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# Progress and Challenges in Improving Nutritional Quality in Wheat

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Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/67230>

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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

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## 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

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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 bio-availability 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  $\text{Ca}^{2+}$ ,  $\text{Zn}^{2+}$  and  $\text{Fe}^{2+}$  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

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  $\beta$ -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].

### 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 *Xuhtw89* 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 tocopherols, including  $\alpha$ -tocopherol and  $\beta$ -tocopherol, in a study that screened the distribution of tocopherols 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 over-expression 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 phenylpropanoid 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-methyltransferase (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**

Omic 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 omic data quite a challenging task if the experimental analyses were not designed to contribute to downstream data analyses. The integration of omic 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 omic 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 omic technologies to have an integrated biological system that will allow easier manipulation of data. Integrating multiple omic 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.

## Acknowledgements

The authors would like to acknowledge funding from the Department of Agriculture, Fisheries and Forestry through the National Research Foundation of South Africa and also Agricultural Research Council.

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