9 Biofortification of Barley for Nutritional Security

Kiran Khandagale, Dhananjay Shirsat, and Avinash Ade

9.1 Introduction

The worldwide continuous growth of the human population resulted in increased demands for food. The green revolution has increased food production significantly (Evenson and Gollin 2003). But this increase in yield was often accompanied by reduced nutritional quality (Simmonds 1995; Oury et al. 2003). More than one billion people suffer from the low intake of proteins, minerals, and vitamins especially in developing and underdeveloped countries (WHO 2016), and thus biofortification of crops is a very important approach to overcome it (Wiegmann et al. 2019). Biofortification is the practice of enhancing the amount or bioavailability of vital nutrients in food using agronomic, genetic, and biotechnological methods (Bouis et al. 2011).

Generally, staple food crops are targeted for biofortification as they are the major portion of the diet in poor people. Vitamins and minerals are required by humans in very minute amounts (less than 1 mg/day) and thus are the main focus of the biofortification program. These compounds govern several vital biological processes in the body, and therefore biofortification approaches can improve the content and availability of nutrients in the human diet to improve the nutritional security of vulnerable communities around the globe. Three main strategies were followed for biofortification: conventional breeding, agronomic, and biotechnological/transgenic approach. Plant breeding strategy involves crossing of elite variety with genotype having higher micronutrient content, and after several generations, we get the ideal genotype with higher mineral level and other desired characters. Further, agronomic

K. Khandagale · A. Ade (✉)
Department of Botany, Savitribai Phule Pune University, Pune, India

D. Shirsat
ICAR-Directorate of Onion and Garlic Research, Rajgurunagar, India

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methods are comprised of applying fertilizer to soil or foliar application to improve the level of a particular nutrient in the edible part of the crop. The transgenic approach is used where a specific nutrient doesn't exist naturally in that crop or breeding for that trait is not effective. Gene for that trait can be sourced from any organism from bacteria to animals and inserted in the desired crop to get the particular nutrient at a higher level. Acceptability of GM crops, the stability of gene insert, and biosafety regulations are the main hurdles in the transgenic approach.

Barley (*Hordeum vulgare* ssp. *vulgare*) is the fourth most important cereal crop globally. The average annual production of barley is more than 140 million tons from the 50 million hectares of area. About 70% of barley produced is used as animal feed, 21% is used for beer making in distilleries, 6% is used as food for human beings, and the remaining is used in biofuel production (www.fao.org/faostat/en/#data/QC). Nutritionally, barley grain is comprised of 70% starch, 10–20% protein, 2–3% lipids, 5–10% β-glucan, 2–5% minerals, and 11–34% dietary fibers (Sullivan et al. 2013). Barley was the earliest cereal that was domesticated and used for the preparation of bread (Tiwari 2010). Barley is also used as model species for the members of *Triticeae* such as soft wheat, durum wheat, and rye; as these species are closely related, genetic information from barley can be used for the research in these *Triticeae* species (Sreenivasulu et al. 2008). Barley grain harbors several bioactive compounds like β-glucans, lignans, tocotrienols, folate, fructans, phytosterols, polyphenols, policosanol, phytates, etc.; therefore, consumers show interest in barley as a food. It is a rich source of dietary fiber and functional food; β-glucans in barley are known to lower the blood cholesterol and has low glycemic index (Baik and Ullrich 2008). Further biofortification of barley with different nutrients will increase its nutritional value and will help in overcoming malnutrition.

9.2 Biofortification Approaches

Generally, biofortification strategies are comprised of these main approaches: genetic/breeding, biotechnological/transgenic, and agronomic approaches (Fig. 9.1).

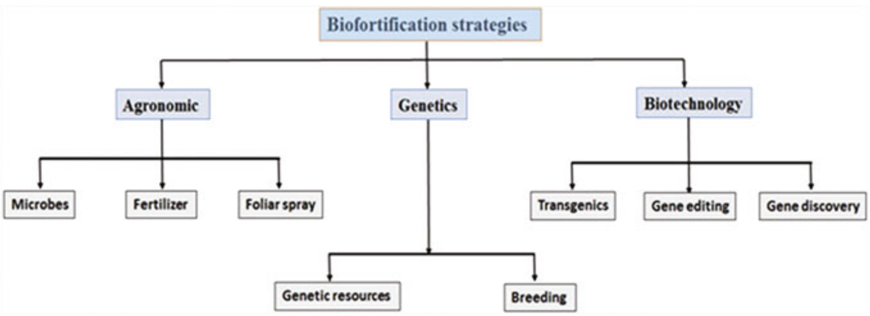

Fig. 9.1 Different strategies for biofortification of barley

9.3 Genetic and Plant Breeding Approach

Genetic and plant breeding is the most believed approach which is more sustainable and economical compared to the other two approaches. Screening of available germplasm for a trait of interest is performed, and the success relies on the availability of enough diversity in that trait (Velu et al. 2014). Further, biofortification by the genetic approach is also influenced by accurate phenotyping and gene-environment interactions. Genetically fortified plants need to be supported with optimal agronomic practices to get the best out of them.

For any breeding experiment, one should have suitable parents for crossing, and getting suitable parents depends upon the availability of diversity in germplasm and the extent of screening. Selection for mineral content is a complex process as this nutrient content is affected by many physiological factors (Narwal et al. 2020). QTL mapping in crops is done to understand the complex biological traits. Transport of Zn from vegetative parts to grain via phloem is a major barrier in the loading of Zn in grain endosperm. Hussain et al. (2016) performed double haploid mapping to genetic characterization of Zn remobilization. They found a large variation in Zn content from 27 to 75 μg g^{-1}, and this variation was correlated with the remobilization of Zn to grain. Three QTLs associated with leaf and two QTLs linked with stem were found to involve in Zn remobilization. Such studies will help in Zn biofortification of barley. Further, QTLs for mineral content were detected using 193 recombinant inbred lines. Seventeen QTLs were detected in barley grain which contributed 6.36–64.08% diversity in Zn, Mg, Ca, K, Na, Mn, Fe, and P. They also detected pleiotropic QTLs having an additive effect on mineral content (Zeng et al. 2016). Such efforts further will help us with marker-assisted selection for mineral biofortification. QTLs for grain and malt β-glucan amounts were mapped with the help of the 123 marker linkage map. They identified three QTLs for grain β-glucan, six for malt β-glucan, and eight for malt β-glucanase using interval mapping (Han et al. 1995). Such dissection of β-glucan content is further useful in the breeding as well as the selection of high- and low-β-glucan-containing genotypes (Li et al. 2008). Genome-wide association studies (GWAS) were performed using 336 spring barley accessions for element amounts in the grain with the help of 6519 SNP markers and TASSEL software. Several QTLs for different minerals were identified that will be used in the future for breeding nutrient-rich barley (Gyawali et al. 2019). Grain protein content (GPC) is the main grain quality character. *Gpc-B1*, a wheat GPC QTL, is an NAC transcription factor (TtNAM-B1) involved in higher levels of protein, zinc, and iron in grain. A similar QTL in barley was identified on chromosome 6H. The colinearity of GPC regions indicated its role in GPC QTL in barley (Distelfeld et al. 2008). Xue et al. (2016) studied the nutrient level in barley grain under different environmental conditions and reported high genetic and environmental interactions. Total nitrogen exhibited high genotype contribution; thus nitrogen remobilization might have increased the Zn and Fe transport to grain indicating the genetic effect of GPC locus on Zn and Fe translocation. Further, Fan et al. (2017) identified environmentally stable QTLs using SNP and SSR marker 190 recombinant inbred lines. These QTLs were identified on chromosomes 2H (1), 4H (1), 6H (1), and 7H (3). These studies could be used in developing high-grain protein barley genotypes.

9.4 Transgenic and Biotechnological Approach

Trangenic and biotechnological methods for biofortification are generally used in such conditions where limited or no variation in the trait of interest was present in germplasm (Zhu et al. 2007). It involves genetic engineering where genes from different sources for a trait of interest are introduced in the target variety of crop. This approach enables us to transfer traits across the species boundaries independent of taxonomic status. Similarly, transgenics is the only method to fortify any crop with a micronutrient that is not present naturally in crops (Perez-Massot et al. 2013); thus transgenic approach of biofortification has the potential to significantly contribute toward the improvement of nutrition and health.

9.5 Transgenics

Transgenic strategies generally target the enhancement of nutrient uptake, biosynthesis of nutrients, and bioavailability of nutrients. It can be achieved by following these key steps: enhancing uptake, increasing translocation to grain, targeting storage toward endosperm, decreasing anti-nutritional factors, and increasing the bioavailability of nutrients of interest (Mulualem 2015). There are only a few attempts taken in barley biofortification using a transgenic approach. Ramesh et al. (2004) reported that overexpression of the known zinc transporters from *Arabidopsis* in barley under ubiquitin promoter increased Zn concentration in transgenic barley. Menguer et al. (2018) improved the zinc content in grains by overexpressing a transition metal transporter (*HvMTP1*) gene under the endosperm-specific promoter. They found higher grain content in the endosperm of transgenic lines of barley. Similarly, transgenic barley expressing cytokinin oxidase/dehydrogenase (CKX) gene in roots led to the development of a larger root system which also accumulated a higher amount of zinc in barley grain than wild type (Ramireddy et al. 2018). Cereal grains are containing an insufficient amount of essential amino acid-like lysine. Ohnoutkova et al. (2012) developed transgenic barley expressing dihydrodipicolinate synthase from *E. coli*. The resulted T1 generation plants were having a more than 50% increase in lysine over the wild type. C-hordein in barley is a member of prolamin protein families and is composed of mainly nonessential amino acids like proline and glutamine and thus has low nutritional value. Therefore, Sikdar et al. (2016) silenced the C-hordein gene in barley using RNAi technology, and quadrupole-time-of-flight mass spectrometry analyses of protein fraction revealed a reduction in C-hordein, and the level of essential amino acids was increased. Earlier, Lange et al. (2007) suppressed the C-hordeins in barley using antisense construct, and amino acid analyses revealed that levels of nonessential amino acids (proline, glutamic acid/glutamine, and phenylalanine) were decreased by 12%, 6%, and 9%, respectively, while the amount of essential amino acids like lysine, threonine, and methionine was elevated by 16%, 13%, and 11%, respectively. Therefore modulation of prolamin levels in barley grains is a promising way to improve protein quality. Further, overexpression of homogentisate geranylgeranyl transferase (*HvHGGT*) gene in barley resulted in an increase of tocotrienol content and antioxidant activity in barley grain (Chen et al. 2017). The cisgenesis concept was used in barley to increase phytase activity in grain (Holme et al. 2012). They expressed a barley phytase gene (*HvPAPhy_a*) during grain filling stages, and homozygous lines showed more than 2.5-fold increase in phytase activity. This enhanced phytase activity was stable for three generations analyzed. The marker elimination method was used in this study to obtain marker-free transgenic plants. Cisgenesis along with marker-free technique might increase the acceptability of genetically engineered crops. The polysaccharides like (1,3;1,4)-b-D-glucans are useful components in the diet of a human being, which decreases the risk of diabetes, obesity, and cancer. Overexpression of barley cellulose synthase-like family (*CslF6*) gene under endosperm-specific promoter led to an 80% increase in (1,3;1,4)-b-D-glucan content in transgenic barley grain (Burton et al. 2011).

9.6 Genome Editing

Recently, genome editing techniques are also being used for the creation of new alleles and gene editing independent of genome sequences (Khandagale and Nadaf 2016) which could be used for biofortification of barley. CRISPR/Cas9 and TALENs were used for the evaluation of the *HvPAPhy_a* gene in barley. It was found that *HvPAPhy_a* is the main contributor to mature grain phytase activity. Thus higher expression of *HvPAPhy_a* led to fast germination as well as higher phosphate utilization (Holme et al. 2017). Pathway of vitamin E biosynthesis in monocots was not studied in detail due to the lack of functional mutants. Zeng et al. (2020) used CRISPR for the generation and characterization of the functional mutants of barley for *HvHPT* and *HvHGGT* genes which revealed that in barley, *HvHGGT* is the only major gene for the biosynthesis of tocotrienols and *HvHPT* plays a minor role. Inositol trisphosphate 5/6 kinases (ITPK) is an enzyme involved in the production of inositol hexakisphosphate which is the main form of storage phosphate in cereal grains. The creation of lines containing less inositol hexakisphosphate would increase the phosphate and mineral bioavailability. CRISPR/Cas9-mediated editing of *HvITPK1* increased phosphate in grains by 65–174% over wild type. In barley, D-hordein is one of the storage proteins in the barley which negatively impacts malting quality. Li et al. (2020) used CRISPR/Cas9 genome editing and created new alleles of D-hordein gene; transcriptome analysis and SDS-PAGE revealed reduced D-hordein content in mutant lines. These new alleles provided the new germplasm resource for breeding barley for malt quality.

9.7 Omics in Better Understanding Nutrient Uptake, Storage, and Bioavailability

For successful biofortification of any crop, a thorough understanding of nutrient homeostasis is needed. Omics approaches such as genomics, transcriptomics, proteomics, metabolomics, and metagenomics will help us to elucidate the complex phenomenon behind mineral homeostasis. Darbani et al. (2015) attempted the elucidation of the mineral homeostasis in barley seed transfer cells using the RNA-seq approach. Seed transfer cells were isolated using laser capture microdissection from the grain cryosections. The number of genes such as auxin and ethylene signaling factors, sulfur homeostasis components, mineral trafficking components, vacuole organization factors, protein sorting, and recycling factors, etc. were differentially expressed in changes in mineral content. Earlier, Tauris et al. (2009) also demonstrated the road map for zinc transport in the developing grain with the help of barley microarray, Affymetrix 22k GeneChip and proposed a model for zinc trafficking from the phloem to the developing grains.

9.8 Agronomic Approach

Agronomic strategies for biofortification involve the application of nutrients to soil or plants to enhance the content of particular nutrient in the edible part of that crop so that after consumption it will improve human nutrition. Micro minerals, like Zn, Fe, Se, copper, manganese, I, Mo, etc. when applied in the soil to improve the nutritional status of soil, are absorbed by plants which results in alleviating the micronutrient deficiency in humans. This approach is simple but provides short-term solutions, and care should be taken for the selection of the source of nutrients, application method, and effects on the environment. Along with chemical fertilizers, plant growth-promoting microbes like N2 bacteria and P- and K-solubilizing microbes and other microbes which enhance the phyto-availability of nutrients are also used in this approach (Garg et al. 2018).

9.9 Fertilization Application

Agronomic biofortification involves the application of micronutrients in the form of fertilizer to elevate the micronutrient level in grains or edible parts.

Micronutrient fertilizers when applied in combination with NPK and organic fertilizers showed a good response in the uptake of micronutrients (De Valenca et al. 2017). The low solubility of Zn in the soil is the main reason for Zn deficiency in plants; thus sufficient amount of available zinc needs to be maintained in the soil during the grain filling stage to achieve a higher level of Zn in grain. Yadav and Sharma (2018) reported that the application of zinc sulfate along with NH_4NO_3 increased yield as well as Zn content in grain of barley; it was due to the acidifying effect of NH_4NO_3. It was found that the foliar-applied Zn gets easily translocated to grain during development and the localization studies also showed the interaction between Zn and grain proteins (Cakmak and Kutman 2018). Several studies showed that the use of Zn fertilizers elevated the Zn level in grains of some cereals including barley (Cakmak 2010). Similarly, zinc fertilizer applications have been reported to increase the Zn content in barley grain (Uddin et al. 2014). Sulfur is an important element that plays a role in plant development and biotic stress. Its application also increases the uptake of Mn, Fe, Zn, and Cu. The application of sulfur in the form of ammonium and potassium sulfate was found effective in the elevation of microelements in barley (Barczak et al. 2019). For selenium biofortification, Rodrigo et al. (2013) sprayed two-rowed barley with four different concentrations of sodium selenate and sodium selenite for two seasons. It was found that sodium selenite was more efficiently absorbed by the plant. For every gram of sodium selenite and selenate sprayed, the Se concentration in grain was increased by 9 and 44 μg/kg dry weights, respectively.

9.10 Microbes in Biofortification

The continuous chemical fertilizer application in high doses leads to soil and environmental pollution as well as toxicity to plants and animals. Further transgenic crops are not easily accepted by the public which is seen in the case of GM brinjal and mustard. An alternate way is the use of microbe for biofortification; despite its huge potential this approach did not receive enough attention. Rhizospheric or endophytic microbes are known to increase the availability and absorption of micronutrients by plants and ultimately led to the enhancement of micronutrient content in the edible part of the crop (Ku et al. 2019). Soil microbes play a key role in maintaining soil health and fertility (Barret et al. 2011). Farmers are using N-fixing and P- and K-solubilizing microbes to increase the availability of major nutrients to increase the yield. These microbes could be used for biofortification as present in soil and increase the availability of nutrients to crops (Prasanna et al. 2016). Several micronutrients are present in fixed form as a precipitate or adsorbed on soil mineral and organic surfaces. These nutrients are solubilized by PGPR by secreting some enzymes. In wheat, it was found that the application of biofertilizers enhanced the acquisition of minerals (Rana et al. 2012). The iron level in paddy was elevated by application of PGPR inoculum comprised of *P. putida*, *P. fluorescens*, and *Azospirillum lipoferum* (Sharma et al. 2013a). Similarly, Fe content in lentils was found to be doubled by the treatment of biofertilizer containing *Pseudomonas* species (Mishra et al. 2011). Inoculation of *Pseudomonas* and *Acinetobacter* strains significantly increased the Zn, Fe, Mg, Ca, K, and P in crops (Tariq et al. 2007; Khan 2005). These PGPRs could be used in barley to achieve sustainable enrichment of micronutrients.

Arbuscular mycorrhizal fungi (AMF) also solubilize different minerals in the soil and have potential use in biofortification (Martino et al. 2003). Ingra et al. (2019) studied the eight different species of AMF in wheat and reported an increase in the uptake of P, Fe, and Zn along with better root lengths and density. Further selenium level was found to be increased in wheat grain after co-inoculation of *Glomus clarideum*, *Pseudomonas* sp., and *Bacillus* sp. (Duran et al. 2013). The use of PGPRs and AMF for biofortification has been attempted in several crops, but microbe-mediated biofortification is little studied in barley. Watts-Williams and Cavagnaro (2018) demonstrated the increased grain and straw zinc concentration in modern barley after inoculation with AMF *Rhizophagus irregularis*. This increase in Zn concentration was due to the increased uptake of Zn from the soil under the upregulation of ZIP transporters; it interestingly did not increase the yield of grains. Similarly, Coccina et al. (2019) showed AMF-mediated Zn uptake in wheat and barley.

9.11 Biofortification for Minerals

Micronutrient deficiencies are an important form of human malnutrition, known as hidden hunger. Globally micronutrient malnutrition is recognized as an enormous and speedily growing public health problem, especially in developing countries (Zou et al. 2019; Sazawal et al. 2018). Deficiencies of mineral micronutrient such as zinc (Zn), iodine (I), selenium (Se), and iron (Fe) denote the global health problems because these affect more than one-third of the world population (Zou et al. 2019; Lyons 2018). The zero hunger is the Sustainable Development Goal 2 which aims to end hunger through enhanced food and nutritional security, and biofortification of food crops is the most sustainable and cost-effective method to provide nutrition to the target population in natural form fulfilling this goal (Yadava et al. 2018).

9.12 Genetic Diversity for Mineral Content in Barley

Besides "calories," various essential micronutrients are important in the health and nutrition of organisms. These nutrients are divided into *macro*nutrients and *micro*nutrients. Micronutrients are needed in minute quantity which makes up only 0.05% of human body, whereas macroelements constitute 99.5% of human body (Kotz et al. 2006). Several micronutrients function as a cofactor of enzymes that regulate crucial life processes in the organism. Genetic variation of micronutrient content is crucial for the breeding of high-nutrient crops. Wild barley is known to harbor the highest amount of micronutrients. Iron content in wild barley ranged from 10.8 to 329.1 mg kg^{-1}, and zinc content was 66.3–493.9 mg kg^{-1} (Yan et al. 2012). Recently, the International Center for Agricultural Research in the Dry Areas (ICARDA) has analyzed 336 accessions for 13 different micronutrients. Some genotypes showed a high amount of these minerals, which are suitable candidates for the biofortification program in barley (Gyawali et al. 2019).

These micronutrients are not evenly distributed in grain; some are concentrated in husk and aleurone layers which get removed during the milling and polishing process in many cereals including barley. This distribution of nutrients is genotype-dependent. Therefore, one should have a thorough knowledge of diversity and the mechanism of micronutrient uptake and accumulation for biofortification. Detterbeck et al. (2016) studied the micronutrient diversity and distribution and found that more than 120 lines showed good variation in Zn content, and the majority of this diversity is due to genetic differences. Micro-proton-induced X-ray emission (l-PIXE) was used for a detailed study of micronutrient distribution within the grains' four tissues: embryo, aleurone, endosperm, and husk. Further, it is also found that the cultivation of high Zn lines in Cd-contaminated soils resulted in higher Cd accumulation which exceeded the *Codex Alimentarius* threshold. Thus, along with genetic variations for the desired micronutrient, one should consider the levels of toxic elements while planning for the biofortification of barley.

Domestication and repetitive selections led to genetic erosion in several modern crops (Zamir 2001; van de Wouw et al. 2010). A wild relative could be used to replenish the gene pools of modern crops. Wild barley (*Hordeum vulgare* ssp. *spontaneum*) can be used for introgression of fertile barley cultivars (Morrell and Clegg 2007). Such a successful example is the introgression of *Gpc-B1* locus from wild emmer into bread wheat through chromosomal substitution technique (Distelfeld et al. 2006). This locus elevated the micronutrient levels in mature grains (Distelfeld et al. 2007). Wild barley also has huge variations for agronomic traits but has been paid limited attention as a source for biofortification. Wiegmann et al. (2019) studied the interplay between plant development, yield, and nutrient concentrations in wild barley nested association mapping population HEB-25. They observed a huge variation in nutrient concentrations; some lines have more than 50% higher levels of protein, iron, and zinc than a recurrent parent. It was found that grain yield and nutritional value are negatively correlated in barley. Analyses of genetic elements in nutrient content revealed that wild alleles were often linked with the higher nutrient level which indicated that the targeted introgression of wild barley alleles may help us in the biofortification of barley (Wiegmann et al. 2019).

Selenium is a vital trace element important for the health of humans, and the main source of selenium is a plant-based diet (Rayman 2000). Most of the soils are deficient in selenium content, and thus ultimately selenium in the food system is also low, and thus a large number of people suffer from Se deficiency (Combs 2001; Jones et al. 2017). Jun et al. (2011) studied the diversity in grain Se concentration of 92 *H. spontaneum* genotypes representing different habitats in Israel. The grain selenium content ranged from 0 to 0.387 mg kg^{-1}. *H. spontaneum* populations exhibited higher Se content due to their abilities for Se uptake and accumulation.

9.13 Transporters for Mineral Uptake and Transport

The plant roots have an important role in the uptake of essential nutrients from the soil and are used in growth and development functions. The mineral uptake is facilitated by the different transporters in plants belonging to the different transporter families (Sasaki et al. 2016). Fe and Zn are known as essential cellular element which plays a critical role in metabolic processes in all living organisms (Darbani et al. 2015). However many of the metabolic pathways are activated by iron; also it is a prosthetic group constituent of many enzymes (Rout and Sahoo 2015). To date, a large number of iron transporters in plants are known. That involves the yellow stripe 1-like (YSL) subfamily of the oligopeptide transporter (OPT) superfamily, the copper transporter (COPT) family, the natural resistance-associated macrophage protein (NRAMP) family, the zinc-/iron-regulated transporter-like protein (ZIP) family, the Ca^{2+}-sensitive cross complementer 1 (CCC1) family, and the iron-regulated protein (IREG) family (Borg et al. 2009). Darbani et al. (2015) reported vacuolar zinc transporter of Cation Diffusion Facilitator *Mtp*1 with higher expression in barley plants treated by zinc.

Manganese (Mn) is another essential mineral element for plants. Mn deficiency is a serious problem of crop productivity worldwide, which leads to reduced photosynthetic activity and lowers the lignin content, and other structural carbohydrates, ultimately hampering plant growth (Long et al. 2018). However, it is also essential for human health where it regulates the enzymes of glucose and lipid metabolism (Li and Yang 2018). Transporters for Mn absorption and Mn homeostasis are much less known for barley. However, Long et al. (2018) reported the role of the iron-regulated transporter 1 for absorption and transport of Mn in barley. Pedas et al. (2008) reported that *HvIRT1* contributes to genetic diversity in Mn kinetics.

In spite of the mineral uptake, some transporters are involved in mineral translocation activities. Fe deficiency activates Fe^{3+}-mugineic acid family phytosiderophores (MAs) transporter in barley (Murata et al. 2006) and rice (Inoue et al. 2009). These transporters are involved both in Fe uptake and translocation under Fe deficiency (Tsukamoto et al. 2009). ZIP family proteins have a specific role in Zn^{2+} uptake and translocations to the specific organelles of plants and are reported to have essential roles in rice and barley *OsZIP4* having an important role in the translocation of Zn^{2+} from roots to developing young leaves and in long-distance transport of Zn^{2+} between old and young leaves of rice. HvZIP proteins are also found to have very specific roles in translocation of Zn^{2+} to specific organelles in barley (Pedas et al. 2009).

9.14 Zinc

In developing countries, cereals comprise a large portion that contains a low amount of and has less bioavailability of zinc. The inadequate dietary intake of Zn signifies major health problems in the population (Cakmak and Kutman 2018). Zn acts as a prosthetic group for more than 3000 proteins (Sharma et al. 2013a, b) for their activity and thus is essential for growth and cell division (Brown et al. 2004). More than 25% of the human population is facing Zn deficiency across the world (Maret and Sandstead 2006). In animals, Zn is required for normal development and proper function of the immune system. Similarly, in plants, it plays a key role in vital developmental processes (Chattha et al. 2017). The foliar application of Zn on wheat at a late stage of growth recorded increased grain zinc concentration by 61% and 65% with foliar application of micronutrient cocktail (Zou et al. 2019). Wheat is inherently low in zinc concentration and high in phytate, which further limits zinc

Table 9.1 Biofortification of barley to increase the micronutrient concentration in grain with the application of mineral fertilizers under field conditions

Sr. no.	Biofortification of element	Agronomic practices	Success/level of improvement	References
1	Zn	Fertilizer application of ZnCHE	Zn content increased in grains up to 30%	Almendros et al. (2019)
		Application of $ZnSO_4$ foliar spray	33.12% increase in grain Zn content	Gonzalez et al. (2019)
2	Mn	Fertilizer application of ammonium sulfate ($(NH_4)_2SO_4$)	Mn content increased in grains up to 19.2%	Barczak et al. (2019)
3	Fe	Fertilizer application of ammonium sulfate ($(NH_4)_2SO_4$)	Fe content increased in grains up to 19.5%	Barczak et al. (2019)
4	Cu	Fertilizer application of ammonium sulfate ($(NH_4)_2SO_4$)	Cu content increased in grains up to 6.5%	Barczak et al. (2019)
5	Se	Sodium selenate foliar spray	Se concentration in grain increased up to 44 μg/kg dry weight	Rodrigo et al. (2013)
		Sodium selenite foliar spray	Se concentration in grain increased up to 9 μg/kg dry weight	Rodrigo et al. (2013)
		Soil application of Se at 10 to 20 g Se ha^{-1} as selenate	Barley grain Se concentration increase from 100 to 200 μg kg^{-1}	Ylaranta (1985)
		Soil application of 30–60 g ha^{-1} selenate and 4.5–10 g ha^{-1} foliar selenate	Increase in barley grain Se from 7 to 100 μg kg^{-1}	Ros et al. (2016)

bioavailability, which is the major reason for zinc malnutrition in humans where wheat is a staple food (Welch and Graham 2004). Chattha et al. (2017) recorded an increase in the wheat grain zinc concentration by 48.33% under the soil and foliar application and a 47.20% increase in only foliar application. Saha et al. (2017) reported an increase in the Zn concentration in rice by soil and foliar application. However, the loss of Zn on the processing of rice grains increased because of the preferential allocation of applied Zn into bran and aleurone of the rice grains. Despite such losses, Zn application increased bioavailability by 52.2% in cooked rice. Similarly, zinc-containing fertilizers have been used in increasing the Zn level in barley also (Yadav and Sharma 2018; Cakmak and Kutman 2018; Cakmak 2010; Gonzalez et al. 2019; Uddin et al. 2014). Further Watts-Williams and Cavagnaro (2018) used mycorrhizal fungi for Zn enrichment of barley which proved the agronomic approach as an efficient method for Zn fortifications in cereal crops (Table 9.1).

9.15 Iodine

Iodine is an essential micronutrient for humans, involved in functioning of the thyroid system. Salt iodization was inadequate to ensure global iodine adequacy as one-third of the world population may face hypothyroidism and iodine deficiency (Lyons 2018). Agronomic biofortification of cereal crops, which are consumed widely as a staple food, is an effective approach to reduce iodine deficiency (De Valenca et al. 2017). Zou et al. (2019) reported an increase in grain iodine concentration by 13.1-fold on foliar iodine spray; however increase of 10.3-fold was recorded in foliar micronutrient cocktail spray in wheat. Iodine in plants is transported mostly in xylem tissues; hence it is relatively easy to biofortify the leafy vegetable crops (Smolen et al. 2014). Comandini et al. (2013) reported vegetables biofortified with foliar I showed a high I stability during cooking. Till today there is no single report on iodine biofortification in barley.

9.16 Selenium

In the view of global health issues, selenium (Se) deficiency in the diet is the major problem as it is an essential element for mammals. Plants represent a major source of selenium as it is a beneficial element for them as an antioxidant and a growth promoter (Schiavon et al. 2020; Garcia-Banuelos et al. 2011). Methyl-selenocysteine (MeSeCys), the organic form of Se, appears to be a predominantly effective source of dietary Se; however, Se is incorporated as selenocysteine (SeCys) at the active site of selenoproteins involved in major metabolic pathways such as antioxidant defense and immune functions (Malagoli et al. 2015). The ability of the plants to accumulate and transform the Se into bioactive compounds tends to be an important implication for human nutrition. Se deficiency is a global problem, and plants are the essential source of dietary Se that can help to solve this problem (Garcia-Banuelos et al. 2011). Agronomic biofortification intends to enrich crops with Se (Schiavon et al. 2020); it can be done by applying the foliar application of sodium selenite or selenite fertilizers (Lidon et al. 2018).

Foliar application of Se was proved very effective to increase wheat grain Se from 90 to 338 μg kg^{-1} (Zou et al. 2019). Lyons et al. (2005) reported Se applied as sodium selenate at rates of 120 g Se/ha sprayed on the soil at seedling stage increased grain Se concentration up to 133-fold, while it increased up to 20-fold when applied as a foliar spray after flowering. The application of sodium selenite 25 g Se/ha was found to increase from 0.02 to 0.38 mg kg^{-1} in rice grains (Reis et al. 2018). Two-rowed barley was sprayed with different concentrations of sodium selenite; selenium level was found to be significantly increased in barley grain (Rodrigo et al. 2013). Sodium selenate application during the anthesis and germination stage of the malting enhanced level of Se in barley grains as well as in final beer products (Gibson et al. 2006).

9.17 Iron

Iron deficiency is the sixth most serious problem for human health in the world and is also known as hidden hunger (Masuda et al. 2020). Fe deficiency causes anemia, poor pregnancy outcomes, and lower immunity (Connorton and Balk 2019). Most of the world's population have monotonous diets consisting mainly of cereals predominantly starch-rich but nutrient-poor such as rice, corn, wheat, and the tubers like potato and cassava which are deficit in iron, which have affected two billion population globally (Connorton and Balk 2019; Sperotto et al. 2012). For humans, plants are the ultimate source of the Fe either directly as a staple food or indirectly from animal fodder. Fe fertilization to crops is not a very effective technique to enrich Fe in crops as Fe is insoluble in soil; therefore Fe biofortification is the most suitable alternative solution to enrich Fe in food grains. It is possible by generating cultivars that can efficiently mobilize, uptake, and translocate Fe to its edible parts (Sperotto et al. 2012). The foliar application of Fe takes 10–20 days to absorb 50% of the micronutrient as it is affected by different factors such as endogenous (leaf anatomy), exogenous (soil, pH), and environmental factors (Ludwig and Slamet-Loedin 2019). Therefore the foliar application to reach a significant enrichment in grain Fe for biofortification remains quite challenging. Dragicevic et al. (2016) demonstrated an increase in bioavailability of iron in barley after foliar spray of nonstandard fertilizers, hormonal growth stimulators.

Genetic engineering has been used in various crops to enrich mineral content like Fe and Zn. The transgenic strategies for the enrichment of Fe content have been focused on the intake and utilization efficiency of the plants by regulating and modulating the expression of the transporter (Kumar et al. 2019). Takahashi et al. (2001) reported enhanced iron uptake in low iron availability in soil by transgenic rice with two *naat* genes, coding for crucial enzymes for phytosiderophores. Drakakaki et al. (2000) reported that recombinant ferritin significantly increases iron in rice and wheat. However, the ferritin hyper-expressing rice lines were reported with a 30% higher iron rice (Qu et al. 2005). Such attempts can be made in barley to improve grain iron content in barley.

9.18 Biofortification for Antioxidants and Vitamins

Antioxidants are health-promoting molecules that nullify the reactive oxygen species and protect the cellular components and nucleic acids from oxidative damage. During the metabolic process and stress, reactive oxygen species and free radicals are produced; antioxidant molecules present in natural foods like fruits, vegetables, and grains scavenge these reactive oxygen species protecting our body (Zhu et al. 2013). Phytochemicals like flavonoids, carotenoids, phenolics, lignans, vitamins, minerals, and phytates present in food act as an antioxidant. Antioxidants are grouped into two categories: lipophilic (carotenoids, tocochromanols, coenzyme Q10, etc.) and hydrophilic (ascorbate, flavonoids, melatonin, etc.).

Vitamin E is one of the lipid-soluble antioxidants essential for human health. The seeds of most monocots contain the majority of vitamin E in the form of tocotrienols. Chen et al. (2017) produced transgenic barley overexpressing HvHGGT under endogenous D-hordein promoter (proHor) which led to an increase in tocotrienol content by 10–15% in seeds of transgenic lines. The radical scavenging activity of transgenic seed extracts was also enhanced by 17–18% over wild type. Similarly, other vitamins and antioxidant levels could be enhanced in barley by regulating individual rate-limiting steps or key branch points or modification of regulatory elements that may help in the biofortification of some antioxidants. Naqvi et al. (2009) developed modified three distinct metabolic pathways and developed multivitamin-rich corn. The levels of the β-carotene, ascorbate, and folate were increased in transgenic kernels by 169-fold, 6-fold, and 2-fold, respectively. Such attempts need to be done in barley to develop vitamin- and antioxidant-rich barley. Ascorbate or vitamin C is a potent water-soluble antioxidant. Hormones can also regulate ascorbate biosynthesis. The increased level of abscisic acid reduces the expression of NADPH oxidases which is the main producer of ROS in seeds (Ishibashi et al. 2017). Biofortification of durum wheat for provitamin A was performed using a tilling approach which resulted in an increase of roughly 75% in β-carotene in the grains (Sestili et al. 2019). The amino acid sequences of lycopene epsilon cyclase of wheat revealed that it has great homology with barley but differs from other cereals. Wicker et al. (2009) showed that the gene structure and order are strongly conserved in wheat and barley despite their divergence about 11 million years ago. Thus, this strategy for provitamin A biofortification could be used in barley.

9.19 Factors Affecting Biofortification

Several pre-harvest and postharvest factors affect the success of the biofortification program (Fig. 9.2).

9.20 Mineral-Deficient Soil

Mineral nutrient-deficient soil is the major factor affecting the biofortification in crop plants. More than 90% of the zinc (Zn) in the soils exists as an insoluble Zn, therefore poorly available to the plants (Singh 2011). The rapid absorption of Zn on clay minerals reduces the mobility of Zn in soil by making it unavailable to plants. However, in India, Arunachalam et al. (2013) reported that 49% of lands under cultivation are having Zn-deficient soils together with 12% deficiency in iron, 3% in copper, 5% in manganese, 33% in boron, and 13% in molybdenum. It limits the crop productivity and nutritional quality of the crops.

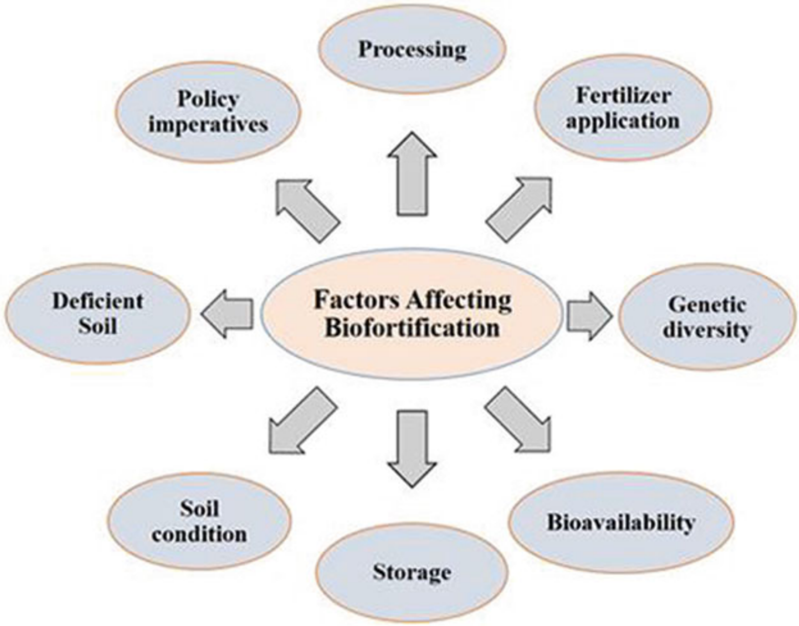

Fig. 9.2 Factors affecting the biofortification

9.21 Soil Condition

Soil pH, moisture status, organic matter content, salinity, and other factors affect micronutrient uptake by roots from the soil. A change in soil pH from 6 to 7 resulted in a 30-fold decrease in the chemical solubility of Zn in soil (Marschner 1993). Similarly, salinity (Cakmak 2008) and moisture stress also negatively affect the mineral availability and uptake by crop plants (Waters and Grusak 2008). Therefore, to achieve efficient and sustainable biofortification of crops, we need to maintain good fertility as well as physical-chemical properties of soil.

9.22 Fertilizer Application

The optimum amount of fertilizer containing the desired nutrient is needed to be applied before or during the growth of plants. These fertilizers can be applied directly to the soil or in the form of foliar applications. Micronutrient uptake was found to be increased when fertilizer containing micronutrients was supplied in combination with NPK and organic fertilizers (De Valenca et al. 2017). Therefore integrated practices are needed to follow to increase soil fertility to achieve successful biofortification using an agronomic approach.

9.23 Soil Microflora

The soil microflora such as bacteria, fungi, mycobacteria, and cyanobacteria helps the plants for precise nutrient acquisition. Increasing the soil microbial diversity is the best approach for the fortification of essential elements such as zinc, iron, and selenium in crop plants (Dapkekar et al. 2020). The long-term and excessive input of chemical fertilizers and pesticides cause chemicals to persist in the soil, which is so bound to affect the soil microflora (Prashar and Shah 2016). As discussed earlier soil microflora also plays a key role in micronutrient uptake by the root system from the soil. Therefore, we need to maintain rich microflora in soil by application of organic inputs and biofertilizers.

9.24 Bioavailability of Nutrients

Accumulation of nutrient in the crop is not enough; it should have good bioavailability character. Bioavailability largely depends upon the biochemical nature of the nutrient, anti-nutritional factors, as well as the health of the individual consuming the biofortified food; these factors can promote or delay the absorption of nutrients (Diaz-Gomez et al. 2017). Pfeiffer and McClafferty (2007) reported that 5% iron and 25% zinc are present in a bioavailable form in several crops. Phytate and phenolic compounds act as anti-nutritional factors that need to be considered during the biofortification of barley. Thus, the bioavailability of nutrients in biofortified crops needs to be assessed before large-scale adoption.

9.25 Storage and Processing

The stability of biofortified nutrients in the storage period is an important factor governing the success of biofortification. It has been reported that in provitamin A-biofortified maize, a large number of carotenoids were lost during storage (Mugode et al. 2014). The nutritional quality of barley was also reduced during the storage period by 1.74% and 2.82% (Polat 2015). Malting quality is known to increase in storage for 1 year.

A biofortified nutrient should be stored in grain endosperm to avoid or minimize losses during postharvest processing like pearling and milling. Generally, cereals are dehulled and polished which results in substantial losses of nutrients like minerals and vitamins (Raes et al. 2014; Dunn et al. 2014). Therefore, the accumulation of biofortified nutrients in endosperm with the help of genetic engineering will be useful in maintaining the quality of grain after milling (De Steur et al. 2015). β-glucan levels in barley were not affected after pearling as it is concentrated in endosperm; also thus even 30% pearling has no significant effect on the β-glucan content. Further, heat treatment during processing also adversely affects nutrients like vitamins and phenolics (Sharma and Gujral 2010). To take good benefit of biofortified crops, the storage and postharvest processing conditions should be optimized to maintain the higher level of nutrient in the processed final product.

9.26 Advantage of Biofortification

There are three main advantages of biofortification of crops: effective outreach, cost-effectiveness, and sustainability.

Biofortification of staple food will help to reach nutritious food to the targeted population as staple foods like cereals are a major part of their diet. Seeds of biofortified high-yielding varieties developed by research institutes and universities can be distributed to the targeted population or poor farmers at a reasonable cost to achieve the nutritional security of targeted people (Bouis et al. 2011). Several processed and fortified foods are available in the market but are unavailable to poor people due to high cost, lack of awareness, and lack of education.

Biofortification is a cost-effective strategy to lift the large population over the threshold from malnourishment to micronutrient sufficiency. A high-yielding variety is fortified with particular micronutrients like Fe and Zn; it will continuously produce nutritionally rich food for several generations in a cost-effective manner compared to processed and physically fortified food. The benefits of this biofortification will be far higher than the cost of development of biofortified variety.

Sustainability is another advantage of biofortification as once the gene or trait of interest is transferred in a variety, it will continue to be grown by farmers and consumed by the needy population year after year. There will be very less investment needed to monitor and maintain biofortified traits as compared to other fortification programs.

9.27 Conclusion

Hidden hunger or micronutrient malnutrition has a severe impact on the health of the population. Therefore, the prevention of micronutrient malnutrition is one of the major goals of scientists and policymakers worldwide. Thus biofortification of staple crops like cereals is considered a sustainable strategy for delivering nutritional food to target at-risk population.

Barley is the fourth most important cereal crop, and thus increasing its nutritional value by biofortification will play important role in the reduction of hidden hunger. Enrichment of barley with minerals like Zn, Fe, and Se and vitamins will help provide sufficient amount of trace elements and vitamins to the target population. Biofortification of barley with essential amino acids like lysine and β-glucan increased the nutritional quality and health-promoting nature of barley. Strategies for biofortification are based on breeding, agronomic practices, and genetic engineering that will help in addressing malnutrition. Transgenics is a promising method of biofortification but the acceptance and biosafety issues are the major hindrance. This hindrance can be overcome by the use of genome editing tools like CRISPR;

these tools are very precise and rapid and produce stable mutants for sustainable use. Further, enrichment with nutrients is not sufficient, the bioavailability of that nutrient is also an important factor, and it depends upon the intrinsic qualities of nutrients, food matrix, and health of the consumer. Adoption of biofortified varieties by the farmers is only possible if the cultivation of these varieties does not require additional inputs and has no yield penalty and if farmers get a premium price for their harvest. Finally, we need to run awareness programs for the target population about the benefits of these biofortified crops.

References

Almendros P, Obrador A, Alvarez JM, Gonzalez D (2019) Zn-DTPA-HEDTA-EDTA application: a strategy to improve the yield and plant quality of a barley crop while reducing the N application rate. J Soil Sci Plant Nutr 19(4):920–934

Arunachalam P, Kannan P, Prabukumar G, Govindaraj M (2013) Zinc deficiency in Indian soils with special focus to enrich zinc in peanut. Afr J Agric Res 8(50):6681–6688

Baik BK, Ullrich SE (2008) Barley for food: characteristics, improvement, and renewed interest. J Cereal Sci 48:233–242. https://doi.org/10.1016/j.jcs.2008.02.002

Barczak B, Jastrzębska M, Kostrzewska MK (2019) Biofortification of spring barley grain with microelements through sulfur fertilization. J Chem 2019:8214298

Barret M, Morrissey JP, O'Gara F (2011) Functional genomics analysis of plant growth-promoting rhizobacterial traits involved in rhizosphere competence. Biol Fertil Soils 47:729–743

Borg S, Brinch-Pedersen H, Tauris B, Holm PB (2009) Iron transport, deposition and bioavailability in the wheat and barley grain. Plant Soil 325(1–2):15–24

Bouis HE, Hotz C, McClafferty B, Meenakshi JV, Pfeiffer WH (2011) Biofortification: a new tool to reduce micronutrient malnutrition. Food Nutr Bull 32:S31–S40

Brown KH, Rivera JA, Bhutta ZA, Gibson RS, King JC, Lonnerdal B et al (2004) International Zinc Nutrition Consultative Group (IZiNCG) technical document no.1. Assessment of zinc deficiency in populations and options for its control. Food Nutr Bull 25:S99–S199

Burton RA, Collins HM, Kibble NA, Smith JA, Shirley NJ, Jobling SA, Henderson M, Singh RR, Pettolino F, Wilson SM, Bird AR (2011) Over-expression of specific HvCslF cellulose synthase-like genes in transgenic barley increases the levels of cell wall (1, 3; 1, 4)-β-d-glucans and alters their fine structure. Plant Biotechnol J 9(2):117–135

Cakmak I (2008) Enrichment of cereal grains with zinc: agronomic or genetic biofortification? Plant Soil 302(1–2):1–17. https://doi.org/10.1007/s11104-008-9584-6

Cakmak I (2010) Biofortification of cereals with zinc and iron through fertilization strategy. In: 19th world congress soil sci soil solutions changing world, vol 5, pp 1–6

Cakmak I, Kutman UÁ (2018) Agronomic biofortification of cereals with zinc: a review. Eur J Soil Sci 69(1):172–180

Chattha MU, Hassan MU, Khan I, Chattha MB, Mahmood A, Nawaz M, Subhani MN, Kharal M, Khan S (2017) Biofortification of wheat cultivars to combat zinc deficiency. Front Plant Sci 8: 281

Chen J, Liu C, Shi B, Chai Y, Han N, Zhu M, Bian H (2017) Overexpression of HvHGGT enhances tocotrienol levels and antioxidant activity in barley. J Agric Food Chem 65(25):5181–5187

Coccina A, Cavagnaro TR, Pellegrino E, Ercoli L, McLaughlin MJ, Watts-Williams SJ (2019) The mycorrhizal pathway of zinc uptake contributes to zinc accumulation in barley and wheat grain. BMC Plant Biol 19:133

Comandini P, Cerretani L, Rinaldi M, Cichelli A, Chiavaro E (2013) Stability of iodine during cooking: investigation on biofortified and not fortified vegetables. Int J Food Sci Nutr 64(7): 857–861

Combs GF (2001) Selenium in global food systems. Br J Nutr 85:517–547

Connorton JM, Balk J (2019) Iron biofortification of staple crops: lessons and challenges in plant genetics. Plant Cell Physiol 60(7):1447–1456

Dapkekar A, Deshpande P, Oak MD, Paknikar KM, Rajwade JM (2020) Getting more micronutrients from wheat and barley through agronomic biofortification. In: Wheat and barley grain biofortification. Elsevier, Amsterdam. https://doi.org/10.1016/B978-0-12-818444-8.00003-1

Darbani B, Noeparvar S, Borg S (2015) Deciphering mineral homeostasis in barley seed transfer cells at transcriptional level. PLoS One 10(11):e0141398. https://doi.org/10.1371/journal.pone.0141398

De Steur H, Blancquaert D, Strobbe S, Lambert W, Gellynck X, Van Der Straeten D (2015) Status and market potential of transgenic biofortified crops. Nat Biotechnol 33:25–29

De Valenca AW, Bake A, Brouwer ID, Giller KE (2017) Agronomic biofortification of crops to fight hidden hunger in sub-Saharan Africa. Glob Food Sec 12:8–14

Detterbeck A, Pongrac P, Rensch S, Reuscher S et al (2016) Spatially resolved analysis of variation in barley (*Hordeum vulgare*) grain micronutrient accumulation. New Phytol 211:1241–1254

Diaz-Gomez J, Twyman RM, Zhu C, Farré G, Serrano JC, Portero-Otin M et al (2017) Biofortification of crops with nutrients: factors affecting utilization and storage. Curr Opin Biotechnol 44:115–123

Distelfeld C, Uauy T, Fahima J (2006) Dubcovsky, physical map of the wheat high grain protein content gene Gpc-B1 and development of a high-throughput molecular marker. New Phytol 169:753–763

Distelfeld I, Cakmak Z, Peleg L, Ozturk AM, Yazici H, Budak Y, Saranga T (2007) Fahima, multiple QTL-effects of wheat Gpc-B1 locus on grain protein and micronutrient concentrations. Physiol Plant 129:635–643

Distelfeld A, Korol A, Dubcovsky J, Uauy C, Blake T, Fahima T (2008) Colinearity between the barley grain protein content (GPC) QTL on chromosome arm 6HS and the wheat Gpc-B1 region. Mol Breed 22(1):25–38

Dragicevic V, Nikolic B, Radosavljevic M, Djuric N, Dodig D, Stoiljkovic M et al (2016) Barley grain enrichment with essential elements by agronomic biofortification. Acta Periodica Technologica 47:1–9

Drakakaki G, Christou P, Stöger E (2000) Constitutive expression of soybean ferritin cDNA intransgenic wheat and rice results in increased iron levels in vegetative tissues but not in seeds. Transgenic Res 9(6):445–452

Dunn ML, Jain V, Klein BP (2014) Stability of key micronutrients added to fortified maize flours and corn meal. Ann N Y Acad Sci 1312:15–25

Duran P, Acuna JJ, Jorquera MA, Azcón R, Borie F, Cornejo P, Mora ML (2013) Enhanced selenium content in wheat grain by co-inoculation of selenobacteria and arbuscular mycorrhizal fungi: a preliminary study as a potential Se biofortification strategy. J Cereal Sci 57:275–280

Evenson RE, Gollin D (2003) Assessing the impact of the green revolution, 1960 to 2000. Science 300:758–762

Fan C, Zhai H, Wang H, Yue Y, Zhang M, Li J et al (2017) Identification of QTLs controlling grain protein concentration using a high-density SNP and SSR linkage map in barley (Hordeum vulgare L.). BMC Plant Biol 17(1):122

Garcia-Banuelos ML, Hermosillo-Cereceres MA, Sanchez E (2011) The importance of selenium biofortification in food crops. Curr Nutr Food Sci 7(3):181–190

Garg M, Sharma N, Sharma S, Kapoor P, Kumar A, Chunduri V, Arora P (2018) Biofortified crops generated by breeding, agronomy, and transgenic approaches are improving lives of millions of people around the world. Front Nutr 5:12. https://doi.org/10.3389/fnut.2018.00012

Gibson C, Park YH, Myoung KH, Suh MK, McArthur T, Lyons G, Stewart D (2006) The biofortification of barley with selenium. In: Proceedings of the Institute of Brewery & Distillating (Asia-Pacific Section) Carventron, pp 19–24

Gonzalez D, Almendros P, Obrador A, Alvarez JM (2019) Zinc application in conjunction with urea as a fertilization strategy for improving both nitrogen use efficiency and the zinc biofortification of barley. J Sci Food Agric 99(9):4445–4451

Gyawali S, Ottec ML, Jacob DL, Abderrazek J, Verma RPS (2019) Multiple element concentration in the grain of spring barley (*Hordeum vulgare* L.) collection. J Plant Nutr 42(9):1036–1046

Han F, Ullrich SE, Chirat S, Menteur S, Jestin L, Sarrafi A, Hayes PM, Jones BL, Blake TK, Wesenberg DM, Kleinhofs A (1995) Mapping of β-glucan content and β-glucanase activity loci in barley grain and malt. Theor Appl Genet 91(6–7):921–927

Holme IB, Dionisio G, Brinch-Pedersen H, Wendt T, Madsen CK, Vincze E, Holm PB (2012) Cisgenic barley with improved phytase activity. Plant Biotechnol J 10(2):237–247

Holme IB, Wendt T, Gil-Humanes J, Deleuran LC, Starker CG, Voytas DF, Brinch-Pedersen H (2017) Evaluation of the mature grain phytase candidate HvPAPhy_a gene in barley (*Hordeum vulgare* L.) using CRISPR/Cas9 and TALENs. Plant Mol Biol 95:111. https://doi.org/10.1007/s11103-017-0640-6

Hussain S, Rengel Z, Mohammadi SA, Ebadi-Segherloo A, Maqsood MA (2016) Mapping QTL associated with remobilization of zinc from vegetative tissues into grains of barley (Hordeum vulgare). Plant Soil 399(1–2):193–208

Ingra R, Amato G, Frenda AS, Giambalvo D (2019) Impacts of arbuscular mycorrhizal fungi on nutrient uptake, N2 fixation, N transfer, and growth in a wheat/faba bean intercropping system. PLoS One 14:e0213672

Inoue H, Kobayashi T, Nozoye T, Takahashi M, Kakei Y, Suzuki K, Nakazono M, Nakanishi H, Mori S, Nishizawa NK (2009) Rice OsYSL15 is an iron-regulated iron (III)-deoxymugineic acid transporter expressed in the roots and is essential for iron uptake in early growth of the seedlings. J Biol Chem 284(6):3470–3479

Ishibashi Y, Aoki N, Kasa S, Sakamoto M, Kai K, Tomokiyo R et al (2017) The interrelationship between abscisic acid and reactive oxygen species plays a key role in barley seed dormancy and germination. Front Plant Sci 8:275. https://doi.org/10.3389/fpls.2017.00275

Jones GD, Droz B, Greve P, Gottschalk P, Poffet D et al (2017) Selenium deficiency risk predicted to increase under future climate change. Proc Natl Acad Sci 114(11):2848–2853

Jun Y, Fang W, Haibo Q, Guoxiong C, Eviatar N, Fahima T, Jianping C (2011) Natural variation in grain selenium concentration of wild barley, *Hordeum spontaneum*, populations from Israel. Biol Trace Elem Res 142(3):773–786

Khan AG (2005) Role of soil microbes in the rhizospheres of plants growing on trace metal contaminated soils in phytoremediation. J Trace Elem Med Biol 18:355–364

Khandagale K, Nadaf A (2016) Genome editing for targeted improvement of plants. Plant Biotechnol Rep 10:327–343. https://doi.org/10.1007/s11816-016-0417-4

Kotz JC, Treichell PM, Weaver G (2006) Chemistry and chemical activity, 6th edn. Thomas Books, Cole

Ku YS, Rehman HM, Lam HM (2019) Possible roles of rhizospheric and endophytic microbes to provide a safe and affordable means of crop biofortification. Agronomy 9(11):764

Kumar S, Palve A, Joshi C, Srivastava RK (2019) Crop biofortification for iron (Fe), zinc (Zn) and vitamin A with transgenic approaches. Heliyon 5(6):e01914

Lange M, Vincze E, Wieser H, Schjoerring JK, Holm PB (2007) Suppression of C-hordein synthesis in barley by antisense constructs results in a more balanced amino acid composition. J Agric Food Chem 55(15):6074–6081

Li L, Yang X (2018) The essential element manganese, oxidative stress, and metabolic diseases: links and interactions. Oxidative Med Cell Longev 2018:1

Li J, Baga M, Rossnagel BG, Legge WG, Chibbar RN (2008) Identification of quantitative trait loci for β-glucan concentration in barley grain. J Cereal Sci 48(3):647–655

Li Y, Liu D, Zong Y, Jiang L, Xi X, Cao D et al (2020) New D hordein alleles were created in barley using CRISPR/Cas9 genome editing. Cereal Res Commun 48(2):131–138

Lidon FC, Oliveira K, Ribeiro MM, Pelica J, Pataco I, Ramalho JC, Leitao AE, Almeida AS, Campos PS, Ribeiro-Barros AI, Pais IP (2018) Selenium biofortification of rice grains and implications on macronutrients quality. J Cereal Sci 81:22–29

Long L, Persson DP, Duan F, Jorgensen K, Yuan L, Schjoerring JK, Pedas PR (2018) The iron-regulated transporter 1 plays an essential role in uptake, translocation and grain-loading of manganese, but not iron, in barley. New Phytol 217(4):1640–1653

Ludwig Y, Slamet-Loedin IH (2019) Genetic biofortification to enrich rice and wheat grain iron: from genes to product. Front Plant Sci 10:833

Lyons G (2018) Biofortification of cereals with foliar selenium and iodine could reduce hypothyroidism. Front Plant Sci 9:730

Lyons GH, Judson GJ, Ortiz-Monasterio I, Genc Y, Stangoulis JC, Graham RD (2005) Selenium in Australia: selenium status and biofortification of wheat for better health. J Trace Elem Med Biol 19(1):75–82

Malagoli M, Schiavon M, Pilon-Smits EA (2015) Effects of selenium biofortification on crop nutritional quality. Front Plant Sci 6:280

Maret W, Sandstead HH (2006) Zinc requirements and the risks and benefits of zinc supplementation. J Trace Elem Med Biol 20:3–18

Marschner H (1993) Zinc uptake from soils. In: Robson AD (ed) Zinc in soils, plants. Kluwer Academic, Dordrecht, pp 59–78

Martino E, Perotto S, Parsons R, Gadd GM (2003) Solubilization of insoluble inorganic zinc compounds by ericoid mycorrhizal fungi derived from heavy metal polluted sites. Soil Biol Biochem 35:133–141

Masuda H, Aung MS, Kobayashi T, Nishizawa NK (2020) Iron biofortification: the gateway to overcoming hidden hunger. In: The future of rice demand: quality beyond productivity. Springer, Cham, pp 149–177

Menguer PK, Vincent T, Miller AJ, Brown JK, Vincze E, Borg S, Holm PB, Sanders D, Podar D (2018) Improving zinc accumulation in cereal endosperm using *Hv MTP 1*, a transition metal transporter. Plant Biotechnol J 16(1):63–71

Mishra PK, Bisht SC, Ruwari P, Joshi GK, Singh G, Bisht JK, Bhatt JC (2011) Bio associative effect of cold tolerant Pseudomonas spp. and rhizobium leguminosarum-PR1 on iron acquisition, nutrient uptake and growth of lentil (Lens culinaris L.). Eur J Soil Biol 47:35–43

Morrell PL, Clegg MT (2007) Genetic evidence for a second domestication of barley (*Hordeum vulgare)* east of the Fertile Crescent. Proc Natl Acad Sci U S A 104:3289–3294

Mugode L, Ha B, Kaunda A, Sikombe T, Phiri S, Mutale R, Davis C, Tanumihardjo S, De Moura FF (2014) Carotenoid retention of biofortified provitamin A maize (*Zea mays* L.) after Zambian traditional methods of milling, cooking and storage. J Agric Food Chem 62:6317–6325

Mulualem T (2015) Application of biofortification through plant breeding to improve the value of staple crops. Biomed Biotechnol 3:11–19

Murata Y, Ma JF, Yamaji N, Ueno D, Nomoto K, Iwashita T (2006) A specific transporter for iron (III)–phytosiderophore in barley roots. Plant J 46(4):563–572

Naqvi S, Zhu C, Farre G, Ramessar K, Bassie L, Breitenbach J, Conesa DP, Ros G, Sandmann G, Capell T, Christou P (2009) Transgenic multivitamin corn through biofortification of endosperm with three vitamins representing three distinct metabolic pathways. Proc Natl Acad Sci 106(19): 7762–7767

Narwal S, Gupta OP, Pandey V, Kumar D, Ram S (2020) Effect of storage and processing conditions on nutrient composition of wheat and barley. In: Wheat and barley grain biofortification. Woodhead Publishing, Sawston, pp 229–256

Ohnoutkova L, Zitka O, Mrizova K, Vaskova J, Galuszka P, Cernei N, Smedley MA, Harwood WA, Adam V, Kizek R (2012) Electrophoretic and chromatographic evaluation of transgenic barley expressing a bacterial dihydrodipicolinate synthase. Electrophoresis 33(15):2365–2373

Oury FX, Berard P, Brancourt-Hulmel M, Depatureaux C, Doussineaux G, Galic N, Giraud A, Heumez E, Lecomte C, Pluchard P, Rousset M, Trottet M (2003) Yield and grain protein

concentration in bread wheat: a review and a study of multi-annual data from a French breeding program. J Genet Breed 57(1):59–68
Pedas P, Ytting CK, Fuglsang AT, Jahn TP, Schjoerring JK, Husted S (2008) Manganese efficiency in barley: identification and characterization of the metal ion transporter HvIRT1. Plant Physiol 148(1):455–466
Pedas P, Schjoerring JK, Husted S (2009) Identification and characterization of zinc-starvation-induced ZIP transporters from barley roots. Plant Physiol Biochem 47(5):377–383
Perez-Massot E, Banakar R, Gomez-Galera S, Zorrilla-Lopez U, Sanahuja G, Arjo G et al (2013) The contribution of transgenic plants to better health through improved nutrition: opportunities and constraints. Genes Nutr 8(1):29–41. https://doi.org/10.1007/s12263-012-0315-5
Pfeiffer WH, McClafferty B (2007) Harvest plus: breeding crops for better nutrition. Crop Sci 47: S88–S105
Polat HE (2015) Effects of different storage conditions on the nutritional qualities of barley and maize. J Agric Sci 21:246–255
Prasanna R, Nain L, Rana A, Shivay YS (2016) Biofortification with microorganisms: present status and future challenges. In: Biofortification of food crops. Springer, New Delhi, pp 249–262
Prashar P, Shah S (2016) Impact of fertilizers and pesticides on soil microflora in agriculture. In: Sustainable agriculture reviews. Springer, Cham, pp 331–361
Qu QL, Yoshihara T, Ooyama A, Goto F, Takaiwa F (2005) Iron accumulation does not parallel the high expression level of ferritin in transgenic rice seeds. Planta 222(2):225–233
Raes K, Knockaert D, Struijs K, Van Camp J (2014) Role of processing on bioaccessibility of minerals: influence of localization of minerals and anti-nutritional factors in the plant. Trends Food Sci Technol 37:32–41
Ramesh SA, Choimes S, Schachtman DP (2004) Over-expression of an Arabidopsis zinc transporter in *Hordeum Vulgare* increases short-term zinc uptake after zinc deprivation and seed zinc content. Plant Mol Biol 54:373–385. https://doi.org/10.1023/B:PLAN.0000036370.70912.34
Ramireddy E, Galuszka P, Schmülling T (2018) Zn-fortified cereal grains in field-grown barley by enhanced root cytokinin breakdown. Plant Signal Behav 13(11):e1530023
Rana A, Joshi M, Prasanna R, Shivay YS, Nain L (2012) Biofortification of wheat through inoculation of plant growth promoting rhizobacteria and cyanobacteria. Eur J Soil Biol 50: 118–126
Rayman MP (2000) The importance of selenium to human health. Lancet 356(9225):233–241
Reis HPG, de Queiroz Barcelos JP, Junior EF, Santos EF, Silva VM, Moraes MF, Putti FF, dos Reis AR (2018) Agronomic biofortification of upland rice with selenium and nitrogen and its relation to grain quality. J Cereal Sci 79:508–515
Rodrigo S, Santamaría O, López-Bellido FJ, Poblaciones MJ (2013) Agronomic selenium biofortification of two-rowed barley under Mediterranean conditions. Plant Soil Environ 59(3):115–120
Ros GH, Van Rotterdam AMD, Bussink DW, Bindraban PS (2016) Selenium fertilization strategies for bio-fortification of food: an agro-ecosystem approach. Plant Soil 404(1–2):99–112
Rout GR, Sahoo S (2015) Role of iron in plant growth and metabolism. Rev Agric Sci 3:1–24
Saha S, Chakraborty M, Padhan D, Saha B, Murmu S, Batabyal K, Seth A, Hazra GC, Mandal B, Bell RW (2017) Agronomic biofortification of zinc in rice: influence of cultivars and zinc application methods on grain yield and zinc bioavailability. Field Crop Res 210:52–60
Sasaki A, Yamaji N, Ma JF (2016) Transporters involved in mineral nutrient uptake in rice. J Exp Bot 67(12):3645–3653
Sazawal S, Dhingra U, Dhingra P, Dutta A, Deb S, Kumar J, Devi P, Prakash A (2018) Efficacy of high zinc biofortified wheat in improvement of micronutrient status, and prevention of morbidity among preschool children and women—a double masked, randomized, controlled trial. Nutr J 17(1):86
Schiavon M, Nardi S, Dalla Vecchia F, Ertani A (2020) Selenium biofortification in the 21st century: status and challenges for healthy human nutrition. Plant Soil 453:1–26

Sestili F, Garcia-Molina MD, Gambacorta G, Beleggia R, Botticella E, De Vita P, Savatin DV, Masci S, Lafiandra D (2019) Provitamin A biofortification of durum wheat through a TILLING approach. Int J Mol Sci 20(22):5703. https://doi.org/10.3390/ijms20225703

Sharma P, Gujral HS (2010) Antioxidant of polyphenol oxidase activity of germinated barley its milling fractions. J Food Chem 120:673–678

Sharma A, Patni B, Shankhdhar D, Shankhdhar SC (2013a) Zinc—an indispensable micronutrient. Physiol Mol Biol Plants 19:11–20

Sharma A, Shankhdhar D, Shankhdhar SC (2013b) Enhancing grain iron content of rice by the application of plant growth promoting rhizobacteria. Plant Soil Environ 59:89–94

Sikdar MSI, Bowra S, Schmidt D, Dionisio G, Holm PB, Vincze E (2016) Targeted modification of storage protein content resulting in improved amino acid composition of barley grain. Transgenic Res 25:19–31

Simmonds NW (1995) The relation between yield and protein in cereal grain. J Sci Food Agric 67: 309–315

Singh MV (2011) Assessing extent of zinc deficiency for soil fertility mapping and nutrition security in humans and animals. Indian J Fertilizer 7:36–43

Smolen S, Sady W, Ledwozyw-Smolen I, Strzetelski P, Liszka-Skoczylas M, Rożek S (2014) Quality of fresh and stored carrots depending on iodine and nitrogen fertilization. Food Chem 159:316–322

Sperotto RA, Ricachenevsky FK, de Abreu Waldow V, Fett JP (2012) Iron biofortification in rice: it's a long way to the top. Plant Sci 190:24–39

Sreenivasulu N, Graner A, Wobus U (2008) Barley genomics: an overview. Int J Plant Genomics 2008:486258

Sullivan P, Arendt E, Gallagher E (2013) The increasing use of barley and barley by-products in the production of healthier baked goods. Trends Food Sci Technol 29:124–134

Takahashi M, Nakanishi H, Kawasaki S, Nishizawa NK, Mori S (2001) Enhanced tolerance of rice to low iron availability in alkaline soils using barley nicotianamine aminotransferase genes. Nat Biotechnol 19(5):466–469

Tariq M, Hameed S, Malik KA, Hafeez FY (2007) Plant root associated bacteria for zinc mobilization in rice. Pak J Bot 39:245–253

Tauris B, Borg S, Gregersen PL, Holm PB (2009) A roadmap for zinc trafficking in the developing barley grain based on laser capture microdissection and gene expression profiling. J Exp Bot 60(4):1333–1347

Tiwari V (2010) Growth and production of barley. In: Verheye W (ed) Soils, plant growth and crop production, Encyclopedia of life support systems (EOLSS), vol 1. UNESCO-EOLSS, Oxford, pp 267–297. http://www.eloss.net

Tsukamoto T, Nakanishi H, Uchida H, Watanabe S, Matsuhashi S, Mori S, Nishizawa NK (2009) 52Fe translocation in barley as monitored by a positron-emitting tracer imaging system (PETIS): evidence for the direct translocation of Fe from roots to young leaves via phloem. Plant Cell Physiol 50(1):48–57

Uddin MN, Kaczmarczyk A, Vincze E (2014) Effects of Zn fertilization on Hordein transcripts at early developmental stage of barley grain and correlation with increased Zn concentration in the mature grain. PLoS One 9(9):e108546. https://doi.org/10.1371/journal.pone.0108546

van de Wouw M, Kik C, van Hintum T, van Treuren R, Visser B (2010) Genetic erosion in crops: concept, research results and challenges. Plant Genet Resour 8:1–15

Velu G, Ortiz-Monasterio I, Cakmak I, Hao Y, Singh RÁ (2014) Biofortification strategies to increase grain zinc and iron concentrations in wheat. J Cereal Sci 59(3):365–372

Waters BM, Grusak MA (2008) Whole-plant mineral partitioning throughout the life cycle in *Arabidopsis thaliana* ecotypes Columbia, *Landsberg erecta,* Cape Verde Islands, and the mutant line ysl1ys. New Phytol 177:389–405

Watts-Williams SJ, Cavagnaro TR (2018) Arbuscular mycorrhizal fungi increase grain zinc concentration and modify the expression of root ZIP transporter genes in a modern barley (*Hordeum vulgare*) cultivar. Plant Sci 274(2018):163–170

Welch RM, Graham RD (2004) Breeding for micronutrients in staple food crops from a human nutrition perspective. J Exp Bot 55(396):353–364

WHO (2016) Vitamin and mineral nutrition information system. World Health Organization. www.who.int

Wicker T, Taudien S, Houben A, Keller B, Graner A, Platzer M, Stein N (2009) A whole-genome snapshot of 454 sequences exposes the composition of the barley genome and provides evidence for parallel evolution of genome size in wheat and barley. Plant J 59:712–722. https://doi.org/10.1111/j.1365-313X.2009.03911.x

Wiegmann M, Thomas WTB, Bull HJ, Flavell AJ, Annette Zeyner A et al (2019) Wild barley serves as a source for biofortification of barley grains. Plant Sci 283:83–84

Xue WT, Gianinetti A, Wang R, Zhan ZJ, Yan J, Jiang Y, Fahima T, Zhao G, Cheng JP (2016) Characterizing barley seed macro- and micro-nutrients under multiple environmental conditions. Cereal Res Comm 44(4):639–649

Yadav N, Sharma YK (2018) Enhancement of Zn density in barley (*Hordeum vulgare*) grain: a physiological approach. Asian J Plant Sci Res 8(4):13–17

Yadava DK, Hossain F, Mohapatra T (2018) Nutritional security through crop biofortification in India: status & future prospects. Indian J Med Res 148(5):621

Yan J, Wang F, Yang R, Xiao T, Fahima T et al (2012) Natural variation in grain iron and zinc concentrations of wild barley, Hordeum spontaneum, populations from Israel. In: Zhang G, Li C, Liu X (eds) Advance in barley sciences. Proceedings of 11th international barley genetics symposium. Springer, Cham, pp 169–183

Ylaranta T (1985) Increasing the selenium content of cereals and grass crops in Finland. Academic dissertation. Agricultural Research Centre, Institute of Soil Science, Jokioinen, p 72

Zamir D (2001) Improving plant breeding with exotic genetic libraries. Nat Rev Genet 2:983–989

Zeng YW, Du J, Yang XM, Pu XY, Wang LX, Yang JZ, Du LJ, Yang T, Yang SM, Sun ZH (2016) Identification of quantitative trait loci for mineral elements in grains and grass powder of barley. Genet Mol Res 15(4)

Zeng Z, Han N, Liu C, Buerte B, Zhou C, Chen J et al (2020) Functional dissection of HGGT and HPT in barley vitamin E biosynthesis via CRISPR/Cas9-enabled genome editing. Ann Bot 126:929

Zhu C, Naqvi S, Gomez-Galera S, Pelacho AM, Capell T, Christou P (2007) Transgenic strategies for the nutritional enhancement of plants. Trends Plant Sci 12:548–555. https://doi.org/10.1016/j.tplants.2007.09.007

Zhu C, Sanahuja G, Yuan D, Farré G, Arjó G, Berman J, Zorrilla-López U, Banakar R, Bai C, Pérez-Massot E, Bassie L (2013) Biofortification of plants with altered antioxidant content and composition: genetic engineering strategies. Plant Biotechnol J 11(2):129–141

Zou C, Du Y, Rashid A, Ram H, Savasli E, Pieterse PJ, Ortiz-Monasterio I, Yazici A, Kaur C, Mahmood K, Singh S (2019) Simultaneous biofortification of wheat with zinc, iodine, selenium, and iron through foliar treatment of a micronutrient cocktail in six countries. J Agric Food Chem 67(29):8096–8106