



Review

Biofortification strategies to increase grain zinc and iron concentrations in wheat



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ABSTRACT

Micronutrient deficiencies, especially those arising from zinc (Zn) and iron (Fe), pose serious human health problems for more than 2 billion people worldwide. Wheat is a major source of dietary energy and protein for the world's growing population, and its potential to assist in reducing micronutrient-related malnutrition can be enhanced via integration of agronomic fertilization practices and delivery of genetically-manipulated, micronutrient rich wheat varieties. Targeted breeding for these biofortified varieties was initiated by exploiting available genetic diversity for Zn and Fe from wild relatives of cultivated wheat and synthetic hexaploid progenitors. The proof-of-concept results from the performance of competitive biofortified wheat lines showed good adaptation in target environments without compromising essential core agronomic traits. Agronomic biofortification through fertilizer approaches could complement the existing breeding approach; for instance, foliar application of Zn fertilizer can increase grain Zn above the breeding target set by nutritionists. This review synthesizes the progress made in genetic and agronomic biofortification strategies for Zn and Fe enrichment of wheat.

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1. Introduction

Dietary deficiency of essential micronutrients such as zinc (Zn) and iron (Fe) affects more than two billion people worldwide (White and Broadley, 2009; WHO, 2012), mostly pregnant women and children below the age of five who suffer from severe acute malnutrition. In many parts of the world, micronutrient deficiency is a more widespread problem than poor dietary quality and low energy intake (Stewart et al., 2010), and about 20% of deaths in children under five can be attributed to vitamin A, Zn, Fe, and/or I deficiency (Prentice et al., 2008). In countries with a high incidence of micronutrient deficiencies, cereal-based foods represent the largest proportion of the daily diet (Cakmak et al., 2010a; Bouis et al., 2011). The Harvest Plus initiative of the CGIAR consortium is working with national and international partners to alleviate deficiencies of these mineral nutrients by biofortifying staple food crops with essential minerals and vitamins; an approach considered to be the most economical solution to human micronutrient

deficiency (Welch and Graham, 2004; Bouis, 2007; Cakmak, 2008; Peleg et al., 2009).

Biofortified staple foods may not deliver equally high levels of minerals and vitamins per day, compared to supplements or fortified food products, but they can increase micronutrient intake for the resource-poor people who consume them daily, and therefore complement existing approaches (Bouis et al., 2011). One common agricultural strategy is the agronomic approach using micronutrient fertilizers, but this involves some technology and costs. On the other hand, crop biofortification via traditional cross breeding offers a sustainable and low-cost way to provide essential micronutrients to people in both developing and developed countries (Graham et al., 2007). Genetic biofortification involves classical breeding approaches to characterize and exploit genetic variation for mineral content, as well as new approaches involving gene discovery and marker assisted breeding (Grusak, 2002). It is expected that adoption of micronutrient-dense wheat varieties will be driven by their improved agronomic properties, higher yield potential, resistance to new strains of rusts, and tolerance to climate change induced heat and drought stresses. The provision of wheat grains with higher micronutrient levels is a challenging task for wheat breeders, but one that would complement the use of supplemental fertilizers, particularly on soils inherently low in these nutrients. This review synthesizes the progress made in wheat biofortification approaches, including genetic and

Abbreviations: CENEB, Campo Experimental Normal E Borlaug; CIMMYT, Centro Internacional de Mejoramiento de Maiz y Trigo; Fe, iron; G × E, genotype by environment interaction; HPYT, HarvestPlus Yield Trial; QTL, quantitative trait loci; Zn, zinc.

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Table 1
Variation for Zn concentration in bread wheat, durum wheat, and their wild relatives, documented in various studies.

Germplasm	Number of genotypes	Zn concentration (mg/kg)		Source
		Mean	Range	
Bread wheat	25		26–32	Pomeranz and Dikeman, 1983
Bread wheat	132		25–53	Graham et al., 1999
<i>Triticum boeoticum</i>	12	89	45–177	Cakmak et al., 2000
Bread wheat	8	26	23–28	
All wheats	>3000		16–142	Monasterio and Graham, 2000
Durum wheat	65	21	17–28	Cakmak et al., 2001
Hard red winter wheat	1605	33.3	14.3–74.4	Eugene et al., 2002
<i>Triticum dicoccoides</i>	518	61	30–98	Cakmak et al., 2004a,b
Spring and winter wheat	66	28	20–32	Morgounov et al., 2007
Bread wheat	150	21.4	13.5–34.5	Zhao et al., 2009
Durum wheat	10	21.4	14.0–26.9	
Einkorn wheat	5	22.4	20.1–27.8	
Emmer wheat	5	22.8	15.8–30.3	
<i>Triticum spelta</i>	5	22.9	16.8–28.0	
Winter wheat	137	31.6	11.7–64.0	Karami et al., 2009
Durum wheat (old)	10	36.4	33.7–41.4	Ficco et al., 2009
Durum wheat (modern)	57	33.9	28.5–46.3	
Durum wheat (advanced)	17	32.7	29.1–40.9	
Wild emmer wheat	19		39–115	Gomez-Becerra et al., 2010
Bread wheat (advanced)	20	33.6	32.6–34.8	Joshi et al., 2010
Bread wheat (advanced)	1300	30.5	23–52	Velu et al., 2011a
Bread wheat (advanced)	600	30.4	16.9–60.8	Velu et al., 2011b
Bread wheat (advanced)	40	32.5	29.0–39.5	Velu et al., 2012

agronomic biofortification strategies by traditional cross breeding and fertilizer management strategies, respectively.

2. Genetic biofortification

Genetic biofortification is a strategy that uses plant breeding techniques to produce staple food crops with higher micronutrient levels, reducing levels of anti-nutrients and increasing the levels of substances that promote nutrient absorption (Bouis, 2003). It offers a sustainable solution to malnutrition problems by exploring natural genetic variation to develop mineral-dense crop varieties (Pfeiffer and McClafferty, 2007). Plant breeders screen existing accessions in global germplasm banks to determine whether sufficient genetic variation exists to breed for a particular trait. They then selectively breed nutritious cultivars of major staples, rich in Zn and Fe concentrations and with substances that promote the bioavailability of Zn and Fe.

2.1. Germplasm screening

Germplasm screening of wheat and its wild relatives has revealed substantial genetic variation for grain Fe and Zn concentrations (Cakmak et al., 2000). More than 3000 germplasm accessions, including hexaploid, tetraploid, and diploid sources from the International Maize and Wheat Improvement Center (CIMMYT) gene bank have been screened for Zn and Fe variation (Monasterio and Graham, 2000). Materials with the highest Zn and Fe

concentrations are progenitors of wheat such as einkorn wheat and wild emmer wheat, and landraces (Cakmak et al., 2000; Ortiz-Monasterio et al., 2007). Unfortunately little variation exists in improved adapted wheat varieties. Researchers therefore focused on a more in-depth evaluation of wheat landraces (Monasterio and Graham, 2000) and, then, the secondary gene pool, i.e. tetraploid and diploid progenitors of hexaploid wheat, was evaluated for micronutrient concentration. *Triticum dicoccoides*, *Aegilops tauschii*, *Triticum monococcum*, and *Triticum boeoticum* were among the most promising sources of high Fe and Zn grain concentration (Cakmak et al., 2000). Some of these genotypes gave Zn values up to 142 mg/kg, but these high Zn values may have been influenced by the previous application of manure at some locations (Table 1). In case of *T. dicoccoides* genetic variation for Zn was substantial. In a *T. dicoccoides* germplasm covering 518 lines, Zn concentrations ranged between 30 and 98 mg/kg (Cakmak et al., 2004a,b). Another study of a set of high yielding genotypes showed Zn values of 15–35 mg/kg and Fe concentrations of 20–60 mg/kg (Oury et al., 2006). Several hundreds of CIMMYT core-collection accessions were screened for Zn and Fe nearly a decade ago, resulting in the identification of *Triticum turgidum* ssp. *dicoccum* accessions with elevated Zn and Fe levels. These tetraploids were used to develop synthetic hexaploid wheats in the CIMMYT wide-crossing unit, and those stocks (*T. turgidum* ssp. *dicoccum*/*Ae. tauschii*) entered the wheat breeding program at CIMMYT (Ortiz-Monasterio et al., 2007).

2.2. Breeding target and target population

General estimates of breeding targets for Fe and Zn were derived based on the expected bioavailability percentage, daily intake of wheat per capita, type of food preparation, and estimated average requirements (EAR). The preliminary breeding target for primary target countries of Pakistan and northern India is to increase Fe and Zn levels by 25 and 10 mg/kg, respectively, above the baseline, which is the mean of popular varieties currently grown in the region. Within the wider gene pool, there is sufficient genetic variability for Zn concentration to develop wheat varieties with increased Zn levels in the grain (Table 1). There is also promising genetic variability for Fe, but due to its lower bioavailability, the target levels for Fe need to be high, and achieving them may not be easy. Our review therefore focuses primarily on Zn improvement, though several studies have shown significant positive associations between Zn and Fe. Fe data is therefore provided where necessary or relevant.

2.3. Breeding strategies

In the past 50 years, the primary objective of modern wheat breeding programs has been to increase productivity by increasing yields. This has been achieved largely by selecting for resistance to diseases, short plant height, and increased biomass and harvest index, among other essential traits (Ortiz et al., 2007; Trethowan et al., 2007). Further yield increases are essential to feed the world's growing population. However, the nutritional composition of staple crops, especially micronutrients and protein quality, is equally important but often overlooked.

Studies have demonstrated genotypic variation in wheat grain Zn concentration (Table 1), and the available genetic variation for Zn is being exploited through breeding at CIMMYT, in collaboration with national programs, in order to develop and disseminate high-yielding, disease-resistant wheat varieties with significantly increased Zn and Fe concentrations. Past evidence has shown that grain Zn and Fe are quantitatively inherited traits in wheat (Trethowan et al., 2005; Trethowan, 2007). In number of

germplasms of wild and modern wheats and also spelt wheat, very close positive correlations between seed Zn and Fe concentrations have shown, indicating that physiological and genetic factors involved in Zn and Fe deposition in the seeds are same or very similar (Cakmak et al., 2004a,b; Morgounov et al., 2007; Gomez-Becerra et al., 2010b). Current breeding efforts at CIMMYT have focused on transferring genes governing increased Zn from *Triticum aestivum* ssp *spelta*- and *T.turgidum* ssp *dicocon*-based synthetics, landraces, and other reported high Zn sources, to high yielding elite wheat backgrounds. The first proof of concept results from the 1st HarvestPlus Yield Trial (HPYT), comprised of 40 high-yielding, biofortified, CIMMYT-derived wheat lines tested at target environments, showed high heritability along with high genetic correlations between locations. A considerable number of entries across diverse environments exceeded the intermediate to full target level of Zn in trials, endorsing the feasibility of developing competitive biofortified varieties, directly adapted to target environments, with farmer- and consumer-preferred agronomic and end-use traits (Velu et al., 2012). During the 2011–12 crop season, the 2nd HPYT trial of 50 biofortified wheat lines deployed in target environments identified 6–7 ‘best bets’, with grain Zn 75–150% above checks, along with higher yield potential, resistance to rusts, and preferred end-use quality traits. This indicates a positive trend over the years for the emergence of candidate varieties with high Zn and essential agronomic features (Velu and Singh, 2013).

2.4. Precision phenotyping

Several soil and environmental variables such as pH, temperature, radiation, precipitation, organic matter, and soil texture have the potential to influence concentration and solubility of micronutrients to plant roots (Tisdale and Nelson, 1975; Cakmak, 2008; Joshi et al., 2010). Precision phenotyping is a vital tool in breeding wheat germplasm with stable and high Zn concentration. Early and advanced generation materials should be evaluated in locations where soil Zn is homogeneous and not limiting. This can be achieved in at least two ways: (1) by applying a high rate of Zn fertilizer to homogenize the area and ensure that Zn is not limiting; and (2) by identifying areas that are naturally homogeneous, with non-limiting levels of soil Zn. The latter may be done by planting a systematic check cultivar in a given area and developing maps using geo-statistics that show variability for Zn grain concentration (Ortiz-Monasterio et al., 2010). It is important to maintain an adequate amount of available Zn and Fe in the soil during the crop growth period. Soil application of Zn-containing fertilizers may improve grain Zn concentrations in wheat depending on the severity of Zn deficiency in soil (Cakmak, 2008; Zou et al., 2012); for example, soil application of ZnSO₄ at the CENEB experimental station in Sonora, northwest Mexico, more than doubled grain Zn concentrations and reduced the soil Zn heterogeneity. Similar increases in grain Zn concentration following soil Zn applications have also been observed in other parts of the world (Cakmak et al., 2010a).

2.5. High throughput screening methodology

Selecting genotypes with higher micronutrient concentrations requires fast, accurate, and inexpensive methods of identifying nutrient dense genotypes. Traditionally, elemental analysis was conducted using inductively coupled plasma-optical emission spectrometry (ICP-OES) (Zarcinas et al., 1987), but this method requires expensive equipment, highly trained analysts, contamination free reagents, and extensive sample preparation. Consequently, many biofortification programs, especially those in more remote locations, have not been able to conduct their own analyses and

have had to send samples to better-equipped laboratories elsewhere.

Alternative, colorimetric approaches have been developed for Zn and Fe analysis in different cereal crops (Prom-u-thai et al., 2003; Ozturk et al., 2006; Choi et al., 2007; Velu et al., 2006, 2008). Though simpler to perform than ICP-based methods, these approaches are only semi-quantitative and laborious when applied in large scale screens. Furthermore, energy-dispersive X-ray fluorescence spectrometry (EDXRF) was standardized by Paltridge et al. (2012) for measuring Zn, Fe, and selenium (Se) concentrations in whole grain wheat. The high throughput, low cost XRF screening technique allows screening large number of breeding lines to discard low Zn/Fe lines and the selected high Zn/Fe lines could be tested with ICP for confirmation. The promising genotypes with significantly high Zn and Fe levels along with other essential agronomic features will be considered for large scale multi-locational testing and variety release.

2.6. Genotype \times environment ($G \times E$) interaction

Breeding for high Zn concentration is complicated by environmental conditions, particularly soil composition (Trethowan, 2007). Therefore, despite advances in breeding for uptake efficiency or mobilization to the grain, grain Zn concentration is limited by Zn availability in the soil (Ortiz-Monasterio et al., 2007; Ortiz-Monasterio et al., 2011). Significant genotype \times location interactions have been observed for Zn and Fe in wild and improved wheat cultivars (Oury et al., 2006; Ortiz-Monasterio et al., 2007; Trethowan, 2007; Gomez-Beccara et al., 2010a). The best high Zn lines developed at CIMMYT, Mexico, and evaluated in a multi-location trial in India's Eastern Gangetic Plains (EGP), revealed that wheat grain Zn concentrations were highly unstable (Joshi et al., 2010) as the performance of the elite lines varied across locations and years. Another reason for greater $G \times E$ interaction for Zn concentration may be its quantitative inheritance, as reported in maize and rice (Gregorio et al., 2000; Long et al., 2004). Other reports also suggest quantitative control in wheat (Trethowan, 2007). A recent study tested biofortified wheat lines at multiple locations in South Asia and revealed high heritability and high genetic correlation between locations for grain Zn, suggesting that $G \times E$ may not be a serious issue in breeding high Zn wheat genotypes (Velu et al., 2012).

2.7. Associations between micronutrients and protein

Understanding the associations between micronutrients and grain yield, plant height, grain size, and end-use quality parameters would facilitate the selection of mineral dense progenies through breeding with desired phenological and consumer preferred traits. As indicated above, previous studies have indicated that grain Zn and Fe are positively correlated in wheat (Morgounov et al., 2007; Peleg et al., 2009; Genc et al., 2009; Zhang et al., 2010; Gomez-Becerra et al., 2010b; Velu et al., 2011a, 2011b, 2012), implying that the alleles for Zn and Fe deposition in the grain co-segregate or pleiotropic, and therefore that Zn and Fe can be improved simultaneously. Further studies have shown that Zn and Fe in the flag leaves of *Aegilops* species are positively correlated with grain Zn and Fe (Rawat et al., 2009a, 2009b). More studies are needed to define these relationships before we begin using flag leaves for indirect selection of plants with significantly high grain Zn and Fe in breeding programs.

There was no negative linkage of grain Zn and Fe with grain yield (Graham et al., 1999; Welch and Graham, 2004; Velu et al., 2012). On contrary, some reports showed slightly negative association between Zn and grain yield in wheat (Morgounov et al., 2007; Peleg

et al., 2009; Zhao et al., 2009; Gomez-Becerra et al., 2010a). Furthermore, there seems to be no correlation between thousand grain weight and grain Zn in adapted wheat lines (Velu et al., 2012), suggesting no concentration effect due to small grain size; however, there is a significant negative correlation in unadapted wheat (Morgounov et al., 2007). Significant positive correlations were found between grain protein with Zn and Fe concentrations (Oury et al., 2006; Morgounov et al., 2007; Peleg et al., 2008; Zhao et al., 2009; Velu et al., 2011a,b). A very strong correlation between grain Zn and grain protein was also shown previously (Peterson et al., 1986; Feil and Fossati, 1995), indicating that grain Zn, Fe, and protein might have the same genetic base to some extent, and could be simultaneously improved by breeding (Welch and Graham, 2004). Recent publications indicate that nitrogen (N) nutritional status of plants has also positive effects on root uptake and shoot transport, retranslocation from vegetative tissues into seed and seed allocation of Zn and Fe (Aciksoz et al., 2011a; Kutman et al., 2010; Erenoglu et al., 2011). Experiments on wheat showed that increasing soil or foliar application of N was highly effective in improving root uptake and shoots and grain accumulation of Zn and Fe (Aciksoz et al., 2011a; Kutman et al., 2011). For example, under high N supply, around 60% of total shoot Fe was allocated into seeds, while in case of low N supply this value was around 38% (Kutman et al., 2011). In a radiolabeled experiment with ^{65}Zn in wheat, soaking flag leaves into ^{65}Zn -labelled ZnSO_4 solution showed that plants with high N translocated more ^{65}Zn from flag leaves into seeds than the plants with low N supply (Erenoglu et al., 2011). Cereals are known to release Zn- or Fe-mobilizing compounds from roots, so-called phytosiderophores, when they suffer from Fe or Zn deficiency (Cakmak et al., 1994). Phytosiderophores play a critical role in Zn and Fe uptake of plants (Murata et al., 2006; Suzuki et al., 2006). Release of phytosiderophores from roots and root uptake of Fe-complexed phytosiderophores were found to be promoted by improving N nutritional status of wheat plants (Aciksoz et al., 2011b). It seems that the N nutritional status of plants is an important factor in improving root uptake, shoot transport and seed accumulation of Zn and Fe. Most probably, the activity and pool of Fe and Zn transporter proteins and the level of Zn- and Fe-complexing compounds for transport such as amino acids and nicotianamine are positively affected by improving N status of plants (Cakmak et al., 2010a,b). Therefore, N fertilization might influence directly or indirectly to Zn and other micronutrients uptake and translocation, hence a special attention should be paid to the N status of plants and N fertilization regime in enrichment of food crops with Zn and Fe and in related breeding programs.

Significant negative correlations have been observed between glutenin content and Zn and Fe concentrations (Gomez-Becerra et al., 2010a); strong negative significant correlations occurred between Fe and plant height, and Fe and glutenin content, indicating that shorter plants with lower glutenin content favor higher grain-Fe concentration.

2.8. Gene discovery

By identifying molecular markers linked to loci determining variation for micronutrients, we can select favorable genotypes without having to determine mineral levels in field conditions. Only a few studies have identified quantitative trait loci (QTL) linked to Zn in cereals. Wild emmer wheat holds rich allelic diversity including for grain Zn and Fe concentrations (Xie and Nevo, 2008). A major locus GPC-B1 (250 kb-locus), mapped as a simple Mendelian locus (Distelfeld et al., 2007) associated with increased protein, Zn, and Fe from *T. dicoccoides*, encodes a NAC transcription factor (NAM-B1) that accelerates senescence and increases nutrient

remobilization from leaves to grain (Uauy et al., 2006; Distelfeld et al., 2007). Peleg et al. (2009) mapped 82 QTL for 10 different minerals with most of the positive alleles contributed by wild emmer and many QTLs mapped to homoeologous positions, reflecting synteny between the A and B genomes. The TtNAM-B1 gene, originated from *T. dicoccoides*, affecting grain protein, Zn, and Fe has been cloned (Distelfeld et al., 2007). Another QTL mapping study conducted on diploid A-genome wheat led to the identification of two QTLs for grain Fe on chromosomes 2A and 7A, and one QTL for grain Zn on chromosome 7A (Tiwari et al., 2009). In a *T. monococcum* mapping population, a major QTL has been identified on chromosome 5B that is associated with high grain content of Zn, Fe, copper (Cu), and manganese (Mn) (Ozkan et al., 2006). Furthermore, Singh et al. (2010) identified two QTL (*QFe-pau2A* and *QFe.pau-7A*) for Fe, and a QTL (*QZn.pau-7A*) for Zn, which they transferred from *Aegilops kotschy* and *Ae. peregrina* (both UUSS genome species). In a doubled haploid population, four QTLs for grain Zn concentration and a single QTL for grain Fe concentration were identified (Genc et al., 2009). Major QTLs that described 92% of the genetic variation in grain Zn concentration were located on chromosomes 3D, 4B, 6B, and 7A. A recent study of a Chinese winter wheat population evaluated under two different environments revealed nine additive and four epistatic QTLs, among which six additive QTLs and all of the four epistatic QTLs were effective in the two environments. A locus affecting both Zn and Fe was found on chromosome 5A, as well as two loci on chromosomes 4B and 5A that affected grain Zn and Fe concentrations, respectively, indicating a common genetic basis for both traits. The locus on chromosome 5A for grain Fe and protein concentrations was co-located with QTLs controlling the same traits in a previous study near the marker *Xgwm154* (Peleg et al., 2009). The marker interval *Xcfd21641-NP21.1* on chromosome 6A may be an ortholog of *Gpc-B1*. The QTLs identified from these studies will facilitate better understanding of the genetic basis of grain Zn, Fe, and the molecular markers closely linked to the QTLs can be used in large scale marker assisted breeding (Xu et al., 2012).

3. Agronomic biofortification

Soil Zn deficiency in major wheat growing areas leads to inherently low grain Zn concentration and is considered as a major factor in low human Zn intake (Alloway, 2009). Compared to the breeding approach, agronomic biofortification (e.g. application of Zn fertilizers) represents a short-term solution to the problem (Cakmak, 2008). Soil Zn applications are, however, less effective in increasing grain Zn, while foliar Zn applications result in remarkable increases in grain Zn concentration in wheat (Cakmak et al., 2010a,b). By optimizing the timing and the solute concentration of foliar Zn application, wheat grain Zn concentration could be further increased, not only in whole grains but also in the endosperm (Cakmak et al., 2010b; Zhang et al., 2010). Most Zn fertilization studies have focused on increasing grain yield, though grain Zn concentration is also starting to be addressed (Cakmak, 2009). The various methods of Zn application may differentially influence yield and grain Zn concentration.

Knowledge of the different forms of Zn fertilizer and timing of foliar Zn application is crucial for enhancing grain Zn. The most effective method for increasing grain Zn is the soil + foliar application method, which may result in an about 3-fold increase in grain Zn concentration (Cakmak et al., 2010a). When a high concentration of grain Zn is targeted, in addition to a high grain yield, combined soil and foliar application is recommended. Alternatively, using seeds with high Zn concentrations, together with foliar application of Zn, is also an effective way to improve both grain yield and grain Zn concentration. Applying Zn during the grain

development stage contributes to increased grain Zn concentration (Zhang et al., 2010) as foliarly-applied Zn can be absorbed by the leaf epidermis and then transported to other plant parts via the xylem and phloem (Haslett et al., 2001).

The timing of foliar Zn application is an important factor determining its effectiveness in increasing grain Zn concentration; large grain Zn increases are most likely when foliar Zn fertilizers are applied to plants at a late growth stage. Ozturk et al. (2006) studied changes in grain Zn concentration in wheat during the reproductive stage and found that the highest concentration of grain Zn occurs during the milk stage of grain development. Foliar application of Zn during reproductive growth seems to be more effective in increasing grain Zn concentration than spraying of Zn at earlier growth stage (Fig. 1). In addition to increasing the concentration of Zn in the whole grain, foliar application also increased the concentration in the starchy endosperm. As shown in Fig. 1, late season foliar application of Zn increased the concentration in the starchy endosperm by up to 3 fold. Since the concentration of phytate in the starchy endosperm (ie, white flour) of wheat is very low, or even not measurable (Pomeranz, 1988), such an increase in Zn implies a positive effect on the use of the grain for human nutrition. The increased Zn in the starchy endosperm resulting from foliar application should also be highly bioavailable due to the low phytate content.

Among the different forms of Zn fertilizer that were tested, the application of Zn as ZnSO₄ was most effective in increasing grain Zn, compared to other forms of Zn. The HarvestZinc (www.harvestzinc.org) initiative has been investigating different fertilizer strategies and the most efficient Zn application method for promoting Zn uptake and maximizing grain Zn accumulation. Increasing grain Zn by soil and/or foliar applications also provides additional positive impacts in terms of seed vitality and seedling vigor. Priming seeds in Zn-containing solutions is an alternative

way to increase seed Zn prior to sowing. High seed Zn concentrations ensure good root growth and contribute to better protection against soil borne pathogens (Cakmak, 2012). Preliminary studies showed that ZnSO₄ could be mixed with some wheat herbicides, insecticides and fungicides without affecting the effectiveness of foliar application for increasing grain Zn concentration. This would increase the possibility that farmers may be willing to apply ZnSO₄ in their fields by reducing the cost and time of application (Cakmak and Ortiz-Monasterio, unpublished results).

4. Bioavailability

The candidate biofortified wheat genotypes from CIMMYT and its partners is being characterized for bioavailability of Zn relative to improved Zn levels in humans, using different milling rates of wheat grain. A feeding trial was conducted to determine the bioavailability of Zn-biofortified wheat and control wheat in Mexican women (Rosado et al., 2009). They found that absorption of Zn was greater from Zn-biofortified wheat than from control wheat, when consumed by adult women as their primary source of energy and nutrients. Potentially valuable increases in Zn absorption were therefore achieved from biofortified wheat with high Zn.

An important consideration is the negative correlation between phosphorus (P) and both Fe and Zn. Approximately 75% of the total P in the wheat grain is stored as phytic acid, particularly in the germ and aleurone layers (Lott and Spitzer, 1980). Fewer genes are involved in the biosynthesis and metabolism of inhibitors and promoters, compared with the uptake, transport, and deposition of Fe and Zn. Thus, improving the bioavailability of Fe and Zn should be much easier than increasing their concentrations in grains (Bouis and Welch, 2010). Enhancing the promoters and decreasing the inhibitors could improve micronutrient bioavailability (Welch and Graham, 2004). However, breeders should be cautious of the

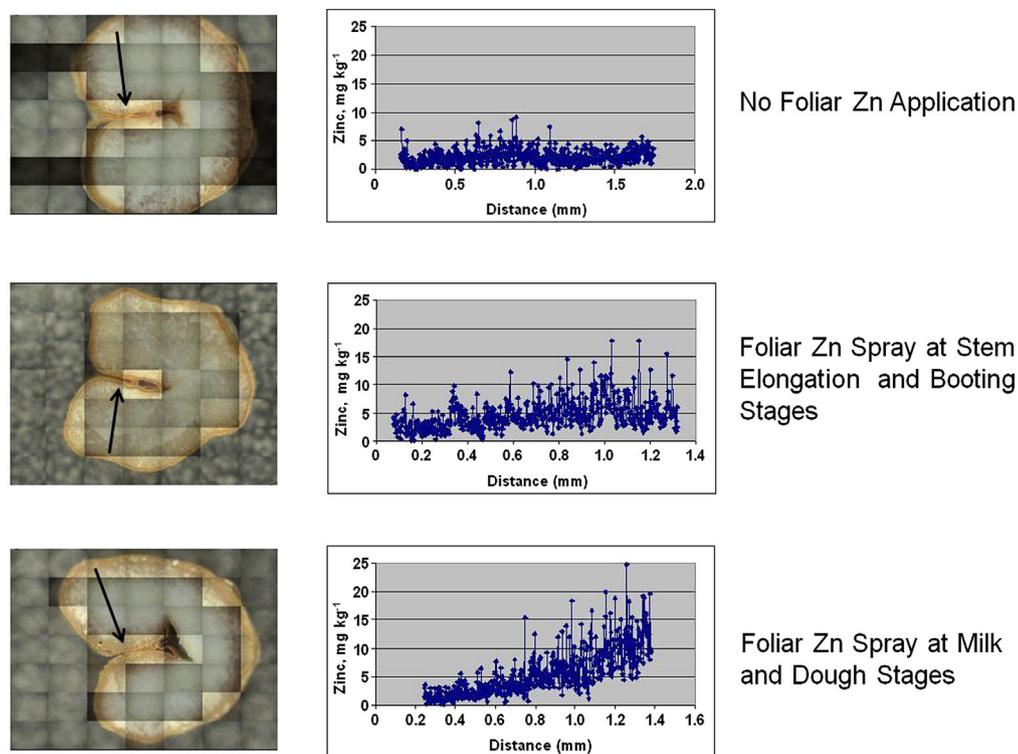


Fig. 1. Changes in Zn concentration of endosperm part of wheat seeds from plants sprayed with ZnSO₄ at different growth stages in field. Monitoring Zn localization in endosperm part has been realized by using LA-ICP-MS (Laser Ablation Inductively Coupled Plasma Mass Spectrometry). Black arrow on seeds (left side) shows the studied area on the endosperm. Distance on the x-axis shows the length of the studied endosperm section of the seeds (for more detail see Cakmak et al., 2010b; Cakmak, 2012).

possible negative consequences of altering anti-nutrients because of the important roles phytate and some other anti-nutrients play in plant metabolism and human diets. Interestingly, a fertilizer management study showed that soil Zn application increased estimated Zn bioavailability to humans (Hussain et al., 2012).

4.1. Increased synthesis of enhancers for Zn and Fe absorption

Dietary substances that promote Zn bioavailability in the presence of anti-nutrients are also known; their levels are influenced by both genetic and environmental factors. Inulin type-fructans are considered as a potential enhancers for micronutrients. Several studies in humans and animals (e.g. Scholz-Ahrens et al., 2001) have shown that inulin and other fructans can increase the intestinal absorption of minerals. Huynh et al. (2008) measured fructan content in 29 international wheat landraces and 14 new wheat lines from CIMMYT. There was significant genotypic variation among these materials, with grain fructan content ranging from 0.7 to 2.9% of grain dry weight, with no evidence of strong G × E interaction.

Phytases (myo-inositol hexakisphosphate phosphorylase) represent a subgroup of phosphatases that are capable of initiating the stepwise dephosphorylation of phytate, the most abundant inositol phosphate in nature. Development of plants with higher phytate-degrading activity may result in more extensive phytate degradation in the human stomach. For example, varieties that had greater mineral bioavailability due to improved breakdown of phytate was related to higher phytase levels (Lopez et al., 2003). Variability in phytase activity was studied in diverse wheat genotypes including synthetic wheats, advanced breeding lines, and modern Indian varieties (Ram et al., 2010). There were 3.4-fold differences in varieties developed in India and 5.9-fold variation in synthetic hexaploids. It is interesting to note that greater variability was observed in this set of synthetic hexaploids. Synthetic hexaploids with higher phytase levels can be used to enhance diversity in enzyme levels in bread and durum wheats. Significant positive correlations have been reported between native phytase activity and phosphorus utilization and micronutrient bioavailability (Lopez et al., 2003). Wheat phytases may therefore be a good alternative for application in food processing, where microbial phytases are used, because of their higher acceptance among consumers and their assumed lower allergenic potential (Greiner and Konietzny, 2006). Therefore, successful application of higher phytase levels in wheat can be beneficial, both economically and environmentally.

5. Conclusion

It is clear that genetic and agronomic biofortification offer sustainable solutions to the escalating micronutrient-related malnutrition problems. Genetic and agronomic biofortification approaches are actually not separate solutions; they are complementary and synergistic. There is promising, substantial genetic diversity in wild and primitive wheats, which have wide and useful genetic variation in grain Zn. This genetic variation is being intensively exploited by wheat breeding programs to improve both the concentration and bioavailability of Zn in modern wheat cultivars. A wide range of wheat germplasm is being evaluated at CIMMYT for grain Zn concentration and environmental interactions in target environments. Based on a range of reports and survey studies, the average grain Zn concentration of wheat in various countries ranges between 20 and 35 mg/kg, but large genetic variation exists for Zn in primitive and wild relatives of cultivated wheat. This implies that sufficiently enough genetic variation exists within the wheat gene pool to substantially increase grain Zn concentration. A

combination of fertilizer strategy with genetic biofortification will maximize the enrichment of food crop with micronutrients. Fertilizer strategies can also provide an immediate and effective option for increasing grain Zn concentration and wheat productivity, particularly in soils with severe Zn deficiency. Fertilizer practices can be also combined with other agrochemicals, for example, using Zn-containing N fertilizers for soil application and foliar Zn application will become feasible while combining with herbicides and fungicides, to reduce economic and time costs. Creating awareness among resource-poor farmers in developing world will further enhance adoption of effective Zn application procedures.

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