Enriching Nutrient Density in Staple Crops Using Modern "-Omics" Tools

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Abstract

A sizeable proportion of the global population faces nutritional disorders. Notably, the poorest regions in the developing world share considerably large segment of malnourished people. Given the rising prevalence of nutritional disorders, sustainable solutions urgently need to be in place in order to tackle the menace of hidden hunger. An array of improvement strategies is suggested to meet the growing challenge. These strategies involve dietary diversification, food supplementation/fortification, and biofortification using nutritional breeding approaches, genetic engineering, and agronomic interventions. The mounting concerns about environmental safety and poor economic status of the target population further put a limit on the large-scale use of micronutrient-rich fertilizers. Hence, crop biofortification via conventional and molecular breeding stands to be the most economic, readily accessible, and globally accepted strategy. For some obvious reasons, staple crops that serve the daily dietary needs of the maximum population in the developing world are targeted for nutritional enhancement. As a prerequisite, survey of the germplasm pools is needed to quantify the exploitable genetic variation that exists in the crop gene pool. Further, modern omics approaches like genomics, proteomics, metabolomics, and ionomics will definitely advance our knowledge about the genetic makeup, molecular networks, and physiological alternations involved in the process of mineral accumulation and subsequent partitioning of minerals to edible plant parts. Similarly, engineering

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metabolic pathways through genetic modification holds great relevance for expediting the development of nutrient-dense food crops. We expect that the "omics" assisted nutritional breeding, as the most potential biofortification strategy, will be greatly helpful in achieving the nutritional security of over two billion nutrient-deficient people worldwide.

Keywords
Biofortification • Diversity • Genome • DNA marker • Linkage • QTL • Molecular map

8.1 Introduction

The number of people that populate the earth is constantly increasing, and based on the current trajectory, over two billion is to be additionally incorporated to the present figure of seven billion by the first half of this century, i.e., 2050 (Evans 2009; Varshney et al. 2015). Obviously, a dramatic increase is also expected in the size of population that faces undernourishment and malnutrition (in particular micronutrient-malnutrition)-related issues (Bohra et al. 2014a, b). A comprehensive report on "The State of Food Insecurity in the World 2014" documented recently by FAO, IFAD, and WFP indicates that about 800 million people encountered the problem of undernourishment during 2012–2014, a substantial proportion of which inhabits the developing world. The main reason behind the prevalence of this micronutrient deficiency in developing nations is the dependence of inhabiting people on staple food dominated by crops like wheat and rice, which are intrinsically deficient in mineral micronutrient that is essentially required to maintain the metabolism (Graham et al. 2001; White and Broadley 2009; Borill et al. 2014).

In a similar manner, elemental dietary deficiencies characterized by insufficient supply of essential minerals and micronutrients or trace elements are known to affect almost three billion people worldwide (Carvalho and Vasconcelos 2013; Tako et al. 2013). For example, iron (Fe) and iodine (I) deficiencies are shown by over 30 % and 17 %, respectively, of the current global population. Scarcity of these essential elements in turn predisposes deficient individuals to a range of diseases (Bohra et al. 2014a). A major fraction (~2 billion) of today's global population suffers from anemia which principally appears as a direct outcome of prolonged feeding on iron-limiting diets. Severity of iron-deficiency anemia (IDA) is much more evident in the case of developing countries where this nutritional disorder is reported to afflict half of the pregnant women and 40 % of the preschool children (Murgia et al. 2012).

To improve the nutritional status of the global population, a variety of strategies is proposed including dietary diversification, food fortification and supplementation, and biofortification through different means (Graham et al. 2001; White and Broadley 2009; Carvalho and Vasconcelos 2013). Widespread implementation of the methods like dietary diversification and food fortification/supplementation is constrained by the poor access of the target population to the market coupled with marginal nature of their income. Therefore, biofortification of crop plants is an attractive and economic strategy to develop nutrient-rich crops. Biofortification through breeding offers sustainable solution in comparison to indiscriminate application of fertilizers containing micronutrients since regular use of such fertilizers poses a considerable threat to the environment. In addition, growing concerns about environmental safety coupled with the increasing rigidity of the legislative regulations also limit the use of such agronomic interventions (Borill et al. 2014).

Cereals and food legumes constitute the dominant portion of human diets (particularly, in the developing nations) complementing each other in a synergistic fashion (Graham et al. 2001;

Bohra et al. 2014b, 2015). Further, concerning nutritional enrichment of crops, extensive examination of the natural variation for minerals/micronutrients that is present in the crop gene pool sets the initial step while executing crop-based biofortification (Bohra et al. 2015). This is accompanied by accelerated use of exploitable genetic variation in the nutritional breeding programs. In this regard, high-throughput modern omics tools and technologies have been available in recent years to augment the crop biofortification program. The unprecedented amount of the information that has been generated using various omics platforms like proteomics, metabolomics, and ionomics is expected to greatly advance our understanding of elemental composition and the underlying causative genes and their elaborate network. Taken this into consideration, here we review the recent progress made in the area of crop biofortification (especially the staple crops like cereals and food legumes) along with exploring the prospects and challenges that lie ahead.

8.2 Identifying Nutrient-Dense Crop Genotypes Through Analyzing Crop Gene Pool

As has been described in various published reports, cultivated *Triticum aestivum* L. and *T. turgidum* L. ssp. *durum* (Desf.) Husn. species contain lesser quantities of grain iron (Fe) and zinc (Zn) than the wild *Triticum* and *Aegilops* species (Chhuneja et al. 2006; Cakmak et al. 2000; Monasterio and Graham 2000; Rawat et al. 2009; Velu et al. 2014). Importantly wild emmer wheat (*T. turgidum* ssp. *dicoccoides*) serves as rich reservoir of micronutrients especially Fe and Zn (Cakmak et al. 2004; Peleg et al. 2008a, b; Chatzav et al. 2011). Moreover, *T. dicoccoides*, *Ae. tauschii*, *T. monococcum*, and *T. boeticum* are also worth mentioning while considering the potential sources of Fe and Zn (Cakmak et al. 2000; Tiwari et al. 2009; Rawat et al. 2008). Notably, various researchers (Chhuneja et al. 2006; Rawat et al. 2009; Tiwari et al. 2010) have reported wild *Triticum* and *Aegilops* species as the storehouse of high Fe and Zn content offering 75 % and 60 % higher Fe and Zn, respectively, than the cultivated bread wheat cultivar. Likewise, wild *Ae. kotschyi* acc. 3790 contained three times higher grain Fe in comparison to *T. aestivum* Cv. WH291 and *T. aestivum* Cv. WL711 (Singh et al. 2013). Synthetic hexaploids derived from *T. turgidum* spp. *durum* × *Aegilops tauschii* showed 30 % higher grain Fe and Zn (Calderini and Ortiz-Monasterio 2003). Attempts are under way at International Maize and Wheat Improvement Center (CIMMYT), Mexico (Velu et al. 2011a, b), for introgressing beneficial genes that contribute high Fe and Zn in synthetics derived from *T. spelta* × *T. dicoccon* and *Ae. tauschii* × *T. dicoccon* to high-yielding cultivars in wheat. In rice, after screening 46 rice genotypes (including cultivated and wild accessions), Banerjee et al. (2010) have reported that wild accessions of rice harbor greater amount of Fe than found in the cultivated genotypes. Genetic variation for Fe content varying from 15 to 109 mg/kg (based on dry weight) was reported in wild emmer wheat (Cakmak et al. 2004). Therefore, given the existence of tremendous germplasm variability for grain micronutrients (especially Fe and Zn) in the wild species, focused strategies are required to be undertaken that enable faster transfer of these micronutrient accumulating genes/QTLs into high-yielding popular cultivars.

Considering the importance of landraces vis-à-vis grain Fe content, a set of 52 accessions (commercial cultivars + landraces) was surveyed for variation in Fe content revealing a wide range between 1.32 ppm and 100.45 ppm. The landrace 'Lal Gotal' was observed to contain the highest amount of Fe (100.45 ppm) (Jahan et al. 2013). Likewise, analysis of 126 brown rice genotypes uncovered ample variability (varying from 6.2 to 71.6 ppm) for Fe content, and interestingly, the local accession had the highest value of Fe content (Anuradha et al. 2012a). The greater genetic variation in landraces for Fe content was also supported by Anandan et al. (2011). A higher Fe content was noted in the case of traditional cultivars, viz., 'Kalabath', 'Noothipattu', 'Koomvalazhi', and 'KDML

105', than the improved rice cultivars. Earlier, traditional rice cultivars (CMU122, CMU123, and CMU124) among the Thai brown rice cultivars had greater Fe content ranging from 7 to 22 mg Fe/kg (Prom-teru-thai and Rerkasem 2001). Up to threefold variation in Fe content (7.5–24 mg Fe/kg) was reported in cultivated brown rice (Senadhira et al. 1998; Graham et al. 1999; Prom-u-thai and Rerkasem 2001), whereas the range was found to be between 3 and 11 mg Fe/kg in white rice (Prom-u-thai et al. 2007). Likewise, by screening 15 Fe-dense and Fe-normal genotypes of unpolished rice through in vitro digestion/Caco-2 cells, variation in Fe content (14–39 μg/g) was recorded (Glahn et al. 2002). Applying the similar method, significant variation for kernel Fe content (ranging from 15.5 to 19.1 mg/kg) was obtained in maize (Oikeh et al. 2003), and particularly, the two genotypes 'ACR90POOL16-DT' and 'ACR86TZESR-W' were found to be promising relating to kernel Fe content in maize. Meng et al. (2005) have suggested that black rice is superior to the other forms of rice such as red rice, sticky rice, and fine rice concerning Fe content. And also, husk and chaff contain more Fe content. The grain Fe content of white rice varied between 0.05 and 0.2 μg/grain (Prom-u-thai et al. 2007). In vitro digestion/Caco-2 model in wheat aided in the identification of *Aegilops* derivatives with a 1.5-fold increase in the bioavailable Fe. Further, positive correlation of Fe content with protein and phytate contents was also obtained (Salunke et al. 2011). In a similar way, a threefold increase in Fe bioavailability was recorded in 11 Chinese rice genotypes tested using Caco-2 cell culture model. Moreover, impacts of ascorbic acid application on Fe bioavailability were also examined, and enhanced Fe bioavailability was noticed in the polished rice due to ascorbic acid (He et al. 2013).

Up to threefold difference in Fe content was reported after elemental evaluation of a worldwide wheat collection (Oury et al. 2006). Similarly, analyzing the Fe content in core collection of wheat showed the range of Fe content varied from 26.26 to 68.78 mg/kg, and 'Andalucia 344' accession was found to carry highest Fe content (Velu et al. 2011b). While investigating Fe accumulation in 20 wheat genotypes in response to Fe treatment, it was reported that selenate enhanced the Fe accumulation (de Souza et al. 2013). Besides, two Fe-rich genotypes, viz., 'EMB 38' and 'BRS 264' were recovered with higher grain Fe content.

Noteworthy genetic variation for Fe content was observed across 109 sub-Saharan African inbreds, in particular mid-altitude (15–159 ppm) and lowland (14–134 ppm) inbreds (Maziya-Dixon et al. 2000). In a study based on 3-year trial data, Prasanna et al. (2011) analyzed variation for kernel Fe concentration in 30 maize genotypes and observed a range from 11.28 to 60.11 mg/kg. They also identified two genotypes 'HP2' and 'BAJIM 06-17' which remained stable across multiple environments. Recent studies at ICRISAT showed Fe content varying from 29.8 to 44.2 mg/kg in sorghum (Kumar et al. 2010) and 30.1–75.7 mg/kg in pearl millet (Velu et al. 2007). Similarly, Rai et al. (2012) also reported adequate Fe variability (ranging from 18 to 97 ppm) in advanced breeding lines of pearl millet.

In wheat, wild emmer, einkorn, and landraces are known as the richest source of grain Zn content (Cakmak et al. 2000; Ortiz- Monasterio et al. 2007). Further, Cakmak et al. (2000) reported that other wheat species such as *T. dicoccoides*, *Ae. tauschii*, *T. monococcum*, and *T. boeticum* also contain higher Zn content. High variation for Zn content ranging from 30 to 118 mg/kg was recorded after screening 825 accessions of *T. dicoccoides* (Cakmak et al. 2004). In hexaploid wheat, the Zn concentration differed (15–35 ppm) in wheat germplasm collection that comprised of elite breeding lines and germplasm collected across the globe (Oury et al. 2006). Significant variation was also reported from wheat core collection with the Zn content varying between 16.85 and 60.77 mg/kg. Specifically, Chinese spring bread cultivar (Hong Duan Mang) showed the highest Zn content (Velu et al. 2011b). Similarly, genotypic variation for grain Zn was also noticed among 20 Brazilian wheat genotypes manifesting a twofold difference within the sample studied.

Further, addition of selenium led to an increase in the grain Zn content (Souza et al. 2014). To elucidate the role of genotype × environment (G × E) interaction in determining grain Zn and Fe contents, elite lines of wheat from CIMMYT were tested in Eastern Gangetic Plains (EGP) of India under multiple environments. The results revealed significant G × E effects operating on grain Fe and Zn concentrations, and more variation across locations was noted in the case of Zn (Joshi et al. 2010). Multilocation testing of advanced wheat lines (developed from *T. spelta*, landraces, and synthetic wheat) in South Asia and Mexico showed high heritabilities for Zn and Fe contents across multiple sites. The greater extent of variation generates new avenues for selection of Zn- and Fe-dense lines for future use (Velu et al. 2012). Based on phytosiderophore release, Daneshbakhsh et al. (2013) recorded two Zn efficient wheat genotypes, viz., Cross and Rushan.

Regarding Zn content in other staple crops, grain Zn variation of 13.5–58.4 mg/kg was recorded in rice germplasm (Gregorio et al. 2000; Welch and Graham 2002), while it ranged between 29 and 37 mg/kg in the case of aromatic rice (Graham et al. 1999), 24.5–64.8 mg/kg in pearl millet (Velu et al. 2007), and 22.2–32.9 mg/kg in sorghum (Kumar et al. 2010). Recently, Rai et al. (2012) have reported large variation for Zn content (from 22 to 69 ppm) in advanced breeding lines of pearl millet. Considering genotypic difference in the micronutrient lost due to milling process, variable degrees of loss were reported for Fe (24–60 %) and Zn (10–58 %) in 15 Thai rice genotypes (Saenchai et al. 2013). In maize, high degree of variation for Zn content was observed across 109 inbreds of sub-Saharan African maize ranging from 12 to 96 ppm for mid-altitude inbreds and 24 to 96 ppm for lowland inbred line (Maziya-Dixon et al. 2000). Prasanna et al. (2011) also reported variation (15.14–52.95 mg/kg kernel Zn) in maize and also identified a genotype (IML467) due to its kernel Zn concentration remaining stable over the environments.

Concerning the genetic variability for calcium (Ca) content, Graham et al. (1999) reported the Ca content varying from 0.25 to 0.73 g/kg (on dry weight basis) among 132 wheat genotypes. In durum wheat, the Ca content ranging from 388 to 640 mg/kg was obtained through analyzing 84 Italian wheat for two years under multiple environments (Ficco et al. 2009). In hexaploid wheat, cultivar 'PBW-396' showed the highest Ca content (76.67 mg/100 g dry weight) among the 10 genotypes tested (Mallick et al. 2013). Based on six field trials, Feil and Fossati (1995) recorded the range of Ca from 0.35 to 0.50 g/kg in triticale grains.

In relation to provitamin A, orange maize varieties are known to contain higher provitamin A than the yellow varieties (Li et al. 2007; Ortiz-Monasterio et al. 2007; Menkir et al. 2008). Based on the visual scoring of orange kernel color (as a marker for high provitamin A), Chandler et al. (2013) noticed moderate heritability in nested association mapping (NAM) maize panel. Similarly, yellow endosperm varieties were also reported to carry higher amount of provitamin A carotenoids in sorghum (Fernandez et al. 2009). Greater genotypic differences for provitamin A (0.24–8.80 μg/g) were manifested across 1000 tropical maize germplasm at CIMMYT (Ortiz-Monasterio et al. 2007). Likewise, Babu et al. (2012) estimated the range of provitamin A (15–20 μg/g) in the grains of improved lines. Genetic variability for kernel β-carotene varying from 0.02 to16.50 μg/g was discovered from 105 maize inbreds from India and CIMMYT (Vignesh et al. 2012). The authors also suggested the wide variation due primarily to the allelic variation in *crtRB1* 3'TE gene. More recently, Azmach et al. (2013) reported that the tropical maize lines harboring the functional markers, viz., crtRB1-5′TE and 3′TE, could act as rich source of provitamin A. To decipher the gene action, both the general combining ability (GCA) and specific combining ability (SCA) for the grain yield and provitamin A were examined

in hybrids, and the results revealed existence of significant GCA effects for provitamin A, suggesting the presence of an additive gene action (Suwarno et al. 2014). Notably, significant G × E effects were observed for the grain carotenoid concentration, and associated carotenoid traits in maize population derived from the cross DEexp × CI7. Further, the tested population showed higher variation for the carotenoid under subtropical condition than temperate condition (Kandianis et al. 2013). Similarly in wheat, 'RSP-561' genotype was reported to contain highest carotenoid content (105.67 µg/100 g) among the 10 genotypes tested (Mallick et al. 2013).

Focus needs to be given not only towards evaluation of nutrients but also to a variety of so-called anti-nutritional factors like phytates. Phytate content ranged from 1.98 to 2.46 g/kg in maize while evaluating 90 S1 families derived from the BS31 population at two locations (Lorenz et al. 2008). Average value of phytate content was recorded to be 2.91 g/kg from 54 land races of maize (Drinic et al. 2009). Phytate content was measured varying from 800 to 1000 mg/ 100 g seed in inbred lines and F_1 hybrids. Based on the phytic acid (PA) content, Ki inbred lines were grouped into low PA group (<900 mg/100 g seed) and medium/high group (≥900 mg/100 g seed) (Chiangmai et al. 2011). Additionally, the population stemming from "derived flints" showed lower (1.95 g/kg) phytate content. The "Mediterranean flint"-based populations had the lowest (0.46 g/kg) phytate content in contrast to the 'flinty dents'-derived population which exhibited the highest value (3.43 g/kg) for phytate (Drinic et al. 2012).

In wheat, PA content varied from 200 mg/ 100 g to 400 mg/100 g in refined flour and 600–1000 mg/100 g in the whole flour (Febles et al. 2002). Two Iranian cultivars 'Pavarus' and 'Niknejad' were reported to contain low PA, while 'Estar', 'S-78-11', 'S-79-10', and 'Niknejad' had maximum phytase activity (Tavajjoh et al. 2011). Mallick et al. (2013) reported PA content ranging from 0.35 to 1.60 mg 100 g^{-1} across 10 genotypes investigated, and they identified 'HD2687' genotype exhibiting the lowest PA content. Notably, by performing a diallel analysis, Ahmad et al. (2013) recorded the PA up to 3.43 % among the F_1 hybrids of wheat; on the other hand F_1s of crosses Ps-2005 × Ghaznavi, AUP-4006 × Ps-2004, Janbaz × Ps-2004, and Janbaz × Ps-2005 exhibited lower PA. Likewise in barley, Dai et al. (2007) observed genetic variation in PA ranging from 3.85 mg/g to 9.85 mg/g across 100 genotypes. They suggested the significant variation was primarily accounted to effects exerted by both location and time.

In relation to selenium (Se), immense variability in Se content (5–720 µg/kg) was reported in wheat germplasm (Lyons et al. 2005). The authors also reported that majority of this variation could be credited to the soil factor. Based on an extensive survey involving 88 different sites in Malawi, Se concentration of maize grain was observed to range between 0.005 mg/kg and 0.533 mg/kg (Chilimba et al. 2011).

Considering nutrient uptake efficiency, wheat genotype "Maris Butler" was reported to be highly efficient of extracting manganese (Mn) from Mn-deficient soil (Jiang and Ireland 2005). Further, 'Maris Butler' and 'C8MM' genotypes of wheat were also reported to be highly efficient for Mn uptake (Jiang 2008). During reproductive phase, the wheat genotypes PBW550, BW9178, and HD2967 were identified based on the utilization efficiency of Mn. Therefore, these genotypes can be potentially exploited for the development of Mn-rich wheat cultivars in the near future.

More recently, Pinson et al. (2014) from USDA and other collaborating institutes comprehensively examined a worldwide collection comprising 1763 greatly diverse germplasm accessions (majorly from USDA core collection) in rice for 16 mineral nutrients in flooded and non-flooded conditions. They used inductively coupled plasma mass spectrometry (ICP-MS) technology to assess the level of elemental variation. Resultantly, in view of moderate to high heritability estimates obtained for these mineral [except for nickel (Ni)], the authors strongly advocated for the possibilities of rice nutritional

enhancement using selection and breeding. Similarly, a set of 72 pearl millet genotypes including landraces and open-pollinated varieties was examined for mineral densities across multiple environments at Niger (Pucher et al. 2014). This compositional survey revealed a greater range of variation for Ca, Fe, copper (Cu), and Zn existing within the material studied. On the other hand, moderate level of variation was noticed for Mg, P, K, and Mn. Owing to the considerable extent of G × E interactions recorded particularly Fe and Zn densities, authors advocated multi-environmental testing of diverse accessions while measuring variations in mineral and micronutrient contents.

8.3 Elucidating the Genetic Architecture of Nutrient Accumulation via QTL Mapping

Detecting the causative loci (genes or QTLs) that determine the mineral/nutrient content is a crucial step while understanding the genetic makeup of the nutrient-related traits. Molecular mapping of the genome segments that govern nutrient content/concentration has been practiced in many crops (Table 8.1). In this section, we update the existing literature about mapping of QTL associated with nutrient traits. By applying inductively coupled plasma optical emission spectroscopy (ICP-OES) technique to F_4 lines derived from the cross (B84 × Os6-2) in maize, Simić et al. (2012) detected significant QTLs controlling concentrations of four prime micronutrients, i.e., phosphorus (P), Fe, Zn, and Mg. The QTL analysis revealed occurrence of a total of 32 QTLs associated with seven different traits and the phenotypic variances (PVs) of these QTLs ranged between 6.7–19.9 %. More importantly, co-localization of some of these QTLs in chromosome 3 offers a promising marker-delimited chromosomal region for immediate utilization in nutritional breeding. Similarly, the QTLs were discovered from $F_{2:3}$ lines stemming from the crosses Mu6 × SDM and Mo17 × SDM that determined Zn and Fe concentration in maize kernels and cobs (Qin et al. 2012). More importantly, a joint QTL analysis was performed using data from the two populations which eventually provided a set of 12 QTLs, majority of which were observed in both joint- and single-environment analyses. Lung'aho et al. (2011) reported three QTLs for grain Fe concentration in a maize recombinant inbred line (RIL) population B73 × Mo17 (popularly known as IBM). On the other hand ten QTLs were found, controlling Fe bioavailability accounting over 50 % of the total PV. Similarly, a 178 × P53-based $F_{2:3}$ population in maize was examined using atomic absorption spectrophotometer (AAS), and consequently, five QTLs (four for Zn and one for Fe content) were detected which accounted to 17.5 % (Zn) and 16.8 % (Fe) of observed PV (Jin et al. 2013). Furthermore, QTL data collected from five different studies were subjected to an integrated analysis or, more appropriately, the meta-QTL analysis, and as a result, 10 meta-QTLs (M-QTLs) were detected which governed up to 28 % of the observed PV. The investigation highlighted the underlying importance of *bins* 2.07 and 2.08 in deciphering the genetic architecture of mineral concentration in maize. Given the significance of "ionome" characterization in maize leaves particularly concerning the silage preparation, Zdunic et al. (2014) performed ionome profiling of over 300 intermated RILs belonging to IBM population. The QTL analysis using a high-resolution genetic map (comprising 2161 DNA markers) facilitated mapping of major QTLs (≥10 % PV) for cadmium (Cd), potassium (K), and strontium (Sr) accumulation, whereas QTLs related to other metals like Cu, Fe, and Mg showed lesser R^2 values (<10 % PV). As a result of elemental examination conducted across two different environments, significant QTL × environment interactions were revealed in this study. Importantly, QTLs for Cd were noted to be consistent with previous QTL studies. The same population was earlier analyzed by Baxter et al. (2012) to profile grain ionome in maize using ICP-OES. Based on the experimental data collected across multiple locations during different years, i.e., 2003, 2005, and 2007, potential QTLs were

Table 8.1 List of some important QTLs that are associated with mineral/nutrient traits in different crops

Crop	Nutrient trait	Potential genomic region	Mapping population	Associated DNA marker	References
Rice	Zn	One major effect of QTL on chromosome 8	IL: *O. sativa* × *O. rufipogon*	SSR	Garcia-Oliveira et al. (2009)
	Zn, Fe	14 QTLs for Zn and Fe on chromosomes 1, 3, 5, 7, and 12	RIL: Madhukar × Swarna	SSR	Anuradha et al. (2012b)
	Zn, Fe	OsYSL6, OsYSL8, OsYSL14, OsNRAMP1, OsNRAMP7, OsNRAMP8, OsNAS1, OsFRO1, and OsNAC5 genes	–	–	Sperotto et al. (2010)
	Zn, Fe	5 QTLs for Fe and 3 QTLs for Zn present on 2, 3, 8, and 12	BC: Swarna × *O. nivara*	SSR	
		7 QTLs for Fe and 5 QTLs for Zn present on 7 and 12	RIL: Madhukar × Swarna	SSR	
	Zn, Fe	*OsYSL1*,*OsNAC*, *OsYSL16*, *OsZIP4*, *OsYSL17*, and *OsNAAT1*	BC: Swarna × *O. nivara*	SSR	
	Zn, Fe	*OsYSL1*, *OsMTP1*, *OsNAS1*, *OsNAS3*, *OsNRAMP1* candidate genes	RIL: Madhukar × Swarna	SSR	
	Zn	*qZnc4*, *qZnc6* on chromosomes 4 and 6	JX17 × ZYQ8	RFLP, SSR	Zhang et al. (2011)
	Fe	qFe1, qFe3, qFe4, qFe7 on 1, 3, 4, 7	Bala × Azucena	–	Norton et al. (2010)
	Zn	qZn6, qZn7, qZn10.1, qZn10.2 on 6, 7, 10			
	Cu	qCu1, qCu2, qCu12.1, qCu12.2 on 1, 2, 12			
	Mn	qMn10, qMn11 on 10 and 11			
	Se	qSe1.1, qSe1.2, qSe3, qSe6, qSe7, qSe9 on 1, 3, 6, 7, 9			
	Mn	Four QTLs: qGMn1, qGMn2, qGMn7, qGMn12 on chromosomes 1, 2, 7, 12	Sasanishiki × Habataki	–	
	Fe	QTL (qGFe4) on chromosome 4			
Maize	Fe	Three FeGC QTLs, 10 QTLs for FeGB	RIL: B736 × Mo17 (IBM)		Lungaho et al. (2011)
	Fe/P, Zn/P, and Mg/P	Three QTLs on chromosome 3	F4: B84 × Os6-2	SNP, SSR	Simić et al. (2012)
	Zn, Fe	QTL for ZnK, ZnC, FeK, and FeC on chromosome 2	F2:3: Mu6 × SDM and Mo17 × SDM		Qin et al. (2012)
		QTL for Znk, FeK, and FeC on chromosome 9			
		QTL for ZnK and ZnC on chromosome 7			
	Zn, Fe	Five QTLs and 10 Meta-QTLs	F2:3: 178 × P53	SSR	Jin et al. (2013)
	Zn and Fe	17 QTLs on chromosomes 1, 2, 3, 4, 6, 7, 10	DH: DH8 × DH40 and DH86 × S137	–	Zhou et al. (2010)
	Fe		B73 × Mo17	–	

(continued)

Table 8.1 (continued)

Crop	Nutrient trait	Potential genomic region	Mapping population	Associated DNA marker	References
		Three candidate regions on chromosomes 3, 6, and 9			Tako
Wheat	Fe	*QFe.pau-2A and QFe.pau-7A.*	RIL: *Triticum boeticum* × *T. monococcum*	SSR and RFLP	Tiwari et al. (2009)
	Zn	*QZn.pau-7A* (Zn QTL)			
	Zn	Four QTLS on chromosomes 3D, 4B, 6B, and 7A	RAC875-2 × Cascades	RFLP, AFLP, SSR, and DArT	Genc et al. (2009)
	Fe	One QTL	RAC875-2 × Cascades	RFLP, AFLP, SSR, and DArT	Genc et al. (2009)
	Zn	Five QTLs on seven different chromosomes	RIL: *T. spelta* × *T. aestivum*	DArT, SNP	Srinivasa et al. (2014)
	Fe	Five QTLs on seven different chromosomes	RIL: *T. spelta* × *T. aestivum*	DArT, SNP	
	Fe, Zn, and Protein	Nine additive and four epistatic QTLs on chromosomes 4B and 5A	RIL	–	Xu et al. (2012)
	Zn, Fe, Mn, and Cu	Major QTL on chromosome 5	RIL	–	Ozkan et al. (2007)
	Zn and Fe	QTL on chromosome 6B	*T. turgidum* ssp. *dicoccoides*	–	Distelfeld et al. (2007)
	Zn	Six QTLs on 2A, 5A, 6B, 7A, 7B	Durum wheat × wild emmer	–	Peleg et al. (2008b)
	Fe	11 QTLs			
	Cu	10 QTLs			
	Mn	Two QTLs			
	Zn	Four QTLs chromosomes 4A, 4D, 5A, and 7A	Hanxuan10 × Lumai 14	–	Shi et al. (2008)
	Zn	Seven QTLs for grain Zn on chromosomes 1A, 2D, 3A, 4A, 4D, 5A, and 7A			
	Ca	Nine QTLs on 1A, 4A, 5B, 6B, 2B, 4B, 6A, 6B, 7B	Langdon × G18-16	–	Peleg et al. (2009)
	Fe	11 QTLs on 2A, 3B, 5A, 6B, 7A, 2B, 3A, 4B, 6A, 7B, 5A, 6A			
	Zn	Six QTLs on chromosomes 2A, 5A, 6B, 7A, 7B			
	Mn	Two QTLs			
	Cu	10 QTLs on 2A, 4A, 4B, 5A, 6B, 7A, 1A, 3B, 6A, 7B			
Barley	Zn	Five QTLs on 1HS, 1HL#1, 2HS, 2HL#2, and 5HL	DH: Clipper × Sahara	–	Lonergan et al. (2009)

discovered for a variety of elements including Ca, Cu, Mg, Fe, K, Mn, P, Zn, and S, which controlled up to 46 % of the PV.

A recent study discovered a total of 134 QTLs that influenced grain element concentration in two rice mapping populations including RILs and introgression lines developed from the cross Lemont × TeQing. It is important to note that 34 of these QTL-containing regions were found consistent in more than one experimental population. Noticeably, the authors suggested the presence of an intricate network of Mg, P, and K (Zhang et al. 2014). In a similar fashion, candidate simple sequence repeat (SSR) markers associated with grain Zn content were identified from a RIL population (IRRI38 × Jeerigesanna) comprising 160 individuals. The phenotypic contributions of these QTLs varied between 4.5 % and 19 %. Additional three of these candidate markers were experimentally confirmed in panel of 96 accessions (Gande et al. 2014). Using 110 DNA markers, a total of 14 QTLs associated with Fe and Zn concentrations in rice grains were identified from 168 RILs (Madhukar × Swarna). The QTLs were mapped to different chromosomes, viz., 1, 3, 5, 7, and 12 (Anuradha et al. 2012b). Later, two inbred lines from this RIL population, viz., RP Bio5478-185 M (high Fe and Zn) and RP Bio5478-270 M (low Fe and Zn), and one of the parents (Swarna) were used for quantitative real-time PCR (qRT-PCR)-based expression profiling using 15 candidate genes. The QTL allele leading to an increase in Fe concentration was also found to be associated with enhanced upregulation (Agarwal et al. 2014).

In wheat, QTL analysis of more than 100 RI individuals (Tabassi × Taifun) led to the discovery of six and two QTLs respectively for Fe and Zn grain concentrations. The Fe-QTLs explained 29 % of the PV, while Zn-QTLs accounted to 51 % of the total PV. A major QTL for Cl^- influencing 32 % of the variation was detected from a DH population (Berkut × Krichauff). Mapped on chromosome 5A, the underlying genomic region was delimited by SSR markers *gwm304*and *barc141* (Genc et al. 2014). The Cl^- was analyzed with ICP-OES technique. More recently, Pu et al. (2014) created two RIL populations in wheat, i.e., SHW-L1 × Chuanmai 32 and Chuanmai 42 × Chuannong 16, which showed quantitative variation for Se, Fe, Zn, Cu, and Mn. The QTL analysis in SHW-L1 × Chuanmai 32 population facilitated identification of a series of QTLs contributing up to 28.5 % of the phenotypic variance. Similarly, 13 QTLs were obtained from Chuanmai 42/Chuannong 16 population with the PV varying between 7.5 % and 35.1 %. Grain protein content B1 (Gpc-B1) is a QTL detected in wheat and it was found to be associated with enhanced grain protein, Zn, and Fe contents (Uauy et al. 2006). An increment of 10–34 % in concentrations of grain Zn, Fe, Mn, and protein was observed in cultivated wheat after introgression of Gpc-B1 locus from the wild tetraploid wheat *T. turgidum* ssp. *dicoccoides* into different recombinant chromosome substitution lines (Distelfeld et al. 2007). Authors proposed that the Gpc-B1 locus promoted remobilization of protein, Zn, Fe, and Mn from the leaves to the grains.

In soybean, QTLs were discovered for seed Ca content from $F_{2:3}$ families obtained by crossing low- and high-calcium-containing genotypes (Zhang et al. 2009). In a similar fashion, Jegadeesan et al. (2010) analyzed a RIL population (AC Hime × Westag-97) and they reported a major QTL on LG K that accumulates Cd in soybean seeds and accounted for 57.3 % of the total PV. Further, in order to validate the QTLs detected from the population 'AC Hime × Westag-97', another RIL population (Leo × Westag-97) was employed for QTL mapping. Notably, the SSR markers found linked with the Cd locus or *Cda1* gene were also mapped in the Leo × Westag-97 population, and the mapping positions of these SSR markers manifested the same marker order. In addition, three of these linked markers, viz., SatK 147, SatK 149, and SattK 152, successfully discriminated between the low- and high-Cd-containing lines, thereby offering robust DNA markers for exercising marker assisted selection (MAS) for isolating low Cd genotypes or for the precise introgression of the candidate genomic

region into diverse genetic backgrounds. Likewise, QTL analysis of 144 RILs [OAC Bayfield (high vitamin E content) × Hefeng 25 (low vitamin E content)] revealed candidate chromosomal regions within the soybean genome that determine vitamin E content in soybean seeds. Major QTLs were detected for alpha-, gamma-, and delta-tocopherols as well as for the total vitamin E. The percent PV (% R^2) of these QTLs ranged between 2.4 and 16.7 (Li et al. 2010). The QTLs and other genomic-based tools that are currently being used for legume biofortification are reviewed recently by Bohra et al. (2015).

8.4 Genome-Wide Association Study (GWAS)-Based Dissection of Elemental Composition and Nutrient-Related Traits

The transformational potential of GWAS for elucidating the architecture of important nutritional quality traits was experimentally demonstrated in rice using 44,100 single nucleotide polymorphism (SNP) markers and 413 diverse accessions collected from 82 countries (Zhao et al. 2011). Likewise, to finely resolve the genetic makeup of four mineral nutrients (As, Cu, Mo, and Zn), genomes of ~300 rice cultivars were scanned with genome-wide DNA markers to build significant marker trait associations (SMTAs). The genotyping of the entire diversity panel was performed using 36,901 SNPs, while elemental compositions were investigated through ICP-MS. The SNP markers displaying SMTAs with the grain nutrient contents were detected on different rice chromosomes [As (chromosome 5), Cu (chromosomes 1 and 5), Mo (chromosome 10), and Zn (chromosomes 7 and 9)] with a greater extent of QTL × environment interactions influencing the traits under consideration. Interestingly, the candidate genomic segments near these SNP markers were observed to be residing in close association with mineral transporters especially for Cu and Mo (Norton et al. 2014). More recently, a panel of accessions composed of 298 barley landraces belonging to African countries (Ethiopia and Eritrea) was subjected to genome-wide marker genotyping with almost 8 K SNPs of iSelect SNP chip (Mamo et al. 2014). GWAS of the micronutrient variation existing in the association panel allowed mapping of QTLs conferring enhanced Zn concentration in barley genome, more precisely to the chromosome 6HL (*Zn-qtl-6H_SCRI_RS_*10655). It is interesting to note that even a high-density genotyping using 8 K DNA markers could not be able to detect the QTLs for Fe concentration which remains in concordance with the earlier published reported that could not map QTL related to Fe concentration (Mamo et al. 2014). Notably, positive correlation between Zn and Fe concentrations was observed in both field and greenhouse experiments.

A joint linkage analysis and GWAS using nested association mapping (NAM) population provides valuable insights about the carotenoids in maize grains. NAM panel comprises of a total of ~5000 RILs which were produced by crossing 25 diverse parents to a common parent (B 73) (Yu et al. 2008). A total of 28 million SNPs derived from ultra high-resolution maize HapMaps were used to execute the genome-scale association analysis (Lipka et al. 2013a). Given the immense health implications of vitamin E and antioxidants (particularly in the developing world), a set of 281 inbreds was chosen to perform GWAS in order to illuminate the genetic architecture of composition as well as the content of tocopherols and tocotrienols in maize grains. A total of 591,822 SNPs were initially targeted for analysis; however, 294,092 were finally chosen for conducting the GWAS. Besides advancing understanding about the genetic association between *ZmVTE4* haplotypes with α-tocopherol content, novel insights were gained about relationship between the *ZmVTE1* and tocotrienol composition. In addition, candidate gene-based approach was also implemented to generate additional experimental evidences about molecular mechanism that underlies tocochromanol biosynthesis. A set of 60 candidate genes including *VTE* genes was targeted for pathway-level analysis

(Lipka et al. 2013b). A similar strategy was used for detecting significant association for carotenoid composition in maize kernels. The GWAS, as well as pathway-level analysis, was performed. The GWAS was applied with 284,180 SNP and seven insertion-deletion (indel) markers, whereas 7 K SNPs and seven indels were chosen for pathway-level analysis. The genome-scale and pathway-level analyses provided novel association within *zep1* and *lut1* and *dxs2* and *lut5*, respectively, which were not reported earlier (Owens et al. 2014).

Concerning association mapping, mapping populations derived from multiple founders such as NAM and multi-parent advanced generation intercross (MAGIC) offer advantages over diversity panel as the latter owing to the population structure is prone to generate spurious associations or false positives (Cavanagh et al. 2008). The MAGIC and NAM populations are increasingly generated in several crops such as rice, wheat, sorghum, etc. The most recent examples of MAGIC populations include an eight-parent wheat MAGIC population developed at National Institute of Agricultural Botany (NIAB), Cambridge, UK, which comprises a total of 1091 lines (Mackay et al. 2014). Coupling multi-founder populations with GWAS promises to be an attractive means to overcome the caveats routinely faced with the GWAS. Multi-parent populations have also been found promising in generating genome-wide predictions (GWPs) in crop plants.

8.5 Genomic Selection (GS) or GWPs to Develop Nutritionally Enriched Staple Crops

GS was proposed by Meuwissen et al. (2001) as a genome-level improvement strategy, which unlike MAS does not target a specific set of significant markers but exploits the genome-wide LD using high-density genetic variants spanning the entire genome. In other words, GS resembles a *black box* approach that does not intend to elucidate the detailed genetic architecture of complex traits; instead GS aims to provide the estimated breeding values (EBVs) of individuals using genome-wide marker data (Hamblin et al. 2011; Jonas and Koning 2013). In conventional plant breeding, EBVs derived from best linear unbiased predictions (BLUPs) have always been important criteria for selecting worthy individual which is based on the premise that performance of progenies acts as a better indicator of one's genetic merit than its own performance (Goddard and Hayes 2007; Jonas and Koning 2013; Meuwissen et al. 2001; Heffner et al. 2010).

In GS scheme, phenotyping which has always been a costly and time-consuming operation in plant breeding is exercised only to train the models, i.e., training population(Varshney et al. 2015). On the other hand, genotyping of both training and breeding populations was exercised using high-density DNA markers (Goddard and Hayes 2007). Several factors are known to affect the accuracy of genomic EBVs which include genetic composition and size of the training population, types and optimum number of DNA markers to be assayed, appropriate statistical methods used to generate GWPs, and, indeed, the heritability of the traits (Hamblin et al. 2011). Though extensively implemented in livestock breeding, GS is in developing stage in plants (Hamblin et al. 2011). Nevertheless, encouraging empirical results are being increasingly made available from a wide range of crops including maize (Lorenzana and Bernardo 2009; Crossa et al. 2013), barley (Zhong et al. 2009), wheat (Heffner et al. 2011; Poland et al. 2012; Crossa et al. 2010, 2013), soybean (Jarquín et al. 2014), sugar beet (Würschum et al. 2013), etc. While exploring the potential of GS in plant breeding, Jonas and Koning (2013) highlighted the immense potential of the GEBVs in increasing the gain per unit time; however, the authors have also opined that the implementation of GS in plant breeding may not be as simple as it has been in the case of livestock breeding. In addition, authors have also offered a set of valuable questions that need to be taken into consideration while executing GWP-based selection for crop improvement.

Desta and Ortiz (2014) recently reviewed the various prediction models used in GS including ridge regression best linear unbiased prediction (RR-BLUP), least absolute shrinkage and selection operator (LASSO), elastic net (EN), random forest (RF), reproducing kernel Hilbert spaces regression (RKHS), Bayesian LASSO, and other Bayesian methods like Bayes A, B, C, and so forth. Recently, Heslot et al. (2012) have compared the predictive abilities of various models that are currently being used in GS. Concerning provitamin A biofortification, Owens et al. (2014) used ~200 maize lines for prediction analysis using three statistical approaches, viz., RR-BLUP, LASSO, and EN. Apart from using various methods, three different marker sets were considered for assessing the prediction accuracies: first set included genome-wide DNA markers [284,180 SNP and seven insertion-deletion (indel) markers] which provided genome-scale predictions. The second set comprised of markers specific to carotenoid biosynthesis in maize (7 K SNPs and seven indels), thus generating pathway-level predictions. The remaining third set aimed to deliver QTL-targeted predictions as it focused on eight candidate genes (944 SNPs and seven indels) that were found associated with the genomic regions controlling carotenoid content in maize grain. Interestingly, the three statistical approaches offered more or less similar prediction accuracies. As a result of prediction analysis, an average GWP accuracy of 0.43 was recorded, with the highest (0.71) obtained for β-xanthophylls (Owens et al. 2014).

Ever-increasing capacities of next-generation sequencing (NGS) assays would likely to bolster these genome-wide techniques including GWAS and GS that rely on extensive exploration of linkage disequilibrium spanning the entire crop genome (Poland et al. 2012; Poland and Rife 2012). Further, the next-generation phenotypic screens driven largely by high-throughput automated platforms would radically expand the potential of such whole-genome strategies (Cobb et al. 2013).

8.6 Rising "-Omics" Technologies to Reinforce Plant Nutritional Genomics and Breeding

In conjunction with the rapid development in the field of plant genomics, the recent technological advances driving the "omics" science are also noteworthy (Bohra 2013). It is anticipated that the growing fields of proteomics, metabolomics, ionomics, and so forth would spectacularly supplement the crop biofortification. Interrogating "metabolic phenotypes" or "metabotype" in detail via genome-wide genomics approaches intends to illuminate the underlying genetic mechanism and intricate molecular network (Fiehn et al. 2000; Wen et al. 2014). According to Fernie and Schauer (2009), metabolomics involves metabolite profiling which is defined as the detailed characterization of the entire metabolites extracted from the cell. An array of strategies is being adopted to quantify the plant metabolites which primarily are based on either mass spectrometry (MS) or nuclear magnetic resonance (NMR). Fernie and Schauer (2009) have provided a brief review on these metabolite-profiling methods and have proposed metabolomics-assisted breeding as a compelling supplement to various breeding strategies owing to its relatively cost-effective nature compared to transcriptomics and other emerging "-omics" technologies.

Although majority of the metabolite QTLs identified to date have used model plant systems like *Arabidopsis*, this approach is rapidly expanding towards other plant species especially the staple crops. Recently, over 1000 SMTAs were established through applying metabolite-based GWAS across 702 maize lines, and the detected loci were further validated by resequencing, expression analysis (e-QTL), and family-based linkage analysis in two RILs (Wen et al. 2014). Collaborative attempts such as *META*bolomics for *P*lants, *H*ealth and *O*ut*R*each (META-PHOR) (http://www.meta-phor.eu/) were initiated in recent years to establish community-driven platforms that enable large-scale and rapid metabolite profiling and to

develop public databases that serve as a global inventory for a wide variety of plant metabolites. In a similar fashion, ionome profiling has been suggested by Salt et al. (2008) as a powerful and high-throughput technique to assist functional analysis of "ionome" influencing genes and their complex network and to offer deeper insights about the overall physiological mechanism. Increasing attention is being placed towards development of high-throughput ionomics platforms such as available at IPK, Gatersleben, Germany, and The Purdue Ionomics Information Management System (PiiMS) (Baxter et al. 2007).

8.7 Conclusion

Taken the context of expanding volume of malnourished people worldwide, the crop biofortification offers the economic, easily accessible, and reasonable solution to develop the improved crop variety with nutritionally dense grains. Obviously, staple crops that satisfy the dietary needs of the maximum population in the developing world have been targeted for biofortification using various modern tools and technologies. The recent advances in omics science including genomics, proteomics, metabolomics, and ionomics greatly improve our understanding about causative genomic regions and the associated molecular network and further developments are likely to refine the existing knowledge. As already mentioned earlier, greater extent of exploitable genetic variation has been documented across gene pools in a wide range of crops. An accelerated incorporation of the gene(s)/QTLs that enhance the nutrient content in staple crops will largely determine the success of various nutritional breeding schemes. Consideration should also be taken about the bioavailability of the enhance nutrient. Therefore, collaborative research efforts involving breeders, biotechnologists, physiologists, biochemists, and, importantly, the nutritionists are needed to strengthen the biofortification programs several-fold in order to meet the challenge of attaining the nutritional security worldwide.

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