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MARSCHNER REVIEW

Enrichment of cereal grains with zinc: Agronomic or genetic biofortification?

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Abstract Zinc deficiency is a well-documented problem in food crops, causing decreased crop yields and nutritional quality. Generally, the regions in the world with Zn-deficient soils are also characterized by widespread Zn deficiency in humans. Recent estimates indicate that nearly half of world population suffers from Zn deficiency. Cereal crops play an important role in satisfying daily calorie intake in developing world, but they are inherently very low in Zn concentrations in grain, particularly when grown on Zn-deficient soils. The reliance on cereal-based diets may induce Zn deficiency-related health problems in humans, such as impairments in physical development, immune system and brain function. Among the strategies being discussed as major solution to Zn deficiency, plant breeding strategy (e.g., genetic biofortification) appears to be a most sustainable and cost-effective approach useful in improving Zn concentrations in grain. The breeding approach is, however, a long-term process requiring a substantial effort and resources. A successful breeding program for biofortifying food crops with Zn is very much dependent on the size of plant-available Zn pools in soil. In most parts of the cereal-growing areas, soils have, however, a variety of chemical and physical problems that significantly reduce availability of Zn to plant roots. Hence, the genetic capacity of the newly developed (biofortified) cultivars to absorb sufficient amount of Zn from soil and accumulate it in the grain may not be expressed to the full extent. It is, therefore, essential to have a short-term approach to improve Zn concentration in cereal grains. Application of Zn fertilizers or Zn-enriched NPK fertilizers (e.g., agronomic biofortification) offers a rapid solution to the problem, and represents useful complementary approach to on-going breeding programs. There is increasing evidence showing that foliar or combined soil+foliar application of Zn fertilizers under field conditions are highly effective and very practical way to maximize uptake and accumulation of Zn in whole wheat grain, raising concentration up to 60 mg Zn kg^{-1}. Zinc-enriched grains are also of great importance for crop productivity resulting in better seedling vigor, denser stands and higher stress tolerance on potentially Zn-deficient soils. Agronomic biofortification strategy appears to be essential in keeping sufficient amount of available Zn in soil solution and maintaining adequate Zn transport to the seeds during reproductive growth stage. Finally, agronomic biofortification is required for optimizing and ensuring the success of genetic biofortification of cereal grains with Zn. In case of greater bioavailability of the grain Zn derived from foliar applications than from soil, agronomic biofortification would be a very attractive and useful strategy in solving Zn deficiency-related health problems globally and effectively.

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Introduction

Increasing the Zn and Fe concentration of food crop plants, resulting in better crop production and improved human health is an important global challenge. Among micronutrients, Zn deficiency is occurring in both crops and humans (White and Zasoski 1999; Hotz and Brown 2004; Welch and Graham 2004). Zinc deficiency is currently listed as a major risk factor for human health and cause of death globally. According to a WHO report on the risk factors responsible for development of illnesses and diseases, Zn deficiency ranks 11th among the 20 most important factors in the world and 5th among the 10 most important factors in developing countries (Table 1). In a comprehensive study, Hotz and Brown (2004) reported that Zn deficiency affects, on average, one-third of world's population, ranging from 4 to 73% in different countries. Zinc deficiency is responsible for many severe health complications, including impairments of physical growth, immune system and learning ability, combined with increased risk of infections, DNA damage and cancer development (Hotz and Brown 2004; Gibson 2006; Prasad 2007).

The regions with Zn-deficient soils are also the regions where Zn deficiency in human beings is widespread, for example in India, Pakistan, China, Iran and Turkey (Cakmak et al. 1999; Alloway 2004; Hotz and Brown 2004). Zinc deficiency in soils and plants is a global micronutrient deficiency problem reported in many countries (Sillanpaa 1982; Alloway 2004). Low solubility of Zn in soils rather than low total amount of Zn is the major reason for the widespread occurrence of Zn deficiency problem in crop plants. Figure 1 shows global distribution of the regions where Zn deficiency problem has been reported in crop plants. Possibly, there are many other regions or countries where Zn deficiency problem has not been reported or diagnosed. Nearly 50% of the cereal-grown areas in the world have soils with low plant availability of Zn (Graham and Welch 1996; Cakmak 2002). Cereal crops represent a major source of minerals and protein in developing world. For example, in most of Central and West Asian countries, wheat provides nearly 50% of the daily calorie intake on average, likely increasing to more than 70% in the rural regions (Fig. 2; Cakmak et al. 2004). As presented in Fig. 2, wheat plays a particular role in covering daily caloric requirements of humans in Tajikistan. According to a report published by Hotz and Brown (2004), among all countries in the world, Tajikistan has been listed as the country having the highest percentage of population living under risk of Zn deficiency. Wheat inherently low in concentrations of Zn in grain, particularly when grown on Zn-deficient soils. In Turkey, grain Zn concentrations of wheat grown on Zn-sufficient soils range, generally, between 20 and 30 mg/kg, whereas on the Zn-deficient soils this range is between 5 and 12 mg/kg (Erdal et al. 2002; Kalayci et al. 1999). Based on a range of reports and survey studies, the average concentration of Zn in whole grain of wheat in various countries is between 20 to 35 mg kg^{-1} (Rengel et al. 1999; Cakmak et al. 2004). The Zn concentrations reported are too low to meet daily human requirement, especially for those consuming a high proportion of cereal-based diets. For a measurable biological impact on human health, the concentration of Zn in whole wheat grain needs to be increased at least by approximately 10 mg kg^{-1}, assuming a 400 g per day intake for adult woman in the countries where whole grain flour is used for making food like chapatti in India (Pfeiffer and McClafferty 2007; and http://www.harvestplus.org). Generally, recommended dietary allowance for Zn is around 15 mg per day (National Research Council 1989).

Table 1 Ten leading causes of illness and disease in low-income countries (WHO 2002)

Risk factors	Ratio (%)
Underweight	14.9
Unsafe sex	10.2
Unsafe water	5.5
Indoor smoke	3.7
Zinc deficiency	3.2
Iron deficiency	3.1
Vitamin A deficiency	3.0
Blood pressure	2.5
Tobacco	2.0
Cholesterol	1.9

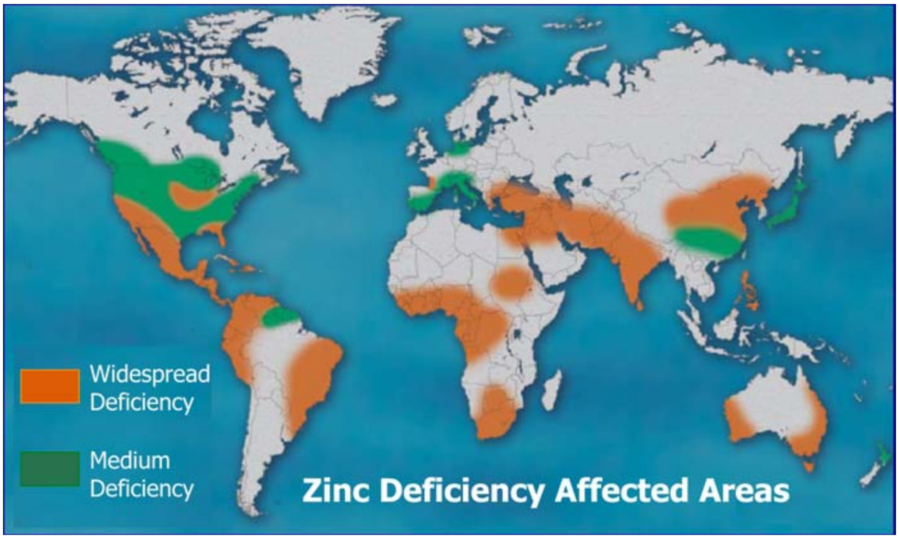

Fig. 1 Global distribution of Zn deficiency-affected areas (Alloway 2004)

Besides having inherently low levels of Zn, wheat grain is also rich in substances limiting utilization (bioavailability) of Zn in the human digestive tract, such as polyphenols and phytic acid (Welch and Graham 2004). Phytic acid is the major storage compound of phosphorus in grain. By binding Zn, phytic acid reduces solubility of Zn in food and restricts its utilization and retention in human body.

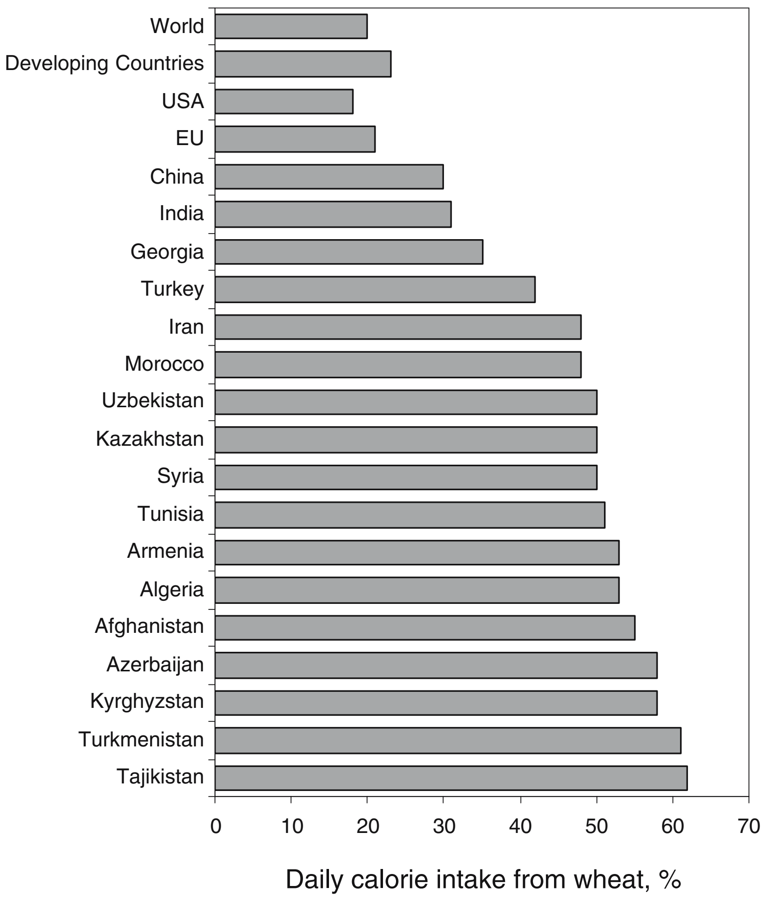

Fig. 2 Daily calorie intake from wheat in selected countries and regions (FAO Database, 2003)

However, these negative effects of phytic acid are balanced to a certain extend by beneficial effects of phytic acid on human health (e.g., inhibition of different type of cancers; Somasundar et al. 2005; Vucenik and Shamsuddin 2003). Seed phytic acid is also of great importance to good seed germination and seedling vigor (Oltmans et al. 2005; Guttieri et al. 2006). Most of the seed-Zn is located in the embryo and aleurone layer, whereas the endosperm is very low in Zn concentration (Ozturk et al. 2006). The embryo and aleurone parts are also rich in protein and phytate (Lott and Spitzer 1980; Mazzolini et al. 1985), indicating that protein and phytate in seeds could be sinks for Zn. According to a Zn-staining study in wheat seed (Fig. 3), Zn concentrations were found to be around 150 mg kg^{-1} in the embryo and aleurone layer and only 15 mg kg^{-1} in the endosperm (Ozturk et al. 2006). The Zn-rich parts of wheat seed are removed during milling, thus resulting in a marked reduction in flour Zn concentrations. Consequently, heavy consumption of high proportion of milled wheat and other cereal products may result in reduced intake of Zn. Enrichment of cereal grains with Zn is, therefore, a high priority area of research and will contribute to minimizing Zn deficiency-related health problems in humans.

Among the interventions currently being used as major solution to Zn deficiency in humans, food fortification and supplementation are being widely applied in some countries. However, these approaches appear to be expensive and not easily accessible by those living in developing countries (Bouis 2003; Stein et al. 2007; Pfeiffer and McClafferty 2007). For example, to eliminate micronutrient deficiencies in a nation with 50 million affected people using food fortification program US$ 25 million is needed annually (Bouis et al. 2000). Alternatively, agricultural strategies (e.g., breeding and fertilization) appear to be cost-effective approaches useful in improving micronutrient concentrations in grain, and thus contributing to human health. There are several examples demonstrating that applying Zn fertilizers to cereal crops improve not only productivity, but also grain Zn concentration of plants. Depending on the soil conditions and application form, Zn fertilizers can increase grain Zn concentration up to fourfold under field conditions (Yilmaz et al. 1997).

This paper is aimed at (1) evaluating the potential role of Zn fertilization strategy (e.g., agronomic biofortification) in increasing Zn concentration in cereal crops and (2) discussing the constraints of breeding strategy. Molecular biological tools also offer a major promising research area to assure a rapid progress towards enriching cereals with Zn. Readers interested in the role of molecular biology and transgenic approaches in biofortification of cereal

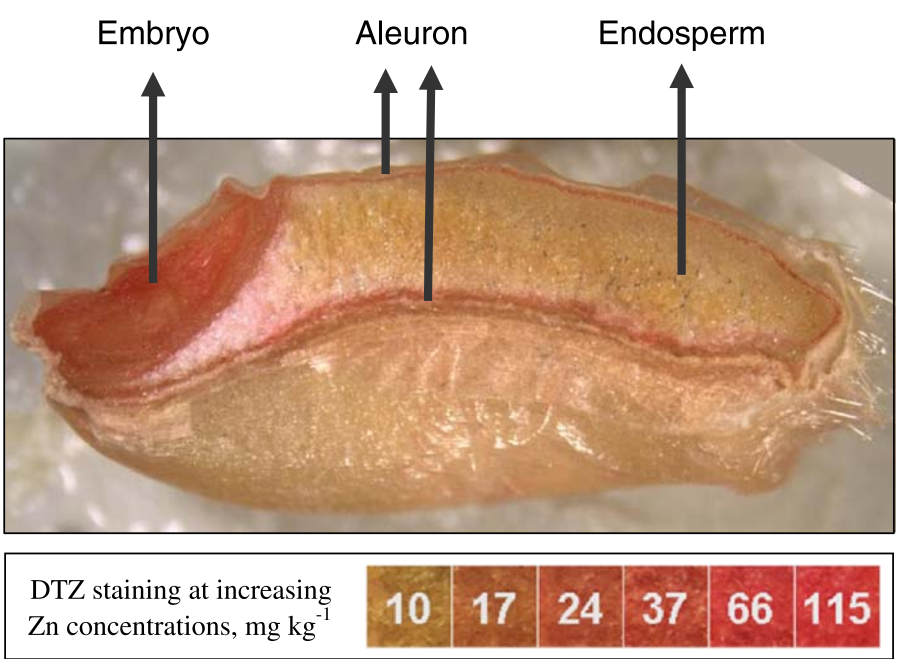

Fig. 3 Diphenyl thiocarbazone (DTZ) staining a wheat seed. When reacting with Zn, DTZ forms a red DTZ-complex which indicates localization of Zn (for further details see Ozturk et al. 2006)

crops with Zn and other micronutrients are referred to White and Broadley (2005), Lucca et al. (2006) and Ghandilyan et al. (2006). Some results and parts of the discussion documented in this paper have been presented at the International Workshop on Micronutrients organized by IFA (International Fertilizer Industry Association; http://www.fertilizer.org) in Kunming, China (27 February–2 March 2006).

Breeding approach

In an effort coordinated by the HarvestPlus project, CGIAR (The Consultative Group on International Agricultural Research) Centers are taking a leading role in breeding for increasing concentration and bioavailable levels of Zn and Fe in seeds of major stable food crops (Bouis 2003; Pfeiffer and McClafferty 2007). Plant breeding (e.g., genetic biofortification) approach to minimize the extent of Zn deficiency is thought to be cost-effective, easily applicable and affordable in the target populations. A breeding program aiming at development of new genotypes with high Zn concentration first requires existence of useful genetic variation for Zn accumulation in grain. However, as indicated above, cultivated wheat contain very low levels of Zn and shows a narrow genetic variation for Zn. Compared to cultivated wheats, wild and primitive wheats represent a better and more promising genetic resource for high Zn concentrations. Among wild wheats tested so far, the collections of wild emmer wheat, *Triticum turgidum* ssp. *dicoccoides*, showed impressive genetic variation and the highest concentrations of Zn (14 to 190 mg Zn kg^{-1}, Cakmak et al. 2004). Very recently, new wild emmer wheat accessions have been identified showing simultaneously both very high concentrations of Zn (up to 139 mg kg^{-1}), Fe (up to 88 mg kg^{-1}) and protein (up to 380 g kg^{-1}) in seeds and high tolerance to drought stress and Zn deficiency in soil (Peleg et al. 2008). In addition, synthetic wheats derived from *Aegilops tauschii* have also a high genetic potential for increasing grain Zn concentration of cultivated wheat (Calderini and Ortiz-Monasterio 2003). A large genetic variation for grain Zn has also been shown in different germplams of rice and maize and this variation is being exploited in breeding programs (Graham et al. 1999). White and Broadley (2005) published a comprehensive review on natural variation of micronutrients in different crop species and reported recent advances in development of new genotypes with high levels of micronutrients, including Zn.

Little information is, however, available about the genetic control and molecular physiological mechanisms contributing to high accumulation of Zn and other micronutrients in grain of different genetic materials (Ghandilyan et al. 2006; Lucca et al. 2006; White and Broadley 2005). Studies with the substitution lines of *Triticum dicoccoides* showed that the *dicoccoides* chromosome 6B is the most relevant chromosome carrying the genes determining high levels of Zn in grain (Cakmak et al. 2004). In studies with different recombinant substitution lines derived from *Triticum dicoccoides* a locus *Gpc-B1* has been identified on the short arm of the chromosome 6B that affects both protein and Zn concentrations (Fahima et al. 2006; Distelfeld et al. 2007). The enhancing role of *Gpc-B1* on grain Zn and protein concentrations has been shown consistently in five different environments, suggesting that the effect of GxE on the Zn and protein levels of the lines carrying the Gpc-B1 allele is small (Distelfeld et al. 2007). These results indicate that the genes responsible for high levels of Zn and protein are, most probably, closely related, and breeding for high protein in grain may result in simultaneously high grain Zn. Very high positive correlations reported between grain Zn and protein (Peterson et al. 1986; Feil and Fossati 1995; Morgonuov et al. 2007; Distelfeld et al. 2007) support the idea that the genetic factors affecting grain Zn and protein concentrations are possibly co-segregated. Breeding efforts are, now, ongoing to introgress the *Gpc-B1* locus into genetic background of high yielding elite cultivars.

Transgenic approaches could be a further option in improving food crops with Zn. Currently, impressive progress is being made in developing transgenic plant genotypes with increased concentrations of Zn and Fe. Evidence is available showing a potential role of ZIP family Fe and Zn transporter proteins in improving micronutrient density in grain (Schachtman and Barker 1999; Eide 2006). These proteins are involved in uptake and transport of cationic micronutrients in cells. In most cases, the genes encoding the Fe and Zn transporter proteins are expressed in response to Fe and Zn deficiencies, respectively. However, the role of these transporter proteins in genotypic variation for

Zn deficiency tolerance or grain Zn accumulation is not clear. Expression of the genes encoding a Zn-transporter protein from *Arabidopsis thaliana* in roots of a barley genotype resulted in an increase in grain Zn concentration (Ramesh et al. 2004). Several reports have investigated the role of ferritin protein in seed accumulation of Fe and Zn. Ferritin is a major Fe protein existing in most living organisms (Harrison and Arosio 1996). Vasconcelos et al. (2003) reported that overexpression of soybean ferritin genes in rice was effective in increasing both Fe and Zn concentrations of seeds. Transforming rice with ferritin gene from soybean (Goto et al. 1999; Qu et al. 2005) or French bean (Lucca et al. 2001) increased grain Fe concentrations. As in rice, over-expression of the ferritin gene was also effective in improving seed Fe concentration of transgenic maize plants expressing ferritin gene from soybean (Drakakaki et al. 2005). Very recently, it has been shown that the Gpc-B1 locus from *Triticum diccocoides* encodes a NAC transcription factor (NAM-B1) that increases grain Zn and Fe concentrations, possibly by stimulating leaf senescence and thus remobilization of Zn and Fe from flag leaves into seeds (Uauy et al. 2006). Reduced expression of the NAM genes delayed senescence and reduced Zn and Fe concentrations. However, in all these studies dealing with overexpression of ferritin (Goto et al. 1999; Lucca et al. 2001; Vasconcelos et al. 2003; Drakakaki et al. 2005; Qu et al. 2005), Zn-transporter protein (Ramesh et al. 2004) and NAM genes (Uauy et al. 2006), no data on grain yield per plant or per spike were presented, making it impossible to assess any potential dilution or concentration effects on the reported changes in grain Fe or Zn concentrations. Genotypic variation for grain yield or the number of seeds per plant may cause significant “dilution” or “concentration” effects on the reported amount of micronutrients, despite a similar 1,000-kernel weight (Marschner 1995; Abbate et al. 1998; Calderini et al. 2006).

Although it is a powerful and sustainable strategy, breeding approach has some limitations: it is a long-term process requiring variety of breeding activities and huge resources. In addition, it is uncertain whether this strategy will effectively work after all the long-term efforts. The breeding steps include at least (1) identification of a useful genetic variation and the most promising parents, (2) long-term crossing and back-crossing activities, (3) stability of the target traits (e.g., high grain Zn concentrations) across the different environments that feature huge variation in soil and climatic conditions, and finally (4) adaptation of the newly developed biofortified genotypes over a range of crop and soil management practices applied in the target regions or countries. The acceptance of biofortified crops by producers is a further issue that needs a special attention. Most importantly, newly developed genotypes should be able to extract sufficiently large amounts of Zn from potentially Zn-deficient soils and accumulate it in whole grain at sufficient levels for human nutrition (e.g., up to 40–60 mg kg^{-1}). As discussed below in detail, the soils widespread in major cereal-growing regions have several adverse soil chemical factors (i.e., high pH values and low soil moisture and organic matter) that could potentially diminish the expression of high grain Zn trait and limit the capacity of newly developed (biofortified) cultivars to absorb adequate amount of Zn from soils to contribute to daily Zn requirement of human beings.

Constraints to the breeding approach

Adverse soil chemical properties

A successful breeding program for biofortifying cereals and other food crops with Zn is dependent on the size of plant-available Zn pools in soil. In order to increase Zn accumulation in grain required for a measurable biological impact, sufficient amount of plant-available Zn must be maintained in soil. However, in many cereal-growing areas, soils have a variety of chemical and physical problems that significantly reduce solubility and impair root absorption of Zn (Fig. 4). Among the soil chemical factors, soil pH plays the most important role in Zn solubility in soil solution. In a pH range between 5.5 and 7.0, Zn concentration in soil solution is decreased by 30-fold to 45-fold for each unit increase in soil pH, thus increasing a risk for development of Zn deficiency in plants (Marschner 1993). Increasing soil pH stimulates adsorption of Zn to soil constituents (e.g., metal oxides, clay minerals) and reduces the desorption of the adsorbed Zn (Fig. 4). Lindsay (1991) reported that at pH 5.0 the concentration of Zn^{2+} in soil solution is sufficiently high, about 10^{-4} M (6.5 mg kg^{-1}). When soil pH increased from 5 to 8, concentration of soil

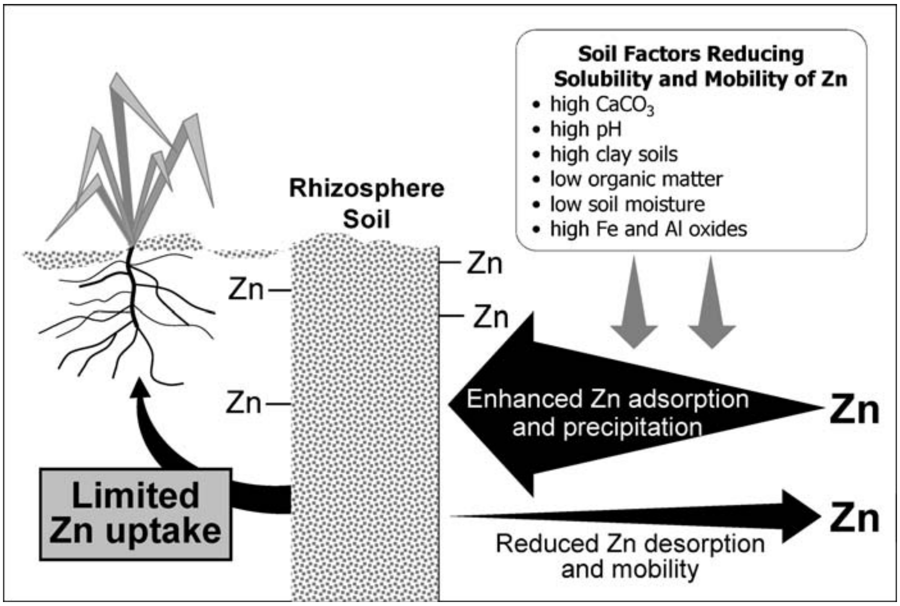

Fig. 4 Major soil chemical and physical factors affecting availability of Zn to roots

solution Zn^{2+} is reduced 1,000 times and becomes nearly 10^{-10} M (approx. 0.007 mg kg^{-1}). Consequently, an increase in soil pH is associated with very sharp decreases in concentrations of Zn in plant tissues (Sarkar and Wyn Jones 1982; Marschner 1995).

Transport of Zn to root surface in soils occurs predominantly via diffusion (Wilkinson et al. 1968), and this process is highly sensitive to soil pH and moisture. Soil moisture is a key physical factor providing suitable medium for an adequate Zn diffusion to plant roots. This role of soil moisture is very critical in soils with low Zn availability (Rattan and Deb 1981; Marschner 1993). Zinc nutrition of plants is, therefore, adversely affected under water-stressed conditions, particularly in regions where topsoils are usually dry during the later stages of crop growth (Fig. 4). Thus, development of Zn deficiency stress and decreases in grain yield due to Zn deficiency were found to be more severe under rainfed compared to irrigated conditions, as shown in Central Anatolia under field conditions (Ekiz et al. 1998; Bagci et al. 2007).

Soil organic matter plays a critical role in solubility and transport of Zn to plant roots (Marschner 1993; Obrador et al. 2003). In a study with 18 Colorado soils, there was a strong inverse relationship between the contents of soil organic matter and soluble Zn concentrations in the rhizosphere (Catlett et al. 2002). Turkish soils, especially the soils in Central Anatolia, are characterized by low levels of organic matter and high soil pH (Eyupoglu et al. 1994; Cakmak et al. 1996). In a survey of 1,511 soil samples from Turkey conducted by Eyupoglu et al. (1994), it has been found that diethylenetrinitrilopentaacetic acid (DTPA)-extractable Zn concentrations are inversely related to soil pH and soil organic matter (Fig. 5). In general, soils containing less than 0.5 mg DTPA-extractable Zn are considered potentially Zn deficient that may respond well to Zn fertilizers (Lindsay and Norvell 1978). The percentage of the soils containing DTPA-Zn higher than 0.5 mg kg^{-1} is much greater in soils with pH values between 4 to 6 than in soils with pH between 7 to 8 or higher (Fig. 5). A similar relationship was also found between DTPA-extractable Zn and soil organic matter: the higher the soil organic matter, the greater the DTPA-extractable Zn concentrations in soil (Fig. 5).

These results indicate that the pool of readily available Zn to plant roots may be extremely low in soils with high pH and reduced levels of organic matter and soil moisture. Majority of the soils cropped to cereals in the world have such adverse soil chemical properties as reported for Turkey, India, Pakistan, China and Australia, leading to impaired Zn nutrition of cereals (Graham et al. 1992; Takkar and Walker 1993; Cakmak et al. 1999; White and Zasoski 1999; Alloway 2004). Hence, the genetic capacity of the newly developed (biofortified) cultivars to absorb

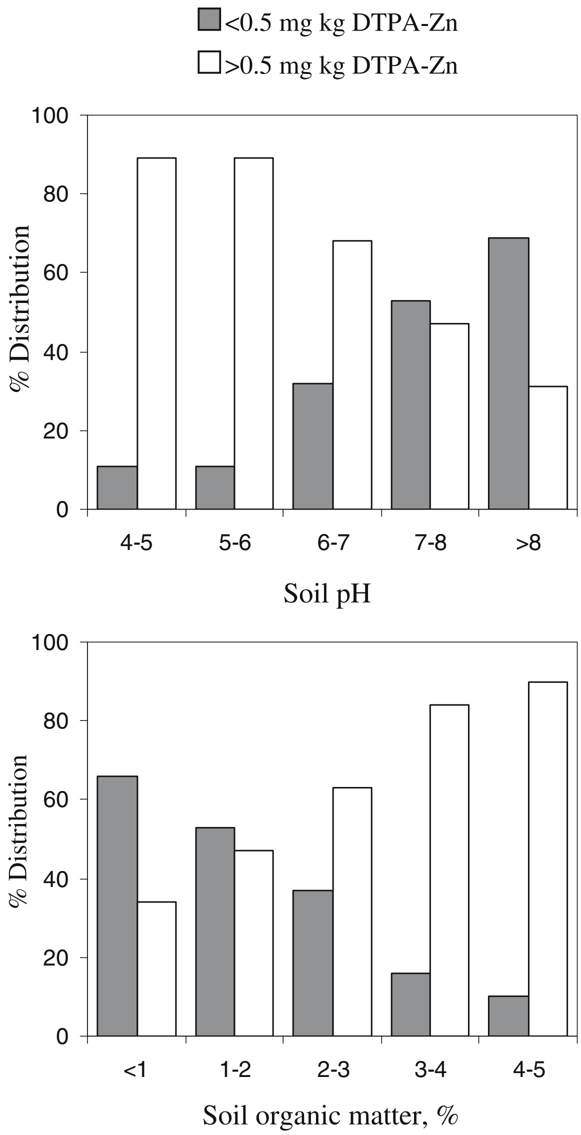

Fig. 5 Percentage of DTPA-extractable Zn that is lower or higher than 0.5 mg kg^{-1} in a given range of soil pH and organic matter. Based on 1,511 soil samples collected in different parts of Turkey (Eyupoglu et al. 1994)

sufficient amount of Zn from soil and accumulate it in the grain to achieve the nutritional benefit may not be expressed to the full extent.

Release of high-yielding cultivars and large offtake of Zn from soils

A steady increase in cereal yield capacity due to availability of new cultivars with a high yield potential may further aggravate Zn deficiency in soils by progressively depleting available soil-Zn pools. This depletion of available Zn pools by large offtake in agricultural produce may occur to a greater extent in soils with adverse chemical properties (e.g., high $CaCO_3$, low levels of organic matter and soil moisture). In such soils, the supply of Zn to roots would be lower than the root capacity to take up Zn. Therefore, under such conditions, adding Zn to soil and/or foliar Zn applications are important for biofortification of grains with Zn. The soil depletion of nutrients is a growing concern, especially pronounced in soils of some African countries where continuous monoculture cropping systems takes places without applying micronutrients and even some macronutrients (Tan et al. 2005; Hartemink 2006). This leads to rapid depletion of mineral nutrients including Zn. Replenishment of Zn is needed not only for improving Zn concentrations of edible parts of plants, but also for sustaining high yields (Tandon 1995; Cakmak 2002).

Agronomic biofortification

In view of the discussion above, providing Zn to plants (for example, by applying Zn-fertilizers to soil and/or to foliar) appears to be important to ensure success of breeding efforts for increasing Zn concentration in grain. Fertilizer strategy could be a rapid solution to the problem and can be considered an important complementary approach to the on-going breeding programs. Fertilizer studies focusing specifically on increasing Zn concentration of grain (or other edible parts) are, however, very rare, although a large number of studies are available on the role of soil and foliar applied Zn fertilizers in correction of Zn deficiency and increasing plant growth and yield (Martens and Westermann 1991; Mortvedt and Gilkes 1993; Rengel et al. 1999). Zinc can be directly applied to soil as both organic and inorganic compounds. Zinc sulfate ($ZnSO_4$) is the most widely applied inorganic source of Zn due to its high solubility and low cost. Zinc can also be applied to soils in form of ZnO, ZnEDTA and Zn-oxysulfate. The agronomic effectiveness (e.g., magnitude of the crop response per unit applied micronutrient) of Zn fertilizers is higher with ZnEDTA than the inorganic Zn fertilizers (Mortvedt 1991; Martens and Westermann 1991). However, due to its high cost, use of ZnEDTA in cereal farming is limited.

Convincing evidence about the role of Zn fertilizer strategy in improving grain Zn concentration in wheat (e.g., agronomic biofortification) has been obtained in field trials in Turkey. Applying Zn fertilizers to wheat grown in field in Central Anatolia improved not only productivity, but also grain Zn concentration (Yilmaz et al. 1997). Depending on the application method, Zn fertilizers can increase grain Zn concentration up to three- or fourfold (Table 2). The most effective method for increasing Zn in grain was the soil+foliar application method that resulted in about 3.5-fold increase in the grain Zn concentration. The highest increase in grain yield was obtained with soil, soil+foliar and seed+foliar applications (Table 2). When a high concentration of grain Zn is aimed in addition to a high grain yield, combined soil and foliar application is recommended. Alternatively, using seeds with high Zn concentrations at sowing together with foliar application of Zn is also an effective way to improve both grain yield and grain Zn concentration (Table 2).

In the field trial described in Table 2, Zn has been applied foliarly before anthesis at the tillering and stem elongation stages. Timing of foliar Zn application is an important factor determining the effectiveness of the foliarly applied Zn fertilizers in increasing grain Zn concentration. It is expected that large increases in loading of Zn into grain can be achieved when foliar Zn fertilizers are applied to plants at a late growth stage. Ozturk et al. (2006) studied changes in grain concentration of Zn in wheat during the reproductive stage and found that the highest concentration of Zn in grain occurs during the milk stage of the grain development. Data obtained from a recent field trial showed that when Zn was applied foliarly both before and after anthesis, grain Zn concentrations increased up to 60 mg kg^{-1} (Fig. 6). Among the forms tested, the application of Zn as $ZnSO_4$ was most effective in increasing grain Zn compared to ZnEDTA and ZnO (N. Aydin and I. Cakmak, unpublished results). These results show a high potential of Zn fertilizer strategy for rapid improvement of grain Zn concentrations, especially in the case of late foliar Zn application. In practical agriculture, it is known that foliar uptake of Zn is stimulated when Zn fertilizer is mixed with urea (Mortvedt and Gilkes 1993). Urea fertilizers containing Zn (e.g., zincated urea) may represent a good foliar N fertilizer to improve both grain Zn and protein concentrations.

There are different methods for adding Zn into NPK fertilizers. Zinc can be added to a compound fertilizer either by incorporating it into granules during the manufacture, or by coating Zn onto granular compound fertilizer, or by bulk blending Zn fertilizers with granular NPK fertilizer (Mortvedt 1991). Little information is available in the literature about the effectiveness of various Zn-supplemented compound fertilizers and the type of the Zn-supplementation process (e.g., incorporation, coating or bulk-blending) on Zn uptake and accumulation in plants. In a study with bean, different types of Zn-supplemented compound fertilizers have been tested for their effect on

Table 2 Effect of different Zn application methods on Zn concentration in whole shoots and grain, and the increases in shoot biomass and grain yield by Zn applications

Zn application methods[a]	Zn concentration		Increases in yield by Zn application	
	Whole shoot (mg kg^{-1})	Grain	Whole shoot (%)	Grain
1 – Control	10	10	–	–
2 – Soil	19	18	109	265
3 – Seed	12	10	79	204
4 – Foliar	60	27	40	124
5 – Soil+foliar	69	35	92	250
6 – Seed+foliar	73	29	83	268

Shoots were sampled at the beginning of stem elongation. The values represent the average values of one durum and three bread cultivars grown on a Zn-deficient soil in Central Anatolia (Yilmaz et al. 1997)

[a] *1*, control (no Zn application); *2*, 23 kg Zn ha^{-1} as broadcast to soil; *3*, seed coating (1 l 30% $ZnSO_4$ sprayed on to 10 kg seeds and then the seeds dried and sown); *4*, foliar application (2×220 g Zn ha^{-1} as $ZnSO_4$ in 450 l at tillering and stem elongation); *5*, combination of the methods *2* and *4*; *6*, combination of the methods *3* and *4*

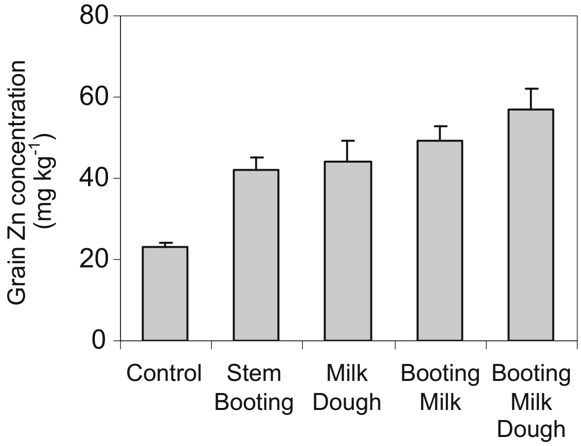

Fig. 6 Effect of foliar application of Zn on grain Zn concentration in wheat grown under field conditions in Black Sea region of Turkey. Zinc has been sprayed on foliar in form of $ZnSO_4$ at the rate of 0.5% (*w*/*v*). Zinc applications have been realized at the following growth stages: (1) stem elongation and booting stages, (2) milk and dough stages, (3) booting and milk stages and (4) booting, milk and dough stages. Control treatments have been sprayed with water. Data show the means±SD of four independent replications (N. Aydın and I. Cakmak, unpublished)

yield and plant Zn concentration. As shown in Table 3, all type of the Zn-supplementation procedures were equally effective in increasing yield, but the fertilizers with Zn blending were more effective in increasing plant Zn concentration, most probably due to less reactions of the blended Zn with the NPK fertilizer and/or soil constituents (Ellis et al. 1965 cited in Mortvedt and Gilkes 1993).

Success stories in Finland and Turkey

A very successful fertilizer strategy has been applied to improve grain Se concentration in Finland. Selenium is important anti-carcinogenic compound, prevents development of cardiovascular diseases and improves human immune system (Combs and Gray 1998; Rayman 2005). Application of Se-enriched NPK fertilizers was highly successful in both increasing Se concentration of food crops and improving daily Se intake and serum Se concentrations in the Finnish population to targeted levels (Fig. 7). The amount of Se added into regular NPK fertilizers in Finland was initially 16 mg Se per kg fertilizer. Then, to avoid any possible Se toxicity associated with unnecessarily high Se intake, the amount of Se added was reduced to 6 mg per kg fertilizer. This decision led to a significant decrease in Se concentration of food crops and daily Se intake. Consequently, the level of Se in all fertilizers was increased to the current level of 10 mg per kg fertilizer (Fig. 7; Aspila 2005). The Finnish Se fertilization program that was conducted in the last 20 years is an excellent example of the role of agronomic biofortification in achieving high levels of micronutrients in cereal grains required for improved human nutrition. In addition, low dietary intake of Se in UK has been ascribed to low levels of Se in cereal-based foods (Arthur 2003). Recently, agronomic biofortification strategy has been suggested as a promising way for enriching cereal grains with Se in UK (Broadley et al. 2006).

A similar agronomic biofortification strategy has been initiated in Turkey nearly 12 years ago by applying Zn-enriched NPK fertilizers (Cakmak et al. 1999; Cakmak 2004). In a previous survey study initiated by FAO it has been shown that plant available Zn concentrations in Turkish soils are some of lowest ever recorded (Sillanpaa 1982). After demonstration of the impressive increases in plant growth and grain yield caused by application of Zn fertilizers to wheat in early 1990s, an increasing amount of Zn-enriched NPK fertilizers have been produced and applied in Turkey. Zinc was added into NP or NPK fertilizers at the rate of 1% *w*/*w* by incorporation into granules during the manufacture. In addition to these compound fertilizers, increasing amounts of $ZnSO_4$ and ZnO are being applied in crop production in Turkey. Today, after the Zn deficiency problem was first diagnosed as a critical micronutrient deficiency problem for wheat production in Central Anatolia (Cakmak et al. 1996, 1999), the total amount of Zn-containing compound fertilizers applied in Turkey rose from zero in 1994 to a

Table 3 Grain yield and leaf Zn concentrations of bean plants as affected by application method of $ZnSO_4$ together with a granular NPK fertilizer (Ellis et al. 1965; cited in Mortvedt and Gilkes 1993)

Method of Zn application	Yield (kg ha^{-1})	Leaf Zn concentration (mg kg^{-1})
No Application	1,230	20
Blended	1,660	40
Incorporated	1,640	31
Coated	1,670	34

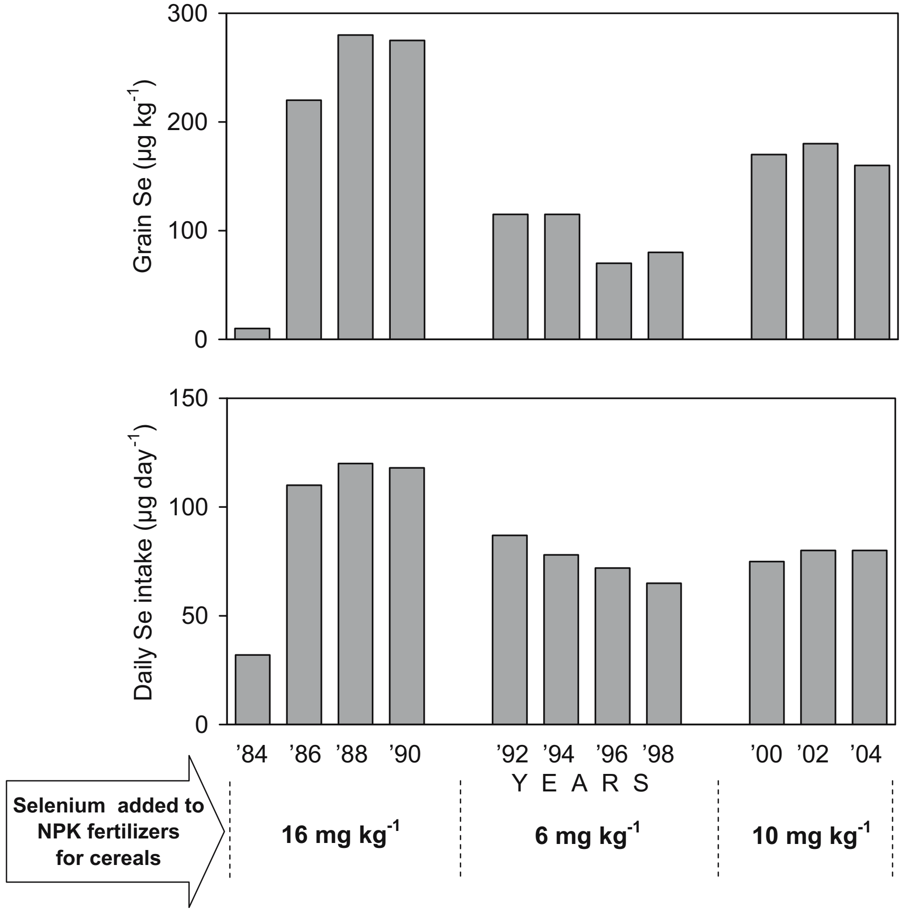

Fig. 7 Changes in Se concentration in cereal grains and daily Se intake by people in Finland during nationwide application of Se-enriched NPK fertilizers between 1984 and 2004 (re-drawn from the presentation of Dr. G.F. Combs, Jr at "Farming for Health", Oslo, 17-18 October, 2005; see also Ekholm et al. 2005)

record level of 350,000 tonnes per annum (Fig. 8; Cakmak 2004). Unfortunately, there is no impact study showing the effects of applying Zn-enriched fertilizers on daily Zn intake of people, especially in rural regions in Central Anatolia. Most probably, increases in grain Zn concentration by Zn fertilization may have positive contributions to human health.

Agronomic benefits resulted from Zn fertilization

Increasing seed concentration of Zn by soil and/or foliar applications of Zn also brings several agronomic benefits for crop production. Applying Zn to plants grown under potentially Zn-deficient soils is effective in reducing uptake and accumulation of P (and thus phytate) in plants. This agronomic side effect of Zn fertilization may result in better bioavailability of Zn in the human digestive system. In addition, seedlings from seeds containing high Zn have better ability to withstand adverse environmental conditions. These benefits are discussed in detail below.

Zinc reduces phosphorus uptake by roots and accumulation of phytate in grain

Total amount of Zn in grains is not always a good indicator of its utilization (bioavailability) in the human digestive system. Therefore, besides high levels of Zn in grain (or diet), bioavailability of Zn is also an important nutritional aspect. Among the factors decreasing bioavailability of Zn, phytic acid (or phytate) is most often studied (Egli et al. 2004; Gibson 2007; Hotz and Gibson 2007). From 65 to 85% of the total P in the grain is present as phytate, which is a major compound binding Zn and making it poorly bioavailable in the human digestive tract. The phytate-Zn molar ratio is a widely used criterion for estimating bioavailability of Zn in diets (Morris and Ellis 1989; Gibson 2006). Generally, keeping this

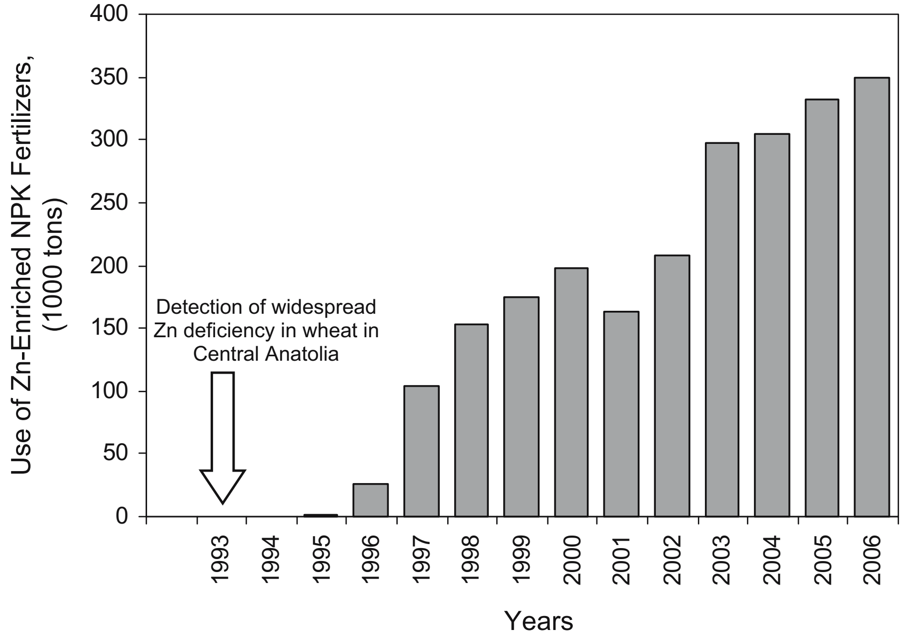

Fig. 8 Use of Zn-containing NP or NPK fertilizers in Turkey (Turkish Ministry of Agriculture, 2005; TOROS Fertilizer and Chemical Industry-Istanbul, 2007)

ratio lower than 15–20 by increasing Zn or reducing phytate concentrations improves bioavailability of Zn in the human body. As expected, increased root uptake and shoot accumulation of P is accompanied by corresponding increases of P in grain due to the high phloem mobility of P (Buerkert et al. 1998). When transported into grain, most of the inorganic P is converted into phytic acid (phytate).

It has been well-documented that adequate Zn nutrition is important in controlling the uptake of P by roots. Numerous reports show that low Zn supply in a growth medium promotes root uptake and root-to-shoot transport of P in different crops (Loneragan et al. 1982; Cakmak and Marschner 1986; Parker 1997). Generally, Zn deficiency is associated with excessive accumulation of P in shoots that may even induce P toxicity symptoms in leaves (Loneragan et al. 1982; Cakmak and Marschner 1987). This enhancement effect of Zn deficiency on P uptake is very specific and was not found under deficiency of Fe, Cu or Mn (Cakmak and Marschner 1986). Zinc deficiency also enhanced expression of P-transporter genes in root cells and enhanced P accumulation in plants (Huang et al. 2000). In field trials on Zn-deficient soils in Central Anatolia, it has been shown that soil and/or foliar application of Zn fertilizers reduced shoot and grain P concentrations and thus grain phytate concentrations. As expected, the reducing effect of Zn on grain P and phytate was associated with a large decrease in phytate-to-Zn molar ratios (Erdal et al. 2002). Reduction in grain P or phytate by Zn fertilization was not related to increases in grain yield (and thus dilution of the Zn concentration) because similar results were also found in rye (I. Erdal et al., unpublished results) that is highly tolerant to Zn deficiency and its yield is not affected by Zn deficiency (Cakmak et al. 1997). These results show an important potential benefit of Zn fertilizers in increasing the level of Zn bioavailability in grains by reducing phytate/Zn molar ratio in grain.

Benefits of high seed-Zn on plant growth

Increasing grain Zn can also result in several agronomic benefits besides improvements in human health (Fig. 9). When seeds with low concentration of Zn are re-sown, the ability of the new crop to withstand environmental stresses at the early growth stages is greatly impaired (Welch 1999). Seeds with low Zn concentrations might not be viable despite their normal appearance. This indicates existence of a potential cellular defect or damage in seeds associated with low levels of Zn. It is, therefore, important to maintain the sufficient amount of Zn in soils during seed germination and early seedling development (Welch 1999). Maintenance of the adequate amount of readily available Zn in soils or high Zn concentrations in seeds ensure good root growth and

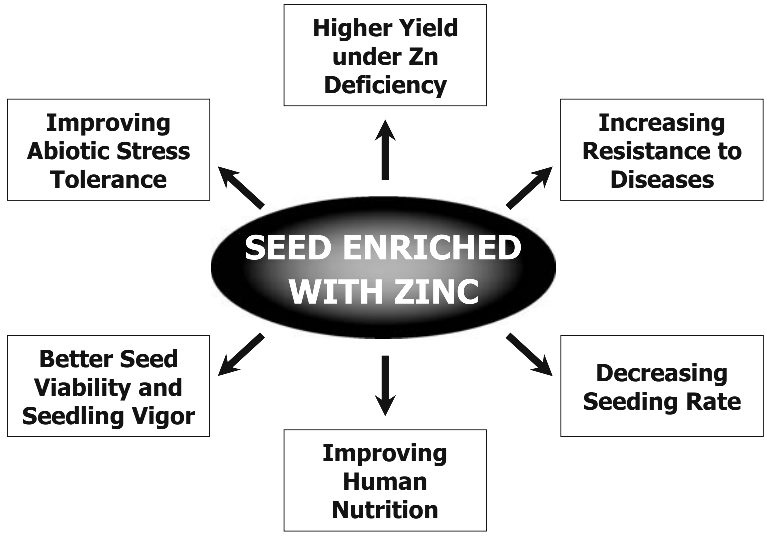

Fig. 9 Agronomic and human nutritional benefits resulting from use of Zn-enriched seeds

contribute to protection against soil-borne pathogens. An increasing Zn transport from leaves into seeds can be achieved by foliar applications of Zn, particularly under environmental stress conditions (e.g., drought) and on potentially Zn-deficient soils (Yilmaz et al. 1997; Welch 1999).

Plants emerging from seeds with low Zn have poor seedling vigor and field establishment on Zn-deficient soils (Fig. 9; Yilmaz et al. 1998). Under rainfed conditions, wheat plants derived from seeds containing 1.5 μg Zn per seed had better seedling establishment and twofold higher grain yields than the wheat plants that emerged from seeds containing only 0.4 μg Zn per seed (Yilmaz et al. 1998). Similar to results obtained in the field, Rengel and Graham (1995) showed in pot experiments that increasing seed-Zn contents from 0.25 μg per seed to 0.70 μg per seed significantly improved root and shoot growth of wheat plants under Zn deficiency, and concluded that high seed-Zn acts similarly to a starter-fertilizer effect.

Priming seeds in Zn-containing solutions is a practical way to increase seed Zn prior to sowing and to contribute to better seedling growth (Harris et al. 2007). For example, priming maize seeds in 1% w/v $ZnSO_4$ solution for 16 h significantly increased seed Zn concentration, and the seedlings derived from these seeds showed greater biomass and significantly greater grain yield (Harris et al. 2007). Based on the average grain yield values from seven field trials, grain yield of maize plants from non-primed seeds was 3 ton ha^{-1}, while in plants derived from seeds primed with 1% *w/v* Zn, grain yield was 3.8 ton ha^{-1}. The benefit-cost value calculated is reached 236 in the case of 1% *w/v* Zn priming compared to the benefit-cost value of 15 with soil Zn applications (Harris et al. 2007). Slaton et al. (2001) showed that treatments of rice seeds with Zn greatly increased grain yield and concluded that this type of Zn application method is a very economical alternative to more expensive broadcast Zn fertilizer applications. Seed priming with Zn was also very effective in improving seed germination and seedling development in barley (Ajouri et al. 2004).

These results may indicate that high seed-Zn has very important physiological roles during seed germination and early seedling growth (Fig. 9). Recently, we found that during seed germination Zn concentration of newly developed radicle and coleoptile is extremely high (up to 200 mg kg^{-1}), indicating critical physiological roles of Zn during early seedling development (Ozturk et al. 2006). In such highly metabolically active root and coleoptile tissues, Zn is most probably used for protein synthesis, membrane function, cell elongation and tolerance to environmental stresses (Cakmak 2000). High Zn concentrations in seeds might be important for protection of newly germinating seeds and developing seedlings from attack of different soil-borne pathogens. An important aspect in infection of plant tissues by pathogens is the availability of exudates in leaf or root apoplast released from cells. Exudates such as carbohydrates and amino acids are critical compounds in attracting pathogens and their rapid invasion of root or leaf tissue (Marschner 1995). The release rate of exudates is affected by structural integrity of cell membranes. During development of root tissue, any alterations in structural integrity of membranes resulting from Zn deficiency can increase the rate of exudate release from cells, with these exudates representing a suitable feeding medium for pathogens (Marschner 1995). Zinc plays a fundamental role in protecting and maintaining structural stability of cell membranes (Welch et al. 1982; Cakmak 2000). Under Zn deficiency, cell membranes become leaky and release high amounts of organic compounds which cause pathogen attraction (Cakmak and Marschner 1988). Zinc has been shown to suppress root-rotting pathogens, root nematode infestation and take-all infections (Brennan 1992; Rengel 1997; Streeter et al. 2001; Siddiqui et al. 2002) possibly by reducing exudation of organic compounds from roots in the rhizosphere.

Increased amount of Zn in seeds could be also important for seeding rates (Fig. 9). In some Zn deficient locations of Central Anatolia, seeding rates are much higher (e.g., 200–300 kg per hectare) than the usually recommended rates (e.g., 100 kg per hectare) in the comparable agro-ecological regions (Braun 1999). High seeding rates are required because of poor seedling establishment and a high level of "winter killing". It is estimated that with an increase in Zn concentration of seeds, seeding rate can be significantly reduced and at least 500 000 tons of seeds per year can be saved in Central Anatolia, leading to an annual economic benefit of more than 100 million USD (Braun 1999).

Environmental aspects

In application of Zn fertilizers or Zn-containing NPK fertilizers a special attention should be paid to environmental aspects regarding the possibility of Zn toxicity in soils. To our knowledge, Zn toxicity problem has not been reported in practical agriculture despite the common application of Zn-enriched fertilizers over many years (e.g., superphosphate supplemented with Cu, Zn and Mo in Australia). After application of Zn fertilizers to Zn-deficient soils, Zn is rapidly fixed by soil. Using Zn-deficient soils from Central Anatolia, it was found that Zn added to soils (up to 3.9 mg kg^{-1} soil equivalent to up to 1 ton Zn per ha) was almost completely adsorbed (Erenoglu 1995). It appears that application of high rates of Zn fertilizers over many years may be required before a Zn toxicity problem may occur. Nevertheless, in areas where Zn fertilizers will be applied regularly, concentrations of Zn in soils and plants should be periodically monitored to avoid possible development of Zn toxicity problem. Zinc fertilizers are effective up to 3–4 years in correcting Zn deficiency (Martens and Westermann 1991), and should not be re-applied every year in case of soil applications.

Conclusions

Agronomic biofortification is of great importance in enriching seeds with Zn. Due to some degree of uncertainty whether the breeding strategy will be efficacious in enriching grains with Zn, the short-term agricultural tools like applying Zn fertilizers should be considered. In the target countries with high incidence of Zn deficiency, fertilizer strategy should be applied nationwide as a quick solution to the Zn deficiency problem in human populations. For the long-term, agronomic biofortification is a complementary approach to breeding strategy and is likely to be required for ensuring success of breeding efforts. In future, new research programs should be initiated focusing on development of most efficient Zn application methods for promoting Zn uptake and maximizing Zn accumulation in grain. Studying the bioavailability of grain Zn derived from foliar applications would be an important research topic in future. In case of greater bioavailability of the grain Zn derived from foliar application than from soil, agronomic biofortification would be a very attractive and useful strategy in solving Zn deficiency-related health problems globally and effectively.

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