# Biofortification of Wheat Using Current Resources and Future Challenges

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## Abstract

Wheat is the second most significant staple food grain crop after rice; however, its grains mostly contain suboptimal levels of provitamins, proteins, and essential micronutrients, including zinc, iron, selenium, and iodine. However, during processing, wheat flour is enriched or fortified with several required nutrients. The most reasonable, long-lasting, and viable solution for this problem is biofortification that can be performed through either agronomic approaches, breeding efforts, or transgenic techniques. Agronomic fertilization techniques for wheat biofortification include basal application, foliar spray, and seed priming with the appropriate nutrient sources. Recently, various potent bacterial strains have been used, and these techniques can be used in combination with agronomic and genetic techniques to significantly enhance the density of the nutrients that require to be supplemented in wheat grains. Compared to agronomic approaches, breading techniques are more sustainable and include conventional and marker-assisted breeding. Transgenic approaches for micronutrient biofortification of

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R. Deshmukh et al. (eds.), *Biofortification in Cereals*,
https://doi.org/10.1007/978-981-19-4308-9_7

wheat include modulation of the gene expression of transporters to improve the absorption rate and assimilation capability of the wheat plant while lowering antinutrient content. In this chapter, along with the possible techniques of biofortification, we discuss the mode of uptake and deposition of the desired nutrients in the grain at molecular and physiological levels. We discuss the possible wheat genomic obstacles that hinder wheat biofortification as well as the economic and social challenges for the release of biofortified wheat.

**Keywords**

Wheat biofortification · Micronutrients · Provitamin · Transgenic approaches · Breeding tools · Malnutrition · Hidden hunger

## 7.1 Introduction

Wheat (*Triticum* spp.) is one of the very important staple cereal grain crop that is produced, consumed, and traded globally. This grain is a major source of food, feed, protein, and nutrition for human beings and animals (Mayer et al. 2014). Therefore, the consumption rate of wheat is on the rise across the globe. China is the largest consumer of wheat, followed by the European Union (EU), and India. The cultivation of wheat has been increasing following its domestication about 8000 years ago; presently, it is widely grown in the world. About 214 million ha area is currently being used for wheat cultivation across the world, and wheat is considered the single largest trade crop in the world, in comparison to all other crops together (FAO 2018). Wheat is a widely consumed crop that can be used for several food products, such as alcohol, bread, and baked goods. Thus, wheat production is an important contributor to the agriculture economy and influences the gross domestic product (GDP) of developing countries (Kiss 2011). However, the world wheat export is dominated by a few countries and is directly connected to the international wheat trade and price determination. Price changes in the wheat market may increase the overall food prices in the future.

The production yield of wheat in the world during the period of 2001–2019 was accessed and showed the declining trend in the cultivation area; however, the production is on the rise, relative to the planted area, probably owing to modern cultivation practices and the use of technology (Fig. 7.1). Figure 7.2 shows information about wheat production in the world's major areas; Asia produces a major proportion (43.7%) of the total wheat produced in the world followed by Europe and America. Wheat cultivation is largely classified in developing and developed countries. When ranked as per the volume of wheat produced during the period from 2001 to 2019, China ranked first (114 M ton), followed by India (83 M ton), and the USA (56 M ton); these three countries account for roughly 54% of the total global wheat production (Fig. 7.3). The next-largest wheat producers are Russia, France, Canada, Germany, Pakistan, Australia, and Ukraine that together account for 45% of the global wheat production (FAO 2020). However, it is noteworthy that

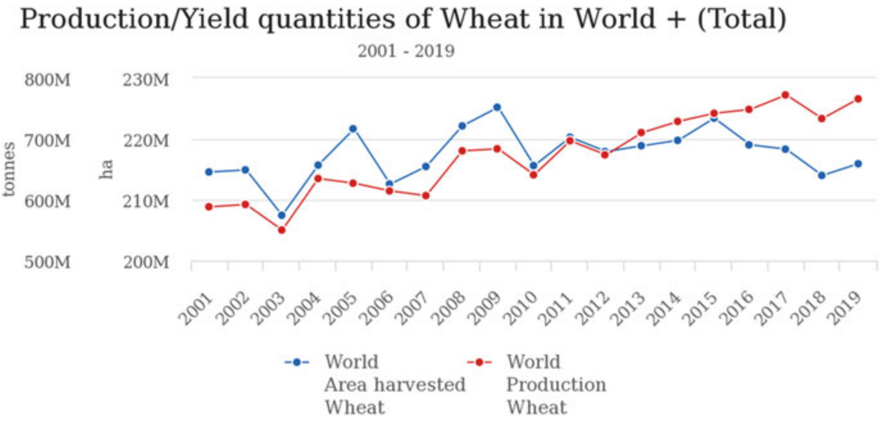

**Fig. 7.1** Worldwide wheat production and cultivation area during the period from 2001 to 2019

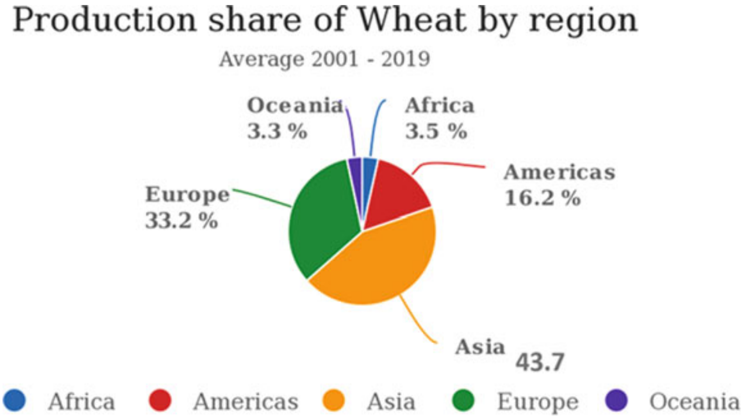

**Fig. 7.2** Region-wise average wheat production during the period from 2001 to 2019

wheat production in the USA has reduced significantly during the previous decades. The wheat trade is worth about US $50 billion annually across the world. Among the world regions, in 2019 South East Asian countries earned their most revenue from overall wheat imports, contributing $16 billion (40.6%) of the world total imports. Africa ranked second, followed by Europe with wheat revenue importers at 22.2% and 20.9%, respectively. The per capita GDP of the top 10 wheat-growing countries showed significant change (Table 7.1). The major wheat exporter countries Argentina, Australia, Canada, EU, Russia, Ukraine, and the USA showed higher per capita GDP. However, an increasing trend has also been observed for the per capita GDP of China, India, and Pakistan during the previous two decades (World Bank Data).

Wheat production is divided in developed and underdeveloped countries; irrespective of the wheat-producing group, mostly hunger is a huge issue across

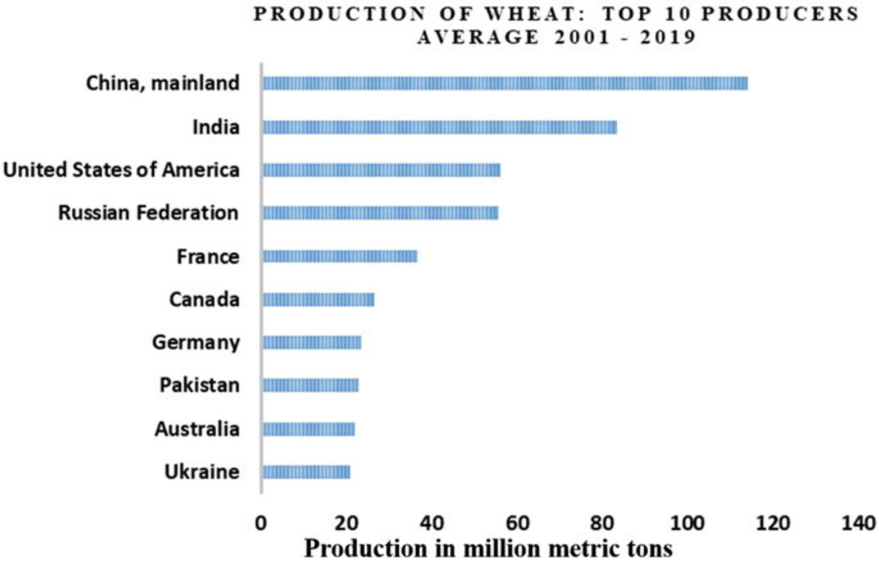

**Fig. 7.3** Top ranked average wheat-producing countries during the period from 2001 to 2019

**Table 7.1** Details of economic status and hunger index of major wheat-growing countries worldwide

| Country name | Country code | Population[a] | Wheat production[b] | GDPA per capita[c] | Hunger index[d] | Child[e] |
|---|---|---|---|---|---|---|
| China, mainland | CHN | 1,427,647.79 | 114,333,057.9 | 10,261.7 | <5 | 5.2 |
| India | IND | 1,352,642.28 | 83,480,287.68 | 2104.1 | 27.2 | 37.9 |
| United States of America | USA | 327,096.27 | 56,257,303.11 | 65,118.4 | — | — |
| Russian Federation | RUS | 145,734.04 | 55,764,659.42 | 11,585.0 | 5.2 | 10.7 |
| France | Fra | 64,990.51 | 36,814,143.16 | 40,493.9 | — | — |
| Canada | CAN | 37,074.56 | 26,772,996.58 | 46,194.7 | — | — |
| Germany | GER | 83,124.42 | 23,530,102.32 | 10,006.1 | — | — |
| Pakistan | PAK | 212,228.29 | 22,953,457.68 | 1284.7 | 24.6 | 37.6 |
| Australia | AUS | 24,898.15 | 22,064,214.05 | 55,060.3 | — | — |
| Ukraine | UKR | 44,246.16 | 20,916,046.53 | 3659.0 | <5 | 6 |

Note: —, Data not available or not presented

[a] 1000 persons (unit)

[b] Average wheat production in tons (2001–2019)

[c] GDP per capita (current US$) (2019)

[d] Hunger index (2019)

[e] Child malnutrition index; source of information FAO http://www.fao.org/faostat/en/#data/OA, and https://databank.worldbank.org/

the globe. The severity of this problem is more prominent in developing countries, with a higher impact observed in rural areas (von Grebmer et al. 2019). Poverty is a major cause of hunger in most countries. As per the Global Hunger Index (GHI) 2019, among the top 10 wheat-producing countries, India ranks the first in terms of hunger, with a hunger index of 27.2, followed by Pakistan (Table 7.1) (von Grebmer et al. 2019). Although abundant natural and agricultural resources are available in both of these countries, they face the problem of hunger, owing to several reasons, such as high population, poverty, and less per capita income in the region (Table 7.1). In addition, child mortality and malnutrition are also associated with the hunger index; India and Pakistan have the highest prevalence of child mortality and malnutrition among the top 10 wheat-growing or producing countries (Table 7.1). In contrast, most countries that belong to the wheat export group showed lower hunger index, child mortality, and malnutrition.

About 98% of the population with a higher hunger index belongs to developing regions. Furthermore, <780 million people from the Asia Pacific region and sub-Saharan Africa still face major hunger crises and go to bed without food every night (McGuire 2015). Malnutrition is presently a growing problem affecting children and adults all over the world (Dukhi 2020). Despite the record production of cereal grains, vegetables, and other food products, malnutrition (including overweight, fat, and undernourishment) and hunger remain to be the major problems across the world. In fact, world hunger and malnutrition cannot be resolved only by increasing food production and supply. The eradication of world hunger and malnutrition can only be achieved if we precisely identify the root cause of the problems. The major cause of these issues is poverty. Several efforts, as discussed below, are required to completely eradicate hunger and malnutrition.

It was considered that growth potential is higher for the sector of agriculture than for any other sector for reducing poverty. Therefore, the role of small-scale farmers in agricultural development in concurrence with policies that facilitate the poor increase of access to food will be vitally important for reducing mass poverty and hunger in the world. Furthermore, improving the economic level of people by establishing government policies, developing systems that enable secure access to food, increasing awareness, and changing behaviors toward the use of a nutritional diet can help to reduce hunger in the world. We need to accelerate progress in raising the income of the poor, ending hunger, and ensuring a more sustainable system for food distribution and use. Recently, the World Bank Group presented the action plan to "Ending Poverty and Hunger by 2030: An Agenda for the Global Food System." In addition, food fortification and biofortification are important for handling malnutrition across the globe (Bouis and Saltzman 2017). Moreover, there is a need to enhance the existing health system policies and upgrade and revise the child malnutrition research and interventions, starting from the community leveling up to the regional and national levels. Further, the need of the hour is to close the sex gap, sex-based inequality, and give equal opportunities to women in employment, assets, and decision inputs. The participation of women in agriculture services could increase the farm yield by 20–30% and could eliminate hunger in 12–17% of all hungry people across the world (Doss 2018). In addition, providing health support and micronutrient supplementation to underweight mothers is crucial for healthy childbirth; varied forms of micronutrient supplementation are also crucial for ensuring the health of the population (Caron et al. 2017). Moreover, stable political conditions and leadership with the required political will can influence economic growth and social reforms and help protect targeted vulnerable population groups. Medium- and long-term participation of all the abovementioned stakeholders is an urgent need in tackling the issues of hunger and malnutrition.

Developing countries have a higher population of individuals who are below the poverty line and cannot afford a fortified diet, consisting of fruits and animal food products. Moreover, most of these people consume staple crops, including wheat, rice, and maize. Wheat is consumed by >2.5 billion people worldwide, with most of them being from developing countries (CIMMYT 2017; Lobell et al. 2011). Wheat accounts for almost 20% of the overall calorie intake received by humans (FAO 2017). However, the current high-yielding wheat varieties are deficient in basic and essential elements (minerals or micronutrients), such as zinc (Zn), iron (Fe), selenium (Se), and iodine (I). As per the WHO, one in every three women of reproductive age has Fe deficiency (FAO 2018). During 2010, Fe deficiency alone caused a loss of >45,000 disability-adjusted life years (Murray and Lopez 2013). Furthermore, Zn malnutrition affects around 17% of the worldwide population, subsequently leading to malnutrition and increased child mortality (Wessells and Brown 2012). Therefore, a population where wheat grain is used as the prime source of diet can develop micronutrient deficiencies that subsequently lead to malnutrition. To address this serious issue, biofortification is an attractive, sustainable, and long-term effective strategy that can overcome micronutrient deficiency. This approach is more cost-effective for staple crops than other fortification approaches or dietary supplements (de Valença et al. 2017). Biofortification can be achieved via breeding, genomics, or transgenic strategies (Bouis and Saltzman 2017; Ludwig and Slamet-Loedin 2019). Considering the human health implications, biofortification with micronutrients has become the prime focus of several staple crop breeding research.

## 7.2 Top Priorities for Wheat Biofortification

All organisms including plants and animals require most micronutrients and proteins because of their pivotal roles as cofactors in enzymatic activities and regulators in a number of significant biochemical activities. The importance of Zn, Fe, Se, I, provitamins, and protein in plants and humans is discussed in the following sections.

### 7.2.1 Zinc

Basic and essential micronutrients, such as Zn, Fe, Se, and I, are important elements for animals as well as plants. These micronutrients are required by all organisms in small amounts; however, they play a very crucial role via several mechanisms. Zn is the most abundant transition element and is considered a key micronutrient that plays a diverse role in maintaining physical health, growth, development, immunity, reproductive health, and mental health in humans (Dapkekar et al. 2020). In a similar manner, in plants, Zn regulates several metabolic and physiological process, acts as a cofactor in several enzymatic pathways, and participates in lipid, protein, carbohydrate, chloroplast, and nucleic acid synthesis (Palmer and Guerinot 2009; Hänsch and Mendel 2009). Deficiencies of Zn in animals can cause growth stunting, malnutrition in adults and children, and other severe implications, such as weak immunity, learning disabilities, diarrhea, and impaired wound healing. Likewise, it causes a decrease in growth and development, stress tolerance, as well as photosynthesis in plants (Kawachi et al. 2009; Lee et al. 2010).

### 7.2.2 Iron

Like Zn, Fe is also an abundant mineral element in nature that is required for all organisms for several functions. In both plants and animals, it participates in a variety of biochemical and physiological activities. Being an active redox element, it involves several plant functions, such as plant hormone regulation, nitrogen assimilation, photosynthesis, and mitochondrial respiration. In addition, it participates in electron transport, scavenging, and the production of reactive oxygen species and thereby protects the plants from abiotic stress (Palmer and Guerinot 2009). Fe deficiency in plants can cause leaf chlorosis, inhibit growth, and increase the susceptibility for several plant diseases and yield (Chatterjee et al. 2006; Cakmak 2002). Similarly, in humans, Fe is involved in electron transfer and the transport of oxygen which is important for myoglobin and hemoglobin synthesis. Fe deficiency has several implications in humans and leads to malnutrition, stunted height, reduced learning ability, fatigue, and nutritional anemia in children and adults (Dapkekar et al. 2020; Thomas and Frankenberg 2002). Moreover, reproductive-age women are severely affected by Fe deficiency that may lead to poor pregnancy outcomes and high child mortality (Bailey et al. 2015).

### 7.2.3 Selenium

Selenium (Se) is another element found in soil (1.0 and 1.5 μg $g^{-1}$). Commonly, it occurs in the form of organically bound selenium, selenide, and selenite. Although Se is considered nonessential to plants, selenate is a dominant and available form to plants. In several regions of the world, Se deprivation has been recorded (Combs 2001; Fordyce et al. 2000; Lin et al. 2004; Sempértegui et al. 2003). Se insufficiency is not a common or major social problem in humans. However, insufficient Se intake has been identified in several parts of the world, especially Africa, Europe, Finland, New Zealand, Australia, and Russia (Combs 2001; Dorea 2002; Lyons et al. 2003; Rayman 2000). In humans, Se is involved in several metabolic regulatory processes that involve proteins, hormones, and the antioxidant defense system (Fordyce et al. 2000; Rayman 2000; Tan et al. 2002). Inadequate intake of Se may cause several health issues, such as cardiovascular diseases, hypothyroidism, reduced male fertility, cancer, and HIV/AIDS (Rayman 2000, 2002; Kupka et al. 2004). Furthermore, Se consumption is linked to Keshan illness and Kashin-Beck disease (Lyons et al. 2003). In addition to that in humans, other creatures, such as goats, sheep, cows, and pigs, require Se for a variety of functions (Gupta and Gupta 2000; Meschy 2000; Tinggi 2003).

### 7.2.4 Iodine

Broadly, iodine (I) is a trace mineral that is considered a nonessential element for plants; however, in some aquatic plants, it is involved in antioxidant metabolism. Plants grown in soil that is rich in iodine content can absorb the I and provide it to humans via diet. Sufficient intake of I is very essential for humans because I is required for thyroid metabolism, which is essential for the growth and development. In addition, I shortage causes a wide range of health problems in humans. For example, it has been linked to the development of several types of cancers, nervous system impairments, mental retardation, cretinism diseases, deaf-mutism, stunting, and death in children (Bailey et al. 2015; Cakmak et al. 2017).

### 7.2.5 Provitamins

In addition to essential micronutrients, provitamins are essential for organisms. A provitamin is a substance that can be converted within the body to a vitamin, such as a plant that synthesizes ergosterol that when eaten as plant food by animals can form ergocalciferol (vitamin D2) under the exposure of ultraviolet light (Brody 1999). Widely known provitamins are “provitamin A” which is a name for β-carotene, “provitamin D2,” “provitamin D3,” and menadione (vitamin K). Here we discuss vitamin A.

Vitamin A essential nutrient is commonly known as β-carotene. In plants, tocopherols and β-carotene, two precursors of vitamin A, are found in the leaf or photosynthetic organ in most plants. However, other forms (α-carotene and β-cryptoxanthin) and tocotrienols are not consistently found in the plant across the species. It is mainly synthesized in the plastids and plays distinct functions in plants and animals (Asensi-Fabado and Munné-Bosch 2010; Medrano-Macías et al. 2016). All the provitamins, including vitamin A, are important regulators of cellular metabolism in plants, are involved in several enzymatic reactions, and act as antioxidants. Particularly, vitamin A is involved in improving light harvesting and photoprotection, stress signaling, and growth and development of the root and the shoot. Vitamin A function in humans is greatly related to vision, dark adaptation, and enhances immunity (Lima et al. 2010; Wiseman et al. 2017). In humans, deficiency of vitamin A may lead to anemia via infusion of Fe metabolism, reproductive health issues, susceptibility toward several infections, and gastric issues (Wiseman et al.

2017; Brown and Noelle 2015; Hogarth and Griswold 2010; Clagett-Dame and Knutson 2011; West and Mehra 2010; Semba et al. 1992).

### 7.2.6 Protein

Protein commonly comprises 20 different amino acids (AA) in different concentrations; peptide linkages connect these amino acids (Wu 2013). The word "protein" derived from the Greek word "proteios," which means "principal" or "first." It is a crucial component of both human and animal cells. Protein is considered an essential macronutrient, and unlike minerals and vitamins that can be stored in the human body, proteins cannot be stored based on daily intake. Proteins are sources of AA (indispensable and dispensable) and provide nitrogen, hydrocarbon skeletons, and sulfur. Proteins are needed in larger amounts in the body for the maintenance of homeostasis. The two main sources of protein are animals and plants. Plant protein is relatively less digestible than animal protein. Proteins are important for human cell function; they provide AA (indispensable and dispensable) for humans, for physical and normal metabolic functions. Protein deficiency is associated with several diseases, such as Kwashiorkor, that increase an individual's susceptibility to metabolic and infectious diseases (Dasgupta et al. 2005; Wu 2016). In addition, excess protein intake causes hepatic or renal dysfunction (Hoffer and Bistrian 2012).

Globally, the deficiencies of Zn, Fe, Se, I, provitamin, and protein have serious implications on human health. Malnutrition due to deficiency of these essential micronutrients and protein causes serious health issues in billions of individuals, most predominantly affecting those in developing countries (Ritchie 2017). Individuals in these developing nations mainly consume cereal-based staple food. It has been well documented that wheat, rice, and maize have a low content of micronutrients and inadequate protein concentration (Dapkekar et al. 2020; Zou et al. 2019). Wheat is a prominent staple grain crop; yet, the currently developed varieties of wheat inherently have lower micronutrients and protein concentrations (Cakmak et al. 2017; Zou et al. 2019; Zia et al. 2015). Therefore, enhancing the nutritional composition of wheat as regards Zn, Fe, Se, I, vitamin A, and protein concentrations is widely recognized as the top priority for improving public health. The best suitable approach for improving the concentration of these vital elements is biofortification via conventional breeding or biotechnological and genomic approaches.

## 7.3 Agronomic Biofortification of Wheat

In the previous years, there has been a surge of enthusiasm in boosting the health advantages of cereal, such as improving its mineral and vitamin content, improving their production, and disease resistance (Vasconcelos et al. 2017). Biofortification is performed to improve the micronutrient content of the grain, rather than the endosperm tissue. The two main biofortification approaches are agronomy and genetics, which comprise traditional breeding, gene editing, and genetic manipulation. Agronomic biofortification can increase Zn content in crops by supplementing with Zn fertilizers; for instance, foliar application of $ZnSO_4$ can increase Zn content in grains by about 60% (Zhang et al. 2012). However, these agronomic techniques are not much beneficial for Fe biofortification, unless supplemented with enhanced chemical fertilizers (Aciksoz et al. 2011) that are not economically and environmentally suitable.

The International Crops Research Institute for the Semi-Arid Tropics (India) is employing traditional breeding to develop cereal (sorghum and pearl millet) cultivars with higher nutritional content, particularly Fe. Moreover, the International Maize and Wheat Improvement Center (Mexico) is working to improve the Zn content of wheat (Velu et al. 2018). The CIMMYT developed Zn biofortified lines that are currently being grown in India and Pakistan. These lines provide around 20–40% higher level of Zn, with overall production comparable to that of the best local cultivars (Velu et al. 2018). Furthermore, in Pakistan, human interference efforts to evaluate the bioavailable Zn in biofortified lines are presently underway (Lowe et al. 2018). However, no improved Fe or other micronutrient wheat lines have been developed thus far with conventional breeding across the globe, including at CIMMYT, irrespective of several research programs having been conducted.

## 7.4 Breeding Efforts for Wheat Biofortification

Biofortification of essential micronutrients into crops is generally achieved using the following approaches: transgenic, genetic, and agronomic approaches, which use biotechnology, plant breeding, and fertilizer methods, correspondingly (Garg et al. 2018). Latest developments in plant molecular breeding science, as well as the advent of contemporary genomics technologies, have greatly enhanced our knowledge of the genetic components to ultimate crop growth, evolution, and performance, including yield. It is well understood that qualitative traits are controlled by a single gene, whereas quantitative traits, like yield, are typically influenced by multiple genes (Sempértegui et al. 2003; Dorea 2002; Lyons et al. 2003). The Zn, Fe, Se, I, provitamin, and protein content traits in wheat are quantitative. It is easier to breed the crop for qualitative traits than for quantitative traits via conventional breeding. Using conventional breeding methods, enriched Zn content-biofortified wheat varieties have been successfully developed by CIMMYT. These released Zn-biofortified varieties are "Zn Shakti" (Chitra), "Znol 2016," "WB02," "HPBW-01," and "BARI Gom 33" that have been created using different background varieties, such as PBW343 and NARC2011 (Bouis and Saltzman 2017; Das et al. 2019; HarvestPlus 2019).

Several genetic methodologies are well established for wheat biofortification, such as genome-wide association study (GWAS), quantitative trait loci (QTL) mapping, marker-assisted selection (MAS), and omics (Abid et al. 2017; Adams et al. 2002; Bohra et al. 2016, 2019; Mérida-García et al. 2019). In addition, the advent of genome sequencing technologies has offered new opportunities for determining the significance of functional genetic links of plants in phenotype heterogeneity as well as the unpredictability of complex traits across and within species. In wheat, grain is the primary objective component for human utilization and the food business. However, given the micronutrient deficiency or low levels of micronutrients (Fe, Zn, Se, I, etc.) in several modern wheat cultivars, many wheat-breeding programs have employed diverse strategies for wheat biofortification to enhance the nutrient quality and nutraceutical compounds of wheat grain.

To achieve this, plant breeders and other research groups have identified multiple putative QTLs associated with wheat with varying content of micronutrients (Fe, Zn, Se, I, etc.) and proteins (Blanco et al. 1996; Guo et al. 2020; Olmos et al. 2003; Prasad et al. 1999). These QTLs were identified using linkage mapping and the GWAS approach (Mérida-García et al. 2019; Alomari et al. 2018; Bhatta et al. 2018; Crespo-Herrera et al. 2016). These approaches used various types of mapping populations that include recombinant inbred lines (RILs) (Crespo-Herrera et al. 2017; Krishnappa et al. 2017; Wang et al. 2017), with single seed descent (SSD) (Parker et al. 1998), doubled haploid (DH) (Perretant et al. 2000; Shi et al. 2008), and near-isogenic lines (NILs) (Krishnappa et al. 2017). In order to identify the desired traits, researchers used a variety of molecular markers, like restriction fragment length polymorphism (RFLP) (Parker et al. 1998), amplified fragment length polymorphism (AFLP) (Elouafi et al. 2001), single sequence repeat (SSR) (Krishnappa et al. 2017), and single nucleotide polymorphism (SNP) (Alomari et al. 2018; Bhatta et al. 2018). The identified QTLs for micronutrients and grain proteins in wheat are mapped across the genome under specific environments, each with a small effect on the phenotypic variation explained (PVE), as shown in Table 7.2. Furthermore, multiple QTLs in wheat have been found to influence micronutrient absorption by plant roots from the soil and subsequent transfer to the shoot.

Among the numerous QTLs identified, few have been fine mapped, and candidate genes have been proposed (Olmos et al. 2003; Alomari et al. 2018; Uauy et al. 2006) for MAS using specific DNA markers or cloned using the reverse genetic approach (Uauy et al. 2006). Unlike genetic approaches, agronomic biofortification, often known as a fortification, is a fertilizer-based exogenous method for soil or plant foliar utilizing a composite micronutrient mixture (Zou et al. 2019; Cakmak and Kutman 2018; Garcia-Oliveira et al. 2018). This technique is based on inorganic elements being taken up by the roots from soil or the application site and mobilized inside the plants to the source or sink functions (White and Brown 2010). Previously, the potential of agronomic biofortification in alleviating micronutrient inadequacy in wheat was investigated (Ram et al. 2016; Cakmak et al. 2010). However, this approach is regarded as a short-time solution that is used as a complement to genetic biofortification, singularly when the soil in a specific region has poor micronutrient content (Cakmak et al. 2010, 2018). For example, in soil with an alkaline pH, Zn adheres with soil particles, like ferric oxides and calcites, which leads to decreasing Zn availability to plant roots. Similarly, soil with decreased humidity or moisture and organic compound level leads to the decrease of Zn in the root environment significantly (Graham et al. 1992; Marschner 1993; Alloway 2009). Furthermore,

**Table 7.2** List of significant studies performed for wheat biofortification

| Biofortification trait | Mapping population | QTL ID | Flanking marker[a] | Marker interval/ position (cM)[b] | LOD[c] | PVE (%)[d] | Ref.[e] |
|---|---|---|---|---|---|---|---|
| Grain iron (Fe) content | RILs | *QGFe.iari-2A* | Xgwm359–Xgwm249 | 21.9–34.9 | 4.1 | 6.8 | Kiss (2011) |
| | | *QGFe.iari-5A* | Xgwm126–Xgwm595 | 11.3–25.2 | 3.0 | 2.3 | |
| | | *QGFe.iari-7A* | Xbarc49–Xwmc525 | 7.8–29.4 | 2.9 | 4.3 | |
| | | *QGFe.iari-7B* | Xgwm364–Xgwm396 | 44.0–65.4 | 2.5 | 3.5 | |
| | | *QGFe.iari-7B* | Xgwm364–Xwmc396 | 49.5–65.3 | 2.8 | 2.5 | |
| | | *QGFe.iari-7B* | Xgwm577–Xbarc264 | 60.2–74.5 | 3.4 | 6.0 | |
| | | *FeE1-4A* | Xwmc468–Xbarc170 | 50 | 3.4 | 10.3 | von Grebmer et al. (2019) |
| | | *FeE1-5A** | Xsrap97–Xbarc330 | 163 | 3.5 | 10.4 | |
| | | *FeE2-2B** | wPt–7004–wPt–4210 | 171 | 4.6 | 9.5 | |
| | | *FeE2-4D* | Xgwm154–Xbarc108 | 160 | 5.0 | 19.1 | |
| | | *FeE2-5B** | Xbarc216–Xbarc74 | 92 | 3.4 | 9.2 | |
| | | *FeE2-5B** | wPt–7237–wPt–0708 | 171 | 3.8 | 7.4 | |
| | | *FeE2-5D* | wPt–667104–wPt–731590 | 8 | 3.5 | 8.3 | |
| | | *FeE2-7D* | wPt–663949–wPt–7068 | 182 | 2.8 | 5.4 | |
| | | *QGFe.co-1A* | 100008269\|F\|0–134239\|F\|0 | 179.5–184.5 | 2.6 | 2.2 | McGuire (2015) |
| | | *QGFe.co-2A* | 4993302\|F\|0–3954215 | 146.5–147.5 | 4.1 | 3.0 | |
| | | *QGFe.co-3B.1* | 1089107–1127875\|F\|0 | 199.5–200.5 | 3.7 | 14.6 | |
| | | *QGFe.co-3B.2* | 1233878–4262223\|F\|0 | 262.5–263.5 | 3.1 | 5.6 | |
| | | *QGFe.co-3D* | 1372776–100008980\|F\|0 | 124.5–125.5 | 2.6 | 2.2 | |
| | | *QGFe.co-4B* | 1242543–1125612\|F\|0 | 120.5–124.0 | 5.3 | 4.4 | |
| | | *QGFe.co-5A.1* | 14394095\|F\|0–4543804 | 43.5–46.5 | 2.7 | 2.1 | |
| | | *QGFe.co-5A.2* | 1102433–988523 | 96.5–97.5 | 3.1 | 6.9 | |
| | | *QGFe.co-6B* | 5332918–7342703 | 292.5–295.5 | 3.2 | 6.2 | |
| | | *QGFe-2A* | Xwmc382–Xbarc124 | 23.6 | 3.7 | 14.3 | Dukhi (2020) |
| | | *QGFe-7A* | Xgwm473–Xbarc29 | 153.8 | 3.3 | 12.6 | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | *QFe. Across_7DS* | TP43715-TP37547 | 2.0–5.4 | 6.6 | 14.5 | Bouis and Saltzman (2017) |
| | | *QFe.Y13-14_5BS* | TP91631-TP81797 | 23.1–23.7 | 5.3 | 12.0 | |
| | | *QFe. Across_4BS* | TP91631-TP81797 | 23.1–23.7 | 5.1 | 10.7 | |
| | | *QFe. Across_6AL* | TP5797-TP5676 | 86.9–89.5 | 4.2 | 9.6 | |
| | | *QFe.Y13-14_6BL* | TP68423-TP54256 | 36.6–37.9 | 3.9 | 8.9 | |
| | | *QFe. Across_2DS* | TP16058-TP69509 | 39.4–41.1 | 3.6 | 7.8 | |
| | | *QFe. Across_2BL* | TP54992-TP7812 | 103.5–106.5 | 3.5 | 7.5 | |
| | | *QFe.Y13-14_5BL* | TP17691-TP85478 | 62.7–64.4 | 3.4 | 7.7 | |
| | | *QFe.Y12-13_6DS* | TP52164-TP31712 | 19.5–20.5 | 3.2 | 9.1 | |
| | | *QGFe.cimmyt-4A_P2* | 3385350–1211533 | 198.5–199.5 | 9.7 | 21.1 | Doss (2018) |
| | | *QGFe.cimmyt-3A_P1* | 1234521–3034169F0-11AG | 85.5–87.5 | 7.1 | 10.4 | |
| | | *QGFe.cimmyt-3B_2P2* | 4394657–3947677 | 511.5–513.5 | 6.5 | 7.2 | |
| | | *QGFe.cimmyt-4D_P2* | 2363822–3961236 | 0–2.5 | 6.5 | 14.6 | |
| | | *QGFe.cimmyt-2A_P2* | 4262668–1226245 | 420.5–428.5 | 6.4 | 14.2 | |
| | | *QGFe.cimmyt-5B_P2* | 4407677–1129284 | 168.5–174.5 | 5.4 | 11.6 | |

(continued)

**Table 7.2** (continued)

| Biofortification trait | Mapping population | QTL ID | Flanking marker$^{a}$ | Marker interval/ position (cM)$^{b}$ | LOD$^{c}$ | PVE (%)$^{d}$ | Ref.$^{e}$ |
|---|---|---|---|---|---|---|---|
| | | *QGFe.cimmyt-3B_1P2* | 3533713–1007339 | 275.5–281.5 | 5.1 | 5.8 | |
| | | *QGFe.cimmyt-2B_P2* | wPt–0289–1026059 | 170.5–173.5 | 5.0 | 5.8 | |
| | | *QGFe.cimmyt-5B_P1* | 4989996–5410720 | 96.5–97.5 | 4.7 | 5.5 | |
| | | *QGFe.cimmyt-4B_P1* | 1008589F0–58TC-2256263 | 43.5–44.5 | 4.6 | 6.7 | |
| Grain zinc (Zn) content | DH | *Zn-2D* | P3470.3–P3176.1 | – | 5.6 | 13.4 | Caron et al. (2017) |
| | | *Zn-3A* | Xgwm391–P8422 | – | 3.0 | 7.1 | |
| | | *Zn-4A* | P3446–205–CWM145 | – | 3.2 | 8.3 | |
| | | *Zn-4D* | WMC331–Xgwm624 | – | 4.0 | 8.6 | |
| | | *Zn-4D* | Xgwm192–WMC331 | – | 4.2 | 11.9 | |
| | | *Zn-5A* | Xgwm291–Xgwm410 | – | 3.6 | 10.9 | |
| | | *Zn-7A* | WMC488–P2071–180 | – | 6.0 | 14.6 | |
| | RILs | *QGZn.iari-2A* | Xgwm359–Xwmc407 | 30.0–38.5 | 13.5 | 11.1 | Kiss (2011) |
| | | *QGZn.iari-2A* | Xgwm359–Xgwm249 | 22.0–34.7 | 11.8 | 14.4 | |
| | | *QGZn.iari-2A* | Xgwm359–Xgwm249 | 23.6–32.9 | 6.5 | 8.5 | |
| | | *QGZn.iari-4A* | Xbarc184–Xbarc106 | 58.0–76.5 | 2.6 | 4.7 | |
| | | *QGZn.iari-5A* | Xgwm126–Xbarc144 | 5.8–17.5 | 3.7 | 6.2 | |
| | | *QGZn.iari-7A* | Xbarc49–Xwmc525 | 7.0–25.2 | 2.6 | 3.2 | |
| | | QZnE1-2D* | wPt-730057–wPt-671700 | 174 | 5.0 | 8.6 | von Grebmer et al. (2019) |
| | | *QZnE1.2-3D* | wPt-6191–wPt-8658 | 218 | 2.7 | 7.7 | |
| | | QZnE1-4D* | wPt-671648–wPt-667352 | 41 | 3.2 | 5.5 | |
| | | QZnE2-5B* | wPt-7237–wPt-0708 | 81 | 2.8 | 7.8 | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | *QZnE2 3D* | Xbarc6–Xcfe172 | 33 | 4.0 | 14.5 | |
| | | *QZnE2-4D** | Xcfa2149–Xbarc48 | 194 | 3.3 | 15.9 | |
| | | *QZnE1-5B** | Xgwm213–Xbarc216 | 75 | 3.4 | 13.8 | |
| | | *QGZn.co-1B* | 124470–3028438lFl0 | 382.5–383.5 | 2.8 | 5.4 | McGuire (2015) |
| | | *QGZn.co-2B* | 230380–2275590 | 516.5–517.5 | 2.8 | 7.1 | |
| | | *QGZn.co-3A* | 3022261–3936326 | 99.5–100.5 | 3.6 | 5.2 | |
| | | *QGZn.co-3B* | 1002594lFl0–1103633 | 315.5–316.5 | 2.8 | 3.6 | |
| | | *QGZn.co-3D* | 1372776–100008980lFl0 | 123.5–126.5 | 2.8 | 2.7 | |
| | | *QGZn.co-4B* | 2277812–1242543 | 120.5–124 | 3.3 | 7.2 | |
| | | *QGZn.co-5A* | 124421–1272027lFl0 | 53.5–54.5 | 2.7 | 14.2 | |
| | | *QGZn.co-6B.1* | 1252668–100005882lFl0 | 277.5–278.5 | 3.6 | 4.2 | |
| | | *QGZn.co-6B.2* | 3941131–990183 | 177.5–180.5 | 3.4 | 4.1 | |
| | | *QGZn.co-7A* | 5356706–5325178lFl0 | 285.5–288.5 | 5.5 | 7.8 | |
| | | *QGZn.cimmyt-1A_P2* | 4543935–3937719 | 133.5–136.5 | 8.9 | 10.8 | Doss (2018) |
| | | *QGZn.cimmyt-1B_P1* | 3934172–3934936 | 83.5–84.5 | 8.3 | 15.1 | |
| | | *QGZn.cimmyt-1B_P2* | 4991478–3937490 | 226.5–228.5 | 8.6 | 11.3 | |
| | | *QGZn.cimmyt-3B_1P2* | 3533713–1007339 | 277.5–284.5 | 5.4 | 4.4 | |
| | | *QGZn.cimmyt-3B_2P2* | 4394657–3947677 | 512.5–513.5 | 7.9 | 10.9 | |
| | | *QGZn.cimmyt-3D_P2* | wPt–741157–1297057 | 191.5–193.5 | 5.8 | 7.5 | |
| | | *QGZn.cimmyt-4A_P2* | 4543988–3533871 | 123.5–124.5 | 5.2 | 3.8 | |

(continued)

**Table 7.2** (continued)

| Biofortification trait | Mapping population | QTL ID | Flanking marker$^{a}$ | Marker interval/ position (cM)$^{b}$ | LOD$^{c}$ | PVE (%)$^{d}$ | Ref.$^{e}$ |
|---|---|---|---|---|---|---|---|
| | | *QGFe.cimmyt-4D_P2* | 2363822–3961236 | 0–2.5 | 6.5 | 14.6 | |
| | | *QGZn.cimmyt-5B_P2* | 1078595–4538122 | 102.5–108.5 | 6.6 | 5.1 | |
| | | *QGZn.cimmyt-6A_P1* | 1238392–4990410 | 108.5–109.5 | 7.9 | 9.7 | |
| | | *QGZn.cimmyt-6A_P2* | 1697218–1082136 | 178.5–179.5 | 11.9 | 8.5 | |
| | | *QGZn.cimmyt-7B_1P1* | 3945822–1132640F0–5CG | 61.5–62.5 | 7.1 | 16.8 | |
| | | *QGZn.cimmyt-7B_1P2* | 1079651–1262636 | 43.5–44.5 | 20.8 | 32.8 | |
| | | *QGZn.cimmyt-7B_2P1* | 989723F0–48CT–1204955F0–26CT | 85.5–86.5 | 5.4 | 2.9 | |
| | | *QGZn.cimmyt-7B_2P2* | 4003947–3532745 | 95.5–98.5 | 7.8 | 3.3 | |
| | | *QGZn.cimmyt-7B_3P2* | 4009608–5411574 | 146.5–148.5 | 7.0 | 5.4 | |
| | | *QGZn.cimmyt-7D_P2* | wPt–733859–3033815 | 41.5–53.5 | 5.2 | 5.8 | |
| | | *QGZn-7A.1* | Xcfd31–Xcfa2049 | 72.6 | 4.4 | 21.1 | Dukhi (2020) |
| | | *QGZn-7A.2* | Xgwm473–Xbarc29 | 153.8 | 3.5 | 14.7 | |
| | | *QZn. Across_4BS* | TP91631-TP81797 | 23.1–23.7 | 6.6 | 17.3 | Bouis and Saltzman (2017) |
| | | *QZn. Across_6AL* | TP38159-TP75088 | 50.2–50.7 | 3.3 | 8.4 | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | *QZn.Y12-13_4BS* | TP73864-TP71929 | 17.1–19.4 | 4.0 | 11.7 | |
| | | *QZn.Y13-14_4BS* | TP91631-TP81797 | 23.1–23.7 | 7.0 | 19.6 | |
| | | *QZn.Y13-14_6BL* | TP54256-TP29689 | 37.9–39.9 | 3.3 | 9.0 | |
| Grain selenium (Se) content | RILs | *QGsec-5B* | gwm67Đ–swes100 | – | 8.0 | 17.7 | Lobell et al. (2011) |
| | | *QGsec-5B* | BS00082312–51ĐwPt-5120 | – | 7.0 | 14.5 | |
| | | *QGsece-5B.1* | D-3953407ĐD–1071681 | – | 4.6 | 9.0 | |
| | | *QGsece-5B.2* | D-2289135ĐD–1236560 | – | 3.8 | 7.4 | |
| | | *QGsece-5B.3* | RAC875c33387_888ĐD–3936732 | – | 7.0 | 13.9 | |
| | | *QGsece-2B.1* | D-3956657ĐS–1105975 | – | 3.7 | 12.6 | |
| | | *QGsece-2B.2* | S-1120640ĐD–3024250 | – | 4.3 | 10.4 | |
| | | *QGsec-5D* | S-3958480Đ–S-2347952 | – | 4.4 | 8.9 | |
| | | *SeE1-4D* | Xbarc241–Xsrap15a | 100 | 10.4 | 35.1 | von Grebmer et al. (2019) |
| | | *SeE2-7D* | wP-t671684–wPt-667506 | 215 | 10.8 | 28.5 | |
| | | *SeE1-3D* | wPt-6191–wPt-8658 | 218 | 5.2 | 23.4 | |
| | | *SeE1-5B* | wPt-5120–wPt-0708 | 169 | 3.8 | 10.1 | |
| | | *SeE1-4A* | wPt-731120–wP-t1642 | 91 | 2.8 | 6.4 | |
| Grain protein content | RILs | *QGpc.iari-2A* | Xgwm359–Xwmc407 | 29.6–37.2 | 14.6 | 18.8 | Kiss (2011) |
| | | *QGpc.iari-2A* | Xgwm359–Xwmc407 | 25.3–38.1 | 7.0 | 12.1 | |
| | | *QGpc.iari-2A* | *Xgwm359–Xwmc407* | 26.1–38.8 | 5.9 | 9.9 | |
| | | *QGpc.iari-2A* | Xgwm359–Xwmc407 | 24.4–39.3 | 5.8 | 9.2 | |
| | | *QGpc.iari-2A* | Xgwm359–Xwmc407 | 22.0–45.1 | 3.0 | 5.4 | |
| | | *QGpc.iari-2A* | Xgwm359–Xwmc407 | 23.5–45.6 | 2.9 | 4.3 | |
| | | *QGpc.iari-3A* | Xbarc45–Xbarc57 | 61.1–81.9 | 2.9 | 5.3 | |

(continued)

**Table 7.2** (continued)

| Biofortification trait | Mapping population | QTL ID | Flanking marker[a] | Marker interval/ position (cM)[b] | LOD[c] | PVE (%)[d] | Ref.[e] |
|---|---|---|---|---|---|---|---|
| | | *QGpc.co-2A* | 1267600–1138191 | 302.5–308.5 | 3.5 | 4.2 | McGuire (2015) |
| | | *QGpc.co-2B.1* | 1083804–1117983 | 304.5–312.5 | 3.1 | 6.3 | |
| | | *QGpc.co-4A* | 3942314–5323574\|F\|0 | 0–18.5 | 2.8 | 3.2 | |
| | | *QGpc.co-2A* | 1267600–138191 | 302.5–308.5 | 2.8 | 5.8 | |
| | | *QGpc.co-2B.2* | 2303802–2275590 | 516.5–518.5 | 2.8 | 10.8 | |

[a] DNA markers flanking the detected QTL
[b] The absolute position of the QTL
[c] The logarithm of the odd profile
[d] Phenotypic variation explained by the QTL
[e] Reference

agronomic biofortification is also less effective due to the limited mobility of Fe and Zn in the root system, resulting in reduced concentration in the plant's edible portions (Cakmak et al. 2010; White and Broadley 2005). Several studies have found that the amount of elements like zinc varies across current wheat cultivars and wild ecotypes. For instance, the Zn content in high-yielding wheat cultivars is significantly lower (two- to threefold) than that recorded in the primitive or wild wheat genetic resources. Thus, wild wheat genotypes are widely used as important genetic resources in many breeding programs on wheat biofortification, among other quality traits (Cakmak et al. 2010; Fan et al. 2008; Zhao et al. 2009). Thus, using diverse breeding approaches, a panel of wheat varieties has been developed and released to the public to address the issue of malnutrition or hidden hunger caused by micronutrient deficiency.

## 7.5 Challenges, Limitations, and Success of Breeding Approaches for Wheat Biofortification

Biofortification of crops has been performed for a long time, and numerous strategies have been considered to grow biofortified crops. Wheat has ample natural wild germplasm and landrace resources with Zn, Fe, and Se content. However, to develop the biofortified wheat grain for micronutrient, provitamin, and protein content is very challenging and has some limitations to accomplish using breeding strategies. First, agronomic or fertilizer approach implementation is costly and non-sustainable and does not offer long-term solutions for the biofortification of wheat. Similarly, the conventional breeding approach of wheat biofortification is a conventional and phenotyping-based long-term, expensive method for increasing micronutrient, provitamin, and protein content. Furthermore, the inverse relationship between grain yield and grain mineral composition is seen as a key barrier to biofortification of wheat using the traditional breeding approach (Garvin et al. 2006; Shi et al. 2013). This limitation could be overcome via the selection of desired traits with MAS or a genetic approach. However, this approach also poses some challenges owing to complex polyploidy; large (16Gb), high riddance sequence (>85%) of a genome; and risk of linkage drag associated with the traits. In order to design genetic markers, map loci that are responsible for micronutrient, vitamin, and protein content prerequisite are well-annotated reference genome sequence, and multiple cultivars need to a sequence.

The availability of multiple diploid, tetraploid progenitors of wheat, the discovery of genes in the Chinese Spring reference landrace, and perhaps other modern genomic resources, will aid efforts to increase wheat grain micronutrient, protein, and provitamin composition. This would also facilitate the mapping of micronutrient, protein, and provitamin composition genomic regions. However, efforts need to be put for the integration of data on standard new reference, and multiple accessions of wild cultivars should be sequenced. In addition, previous studies related to biofortification traits need to combine on a standard reference sequence that would improve the understanding of wheat biofortification traits. Another important challenge is to look beyond higher Zn, Fe, and Se content resources in wheat and to use them in breeding programs to develop new cultivars with accumulated micronutrients. Deficiency of I is also a prevalent issue; however, salt iodization has its limitations, such as volatilization while cooking, inaccessibility of iodized salt for all households, and inadequate intake because of health concerns (Medrano-Macías et al. 2016; White and Broadley 2009). Thus, it's crucial to focus on identifying desirable traits that control uptake, mobilization, and accumulation of I and other minerals in wheat grain. This can be achieved via plant breeding and genetic or biotechnological approaches (Cakmak et al. 2017).

Despite the challenges in the conventional breeding, genomic, and agronomic approaches, some Zn-biofortified wheat varieties have been successfully developed and released ["Zn Shakti" (Chitra), "Znol 2016," "WB02," "HPBW-01," and "BARI Gom 33"] (Bouis and Saltzman 2017; Das et al. 2019; HarvestPlus 2019). However, there is a need to create awareness about commercially available biofortified cultivars, and these cultivars must be accessible to the most vulnerable sections of the world. In this context CIMMYT and HarvestPlus are raising awareness about the importance of biofortified wheat and acting toward the accessibility of these biofortified released varieties in India, Pakistan, and Bangladesh.

Furthermore, it is well recognized that traditional breeding-based fortification increases the nutritive value of rice and wheat (Garg et al. 2018; To 2014). The current trend shows a growing interest in the use of genetic engineering techniques (transgenic, gene editing, or the use of sequenced mutants) that target specific metabolic pathways to improve the genotypes for the desired trait (Vanderschuren et al. 2013) for animal consumption and, progressively, for human diet (Graybosch et al. 2013). In addition, increasing multiple genome sequences and modern genomic or well-annotated gene models offer gene-centric methodologies for wheat biofortification.

## 7.6 Molecular Understanding of Essential Micronutrient Uptake and Deposition in Wheat Grain

Uptake, transport, mobilization, and deposition of essential micronutrients in cereals are important research topics. Usually, essential minerals, such as Fe and Zn, are available from the soil. In order to increase the essential micronutrient content in wheat grain, it is crucial to precisely understand the absorption and translocation of elements from the soil to the plant and their subsequent accumulation in the grain. Here, we briefly discuss the prospect of Zn and Fe absorption, translocation, and accumulation in wheat grains.

### 7.6.1 Factors Affecting Micronutrient Availability to Wheat Grains

In cereal cultivation areas, the soil's physical and chemical properties (moisture, temperature, organic matter, and soil) determine the Zn and other metal solubility and the ability of roots to absorb adequately (Cakmak 2008; Nadeem and Farooq 2019). Here, we briefly discuss the prospect of Zn absorption and availability to wheat grains.

Commonly, soil pH ranges between 5.5 and 7.0, every single unit increase in pH that influences available soil Zn concentration decreases by 30–45-folds to plants leading to a deficiency of Zn in plants. Moreover, increasing pH causes Zn adsorption to soil clay minerals, etc. $Zn^{2+}$ concentration in the soil solution is adequately high at a pH of 5. Zn is mainly transported in the soil to the root surface through diffusion. This mineral is very sensitive to soil moisture and pH (Marschner 1993; Cakmak 2008; Lindsay and Collins 1991). Similarly, reduced organic matter content and soil moisture ratio hinder the absorption and availability of Zn in the root environment (Graham et al. 1992; Marschner 1993; Alloway 2009). Plant Zn nutrition is also negatively affected by water deficit, mainly in areas where the topsoil is dry during the reproductive stages of crops. Zn deficiency stress was more severe in rain-fed areas than in irrigated areas in field trial conditions. Thus, the rate of transportation of Zn to the plant roots and Zn solubility is considerably influenced by the percent content of organic matter in the soil (Catlett et al. 2002). A successful genetic crop biofortification program with Zn and other essential micronutrients relies on its concentration of availability for crops in the soil. A continuous supply of sufficient quantities of available essential micronutrients for crops is necessary for significant biological impact in genetic biofortification programs.

### 7.6.2 Molecular-Level Translocation of Micronutrients from Soil to Grain in the Wheat

The essential elements' uptake in plants from the soil occurs via two methods, direct and indirect. The direct uptake comprises $Fe^{2+}$ and $Zn^{2+}$ of ZRT/IRT-related protein (ZIP) family, while the indirect method occurs via phytosiderophores that chelate Fe cations, with consequent uptake via yellow stripe-like transporters (YSL) (Dempski 2012; Guerinot 2000; Sperotto et al. 2012; Milner et al. 2013). Monocot crops, such as wheat, barley, and maize, usually follow chelation for Fe uptake. Same protein families generally regulate the transport of essential micronutrients (Fe and Zn) to plants. However, plants, with the help of multigene family members, treat different elements differently. In addition, metal chelators, such as metallothioneins, nicotianamine, low-molecular-weight proteins, and non-proteinogenic AA also play a crucial role in chelation, detoxification, and circulation of Fe and Zn from the root to the other parts of plants (Rellán-Álvarez et al. 2010; Deinlein et al. 2012). Vacuolar nicotinamide is observed to play a critical and distinct role in Fe deficiency and for Zn sequestration in *Arabidopsis* (Haydon et al. 2012*)*. Some other transporter proteins are also involved and play important roles in metal and micronutrient cellular homeostasis. These transporter proteins belong to the cation diffusion facilitator (CDF) family, vacuolar iron transporter family, natural resistance-associated macrophage protein (NRAMP) family, P1B-like heavy metal ATPase

(HMA) family, major facilitator superfamily (MFS), ATP-binding cassette protein (ABC) transporter family, and cation exchange (CAX) family (Gustin et al. 2011; Montanini et al. 2007; Connorton et al. 2017; Nevo and Nelson 2006; Rice et al. 2014; Pittman and Hirschi 2016; Verrier et al. 2008). In particular, the ZIP and YSL family proteins facilitate the transport of Fe and Zn from the xylem to the phloem in the root and shoot or during remobilization from the leaves to the grains. Wheat has xylem discontinuity; thus, all minerals and nutrients must pass through the phloem to be obtained in the grains (Zee and O'brien 1970).

The majority of Fe and Zn intakes from the root to the seed grains have been studied in cereal crops (maize, rice, and barley). The information of these cereal crops facilitated the proposed Fe and Zn transport in wheat where information is partial. The uptake and transport of Fe and Zn from the soil to the grains and its homeostasis in plants, including wheat, is reviewed and covered in detail in previous trials (Ludwig and Slamet-Loedin 2019; Borrill et al. 2014; Olsen and Palmgren 2014; Sinclair and Krämer 2012; Nakagawa et al. 2018; Sperotto et al. 2018; Curie and Mari 2017). Based on these data, we illustrated the putative framework of protein families/genes involved in micronutrient (Fe/Zn) translocation from the roots to the seed grains in wheat (Fig. 7.4).

The literature survey determined the probable groups of transport proteins, which are represented in red font. Unknown transporters are represented by question marks. Bioavailable elements in the soil (free $Zn^{2+}$ and phytosiderophore (PS)-bound Fe and Zn) are assimilated in the root epidermal cells. Fe and Zn travel to the pericycle via apoplast and symplast; on the way they may be trapped in vacuoles. Further, Fe and Zn are transported into the xylem and subsequently transferred into the phloem in the root, basal shoot, or leaf tissues (not shown). Remobilization of Fe and Zn takes place from leaf cell plastids (P) → vacuoles (V) → phloem→ ear. Further, Fe and Zn are transferred into the embryonic cavity from parent tissue. After absorption into the aleurone layer, mostly Fe and Zn are sequestered in protein storage vacuoles (PSVs) attached to phytate (Phy). A limited amount of Fe and Zn may penetrate the endosperm and be stored or aggregated to bind with ferritin (Fer) in starch bodies, also known as amyloplasts (A). ZIP, ZRT-, IRT-like protein; YSL, yellow stripe-like transporter; MFS, major facilitator superfamily transporter; MTP, metal tolerance protein; HMA, heavy metal ATPase; FPN, ferroportin; NRAMP, natural resistance-associated macrophage protein; VIT, vacuolar iron transporter; NA, nicotianamine; Cit, citrate; SP, small proteins.

## 7.7 Transgenic Efforts for the Development of Biofortified Wheat

The nutritional value of food crops can be enhanced with transgenic approaches. Transgenic approaches differ from other strategies. New specific gene is added directly into the organism or plants using transgenic approaches, and plants can be converted for the production of preferred compounds. This strategy is dependent on the compound source and whether it is produced by plants or obtained from the

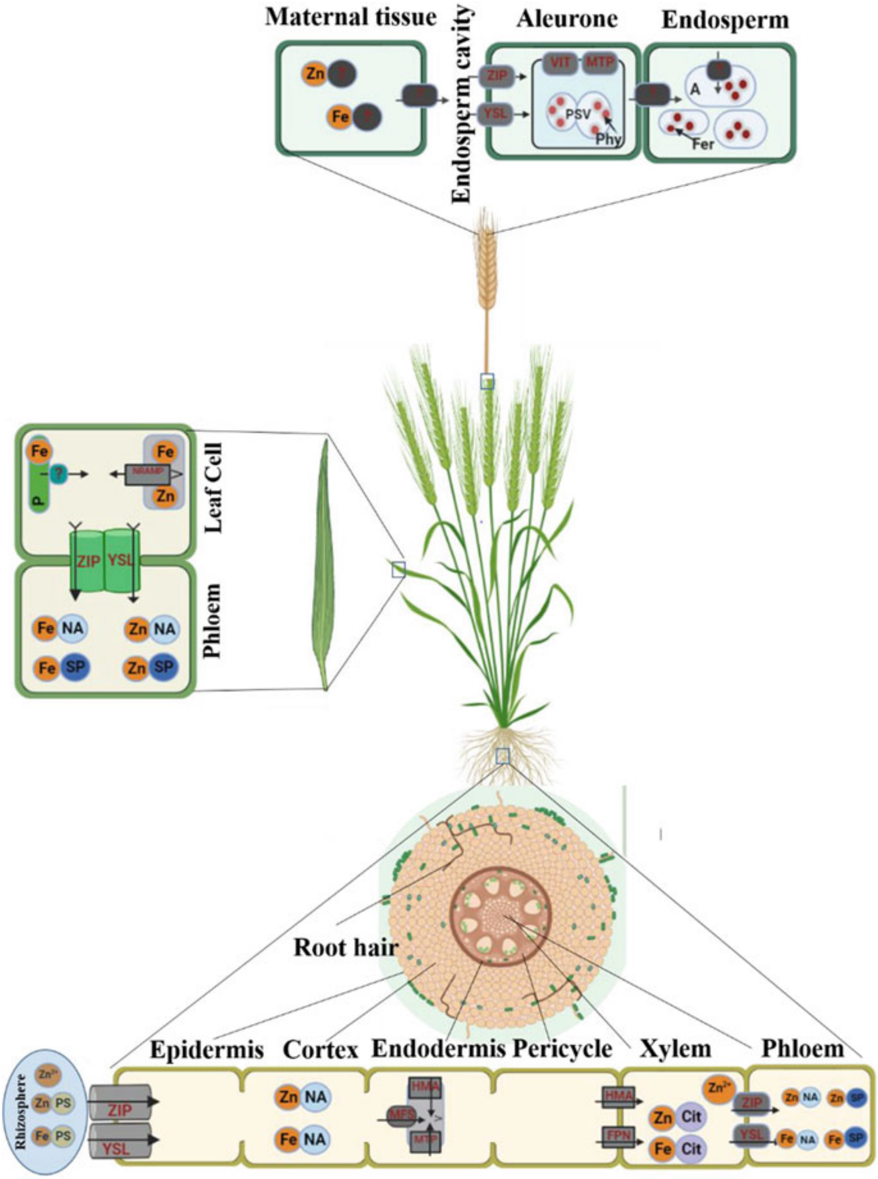

**Fig. 7.4** Micronutrient (Fe and Zn) uptake and translocation from the root to the grain in wheat is depicted schematically. (Modified from Borrill et al. 2014)

atmosphere. The amino acid, micronutrients, essential fats or lipids, and vitamins are produced by plants, and these molecules can increase the nutrient content. However, to increase the cumulative amount of these required nutrients, metabolic engineering is necessary.

Some transgenic techniques increase total absorption and transport of the Zn and Fe to edible parts as well as efficiently enhance bioavailable minerals added to the plants. Minerals are absorbed into the roots by particular enzymes or proteins and are transferred to sink organs through the phloem. The formation of these transport and chelating proteins is aided by the presence of Fe(Connolly 2002). Alternatively, the generation of phytosiderophores can promote Fe concentration and accumulation; for example, the expression of the barley genes (naat-A and naat-B) encoding nicotinamide aminotransferases in rice crops resulted in higher Fe uptake. Remarkably, mutants and transgenic plants with overexpressed Fe reductases have shown that the Fe and Zn transport networks share some interaction. Fe transporters also show improved Zn accumulation, leading to increased synthesis of nicotinamide that improves the mobilization of metals in the vascular tissue. Accordingly, the overexpression of nicotianamine synthases also indicates Fe and Zn accumulation; for example, the expression of HvNAS1 in *Nicotiana tabacum* doubled the Fe and Zn content of leaves (Ma et al. 2001; Takahashi et al. 2003).

Another way for improving minerals is to overexpress proteins using an endosperm-specific promoter which allows elements to be retained in an accessible form, such as soybean ferritin (ferritin is a Fe storage protein) in rice crops. This type of rice has threefold the amount than the wild-type rice. The Fe concentration was also measured in polished grains; however, the levels of Fe and Zn remained higher than those of unpolished rice grains. A comparison of the use of a constitutive promoter to initiate ferritin expression resulted in higher Fe levels in transgenic rice leaves but not in the grains owing to higher ferritin expression in the tissues (Goto et al. 1999). An additional bioavailability is another difficulty with nutritional availability; minerals must be accessible in some kind of a category that could be eaten then assimilated by the body. Phytate seems to be an antinutritional component that chelates Fe and Zn and decreases their absorption in the human gut. Consequently, a transgenic method involving the expression of both phytase and ferritin was developed. Experiments on transgenic maize and rice crops showed how rice grains had twofold more Fe content than wild type. Further, predictions of absorption consuming the maize kernels revealed that the quantity of bioavailable Fe had improved (Drakakaki et al. 2000, 2005). Thus, the combined use of different Fe fortification techniques can yield the highest levels of bioavailable Fe. Similar approaches can be adopted for other nutrients. In Fig. 7.5, a comprehensive strategy for wheat biofortification (micronutrients, provitamin A, and protein) is proposed based on the existing literature (Tong et al. 2020), using genetic engineering, reverse genetic, and breeding approaches.

### 7.7.1 Challenges for the Public Release of Transgenic Wheat

In wheat, very few agronomical genes have been introduced via the transgenic approach. Although the development of transgenic wheat is increasing rapidly, some important challenges remain. Primarily, there is considerable misinformation in the general public about the consumption of transgenic food grains, such as it may

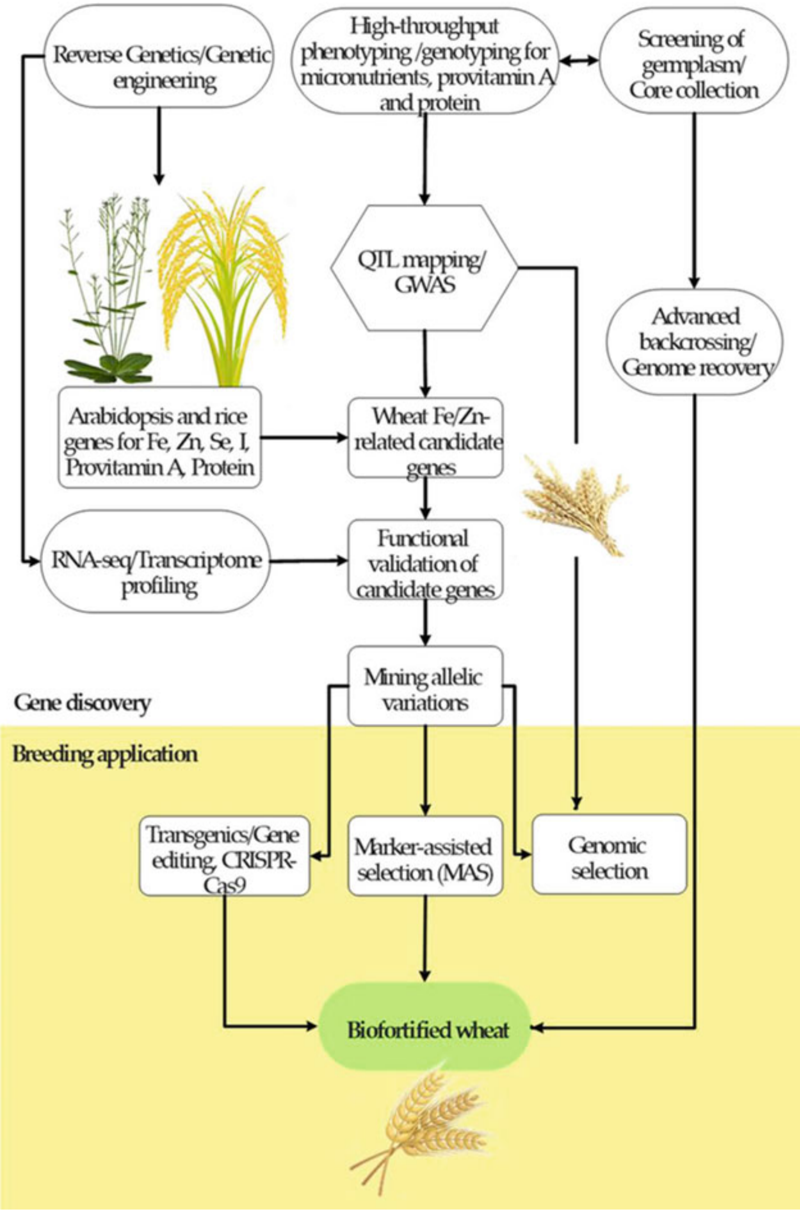

**Fig. 7.5** A comprehensive strategy for wheat biofortification

harm human health (Dale 1999). Transgenic wheat developed in Bobwhite or Fielder background which is not a commercially preferable transgenic wheat (Li et al. 2012). Secondly, not providing better consumer benefits, all the outlook is controlled by multinational private organizations of the main crops, leading to rejection of genetically modified (GM) food by the regulatory authorities of most European countries (Bhalla 2006). There are complex or confusing regulations and government policies regarding transgenic crop utilization in several countries. Lack of political wills toward transgenic or GM wheat and other food crops is another crucial factor involved in the release of transgenic wheat.

Despite all the challenges, the majority of the scientific community believes that transgenic technology can offer a very exciting future to plant breeders, farmers, consumers, and the general public. The acceptance and mainstream use of transgenic wheat can lead to substantial benefits and provide numerous opportunities for crop improvement.

## 7.8 Economic and Social Constraints for the Use of Biofortified Wheat

The micronutrient deficiency confronted by the poor all over the world and particularly in Asia and Africa revealed that scientists have to work out the resolution for the eradication of micronutrient deficiency. Biofortification of food crops resulting from genetic techniques has the potential to be cost-effective and provides numerous advantages to 40% of the population that rely solely on food for nourishment. It is recommended that a one-time financial investment be made in the production of food crop seeds that uptake micronutrients efficiently, ensuring adequate Zn and Fe intake by rural communities. Genetic biofortification is more cost-effective than other strategies, such as agronomic fortification, dietary modification, and supplementation programs. Primary commercial training for Zn biofortification suggests a cost-to-benefit proportion of greater than 20% over two decades and cost-to-benefit proportions between 20% and 30% for Fe biofortification of rice in South Asia (Bouis 2003). Informal assessments of cost-to-benefit proportions for fertilization with Se or I also recommend high yields on financial investments (Nestel et al. 2006).

The potential impact of biofortification is measured in terms of reduction in the disability-adjusted life years in India. Further, the annual burden of Fe deficiency (anemia) may be significantly reduced with biofortification. Similarly, the annual burden of Zn deficiency is 2.8 million lost disability-adjusted life years in India, and the Zn biofortification of wheat and rice may overcome this burden by 20–51% (Stein et al. 2007). The cost of saving one disability-adjusted life year with biofortification of major food crops (maize, wheat, and rice) with Zn and Fe in African countries is estimated to be US $2–20.

## 7.9 Genome Editing Approaches for Wheat Biofortification

In the lack of stable genetic diversity for the particular trait in a single plant species, genetic engineering technologies offer a feasible edge over traditional breeding strategies (Bhalla 2006; Hu et al. 2003; Vasil et al. 1992). The use of genome resequencing and genome manipulation have contributed to the understanding of functional genetic components underlying the biochemical regulation and physiological and molecular developments in plants, including the nutritional and nutraceutical properties of food crops. Although there has been considerable progress in the use of QTL mapping and the development of gene-transformation technologies, this remains an important challenge for wheat biofortification. Recent advancements in plant biotechnology have offered various possibilities to uphold the level of biofortification programs. The availability of complete newly sequenced genomes in staple food crops and novel gene-editing techniques, such as transcription activator-like effector nucleases and cluster regularly interspaced short palindromic repeats (CRISPR)/Cas9, have opened new doors for the biofortification of cereal crops (Ricroch et al. 2017). In wheat, CRISPR/Cas9 has been employed in various studies, to improve abiotic stress tolerance (Kim et al. 2018) and biotic stress resistance (Shan et al. 2014; Wang et al. 2014; Zhang et al. 2017). However, few studies targeting micronutrients or protein contents have been reported (Ludwig and Slamet-Loedin 2019; Connorton et al. 2017; Liang et al. 2017; Sánchez-León et al. 2018; Zhang and Gao 2017). Although the CRISPR/Cas9 system is a powerful tool for crop genome editing, achieving highly efficient and specific editing in polyploidy species, such as wheat, this technology can be challenging because its efficiency depends on the gRNA used, as reported previously (Arndell et al. 2019). Moreover, the progress in the CRISPR technique (Kim et al. 2006; Lapinskas et al. 1996; Li et al. 2001) is underutilized for the editing of the genes associated with micronutrient levels. This might be due to the uncertain stand on its regulation and political will in many countries and international organizations.

## 7.10 Improving the Nutraceutical Properties of Wheat

Recent studies that have investigated the health benefits of functional products in wheat have shown the importance of introducing phytochemicals with a high nutraceutical potential using different varieties and ecotypes. Thus, there is renewed interest in the ancient genetic resources of wheat, specifically those with high nutraceutical properties (Dinelli et al. 2007, 2011; Adom et al. 2003; Heimler et al. 2010; Behall et al. 2006; Fardet 2010; He et al. 2010). Phenolic compounds have emerged as health-promoting phytochemicals in wheat grains and have gained attention owing to their high antioxidant capacity and can protect against various degenerative diseases (Heimler et al. 2010; Laddomada et al. 2015). A study that aimed to investigate the phenolic profile in grains of modern and old common wheat varieties suggested that the flavonoid (both bound and free) content and the ratio between flavonoids and polyphenols influence the antioxidant activity of FRAP and

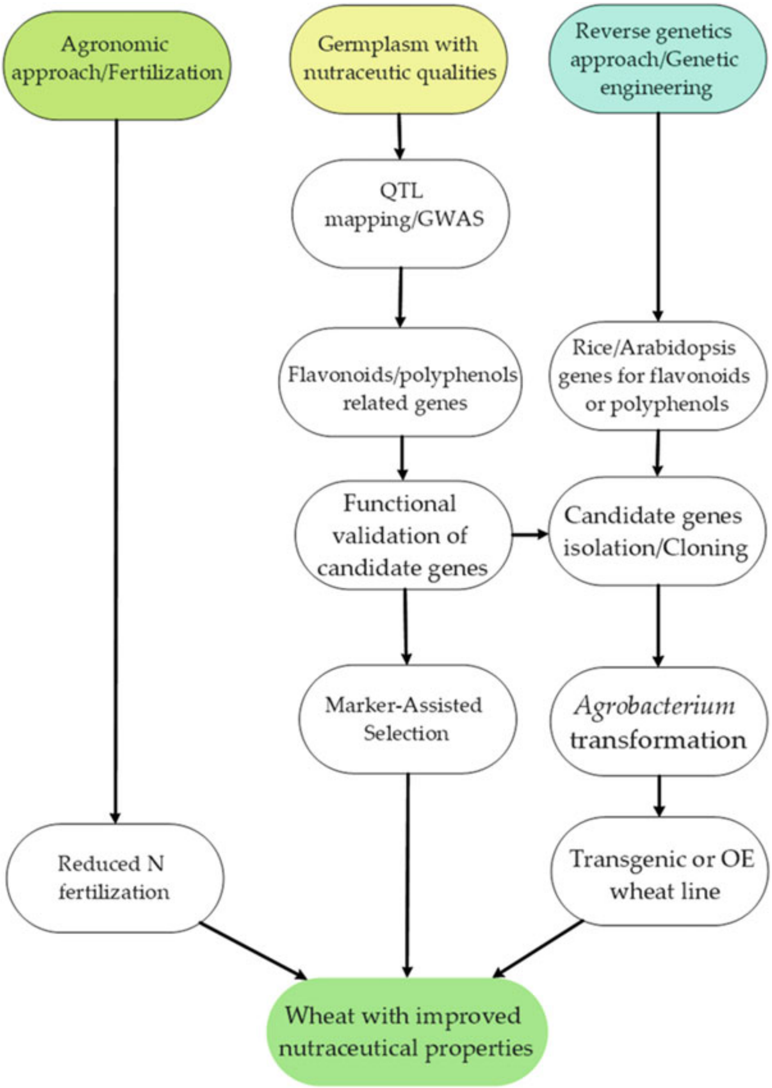

**Fig. 7.6** Different approaches for the improvement of nutraceutical properties of wheat grain

DPPH (Leoncini et al. 2012). The authors also supported that phenolic extracts from wheat exerted a cytoprotective and antiproliferative effect on cardiomyocytes and leukemic cell (HL60) line, respectively, suggesting that an increased intake of wheat grain-derived products could help achieve both chemoprevention and protection against oxidative stress-related diseases. Thus, to improve the nutraceutical properties of wheat varieties, while maintaining an optimum level of productivity, different approaches can be used, as illustrated in Fig. 7.6. These approaches include agronomic (reduced nitrogen supply without hampering plant growth and development and yield), conventional breeding (employing the available genetic resources with a high potential for improving the nutraceutical qualities of wheat), and genetic engineering targeting specific genes.

## 7.11 Conclusion and Future Prospects

Biofortification approaches are based on plant breeding and targeted genetic manipulation to address malnutrition in humans. Genotype and micronutrient interactions in grain production and nutrient density remain unclear. In most studies, enhancements in nutrient use effectiveness are limited by the costly and laborious phenotyping. Moreover, the bioavailability of minerals is another important factor to measure grain quality. Because more starch accumulates in grains, a quest for better yield is typically followed by a diluting effect of minerals. Therefore, more focused efforts are needed to achieve these goals. In this situation, it is recommended that researchers and breeders work very closely not only to attain the maintained Fe and Zn contents but also to improve Fe, Zn, Se, I, provitamin, and protein contents required in the edible parts. Considering the problems outlined above, we believe that wheat researchers have the tools and resources necessary to greatly improve the concentration of important micronutrients, provitamins, and proteins in wheat grains as well as transfer these developed varieties to fields. These improved varieties with higher nutrient contents could make a vital contribution to the health of the global population by preventing micronutrient deficiency, but still nearly 30% of the global population face deficiency for one or more crucial micronutrients.

Ultimately, the human diet depends on the sufficient consumption of several different minerals and other bioactive compounds in combinations and within ranges that are not entirely understood. Therefore, the best solution to eradicate malnutrition as a community health issue in developing countries is a more appropriate consumption of a variety of foodstuff. However, achieving this requires several years for awareness creations, the formation of informed management policies, and a comparatively huge investment in agricultural research and on-farm infrastructure. Human nutrition scientists need to be informed about the levels to which the mineral concentrations of the edible parts of plants and compounds that stimulate and hinder their bioavailability can be amended via the processes of plant breeding and genetics. Plant geneticists need to be knowledgeable about the major effects that plant research may have previously had on micronutrient utilization. Further, they should also be aware of the differences in mineral bioavailability between current varieties and traditional cultivars, as well as the potential of plant genomes to improve human nutrition in the future.

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