Special issue article

Agronomic biofortification of cereals with zinc: a review

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Summary

Zinc (Zn) still represents an important health problem in developing countries, caused mainly by inadequate dietary intake. A large consumption of cereal-based foods with small concentrations and low bioavailability of Zn is the major reason behind this problem. Modern cultivars of cereals have inherently very small concentrations of Zn and cannot meet the human need for Zn. Today, up to 50% of wheat-cultivated soil globally is considered poor in bioavailable Zn. Agricultural strategies that are used to improve the nutritional value of crop plants are known as biofortification strategies. They include genetic biofortification, which is based on classical plant breeding and genetic engineering for larger nutrient concentrations, and greater agronomic biofortification, which is based on optimized fertilizer applications. This review focuses on agronomic biofortification with Zn, which has proved to be very effective for wheat and also other cereal crops including rice. Molecular and genetic research into Zn uptake, transport and grain deposition in cereals are critically important for identifying 'bottlenecks' in the biofortification of food crops with Zn. Transgenic plants with large Zn concentrations in seeds are often tested under controlled laboratory or glasshouse conditions with sufficient available Zn in the growth medium for the entire growth period. However, they might not always show the same performance under 'real-world' conditions with limited chemical availability of Zn and various stress factors such as drought. What purpose can an upgraded transport and storage system serve if the amount of goods to be transported and stored is limited anyway? Given the fact that the Zn concentrations required to achieve a measurable impact on human health are well above those required to avoid any loss of yield from Zn deficiency, providing crop plants with sufficient Zn through the soil and foliar fertilizer strategy under field conditions is critically important for biofortification efforts.

Highlights

- Zinc malnutrition is a major global health issue associated with cereal-based diets.
- Agronomic biofortification with Zn aims to provide edible parts of crop plants with sufficient Zn.
- Biofortification with Zn fertilizers, particularly foliar applications, works well for wheat and other cereals.
- Agronomic biofortification with Zn provides a practical and cost-effective option to tackle the global Zn malnutrition problem.

Why wheat grain needs to be enriched with Zn for better human nutrition

There are several critical facts that highlight the importance of increasing the Zn concentration in wheat grain for human consumption. These are discussed below, starting with the human aspect and continuing with the relevant plant and soil factors.

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Roles of Zn in human physiology and health

Zinc has diverse physiological functions in biological systems. It interacts with a large number of enzymes and other proteins in the body and performs critical structural, functional and regulatory roles. It is estimated that about 10% of all the proteins in the human body, corresponding to nearly 3000 proteins, are Zn-dependent (Andreini *et al.*, 2006; Krezel & Maret, 2016). Therefore, clinical or subclinical Zn deficiency is associated with a wide array of physiological issues, including growth retardation, impaired brain development, increased susceptibility to infectious diseases such as pneumonia and diarrhoea, reduced physical performance and work productivity, and poor birth outcomes in pregnant women (Black
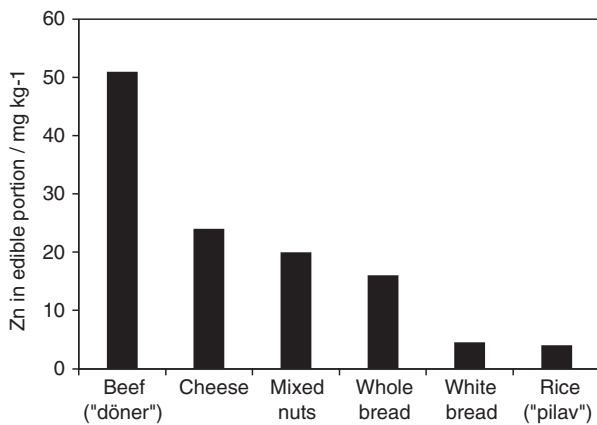
Figure 1 Concentration of Zn in edible portion of different foods collected in Istanbul (I. Cakmak, unpublished results).

et al., 2008; Gibson, 2012; Krebs *et al.*, 2014; Terrin *et al.*, 2015). About a third of the world's population is estimated to be at risk of Zn deficiency, which is especially prevalent in children under 5 years of age because of their relatively large demand for Zn to support growth and development (Wessells & Brown, 2012). Every year, about half a million children under 5 years of age die from causes related to Zn deficiency (Black *et al.*, 2008; Krebs *et al.*, 2014). Deficiencies of Zn and other micronutrients in developing countries are also reported to cause great economic losses and have a considerable effect on the gross national product by decreasing productivity and increasing the health care costs (Darton-Hill *et al.*, 2005; Stein, 2014).

Contribution of cereals to daily calorie and zinc intake

Zinc deficiency is often caused by low dietary intake that is associated with a large consumption of cereal-based foods. In comparison to animal-based foods, cereal-based foods have very small Zn concentrations (Figure 1). The majority of people in developing countries, however, especially in rural regions, rely on cereal-based foods as the major source of energy and minerals because of widespread poverty, high food prices and cultural preferences (Bouis, 2003; Bouis & Welch, 2010). Therefore, the contribution of animal-based foods to the daily Zn intake is much less in developing countries than in high-income countries (Wessells & Brown, 2012; Figure 2). At present, three cereals, wheat, rice and maize, provide up to 60% of the daily energy intake of human populations (Tilman *et al.*, 2002), and bread wheat alone is the staple food for 35% of the world's population (Poursarebani *et al.*, 2014). These rates are even higher for developing countries. For example, in several Asian countries with a high incidence of Zn and Fe deficiencies, rice and wheat alone provide over 70% of the daily calorie intake in rural areas (Cakmak, 2008a; Timmer, 2014). Wheat and rice are categorized as very poor sources of Zn and Fe, in terms of both content and bioavailability (see below).

In general, concentrations of Zn in grain in wheat-cultivated regions range between 20 and 35 mg kg^{-1}, with an average value
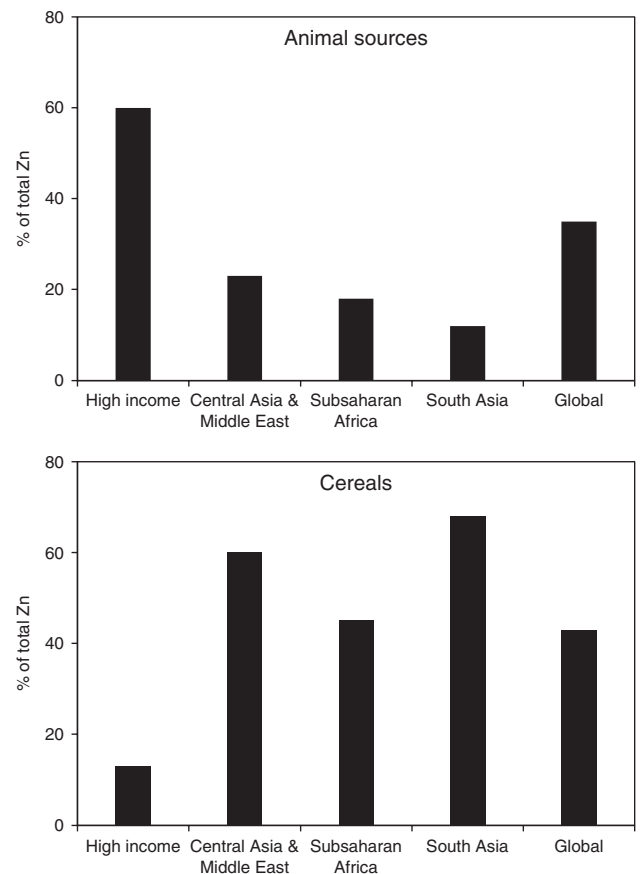

Figure 2 Regional data on the contribution of animal-based foods (above) and cereals (below) to daily Zn intake (redrawn from Wessells & Brown, 2012).

of around 28–30 mg kg^{-1} (Rengel *et al.*, 1999; Graham *et al.*, 2007; Fardet *et al.*, 2008; Cakmak *et al.*, 2010a). The Zn in grain can be far below the 20 mg kg^{-1} concentration, however, when wheat is grown on Zn-deficient or Zn-poor soil. For example, in Zn-deficient soil of Australia and Turkey, Zn concentrations of grain are < 10 mg kg^{-1}, whereas in Zn-sufficient or Zn-fertilized soil, grain concentrations of Zn are over 20 mg kg^{-1} (Graham *et al.*, 1992; Cakmak *et al.*, 1999a, 2010a). These Zn concentrations are too small to meet the daily required intake of Zn. There is clearly a large gap between the actual average Zn concentration of grain and the target range for human health, which is 40–50 mg kg^{-1} (Graham *et al.*, 2007; Zhao *et al.*, 2009; Cakmak *et al.*, 2010a).

Dilution of Zn in wheat grain because of increases in yield

There have been marked increases in the grain yield of major cereal crops over the past 100 years, especially during the Green Revolution, which began most markedly in the 1960s (Grassini *et al.*, 2013). In the past, plant breeders were almost exclusively interested in the development of new cereal varieties with larger grain yield. With breeding efforts and improved soil and crop management practices, the average yields of major crops has

increased more than twofold during the past 50–60 years (Tilman *et al.*, 2002; Davis, 2009; Curtis & Halford, 2014). These large increases in yield, however, have caused considerable decreases in the concentrations of essential nutrients such as Zn because of the so-called 'dilution effect' (Garvin *et al.*, 2006; Fan *et al.*, 2008; Davis, 2009; Shewry *et al.,* 2016). Cakmak *et al.* (1999b, 2010b) reported that primitive and wild relatives of cultivated wheat contain two- or threefold more Zn than modern wheat cultivars, which can produce large grain yields. Smaller grain Zn concentrations in high-yielding wheat cultivars than in older varieties were also reported by Fan *et al.* (2008) and Zhao *et al.* (2009).

Adverse soil conditions that affect grain Zn

Up to 50% of wheat-cultivated soil in the world is considered poor in plant-available Zn. Under such conditions, wheat varieties cannot realize their full potential in Zn absorption and accumulation, and therefore, grain Zn concentrations are reduced further (Cakmak, 2008a). The availability of Zn to plant roots is very low in alkaline soils, which cover at least 30% of the arable land globally (Chen & Barak, 1982; Cakmak, 2002; Alloway, 2009). The major soil types that are frequently associated with Zn deficiency are calcareous soil (Calcisols), sandy soil (Arenosols), weathered tropical soil (Ferralsols), saline soil, waterlogged soil (Gleysols) and heavy cracking clay soil (Vertisols) (Alloway, 2008). The potentially bioavailable Zn in the soil comprises the soluble, exchangeable and organically-bound pools, and plant roots take up Zn mainly from the soil solution. In the soil solution, Zn concentration decreases by 30-fold for each unit increase in soil pH between 5 and 7 (Marschner, 1993). When soil pH is above 8, Zn is bound to soil particles (such as Fe oxides and calcites) more strongly, causing very poor availability of Zn to plant roots. Like high soil pH, small soil moisture and organic matter contents limit the amount of soluble Zn considerably in the root environment. Small moisture and organic matter contents are common features of soil under wheat cultivation (Graham *et al.*, 1992; Marschner, 1993; Alloway, 2009). Zinc in the soil reaches plants roots through diffusion, and this process is severely impaired when soil moisture and organic matter decline (Marschner, 1993; Rengel, 2015). Consequently, the capacity of roots to take up Zn is hampered, leading to low Zn accumulation in plants. This causes losses of yield, depending on the severity of Zn deficiency. Under such soil chemical conditions, Zn fertilizer application to soil is of great importance to ensure a sufficient uptake of Zn by roots (Cakmak *et al.*, 1996; Rengel, 2015). Wheat is most commonly cultivated in semi-arid regions where the topsoil often remains dry during the grain-filling stage. It is not surprising that Zn deficiency in wheat often occurs when soil moisture is limited because of the low and irregular precipitation reported for Australia (Graham *et al.*, 1992), Turkey (Ekiz *et al.*, 1998; Bagci *et al.*, 2007) and several Asian countries (Rafique *et al.*, 2006; Karim & Rahman, 2015). Therefore, maintaining a sufficiently large concentration of plant-available Zn in the soil of semi-arid regions is particularly important to achieve desirable Zn concentrations in grain for human nutrition. The geographical distribution of Zn deficiency in human
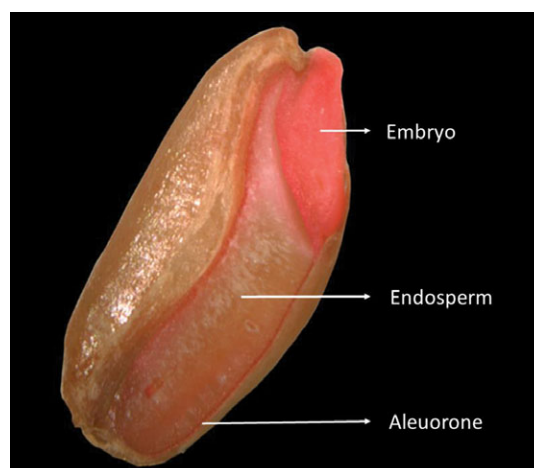

Figure 3 Localization of Zn in a wheat grain, visualized with dithizone as a Zn-sensing dye that develops a red complex with Zn. The intensity of the red colour is associated with the Zn concentration (see Ozturk *et al.*, 2006).

populations overlaps with the distribution of Zn-deficient soil and low socioeconomic status of the population.

Localization and bioavailability of Zn in wheat grain and the effects of milling

Zinc is mainly localized and concentrated in the aleurone and embryo parts of wheat grain. Figure 3 shows that the Zn concentration of the endosperm (white flour) is very small. According to Ozturk *et al.* (2006), whereas the endosperm contains around 10 mg Zn per kg, the embryo and aleurone layer may contain over 100 mg Zn kg^{-1}. Wheat grain is often consumed after milling, which removes the Zn-rich parts and leaves just the Zn-poor endosperm behind. Depending on the extraction rate, typical white wheat flour contains about 5–10 mg Zn per kg only (Peterson *et al.*, 1983; Cakmak *et al.*, 2010b), and these concentrations cannot meet the dietary requirement for Zn. The data on dietary Zn requirement and the methods used to estimate them have continued to evolve since the 1970s together with our understanding of Zn absorption and bioavailability (Gibson *et al.*, 2010). The recommended dietary allowance (RDA) for Zn suggested by the International Zinc Nutrition Consultative Group (IZiNCG) varies between 9 and 19 mg per day for adults who consume an unrefined, cereal-based diet, depending on gender and special conditions such as pregnancy and lactation (Brown *et al.*, 2004). Assuming that about 400 g of wheat flour is eaten daily by the target individuals who live in the major wheat-consuming countries and the average concentration of Zn in flour is about 8 mg Zn per kg, the average daily intake of Zn will be around 3.2 mg only. Without any intervention such as food fortification, supplementation or dietary diversification, these people must suffer from severe Zn deficiency and its consequences. For target countries where the Zn concentrations in flour are very small, fortification of flour with Zn at concentrations of 15–30 mg Zn per kg has been suggested as an intervention strategy (Brown *et al.*, 2010).

In addition, wheat grain is rich in compounds that reduce the bioavailability and limit the intestinal absorption of Zn such as phytate and phenolic compounds. Published evidence is available showing that a reduction in dietary phytate concentrations by dephytinization is associated with greater bioavailability and intake of Zn (Egli *et al.*, 2004; Gibson *et al.*, 2010). Phytate is, however, localized mainly in the aleurone and embryo parts of wheat and rice grains, and occurs in very small concentrations only (if detectable at all) in the endosperm (Pomeranz, 1988; Lehrfeld & Wu, 1991; Prom-u-Thai *et al.*, 2008), which suggests that the small concentrations of Zn in the endosperm are potentially bioavailable. More research is required to investigate Zn bioavailability in the white flour fraction of wheat.

Agronomic biofortification with zinc through fertilizer application

Conventional and molecular plant breeding, genetic modification (transgenic technologies) and agronomic interventions including appropriate fertilizer applications are the major tools that are used and investigated for the biofortification of food crops with Zn. As described above, most of the cultivated soil, especially that used for wheat and other cereals in the target countries, has a diversity of chemical and physical problems that reduce the plant-availability of Zn. Under such conditions of inadequate Zn availability, newly developed genotypes biofortified with Zn by breeding or genetic engineering might be unable to realize their full potential and to accumulate the amount of Zn in their grains that would achieve a marked biological effect in target populations (Cakmak, 2008a). Although molecular and genetic studies for investigating Zn uptake, transport and grain deposition phenomena in cereals are critically important for understanding the physiology of these processes and identifying the bottlenecks, transgenic strategies for the biofortification of cereals with Zn are still in their infancy. Although there are quite a few encouraging examples of transgenic cereals developed for enhanced root uptake, transport and grain accretion capacity for Fe or Zn or both in the recent literature (Suzuki *et al.*, 2008a,b; Borg *et al.*, 2012; Gomez-Galera *et al.*, 2012; Masuda *et al.*, 2013; Borrill *et al.*, 2014; Trijatmiko *et al.*, 2016), genetically engineered plants are often tested under controlled laboratory or glasshouse conditions only with sufficient micronutrient availability. They might not always be able to show the same performance under 'real world' conditions with limited micronutrient availability and various other stress factors such as water deficit, heat stress and disease pressure. What purpose can an upgraded transport and storage system serve if the amount of goods to be transported and stored is limited anyway?

Figure 4 shows that there are two major sources of Zn in the grain: (i) Zn that is absorbed continuously by roots from soil and translocated into grain and (ii) Zn that is deposited in vegetative tissues (leaves and stems) and then remobilized to be translocated into grain during the reproductive stage (Waters *et al.*, 2009; Kutman *et al.*, 2012; Sperotto, 2013). The relative contributions of these two sources to the accumulation of Zn in grain vary depending
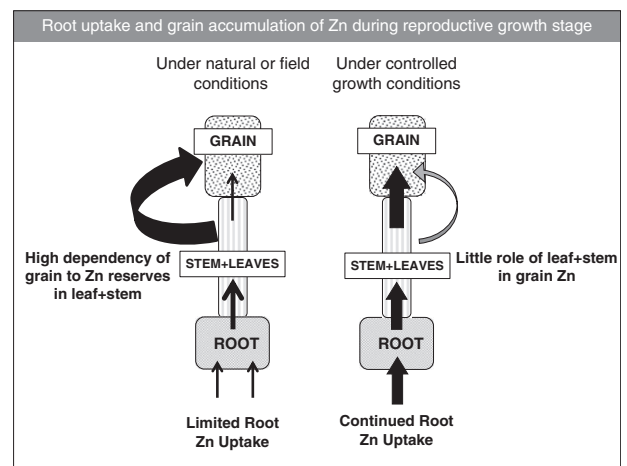

Figure 4 Illustration of the roles of root Zn uptake and Zn retranslocation from vegetative organs (leaves and stems) into grain of wheat plants grown under field ('real-world') conditions with limited water or Zn availability or both during seed-filling (left) or under controlled growth conditions with adequate water and Zn supply (right).

on several plant and soil factors, including micronutrient and water availability during grain-filling, timing of senescence, length of the grain-filling period and the nitrogen (N) nutritional status of the plant. For the biofortification of cereals with Zn, it is critical to maintain an adequate level of plant-available Zn in soil or a large and readily available pool of Zn in vegetative organs during seed-filling or both.

Since the discovery of Zn as an essential micronutrient for plants (Sommer & Lipman, 1926), fertilization of crop plants with Zn fertilizers either through soil or foliar application has become an increasingly common practice in agricultural soil where Zn deficiency limits crop productivity. The main aim of Zn fertilization was typically to prevent or correct Zn deficiency and thus to improve the yield; however, very little or no attention was paid, from a human nutritional perspective, to the Zn concentrations of the edible parts of food crops such as seeds and grains or starchy roots. Finally, since the start of the International HarvestPlus (www.harvestplus .org) programme and its sub-project HarvestZinc (www.harvestzinc .org), there has been an increase in global interest to enhance Zn concentrations in the edible parts of food crops.

In the framework of the HarvestZinc project (www.harvestzinc .org), several field experiments have been carried out during the past 7–8 years in 12 countries on wheat, rice and maize with applications of several soil- and foliar-applied fertilizers. Clearly, soil Zn applications at the time of sowing had little effect on the concentration of Zn in the grain under field conditions, whereas foliar Zn sprays were very effective in improving the grain Zn. Figure 5 shows that among the cereals studied, wheat was by far the most responsive to foliar Zn spray in terms of increases in grain Zn (up to 83%). Rice showed an intermediate response to foliar Zn applications and exhibited moderate increases in grain Zn (up to 27%), whereas maize appeared to be less responsive (Figure 5). Research is now going on to elucidate the physiological reasons
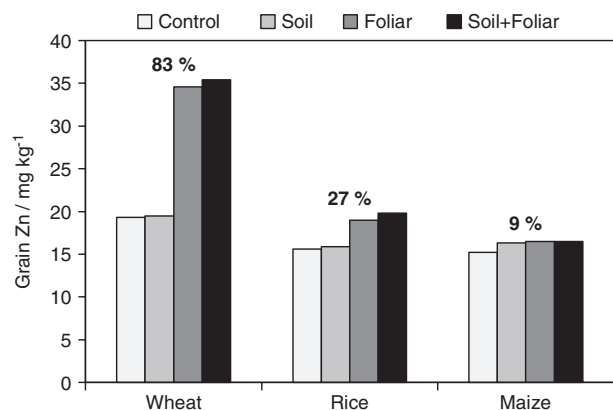
Figure 5 Average changes in grain Zn concentration caused by soil, foliar and soil + foliar applications of Zn in the form of $ZnSO_4$ to wheat, rice and maize grown in Turkey at four locations. (For more detail about the applications, please refer to Zhou *et al.*, 2012 and Phattarakul *et al.*, 2012.) The percentages above the columns show the increases in grain Zn following the soil + foliar Zn relative to the control (no Zn application).

behind the different responses of these three major cereal crops to foliar Zn applications.

It is well documented that foliar-applied Zn is phloem-mobile and can be readily translocated into developing grains in wheat (Haslett *et al.*, 2001; Erenoglu *et al.*, 2011). For foliar Zn applications to wheat, the options are zinc sulphate ($ZnSO_4$) and EDTA-chelated Zn. Zinc sulphate is at least as effective as Zn-EDTA for correcting Zn deficiency and increasing Zn concentrations in tissues, which means that it is the most cost-effective option compared with the relatively highly priced Zn-EDTA. The timing of foliar Zn fertilizer application is an important determinant of its effectiveness in terms of biofortification (Welch *et al.*, 2013). In both wheat and rice, foliar Zn applications are particularly effective in enriching the grain with Zn if they are applied at a later rather than an earlier developmental stage, preferably during grain-filling (Cakmak *et al.*, 2010a; Boonchuay *et al.*, 2013; Abdoli *et al.*, 2014). The fertilizer strategy for the agronomic biofortification of wheat with Zn enhances the Zn concentrations not only at the whole-grain level but also specifically at the endosperm level, which is critical for target populations that consume large quantities of white flour (Cakmak *et al.*, 2010a; Kutman *et al.*, 2011a).

Although Zn fertilization is central to agronomic biofortification of wheat with Zn, the implementation of an integrated mineral nutrient management strategy offers even more benefits in terms of increasing the Zn concentration of grain. Specifically, interactions between nitrogen (N) and Zn applications have been studied extensively in this context. It has been shown that the effects of soil and foliar Zn fertilizers on grain Zn were enhanced by the addition of adequate N fertilizer (Kutman *et al.*, 2010). Whole-plant partitioning and Zn radioisotope (65Zn) studies on wheat revealed that suboptimal N supply limited the root uptake and root-to-shoot translocation as well as the remobilization of Zn, which reduced the accumulation of Zn in the grain (Erenoglu *et al.*, 2011; Kutman *et al.*, 2011b). Clearly, optimized N management is also required to

realize the yield potential, which is critical from an economic point of view and to maximize the grain protein concentration (Kutman *et al.*, 2010; Abedi *et al.*, 2011), which is valuable in itself from a nutritional point of view, given the large prevalence of protein malnutrition in the world (de Onis *et al.*, 1993). Speciation and localization studies on cereal grains indicate that Zn interacts with proteins in the grain, and therefore, grain proteins constitute a physiological sink for Zn (Ozturk *et al.*, 2006; Persson *et al.*, 2009, 2016; Cakmak *et al.*, 2010b).

Possible economic and environmental concerns of the fertilizer strategy

A possible drawback of the fertilizer strategy for biofortification is the extra cost of Zn fertilizer application, which might not have a clear economic return unless crop productivity is limited by Zn deficiency or there is a premium price for biofortified grain. Depending on the severity of the Zn deficiency problem in soil, Zn fertilization can contribute considerably to better crop production in addition to the increases in grain Zn concentration. Published reports show that the costs of Zn fertilizer application are small compared with the economic returns through increases in yield and the public health benefits (Harris *et al.*, 2007; Shivay *et al.*, 2008; Manzeke *et al.*, 2014; Joy *et al.*, 2016). Based on the meta-analysis of published data, Joy *et al.* (2015) reported that foliar Zn application is a cost-effective strategy to improve grain Zn in cereal crops, and the costs associated with foliar Zn treatments seem to be similar to the cost of flour fortification with Zn.

It is estimated that 90% or more of the total cost of foliar Zn fertilizer application is the cost of application itself, which can be avoided by applying $ZnSO_4$ together with pesticides that are applied anyway (Ortiz-Monasterio *et al.*, 2015; Wang *et al.*, 2015, 2016; Ram *et al.*, 2016). There are no apparent compatibility issues, and the pesticides tested do not reduce the effectiveness of foliar Zn fertilizer. Farmers can be motivated to adopt the fertilizer strategy for agronomic biofortification even in the absence of an increase in yield because of the other agronomic benefits provided by Zn fertilization. These include improved germination and seedling vigour, and enhanced tolerance to abiotic stress and disease resistance (Huber & Graham, 1999; Welch, 1999; Cakmak, 2008a).

Seeds with a larger Zn content perform distinctly better than seeds with a smaller Zn content, especially in Zn-deficient soil (Rengel & Graham, 1995; Yilmaz *et al.*, 1998). Improved seedling establishment might decrease the required seeding rates and thus provide considerable economic benefits to farmers (Braun, 1999). In addition, Zn suppresses important fungal diseases of wheat, including Fusarium crown rot, *Rhizoctonia cerealis* 'winter-kill' and 'take-all' caused by *Gaeumannomyces graminis* (Brennan, 1992; Grewal *et al.*, 1996; Braun, 1999).

Another potential health benefit of the fertilizer approach for bio-fortification of wheat with Zn stems from the fact that Zn competes strongly with cadmium (Cd), which is a very toxic, non-essential heavy metal that threatens human health at every level from root

uptake from soil to absorption in the digestive tract (Welch *et al.*, 1999; Reeves & Chaney, 2008; Cakmak, 2009). Cereals, especially durum wheat and rice, are reported to contribute the majority of dietary Cd, and when wheat is grown in agricultural soil with slightly elevated Cd concentrations, the grain Cd concentration can exceed the maximum level set for wheat (0.2 mg kg^{-1}) by Codex Alimentarius (FAO/WHO, 2011; Harris & Taylor, 2013). Because of the chemical similarity of Cd to Zn, plant Zn transporters such as those in the zinc-regulated transporter (ZRT), iron-regulated transporter (IRT)-like protein (ZIP) and heavy metal ATPase (HMA) families also work as Cd transporters (Guerinot, 2000; Takahashi *et al.*, 2012; Uraguchi & Fujiwara, 2012; Cun *et al.*, 2014). Genetic interventions that alter the expression levels of the genes that encode for these transporters often affect tissue Cd concentrations in addition to Zn concentrations. Recently, a study on a diverse collection of barley genotypes demonstrated that large Zn accumulation in grain was associated with large Cd accumulation (Detterbeck *et al.*, 2016). Therefore, genetic biofortification of wheat with Zn through breeding or transgenic strategies might be risky in terms of grain Cd concentrations, especially if it is not complemented with agronomic biofortification through optimized Zn fertilizer application. It has been well documented that Zn applications to wheat have a marked inhibitory effect on the root uptake, root-to-shoot translocation, remobilization and seed deposition of Cd (Welch *et al.*, 1999; Cakmak *et al.*, 2000; Jiao *et al.*, 2004). In addition to increasing the Zn concentration of wheat grain, proper Zn fertilization can also contribute considerably to minimizing its Cd concentration and the associated health risks.

Zinc is not only an essential mineral nutrient but it is also a heavy metal. Therefore, one of the possible concerns about Zn fertilizer application is its toxicity. Although crop plants and soil organisms might be affected by Zn toxicity, it is quite rare in practice and unlikely to be an important problem in most agricultural soil (Broadley *et al.*, 2007; Alloway, 2009). Zinc toxicity in crop plants is usually limited to soil contaminated by mining and smelting activities, polluted with industrial wastewater or treated excessively with high-Zn sewage sludge. Although the threshold for Zn toxicity in tissues varies widely among and within plant species, a typical value for potentially toxic Zn concentration in leaves is 300 µg per g dry weight (Marschner, 2012). Even for soil microorganisms, which are more sensitive to heavy metal toxicity than crop plants because of less advanced homeostatic mechanisms, Zn is reported to be far less toxic than copper (Cu) or Cd (Saviozzi *et al.*, 1997; Alloway, 2008).

To correct Zn deficiency and prevent yield losses in crop plants, Zn is applied to deficient soil, typically in the form of ZnSO$_4$, at rates that range typically from 5 to 25 kg Zn ha^{-1} (Yilmaz *et al.*, 1997; Cakmak, 2008b; Abid *et al.*, 2013; Zhao *et al.*, 2014). The rates of soil Zn application vary depending on the crop species, soil characteristics and method of application; higher rates are associated with crops sensitive to Zn deficiency, alkaline or calcareous soil and broadcasting rather than banding (Alloway, 2008). Because a small percentage only of Zn applied to soil is taken up in a single season by an annual crop, Zn fertilization has residual effects for up to 10 years and is not needed every year (Brennan, 2001; Alloway, 2008; Cakmak, 2008b; Singh, 2008). A typical foliar Zn fertilizer solution, on the other hand, contains 2–5 g zinc sulphate heptahydrate (ZnSO$_4$·7H$_2$O) per litre. Therefore, the amount of Zn applied as foliar spray is usually about 1 kg ha^{-1} only or less (~23% of 500–1000 l ha^{-1} 2–5 g l^{-1} ZnSO$_4$·7H$_2$O), which is at least five times less than that applied at the smallest rate of application to the soil (5 kg Zn ha^{-1}) and is considered completely safe for the ecosystem (Cakmak *et al.*, 2010a; Boonchuay *et al.*, 2013; Ram *et al.*, 2016). Accidental over-applications of Zn and contaminants such as Cd in low-quality Zn fertilizers only might pose a toxicity risk to soil, crop plants and other organisms.

Conclusions

It is clear that the Zn fertilizer strategy is an effective way to biofortify food crops with Zn, and it is also advantageous because it might also contribute to (i) better yields depending on the extent of soil Zn deficiency, (ii) improved seed and seedling vigour and (iii) reduced root uptake and shoot (or grain) accumulation of Cd. The Zn fertilizer strategy has important synergistic effects on classical and molecular plant breeding approaches. The genetic capacity of the newly developed (biofortified) genotypes or lines through conventional breeding or genetic engineering to absorb Zn from soil or to translocate Zn from vegetative tissues into grain at desirable amounts for human nutrition or both might not be expressed to the full extent if the soil has inadequate concentrations of bioavailable Zn. Applications of Zn to soil to ensure sufficient availability of Zn for root uptake and foliar applications of Zn to enrich vegetative tissues with Zn and thus enhance Zn remobilization into grains are key agronomic interventions for achieving successful biofortification of food crops with Zn.

Acknowledgement

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