

REVIEW ARTICLE

Zinc biofortification in *Triticum aestivum* L. – from grains to bakery products

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Abstract

Micronutrient deficiencies, namely those arising from zinc, pose serious human health problems. As a staple food crop, wheat is a major source of dietary energy and protein for the world's growing population, and a main target to reduce malnutrition via integration of agronomic fertilization practices. In this context, agronomic biofortification through fertilizer approaches, namely foliar application of Zn can increase grain Zn above the breeding target set by nutritionists. Nevertheless, zinc uptake and translocation, as well as milling yields and bioassimilation in the human intestinal mucosa must be also considered. This review synthesizes the progress made in the framework of the agronomic biofortification strategies for Zn enrichment of wheat, further considering the flour production and bioassimilation limitations.

Key words: Biofortification, Wheat flour, Zn absorption, Zinc application on plants, Zn bioavailability

Introduction

Zinc is the most abundant intracellular element responsible for the genetic stability and cellular proliferation. It is essential to some antioxidant defence enzymes, mainly the CuZn containing superoxide dismutase (CuZnSOD), which limits against oxidative stress allowing DNA integrity (Ho, 2004; Bruno et al., 2007; Song et al., 2010). Zinc deficiency can lead to DNA damage, followed by degenerative diseases and some kinds of cancers, such as prostate cancer (Ho, 2004; Franklin and Costello, 2007; Song et al., 2010). Zinc also exerts its action on cellular division mechanisms and, thus, it has an important role in

body growth. Zinc also participates on the neurocerebral mechanisms, as its deficiency on the hippocampus can affect the learning capabilities, memory and recognition (Ho, 2004; Takeda and Tamano, 2009). In extreme situations of no zinc intake some dermatitis, hypotension, hair losing, sensorial dysfunctions can develop, whereas the immune system also becomes weak, especially during the pregnancy (Takeda and Tamano, 2009; da Costa et al., 2013).

Staple foods have considerably high levels of zinc. However, they have also high levels of phytate which hampers the zinc uptake because it creates soluble complex with zinc and these make zinc unavailable to the organism (Song et al., 2010; Hussain et al., 2013). Malnutrition of zinc is higher in developing countries because the main food sources of zinc, such as, red meat or seafood, are scarce than staple foods which have poor zinc bioavailability (Ho, 2004; Bruno et al., 2007).

In this review a global glance about zinc uptake and translocation by plants, as well as the wheat milling yields and characteristics and bioassimilation in mammals is discussed.

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Zinc enrichment in crops

Augmentation of zinc levels can be attained by supplementation or agronomic fortification (Bouis and Welch, 2010). Nevertheless, supplementation is an expensive process and is hard to apply in a scale up system, especially in rural areas (Cakmak et al., 2004). The enrichment of cultures through genetic variation aims the traditional vegetal improvement, which is called biofortification (Cakmak et al., 2004; Welch and Graham, 2004; Cakmak, 2008; Bouis and Welch, 2010; Velu et al., 2014). Biofortification success depends on, as much the capacity of increase the micronutrients in plants edible parts, as on the bioavailability of micronutrients in human body (White and Broadley, 2009; Bouis and Welch, 2010). Yet, natural fortification can further be achieved through genotype's selection, fertilizer application and genetic manipulation, without losing sight crop yield (Hussain et al., 2010).

Primordial wheat cultures accumulate higher zinc concentrations rather domestic ones (Cakmak, 2008; Velu et al., 2014). *Triticum turgidum* ssp. *dicoccoides* showed a good genetic variation to zinc concentration between 14 to 190 mg Zn/kg (Cakmak et al., 2004).

Biofortification based on agriculture is a quick solution to minimize zinc deficiencies in soils through fertilizers application (Cakmak, 2008; Bouis and Welch, 2010; Hussain et al., 2010). Fertilizer application allows overcoming a zinc deficiency in soils with a consequent increase of production and yield (ranging between 11-109% and 9-256%, respectively) and increasing zinc concentration in grains (9-912%) (Hussain et al., 2010). However, the endurance to zinc levels in soils is different among genotypes, despite of the optimum amount of zinc, as accumulation in grains is higher than the plants growth requires (Hussain et al., 2010).

Genetic manipulation focus on gene suppression of anti-nutrients, such as phytic acid, or/and gene overexpression which are responsible for zinc accumulation. However, some anti-nutrients also have an important role on human diet. Some of them work as anticarcinogenic or health promoters, reducing the risk of heart disease or diabetes (Bouis and Welch, 2010; Hussain et al., 2010). On the other hand, gene overexpression responsible by ferritin shows an effective augmentation of zinc concentrations in the grains (Cakmak, 2008). Uauy et al. (2006) found that GPC-B1 gene from *Triticum dicoccoides* codifies a NAC (NAM-B1) transcription, which increases zinc levels in the grain. This probably happens

because the zinc levels upturn in grains also stimulates leaf senescence and consequently remobilization to the seeds from the flag leaf (cf. Cakmak, 2008; Velu et al., 2011).

Zinc uptake and translocation in plants

Biofortification through agricultural techniques has been related as a good strategy to improve mineral levels on staple food crops. Zinc levels can be augmented threefold or fourfold concentration depending on the kind of fertilizers applied (Cakmak, 2008). Zinc can be directly applied in soils using organic and inorganic compounds (Cakmak, 2008). Inorganic fertilizers are available as oxides, nitrates and sulphates, but zinc sulphate is the most used because is inexpensive and has a high solubility (Cakmak, 2008; White and Broadley, 2009; Hussain et al., 2010). Indeed, to be efficient a fertilizer should have a solubility of about 40% (Hussain et al., 2010).

Zinc application by ZnSO_4 presents better results on its accumulation in grains than ZnO or ZnEDTA (Cakmak, 2008; White and Broadley, 2009). ZnSO_4 use can increase the zinc level from 11.8 up to 17.4 mg/Kg on flour, thus 0.47 fold increase. Mineral level in the grains increased to 28-68% by foliar application. This method has shown a rise zinc concentration from 29 mg/Kg on the control to 45.7 mg/Kg through ferric citrate mixed with ZnSO_4 application and to 39.6 mg/Kg by application of all nutrients, which represents 58% and 37% increase, respectively (Zhang et al., 2010).

Soil and foliar-pooled application has been considered the most efficient method with 3.5 fold concentration (Cakmak, 2008). To obtain greater effects on yield soil fertilizers application are recommended or grow a new crop from biofortified seeds with high zinc levels and foliar application (Cakmak, 2008; Cakmak et al., 2010; Zou et al., 2012). The last method has shown the most able to increase zinc level in grains (Table 1), however its success is not the same during the whole plant growth period. The last stage of growth reveals the best results (Cakmak, 2008; Cakmak et al., 2010). On the other hand, foliar zinc application on the last growth stage, combined with high soil nitrogen, or soil zinc application, increases zinc content in the grain (from 23 to 55 mg kg^{-1} with nitrogen application and from 12 to 29 mg kg^{-1} with soil zinc application) (Cakmak et al., 2010). Additionally, increases in the zinc concentration in the whole grain through soil and/or foliar zinc applications were reflected proportionally among the grains fractions (Cakmak et al., 2010).

The wheat grain normally contains 25-30 µg/g dry weight and the ideal quantity to have health impact should be higher than 50 µg/g dry weight (Cakmak, 2008; Hussain et al., 2010). However, is it possible to obtain more than 100 µg zinc/g dry weight grain, without yield loss (Bouis and Welch, 2010; Hussain et al., 2010).

Table 1. Effect of different zinc applications on zinc concentration in grain and shoots and yield plant (adapted from Cakmak, 2008).

Zinc application	Zinc concentration (mg/Kg)		Yield augmentation (%)	
	Shoots	Grain	Shoots	Grain
Control	10	10	-	-
Seed	12	10	79	204
Soil	19	18	109	265
Foliar	60	27	40	124
Seed + Foliar	73	29	83	268
Soil + foliar	69	35	92	250

Environmental factors, such as soil properties, water availability and fertilizers application have a huge impact on genotypes factors and thereby on the mineral concentrations (Cakmak et al., 2004; White and Broadley, 2009; Bouis and Welch, 2010). Soil pH presents a great impact on micronutrients solubility. When the soil is alkaline solubility is low, the opposite occurring with acid soils (Cakmak, 2008; Velu et al., 2014). Additionally, alkaline soils with high calcium carbonate levels, salinity, high phosphate levels and waterlogging, show high zinc deficiencies (Cakmak, 2008; Hussain et al., 2010). On the other hand, at the last growing stage, uptake by the roots is limited and the minerals are supplied to plant by foliar application (White and Broadley, 2009). Furthermore, fertilizers application shows better results on increasing zinc levels without adverse effects on culture yield (Zhang et al., 2010; Zou et al., 2012), probably due to a quick zinc remobilization from vegetative organs to grain (Zhang et al., 2010).

Mineral concentrations on plant organs begin with mineral uptake by roots, followed by translocation to the shoots and deposition in grains (Bouis and Welch, 2010). This whole process is not only commanded by genetic variation but it is also affected by genotype vs environment interactions (Cakmak et al., 2004; White and Broadley, 2009; Bouis and Welch, 2010).

Micronutrients uptake can improve by changing roots morphology or changing the

solubility and movement of metallic elements (Welch and Graham, 2004). Additionally, mineral accumulation in the rhizosphere occurs mainly by diffusion, with soil moisture having an important role (Cakmak, 2008; Lidon et al., 2013; Velu et al., 2014).

Micronutrients uptake mechanisms include ions transporters located on plasma membrane of roots cells that allow metallic elements enter on apoplasm (Welch and Graham, 2004; White and Broadley, 2009; Lidon et al., 2013). Micronutrients show more phytoavailability to be absorbed by roots under cations form (White and Broadley, 2009), being about 80% of zinc levels in the xylem sap of hyperaccumulator plants in the form of Zn²⁺ (Lidon et al., 2013).

Zinc uptake and accumulation kinetics begin with bioavailable Zn²⁺ transportation across the plasma membrane (Lidon et al., 2013). The cation uptake results on inside-negative membrane potential, where Zn²⁺ membrane transport seems to be dominated by ion channels (where proteins are formed and the voltage gradient is established and controlled across the plasma membrane of cells) (Lidon et al., 2013).

Zinc transportation to the shoots requires diffusion root cells and active loading across the plasma membrane of the xylem parenchyma into the apoplastic. Yet, zinc translocation rate from roots to shoots depends on the accessibility and mobilization of the metal sequestration in the root vacuoles, whose efficiency requires nicotianamine and zinc ligand availability (Gramlich et al., 2013; Lidon et al., 2013). Citrate and histidine further enhance Zn uptake into the roots and shoots (Gramlich et al., 2013). In the phloem Zn²⁺ becomes actively loaded and highly fit to form complexes with transportation proteins (Lidon et al., 2013).

Within the xylem zinc makes preferentially complexes with carboxylic acids, such as citrate and malate, and on the phloem sap zinc makes often complexes with nicotianamine or small proteins (Gramlich et al., 2013; Lidon et al., 2013). In the cereal grain, zinc is mainly stored in the aleurone and the embryo, persisting lowest concentrations is in the endosperm, with zinc forming complexes with phytate (Lidon et al., 2013).

From bread wheat grains to zinc biofortified flour

Wheat is a good nutrient source (Zhang et al., 2010; Zou et al., 2012), as despite the low protein level in wheat (8-16%), its high intake makes it a

good source for that nutrient (Shewry, 2009; Scheuer et al., 2011). Starch, quantitatively the most compound of wheat (about 60-70%) (Shewry, 2009; Scheuer et al., 2011), also represents 65-75% of flour wheat constitution (Shewry, 2009). Additionally, wheat also has about 12-14% of relative moisture content, non-starch polysaccharides (2-3%), lipids (2%) and ash (1%) (Shewry, 2009). Moreover, the mineral composition of staple food in general and wheat in particular, is dependent of soil mineral composition (White and Broadley, 2009; Galinha et al., 2013; Lidon et al., 2013). Phosphorus is the most abundant mineral compound in wheat grain (16-22% of total ash) (Scheuer et al., 2011). Wheat has own low zinc levels, which do not supply the amount required by human body (Hussain et al., 2013), mainly as consequence of deficiency in a large amount of soils (Zhang et al., 2010; Lidon et al., 2013). In this context, wheat therefore might trigger zinc deficiency (Hussain et al., 2013; Velu et al., 2014).

Triticum aestivum L. has lower protein content than durum wheat (*Triticum turgidum* durum) and it has few protein-starch links, which provides it less hardness and makes the grinding easier (Greffeuille et al., 2007; Peck et al., 2008; El-Porai et al., 2013). Wheat protein are divided in soluble (albumins and globulins) and storage protein or gluten (gliadin and glutenin) (Shewry, 2009; Scheuer et al., 2011). Moreover, wheat functionality depends on its viscoelastic properties, which are responsibility of gluten proteins. Gliadin has a plasticizing function which provides viscosity to dough and glutenin provides resistance giving more elasticity to dough (Scheuer et al., 2011). In this framework, dough production includes several steps according grain quality, such as debranning, grinding, purifying, among others. The grinding aims to reduce of endosperm to very small and thin particles and to remove external layers of grain mainly bran (Mousia et al., 2004; Lijuan et al., 2007). Nevertheless, not always is possible to remove bran from endosperm and in some cases is necessary to purify flour to minimize the negative effects in dough colour caused by bran. Besides the dough colour effects, bran also has impact on dough volume, texture and taste due its interaction with gluten-starch links (Mousia et al., 2004). Germen is eliminated during the milling step and hence most of minerals and lipids content are lost (Cakmak, 2008; Scheuer et al., 2011). Wheat functional compounds such as vitamins, minerals, fibre and phenolic compounds are mainly located on external layers of wheat grain which are removed during

milling (Liu et al., 2008). Most of minerals amount is located in kernel and aleurone while less mineral content is on endosperm (Hussain et al., 2010).

Biofortification using foliar fertilization can increase zinc concentration in the grain and therefore in the zinc flour (Zhang et al., 2010). Zhang et al. (2010) found zinc rise about 26-48% in flour and 24-58% in bran. Yet, although it is possible to increase zinc levels in seeds by fertilizers application, most of zinc is concentrated on pericarp, being therefore lost during grinding (Cakmak et al., 2004; Hussain et al., 2010). So, it is important to evaluate different grinding process in order to find the best method to provide the fewest nutritional losses (Cakmak et al., 2004; Hussain et al., 2010).

Debranning technique allows removing external layers of grain in a sequential way by abrasion and friction, which therefore increases the pericarp levels in flour and thereby its mineral content (Lijuan et al., 2007). However, this method must be controlled because high bran levels affect rheological and technological dough functions (Lijuan et al., 2007). Additionally, milling process becomes easier on each step of debranning (Greffeuille et al., 2007). In this context, Liu et al. (2008) evaluated the debranning effect on phytate levels, phytase activity and iron and zinc level during dough grinding and found that flour had higher levels of those compounds.

The success of biofortification also depends on the stability of micronutrients and food where they are added (Akhtar et al., 2011). Micronutrients stability is important during food processing, which includes physical and chemical factors and food storage. The quality of biofortified wheat to bakery products is evaluated by rheological properties of dough and nutritional value (Akhtar et al., 2009). There is a very limited published studies about the effect of zinc fortification on the quality of dough and bread (Khoshgoftarmanesh et al., 2010). Changes on water absorption, dough development time, dough stability time and peak viscosity as a function of mineral fortification with different treatments (60 mg NaFeEDTA/Kg flour plus 30 mg ZnSO₄/kg flour, 40 mg NaFeEDTA/kg flour plus 20 mg ZnO/Kg flour, 40 mg elemental iron/Kg flour plus 30 mg ZnSO₄/Kg flour and 40 mg elemental iron/Kg flour plus 20 mg ZnO/Kg flour) were evaluated by Akhtar et al. (2009), being found that zinc fortification do not appear to impart any significant change in dough rheological characteristics, where ZnSO₄ shown the fewest changes in dough quality.

Despite zinc fortification can enhance the nutritional value of food products and doesn't impart rheological properties changes, it needs to be approved by consumers and they do it based on organoleptic characteristics (Khoshgoftarmanesh et al., 2010). In this context, some organoleptic characteristics (colour, texture, taste and overall acceptability) of bread prepared from flours obtained by different extraction rates, 87 and 68% and five fortifications treatments (60 mg Fe/Kg flour, 60 mg Fe/Kg flour plus 2 mg folic acid/Kg flour, 60 mg Fe/Kg flour plus 30 mg Zn/Kg flour and 30 mg Zn/Kg flour) were evaluated by a trained panel, using Hedonic scale. The bread from flour with higher extraction and only submitted to zinc fortification obtained the best results in all characteristics (Khoshgoftarmanesh et al., 2010). However, some biofortified bread can display an undesirable flavour mostly due to the type of fertilizer applied. Indeed, zinc oxide is known by supply a bitter flavour and sulphate of zinc supplies an astringent flavour (Akhtar et al., 2011).

Zinc absorption in animals and its inhibitors/promoters

Biofortified wheat flour in iron and zinc facilitates the absorption of these chemical elements in the intestinal mucosa (Khoshgoftarmanesh et al., 2010; Ahmed et al., 2012), yet some interactions among minerals might change their bioavailability (Ahmed et al., 2012). In this context, a study was developed to measure bioavailability and the extent of interaction among minerals such as calcium, iron and zinc, obtained from 72% extraction fortified wheat flour bread, using animal's model. The authors (Ahmed et al., 2012) found that the diet with zinc, in combination with calcium or iron, showed less zinc retention in plasma and liver, whereas the diet which contained zinc alone presented higher zinc levels in plasma and liver of rats. These data can be explained considering antagonistic Ca-Zn interactions, suggesting that higher dietary calcium can provide lower zinc absorption (Ahmed et al., 2012).

Another study, where zinc absorption was evaluated from zinc biofortified wheat, in adult women, with different extraction grain rate (whole grain, 95% and 80% extraction) (Rosado et al., 2009), showed that the amount of zinc absorbed from 80% extracted wheat was similar to the quantity of zinc absorbed from 95% extracted wheat. The results are consequence of a high phytate reduction from moderate extraction. The same interactions with zinc absorption and phytate content from different wheat extraction rates was

observed by Sreenivasulu et al. (2008), which suggest an higher inhibitor effect of phytate on zinc absorption.

Zinc absorption depends on the food matrices and on the effect of several dietary ligands. Tannic acid or some polyphenolic compounds from beverages sources, such as tea or grape juices, increased zinc uptake in Caco-2 Cells, where polyphenols rich foods don't show adverse effect on zinc absorption (Sreenivasulu et al., 2008). However, tannic acid required much higher amount to enhance zinc uptake in Caco-2 Cells from rice and wheat based meals, than from zinc salt, which suggest possible interactions between tannic acid and others components of food matrix. In another study supplementation of zinc-methionine complex further improved zinc levels in the plasma, comparatively to ZnSO₄ in mice (Sreenivasulu et al., 2008). Still further, histidine at higher concentrations showed a decreased zinc uptake in Caco-2 Cells, but arginine enhanced zinc uptake in Caco-2 Cells (Sreenivasulu et al., 2008).

Conclusion

Biofortification of staple food crops is a theme of increasing importance mostly due to the implications on human health and nutrition. Accordingly, it is becoming a source of research for application of several technologies that can allow the production of different types of biofortified foods, namely zinc enrichment. However, although the increase of food nutritional value is the goal of biofortification, hence is extremely important to evaluate the bioavailability and uptake of the nutrients by plants as well as the implication on the bioassimilation of derived products in the intestinal mucosa.

Author Contributions

M. P. M., I. M. P., K. O., C. A. and J. P. made a major contribution to the writing of the paper. F. C. L., F. H. R., M. F. P., J. C. R., P. S. C., M. M. A. S., I. P. P. and A. E. L. were involved in the overall planning and supervision of the work and paper review.

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