azospirillum brasilense* Improves Nutrition and Increases Wheat Yield in Association with Nitrogen Fertilization

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Additional information is available at the end of the chapter

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**Abstract**

The management of nitrogen fertilization is performed in order to ensure adequate productivity, and depending on the N dynamics in the soil, large amount of N is added to the soil, raising production cost for the farmers. Considering the benefits attributed by seed inoculation with *Azospirillum brasilense* (diazotrophic bacteria), with emphasis on biological nitrogen fixation (BNF), greater development of the root system, and, consequently, greater absorption of water and nutrients, it infers that inoculation can improve crop performance allowing greater efficiency of nitrogen fertilization. Thus, the research that evaluates nutritional status and wheat yield, in terms of nitrogen rates in association with inoculation with *A. brasilense* is important. We found that increment of N rates in association with *A. brasilense* inoculation increases the wheat yield up to 139 kg $ha^{-1}$ N, whereas without this inoculation linear increase occurred with lower maximum yield of wheat. That is, the inoculation afforded higher grain yield applying less nitrogen fertilizer in topdressing. This research demonstrated that inoculation with *A. brasilense* associated with nitrogen fertilization in topdressing is beneficial to nutrition and wheat yield. Therefore, inoculation is a low-cost technique, easy to apply and use, and nonpolluting, which fall within the desired sustainable context in actuality.

**Keywords:** diazotrophic bacteria, nitrogen, nutrient concentrations, bacterial promoters of plant growth, agronomic efficiency

## 1. Introduction

Wheat (*Triticum aestivum* L.) is an annual cycle plant, considered among the cool season cereal, one that has greater economic importance, with large grain yield capacity [1].

The final crop yield is defined according to the cultivar used, the amount of agricultural supplies, and management techniques employed. The increasing use of high-yield potential wheat has implicated in more frequent use of agricultural supplies, among which nitrogen fertilization shown to be important in defining the grain yield [2]. Therefore, there is a need to study wheat cultivars verifying their response to the uptake and utilization of nutrients in the soil and their performance and cultural practices in different environments [3].

Nitrogen fertilization is one of the highest costs of the production process of nonleguminous crops [4]. Wheat, corn, and rice crops utilize approximately 60% of the N fertilizer produced in the world [5]. The use of N fertilizer must be carefully controlled to ensure good yield and manage N in the soil; N fertilizer increases production costs for farmers [6].

Several authors reported a positive response of nitrogen fertilization on grain yield of wheat [3, 7–11]. Due to the high cost of fertilizers and awareness in support of sustainable agriculture and less polluting, in which the research is growing, one possibility would be to use inoculants containing bacteria that promote growth and increase the productivity of plants. Studies on biological nitrogen fixation (BNF) by *Azospirillum* in grass have been carried out in Brazil. Until recently, no commercial inoculants with these bacteria are available in the country [12].

Although the plant genotype performs an essential role in the colonization of bacteria, cultivars with high and low potential of association exist [13]. Several studies have been published confirming that *Azospirillum* produces phytohormones that stimulate root growth in many plant species. The components released by *Azospirillum brasilense* responsible for stimulating root growth are indoleacetic acid (IAA), gibberellins, and cytokinins [14]. Inoculation with *Azospirillum* can improve the leaf photosynthetic parameters, including chlorophyll content and stomata conductance, greater proline content in shoots and roots, improvement in water potential, an increase in water content in the apoplast, more elasticity of the cell wall, more biomass production, and greater plant size which were reported by Barassi et al. [15]. Increases in photosynthetic pigments such as chlorophyll a and b and auxiliary photoprotective pigments, such as violaxantine, zeaxantin, ateroxantine, lutein, neoxanthin, and beta-carotene, which result in greener plants without water-related stress, were verified by Bashan et al. [16].

In addition, the increase in root development caused by inoculation with *Azospirillum* is involved with several other effects. Increases in water and mineral uptake have been reported, as well as greater tolerance to stresses, such as salinity and drought, resulting in a more vigorous and productive plant [17, 18]. According to Dobbelaere et al. [19], positive responses to inoculation with *A. brasilense* are obtained even when the crops are grown in soils with high N content available, which indicates that the plant responses occur not only due to the $N_2$ fixed but also mainly depending on the production of phytohormone growth promoters

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such as cytokinin, gibberellin, and indoleacetic acid. Lemos et al. [20], studying five wheat cultivars, found a positive interaction of *A. brasilense* and nitrogen fertilization only for one wheat cultivar (CD 150). Increases in nitrogen fertilization efficiency associated with inoculation with *A. brasilense* were reported by Galindo et al. [21] but in the grain yields of corn in the Brazilian Cerrado.

Considering the benefits attributed to several crops by inoculation with *A. brasilense*, with emphasis on biological nitrogen fixation, greater development of the root system, and, consequently, greater absorption of water and nutrients, therefore, the inoculation can improve crop performance allowing greater efficiency of nitrogen fertilization. Thus, research that evaluates the nutritional status and wheat yield, in terms of nitrogen rates in association with inoculation with *A. brasilense*, is important.

## 2. Materials and methods

The wheat experiment was conducted in 2014, in an experimental area that belongs to the UNESP Engineering Faculty, located in Selvíria—MS/Brazil—with the following geographical coordinates 20°22′S and 51°22′W and an altitude of 335 m. Soil in this experimental area was classified as Distroferric Red Oxisol with clay texture (with values of particle size of 420, 50 $kg^{-1}$, and 530 g of sand, silt, and clay, respectively), according to Embrapa [22], which has been cultivated with annual cultures over 27 years and the last 11 years with no-tillage system. The area was under corn cultivation before sowing wheat. The annual average temperature was 23.5°C, annual average pluvial precipitation was 1370 mm, and annual average relative air humidity was between 70 and 80%.

Glyphosate [1800 g $ha^{-1}$ of active ingredient (a.i.) and 2,4-D (670 g $ha^{-1}$ of a.i.)] herbicides were used for desiccation and applied in 2 weeks prior to sowing wheat. Chemical attributes of the soil in the tillable layer were determined before the wheat experiment began. The methods proposed by van Raij et al. [23] showed the following results: 13 mg $dm^{-3}$ of P (resin), 6 mg $dm^{-3}$ of $S{=}SO_4$, 23 g $dm^{-3}$ of organic matter (OM), pH ($CaCl_2$) of 4.8, 2.6 $mmol_c$ $dm^{-3}$ of $K^+$, 13.0 $mmol_c$ $dm^{-3}$ of $Ca^{2+}$, 8.0 $mmol_c$ $dm^{-3}$ of $Mg^{2+}$, 42.0 $mmol_c$ $dm^{-3}$ of H + Al, 5.9 mg $dm^{-3}$ of Cu, 30.0 mg $dm^{-3}$ of Fe, 93.9 mg $dm^{-3}$ of Mn, 1.0 mg $dm^{-3}$ of Zn (DTPA), 0.24 mg $dm^{-3}$ of B (hot water), and 36% base saturation. After soil chemical analysis, 2.5 t $ha^{-1}$ of dolomitic limestone (with 88% relative total neutralizing power) was directly applied as topdressing 80 days before the wheat was sown in 2014 in order to elevate base saturation to 70%, as recommended by Cantarella et al. [24].

The experimental design was a randomized block with four replications, in a factorial scheme 5 × 2, with five N rates (0, 50, 100, 150, and 200 kg $ha^{-1}$, as urea) applied as topdressing at the growth stage 3.2 on Zadok's scale [25], with and without seed inoculation with *A. brasilense*. Wheat seeds were inoculated with 300 mL $ha^{-1}$ of inoculant liquid of *A. brasilense* bacteria AbV5 and AbV6 strains (guaranteed minimum analysis of $2 \times 10^8$ UFC $mL^{-1}$). The inoculant was mixed with the seeds using a cement mixer, 1 h before planting and after the seed treatments with carbendazim + thiram fungicides (45 + 105 g

a.i. per 100 kg of seed) and thiodicarb + imidacloprid insecticides (45 + 135 g a.i. per 100 kg of seed). Each plot consisted of 6 m in length with 12 lines and an inter-row spacing of 0.17 m. The usable area of the plot was eight center lines, excluding 0.5 m extremities. The plot size was 10.20 $m^2$.

Were applied 350 kg $ha^{-1}$ of the 08-28-16 formulation in the forms of urea, triple superphosphate, and potassium chloride, respectively, at wheat sowing was applied. The experiments were conducted in a no-tillage system. The area in both crops was irrigated by a central pivot sprinkler system. The water coverage was 14 mm over a period of around 72 h. The cultivar used was the CD 116, and sowing was done with an experimental machine on 05/16/14, with 80 seeds being sown per meter. Metsulfuron-methyl (3.0 g a.i. $ha^{-1}$), a postemergence herbicide, was applied 20 days after emergence (DAE) to control weeds, like *Ipomoea grandifolia*, *Tridax procumbens*, and *Spermacoce latifolia*. The seedling emergence was 6 days after sowing. Topdressing with nitrogen fertilization was performed at 35 DAE, manually distributing the fertilizer on the soil surface (no incorporation) beside and approximately 8 cm of sowing lines in order to avoid the contact of the fertilizer with the plants. After this topdressing, the area was irrigated by sprinkling (depth 14 mm) at night to minimize N losses by volatilization of ammonia, a procedure common in the irrigated wheat crop. The plants were harvested 110 days after wheat emergence.

Concentrations of N, P, K, Ca, Mg, S, Cu, Fe, Mn, and Zn were measured in the grain and straw (above the soil) of wheat at harvest occasion (the end of the crop cycle), in 10 plants per useful area of plot. The determination of nutrients was carried out as described by Malavolta et al. [26]. The wheat was harvested from the plants in the useful area of each plot, and grain yield was calculated after mechanical threshing. Data were transformed into kg $ha^{-1}$ and corrected for 13% moisture (wet basis). The agronomic efficiency of the treatments was determined:

$$AE = \frac{\text{grain yield with fertilizer} - \text{grain yield without fertilizer}}{\text{amount of N applied}}. \quad (1)$$

The results were subjected to analysis of variance and Tukey's test at 5% probability to compare the averages of plants that had been inoculated with *A. brasilense* with those that had not been inoculated. Regression equations were fitted for the effect of N rates using the Sisvar program [27]. For the Pearson correlation analysis, separated from inoculated and non-inoculated treatments, we used SAS program [28].

## 3. Results and discussion

The increase in nitrogen rates isolated did not influence the nutrients concentrations in irrigated wheat grains, inclusive of N (**Table 1**). However, it is worth noting that the nutrients N, P, and S concentrations presented in the diagnosis leaf (data not shown) were higher

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than average recommended by Cantarella et al. [24], whose ranges for these nutrients are 20–34, 1.5–3, and 2.1–3.3 g $kg^{-1}$, respectively. For average of Ca and Mg, leaf concentrations are within the recommendation by Cantarella et al. [24] as appropriate, whose ranges for such nutrients are 2.5–10.0 and 1.5–4.0 g $kg^{-1}$. The K leaf concentration was slightly below 15 (13.5 g $kg^{-1}$), being the critical level considered as appropriate. However, the average of leaf concentrations of Cu, Fe, Mn, and Zn were suitable, whose ranges for these nutrients are 5–25; 10–300, 25–150, and 20–70 mg $kg^{-1}$ [24], respectively.

With regard to inoculation with *A. brasilense*, the concentrations of P, Ca, andMg were positively influenced by the use of bacteria, where inoculated treatments showed higher concentrations of these nutrients in wheat (**Table 1**). Increasing concentrations of P, Ca, and Mg in the grain raise the possibility of partial immobilization of nutrients in the plant by the bacteria and subsequent release of the same for plants. These bacteria can act on plant growth by producing substances promoting development (auxins, gibberellins, and cytokinins) which provide better root growth [29] and, therefore, have greater uptake of water and nutrients [30] resulting in a more vigorous and productive plant [12, 18], and to be free-living organisms with endophytic characteristics, it is possible to perform some of the metabolic and vital process use of nutrients in the plant, which would then be made available to reflect in increased concentrations in the grains.

Inoculation with *A. brasilense* provided higher concentrations of Mn and Zn in the grains compared to treatments not inoculated, probably due to the possibility of temporary immobilization and subsequent greater redistribution of nutrients to the grain filling (**Table 1**). This result is very interesting, because the increase of Zn in cereal grains such as wheat is the target of a series of research related to agronomic biofortification, since many people are deficient in zinc, especially in less developed countries of the world.

The interaction between nitrogen rates and inoculation with *A. brasilense* was significant for the Mg concentration in the grains. In the absence of N and at doses of 50 and 100 kg $ha^{-1}$, the treatments inoculated with *A. brasilense* via seed showed higher Mg concentration in the grains compared to treatment that was not inoculated (**Table 2**). There was linear increasing function adjusted for nitrogen rates where there was no inoculation (**Figure 1**), but these values always were lower compared to treatments with inoculation.

The increase in nitrogen rates did not influence the concentration of macronutrients and Cu, Mn, and Zn in wheat straw (**Table 1**). Only the Fe straw concentration was influenced by N rates, adjusting the increasing linear function (**Figure 2**).

Inoculation with *A. brasilense* influenced the concentrations of P and S in the wheat straw in distinct forms. For the P concentration, inoculation promoted lower concentrations of this nutrient in the straw, which is explained by the greater redistribution and accumulation of P in wheat grain filling, as previously reported. However, for the S concentration, in treatments that were performed, the inoculation showed higher concentration of nutrients in the straw (**Table 1**), which can be explained by the greater S uptake in the subsurface soil layers, due to further deepening of the system root of inoculated wheat.

| N rates (kg ha$^{-1}$) | N (g kg$^{-1}$) | | P (g kg$^{-1}$) | | K (g kg$^{-1}$) | | Ca (g kg$^{-1}$) | | Mg (g kg$^{-1}$) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Grain | Straw | Grain | Straw | Grain | Straw | Grain | Straw | Grain | Straw |
| 0 | 27.12$^{ns}$ | 5.32$^{ns}$ | 4.84$^{ns}$ | 0.62$^{ns}$ | 6.77$^{ns}$ | 23.22$^{ns}$ | 0.48$^{ns}$ | 1.36$^{ns}$ | 1.48$^{ns}$ | 0.76$^{ns}$ |
| 50 | 25.81 | 4.79 | 4.67 | 0.66 | 6.47 | 21.36 | 0.46 | 1.40 | 1.43 | 0.64 |
| 100 | 26.55 | 4.76 | 5.18 | 0.61 | 7.14 | 22.09 | 0.53 | 1.26 | 1.46 | 0.68 |
| 150 | 28.28 | 5.28 | 5.33 | 0.58 | 7.33 | 23.47 | 0.53 | 1.34 | 1.45 | 0.68 |
| 200 | 25.27 | 4.67 | 4.86 | 0.60 | 6.89 | 21.13 | 0.49 | 1.38 | 1.53 | 0.68 |
| Inoculation | | | | | | | | | | |
| With *A. brasilense* | 26.92 a | 5.23 a | 5.23 a | 0.35 b | 7.08 a | 22.09 a | 0.56 a | 1.30 a | 1.65 a | 0.70 a |
| Without *A. brasilense* | 26.29 a | 4.70 b | 4.72 b | 0.88 a | 6.76 a | 22.42a | 0.43 b | 1.40 a | 1.30 b | 0.67 a |
| LSD (5%) | 0.99 | 0.45 | 0.33 | 0.11 | 0.39 | 1.21 | 0.05 | 0.14 | 0.14 | 0.05 |
| Overall mean | 26.61 | 4.96 | 4.98 | 0.61 | 6.92 | 22.25 | 0.50 | 1.35 | 1.47 | 0.69 |
| CV (%) | 7.09 | 17.38 | 12.79 | 23.77$^{\#}$ | 10.81 | 10.38 | 20.73 | 20.06 | 18.33 | 13.80 |
| **N rates (kg ha$^{-1}$)** | **S (g kg$^{-1}$ )** | | **Cu (mg kg$^{-1}$)** | | **Fe (mg kg$^{-1}$)** | | **Mn (mg kg$^{-1}$)** | | **Zn (mg kg$^{-1}$)** | |
| | **Grain** | **Straw** | **Grain** | **Straw** | **Grain** | **Straw** | **Grain** | **Straw** | **Grain** | **Straw** |
| 0 | 2.59$^{ns}$ | 1.76$^{ns}$ | 7.25$^{ns}$ | 8.42$^{ns}$ | 45.08$^{ns}$ | 235.25$^{**}$ | 63.83$^{ns}$ | 82.17$^{ns}$ | 39.92$^{ns}$ | 9.33$^{ns}$ |
| 50 | 2.58 | 1.67 | 6.25 | 10.33 | 39.67 | 275.67 | 59.50 | 84.42 | 37.08 | 9.42 |
| 100 | 2.55 | 1.65 | 7.25 | 6.50 | 44.58 | 232.25 | 76.58 | 78.42 | 41.83 | 8.33 |
| 150 | 2.67 | 1.72 | 7.83 | 8.33 | 50.67 | 263.08 | 79.58 | 78.83 | 47.83 | 9.42 |
| 200 | 2.62 | 1.74 | 7.67 | 9.17 | 42.17 | 321.33 | 67.58 | 88.42 | 39.50 | 8.75 |

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| N rates (kg ha$^{-1}$) | S (g kg$^{-1}$ ) | | Cu (mg kg$^{-1}$) | | Fe (mg kg$^{-1}$) | | Mn (mg kg$^{-1}$) | | Zn (mg kg$^{-1}$) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Grain | Straw | Grain | Straw | Grain | Straw | Grain | Straw | Grain | Straw |
| Inoculation | | | | | | | | | | |
| With *A. brasilense* | 2.57 a | 1.92 a | 6.87 a | 7.87 a | 46.33 a | 270.30 a | 76.30 a | 82.47 a | 44.53 a | 9.40 a |
| Without *A. brasilense* | 2.64 a | 1.49 b | 7.63 a | 9.23 a | 42.53 a | 260.73 a | 62.53 b | 82.43 a | 37.93 b | 8.70 a |
| LSD (5%) | 0.07 | 0.09 | 0.86 | 1.95 | 4.73 | 31.06 | 10.86 | 9.91 | 4.60 | 1.38 |
| Overall mean | 2.60 | 1.71 | 7.25 | 8.55 | 44.43 | 265.52 | 69.42 | 82.45 | 41.23 | 9.05 |
| CV (%) | 5.42 | 9.94 | 22.73 | 23.58[#] | 20.39 | 22.38 | 29.92 | 23.00 | 21.34 | 29.22 |

Means followed by the same letter in the column do not differ by Tukey's test at 5%.

[**]significance at $p < 0.01$.

ns significance at not significant.

[#]data fitted by following equation $(x + 0.5)^{0.5}$.

**Table 1.** Means and Tukey's test concerning nitrogen, phosphorus, potassium, calcium, magnesium, sulfur, cooper, iron, manganese, and zinc grain and straw concentrations of wheat affected by nitrogen rates, with or without inoculation by *A. brasilense*.

| Inoculation | N rates (kg ha$^{-1}$) | | | | |
|---|---|---|---|---|---|
| | **0** | **50** | **100** | **150** | **200** |
| With *A. brasilense* | 1.77 a | 1.65 a | 1.68 a | 1.47 a | 1.62 a |
| Without *A. brasilense* | 1.18 b | 1.22 b | 1.23 b | 1.43 a | 1.43 a |
| LSD (5%) | 0.31 | | | | |

Means followed by the same letter in the column do not differ by Tukey's test at 5%.

**Table 2.** Inoculation by *A. brasilense* and nitrogen rate interaction for magnesium grain concentration of wheat.

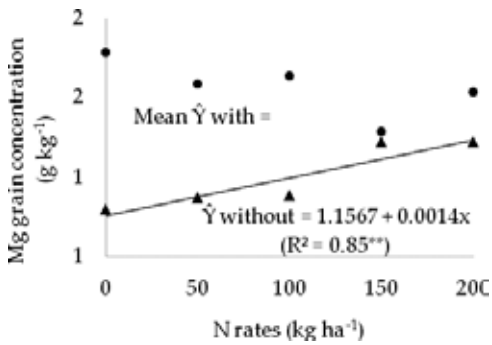


**Figure 1.** Magnesium grain concentration of wheat in regard to nitrogen rate interaction within inoculation.

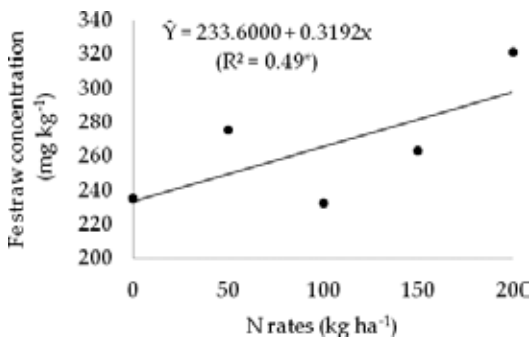


**Figure 2.** Iron straw concentration of wheat in regard to nitrogen rate.

The *Azospirillum* genus encompasses a group of bacteria that promote plant growth, free life that is found in almost all places of the earth; there are reports also that bacteria of this kind can be facultative endophytic [31]. *Azospirillum* genus of bacteria can act on plant growth by reducing nitrate to ammonia; this energy can be made available to other vital metabolic processes [32]. Nevertheless, this fixation process also requires energy in the form of adenosine triphosphate (ATP) to occur [33], which raises the possibility that these bacteria temporarily immobilize some

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plant nutrients such as K, Ca, Mg, S, Mn, Zn, and especially P for their metabolic processes and subsequently make available again to plants, reinforcing the results obtained in the nutritional assessment, which observed lower concentrations of P, K, and Ca in leaf tissue and a smaller concentration of P in the straw but increases in the concentrations of P, Ca, Mg, Mn, and Zn in the grains, being interesting from the point of view of human or animal nutrition.

The interaction between nitrogen rates and inoculation was significant for the N concentration in the straw. The treatments that were inoculated by seed with the bacteria *A. brasilense* at the rate of 100–150 kg $ha^{-1}$ N showed higher N concentration in the straw compared to uninoculated treatments (**Table 3**) and, therefore, contributed more to the supply of this important nutrient for subsequent crops. There was linear decreasing function adjusted for nitrogen rates in treatments that were not inoculated, that is, the increase of N rates resulted in decreased concentration of N in the straw (**Figure 3**), indicating a greater need to redistribute N of the straw for grains. Thus, it appears that there was a greater contribution to the absorption of N due to the further development of the root system in relation to biological $N_2$ fixation when there was seed inoculation with *A. brasilense*. This bacterium can act on plant growth by producing substance promoters for development of (auxins, gibberellins, and cytokinins), which provide improved root growth [29] and consequently

| Inoculation | Doses de N (kg $ha^{-1}$) | | | | |
|---|---|---|---|---|---|
| | 0 | 50 | 100 | 150 | 200 |
| With *A. brasilense* | 5.23 a | 4.62 a | 5.32 a | 5.88 a | 5.08 a |
| Without *A. brasilense* | 5.40 a | 4.97 a | 4.20 b | 4.67 b | 4.25 a |
| LSD (5%) | 1.01 | | | | |

Means followed by the same letter in the column do not differ by Tukey's test at 5%.

**Table 3.** Inoculation by *A. brasilense* and nitrogen rate interaction for nitrogen straw concentration of wheat.

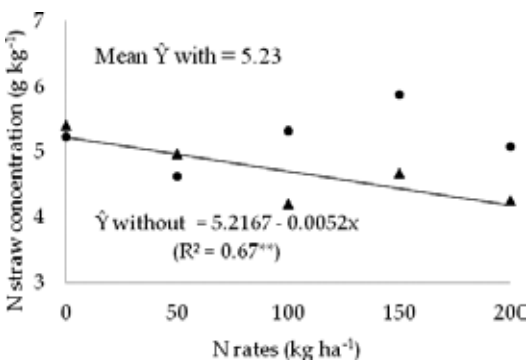


**Figure 3.** Nitrogen straw concentration of wheat in regard to nitrogen rate interaction within inoculation.

greater absorption of water and nutrients [30], resulting in more vigorous and productive plant [12, 18].

For treatments not inoculated, the Pearson correlation was significant between the concentration of N in the grains and K concentration in straw (0.5131*), Cu in straw (−0.5584*), and Zn in grains (0.4573*). For the treatments inoculated with *A. brasilense*, the correlation was significant between the concentration of N in grains and Fe concentrations in the straw (−0.4440*), K in grains (0.4547*), Ca in grains (0.4994*), and Mg in the grain (0.5087*).

The Pearson correlation was significant between the concentration of N in straw and concentrations in grains of N (1.0000**), of K (0.4547*), of Ca (0.4994*), and of Mg (0.5087*) in the treatments inoculated with *A. brasilense*. However, there was no correlation between the N concentration in straw and the other variables in the non-inoculated treatments.

For the agronomic efficiency of wheat, there was no significant difference between with or without inoculation by *A. brasilense* (**Table 4**), even though numerically in inoculated wheat, the efficiency of nitrogen fertilization has been higher. The interaction between N rates and inoculation was significant for the grain yield of wheat. Inoculated treatments at the rate of 150 kg $ha^{-1}$ N were greater in grain yield of non-inoculated treatments (**Table 5**). There was linear increasing function adjusted for nitrogen rates in the treatments without inoculation and the quadratic function adjusted for the treatments inoculated with positive response up to the dose of 139 kg $ha^{-1}$ N (**Figure 4**). However, *A. brasilense* alone is not effective enough to replace the entire nitrogen fertilization but, associated with N fertilization, makes it possible to achieve the highest yields of irrigated wheat grains in the Brazilian Cerrado.

| N rates (kg $ha^{-1}$) | Grains yield (kg $ha^{-1}$) | Agronomic efficiency (kg grain $kg^{-1}$ N) |
|---|---|---|
| 0 | 2269 | – |
| 50 | 3004 | 13.92 |
| 100 | 3132 | 8.59 |
| 150 | 3266 | 6.75 |
| 200 | 3161 | 4.76 |
| Inoculation | | |
| With *A. brasilense* | 2996 | 9.40 a |
| Without *A. brasilense* | 2937 | 7.61 a |
| LSD (5%) | 227 | 3.33 |
| Overall mean | 2966 | 8.51 |
| CV (%) | 17.12 | 24.78[#] |

Means followed by the same letter in the column do not differ by Tukey's test at 5%.
[#]data fitted by following equation $(x + 0.5)^{0.5}$.

**Table 4.** Means and Tukey's test concerning grain yields and agronomic efficiency of wheat affected by nitrogen rates, with or without inoculation by *A. brasilense*.

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| Inoculation | N rates (kg ha$^{-1}$) | | | | |
|---|---|---|---|---|---|
| | **0** | **50** | **100** | **150** | **200** |
| With *A. brasilense* | 2196 a | 2916 a | 3205 a | 3544 a | 3119 a |
| Without *A. brasilense* | 2342 a | 3092 a | 3060 a | 2989 b | 3203 a |
| LSD (5%) | 508 | | | | |

Means followed by the same letter in the column do not differ by Tukey's test at 5%.

**Table 5.** Inoculation by *A. brasilense* and nitrogen rate interaction for grains yield of wheat.

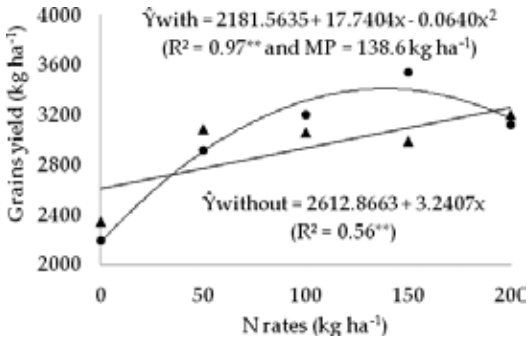


**Figure 4.** Grain yield of wheat in regard to nitrogen rate interaction within inoculation.

Regarding grain yield, several authors also reported a positive response to nitrogen fertilization on wheat [2, 3, 7–10]. In similar climatic conditions for the cultivation of wheat as a winter crop in the Cerrado region with low altitude, Cazetta et al. [7] and Teixeira Filho et al. [3, 8, 9] suggested maximum grain yield with N doses ranging from 78 kg ha$^{-1}$ [7], 90 kg ha$^{-1}$ [3, 8] to 120 kg ha$^{-1}$ [9]. These differences in rates of N that provide maximum productivity of wheat are due to different requirements of N from the cultivars, as well as the variation in climate and soil conditions.

Lemos et al. [20] studied five wheat cultivars (CD 104, CD 108, CD 119, CD 120, and CD 150), with and without inoculation and, associated with nitrogen rates, found that response to inoculation with *A. brasilense* in wheat crop occurs satisfactorily when held in conjunction with the nitrogen fertilization, as observed in this study at a dose of 150 kg ha$^{-1}$ N (**Table 5**). On the other hand, Ferreira et al. [34], working with foliar application of *A. brasilense* and nitrogen rates in the wheat crop in the Brazilian Cerrado, observed that inoculation had no effect on grain yield. Similarly, Nunes et al. [4] studied inoculation with *A. brasilense* in soils with high and low availability of N, and Galindo et al. [35], in research with application times by leaf of *A. brasilense* with the application of 100 kg ha$^{-1}$ N, found no effect of inoculation in the production components and grain yield of wheat in the Cerrado region.

Tarumoto et al. [36], analyzing inoculation with *A. brasilense* and seed treatment with pesticides on irrigated wheat yield in the Cerrado region and agreeing with the results obtained

in this study, also did not verify influence of inoculation alone, on yield of irrigated wheat crop. However, Santa et al. [37] found significant effects on the wheat yield (average increase of 23.9% compared to the control) in the treatment inoculated with *A. brasilense*, both with and without the addition of nitrogen fertilization. While Piccinin et al. [38] suggested that the use of N can be reduced by up to 50% when inoculation with *A. brasilense* is performed. Zorita and Caniggia [39] also reported significant increases on grain yield after inoculation of *A. brasilense* on wheat seeds. Hungria [12] also observed a mean increase in grain yield of 31% for wheat. However, it is noteworthy that the affinity of cultivar with the strains of this bacterium diazotrophic may vary and determine the success or failure of inoculation with *A. brasilense*.

Agronomic efficiency was negatively affected by the increase of N rates (**Table 4**), with adjustment to decreasing linear function due to higher losses of N in the soil (**Figure 5**), as we know, the higher the dose, the greater will be the loss. Increases in the efficiency of nitrogen fertilization associated with inoculation with *A. brasilense* were reported by Galindo et al. [21] but in the corn crop in the Brazilian Cerrado. According to Dobbelaere et al. [19], positive responses to inoculation with *A. brasilense* are obtained even when the crops are grown in soils with high N content available, which indicates that the plant responses occur not only due to the $N_2$ biological fixation but also mainly depending on the production of phytohormones growth promoters such as the cytokinin, gibberellin, and indoleacetic acid. This fact may possibly has favored root development of wheat, which according to Novakowiski et al. [40] improved the utilization efficiency of the residual N, uptake of water, and other nutrients directly reflected in a higher agronomic efficiency of the plant with *A. brasilense* inoculation as found in the present study for grain yield.

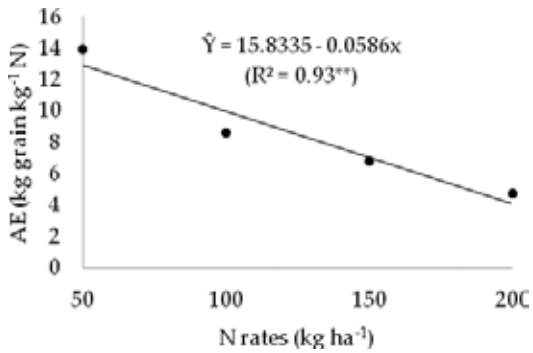


**Figure 5.** Agronomic efficiency (EA) of wheat in regard to nitrogen rate.

## 4. Conclusion

Inoculation with *A. brasilense* increased concentrations of Ca, Mg, Mn, and Zn in grain and concentrations of N and S in wheat straw. This bacterium decreases the straw concentration of P, but it increases grain concentration of P.

The straw concentration of N decreased linearly with the increase of N doses, only without inoculation with *A. brasilense*. That is, when inoculation with these bacteria occurred, there was a lower N redistribution from leaves and culms into the grain filling, without the reduction in the N grains concentration. So, it is a great interest for the supply of N to subsequent crops.

The increase in N rates in association with *A. brasilense* inoculation increases the wheat yield up to 139 kg $ha^{-1}$ N, whereas without this inoculation, linear increase occurred with lower maximum yield of wheat. That is, the inoculation afforded higher grain yield applying less nitrogen fertilizer in topdressing.

This research demonstrated that inoculation with *A. brasilense* associated with nitrogen fertilization in topdressing is beneficial to nutrition and wheat yield. Therefore, inoculation is a low-cost technique, easy to apply and use, and nonpolluting, which fall within the desired sustainable context in actuality; the trend is that this technology be increasingly used in wheat crop.

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Chapter 7

# The Role of Soil Beneficial Bacteria in Wheat Production: A Review

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Additional information is available at the end of the chapter

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**Abstract**

Free-living plant growth-promoting rhizobacteria (PGPR) have favourable effect on plant growth, tolerance against stresses and are considered as a promising alternative to inorganic fertilizer for promoting plant growth, yield and quality. PGPR colonize at the plant root, increase germination rates, promote root growth, yield, leaf area, chlorophyll content, nitrogen content, protein content, tolerance to drought, shoot and root weight, and delayed leaf senescence. Several important bacterial characteristics, such as biological nitrogen fixation, solubilization of inorganic phosphate and mineralization of organic phosphate, nutrient uptake, 1-aminocydopropane-1-carboxylic acid (ACC) deaminase activity and production of siderophores and phytohormones, can be assessed as plant growth promotion traits. By efficient use, PGPR is expected to contribute to agronomic efficiency, chiefly by decreasing costs and environmental pollution, by eliminating harmful chemicals. This review discusses various bacteria acting as PGPR, their genetic diversity, screening strategies, working principles, applications for wheat and future aspects in terms of efficiency, mechanisms and the desirable properties. The elucidation of the diverse mechanisms will enable microorganisms developing agriculture further.

**Keywords:** PGPR, wheat, abiotic stress, enzymes, nitrogen fixation

## 1. Introduction

Wheat (*Triticum aestivum* L.) is one of the three major cereals (together with maize and rice), major source of energy, renewable resource for food, feed and industrial raw material, protein and fibre source in human diet, staple food crop for more than one-third of the world population [1], grown both as a spring and winter crop.

Plant growth-promoting bacteria (PGPR), typically colonizing at the rhizosphere, is known to increase the yield and help alleviating the effects of biotic or abiotic stresses [2]. The practice of PGPRs is promising in reducing the use of chemical fertilisers, at the same time maintaining yields at commercially viable levels and/or maintaining grain protein content [3]. As such, PGPR contributes to the improvement of both local and global environments, reducing dependence on non-renewable resources while still being economically competitive (both price and quality aspect) [4–6].

Several beneficial free-living rhizobacteria have been termed as PGPR, including, but not limited to, *Acinetobacter, Acetobacter, Alcaligenes, Arthrobacter, Azotobacter, Azospirillum, Bacillus, Burkholderia, Beijerinckia, Enterobacter, Flavobacterium, Methylobacterium, Pseudomonas, Rhizobium, Paenibacillus* and *Pantoea* [7–10]. These bacteria enhance growth through numerous mechanisms [2, 11–15]. A short list of mechanisms cover:

- The biological nitrogen fixation (BNF) and phosphate solubilization
- Secretion of hormones, for example, auxins, indole acetic acid (IAA), cytokinins, gibberellins and ethylene
- Facilitating the uptake of essential nutrients (N, P, Fe, Zn, etc.) from the atmospheric air and soil
- Zinc and iron solubilization and organic matter mineralization
- Secretion of certain volatiles and lowering of plant ethylene level
- Induction of systemic resistance
- Production of 1-aminocyclopropane-1-carboxylate deaminase (ACC)
- Quorum sensing (QS) signal interference and inhibition of biofilm formation
- Promoting beneficial plant-microbe symbioses
- Exhibiting antifungal activity, exhibition of antagonistic activity against phytopathogenic microorganisms by producing siderophores, b-1,3-glucanase, chitinases and antibiotics
- Interference with pathogen toxin production.

A non-exhaustive list of Plant Growth Promoting Rhizobacteria (PGPR)s used to alleviate various stresses is given in **Table 1**, and the various other uses of these bacteria are listed in **Table 2**. Two important mechanisms employed by PGPR are the production of different phytohormones,

| Stress type | Bacterial inoculate | Properties of the crop | Reference |
|---|---|---|---|
| Drought/water | *Azospirillum brasilense* Sp245 | Wheat, growth rate of coleoptiles | [132] |
| Drought | *Azospirillum brasilense* INTA Az-39 wheat roots | Wheat (*T. aestivum*) | [133] |
| Drought | *Azospirillum lipoferum* | Wheat (*T. aestivum* L.) | [134] |
| Drought | *Burkholderia phytofirmans* | Wheat (*T. aestivum*)<br>Grain yield, photosynthetic rate, water use efficiency, chlorophyll content | [135] |
| Drought | *Bacillus safensis, Ochrobactrum pseudogregnonense* | Wheat (*T. aestivum*) | [137] |
| Heavy metal-stressed | *Bacillus sp* | Wheat (*T. aestivum*)<br>Indole-3-acetic acid<br>Antioxidant defence system<br>SOD shoots and roots<br>Shoot POD and CAT | [198] |
| Heavy metal | *Bacillus thuringiensis, Azotobacter chroococcum, Paenibacillus ehimensis, Pseudomonas pseudoalcaligenes* | Higher heavy metal resistance<br>Siderophore,, indole acetic acid, HCN, P solubilization | [151] |
| Osmotic stress | *Azospirillum* | Wheat (*T. aestivum*) | [199] |
| Osmotic stress | *Azospirillum brasilense* sp. 245 | Wheat (*T. aestivum*) | [200] |
| Cold | *Pseudomonas* spp. | IAA, P solubilization, rhamnolipids, siderophores | [201] |
| Cold | *Bacillus megaterium* M3, *Bacillus subtilis* OSU142, *Azospirillum brasilense* Sp245, *Raoultella terrigena* | Root and shoot dry weight, leaf total chlorophyll content, stomatal conductance, leaf relative water content | [171] |
| Temperature | *Bacillus amyloliquefaciens* and *Azospirillum brasilense* | Wheat (*T. aestivum*) | [202] |
| Heat stress | *Pseudomonas putida* AKMP7 | Wheat (*Triticum* spp) | [98] |
| Temperature | *Pseudomonas fluorescens, Pantoea agglomerans, Mycobacterium* sp | Wheat (*T. aestivum*) | [203] |
| Salinity | *Azospirillum* | Wheat (*T. aestivum*) | [62] |
| Salinity | *Pseudomonas putida, Pseudomonas extremorientalis, Pseudomonas chlororaphis* and *Pseudomonas aurantiaca*. | Wheat (*T. aestivum* cv. Turon)<br>wheat root tip coloniser, tolerated salt | [125] |
| Salinity | *Pseudomonas fluorescens* 153, 169, *Pseudomonas putida* 108 | Wheat (Triticum aestivum) grain yield, 1000 grain weight, grain yield | [143] |
| Salinity | *Pseudomonas putida* N21, *Pseudomonas aeruginosa* N39 and *Serratia proteamaculans* M35 | Wheat (*Triticum aestivum* L.) | [142] |
| Salinity | *Azospirillum* sp | Durum wheat (*Triticum durum*) | [128] |

| Stress type | Bacterial inoculate | Properties of the crop | Reference |
|---|---|---|---|
| Salinity | *Pseudomonas putida, Enterobacter cloacae, Serratia ficaria*, and *Pseudomonas fluorescens* | Wheat | [204] |
| | *Bacillus, Burkholderia, Enterobacter, Microbacterium, Paenibacillus* | Wheat (*T. aestivum*) | [46] |
| Salinity | *Bacillus pumilus, Pseudomonas mendocina, Arthrobacter* sp., *Halomonas* sp., and *Nitrinicola lacisaponensis* | P solubilization, indole acetic acid (IAA), siderophore, ammonia,proline accumulation, salt tolerance, choline oxidase activity | [140] |
| | *Streptomyces* sp | Wheat (*T. aestivum*) | [85] |
| Salinity | *B. subtilis, Arthrobacter* sp. | Wheat (*T. aestivum*) | [97] |
| | *Azospirillum* sp. | Wheat (*T. aestivum*) | [95] |
| Salinity | *Pseudomonas putida, Enterobacter cloacae, Serratia ficaria* and *P. fluorescens* | Wheat (*T. aestivum*) Germination rate percentage and index and improved nutrient status | [205] |
| Salinity | *Hallobacillus* sp. SL3 and *Bacillus halodenitrificans* PU62 | Root length, root elongation, dry weight | [1] |
| Salinity | *Enterobacter asburiae, Moraxella pluranimalium, Pseudomonas stutzeri* | Number of tillers, grain weight, growth and yield | [138] |

**Table 1.** PGPB-mediated IST against abiotic stress.

| PGPR | Source | Plant growth regulation | Results of addition of bacteria to plants | References |
|---|---|---|---|---|
| *Azospirillum* sp. | Wheat rhizospheric | N2 fixation | Grain yield, dry matter, N content | [32] |
| *Azospirillum brasilense* | Mutant | Indole-3-acetic acid (IAA) | Number and length of lateral roots, distribution of root hairs. | [206] |
| *Cyanobacteria* | Rhizospheric | N2 fixation | Root dry weight, N content root and hoot | [207] |
| *Azorhizobium caulinodans* | Wheat | N2 fixation | Dry weight, nitrogen content | [192] |
| *Azotobacter chroococcum* | Wheat Rhizospheric | P solubilization, N2 fixation, IAA | Seed emergence radicle and plumule length | [114] |
| *Azotobacter* sp. *Azotobacter chroococcum* | Wheat Rhizospheric | N2 fixation | Growth | [73] |
| *Paenibacillus polymyxa* | Wheat | Cytokinin, N2 fixation | Plant growth | [208] |
| *Azospirillum brasilense* Sp7 | Digitaria decumbens | Lectins, $N_2$ fixation | Activities of a-glucosidase, b-glucosidase and b-galactosidase in wheat-seedling | [209] |
| *Azospirillum brasilense* 75, 80 and Sp245 | Non-sterilised and surface-sterilised wheat roots | N2 fixation | Root-hair deformation colonization | [99] |

| PGPR | Source | Plant growth regulation | Results of addition of bacteria to plants | References |
|---|---|---|---|---|
| *Azospirillium brasilense* | Wheat rhizospheric | N2 fixation | Plant growth, N accumulation and content, biomass, grain yield and protein concentration | [164] |
| *Klebsiella pneumoniae* 342 | Maize | N2 fixation | Dry weight of roots and shoots, total N per plant colonized the interior of wheat roots | [26] |
| *Pseudomonas denitrificans Pseudomonas rathonis* | | Auxin | Plant growth | [210] |
| *Bacillus simplex* BS BNM-10, *Bacillus firmus* BF BNM-4 | Wheat roots, | | Biomass number of ears nitrogen accumulation, N content | [172] |
| *Azotobacter chroococcum Pantoea agglomerans* | Geographically and climatically diverse locations | Gibberellic acid (GA), IAA | Increase in number root hairs, thickening of roots, root and shoot biomass | [11] |
| *Pseudomonas* spp | Rhizosphere of wheat | P solubilization, siderophore Indole acetic acid ACC deaminase, diacetyl-phloroglucinol | Protein content, yield and grain quality | [162] |
| *Bacillus* RC01, *Bacillus* RC03 | Rhizosphere of wheat | P solubilization, $N_2$ fixation | Root and shoot weight, total biomass | [111] |
| *Azotobacter chroococcum* | Various sources | $N_2$ fixation | Grain and straw yield, N content in grain and straw | [211] |
| *Rhizobium leguminismarum* Thal-8/ SK8), *Pseudomonas* sp. 54RB | Rice | $N_2$ fixation P solubilization | Root and shoot weight, plant height, spike length, grain yield, seed P content, leaf protein and sugar content | [185] |
| *Pseudomonas putida, P. extremorientalis, P. chlororaphis, P. aurantiaca* | Rhizosphere of wheat grown in saline soil | Hydrogen cyanide (HCN), IAA, ACC deaminase, protease, cellulases competitive colonisers, tolerated salt | Shoot and root length, shoot, root and dry matter of wheat | [125] |
| *Acinetobacter calcoaceticus* | Rhizospheres of wheat. | P solubilization, siderophore IAA | Wheat growth, increase in the rate of germination, in the root length and dry weight | [106] |
| *Azospirillum brasilense* | | $N_2$ fixation | uptake of several macro and micronutrients | [5] |
| *Acinetobacter calcoaceticus, A. baumannii, A. lwoffii* | | $N_2$ fixation, siderophores, P solubilization | Root growth Root length | [45] |
| *Bacillus simplex* KBS1F-3, *Bacillus megaterium* NAS7-L, *Bacillus cereus* KFP9-F, *Paenibacillus alvei* NAS6G-6 | Grass | IAA, siderophores P solubilization | Shoot and root Weight colonisation | [212] |

| PGPR | Source | Plant growth regulation | Results of addition of bacteria to plants | References |
|---|---|---|---|---|
| *Pseudomonas* sp. | Wheat | P solubilization, ACC deaminase, siderophores, IAA | Increased soil enzyme activities, total productivity, and nutrient uptake, nutrient assimilation | [102] |
| *Pseudomonas jessenii* R62; *Pseudomonas synxantha* R81 and arbuscular *mycorrhizal* fungi (AMF) | Wheat roots | P solubilization, IAA, siderophores, ACC deaminase, diacetyl-phloroglucinol | Grain yield Protein and mineral nutrient concentration (P, K, Cu, Fe, Zn, Mn) alkaline and acid phosphatase, urease, dehydrogenase. | [163] |
| *Pseudomonas putida* AKMP7 | sorghum | Phytohormones(IAA, GA), HCN, ammonia, Siderophore, P-solubilization | Increased root, shoot length, dry biomass, chlorophyll content | [98] |
| *Bacillus* sp. (AW1), *Providencia* sp. (AW5), *Brevundimonas diminuta* (AW7) | Rhizosphere of wheat | P-solubilization, $N_2$ fixation, ACC deaminase siderophore, ammonia, HCN | Seedling length, germination, plant height, panicle weight, root weight | [40] |
| *Pseudomonas fluorescens* 153 and 169, *P. putida* 4 and 108 | | ACC deaminase, IAA-like products, P solubilization | Height, tillers, number of grains/spike, garain and straw yield, N, P and K uptake | [54] |
| *Pseudomonas lurida* | Radish | P solubilization IAA, HCN, siderophores | Growth and nutrient uptake parameters | [64] |
| *Providencia sp.* PW5 | Wheat rhizosphere | Ammonia siderophore, HCN, IAA, P solubilization Zn solubilization | N uptake in wheat grain. protein content grain Fe, Zn, Mn, and Cu content | [161] |
| *Pseudomonas fluorescens* MKB37 | Barley | Siderophore ACC deaminase. Protease phytate | Grain number, weight and yield | [23] |
| *Azospirillum sp., Azotobacter* sp. *Bacillus megaterium* | Wheat | $N_2$ fixation, P solubilization | Plant height, number spikes, grain yield, protein content | [213] |
| *Azospirillum brasilense* | Wheat | $N_2$ fixation | Agronomic performance and yield of wheat | [35] |
| *P. fluorescens* and *Serratia* sp. | Rubus and wheat | P solubilization | Shoot length, root and shoot dry weight, P uptake | [214] |
| *Hallobacillus* sp. SL3, *Bacillus halodenitrificans* PU62 | Naturally saline habitats | ACC deaminase, IAA, HCN, siderophores, P solubilization, | Seed germination, root length, root elongation, dry weight root biomass | [1] |
| *A. chroococcum* (W5), *Mesorhizobium ciceri* (F 75), *P.striata* (P27), *S.marcescens* (L11) *A.torulosa* | | $N_2$ fixation P solubilization, | Nutrient status of soil and plants, plant biomass, N and P uptake | [177] |

| PGPR | Source | Plant growth regulation | Results of addition of bacteria to plants | References |
|---|---|---|---|---|
| *Bacillus* OSU-142, *Bacillus* M3, *A. brasilense* sp. 245, *B. megaterium* RC07, *P. polymyxa* RC05, *B. licheniformis* RC08, *R. terrigena, B. cepacia* FS Tur | | $N_2$ fixation P solubilization | Grain and straw N content, root and shoot weight. grain and total biomass yield, protein content, grain weight per spike | [3] |
| *Bacillus* spp. | Rhizospheres of wheat and tomato | IAA, lipase, protease, siderophore, P solubilization salt tolerant | Germination, root length, root weight, panicle weight | [52] |
| *B. subtilis* IB-22 *B. subtilis* IB-21 | | Zeatin type cytokinins | Shoot concentrations of zeatin, total chlorophyll and nitrogen contents of wheat leaves | [90] |
| *Streptomyces* spp. | Wheat roots | P solubilization, phytase, chitinase, IAA, siderophore | Growth, biomass, Fe, Mn and P content antifungal activity | [43] |
| *P. brassicacearum* subsp. *brassicacearum* RZ310 *Pseudomonas* sp. PO283, *Pseudomonas* sp. PO366 | Rhizosphere of wheat | IAA, ACC deaminase | Both coleoptiles and root elongation, root length, wheat seedling growth, growth and biomass of Wheat coleoptiles | [215] |
| *S.liquefaciens, S. marcescens, B. thuringiensis* | Wheat rhizosphere | Zn solubilizing | Enhance grain yield and Zn content of wheat | [160] |
| *Bacillus* sp., *Pseudomonas*, sp., *Arthrobacter* sp. | Wheat rhizosphere | P solubilization | Plant biomass P, K, Mg, Zn and Mn contents at harvest | [216] |

**Table 2.** Examples of plant growth-promoting substances released by some commonly employed PGPR.

including auxins, cytokinins and gibberellins, and the synthesis of several enzymes, such as phosphatase and catalase, modulating plant growth and development as well as strengthening their immune system [16, 17]. In a review, Palacios et al. compiled many molecules facilitating interactions of PGPB with plants [18]. The list includes plant hormones, hydrolytic enzymes, antibiotics, flavonoids, other signal molecules, toxic molecules, siderophores, exopolysaccharide, volatiles, polyamines, lectins and vitamins. The PGPR efficiency, in turn, depends upon a number of factors like soil mineral content, type of crop and its genotype, specific PGPR strain and its combination with the plant, competition with indigenous strains, environmental conditions and the growth parameters evaluated, as illustrated in greenhouse and field trials [3] and other studies [19–22].

Despite the promising features from agronomic efficiency and crop yield perspective, the key bottleneck for the commercial use of PGPRs is their varying performance under field conditions: the results obtained in a field are not always similar to those of laboratory [23], which calls for immediate further research on the agricultural use of these PGPRs.

## 2. Mechanisms of plant growth promotion

### 2.1. Biological nitrogen fixation

PGPR improve plant growth by multiple mechanisms. A well-established mechanism is the biological nitrogen fixation (BNF), as described in extensive literature available on diazotrophic association in wheat and subsequent addition of nitrogen to the ecosystem [24], contributing to the total $N_2$ requirement of wheat [25–27]. Nitrogen fixation is considered to be a direct plant growth-promoting trait and the nitrogen-fixing rhizobacteria provide an alternative source to inorganic nitrogen fertilizers.

*Azospirillum* is a kind of nitrogen-fixing bacterium that lives in close association with plants in the rhizosphere. Its beneficial effects on wheat yields in both greenhouse and field conditions have been reported [28, 29]. Balandreau found that *Azosprillum lipoferum* inoculation increased yield around 1.8 t/ha and wheat grain by up to 30% [30, 31]; Okon and Labandera-Gonzalez by inoculation with *Azospirillum brasilense* [31]. In an earlier study, Boddey et al. were unable to observe fixed N in wheat from similar organisms [32]. Further, Ruppel and Merbach investigated the dinitrogen-fixing ability strain of *Pantoea agglomerans* and *Azospirillum* spp. and in hydroponic experiments with wheat found that bacterial strain inoculation affected plant growth, by nitrogen uptake and the amount of biologically fixed dinitrogen. In this sense, when *Azospirillum* brasilense is inoculated using seed inoculation, it increases the productivity of wheat [33–35]. *P. agglomerans*, as a diazotroph, is able to fix molecular $N_2$ with wheat [36]. Ruppel et al. reported *P. agglomerans* to be superior strain for winter wheat, reporting a grain yield increase for different wheat cultivars ([37], also in Ref. [38]). Moreover, a nitrogen-fixing *P. agglomerans* Lma2 was isolated from wheat rhizosphere, it was found to have the ability to produce IAA, siderophores and solubilize P, and growth performance of plant was significantly better in the presence of salt [39].

*Acinetobacter* strains also possessed BNF properties, siderophore and ammonia production as well as mineral solubilization. Rana et al. reported a positive correlation of BNF potential of *Providencia* spp. AW4 and *Brevundimonas diminuta* AW7 strains with panicle weight and plant height in wheat, indicating the enhancing plant growth role of BNF [40].

### 2.2. Phosphate solubilization and mineralization

Soil stores several structures and forms of phosphate, both organic and inorganic. Phosphorus plays a key role in photosynthesis, respiration, root development, signal transduction, energy transfer, macromolecular biosynthesis and the resistance ability of plants to diseases and adverse conditions. However, majority of soil phosphorus is insoluble that is not available to plants. The secondary significant contributing factor to promoted growth is the availability of phosphorous in the rhizospheric region, as a result of phosphate solubilization by the PGPR [41].

PGPRs serve as phosphate (and zinc) solubilizer (PSB). This is due to the decreased pH of the medium, indicating the possible involvement of organic acids such as gluconic acid. Plant growth promotion can be achieved through solubilization of inorganic phosphates by these

organic acids. de Werra et al. showed that this happens with not only gluconate but also malate [42, 43]. These results were consistent with earlier report on the P and Zn solubilizing properties of *Acinetobacter* sp. [44]. Nearly all the *Acinetobacter* species isolated from rhizosphere soil of the three wheat varieties in the present study were efficient phosphate and zinc solubilizers and produced iron chelating siderophores [45]. Phosphate solubilizing bacteria (PSB) belong largely to the genera pseudomonads, bacilli and rhizobia [46].

Phosphorus-solubilizing *Bacillus* strains have been reported to increase the plant biomass and yield of wheat as well as uptake of nutrients [47]. Similar results have been reported by Afzal et al. when a combination of nitrogen-fixing Rhizobium leguminosarum with P-solubilizing *Pseudomonas* sp. strain 54RB have been used [48]. Similarly, several *Pseudomonas* spp. strains have been tested in the field for their efficacy to increase growth and yield of wheat [49]. Four P solubilizer (*Arthrobacter* WP-2, *Bacillus* MP5, *Rhodococcus* M28 and *Serratia* 5D) and one phytohormone producer (*Azospirillum* WS1) strains tested as single-strain inocula resulted in improved growth of wheat plants [50]. Some *Bacillus* species can improve phosphate solubilization of the soil [51, 52]. On the other hand, Baig et al. reported a positive correlation between P-concentration in soil, P-solubilization activity of the Bacillus strains and P uptake by wheat plants [53]. Along the same line, improvement of growth and yield of wheat was observed and reported upon inoculation with P-solubilizing microorganisms. Both PGPR (*Bacillus* and *Pseudomonas* spp.) are similar in effectively solubilizing phosphate. A short list of phosphate-solubilizing bacteria (PSB) includes *P. fluorescens* 153, *P. fluorescens* 169, *P. putida* 4 and *P. putida* 108 together with their capability in natural soil ecosystem to synthesize ACC deaminase and IAA-like products [54].

Combined application of PSB with conventional fertilizer (50% PSB, 25 kg/ha $P_2O_5$) improves plant growth. Similarly, a combination of PGPRs are more effective when compared with isolated applications as reported by Hassan et al. for wheat crops and by Baig et al. for wheat yield and P uptake [53, 55].

### *2.2.1. Mineralization*

Mineralization of most organic phosphorous compounds is carried out by means of phosphatase enzymes. The conversion of insoluble inorganic P to a form accessible by plants is achieved by PSB via organic acids, chelation and exchange reactions [56]. However, organic P forms, particularly phytates, are predominant in most soils (10–50% of total P) and must be mineralized by phytases (myo-inositol hexakisphosphate phosphohydrolases) to be available P for plants [57, 58]. Previous research has shown that *Bacillus* sp., *Providencia* sp., *Brevundimonas* and *Alcaligenes* were recorded positive for P solubilization [40, 59].

Singh et al. reported that phytase-producing bacteria from Himalayan soils showed ability to solubilize inorganic phosphate, producing phytase, siderophores, ammonia and IAA and increased availability of P, IAA and ammonia leading to increased plant growth [57]. The role of PGPR in production of phosphataes, β-gluconase, dehydroginase, antibiotic, solubilization of phosphates and other nutrients, stabilization of soil aggregates, improved soil structure and organic matter contents has been recognized.

### 2.3. Production of plant hormones and other beneficial plant metabolite

There are five groups of plant hormones of well-known PGRs, namely auxins, gibberellins, cytokinins, ethylene and abscisic acid [60]. Direct plant growth promotion includes symbiotic and non-symbiotic PGPR, which functions through production of these plant hormones [11, 61–63]. Much attention has been given on the role of phytohormone auxin. Production of indole-3-ethanol or indole-3-acetic acid (IAA), the compounds belonging to auxins, which is known to stimulate in cell elongation, division and differentiation responses in plants, has been reported for several bacterial genera [12, 17, 64]. PGPR promote root growth by increasing root surface area, which, in turn, promotes nutrient uptake, thereby indirectly stimulating plant growth positively [52, 65]. Khalid et al. reported a correlation between *in vitro* auxin production and increase in early growth parameters of inoculated wheat seeds [66].

Inoculation with *A. brasilense* Cd and the application of pure IAA to the roots both increased root length, number of lateral roots and number of root hairs in wheat as observed by earlier workers [67, 68]. IAA-producing *Azospirillum* sp. also promoted alterations in the growth and development of wheat (*Triticum aestivum* L.) plants [69–72]. Bacteria of the *Azotobacter* genus synthesize auxins, cytokinins and GA-like substances, and these growth materials are the primary substances controlling the enhanced growth [73]. These hormonal substances, which originate from the rhizosphere or root surface, affect the growth of the closely associated higher plants. The highest concentration of IAA is produced by bacterial strain *P. fluorescens* and *Kocuria* varians [74]. Specifically for wheat, the positive effect of PGPR via IAA has been reported [75–78].

When applied in optimum concentrations, bacterial indole-3-acetic acid (IAA), synthesized by gram-positive and -negative, photosynthetic, methylotrophic and cyanobacteria, is reported to stimulate root hair formation, at the same time increasing the length and the number of primary and lateral roots [66, 72, 79]. IAA synthesis by these bacteria is reported to be affected by tryptophan, vitamins, salt and oxygen levels, as well as pH, temperature, carbon and nitrogen source. For example, IAA from *Azospirillum brasilense* Sp245 stimulates early plant development and increases significantly the plants and roots yield (in dry weight) and the N-uptake efficiency of wheat [71, 80]. The ability to synthesize ABA, particularly under stressful conditions, for example, salinity, and to affect the ABA level in plants was detected in PGPB from the genera *Azospirillum, Bacillus, Pseudomonas, Brevibacterium* and *Lysinibacillus* [15, 81, 82]. Both plants and bacteria can be synthesized via several pathways, including the indole-3-pyruvic acid (IPA), indole-3-acetamide (IAM) and indole-3-acetonitrile (IAN) pathways, which are often regulated by tryptophan, carbon and nitrogen availability, a reduction in growth rate and abiotic factors such as temperature, pH and oxygen [79].

As a PGPR application to wheat seedlings, Sachdev et al. reported that IAA producing *Klebsiella* strains significantly increased the root length and shoot height, when compared with the control, in pot experiments [83, 84]. Similarly, Khalid et al. reported up to 28% higher grain yields in wheat grown in field as a result of seed inoculation in peats with high auxin-producing rhizobacteria [66]. The capability of auxin synthesis detected in many bacterial strains from the genera *Azospirillium, Pseudomonas, Bacillus*, etc., is thought to underlie the activation of plant root growth by these microorganisms [81]. Sadeghi et al. demonstrated

that a *Streptomyces* isolate increased plant growth in wheat and produced indole acetic acid and auxin in presence of salt [85]. Phytohormone-producing *Bacillus* sp. and *B. subtilis* have potential at field level to improve wheat productivity and may be helpful in formulation of an effective biofertilizer for wheat [52, 79, 86–89]. A complete understanding of the IAA system can further mediate the efficient use of these PGPRs for biofertilizer.

Cytokinins can be produced by representative strains of *Bacillus*, *Rhizobium*, *Arthrobacter*, *Azotobacter*, *Azospirillium* and *Pseudomonas*. The plants inoculated with cytokinin-producing bacteria *B. subtilis* showed the increased chlorophyll content and cytokinin accumulation, which led to the increase in weight of shoots and roots [90, 91]. On the other hand, treatment of plant with a substance obtained from cytokinin-producing microorganisms, typically colonizing in wheat roots [92, 93], increased chlorophyll content in leaf; in this case, the level of chlorophyll was comparable to that observed in the plants treated with a synthetic cytokinin benzyladenine. Cytokinins can promote stomatal opening, stimulate shoot growth and decrease root growth.

#### *2.3.1. Accumulation of osmolytes*

Proline is a known osmoprotectant, promoting the protection of the plant from drought, salt and other stresses [94]. Alternative to proline accumulation, another defence strategy is to increase total soluble sugar level in plants under salinity stress. PGPRs have been demonstrated to enhance wheat stress tolerance via osmolyte accumulation as reported in Refs. [95–97]. Ali et al. used *P. putida* AKMP7 resulting in significant increase in proline levels in heat-stressed wheat plants [98].

Yegorenkova et al. suggested that lectin-carbohydrate interactions are involved in the initial stages of bacterial-plant root attachment [99]. Additionally, PGPR producing extracellular polymeric substance are reported to enhance greatly the soil volume macropores and the rhizosphere aggregation of soil, which results in increased water and fertiliser availability to plants [46].

### 2.4. Siderophore and exopolysaccharide production by PGPR

With its unique physico-chemical properties, iron (Fe) has a key role in plant growth, taking part in several metabolic pathways including TCA cycle, nitrogen fixation, respiration and ETC, oxidative phosphorylation and photosynthesis, biosynthetic regulation (chlorophyll, toxin, vitamins, antibiotic, cytochrome and pigment) and as cofactor for numerous enzymes [100]. Following this, iron deficiency (typically caused by low iron bioavailability) is frequently seen at elevated pH, alkali soils in dry regions, as well as in case of excessive fertilizer and pesticides application.

Siderophores are small iron carriers, chemically high-affinity iron chelating compounds secreted by PGPRs and are among the strongest soluble $Fe^{3+}$ binding agents known. Comprehensive information on the role of siderophores in increasing iron oxide solubility and promoting dissolution in soils requires the consideration of the rates of various processes such as siderophore exudation, the uptake, and the degradation rates [101]. In BNF, siderophores are expected to play significant role, since in its very essence, nitrogenase requires Fe [102], also supported by a high correlation between N and Fe uptake.

Siderophore productions promote the crop growth, or protect the plant against pathogens. Produced by microorganisms, these are found in soil solutions and influence Fe nutrition of plants [103]. The role of siderophores has been reported as signalling molecules and as such, their use points to avenues for novel agricultural applications [54].

The wheat seed inoculation was tested for their effect on wheat in terms of healthier germination and productivity. The organisms used were siderophoregenic pyoverdin-producing *Pseudomonas putida* and *Pseudomonas aeruginosa* strains from two diverse habitats. Inoculation with siderophoregenic PGPR increased percentage germination, shoot height, shoot and root length, weight of spikelets, chlorophyll content, grain yield and iron content [100, 104, 105]. Inoculated wheat plants showed increase in total iron uptake and physiologically available iron contents. *Acinetobacter calcoaceticus* obtained from wheat rhizosphere produces catechol type of siderophores during exponential phase, which is influenced by iron content of medium [106]. Ca, Cd and Mg ions and succinic acid stimulated the synthesis of the siderophore examined, whereas Zn and Pb ions partially decreased its level.

Some PGPR strains may also protect plants from salt and drought stress by producing exopolysaccharides (EPS), binding, in turn, Na+ or by biofilm formation [107]. Resultingly, reduced Na+ results in lower Na+ uptake and high K+/Na+ ratio, promoting survival in salt-stressed conditions [107, 108]. Another example is the wheat seedling inoculation by EPS producing strain of *Pantoea agglomerans* (NAS206) isolated from the wheat rhizosphere, growing in a Moroccan vertisol. It had a positive effect on aggregation and stabilization of root-adhering soil, by increased mean aggregate diameter and macroporosity [109].

### 2.5. PGPR and plant nutrient uptake

Seed inoculation with the bacterium has been found to improve the growth and nutrient uptake of wheat seedlings via promotion of the plant growth and increased root surface area or the general root architecture [110]. With enlarged root hairs, nutrient uptake is promoted [21, 71, 77, 111].

The PGPR effects also increase N and P uptake in field trials [112], presumably, by stimulating greater plant root growth. Both *A. chroococcum* and *P. agglomerans* were found to increase plant growth, plant dry matter, as well as N and P uptake [25, 113]. *Azospirillum*-inoculated plants under drought conditions had increased Mg, K and Ca contents compared to non-inoculated plants [62, 114–117]. The increase in nutrient accumulation/uptake due to biofertilizers/PGPR was previously reported in wheat [118–120]. Sharma et al. reported that the majority of 13 tested *Pseudomonas* spp. strains increased the macro (N, P, K and S) and micronutrients uptake (Cu, Fe, Zn and Mn) in wheat [102, 121].

Inoculation of efficient plant-growth-promoting actinobacterial *Streptomyces* species significantly improved the Fe, Mn and P content of wheat plants when compared with an uninoculated control [43, 105]. Yasin et al. investigated the effects of selenate fertilization and bacterial inoculation on Se uptake and plant growth [122]. They found that *Bacillus pichinotyi* enhanced wheat growth, dry weight, shoot length and spike length, Se and Fe concentration in wheat kernels and stems. Selenium (Se) is an essential trace element for humans [123], and

they reported that inoculation with rhizospheric microorganisms significantly enhanced wheat Se content.

### 2.6. Alleviation of abiotic stress in wheat by PGPR

Abiotic stress is the major cause of decreasing crop productivity worldwide. The application of the combination of PGPR and mycorrhizal fungi alleviates the stress conditions, as reported by Nadeem et al., via the regulation of hormones, nutrition uptake and growth [124]. Similar outcomes have been reported by Cakmakci et al. for wheat and spinach plants [77]. Enzymatic activities in the leaves of these plants such as glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, glutathione reductase and glutathione S-transferase have been observed.

Additionally, numerous studies suggested that both IAA and ACC deaminase-producing bacteria protect plants most effectively, against a wide range of different stresses [125]. Notable reports among those are *Azospirillum* strains helping to cope with salt stress [126–128] and *Bacillus* and *Azospirillum* leading to improve heat tolerance in wheat [129].

#### *2.6.1. Drought*

Drought stress, exhibited as limited water supply, usually causes a severe loss in plant yield, where the combination of severity and duration are critical factors for plant survival [130]. The application of PGPR can counteract damaging effects of moisture stress, and therefore boost crop yields. Creus et al. reported that growing *Azospirillum brasilense* Sp245-primed wheat under drought stress conditions resulted in large increase in water content and potential, and apoplastic water function in both shoots and roots compared to the non-primed plants [62].

Moreover, Pereyra et al. reported that *Azospirillum* inoculation provided a better water status in wheat seedlings under osmotic stress due to morphological modifications of the coleoptile xylem architecture [131]. *Azospirillum*-inoculated wheat seedlings subjected to osmotic stress developed significant higher coleoptiles, with higher fresh weight and better water status than non-inoculated seedlings [132]. In this regard, ABA-producing bacteria *Azospirillum* promoted resistance of *Arabidopsis*, maize and wheat plants to soil drought [81]. *Azospirillum brasilense* INTA Az-39-inoculated wheat plants under typical dry land farming conditions exhibited better growth and increased vegetative growth, shoot and root dry matter accumulation, grain number and grain yield [133]. According to Arzanesh et al. results, inoculation of wheat with *Azospirillum* spp. can alleviate drought stress on plant growth and yield through adjusting plant water characters [134].

Inoculation of wheat with *Burkholderia phytofirmans* PsJN significantly diluted the adverse effects of drought on relative water contents and $CO_2$ assimilation rate, thus improving the photosynthetic rate, water use efficiency and chlorophyll content over the uninoculated control [135]. In a similar study conducted on wheat under water stress environment showed that mycorrhizal inoculation enhanced the activities of antioxidant enzymes such as peroxidase and catalase compared to those in uninoculated control plants [136]. Several other studies report similar outcomes [137].

### *2.6.2. Salinity*

Salinity decreases the yield of many crops because salt inhibits plant photosynthesis, protein synthesis and lipid metabolism. Nutrient contents decrease in the roots and shoots with increasing NaCl concentration in the growth medium. PGPR counteract osmotic stress and help plant growth. Investigations on interaction of PGPR with other microbes and their effect on the physiological response of crop plants under different soil salinity regimes are still in incipient stage.

Rhizobacteria that are residing within the rhizosphere of plants growing in saline habitats may have already been adapted to salt stress that may be a valuable resource to develop crop inoculants. Raheem and Ali isolated rhizobacteria that were producing beneficial plant growth-promoting metabolites such as IAA and ACC-deaminase activity [138]. The isolation of indigenous microorganisms from the stress-affected soils and screening on the basis of their stress tolerance and PGP traits may be useful in the rapid selection of efficient strains that could be used as bio-inoculants for stressed crops [139, 140]. For several durum cultivars, PGPR efficacy in mitigating salt stress in tetraploid wheat is salt level and bacterial strain-specific [128, 141, 142]. There are some instances of ameliorating salt-stricken cereal crops by PGPR's. Salinity stress in the wheat was alleviated by inoculations with four strains of PGPR, *Pseudomonas fluorescens* 153, 169, *Pseudomonas putida* 108 and 4 [143]. Upadhyay et al. considered the impact of PGPR inoculation on the growth and antioxidant of wheat under saline conditions [46]. In a follow-up study, Upadhyay et al. investigated the effects of two salt-tolerant PGPR (*B. subtilis* and *Arthobacter* sp.) on wheat plants under different salinity regimes and the results obtained demonstrated alleviation of the salinity stress effects on plants treated with bacteria [97]. Similar outcome has been reported by Nia et al. for *Azospirillum* strains on wheat plants [144]. Several PGPR of the genus *Pseudomonas* contain ACC-deaminase enzyme, and when inoculated into plant roots may sustain plant growth under salinity [125, 142].

### *2.6.3. Mitigation of cold stress in wheat by PGPR*

The over-wintering ability of PGPR is fundamental when considering uses in colder climates. De Freitas and Germida reported that *Pseudomonas* species are able to over-winter in sufficient quantities on the roots of winter wheat [145]. It has also been argued that antifreeze protein activity of many bacterial species may contribute to their survival in colder climates [146–148].

The effect of inoculation with 12 psychrotolerant *Pseudomona* strains on cold alleviation and growth of wheat seedling at cold temperature was investigated in Ref. [105]. Psychrotolerant PGPR inoculation improved metabolite levels, such as chlorophyll, anthocyanin, free proline, total phenolics, starch content, physiologically available iron, proteins and amino acids that are sign of alleviation of cold stress in wheat plants.

Higher chlorophyll content in leaves of cold acclimated winter wheat over control plants was also reported [105]. Proline is a dominant amino acid that accumulates in many organisms upon exposure to environmental stress and plays multiple roles in plant adaptation to stress. Also increased proline content in wheat plant at low temperature with the bacterial inoculation is an indication to chilling tolerance [105].

Turan et al. conducted greenhouse experiments in wheat and barley under cold stress conditions to determine the growth, freezing injury, antioxidant enzyme activity effect of four different rhizobacteria and boron [149]. The authors showed that boron+PGPR treatments have positive effect on root and shoot growth, $H_2O_2$, and SOD, POD and CAT antioxidant enzyme activities of wheat and barley plants under cold and control conditions. This suggests that the PGPB application can ameliorate the deleterious effects of cold stress by increasing chlorophyll content, photosynthetic activity and relative water content, altering mineral uptake, and decreasing membrane damage, increasing cold tolerance in wheat and barley.

*2.6.4. Metal stress tolerance in wheat*

Plant growth-promoting bacteria are able to also grow in heavy metal-contaminated environment and protect plants against heavy metals toxicity in contaminated soils [150, 151]. Hasnain and Sabri reported that upon *Pseudomonas* sp. inoculation of wheat in Pakistan, growth was stimulated, less toxic ions were taken up and increased auxin content was observed [152].

Under Cr stress conditions, Shahzadi et al. reported root length, shoot length, root dry weight and shoot dry weight, respectively, as compared to uninoculated control plants upon inoculation of wheat seeds with *Pseudomonas fluorescens* Q14 and *Bacillus thuringiensis* KAP5 [153]. In this context, ACC-deaminase producing PGPR could play vital role in improving the plant growth under metal-stress condition and they may enhance bioremediation process in Cr-contaminated environment. Similarly, Jamali et al. studied the relationship of bacterial Cr mobilization in soil with total Cr accumulation in wheat [154]. Hassan et al. reported that inoculation with PGPR decreased the deleterious effects of cadmium pollution by chelating and influencing its bioavailability and increased the wheat growth [155]. Singh et al. found that PGPR having ACC-deaminase activity were resistant against Cd, Cr, Pb and Cu toxicity, and increased the wheat and pigeon pea growth [156]. Consequently, uses of rhizospheric microorganisms are generally considered as safe, cost effective and reliable technique for elimination of heavy metals from environmental compartments [150, 157, 158]. Govindasamy et al. observed that growth-promoting ability of rhizoacteria containing ACC deaminase in wheat seedlings through modulation of stress ethylene synthesis enhanced root elongation significantly and minimized ethylene synthesis in wheat seedlings under induced cadmium stress condition [159].

## 2.7. Improve yield and quality of wheat

Beneficial rhizobacteria associated with cereals has increased recently and several studies clearly demonstrated the positive and beneficial effects of PGPR on growth and yield of wheat at different environment under variable ecological conditions (Turan et al., 2010).

Zn solubilizing rhizobacteria significantly influenced the growth, yield and Zn concentration of wheat grain over uninoculated control and Zn fertilizer [160, 161]. Similarly, increased nutrient concentrations in wheat due to inoculation were reported in Refs. [5, 118, 162–165]. It is pointed out by Mäder et al. that microbial inoculants have been shown to be a valid option for sustainable high quality wheat production in low-input areas, promising to improve the nutritional status and health of the rural population [163]. In a survey of 20 years of experiments, Okon

and Labandera-Gonzalez reported that 60–70% of the experiments showed yield increases due to inoculation, with statistically significant increases in yield from 5 to 30% [31].

*Pseudomonas* strains significantly increased grain yield of wheat [23, 49, 143, 166]. Similarly, Shaharoona et al. reported that N use efficiency increased in response to inoculation with *Pseudomonas fluorescens* at all fertilizer levels in wheat [167]. PGPR isolates significantly increased shoot and root length, shoot and root dry weight, grain weight per spike, shoot and root N content and also enhanced the N contents of inoculated wheat seedlings [168]. Barneix et al. reported that inoculation of wheat with *Bacillus simplex* and *Bacillus firmis* resulted in consistent increase in dry matter and wheat grain quality. A number of other *Bacillus* spp. isolated from wheat rhizosphere have also been investigated for their growth-promoting property in wheat having similar effects on dry weight [10, 40, 169], the latter focusing on isolating and characterizing PGPRs. Trials with rhizosphere-associated plant growth-promoting $N_2$-fixing and P-solubilising *Bacillus* and other species indicated yield increases in many crops such as wheat [43, 51, 170, 171]. In wheat, several rhizobacteria have been reported as improving grain yield, grain protein concentration or both [3, 135, 140, 164, 172].

## 3. Co-inoculation of multiple PGPRs

Inoculation with mixed different strains could be an alternative to inoculation with individual strains, likely reflecting the different mechanisms used by each strain in the consortium [173]. Combined inoculation with $N_2$-fixing and phosphate solubilizing bacteria were more effective than a single microorganism for providing a more balanced nutrition for plants [19, 174]. There are numerous examples in wheat whereby synergistic effects of multiple PGPRs are observed [97, 175, 176]. Among those, notable is the combined inoculation of mixtures and biofilmed bio-inoculants (*Anabaena torulosa* + *Pseudomonas striata* and/or *Anabaena torulosa* + *Azotobacter chroococcum*) were superior over single inoculation and chemical fertilizer control in term of plant growth and nutrient uptake [177]. The benefits can be on nutrient uptake, but also in root physiology as exemplified by Manjunath et al. as co-inoculation of wheat with two proteobacterial (*Providencia* sp. and *Alcaligenes* sp.) and two cyanobacterial (*Anabaena oscillarioides* and *Anabaena torulosa*) inoculants, similarly in Ref. [178, 179].

Seed bacterization with both strains, *P. fluorescens* BAM-4 and *B. cepacia* BAM-12 single or combined significantly enhanced growth and yield, but increase in bacterial population, spike length, P content of shoots and grain yield was more in co-inoculation treatment than single. The best among the bioinoculation treatments was *B. cepacia* BAM-12 + TCP and *B. cepacia* BAM-12 + *P. fluorescens* BAM-4 + TCP for P content with free and immobilized cells [180].

Several authors conducted experiments on wheat either under pot and field conditions to examine the effect of co-inoculations of PGPR on the growth and yield of wheat. Kumar et al. found that *B. megaterium, A. chlorophenolicus* and *Enterobacter* significantly increased plant height, grain yield and straw yield [181]; Baris et al. concluded that *Bacillus megaterium* M3 and Mixed (*Bacillus subtilis* 05U142, *B. megaterium* M3, *Azospirillum brasilense* Sp245) inoculation provided greater plant nutrient element concentrations than mineral fertilizer application

[182]. Similar outcomes also compared with chemically fertilized soils are reported in Refs. [53, 183–185].

Nowadays, there is a greater awareness to use biological components such as PGPR and mycorrhizal fungi as a component of integrated nutrient management strategies to obtain higher input use efficiency, to maintain the desired productivity through optimization of the benefits from all possible sources, to cope with increasing fertilizer costs and their long-term adverse effects on agricultural ecosystems such as increased nutritional imbalances, declining productivity, adverse conditions prevailing in this ecosystem, and or a combination of these factors, as reported in Refs. [113, 177]. Note that some PGPR inoculants may adversely affect mutualistic associations between plants and indigenous soil microorganisms and suggest a possible reason as to why spring wheat growth was not consistently enhanced by these *pseudomonad* PGPR [186]. Co-inoculation of *Azotobacter* and *Mycorrhiza* increased grain yield and yield components of wheat [187].

Wheat rhizobacterial community structure is highly dynamic and influenced by different factors such as wheat cultivar line ages, plant's age, growth stage, distance from the soil to the root, root exudation pattern, multiple soil properties and agronomic practises [162, 188, 189]. Roesti et al. employed a consortia formed by a PGPR *Pseudomonas* spp. and an indigenous AMF to study their effect on the bacterial community structure and wheat growth [162].

All in all, greater attention should be paid to new combinations of different types and properties organisms such as $N_2$-fixing and P-solubilizing bacteria for improvement of biofertilizers efficiency [19].

## 4. PGPR reduce chemical fertilization

Due to high cost of chemical fertilizers and negative environmental effects, the use of PGPR as biofertilizer is advantageous for development of sustainable agriculture, increasing agronomic efficiency, once the use of chemical fertilizers can be reduced or eliminated if the inoculants are efficient [6]. The use of bio-fertilizers with a good management can decrease the leaching loss of nitrate and phosphate from the agricultural land and improve the ground water quality [190]. Also, the use of PGPR with low-fertilizer rate is also an environment friendly step and would be a viable supplementary strategy for further increasing crop yields [71, 78, 191].

Trials conducted under greenhouse conditions showed that most of PGPR in the absence of any fertilizer application achieved increases in root and shoot weight [3], corresponding to nitrogen treatment at the rate of 40 and 80 kg N $ha^{-1}$ in wheat. Furthermore, co-inoculation of $N_2$-fixing and P-solubilizing bacteria always gave equal or higher grain yield than conventional application of nitrogen.

Rosas et al. studied the promotion effect of *Pseudomonas aurantiaca* SR1 on maize and wheat in field treatments that included phosphorus and nitrogen fertilization [166]. Both crops, when inoculated with the SR1 strain, presented significant promoting effect in growth parameters and higher yields with lower fertilization doses than conventionally applied. Additionally,

PGPR are also important with respect to an efficient use of resources such as P and N, as illustrated by a 95% increased P use efficiency of wheat grains [163].

It could be concluded that application of PGPR with low-fertilizer rates could be a viable supplementary strategy for maximum benefits and should be employed with appropriate doses of fertilizers to get maximum benefit in terms of fertilizer savings and better growth in any yield of crops. Experiments as field trials with dry land areas, the co-inoculations of PGPR strains for wheat, maize and barley with chemical fertilizers gave improved response [3, 183, 192–197].

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Chapter 8

# Approaches to Enhance Salt Stress Tolerance in Wheat

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Additional information is available at the end of the chapter

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**Abstract**

Wheat is consumed as a staple food by more than 36% of world population. Wheat provides nearly 55% of the carbohydrates and 20% of the food calories consumed globally. The productivity of wheat is often adversely affected by salt stress which is associated with decreased germination percentage, reduced growth, altered reproductive behavior, altered enzymatic activity, disrupted photosynthesis, damage of ultrastructure of cellular components, hormonal imbalance, and oxidative stress. Different approaches have been adopted to improve plant performance under salt stress: introduction of genes, screening of better performing genotypes, and crop improvement through conventional breeding methods which are often not so successful and suitable due to time-consuming or reduction of plant vigor with the succession of time. Uses of exogenous phytoprotectants, seed priming, nutrient management, and application of plant hormone are convenient for improving plant performances. This chapter reviews the mechanism of damage of wheat plants under salt stress and also the recent approaches to improve growth and productivity of salt-affected wheat plants emphasizing the use of exogenous phytoprotectants from the available literature.

**Keywords:** abiotic stress, antioxidant defense, cereal crop, stress tolerance, phytohormones

## 1. Introduction

Worldwide, more than 20% of the cultivable land is affected by salinity. Due to climate change and anthropogenic activities, the salt affected area is tended to increase day by day [1]. Abiotic stresses (including salinity) are accountable for more than 50% yield reduction [2]. In contrary, due to rapid increase of global population, food production should be increased by more than 70% by 2050 [3]. Wheat (*Triticum* spp.) ranks first in the world's grain production. Wheat is consumed as staple food by more than 36% of world population. Wheat provides nearly 55% of the carbohydrates and 20% of the food calories consumed globally [4, 5]. The productivity of wheat is often adversely affected by salt stress. The yield of wheat starts to decline at 6–8 dS $m^{-1}$ [6]. Under salt stress, hyperosmotic and hyperionic (ion toxicity) stresses occur due to low water potential of soil and excess sodium ion accumulation within the plant. Ionic stress is also associated with nutritional imbalance [7, 8]. Decreased germination percentage, reduced growth, altered reproductive behavior, and reduced yield are general effects on plants under salt stress [9]. Altered enzymatic activity, disrupted photosynthesis, oxidative stress, disrupted biomembrane structure and function, damage of ultrastructural cellular components, and hormonal imbalance are some reasons for decreasing overall growth and development of plants under salt stress [10–12].

Salt stress tolerance is a polygenic trait regulated by multiple factors/genes. Exclusion of $Na^+$, cytosolic $K^+$ retention and maintenance of $K^+/Na^+$ homeostasis, osmotic adjustment, transpiration efficiency, and enhanced antioxidant defense system are vital for better plant performance under salt stress [13–15]. Different approaches have been adopted to improve plant performance under salt stress; introduction of genes, screening of better performing genotypes, and crop improvement through conventional breeding methods which are often not so successful and not suitable due to time consuming or reduction of plant vigor with the succession of time. Uses of exogenous phytoprotectants, seed priming, nutrient management, and application of plant hormones are convenient for improving plant performances. These approaches are being also popular for stress management practices including the salt stress [16–25]. In this chapter, we will review the recent research works on different approaches of salt stress tolerance in wheat plants emphasizing the use of exogenous phytoprotectants.

## 2. Wheat responses to salt stress

Salinity is one of the most devastating abiotic stresses having enormous negative effects on morphological, physiological, and biochemical attributes of plant including germination, growth, water uptake, photosynthesis, nutrient uptake, enzymatic activities, and yield. A number of studies revealing the effects of salt stress on different wheat cultivars, among which some are tolerant and some susceptible to salt stress. Higher salinity causes lower germination rate, photosynthesis, transpiration, and higher accumulation of $Na^+$ and $Cl^-$ ions which disturb the normal metabolic processes of wheat plants (**Table 1**; **Figure 1**).

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| Cultivars | Salinity level | Effects | Reference |
|---|---|---|---|
| MH-97 and AUQAB-2000 | 15 dS $m^{-1}$ | • Decreased DW and FW of root and shoot<br>• Increased activities of catalase (CAT) and superoxide dismutase (SOD)<br>• Increased protein and AsA contents | Afzal et al. [43] |
| KRL-19 and WH-542 | 100 mM NaCl, 6 d | • Decreased leaf relative water content (RWC) and leaf osmotic potential<br>• Increased $H_2O_2$, malondialdehyde (MDA) contents and electrolyte leakage<br>• Increased activities of CAT, glutathione reductase (GR), SOD, ascorbate peroxidase (APX), and peroxidase (POD)<br>• Increased $Na^+$ accumulation, decreased $K^+$ accumulation, and increased $K^+/Na^+$ ratio | Mandhania et al. [37] |
| S-24 and MH-97 | 150 mM NaCl, 7 d | • Decreased shoot and root FW<br>• Decreased grain yield, 1000 grain weight and transpiration rate<br>• Increased $Na^+$ and $Cl^-$ contents and decreased $K^+$ and $Ca^{2+}$ contents in both leaf and root | Arfan et al. [19] |
| MH-97 | 150 mM NaCl | • Increased mean germination time<br>• Decreased FW and DW of shoot and leaf area<br>• Increased $Na^+$ and $Cl^-$ contents, and decreased $K^+$ and $Ca^{2+}$ contents and $K^+/Na^+$ ratio<br>• Decreased $NO_3^-$ and $PO_4^{3-}$ contents | Wahid et al. [35] |
| Inqlab-91 and SARC-1 | 125 mM NaCl, 7 d | • Increased $Na^+$ and $Cl^-$ contents, and decreased $K^+$ and $Ca^{2+}$ contents<br>• Decreased FW and DW | Afzal et al. [28] |
| Banysoif 1 | 320 mM NaCl, 155 d | • Decreased contents of photosynthetic pigments and rate of transpiration<br>• Increased Pro content and decreased amino acid content<br>• Higher accumulation of $Na^+$ in root, shoot, and spike | Tammam et al. [36] |
| Hirmand, Chamran, Hamoon, Bolani, Sorkhtokhm, and Kavir | 12.5 dS $m^{-1}$ | • Delayed and decreased seed germination<br>• Increased $Na^+$ accumulation and decreased $K^+$ content in both shoot and root | Akbarimoghaddam et al. [30] |
| Tatara-96, Ghaznavi-98, Fakhri Sarhad, Bakhtawar-92, Pirsabaq-2004, and AUQAB-2000 | 120 mM NaCl | • Decreased shoot FW and DW<br>• Increased $Na^+$ and $K^+$ contents, and decreased $K^+/Na^+$ ratio | Jamal et al. [32] |

| Cultivars | Salinity level | Effects | Reference |
|---|---|---|---|
| Tajan, Rasoul, Atrak, and Kouhdasht | 16 dS $m^{-1}$ | • Decreased grain yield and 1000-grain weight<br>• Increased $Na^+$ and $Cl^-$ contents, and decreased $K^+$ and $Ca^{2+}$ contents | Asgari et al. [40] |
| Caxton | 200 mM NaCl, 8 d | • Decreased germination percentage | Fuller et al. [31] |
| MH-97 and Inqlab-91 | 15 dS $m^{-1}$ | • Decreased net $CO_2$ assimilation rate, stomatal conductance, and transpiration rate<br>• Decreased shoot FW | Iqbal and Ashraf [44] |
| Dan-4589 | 80 mM (NaCl and $Na_2SO_4$ at a molar ratio of 9:1) | • Increased $Na^+$ content and decreased $K^+$ content<br>• Decreased rate of photosynthesis and transpiration<br>• Decreased chl content and intercellular $CO_2$ concentration | Guo et al. [33] |
| HD 2329 and Kharchia-65 | 200 mM NaCl, 9 d | • Decreased activity of SOD and increased activities of POD, APX, CAT, and GR | Singh et al. [45] |
| Transgenic lines: T1, T4, and T6 | 200 mM NaCl, 4 d | • Decreased net photosynthetic rate, stomatal conductance, and increased intercellular $CO_2$ concentration in leaves<br>• Decreased chl and carotenoid contents | Tian et al. [46] |
| Yangmai 16 | 0.75% NaCl | • Higher accumulation of $Na^+$ and decreased $K^+$/$Na^+$ ratio | Zhang et al. [47] |
| Jimai 22 | 100 mM NaCl, 10d | • Increased MDA content<br>• Increased activities of SOD, POD, CAT, GR, APX, and dehydroascorbate reductase (DHAR) | Zou et al. [34] |

**Table 1.** Responses of *T. aestivum* plants to salt stress.

## 2.1. Germination

Germination is one of the most important and vital processes of plant life cycle. It is the determinant of the subsequent growth and yield characteristics of plants. Available literature showed that wheat seeds tended to germinate at a lower rate and consumed longer time when exposed to salt stress. The reasons underlying this fact are higher concentrations of salt create lower osmotic potential of germination media which hampers the imbibition of water by seed, creates an imbalance in the normal activities of enzymes responsible for nucleic acid and protein metabolism, causes hormonal imbalance, and deteriorates the food reserves of seed [26]. But, there are many other factors related to the plant and environment which also have effects on germination process. These include age of seed, seed dormancy, seed coat hardness, seed polymorphism, vigority of seedling, moisture, temperature, gasses, and light, etc. [27]. Germination also varies with different cultivars considering whether tolerant or susceptible type. Afzal et al. [28] reported that under saline condition (125 mM NaCl), wheat seeds required longer time for germination than seeds under nonsaline condition. Similar results were presented by Ghiyasi et al. [29] with upto 16 dS $m^{-1}$ salinity levels. Mean germination

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time increased and germination rate and germination index decreased with increasing levels of salinity. Akbarimoghaddam et al. [30] reported delayed and decreased wheat seed germination at 12.5 dS $m^{-1}$ salinity. Fuller et al. [31] also showed a distinct relationship of the decreasing germination percentage with increasing salinity levels (upto 200 mM NaCl).

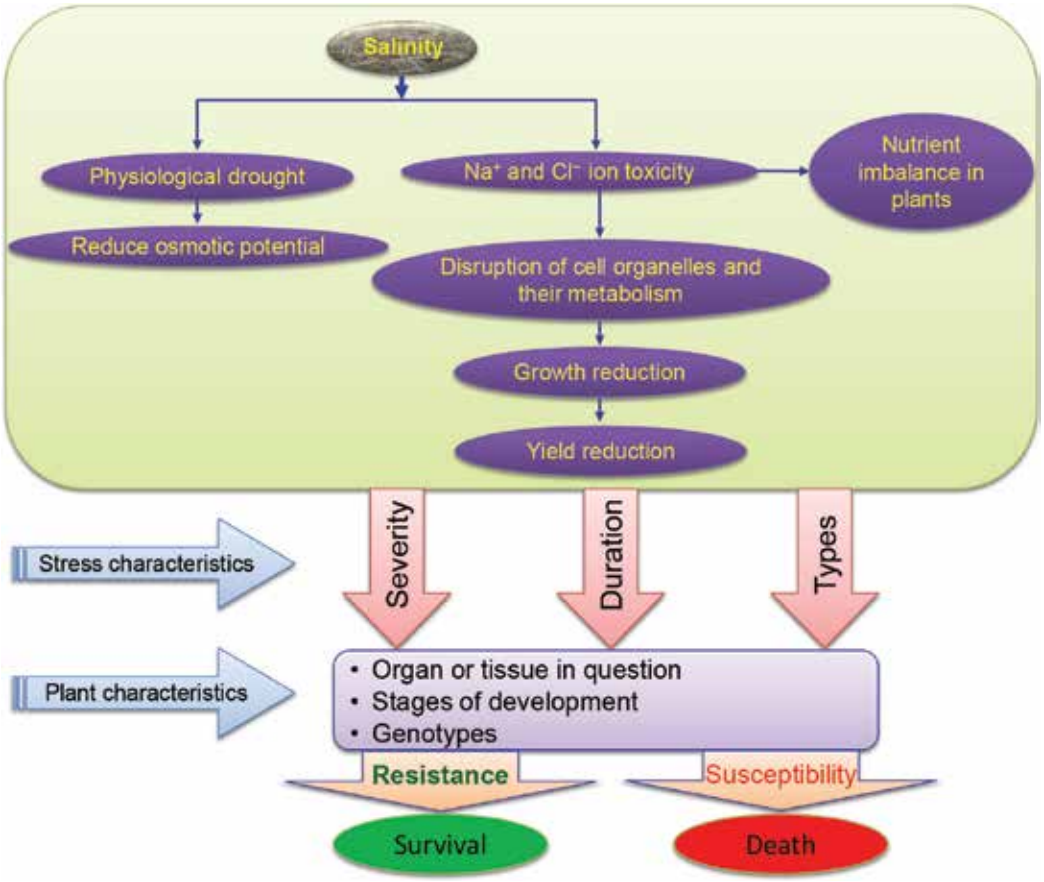


**Figure 1.** General scheme of salt stress responses and adaptation in plants.

### 2.2. Growth

Salt stress affects the growth of wheat seedlings remarkably. Root and shoot length, plant height, leaf area, number of effective tillers, and number of spike, etc. are considered to be growth parameters. There are many reports that show the evidence of hampering these characters under saline condition. Moreover, in the seedling stage, plants are more sensitive to adverse environmental conditions. So, in this stage, high salinity may even cause death of seedlings. Fresh and dry mass of shoot, leaf area, etc. of both sensitive and tolerant cultivars declined under salt stress in wheat seedlings [19]. Length, fresh weight (FW), and dry weight (DW) of both root and shoot of wheat seedlings were negatively affected by different levels

of salinity as 150 mM NaCl [20]; 125 mM NaCl [28], 16 dS $m^{-1}$ salinity [29], and 120 mM NaCl [32]. Guo et al. [33] showed decreased growth of leaves of wheat seedlings and roots under salt stress, compared to the nonstressed control. Similarly, reduced shoot length, root length, wet weight, and DW after 10 d with 100 mM NaCl treatment were observed by Zou et al. [34].

### 2.3. Photosynthesis

Photosynthesis is the major physiological process for plant survival and greatly influenced by environmental factors. As salinity reduces water potential and increases accumulation of $Na^+$ and $Cl^-$ ions in the chloroplast, the rate of photosynthesis gets inhibited [26]. According to the experiment conducted by Arfan et al. [19], exposure to salt stress reduced the transpiration rate, net $CO_2$ assimilation rate, stomatal conductance, and substomatal $CO_2$ concentration of both cultivars. Similarly, net photosynthetic rate, transpiration rate, stomatal conductance, and substomatal $CO_2$ concentration were decreased significantly at 150 mM NaCl stress [35]. Tammam et al. [36] reported that amount of photosynthetic pigments were significantly deceased in seedlings under 320 mM NaCl stress. Reduction of stomatal conductance and transpiration rate were also reported by Guo et al. [33]. Significant decrease of chlorophyll (chl) content was recorded in wheat seedlings at 100 mM NaCl, for 10 d [34].

### 2.4. Water relation

Availability of moisture in plants is a crucial factor for all physiological and metabolic processes of plants. Higher salt concentrations induce osmotic stress to plants, which ultimately causes low water potential. Relative water content (RWC) declined by 3.5 and 6.7%, compared to their controls in the salt-tolerant and salt-sensitive cultivars, respectively, after 6 d of 100 mM NaCl exposure [37]. They also reported lowering of osmotic potential with increasing salt concentrations. Arfan et al. [19] showed reduced water use efficiency (WUE) of both sensitive and tolerant cultivars under saline condition. Leaf water potential also decreased under salt stress of 150 mM NaCl [35] and 16 dS $m^{-1}$ [38]. Percentage of water content decreased in root, but increased in shoot and spike of Banysoif 1 cultivar of wheat [36]. Lv et al. [39] recorded lower RWC in leaves of *T. monococcum* seedlings exposed to salt stress of 320 mM NaCl.

### 2.5. Cellular damage

Inconsistent growth and improper uptake of water and nutrients ultimately result in deterioration of cell membrane properties of plants. Lipid peroxidation, accumulation of hydrogen peroxide ($H_2O_2$), and increased membrane permeability are some common phenomenon of wheat seedlings under salt stress. Mandhania et al. [37] reported higher damage of cellular membranes of salt-sensitive cultivar due to higher $H_2O_2$ accumulation and lipid peroxidation which enhanced the electrolyte leakage compared to the tolerant one. Higher accumulation of $H_2O_2$ in salt-stressed wheat seedlings was also proved by Wahid et al. [35] which was responsible for the increased relative membrane permeability. Lipid peroxidation increased by 68% under NaCl treatment of 100 mM for 10 d compared to control [34].

### 2.6. Ion uptake

Higher accumulation of $Na^+$ and $Cl^-$ ions interferes with the uptake of other necessary ions which disturbs plant processes. Salt-sensitive cultivars tend to uptake more $Na^+$ compared to the tolerant one and this uptake rate increases with increasing concentration of salt [37]. Lower accumulation of $NO_3^-$ and $PO_4^{3-}$ ions were recorded by Wahid et al. [35]. They also reported higher uptake of $Na^+$ and $Cl^-$, and reduced uptake of $K^+$ and $Ca^{2+}$ by salt stressed wheat seedlings. Similar results were published by Afzal et al. [28] in wheat seedlings exposed to 125 mM of NaCl stress for 7 d. But, Jamal et al. [32] reported increased uptake of $Na^+$ and $K^+$ both ions, and decreased $K^+/Na^+$ ratio in wheat shoots when exposed to 120 mM of NaCl. On the other hand, both Asgari et al. [40] and Afzal et al. [41] recorded significant decrease of $K^+$ uptake under saline condition (15–16 dS $m^{-1}$). Under medium salinity, higher accumulation of both $Na^+$ and $Cl^-$, and lower uptake of $K^+$, $Ca^{2+}$, and $Zn^{2+}$ ions were reported by Guo et al. [33].

### 2.7. Yield

All the above mentioned factors are responsible directly or indirectly for the subsequent yield reduction of wheat plants. Yield of almost all crops, except some halophytes, is reduced under salt stress. The amount of yield reduction may vary upon the sensitivity and tolerance of the wheat cultivars. Chinnusamy et al. [42] indicated that above the threshold level of salinity of 6 dS $m^{-1}$, wheat yield can reduce at a rate of 7.1% per dS $m^{-1}$ increase of salinity. Asgari et al. [40] reported that the spikes number per plant, spike length, number of spikelets per spike, straw weight, grain yield, 1000-grain weight, and harvest index declined with the increasing level of salinity, which ultimately caused yield loss. A significant decrease in number of grains per spike, 1000-grain weight, and grain yield were reported in both tolerant and sensitive cultivars of wheat seedlings under 15 dS $m^{-1}$ salinity [41].

## 3. Salt-induced oxidative stress in wheat

Salt stress can lead to stomatal closure, which reduces $CO_2$ availability in the leaves and inhibits carbon fixation, exposing chloroplasts to excessive excitation energy which in turn increase the generation of reactive oxygen species (ROS) such as superoxide ($O_2^{\bullet -}$), $H_2O_2$, hydroxyl radical (OH•), and singlet oxygen ($^1O_2$) [26, 48, 49] (**Figure 2**). Since, salt stress is complex and imposes a water deficit because of osmotic effects on a wide variety of metabolic activities [50]; this water deficit leads to the formation of ROS that are highly reactive and may cause cellular damage through oxidation of lipids, proteins, and nucleic acids [51]. If there is a serious imbalance in any cellular compartment between the production of ROS and antioxidant defense, oxidative stress and damage occur [52] (**Figure 2**). Enhanced production of ROS under salinity stress induces phytotoxic reactions such as lipid peroxidation, protein degradation, and DNA mutation [53]. When a plant faces harsh conditions, ROS production overcomes scavenging systems and oxidative stress will burst. In many plant studies, it was observed that production of ROS increased under saline conditions [54]

and ROS-mediated membrane damage has been demonstrated to be a major cause of the cellular toxicity by salinity in different crop plants ([49]; **Table 2**). Long-term salinity treatments (5.4 and 10.6 dS $m^{-1}$, 60 d) caused significant increase in $H_2O_2$ and lipid peroxidation in wheat seedlings, which were higher in salt-sensitive cultivar than salt-tolerant cultivar [55]. Increased lipid peroxidation and $H_2O_2$ levels with increased salinity stress in *T. aestivum* were observed in our study [24]. Wheat seedlings exposed to 300 mM NaCl resulted in 60 and 73% increase in $H_2O_2$ and MDA contents. Salt stress also decreased ascorbic acid (AsA) content by 52%. According to Zou et al. [34], *T. aestivum* leaves showed 35% increase in MDA content upon 100 mM NaCl treatment for 5 d which further increased by 68% after 10 d of treatments. Rao et al. [56] observed dose dependent increase in lipid peroxidation in wheat exposed to salt (2, 4, 8, and 16 EC) and these effects were variable among the cultivars. They found increased MDA content in cultivars, ZARDANA (55.9%), ROHTAS-90 (42.26%), SAUGHAT-90 (51%), and SHAHEEN-94 (52%), and hence they were designated as salt sensitive, whereas PUNJAB-85 (33%), BHAKAR 2002 (35%), PIRSBAK-05 (31%), and AUQAB (28%) showed decreased levels of lipid peroxidation and were categorized as salt tolerant [57].

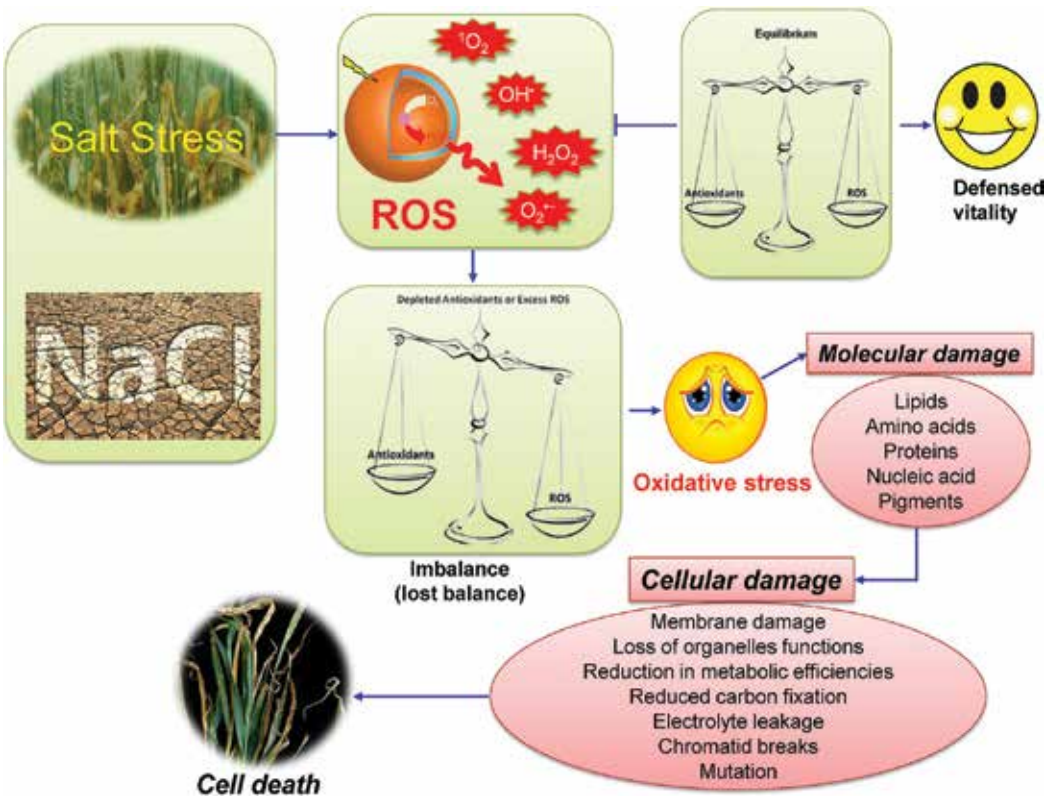


**Figure 2.** Generalized scheme of salt-induced oxidative stress in plants.

| Name of cultivars | Dose and duration of salt | Level of oxidative stress | References |
|---|---|---|---|
| Pradip | 300 mM NaCl, 4 d | • Increased $H_2O_2$ and MDA content by 60 and 73%, respectively | Hasanuzzaman et al. [24] |
| Kharchia-65 | 6.85 dS $m^{-1}$ NaCl | • Enhanced lipid peroxidation (TBARS) and $H_2O_2$ content by 21 and 38%, respectively | Sairam and Srivastava [58] |
| Jimai 22 | 100 mM NaCl, 10 d | • MDA content increased by 68.3% in leaves | Zou et al. [34] |
| ZARDANA BAKHAR-2002, SAUGHAT-90, and AUQAB-2000 | 16 dS $m^{-1}$ NaCl | • Lipid peroxidation enhanced by 56, 35, and 51% in ZARDANA BAKHAR-2002, and SAUGHAT-90 cultivars, respectively<br>• DPPH radical scavenging activity decreased by 47% in AUQAB-2000 | Rao et al. [56] |
| Kızıltan-91 | 100 mM NaCl, 5 d | • Increased lipid peroxidation level by 53% | Seckin et al. [59] |
| Yongliang 4 | 150 mM NaCl, 16 h | • Increased MDA content in leaves by 50% | Zhang et al. [60] |
| Altındane | 100 mM NaCl, 3 d | • Elevated MDA, $O_2^{\bullet-}$, and $H_2O_2$ contents by 26, 43, and 53%, respectively | Gorcek and Erdal [61] |
| Waha | 150 mM NaCl, 14 d | • MDA content increased by 10% and fourfold increase of $H_2O_2$ content | Fercha [62] |
| WenmaiNo.6 | 150 mM NaCl, 4 d | • Increased MDA, $O_2^{\bullet-}$, and $H_2O_2$ contents by 47, 38, and 33%, respectively | Qiu et al. [63] |
| | 150 mM NaCl, 72 h | • Increased MDA and $H_2O_2$ contents by 52 and 47%, respectively | Genişel and Erdal [64] |

**Table 2.** Salt-induced oxidative stress in *T. aestivum* compared to control.

Plants have antioxidative mechanism to fight against stress under adverse conditions. So, they naturally produce higher amount of antioxidant enzymes, e.g., CAT, GR, SOD, APX, POD, and DHAR, etc. to minimize the damage due to stress. Mandhania et al. [37] reported that the activities of CAT, GR, SOD, APX, and POD enzymes increased with the increasing concentration of salt irrespective to tolerance or sensitivity of the cultivar. In another experiment with sensitive and tolerant type of cultivars, ascorbic acid (AsA) content and activities of SOD, CAT, and POD also increased in both under salt stress [20]. But, in another experiment by Singh et al. [45], SOD activity was recorded to decrease with the increasing concentration of salt in a salt-sensitive cultivar named HD2329; while activities of POD, APX, CAT, and GR increased with the same treatments. Significantly, higher activities of SOD and POD were presented by Zou et al. [34] with NaCl treatment of 100 mM for 10d, but they showed insignificant increase of CAT and APX activities, and significant decrease of GR and DHAR activities under same treatment. The activities of SOD and POD were increased with increasing the levels of salt concentrations in *T. monococcum* seedlings [39].

## 4. Salt tolerance approaches

Considering the adverse effects of salt stress in wheat plant, biologists are trying to find out the salt-tolerant strategies in plants by different approaches. Many researchers found positive effect in using exogenous phytoprotectants in alleviating salt-induced damages in wheat. In this section, some of the evidences are discussed.

### 4.1. Use of osmoprotectants

To prevent the adverse effects of various environmental stresses including salt stress, plants demonstrate a variety of adaptive mechanisms both at the cellular and organismal levels. Under salt stress conditions, to cope with the salt-induced osmotic, ionic as well as oxidative stresses, plant synthesizes and accumulates organic compatible solutes or osmolytes [48, 65, 66]. Accumulation of these compatible solutes is one of the most important physiological strategies employed by plants under salt stress conditions. Osmoprotectants or osmolytes are small, highly soluble, uncharged, and nontoxic organic molecules which help to survive organisms in extreme osmotic stresses. Osmoprotectants comprise of (i) $\alpha$-amino acids such as proline (Pro) and ectoine; (ii) ammonium compounds such as glycine betaine (GB), $\beta$-alanine betaine, dimethylsulfoniopropionate (DMSP), and choline; and (iii) polyols, sugars, and sugar alcohols such as trehalose (Tre), sorbitol, and mannitol, etc. These osmoprotectants perform vital functions in osmotic adjustment, stabilizing proteins and membranes. Thus, enhanced salt stress tolerance is observed in plants overexpressing the osmoprotectants biosynthetic and metabolic genes. Enhanced salt exposure causes increased biosynthesis of osmoprotectants (Pro, GB, Tre, ecotine, and sorbitol, etc.) which provides enhanced osmotic stress tolerance generated from salt stresses [67, 68] (**Table 3**). For mitigating salt-induced damages, in recent decades, the use of exogenous osmoprotectants has been found effective [12, 69]. Several research findings demonstrated that the use of osmoprotectants provided significant protection against adverse effects of salt stresses in *T. aestivum* seedlings (**Table 3**). At the same time, several research studies proved Pro as a potent protectant against the adverse effects of salt. Proline acts not only in osmotic adjustment as a compatible solute, but also in scavenging ROS, chelating metal, activating detoxification pathways, balancing cells redox status, buffering cytosolic pH, storing energy (carbon and nitrogen), stabilizing subcellular membranes and structures including photosystem II (PS II), and as signaling molecule [70–74]. Raza et al. [75] demonstrated the effect of exogenous GB (50 mM and 100 mM) in moderately salt-sensitive (MH-97) and salt-tolerant (S-24) wheat cultivars grown under salt stress (15 dS $m^{-1}$ NaCl). Glycine betaine treatment ameliorated the salt-induced photosynthetic reduction as well as increased the photosynthetic capacity, water use efficiency, and osmotic adjustment where salt-tolerant (S-24) cultivar showed better performance against salt stress compared to moderately salt-sensitive (MH-97) cultivar. Later, with the same experimental procedure, they again suggested that the exogenous GB modulated the activities of antioxidant enzymes such as SOD, CAT, and POD which contributed significantly to salt stress tolerance in *T. aestivum* [76]. It has been reported that accumulation of Pro protects *T. aestivum* from the salt-induced damages by maintaining a higher $K^+/Na^+$ ratio and reducing ionic toxicity [38], increasing the major antioxidant enzymes (CAT, APX, SOD, and POD) activities [77]. In *T. aestivum*, GB (10 mM and

30 mM) supplementation with salt stress (150 mM NaCl) increased the germination percentage, shoot Ca content, total chl content, and thus confer salt stress tolerance [78]. Khan et al. [79] reported that increased grain yield in *T. aestivum* associated with the increased Pro, chl content and $K^+/Na^+$ ratio. Overexpression of GB in transgenic *T. aestivum* lines T1, T4, T6, and Shi 4185 (wild type line) caused enhanced salt stress (200 mM NaCl) tolerance by enhancing ROS scavenging, osmotic adjustment and regulating ion homeostasis [80]. Salt stresses (10 dS $m^{-1}$ NaCl) were imposed in two wheat cultivars (cv. Seher and Lasani). In both wheat cultivars, salt stresses caused significant reduction in the germination percentage, chl contents and growth. Exogenous Pro (50 and 100 mM) application alleviated the adverse effects of salt stress by improving the germination percentage, seedling growth and chl contents of wheat plants but 100 mM Pro was found more effective compared to 50 mM Pro [81]. Mahboob et al. [82] reported that the supplementation of Pro (50 and 100 mM) ameliorated the salt (60 and 120 mM NaCl) induced reduction of plant growth, photosynthetic pigments and ionic balance by increasing shoot and root length, chl *a*, *b* contents, FW and DW of seedlings and endogenous Pro, GB, and $K^+/Na^+$ ratio in *T. aestivum* seedlings. Exogenous Pro (60 ppm) upregulated the endogenous hormones (gibberelic acid ($GA_3$) and indole acetic acid (IAA)), ammonium compounds (GB and choline) and downregulated the MDA content and growth inhibitor abscisic acid (ABA) in salt stressed *T. aestivum* [83]. Salt (50, 150, and 300 mM) induced disruption of photosynthetic pigments and protein polypeptide synthesis in *T. aestivum* were prevented by the exogenously applied Pro (50 ppm) and at the same time by protecting the turnover machinery of proteins [84]. Besides osmotic adjustment, GB is also involved in ROS scavenging, stabilizing macromolecules (nucleic acids, proteins, and lipids) and various components of photosynthetic machinery such as PS II complexes and RuBisCO and acts as reservoir of carbon and nitrogen sources [85–87]. Upon salt exposure (150 mM NaCl), reduced lipid peroxidation, increased glutathione (GSH) and GB concentrations, enhanced plasma membrane protection, increased cell solute potential and improved ion homeostasis were observed when caryopsis of *T. aestivum* were primed with GB (25, 50, 100 mM) [88]. Increasing the $K^+/Na^+$ and $Ca^+/Na^+$ ratios, reducing MDA content, protecting photosynthetic apparatus, improving plasma membrane integrity and stabilizing macromolecules (proteins, PS II and transporters) GB (20 mM) imparted in salt stress tolerance in *T. aestivum* [83]. Exogenous GB (5 mM) application improved chl *a*, total chl and $K^+$ content of roots, increased root length, plant height, FW and DW of *T. aestivum* under salt stresses (100 and 200 mM NaCl) [89]. Rao et al. [57] suggested that the enhanced production of Pro and GB in six salt-tolerant cultivars (*T. aestivum* cv. AUQAB-2000, PUNJAB-85, PIRSABAK-05, BAKHAR-2002, FARKHARE-SARHAD and KAGHAN-94) alleviated the damaging effects of salt stress by activating their antioxidant enzymes. Endogenous Pro and GB mediated salt stress (8 EC, 16 EC) mitigation in fifteen *T. aestivum* cultivars were further reported by Rao et al. [57]. They suggested that the five cultivars of wheat (SEHAR-2006, LU26-CTR, NARC-2009, BARS-2009, PIRSABAK-09) showed obvious salt stress tolerance by increasing the production of Pro and GB. Yan and Zheng [90] demonstrated that pretreatment with Tre (2, 20, and 40 mM) alleviated the adverse effects of salt stress (3 g $L^{-1}$ NaCl) in *T. aestivum* cv. Yangmai-19. Various beneficial effects were observed in different physiological parameters. Increased relative growth rate, relative chl content, N content, DW and biomass $plant^{-1}$ were observed with Trehalose supplementation. Trehalose application also improved Pro accumulation, $K^+$ accumulation and $K^+/Na^+$ ratio. In addition,

Tre has functions in stabilizing the biomolecules and structures like membrane lipids, proteins under salt stress [91–93]. Salt-sensitive wheat cultivar (*T. aestivum* cv. Kızıltan-91) under salt stress (100 mM NaCl) showed physiological alteration. However, pretreatment with exogenous mannitol (100 mM) reversed the deleterious salt effects by increasing antioxidant enzymes (such as SOD, POD, CAT, APX, and GR) activities, appearance of SOD and POD isozyme activity bands and reducing lipid peroxidation [59].

| Cultivars | Salinity doses and duration | Doses of osmolytes | Protective effects | References |
|---|---|---|---|---|
| ESW-9525 and kherman | 60 and 120 mM NaCl, 7 d | 50 and 100 mM Pro, foliar spray | • Increased shoot and root length<br>• Increased FW and DW of seedlings<br>• Increased chl *a*, *b* contents<br>• Improved Pro, GB, $K^+$ contents, and $K^+/Na^+$ ratio | Mahboob et al. [82] |
| Seher and Lasani | 10 dS $m^{-1}$ NaCl, 6 d | 50 mM and 100 mM Pro, foliar spray | • Improved gaseous exchange parameters (net $CO_2$ assimilation rate, stomatal conductance, substomatal $CO_2$ concentration, and transpiration rate)<br>• Increased chl *a*, *b*, and total chl contents | Talat et al. [81] |
| MH-97 and S-24 | 15 dS $m^{-1}$ NaCl | 50 mM and 100 mM GB, foliar spray | • Improved WUE<br>• Increased photosynthetic capacity<br>• Increased stomatal conductance | Raza et al. [75] |
| Kızıltan-91 | 100 mM NaCl, 5 d | 100 mM mannitol, pretreatment, 24 h | • Increase activities of SOD, POD, CAT, APX, and GR<br>• Reduced lipid peroxidation and membrane damage | Seckin et al. [59] |
| Gomeza 7 | 150 mM NaCl, 38 d | 25, 50, and 100 mM GB, caryopsis priming, 24 h | • Reduced lipid peroxidation<br>• Increased the GSH and GB concentrations<br>• Enhanced plasma membrane (PM) protection<br>• Increased the cell solute potential<br>• Improved ion homeostasis | Salama et al. [88] |
| Sakha 93 and Gimmeza7 | 10.04 dS $m^{-1}$ (soil), 35–65 d 7.33 dS $m^{-1}$ (irrigation water), 35–65 d | 60 ppm Pro, foliar spray | • Increased chl *a* and *b*<br>• Increased endogenous hormones (GA and IAA)<br>• Increased GB and choline<br>• Decreased MDA content and ABA | Hendawey et al. [83] |
| Sakha 93 and Gimmeza7 | 10.04 dS $m^{-1}$ (soil) 7.33 dS $m^{-1}$ (irrigation water), 35 to 65 d | 20 mM GB, foliar spray | • Increased $K^+/Na^+$ and $Ca^+/Na^+$ ratios<br>• Improved K, Ca, and Zn contents<br>• Reduced MDA content<br>• Protected photosynthetic apparatus<br>• Improved PM integrity and stabilization of macromolecules (proteins, PS II, and transporters) | Hendawey et al. [83] |

| Cultivars | Salinity doses and duration | Doses of osmolytes | Protective effects | References |
|---|---|---|---|---|
| Yangmai-19 | 3 g $L^{-1}$ NaCl | 2, 20, and 40 mM Tre, seed soaking | • Increased relative growth rate, relative chl content, N content, DW, and biomass per plant<br>• Increased Pro accumulation<br>• Increased $K^+$ accumulation and $K^+/Na^+$ ratio, and decreased $Na^+$ content | Yan and Zheng [90] |
| S-24 and MH-97 | 150 mmol $L^{-1}$ NaCl, 15 d | 10 and 30 mM of GB, presoaking | • Increased germination percentage<br>• Increased shoot Ca content<br>• Increased total chl content | Akhter et al. [78] |
| Gomeza | 150 mM NaCl, 21 d | 5 and 10 mM choline, caryopsis priming | • Increased $K^+$, $Ca^{2+}$, and decreased $Na^+$ and $Cl^-$ in both shoot and root<br>• Improved PM permeability<br>• Remarkably reduced lipid peroxidation<br>• Increased GB accumulation and improved ion homeostasis | Salama et al. [94] |
| S-24 and MH-97 | 15 dS $m^{-1}$ NaCl, 47 d | 50 and 100 mM GB, foliar spray | • Modulated activities SOD, CAT, and POD<br>• Significantly increased $K^+/Na^+$ ratio in leaves and roots, and $Ca^{2+}/Na^+$ ratio in leaves<br>• Enhanced endogenous GB and $K^+$ | Raza et al. [76] |

**Table 3.** Protective effects of various exogenously applied osmoprotectants under salt stress in *T. aestivum*.

Priming of *T. aestivum* seeds with choline (5 and 10 mM) reduced the damaging effects of NaCl (150 mM) by increasing the $K^+$, $Ca^{2+}$, GB accumulation, improved ion homeostasis and decreased $Na^+$ and $Cl^-$ in both shoot and root, mitigated PM permeability and reduced lipid peroxidation of leaf [94]. Expression of *mtlD* gene encoding mannitol-1-phosphate dehydrogenase resulted in enhanced salt stress tolerance in *T. aestivum* due to defensive roles of mannitol against salt stress [95]. The *mtlD* gene encoding mannitol-1-phosphate dehydrogenase transformation in *T. aestivum* cv. Giza 163 conferred salt stress tolerance by inducing mannitol and reducing sugars in tissues of plant [96]. Kerepesi et al. [97] demonstrated that increased fructan contents in salt resistant (Sa) and moderately salt-tolerant (Ch) varieties of *T. aestivum* showed increased tolerance against salt stress (200 mM NaCl). Sharbatkhari et al. [98] investigated the role of fructan in salt-tolerant (Bam) and salt-sensitive (Ghods) cultivars of *T. aestivum*. They found higher fructan accumulation and remobilization in salt-tolerant Bam cultivar, which contributed to the higher salt stress tolerance by increasing the photosynthetic capacity and decreasing the salt induced severe yield loss.

### 4.2. Plant hormone

Plant hormones are chemicals produced within the plants at low concentration involved in regulation of plant development and tolerance towards various stresses including salinity [99]. Now-a-days, various kinds of plant hormones such as ABA, auxin, cytokinins (CK),

brassinosteroids and $GA_3$ are externally used for alleviating various kinds of abiotic stresses including salinity (**Table 4**). The plant growth hormone auxin increased the germination percentage, shoots DW and maintained ion homeostasis under salt stress condition [100]. Iqbal and Ashraf [101] reported that seed priming with different auxins alleviated salt stress (15 dS $m^{-1}$) by maintaining hormonal balance and assimilation rate and improved growth and yield of both tolerant and sensitive cultivars under salt stress condition. Seed priming with $GA_3$ alleviates the drastic effect of salinity and increases grain weight and grain quality by improving photosynthetic pigments, leaf area and plant growth [102]. Foliar application of $GA_3$ also confers salt stress tolerance by increasing germination percentage, plant growth and upregulating antioxidant enzyme [103]. Seed priming with cytokinin such as kinetin and benzylaminopurine (BAP) increase germination percentage and grain yield by increasing plant growth, productive tiller and 1000-grain weight under salt stress condition [104, 105]. Gurmani et al. [106] noted that, seed priming with ABA improved salt stress tolerance by increasing net assimilation rate, chl content and decreasing Na uptake. It is also evident that phytohormone brassinosteroid plays role in alleviating salt stress. Ali et al. [107] reported that brassinosteroid increased grain yield by improving photosynthetic attribute, assimilation rate and transpiration rate under salt stress condition (150 mM NaCl). Eleiwa et al. [22] also showed brassinosteroid-induced positive response in wheat seedlings under salt stress conditions (**Table 4**).

| Cultivars | Salinity dose and duration | Dose of phytohormones | Protective effects | References |
|---|---|---|---|---|
| MH-97 (salt intolerant), Inqlab-91 (salt tolerant) | (15 ds $m^{-1}$) 150 mM NaCl, entire growth period | Auxin (Tryptopan) $4.89 \times 10^{-4}$ mM, 12 h seed priming | • Increased $CO_2$ assimilation rate<br>• Increased net assimilation rate<br>• Increased growth<br>• Increased productive tiller and grain yield | Iqbal and Ashraf [44] |
| MH-97 (salt intolerant), Inqlab-91 (salt tolerant) | 150 mM NaCl, entire growth period | $4.89 \times 10^{-1}$ mM auxin (tryptophan), 12 h seed priming | • Increased germination percentage<br>• Improved ion homeostasis<br>• Increased shoot DW | Iqbal and Ashraf [100] |
| Sohag 3 (sensitive), Giza 168 (tolerant) | 50, 100, 150, and 200 mM NaCl),entire life cycle | 150 mg $L^{-1}$ $GA_3$, foliar spray | • Improved leaf area, photosynthetic pigment, carobohydrate, protein, amino acid and Pro content, grain weight | Shaddad et al. [102] |
| MH-97, Inqlab-91 | 15 dS $m^{-1}$, 8 d | 100, 150 and 200 mg $L^{-1}$ cytokinins (kinetin and BAP), 12 h seed priming | • Increased germination rate<br>• Increased early seedlings growth such as shoot DW and root DW | Iqbal et al. [105] |
| MH-97, Inqlab-91 | 15 dS $m^{-1}$, entire life cycle | 100, 150 and 200 mg $L^{-1}$ cytokinins (kinetin and BAP), 12 h seed priming | • Increased plant height, shoot dry biomass<br>• Increased fertile tiller, 1000-grain weight, grain yield | Iqbal and Ashraf [104] |

| Cultivars | Salinity dose and duration | Dose of phytohormones | Protective effects | References |
|---|---|---|---|---|
| Mehran-89 | 0.13 M NaCl, 8 d | $10^{-6}$ M ABA, 8 d | • Increased germination percentage, and shoot and root biomass | Naqvi et al. [108] |
| Kharchia-65, PUNJAB-85 | 100 mM NaCl 16 d | 10 mM ABA, seed priming 24 h | • Increased plant height, root length<br>• Improved root and shoot dry weight<br>• Increased chl content<br>• Increased net assimilation rate<br>• Decreased Na uptake | Gurmani et al. [106] |
| Giza 164 | 2000–6000 ppm NaCl, irrigation water entire life cycle | 0, 50, 100 and 200 mg $L^{-1}$ 28-homobrassinoloide, foliar application, | • Increased chl, carotenoids and total pigments<br>• Increased plant height, leaf area<br>• Improved tiller number, weight of 1000 grain, grain yield and biological yield | Eleiwa et al. [22] |
| S-24, MH-97 | 150 mM NaCl, 45 d | 0, 0.052, 0.104, 0.156 μM 24-epibrassinolide | • Increased photosynthetic attribute and chl content<br>• Increased net $CO_2$ assimilation rate, stomatal conductance and transpiration rate<br>• Increased root and shoot weight and length<br>• Increased number of grain $plant^{-1}$ and grain yield | Ali et al. [107] |

**Table 4.** Protective effects of various exogenously applied phytohormones under salt stress in *T. aestivum*.

### 4.3. Plant nutrient

Along with other physiological and biochemical functions, plant nutrients play positive roles in alleviating damage effects of abiotic stresses including salinity (**Table 5**). Exogenous application of K enhanced salt stress tolerance in wheat seedlings by improving photosynthetic pigments, antioxidant enzyme activity, K uptake and decreasing Na uptake [109, 110]. Foliar application of phosphorus (P) also alleviated salt-induced damage by increasing plant biomass, leaf area and decreasing Na uptake [111]. Application of $CaSO_4$ increased plant growth, water status and K and Ca uptake under salt stress condition [112]. Later on, Tian et al. [113] noted that application of $Ca(NO_3)_2$ reduced salt-induced oxidative damage by decreasing lipid peroxidation and electrolyte leakage in wheat seedlings.

| Cultivars | Salinity dose and duration | Plant nutrients | Protective effects | References |
|---|---|---|---|---|
| NAYAB-11 and MILLAT-11 | 150 mM NaCl, 113 d | 50, 100, 150 and 200 mM $K_2SO_4$, 106 d | • Increased root length and biomass<br>• Increased plant height and biomass<br>• Increased $K^+$ uptake and decreased $Na^+$ uptake | Kausar and Gull [110] |
| Gemiza 9, Sakha 93 | 40, 80, and 120 mM NaCl, 90 d | 25 and 150 mg $K_2O$ $kg^{-1}$ soil, 110 d | • Increased plant height and biomass<br>• Increased chl *a*, chl *b* and carotenoid content<br>• Increased SOD and POD activity | El-Lethy et al. [109] |
| | 150 mM NaCl | 400 and 800 mg P $L^{-1}$, foliar application | • Increased plant height, root length, root and shoot biomass<br>• Increased leaf number, leaf area and chl content<br>• Decreased Na uptake and increase K uptake | Khan et al. [111] |
| PUNJAB-85 | 50 mM NaCl, 34 d | 3 and 6 mM $CaSO_4$ | • Increased root and shoot biomass<br>• Increased root and leaf RWC<br>• Increase K and Ca uptake | Zaman et al. [112] |
| Jimai 22 | 100 mM NaCl, 15 d | 17.5 mM $Ca(NO_3)_2$, 15 d | • Decreased $O_2^{\bullet-}$ and $H_2O_2$ contents<br>• Decreased lipid peroxidation, electrolyte leakage<br>• Increased SOD, POD, and CAT activities | Tian et al. [113] |

**Table 5.** Protective effects of plant nutrients under salt stress in *T. aestivum*.

### 4.4. Antioxidant

Antioxidants are important for plants to maintain the ROS level lower. Plant possesses various non-enzymatic antioxidants in their cellular components to protect themselves from oxidative stress. The major antioxidant includes AsA, GSH, tocopherol and some phenolic compounds. Some of these antioxidants showed advanced protection against salt-induced oxidative stress when they were applied exogenously (**Table 6**). However, these are mostly dose dependent. A number of studies have been reported the positive effects of AsA in mitigating salt stress in wheat. Athar et al. [20] studied the effect of AsA on wheat plants subjected to salt stress. Salt stress (150 mM NaCl) caused reduction in growth and photosynthesis which were associated with decrease in tissue $K^+/Na^+$ ratio in both sensitive and moderately tolerant varieties. However, root applied AsA (100 mg $L^{-1}$) counteracted the adverse effects of salt stress on the growth of tolerant variety which was due to the

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enhanced endogenous AsA level and CAT activity, and higher photosynthetic capacity, and accumulation of $K^+$ and $Ca^{2+}$ in the leaves. Their study supports the notion that exogenous AsA counteracts the adverse effects of salt stress on growth of wheat by improving photosynthetic capacity of wheat plants against salt-induced oxidative stress and maintaining ion homeostasis, however, these effects were cultivar specific [20]. Ascobin (compound composed of ascorbic acid and citric acid) was found to be effective in mitigating salt-induced damages in wheat as reported by Elhamid et al. [114]. Salt stress markedly increased the lipid peroxidation while the activities of antioxidant enzymes (SOD, CAT, POD, APX and GR) dramatically increased. However, foliar treatment of wheat cultivars with ascobin could partially alleviate the harmful effect of salinity especially at the lower levels of salinity imposed in the two cultivars of wheat at most of the studied parameters [114]. Apart from the dose, mode of application is also a factor to initiate the protective effect by exogenous AsA. In their study Athar et al. [115] found differential effects when AsA was applied through the rooting medium, or as seed soaking or as foliar spray to salt stressed (120 mM NaCl) wheat plants. Exogenous AsA mitigated the adverse effect, e.g. improved leaf ascorbic acid, activities of CAT, POD, and SOD. Root applied AsA caused more enhancements in photosynthetic capacity and more reduction in leaf sodium ($Na^+$) compared with AsA applied as seed soaking or foliar spray. However, the effects were also cultivar specific [115]. In a hydroponic experiment Khan et al. [116] showed that foliar applied AsA (50 and 100 mg $L^{-1}$) could not alleviate the adverse effects of salt stress on plants, but it improved the growth of nonstressed plants. Since AsA failed to enhance the antioxidant defense, it enhanced the $Na^+$ accumulation in the leaves but did not change the $K^+$ accumulation in the salt-stressed plants. Azzedine et al. [21] observed that the exogenous AsA improved the plant growth under salt stress condition which was partly due to the increased leaf area, improved chl and carotenoid contents, enhanced Pro accumulation, and decreased $H_2O_2$ content. Melatonin (*N*-acetyl-5-methoxytryptamine) is also considered a potential antioxidant in plants which is distributed in many parts of the plant. Due to its universal hydrophilic and hydrophobic nature and solubility in both water and lipid, it can cross cell membranes easily and enter subcellular compartments and hence, considered as an antioxidant and a modulator in multiple plant developmental processes and various stress responses [117]. In their pot experiment, Sadak et al. [117] observed that wheat seeds presoaked with melatonin (100 and 500 μM) provided better growth, photosynthetic pigments, yield, and quality in wheat under salinity (3.85 and 7.69 dS $m^{-1}$). Melatonin treatments at different levels caused significant increase in yield and yield attributes, carbohydrate, protein, N, P, K, flavonoids, phenolic contents, and antioxidant activity either in nonstressed and salinity-stressed plants relative to their corresponding controls. Importantly, 500 μM melatonin was more effective than 100 μM. Farouk [118] reported that both AsA and $\alpha$-tocopherol minimized salt-induced senescence of flag leaves of wheat. This was due to enhanced activities of antioxidant enzymes which led to the lower lipid peroxidation and $H_2O_2$ accumulation. Exogenous antioxidants also decreased membrane permeability, Na and Cl content. These higher levels of antioxidants and lower level of $H_2O_2$ in flag leaf might be the prerequisite for delayed leaf senescence in antioxidants-sprayed plants [118].

| Cultivars | Dose and duration of stress | Antioxidants | Major effects | References |
|---|---|---|---|---|
| S-24 and MH-97 | 150 mM NaCl, 58 d | 50, or 150 mg $L^{-1}$ AsA | • Decreased $Na^+$ content, and increased $K^+$ and $Ca^{2+}$ content<br>• Improved photosynthesis<br>• Increased AsA content and CAT activities<br>• Improved growth | Athar et al. [20] |
| Sids 1 and Giza 168 | 3000 and 6000 mg $L^{-1}$ NaCl, 75 d | 200-600 mg $L^{-1}$ ascorbin (ascorbic acid and citric acid 2:1) | • Decreased MDA content<br>• Decreased activities of antioxidant enzymes | Elhamid et al. [114] |
| S-24 and MH-97 | 120 mM NaCl, throughout the growth duration | 100 mg $L^{-1}$ AsA | • Increased activities of CAT, POD, and SOD<br>• Improved photosynthesis<br>• Decreased $Na^+$ content | Athar et al. [115] |
| S-24 and MH-97 | 150 mM NaCl, 4 weeks | 50 and 100 mg $L^{-1}$ AsA | • Lower $Na^+$ accumulation<br>• Protection of photosynthesis machineries | Khan et al. [116] |
| Waha | 150 mM NaCl, 2 weeks | 0.7 mM AsA | • Increased leaf area<br>• Improved chl and carotenoid contents<br>• Enhanced Pro accumulation<br>• Decreased $H_2O_2$ content | Azzedine et al. [21] |
| Giza 168 | 0.23, 3.85, and 7.69 dS $m^{-1}$ salinity, 75 d | 500 μM melatonin | • Improved shoot height, number of leaves per plant, FW and DW of shoot<br>• Increased photosynthetic pigments<br>• Increased carbohydrate, protein, N, P, K, flavonoids, phenolic contents, and antioxidant activity | Sadak et al. [117] |
| Giza 168 | 0.8, 7.5, and 11.5 dS $m^{-1}$ salinity, 65 d | 100 mg $L^{-1}$ AsA or α-tocopherol | • Enhanced antioxidant enzymes activities<br>• Reduced $H_2O_2$ accumulation, lipid peroxidation, and membrane permeability<br>• Decreased $Na^+$ and $Cl^-$ contents | Farouk [118] |
| Huaimai 17 | 300 mM NaCl, 7 d | 100 μM SNP (sodium nitroprusside, a nitric oxide/NO donor) | • Improved germination<br>• Deceased Na content and increased K content<br>• Enhanced CAT and SOD activities | Zheng et al. [119] |

| Cultivars | Dose and duration of stress | Antioxidants | Major effects | References |
|---|---|---|---|---|
| S-24 | 150 mM NaCl, 2 weeks | 0–150 μM SNP | • Increased FW<br>• Increased leaf area<br>• Increased photosynthetic parameters | Kausar and Shahbaz [120] |
| Pradip | 100–200 mM NaCl, 48 h | 1 mM SNP | • Decreased MDA and $H_2O_2$ content<br>• Increased AsA and GSH content<br>• Enhanced activities of antioxidant enzymes<br>• Increased activities of glyoxalase enzymes | Hasanuzzaman et al. [24] |
| Sakha | 2000–8000 ppm NaCl, 75 d | 1.25–5.0 mM Arg (arginine) | • Decreased growth<br>• Decreased yield components<br>• Decreased grain and straw yield<br>• Lower amount of Pro, secondary metabolites and mineral contents | Qados et al. [121] |
| Sepahan and Neyshabour | 100 and 200 mM NaCl, 41 d | 0.5 and 1.0 mM Spm | • Increased chl content<br>• Enhanced antioxidant enzymes' activities | Saeidnejad et al. [122] |
| Zhengmai No. 004 | 150 mM NaCl, 48 h | 0.05 μM $H_2O_2$ | • Increased activities of SOD, CAT, APX, and POD<br>• Increased AsA and GSH level<br>• Decreased MDA and $O_2^{\bullet-}$ level<br>• Improved plant height and biomass | Li et al. [123] |

**Table 6.** Protective effects of various exogenously applied antioxidants under salt stress in *T. aestivum*.

### 4.5. Signaling molecules

Although there are specific signaling roles of phytohormones and antioxidants present in plants, which have been discussed in previous sections, this part will discuss the role of exogenously applied signaling molecules. Among the signaling molecules, nitric oxide (NO) has been widely studied in recent decades, due to its diverse role in tolerance to several abiotic stresses including salinity. Nitric oxide exerts its signaling role through various pathways and through interaction with other molecules (**Figure 3**) [26]. In the last decade, exogenous application of NO through different donors was found to enhance crop growth and productivity under stressful conditions [26]. Zheng et al. [119] observed great improvement in seed germination of wheat under high salinity (300 mM NaCl). Wheat seeds soaked in SNP solution provided better germination under salinity which was associated with decreased $Na^+$ concen-

tration and increased $K^+$ concentration in the seeds. Exogenous SNP also helped in increasing starch and amylase content in seeds which increased the weights of coleoptile and radical. Moreover, exogenous NO enhanced the activities of SOD and CAT which decreased the oxidative damages evident with lower level of lipid peroxidation, $O_2^{\bullet-}$, and $H_2O_2$ [119]. Kausar and Shahbaz [120] found the positive effect of foliar applied NO in mitigating salt stress in wheat. Wheat seedlings grown under 100 mM NaCl exhibited reduced growth and photosynthetic rate. However, NO spray ameliorated the effect by enhancing FW of plants, leaf area, stomatal conductance, and internal $CO_2$ concentration. However, NO could not take part role in enhancing PS II activity [120]. In our laboratory, we examined the effect of exogenous NO in conferring salt stress tolerance in wheat [24]. Wheat plant exposed to any level of salt (150 and 300 mM NaCl) caused significant increase in oxidative stress (as indicated by MDA and $H_2O_2$ content). Salt stress-induced oxidative stress was due to the disruption of antioxidant defense. However, the seedlings which were pretreated with NO donor (1 mM SNP) showed enhanced tolerance which was due to increased nonenzymatic antioxidants (AsA and GSH pool) and the activities of monodehydroascorbate reductase (MDHAR), DHAR, GR, glutathione *S*-transferase (GST), GPX, glyoxalase (Gly) I, and Gly II. Therefore, we concluded that both antioxidant defense and glyoxalase systems worked together in enhancing salt stress tolerance as induced by NO [24]. As shown in **Figure 3** Arg is one of the precursors of NO production. Few studies have indicated the role of exogenous Arg in salt stress tolerance in wheat. Qados et al. [121] observed that Arg could alleviate the salt-induced adverse effects in wheat. When wheat plants were exposed to different levels of salinity (2000–8000 ppm NaCl), plant mass, relative water content, yield components (spike length, spike weight, and spikelets per spike), grain yield, straw yield, biological yield, and harvest index decreased in dose dependent manners. Salt stress also deteriorated the chemical constituents of the grains. However, when the grains were presoaked with Arg, they provided better growth, yield components, yield as well as the quality aspects (nutrient content) at harvest [121]. Polyamines are often considered as signaling molecules which interact with NO and also exert direct beneficial effects [124–126]. Saeidnejad et al. [122] found the positive effect of spermine (Spm) in mitigating salt stress (100 and 200 mM NaCl) effect in wheat. In general, although seed priming with Spm showed a slight effect on germination process on both susceptible and tolerant cultivars, Spm application was an effective approach in salinity tolerance induction of wheat cultivars mostly through the activation of enzymatic antioxidants and increasing osmolytes production [122]. $H_2O_2$, which was previously thought to be a toxic substance and a major ROS recently been considered as signaling molecules. The double role of $H_2O_2$ is now an interesting topic of research of many plant scientists. However, as exogenous application, most of the experiments were conducted using $H_2O_2$ as priming agents or pretreatments rather than using as cotreatment. Signaling cross talk of $H_2O_2$ with NO is also well established since last two decades [127]. Exogenous $H_2O_2$ protected wheat plants from salt-induced damages by enhancing antioxidant defense as reported by Li et al. [123]. The seedlings supplemented with $H_2O_2$ (0.05 μM) decreased the levels of MDA and $O_2^{\bullet-}$, which was associated with the increased activities of SOD, POD, CAT and APX and the concentration of GSH and carotenoid under salt stress (150 mM NaCl). Exogenous $H_2O_2$ also increased plant height, shoot length, root length, and biomass under saline condition. The results were reversed when $H_2O_2$ scavenger was used that indicated a clear role of $H_2O_2$ in initiating its signaling role when applied at lower concentration [123].

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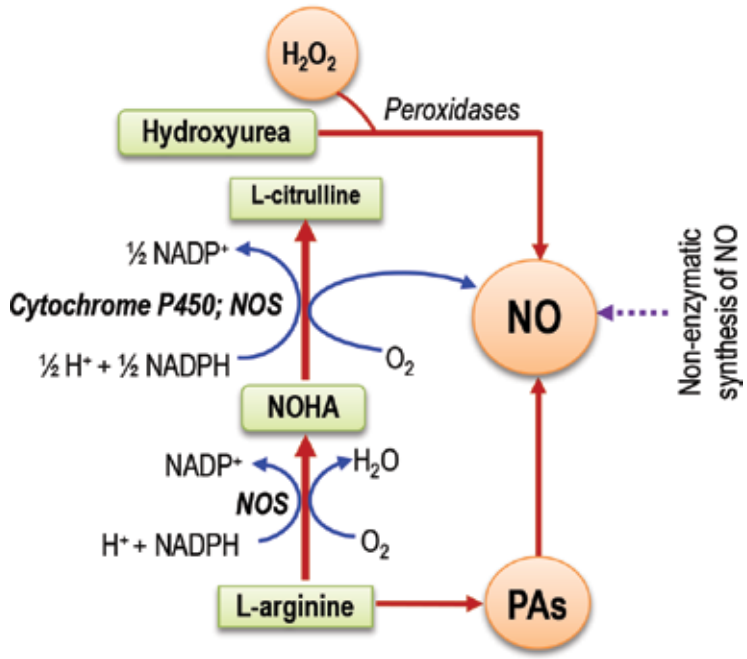


**Figure 3.** Interaction with PA, $H_2O_2$, and Arg during NO biosynthesis.

### 4.6. Seed priming

Seed priming is one of the easiest and cheapest techniques for successful crop production under various abiotic stress conditions including salinity [128, 129]. Seed priming is a presowing, controlled hydration technique that regulates and increases pregermination metabolic activity during early germination stage, but before radical projection [130, 131]. Seed priming has been effectively affirmed to improve germination percentage and seedling establishment in many crops such as wheat, rice, maize, soybean, canola, sunflower, sugarbeet, etc. [29, 132, 133]. Positive effects of seed priming might originate from de novo synthesis of certain germination-promoting substances, enhancing pregermination metabolites [131], early DNA replication, greater ATP availability, enzyme activation, osmotic adjustments [134], and membrane reorganization through restoring their original structures and reducing leakage of metabolites. Along with synchronous and fast emergence, primed seeds show reduced photo and thermodormancy, a wider range of germination temperatures and better capacity to compete with weeds and pathogens [135, 136]. Seed priming can be an easy solution for crops to overcome adverse environmental situations; it is reliable, simple, low cost, and also low risk technique [128, 137]. Various priming techniques such as hydropriming (soaking seed in water), osmopriming (soaking seed in nutrient, hormone, or chemicals), and halopriming (soaking seed in salt solution) have been developed to increase speed of germination, uniform seedling establishment, and crop production [138].

Seed priming has been effectively shown to increase germination and emergence of seeds of many crops in the tropical and subtropical areas, especially under salt stress conditions [139]. Increased germination rates and better seedling establishment resulted in higher levels of salt stress tolerance and crop yields when seeds were primed. Seed priming has recently been applied to overcome the salt stress problem on agricultural land [137]. Several research findings evidenced the role of seed priming to improve salt stress tolerance in wheat (**Table 7**). Hydropriming for 12 h on six Indian wheat cultivars showed 50% reduction of mean germination time under saline condition [140]. Effect of hydropriming was studied in salt-sensitive (MH-97) and salt-tolerant (AUQAB-2000) cultivars of wheat under salt stress (15 dS $m^{-1}$) condition [16]. It is well documented that seed osmopriming helps to improve salt stress tolerance in wheat seedlings. Seed osmopriming with PEG-8000 solution showed increased germination percentage, germination index, root and shoot length, and seedling FW and DW than salt-affected wheat seedlings at different salinity levels (4, 8, 12, and 16 dS $m^{-1}$). It has been reported that seed osmopriming with AsA helped to increase the endogenous AsA content and CAT activity which increased the salt stress tolerances in wheat [141]. Increased germination percentage, early seedling establishment, accumulation of ABA and Pro, and plant growth were featured due to seed osmopriming with 0.05 mM SA in wheat under salt stress condition [142]. Seed halopriming improves plant salt-tolerance by maintaining ion homeostasis mechanism. Salt stress increases the accumulation of $Na^+$ concentrations in the roots and shoots of wheat plants and decreases the uptake of beneficiary nutrients. However, seed halopriming helps to maintain the ion homeostasis by decreasing $Na^+$ concentration and increasing $K^+$, $Ca^{2+}$ concentration, and $K^+/Na^+$ ratio in roots and shoots. Increasing $K^+$ and $Ca^{2+}$ absorption, $K^+/Na^+$ ratio due to seed halopriming under salt stress was connected with vigorous seedling growth and crop production, increased photosynthetic activity, and reduced electrolyte leakage. Seed halopriming with $CaCl_2$ helps in the maintenance of ionic balance by reducing the $Na^+$ and increasing the $K^+$ absorption consequently improves salt stress tolerances [143]. Salt stress also induced oxidative damage by producing ROS. Seed halopriming detoxifies the ROS by increasing the activity of enzymatic antioxidant such as SOD and CAT [43]. Iqbal and Ashraf [100] demonstrated that halopriming with 100 mM KCl, NaCl, and $CaCl_2$ reduced the salt stress affect on growth and grain production of two wheat cultivars. Priming with phytohormone increased germination with better seedling establishment and tolerance to various stresses including salinity. Seed priming of wheat with IAA increased germination percentage by improving amylase activity [144] and mitigated the growth inhibitory effect of salinity [16]. Seed priming of three wheat cultivars with auxin (0, 1, and 2 mg $L^{-1}$) increased germination percentage, root and shoot length, seedling FW and DW, and yield under salt stress condition [18]. Priming with SA (100 mg $L^{-1}$) solution for 24 h enhanced growth, photosynthetic pigments such as chl *a*, chl *b* and also increased total soluble and reducing sugar for maintaining osmotic adjustment during salt stress [145]. Iqbal and Ashraf [101] reported that seed priming with GA (150 mg $L^{-1}$) played a potential role in alleviating salt stress damages by reducing $Na^+$ and $Cl^-$ concentrations, $Na^+/K^+$ ratio, and increasing $K^+$ and $Ca^{2+}$ contents. Moreover, seed priming with GA increased germination percentage, seedling growth and yield contributing components under salt stress condition.

| Cultivar | Priming agent | Duration of priming | Salinity doses and duration | Major responses | References |
|---|---|---|---|---|---|
| AUQAB-2000, MH-97 | Hydropriming, 50.0 mM $CaCl_2$. $2H_2O$, 50 mg $L^{-1}$ AsA | Seeds soaked for 12 h | 15 dS $m^{-1}$, 12 d | • Increased germination percentage<br>• Reduced mean germination time<br>• Increased root and shoot FW and DW<br>• Enhanced activities of CAT, SOD, and POD | Afzal et al. [43] |
| Gomeza 7 | 25, 50, and 100 mM GB | Seed soaked for 24 h | 150 mM NaCl, 38 d | • Decreased lipid peroxidation<br>• Increased PM stability and eventually ion homeostasis | Salama et al. [88] |
| MH-97 | 1, 40, 80, and 120 μM $H_2O_2$ | Seed soaked for 8 h | 150 mM NaCl | • Increased photosynthetic capacity<br>• Enhanced the leaf gas exchange<br>• Increased $K^+/Na^+$ ratio | Wahid et al. [35] |
| | 0.6 mM AsA and sodium salicylate, 0.3 mM thiamine | Seed soaked for 6 h | 40, 80, 120, and 160 mM NaCl, 30 d | • Stimulated starch accumulation<br>• Inhibited production of soluble protein<br>• Reduced water soluble Pro accumulation | Al-hakimi and Hamada [141] |
| Gomeza 7 | 5 and 10 mM choline chloride | Seed soaked for 24 h | 150 mM NaCl, 21 d | • Increased stigmasterol<br>• Decreased cholesterol and campesterol<br>• Increased the plasma membrane stability | Salama et al. [146] |
| AUQAB-2000 | 10 ppm ABA, 50 ppm SA, 50 and 100 ppm AsA | Seed soaked for 12 h | 15 dS $cm^{-1}$ | • Increased seed germination time<br>• Decreased electrolyte leakage by modulating antioxidant enzymes | Afzal et al. [147] |
| AUQAB-2000 | 25 ppm IAA, 50 ppm $GA_3$, 100 ppm kinetin, and 1% prostart | Seed soaked in IAA, $GA_3$, and kinetin for 12 h; and in prostart for 2 h | 15 dS $cm^{-1}$, 21d | • Decreased electrolyte leakage<br>• Increased invertase, α-amylase and starch synthetase activities which helped in better seedling growth | Afzal et al. [16] |
| PUNJAB-11 | 10, 20, 30, 40, and 50 mM $Na_2SiO_3$ | Seed soaked for 12 h | 15 dS $cm^{-1}$ | • Reduced accumulation of $Na^+$<br>• Increased $Ca^{2+}$ content<br>• Increased germination percentage, and root and shoot length<br>• Vigorous seedling establishment | Azeem et al. [148] |
| DK961 | 0.06 mM SNP | Seed soaked for 24 h | 100 mM NaCl | • Increased germination percentage by increasing α-amylase, β-amylase isoenzymes activities<br>• Decreased MDA content, $Na^+$ content<br>• Increased SOD, CAT, APX activities | Duan et al. [149] |

| Cultivar | Priming agent | Duration of priming | Salinity doses and duration | Major responses | References |
|---|---|---|---|---|---|
| Kakaba and Paven-76 | 1 and 2% $CaCl_2$ and $KNO_3$ | Seed soaked for 12 h | 5.97, 9.62, 13.28, and 16.9 dS $m^{-1}$ | • Increased germination with uniform seedlings<br>• Increased tillers per plant<br>• Shortened the physiological maturity period | Dugasa et al. [150] |
| Tatara-96, Ghaznavi-98, Fakhri Sarhad, Bakhtawar-92, Pirsabaq-2004 and AUQAB-2000 | 30 mM NaCl | Seed soaked | 0, 40, 80, and 120 mM NaCl, 55 d | • Enhanced the activities of enzymatic antioxidants<br>• Maintained ionic balance by increasing $K^+$ and $Ca^{2+}$ accumulation<br>• Increased tillers per plant and grain yield | Jamal et al. [32] |
| MH-97, Inqlab-91 | 100, 150, and 200 mg $L^{-1}$ kinetin and BAP | Seed soaked for 12 h | 15 dS $m^{-1}$ | • Increased germination and early seedling establishment<br>• Increased shoot dry weight and grain yield<br>• Enhanced the endogenous growth hormones<br>• Maintained hormonal homeostasis | Iqbal et al. [105] |
| MH-97, Inqlab-91 | 100, 150, and 200 mg $L^{-1}$ $GA_3$ | Seeds primed for 12 h | 15 dS $m^{-1}$ | • Maintained ionic balance by decreasing $Na^+$ and $Cl^-$ ions in roots and shoots<br>• Increased $Ca^{2+}$ and $K^+$ in roots and shoot<br>• Increased leaf salicylic acid concentration<br>• Increased fertile tiller per plant and grain yield | Iqbal and Ashraf [101] |
| MH-97, Inqlab-91 | 2.5 mM Spd and 5 mM Spm | Seeds soaked for 12 h | 15 dS $m^{-1}$ | • Increased shoot growth and grain yield<br>• Enhanced beneficial mineral nutrient uptake by maintaining ion homeostasis<br>• Increased biomass production and photosynthesis rate | Iqbal [151] |
| Inqlab-91 and SARC-1 | 50 mM NaCl, $CaCl_2$, and $CaSO_4$ | Seeds soaked for 12 h | 125 mM NaCl | • Increased germination percentage by increasing total soluble and reducing sugar<br>• Increased shoot and root length under $CaCl_2$ and $CaSO_4$ priming<br>• Increased biomass production<br>• Improved $K^+$ and $Ca^{2+}$ accumulation, and reduced $Na^+$ concentration | Afzal et al. [28] |

| Cultivar | Priming agent | Duration of priming | Salinity doses and duration | Major responses | References |
|---|---|---|---|---|---|
| SARC-1 and MH-97 | 50 mg $L^{-1}$ AsA, $CaCl_2$, kinetin, and SA | Immersed seed in solutions for 12 h | 20 dS $m^{-1}$ | • Decreased emergence time by inducing biochemical changes and antioxidant enzymes activity<br>• Reduced $Na^+$ absorption, and increased $K^+$ and $Ca^{2+}$ absorption<br>• Improved protease and $\alpha$-amylase activities<br>• Enhanced all agronomic and yield characteristics such as plant height, number of tillers, number of spikelets, grain yield, biological yield, and harvest index | Jafar et al. [143] |
| Caxton | 22% PEG-6000 | Seed soaked for 6 h | 50, 100, 150, and 200 mM of NaCl | • Improved germination related metabolic activity such as synthesis of nucleic acids, proteins, and enzymes, and enhanced respiratory activity upto 150 mM level of salt stress but at 200 mM salt stress priming effect becomes reduced | Fuller et al. [31] |
| Sakha-93, Gemmiza-9 | 0.2 mM SNP, 9% diluted sea water, diluted sea water + SNP | Seeds soaked for 10 h | 9 dS $m^{-1}$ | • Increased leaf pigment concentration<br>• Enhanced membrane stability by decreasing lipid peroxidation<br>• Increased total soluble sugar, $K^+$ and $Ca^{2+}$ concentration which decreased $Na^+$ uptake | Maswada and Abd El-Kader [152] |
| Inqlab and S-24 | 100 mg $L^{-1}$ SA | Seeds soaked for 24 h | 50 or 100 mM NaCl; 14 d | • Increased root and shoot length, root and shoot dry weight, total soluble sugar, and carbohydrate metabolism<br>• Increased chl *a* and *b* content | Hamid et al. [145] |
| Azar 2 | 3% NaCl, 5% mannitol, 25% sugar beet extract and hydropriming | Seeds soaked for 4, 8, and 10 h | 3.6 dS $m^{-1}$ | • Increased hypocotyle length, root number and leaf length, shoot and root fresh weight<br>• Increased photosynthesis rate<br>• Enhanced biomass production | Amoghein et al. [153] |

**Table 7.** Beneficial effects of seed priming in improving salt stress tolerance in *T. aestivum*.

## 5. Conclusions and future perspectives

Wheat is the most popular and widely consumed cereal crops in the world due to its diverse uses. Most of the cultivated wheat is hexaploid which has some acquired tolerance to salt stress. However, increasing levels of salinity in irrigated lands make wheat production difficult because plant growth and productivity of wheat are severely affected by high salinity. Salt stress adversely

affects seed germination, plant growth, photosynthesis, water relations, nutrient uptake, and yield. Oxidative stress is one of the most common effects of salt stress in wheat. However, salt stress effects depend on the dose and duration of stress, and mostly on genotypes. Considering the importance of wheat and the adverse effects of salt stress, plant biologists are trying to develop strategies to improve salt tolerance in wheat. Some of the strategies are related to the genetic manipulation of salt-tolerant traits. Physiologists are also trying to find the adaptive mechanisms to cope with the salt stress. However, the actual physiological mechanism of salt stress tolerance is yet to be revealed. Therefore, coordinated attempts by plant physiologists, breeders, and agronomists are essential to find out a sustainable strategy to enhance salt tolerance in wheat.

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# Wheat Management

Chapter 9

# Winter Wheat Response to Weed Control and Residual Herbicides

Timothy L. Grey and Larry J. Newsom

Additional information is available at the end of the chapter

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**Abstract**

Italian ryegrass has become one of the most common and troublesome weeds of wheat production in the Southern United States. There are multiple reports in this region of Italian ryegrass herbicide resistance to acetyl-CoA carboxylase (ACCase), acetolactate synthase (ALS), and glyphosate herbicides. One commonality for Italian ryegrass resistance in this area is that most of these mechanisms of action for these herbicides are all postemergence (POST) applied. In order to have profitable soft red winter wheat production, applications of preemergence (PRE) herbicides with residual control of Italian ryegrass and other winter weed species would benefit growers. There are a very limited number of herbicides that can be applied at the time of wheat planting, primarily only when pyroxasulfone is registered for this timing. Research was conducted to establish weed control information when herbicides were applied to soft red winter wheat PRE, at wheat emergence (AE), or POST at Feekes stages 1.0–1.9, depending on herbicide label recommendations. Injury from any pyroxasulfone PRE treatments up to 120 g a.i. $ha^{-1}$ was transient and did not affect wheat yield for any experiment. Italian ryegrass control was variable depending on location and year. Susceptible and diclofop-resistant Italian ryegrass control was 86% or greater with pyroxasulfone at 60 g a.i. $ha^{-1}$ and greater with applied PRE. Italian ryegrass control was variable ranging from 27 to 49% with pendimethalin ME-applied PRE, diclofop at Feekes sage 1.0, and pinoxaden applied at Feekes stage 1.9.

**Keywords:** crop tolerance, Italian ryegrass, wild radish, diclofop, 2,4-diclorophenoxy acetic acid, pyroxasulfone, pendimethalin ME, metribuzin, thifensulfuron, tribenuron, MCPA, pinoxaden, mesosulfuron

## 1. Introduction

Information about wheat production in the United States has been recorded since 1867 with respect to hectares planted (black dots on **Figure 1A**) and yield (bars on **Figure 1B**). While production in hectares first increased until 1950, it then decreased as yield in kg per hectare increased.

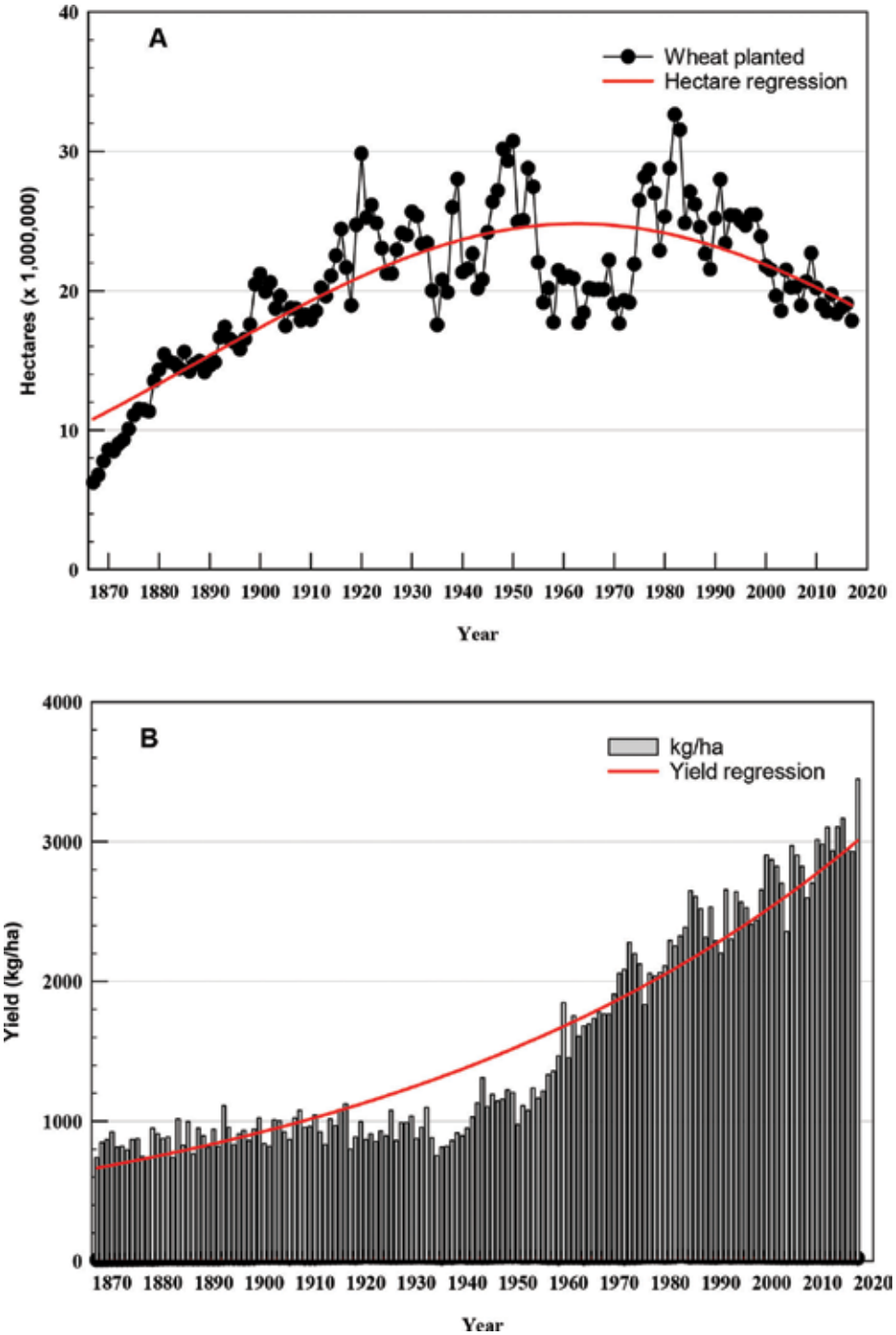


**Figure 1.** Hectares of wheat planted (A) and grain yield (B) in the United States (National Agricultural Statistics Service, USDA. Hectare and kg/ha data available at https://www.nass.usda.gov).

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Improved genetics, fertility, disease, insect, and weed control options contributed to increased yield. As **Figure 1A** indicates, hectares planted decreased from 1950 to 1955, then stayed relatively constant until 1980, and then began to decline in 2000 and have continued this downward trend into the twenty-first century. However, wheat yield doubled from approximately 1000 to 2000 kg/ha from 1950 to 1980 (**Figure 1B**). Then, from 1980 to 2010, yield increased from 2000 to 3000 kg/ha as the overall number of hectares planted again declined to the 1950s level. The mid-1950s increase is significant as it occurred with the introduction of herbicides. Multiple weed species have become an issue in wheat all across this production region. However, Italian ryegrass [*Lolium perenne* L. ssp. *multiflorum* (Lam.) Husnot] is one of the most common and troublesome weeds in wheat. As a winter forage, Italian ryegrass is planted and then becomes a problematic weed in small grains due to escapes [1–3]. While herbicides can be used to control this weed, there are also herbicide-resistant issues. There are multiple reports of Italian ryegrass herbicide resistance to acetyl CoA carboxylase (ACCase), acetolactate synthase (ALS), and glyphosate herbicides [1, 4]. A commonality for Italian ryegrass resistance is that most of these herbicides mechanisms of action that have resistance issues are generally all postemergence (POST) applied to the weed. In order to have profitable wheat production, applications of preemergence (PRE) herbicides with residual control of Italian ryegrass and other winter weed species would benefit growers. Currently, there are limited herbicides that can be applied at the time of wheat planting.

## 2. Importance

Soft red winter wheat is an autumn-seeded crop in the Mid-South and Southeastern United States where it is double-cropped with cotton (*Gossypium hirsutum* L.), peanut (*Arachis hypogaea* L.), or soybean (*Glycine max* (L.) Merr.). Italian ryegrass is a vigorous erect winter annual native to temperate Europe where it was grown as a forage with reports of its presence in France, Switzerland, and England from 1818 to 1831 [3]. This use led to its migration to the Western Hemisphere with reports by Henderson on its quality [5]. Because of easy establishment, it was adapted for forage production. However, volunteer Italian ryegrass seed can become a weedy plant in small grains such as wheat [2, 3]. It can grow to over 1 m in height protruding above the wheat canopy, producing multiple tillers and seed heads from a single plant (**Figure 2**). It has long, clasping auricles, and awned seeds (**Figure 2**). Over time, it has become a major weed species for this region due to its aggressive growth and seed production. It has consistently ranked as being one of the most common and troublesome weeds in small grains and wheat for over 20 years [6–10]. Stone et al. [11] reported that Italian ryegrass interference with wheat was the result of its greater root density relative to the crop, which creates excess competition for moisture and nutrients. With respect to aboveground development, Ball [12] determined that leaf production rate was greater for Italian ryegrass as compared to wheat. Liebl and Worsham [13] noted that wheat grain yields were reduced by 4% for every ten Italian ryegrass plants per $m^2$ and that declining yields could be primarily attributed to reductions in crop tillering. According to Appleby et al. [14], Italian ryegrass infestations of 29–118 plants per $m^2$ reduced wheat yields between 7 and 50%. Italian ryegrass has similar growth stages to soft red winter wheat (**Figure 3**) and thus competes for resources in terms of space, sunlight, nutrients, and moisture.

**Figure 2.** Italian ryegrass [*Lolium multiflorum* L. ssp. multiflorum (Lam.) Husnot] in soft red winter wheat field, spikelet, and single seed, respectively (photos by Sidney Cromer).

**Figure 3.** Italian ryegrass [*Lolium multiflorum* L. ssp. multiflorum (Lam.) Husnot] in seedling, tillering, and reproductive phases, respectively [photos by Timothy Grey (center) and Sidney Cromer (left and right)].

Wild radish (*Raphanus raphanistrum* L.) is another common and troublesome winter annual weed in soft red winter wheat production regions of the Southeastern United States [6]. Cruciferous species compete vigorously with wheat, and data indicate that significant yield losses can occur if these weeds are not controlled soon after crop emergence [15]. Seeds of cruciferous species are high in erucic acid and glucosinolates that can pose quality problems in harvested wheat [16]. Once wild radish is established in wheat, it can be controlled with POST-applied herbicides, but these herbicides are not always used for economic, management, or even herbicide-resistant reasons [17–19]. Other winter weeds in soft red winter wheat production include henbit (*Lamium amplexicaule* L.), swine cress [*Coronopus didymus* (L.) Sm.], and cutleaf evening primrose (*Oenothera laciniata* Hill.) [6].

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## 3. Background information on wheat herbicides

As the records for the US wheat production indicated in **Figure 1**, yield and hectares increased from the 1870s to the 1950s due to improved agronomic practices. Herbicides were introduced in small grain production in the 1940s for broadleaf weed control [20] and marked the beginning for the trend of reduced hectares producing greater yields. These two facts are born out in regression of the data over this era, with a negative regression for hectares planted beginning in the 1960s. In contrast, yield in kg per ha has maintained a positive slope, with slight declines in production during the 1930's Dust Bowl. With the introduction of improved farming techniques, pesticides, fertility, and improved cultivars, wheat production after World War II began to increase significantly as herbicides were incorporated into production practices.

### 3.1. Herbicides

Herbicides are used for PRE and POST control of grass and broadleaf weed species in wheat. However, control with POST herbicide applications is often the most commonly used as noted by **Figure 4**. Herbicide-applied POST can have less than desired weed control. Reduced efficacy has been associated with variables such as delayed application, suboptimum rates, not including a suitable adjuvant, including in tank mixture with other herbicides that are antagonistic, or during environmentally induced plant stress. The second factor that contributes to control failure is herbicide-resistant weeds. Herbicides that inhibit ACCase include the aryloxyphenoxypropionates and cyclohexanediones. Within the United States, there has been a rapid increase in ACCase-resistant Italian ryegrass biotypes since 1990 [21]. For example, Italian ryegrass resistant to diclofop was first reported in 1987 in Oregon [22, 23]. It has subsequently been reported in the Southeastern United States [21] and throughout the world [22, 24–27]. The widespread development of herbicide resistance in Italian ryegrass will reduce control options in wheat. While wild radish herbicide resistance has been reported in multiple wheat production regions including Australia, Brazil, and South Africa [4], no reports have occurred in North America.

### 3.2. Synthetic auxin herbicides

The first herbicide to be introduced for chemical weed control in any crop was 2,4-(dichlorophenoxy)acetic acid (2,4-D). Reports of the plant growth regulatory effects were first noted by Marth and Mitchell [25] in the journal *Botanical Gazette*. They reported via a personal communication that 2,4-D could potentially be used for weed control. Marth and Mitchell [28] reported on the delivery of 2,4-D specifically via POST aqueous spray solutions at 500 and 1000 ppm, with efficacy on several broadleaf weed species including dandelion (*Taraxacum officinale* F.H. Wigg.) and plantain (*Plantago lanceolata* L.) that were controlled in Kentucky bluegrass (*Poa pratensis* L.). Klingman [29] experimented with wheat and noted 2,4-D tolerance when applied with postemergence to the crop. After decades of further research on wheat evaluating rate and timing of applications, 2,4-D became a standard herbicide used for broadleaf weed control and is still currently used as a POST treatment. Other auxin herbicides which used POST in wheat for broadleaf weed control include (4-chloro-2-methylphenoxy) acetic acid (MCPA) and 3,6-dichloro-2-methoxybenzoic acid (dicamba). These herbicides have

had consistent use patterns for the past 25 years in winter wheat with 2,4-D averaging over 1,000,000 kg applied in the United States annually (**Figure 4**). Dicamba and MCPA have averaged between 200,000 and 400,000 kg annually since 2006 (**Figure 4**) in winter wheat [30].

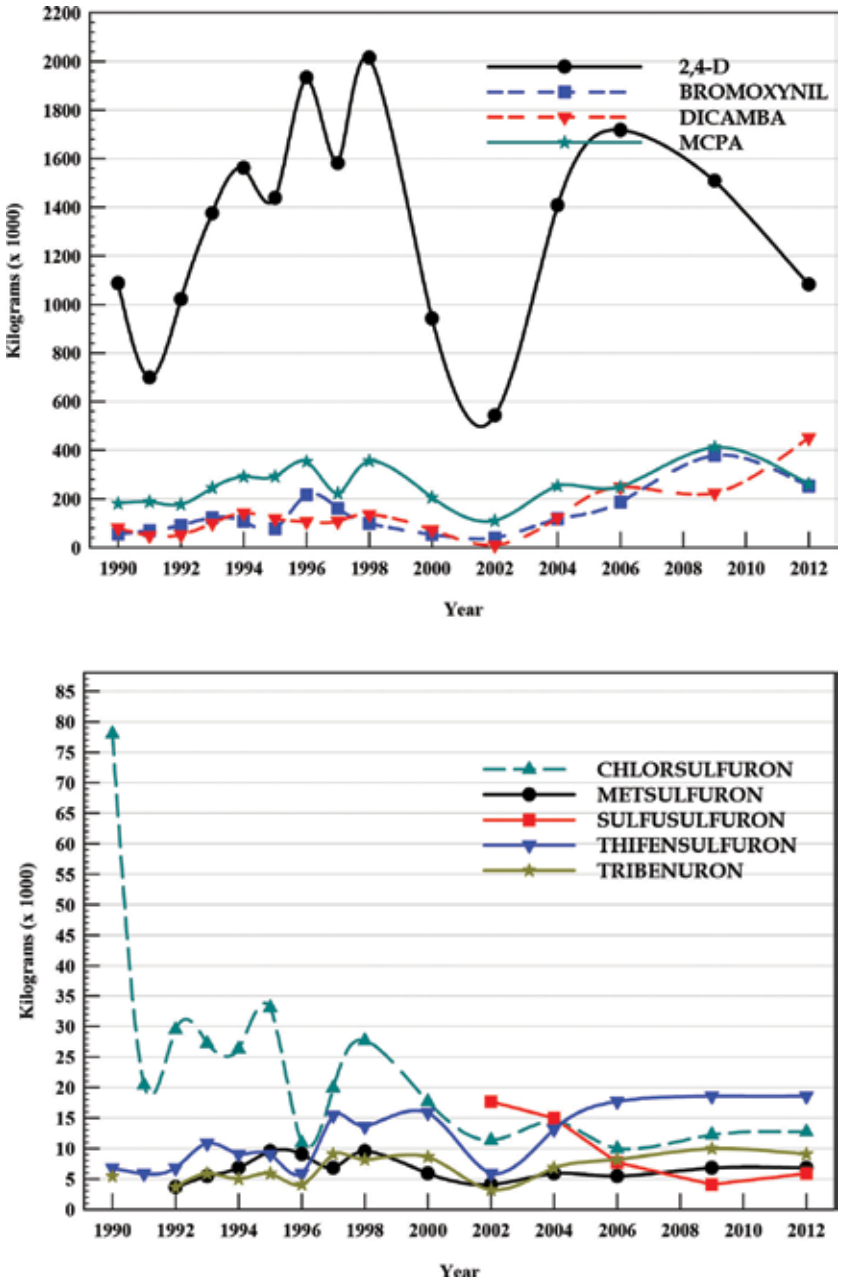


**Figure 4.** Herbicide use in winter wheat from 1990 to 2012 in the United States for multiple mechanisms of action. Data available at http://www.nass.usda.gov/Statistics_by_Subject/Environmental/index.asp.

### 3.3. Photosystem II herbicides

Photosystem II (PS II) herbicides used in winter wheat include metribuzin and bromoxynil and are utilized in the Southeastern United States [16]. Metribuzin can be POST applied to winter wheat for control of annual grasses and dicot weeds including Italian ryegrass and wild radish [16] just as the coleoptile is emerging from soil. While metribuzin can control Italian ryegrass effectively, careful management, including cultivar selection and timely application, is required to achieve acceptable crop tolerance and weed control. Many agronomically desirable, high-yielding wheat cultivars are sensitive to metribuzin and cannot be planted if metribuzin is to be applied and some cultivars are extremely sensitive [31–33]. Bromoxynil in wheat will control wild radish but is ineffective on Italian ryegrass. Bromoxynil use in soft red winter wheat has averaged over 200,000 kg in the United States since 2006 (**Figure 4**).

### 3.4. Acetolactate synthase (ALS) herbicides

Sulfonylurea (SU) herbicides were first synthesized by E.I. DuPont Corp. in the mid-1950s and screened for pesticide properties, but first attempts revealed no significant biological activity [34]. It was not until the 1970s that the analogs of SUs began to be synthesized and their herbicidal activity evaluated. Prior to this there was no precedence for high potency and extremely low use rates in the g $ha^{-1}$ range for weed control. One example described by Bhardwaj [34] was that university researchers would move the decimal two places as they could not believe that herbicides could be effectively applied at g $ha^{-1}$, rather than kg $ha^{-1}$. The result was that weeds would not grow in treated test plots after 2 years, despite half-lives of 6–8 weeks. Thus, the potency of the SUs was recognized, and their use in plant production systems, including wheat, was quickly established. The key components to SUs are two moieties (R1 and R2) on either side of a sulfonylurea bridge. Generally, the moieties are composed of an aryl group, a pyrimidine ring, or a triazine ring [35, 36]. Variation in herbicidal activity occurs by substitutions made to branches on these rings. Chlorsulfuron was the first SU herbicide released by E.I. DuPont for weed control in small grains [37]. LaRossa and Schloss [38] reported that sulfometuron methyl was a potent acetolactate synthase (ALS) isozyme II inhibitor by testing of *Salmonella typhimurium*. Since then, all SUs have been identified as ALS inhibitors [39]. There are currently several SUs used in wheat weed control including chlorsulfuron, metsulfuron, sulfosulfuron, mesosulfuron, thifensulfuron, and tribenuron. Use rates vary but fall primarily within a range of 4–280 g $ha^{-1}$. These use patterns are reflected in the masses of herbicides used when comparing the auxin and PS II inhibitors combined to average over 2,450,000 kg in 2012, versus the ALS herbicides at 53,000 kg (**Figure 4**): a 46 times greater application mass. This comparison reflects the potency and reduces environmental impact aspect of the ALS herbicides. Another POST ALS wheat herbicide is the triazolopyrimidine pyroxsulam that is specifically preferred in the Southeastern United States because it controls Italian ryegrass and wild radish. However, there are multiple reports of ALS resistance in Italian ryegrass that make these herbicides less viable options and essentially render those useless [40].

### 3.5. Soil residual herbicides

New herbicide chemistries and new formulations of older compounds are available for weed control in soft red winter wheat. These include options for grass and broadleaf weed species. Pendimethalin [*N*-(1-ethylpropyl)-3,4-dimethyl-2,6-dintrobenzenamine] formulated as a micro-encapsulated (ME) aqueous capsule suspension contains 38.7% (0.47 kg $L^{-1}$) active ingredient and can be applied after wheat has the first true leaf. This will provide residual weed control to later emerging weeds, but does not overcome the issue of weeds emerging right after wheat planting.

Pyroxasulfone (3-[5-(difluoromethoxy)-1methyl-3-(trifluoromethyl)pyrazol-4-ylmethylsulfonyl-4,5-dihydro-5,5-dimethy-1,2-oxazole) is an isoxazoline PRE soil residual herbicide registered for soft red winter wheat since 2014 in the United States [41]. It has been researched and registered in multiple wheat production regions of the world including Australia [42], Japan, Canada, Saudi Arabia, South Africa, and the United States [43]. Pyroxasulfone inhibits the biosynthesis of very-long-chain fatty acids (VLCFAs) leading to the buildup of fatty acid precursors, specifically inhibiting many elongation steps catalyzed by VLCFA elongases, as a Group 15 (WSSA)/Group $K_3$ (HRAC) herbicide [39, 44]. Nakatani et al. [43] noted that the herbicide benthiocarb (*S*-[(4-chlorophenyl)methyl]diethylcarbamothioate) was used as the basis for research development of pyroxasulfone by developing a novel chemical structure by using various substitutions. This resulted in a compound with low water solubility (3.49 mg $L^{-1}$), no pKa, and hydrolytically stable at all pH values at 25 C, allowing less susceptibility to decomposition and thus providing extended soil residual activity [39, 43]. Dissipation rates ($DT_{50}$) for pyroxasulfone have ranged from 8 to 71 days in the top 8 cm of Tennessee soils [45] and 54 to 94 days in the top 7.5 cm of Colorado soils [46]. Pyroxasulfone's soil residual activity and utility have allowed it to be registered for multiple uses including corn (field, sweet, and pop) (*Zea mays* L.), soybean, cotton, fallow land, and non-crop areas [47–49]. Winter wheat tolerance has been well documented with only minor injury in the form of stunting with no negative effects on yield [50–52]. With PRE soil activity on broadleaf and grass species including ALS- [52], ACCase- [41], and glyphosate- [1] resistant Italian ryegrass biotypes, pyroxasulfone use in wheat will afford growers an early season weed control option that was previously unavailable.

## 4. Research

While auxins, PS IIs, and SUs are effective in wheat production, they have traditionally been POST applied for weeds that have already emerged. By applying after the crops emergence, the potential for weed infestation increases leading to a greater production costs, resulting in yield loss and potential quality issues. By utilizing herbicides either as PRE or soon after emergence, weed control could be enhanced in soft red winter wheat. Pyroxasulfone is labeled for delayed PRE or early POST application. The registrations for application of pyroxasulfone differ by company. One company defines that applications of pyroxasulfone must be delayed PRE as to when wheat has 80% germinated seed with a 1.2 cm long shoot, as well as having an early POST from spike to the fourth tiller timing [44]. Other companies have regional and state requirements that wheat must be planted 2.5–3 cm deep for PRE application (Pacific

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north western region of the United States) [45] but can also have POST applications for specific states [46]. Therefore, this chapter will emphasize pyroxasulfone, pendimethalin ME, and other herbicides for PRE and POST weed control and wheat response.

### 4.1. Field studies

Field studies were conducted to evaluate herbicides used for soft red winter wheat production focusing on residual and contact active ingredients, as well as timing when applied either PRE or POST with respect to crop emergence. All studies were conducted as described in **Table 1** for soil nomenclature, soil texture, soil pH, organic matter content, wheat cultivar, and dates associated with seeding, herbicide application timings, and harvest. Experiments were conducted from autumn to spring in 2009–2010 (**Table 2**), 2010–2011 (**Tables 2** and **3**), 2011–2012 (**Tables 2** and **3**), 2012–2013 (**Tables 4** and **5**), and 2013–2014 (**Table 4**). Experiments were conducted on the University of Georgia property at the Bledsoe Research Farm near Williamson, at the Southwest Georgia Branch Experiment Station located near Plains, or at the Ponder and Lang Research Farms near Tifton. Treated plots included eight rows of wheat on 19 cm spacing (1.8 m wide), in plots 7.6 or 9.1 m long, with wheat seeding rates of 90 kg $ha^{-1}$. A randomized complete block design with four replications was used for all experiments. Herbicides were applied with a $CO^2$-pressurized sprayer calibrated to deliver 187 L $ha^{-1}$ at 210 kPa for all experiments. PRE applications were made prior to wheat emergence; at emergence (AE) applications were made at Feekes 0.9 [53] when the coleoptile was soil emerged. POST applications were applied between Feekes 1.0 and 1.9. Fertilizer and liming requirements were based on the University of Georgia Extension recommendations for wheat. Insects and plant diseases were monitored and sprayed when necessary. Wheat stand counts were made multiple times during the season on 1 m of length of row. Wheat injury and natural infestations of weeds were evaluated for each location at multiple times during the growing season. Wheat injury and weed control were visually estimated on a scale of 0 (no injury) to 100% (death). Data for experiments that were identical were combined for analysis. Weed control, wheat stand counts, wheat injury, and wheat yield were subjected to mixed model analysis of variance (ANOVA) in SAS 9.2 [54]. Complete treatment description for all 15 experiments is listed in **Table 1**.

| | **Griffin** | | **Plains** | | **Tifton** | | **Plains** | | **Tifton** |
|---|---|---|---|---|---|---|---|---|---|
| | **2009–2010** | **2010–2011** | **2009–2010** | **2010–2011** | **2009–2010** | **2010–2011** | **2010–2011** | **2011–2012** | **2010–2011** |
| Soil nomenclature | Clayey, kaolinitic, thermic, Typic Hapludult | | Clayey, kaolinitic, thermic, Typic Kandiudults | | Fine-loamy, kaolinitic, thermic, Plinthic Kandiudult | | Clayey, kaolinitic, thermic, Typic Kandiudults | | Fine-loamy, kaolinitic, thermic, Plinthic Kandiudult |
| Soil texture | Cecil sandy clay loam | | Faceville sandy loam | | Tifton loamy sand | | Faceville sandy loam | | Tifton loamy sand |
| Soil pH | 6.3 | 6.2 | 6.0 | 6.2 | 5.9 | 6.1 | 6.2 | 5.9 | 6.2 |
| Organic matter (%) | 1.5 | 1.0 | 1.0 | 0.5 | 1.1 | 0.5 | 1.0 | 1.3 | 0.5 |
| Wheat cultivar | AGS 2031 | AGS 2026 | AGS 2000 | AGS 2020 | Gore | AGS 2031 | Gore | CL 7 | AGS 2020 |

| | Griffin | | Plains | | Tifton | | Plains | | Tifton |
|---|---|---|---|---|---|---|---|---|---|
| | **2009–2010** | **2010–2011** | **2009–2010** | **2010–2011** | **2009–2010** | **2010–2011** | **2010–2011** | **2011–2012** | **2010–2011** |
| Seeding date | 5 Nov 2009 | 2 Nov 2010 | 18 Nov 2009 | 19 Nov 2010 | 5 Nov 2009 | 2 Nov 2010 | 19 Nov 2010 | 22 Nov 2011 | 23 Nov 2010 |
| Number of treatments | 12 | 12 | 12 | 12 | 12 | 12 | 14 | 14 | 14 |
| PRE application(s) | 7 Nov 2009 | 8 Nov 2010 | 18 Nov 2009 | 19 Nov 2010 | 6 Nov 2009 | 8 Nov 2010 | 8 Nov 2010 | 9 Nov 2011 | 12 Nov 2010 |
| | —— | —— | —— | —— | —— | —— | 19 Nov 2010 | 22 Nov 2011 | 23 Nov 2010 |
| POST application(s) | 14 Jan 2010 | 17 Jan 2011 | 13 Jan 2010 | 24 Jan 2011 | 12 Jan 2010 | 17 Jan 2010 | 26 Nov 2010 | 1 Dec 2011 | 3 Dec 2010 |
| | —— | —— | —— | —— | —— | —— | 24 Jan 2011 | 14 Dec 2011 | 10 Jan 2011 |
| Harvest date | 4 June 2010 | NY[a] | 27 May 2010 | 6 June 2011 | 28 May 2010 | 2 June 2010 | 12 May 2011 | 1 May 2012 | 6 June 2011 |

| | Griffin | | Plains | Tifton | Griffin | Plains |
|---|---|---|---|---|---|---|
| | **2012–2013** | **2013–2014** | **2012–2013** | **2012–2013** | **2012–2013** | **2012–2013** |
| Soil name | Clayey, kaolinitic, thermic, Typic Hapludult | | Clayey, kaolinitic, thermic, Typic Kandiudults | Fine-loamy, kaolinitic, thermic Plinthic Kandiudult | Clayey, kaolinitic, thermic, Typic Hapludult | Clayey, kaolinitic, thermic, Typic Kandiudults |
| Soil texture | Cecil sandy clay loam | | Faceville sandy loam | Tifton loamy sand | Cecil sandy clay loam | Faceville sandy loam |
| Soil pH | 6.0 | 6.3 | 6.5 | 5.9 | 6.0 | 6.5 |
| Organic matter | 1.0 | 1.5 | 2.0 | 1.1 | 1.0 | 2.0 |
| Wheat cultivar | SS 8641 | SS8461 | AGS 3035 | AGS 2020 | SS 8641 | AGS 3035 |
| Seeding date | 1 Nov 2012 | 31 Oct 2013 | 20 Nov 2012 | 14 Nov 2012 | 1 Nov 2012 | 20 Nov 2012 |
| Number of treatments | 15 | 15 | 15 | 15 | 10 | 10 |
| PRE application | 2 Nov 2012 | 4 Nov 2013 | 20 Nov 2012 | 14 Nov 2012 | 2 Nov 2012 | 20 Nov 2012 |
| POST application(s) | 13 Nov 2012 | 9 Nov 2013 | 3 Dec 2012 | 26 Nov 2012 | 13 Nov 2012 | 3 Dec 2012 |
| | 6 Dec 2012 | 20 Nov 2013 | 14 Dec 2012 | 18 Dec 2012 | 6 Dec 2012 | 14 Dec 2012 |
| | —— | —— | —— | —— | 15 Jan 2013 | 11 Jan 2013 |
| Harvest date | NY[a] | NY | 28 May 2013 | 29 May 2013 | NY | 28 May 2013 |

[a]No yield (NY) taken as natural infestations of ryegrass populations made harvest impossible.

**Table 1.** Location information by table for soft red winter wheat herbicide trials and weed control evaluations in Georgia.

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| Herbicide | Timing | Rate (g a.i. ha$^{-1}$)[b] | Injury (%) | | | | Italian ryegrass (%) | | | | Yield (kg ha$^{-1}$) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 15 DAP | | 90 DAP | | 30 DAP | | 175 DAP | | | |
| Nontreated control | | | 0 | c | 0 | e | 0 | e | 0 | e | 3730 | a |
| Pyroxasulfone | PRE | 40 | 0 | c | 4 | cde | 72 | abc | 75 | bc | 4110 | a |
| Pyroxasulfone | PRE | 60 | 3 | c | 8 | bcd | 83 | abc | 90 | ab | 4230 | a |
| Pyroxasulfone | PRE | 80 | 9 | b | 14 | b | 91 | ab | 92 | ab | 4180 | a |
| Pyroxasulfone | PRE | 160 | 20 | a | 23 | a | 93 | a | 97 | a | 4000 | a |
| Pendimethalin ME | PRE | 1064 | 3 | c | 5 | cd | 66 | c | 54 | cd | 4050 | a |
| Pinoxaden | POST | 119 | – | – | 11 | bc | 75 | abc | 57 | cd | 3930 | a |
| Pendimethalin ME + pinoxaden | POST | 1064 + 119 | – | – | 8 | bcd | 84 | abc | 51 | cd | 4000 | a |
| Pyroxasulfone + pinoxaden | POST | 40 + 119 | – | – | 5 | cd | 70 | bc | 96 | a | 4110 | a |
| Diclofop | POST | 559 | – | – | 6 | cd | 27 | d | 50 | d | 3970 | a |
| Pyroxsulam | POST | 18 | – | – | 6 | b | 92 | ab | 86 | ab | 3987 | a |
| Mesosulfuron | POST | 15 | – | – | 9 | bcd | 94 | a | 79 | ab | 3970 | a |

[a] Site-year locations: Griffin, Plains, and Tifton, Georgia

[b] Abbreviations: a.i., active ingredient; DAP, days after planting; ME, microencapsulated; PRE, preemergence; POST, postemergence applied 65–70 DAP at Feekes scale 1.5–1.9

**Table 2.** Herbicide, rates, and timing of applications for evaluating weed control and soft red winter wheat growth response in Georgia, 2010–2011 and 2011–2012: data represents six site-year locations[a].

| Herbicide | Timing | Rate (g a.i. ha$^{-1}$)[b] | Injury (%) | | | | Italian ryegrass (%) | | | | Henbit[c] | | Yield (kg ha$^{-1}$) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 14 DAP | | 28 DAP | | 30 DAP | | 175 DAP | | 30 DAP | | | |
| Nontreated control | | | 0 | b | 0 | d | 0 | d | 0 | h | 0 | b | 4370 | a |
| Pyroxasulfone | 12 DPRE | 40 | 0 | b | 1 | cd | 88 | a | 55 | def | 95 | a | 4570 | a |
| Pyroxasulfone | 12 DPRE | 60 | 0 | b | 2 | cd | 98 | a | 35 | fg | 97 | a | 4810 | a |
| Pyroxasulfone | 12 DPRE | 80 | 4 | b | 5 | bc | 97 | a | 74 | abcd | 98 | a | 4600 | a |
| Pyroxasulfone | 12 DPRE | 100 | 5 | b | 5 | bc | 98 | a | 66 | bcd | 97 | a | 4670 | a |
| Pyroxasulfone | 12 DPRE | 120 | 11 | a | 7 | ab | 98 | a | 65 | bcd | 99 | a | 4760 | a |
| Pyroxasulfone | PRE | 40 | 3 | b | 2 | cd | 88 | a | 63 | bcd | 91 | a | 4620 | a |
| Pyroxasulfone | PRE | 60 | 3 | b | 1 | cd | 96 | a | 84 | abc | 91 | a | 4660 | a |
| Pyroxasulfone | PRE | 80 | 2 | b | 3 | bcd | 96 | a | 91 | a | 98 | a | 4860 | a |
| Pyroxasulfone | PRE | 100 | 10 | a | 9 | a | 98 | a | 92 | a | 98 | a | 4650 | a |
| Pyroxasulfone | PRE | 120 | 14 | a | 10 | a | 99 | a | 87 | ab | 91 | a | 4590 | a |
| Saflufenacil | PRE | 60 | 1 | b | 2 | cd | 97 | a | 54 | def | 98 | a | 4740 | a |

| Herbicide | Timing | Rate (g a.i. ha$^{-1}$)[b] | Injury (%) | | | | Italian ryegrass (%) | | | | Henbit[c] | | Yield (kg ha$^{-1}$) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 14 DAP | | 28 DAP | | 30 DAP | | 175 DAP | | 30 DAP | | | |
| Pendimethalin ME | AE | 1064 | 0 | b | 1 | cd | 48 | c | 27 | g | 91 | a | 4650 | a |
| Pinoxaden | POST | 119 | 0 | b | 3 | bcd | 68 | b | 40 | efg | 0 | b | 4450 | a |

[a] Site-year locations: Griffin, Plains, and Tifton, Georgia

[b] Abbreviations: a.i., active ingredient; DAP, days after planting; ME, microencapsulated; 12 DPRE, 12 days before planting; PRE, preemergence; AE, at wheat emergence; POST, postemergence applied 65–70 DAP at Feekes scale 1.5–1.9

[c] Henbit at Plains location 2010–2011 and 2011–2012

**Table 3.** Herbicide, rates, and timing of applications for evaluating weed control and soft red winter wheat growth response in Georgia, 2010–2011 and 2011–2012: data represents three site-year locations[a].

| Herbicide | Timing | Rate (g a.i. ha$^{-1}$)[b] | Injury (%) | | Italian ryegrass (%) | | | | Henbit[c] | | Yield (kg ha$^{-1}$) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 30 DAP | | 75 DAP | | 175 DAP | | 30 DAP | | | |
| Nontreated control | | | 0 | b | 0 | f | 0 | h | 0 | f | 5120 | b |
| Pyroxasulfone | PRE | 60 | 0 | b | 95 | a | 93 | a | 75 | e | 5680 | ab |
| Pyroxasulfone | PRE | 80 | 1 | b | 95 | a | 95 | a | 69 | e | 5762 | ab |
| Pyroxasulfone + saflufenacil | PRE | 60+119 | 0 | b | 95 | a | 92 | ab | 95 | ab | 6080 | a |
| Pyroxasulfone + saflufenacil | PRE | 80+119 | 3 | b | 97 | a | 96 | a | 90 | abcd | 6310 | a |
| Pyroxasulfone | AE | 60 | 0 | a | 74 | bcd | 54 | efg | 75 | e | 6070 | a |
| Pyroxasulfone | AE | 80 | 0 | b | 78 | bc | 72 | cde | 81 | bcde | 6320 | a |
| Metribuzin | AE | 476 | 18 | a | 85 | abc | 73 | cde | 97 | a | 5670 | ab |
| Pyroxasulfone + pendimethalin ME | AE | 60+1064 | 0 | b | 74 | cde | 69 | ed | 98 | a | 6160 | a |
| Pyroxasulfone + pendimethalin ME | AE | 80+1064 | 0 | | 76 | bcd | 67 | edf | 98 | a | 6440 | a |
| Metribuzin + pendimethalin ME | AE | 476+1064 | 17 | a | 87 | ab | 75 | bcd | 98 | a | 5350 | b |
| Pyroxasulfone | POST | 60 | 0 | b | 60 | de | 50 | fg | 76 | de | 6260 | a |
| Pyroxasulfone | POST | 80 | 0 | b | 70 | cde | 62 | ed | 78 | cde | 6020 | a |
| Diclofop | POST | 840 | 6 | b | 58 | e | 42 | g | 0 | f | 5750 | ab |
| Pyroxsulam | POST | 18 | 0 | b | 62 | de | 49 | fg | 92 | abc | 6330 | a |

[a] Site-year locations: Tifton and Griffin, Georgia.

[b] Abbreviations: a.i., active ingredient; DAP, days after planting; ME, microencapsulated; 12 DPRE, 12 days before planting; PRE, preemergence; AE, at wheat emergence; POST, postemergence applied 65–70 DAP at Feekes scale 1.5–1.9.

[c] Henbit at Griffin location 2012–2013 and 2013–2014.

**Table 4.** Herbicide, rates, and timing of applications for evaluating weed control and soft red winter wheat growth response in Georgia, 2012–2013 and 2013–2014: data represents four site-year locations[a].

| Herbicide | Timing | Rate (g a.i. ha$^{-1}$) | Injury (%) | | Italian ryegrass (%) | | | | Yield (kg ha$^{-1}$) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 30 DAP | | 75 DAP | | 175 DAP | | | |
| Nontreated control | | | 0 | b | 0 | d | 0 | e | 6750 | bcd |
| Pyroxasulfone Thifensulfuron + Tribenuron + MCPA | PRE[b] POST | 45 17.5+4.3+420 | 0 | b | 88 | ab | 74 | ab | 7180 | abc |
| Pyroxasulfone Thifensulfuron + Tribenuron + MCPA | PRE POST | 60 17.5+4.3+420 | 0 | b | 95 | a | 94 | a | 6500 | cd |
| Pyroxasulfone Thifensulfuron + Tribenuron + MCPA | PRE POST | 120 17.5+4.3+420 | 0 | b | 97 | a | 95 | a | 6660 | cd |
| Pyroxasulfone Thifensulfuron + Tribenuron + MCPA | AE POST | 45 17.5+4.3+420 | 0 | b | 40 | c | 16 | de | 7410 | a |
| Pyroxasulfone Thifensulfuron + Tribenuron + MCPA | AE POST | 60 17.5+4.3+420 | 0 | a | 53 | c | 26 | d | 7150 | abc |
| Pyroxasulfone Thifensulfuron + Tribenuron + MCPA | AE POST | 120 17.5+4.3+420 | 0 | b | 74 | b | 54 | bc | 7130 | abc |
| Pinoxaden Thifensulfuron + Tribenuron + MCPA | EPOST POST | 60 17.5+4.3+420 | 6 | a | 77 | b | 54 | bc | 7290 | ab |
| Pyroxasulfone Thifensulfuron + Tribenuron + MCPA | EPOST POST | 60 17.5+4.3+420 | 0 | b | 42 | c | 34 | cd | 7370 | a |
| Pyroxasulfone Thifensulfuron + Tribenuron + MCPA | EPOST POST | 120 17.5+4.3+420 | 0 | b | 47 | c | 38 | cd | 7200 | abc |

[a] Site year locations: Plains and Griffin, Georgia.

[b] Abbreviations: a.i., active ingredient; DAP, days after planting; PRE, preemergence; AE, at wheat emergence; POST, postemergence applied 65–70 DAP at Feekes scale 1.5–1.9.

**Table 5.** Herbicide, rates, and timing of applications for evaluating weed control and soft red winter wheat growth response in Georgia, 2012–2013: data represents two site-year locations[a].

## 5. Crop response and weed control

Treatments were applied at times typically occurring in Georgia soft red winter wheat production (**Table 1**) and are thus representative of producer practices and label recommendations for the PRE herbicides evaluated. For AE and POST herbicide treatments, applications that included surfactants when needed were made based on label recommendations.

An important factor for any PRE-applied herbicide in soft red winter wheat is stand establishment. Crop injury or stand reduction can lead to weed infestations, promote disease proliferation, and thus reduce yield and quality. Three herbicides were PRE applied over the course of

these experiments: pyroxasulfone (40 to 160 g $ha^{-1}$), pendimethalin microencapsulated (ME) (1064 g $ha^{-1}$), and saflufenacil (60 g $ha^{-1}$). There was no stand reduction in any of the experiments for any PRE herbicide treatment where the average wheat stand was 21 (**Table 2**), 21 (**Table 3**), 22 (**Table 4**), and 24 (**Table 5**) plants per meter of row (data not shown). Even when pyroxasulfone was applied 12 days PRE (12 DPRE), no reduction in stands occurred (**Table 3**) (data not shown). These data indicate the crop safety which these herbicides, pyroxasulfone, pendimethalin ME, and saflufenacil PRE, have toward soft red winter wheat in this region. The AE and POST for these herbicide applications did not affect wheat stand.

Soft red winter wheat injury ranged from 0 to 20% across PRE treatment timings for all studies when evaluated at 14, 15, or 30 DAP (**Tables 2–5**). Pyroxasulfone PRE at 160 or 120 g/ha injured wheat 20 and 11% (**Tables 2** and **3**), respectively. This injury was in the form of stunting. Some stunting from pyroxasulfone was still visible at 90 DAP for the 80 and 160 g $ha^{-1}$ rates (**Table 2**). However, this injury was transient by the end of the season and not observed. Metribuzin applied alone or in combination with pendimethalin ME at emergence resulted in significant injury, 18%, at 30 DAP (**Table 4**). The soils for the present studies were a sandy loam, loamy sand, or sandy clay loam with less than 2.0% organic matter. Hulting et al. [52] noted 3% or less wheat injury from pyroxasulfone rates up to 100 g $ha^{-1}$ on a silt loam soil. Previous research indicated decreased pyroxasulfone injury with legumes grown in soils with greater clay contents [19]. Canadian dry bean research indicated that pyroxasulfone injury at 210 g/ha was 11% or less [55]. These data indicate that at rates up to 160 g $ha^{-1}$ wheat had tolerance in sandy loam, loamy sand, and sandy clay loam soils of the Southeastern United States. When pyroxasulfone was POST applied at Feekes scale 1.0–1.9 (**Tables 2**, **4**, and **5**), no injury was ever observed. Pyroxasulfone has limited POST activity but can be applied after wheat emergence per label recommendation [47–49]. This will provide growers an opportunity to incorporate a residual herbicide to promote weed control. When pinoxaden, diclofop, or mesosulfuron was POST applied, wheat injury did occur but was consistently less than 9% (**Tables 2–4**).

Wheat yield varied by location and by year (**Tables 2–5**). There were no differences for yield when pyroxasulfone was PRE applied (**Table 2**) or 12DPRE and PRE (**Table 3**) as compared to AE or POST applications of diclofop, pyroxsulam, mesosulfuron, or pinoxaden. For these experiments, yield exceeded 4000 kg $ha^{-1}$ for all pyroxasulfone treatments and was always greater than the nontreated control. There was no rate response for wheat yield for pyroxasulfone rates of 40, 60, 80, 100, or 120 g $ha^{-1}$ (**Tables 2** and **3**). There were no differences in wheat yield as compared to the nontreated control when pyroxasulfone was applied alone or in combination with saflufenacil PRE, AE, or POST (**Table 4**). Wheat yields in this set of experiments (four totals) were consistent with early season injury, in that metribuzin alone or in combination with pendimethalin ME-applied AE had significant injury 30 DAP, and this translated into reduced yields of 5670 and 5350 kg $ha^{-1}$, respectively. Previous research indicated that metribuzin reduced yield demonstrating the risks growers take when using this herbicide for weed control [16, 31, 33].

Early-season Italian ryegrass control for pyroxasulfone application at 40 to 160 g $ha^{-1}$ 12DPRE or PRE was 72 to 99% when evaluated at 30 DAP (**Tables 2–4**). However, by 175 DAP Italian ryegrass control began to decline to 83% and less for the 40 and 60 g $ha^{-1}$ rates of pyroxasulfone. Pyroxasulfone at 80 g $ha^{-1}$ or greater provided 87% or greater season-long control (**Tables 2–4**). Previous research has noted similar Italian ryegrass response to pyroxasulfone at 50–150 g/ha

with control ranging from 63 to 100% [52]. Bond et al. [1] noted a significant difference of 37 versus 99% control of glyphosate-resistant Italian ryegrass for pyroxasulfone at 50 versus 160 g $ha^{-1}$, respectively. These data indicate that for season-long Italian ryegrass control, pyroxasulfone at 100 g/ha will be required in the Southeastern US soft red winter wheat production. As a PRE herbicide, soil dissipation of pyroxasulfone will occur over time [45, 46], thus requiring the appropriate rate to be utilized for season-long weed control. Applying pyroxasulfone 12DPRE prior to wheat planting resulted in 74% and less Italian ryegrass control. This could be potentially contributed to soil disruption in the planting process via the tractor wheels and planter disk blades.

Although no attempt was made to quantify the level of herbicide resistance in these Italian ryegrass populations, ACCase resistance is suspected in the Griffin GA population (unpublished data). Diclofop and pinoxaden are ACCase herbicides that failed to control Italian ryegrass effectively (**Tables 2–4**) when POST applied. Similarly, the ALS herbicide pyroxsulam exhibited variable Italian ryegrass control at 86 and 49% at 175 DAP (**Tables 2** and **4**, respectively) indicating potential ALS-susceptible and potential ALS-resistant populations. This was established even further when the ALS herbicides thifensulfuron plus tribenuron were used as sequential POST applications following AE or POST pinoxaden applications in that Italian ryegrass control was 54% at 175 DAP (**Table 5**). Multiple herbicide-resistant Italian ryegrasses to ALS and ACCase herbicides have been confirmed in Georgia [4]. Previously, growers relied on AE or POST herbicide combinations for weed control, but the addition of pyroxasulfone for PRE application in soft red winter wheat will provide much greater potential for successful crop production. However, pyroxasulfone must be applied prior to Italian ryegrass establishment, as noted by AE and POST applications in **Tables 4** and **5**. Italian ryegrass control declined significantly to 72% and less for any rate of pyroxasulfone AE or POST alone or when used with other POST-applied herbicides at 175 DAP.

Pyroxasulfone PRE controlled henbit 91% and greater (**Table 3**) or provided suppression (**Table 4**). Combinations with other herbicides improved control of this winter annual species (**Table 4**). These data indicate that henbit can be controlled with currently registered herbicides for wheat production. There is limited information about pyroxasulfone winter weed species control in wheat, other than Italian ryegrass, in the literature.

The complexity and difficulty of managing winter weed species in soft red winter wheat have increased with the discovery of herbicide-resistant weeds, specifically ACCase- and ALS-resistant Italian ryegrass [4]. Additionally, glyphosate-resistant Italian ryegrass is now an issue in this same region [1]. Successful management of Italian ryegrass resistant to multiple mechanisms of action will require diligent control programs utilizing PRE residual herbicides prior to wheat emergence, during the cropping season, and after crop rotation, in order to extend the use of pyroxasulfone's mechanism of action, which is different from all other previous wheat herbicides.

## 6. Conclusion/recommendations

This research indicated that using the appropriate rates of pyroxasulfone PRE could provide season-long control of Italian ryegrass in wheat. However, variability in Italian ryegrass control was observed when low rates or improper timing of application were used, which

indicates the need for further development as growers incorporate this herbicide. Eight different soft red winter wheat cultivars were used in this research, and all exhibited tolerance to pyroxasulfone alone and with other herbicide combinations. Future research should be conducted with the currently evaluated herbicides for control of other weed species. Italian ryegrass control was attained and maintained with the appropriate herbicide applications, but variability can be an issue if proper rates and timings are not adhered to. This should be considered as an area for future research efforts in soft red winter wheat production using combinations of these herbicides. Growers should follow registration recommendations for the herbicides evaluated in this research, along with crop rotation and using different mechanisms of action herbicides to limit exposure and reduce potential for resistance to proliferate.

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Chapter 10

# The Occurrence of Cereal Aphids in Rainfed Wheat in Kenya: The Problem and Possible Integrated Pest Management Strategies

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Additional information is available at the end of the chapter

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**Abstract**

Cereal aphids cause direct damage to rainfed wheat through sucking of plants sap and cause losses of up to 90%, particularly in dry years in Kenya. The Russian wheat aphid (RWA) is the most destructive and may account for up to 50% yield loss or more depending on the severity and length of infestation. Current control strategies mainly rely on the use of insecticides to control cereal aphids' infestations. Chemicals improve yields in the short term, but adversely affect the environment, hence the need for development of effective IPM strategies. Early planted crops escape heavy aphid attacks and give good yields. A combination of seed rate of 100 kg and 100 kg N/ha provided the best cultural management of RWA. Ladybird beetles *Adonia variegata*, lacewings (*Chrysoppa* spp.) and parasitic wasp *Aphidius* sp. were the most important natural enemies. Control of cereal aphids can be achieved with systemic insecticides applied as seed dressings or foliar applied insecticides. Four lines of wheat were found to show RWA resistance and crosses with Kenyan wheat made and populations are being evaluated for resistance to cereal aphids.

**Keywords:** wheat, cereal aphids, natural enemies, cultural strategies, insecticides, host plant resistance

## 1. Introduction

Grain cereals contribute significantly to food security in Kenya. Bread wheat (*Triticum aestivum* L.) is the second most important cereal crop in Kenya, after maize. It was grown on 147,210 ha producing 328,637 tons of grain with a yield of 2232 kg/ha [13]. However, there

is still a gap between production and consumption of wheat in Kenya [44]. Increased population growth, urbanization and change in eating habits have led to increased wheat demand. The national demand is estimated at more than 990,000 tons per year while production is as low as 360,000 leading to importation to meet the difference [52]. It is mainly grown in Nakuru, Trans Mara, Uasin Gishu, Nyandarua, Narok, Meru Central, Trans Nzoia, Keiyo and Laikipia counties. The crop is grown in diverse agro-ecological zones at altitudes ranging from 1500 to 2900 m.a.s.l. in areas with 700–1000 mm rain per year.

The country in its strategic plan is struggling to become self-sufficient in wheat production either by increasing yields per unit area or by expanding the area under production in the marginal areas. This is possible when the gap between potential yield of wheat varieties (6–7 t/ha) and the actual yields (2.3 t/ha) realized by wheat growers in Kenya is filled. This gap is attributed to the lack of good quality seed, lack of appropriate technology and attack by diseases and pests, which have presented a continuous challenge to wheat productivity in Kenya. Cereal aphids are considered as one of most serious insect pests attacking rainfed wheat [8, 60]. Cereal aphid outbreaks are frequent in Kenya and are responsible for most of the control interventions on wheat. They are capable of completely devastating the crop by making it a total failure during years of severe infestation [59]. Aphids damage the cereals by direct feeding and transmitting barley yellow dwarf virus (BYDV) diseases, both causing losses in yield [22, 57]. Outbreaks of cereal aphids that are vectors of barley yellow dwarf are frequent in Kenya and often result in extensive use of insecticides [39, 58]. Their relative importance as pests/vectors varies considerably from one area with losses in the range of 1–100% [28]. Yield losses of 47% due to BYDV have been reported in wheat [57]. It is transmitted by cereal aphids in a persistent, circulative but nonpropagative manner. Five strains occur in Kenya and their principal vectors are RPV (*Rhopalosiphum padi*), RMV (*R. maidis*), MAV (*Sitobion avenae*), SGV (*Schizaphis graminum*) and PAV (*R. padi*, *S. avenae* and others) [25]. In the field, symptoms appear as yellow or red patches of stunted plants. In general, PAV causes severe symptoms, MAV moderately severe and RPV, RMV and SGV produce mild symptoms [16]. This chapter reports on cereal aphids affecting wheat in Kenya with emphasis on Russian wheat aphid *Diuraphis noxia*, their problem and possible integrated pest management strategies.

## 2. Cereal aphids occurring on rainfed wheat in Kenya

The six most important cereal aphid species reported as pests that attack wheat and barley include: Greenbug *Schizaphis graminum* R., English grain aphid *Sitobion avenae* F., Oat bird cherry aphid *Rhopalosiphum padi* L, Cereal leaf aphid *R. maidis* F., Rose grain aphid *Metopolophium dirhodum* W. and Russian wheat aphid *D. noxia* M. [31, 32, 42]. Of these species, the Russian wheat aphid which is a recent introduction in Kenya in 1995 is the most destructive followed by Greenbug *S. graminum* [25]. The other species are less serious and usually cause no significant yield reduction. In reality, there are usually two or more aphid species present at one time.

The Russian wheat aphid was first officially detected in June 1995 [25] and affected areas experienced damaging infestations resulting in 90–100% crop loss. Since then most reports

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from major wheat and barley growing areas of Narok, Nakuru, Uasin Gishu, Trans Nzoia and Mt Kenya region indicate that *D. noxia* has become a serious pest causing estimated crop losses of 25–80% depending on the stage of infestation. The pest is now a major constraint to wheat production in eastern Africa region [54]. The appearance of these aphids resulted in a dramatic increase in use of insecticides in cultivated wheat crops. According to the economic losses attributable to Russian wheat aphid [25], it can be categorized as reduced grain yields, loss of kernel weight and quality and increased costs of production due to application of insecticides.

The Russian wheat aphid causes damage to host plants through direct feeding and by injecting toxins during feeding which cause leaf rolling and unfolding thus making it difficult to control by application of contact insecticides [21, 54, 56]. Visible damage to the susceptible host plants is manifested as chlorotic lesions, white streaking, purple discoloration and tightly curled leaves. The level of infestation, the growth stage of host plant and the duration of the infestation, all influence the severity of the damage caused by *D. noxia*.

## 3. Distribution of cereal aphids in Kenya

National aphid surveys were conducted in farmers' fields [32, 42] during wheat cropping seasons in Mt Kenya area, Mau Narok, Nakuru, Narok, Trans Nzoia and Uasin Gishu in order to determine the abundance and distribution of cereal aphids. In each area, 10 random assessments were taken. On every plant, the sampled aphid species were identified using taxonomic keys [37] and recorded. The different species of aphids found in the wheat fields were Russian wheat *aphid D. noxia*, which was the most predominant species with the highest overall density of 80.0%, followed by *M. dirhodum* (10.0%). The least species appeared were *R. padi* (5.3 %) and *R. maidis* (3.7%), while infestations of *S. graminum* was relatively low (1.0%) in all the areas (**Table 1**). It has also been reported elsewhere that wheat cultivars are usually attacked by a complex of cereal aphids' species [14]. The high incidence of Russian wheat aphids on the crops made the leaves to remain furled for a long time, thus creating conducive environment for other species such as *R. padi*, *R. maidis* and *S. graminum* to stay longer in the wheat crops after crop heading. The surveys complemented the Cereal aphid Forecast Bulletins in advising growers on control decisions [27].

Alternate host plants of RWA and other cereal aphids found in Kenya during the surveys included wild oats *Avena fatua*, brome grass *Bromus* spp., wild rye grass *Elymus* spp and foxtail grass *Setaria* spp. These grass weed are common in high altitude wheat growing areas in Mt Kenya and west Mau regions of Kenya and serve as reservoirs of cereal aphids during dry weather. Neglected volunteer wheat, barley and oats plants were also important for the survival of Russian wheat aphids and other cereal aphids. These host plants have also been reported [2, 6, 33] supporting RWA and thus provide a bridge for infestation of the next season wheat crop. These alternate host plants play an important role in supporting cereal aphids between crop harvest and emergence of the new crop in the next planting season.

| Region | Incidence of cereal aphids species | | | | | |
|---|---|---|---|---|---|---|
| | **Dn*** | **Md** | **Sa** | **Rp** | **Rm** | **Sg** |
| **Mt Kenya area** | +++ | ++ | ++ | ++++ | ++ | + |
| **Mau Narok** | ++ | + | + | ++ | ++ | + |
| **Nakuru** | ++++ | ++ | ++ | +++ | ++++ | + |
| **Narok** | ++++ | ++ | ++ | +++ | ++++ | + |
| **Trans Nzoia** | +++ | ++ | ++ | +++ | + | + |
| **Uasin Gishu** | +++ | ++ | +++ | +++ | +++ | + |

Key: *Dn – *Diuraphis noxia*; Md – *Metopolophium dirhodum*; Sa – *Sitobion avenae*; Rp – *Rhopalosiphum padi*; Rm – *Rhopalosiphum maidis*; Sg – *Schizaphis graminum*.

*Incidence:* ++++, highly abundant; +++, moderately abundant; ++, abundant; +, low abundance.

**Table 1.** Incidence of Russian wheat aphid and other cereal aphids in wheat growing regions of Kenya.

Continuous cropping of wheat was practiced by wheat growers in Mt Kenya region, eastern Aberdares ranges and West Mau areas of Kenya [31, 33]. This enabled cereal aphids to migrate from one field to another and survive from one season to the next. Similar observations have been reported in wheat growing areas in the highlands of Ethiopia [40]. It was also observed that the different crop planting dates and eco-zones across the country as well as the presence of volunteer cereals and alternate host grasses provide a continuous source of alternative host plants and consequent spread source of cereal aphids to wheat crops planted the following season. The information being generated from field surveys has been used to advice farmers when to plant and which control options to adopt in order to escape the damaging species of cereal aphids.

Cereal aphids also have preferential performance on different hosts. Results observed in Kenya [49] revealed that aphid species differed in their time of colonization on wheat varieties. Aphid abundance differed among the species, wheat varieties and crop growth stages. *R. padi* appeared at two leaf stage, followed by *S. graminum* at the two tiller stage and *M. dirhodum* appeared at stem elongation stage. The aphids also differed in their points of colonization, thus the studies confirmed crop growth stage and feeding preferences among cereal aphids in wheat.

## 4. Cultural control of cereal aphids

Cultural practices can play a significant role in IPM strategies targeting cereal aphids. Cultural controls are generally the cheapest of all control measures because of their preventive measure and only require modifications to normal production practices. They do not possess some of the detrimental side effects of pesticides, such as killing of beneficial insects, no contamination of environment and development of resistance to pesticides [17]. Modified cultural practices can be important in minimizing cereal aphid infestations which include dates of planting, seed rates and fertilization to produce the healthiest crop possible.

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In Kenya, studies on use of cultural control practices such as time of planting as alternative strategies for control of aphids to reduce cereal aphid populations have been assessed. Cultural control of aphids and its effect on the incidence of cereal aphids was addressed through research, especially the assessment of time of planting and subsequent cereal aphid population levels. Three planting dates were evaluated, namely, early planting (April), medium planting (May) and late planting (June). The data (**Table 2**) indicates that for late planted wheat crops, Russian wheat aphid density levels were very high and the resultant grain yields very low [30]. Seasonal mean aphid population was 7.4 and 2.1 times higher on late as compared to that on early and timely sown crop, respectively (**Table 2**). This indicates that changing sowing date of wheat will affect cereal aphids infestation, thus planting date may be adjusted to minimize cereal aphid infestations. The study also showed that early planted wheat crops escape heavy cereal aphid infestations and give good yields. It has been recommended that wheat growers should sow their crops early to avoid damaging levels of Russian wheat aphid and other cereal aphids. In addition, it is important to note that sowing should be done early in the season so that the crop benefits from the early rains and it should be timed such that harvesting coincides with the dry spell. The potential of cereal aphid infestations can be reduced by sowing wheat crops early in the season [1] and also cereal aphid infestation increases on late plantings and reduces yield as compared to normal planting [3].

| Time of Planting | Mean no. of Russian wheat aphids/plant | Mean grain yield (kg/ha) |
|---|---|---|
| Early planting (April) | 1.37a* | 4822.0a |
| Medium planting (May) | 5.13b | 4226.0b |
| Late planting (June) | 15.71c | 3026.0c |
| Seasonal mean | 7.4 | |

* Means in the same column followed by the same letter are not significantly different at the 5% level of the LSD test.

**Table 2.** Effects of time of planting on Russian wheat aphid densities and wheat yields at Njoro, Kenya (2160 m.a.s.l.).

The effects of sowing dates of planting dates and insecticide sprays on aphid populations and barley yellow dwarf incidence in Kenya were also assessed [57]. The most common important cereal aphids observed included *M. dirhodum*, *R. maidis*, *R. padi*, *Sitobion avenae* and *S. graminum*. BYD incidence was significantly decreased in plots sown with seed that had been treated with imidacloprid and later sprayed with cypermethrin foliar insecticide. Yield losses due to BYD were also significantly different between the treatments and between the early planted and late planted crop.

Field trials have also been conducted in Njoro, Kenya [47, 48] on development of environment-friendly and cost-effective cultural practices (utilizing seed rates and fertilization levels) for management of RWA in order to reduce/avoid use of chemicals in wheat production. It was observed that a combination of moderate seeding rate (100 kg/ha) and application of nitrogen (100 kg N/ha) was observed to be the best for the cultural management of Russian wheat aphids in wheat production. To minimize dependence on insecticides for cereal aphids' control, cultural practices like adjustment of planting dates and seed rates may be helpful.

## 5. Survey of natural enemies of cereal aphids

All commercial wheat varieties are susceptible to cereal aphid's damage and control is achieved by extensive use of insecticides. Chemicals improve yields in the short term, but adversely affect ecological and human health. There is a lot of concern about the expense and possible environmental pollution from insecticidal applications and farmers would prefer to minimize losses through the use of resistant cultivars and effective natural enemies (predators and parasites) because of sustainability and environmentally friendly action. Most farmers in Kenya are not aware of biological control and therefore do not perceive it to be effective. However, majority of farmers will be willing to stop spraying should the biological control strategy be effective.

In view of the large wide range of aphid species that attack wheat in Kenya, causing substantial yield losses, biological control strategies must be developed that will enhance the integration of these control agents in an IPM control strategy. Therefore, surveys were initiated to document the natural enemies that attack the Russian wheat aphid and other cereal aphids on infested wheat crops in farmers' fields.

A number of predators and parasitoids were observed to attack cereal aphids (**Tables 3** and **4**) but none of these biocontrol agents exerted adequate controls. The absence of successful aphid predators and parasitoids may be a prime reason for the rapid spread of Russian wheat aphid. Field observations also revealed that the natural enemies of Russian wheat aphid were only present late in the crop season when damage to wheat had already taken place. Ladybird beetles *Adonia variegata* and parasitic wasp *Aphidius* sp. were the most important natural enemies.

| **Scientific name** | **Cereal aphid species*** | | | | | |
|---|---|---|---|---|---|---|
| **Predators** | | | | | | |
| • Coleoptera (beetles) | | | | | | |
| 1. *Adonia variegata* | a | b | c | d | e | – |
| 2. *Cheilomenes* spp. | a | b | c | d | e | – |
| • Diptera | | | | | | |
| 1. Syrphidae (hover flies) | a | – | c | d | e | f |
| • Arachnoidea (spiders) | a | b | c | d | e | f |
| • Neuroptera (lacewings) | a | b | c | d | e | f |
| **Parasitoids** (Hymenoptera) | | | | | | |
| 1. *Aphidius* spp. | a | – | c | d | e | f |
| 2. *Aphelinus* spp. | a | – | c | d | e | f |

*Key*: a, Russian wheat aphid *D. noxia*; b, *R. maidis*; c, *S. graminum*; d, *Sitobion* spp.; e, *M. dirhodum*; f, *R. padi*.

**Table 3.** Predators and parasitoids recorded attacking cereal aphids in wheat in Kenya in 2004 cropping season.

The Russian wheat aphid was the most prevalent insect pest of rainfed wheat [31, 32]. The maximum number of RWA per tiller was 58.0 while rose grain (*M. dirhodum*), oat-bird-cherry

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aphid (*R. padi*) and corn leaf aphid (*R. maidis*) were prevalent at low density ranging from 0.1 to 9.0 per tiller. The general aphid predators *Cheilomenes* spp., spiders, lacewings and the parasitoid *Aphidius* spp. were the natural enemies of cereal aphids found in Kenyan wheat.

| **Predators and parasitoids** | **Cereal aphid species** | | | |
|---|---|---|---|---|
| | *Diuraphis noxia* | *Metopolophium dirhodum* | *Rhopalosiphum maidis* | *Rhopalosiphum padi* |
| **Predators** | | | | |
| 1. Coleopteran beetles *Cheilomenes* spp. | x | x | x | – |
| 2. Neuroptera (lacewings) | x | x | x | x |
| 3. Arachnoidea (spiders) | x | x | x | x |
| **Parasitoids** | | | | |
| 4. Hymenoptera *Aphidius* spp. | x | x | – | x |

**Table 4.** Predators and parasitoids attacking cereal aphid species in Kenya 2015 cropping season.

Generalist predators, namely, Coccinellid beetle (*Cheilomenes* spp.), spiders (Arachnidea) and lacewings (*Chrysoppa* spp.), were observed to occur at very low population densities from tillering stage to heading growth stages (**Table 4**). Similar observations have been reported in Ethiopia [2].

The natural enemies such as *Aphidius* spp., appeared late in the crop season when the cereal aphids population levels had passed damaging levels. Therefore, they may not contribute to season long control of RWA and other cereal aphids in the wheat crop.

The survey data revealed that the Russian wheat aphid is the most important and predominant cereal aphid. Its feeding habit led to leaf rolling, which enabled the other cereal aphids to stay longer on the crop thereby increasing their inoculation period of viral diseases such as barley yellow dwarf virus. Leaf rolling particularly the rolling of flag leaf interferes with the pollination of wheat flowers. The survey also revealed that grass weeds support cereal aphids; hence, good control of grass weeds is essential. Moreover, though the number of predator and parasitoid species recorded were sufficient, because of their low density, they are unable to keep cereal aphid populations below damaging levels. However, efforts should therefore be made to conserve these natural enemies as they are of great importance in controlling the cereal aphids. For development of an effective cereal aphids IPM package, there is need for comprehensive studies on population dynamics of cereal aphids' species and their natural enemies in wheat crops.

## 6. Chemical control of Russian wheat aphid and other cereal aphids

All commercially available wheat varieties in Kenya are susceptible to Russian wheat aphid and other cereal aphids and chemical control of cereal aphids has been the only option for many growers [25]. Research has focused on screening for more effective insecticides, application methods and development of recommendations for wheat growers on economical control

measures. Control of cereal aphids can be achieved with systemic insecticides applied as seed dressings or foliar spray and contact insecticides applied with aerial or ground equipment. The seed dressing insecticides controls the colonizing migrant aphids and prevents primary infestation. The foliar applied insecticides controls primary spread.

Control recommendations in Kenya include the use of systemic insecticides as shown in **Table 5** [33]. The rolling of leaves as a result of the feeding habit of RWA causes the leaves to roll around *D. noxia* aphid colonies thus protecting the aphids from being reached by the contact insecticides. A characteristic behavior of RWA is to feed and develop inside the rolled leaf whorl confining insecticide options to active ingredients with systemic action able to penetrate the rolled leaf [23]. Systemic insecticides presented in **Table 5** have proven to be effective against Russian wheat aphid and other cereal aphid species in rainfed wheat crops with resultant high grain yields [33].

| Trade name of chemical | Active ingredient (a.i.) | Application rate |
|---|---|---|
| ***Seed dressing insecticides*** | | |
| 1. Gaucho 350FS | Imidacloprid 350 g/L | 200 mL/100 kg seed |
| 2. Cruiser 350FS | Thiamethoxam 350 g/L | 150 mL/100 kg seed |
| 3. Redigo Deter 300FS | Clothianidin 250g/L + prothioconazole 50g/L | 200 mL/100 kg seed |
| 4. Celest Top 312FS | Thiamethoxam 262.5 g/L + fludioxonil 25 g/L + difenoconozole 25 g/L | 150 mL/90 kg seed |
| ***Foliar applied insecticides*** | | |
| 1. Pirimor 50WG | Pirimicarb 500 g/kg | 0.75 kg/ha |
| 2. Bulldock star 262.5EC | Betacyfluthrin 12.5 g/L + chlorpyrifos 250 g/L | 0.5 L/ha |
| 3. Thunder OD 145 | Imidacloprid 100 g/L + betacyfluthrin 45 g/L | 0. 3 L/ha |
| 4. Nurelle* D 50/500 EC | Cypermethrin 50 g/L + chlorpyrifos 500 g/L | 0.5 L/ha |
| 5. Engeo 247SC | Thiamethoxam 141 g/L + lambda-cyhalothrin 106 g/L | 150 mL/ha |

**Table 5.** Recommended insecticides for control of cereal aphids in rainfed wheat in Kenya.

Seed dressing is an insurance against infestation by early seedling pests such as the Russian wheat aphid. The use of seed dressers ensures a better crop establishment, more uniform and healthier crops with increased yields and quality. Cereal aphids occurring during other growth stages of wheat are controlled using foliar applied insecticides (**Table 5**). Applications of Gaucho 350FS followed by applications of systemic foliar insecticides achieved very good control of the Russian wheat aphid [26, 29, 33]. Contact insecticides are not effective against Russian wheat aphid but they are effective against *R. maidis*, *S. graminum*, *Sitobion* spp., *M. dirhodum* and *R. padi*. Satisfactory control of RWA using foliar applied aphicides depends on early detection of infestation through periodic scouting. This approach will therefore offer a cheaper strategy for cereal aphid and BYD control. Farmers are advised to scout their fields weekly in order to make an accurate decision on whether or not treatment is required. It is important for wheat growers to know that not all insects are pests. One should know the insects, which are beneficial to mankind. Ladybird beetles, hoverflies, lacewings, spiders, dragonflies and praying mantis feed on other insects. Using insecticides indiscriminately

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can cause harm to the beneficial insects too. Farmers should monitor and consider beneficial insects when making control decisions and after treatment application, continue monitoring to assess pest populations and their control.

BYDV is a virus disease vectored by cereal aphids during feeding. The best control is of this disease is by use of resistant varieties. However, majority of the current wheat varieties are susceptible to the disease and control is by use of insecticides to control the cereal aphid vector. Seed dressings control early cereal aphids' infestations and later infestations are controlled by use of foliar applied insecticides. Seed treatment is a good insurance against infection by seed borne, soil borne and early seedling pests. Seed dressings with insecticides also controlled early seedling pests such as barley bulb fly, cutworms, chaffer grubs and cereal aphids that also transmit BYDV [7]. The seed treatments provided early protection against cereal aphids, while the foliar applied aphicides provided good control for management of later infestations on wheat crops. Therefore, utilization of both seed dressing and foliar applied aphicides could be used in integrated pest management programs for controlling the cereal aphids.

However, controlling cereal aphids with insecticides has many risks, including destruction of natural enemies and accelerated development of insecticide resistance in cereal aphid species. In addition, chemical control of cereal aphids has proven expensive and there is need for development of resistant varieties.

## 7. Host plant resistance

Host plant resistance is an integral part of IPM of cereal aphids and is one of the most important alternative methods of management of cereal aphids. BYD resistance in wheat has been more difficult to assess and screening programs have yielded only a few possible sources of resistance, many of which showed susceptibility upon repeated testing due to the wide variation of BYDV strains. In addition, severe epidemics may render genotypes with useful resistance as being apparently highly susceptible [50]. All commercially available wheat varieties are susceptible to Russian wheat aphid and other cereal aphids and have to be chemically protected [20, 26, 35]. In Kenya, the development of RWA-resistant varieties has been constrained by variation in resident RWA populations and by concerns of possible existence of virulent biotypes [21, 36, 46, 45]. Collapse of resistant crop varieties due to biotype development is a major threat to food security and even a greater catastrophy would be caused by the unavailability of advanced breeding lines containing genetic variability potentially resistant to future biotypes [23]. Investigations to evaluate seven commercial bread wheat cultivars (Pasa, Mbuni, Kenya Heroe, Kenya Fahari, Chozi, Duma and Kwale) in five different environments in Kenya revealed that varieties K. Fahari and Duma suffered the lowest RWA damage [35]. K. Fahari which had been previously reported to be resistant to green bug *Schizaphis graminum* was observed to have some resistance to RWA.

While some protection against Russian wheat aphid can be realized by crop management practices, resistant varieties offer the greatest opportunity for reduction of crop losses. The sudden appearance of Russian wheat aphid has made resistance breeding program of high

priority now as farmers are currently relying on pesticides to control the aphids. The high cost of chemical control and concern for extensive and frequent use of insecticides has led to search for Russian wheat aphid resistance. Using host plant resistance instead could be economical, effective throughout the growing season, environmentally safe and it will require no elaborate technology transfer to farmers. Natural enemies and host plant resistance are considered as more desirable alternatives to insecticides because of their low cost and environmentally friendly mitigation strategy [12, 51] for effective management of cereal aphids in wheat.

Research has focused on screening wheat genotypes for possible source or sources of resistance against Russian wheat aphid for use in our national wheat breeding program as an alternative to chemical control. A search for sources of resistance from among introductions collected from other countries (CIMMYT, Mexico, South Africa and Turkey) identified four sources of resistance, viz., RWA 9, RWA 8, RWA 16 and RWA 230 [34]. These sources have been incorporated into the breeding program using back-crossing technique. In addition, doubled haploid technique is being used to shorten the breeding cycle by about 5 years. Host plant resistance should be used as on more control strategy with IPM, as the strategy is nonpollutant to the environment and does not demand specific knowledge by the wheat farmers. The use of host plant resistance that rarely requires treatment by application of aphicides has also been reported elsewhere as one of the most important methods for control of cereal aphids [10, 11, 19, 38, 61].

## 8. Future research needs

Action thresholds are lacking and there is urgent need to develop economic action levels (economic injury levels and economic threshold levels) for cereal aphid species infestation in wheat growing areas of Kenya during different seasons. They will be used as basis for making recommendation for cereal aphids control programs in wheat crops which will improve the development of integrated pest management strategies.

Breeding for resistance to cereal aphids is the most effective means of reducing yield losses associated with cereal aphids' infestation. Recently acquired wheat germplasm with resistance to cereal aphids especially Russian wheat aphid are now being screened and will be utilized in the breeding program. Resistance information of wheat genotypes to cereal aphids is important to be known by wheat breeders as a guide in selecting source of resistance genes to be used in the improvement of wheat varieties resistant to cereal aphids. However, since breeding is a slow procedure, it is necessary to consider other strategies to minimize yield losses. The use of one or more applications of aphicides, together with cultural practices such as early planting, application of right amounts of fertilizers and good seed rates could be very effective in providing considerable control of cereal aphids.

## 9. Conclusion

Cereal aphids are major insect pests of wheat in Kenya. Their outbreaks have significant economic impact through increased production costs due to the need to control barley yellow dwarf virus and aphid infestations with insecticides. In order to reduce the crop losses and minimize

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production costs, the ultimate goal of KALRO—Food Crops Research Centre—Njoro national wheat program is to develop a sound integrated management strategy for cereal aphids. It was observed that at earlier seedling stages, the population of natural enemies was too low to exert effective cereal aphids' control. The populations of these natural enemies increased only with the rise of aphids. The increased numbers of natural enemies were observed not to have any effective impact on the population of RWA. However, efforts should therefore be made to conserve these natural enemies as they are of great importance in controlling cereal aphids in wheat.

Applications of either Gaucho 350FS or Cruiser350FS followed by applications of systemic foliar insecticides achieved very good control of the Russian wheat aphid. Contact insecticides are not effective against Russian wheat aphid but they are effective against *R. maidis*, *S. graminum*, *Sitobion* spp., *M. dirhodum* and *R. padi*. Seed dressing is an insurance against infestation by early seedling pests such as the Russian wheat aphid. The use of seed dressers ensures a better crop establishment, more uniform and healthier crops with increased yields and quality. Satisfactory control of RWA using foliar applied aphicides depends on early detection of infestation through periodic scouting. This approach will therefore offer a cheaper strategy for cereal aphid and BYD control.

While some protection against cereal aphids can be realized by crop management practices, resistant varieties offer the greatest opportunity for reduction of crop losses. The sudden appearance of Russian wheat aphid has made resistance breeding program of high priority now as farmers are currently relying on insecticides to control the aphids. The high cost of chemical control and concern for extensive and frequent use of insecticides has led to search for Russian wheat aphid resistance. Using host plant resistance instead could be economical, effective throughout the growing season, environmentally safe and it will require no elaborate technology transfer to farmers and is a good strategy for effective strategy for effective management of cereal aphids in wheat by wheat growers.

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Chapter 11

# Mycotoxins in Wheat and Mitigation Measures

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Additional information is available at the end of the chapter

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**Abstract**

Latest estimates for world cereal production in 2015 and EU-28 production in 2014 are approximately 2540 and 323 mil tons, respectively. The FAO estimated that the global wheat consumption is about 66 kg/per capita. Among the most important risks associated with wheat consumption are mycotoxins. It has been estimated that up to 25% of the world's crops grown for food and feed may be contaminated with mycotoxins. Despite efforts in controlling fungal growth, mycotoxin co-contamination represents an unavoidable risk, occurring pre- and postharvest and resulting in reduced nutritional value and possible risks for human and animal health. In addition to health risks, mycotoxins have a detrimental effect on the quality and the processing performance of wheat. Mitigation measures to manage the challenge of mycotoxins in wheat include strategies at pre- and postharvest. Preharvest events are predominantly dictated by environmental factors and good agronomic/cultural practices, whereas storage and processing are the major areas where contamination can be prevented at postharvest. Integrating as many management options as possible may minimize the risk of mycotoxin contamination in wheat and wheat products.

**Keywords:** wheat, mycotoxins, mitigation strategies, preharvest, postharvest

## 1. Introduction

Cereals and cereal by-products constitute a major part of the daily human and animal diet. Latest estimates for world cereal production in 2015 and EU-28 in 2014 are approximately 2540 and 323 mil tons, respectively [1]. According to the Food and Agriculture Organization of the United Nations (FAO), rice, maize, and wheat are staple foods for 4 bn people and make up about 60% of the world's food energy intake [2]. The FAO estimated that the global

consumption for wheat is about 66 kg/per capita [3]. Among the most important risks associated with cereal consumption are mycotoxins, heavy metals, pesticide residues, and alkaloids. Richard et al. [4] estimated annual losses of $932 million in stored grain in the United States due to mycotoxin contamination. Cereal and cereal products can be contaminated with mycotoxins produced by a variety of fungi that colonize crops in the field or postharvest [5–8]. Mycotoxins are toxic secondary fungal metabolites that can cause a variety of adverse health effects in humans and animals, depending on the type of mycotoxin and the contamination levels. There are 300–400 mycotoxins known today. However, for practical consideration in food manufacturing, because of their worldwide occurrence and concern regarding human and animal diseases, the number is considerably less. The most important mycotoxins in wheat are mainly *Fusarium* toxins, such as deoxynivalenol (DON), zearalenone (ZEA), nivalenol (NIV), fumonisins (FUM), T-2, and HT-2 toxins [8–14]. Moreover, recent studies provided increased evidence for the presence of modified *Fusarium* mycotoxins and so-called emerging mycotoxins, particularly enniatins [15, 16]. Multi-mycotoxin contamination is the most common type of contamination [10, 14, 17–22]. This is a topic of great concern, as co-contaminated samples might still exert adverse health effects due to additive/synergistic interactions of the mycotoxins.

Mycotoxin regulations have been established in more than 100 countries, and the maximum acceptable limits vary greatly from country to country. The globalization of the trade in agricultural commodities and the lack of legislative harmonization have contributed significantly to the discussion about the awareness of mycotoxins entering the food supply chain. The European Union harmonized regulations for the maximum levels of mycotoxins in food and feed [23, 24]. Moreover, two EFSA scientific opinions recommended that the presence of modified and emerging mycotoxins must be considered by the European legislation in the near future [25, 26].

Fungal growth and mycotoxin contamination can occur during several steps of the food supply chain. Despite efforts in controlling fungal growth, mycotoxin co-contamination represents an unavoidable risk, occurring pre- and postharvest and resulting in reduced nutritional value and possible risks for human and animal health. In addition to health risks, fungal growth and mycotoxins have a detrimental effect on the quality and the processing performance of wheat. *Fusarium* damage may reduce wheat milling performance and affect flour yield and flour ash, with a strong negative effect on flour brightness, and baking performance [27–29].

Many factors with pre- and postharvest origins must be taken into account to manage the challenge of mycotoxins in wheat. Preharvest events are predominantly dictated by environmental factors and good agronomic/cultural practices, whereas storage and processing are the major areas where contamination can be prevented at postharvest level. The aim of this chapter is to present an overview of the most recent findings on wheat mycotoxin contamination and of the main pre- and postharvest strategies as mitigation measures, focusing on those more consolidated and used by the wheat industry chain. Other promising measures, but still studied at research level, will be presented with papers and reviews to which the reader is directed for specific insights.

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## 2. Mycotoxin occurrence in wheat

The major mycotoxins occurring in wheat, at levels of potential concern for human and animal health, are *Fusarium* mycotoxins [8–14] (**Figure 1**).

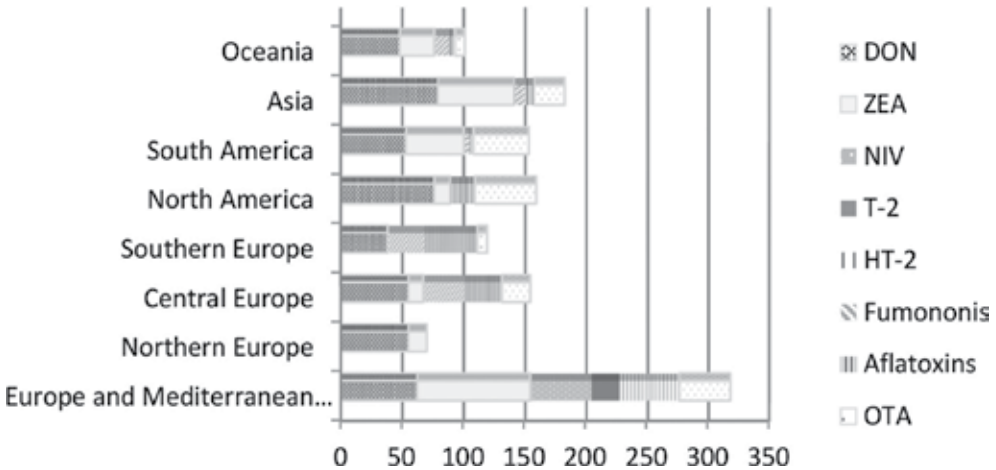


**Figure 1.** World mycotoxin occurrence (% of positive samples) in wheat and wheat bran (modified from Ref. [8]).

Results from worldwide mycotoxin occurrence studies indicate that DON is the most common mycotoxin contaminant of wheat and wheat-based products. Moreover, results highlighted the presence of considerable differences regarding the type and prevalence of mycotoxin contamination in different regions of the world, confirming that contamination is strongly dependent on regional climatic conditions [10, 14, 17–22]. Differences in mycotoxin occurrence and concentration between distant geographical areas are uncontroversial. Within each geographical area, seasonal and local weather conditions during critical crop growing stages are of great importance to explain the variation in mycotoxin occurrence. In general, environmental conditions, such as excessive moisture, temperature extremes, humidity, drought conditions, insect damage, crop systems, and some agronomic practices, can cause stress and predispose wheat in the field to mold and determine the severity of mycotoxin contamination [20, 30–32]. Moreover, the high variability in the occurrence and level of mycotoxins may be the results of several factors, such as the years of the surveys, the annual weather fluctuations, and the storage conditions (**Figure 2**).

Data on the occurrence of *Fusarium* mycotoxins in durum wheat are quite limited. Available data indicated that durum wheat was generally more contaminated than common wheat, but, with the exception of a few samples, no durum wheat sample was noncompliant to the maximum permitted level for DON and ZEA [33].

Another important point highlighted from studies on the worldwide mycotoxin occurrence in wheat and cereals is that the levels of detected mycotoxins are extremely variable. Average

levels of mycotoxin contamination may be low and rarely exceed risk threshold levels, but as the content range is very wide, several samples may exceed the maximum or recommended levels for mycotoxin contamination (**Table 1**) [11, 14, 17, 18, 20, 22, 34].

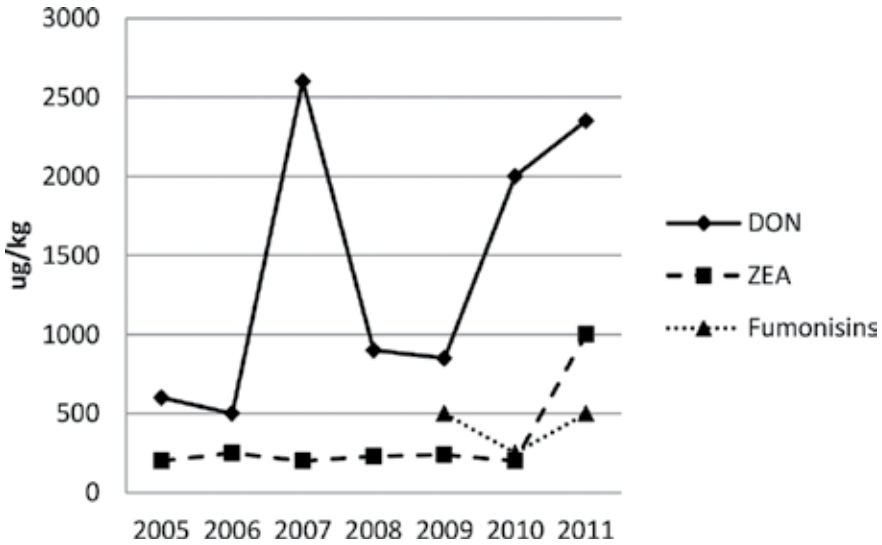


**Figure 2.** Year-by-year average mycotoxin concentration in wheat and wheat bran samples (modified from Ref. [20]).

| Mycotoxins | Contaminated samples, % (n of tested samples) | Content, average of positive (ppb) | Maximum level (ppb) | EU maximum levels* (ppb) |
|---|---|---|---|---|
| DON | 68 (770) | 960 | 15976 | UW: 1250<br>W: 750 |
| ZEA | 37 (645) | 98 | 3274 | UW: 100<br>W: 75 |
| T-2 | 22 (342) | 21 | 163 | T-2+HT-2**<br>UW: 100<br>W: 50 |
| FUM | 14 (331) | 356 | 5334 | – |
| AFLA | 16 (396) | 5 | 161 | 4 |
| OTA | 14 (278) | 3 | 9 | UW: 5<br>W: 3 |

AFLA, aflatoxins; DON, deoxynivalenol; FUM, fumonisins; OTA, ochratoxin A; T-2, T-2 toxin; ZEA, zearalenone; $A_w$, water activity; n.a., not available; W, wheat for direct human consumption; UW, unprocessed wheat.

*Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs.

**Indicates recommendations (2013/165/EU: Commission Recommendation of 27 March 2013 on the presence of T-2 and HT-2 toxin in cereals and cereal products).

**Table 1.** Results of mycotoxin occurrence in wheat in 2015 (modified from Ref. [22]).

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Another important point highlighted from mycotoxin researches is that mycotoxin co-contamination is more the rule than the exception. Several studies reported a high incidence of multi-mycotoxin contamination in cereals and agricultural commodities [10, 14, 17–22]. A recent survey showed that in 2015, 46% of wheat samples were co-contaminated by two to six mycotoxins [35]. A study carried out in Italy showed that at least 80% of wheat samples were contaminated with one mycotoxin, while two mycotoxins were found in 27% of contaminated samples; 38% of the analyzed samples were contaminated with three or more mycotoxins [36]. Multi-mycotoxin contamination is a topic of great concern, as co-contaminated samples, although at lower levels than those indicated by EU regulations, might still exert adverse effects on animals due to additive/synergistic interactions of the mycotoxins.

A further scenario is represented by the climate changes. Estimates suggest that climate change will reduce wheat production globally by 29–34% by 2050 in developing countries [37]. This will have a great impact on food security. In terms of food safety and mycotoxin contamination, although aflatoxin is the mycotoxin that is most likely to increase under near-future climate scenario, problems concerning also *Fusarium* toxins may represent a challenge if the temperature increases in cool or temperate climate countries [38, 39].

In terms of mycotoxin contamination, new issues for cereal safety include both emerging mycotoxins and modified forms [15, 16, 25, 26, 40]. Mycotoxin contamination by emerging *Fusarium* mycotoxins, such as beauvericin and enniatins, represents a problem of global concern, especially in Northern Europe [15, 25, 36, 40]. Modified mycotoxins represent another emerging topic. Plant metabolites have been identified so far for DON, NIV, fusarenon-X, T-2 toxin, HT-2 toxin, ZEA, ochratoxin A (OTA), destruxins, fusaric acid, and modified fumonisins have been found, especially in wheat and other cereal commodities [41–46]. The acetylated derivatives of DON, 3-ADON, and 15-ADON are frequently detected in DON-contaminated grains [47].

## 3. Strategies to mitigate mycotoxin contamination

Fungi can invade, colonize, and produce mycotoxins during either preharvest or postharvest stages [5–8]. Therefore, to properly manage mycotoxin contamination in wheat, the primary strategy is the prevention, by reducing fungi proliferation in field and during storage [48–51]. Commonly and usually, mycotoxinogenic fungi are divided into two groups: preharvest (mainly *Fusarium* species) and postharvest (mainly *Aspergillius* and *Penicillium* species) fungi. During storage, fungi and insects may cause further deterioration. Fungi, such as *A. clavatus*, *A. fumigatus, Chaetomium, Scopulariopsis, Rhizopus*, *Mucor*, and *Absidia,* do not infect intact crops, but can easily attack damaged grains and, in the presence of high moisture content, may be responsible of advanced deterioration [52].

There are several possibilities for mitigating mycotoxin contamination. Preharvest events are predominantly dictated by environmental factors and good agronomic/cultural practices. Conditions, such as excessive moisture, temperature extremes, humidity, drought conditions, insect damage, crop systems, and some agronomic practices, can cause stress and predispose

plants in the field to mold and determine the severity of mycotoxin contamination [5, 31, 53]. *Fusarium* sp. are generally associated with a cool and excessively wet growing season [31, 54]. Wheat storage and processing are the major areas where contamination can be managed and mitigated at postharvest level, keeping in mind that postharvest contamination is also the result of preharvest presence of fungal contamination. The main strategies that need to be considered and implemented to mitigate mycotoxin accumulation pre- and postharvest are summarized in **Figure 3**.

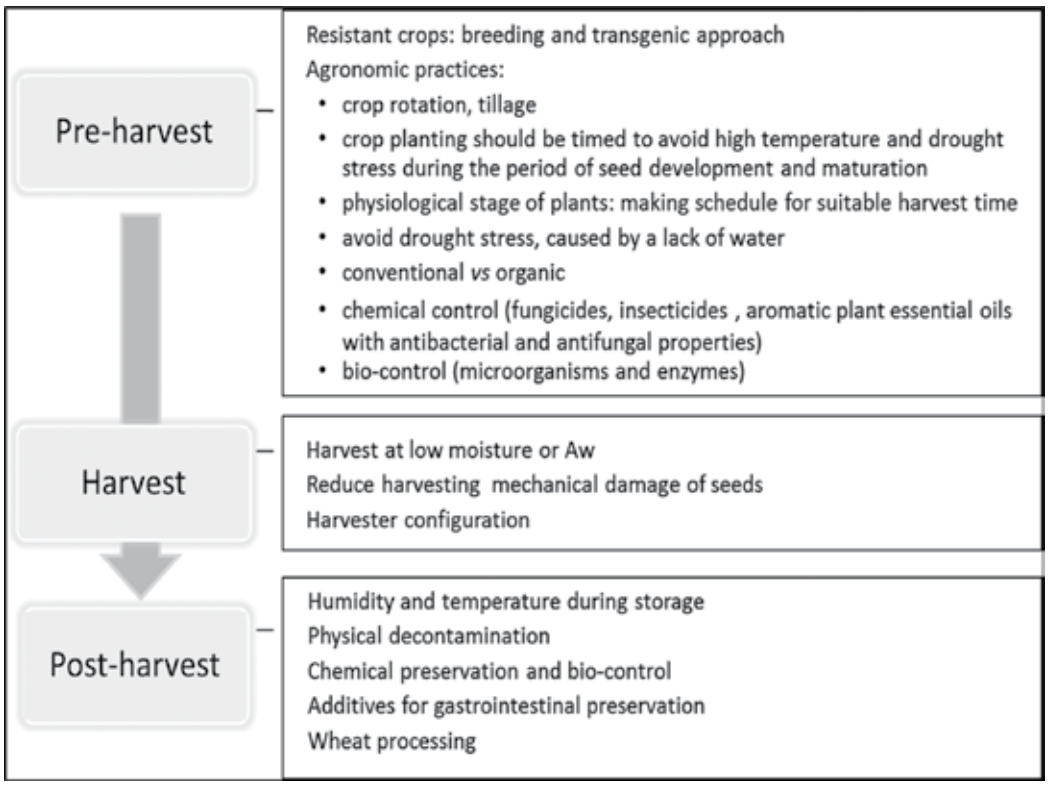


**Figure 3.** More consolidated and emerging strategies to reduce mycotoxigenic fungi and mycotoxin contamination in wheat.

## 4. Preharvest mitigation measures and management

One of the main wheat diseases associated with mycotoxin contamination is *Fusarium* head blight (FHB) caused by several species of *Fusarium* fungi, mainly *Fusarium graminearum, Fusarium culmorum,* and *Fusarium avenaceum.* The control of infection by *Fusarium* fungi in field is the first critical step in mitigating mycotoxin accumulation in the harvested products. To reduce the risk of *Fusarium* fungi and mycotoxin contamination, the most important pre-harvesting strategy is the application of appropriate good agriculture practices, such as crop selection, crop rotation, tillage, irrigation, and the proper use of chemicals [53].

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*Crop selection:* The use of genetic varieties more resistant to *Fusarium* sp. represents an effective management strategy to mitigate the mycotoxin challenge in wheat. There are differences in the susceptibility of wheat variety to *Fusarium* and differences in the degree of mycotoxin contamination. Moreover, differences between crops appear to differ between countries which can be related to differences in the genetic pool within each country and the different environmental and agronomic conditions in which crops are cultivated [48]. Wheat lines have been produced and provide good resistance to *Fusarium* sp. [55, 56]. For an important impact in terms of wheat security and safety, breeding for resistance must provide good resistance to *Fusarium* sp. without adversely affecting quality and agronomic properties. In addition to breeding programs, the increase in *Fusarium* resistance through developing genetically modified plants is another approach. It is well documented that transgenic resistance against toxigenic fungi or their toxins may be improved by using three basic strategies: enhance resistance to insect attack, induce mycotoxins detoxification pathways, and reduce mycotoxin accumulation by interfering with the biosynthetic pathway [57]. The topic of breeding for resistance and transgenic resistance would require a full manuscript. These topics have been specifically and extensively reviewed by several Authors to which the reader is directed [58, 59]. Despite progress made in prevention through breeding of resistant varieties and improvement in agronomic practices [31, 57], hazardous concentrations of mycotoxins may further occur as a result of annual weather fluctuations.

*In field management:* Appropriate field management practices may be effective to mitigate mycotoxin contamination in wheat [60]. When crop rotation is considered, maize should be avoided in the rotation, as maize is very susceptible to *Fusarium* sp. and the presence of maize residues appears to be an important factor contributing to DON contamination of wheat [57, 61]. The incidence and severity of *Fusarium graminearum* and DON contamination levels are higher in wheat grown after maize or wheat compared with wheat grown after soybeans [61, 62]. Moreover, the great differences in the frequency of isolation of *Fusarium* sp. and *F. graminearum* among years suggest the importance of annual climatic conditions in promoting the colonization and survival of these fungi. Other studies found no evidence that wheat following wheat is more at risk than wheat following a non-cereal crop, since some pathogenic *Fusarium* species isolated from cereals can also have pathogenicity toward non-cereal crops [63, 64]. The incidence of *F. avenaceum*, which is another of the most commonly isolated *Fusarium* species from FHB-infected ears of wheat in Canada, was lower in wheat grown continuously compared to wheat grown in crop rotation [65]. Crop rotation in conjunction with tillage techniques may further mitigate *Fusarium* and mycotoxin contamination. Higher levels of *Fusarium* and DON contamination in wheat have been reported with minimum tillage or no-till compared to conventional tillage [61, 63]. This effect can be attributed to inoculum survival and the concentration of *Fusarium* sp. in the soil [66, 67]. However, not significant effect of tilling has been reported when wheat was grown after soybeans [60].

Irrigation management is another critical point to mitigate preharvest mycotoxin contamination. All plants in the field need adequate water supply. Drought stress and also an excess irrigation are favorable conditions for *Fusarium* infection. Drought stress should be avoided during the period of wheat seed development and maturation; therefore, crop planting should be timed accordingly. Excessive moisture in irrigated wheat fields during flowering and early

grain fill period is a favorable condition for *Fusarium* infection [68, 69]. Nevertheless, the effect of moisture in increasing the levels of DON contamination is not consistent among published studies [69–73].

*Use of chemical and biological compounds:* Mold infection can be controlled by the appropriate use of fungicides. Fungicide treatment reduces wheat *Fusarium* infection and DON contamination [74–76]. Recently, Scarpino et al. [77] reported that azole fungicides, the most effective active substances in the reduction of DON, also consistently reduce the main emerging and modified mycotoxins of winter wheat in temperate areas. However, as far as the effectiveness of fungicide application to control mycotoxin contamination by *Fusarium* species, conflicting evidence has been reported. A meta-analysis carried out by Paul et al. [78] reported results ranging from no detectable effects to substantial reduction in both *Fusarium* head blight and DON with triazole-based fungicides. Overall results indicate that the variability of fungicide effects is related to several factors, such as cultivar resistance, the type of fungicide used, fungicide timing, pathogen aggressiveness, and different environmental and agronomic conditions. A greater fungicide efficacy in reducing FHB and DON has been reported in moderately resistant cultivars than in susceptible ones [79]. These results confirm that the efficacy of each mitigating approach must be considered within an integrated strategy for an effective management of *Fusarium* and mycotoxin control in wheat. As a tool of chemical control, several aromatic plant essential oils have been tested for their antibacterial and antifungal properties [80–83]. Results demonstrated a different antifungal activity and efficacy of these compounds, but more research is needed on this topic.

The chemical control of fungal infection and mycotoxin contamination may be only partly effective; therefore, biological control as an additional strategy has been considered and evaluated [53]. The efficacy of bacterial and fungal antagonist against *Fusarium* sp. has been reported in vitro, in the greenhouse, and in the field [84–92]. Biological antagonists can be sprayed directly at the flowering stage to limit the growth of fungal toxin producers. Wegulo et al. [53] concluded that the application and efficacy of the biological control for *Fusarium* infection and mycotoxin control pose challenges similar to those posed by fungicide application.

The use of biological control strategies to reduce mycotoxin challenge in wheat can be especially useful in organic production where synthetic fungicides cannot be used. The increased demand for organically produced food asks for scientific assessments of the safety of products from different farming systems, such as organic *vs.* conventional. Brodal et al. [93] published very recently an extensive review of studies comparing the content of DON, HT-2+T-2 toxins, ZEA, NIV, OTA, and fumonisins in cereal grains from organic and conventional farming systems. Inconsistent results have been reported regarding the DON, ZEA, NIV, and T-2+HT-2 content in wheat from the two farming systems (**Figure 4**).

Although no significant differences have been found in the majority of mycotoxin comparisons, several studies showed a tendency of a lower mycotoxin content in organically than in conventionally produced wheat. Moreover, results indicate that organic systems appear generally able to maintain mycotoxin contamination at low levels, despite no use of fungicides. The inconsistency of the results confirm that several preharvest factors, such as those previously described, may have more influence on the mycotoxin levels than the type of farming.

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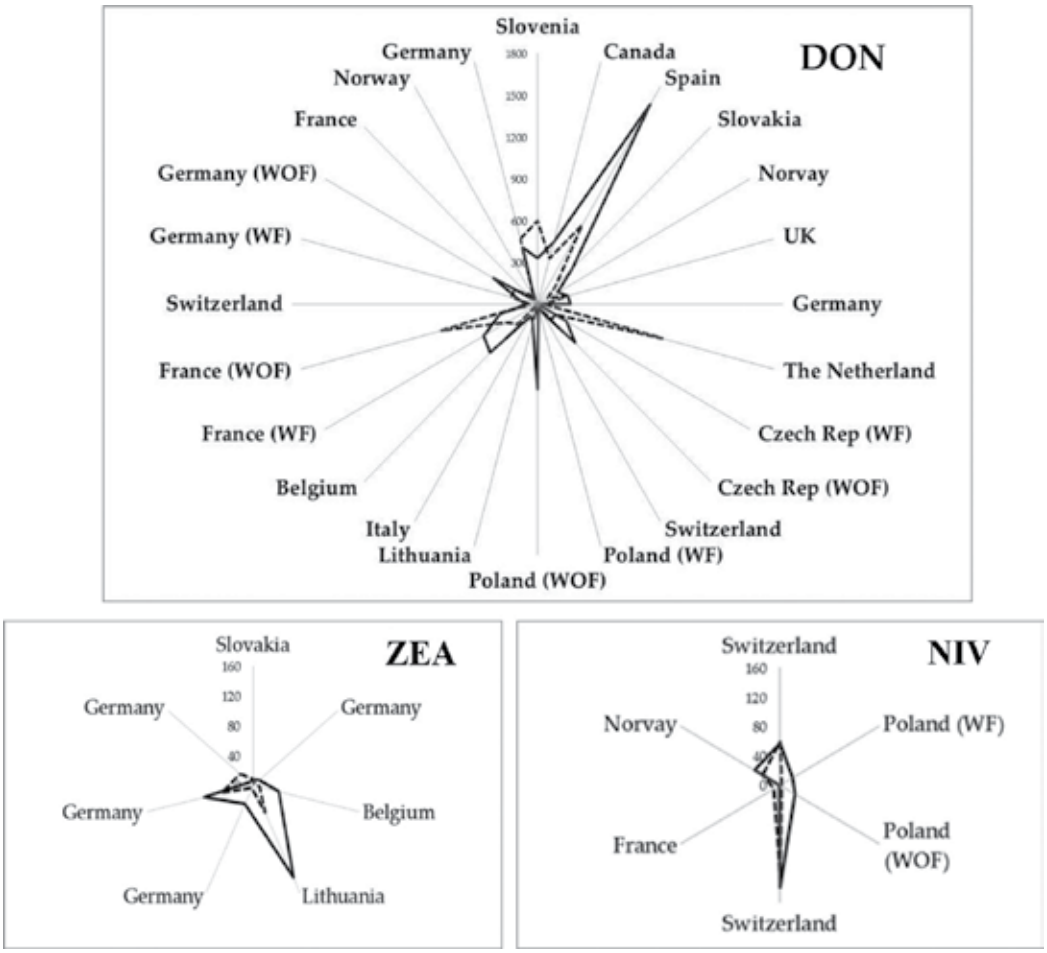


**Figure 4.** Mycotoxin contamination (μg/kg) in wheat from organic (- - -) and conventional (—) production (W: with fungicide; WOF: without fungicide) (modified by Ref. [93]).

To conclude, there are several preharvest practices and management approaches to reduce the risk of mycotoxin contamination in wheat, whose combination in an integrated strategy represents the best mitigation measure. All preharvest practices can be controlled, while climatic and environmental conditions cannot. Computer models, integrating field parameters and weather variables (temperature, rainfall, and moisture level) have been developed to predict the occurrence and risk of *Fusarium* and mycotoxin contamination in wheat [94–98]. Moreover, forecasting systems have been developed to optimize the use and application of chemical treatments [53].

## 5. Harvest and postharvest mitigation measures and management

Controlling harvest and storage conditions is critical to effectively prevent mold growth and mycotoxin production in wheat postharvest. Harvesting strategies, moisture, water activity

($A_w$), temperature, storage period, contamination rate, broken grains, insect presence, and oxygen rate are the main critical points to manage in order to mitigate the mycotoxin risks postharvest [48, 50–52, 99].

*Harvest management:* Wheat should be harvested as soon as possible to reduce fungal growth and spread during favorable weather conditions. Management strategies during harvest include wheat harvest at low moisture or $A_w$, reduced mechanical seed damage, and the use of different grain harvest strategies to remove diseased kernels which are often lighter than the healthy ones. The use different harvesting configurations, with varying fan speeds and shutter openings, resulted in lower *Fusarium*-damaged wheat kernels and DON content in harvested wheat [99, 100]. The removal of damaged grain implies a loss in the yield of harvested grain, but results in better storage conditions and improvement in grain safety offsetting the economic losses.

*Postharvest management:* Efficient drying and storage of wheat in silos free of insect pests and moldy material are critical points to reduce mycotoxin contamination. Harvested grain must be dried to <14.5% moisture content and at a relative humidity of 70% to avoid mold spoilage or increase of preharvest contamination with mycotoxins [48, 51, 101, 102]. Besides humidity, the temperature during storage is another critical point for fungal growth and activity. During storage, humidity and temperature are strictly related and cause changes in the microclimate conditions favoring or inhibiting fungal growth and colonization and influencing the pattern of mycotoxin contamination [49, 51, 103]. A comparison of environmental conditions for fungal growth and toxin production by some common fungal species is reported in **Table 2**.

| | **T, °C** | | **pH** | | **Optimal $A_w$** | |
|---|---|---|---|---|---|---|
| **Species (mycotoxins)** | **G** | **TP** | **G** | **TP** | **G** | **TP** |
| *A. parasiticus* (AFLA) | Range: 10–43<br>Optimum: 32–35 | 12–40 | Range: 2.1–11.2<br>Optimum: 3.5–8.0 | Range: 3.5–8.0<br>Optimum: 6.0 | 0.84 | 0.87 |
| *A. flavus* (AFLA) | Range: 10–43<br>Optimum: 32–35 | 12–40 | Range: 2.1–11.2<br>Optimum: 3.5–8.0 | Range: 3.5–8.0<br>Optimum: 6.0 | 0.80 | 0.82 |
| *Fusarium* species (T-2, DON, NIV, ZEA) | 24–26 | 24–26 | 2.4 at 30°C and 3.0 at 25°C and 37°C | 2.4–3.0 | 0.90 | 0.90 |
| *P. verrucosum* (OTA) | Range: 0–31<br>Optimum: 20 | 4–20 | Range: 2.0–10.0<br>Optimum: 6.0–7.0 | n.a. | 0.80 | 0.86 |

AFLA, aflatoxins; DON, deoxynivalenol; NIV, nivalenol; OTA, ochratoxin A; T-2, T-2 toxin; ZEA, zearalenone; $A_w$, water activity; n.a., not available.

**Table 2.** Comparison of environmental conditions for fungal growth (G) and toxin production (TP) by some common fungal species (modified from Ref. [104]).

In wheat, positive relationships between dry matter losses caused by *F. graminearum* under different environmental conditions (temperature, humidity, $A_w$) and the level contamination with DON have been reported [49, 51]. Moreover, it has been shown that the pattern and the

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levels of mycotoxin production in wheat grains by various *Aspergillus* sp. are different in relation to different relative humidity values and storage periods [101].

*Use of physical, chemical, and biological decontaminating methods:* Despite efforts to control, mitigate, and reduce fungal and mycotoxin contamination, wheat mycotoxin contamination is unavoidable and unpredictable, and postharvest decontaminating approaches can offer a last resort. Different decontaminating methods can be used to eliminate or reduce mycotoxin content in cereals before their entry in the food supply chain (**Table 3**).

| Strategy | Effects | References |
|---|---|---|
| Physical decontamination | | |
| - Sorting, dehulling, debranning, milling, irradiation, heating, or combined approaches | Removing of highly contaminated fractions or mycotoxin repartitioning from bulk wheat | [8, 105–112] |
| - Inorganic or organic mycotoxin binders | Reduced food mycotoxin bioavailability | [113–116] |
| Chemical decontamination | Conversion of mycotoxins via chemical reactions | [48, 51, 80, 106–118] |
| Microbial based methods | Microbial transformation, biodegradation | [51, 84, 106, 119, 120] |

**Table 3.** Mycotoxin contamination: main post-harvest physical, chemical, and biological based decontamination strategies.

Jard et al. [120] underlined that the decontaminating approaches must consider several topics concerning safety issues: they must not generate toxic products, ensure the nutritional value of the food, and should not induce negative modification for food processing.

A wide variety of chemical decontamination processes including oxidation, reduction, ammonization, alkalization, acidification, and deamination has been reported [48, 121]. These methods have some limitations concerning safety issues, efficacy coupled with cost and regulatory implication. The use of chemical methods for the decontamination of cereals that exceed the mycotoxin threshold limits are not allowed in the European Union [122]. In the United States of America, only ammonization is licensed for detoxifying aflatoxins [123, 124]. In addition to chemical methods, natural plant extracts and spices are known to prevent mold growth and mycotoxin production. In recent years, the use of essential oils as natural food preservatives to control mold and mycotoxin contamination is gaining interest [117]. Several essential oils have been found to be effective in controlling growth of several *Fusarium* sp. and production of mycotoxin in stored wheat [125, 126]. However, more studies should be performed to identify the components of essential oils with modulatory activity on the growth and toxin production of *Fusarium* sp.

Currently, many researches have been carried out to evaluate the possible use of biological agents or biological transformations for mycotoxin detoxification, as an alternative to the chemical one. This approach includes fungal, microbial, and enzymatic degradation of mycotoxins. Several very recent reviews on this topic can be found in the literature to which the

reader is directed for specific insights [84, 118, 119, 127, 128]. Despite the many publications on this topic, this promising approach is still at a research level and far from an immediate outcome and application in practice for mycotoxin detoxification of food at industrial level. More research is needed to fully understand mycotoxin biotransformation mechanisms, to evaluate the toxicity of metabolites and the feasibility of application in wheat industry. All these topics must be considered and evaluated keeping in mind the existing regulatory issues for food safety.

Physical decontamination reducing mycotoxins in wheat can be carried out during industrial processing. For the wheat milling industry, the precise knowledge of the fate of mycotoxins during milling is vital and may provide a sound technical basis to conform to legislation requirements, support risk management and regulatory bodies in order to reduce human and animal exposure to mycotoxins, and reduce the risk of severe adverse market and trade repercussions. Wheat sorting, cleaning, debranning, and milling influence mycotoxin repartitioning in wheat milling fractions entering the food chain. The effects of wheat milling and thermal processes on the fate of mycotoxins have been extensively studied [8, 33, 105–112, 121, 129–133]. Published data confirm that milling reduces mycotoxin concentration in fractions used for human consumption, but concentrates mycotoxins into fractions commonly used as animal feed. Physical and mechanical processes, such as sorting and cleaning prior to milling, reduce mycotoxin contamination in wheat by removing kernels with extensive mold growth, broken kernels, fine materials, and dust. The results indicate that the effect of pre-milling processes and the efficiency of mycotoxin removal are extremely variable. The concentration of mycotoxins in cleaned wheat ranges from 7 to 63% for DON, from 7 to almost 100% for NIV, and from 7 to 40% for ZEA, of the contamination level in unclean grains [28, 134, 135]. A reduction of 62 and 53% of T-2 and HT-2, respectively, has been reported in wheat grains after cleaning [136]. Several factors may be involved in this response, such as the initial condition of the grains, the type and extent of the contamination, and the type and efficiency of the cleaning process. Debranning before cleaning is used in industrial processing to enhance the milling performance of wheat and the degree of refinement of flour and semolina [137]. Debranning before milling further reduces the level of mycotoxin content in wheat grain. As for the cleaning and sorting procedures, the effect of debranning and the efficiency of mycotoxin removal are extremely variable. A reduction of DON in debranned wheat ranging from 15 to 78% has been reported [134, 138–140]. Despite the high variability in removal efficiency of mycotoxin, overall results indicate that the physical processes that are carried out before milling (such as sorting, cleaning, and debranning) are very efficient methods to reduce wheat mycotoxin content before milling. As in cleaning and debranning, in the milling process there is no step that destroys mycotoxins; however, mycotoxin contamination may be redistributed in milling fractions [141–143].

Overall results regarding the efficacy of mycotoxin reduction/repartition wheat industrial processing showed a high variability and sometimes appear conflicting. This is related to the type of mycotoxins, the level and extent of fungal contamination, and a failure to understand the complexity of the milling technology. The knowledge of mycotoxin repartitioning in wheat milling fractions is largely limited to DON, using different approaches (artificially vs. naturally contaminated wheat; wide range of mycotoxin contamination levels; laboratory;

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semi-industrial; and industrial milling), but there is still a lack of data for other mycotoxins. Fewer data are available regarding the distribution of other mycotoxins and modified mycotoxins in milling fractions [45, 142–146], but a similar scenario has been found, such as mycotoxins concentration in milling fractions intended for animal feed.

## 6. Conclusions and future perspectives

Mycotoxins in wheat represent a significant health risk to animal health and significant issues for a safe food supply chain. Regarding this topic, mycotoxin regulations have been established in more than 100 countries, and maximum acceptable limits have been fixed for food and feed. Mycotoxin co-contamination in wheat is a reality, and future attention should be paid not only to the mycotoxins believed to be the most likely to occur, but also to emerging and modified mycotoxins. The co-occurrence of several mycotoxins, with specific chemical traits and modes of action, is a serious health problem because of potential additive and/or synergistic effects. The impact of mycotoxins entering the food chain could increase in the next future. Most predictions indicate that the climate change scenarios, with global warming, could affect agriculture and increase the threat from fungal invasion of crops. Regarding this topic, there is a need to improve predictive models for mycotoxin contamination in wheat, integrating field parameters and weather variables.

Strategies to mitigate and reduce mycotoxin contamination in wheat include approaches at pre- and postharvest levels. The efficacy of each mitigating approach is highly variable depending on several factors, such as the type of approach, the type and level of mycotoxin contamination, the crop variety and agronomic practices, storage condition, etc. Integrating as many management options as possible is the key to minimize the risk of mycotoxin contamination in wheat and wheat products. However, it must be underlined that even if pre- and postharvest practices can be controlled, there is an unpredictable factor that influence mycotoxin occurrence in wheat, namely the climatic and environmental conditions. Therefore, despite efforts to control and reduce fungal and mycotoxin contamination, wheat mycotoxin contamination is unavoidable and unpredictable and postharvest decontaminating approaches can offer the last resort. The use of these strategies must not be detrimental for the wheat quality and safety, and must comply with the existing regulatory requirements.

The high variability in the efficacy of mitigating strategies increases awareness and ongoing surveillance for mycotoxins. At industrial level, an effective approach to manage the mycotoxin challenge in wheat requires regular, effective, economical, and straight forward wheat sampling and analytical diagnostic tools which can be used to monitor mycotoxin contamination, rapidly identify material below specified standards, and make justified management decisions regarding what to do with wheat lots that may be contaminated with mycotoxins. Sampling is the greatest source of error in quantifying mycotoxin contamination because of the difficulty in obtaining samples from large grain consignments and of the uneven distribution of mycotoxins within a commodity [147]. The Commission Regulation 401/2006/EC provides precise details regarding the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs [148]. The development of rapid methods for use in

the field represents a future challenge, but such methods would allow for "decision-making" regarding the safe use of wheat and wheat by-products. Moreover, more research on the development and application of multi-mycotoxin analytical methods should be encouraged in order to obtain a more accurate picture of the extent of multi-mycotoxin contamination.

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Section 4

# Wheat Processing and Utilization

Chapter 12

# Rheological and Technological Quality of Minor Wheat Species and Common Wheat

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Additional information is available at the end of the chapter

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**Abstract**

Wheat is an important food grain source that nurtures millions of people around the world. Not only does wheat contain a large number of nutrients such as protein, wet gluten, etc., but also it has a lot of antioxidants such as dietary fibre, tocopherols, tocotrienols, etc. In a majority of cases, attention has been drawn to evaluate the grain yield and its components rather than its quality. The present investigation was carried out to evaluate the differences between minority wheat species and common wheat to determine the best rheological characteristics, technological quality as well as correlations between rheological and technological traits. The results revealed that hulled wheat species had a high protein content and wet gluten content. Einkorn and emmer were not suitable for 'classical' baking processing. But there is potential for other products, e.g. wheat rice (einkorn) or pasta (emmer). Spelt should be possible to be used in 'classical' baking industry, but the best solution is to use grain as a mixture with bread wheat. Also, this study showed a genotype variation in the antioxidant activity of einkorn, emmer, spelta and *Triticum aestivum*.

**Keywords:** wheat, quality, rheological properties, antioxidant capacity, organic farming

## 1. Introduction

Wheat is a plant grown on more land area than any other commercial crop. It is also one of the most important food grain sources for people all over the world because of the universal

use of wheat for a wide variety of products such as bread, noodles, cakes, biscuits, etc. Wheat kernel is composed of endosperm (81–84%), bran (14–16%) and germ (2–3%) [1]. Endosperm is the inner part playing a role as storage of energy and functioning protein. Bran is the outer layer protecting the grain, and germ is the kernel's reproduction system. Whereas wheat endosperm contains mostly starch and protein, bran and germ are rich in dietary fibre, vitamins, minerals and phytochemicals playing an important role in nutrition and health benefits for humans [2]. The customers are, therefore, strongly recommended to consume whole grain foods with at least three servings per day. Recent studies have shown that regular consumption of whole-wheat grain has been found to be associated with reduced total mortality, as well as reduced risk of coronary heart disease, ischemic stroke, type 2 diabetes [3], hypertension in women and colorectal cancer [4].

Over the last few years, despite the development of organic farming throughout Europe, there are not enough varieties that have been purposely bred for organic farming [5]. Conventional bred and tested varieties which were reproduced under the organic farming conditions are grown there [6]. But there are many references from different authors [7] that reported lower baking quality of bread wheat within organic farming. On the other hand, there are many neglected wheat species which have potential to be grown in organic farming and provide high-quality grain [8].

Original cultivars and landraces (e.g. spelt wheat) are the most usual organically cultivated cereal species. Their yield rate is supposed to be lower. Therefore, they have been pushed out of the conventional farming system and replaced by common wheat species. Obsolete cultivars and landraces are also highly appreciated as valuable genetic resources because they are unique and irreplaceable genetic resources for further development of the biological and economic potential of cultural crops. The neglected cereal species have become attractive in the Czech Republic. Spelt wheat (*Triticum spelta* L.) was created by interbreeding of the Tausch's multigraft (*Aegilops tauschii* syn. *squarrosa* L.) with emmer wheat. It is a cultural hulled wheat species and has got 42 chromosomes. There are winter and spring forms of spelt wheat [9]. In 2001, a winter spelt wheat variety called Rubiota was bred in the Crop Research Institute in Prague-Ruzyne and registered. Nowadays, the largest areas of spelt wheat can be found in the Western European countries, such as Germany, Belgium, northern France and Switzerland. There are about 30,000 ha of spelt wheat areas in all of these countries and regions [10]. Spelt wheat has become more attractive in the Czech Republic too – thanks to the development of organic farming. In 2014, spelt wheat crop stands at 2058 ha in the Czech Republic, and the average yield rate attained is 2.81 t/ha. Having the origin from Turkey, *Triticum macha* Dekapr. and Menabde. has got 42 chromosomes as well. This variety ranks among hexaploid wheat species and is cultivated only in the Caucasus region and currently in Russia. It was not grown commercially in Europe or the USA either [11]. It has not been explored too much. Winter varieties are frost proof. This wheat species prefers mid-dry soil types with neutral pH. This is a late winter wheat species and plants have got long stalks. Grains stay in spikelet for a long time; they are kept there even if threshed. They are elliptical, red and mid-hard [11]. Based on foreign literature data, both hulled wheat species are attractive because of their nutritional parameters. Both species contain more proteins (13.5–19%) [12]. Wet gluten content varies from 35 to 45% (but it can be up to 48%) [13]. SDS test values are similar to common wheat values (40–60 mL). Digestible starch content in spelt wheat plants is also similar to the

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one in common wheat plants. Digestible saccharide content in spelt wheat plants is much lower than the one in common wheat plants. There are fairly less insoluble fibres in the spelt wheat plants than in the common wheat plants [12].

Our chapter is aimed at comparing the baking quality of grains of the different species with the baking quality of grains of modern common wheat. It is also partly aimed at assessing individual parameters of the dough rheology and comparing it with the results of usual grain quality measurement and assessment. The second aim of this chapter is to determine the contents of antioxidant activity (tocopherols) in varieties of einkorn (*Triticum monococcum* L.), emmer (*T. dicoccum* Schuebl [Schrank]), spelt (*T. spelta* L.) and *Triticum aestivum* L. and identify the richest sources for improving the nutritional value of bread, pasta and other wheat products.

## 2. Materials, methods and results

### 2.1. Materials and methods

#### *2.1.1. Baking quality and rheological properties*

The used varieties were from the Gene Bank of the Crop Research Institute in Prague-Ruzyne, including *T. macha* Dekapr. and Menabde, *T. spelta* L. and control varieties of *T. aestivum* L. – variety SW Kadrilj. Varieties were sown on the organic certified research area of the University of South Bohemia in Ceske Budejovice, Czech Republic, and the University of Natural Resources and Life Sciences, Vienna, Austria, during 2014. The seeding rate was adjusted for a density of 350 germinable grains per $m^2$. The crop stands were treated in compliance with the European legislation (the European Council (EC) Regulation No. 834/2007 and the European Commission (EC) Regulation No. 889/2008).

Characteristics of the conditions of the University of South Bohemia in Ceske Budejovice research area: mild warm climate, soil type – pseudo gley cambisols, kind of soil – loamy sand soil and altitude of 388 m. Characteristics of the conditions of the University of Natural Resources and Life Sciences research area: located in Raasdorf, the soil was Calcaric Phaeozems (WRB) from loess with a silty loam texture, with the altitude of 156 m.

Quality analysis: The following parameters were tested after harvesting and dehulling of the grains by the International Association for Cereal Chemistry (ICC) methods: crude protein content (ICC 105/2); index of sedimentation – SDS test (ICC 151); wet gluten content (ICC 106/2), gluten index (ICC 155) and baking experiment [14]. For the detailed evaluation of baking quality, Mixolab II. System (accepted as the ICC standard method No. 173 – ICC 2006) was used, which makes possible to evaluate physical dough properties such as dough stability or weakening, and starch characteristics in one measurement (**Table 1**).

Statistical analysis: data were analysed by the Statistica 9.0 (StatSoft Inc., USA) programme. Regression and correlation analyses provided the evaluation of interdependence. The comparison of varieties and their division into statistically different categories were provided by Tukey's HSD test.

| | |
|---|---|
| Time for C1 (min) | The time evolution of the dough. The stronger the flour, the longer the time evolution (time to reach C1) |
| Amplitude (Nm) | The elasticity of the dough. The higher the value, the greater the flexibility of flour |
| Stability (min) | Resistance against kneaded dough. The longer the duration, the more the flour is considered stronger |
| C2 | Measured attenuation of protein due to mechanical work and temperature |
| C3 | Measures the gelling starch |
| C4 | It measures the stability of the hot gel |
| C5 | Measured starch retrogradation in cooling phase |
| Guideline $\alpha$ (C1–C2) | Attenuation rate of protein in warming |
| Guideline $\beta$ (C3–C4) | Speed starch gelatinisation |
| Guideline $\gamma$ (C5–C4) | The rate of enzymatic degradation |

**Table 1.** Description of Mixolab II. phases.

### *2.1.2. Quality of pasta*

A mixture of tested wheat varieties – einkorn wheat, emmer wheat, spelt wheat and bread wheat (the SW Kadrilj cultivar) – was milled into semolina and flour. The semolina was used to make pasta and the selected baking properties of the flour were determined. A reference method was used for the determination of moisture content of flour; the Falling Number method according to Hagberg-Perten was used, as well. The amount of nitrogen in the flour was measured according to Kjeldahl; the sedimentation index was calculated on the basis of Zeleny's test, and the wet gluten quantity and quality were determined with the Glutomatic System.

Semolina pasta was prepared in the pasta machine MPF2.5, and subsequently, a cooking test was performed. The cooking test focused on the determination of the boiling properties, binding and swelling capacity as well as the amount of sediment. The sensory evaluation of the cooked pasta samples was carried out by a group of 10 evaluators.

### *2.1.3. Antioxidant capacity*

The used varieties came from the Gene Bank of the Crop Research Institute in Prague-Ruzyne. In the precise, 3-year field experiments in 2010, 2011 and 2012, four varieties of wheat einkorn *T. monococcum* L., eight varieties of emmer (*T. dicoccum* Schuebl [Schrank]), seven varieties of spelt (*T. spelta* L.), four varieties of landraces of bread wheat (*T. aestivum* L.) and three varieties of spring wheat (*T. aestivum* L.) as control (SW Kadrilj, Vanek, Jara) were used.

Varieties were sown on the organic certified research area of the University of South Bohemia in Ceske Budejovice, Czech Republic. The seeding rate was adjusted for a density of 350 germinable grains per $m^2$. The crop stands were treated in compliance with the European legislation (the

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European Council (EC) Regulation No. 834/2007 and the European Commission (EC) Regulation No. 889/2008). Characteristics of the conditions of the University of South Bohemia in Ceske Budejovice research area: mild warm climate, soil type – pseudo gley cambisols, kind of soil – loamy sand soil and altitude of 388 m.

The following methodology is based on the description in a paper by Lachman et al. [14]. Laboratory analysis of composed finely ground wheat samples (ca 5.0 g) were weighed into 100 mL volumetric flasks and dissolved in methanol. The flasks were filled up with methanol to a volume of 100 mL. For AOA determination, 100 μL aliquots of sample solutions were pipetted. Indirect method described by Roginsky and Lissi was used [15]. Sample containing antioxidants reacts with a solution of stable synthetic radical being converted to a colourless product (DPPH assay). Methanolic DPPH solution [absorbance (t0) 0.600 ± 0.01] was prepared and 100 μL of the sample were added. Reaction time was 20 min. Absorbency was measured at wavelength $\lambda$ = 515 nm. AOA was calculated as the decrease of absorbency according to the equation (1): AOA (%) = 100−[(At20/At0) × 100] (1) where At20 is the absorbency in time 20 min and At0 is the absorbency in time 0 min. Calculated AOA was expressed in mg Trolox/kg DM. At0 and At20 were determined from the standard calibration curve ($r2 \geq 0.9945$). Calibration curves were prepared using working solutions of Trolox in methanol between 5 and 25 μg Trolox/mL (LOD = 0.601 μg Trolox/mL, LOQ = 2.000 μg Trolox/mL, RSD = 1.83%). All samples were analysed in duplicates.

### 2.2. Results

#### *2.2.1. Baking quality and rheological properties*

Part of our work focuses on finding any differences in the baking quality between the tested varieties. It is also aimed at evaluating correlations between the baking quality parameters determined by common methods and in every single stage of Mixolab II. **Table 2** shows the tested varieties and their average values do not differ statistically from each other in the amplitude, stability, C2–C5, Gamma directive and Falling Number (the Mixolab II. stages are explained in **Table 2**). On the other side of the coin, there were statistically significant differences in C1 stage, Alpha and Beta directives, protein content, wet gluten, gluten index and SDS test. Statistically, significant differences and correlations existed between the following stages. C1 stage had a positive correlation with gluten index and dough stability. According to **Table 2**, a control variety of *T. aestivum* L. was different from the other varieties in C1 stage, which was confirmed by a high gluten index value and more stable dough as well. A positive correlation existed between protein content and C4 + C5 stages, wet gluten content and Gamma directive. *T. macha* Dekapr. and Menabde contained the highest amount of proteins. Wet gluten had a negative correlation with C1 stage. If dough contains more wet gluten, it does not need to be worked so hard mechanically [16]. A positive correlation existed between wet gluten content and protein content. On the other hand, higher gluten index value enhanced dough to develop and had a negative correlation with protein content and wet gluten content. *T. aestivum* L. attained the highest gluten index values. SDS test had a negative correlation with Alpha directive, which relates to a starch grain size and resistance –

| Species | C1 | Amplitude | Stability | C2 | C4 | C5 | Alpha |
|---|---|---|---|---|---|---|---|
| *T. macha* | 3.08[a] | 0.09[a] | 7.8[a] | 0.36[a] | 1.1[a] | 1.8[a] | −0.08[a] |
| *T. spelta* | 4.45[a] | 0.07[a] | 8.9[a] | 0.39[a] | 1.2[a] | 1.9[a] | −0.09[ab] |
| *T. aestivum* | 6.69[b] | 0.07[a] | 9.8[a] | 0.44[a] | 1.3[a] | 2.0[a] | −0.10[b] |
| **Species** | **Beta** | **Gamma** | **Protein content** | **Wet gluten** | **GI** | **SDS** | **Falling Number** |
| *T. macha* | 0.45[a] | −0.05[a] | 15.2[b] | 36.07[b] | 55.4[a] | 42.75[a] | 526[a] |
| *T. spelta* | 0.63[b] | −0.07[a] | 14.99[a] | 36.9[a] | 56.0[a] | 43.08[a] | 425[a] |
| *T. aestivum* | 0.65[ab] | −0.07[a] | 13.1[a] | 19.3[a] | 96.8[b] | 49.40[b] | 463[a] |

Note: Values marked with the same letter are, based on Tukey's HSD test, statically significantly different at a significance level $P \geq 0.05$.

**Table 2.** Average values of tracked characteristic on Mixolab II. machine and basic parameters of baking quality.

the bigger and the better quality the grains are (prime ones), the more they swell and the less resistant they are to higher temperatures [10].

Statistically non-significant differences and correlations existed in the following stages. C2–C2 stage had a positive correlation with C3–C5 stages and Beta and Gamma directive and Falling Number. This indicates that the baking technology must be adapted to its properties. C3 – the so-called amylase peak – indicates a different composition of starch and size fractions of starch grains [10]. Samples originating from Vienna research locality contained a higher amount of small starch grains (second ones) which are bound tightly to the protein matrix, and they gelatinise in higher temperatures. On the contrary, samples originating from the Ceske Budejovice research locality contained a higher amount of good-quality big starch grains (prime ones) which gelatinise in lower temperatures. A positive correlation existed with C4 and C5, Falling Number and Beta and Gamma directives. C4 – less stable dough – needs to be baked longer at lower temperature. They do not need to be worked so hard mechanically [16]. This parameter had a strong correlation with C2. In C5, starch gets cooler, starch structure changes and starch gets harder. Retrogradation had a positive correlation with Falling Number. For amplitude, stability and Gamma directive, see **Table 2**. Falling Number had a strong correlation with stability, C2–C5 stages, Beta and Gamma directives and protein content. It is one of the most significant features determining flour baking quality [10].

*Triticum macha* Dekapr. and Menabde: there were significant differences in the protein-weakening stage (C2) (see **Figure 1**). These were caused by an increasing temperature and mechanical processing of dough. Large differences between two of our localities existed since the starch gelatinisation stage (C3). Samples originating from Vienna research locality attained the amylase peak at the same stage as samples of the control common wheat. Values, enzymatic activity and stability were lower in Ceske Budejovice. Such differences were kept until C5 stage – retrogradation – solidification. *Triticum spelta* L: There were enormous differences since C2 stage as well. Since C3 stage, samples originating from Vienna research locality attained the amylase peak. Those samples from Vienna kept the same or similar test results in C4 stage too.

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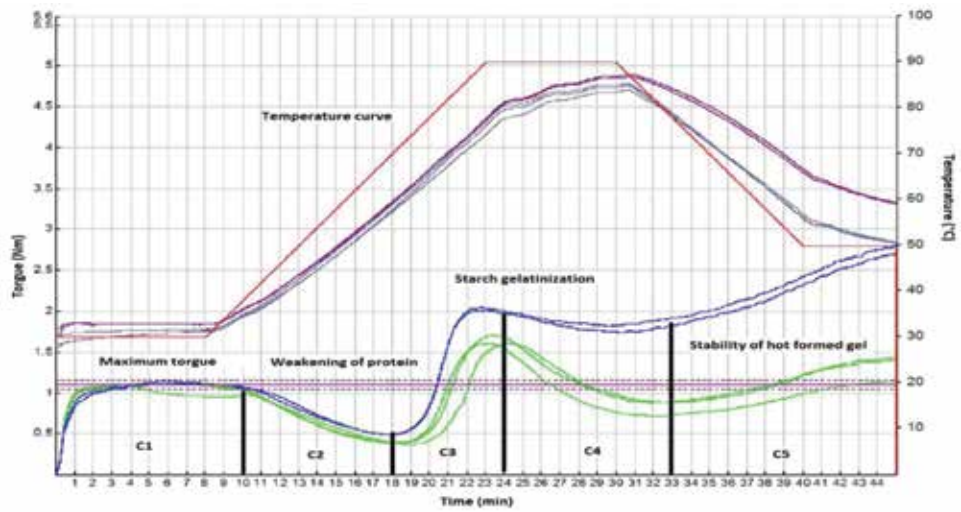
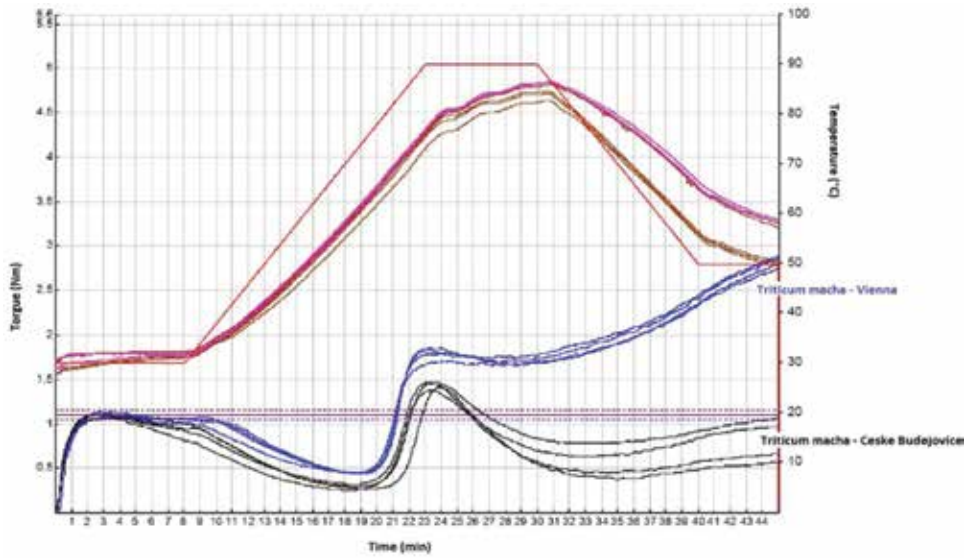



| serie testy | Hyd [%] | Work basis | Vyhřívaná víka | teplota stěn | teplota mísa |
|---|---|---|---|---|---|
| Mendel net 2 SW Kadrilj bez F | 63,0 | b14 | | | |
| Mendel net 3 SW Kadrilj a CB 2014 | 61,8 | b14 | | | |
| Mendel net 4 SW Kadrilj b CB 2014 | 61,8 | b14 | | | |
| Mendel net 47 SWKadrilj a Vídeň 2014 | 62,8 | b14 | | | |
| Mendel net 48 SWKadrilj b Vídeň 2014 | 62,3 | b14 | | | |

**Figure 1.** Rheological properties of control variety of *Triticum aestivum* L. - SW Kadrilj

There were large differences in stability between samples originating from Ceske Budejovice locality. In the last C5 stage, spelt wheat varieties attained higher average values than *T. macha* varieties. *T. aestivum* L. (control variety) – SW Kadrilj: larger differences between common wheat varieties arose during the test, since C3 stage (see **Figure 2**). These were the largest differences during the enzymatic degradation (C4 stage). It meant a very different enzymatic activity in every single sample. It was reflected in the retrogradation of starch as well (C5).

Such differences between Vienna and Ceske Budejovice research samples were caused by different conditions in every research locality (climate, weather changes, soil quality, agro-technology and quality of harvested material and post-harvest arrangements). There are




| serie testy | Hyd (%) | Work basis | Vyhlazená křivka | teplota sklo | teplota mixér |
|---|---|---|---|---|---|
| Mendel net 23 Macha 1a CB 2014 | 82,0 | b14 | | | |
| Mendel net 24 Macha 1b CB 2014 | 82,0 | b14 | | | |
| Mendel net 25 Mach 2a CB 2014 | 84,8 | b14 | | | |
| Mendel net 26 Mach 2b CB 2014 | 85,8 | b14 | | | |
| Mendel net 27 Mach 1 a Vídeň 2014 | 84,0 | b14 | | | |
| Mendel net 29 Mach 2 a Vídeň 2014 | 86,5 | b14 | | | |
| Mendel net 28 Mach1 b Vídeň 2014 | 86,3 | b14 | | | |
| Mendel net 30 Mach 2 b Vídeň 2014 | 83,6 | b14 | | | |

**Figure 2.** Rheological properties of control variety of *Triticum macha* Dekapr and Menabde

more precipitations and irregular rains in Ceske Budejovice. Rain occurs during the harvest period too. Water percentage in dry matter is a significant factor influencing Falling Number and behaviour of proteins in grains. On the other side of the coin, there are minimum precipitations in Vienna, and almost no rain occurs during the harvest period. However, there is a good-quality soil in Vienna and the total amount of nutrients in the soil is balanced (**Figure 3**).

*2.2.1.1. Quality of bread*

The results of determination of baking quality are summarised in **Table 3**. It is evident that the lowest protein content was measured in bread wheat flour. This fact is also confirmed

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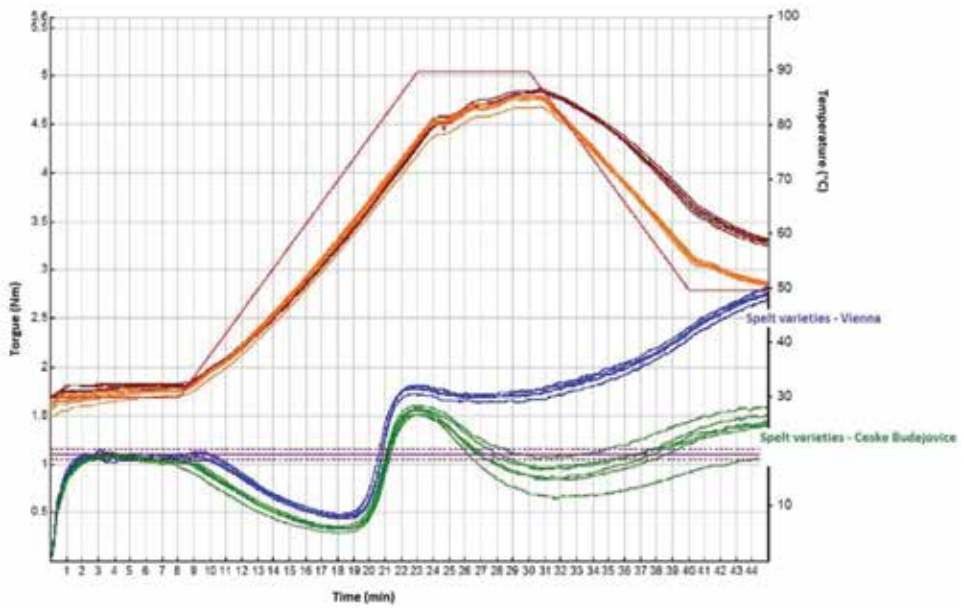


| serie testy | Hyd (%) | Work basis | Vyhlazená křivka | teplota stěn | teplota mísicí |
|---|---|---|---|---|---|
| Mendel net 11 SP6 a CB | 62,7 | b14 | | | |
| Mendel net 12 SP6 b CB | 62,7 | b14 | | | |
| Mendel net 14 SP7 b CB | 63,5 | b14 | | | |
| Mendel net 13 SP7 a CB | 63,5 | b14 | | | |
| Mendel net 16 SP8 b CB | 61,8 | b14 | | | |
| Mendel net 15 SP8 a CB | 61,8 | b14 | | | |
| Mendel net 57 SP8_8_1 Vídeň | 64,3 | b14 | | | |
| Mendel net 56 SP8_3_4 Vídeň | 65,0 | b14 | | | |
| Mendel net 34 SP6_3_2 Vídeň | 62,0 | b14 | | | |
| Mendel net 35 SP6_7_6 Vídeň | 62,8 | b14 | | | |
| Mendel net 37SP7_2_3 Vídeň | 63,7 | b14 | | | |
| Mendel net 38 SP7_6_1 Vídeň | 62,2 | b14 | | | |

**Figure 3.** Rheological properties of *Triticum spelta* L. varieties.

by the classification in a statistically different group ($P < 0.05$). Similarly, the lowest protein content was detected in white spelt flour. The observed result is consistent with the data published in the literature, where the protein content is normally referred above the limit

| Kind of flour | Protein content (%) | Zeleny's test (mL) | Gluten index | Wet gluten content (%) | Falling Number (s) | Bread volume ($cm^3$) |
|---|---|---|---|---|---|---|
| Spelt – whole grain flour | 12.77[a] | 10.0[a] | 57[a] | 40.7[ab] | 441[b] | 1500[a] |
| Spelt – white flour | 14.93[c] | 11.0[a] | 55[a] | 42.4[b] | 560[d] | 1725[c] |
| Bread wheat – whole grain flour | 9.98[b] | 10.0[a] | 52[a] | 40.4[ab] | 406[a] | 2190[d] |
| Bread wheat – white flour | 12.70[a] | 14.0[b] | 83[b] | 30.8[a] | 496[c] | 1610[b] |

Note: Values marked with the same letter are, based on Tukey's HSD test, statistically significantly different at a significance level $P \geq 0.05$.

**Table 3.** Selected parameters of baking quality (mean of two replications).

of 15%. The amount of wet gluten in the samples was in optimum quantity excluding white bread wheat flour that showed a statistically significant difference from white spelt flour. The values resulting from the Zeleny's test were generally low. Only bread wheat reached higher values. Low values of sedimentation are general problems of hulled wheat, which are due to the genetic background to some extent. The gluten index was determined to assess the gluten quality. As expected, the highest amount of gluten was found in bread wheat flour (also confirmed by Tukey's HSD test). However, the value of gluten index in whole-wheat bread flour was surprisingly low. A partial explanation was found based on the correlation analysis (**Table 4**), because the flour had low Zeleny's values. The correlation between these values and the values of gluten index was statistically significant (r = 0.89). Flour of both wheat varieties showed high values of the Falling Number – an indicator of damage to the starch grains due to the pre-harvest sprouting. The values are very high (exceed the standard). Such a high Falling Number may have negative effects on loaf volume, as well as the sensory evaluation of bread crumb.

| Parameter | | Mean ± SD | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|---|---|
| Protein content (%) | (1) | 12.6 ± 1.9 | | | | | |
| Zeleny's test (mL) | (2) | 11.3 ± 1.8 | $0.23^{ns}$ | | | | |
| Gluten index | (3) | 62.0 ± 14.0 | $0.12^{ns}$ | $0.89^{*}$ | | | |
| Wet gluten content (%) | (4) | 38.5 ± 5.2 | $0.11^{ns}$ | $-0.88^{ns}$ | $-0.89^{*}$ | | |
| Falling Number (s) | (5) | 475.8 ± 62.3 | $0.92^{*}$ | $0.42^{ns}$ | $0.24^{ns}$ | $-0.04^{ns}$ | |
| Bread volume ($cm^3$) | | 1756.3 ± 280.9 | $-0.69^{*}$ | $-0.34^{ns}$ | $-0.42^{ns}$ | $0.25^{ns}$ | $-0.44^{ns}$ |

Note: $^{*}P < 0.05$; $^{ns}$, non-significant.

**Table 4.** The results of correlation analysis (mean of a`ll flour kinds).

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The most objective parameter of the baking quality is a determination of the loaf volume. In the present case, a modified methodology was used, and hence the values are for guidance only. Whole-wheat bread showed the highest values. Conversely, the lowest values were found in whole-wheat spelt flour. The results do not fully correspond with the values regarding the gluten index and Zeleny's test (**Table 3**). Correlation analysis results (**Table 4**) indicate a negative correlation (r = −0.69) between the bread volume and protein content. A possible explanation is the fact that spelt is generally higher in protein, but it is of lower baking quality than bread wheat (**Figure 4**).


**Figure 4.** The result of the baking test. A cross-section of bread, in order from left to right: a bread of whole-wheat flour, finely ground flour, whole-wheat spelt flour, finely ground flour, bread wheat flour and spelt flour.

Respondents rated the bread made of whole-wheat, finely ground flour that is the best within the sensory evaluation. The main reason was its high volume. Eight out of 10 respondents described this bread visually appealing. Taste, of course, is a very important indicator of the quality of bread. For all breads, the taste was evaluated as pleasant and less intense. In overall assessment, the bread made of whole-wheat, finely ground flour and bread wheat flour received the highest rating. On the contrary, spelt bread gained only an average rating in the overall evaluation.

*2.2.1.2. Quality of pasta*

The resulting values of baking properties of flour, which may also affect the quality of pasta, were analysed using the correlation analysis. **Tables 5** and **6** show the results of Tukey's HSD test at a level of significance ($P \geq 0.05$). The tables also present the assessment of cooked pasta.

| Species | Protein content (%) | Zeleny's test (mL) | Gluten index | Wet gluten content (%) | Falling Number (s) |
|---|---|---|---|---|---|
| Einkorn | 15.59$^{b}$ | 8.50$^{a}$ | 25.8$^{a}$ | 36.45$^{b}$ | 387$^{a}$ |
| Emmer | 16.04$^{d}$ | 13.00$^{a}$ | 45.9$^{b}$ | 38.40$^{c}$ | 470$^{c}$ |
| Spelt | 15.74$^{c}$ | 21.0$^{b}$ | 76.0$^{c}$ | 42.26$^{d}$ | 403$^{a}$ |
| SW Kadrilj – bread wheat | 12.79$^{a}$ | 34.0$^{c}$ | 86.1$^{d}$ | 30.88$^{a}$ | 187$^{b}$ |

Note: Values marked with the same letter are, based on Tukey's HSD test, statistically significantly different at a significance level $P \geq 0.05$.

**Table 5.** Selected parameters of baking quality of different wheat species.

| Species | Firmness | Binding capacity | Swelling capacity | Sediment | Increase of volume | Increase of weight |
|---|---|---|---|---|---|---|
| Einkorn | 13[a] | 105[a] | 2.48[a] | 180[c] | 120.5[c] | 105[a] |
| Emmer | 10[b] | 100[c] | 2.28[a] | 110[b] | 102[a] | 100[c] |
| Spelt | 13[a] | 108[a] | 2.41[a] | 79.5[a] | 105.0[a] | 108[a] |
| SW Kadrilj – bread wheat | 11[c] | 95[b] | 2.20[a] | 80.0[a] | 94.5[b] | 95[b] |

Note: Values marked with the same letter are, based on Tukey's HSD test, statistically significantly different at a significance level $P \geq 0.05$.

**Table 6.** Selected parameters of quality of different wheat species.

According to CSN 46 1100-2 (the Czech Republic's standard quality), an amount of N-substances in wheat for food use should reach 10.8–13.7%, which corresponds with the sample of bread wheat flour. The fact that hulled wheat is higher in protein has been confirmed within this study. The tested wheat varieties contained 16% of N-substances. Generally, the sedimentation index was low in hulled wheat samples. It may be therefore stated that these varieties are not, in contrast to bread wheat, suitable for baking purpose. On the other hand, these values do not affect the quality of pasta to a great extent. By contrast, gluten index and the amount of sediment have an impact on it. In this case, negative correlation indicates that increased gluten index decreases an amount of sediment with 99.9% probability, when cooking pasta. Gluten index of einkorn wheat is very low, which consequently resulted in the relatively large amount of sediment. The interesting information shown by this statistical method is the dependence of wet gluten on the amount of water bound by pasta during boiling. The amount of water absorbed by pasta thus increases due to the higher wet gluten content together with the weight of pasta. Spelt showed the highest values of wet gluten and thereby the highest binding; conversely, the lowest amounts were found in bread wheat. The Falling Number method did not prove any evidence showing a connection with the quality of pasta.

The sensory evaluation included tasting and filling out a questionnaire. Colour, surface (smooth, rough and floury), edges (sharp and rough), texture (compact and cracked) and firmness (strong, fragile, crumbly and translucent) of uncooked pasta had been evaluated. In consequence, colour, hardness (undercooked, al dente and overcooked), shape (appropriate and deviation of shape), flavour (excellent, good, fair and poor with foreign taste), odour (pleasant and unpleasant) and surface (sticky, slightly sticky and dry) of cooked pasta had been evaluated. Based on the data gained from the questionnaires, it may be assumed that the pasta production of hulled wheat was assessed positively by the respondents on the whole (**Figure 5**).

### *2.2.2. Antioxidant activity of different wheat species*

Whole grain phytochemicals have an antioxidant activity, the ability to scavenge free radicals that may oxidise biologically relevant molecules [17]. Due to this, whole-wheat foods could

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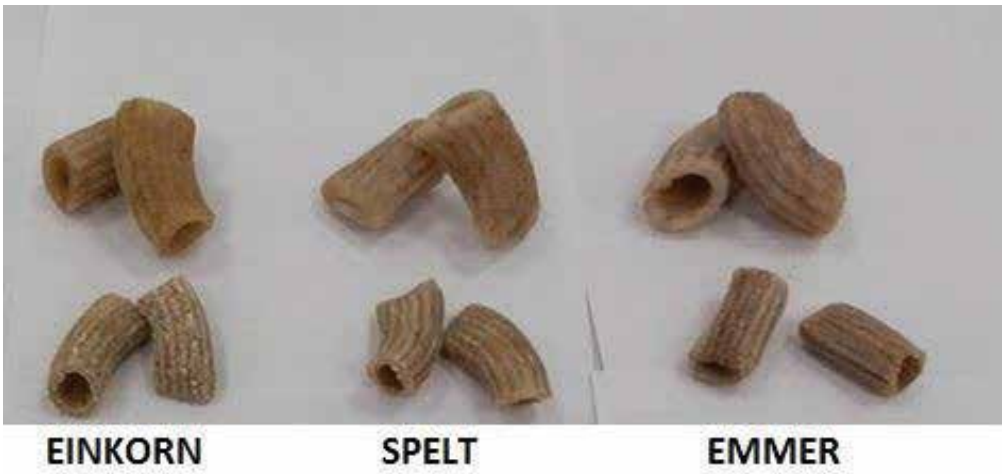


**Figure 5.** Differences in pasta colour.

contribute to the health benefits of people such as reducing the risk of heart disease, diabetes type 2, cancer, etc. In the present study, there were highly significant differences ($p < 0.05$) among 26 varieties for antioxidant activity (**Figure 6** and **Table 7**).

Mean antioxidant activity among varieties ranged from 225.45 mg Trolox/kg DW to 400.83 mg Trolox/kg DW. This demonstrates a broad range of antioxidant content in wheat species. There were eight groups in which the means were not significantly different from one

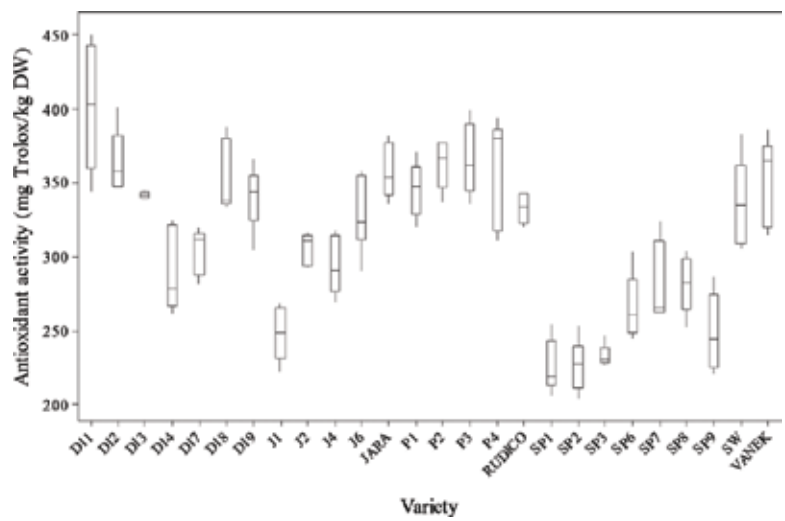


**Figure 6.** Antioxidant activity content of wheat varieties. Values expressed as mg Trolox/kg DM. D11 – Weisser Sommer; D12 – May-Emmer; D13 – *T. dicoccum*; D14 – *T. dicoccum*; D17 – *T. dicoccum*; D18 – *T. dicoccum*; D19 – *T. dicoccum*; J1 – *T.*; J2 – *T. monococcum*; J4 – *T. monococcum;* J6 – *T. monococcum*; P1 – *T. aestivum*; P2 – *T. aestivum*; P3 – *T. aestivum*; P4 – *T. aestivum*; SP1 – *T. spelta*; SP2 – *T. spelta;* SP3 – *T. spelta*; SP6 – *T. spelta*; SP7 – *T. spelta*; SP8 – *T. spelta*; SP9 – *T. spelta*; SW – SW Kadrilj.

| Variety | D11* | D12* | D13* | D14* | D17* | D18* |
|---|---|---|---|---|---|---|
| AOA (mg Trolox/kg DM) | 400.83$^{a}$ | 364.15$^{ab}$ | 341.60$^{bc}$ | 288.36$^{e–g}$ | 304.56$^{c–f}$ | 351.62$^{b}$ |
| Variety | D19* | RUDICO* | J1** | J2** | J4** | J6** |
| AOA (mg Trolox/kg DM) | 339.92$^{bc}$ | 332.90$^{b–d}$ | 247.42$^{gh}$ | 306.16$^{c–f}$ | 293.23$^{df}$ | 327.73$^{b–e}$ |
| Variety | P1*** | P2*** | P3*** | P4*** | SP1**** | SP2**** |
| AOA (mg Trolox/kg DM) | 345.88$^{bc}$ | 362.25$^{ab}$ | 365.26$^{ab}$ | 360.95$^{ab}$ | 225.45$^{h}$ | 226.55$^{h}$ |
| Variety | SP3**** | SP6**** | SP7**** | SP8**** | SP9**** | JARA |
| AOA (mg Trolox/kg DM) | 232.63$^{h}$ | 265.56$^{f–h}$ | 280.63$^{fg}$ | 281.10$^{fg}$ | 248.82$^{gh}$ | 357.36$^{ab}$ |
| Variety | SW | VÁNEK | | | | |
| AOA (mg Trolox/kg DM) | 336.98$^{b–d}$ | 353.70$^{b}$ | | | | |

Values marked with different small letters are significantly different at $P \leq 0.05$.

*Emmer varieties.

**Einkorn varieties.

***Landrace of *T. aestivum*.

****Spelt varieties.

**Table 7.** Content of antioxidant activity in different wheat grains.

another. Having 400.83 mg Trolox/kg DW, D11 variety belonged to the lead group and was significantly different from all other varieties except P3, D12, P2, P4 and JARA. In contrast, the varieties containing the lowest content of antioxidant were SP6, SP9, J1, SP3, SP2 and SP1 with 266.57 mg Trolox/kg DW, 248.82 mg Trolox/kg DW, 247.42 mg Trolox/kg DW, 232.63 mg Trolox/kg DW, 226.55 mg Trolox/kg DW and 225.45 mg Trolox/kg DW, respectively.

The findings of Lachman et al. [14] showed that the antioxidant activity content of seven varieties ranged between 134.0 and 197.5 mg Trolox/kg DW. In this study, our results are approximately two times higher than these ones. This means that the varieties in our experiment are potential for breeding new wheat varieties, as well as essential sources of functional food ingredients.

It is known that antioxidant activity content can be influenced by stress factors of the weather conditions during the vegetation period and genotype effects. Comparing the data of four species collected from 2010 to 2012 (**Figure 7**) show that there is a decrease in the mean of antioxidant during the 3-year period by 23.26 mg Trolox/kg DW. These differences are, however, not statistically significant.

The cultivated diploid (einkorn), tetraploid (durum wheat), hexaploid (bread wheat) and other varieties possess antioxidant activity due to their content of hydrophilic (phenolics, selenium) and lipophilic (carotenoids, tocopherols) antioxidants (**Figure 8**) [18].

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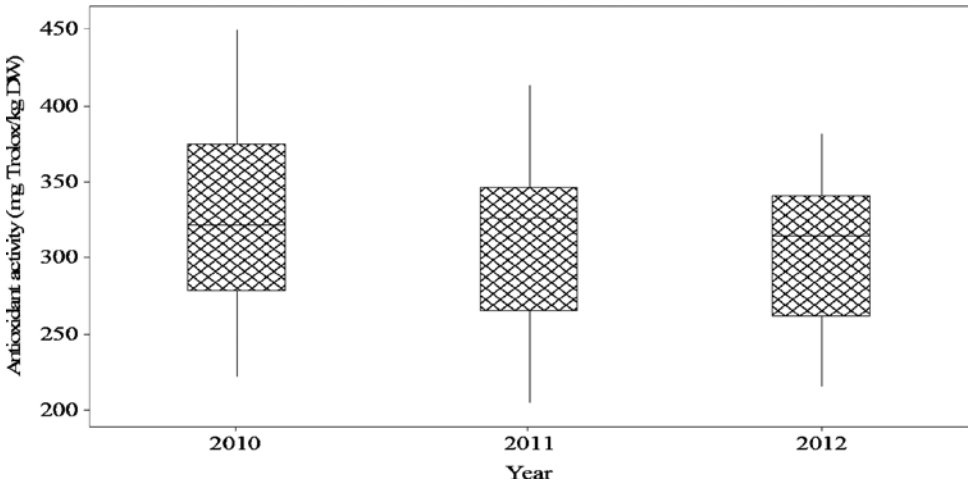


**Figure 7.** Antioxidant activity in 26 varieties harvested in 2010, 2011 and 2012.

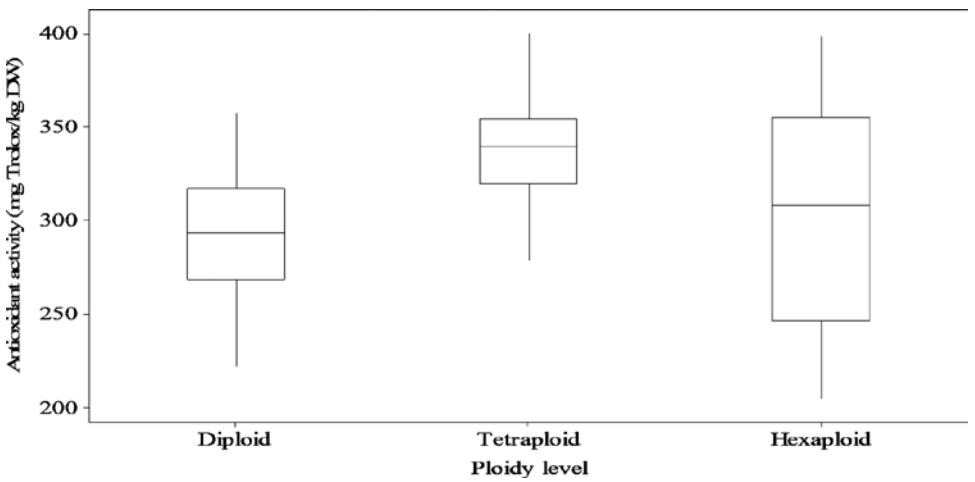


**Figure 8.** Content of antioxidants in wheat grains from the harvests 2010, 2011 and 2012.

Analysing ANOVA, Tukey's HSD revealed statistically significant differences between tetraploid and diploid as well as tetraploid and hexaploid. The mean antioxidant of tetraploid from 2010 to 2012 (340.49 ± 39.11 mg Trolox/kg DW) was higher than the value of diploid and hexaploid (293.64 ± 34.82 mg Trolox/kg DW) and (303.08 mg Trolox/kg DW), respectively. Our results are different from those of Lachman et al. [14]. While antioxidant values in our findings increase from diploid (einkorn) to tetraploid, the reverse is true for Lachman's results. This is because our experiment used 26 varieties in 3 years compared to seven varieties in 2 years.

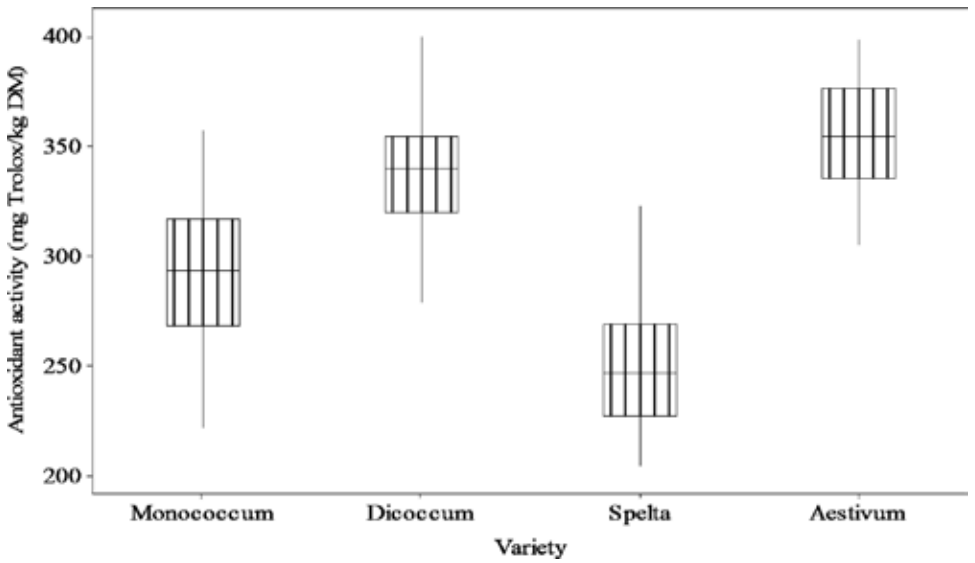


**Figure 9.** Antioxidant activity values of four species.

**Figure 9** illustrates the differences of four varieties. *T. aestivum* and emmer wheat shared the highest value with 354.44 ± 24.97 mg Trolox/kg DW) and 340.49 ± 39.11 mg Trolox/kg DW, respectively, followed by *T. monococcum* (293.64 ± 34.82 mg Trolox/kg DW). Having 251.54 ± 29.60 mg Trolox/kg DW, *T. spelta* had the lowest value in total of four species ($P < 0.05$).

### 2.3. Conclusions

Compared to samples originating from Ceske Budejovice, samples originating from Vienna attained higher and more balanced values in all the stages of Mixolab II. testing. SW Kadrilj was the only common wheat variety that attained a similar protein weakening speed when heated and worked mechanically (C2). There were enormous differences between *Triticum macha* Dekapr. and Menabde and *Triticum spelta* L. Baking technology must be adapted to the requirements of these two wheat species; dough must be worked more sensitively. In spite of this fact, these species can be used for baking purposes.

The working hypothesis, i.e. the use of spelt, einkorn and emmer wheat is technically feasible within the pasta production, was confirmed based on the testing. All wheat varieties are high in protein. Boiling time is not significantly different and pasta swells to the extent close to the pasta commonly available on the market. The taste of evaluated pasta was not assessed negatively, and the consumers, who are used to consuming more whole grain products, could feel the distinctive flavour of products made of einkorn and emmer wheat and spelt, which generally fades fast during milling from pasta made of white durum wheat or bread wheat flour.

Wheat contains huge essential antioxidants such as dietary fibre, tocopherols, tocotrienols, etc. The consumption of wheat is associated with reducing risk of chronic diseases including

type 2 diabetes, obesity and cardiovascular disease. In this study, the content antioxidant activity of 26 varieties of whole wheat is reported. Antioxidant activity was ranged from 225.45 mg Trolox/kg DW to 400.83 mg Trolox/kg DW. The antioxidant activity values were significantly different among varieties, ploidy level and wheat accessions.

The general conclusion is that hulled wheat species had a high protein content and wet gluten content. Einkorn and emmer were not suitable for 'classical' baking processing. But there is potential for other products such as wheat rice (einkorn) or pasta (emmer). Spelt will be possible to be used in 'classical' baking industry. The best solution will be the use of spelt wheat grain mixed with bread wheat grain. Also, this study showed a genotypical variation in the antioxidant activity of einkorn, emmer, spelta and *T. aestivum*.

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Chapter 13

# Grading Factors of Wheat Kernels Based on Their Physical Properties

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Additional information is available at the end of the chapter

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**Abstract**

Cereal grains are biological materials and as such have certain unique characteristics greatly affected by both genetics and environment. Wheat is worldwide considered as the main cereal grain in the average human diet. The aim of this chapter is to provide an overview of the most important grading factors and kernel physical parameters that are involved in the estimation of quality specifications. The determination of the physical properties of wheat kernels gives a first approximation of the structural characteristics useful for the design and selection of equipment for handling, harvesting, aeration, drying, storing and more importantly to functionality, processing and end uses. For instance, physical quality test that directly measure those properties are needed. To get a better prediction, physical evaluation of the wheat kernels offers a first and interesting quality control for their selection as raw materials in order to optimize quality of a large diversity of products. Kernel colour, shape, size, sphericity, porosity and bulk and specific densities and damages incurred due to heat, insects, molds or sprouting are relevant tests related to wheat kernel properties and quality.

**Keywords:** wheat, physical properties, damage, shape-size, volume-weight

## 1. Introduction

Wheat is one of the three most important cereals worldwide in terms of production and consumption. According to FAOSTAT [1], the estimated annual production in 2014 was 728 million tons, which provided daily 178 g per capita to the average human being. Wheat is considered among the oldest crops and is grown in more than 120 countries around the globe [2] and is the

main cereal in the human diet worldwide due to its agronomic adaptability, storability, nutritional value and diversity of products produced from it [3]. Wheat is unique among grains because its protein mixed with water and mechanical work yields a viscoelastic dough or batter capable of trapping gases produced by yeast or baking powders producing and array of leavened products such as breads, cakes and cookies.

Cereals grains are biological materials that differ in features due to many factors such as cultivar or genotype, soil fertility, growing conditions and agronomic practices [4]. The classification and grading play an important and critical role in the market because assures quality control guidelines. Furthermore, the standardization of grain quality allows a better marketing and grain processing to produce different products. Selected grain types for specific uses relate to their physical properties because they affect chemical composition, functionality and optimum industrial end use. The standardization of grain quality allows process a grain's lot with similar grade or quality [5].

Different methods for the classification of wheat grains are based on growth habit, end use and physical characteristics. The use of these methods can be referred to as technological classification [6]. Wheat-based products require different classes of the grain for their processing. Industrial quality is characterized by physical, chemical and rheological analysis related with roller milled refined flour, used especially in the manufacture of yeast-leavened breads (hard wheat) or chemically leavened cookies and cakes (soft wheat) or to produce semolina to elaborate long and short pasta products (durum wheat).

Physical evaluation of the wheat kernels offers a first and interesting quality control for selection as raw materials because the kernel physical features are related with design of equipment, handling, aeration and storage as well as to end use. Increasingly, analyses are implemented to assess the inherent characteristics of the grain to better know their attributes. The study of wheat kernel characteristics is necessary because new cultivars (with new and different properties) are constantly being bred and produced [7].

This chapter reviews the principal physical properties of wheat kernels in three sections. The first covers aspects related to appearance and damage, the second addresses shape and size features and the third grain volume, weight and density.

## 2. Wheat physical properties

Different wheat grain properties are included in the grading systems. Quality parameters are related with *stable* properties, that is, grain hardness, size, shape and color; *variable* properties, that is, moisture content, contamination, damage to grain and bulk density and *permanent* properties, that is, fermented and foreign smells and faults that can be rectified [8]. While there is no uniform approach in the wheat industry to estimate the degree of quality, the stable and variable properties have extensive relationships with physical characteristics and provide a primary basis to determine initial grain quality control parameters.

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Understanding the physical properties using rapid analysis methods is essential to estimate or predict with an acceptable level of certainty grain quality according to postharvest handling, storage and food processing. The market value of wheat grain is determined by various factors such as kernel morphology, texture, test weight and the shape of germ, crease and brush [9]. El Fawal et al. [10] cited that the physical quality of the kernel plays an important role for identifying the engineering characteristics of cereal crop grains, while Dziki and Laskowski [11] discuss that studies concerning the relationships between wheat kernel physical properties and milling properties have been carried out since the beginning of the cereal processing industry.

There are five main categories of data on physical properties of agro-food materials, which responds to physical treatments involving mechanical, thermal, electrical, optical and electromagnetic processes [4, 12]. For granular materials as cereal grains, the physical properties are classified by category or purpose, which in turn include more specific quality assessments; some of the major criteria applied to determine wheat kernel physical quality attributes included appearance and damage, size and shape and weight volume and density test, to cited a few of them.

Broadly, the geometric properties such as size and shape are one of most important physical properties considered during the separation and cleaning of kernels [13]. Study of physical properties in wheat kernels used are more complicated due to the inherent relationship among categories, that is, weight volume and density values are dependent on the shape, size and degree of kernel damage. Besides, physical properties of cereal grains are intrinsically linked to its moisture content level.

### 2.1. Appearance and damage

Appearance is a valuable physical characteristic for selective classification and rating for subsequent handling and processing. It is considered the single most important factor that determines the economic value of a certain lot of grains. A number of grading factors adversely affect the appearance of cereal grains [14]. For purposes of inspection and grading, wheat kernels are considered damaged if the damage is distinctly apparent, therefore recognized as harmful for commercial purposes [15]. More specifically, U.S. Wheat Associates [16] suggested that wheat kernels are damaged if badly ground, weathered, frosted or present heat, insect, mold or sprout damages. The inspection of grain appearance and damaged kernels are recognized to greatly influence wheat kernels quality, because sound kernels could be stored for longer periods of time or processed into better quality intermediate and finished products. A portion of a particular wheat sample is thoroughly examined in order to detect different sorts of damage by physical or biological factors. The most common tests related to quality are presented below.

#### *2.1.1. Color*

Color is one of the first and most relevant characteristics related to grain quality. Wheat kernel color depends on the species and other factors and is mainly dictated to chemical components

presents in the ripe seed coat. Bechtel et al. [17] mentioned that color of wheat caryopses varies from light buff or yellow to red-brown according to the absence or presence of red pigmentation in this layer. Wheat is commonly classed according to color as red or white. Sometimes the perception of grain color is affected by the texture of the endosperm since the soft portion presents air-starch and air-protein interfaces that impart the chalky or dull appearance. On the other hand, the absence of voids or microscopic spaces in the endosperm of hard wheat gives a glassy or vitreous appearance resulting in trends of the red coloration present in the seed coat.

Other factors may affect the natural color of kernels such as mold-infestation in the field or during storage, heat or frost damage and other caused by phytopathogens. Shahin et al. [18] reported that mildew growth on wheat kernels reduces grain quality due to the characteristic gray discoloration which negatively impacts color of refined flours.

Computer-aided image analysis has many applications in agricultural sciences. For assessing grain quality this technique is able to objectively determine shape and color. However, problems are encountered in practical applications of these methods because they rarely correlate with the examined attributes and therefore a multivariate analysis is required. Principal component analysis (PCA)—a variant of the above method—is used when a high number of variables have to be reduced to several components [19]. Image analysis is performed by algorithms that use different color, texture and shape features as input parameters [20].

Actually, interest in white wheat kernels by both the milling and baking industries is increasing, because refined and mainly whole grain flours milled are preferred for different products and applications. Hard white wheat has been reported to have distinct advantages over the conventional hard red counterpart. These advantages include higher flour extraction rate and lighter colored end products [21].

#### *2.1.2. Insect damage*

One of the most relevant wheat kernel quality parameters is insect damage. This particular assessment is considered one of the most critical degrading factors [22], because it relates to flour yield and color and increases the amounts of insect fragments present in flours and processed products which are considered as one of the most important quality factors related to food sanitation. Presence of insects induces losses in quantity and quality by insect consumption, grain weight loss, contamination (toxicity) with excrement, bodily fragments and chemical secretions that disfavor flour flavor and odor. In addition, insects increase heat and kernel moisture due to their metabolic activity. The potent enzymes produced by insects and by the grain respiration system are known to negatively affect milling and baking qualities [23, 24].

The main group of insects that cause serious damage in cereal grains includes beetles such as *Sitophilus granarius, Tribolium castaneum, Trogoderma granarium, Tenebroides mauritanicus* and *Rhyzopertha dominica*. Primary pests infest sound grain whereas secondary pests can attack only broken or cracked grains or milled products [25]. A large portion of the insect's life is spent inside the kernels; therefore, their detection is extremely difficult. Fortunately, the industry has adopted new techniques for identification of insect damage inside grains using Near

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Infrared (NIR) spectroscopy [6]. Needless to say, the internal infestation degrades the quality and value of grains [24].

Pest control begins with a preventive control and disinfection of empty storage containers and storing grain in pest-free conditions. Different improved methods include hermetically closed containers, heating or cooling, ionizing radiation, light and pheromone traps and storage under controlled atmospheres [25]. Postharvest control is essential in many countries and the traditional treatment is chemical fumigation [22]. Unfortunately, the use of synthetic insecticides has problems such as their persistent toxicity, development of resistance in insect populations and other adverse environmental impacts [26, 27].

#### *2.1.3. Sprouted kernels*

Sprouted kernels are easily detected by visual observation such as kernel swelling, growth in the germ area, discoloration of the germ, the split of the bran over the germ and mainly by the detection of the emergence of the radicle or rootlets and coleoptile or acrospires [6, 28]. Sprouting can occur both, in the field or during storage when kernels absorb moisture and are exposed to appropriate temperature conditions [5]. This germination process involves several biochemical changes in the endosperm of the kernel, such as synthesis and release of amylolytic, lipolytic, fibrolitic and proteolytic enzymes that degrade starch, oil, fiber and proteins, respectively [5, 28]. The presence of sprouted damage level usually is determined quantitatively by measuring the amount of $\alpha$-amylase using the Falling Number (FN) [29] or by determining peak viscosity with the Rapid Visco Analyser (RVA) [28]. In the industry, different unfavorable technical factors are associated with the use of sprouted kernels such as reducing milling yield and lowering flour quality, sticky doughs and significant effects in the baking quality of bread wheat [6, 28]. Sprouted kernels usually yield darker colors due to the presence of significant amounts of reducing sugars and degraded proteins that upon heating form higher amounts of Maillard reaction products [5].

#### *2.1.4. Heat damage*

Heat damage in kernels is mainly attributed to two major reasons: first, faulty storage of damp grain and second, as a consequence of an inadequate (high) artificial drying [6]. Grains stored at high moisture-induced elevated respiration rates, consequently metabolic activation of the grain, causing heat, mold growth and possible insect infestation [5]. A darker color in the grain is indicative of heat damage, however sometimes this type of damage is not usually visible and requires testing and it is necessary in most cases to cut the kernels to determine if the color of the cross-section is reddish-brown. Wheat doughs produced from heat-damaged flours are sticky due to the partial degradation of starch granules and gluten proteins by amylases and proteases, respectively. In addition, the bread crumb is darker due to the higher reducing sugars and hydrolyzed proteins that promote browning reactions upon baking. Therefore, the gluten protein and rheological testing are required to evaluate effects of this defect. Heat damaged seeds are usually associated to the loss of viability [5, 6].

#### *2.1.5. Frost damage*

The degree of tolerance shown by wheat kernels on the field to low or freezing temperatures depends largely on the stage of development at which the stress occurs. Wheat is most susceptible to frost damage at flowering, being particularly harmful when it occurs during grain filling [6, 30]. Frosted grains are creased along the long axis and creases are regular or uniform, unlike grains with moisture stress in which this anomaly is not uniform. Sometimes, frosted grains will have a blue-gray appearance [30] and usually lower 1000-kernel weight because they did not fill properly [5]. The premature death of the kernel results in less polymeric protein synthesis and consequently their gluten functionality is compromised affecting negatively baking performance [6].

#### *2.1.6. Mold damage*

Wheat grains represent an important substrate for the development of different types of molds which affect adversely the grain quality. Serna-Saldivar [5] specifies that the genus *Fusarium, Alternaria* and *Penicillum* are the fungus most frequently isolated from wheat infested negatively grains. Growth of fungi in stored wheat affects yield and relevant quality factors such as discoloration, germination, free fatty acid value, falling number and dough rheological properties [31], because molds produce important enzymes such as amylases, proteases and lipases.

Presence of mold in the grain also causes production of undesirable odors and grains infested with *Fusarium* and/or *Aspergillus* will probably contain significant amounts of mycotoxins (secondary metabolites produced by the fungi) that endanger human and animal health [5]. Fungal species that attack cereal grains can be classified into two groups: field fungi (less aggressive) and storage fungi. Temperatures between 30 and 35°C and moisture content in the grain above 15% are the conditions for the optimal development of fungi in storage [31].

### 2.2. Shape and size

#### *2.2.1. Grain morphology*

The wheat grain or kernel—botanically named caryopsis—is a particular dry fruit and indehiscent consisting of three main regions are easily recognizable: pericarp, endosperm and germ (which includes the embryo) [25]. The geometric properties such as size and shape are one of most important physical properties considered during cereal grains processing, due to its morphology can be associated with quality parameters. Grains are considered like spheres or ellipse because of their irregular shapes [13].

Wheat shape can be described as round (approaching spheroid). Morphologically, Evers and Millar [32] describe that wheat kernel presented a marked crease, a re-entrant region on the ventral side, extending along the grain's entire length and deepest in the middle; however, variation occurs in the thickness, large and width of the grain. The shape of the groove is a characteristic feature of some species and cultivars.

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*2.2.2. Axial dimensions*

In a wheat kernel, three principal dimensions are commonly measured: length (*L*), width (*W*) and thickness (*T*) (**Figure 1**), which typically are determined using a micrometer or caliper and reported in millimeters. The principal axial dimensions of grains are useful in selecting sieve separators and for the calculation of extraction rate during size reduction [33]. These measurements can also be used to calculate volume of kernels, which are important during modeling of grain drying, aeration, heating and cooling. The effects of size and surface area on drying rates of particulate materials can also be characterized by using the surface to volume ratio [34]. The kernels at the spikelet had different individual mass. The dimensions of the wheat kernels within a plant varied significantly and the development rates and dimensions of kernels are different [35].

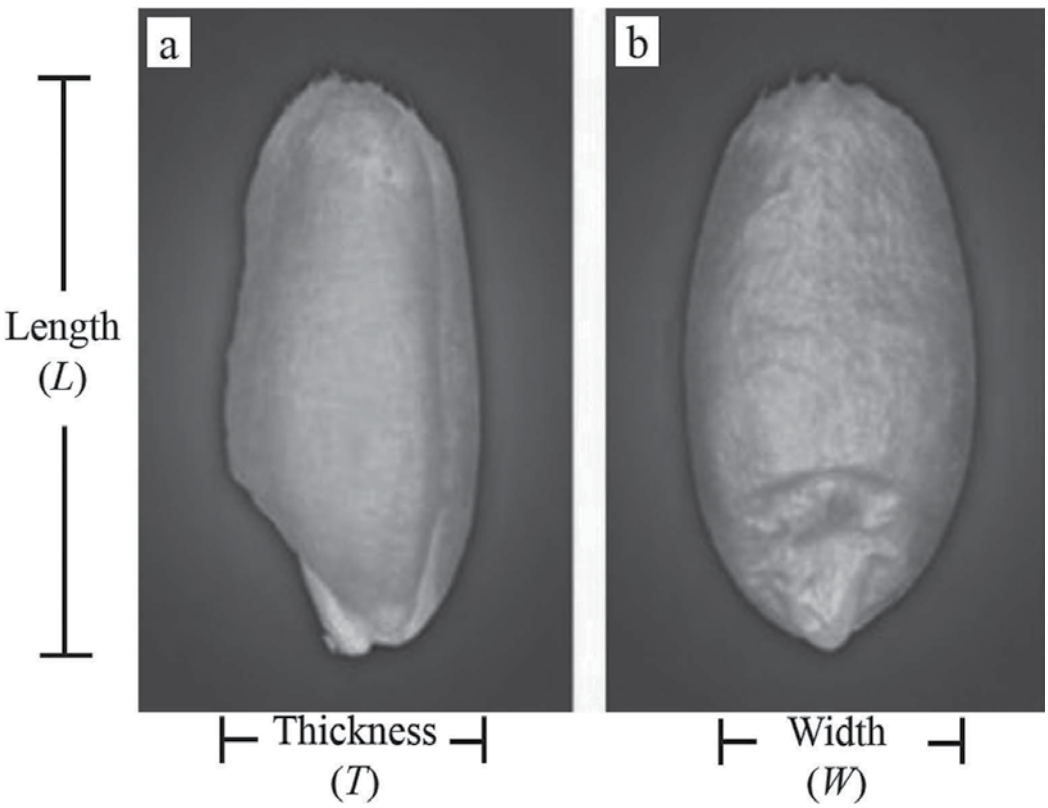


**Figure 1.** Axial dimensions in a wheat kernel. (a) Length (*L*) and thickness (*T*); (b) width (*W*).

Small kernels are considered to have less potential flour yield and inferior milling properties. Gaines et al. [36] discussed that soft wheat cultivars differed in their average kernel size and in the size distribution of their kernels. However, they found that kernel characteristics, milling performance and soft wheat end-use qualities were not influenced by kernel size, except that small kernels tended to be softer. The milling and baking properties of smaller kernels were

not found to be inferior to larger counterparts, but were equivalents. Aversely, Morgan et al. [37] reported that "in general, as kernel size declines, flour yield and flour refinement (ash and color) are adversely affected", but agrees that small kernels were softer than large kernels.

Dholakia et al. [9] proposed an interesting factor-form-density (FFD) for phenotypic measurement on wheat kernels from described the differences in the grain structure (density) and the deviation from the cylindrical form, which was compiled as:

$$\text{FFD} = \frac{\text{Kernel weight}}{\text{Kernel length} \times \text{kernel width}}. \tag{1}$$

*2.2.3. Sphericity*

Sphericity ($\phi$) expresses the characteristic shape of a solid object relative to that of a sphere of the same volume. The longest diameter (major) and shortest diameter (minor) will adequately describe the size of an ellipsoidal object such as the wheat kernel [4]. Bayram [38] suggested that the determination of the sphericity is usually difficult and not practical, due to irregular shape of the granular material and it is the calculation of the exact volume and surface area, involving multiple length measurements. In this sense, this author proposed a novel and easily model to determine the sphericity of granular materials, following the next expression:

$$\phi_s = \frac{\Sigma(D_{i-}\overline{D})^2}{(\overline{D}N)^2}, \tag{2}$$

where $\phi_s$ = sphericity value, $D_i$ = any measured dimension, $\overline{D}$ = average dimension or equivalent diameter and $N$ = number of measurements. Increase in the $N$ increases the accuracy. In Eq. (2), when $\phi_s$ for a sphere is 0, that is, an increase in $\phi_s$ value using Eq. (2) shows the deviation from the sphericity.

*2.2.4. Roundness*

Mohsenin [39] defined roundness as the measure of the sharpness of the corners of a solid, whereas Curray [40] proposed the next equations for estimating roundness under different conditions of geometry and application:

$$\text{Roundness} = \frac{A_p}{A_c}, \tag{3}$$

where $A_p$ = largest projected area of object in natural rest position and $A_c$ = area of smallest circumscribing circle. The object area is obtained using the next equation:

$$\text{Roundness} = \frac{\sum r}{NR}, \tag{4}$$

where $r$ = radios of curvature as defined in **Figure 2**, $R$ = radius of the maximum inscribe circle, $N$ = total numbers of corners summed in numerator.

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$$\text{Roundness} = \frac{r}{R}, \tag{5}$$

where $R$ in this case is the mean radius of the object and $r$ is the radius of curvature of the sharpest corner. The objection to this method is that the radius of curvature of a single corner determines the roundness or flatness (**Figure 2**).

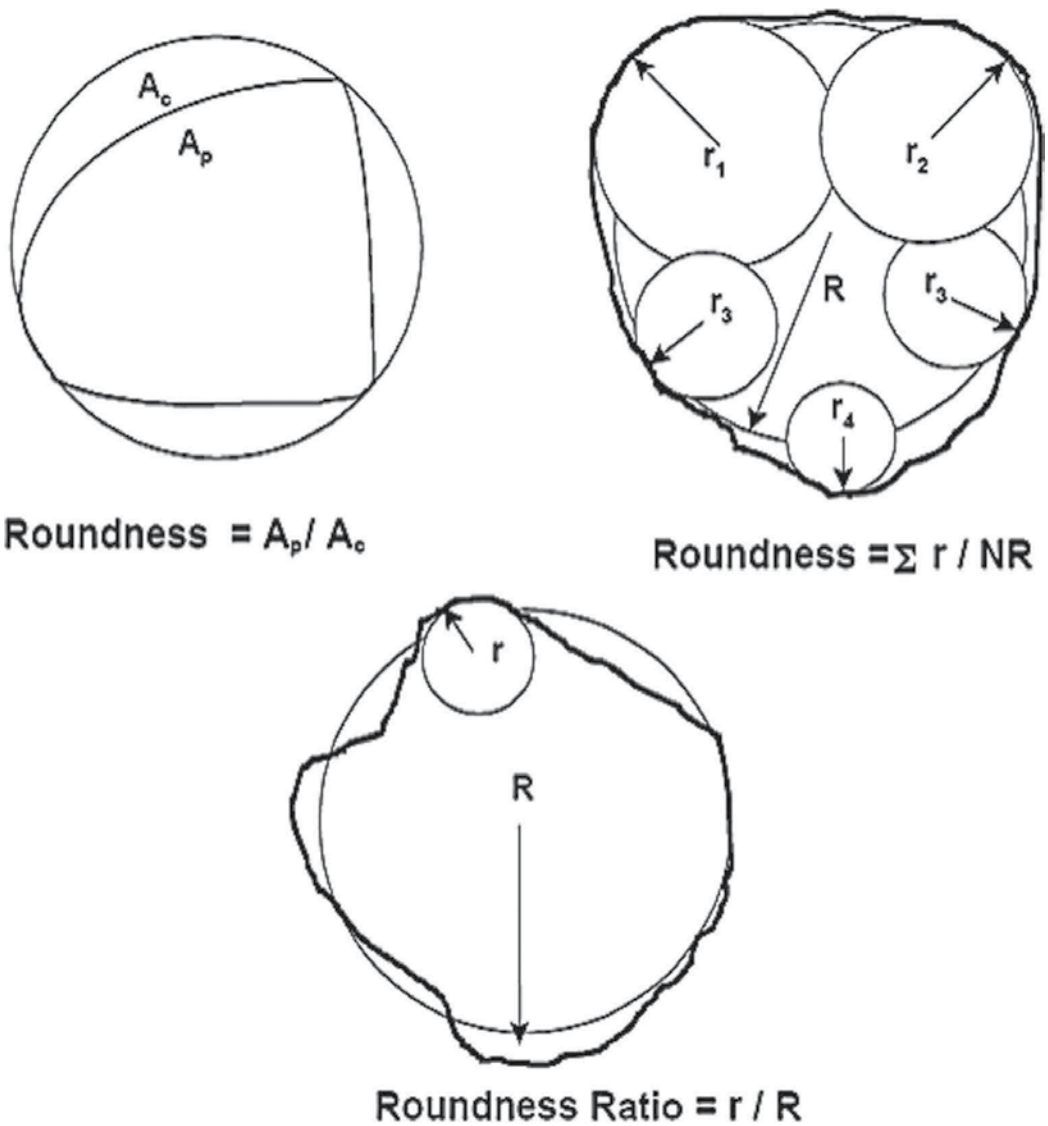


**Figure 2.** Roundness as defined by geologists to describe shape of grains and pebbles (adapted from Mohsenin, 1978).

Higher values of sphericity and roundness indicate that the shape of the kernel is closer being spherical. It is important to know sphericity and roundness—for example, before handling or dryer process—so that this efficiency increases.

It is important noted that the main influence is not the shape and size *per se*, but the degree of variation in these attributes within a sample [17]. Wheat kernel size, like most of the traits of biological interest and agricultural importance, is a complex character and is suggested to be quantitative in nature, although kernel size and shape have emerged as important breeding objectives [9].

### 2.3. Volume weight and density

Unit volume weight indicates the density and compactness for a given volume of grain; a minimum test weight requirement is generally one of the primary specifications used in wheat grading and classification [6]. Test weight or density in wheat kernels is a physical quality characteristic considered mainly by flour and semolina millers. In general, high weight (accordance to wheat class) may indicate a grain sample healthy and optimum appearance, whereas low weight can occur as result of one or more adverse events such as insect damage, heat stress or delayed harvesting [41].

Bulk density and true density can be useful for storage facilities, because affect the rate of heat and mass transfer of moisture during aeration and drying process [33]. In addition of these two parameters, porosity can be useful in sizing grain hoppers and storage facilities [42]. In the grain industry, the ratio weight-volume commonly is report in bushels units or kg/hL (100 L) [4]. Test weight per bushel is the weight of the grain required to fill a level Winchester bushel measure 2150.42 $in^3$ (35.24 L) capacity [16]. The conversion factors of pounds per Winchester bushel and pounds per imperial bushel (2219.36 $in^3$) to kg/hL are 1.297 and 1.247, respectively. This test is related to the true grain density, which is affected by grain condition, grain texture and protein content. Wheat kernel sample affected by insect attack, molds or any other damage had a lower test weight when compared with a healthy sample [5].

#### *2.3.1. Bulk density*

Space occupying by amount of material per volume unit is call density ($\rho$) and is expressed in units of mass per unit volume. True density ($\rho_t$) is defined as the ratio of the volume of particles and can be determined using the water [43] or by gas [33] displacement methods which determine the volume of the sample. Unfortunately simple techniques as water displacement can result in errors especially if the water penetrates into the kernel [39]. In kernel volume ($V$) exists interstitial air spaces with different values of particle density and bulk density. Particle density is the mass divided by the volume of the particle alone. The mass of a group of individual particles divided by the space occupied by the entire mass (volume) including the air space is bulk density ($\rho_b$). This could be calculated from the following relation [42]:

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$$\rho_b = \frac{W_s}{V_s}, \tag{6}$$

where the $\rho_b$ = bulk density (kg/m$^3$), $W_s$ = weight of the sample (kg) and $V_s$ = volume occupied by the sample (m$^3$).

The irregular shape and porous nature of agricultural materials present difficult problems in volume and density measurements. The density of a material has a significant effect on its mechanical characteristics [39]. According to Molenda and Horabik [44], the determination of the bulk density is based on measurement of the mass of a granular material poured freely into a cylindrical container of constant volume, typically 0.25 or 1 dm$^3$. Grain density usually varies within a relatively broad range, depending on the species and cultivar, manner of silo, height of deposit, degree of contamination of the grain and other factors. It is recommended to estimate the density of a granular material in a silo by assuming an average density increase of 6% with relation to the density value determined from the mass of 1 hL.

*2.3.2. Porosity*

Porosity ($\varepsilon$) is the percentage of air between the particles compared to a unit volume of particles [4] and can be calculated from bulk and true density values, using the following relationship proposed by [39]:

$$\varepsilon = \frac{\rho_t - \rho_b}{\rho_b} \times 100, \tag{7}$$

where $\varepsilon$ = porosity (%), $\rho_t$ = true density (kg/m$^3$), $\rho_b$ = bulk density (kg/m$^3$).

Wheat endosperm is mostly composed of starch granules, protein matrix and pores or air voids. Starch granules are bound to one other by the continuous protein matrix. Topin et al. [45] performed an interesting study in which it was determined that two parameters played a major role in the fracture behavior of the wheat endosperm: the matrix volume fraction $\rho^m$ and the particle-matrix adhesion $\sigma^{pm}$. The value $\rho^m$ ranged from 0.04 to 0.2. At $\rho^m$ = 0.2, the whole interstitial space is filled with the protein matrix, corresponding to zero porosity. In this sense, these authors suggested that the crack of the endosperm depends more on protein content than on the starch-granule adherence, because "the stress inhomogeneities, which are responsible for the stress concentration factor, are more sensitive to the porosity than to adherence among the constituents".

In grains, low porosity will have greater resistance to water vapor escape during the drying process, which may lead to higher power to drive the aeration fans [33]. The porosity of the bulk is the ratio of the volume of the internal pores within the kernels to its bulk volume [43]. Porosity values in wheat kernels increased slightly as the moisture content increased [42].

*2.3.3. Thousand kernel weight*

Thousand kernel weight (TKW) measures the mass of the wheat kernel and is an essential parameter for the selection of cultivars with the best physical and physiological seed quality. Generally, higher TKW values are positively related to potential flour extraction or yield [46], because this property is closely related to grain size and proportion of endosperm to germ and pericarp tissues [5]. Wheat breeders and flour millers employ this method as a complement to test weight to better describe wheat kernel composition and potential flour extraction [16]. TKW could be used as an index of wheat milling value and is a good parameter for evaluation of kernels as seed material [11]. When the grain is undamaged may be expected high test weight, due to a greater endosperm to bran ratio [29].

Finally, it is important to highlight that grain moisture content has deep influence on the physical properties, particularly those related with volumetric grain weight and density in bulk as it modifies surface properties of seed-coat as well as the properties of kernel endosperm. Several studies [34, 42, 43, 47] have reported the effect of moisture content on different wheat kernel physical properties and concluded that increasing of moisture content level increased axial dimensions, thousand kernel weight, porosity, kernel volume and sphericity, while bulk density decreased. Higher grain moisture content results in an increase in susceptibility of grains to deformation, thus physical properties of cereal grains vary as a function of moisture content [13, 44].

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## Nomenclature

| | |
|---|---|
| $L$ | Length (mm) |
| $W$ | Width (mm) |
| $T$ | Thickness (mm) |
| FFD | Factor-form-density |
| $\phi_s$ | Sphericity (%) |
| $D_i$ | any measured dimension (mm) |
| $\overline{D}$ | Average dimension or equivalent diameter (mm) |
| $N$ | Number of measurements (Eq. (2)) |
| | Total numbers of corners (Eq. (4)) |
| $A_p$ | Largest projected area ($mm^2$) |
| $A_c$ | Area of smallest circumscribing circle ($mm^2$) |
| $\sum r$ | Sum radios of curvature |
| $R$ | Radius of the maximum inscribe circle (mm) (Eq. (4)) |
| | Mean radius of the object (mm) (Eq. (5)) |

| | |
|---|---|
| $r$ | radius of curvature of the sharpest corner (mm) |
| $\rho$ | Density (kg/m$^3$) |
| $\rho_t$ | True density (kg/m$^3$) |
| $V$ | Kernel volume (mm$^3$) |
| $\rho_b$ | Bulk density (kg/m$^3$) |
| $W_s$ | Weight of the sample (kg) |
| $V_s$ | Volume occupied by the sample (m$^3$) |
| $\varepsilon$ | Porosity (%) |
| $\rho^m$ | Matrix volume fraction |
| $\sigma^{pm}$ | Particle-matrix adhesion |
| TKW | Thousand kernel weight |

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Chapter 14

# Use of Wheat Distiller Grains in Ruminant Diets

Wenzhu Yang and Yanling Li

Additional information is available at the end of the chapter

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**Abstract**

Wheat grain is commonly used to produce ethanol in Canada and Europe. During ethanol production processing, starch in the grain is fermented almost completely, and the remaining protein, fibre, fat, minerals and vitamins increase approximately 3-fold in concentration compared to the original grain. By-product derived from the ethanol production is named distiller grain and primarily used in feeding livestock animals. Wheat-based distiller grain is high in energy, protein and fibre. These properties give wheat distiller grain unique feeding opportunities for various classes of livestock as both energy and protein supplements as well as fibre source. This chapter summarizes some recent research findings published in peer reviewed and extension chapter on the use of wheat distiller grain in ruminant diets. Substantial variation in chemical composition exists among the distiller grain samples, which are mainly influenced by inherent original grain and technology used in ethanol plant. Wheat distiller grain can be used to partly replace grain or forage portion at moderate levels to meet energy and fibre requirements of cattle. A manure management plan needs to be developed that considers the fact that inclusion of wheat distiller grain in the diet will dramatically increase the nitrogen and phosphorus content in manure.

**Keywords:** wheat grain, distiller grain, nutrient content, ruminants, dairy and beef cattle, digestibility, feed efficiency, growth performance, milk production, manure management

## 1. Introduction

Traditionally, wheat grain is primarily used for human food consumption; the milling of wheat produces flour for human use and appreciable quantities of by-products for animal feeds. On average, wheat grain contains 65% starch, 15% protein, 14% fibre, 2.2% oil and 10% moisture [1]. With expansion of fuel ethanol production in North America and other places

in the world during the last decade wheat grain has been used as second feedstock after corn for ethanol production due to its high starch content. Many different classes and types of wheat can be used for ethanol production. In general, soft wheats such as soft white and soft red classes are preferred to hard wheats because they contain higher starch content. Varieties with higher protein are less desirable, but may still be used when blended with one or more high starch varieties.

Increase of fuel ethanol production has resulted in a significant increase in the use of distiller grains in the diets of livestock animals, especially in ruminant feeding. Distiller grains have historically been used as a protein source for dairy cattle. Whereas, increased supply and reduced cost make it also a source of energy to replace grain. The distiller grain has comparable energy value to its original grain, high quality protein and high fibre content but highly digestible which is suitable for ruminant feed but not suitable for monogastric animals or poultry because of high fibre content. Wheat distiller grain is the major by-product of ethanol production when wheat grain is used as a substrate for ethanol production. In the last decade, research has documented the variation in chemical composition of wheat distiller grain, and its feed value as protein, energy or fibre source for dairy and beef cattle as well as small ruminant animals. Studies have frequently focused on comparing the feed value of wheat distiller grain to corn distiller grain and characterizing the impact of inclusion of these by-products on nitrogen and phosphorus excretion in manure. To our knowledge, there is no review article that has addressed these research findings, even though several review articles on the use of corn distiller grain in animal production and one book chapter on use of wheat distiller grain in pigs and poultry have been published [2, 3]. The objective of this chapter is to describe some recently developed knowledge and application of wheat distiller grain in ruminant animal diets.

## 2. Production of distiller grain in ethanol plant

There are two main distillery processes, dry-milling and wet-milling distillery. The dry-milling process is the main process for producing ethanol [4]. The dry-milling process includes primarily the follow steps: grinding or milling, liquefaction, saccharification, fermentation and distillation [5]. The grain is ground to produce bran-free flour, and then mixed with water and enzymes (amylases) to produce a mash (liquefaction). The saccharification is conducted by adding enzymes to the mash to transform starch into dextrose. After saccharification, yeast is added to start the fermentation process to produce a 'beer' and $CO_2$. The beer is separated through a continuous distillation column to yield alcohol [5]. The remaining material is called whole stillage and consists of all the components of the original grain (except the starch), yeast and added water. The whole stillage is centrifuged to produce wet distiller grain (solid fraction) and thin stillage (liquid fraction). The wet distiller grain contains 30–35% dry matter, while thin stillage has only 5–7% solids. The thin stillage is concentrated through evaporation into condensed distiller solubles, which are mixed with wet distiller grain and dried to become dried distiller grains with solubles, which are the most

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produced co-products from bioethanol plants. In general, from each tonne of wheat grain, ethanol production results in approximately 365 l of ethanol, 290 kg of $CO_2$ and 290 kg of distiller grain. With continuing changes of technologies in ethanol plants, it should be noted that wheat distiller grain are still evolving, thus the composition and feed value of distiller grain are changing.

## 3. Chemical composition of distiller grain

During ethanol production process, starch is mostly converted into ethanol and it leaves all other components of grain to be condensed. Therefore, compared to the original wheat grain, starch contents of distiller grain is very low (4.3%), whereas the contents of non-fermentable components including crude protein, neutral detergent fibre, acid detergent fibre, ether extract and phosphorus are considerably higher (**Table 1**). The primary nutrient contents of wheat distiller grain are crude protein and neutral detergent fibre ranging from 30 to 45% or from 25 to 55%, respectively. The chemical composition of wheat distiller grain can vary considerably depending on numerous factors mainly including wheat source and technology used in ethanol plant (**Table 2**). Physical and chemical characteristics of grain vary with

| Item | Wheat | Wheat distiller grain |
|---|---|---|
| Organic matter | 97.9 | 94.4 |
| Starch | 60.2 | 4.3 |
| Neutral detergent fibre | 14.3 | 31.6 |
| Acid detergent fibre | 4.2 | 11.4 |
| Crude protein | 15.1 | 38.8 |
| Ether extract | 2.3 | 3.8 |
| Calcium | 0.05 | 0.12 |
| Phosphorus | 0.39 | 1.0 |

**Table 1.** Chemical composition of original wheat and wheat distiller grain (% of dry matter).

| Item | Mean | STD | Min | Max |
|---|---|---|---|---|
| Organic matter | 94.6 | 0.3 | 93.9 | 95.9 |
| Neutral detergent fibre | 30.4 | 6.5 | 22.7 | 36.5 |
| Acid detergent fibre | 12.3 | 1.6 | 9.7 | 13.7 |
| Crude protein | 37.9 | 3.3 | 30.6 | 44.7 |
| Starch | 4.2 | 1.2 | 2.1 | 6.4 |
| Crude fat | 4.0 | 0.3 | 3.7 | 4.4 |

**Table 2.** Mean values, standard error and range of nutrient content of DDGS.

grain source (variety, growing conditions, etc.), which thus directly affect the composition of distiller grain. Furthermore, the variations in nutrient content of wheat distiller grain have not only been reported from plant to plant, but also from batch to batch [6]. The differences among ethanol plants could be substantial according to the method of grain preparation with or without previous de-hulling, the fermentation conditions, drying method, duration and temperature of drying, amount of solubles added back to wet distiller grain and grinding procedure used. All these can potentially contribute to the product variability. The quantity of solubles added to wet distiller grain pre-drying is easily controlled process but it also can potentially create the variability in wheat distiller grain [6]. Solubles are high in fat (up to 34% of dry matter) and low in neutral detergent fibre, therefore, the more solubles are added to wet distiller grain, the higher the fat and the lower the neutral detergent fibre content. The heat damage is another source of variability and it occurs during the drying process. Wheat distiller grains that have undergone high processing temperature will have a reduced protein degradability in ruminants. The heat damage can be easily checked with the colour of distiller grain which varies from light yellow to dark brown. Cozannet et al. [7] measured the luminance values of 10 European wheat distiller grains and it ranged from 43 (black products) to 63 (yellow products) using a Minolta colorimeter. These authors indicated that wheat distiller grain with luminance values <50 was overheated, which will have a high incidence of Maillard reactions.

The amino acid profile of protein is an important nutrition attribute to ruminant animals. We observed that protein of wheat distiller grain had amino acid profiles partly in agreement with that of the initial grain [8]. Li et al. [8] reported that the changes in amino acid profiles from the original grain to its distiller grain did not follow the same trend as changes in the crud protein; proportion of amino acid increased for some, and decreased, or remained unchanged for others. Han and Liu [9] suggested that the amino acid from yeast source during ethanol fermentation would have important influences on amino acid profiles of distiller grain. Yeasts used for starch fermentation represent an additional protein source equivalent to about 5% of the total distiller grain protein content [10]. Theoretically, yeast cannot hydrolyse protein from grain to free amino nitrogen due to the lack of extracellular proteolytic activity [9]. Li et al. [8] discussed that the differences in amino acid composition between the original grain and its distiller grain also depends on the amino acid composition of the yeast used in ethanol fermentation. In fact, it was reported that yeast protein could contribute up to 20% of the protein in distiller grain, and that amino acid profiles of yeast protein were different from those of grain protein [9]. In addition, the level of soluble fractions added into distiller grain is another source influencing the protein content and the amino acid profile. Cozannet et al. [7] reported that although amino acid profile is quite comparable in wheat and wheat distiller grain, lysine and arginine are lower for wheat distiller grain, and the lysine and arginine levels in the crude protein of wheat distiller grain are highly variable, even in light-coloured products: 1.7–3.0% and 3.7–4.6%, respectively.

The considerable variability in chemical composition of wheat distiller grain is one of the main issues challenging in feed formulation for precisely feed livestock animals. Hence, in practice, a determination of nutrient contents of wheat distiller grain from each delivery is recommended if the nutrient profiles are not provided.

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## 4. Nutrition value of distiller grain

The values of energy and protein are two key nutrient components for a feed ingredient fed to ruminant animal, which ultimately determine whether the nutrient requirement by animal is met. Wheat distiller grain is a good source of energy and protein for ruminants.

### 4.1. Energy value

Wheat DDGS is commonly used as energy source owing to its highly digestible fibre and moderate level of fat. The energy value of a feed depends primarily on its digestibility in the digestive tract of animal. The digestibility of wheat distiller grain in the rumen of beef cattle was 66.5 and 54.8% for dry matter and neutral detergent fibre, which was lower that of dry matter (82.4%) and fibre (67.9%) of original wheat [11]. The lower ruminal digestibility of distiller grain versus its original grain is due to lack starch in the distiller grain and grain starch is highly fermentable in the rumen. It appears that the digestibility of wheat distiller grain varies between studies [11, 12] which could be due to the variation in chemical composition of distiller grain, animal production level or physiology status, etc.; therefore, the energy value of distiller grain varies from study to study. Beliveau and McKinnon [13] did not find the difference in finishing performance of beef cattle fed diets containing increasing replacement of barley grain with wheat distiller grain up to 23% of the dietary dry matter. These authors concluded that wheat distiller grain had similar value of net energy for maintenance (NEm) and net energy for gain (NEg) to barley grain (i.e. 2.00–2.06 Mcal/kg NEm and 1.34–1.40 Mcal/kg NEg, respectively). However, in the study by Gibb et al. [14], increasing substitution of wheat distiller grain for barley grain from 0, 20, 40 to 60% in diets fed beef cattle linearly increased feed consumption but linearly reduced digestibility of dry matter from 76.4 to 68.9%, as a result, dietary energy content (NEg, MCal/kg) declined linearly from 1.15, 1.14, 1.09 to 1.07, and the NEg of wheat distiller grain decreased from 1.36, 1.27 to 1.21%. Although fibre from wheat distiller grain is considered to be highly digestible [6], the lowered digestibility of diets with increasing levels of distiller grain may have resulted from increased passage rate of feed from the rumen and leave the feeds stay shorter in the rumen, and thus not favourable for fibre digestion [15].

### 4.2. Protein value

The protein of wheat distiller grain has lower ruminal degradability (49%) than that of wheat grain (79%), and similar to that of corn distiller grain (47%), but it has more desirable amino acid profile as it contains more arginine, lysine, threonine and valine [8]. Li et al. [8] found that the decrease in ruminal degradability of distiller grain protein versus its original grain resulted from the reduced degradation rate. The distiller grain usually has higher rumen undegradable protein compared to the protein from the original grain [12], and consequently, distiller grain has been historically fed to cattle as a rumen undegradable protein source. Highly degradable feed protein is often not favourably received by the ruminant nutritionist since the highly degradable protein is rapidly broken down by the microbial population in the rumen and the released ammonia nitrogen is absorbed through the rumen wall, converted to urea in the

liver and excreted in the urine. This metabolic pathway has not only an energy cost but also it presents an environmental issue. The urinary urea nitrogen is rapidly hydrolysed to ammonia upon excretion and can contribute to nitrous oxide emissions. In contrast, ruminal undegradable protein resists fermentation in the rumen and a proportion of the amino acids arising from this protein can be directly absorbed in the small intestine. The rumen undegradable protein of wheat distiller grain could vary substantially and range from 38.3 to 71.7% [6, 8]. The variation in rumen undegradable protein content is primarily caused by differences in heat treatment during the drying of distiller grain. Additionally, the inherent characteristics of the protein fractions within the original grain source [6], and the milling process such as conventional versus fractionation [8] also cause this variation. The effective protein degradability of wheat distiller grain (54%) was found to be similar to that of triticale distiller grain (51%) and higher than that of barley distiller grain (49%) but normally higher than corn distiller grain (47.1%) [8, 16]. Therefore, wheat distiller grain can be used as a good source of degradable and undegradable protein in the rumen [17].

The lower rumen degradability of distiller grain protein versus its original grain appears due to protein molecular changes. Yu et al. [18] reported that the grain had higher ratio of protein amide I to II in the protein structure than its distiller grain produced from bioethanol processing (grain vs. distiller grain; 4.58 vs. 2.84). Protein vibration of amide I and II depends on the protein secondary structure of the backbone and is therefore most commonly used for secondary-structure analysis [19]. It was also reported a positive correlation of protein amide I to amide II ratio with the soluble fraction ($r$ = 0.94) or potentially degradable fraction ($r$ = 0.99), but a negative correlation with the undegradable fraction ($r$ = –0.99) [19]. It suggests that lower protein amide I to amide II ratio was associated with a higher undegradable protein in distiller grain. The ethanol production process during fermentation and drying change grain protein molecular structure, and may affect the protein degradation in the rumen.

Ruminal undegradable protein of wheat distiller grain has a good intestinal digestibility and is one of the best sources of metabolizable protein. However, differences may exist between distiller grain from wheat and corn. Li et al. [8] reported that protein quality (i.e. amino acid profile) of the rumen undegradable protein in wheat distiller grain and corn distiller grain was slightly lower compared with that in the original grains. Nuez-Ortin et al. [12] reported that a wheat and corn blend distiller grain was a better source of truly digested and absorbed protein in the small intestine than wheat distiller grain and corn distiller grain alone. The rumen undegradable protein fraction in corn distiller grain may provide similar amounts of intestinally absorbable total amino acid, but greater absorbable essential amino acid, than the undegradable protein in wheat distiller grain.

## 5. Feeding distiller grain for beef cattle

Distiller grain from corn grain fermentation is historically fed to dairy cattle mainly as rumen by-pass protein, and rarely used as feed ingredient in beef production because of limited amount of production and higher price compared to other available feed sources. The inclusion of distiller grain in beef cattle diets, especially feeding wheat-based distiller grain in

Canada has become a common practice only last decade because of increased availability of distiller grain and reduced price along with increased grain cost. Typical beef production in North America includes three different operations: cows and calf production, growing cattle and finishing cattle.

### 5.1. Distiller grain for beef cows

Cow-calf operations are widespread throughout beef-producing countries, and the goal of a cow-calf operation is to produce young beef cattle, which are usually sold. Typically lower-quality forages high in fibre and low in protein are the basis for the beef cows and replacement heifers operations. Cow-calf operations generally raise their stock primarily on pasture and other forms of roughage rather than grain feeds. The cattle require protein, energy and phosphorus supplementation at this feeding system. Because most forage protein is degraded in the rumen, the wheat distiller grain can be an acceptable supplement in an extending grazing system for beef cows. The nutritive profile of wheat distiller grain makes it attractive in forage-based production setting distiller grain as an excellent source of total digestible nutrient containing digestible fibre and relatively high fat. Distiller grain is also high in crude protein with high rumen by-pass protein. Distiller grain is also a good source of phosphorus (0.6%), a nutrient commonly deficient in forage-based diets. The study using distiller grain in cows and calf operation is lacking. Van De Kerckhove et al. [20] reported that wheat distiller grain was an alternative to barley grain as an energy and protein supplement in a chaff and hay grazing system supplemented with rolled barley, wheat distiller grain fed at levels to meet the requirements of total digestible nutrients by cows. Beef cows require maintenance levels of energy and protein, which increase as the animals get close to calving time. The energy from wheat distiller grain is largely in the form of digestible fibre and fat. Therefore, distiller grain fits well as energy supplement in forage-based diet.

### 5.2. Distiller grain for growing cattle

The growing step operation is the process of growing cattle at moderate rates of gain with the goal to develop frame and muscle, and to minimize fat deposition. The daily gains target from 0.9 to 1.2 kg, depending on the type of cattle being fed. Cattle are typically fed either in a feedlot or on-farm by providing a forage-based diet supplemented with protein and energy source. It is evident that growing cattle fed under dry lots or on pasture have the potential to use wheat distiller grain as a supplemental source of energy and protein. Beliveau and Mckinnon [13] conducted a growing study using beef steers fed diets with increasing rates of replacement of barley grain with wheat distiller grain from 0, 8, 16, 24 to 32% (dietary dry matter) and observed a linear improvement of feed consumption and growth performance. Similarly, in another study from the same team, McKinnon and Walker [21] observed a linear improvement of average daily gain and feed efficiency with increasing replacement of wheat distiller grain for barley grain. However, other studies [14, 22] did not find evident beneficial effects of including wheat distiller grain in place of barley grain in growing cattle diets. The discrepancy between studies could be due to dietary factors such as levels and quality of forage used in diets, proportion of distiller grain included and its quality.

Growing beef cattle require protein in the form of amino acids to maximize growth rate. One of the most effective and practical methods of improving feed efficiency, growth performance and reducing nitrogen excretion in beef cattle operations is to optimize protein formulation in the diet of growing cattle. Previous works show the necessity of protein supplements to maintain optimum growth rate in growing cattle diets, when these diets are based on barley or corn grain. Wheat distiller grain has not only the similar content of energy for cattle growth because of highly digestible fibre and relative high fat, but also has high protein content either in total or in the form of rumen by-pass. We previously conducted a growing study using beef steers to compare protein source with canola meal, wheat distiller grain, corn distiller grain or fractionated corn distiller grain, and found an improvement of averaged daily gain and feed efficiency over the control group (no protein supplement) [23]. However, steers fed corn distiller grain performed slightly better than that of steers fed wheat distiller grain, likely because of higher fat in corn than wheat distiller grain. McKinnon and Walker [21] reported that growing steers fed wheat distiller grain at 25 or 50% of dietary dry matter gained faster and were more efficient than steers fed a barley grain diet. In contrast, no benefit was reported when wheat distiller grain was fed at level of 17% [24] or at levels up to 40% [14] in growing diets. It appeared that when the level of wheat distiller grain is too high, dietary protein level can be exceeded to the protein requirement by animal. For example, in the study by Gibb et al. [14], including 40% of wheat distiller grain resulted in a dietary crude protein concentration up to 26%, which considerably exceeded the protein requirement of 12–14% for growing cattle. Although protein can be utilised for energy, the transamination, deamination and excretion of excess nitrogen is physiologically costly to the animals and results in an overall loss of net energy.

### 5.3. Distiller grain for finishing cattle

Following growing period, beef cattle then go into the finishing phase. Rations for finishing beef cattle are high energy rations designed to put gain on as rapidly and efficiently as possible, to lay down adequate marbling, and to maximize carcass yield within a limited time frame. Thus, the finishing diets usually consist of high grain such as barley, corn or wheat at ranging 85–95%, and 5–15% of roughage. The role of roughage in finishing diets primarily serves as fibre source to stimulate chewing activity and to maintain rumen health. Number of studies were attempted to determine the optimum inclusion rate of wheat distiller grain as energy source in finishing diets. In barley grain-based finishing diets, no protein supplement is necessary since the protein requirement is met (12%). Feed consumption was either linearly increased [14, 25], linearly decreased [26] or did not differ [13] with increasing the inclusion rate of wheat distiller grain from 10, 20, 40 to 60%; however, growth performance and feed efficiency were overall not affected with increasing the replacement of grain with wheat distiller grain. These results indicated that wheat distiller grain can be successfully incorporated to substitute a portion of grain within finishing diets with minimal or no adverse impact on cattle growth performance.

The low starch content, but high fibre content of wheat distiller grain is suggested that feeding wheat distiller grain may help reduce the ruminal acidosis and maintain rumen health. It is speculated that a possible reduction in ruminal acidosis by feeding wheat distiller grain

may reduce the requirement for roughage in finishing cattle diets [27]. By entirely replacing roughage with wheat distiller grain, we observed that steers maintained a similar ruminal pH status, but reduced feed intake and improved digestibility compared to a diet containing minimum roughage (i.e. 5%) [11]. Apparently, cattle were able to prevent a further decline in ruminal pH status by adjusting feed intake; thus, cattle fed a roughage-free diet consumed less feed to keep a similar ruminal pH status as cattle fed a standard finishing diet. Our results suggested that wheat distiller grain is less effective than barley silage for maintaining ruminal pH even though the rapidly fermented starch content of diets containing wheat distiller grain is less compared with conventional finishing diets. Based on the previous metabolism study, a growth study was conducted using finishing steers fed diets that were replaced partly for barley grain and entirely for roughage with wheat distiller grain so that up to 35% of distiller grain was incorporated in total. The results showed that final live weight, daily gain and feed efficiency were not affected by increasing levels of wheat distiller grain. Therefore, although substitution of wheat distiller grain for roughage in finishing diets may increase the incidence of ruminal acidosis, this outcome does not appear to adversely impact the performance of the cattle. Such a practice could provide an alternative to roughage source to feedlot producers when the roughage is in shortage or provide a potential saving from reducing acres to roughage production.

Carcass traits and beef quality can be significantly impacted by changing diet formulation and quality of feed ingredients. However, several studies showed that the beef quality from cattle fed wheat distiller grain is comparable with that produced using the diets without wheat distiller grain incorporation. Yang et al. [23] reported that feeding wheat distiller grain to replace a portion of barley grain and barley silage in finishing beef cattle rations had overall no impacts on carcass traits. Actually, substituting wheat distiller grain for barley silage in diets fed to growing beef cattle improved meat fatty acid profiles by increasing content of total polyunsaturated fatty acids, linoleic fatty acids and alpha-linolenic acid in beef [28]. These results suggest that replacement of barley silage with wheat distiller grain cause favourable changes in the fatty acid profile of meat such as omega-3 fatty acids in beef. Similarly, Walter et al. [25] included 40% wheat distiller grain in finishing diets and observed no adverse impact on carcass quality or sub-primal boneless boxed beef yields. Animals fed wheat distiller grain included at 20 or 40% produced backfat, yield, ribeye area and marbling scores consistent with barley-finished cattle with no change in meat quality (chemical composition, cooking time, cooking loss, tenderness, drip loss, colour) or differences in sensory tests (taste, smell, sight) [29]. The addition of 20 or 40% of wheat distiller grain to the diet improves the meat fatty acid profiles by decreasing the fatty acid isomers 10*t*-18:1 (unhealthy trans-fat isomer) and increasing the fatty acid isomer 11*t*:18:1 (health promoting isomer) [29, 30].

## 6. Feeding distiller grain for dairy cows

The co-products from brewing or wet milling corn processing that are similar to the distiller grain from ethanol plant, has been historically fed to dairy cattle as protein supplement, especially as ruminal undegradable protein source. However, with expansion of ethanol

production and consequently increasing distiller grain availability, feeding wheat distiller grain to dairy cattle has been spread recently not only as protein source but also as energy or fibre sources [31]. In fact, high-producing dairy cows are often at risk of subacute rumen acidosis, a common digestive disorder usually caused by feeding a diet containing highly fermentable carbohydrates with insufficient effective fibre to maintain rumen health [32]. Because the distiller grain contain low starch which is highly fermentable in the rumen, and high digestible fibre as well as relative high fat, it was suggested that feeding distiller grain in dairy cow diets could be potentially reduce the incidence of rumen acidosis while maintain milk production. Numbers of studies have been conducted to assess wheat distiller grain as a fibre and energy source to partly replace grain, or roughage or both. Penner et al. [33] evaluated wheat distiller grain to include 10% of wheat distiller grain in the ration showed that feeding wheat distiller grain as a forage substitute increased milk yield by 7% and milk protein content by 9%, whereas milk fat content decreased from 3.36 to 3.04% even though milk fat yield was not affected. Zhang et al. [34] reported that feeding wheat distiller grain in partial replacement of barley grain had no negative effect on dairy cow production. Feeding wheat distiller grain as a partial replacement of barley silage can improve dairy cow production, but, it may decrease chewing time, ruminal pH and milk fat concentration [35]. Overall, substitution of wheat distiller grain for part of concentrate or roughage in dairy cow diets improves milk production as a result of increase of feed consumption without negatively impacting milk fat. In contrast, feeding wheat distiller grain to partly replace roughage may reduce milk fat content due to reduction of chewing activity and rumen pH. Thus, dairy producers and nutritionists formulate dairy rations to ensure cow chewing time is sufficient to maintain rumen pH which is linked to maintaining milk fat concentrations [34].

## 7. Feeding distiller grain for small ruminants

Abundant distiller grain from ethanol production can be used as alternatives to feed grains and other premium ingredients in sheep feeding to reduce feeding costs for sheep farmers. However, most of the studies with feeding wheat distiller grain are with cattle or pigs. With our best knowledge, only one study was conducted using growing lambs fed diets containing wheat distiller grain. O'Hara et al. [36] reported that wheat distiller grain could replace a mixture of barley grain and rapeseed meal at 20% dietary dry matter without negatively affecting feed intake, daily gain and carcass traits of growing lambs. Replacing part of barley grain with 20% of wheat distiller grain in finishing lambs also maintained a healthy rumen function, growth performance and carcass characteristics [36]. McKeown et al. [37] also found that triticale-based distiller grain could replace up to 60% barley grain without adversely affecting on growth performance or carcass traits of lambs. Inclusion of wheat distiller grain in growing or finishing lamb diets is likely a viable feeding management since wheat distiller grain can entirely replace protein supplement to meet protein requirement of growing lambs, and simultaneously used as energy and fibre source because of its high contents of protein, energy and fibre.

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## 8. Manure management consideration

Ammonia emitted from animal feeding operations is a major air and water pollutant contributing to eutrophication, soil acidity, aerosol formation, and impaired visibility. Although ammonia is not a greenhouse gas, it may indirectly contribute to agricultural emissions of nitrous oxide, a potent greenhouse gas with a global warming potential of approximately 300 times that of $CO_2$. During last decade, dramatic increase of high-protein by-products feeding in livestock animals as a result of increased production of corn and wheat distiller grain. Consequently, inclusion of the distiller grain in cattle diets as protein and energy source has been becoming a common practice in cattle production because of high nutritional value. With the increased use of high protein distiller grain in cattle diets, the potential for increased manure nitrogen is a concern. For instance, finishing diets that contain 30% wheat distiller grain have more than 20% (dry matter basis) crude protein, compared to the animal's requirement of about 12%. As a result, the excess nitrogen is excreted in manure (feces, urine and bedding) leading to greater $NH_3$ and $N_2O$ emissions. In feedlot cattle, only a small percentage of the protein consumed by feedlot cattle is retained in animal tissue and as a result 80–90% is excreted in urine and feces, mostly in urine since digestibility of feed protein is relatively high for most types of feeds. Li et al. [38] reported that increased nitrogen intake due to increased distiller grain feeding quantitatively increased nitrogen retention, excretion in feces and urine, whereas, proportionally, nitrogen excretion in urine increased (primarily in the form of urea) and nitrogen excretion in feces decreased. The study clearly identified that urinary nitrogen is the principal source of $NH_3$-N volatilized from cattle manure during the initial 10 days of storage, accounting for an average of 90% of the emitted $NH_3$-N. Thus, from an environmental point, it is important to match dietary protein supplies as closely as possible to rumen microbial and animal needs. However, when the distiller grain is included at high proportion as energy source in cattle diets, high nitrogen excretion is not avoidable, a factor that needs to be considered for manure management.

Wheat distiller grain also contains high concentrations of phosphorus and sulphur [11]. The resulting manure from cattle fed wheat distiller grain, with high phosphorus content, can be beneficial for crop production, but it may also have a negative environmental impact due to increased phosphorus accumulation in crop lands surrounding feedlots [39]. Environmental concerns regarding phosphorus excretion are primarily associated with pollution of surface water. Dietary phosphorus intake was positively associated with the amount of phosphorus excreted in livestock manure [40]. Concentration of sulphur in wheat distiller grain was reported to range from 3.9 to 11.4 g/kg in dry matter [6, 11]. The high sulphur in distiller grain is mostly from chemicals added during the ethanol fermentation to control pH and for cleanup. Excreted sulphur can contribute to $H_2S$ emissions from livestock manure [41]. Li et al. [38] reported that increasing substitution of wheat distiller grain for barley grain and barley silage in diets fed to finishing cattle increased urinary phosphorus excretion. Thus, potential environmental implications of liquid runoff from the feedlot surface and potential phosphorus contamination of surface water need to be considered. In addition, the increased intake and urinary excretion of sulphur as a result of increased inclusion of distiller grain in feedlot diets [38] may increase ammonia and $H_2S$ emissions from the feedlot, in particular

when combined with increased nitrogen excretion. Therefore, cattle producers that replace grains or forages with distiller grain need to take appropriate steps to develop nutrient management programs in order to minimize nutrient loss to the environment and to maximize use of both nitrogen and phosphorus.

## 9. Conclusion

Increase of biofuel ethanol production has resulted in an increase of the production of wheat-based distiller grain, and thus increases in the use of distiller grains in the diets of livestock animals. The chemical composition of wheat distiller grain can vary considerably from plant to plant or between batches within plant depending on the type of wheat fermented and technology of fermentation used in ethanol plants. Direct nutrient analysis of each lot of wheat distiller grain is recommended if such information is not provided to ensure accurate ration formulation for precisely feeding ruminant animals. Wheat distiller grain contains higher protein, fibre, fat and minerals but very lower starch than the original grain. Protein quality in wheat distiller grain is high with moderate rumen degradability, and its fibre is highly digestible in the rumen. Therefore, wheat distiller grain can be used as good protein and energy source in ruminant diets. Wheat distiller grain is commonly fed in beef and dairy cattle feeding as either a protein or energy source or both. It is recommended that wheat distiller grain not be included in dairy rations at levels above 20%, whereas they can be fed to 40% of the diet of growing and finishing cattle. Wheat distiller grain can also be used as fibre source to partly replace roughage in cattle diets, whereas its effectiveness of stimulating chewing activity and maintaining rumen pH status is limited. Thus, feeding wheat distiller grain in place of roughage may increase the risk of rumen acidosis especially if it is used to replace all of the forage in beef cattle diets. With the mandatory inclusion of renewable fuels in gasoline, distiller grain is certain to continue to be an important feed source for ruminants. Development of rapid analysis procedures such as near-infrared spectroscopy may allow this ingredient to be formulated into diets with greater accuracy. The wheat distiller grain is high in nitrogen and phosphorus, and high inclusion in cattle diets, especially when it is used as energy source in cattle diets may exceed the protein requirement, thus increase the manure nitrogen excretion, a factor that needs to be considered for manure management.

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Section 5

# Wheat Nutritional and Food Safety

Chapter 15

# Celiac Disease: Gluten Peptides Characterization after *In Vitro* Digestion

Barbara Prandi

Additional information is available at the end of the chapter

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**Abstract**

Gluten proteins are characterized by the high glutamine and proline content; thus, during gluten digestion, several resistant peptides are produced. Some of them contain sequences that, in celiac patients, are able to trigger an immunological reaction. The prolamin fraction of different wheat samples was submitted to *in vitro* digestion, and the peptides generated were analysed using liquid chromatography coupled to mass spectrometry techniques. Several wheat varieties were analysed, showing large differences in the production of immunotoxic peptides on digestion. After simulated gastrointestinal digestion of wheat, emerged that peptides containing sequences known to elicit the adaptive immune response derived mainly from γ-gliadin, whereas peptides containing sequences involved in the innate immune response were distributed among α-gliadin and γ-gliadin and low-molecular-weight glutenins. From the results, no major differences due to the different cultivation places were observed. On the other hand, statistically significant differences are present among the genotypes tested, especially for the immunogenic peptides. The possible development would be the selection of wheat genotypes with reduced amount of immunogenic sequences, to reduce the exposure of people and decrease the risk of new cases of disease.

**Keywords:** *In vitro* digestion, gluten peptides, celiac disease, LC-MS, wheat protein

## 1. Introduction

Approximately 8% of children and 1–2% of adults suffer from food allergy worldwide, and the perceived prevalence is even much higher, up to 22% of the population, constituting a fast growing health problem [1, 2]. The prevalence of food allergies is continuously increasing in the last decades, especially in the developed countries. The Big-8 of food allergens, namely the foods that are mainly involved in these immunological reactions, are milk, eggs,

fish, crustaceans, peanuts, tree nuts, soybeans and wheat. These foodstuffs can be eaten by most of the population without problems, but they can give a strong immunological reaction with topic and systemic consequences in sensitive people [3, 4]. Thus, the only therapy available for patients suffering from food allergy is the strict avoidance of the offending food. This means that allergic consumers must absolutely avoid eating foods that could provoke potentially life-threatening reactions. Successful avoidance depends on having complete and accurate information on food labels. Thus, huge efforts are made by regulatory agencies, with the collaboration of food industry, to protect allergic consumers, to ensure that all food allergens present in the food are declared on the label and that effective controls are used to prevent the presence of unintended allergens [5]. In the case of children, dietary elimination of nutrient-dense foods may result in inadequate nutrient intake and impaired growth: children with multiple food allergies have a higher risk of impaired growth and may have a higher risk of inadequate nutrient intake than children without food allergies. In addition to this, the social lifestyle of individuals with food allergy and of their families can be severely disadvantaged, since they need to constantly avoid the allergenic ingredient [6]. This task becomes more difficult to manage when people do not eat at home but in restaurants, canteens and other food chains, even if a list of the ingredients of all the dishes must be provided. Moreover, the repercussions of food allergy are not only limited to individuals or households: the food industry must also sustain a lot of extra costs due to food allergy. Primarily, legislative changes, such as the new EU-legislation on food labelling (EU Directive 2003/89/EC amending Directive 2000/13/EC), force the industry to adapt productive processes, food labelling and monitoring to improve allergic consumer protection. The burden of responsibility falls to the food manufacturer, who is required to manage production processes to ensure allergenic ingredients are labelled [7]. Up to now, the potential social impact and economic costs of food allergy on the individual, families, health-related services and food industry are relevant.

Wheat is in the list of the eight main allergenic foods, because the gluten contained in it is the main external trigger of celiac disease. Celiac patients eat several types of gluten-free products, some of them are naturally gluten-free foods (fruits, vegetables and unprocessed meat, fish and poultry) but some others are gluten-free substitute foods (pasta, bread, cereals, crackers and snack foods) where wheat flour is replaced by gluten-free flours. Gluten-free products can be purchased at general and specialty food stores as well as via Internet. Several studies demonstrated that gluten-free food is not always readily available, and it is considerably more expensive than regular, gluten-containing foods [8]. The increasing incidence of celiac disease in the population has negative effects not only on the quality of life but also on the health care system: it has been estimated that the average annual health care costs per-patient in primary care significantly increased by 91% for CD patients after they had been diagnosed with the disease [9]. The impact is also evident for the agricultural and food sectors: wheat is one of the first three cereals for diffusion and cultivation for human nutrition. Gluten, the main trigger of celiac disease, is at the basis of rheological properties of wheat-based products. In fact, the formation of a gluten network in the dough is of outmost importance for air bubbles and starch retention (respectively for leavened products and pasta). A low gluten content of the flour leads to loss of product shape in the case of leavened products and to soft

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and mushy pasta. The consequence is that wheat breeding has been, during the last decades, oriented toward increasing yield and the amounts of amylopectin, gluten and protein [10].

At the moment, no therapies are available for people that are already celiac, so the only treatment is the gluten-free diet. But, on the other hand, efforts can be made in the direction of decreasing celiac disease incidence. Different hypotheses have been made on the reasons of the increased incidence of celiac disease. Since celiac disease affects the gastrointestinal tract, the gut microflora can play a key role in the loss of the immunological tolerance. For example, rod-shaped bacteria in the upper small bowel are present in one-third of the children with CD but in less than 2% of the controls [11]; another study showed that the species *Bacteroides fragilis* is more abundant in the intestinal microbiota of CD patients, whereas *Bacteroides ovatus* is less abundant in comparison to healthy controls [12]. Besides usual microflora, also viral and bacterial gastroenteritis may have a role in celiac disease pathogenesis; in fact, it has been previously demonstrated that a high frequency of rotavirus infections may increase the risk of celiac disease autoimmunity in childhood in genetically predisposed individuals [13]. For what concerns gluten, timing of gluten introduction into the infant diet is associated with risk of celiac disease autoimmunity [14]. Recent studies demonstrated that the oral tolerance to gluten can be lost also in the elderly [15]; the study was conducted after a cohort from 1974 up to now. In parallel, it appears that vital gluten consumption has tripled since 1977. This increase is of interest because it is in the time frame that fits with the predictions of an increase in celiac disease [16]. It seems that massive and early exposure to gluten can be one of the causes of the switch from oral tolerance to celiac disease. Another cause that it has been hypothesized is the transition from sourdough fermentation of bread and baked products to yeast fermentation. So, the bacterial proteolytic activity is rather promising not only as currently demonstrated for eliminating traces of contaminant gluten but probably also in perspective for the manufacture of tolerated baked goods [17].

Thus, trying to decrease these risk factors could help to stop the rising of celiac disease incidence. It is known for a long time that breast feeding has a protective effect against the development of celiac disease, especially when it is still ongoing during gluten introduction in the diet. Also the improvement of infant milk formula, decreasing protein content and osmolarity, has helped to reduce celiac disease incidence [18]. Obviously, the easiest way to reduce the amount of gluten ingestion is the reduction of wheat-derived products consumption, but this would mean a kind of "preventive gluten-free diet", with all the problems and limitations previously described (first of all the decrease in life quality). An alternative way could be the reduction of gluten content in wheat (in contrast with what has been done in the last decades), but this would mean a dramatic decrease in the texture quality of baked products and pasta. Since gluten proteins have a reserve role (nitrogen stock), they underwent to a limited evolutionary pressure, thus showing a high-sequence variability with a lot of different isoforms. This lays the groundwork for a possible varietal selection aimed to have the same total gluten amount (maintaining the same rheological properties) but expressing protein isoforms with a reduced content of sequences involved in celiac disease. In this way, the exposure of the population to immunotoxic sequences will be reduced and, possibly, also the incidence of the disease.

## 2. Characterization of the peptides deriving from gluten digestion

Differences in gluten coding genes have been extensively characterized, besides for their technological and functional implication in baked products, also for evaluating how much the wheat genetic characteristics can impact on the final immunotoxicity of gluten. One of the most studied immunogenic peptides, the 33-mer LQLQPFPQPQLPYPQPQLPYPQPQLPYPQPQPF, has been demonstrated to be encoded by the 6D chromosome, thus being absent in diploid and tetraploid *Triticum* species. The lacking of genes encoding for some immunogenic sequences in diploid/tetraploid varieties has also found evidences in T-cell assays performed on gluten chymotryptic digests that gave different results among the species tested [19]. A lot of efforts have been made in the last years in the direction of decreasing wheat toxicity for celiac patients, for example, making use of *ex vivo* organ cultures and immunohistochemistry assays [20]. The K562 cell agglutinating activity of wheat has been known for many years [21] to screen cereal toxicity for celiac patients [22]. The majority of the studies performed in the past to assess gluten toxicity with immunochemical methods, the main immunotoxic peptides were identified, and in some cases, also a complete characterization of the digestion mixture was obtained [23]. In the recent years, the advances in the field of mass spectrometry allowed more accurate peptides identification, especially in the case of recombinant gliadin and synthetic peptides [24–27]. Other studies took in consideration purified gliadins, to identify the target peptides of tissue transglutaminase [28]. Peptide mixtures obtained by gluten digests are very complex, since than more than 40 components are present both for gliadins and glutenins [29]. The composition of peptide mixtures derived from simulated gastrointestinal digestion of the whole gliadin fraction of wheat was recently investigated [30]. The gliadin fraction was extracted with 70% ethanol in water and then desiccated. The *in vitro* digestion was performed using the three main proteases of the gastrointestinal tract: pepsin in the gastric phase (3 h) and chymotrypsin/trypsin in the intestinal phase (4 h). All the enzymes were used at their optimal temperature (37°C) and pH (respectively 2 and 7.2), using an enzyme:substrate ratio of 1:100. The determination of the amino acid sequence of the gluten-derived peptides was achieved using reverse phase chromatography (High Performance Liquid Chromatography [HPLC], Ultra Performance Liquid Chromatography [UPLC] and Micro-High Performance Liquid Chromatography [μHPLC]) coupled with different types of mass spectrometer (both high and low resolution, e.g., Linear Trap Quadrupole (LTQ) OrbiTrap, single and triple quadrupoles) to achieve the desired resolving power.

This extensive characterization (**Table 1**) gives useful information for a better understanding of the peptides that presumably come in contact with the intestinal mucosa, triggering the immunological response in celiac patients. Immunogenic peptides are those containing sequences known in literature to elicit the adaptive immune response, through recognition by the HLA-DQ2 (or DQ8) of the antigen presenting cells (APC), leading to stimulation of T cell response. As it is shown in **Table 1**, the identified immunogenic peptides derive mainly from $\alpha$-gliadin, in particular from the region between the 56th to the 88th amino acid. Toxic peptides are those containing sequences known in literature to elicit the innate immune response: their interaction with epithelial cells, macrophages and dendritic cells leads to the up regulation of different cell mediators, the most important one being interleukin-15. Also in this case,

most of the peptides were identified as deriving from α-gliadin, more specifically from the N-terminal region. Using the isotopically labelled internal standard method, peptides containing sequences involved in celiac disease can be quantified: these data can be very helpful for interpretation of the results of immunological assays, since the different response can be due both to different epitopes generation in terms of amino acid sequence and to a different relative amount of pathogenic peptides.

| | Gliadin type | Relative amount (durum) | Relative amount (common) |
|---|---|---|---|
| *Immunogenic peptides identified* | | | |
| QLQPFPQPQLPY | α-Gliadin | +++ | + |
| QLQPFPQPQLPYPQPQPF | α-Gliadin | + | + |
| LQLQPFPQPQLPY | α-Gliadin | + | + |
| LQLQPFPQPQLPYPQPQPF | α-Gliadin | ++ | + |
| QLQPFPQPQLPYPQPQLPYPQPQPF | α-Gliadin | nd | + |
| QLQPFPQPQLPYPQPHLPYPQPQPF | α-Gliadin | nd | ++ |
| LQLQPFPQPQLPYPQPQLPYPQPQLPYPQPQPF | α-Gliadin | nd | +++ |
| LPFPQQPQQPFPQPQ | γ-Gliadin | Trace | Trace |
| *Toxic peptides identified* | | | |
| SHIPGLEKPSQQQPLPL | LMW-glutenin | + | + |
| VRVPVPQLQPQNPSQQQPQEQVPLVQQQQF | α-Gliadin | + | + |
| QNPSQQQPQEQVPLVQQQ | α-Gliadin | + | + |
| VPVPQLQPQNPSQQQPQEQVPL | α-Gliadin | ++ | ++ |
| VRVPVPQLEPQNPSQQQPQEQVPL | α-Gliadin | + | + |
| VRVPVPQLQPQNPSQQQPQEQVPL | α-Gliadin | +++ | +++ |
| VRFPVPQLQPQNPSQQQPQEQVPL | α-Gliadin | + | + |
| PSSQVQWPQQQPVPQ | γ-Gliadin | + | + |
| NMQVDPSGQVQWPQQQPF | γ-Gliadin | + | + |

Adapted with permission from Ref. [30]
+++, very abundant; ++, abundant; +, detectable; nd, not detectable.

**Table 1.** Most abundant immunogenic and toxic peptides identified in the digested prolamin extracts (known immunogenic and toxic sequences are underlined), together with an indication of the protein of origin and of their relative abundance in the different types of wheat (durum wheat: *Triticum durum*; common wheat: *Triticum aestivum*).

## 3. Wheat screening through *in vitro* digestion and LC-MS analysis

The *in vitro* digestion of the prolamin extract described in Section 2 was performed on a wide spectrum of wheat samples, to observe eventual differences in the production of immunogenic

and toxic sequences after gastrointestinal digestion. Briefly, the prolamin fraction was extracted with 70% ethanol and submitted to simulated *in vitro* digestion using the three main proteases of the gastrointestinal tract: pepsin in the gastric phase (3 h) and chymotrypsin/trypsin in the intestinal phase (4 h). All the enzymes were used at their optimal temperature (37°C) and pH (respectively 2 and 7.2), in an enzyme:substrate ratio of 1:100. The quantification of the gluten-derived peptides was achieved using reverse-phase ultra-high-performance liquid chromatography (UPLC) coupled with single quadrupole mass spectrometer (SQD), using the isotopically labelled internal standard method (the peptide LQLQPF($d_5$)PQPQLPY, one of the most abundant, was used as standard). In this way, it has been possible to evaluate the influence of the cultivation area (i.e. the soil—climatic conditions) and of the genotype of the wheat (genetic influence).

### 3.1. Influence of the cultivation region

To investigate the role of soil and climatic conditions on the total amount of toxic and immunogenic sequences, durum wheat samples harvested in three different Italian regions were submitted to prolamin extraction and *in vitro* digestion. The three harvesting area were chosen to maximize the soil and climatic differences. Argelato is located in Northern Italy, in the Po plain with a temperate sub-oceanic climate: mean annual temperature is 11.0–13.0°C (months with mean temperature below 0°C: January) and precipitations are 690–1200 mm (May and October are the rainy months, whereas July and August are the driest months). Falconara is located in the hills of the Central Italy, with Mediterranean sub-oceanic climate: mean annual temperature is 12.5–16.0°C (no months with mean temperature below 0°C) and precipitations are 700–1000 mm (November is the rainy month, whereas July and August are the driest). Lucera is located in the Capitanata area, with Mediterranean subtropical climate: mean annual temperature is 12.0–17.0°C (no months with mean temperature below 0°C) and precipitations are 400–800 mm (October and November are the rainiest month, May to September are the driest).

The total amount off peptides containing immunogenic and toxic sequences is reported in **Figure 1**, mediated for each harvesting area. Statistically significant differences were determined with analysis of variance (two ways ANOVA), with $p < 0.05$. Immunogenic and toxic peptides show the same trend: there were no statistically significant differences among the three different locations, with the exception of Argelato (BO), that show a slightly higher content of peptides containing sequences involved in celiac disease. Thus, the soil and climate conditions do not have a determining role on the amount of immunogenic and toxic gluten sequences. The high intra-region variability, indeed, suggests that there are other factors that are playing an important role.

### 3.2. Influence of the genotype

The accurate molecular characterization of the *in vitro* digested prolamin mixtures is an interesting tool for the screening of different wheat lines aimed to identify those producing a smaller amount of pathogenic peptides [31]. Genetic selection operated by breeders, to achieve the desired rheological properties, has led to a decrease in genetic biodiversity of wheat varieties nowadays present on the market. Thus, 25 accessions from a durum wheat

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panel were chosen to maximize genetic biodiversity of the samples: the prolamin fraction was extracted with 70% ethanol and submitted to *in vitro* digestion. From a molecular point of view, the results obtained confirm what has been previously assessed using genetic and immunologic approaches, that there is a strong influence of the genotype in the final amount of peptides containing sequences involved in celiac disease.

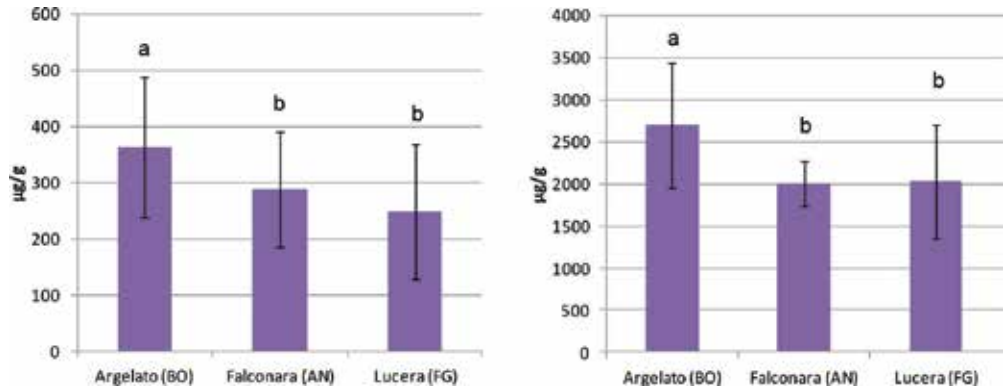


**Figure 1.** Total average content of immunogenic (left panel) and toxic peptides (right panel) of wheat samples harvested in three different regions (Argelato, Falconara and Lucera). Total amount of peptides is expressed in μg of peptide for gram of sample. Bars with different letters mean statistically different samples. Adapted with permission from Ref. [31].

As shown in **Figures 2** and **3**, there are great differences among the different samples. More specifically, the peptides that are more affected by genetic features are those eliciting the adaptive immune system (immunogenic peptides). This relies on the fact that toxic peptides derive from the N-term region of gliadins, which is much more conserved than the region that originates immunogenic peptides. In the latter case, the difference is surprisingly high: there is a 6-fold difference between the highest and the lowest scoring sample (600 μg/g vs 100 μg/g). These data confirm the huge variability in gluten-coding genes, since also among accessions of the same genetic group, there are noticeable differences, for example, in the first group. Recent studies demonstrated that number of subjects that lost the immunological tolerance to gluten in their adulthood is increasing and among the possible causes there is also the amount and the quality of ingested gluten [15]. This means that the use of less immunogenic wheat varieties (especially in the preparation of baby foods) can reduce the exposure to gluten, possibly decreasing the incidence of the disease. And, moreover, it would be possible to operate a varietal selection aimed to have the same gluten content (thus comparable rheological properties), but expressing different gliadin isoforms, with a reduced content of immunogenic and toxic peptides, to reduce the exposure of genetically predisposed subjects, and possibly to reduce the risk of celiac disease development. These data take in consideration the molecular point of view, so it would be really interesting to cross the data with immunological tests (such as T cell proliferation assays or K562 cells agglutination) on the samples to verify the quality of the correlation between pathogenic peptides content and immune response.

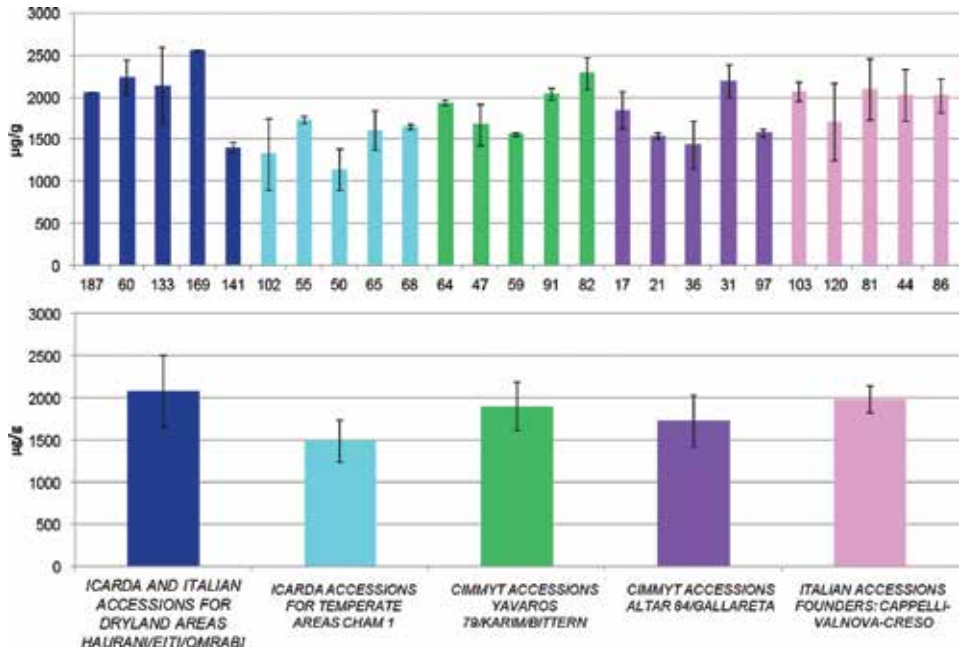


**Figure 2.** Total content of toxic peptides (expressed in µg of peptide for gram of sample) in 25 samples from a Durum Panel collection (upper panel). Samples were grouped on the bases of phylogenetic affinity on dendrogram (lower panel). Adapted with permission from Ref. [31].

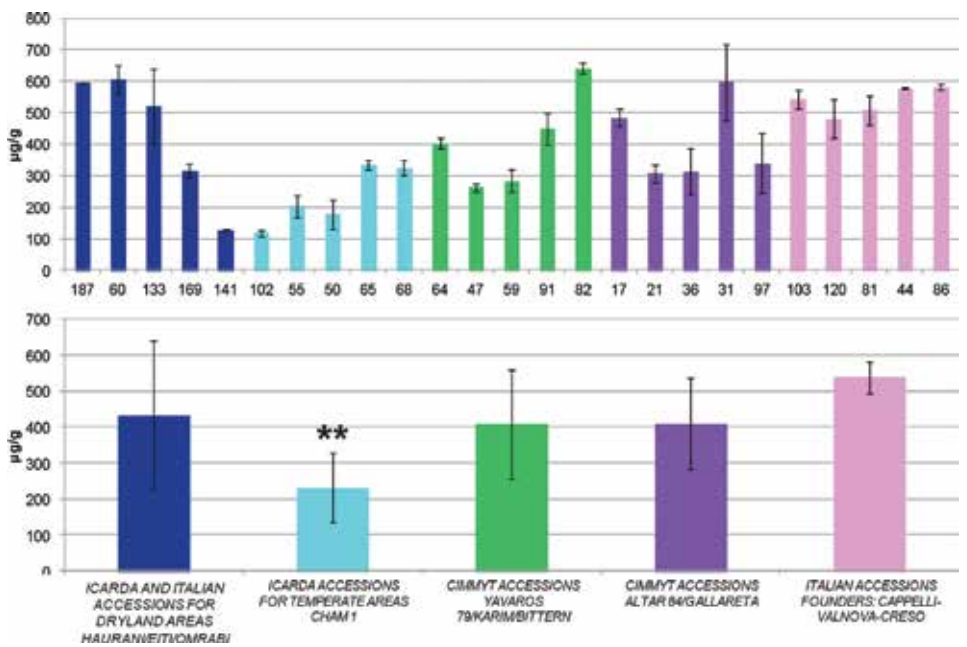


**Figure 3.** Total content of toxic peptides (expressed in µg of peptide for gram of sample) in 25 samples from a Durum Panel collection (upper panel). Samples were grouped on the bases of phylogenetic affinity on dendrogram (lower panel). ** Statistically different group. Adapted with permission from Ref. [31].

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## 4. Wheat digestion: comparison between two different models

To perform immunological assays on gluten peptides, it is necessary to simulate the human gastrointestinal digestion on gliadin/gluten/wheat samples. In literature, *in vitro* digestion models were amply used to study gluten peptides. But the weakness of these approaches is that they are all different and there was no standard method. Previously used models are not consistent with each other for the type of enzyme used (peptic/tryptic digests, peptic/chymotryptic, pancreatin, eventual exoproteases), for the digestion times of the gastric and intestinal phase (from 20 min to several hours), for the buffering agents used (hydrochloric acid, formic acid, bicarbonate, or phosphate buffer), for the protein:enzyme ratio used and so on. All these factors could have a strong influence on the outcome of the digestion, both in terms of peptide sequence and amount, which can be reflected also on subsequent analysis on the gluten digest. In view of this, a qualitative and quantitative comparison of the peptides generated was performed [32] applying two extremely different digestion models: a very simple peptic/tryptic-chymotryptic digestion of a gliadin ethanol extract and a more complex and more physiological method involving the use of artificial digestive juices [33]. These juices strictly reflect the physiological composition of salts and enzymes, involving the use of $\alpha$-amylase, pepsin and pancreatin (a mixture of $\alpha$-amylase, lipase, trypsin, chymotrypsin, elasase and carboxypeptidase). Peptides generated were identified using LC-MS/MS techniques both at high and low resolution and quantified using the isotopically labelled internal standard method.

A qualitative comparison of the peptides generated with the two models is reported in **Table 2**, together with the protein of origin of the peptide and the retention time. Results clearly showed that the peptide composition obtained is completely different. While with the simplified digestion model, quite all the peptides derive from $\alpha$-gliadins; using the physiological digestion model, they are equally distributed among $\alpha$-gliadins, $\gamma$-gliadin and low-molecular-weight glutenins. This fact can be ascribed to the different solubilisation power of the two methods. Ethanol extraction of the prolamin fraction probably leads to a better extractability of $\alpha$-gliadins; on the opposite, in the physiological digestion model, the presence of additional enzymes other than proteases (such as $\alpha$-amylases and lipases), together with the bile salts, contributes to matrix degradation, improving the extractability and digestibility of higher molecular weight proteins such as $\gamma$-gliadins and low-molecular-weight glutenins. Another important difference among the two models is the presence of specific cleavage sites for the enzymes used. In the simplified digestion model, all the peptides show specific cleavage sites for the three enzymes used (pepsin, chymotrypsin and trypsin), for example, tyrosine, phenylalanine and leucine. In the physiological model indeed, in most cases, there are no specific cleavage sites, due to the action of the exoproteases present in pancreatin. Thus, changing the *in vitro* digestion model, the peptide profile is completely different. Using the isotopically labelled internal standard method, the peptides were quantified for both digestion models, and their total amount was plotted to obtain a quantitative comparison. Despite the different identified peptide sequences, the two *in vitro* digestion methods showed a good correlation in terms of immunotoxic sequences. This means that, despite the different amino acid sequence of the peptides generated, the immunotoxicity of a wheat variety is an own intrinsic characteristic of its gluten. In **Figure 4**, the total amount of immunogenic and toxic peptides obtained with the physiological digestion is plotted with those arising from the simplified digestion model.

| Simplified digestion | | | Physiological digestion | | |
|---|---|---|---|---|---|
| **Adaptive immune response** | **Prot** | **Rt** | **Adaptive immune response** | **Prot** | **Rt** |
| QLQPFPQPQLPY | α | 30.5 | TQQPQQPFPQ | γ | 20.5 |
| QLQPFPQPQLPYPQPQPF | α | 32.7 | SQQPQQPFPQPQ | γ | 21.3 |
| LQLQPFPQPQLPY | α | 32.6 | QAFPQQPQQPFPQ | γ | 24.4 |
| LQLQPFPQPQLPYPQPQPF | α | 34.1 | TQQPQQPFPQQPQQPFPQ | γ | 24.9 |
| QLQPFPQPQLPYPQPQLPYPQPQPF | α | 34.1 | PQTQQPQQPFPQFQQPQQPFPQPQQP | γ | 26.8 |
| QLQPFPQPQLPYPQPHLPYPQPQPF | α | 33.3 | FPQQPQLPFPQQPQQPFPQPQQPQ | γ | 29.3 |
| LQLQPFPQPQLPYPQPQLPYPQPQLPYPQPQPF | α | 26.5 | PFPQPQQPQQPFPQSQQPQQPFPQP | γ | 29.3 |
| LPFPQQPQQPFPQPQ | γ | 29.6 | QPQLPFPQQPQQPFPQPQQPQQPSPQSQQPQQPFPQ | γ | 29.8 |
| | | | QQPQQPFPQPQQTFPQQPQLPFPQQPQQPFP | γ | 30.7 |
| **Innate immune response** | **Prot** | **Rt** | **Innate immune response** | **Prot** | **Rt** |
| VRVPVPQLQPQNPSQQQPQEQVPLVQQQQF | α | 28.2 | LQPQNPSQQQPQ | α | 16.6 |
| QNPSQQQPQEQVPLVQQQ | α | 26.8 | RPQQPYPQPQPQ | α | 18.0 |
| VPVPQLQPQNPSQQQPQEQVPL | α | 28.0 | LQPQNPSQQQPQEQVPL | α | 23.9 |
| VRVPVPQLEPQNPSQQQPQEQVPL | α | 26.6 | LGQQQPFPPQQPYPQPQPFPS | α | 27.3 |
| VRVPVPQLQPQNPSQQQPQEQVPL | α | 28.8 | SQQQQPV | γ | 14.5 |
| VRFPVPQLQPQNPSQQQPQEQVPL | α | 29.8 | QQQPL | LMW | 16.5 |
| PSSQVQWPQQQPVPQ | γ | 23.0 | QQQPPFS | LMW | 19.8 |
| NMQVDPSGQVQWPQQQPF | γ | 28.1 | PQQPPFSQQQQPV | LMW | 22.0 |
| SHIPGLEKPSQQQPLPL | LMW | 25.6 | QQPPFSQQQPPPFS | LMW | 25.5 |
| | | | QQQPLPL | LMW | 25.4 |

Adapted with permission from Ref. [32]
α, α-gliadin; γ, γ-gliadin; LMW, low-molecular-weight glutenin; Rt, retention time expressed in minutes. Epitopes and toxic sequences are underlined.

**Table 2.** Pathogenic peptides identified in the digested samples obtained with the two different models, with the protein of origin.

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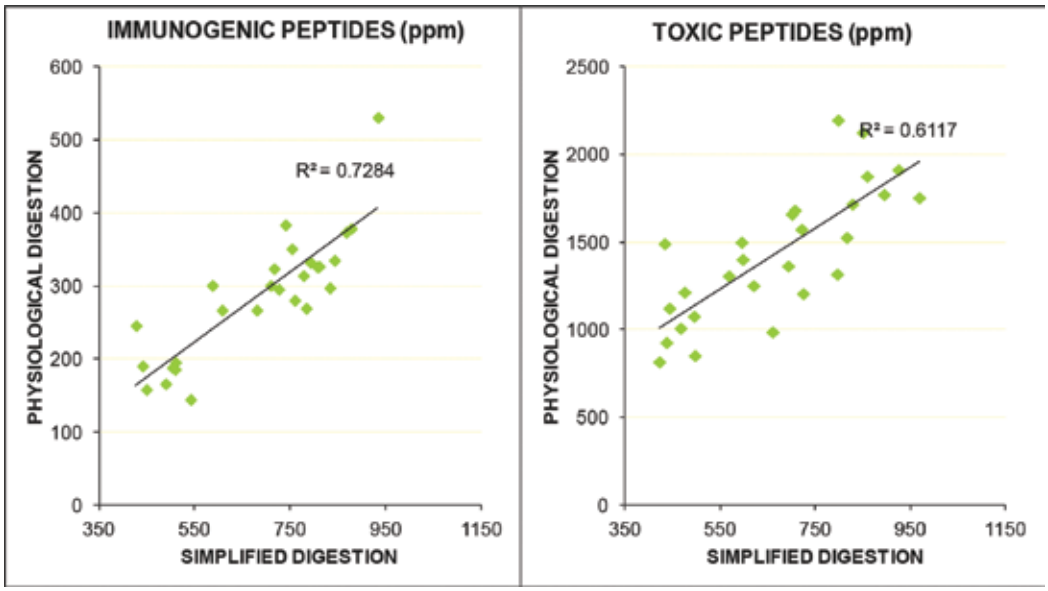


**Figure 4.** Correlation between the amount of immunogenic and toxic peptides generated with the two digestive models. Peptide amounts are expressed as parts per million (ppm).

A good correlation was found using the Pearson test ($p < 0.05$, coefficient of correlation = 0.73 for immunogenic peptides and 0.61 for toxic peptides), as shown in **Figure 4**. This means that for biological experiment, the physiological systems should be suitable, because the peptides generated are similar to those that really come in contact with intestinal cells. Of course, the limit of these models is the lacking of brush border membrane enzymes (that can further proteolyse the peptides) and of the intestinal microflora. A possible interesting continuation of the work could be the use of a physiological model taking into account also these latter variables and studying the effects in terms of peptides produced. However, the good correlation between the total amount of immunogenic and toxic peptides, would suggest to use the simplified method for varietal screening or comparison purposes, where a high throughput, low cost and simple analysis is required.

## 5. Gluten peptides' fate in the pasta production chain

The quantification of gluten peptides reported in **Table 2** was carried out for six steps of pasta production (involving only durum wheat), to verify if some technological treatment have an influence on protein extractability/digestibility. Three different varieties were analyzed to exclude variations exclusively due to the genotype. Results are shown in **Figure 5** for whole wheat, flour, dough, extruded pasta, dried pasta and cooked pasta.

As observed in **Figure 5**, after the physiological digestion method, the amount of toxic peptides is approximately a half than with the simplified method, whereas immunogenic peptides are approximately twice, due to the different type of peptides generated. This should

be taken into account when biological tests are done to assess immunological responses. It can be noted that the amount of toxic and immunogenic peptides remains largely constant along the pasta production chain, so none of the processing steps of pasta is at the moment able to decrease wheat immunogenic potential for celiac patients. In other words, if a varietal screening has to be performed, there is no need to use the end product; it is sufficient to test the basic wheat variety. The difference between the two digestion methods becomes more evident after pasta cooking; in fact, heat causes polymerization of gliadins through intermolecular disulphide bridge formation and to a lesser extent for dehydroalanine formation [34]. Thus, the heat treatment leads to the loss of gliadin extractability, which is the reason of the high underestimation of peptides generated after digestion of the ethanolic extract. It is interesting to note that independently from the method adopted, the differences between varieties maintain the same trend at all the steps of processing. This is an ulterior confirmation that traditional pasta processing leaves gluten immunogenic and toxic peptides unaffected.

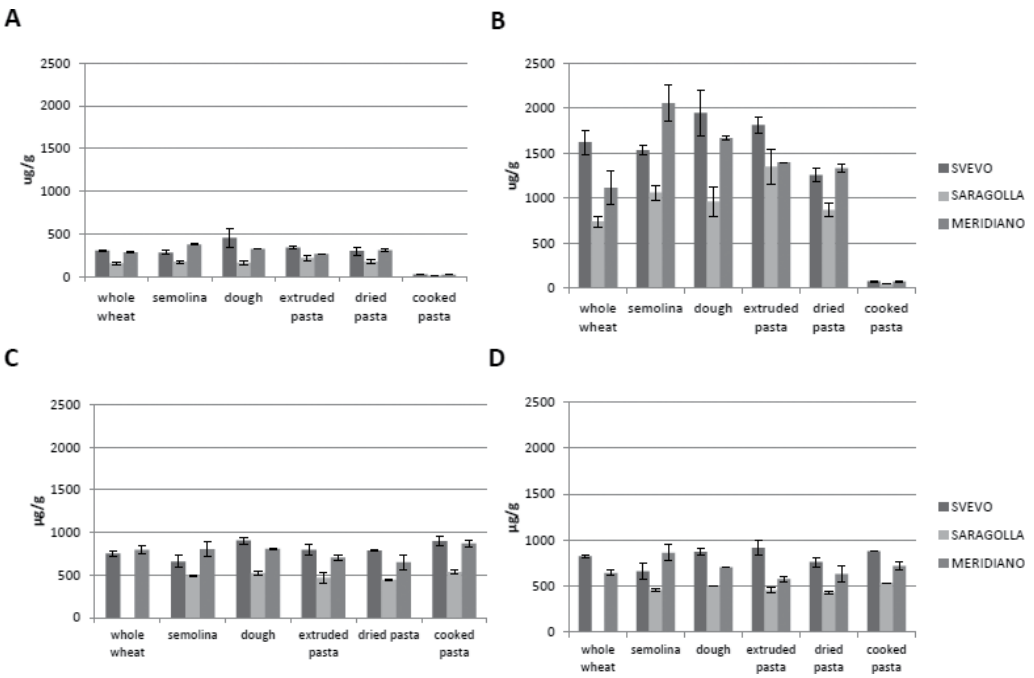


**Figure 5.** Total amount of toxic and immunogenic peptides (upper and lower plots, respectively) quantified after the simplified and the physiological digestion model. Adapted with permission from Ref. [32].

## 6. Conclusion

Several digestion methods applied to gluten proteins are reported in literature. Generally, these models are very simple involving only the use of the main gastric and pancreatic proteases (pepsin, trypsin and chymotrypsin). A buffering agent is also used, to keep the correct pH value at each phase. However, a physiological digestion procedure was previously used

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in literature to assess the release of mycotoxins and heavy metals from food matrices. This method involves the use of digestive juices whose chemical composition strictly reflects the physiological one. These two methods were compared to assess gluten peptides generated. In both cases (simple and complex model), the peptides generated from the digestion were characterized using liquid chromatography coupled with mass spectrometry. In these *in vitro* experiments, the processes occurring in the human gastrointestinal tract during food digestion were simulated, and the outcome of the digestion was assessed by LC-MS techniques. With the use of tandem mass spectrometry, the exact amino acid sequence of the peptides generated by the digestion was determined. Among all the peptides, the ones containing sequences known to be implied in celiac disease were identified. Strong differences were present between the two digestion models. First, with the simplified model almost all the peptides derive from $\alpha$-gliadin, whereas with the physiological method, they are equally distributed among $\alpha$-gliadins and $\gamma$-gliadins and LMW glutenins. This can be explained by the observation that $\alpha$-gliadin-derived peptides of the simplified method are further proteolyzed into shorter peptides in the physiological model and often these shorter peptides did not contain immunotoxic sequences anymore. Moreover, in the physiological model are present enzymes other than proteases (like amylase and lipase) that, even if not directly implied in protein cleavage, can contribute (together with bile salts) to matrix degradation, thus improving the extractability and digestibility of higher molecular weight proteins such as $\gamma$-gliadins and glutenins.

Thus, in the case, a subsequent immunological experiments or biological trials have to be performed, the more physiological method is more suitable than the simplified one, because the peptides generated are really different and the complex method is more similar to what really happens in the human gastrointestinal tract.

The peptides containing immunotoxic sequences were quantified for both the *in vitro* digestion models along the pasta production chain, to evaluate also the suitability of the two methods for processed foods. The samples (kernels, semolina, dough, extruded pasta, dried pasta and cooked pasta) were obtained from three different durum wheat varieties (Svevo, Meridiano and Saragolla). The physiological digestion method produced lesser amount of toxic and a higher amount of immunogenic peptides compared to the simplified one, probably due to the different molecular weight of the peptides generated. A noticeable result is that the difference among the varieties tested remains unchanged, with Saragolla showing a lower content of peptides involved in celiac disease compared to Svevo and Meridiano. Another remarkable result is that the simplified method cannot be applied to thermally treated foods, because heating induces gluten polymerization leading to poor proteins extractability. The two different models are very well correlated in terms of total amount of immunotoxic peptides generated. So, to perform a varietal screening, the simplified method is suitable when a large amount of samples has to be analyzed.

*In vitro* digestion of the prolamin extract was then applied to 45 durum wheat samples belonging to five different varieties and harvested in three different Italian regions (Argelato in the North of Italy, Falconara in the Centre and Lucera in the South). These findings showed no major differences due to the different cultivation place, consistently with the reserve role of that class of proteins (that thus is not affected by environmental factors).

For what concerns genotype influence, since the cultivar selection operated by breeders in the last years to achieve the desired rheological properties has led to a decrease in the genetic biodiversity of durum wheat varieties present nowadays on the market, 25 durum wheat accessions were selected from a durum wheat panel in order to maximize the genetic biodiversity of the samples (and thus eventual differences in immunotoxic peptides production upon digestion). Results obtained from every single accession were mediated in five groups on the basis of phylogenetic affinity on dendrogram.

For toxic peptides, no significant differences were present while strong variability emerged for immunogenic peptides, with accessions of the second groups (International Center for Agricultural Research in the Dry Areas (ICARDA) accessions for temperate areas) showing a significantly lower content of peptides eliciting adaptive immune response.

The higher variability of immunogenic peptides compared to toxic peptides can be explained on the basis of gliadins sequence variability; in fact, toxic peptides usually derive from the N-term region of the protein, which is the most conserved. On the contrary, immunogenic peptides derive from a region of the protein showing a much higher variability. So, different wheat genotypes can express different gliadins isoforms thus showing a different final content of immunogenic sequences.

Then, it is possible to select wheat varieties with good gluten content (and good rheological properties) but with a reduced amount of immunogenic sequences in order to reduce the exposure of people to a possible trigger for celiac disease.

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Chapter 16

# Effect of *Fusarium* spp. Contamination on Baking Quality of Wheat

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Additional information is available at the end of the chapter

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**Abstract**

The effect of *Fusarium* spp. contamination on baking quality of winter common wheat and spelt wheat from different growing systems (organic and conventional) was evaluated by the standard technological quality characteristics and by the rheological system Mixolab. The content of *Fusarium* spp. mycotoxins [deoxynivalenol (DON), deoxynivalenol-3-β-D-glucoside (D3G), 3-acetyldeoxynivalenol (3-ADON), and Zearalenones (ZON)] was determined too. Significantly worse standard technological quality parameters and rheological parameters were determined for artificially inoculated variants of both evaluated wheat species. Statistically significant negative correlation coefficients were discovered between content of mycotoxins and many of technological characteristics, for example, DON content and Zeleny sedimentation for common wheat and spelt (−0.60*; −0.66*) and also between DON content and volume weight (−0.63*; −0.95**) for both wheat species. Resulted Mixolab parameters confirmed that *Fusarium* spp. infection worsens both protein and starch characteristics for both wheat species. However, effect of *Fusarium* spp. contamination in spelt wheat was generally less pronounced in comparison with common wheat. Despite of visible shifts of Mixolab curves of samples from organic and conventional growing systems, resulted Mixolab characteristics were statistically comparable.

**Keywords:** common wheat, spelt wheat, *Fusarium* spp. contamination, mycotoxins, baking quality, organic and conventional growing systems

## 1. Introduction

*Fusarium* head blight (FHB) is a fungal disease of small grain cereals caused by pathogen fungi *Fusarium* spp. and has become a serious danger to the worldwide grain industry. Under favourable weather conditions (moist, warm conditions during flowering), the *Fusarium* spores are spread by wind to cereal spikelet, and then, infection expands within the whole ears. This infection can result in the yield loss and reduction of end-use quality [1]. The most important *Fusarium* species is *Fusarium graminearum* that destroys starch granules, storage proteins, and cell walls and subsequently affects the quality of dough properties [2]. FHB also leads to the accumulation of mycotoxins, which are very stable low-molecular secondary metabolites produced during the fungal infection process [3]. Despite the contradictory reports in the literature concerning the close correlations between FHB and mycotoxins content, it is accepted that overall accumulation of mycotoxins in kernel also would require successful infection and colonization stages of host [4]. Moreover, in the case of very strong infectious pressure, induced by artificial inoculation, it is possible to presume that also content of mycotoxins will be high.

The largest group of *Fusarium* mycotoxins is composed of trichothecenes, which are divided into four groups (types A–D) according to their characteristic functional groups, being the types A and B, the most common. Type A is represented by HT-2 toxin (HT-2) and T-2 toxin (T2), while type B includes nivalenol (NIV), deoxynivalenol (DON), fusarenon-X (FUS-X), 15-acetyldeoxynivalenol (15-AcDON), and 3-acetyldeoxynivalenol (3-AcDON), but the most important is DON. Zearalenones (ZONs)—oestrogenic mycotoxins—cover ZON and its metabolites: a-zearalenol (a-ZOL) and b-zearalenol (b-ZOL) [5].

Regarding huge consumption of cereal product understanding of *Fusarium* infestation impacts not only on health but also on grain properties is essential. The negative effects on baking quality of wheat were already found [6, 7]. Knowledge of the rheological properties of flour is fundamental for specifying baking parameters. Different rheological analyses, for example, farinograph, extensograph, amylograph, or mixograph, are used to evaluate baking properties of cereals [8]. However, these methods evaluate only protein or starch characteristics so lately, the new apparatus Mixolab has been developed and was accepted as the International Association for Cereal Science and Technology (ICC) standard method N°173. This system enables to evaluate physical dough properties such as dough stability or weakening and starch characteristics in one measurement by intense mixing and controlled heating of the kneader to 90°C and ensuing cooling to 50°C [9]. Important researches of predicting the bread and cookie baking quality of different wheat flours were carried out [10, 11]. Reports of efficiency of Mixolab to predict baking quality of various wheat genotypes are verified by significant correlation between some Mixolab and rheological parameters, for example, Zeleny sedimentation and loaf volume [12, 13].

By this time, there are not many studies about a capability of Mixolab to predict rheological parameters of common wheat with various grade of fungi infestation [6]; studies about a capability of Mixolab to predict rheological parameters of other wheat species with various grade of fungi infestation are almost not available.

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The study was focused on the detection of baking quality changes in common wheat and spelt wheat with a different grade of *Fusarium* spp. contamination, using standard methods for technological quality determination and rheological evaluation by the system Mixolab.

## 2. Methodology

### 2.1. Field experiments

Two winter common wheat cultivars (Bohemia and Darwin, both quality group A) and two winter spelt wheat cultivars (Ceralio and Rubiota) from the exact field plot trials, conducted in the years 2010/2011 and 2011–2012 at the experimental station of the Czech University of Life Sciences in Prague (295 m above sea level, average annual temperature 8.4°C, average sum of precipitation 575 mm), were used for the evaluation of the effect of FHB infestation on the *Fusarium* mycotoxins [DON, deoxynivalenol-3-β-D-glucoside (D3G), 3-acetyldeoxynivalenol (3-ADON) and ZON] content in grain, standard technological quality parameters of grain, and rheological parameters of dough. Field plot trials were carried out by the method of randomized blocks in three replications; an average size of experimental plot was 12 $m^2$. Exact field experiments were conducted in an organic farming system on experimental area, certified for organic farming and in comparison also in usual conventional system, using herbicide treatment and nitrogen fertilization −120 kg N $ha^{-1}$, applied in two doses −60 kg N $ha^{-1}$ after wheat overwintering, 60 kg N $ha^{-1}$ at the beginning of shooting. Treatments with natural *Fusarium* spp. contamination were evaluated in both growing systems; to ensure a higher level of *Fusarium* infestation, artificial inoculation of flowering ears was used too.

### 2.2. Artificial inoculation

The isolates of *Fusarium culmorum* and *F. graminearum* used for the artificial inoculation were obtained from the mycological collection of the Crop Research Institute in Prague and cultivated on sterile wheat grains. Wheat grains with the cultures of *F. culmorum* and *F. graminearum* were put into a vessel with water and shaken for 15 min in a laboratory shaker to release the spores into water. The obtained suspension was filtered through the gauze. Then, artificial inoculation was made using the suspension of *F. culmorum* and *F. graminearum* spores in the ratio of 1:1, $10^7$ of spores $ml^{-1}$ (Bürker chamber was used for the verification of inoculums density), 2 l of suspension per experimental plot (12 $m^2$). The suspension was dosed with a hand sprayer at the beginning of the wheat flowering [14]. Harvested grain samples were used for *Fusarium* mycotoxins determination.

### 2.3. *Fusarium* mycotoxins determination

A modified QuEChERS (quick, easy, cheap, effective, rugged, and safe) procedure was used for the isolation of analytes from the wheat grain. It is the multiresidue determination of wide range of mycotoxins applicable within different matrices (cereals, feed, and others), based on the extraction of analytes with acetonitrile; water and further purification of the extracts consists of the division between the two phases by means of inorganic salts (NaCl, $MgSO_4$). Analytes

were transported into the upper acetonitrile layer, while the polar co-extract matrix (e.g. sugars or amino acids) remained in the aqueous phase [15]. The ultra-high performance liquid chromatograph Acquity UPLC System (Waters, USA), coupled with the tandem mass spectrometer LCT Premier XE (Waters, USA) with analyzer time-of-flight MS (TOFMS) was used for the identification and detection of analytes. Wider scale of *Fusarium* mycotoxins was determined—deoxynivalenol (DON) and its conjugated forms, zearalenone (ZON) and its metabolites, enniatins, and so forth, but some of them only in trace amount. Only the mycotoxins, which were detected most frequently in our wheat grain samples—DON, deoxynivalenol-3-β-D-glucoside (D3G), 3-acetyldeoxynivalenol (3-ADON), and ZON—are presented in this study.

### 2.4. Standard technological quality parameters

Crude protein content (CP) in grain dry matter according to the Kjeldahl method (EN ISO 20483; ICC-Standard No. 105/2), wet gluten content (WG) in grain dry matter and gluten index (GI) using the apparatus Glutomatic Perten (ISO 5531), falling number (FN)—ISO 3093, sedimentation index—Zeleny test (ZS)—ISO 5529, volume weigh (VW)—ISO7971-2, and TKW (thousand kernels weight) were determined with the frame of the baking quality.

### 2.5. Rheological characteristics

Protein and starch characteristics of the wheat flour (dough development, protein weakening, starch gelatinization, diastatics activity, and anti-stalling effect) were determined by the apparatus Mixolab (Chopin, Tripette et Renaud, Paris, France) according to the Mixolab protocol Chopin+ [16]. Evaluated flour was obtained by milling the cereal grain samples on a Bühler mill automat MLU 202.

A typical Mixolab curve, which is shown in **Figure 1**, is separated to the five stages represented by five (C1–C5) points [16].

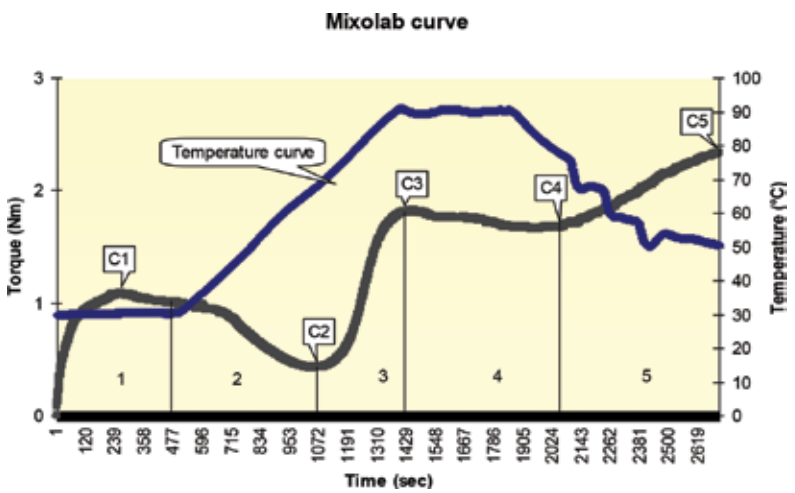


**Figure 1.** Standard Mixolab curve.

The two first stages of the Mixolab curve correspond to the rheological characteristics of proteins—stability, elasticity, and water absorption, whereas the other stages relate mainly to the starch and amylolytic activity. Evaluated characteristics from measured Mixolab curve are C1 (Nm) marks maximum torque during mixing, used to determine water absorption; C1 (min) time required to achieve the maximum torque; C2 (Nm) measures the weakening of the protein based on the mechanical work and the increasing temperature; C3 (Nm) indicates the rate of starch gelatinization; C4 (Nm) represents the stability of the hot-formed gel; C5 (Nm) expresses starch retrogradation during the cooling period; difference C1–C2 represents the protein network strength under the increasing heating; difference C3–C4 shows diastatic activity and relates with falling number; difference C5–C4 correlates with the anti-stalling effects, represents the shelf life of end products; and DS indicates the stability of the dough before weakening [16].

Results were statistically evaluated by the analysis of variance (ANOVA) and correlation analysis with the statistical significance expression on the level $\alpha = 0.05$ and $\alpha = 0.01$ in the "Statistica 9.0 CZ".

## 3. Results

### 3.1. Content of mycotoxins and standard technological quality parameters

An average content of evaluated *Fusarium* mycotoxins in grain is summarized in **Table 1**. Our results show significant differences in evaluated mycotoxins content between variants with natural contamination and artificial *Fusarium* spp. inoculation; on the other hand, differences between organic and conventional growing systems were statistically insignificant. Content of deoxynivalenol (DON) in grain was several times higher than content of other evaluated mycotoxins for both wheat species. Content of mycotoxins in *Triticum aestivum* grain was generally higher in comparison with grain of *Triticum spelta*.

Significant decrease of volume weight and thousand kernels weight but increase of protein and wet gluten content was observed in artificially inoculated variants for both wheat species—higher protein content in smaller grain is expectable. At the same time, general reductions of Zeleny sedimentation and gluten index in artificially inoculated variants were observed (**Table 2**). Similar situation—decreased falling number values were determined in artificially inoculated variants too.

Above mentioned findings were also confirmed by determined correlations between mycotoxins content and evaluated technological parameters (**Table 3**). Negative correlation coefficients were found between content of mycotoxins and most of the technological parameters for both wheat species. The most evident negative effect of mycotoxins content was seen on VW and TKW for the spelt wheat (correlation: −0.95**; −0.97**). On the other hand, positive correlation coefficients were found between content of mycotoxins, crude protein content, and wet gluten content in grain.

| Treatment | Growing system | DON (μg kg$^{-1}$) | D3G (μg kg$^{-1}$) | 3-ADON (μg kg$^{-1}$) | ZON (μg kg$^{-1}$) |
|---|---|---|---|---|---|
| *Triticum aestivum* L. | | | | | |
| Artificial inoculation | Organic | 19411.1$^{b}$ | 2704.6$^{b}$ | 472.3$^{ab}$ | 2965.3$^{b}$ |
| | Conventional | 26729.4$^{b}$ | 4141.3$^{b}$ | 1051.9$^{b}$ | 3191.4$^{b}$ |
| Natural contamination | Organic | 304.7$^{a}$ | 153.8$^{a}$ | 7.5$^{a}$ | 19.0$^{a}$ |
| | Conventional | 257.5$^{a}$ | 66.5$^{a}$ | 7.5$^{a}$ | 22.3$^{a}$ |
| *Triticum spelta* L. | | | | | |
| Artificial inoculation | Organic | 12648.7$^{b}$ | 2154.5$^{b}$ | 149.8$^{b}$ | 117.1$^{b}$ |
| | Conventional | 14433.7$^{b}$ | 2797.8$^{b}$ | 169.8$^{b}$ | 237.0$^{b}$ |
| Natural contamination | Organic | 25.6$^{a}$ | 22.6$^{a}$ | 7.5$^{a}$ | 4.0$^{a}$ |
| | Conventional | 260.1$^{a}$ | 142.4$^{a}$ | 7.5$^{a}$ | 7.1$^{a}$ |

Values with different letter combinations are statistically significant at $p \leq 0.05$; DON—deoxynivalenol; D3G—deoxynivalenol-3-β-D-glucoside; ZON—zearalenone; 3-ADON—3-acetyldeoxynivalenol.

**Table 1.** Content of mycotoxins in the wheat grain of evaluated wheat species (average data of both cultivars).

| Treatment | Growing system | CP (%) | WG (%) | GI | ZS (ml) | FN (s) | VW (kg hl$^{-1}$) | TKW (g) |
|---|---|---|---|---|---|---|---|---|
| *Triticum aestivum* L. | | | | | | | | |
| Artificial inoculation | Organic | 13.7$^{ab}$ | 30.0$^{a}$ | 42.0$^{a}$ | 34.0$^{a}$ | 250.5$^{a}$ | 50.4$^{a}$ | 23.9$^{a}$ |
| | Conventional | 14.1$^{b}$ | 32.5$^{a}$ | 58.5$^{ab}$ | 33.1$^{a}$ | 259.3$^{ab}$ | 47.2$^{a}$ | 22.4$^{a}$ |
| Natural contamination | Organic | 12.0$^{a}$ | 26.1$^{a}$ | 88.3$^{b}$ | 49.3$^{b}$ | 283.8$^{b}$ | 72.8$^{b}$ | 47.3$^{b}$ |
| | Conventional | 12.5$^{ab}$ | 27.3$^{a}$ | 83.8$^{b}$ | 54.8$^{b}$ | 279.8$^{b}$ | 74.1$^{b}$ | 49.5$^{b}$ |
| *Triticum spelta* L. | | | | | | | | |
| Artificial inoculation | Organic | 19.6$^{b}$ | 52.5$^{a}$ | 24.5$^{a}$ | 25.7$^{a}$ | 307.0$^{a}$ | 54.1$^{a}$ | 22.1$^{a}$ |
| | Conventional | 20.6$^{b}$ | 53.2$^{a}$ | 21.0$^{a}$ | 27.1$^{a}$ | 321.0$^{a}$ | 50.1$^{a}$ | 20.9$^{a}$ |
| Natural contamination | Organic | 16.7$^{a}$ | 49.1$^{a}$ | 38.0$^{a}$ | 39.1$^{b}$ | 343.0$^{a}$ | 73.0$^{b}$ | 40.9$^{b}$ |
| | Conventional | 18.8$^{ab}$ | 52.8$^{a}$ | 33.0$^{a}$ | 34.7$^{b}$ | 313.5$^{a}$ | 74.0$^{b}$ | 41.2$^{b}$ |

Values with different letter combinations are statistically significant at $p \leq 0.05$; CP—crude protein content in grain dry matter; WG—wet gluten content in grain dry matter; GI—gluten index; ZS—Zeleny sedimentation; FN—falling number; VW—volume weight; TKW—thousand kernels weight.

**Table 2.** Technological quality parameters of evaluated wheat species (average data of both cultivars).

## 3.2. Mixolab

### *3.2.1. Triticum aestivum L.*

Resulted common wheat Mixolab parameters confirmed that *Fusarium* spp. infection markedly worsenes both protein and starch characteristics (**Table 4**). Average value of C2, which positively correlates with dough strength, was more than half lower after *Fusarium* spp.

| | DON (μg kg$^{-1}$) | D3G (μg kg$^{-1}$) | 3-ADON (μg kg$^{-1}$) | ZON (μg kg$^{-1}$) |
|---|---|---|---|---|
| *Triticum aestivum* L. | | | | |
| CP (%) | 0.48 | 0.37 | 0.44 | 0.22 |
| WG (%) | 0.30 | 0.21 | 0.27 | 0.06 |
| GI | −0.26 | −0.36 | −0.15 | −0.14 |
| ZS (ml) | −0.60* | −0.75** | −0.58* | −0.64* |
| FN (s) | −0.53* | −0.56* | −0.56* | −0.64* |
| VW (kg hl$^{-1}$) | −0.63* | −0.71* | −0.56* | −0.47 |
| TKW (g) | −0.61* | −0.70* | −0.55* | −0.45 |
| *Triticum spelta* L. | | | | |
| CP (%) | 0.69* | 0.64* | 0.64* | 0.35 |
| WG (%) | 0.18 | 0.18 | 0.22 | 0.17 |
| GI | −0.66* | −0.66* | −0.68* | −0.51 |
| ZS (ml) | −0.68* | −0.78* | −0.77** | −0.72* |
| FN (s) | −0.11 | −0.27 | −0.27 | −0.50 |
| VW (kg hl$^{-1}$) | −0.95** | −0.88** | −0.88** | −0.48 |
| TKW (g) | −0.97** | −0.92** | −0.93** | −0.54 |

Statistically significant for $p \leq 0.05^{(*)}$ and for $p \leq 0.01^{(**)}$.

DON—deoxynivalenol; D3G—deoxynivalenol-3-β-D-glucoside; ZON—zearalenon; 3-ADON—3-acetyldeoxynivalenol; CP—crude protein content in grain dry matter; WG—wet gluten content in grain dry matter; GI—gluten index; ZS—Zeleny sedimentation; FN—falling number; VW—volume weight; TKW—thousand kernels weight.

**Table 3.** Correlation between mycotoxins content and technological parameters of evaluated wheat species.

| Treatment | Growing system | C1 (min) | C2 (Nm) | C3 (Nm) | C4 (Nm) | C5 (Nm) |
|---|---|---|---|---|---|---|
| Artificial inoculation | Organic | 2.40$^{a}$ | 0.18$^{a}$ | 2.04$^{a}$ | 1.66$^{a}$ | 2.12$^{a}$ |
| | Conventional | 1.77$^{a}$ | 0.15$^{a}$ | 1.99$^{a}$ | 1.70$^{a}$ | 2.28$^{a}$ |
| Natural contamination | Organic | 3.21$^{a}$ | 0.44$^{b}$ | 2.66$^{b}$ | 1.96$^{a}$ | 2.50$^{a}$ |
| | Conventional | 3.00$^{a}$ | 0.40$^{b}$ | 2.63$^{b}$ | 1.97$^{a}$ | 2.59$^{a}$ |
| Treatment | Growing system | C1C2 (Nm) | C3C4 (Nm) | C5C4 (Nm) | DS (min) | |
| Artificial inoculation | Organic | 0.90$^{b}$ | 0.38$^{a}$ | 0.46$^{a}$ | 6.3$^{a}$ | |
| | Conventional | 0.93$^{b}$ | 0.29$^{a}$ | 0.57$^{a}$ | 5.0$^{a}$ | |
| Natural contamination | Organic | 0.69$^{a}$ | 0.66$^{b}$ | 0.54$^{a}$ | 9.9$^{b}$ | |
| | Conventional | 0.74$^{a}$ | 0.70$^{b}$ | 0.62$^{a}$ | 9.5$^{b}$ | |

Values with different letter combinations are statistically significant at $p \leq 0.05$; C1—time required for maximum torque during mixing; C2—protein weakening; C3—starch gelatinization; C4—stability of gel; C5—starch retrogradation; C1C2—fall of protein strength; C3C4—diastatic activity; C5C4—anti-stalling effect; DS—time of dough stability before weakening.

**Table 4.** Mixolab characteristics of *Triticum aestivum* L. (average data of both cultivars).

inoculation for both growing systems. Therefore, higher rate of protein thermal weakening (C1C2) and visible shorter time of dough stability were found for inoculated variants.

It is evident from **Figure 2** that the inferior effect of infection on protein part of curve is especially obvious for variety Bohemia in 2011, where after dough development, there is rapid fall of the curve which implies low quality of gluten.

Dough heating and thus swelling of starch granules and increasing viscosity cause the increase of the curve—point C3. It was evident from our results that values of artificially inoculated variants were markedly lower than values of variants with natural *Fusarium* spp. contamination. Supposedly, this is due to damaged starch granules of inoculated variants. There was no statistical difference between organic and conventional growing systems. Although C5 parameters in the last section of the curve were slightly higher for naturally contamined variants than for inoculated variants, differences C5C4 representing retrogradation and consequently shelf life of end products were statistically comparable (**Table 4**).

Deteriorated rheological quality of inoculated variants was also confirmed by rated strong negative correlation coefficients between mycotoxins content and Mixolab parameters (**Table 5**).

### *3.2.2. Triticum spelta L*

Final Mixolab characteristics of *T. spelta* L. (**Table 6**) imply slightly better resistance to *Fusarium* spp. infection compare to the reaction of *T. aestivum* variants. It is evident especially in case of Mixolab characteristics, representing protein part of curve (values of C2 and C1–C2).

Average value of C2, which represents the weakening of the protein, was lower in artificially inoculated variants for both wheat species, but in spelt, the difference between naturally contamined and artificially inoculated variants was not so high. At the same time, higher rate of protein thermal weakening (C1C2) and thus shorter time of dough stability were found in

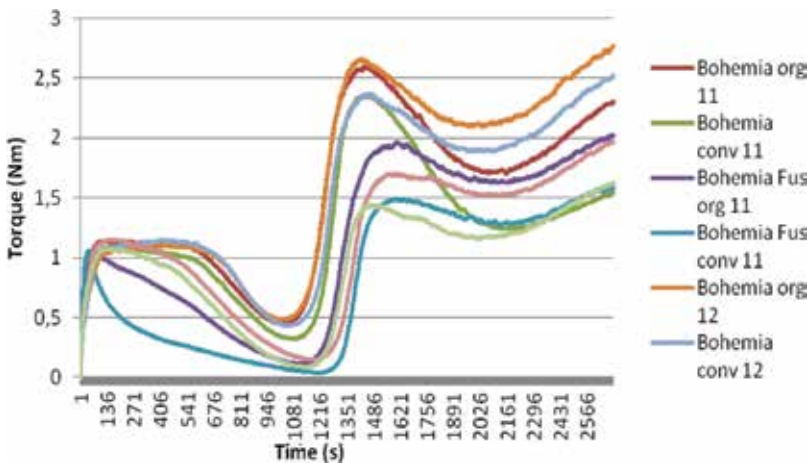


**Figure 2.** An example of Mixolab curve—common wheat cultivar Bohemia.

| | DON (μg kg$^{-1}$) | D3G (μg kg$^{-1}$) | 3-ADON (μg kg$^{-1}$) | ZON (μg kg$^{-1}$) |
|---|---|---|---|---|
| C1 (min) | −0.38 | −0.40 | −0.36 | −0.36 |
| C2 (Nm) | −0.82** | −0.85** | −0.77** | −0.67* |
| C3 (Nm) | −0.74** | −0.66* | −0.72* | −0.54* |
| C4 (Nm) | −0.50* | −0.40 | −0.50* | −0.35 |
| C5 (Nm) | −0.43 | −0.35 | −0.44 | −0.31 |
| C1–C2 (Nm) | 0.73** | 0.74** | 0.69* | 0.56* |
| C3–C4 (Nm) | −0.49 | −0.52* | −0.45 | −0.38 |
| C5–C4 (Nm) | −0.22 | −0.20 | −0.26 | −0.18 |
| DS (min) | −0.89** | −0.93** | −0.90** | −0.89** |

Statistically significant for $p \leq 0.05^{(*)}$ and for $p \leq 0.01^{(**)}$; DON—deoxynivalenol; D3G—deoxynivalenol-3-β-D-glucoside; ZON—zearalenone; 3-ADON—3-acetyldeoxynivalenol; C1—time required for max. torque during mixing; C2—protein weakening; C3—starch gelatinization; C4—gel stability; C5—starch retrogradation; C1C2—fall of protein strength; C3C4—diastatic activity; C5C4—anti-stalling effect; DS—dough stability time.

**Table 5.** Correlation between Mixolab parameters and mycotoxins content for *Triticum aestivum* L. in both growing systems.

inoculated variants for both wheat species, but in spelt, the difference between naturally contamined and artificially inoculated variants was slightly lower compared to common wheat.

Correlation between Mixolab parameters and mycotoxins content for *T. spelta* shows **Table 7**. Although there was a deflection of inoculated variants particularly evident for parameters C2 and C1–C2, affirmed by significant negative correlation between mycotoxins content and C2

| Treatment | Growing system | C1 (min) | C2 (Nm) | C3 (Nm) | C4 (Nm) | C5 (Nm) |
|---|---|---|---|---|---|---|
| Artificial inoculation | Organic | 5.12$^{a}$ | 0.14$^{a}$ | 1.64$^{a}$ | 1.25$^{a}$ | 1.83$^{a}$ |
| | Conventional | 4.20$^{a}$ | 0.14$^{a}$ | 1.65$^{a}$ | 1.33$^{a}$ | 1.89$^{a}$ |
| Natural contamination | Organic | 4.82$^{a}$ | 0.30$^{b}$ | 2.33$^{b}$ | 1.10$^{a}$ | 1.70$^{a}$ |
| | Conventional | 3.72$^{a}$ | 0.28$^{b}$ | 1.78$^{a}$ | 0.85$^{a}$ | 1.30$^{a}$ |
| Treatment | Growing system | C1C2 (Nm) | C3C4 (Nm) | C5C4 (Nm) | DS (min) | |
| Artificial inoculation | Organic | 0.97$^{a}$ | 0.39$^{a}$ | 0.58$^{a}$ | 6.3$^{c}$ | |
| | Conventional | 0.95$^{a}$ | 0.32$^{a}$ | 0.56$^{a}$ | 4.3$^{a}$ | |
| Natural contamination | Organic | 0.83$^{b}$ | 1.23$^{b}$ | 0.60$^{a}$ | 6.0$^{bc}$ | |
| | Conventional | 0.83$^{b}$ | 0.93$^{b}$ | 0.45$^{a}$ | 4.9$^{ab}$ | |

Values with different letter combinations are statistically significant at $p \leq 0.05$; C1—time required for max. torque during mixing; C2—protein weakening; C3—starch gelatinization; C4—stability of gel; C5—starch retrogradation; C1C2—fall of protein strength; C3C4—diastatic activity; C5C4—anti-stalling effect; DS—dough stability time.

**Table 6.** Mixolab characteristics of *Triticum spelta* L. (average data of both cultivars).

| | DON (μg kg$^{-1}$) | D3G (μg kg$^{-1}$) | 3-ADON (μg kg$^{-1}$) | ZON (μg kg$^{-1}$) |
|---|---|---|---|---|
| C1 (min) | 0.18 | 0.12 | 0,20 | 0,02 |
| C2 (Nm) | −0.94** | −0.92** | −0.90** | −0.56* |
| C3 (Nm) | −0.69* | −0.68* | −0.66* | −0.40 |
| C4 (Nm) | 0.28 | 0.25 | 0.30 | 0.19 |
| C5 (Nm) | 0.19 | 0.17 | 0.22 | 0.13 |
| C1–C2 (Nm) | 0.94** | 0.90** | 0.91** | 0.54* |
| C3–C4 (Nm) | −0.51 | −0.49 | −0.52 | −0.32 |
| C5–C4 (Nm) | 0.05 | 0.03 | 0.08 | 0.03 |
| DS (min) | −0.13 | −0.21 | −0.11 | −0.27 |

Statistically significant for $p \leq 0.05^{(*)}$ and for $p \leq 0.01^{(**)}$; DON—deoxynivalenol; D3G—deoxynivalenol-3-β-D-glucoside; ZON—zearalenone; 3-ADON—3-acetyldeoxynivalenol; C1—time for maximum torque during mixing; C2—protein weakening; C3—starch gelatinization; C4—gel stability; C5—starch retrogradation; C1C2—fall of protein strength; C3C4—diastatic activity; C5C4—anti-stalling effect; DS—dough stability before weakening.

**Table 7.** Correlation between Mixolab parameters and mycotoxins content for *Triticum spelta* L. in both growing systems.

and C1–C2 values, generally *Fusarium* effect was less pronounced in comparison with common wheat.

It is evident from **Figure 3** that despite the shifts of individual curves for variety Ceralio, majority of resulting Mixolab parameters for various type of treatment were statistically insignificant. Just characteristics C2 and dough stability for the control from organic treatment were preferable to the conventional variant.

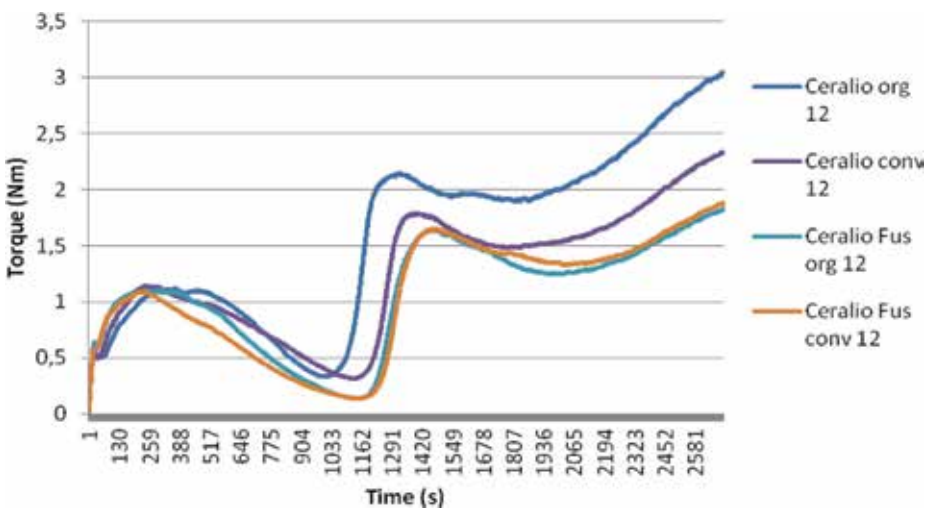


**Figure 3.** An example of Mixolab curve—spelt wheat cultivar Ceralio.

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## 4. Discussion

### 4.1. Content of mycotoxins and standard technological quality parameters

There are contradictory reports on the relationship between the *Fusarium* infection grade and content of mycotoxins in the cereals grain. Some authors did not confirm positive correlation between the infection grade and DON content [17, 18], while others found a high-positive significant correlation [19]. Despite relationship between the *Fusarium* spp. infection grade and mycotoxins content does not have to be too close [18], in the case of a strong infection pressure evoked by artificial inoculation, it is possible to suppose also high mycotoxins content [4, 14–20]. This observation is in accordance with our results, which show significant differences in evaluated mycotoxins content between variants with natural contamination and artificial *Fusarium* spp. inoculation. Differences between organic and conventional growing systems were statistically insignificant, which indicates low dependence of *Fusarium* spp. infestation on cropping system (in the case when fungicidal treatment against FHB was not used). Our results also confirmed, in accordance to [5], that DON was the most occurred *Fusarium* mycotoxin for both evaluated wheat species. According to findings of [21], DON is the most frequent *Fusarium* mycotoxin even in the rye grain.

Several authors mentioned negative effects of *Fusarium* spp. infection on baking quality of wheat [6–22]. On the other hand, there are some contradictory studies where a strong *Fusarium* spp. contamination did not significantly influence the bread making properties [23, 24]. Some authors mentioned an increase of protein content of the wheat grain after the *Fusarium* spp. contamination [25], others mentioned slight decrease of it [2, 6] and [26] concluded that the crude protein content in the wheat grain was not affected by the *F. culmorum* infection. In our case, increase of protein and wet gluten content occurred in artificially inoculated variants. This fact is probably connected with low TKW and volume weight of artificially inocluated variants—in the case of small grains, we can presuppose higher protein concentration.

Zeleny sedimentation index and gluten index measure swelling potential of kernel protein. At the same time, general reductions of Zeleny sedimentation and gluten index in artificially inoculated variants were observed. This indicates that *Fusarium* spp. infection may alter protein quality in grain, in accordance with findings of [6, 14–25].

Decrease of falling number value in artificially inoculated variants was observed too. According to Refs. [27] and [14], fungal infection is expected to increase the degradation of starch due to the activity of enzymes as $\alpha$—amylase in kernels, which is measurable by means of falling number.

Our results showed negative correlation between content of the most mycotoxins and technological quality parameters for both wheat species. The most evident negative effect of mycotoxins content was seen on volume weight and thousand kernels weight. These results are in accordance with [28]—these authors mentioned that FHB can lead to the production of small-sized grains.

### 4.2. Mixolab

Mixolab parameters show, based on the results published up to now, high compatibility with standard rheological analysis (for example, farinograph, extensograph, or amylograph).

Consequently, it is possible to anticipate a potential prediction of bread making quality of wheat from these parameters [10]. But there are not many studies on the efficiency of this Mixolab system to predict rheological parameters of wheat with changed characteristics caused by fungi species, which have become a serious problem in the wheat cultivation during recent years.

#### *4.2.1. Triticum aestivum L*

Some authors [14, 17–20] mentioned that *Fusarium* spp. infection markedly worsens both protein and starch characteristics. Also in our case, average value of C2, which positively correlates with dough strength, was, in accordance with findings of [29], more than half lower after *Fusarium* spp. inoculation for both variants of growing system. Therefore, higher rate of protein thermal weakening (C1C2) and visible shorter time of dough stability were found for these inoculated variants. According to Ref. [30], dough heating and thus swelling of starch granules and increasing viscosity cause the increase of the curve—point C3. It was evident from our results that values of artificially inoculated variants were markedly lower than values of variants with natural *Fusarium* spp. contamination. Supposedly, this is due to damaged starch granules of inoculated variants. The dough with such results is usually stickier and can give a poor baking quality [31]. Mixolab characteristic C5, which represents the rate of starch retrogradation [30], was slightly lower for artificially inoculated variants and verifies the worse quality of the starch part of the wheat grain.

#### *4.2.2. Triticum spelta L*

Despite the fact that common wheat is the most widespread of all cultivated wheat species, at the present time, spelt wheat has growing popularity thanks to the nutritive and pro-health properties. The consummation of spelt products helps to reduce the cholesterol level in blood and fosters the circulatory system [32]. This wheat species has been also known for the high resistance to unsupportive environmental factors. Due to a higher stalk and hard adherent husks, spelt has poor fungal infestation in comparison with common wheat (*T. aestivum* L.) and nowadays is grown mostly by organic methods [33].

Final Mixolab characteristics of *T. spelta* L. show similar trends but imply better resistance to *Fusarium* spp. infection compare to the reaction of *T. aestivum* variants. For example, average values of C2, which represent the weakening of protein, were in spelt as same as in common wheat lower in artificially inoculated variants, but in spelt, the difference between naturally contamined and artificially inoculated variants was not so high. Slightly lesser *Fusarium* effect than common wheat showed correlation coefficients between Mixolab characteristics and mycotoxins content too. This fact was reported by Ref. [33] as well.

## 5. Conclusions

The negative effect of *Fusarium* spp. contamination on the baking quality of wheat (*T. aestivum* L. and *T. spelta* L.) grain and flour was confirmed not only by the standard methods for technological quality determination but also by the rheological system Mixolab. This system

reflected a good sensitivity to the quality changes evoked by *Fusarium* spp. infection. The significantly worsened rheological quality and the negative effect on the protein as well as starch part of the Mixolab curves were observed in wheat artificially inoculated by *Fusarium* spp. compared to wheat with natural *Fusarium* spp. contamination. No significant statistical differences were determined for both growing systems—organic and conventional. There were only visible contrasts between the monitored years.

Our results confirmed that some of the problems related to the rheological properties of flour during processing, which regularly occur in some years and in some areas may be caused by *Fusarium* spp. contamination. However, if the *Fusarium* spp. contamination is not too high and the content of mycotoxins in the kernels does not exceed the allowed hygienic limit, this wheat can be used (after any modification of the processing technology, if necessary).

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Chapter 17

# Progress and Challenges in Improving Nutritional Quality in Wheat

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Additional information is available at the end of the chapter

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**Abstract**

Wheat (*Triticum aestivum* L.) houses a wide range of nutritional components such as iron (Fe), zinc (Zn), vitamins and phenolic acids, which are important for plant metabolism and human health. The bioavailability of these nutritional components is low due to their interaction with other components and low quantity in the endosperm. Biofortification is a more sustainable approach that could improve the bioavailability of essential nutritional components. Substantial progress has been made to improve nutritional quality through the application of conventional, technological and transgenic approaches. This has led to the discovery, cloning and introgression of the *Gpc-B1* gene; the invention of online databases with minimally characterized biosynthetic, metabolic pathways and biological processes of wheat-related species; the establishment of genetic variation in grain Fe and Zn content and the biofortification of wheat with Zn by the HarvestPlus organization. Nonetheless, the biofortification of wheat with micronutrients and phenolic acids is still a challenge due to incomplete understanding of the wheat genome, biosynthesis and translocation of selected nutritional components into different wheat grain compartments. There is a need to integrate selected omics technologies to obtain a holistic overview and manipulate key biological processes involved in the remobilization and biosynthesis of nutritional components into desired wheat grain compartments.

**Keywords:** bioavailability, biofortification, nutritional quality, omics, wheat endosperm

## 1. Introduction

Wheat (*Triticum aestivum* L.) is a major crop grown in many countries. It is predominantly used for the production of products, such as bread, pasta, cereals and cakes, which are consumed on a regular basis [1, 2]. Thus, wheat has the potential to contribute to the reduction

of malnutrition and deficiency-related ailments by contributing to food security and the daily required intake of essential macro- and micro-nutrients in individuals [3].

Nutritional quality, in context of this book chapter, is a collective term that refers to the bioavailability or concentration of desirable nutritional components for human health such as iron (Fe), zinc (Zn), vitamins and selected phenolic acids found in the wheat grain. These key nutritional components are found in different wheat grain compartments at varying concentrations [2]. The wheat germ and bran region contain the highest levels of these nutritional components [1]. Micronutrient deficiencies, especially those arising from Fe, Zn and vitamins pose a serious threat to human health as they affect more than 2 billion people worldwide especially women and children under the age of 5 years [4, 5]. Cardiovascular diseases, diabetes, cancer and malnutrition are among the most dreadful diseases. These diseases could be prevented through regular consumption of selected nutritionally important components, such as wholegrain products and antioxidants, which are acquired as phenolic acids from various foods including the wheat grain [6–11].

There are several challenges encountered in attempts to enhance the levels and bioavailability of some micronutrients, vitamins and phenolic acids in the wheat endosperm [12, 13]. The major challenge is that the complete wheat genome sequence is not available yet. Moreover, biological processes such as nutrient assimilation, translocation and biosynthesis pathway of wheat are not completely understood, in that some pathways have not yet been characterized. In addition, there are a few or no studies aimed at characterizing the process pertaining to micronutrient, vitamin and phenolic acid translocation into the wheat endosperm. Thus, it is difficult to manipulate biological processes involved in the accumulation of micronutrients, vitamins and phenolic acids into the wheat endosperm [14–16]. Therefore, there is a need to characterize the timing at which micronutrients, vitamins and phenolic acids are assimilated, translocated and synthesized in the endosperm. Some wheat grain constituents reduce the bioavailability of Fe through their inhibitory activity [17]. Furthermore, transporter molecules and chelators are mainly localized in the apoplast region, which leave the outer grain compartment layers more concentrated. This prevents desirable nutritional components essential nutrients from being loaded into the endosperm [18].

The NAC transcription factors that are involved in the acceleration of senescence and nutrient remobilization into the grain have also been identified as selected agents that could be used to improve nutritional quality [19]. Gene editing may potentially improve a number of identified traits of interest, thereby resulting in the improvement of the value of wheat [20]. In addition, foliar application has more advantage over other application methods as far as nutritional quality is concerned. Consequently, nitrogen application has enhanced the accumulation of Fe and Zn. Furthermore, soil and foliar application has also been shown to result in an enhancement of Fe and Zn [21]. The bioavailability of $Ca^{2+}$, $Zn^{2+}$ and $Fe^{2+}$ was increased through breaking down phytate by the expression of phytase in transgenic wheat [22].

Additional online resources in the form of databases are also available and have been made public. These databases, including omics viewers for comparative analysis, mainly contain important information regarding various cellular processes, which have been acquired from more than 37,000 publications [23]. The resulting databases include the MetaCyc database

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of metabolic pathways and enzymes, and the BioCyc database is a collection of pathway/genome databases that are currently available to the public [23]. These databases also made a huge contribution to our current understanding of many biological processes involved in nutrient assimilation, accumulation and translocation across different species. Nonetheless, not all pathways on *T. aestivum* are available. In addition, much progress has been made to understand several key biological processes facilitating the uptake of nutrients from the soil, through vascular tissues, then into the grain [14].

Although the strategies deployed to improve the levels and bioavailability of selected nutrients in wheat have been successful, biofortification is a more sustainable approach for improved nutritional quality [12, 13, 24]. It has been rendered sustainable in that it has been used to improve human health through ensuring that the required dietary intake of essential nutrients can reach poor individuals in a more sustainable and cost-effective manner [13]. Biofortification is a process of enhancing the dietary bioavailability or concentration of desirable nutritional components in plants genetically [25, 26]. This process has been used to successfully improve the bioavailability or levels of β-carotene in rice, Zn and Fe in wheat grain as well as levels of other nutrients in other crops [4]. As a result, a number of strategies have been deployed to improve nutritional quality in wheat, such as conventional, technological and transgenic approaches that were undertaken in efforts to improve the levels and bioavailability of micronutrients and phenolic acids, mainly through the biofortification route [17, 21, 27, 28]. This includes several efforts that managed to successfully increase the total grain nutrient content and bioavailability of some micronutrients through genetic biofortification, agronomic biofortification, the use of bioavailability enhancers, including genetic modification through transforming the plants with the ferritin gene, which may not be desirable by the public. Constitutive expression of ferritin, a gene that encodes an iron-rich soybean storage protein reported to be abundant in the endosperm amyloplast region, has largely contributed to Fe bioavailability enhancement [29].

However, there are still some challenges with the biofortification of wheat. The major challenge is enhancing the levels and bioavailability of selected nutritional components in the endosperm region as opposed to increasing the total grain micronutrient or phenolic acid content [15]. This is mainly because most micronutrients and phenolic acids are mainly loaded in the outer layers, which are removed upon milling of the wheat grain [14]. In addition, wheat grain yield, grain protein content and disease resistance are important traits that should not be compromised during new variety development or improvement. Nonetheless, there is little research aimed at characterising the process involved in enhancing the bioavailability and/or loading of micronutrients and phenolic acids into the endosperm region.

The era and deployment of omics technologies has largely contributed to our current understanding of biological functions of many traits in various crops including wheat [30, 31]. This has led to the manipulation and sustainable development of crops with improved traits of interest. These technologies have been widely applied in wheat research and resulted in improved understanding, manipulation and improvement of various complex traits in wheat [31]. Consequently, there is a need to integrate selected omic technologies to improve our current understanding of nutrient loading into different wheat grain compartments further. This will

allow further manipulation of the nutrient loading pathway without affecting other traits of importance [31, 32]. Thereof, the selected omics technological platforms would bring about data outputs that would allow the establishment of a good balance in the expression of selected traits of interest in desired grain compartments [33]. The integration of these technologies would allow researchers to identify novel genes or pathways that could be activated to improve the bioavailability of desired nutritional components in wheat. This chapter aims to highlight the progress and challenges encountered in attempts to improve nutritional quality in wheat in order to recommend strategies that could be deployed to improve nutritional quality in a more sustainable and efficient way. The most important research question that needs to be addressed is, what is the source or origin of the total grain nutrient content of minerals or phenolic acids found in different grain compartments? Thus, there is still a need to conduct a quantitative assessment of the total mineral nutrient use efficiency and the type of mineral used for plant metabolism and seed production.

## 2. Wheat

Wheat, grown in many parts of the world, is a major contributor to food security in that it is a staple food in other countries [1]. It has three main grain compartments such as the bran, endosperm and the germ. The wheat grain as a whole houses a series of nutritional health beneficial components ranging from macronutrients, micronutrients, vitamins, phenolic compounds and other components at different levels across various grain compartments [2]. The wheat grain is also a major contributor to the daily dietary intake required by individuals due to its regular consumption in various forms. Thus, regular consumption of essential nutrients at adequate levels could largely contribute to the reduction of nutrient deficiency-related ailments such as anaemia, growth and development problems, cardiovascular diseases, cancer, diabetes, neurological disorders, etc. [7].

Intriguingly, the endosperm region is the most edible part of the grain reported to contain less contents of Fe and Zn than the outer layers that are removed upon milling [18, 34]. Several efforts to establish the biofortification of wheat have been undertaken and some major challenges have been experienced. Little or no progress has been made to characterize the key biological process involved in the accumulation and bioavailability enhancement of Fe, Zn, vitamins and phenolic acids in this grain compartment [14].

Wheat has a complex genome and the complete genome sequence is not available yet. This makes it challenging to identify and understand the function of many genes in wheat, thereby making it difficult to characterize and manipulate complex traits of interest for the development of improved varieties. Further characterization of some traits is still needed for a continued contribution to better understand various gene networks/pathways and their role within the wheat genome to allow rapid development of improved cultivars with desirable traits of interest for a continued contribution to food and nutrition security. There are various wheat genetic resources ranging from landraces to wild relatives that may carry various genes of interest due to their genetic diversity [1, 35, 36]. Genetic resources have been utilized for crop improvement efforts in cases where information regarding a complex trait is not readily known, the information may then be inferred from closely related species with known biology [1].

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## 3. Progress in improving nutritional quality

The improvement of nutritional quality entails a series of processes to ensure that the nutrients are bioavailable upon consumption. The major process requires a genotypic and phenotypic characterization of key biological processes or pathways that are involved in the assimilation, accumulation, biosynthesis, translocation and remobilization of desired nutritional quality components such as Fe, Zn, vitamins and phenolic acids in the wheat grain [12, 15, 37–39]. The ultimate process involves the application of biofortification, which is the most sustainable approach that can reach the nutritional requirements of the global community in a cost-effective manner. However, the application of biofortification requires rudimentary information regarding the crop's genetic and phenotypic profile across different environments. Substantial progress has been made in attempts to improve nutritional quality in wheat. This includes the deployment of several strategies that involve the application of conventional, technological and transgenic approaches [14, 40].

Conventional-based approaches involve the use of basic genetic and agronomic practices, such as agronomic biofortification, soil+foliar application and genetic biofortification, which involves germplasm screening to reveal the genetic variation for grain Fe and Zn levels across different wheat genotypes grown in different environments [21]. Progress has been made to establish genetic variation of Fe and Zn across various wheat species. Along the process, an important quantitative trait locus (QTL) *Gpc-B1* from wild emmer wheat (*Triticum turgidum* ssp. *dicoccoides*) was discovered and mapped on chromosome arm 6BS [41]. The gene of this locus was then cloned and effectively improved Zn, Fe and protein concentrations by 12%, 18% and 38%, respectively [19]. The *Xuhw89* marker is linked to the *Gpc-B1* locus with a 0.1 cM genetic distance and can be used to identify and select lines with improved levels of selected micronutrients in the wheat grain [42]. In addition, several efforts have also been made to establish the genetic variation in the levels of phenolic compounds in some wheat species.

Technological-based approaches involve the application of advanced high-throughput analytical technologies such as ribonucleic acid sequencing (RNAseq), ribonucleic acid interference (RNAi), genomics, transcriptomics and metabolomics to discover and characterize candidate genes that could be used to improve nutritional quality. This may also include genome editing-based approaches such as the CRISPR Cas9 approach, which has recently been used in wheat [20]. Transgenic-based approaches mainly involve the application of genetic modification to improve nutrient accumulation in the wheat grain. Some minimal progress has been made with the application of transgenic approaches in attempts to improve nutritional quality [43, 44].

Several applications have been deployed to improve nutritional quality in wheat; some applications were successful but not sustainable and others were not successful [12, 13, 28]. Technological applications have also been deployed for wheat improvement. This includes success in increasing the bioavailability of Fe and Zn and decreasing the antinutrients such as phytic acid and polyphenols, which inhibit Fe absorption thereby reducing Fe bioavailability. However, a series of strategies to improve the bioavailability of micronutrients and phenolic acids have been deployed, this includes agronomic biofortification and the use of nutritional enhancers [27, 28, 38]. Micronutrients and phenolic acids have also been reported to be pres-

ent at high concentrations in the outer layers of the seed and in the wheat germ region than in the endosperm region [18, 34].

Nonetheless, there are challenges with the biofortification of wheat with other nutritional components. This is mainly due to an incomplete understanding of pathways involved in the translocation of desirable nutritional components into desired wheat grain compartments such as the endosperm. In addition, the bioavailability of micronutrients such as Fe is reduced due to its interaction with other anti-nutritional components such as phytic acid or the food matrix, which constitutes other nutritional or anti-nutritional wheat grain components [43, 45, 46]. Moreover, the wheat endosperm region was also reported to lack transporters that are essential for the translocation Fe into the endosperm region [14, 47]. little or no research has been conducted to manipulate the transporter proteins to translocate more Fe into the wheat grain. Little or no studies were conducted on the translocation of phenolic acids into the wheat endosperm, and there is less information regarding the translocation or transporters involved in the translocation of phenolic compound and vitamins into the endosperm region.

A number of attempts to address the above-mentioned challenges were undertaken through the application of various conventional, technological and transgenic approaches. Much progress has been made in attempts to understand key processes involved in the assimilation, translocation and biosynthesis of micronutrients into the wheat grain [12–15, 48]. However, there is still a great need to utilize selected omics technologies to further improve our understanding on processes involved in optimising the accumulation of essential nutrients in the wheat grain.

### 3.1. Establishing genetic variation

The establishment of genetic variation entails screening various wheat genotypes grown across different environments for their levels of total Fe, Zn, vitamins and phenolic acids found in the wheat grain with the aid of analytical instruments [49–51]. Most or all studies on genetic variation in grain Fe and Zn concentration reported on the total grain Fe and Zn concentration obtained in wholemeal flour. Little or no reports are available on establishing the genetic variation in grain Fe and Zn concentration in white flour. However, the Agricultural Research Council-Small Grain Institute of South Africa has reported some preliminary data on the levels of Fe and Zn found in white flour among some modern commercial wheat genotypes, which showed some degree of genetic variation at a local conference in 2016 (unpublished data).

Velu et al. [28] reported substantial progress made on screening more than 7800 wheat genotypes for their variation in Zn concentration in bread wheat, durum wheat, wheat landraces and their wild relatives from several studies conducted since 1983 until 2012. The studies from the paper revealed some genotypes that had the highest grain Zn concentration reaching as far as 142 mg/kg, whereas other wheat genotypes especially the improved adapted wheat genotypes showed little variation in grain Zn [28]. Amiri et al. [52] also reported the genetic variation for grain's protein, Fe and Zn concentration among 80 irrigated bread wheat genotypes, which showed some level of genetic variation. Gorafi et al. [53] also reported the assessment of genetic variation in grain Fe and Zn concentrations in more than 40 syn-

thetic hexaploid wheat lines and conducted further development of the wheat lines for use as genetic resources. Thus, various wheat genotypes showed a significant genetic variation in wheat grain Fe and Zn content. Consequently, wheat genotypes that contain the highest levels of Fe and Zn could be selected as donors to improve the levels of Fe and Zn in recurrent parents who have lower levels of Fe and Zn. However, it is imperative to ensure that important traits, such as grain yield, protein content, disease resistance and other agronomic traits, are not compromised upon the development of varieties with improved nutritional quality.

Genetic variation was reported in phenolic acid content of various wheat genotypes [54–56]. Thus, some progress has been made to selectively breed for genotypes with the highest phenolic acid content. However, more studies are needed to further confirm the genetic variation that exists in phenolic acids among different wheat genotypes through germplasm screening of other wheat genotypes including wild relatives and landraces.

Little or no research has been reported on the establishment of genetic variation on the concentration of vitamins, manganese, magnesium, copper, potassium, as well as concentrations of other anti-nutritional components found in the wheat grain. Nonetheless, [49, 50, 57] provided a report on the levels of tocol (vitamin E) content found in various wheat genotypes. However, more studies are needed in this field.

The establishment of genetic variation in minerals has led to the improvement of several wheat germplasms. The selected genotypes were used to improve the levels of Zn by more than twofold in other instances [28]. However, there are some drawbacks with conventional breeding, in that it may take several years to develop a new variety with improved nutritional quality. In addition, only the total grain Fe and Zn can be increased. Therefore, breeders have no control on improving the levels of selected nutrients into desired grain compartments.

### 3.2. Grain nutrient content

Wheat grain houses a number of nutritional components ranging from macronutrients, micronutrients, vitamins, amino acids, arabinoxylans and various other nutritional components [2]. These components vary in quantity due to the manner in which they are incorporated into different grain compartments upon seed formation. Thus, increasing the quantity of selected nutritional component might result in a decrease in other constituents [21, 43]. Hence, it would be ideal to optimize the production of desirable nutrients in a manner that could result in the reduction of non-targeted wheat grain components. However, this would be a major challenge in that some or most traits in certain organisms are quantitative and the expression of a selected trait could depend on the expression of more than one gene, thereby resulting in minimal expression or production of a desired nutritional component.

Starch, protein and cell wall polysaccharides (dietary fibre) are the major grain nutritional components that account for about 90% of the dry weight and minerals, vitamins, lipids, phenolic compounds and terpenoids are among the minor grain nutritional constituents found in wheat. A major component of the endosperm comprises about 80% of starch and about 10% of other constituents, including minerals and some phytochemicals, which are mostly concentrated in the wheat bran area [58].

The levels of minerals in several wheat varieties particularly Fe and Zn have been reported to be declined over more than five decades due to their dilution with starch [2]. Nonetheless, substantial progress has been made in improving the total grain nutrient content with micronutrients such as Fe and Zn. The second HarvestPlus Yield Trial has managed to improve the levels of 50 wheat lines through biofortification with a total grain Zn content, which was 75–150% more than the control lines used for the trial [28, 59]. Hidalgo and Brandolini [60] reported that the wheat bran region of some einkorn accessions and some bread wheat genotype had the highest levels of total tocols, including $\alpha$–tocopherol and $\beta$-tocopherol, in a study that screened the distribution of tocols across different grain compartments.

Agronomic biofortification, a traditional biofortification approach, which involves direct micronutrient uptake from the soil that gets remobilised into the grain, has been applied in wheat to improve the levels of Fe and Zn. Much progress has been made in the application of this strategy for the biofortification of wheat with grain Zn. This was done through the application of Zn fertilizers using the soil and foliar application method, which can result in about threefold increase in the total grain Zn concentration [21]. Several studies that involved the use of radioactive Fe and Zn were carried out to evaluate ways to gain better understanding of the remobilisation of selected minerals [19, 32, 61]. The studies largely contributed to depicting the manner in which micronutrients are translocated into seeds from various tissues. Feil et al. [62] reported that environmental conditions, particularly soil composition, largely influence the total micronutrient concentration of wheat grain. Thus, agronomic biofortification can facilitate nutrient uptake and ultimately improve the total grain Zn content. However, this process is mainly dependent on the availability of minerals in the soil or through provision from the fertilizer, thereby making it an unsustainable approach to utilize in improving nutritional quality.

### *3.2.1. Nutrient translocation into grain*

There are genes that contribute to the translocation of minerals, mainly Fe and Zn into the wheat grain. Nutrient uptake and translocation or remobilization are complex processes that are involved in seed nutrient loading to make up the total grain nutrient content [12, 15, 21]. The two major processes involved in nutrient uptake and translocation and/or remobilization are mainly dedicated for plant metabolism and seed production. In the case of plant metabolism, nutrients would be taken up, translocated or remobilized to specific tissues in response to growth and developmental requirements, including mineral deficiencies. Whereas in the case of seed production, the source of the total nutrient content found in the seed remains unknown because nutrient loading in the seed has been attributed to multiple processes including senescence and direct translocation with the aid of transporters [19, 32]. The process of moving micronutrients from the soil into the seeds is a complex process, which still requires further characterization. Waters and Sankaran [15] provided a review uncovering several processes involved in the improvement of seed mineral biofortification on various species, including wheat, and made a recommendation that the simultaneous enhancement mineral uptake from roots to shoots and ultimately remobilization into seeds would result in successful seed mineral biofortification.

Nutrient remobilization through senescence was reported to be more efficient in cases where the plant carries a Gpc-B1 locus derived from *T. dicoccoides* [21]. Wherein, Distelfeld et al. [42] showed that recombinant substitution lines (RSLs) carrying the *dicoccoides* Gpb-B1 allele had 12%, 18% and 38% more Zn, Fe and grain protein content (GPC), respectively, than (RSLs) carrying a Gpc-B1 locus acquired from durum wheat. Thus, there is a great need to distinguish whether the nutrients that are accumulated in the grain are excess nutrients that were committed for plant development in the leaves, which are translocated into the wheat grain upon senescence or whether they are accumulated and translocated into the wheat grain during different growth developmental stages. Consequently, there is a great need to trace the origin of nutrients found in different grain compartments.

#### *3.2.2. Transgenic approaches*

A transgenic approach that could enhance the Fe concentration in edible plant part is the overexpression of ferritin, an Fe-rich soybean (*Phaseolus vulgaris*) storage protein [63, 64], which completely degrades phytate in seeds. Ferritin is considered a more bioavailable storage form and is abundant in the endosperm amyloplasts, the widely consumed grain compartment [65]. Ferritin genes of soybean were introduced and used to produce transgenic rice lines, and the concentrations of Fe were doubled with the highest Fe level in the transgenic lines [63]. Recombinant soybean ferritin gene also increased seed Fe concentration in rice, under the control of an endosperm-specific promoter [66, 67].

However, ferritin overexpression possesses a disadvantage in transgenic crops as the accumulation of Fe might depend greatly on the soil composition, for example, transgenic tobacco (*Nicotiana tabacum*) continuously overexpresses ferritin under a 35S-GUS promoter [68] and Fe deficiency was widespread in the crop. Metals, such as cadmium, lead and nickel, which are toxic for human health, were found rich in ferritin-overexpressing tobacco plants, when grown in one of the tested soil [68]. Consequently, Fe accumulation within ferritin results in an iron deficiency in these transgenic tobacco plants [68]. Iron deficiency expresses ferrous Fe root transporters, which also uptake cadmium, thereby promoting cadmium accumulation in plants [69–74].

### 3.3. Candidate genes for nutritional quality enhancement

Nutrient biosynthesis and accumulation in the seed involves multiple complex processes. Phenolic acids are mainly synthesized from phenylalanine, a major precursor molecule for the phenlypropanoid biosynthetic pathway [39, 75, 76]. The biosynthesis of phenolic acids is mainly governed by several genes, which encode enzymes to carry out biochemical reactions involved in the production of selected phenolic acids. However, little or no information is known on the process that is involved in the loading of specific phenolic acids into different grain compartments. Micronutrient accumulation in the wheat grain is mainly dependent on the availability of soil mineral nutrients, which are taken up from the roots and then translocated to different plant compartments. In this process, a series of genes and active transport protein families are activated to facilitate in the nutrient translocation and remobilization process. The total quantity of micronutrients found in different grain compartments depend

on environmental circumstances and the growth stage in which micronutrients are taken up, translocated or remobilized from different plant tissues. Thus, it would depend on the nutrient soil status and the stage at which the selected nutrients are taken up. Nonetheless, there is little or no research on the characterization of the origin and starting concentration of the nutritional component attributed to specific concentrations obtained in specific grain compartments.

### *3.3.1. Genes involved in micronutrient accumulation*

Waters and Sankaran [15] reported genes implicated in the uptake of Fe mainly. No gene(s) that are involved in Fe uptake have been reported for wheat. Thus, there is still a need to characterize and identify genes involved in the uptake of Fe from soil to the seeds in wheat. Furthermore, [70, 77, 78] provided a comprehensive overview of genes and pathways involved mainly in Fe uptake from roots to other plant compartments. Waters et al. [61] conducted a more comprehensive investigation on the role of the *NAM-B1* gene, which affects Fe and Zn in wheat.

*Gpc-B1* locus from *Triticum dicoccoides* was mapped and found to enhance Zn and Fe concentrations and encoded a NAC transcription factor that was found responsible to accelerate senescence. Senescence, the programmed degradation of cell constituents makes nutrients available for remobilization from leaves to developing seeds [19, 42]. Kohl et al. [79] reported that some NAC transcription factors were upregulated in the glumes at 14 days after anthesis and were obviously associated with developmental senescence. During senescence, proteases are rapidly activated to degrade leaf proteins into amino acids [80]. Serine proteases are the most important family of proteases participating in nitrogen remobilization (NR) during grain filling, acting as major regulators and executors in wheat and barley [81].

In wheat and barley, the specific NAC and WRKY transcription factors, in combination with hormones (abscisic acid and jasmonic acid), have been shown to be involved in the regulation of transition between early grain filling and developmental senescence [79, 82, 83]. Zhao et al. [84] identified a novel NAC1-type transcription factor, TaNAC-S, in wheat, with gene expression located primarily in the leaf/sheath tissues. Overexpression of TaNAC-S in transgenic wheat plants resulted in delayed leaf senescence, which led not only to increased GPC but also to increased grain yields; thus, this result further verified the improved NR from vegetative organs to growing grain in transgenic lines [84].

### *3.3.2. Genes involved in phenolic acid accumulation*

Very little research has been conducted on the accumulation of phenolic acids. Ma et al. [39] reported five key enzymes, namely phenylalanine ammonia lyase (PAL), coumaric acid 3-hydrolase ($C_3H$), cinnamic acid 4-hydrolase ($C_4H$), 4-coumarate CoA ligase (4CL) and caffeic acid/5-hydroxyferulic acid O-methlytransferase (COMT), which are essential for the biosynthesis of phenolic acids. Ma et al. [39] also characterized gene expression patterns of nine candidate genes associated with phenolic acid biosynthesis during early and late grain filling stages, the most crucial growth stage in polyphenol accumulation [85, 86]. The study revealed that seven genes (*TaPAL1, TaPAL2, Ta4CL1, Ta4CL2, TaCOMT1, TaCOMT2* and *TaC3H2*) are highly expressed during the early stages of grain development among white, red and purple wheat. However, *TaC3H1* was the single gene that was expressed only during the later stage

of grain development. Finally, five genes (*TaC4H, TaPAL1, TaPAL2, Ta4CL2 and TaCOMT1*) showed higher expressions in both early and later grain developmental stages [39]. Hence, there is still a need to conduct studies to further characterize the process of phenolic acid accumulation in seeds.

## 4. The use of -omics technologies to improve nutritional bioavailability

Omics is a multidisciplinary study that refers to studies in applied biology that end with -omics, including but not limited to genomics, transcriptomics, proteomics, metabolomics, phenomics, epigenomics, nutrigenomics, vaccinomics, metagenomics and various others. These studies are mainly conducted through the application of several high-throughput technologies that mainly encompass qualitative and/or quantitative detection of novel or known genes (nucleotides), mRNA transcripts or transcription factors, proteins, metabolites and other parameters through genomics, transcriptomics, proteomics and metabolomics, respectively [87, 88].

The molecular data obtained through high-throughput technological applications are quite intense, comprehensive and may be complex in other instances. This could make the integration of omics data quite a challenging task if the experimental analyses were not designed to contribute to downstream data analyses. The integration of omics data would bring about a comprehensive overview of data on various biological variables, thereby allowing researchers to have a comprehensive manner in which they could study relationships among biological variables within a biological system. Thus, it would be possible for researchers to even predict the quantitative and qualitative effect an introduction or deduction that a selected element or compound could have in the gene network or pathway within a biological system. For example, it would be possible to predict what effect could high concentrations of Ca have on the phenotype or the production of a selected phenolic acid in a selected grain compartment (endosperm) [89]. The integration of these technologies would enable researchers to determine which gene region could be targeted to improve the levels of a desirable metabolite without affecting other biological systems. We would then be able to know what is the optimum level of fertilizer required to drive the translocation of minerals into the wheat grain, at which optimum growth stage, etc.

Biological systems are complex in that there are many biological variables that encompass biological processes, thus, making integrative analyses through omics approaches a major challenge due to several technological limits associated with analysing biological processes that entail a large number of variables [90]. Thus, there is still a great need for researchers to form consortiums aimed at integrating research efforts that contribute towards integrating the data obtained from the application of omics technologies to have an integrated biological system that will allow easier manipulation of data. Integrating multiple omics data is still a major challenge in that there are several computational issues associated with integrating a series of multilayer datasets [91, 92].

Phenomics involve the use of high-throughput non-invasive colour imaging, near infrared imaging, far infrared and fluorescence imaging technologies, which are capable of acquir-

ing several physical traits such as the plant structure, phenology, soil water content, canopy/leaf temperature, physiological state of the photosynthetic machinery as well as automated weighing and water efficiency usage measurement [93]. These technologies have the capability to provide solutions to genomics-enabled crop improvement through the high-throughput platforms that can be integrated with genomics-based platforms [93].

## 5. Concluding remarks and outlook

The era of the omics has largely contributed to our current understanding of various biological and physiological processes in wheat in a faster way through the provision of high-throughput data that have made a provision for researchers to understand and manipulate some complex traits in wheat. The high-throughput data generated from different omics technologies could expedite efforts aimed at improving our current understanding of other complex traits that have not been fully characterized and also allow researchers to easily manipulate complex traits to suit current and future research needs. However, this will depend on whether the output data from a specific omics technology will be in a format that could be linked with that of the other omics technology output data for combined analysis.

Furthermore, there is little or no research on integrating selected omics technologies in order to obtain a holistic overview of physical and biological processes to improve the bioavailability and stability of selected nutritional components, thereby improving nutritional quality in wheat. Nonetheless, there have been some attempts to integrate omics data in other fields of biology with some challenges experienced in trying to integrate omics data. Difficulties that could arise in integrating omics data could mainly arise from the fact that research in these areas is still at elementary stage and research objectives, and outputs from different research programmes were not outlined in such a way that the data could be linked or integrated. Thus, the research outputs should produce data that can be easily used for combined analysis of omics data for a holistic overview of the entire system.

Several research applications involving the use of molecular techniques, analytical techniques and biochemical techniques have been applied in attempt to improve nutritional quality in wheat to establish a platform that has allowed the application of biofortification of wheat with improved grain Zn. However, this only made it possible to improve the total grain Zn, mainly for wholemeal flour and not across specific wheat grain compartments; wherein the wheat endosperm would have been an ideal region that could have been targeted to enhance the concentration of Zn.

One major challenge is that research is mainly conducted independently across the world, and this makes it a major challenge on the turnover in which data are obtained. This leaves a gap in other areas of research in that some research aspects of the same research focus are left uncovered, making it a challenge to obtain a holistic view of the data generated. Thus, should researchers form consolidated consortiums aimed at addressing similar challenges, it would be easier to integrate the data generated in order to allow researchers to obtain a holistic overview of data generated to allow targeted manipulations of the system in a more controllable or desirable manner.

The era of genome editing has also received more attention, wherein recent advances in genome engineering and editing have made provision of a platform that allows scientists to predict and modify an organism's genetic code with more precision. Furthermore, metabolomics, phenomics, genomics and transcriptomics-based approaches may be integrated to address the major challenge in improving nutritional quality, which entails the characterization of the quantity and origin of the nutrient source that gets accumulated in different grain compartments in different levels.

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Chapter 18

# Wheat Antioxidants, Their Role in Bakery Industry, and Health Perspective

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Additional information is available at the end of the chapter

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**Abstract**

Wheat grains and its fractions contain significant level of antioxidant activity and many phytochemicals, such as phenolic acids (ferulic and vanillic acids), carotenoids, and tocopherol are beneficial in curing many disorders. The beneficial phytochemicals are mostly present in aleurone fraction of wheat bran. The phytochemicals and antioxidants present in wheat have several health benefits, such as their ability to act as antioxidants, immunoenhancers, and inhibitors of certain lesions, which have been demonstrated for phenolic. Many wheat antioxidants are similar to the antioxidants present in wheat, but their characteristics are also unique in nature. The regular consumption of these antioxidant compounds in whole grains is associated with a reduced risk of many heart diseases and several forms of cancers and improves the regulation of blood glucose. Wheat antioxidants play a vital role in bakery industry mostly in bread industry. People are getting aware to use the bakery products that are prepared from the white flour due to proper nutrition, healthy lifestyle, improved nutritional composition, and functional properties. In nutshell, wheat antioxidants including phytochemicals synergistically improve the health status of consumers by consuming the products having complete nutrition.

**Keywords:** wheat antioxidants, phytochemicals, bakery products, health perspectives

## 1. Introduction

Wheat (*Triticum aestivum* L.) is used as a staple food by human since the late Stone Age (ca. 6700 BC) [1]. It is also a promising source of bioactive compounds such as phenolic acids, tocotrienols, tocopherols, carotenoids, phytosterols, and flavonoids and antinutritional factors, such as phytic acids and oxalates. Wheat cultivars exert antioxidant activity due to the presence of phytochemicals, such as phenolic acids, carotenoids, anthocyanins, and tocopherols [2]. They are also enriched with basic nutrients, i.e., proteins, vitamins, and minerals including calcium, iron, zinc, phosphorous, etc. The percentages of the phytochemicals are greatly influenced by multiple factors, such as soil type, cultivar type, topography, temperature, and humidity [3].

The population is more diverting toward the consumption of natural antioxidants due to their safe status and effectiveness in the physiological system when compared to synthetic antioxidants. They neutralize the effects of free radicals, act as metal chelators, and terminate the oxidative enzyme inhibitors and reactive oxygen species (ROS) reactions [4]. These free radicals enhance the uncontrolled growth of cells, produce the genetic defects in DNA, and leak the antioxidant enzyme concentration from the cells [5]. Similarly, low-density lipoprotein (LDL) is responsible for the development of coronary diseases [6]. The polyphenols from the wheat have preventive role against reactive oxygen species through neutralizing the hydroxyl and peroxy radicals, thereby suppressing the lipid peroxidation [7]. The human body contains two antioxidant systems: enzymatic including glutathione peroxidase and superoxide dismutase (SOD) and nonenzymatic, i.e., vitamin C, β-carotene, vitamin E, and selenium [8]. Extraction is a process which is used for the recovery and isolation of phytochemicals from wheat cultivars. The quantification of phytochemicals from wheat is affected by multiple factors, i.e., extraction time, solvent, solute/solvent ratio, efficiency of mass transfer, temperature, and particle size [9]. There are frequently methods which are used to determine the antioxidant potential of wheat such as DPPH, ORAC, and FRAP. High-performance liquid chromatography and gas chromatography are direct indicators of antioxidant capacity [10].

## 2. Antioxidant potential of wheat grain

Rising investigations have proved that certain types of cancer, coronary heart disease, and potential health benefits are reduced by the intake of whole wheat. Phytochemicals and nondigestible carbohydrates are the beneficial bioactive factors present in whole wheat grain [11–14]. In wheat grain there are small molecular weight phytochemicals known as bioactive molecules. They consist of but they are not inadequate to carotenoids, phenolic acids, tocopherols, and lignans. Oxidative damage to the most important compounds such as enzymes and DNA by different mechanisms is prevented by these bioactive molecules. To dismiss the attack of reactive oxygen species (ROS) such as singlet oxygen molecule or hydroxyl radicals on biological molecules, bioactive molecules directly react with these ROS molecules [15].

There are an adequate number of bioactive compounds present in the wheat grains instead of aleurone which is mainly consist of protein granules. The wheat bran also contained

the major proteins in the wheat grain [16]. The above investigation indicates the utilization and production of "super bran" and other wheat-based food and components rich in bioactive molecule present in wheat. Other bioactive factors and bioactive molecule-rich wheat grain should be produced after studying the results of old investigations [17]. The bioactive compounds particularly the phenolics are found in the bran portion of the cereal grains and occur in different forms mainly free, soluble conjugated, as well as insoluble-bound forms [18].

In wheat bran, the availability of bioactive molecule potential can be influenced by postharvest treatment, milling practice, and storage conditions investigated in few investigations. The outcomes of the above investigations showed that yeast treatment and postharvest enzymatic particle size of bran and storing practices can considerably change bioactive molecule accessibility in food products containing wheat [19].

Remarkably, the bioactive molecules of selected food have been investigated in food products and the properties of processed food atmosphere on bioactive molecule accessibility in food products containing wheat. To have little health support, bioactive foods are dynamic and are well acknowledged in Ref. [15]. The accessibility and bioavailability of various ingredients from wheat are very beneficial regarding health perspective in targeting specific body organs. Collaboration of wheat bioactive molecule with different food compounds and also the end merchandise to process food environments such as thermal treatment on the universal bioactive molecular properties of wheat products are not understood. Lignans and phenolic acids present as wheat phenolics are known as bioactive molecules [20].

The bioavailability and availability of bioactives can be altered by food matrix. The bioavailability of bioactive molecules presents in wheat such as lignans and phenolic acids from different wheat-based food products and ingredients has been investigated in pilot human and animal investigations. The natural bioactive molecule rich in wheat-based functional food is very important to augment human benefits [21].

There are number of analytical techniques has been established for the determination of phytochemical composition, bioactive molecular properties of wheat grain and their fractions. Among different fluorometric and spectrophotometric methods, electron spin resonance (ESR) spectroscopy considered a better approach because it deals with the presence of free radicals and considered a better analytical method for wheat bioactive molecules. These analytical methods can be used on different botanicals and cereal grains [22].

### 2.1. Phenolic compounds present in wheat

Cereals are used as staple foods due to a promising source of nutrients including carbohydrates, vitamins, proteins, and minerals. They are also consisting of a wide range of bioactive compounds and exert health-promoting effects such as anticancer, cardio-prevention, diabetes, and aging [23]. These bioactive compounds exhibited multiple physiological mechanisms including antioxidant activity, enhancement of immune system, mediation of hormones and facilitation of substance transit via digestive tract, production of butyric acid in the colon, and assimilation of substances in the gut [24, 25].

Among these cereals, wheat (*T. aestivum* L.) is a significant source of minerals, proteins, water-soluble vitamins, and dietary fibers. The wheat grain is divided into three parts such as endosperm (80–85%), bran (13–17%), and germ (2–3%) and comprises all essential nutrients. Generally, wheat grain kernel contains carbohydrates (70%), protein (12%), water (12%), fat (2%), crude fiber (2.2%), and minerals (1.8%), respectively. Moreover, wheat grain kernel is a potential source of minerals, including magnesium, phosphorus, zinc, manganese, iron, selenium, copper, and potassium [26]. Likewise, wheat is also enriched with a wide range of bioactive compounds, including phenolic acids (136.8–233.9 μg/g), alkylresorcinols (AR) (99.9–316.0 μg/g), phytosterols (562.6–1035.5 μg/g), and tocols (19.3–292.7 μg/g) [27, 28]. Phenolic acids are widely distributed in different parts of grains, i.e., testa, pericarp, and aleurone [29]. In wheat, several phenolic acids are present such as chlorogenic acid, ferulic acid, caffeic acid, *p*-coumaric, and sinapic acid, respectively. These compounds are present as bound forms, respectively, as phenolic acids (85%) in maize, wheat and maize (75%), and rice (62%). Cinnamic acids have been categorized as bioactive ingredients of the diet because they are bound to structural compounds of the cell wall [30, 31]. The schematic representation of wheat grain fractions is shown in **Figure 1**.

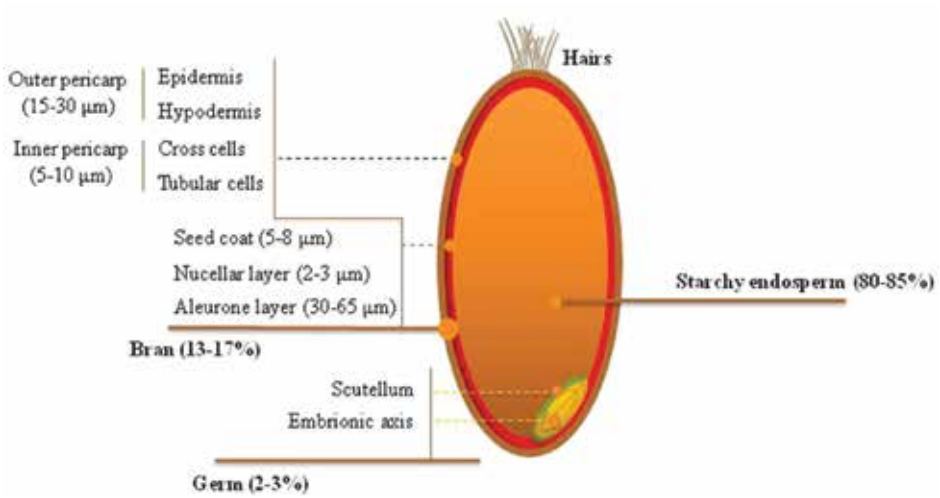


**Figure 1.** Schematic representation of wheat grain fractions.

Ferulic acid (4-hydroxy-3-methoxycinnamic acid) is present in different parts of vegetables, fruits, and grains as well as also endorses the health-promoting perspectives [29, 32]. Wheat flour is the prime ingredient, which is used to prepare different products of bread industry such as pasta. Wheat is also a promising source of dietary fibers along with preventing and curing some digestive disorders [33]. Endosperm is separated from the bran and germ through grinding, sieving, and purifying steps in conventional wheat roller milling. Furthermore, endosperm is also grounded to wheat flour on the basis of refinement and then used to prepare the bread, whereas bran, aleurone layer, starchy endosperm, and germ are used as milling by-products. Similarly, wheat bran is used by animals due to higher nutritional profile which exerts beneficial physiological effects. The bran-based products are shown with more health perspectives when

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compared to refined flour. Consumers are more interested to utilize the bran-based products, such as cookies, bread, pasta, breakfast cereals, cakes, snacks, and more [34].

## 3. Chemistry of wheat polyphenols

Wheat polyphenols are generally involved in defense mechanism against biotic and abiotic stresses which are secondary metabolites [35]. The first substrate of the phenyl propanoid pathway is phenylalanine, which initiates the biosynthesis of phenolic acids and then produces the different phenolic acids and flavonoids [36]. Similarly, wheat phenolic compounds are categorized into derivatives of hydroxybenzoic acid or hydroxycinnamic acid. In hydroxybenzoic acid derivatives, different compounds are present like gallic, vanillic, *p*-coumaric, hydroxybenzoic, and syringic acids, whereas hydroxycinnamic acids contain different derivatives, such as ferulic acid, dehydrotrimers of ferulic acid, *p*-coumaric acids, and dehydrodimers [37, 38].

Wheat antioxidants are located in wheat grain compartments such as the endosperm, bran, and germ [37]. The intermediate layer of wheat grain mostly composed of arabinoxylan and high amounts of ferulic acid monomers, whereas aleurone layer has lower content of ferulic acid dimers and trimers [39]. The phenolic contents present in bran/germ have 15–18 folds higher than that of endosperm, whereas only 17% of the total phenolic content is present in starchy endosperm [40, 41]. The phenolic compounds and antioxidant in wheat grain are shown in **Figure 2**.

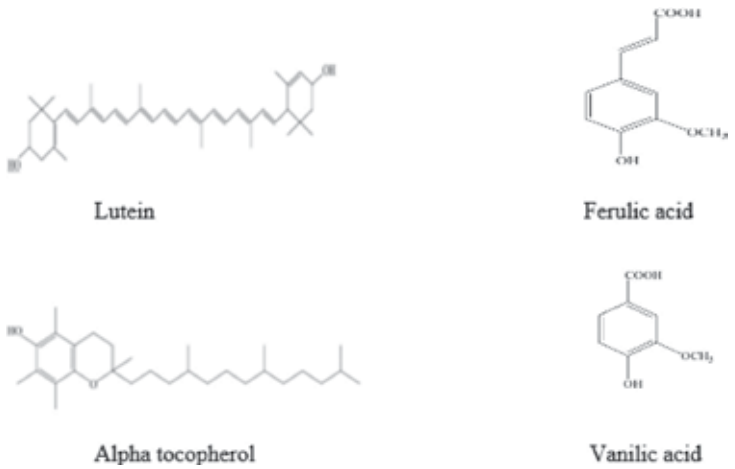


**Figure 2.** Phenolic compounds and antioxidant in wheat grain.

## 4. Wheat antioxidants in bread industry

Bread and bakery products have significant importance in human nutrition through preventing from the human disorders. These products are obtained from the white flour and a promising source of irreplaceable nutrients. People are getting aware to use the bakery products which are

prepared from the white flour due to proper nutrition, healthy lifestyle, improved nutritional composition, and functional properties [42]. The prepared bread is a significant source of dietary fibers, minerals, inulin, vitamins, omega-3 fatty acids, oligosaccharides, β-glucans, and flax seeds. Gluten is a protein which is not present in pseudocereal (buckwheat) [43]. Buckwheat is enriched with carbohydrates, proteins, fiber, and minerals along with reducing the blood pressure, cholesterol levels, blood glucose level, and prevention of cancer [44]. It is also a prominent source of balanced amino acid composition and essential amino acids [45]. Furthermore, buckwheat phenolic compounds comprise significant quantity of rutin, ferulic, and quercetin along with preventing from the lipid peroxidation and activity of free radicals [46]. They also show higher antioxidant activity mainly due to high rutin content [47]. The total phenolic contents of whole wheat and refined flour were reported as 1.58 and 0.87 mg FAE/g, respectively [48]. On baking and phenolic conditions, total phenolic contents were decreased to about 72% and 67% of the average content found in whole wheat and refined flour [49, 50]. The antioxidant activity of bakery products is mainly affected by processing conditions, mixing, fermentation time, baking temperature, and formulations [51]. Phenolic acid recovery after baking was 74–80%. In comparison to baker's yeast wheat bread, sourdough wheat (durum and kamut) offered more antioxidant protection [52].

## 5. Wheat antioxidants in breakfast foods

Wheat-based breakfast is proven effective by promoting the health-endorsing perspectives due to higher concentration of phenolic bioactive moieties. The acid and enzymatic hydrolysis increase the solubility of wheat bioactive compounds. Similarly, food processing conditions mainly affect the stability, distribution, and activity of wheat-based compounds [53]. The resultant breakfast of wheat has been used to prevent from the proliferation of type 2 diabetes mellitus through lowering the glycemic level in the postprandial phase [54]. The utilization of wheat in breakfast foods prevented the individuals from many disorders like obesity, hypertension, oxidative stress, diabetes complications, mental disorders, digestive ailments, and cognition due to the presence of diets higher in minerals and vitamins and lower in fat. These breakfasts are also used to reduce the body mass index and incidences of obesity and overweight [55, 56]. Similarly, wheat antioxidant-based breakfast significantly decreased hunger [57]. They also protect from the bowel disorder due to dietary fiber. They enhanced the hydrated fecal weight between 10 and 20 g/100 g diet from a baseline of 21±1.5 g/100 g diet [58].

## 6. Absorption and bioavailability of wheat antioxidants

### 6.1. Syringic acid, sinapic acid, vanillic acid, and *p*-hydroxybenzoic acid

The information regarding pharmacokinetic parameters or the absorption characteristics of syringic acid, sinapic acid, *p*-hydroxybenzoic acid, and vanillic acid is less. Therefore, it is need of the time to conduct some studies regarding the absorption of these particular acids. Moreover, these compound bioavailability is unknown. Which based on the derived innovative principle of bioavailability and absorption for phenolic acid, nevertheless, we can now assess their bioavailability and absorption effectiveness. The substrate of monocarboxylic acid transporter meets

the structural standards of all these compounds, that is, group of mono-anionic carboxyl and a component of aromatic hydrophobic. Each phenolic acid inhibits the transport of fluorescein, and they increased by the following order: syringic acid (105.9%)<sinapic acid (75.0%)<vanillic acid (56.2%)<*p*-hydroxybenzoic acid (35.5%) [59]. Compared to fluorescein transport inhibition by *p*-coumaric acid (85.2%), ferulic acid (52.4%), and caffeic acid (116.2%), each phenolic acid monocarboxylic acid transporter affinity increased by the following order: *p*-hydroxybenzoic acid>vanillic acid ¼ ferulic acid>*p*-coumaric acid, sinapic acid>syringic acid>caffeic acid. Hence, monocarboxylic acid transporter vigorously absorbed the *p*-hydroxybenzoic acid, vanillic acid, and sinapic acid through a mediated transport system.

Syringic acid absorption is particularly absorbed through paracellular diffusion; lesser amount is absorbed by monocarboxylic acid transporter, same in the case with caffeic acid [60]. Conjugating enzyme susceptibility, for instance, sulfotransferase and glucuronosyltransferase, bioavailability also affects by that enzyme. Vanillic acid, having a group of hydroxymethoxy aromatic ring, may be a good aim for conjugation [61]. The ferulic acid and vanillic acid affinities for monocarboxylic acid transporter, their conjugation susceptibility together, show that these two compounds have similar bioavailability. While the caffeic acid absorption efficiency is alike to the syringic acid, conjugation susceptibility is different. Component of catechol lacks in syringic acid and therefore unlikely to be conjugation [62]. These results propose a syringic acid has a greater bioavailability over caffeic acid. The understood fact, that is, syringic acid, *p*-hydroxybenzoic acid, and sinapic acid bioavailability, is alike to that of *p*-coumaric acid, while the vanillic acid bioavailability is alike to that of ferulic acid. Germano et al. stated that after hydrolyzed extraction from the root of *Trichilia emetica* including numerous phenolic acids which is orally ingested in rats such as free caffeic acid, syringic acid, *p*-coumaric acid, vanillic acid, and gallic acid, free vanillic acid absorption was comparatively efficient and fast [63].

The bran of oat powder rich in phenol-fed hamsters to find the total bioavailability of vanillic acid, sinapic acid, syringic acid, *p*-coumaric acid, *p*-hydroxybenzoic acid, and ferulic acid, including each phenolic acid, is present in conjugated and free forms, calculated by plasma Cmax/oral dose ratio [64]. The expected bioavailability and absorption of these phenolic acids, depend on our new protocol of fluorescein assay, are generally constant with these two reports. Nevertheless, a number of factors influenced by the bioavailability and absorption came from these studies (i.e., impacts of numerous phenolic acid medicated concurrently and considerable differences of bioavailability between intact and total phenolic acid). Both in vivo and in vitro studies must be done for accurate determination of engagement and bioavailability of these phenolic acids. It has already been performed for caffeic acid and *p*-coumaric acid [30, 65, 66].

### 6.2. Soluble, insoluble, and free conjugate-bound phenolic acid present in wheat

In grains, phenolic acid contributes the major portion, for instance, corn, wheat, and rice, which are typically esterified with arabinose or galactose in pectic and hemicellulosis residues in cell wall as well as occur as insoluble fraction (corn 85%, wheat 75%, and rice 62%) [67]. The major phenolic compounds present in grains is ferulic acid with free, soluble conjugated and bound form present in 0.1:1:100 ratios [67]. Furthermore, the major contributors to the total antioxidant activity are the bound phytochemicals e.g. 71% in rice, 90% in wheat, 58% in oats and 87% in corn. The health consequences of dietary phenolic acid in wheat based food

materials depends mainly on the bioavailability and absorption of soluble/ insoluble and free/ conjugate phenolic acids.

In case of insoluble fiber, the greater number of bound ferulic acid, for example, wheat bran, is believed to evade from the stomach or intestinal digestion or absorption to hold out the colon. Microbial enzymes of the colon, for instance, esterase and xylanase, release and solubilize feruloylated oligosaccharides (FOs) (i.e., feruloyl-arabinoxylan, 5-o-feruloyl-arabinofuranose) or free ferulic acid, just after reaching the colon. An esterase from mucosa acts on a part of bound ferulic acid during the flow within the gastrointestinal tract leading to the colon, and feruloylated oligosaccharides or free ferulic acid is released [68, 69]. The newly emancipated phenolic acid is absorbed through the gastrointestinal epithelium into the bloodstream jointly with ingested free phenolic acid and delivered to various tissues. The microbial esterases and mucosal substrates like 5-o-feruloyl-arabinofuranose are examples of soluble free and conjugate phenolic acids which are used for absorption of nutrients [70].

In short, phenolic acid in wheat which is of various forms (i.e., insoluble, soluble, and free conjugated bound forms) is converted by the breakdown reaction of free phenolic acid involving colonial and mucosal enzymes. These free phenolic acids destined to blood stream after distribution and metabolized into the body by the action of different enzymes. Wheat phenolic acids have different stages where various factors affects absorption and bioavailability. These factors include (a) the absorption of phenolic acids in the lumen of digestive system and capacity of the biomembrane and monocarboxylic acid transporter; (b) conjugating enzyme susceptibility, tissue having free phenolic acid metabolism, and transporter of monocarboxylic acid affinity; (c) ingestion of wheat phenolic acid, phenolic acid-bound content in conjugated, free, insoluble, and soluble; and (d) discharge of free phenolic content and soluble and insoluble bound of phenolic acid which are attacked by enzymes and the area of GI tract. In recent studies the effect of ingested food components having phenolic acid has been discussed in Ref. [71]. The health benefits of whole grain such as rice and wheat should be studied in detail to find the effect of different aspects on bioavailability and absorption of phenolic acid present in wheat.

## 7. Health perspectives of wheat antioxidants

There are multiple evidences which prove that utilization of wheat antioxidants is linked with the lower incidence of oxidative stress-related chronic diseases and age-related disorders, such as carcinogenesis, cardiovascular diseases, type II diabetes, and obesity. They perform health-endorsing perspectives due to the presence of vitamin C, vitamin E, carotenoids, phenolic acids, and flavonoids [25, 26]. They also facilitate digestion in human body by allowing the bound phenolics in the colon [67]. Similarly, they improve insulin and inhibit the tumor necrosis factor (TNF) alpha serum levels, lowering the serum cholesterol, fasting glucose, and triglyceride. They also exert anticancer effects on cell growth and apoptosis of human breast cancer cells such as MCF-7 and MDA-MB-231 [72].

The fact is that diet can completely change the life quality and human health. Wheat has numerous essential nutrients, which are important part of diet. It is one of the most dominating nutritious crops. Intake of whole grain or wheat reduces the cardiovascular risk and

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diabetes [73]. The antioxidants of wheat and the insoluble fibers impart the valuable properties. Nevertheless, metabolism and cholesterol biosynthesis are directly standardized through wheat antioxidants as compares to antioxidative agents, which are acting simply. The productive impacts are examined regarding the effect of antioxidants present in wheat on the enzymes which participate in cholesterol biosynthesis.

The expression of genes involved in metabolism and cholesterol biosynthesis are examined by the wheat antioxidants. Wheat antioxidants at a concentration of 0.12 mg/mL are treated with the rat hepatocytes which is equivalent to 2 mg of wheat grain /mL of medium for 24 hours. Ribonuclease protection assay (RPA) are used for the examination of mRNA levels of HMG-CoA reductase (HMG-CoA-R), LDL receptor (LDLR) and cholesterol 7R-hydroxylase (CYP7A1). CYP7A1 and HMG-CoA-R are rate limiting enzymes for the conversion of cholesterol to bile acids and cholesterol biosynthesis respectively. It is precisely evident that wheat antioxidants considerably boosted the breakdown of HMG-CoA-R mRNA due to the progressive studies of impacts of wheat antioxidants on the mRNA stabilities of HMG-CoA-R and CYP7A1 but rise the CYP7A1 mRNA stability. Wheat antioxidants also participate in continuous boosting of bile synthesis. The data concludes that the control of the important genetic factors participating in metabolism and biosynthesis may be illustrated as among basic and important mechanism on molecular/cellular bases due to which the dangers of heart-related disorders are reduced by wheat antioxidants [74].

The polyphenols from wheat have been proven effective against cervical cancer cells (HeLa) and colon cancer cells (HT-29 and Caco-2) due to their antioxidant potential and induction of apoptosis and inhibit the proliferation of uncontrolled growth of cell lines [75]. Similarly, in human neuroblastoma cells (SH-SY5Y), they enhanced the viable cell numbers by 37%, lowered the release of lactate dehydrogenase, suppressed the $H_2O_2$-induced formation of reactive oxygen species, and maintained the mitochondrial transmembrane potential. They also enhanced the Bcl-2/Bax ratio and blocked cleavage poly(ADP-ribose) polymerase by inhibiting caspase-3 activation [76]. They also exert anticancer effects on cell growth and apoptosis of human breast cancer cells such as MCF-7 and MDA-MB-231. Wheat bran is rich in alkylresorcinols (ARs), and having colon cancer cell lines (HT-29 and HCT-116) through increasing or decreasing the side-chain lengths, it diminished the activities. On the aromatic ring of the AR analogue, there are two hydroxyl groups at C-1 and C-3, greatly contributed to their antitumor activity. There is no significant enhancement in activity against HCT-116 cells on the third hydroxyl group at C-2 into the aromatic ring of the AR analogues [77]. Moreover, wheat bioactive compounds caused significant reduction in lipid peroxidation (LPO) and enhancement in glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT) in Swiss albino mice [78]. Similarly, the administration of 2,6-dimethoxy-1,4-benzoquinone (DMBQ) (24 μmol/l) from fermented wheat germ extract (FWGE) exhibited the antiproliferative properties in vitro in nine human cancer cell lines after 24 h of culture by causing cell cycle arrest, inducing apoptotic cell death and neutralizing the effect of reactive oxygen species [79]. Ferulic acid is another promising antioxidant of wheat that induced the apoptotic cell death in human breast cancer cells such as MCF-7, MDA-MB-231, osteosarcoma 143B, and MG63 cells lines in dose-dependent fashion through multiple mechanisms such as (1) induction of apoptosis; (2) caused G0/G1 phase arrest, enhancement of caspase-3 activity; (3) downregulation of

the expression of cell cycle-related protein, CDK 2, CDK 4, and CDK 6; (4) downregulation of Bcl-2 expressions, upregulation of Bax, and inhibition of PI3K/Akt activation [80, 81]. In another study conducted by [82], wheat ethanolic extracts provide protection to hepatic microsomes through reducing the proliferation of the HCT 116 and A549 cancer cell lines. The *in vivo* inclusion of β-Glucans has a great potential against number of conditions like tumor development and infections caused by fungal, viral, prozoal and bacterial pathogens [83]. It also activates the T-helper and natural killer (NK) cells, cytotoxic macrophages, and promotion of T-lymphocyte differentiation and activation [84]. Wheat polyphenols are used to resolve the problem of septic discharge from the ear, relieve ear pain, and eliminate scars [85]. These polyphenols also prevented from the oxidative damage of DNA, proteins, and membrane lipids as well as also protected from the incidence of cardiovascular and cancer [86]. They also suppress the LDL oxidation through binding with apolipoprotein B [87]. Alkylresorcinol from wheat bran suppresses the platelet binding to fibrinogen, stimulates the thromboxane production, and inhibits triglyceride accumulation. In human erythrocytes, they also prevent from the free radical-induced oxidative damage [88, 89]. Wheat antioxidants are also effective to reduce the accumulation of triglycerides, low-density lipoprotein, reactive oxygen species, and concentration of thiobarbituric acid-reactive substances (TBARS) and enhance the high-density lipoprotein, vitamin E, and vitamin A [90]. They also improved insulin, suppressed the tumor necrosis factor (TNF) alpha serum levels, and lowered the serum cholesterol, triglyceride, and blood sugar [72]. The bioactive compounds, such as ferulic acid, tocopherols, and carotenoids, significantly increased the liver glycogen and lowered the glycosylated hemoglobin levels and blood glucose [76, 91, 92].

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The Wheat Improvement, Management, and Utilization book covers some of the most recent research areas that touch on enhancement of wheat productivity. It is obvious that wheat is one of the major staple crops grown globally. This crop has widely been researched on considering that, for instance, it is afflicted by various abiotic and biotic stresses that limit its growth and productivity. Today?s goal of wheat improvement consistently is to develop varieties that are high yielding with good processing and technological qualities, well adapted and tolerant to prevailing biotic and abiotic stresses. Therefore, this is a valuable reference book on wheat improvement, agronomy, and end-use qualities, particularly for those who work in research organizations and higher academic institutions. Moreover, it provides an invaluable resource for readers interested in a quick review of trending topics in wheat.

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