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# Contents

# List of acronyms and abbreviations

| | |
|---|---|
| AMF | Arbuscular Mycorrhizal Fungi |
| DOM | Dissolved Organic Matter |
| DTPA | Diethylene Triamine Pentaacetic Acid |
| EDTA | EthyleneDiamineTetraacetic Acid |
| LMWOA | Low Molecular Weight Organic Acids |
| SOM | Soil Organic Matter |
| ZIP | Zrt- and Irt-related Proteins |

# Abstract


Zinc deficiency is a wide-spread and serious problem both in human populations and in crop production. It is a relevant issue in rice-based systems due to their extent and role in human nutrition worldwide. There is an urgent need to increase human Zn intake through biofortification strategies in these systems.

Particularly in developing countries, there is a spatial correlation between human Zn deficiency and low Zn soils. Main soil factors controlling plant-available Zn are pH, redox condition, organic matter content and concentrations of other trace elements. By far the biggest fraction of total Zn is adsorbed to the soil's solid phase in most soils. This requires management practices that avoid a risk for Zn fertilizer failure and/or a choice of rice cultivars that are able to mobilize Zn adsorbed by the soil's solid phase. Management practices could include balancing Zn with N and P fertilization, seed or foliar application of Zn and effective organic matter management.

On the plant level the rooting density, the root efflux (exudates) and influx (Zn) and Zn translocation are important factors. The translocation from aboveground vegetative tissues to the grain endosperm seems the most limiting step to reach higher Zn concentrations in grains. Genetic variation in several steps leading to grain Zn loading have been found, but options to align these do not seem to have been fully explored. It probably needs combination of breeding with Zn application treatments to both tackle Zn deficiencies at the crop growth stage and at grain Zn loading stages. The role of N fertilization in creating synergy also needs further investigations.

To improve human Zn intake, several strategies can be applied, such as supplementation, dietary diversification and food fortification. Among these, biofortifying rice through breeding or agronomic management is the most sustainable strategy and unlikely to pose any adverse health effects on people. The few case studies available support this conclusion.

# 1 Ecological life cycle of zinc

Zinc (Zn) is among the 30 most abundant elements in the Earth's crust, making up about 75 ppm (0.0075%) of the Earth's crust, which is equal to an amount of 20 x $10^6$ Mg (Emsley, 2003; Rauch, 2011; Rauch and Pacyna, 2009). Zinc has five stable isotopes: $^{64}$Zn (48.63%), $^{66}$Zn (27.90%), $^{67}$Zn (4.90%), and $^{68}$Zn (18.75%) and $^{70}$Zn (0.62%; Broadley et al., 2007). Soils contents vary from 5 to 770 mg $kg^{-1}$ Zn. The concentration in seawater (30 µg $kg^{-1}$) is orders of magnitude lower. The Zn concentration in the atmosphere is 0.1-4 µg $m^{-3}$ (Emsley, 2003; Rauch, 2011).

Zinc is a structural component of enzymes which play a significant role in metabolism of proteins, carbohydrates, lipids and nucleic acids (Alloway, 2008; Broadley et al., 2012). It binds to a vast number of proteins (Graham et al., 2007). The Zn concentration in terrestrial plants varies widely (20-300 mg $kg^{-1}$ dry weight [Kabata-Pendias and Pendias, 1984]) depending on the pedo-geochemical and biogeochemical conditions. Being one of the essential trace elements, it takes part in the synthesis of chlorophyll. Chlorosis and insufficient growth of leaves are the most characteristic symptoms of Zn deficiency in plants (Alloway, 2008). Zinc is also involved in the mechanisms providing for the resistance of plants to frost and drought (Kabata-Pendias and Pendias, 1984).

Zinc toxicity is less widespread than Zn deficiency. An excessive intake and accumulation of Zn leads to toxic effects in all living organisms. An excessive concentration of Zn in humans, e.g., due to pollution caused by Zn mining, smelting or dumping, induces vomiting, nausea, pneumonia and fibrosis of the pulmonary system (Nriagu, 2011).

# 2 Extent and geographic distribution of zinc deficiency

Zinc deficiency is a well-documented problem both in human populations and in crop production globally. It is estimated that about 50% of the cereal-cultivated soils globally are too low in available Zn. This leads to reductions in crop production and nutritional quality of the harvested grains (Cakmak, 2008; Graham et al., 1992; Sillanpää and Vlek, 1985). However, to our best knowledge there is no research underpinning these widely cited statements.

Zinc deficiency in human populations occurs mostly in regions where cultivated soils are low in plant available Zn and where cereal-based foods are the major source of daily calorie intake (Figure 1). Up to 75% of the daily calorie intake of people living in rural areas of the developing world comes only from cereal-based foods with low Zn concentrations and low availability of Zn for humans. Human Zn deficiency ranks therefore third in importance after iron and vitamin A deficiency (Hambidge, 2000).

Zinc deficiency causes severe impairments in human health, including impairments in brain function and development, compromised immune system, increased susceptibility to deadly infectious disease and alterations in physical development. It leads to stunted growth and has been reported to be responsible for deaths of about 450,000 children under 5 years old, annually (Black et al., 2008). Zinc deficiency is also becoming a public health problem in developed countries. Inadequate intake of Zn is reported in the United States, United Kingdom and Australia – especially among women, children and elderly people (Gerrior, 2002; Watt et al., 2001).

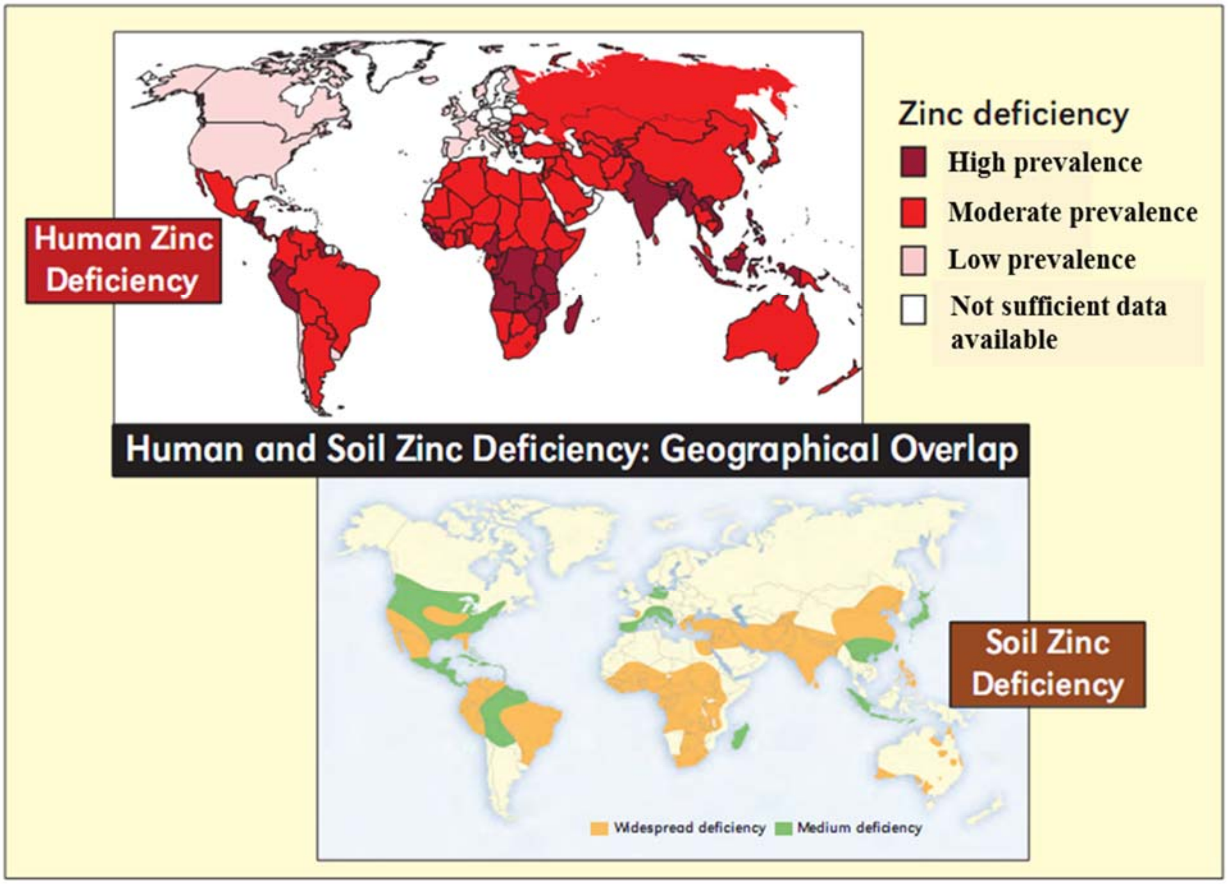


***Figure 1.*** *Geographical overlap of human Zn deficiency (upper map) soil Zn deficiency (lower map) (Sources: http://www.izinc.org; Alloway [2008]).*

# 3 Extent of zinc deficiency in rice cultivation

Rice is grown in a variety of production systems (see text box). We are not aware of any study on the prevalence of Zn deficiency symptoms across these systems. Although Zn deficiency is claimed to be more common in lowland environments because the low redox potential (Rose et al., 2013), Zn deficiency symptoms were observed for aerobic rice in northern China, 2 or 3 years after fields were shifted from anaerobic to aerobic conditions (Wang et al., 2002). We tend to conclude that generalization of Zn availability for rice cultivation systems is not possible. Soil factors such as composition of parent material, pH and soil organic matter (Chapter 3) content probably have an effect on Zn availability that is overriding the effect of cultivation system.

Zinc deficiency in humans has become of much greater concern since the Green Revolution (Welch and Graham, 2002), during which the focus was on yield quantity. Diet diversity was lost (Graham et al., 2007), and draught-animals were replaced by tractors (Mishra et al., 2006), reducing the amounts of manure applied to soil. Cereals were chosen as the cornerstone of the Green Revolution. This effort involved not only new, high-yielding varieties, but also the use of much more nitrogen (N) and phosphorus (P) fertilizers which tended to decrease the Zn plant tissue concentration due to dilution (Graham et al., 2007). The highly productive package of new cereal varieties

**Rice cultivation systems**

Rice production systems differ widely in their cropping intensity and yields, ranging from single-crop rainfed lowland and upland rice with low yields (1-3 Mg $ha^{-1}$) to flooded systems with an annual grain production of up to 15-18 Mg $ha^{-1}$, generally with 2 to 3 crops a year. Flooded and irrigated lowland rice systems account for about 80% of the harvested rice area and 92% of total rice production. Worldwide, the total harvested area of flooded and irrigated rice is about 79 million ha, with 43% (34 million ha) in East Asia (China, Taiwan, Japan, Korea), about 24 million ha in South Asia and 15 million ha in Southeast Asia. The countries with the largest irrigated and flooded rice areas are China (31 million ha), India (19 million ha), Indonesia (7 million ha) and Vietnam (3 million ha). Rain-fed lowland rice grows in bunded fields that are flooded for at least part of the cropping season to water depths that may exceed 50 cm for no more than 10 consecutive days (Dobermann and Fairhurst 2000).

Upland rice is grown at low input levels in unbunded fields. The soil is cultivated when dry and planted by direct seeding. Surface water does not accumulate for any significant amount of time during the growing season. Landforms for upland rice vary from low-lying valley bottoms to undulating and steep sloping lands with high runoff and lateral water movement. Upland rice constitutes only 10% of the global rice area and 3.8% of total world rice production (Dobermann and Fairhurst 2000).

Aerobic rice cultivation is a new rice cultivation system as an alternative to the traditional high water consuming lowland rice cultivation with flooded soils. Aerobic rice varieties are currently developed by crossing lowland with upland varieties and grown as a dry field crop in irrigated but non-flooded and non-puddled fertile soils (Bouman et al., 2005).

Rice can be grown in rotation with other crops. The rice-wheat cropping system is a major producer of India's staple food, for instance. It is applied mainly in the Indo-Gangetic Plain in South Asia (India and Pakistan) and China on a large variety of soils. Both rice and wheat are irrigated, rice typically receiving more irrigations than wheat.

tended to displace Zn-rich pulses such as chickpea or lentil in food systems struggling to meet the needs of a growing population.

In parallel, lower grain Zn densities in modern varieties as a side effect of breeding efforts (Gavin et al., 2008; Murphy et al., 2008; Morgounov et al., 2013) and/or fertilization (Fan et al., 2008) have been demonstrated convincingly for wheat (Figure 2).

# 4 Soil factors controlling zinc availability

The total Zn content of a soil can largely be derived from the geochemical composition of the (weathering rock) parent material on which the soil has developed (Rauch, 2011). Low total Zn concentrations mainly occur in strongly leached tropical soils (e.g., Ferrasols) developed on highly weathered parent materials in the humid tropics on the continental shields of South America and Africa and on easily weatherable rock in hot and humid climates (Figure 1). Sandy soils (e.g., Arenosols, Regosols) developed on sandstones or sandy drift are in general also low in total Zn.

Total soil Zn content can mostly not be directly related to the amount of Zn available to plants and *vice versa*. Zinc forms available to plants are the free ions ($Zn^{2+}$ and $ZnOH^{+}$ [Broadley et al., 2012]) and soluble organic complexes (Wang et al., 2009). The transport and speciation of different Zn forms and therefore the availability of Zn for plant uptake depends on a range of soil properties. The main soil factors controlling the amounts of plant-available Zn forms are pH, redox conditions, organic matter contents, concentrations of other trace elements (especially Fe, Cu and Ni), microbial activity, $CaCO_3$ content and soil moisture (Alloway, 2009).

## 4.1 Soil pH

Hydrogen ion activity (pH) is one main factor governing Zn speciation, solubility from mineral surfaces, transport and bioavailability of Zn to plants in aqueous solutions. The pH affects both solubility of metal hydroxide minerals and adsorption-desorption processes. With increasing pH the adsorptive capacity of the soil's solid phase (mainly

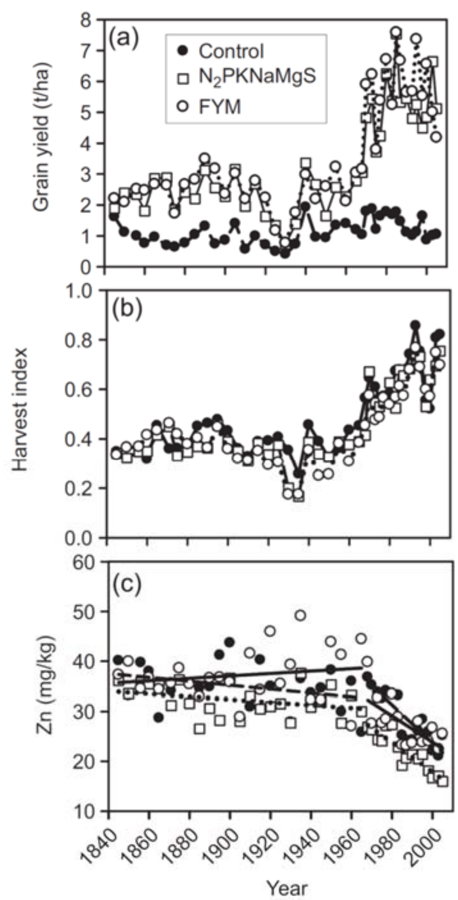


***Figure 2.*** *Decreasing trend in wheat grain Zn (c) coinciding with increased grain yield (a) and harvest index (b) over 160 y in a long-term experiment.*

*The control was without any input. N2PKNaMgS refers to a chemical fertilizer treatment with 96 kg N and 35 kg P ha$^{-1}$ y$^{-1}$ (until 2000); FYM refers to farm yard manure, 35 Mg ha$^{-1}$ y$^{-1}$ fresh weight. In the late 1960s, high-yielding cultivars were introduced with a larger harvest index. Copied from Fan et al. (2008, p. 318).*

organic matter), the formation of hydrolyzed forms of Zn and co-precipitation in Fe oxides increases, and Zn bioavailability decreases. High pH values in soils are the result of a high $CaCO_3$ content (pedogenic or due to liming), high salt contents and reducing (flooded) conditions (Lindsay, 1972).

## 4.2 Soil organic matter

Soil organic matter (SOM) is another soil factor which is relevant in controlling the bioavailability of Zn. In most soils, the largest proportion of Zn is adsorbed to SOM. Especially in sandy soils organic matter is one of the most important solid phases that adsorbs Zn and other metals (Stevenson, 1982). Recently, Duffner et al. (2014) have shown that also in low Zn soils with low SOM contents, organic matter is still the dominant sorbent.

Likewise, dissolved organic matter (DOM) may also bind metal ions like $Zn^{2+}$. Such Zn-DOM complexes enhance Zn mobility as well as their bioavailability (Weng et al., 2002). The concentrations of other trace elements (especially Fe, Cu and Ni) in the soil influence the availability of Zn to the plants, because with increasing concentrations of other trace elements, competition at biotic (e.g., cell walls of soil bacteria or root surface) (Plette et al., 1999) and soil surfaces (Gao et al., 2010) is also increasing. Depending on the affinity of the other tracer metals for the surfaces, the Zn concentration in the soil solution can increase or decrease due to competition with these metals.

A particular fraction of DOM is exuded by plant roots. Ligands such as phytosiderophores, citrate and other low molecular weight organic acids and amino acids can form Zn complexes which increase the mobility of Zn in the solution and the availability to plants (Duffner et al., 2012; Gramlich, 2013; Hoffland et al., 2006; Von Wirén et al., 1996). With increasing concentrations of other trace metal ions the competition increases for the formation of such complexes. Especially Zn and Fe are highly competitive for phytosiderophores, which can be exuded from roots of graminaceous species like rice (Von Wirén et al., 1996). At higher Fe concentrations in the soil solution, less Zn can be bound by organic ligands, potentially resulting in a reduction of Zn bioavailability.

## 4.3 Soil microorganisms

The interaction between plant roots and arbuscular mycorrhizal fungi is highly relevant in the context of Zn deficiency. This interaction will be reviewed in section 5.2.

The microbial activity in the rhizosphere (soil compartment directly surrounding the root and influenced by the roots of plants) could increase mobilization of Zn and thus bioavailability of Zn to the plant (Hu and Li, 2009). Awad and Römheld (2000) have shown that siderophores exuded by microorganisms in the rhizosphere can play a significant role in the mobilization of Zn and other metals for plant uptake. It is unknown, however, whether rice cultivars could select a rhizobiome beneficial for Zn mobilization (Rose et al., 2013).

## 4.4 Soil chemistry interactions during drying of soils

The redox condition of the soil is one of the main factors controlling the availability of Zn to plants. The redox potential plays a crucial role especially in floodplains (Schröder et al., 2008) and rice systems which change from anaerobic to aerobic cultivation (Gao et al., 2012).

Zinc itself is not subject to oxidation or reduction, but it can be associated with other compounds in the soil that are redox active. Giordano and Mortvedt (1973) suggested that flooding would decrease the availability of Zn. Zinc deficiency of flooded rice could be attributed to the formation of insoluble Zn sulfide (ZnS; Kittrick, 1976). Both ZnS (sphalerite) and $FeS_2$ (pyrite) precipitate at approximately the same redox potential, respectively, when $FeCO_3$ (siderite) controls Fe solubility (Lindsay, 1979). However, since soils generally contain much more Fe than sulfides, sulfides will be depleted first and often cannot depress Zn activity through the formation of ZnS (Sajwan and Lindsay, 1988). We conclude therefore that the probability of decreased Zn bioavailability due to flooding is depending on Fe availability but probably relatively low.

During drying of soils in aerobic rice cultivation Zn bioavailability to rice can increase. Johnson-Beebout et al. (2009) reported in a pot study, including calcareous soils, that plant Zn uptake increased in the oxidized soil compared to flooded soil. Contrasting to that, Gao et al. (2006) showed in a field experiment that calcareous soils can cause Zn deficiency in rice under aerobic cultivation. The apparently contrasting results could be due to the difference in soil sulfide content and achievable redox conditions in the two studies. According to Gao et al. (2012) several other factors such as soil pH could also have a dominant impact on Zn bioavailability depending on water regime. Under flooded conditions the soil pH goes towards pH 7 and it goes back again to its original pH after drying.

## 4.5 Soil parameters for zinc availability

It is obvious that in most soils the largest proportion of Zn is not directly plant available because it is present in minerals, precipitates or adsorbed to the solid face (including organic matter). While the total amount of Zn in the soil is usually hundreds to even thousands times the amount needed to support the yield, Zn deficiency may be common as only a fraction of that is directly available to the crop. Most of the Zn is transported to the root by diffusion. After uptake, the amount taken up can potentially be replenished by desorption from the solid phase. This is why it is not straight forward to predict Zn availability to a crop based on soil analysis.

Usually extractants are used to predict Zn availability, of which DTPA is the most common one (Lindsay and Norvell, 1978). The critical soil Zn-DTPA level (below which Zn there is a risk for Zn deficiency in a crop) is supposed to be 0.8 mg $kg^{-1}$ (Dobermann and Fairhurst, 2000). However, the relationship between soil Zn-DTPA levels and yield or Zn uptake is often very poor and statistically not significant (Duffner et al., 2013; Haileselassie, Oenema and Hoffland, unpublished). This was underpinned by the dataset of Phattarakul et al. (2012) and Zou et al. (2012). Plotting their DTPA-extractable Zn data against the grain yield of rice and wheat, respectively, shows an inaccurate estimation of grain yield, especially in the low Zn range (Figure 3). Relating the grain Zn or leaf Zn concentrations to DTPA-extractable Zn showed a comparable result. We are unaware of any similar dataset on other methods.

# 5 Zinc uptake by plants

Zinc enters the root cell wall free space by diffusion and then moves across the plasma membrane via the action of ion transport proteins. In nongraminaceous species Zn is mainly taken up by plant roots as the $Zn^{2+}$ ion. Graminaceous species (including rice) have a second pathway for uptake of Zn-phytosiderophores (Suzuki et al., 2006; Von Wirén et al., 1996). There are at least six different families of transporters that could facilitate plant Zn transport, including ZIP (Zrt- and Irt-related proteins), CDF (Cation Diffusion Facilitator proteins), P-type ATPase (metal transporting ATPases), NRAMP (Natural Resistance-Associated Macrophage Proteins), and CAX (Calcium and other divalent cation exchange antiporter proteins). Recent studies suggest that the ZIP family of micronutrient transporters probably include some of the important Zn transporters that facilitate Zn entry into plants (Guerinot, 2000).

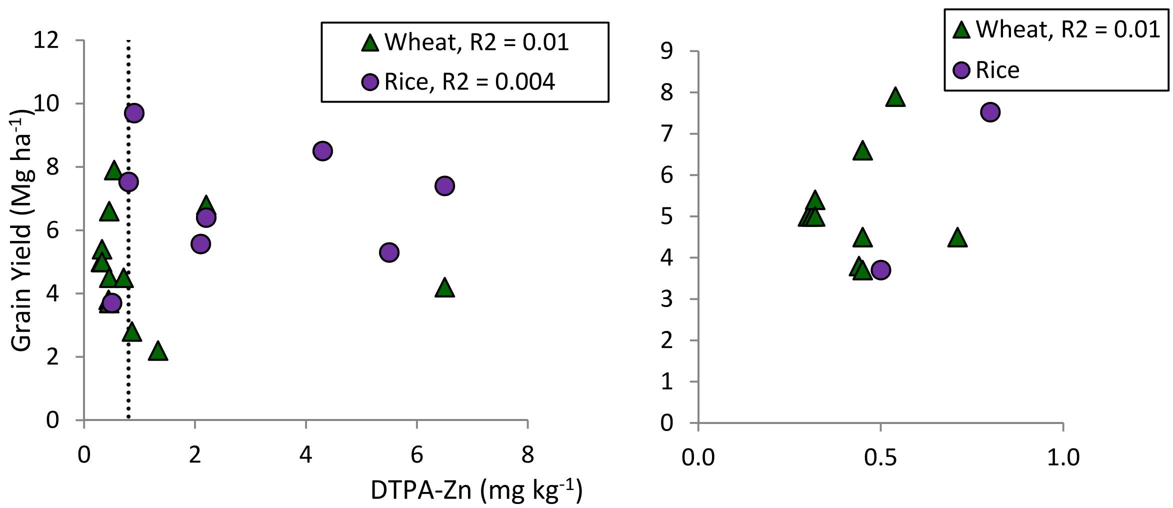


***Figure 3.*** *Absence of any relationship between DTPA-extractable Zn in soil and grain yield of rice (Phattarakul et al., 2012) and wheat (Zou et al., 2012).*

*The dashed line in the left panel represents the critical DTPA-Zn level (0.8 mg kg-1), below which Zn can be a yield-limiting factor. The right panel zooms in on data from soils below this critical level. Rice and wheat were grown under the HarvestPlus program in 18 different field experiments located in seven different countries in Asia.*

Zinc uptake by roots from soil or nutrient solutions at $Zn^{2+}$ concentrations >0.5 µM can quantitatively be well described by the Michaelis-Menten kinetics (Hacisalihoglu et al., 2001; Hart et al., 2002):

$$I(C) = \frac{I_{max}\,C}{K_m + C}$$

Where I(C) is the rate of Zn uptake by roots at a $Zn^{2+}$ concentration C in the solution, $I_{max}$ is the maximum rate of Zn uptake, and $K_m$ is the $Zn^{2+}$ concentration at which the uptake reaches half the maximum rate. Root $Zn^{2+}$ uptake by wheat is mediated by two different transport systems: a high-velocity, low-affinity system ($K_m$ = 2-5 µM; $I_{max}$ 143-521 nmol g $fw^{-1}$ $h^{-1}$), and a low velocity, high affinity system ($K_m$ = 0.6-2 nM; $I_{max}$ 9-31 nmol g $fw^{-1}$ $h^{-1}$) that probably is the dominant transport system under low soil Zn conditions (Hacisalihoglu et al., 2001).

At $Zn^{2+}$ concentrations <0.5 µM (i.e., in most soils) the Michaelis-Menten equation can be reduced to a linear relationship (Degryse et al., 2009). Soil solution Zn concentrations are usually so far below $K_m$, that the response of rice plants to Zn deficiency is determined by root growth maintenance and organic acid exudation, rather than by up-regulation of Zn-transporter activity (Widodo et al., 2010).

Working with low Zn soils, Duffner et al. (2013) showed that Zn concentration in the soil solution or soil extracts (e.g., DTPA or $CaCl_2$) can be related to wheat Zn uptake indirectly and nonlinearly. They applied a stepwise approach, similar to an approach developed for contaminated soils (Kalis et al., 2007). The crucial, pH-dependent step in the statistical relationship between soil Zn and shoot Zn content was adsorption of Zn to the root: Root-adsorbed Zn (i.e., the Zn that could be desorbed from the root surface by washing with EDTA solution) was found to be a good proxy for bioavailable Zn. Although it is not known where in the apoplast exactly this EDTA-desorbed

Zn originates from, it could suggest that pH-dependent adsorption of Zn to cell wall anions in the apparent free space (White, 2012) is either crucial for uptake or at least a good proxy for Zn availability to the plant.

The relationship between soil solution Zn concentration and root-adsorbed Zn, was non-linear and could be described as a two species Freundlich equation (Duffner et al., 2013):

$$[Zn]_{root-adsorbed} = K[Zn^{2+}]^m \, [H^+]^a$$

where $[Zn]_{root\text{-}adsorbed}$ is the quantity of Zn bound to the root surface, $K$ is a constant related to the number of binding sites and their affinity for protons and Zn ions, $[Zn^{2+}]$ represents the Zn concentration in the soil solution, $[H^+]$ is the proton activity, and $m$ and $a$ are empirical parameters. The result is relationship between $Zn^{2+}$ in the soil solution and Zn uptake that is strongly determined not only by bioavailable Zn, but also by soil pH. Since bioavailable Zn itself is also pH dependent (section 3.1), this further emphasizes the crucial role of soil and rhizosphere pH in Zn uptake by crops.

## 5.1 Root traits enhancing zinc uptake at low soil zinc levels

Given the fact that Zn mobility in soils is low, increasing the exploited soil volume by a larger root system seems an obvious strategy (Rose et al., 2013). This probably applies specifically to early growth stages (seedling) where the root system is still poorly developed. This assumption is underpinned by frequent observations of plant recovery from early (seedling stage) symptoms of Zn deficiency. Depending on the set of rice genotypes considered, other mechanisms enhancing Zn uptake may be a more important determinant of the capacity of a genotype to grow well on a low Zn soil (Gao et al., 2005). The major mechanisms for increasing Zn acquisition by rice include the increase of acquisition area by root growth or the involvement of mycorrhizae and root exudation of low molecular weight organic acids (LMWOAs) or phytosiderophores (Gao et al., 2012).

There seems ample proof that Zn deficiency induces exudation of LMWOAs but their effects on Zn availability are inconclusive. Rice can exude citrate under Zn deficiency. The citrate exudation capacity of rice genotypes has been shown to relate to their tolerance to Zn deficiency (Hoffland et al., 2006). Duffner et al. (2012) confirmed that citrate (~0.6 mM) can potentially increase the Zn concentration in the soil solution, but this effect was soil-dependent and probably determined by the presence of ions competing with Zn for complexation with citrate. Hajiboland et al. (2005) and Yang et al. (2003) showed that rice increased malate exudation in response to higher bicarbonate levels in the root growth medium, probably as a consequence of loss of membrane integrity (Rose et al., 2011). Gao et al. (2009) also found increased malate concentrations in the rhizosphere at low Zn supply to rice plants. They detected malate concentrations of approximately 0.5 mM in the rhizosphere soil solution. However, this malate concentration was not sufficient to solubilize significant amounts of Zn, which is in line with the findings of Rose et al. (2011): They found that malate concentrations in the soil solution have to be higher than 100 mM to cause significant Zn mobilization. These concentrations can be found for example in nutrient poor natural ecosystems (Lambers et al., 2009). In lab systems, tomato root exudates have been shown to play a role in Zn uptake, however (Smolders et al., 2013). At the current stage it can be concluded that the effects of root exudates for the Zn bioavailability are soil dependent, and a generalization of the root exudates effects needs further (modeling) work, taking into account relevant soil chemical equilibria.

Though we lack proper evaluation instruments, secretion of phytosiderophores could be more important for rice plants in soil Zn mobilization. Phytosiderophores are organic ligands with similar structure to amino acids, forming stable complexes with metals, especially with Fe and Zn (Dell'mour et al., 2010; Römheld, 1991). Arnold et al.

(2010) used an isotope fractionation technology and a mathematical model to predict realistic rates of secretion of phytosiderophores by rice as well as parameters for the Zn-solubilizing effect of phytosiderophores in soil for Zn uptake. They showed that the complexation of Zn by phytosiderophores and uptake of the complexed Zn by specific root transporters increased the Zn uptake two to five times, depending on exudation rate of the phytosiderophores.

Most studies on the role of phytosiderophores for the Zn bioavailability focused on wheat and barley (Cakmak et al., 1998; Cakmak et al., 1996; Suzuki et al., 2006; Walter et al., 1994; Zhang et al., 1991). Cakmak et al. (1996) and Suzuki et al. (2006) showed that low Zn concentrations in the soil solution increased the secretion of phytosiderophores of wheat and barley, respectively. Although rice exudes smaller amounts of phytosiderophores than other crops at comparable soil conditions, including solution concentration of Zn (Takagi, 1976) phytosiderophores are essential for Zn uptake (Arnold et al., 2010). It is expected that phytosiderophores have a greater impact under aerobic conditions compared to flooded conditions. Due to the reduced transport of phytosiderophores away from the roots under aerobic conditions the exudates should accumulate in the rhizosphere and increase the rhizosphere concentration of bioavailable Zn (Gao et al., 2012).

## 5.2 Arbuscular mycorrhizal fungi

Besides the excretion of root exudates by rice to increase the bioavailability of Zn, the increase of the acquisition area by root growth and the association with mycorrhizae (under aerobic conditions) are also important factors. Direct evidence for uptake of Zn by arbuscular mycorrhizal fungi (AMF) has come from studies employing $^{65}$Zn as a tracer (Jansa et al., 2003; Cavagnaro, 2008). Lehmann et al. (2014) confirmed by a meta-analysis that AMF positively affected Zn concentrations in various crop plant tissues including rice. In aerobic soils AMF can find a favorable environment for their activity. Hajiboland et al. (2009) studied the influence of AMF on the uptake of Zn and P by two contrasting rice genotypes in a pot experiment. After inoculation with mycorrhiza they observed up to twofold higher Zn uptake under Zn deficiency. An enhanced AMF inoculation under aerobic condition has been shown to increase rice Zn uptake by Gao et al. (2007). They conducted a pot experiment with six aerobic rice genotypes inoculated with either *Glomus mosseae* or *G. etunicatum* or without AMF on a low Zn soil. The AMF-inoculated plants produced more biomass and took up more Zn than non-mycorrhizal controls. Mycorrhizal inoculation, however, increased Zn uptake only in genotypes that had a low Zn uptake in the non-mycorrhizal condition, suggesting that AMF association and other Zn mobilization mechanisms are mutually excluding.

There are also a few studies on the influence of AMF on mobilization of Zn in flooded soils (Purakayastha and Chhonkar, 2001; Solaiman and Hirata, 1997). They concluded that AMF-colonized rice plants were more effective in acquiring Zn from either added or native sources than non-colonized plants. However, studies dealing with an increased Zn bioavailability for rice by mycorrhiza in aerobic and flooded soils were mainly conducted in the greenhouse. Validation by results from field studies is pending and hampered by the difficulty to create a nonmycorrhizal control.

## 5.3 Breeding for zinc efficiency

Potentially, breeding for plant traits that increase acquisition of Zn from soil or fertilizer could be a cost effective means to improve nutrient use efficiency in rice production. However, Rose et al. (2013) conclude in an excellent review that few root traits have so far been successfully used in breeding for enhanced Zn uptake by rice in low input systems, despite the fact that Zn acquisition appears to drive Zn efficiency in lowland rice (Wissuwa et al., 2006) and aerobic rice (Gao et al., 2005). They indicate phytosiderophore efflux could be a successful breeding target, provided this trait will prove to increase Zn use efficiency under realistic field conditions.

An avenue that has not been exploited, as far as we are aware, is to revisit traditional rice varieties that perform well on low Zn soils. Some traits related to efficient use of Zn may have been lost during the breeding process. For wheat, it was suggested that modern breeding practices have produced cultivars that are highly dependent on fertilizers and show a reduced dependency on and response to mycorrhizal symbiosis (Hetrick et al., 1992). Similarly, a gene from a traditional rice variety appeared to be involved in early root growth, by which it could increase P efficiency (Gamuyao et al., 2012). This gene was absent in modern varieties.

# 6 Plant zinc allocation and re-allocation

From the start of plant growth, Zn is taken up and distributed among organs. Until the initiation of the panicle all Zn is allocated to vegetative organs. From the moment on generative organs, including grains, are formed, Zn is allocated to these too, as no tissue can be formed without Zn. As Zn is highly phloem and xylem mobile (Marschner, 2002), the element is constantly re-allocated among organs. Recent studies with stable isotopes confirm this (Wu et al., 2010; Stomph et al., 2014). When the plant has a low Zn status during vegetative growth, leaf blades and roots seem to be kept at the higher density at the expense of leaf sheaths and stems. During the reproductive growth phase, also grains are kept at a higher density than other panicle tissues, stems and sheath. Towards grain maturity this happens also at the expense of roots and leaf blades (Stomph et al., 2014). As the plant Zn status improves, the balance among organs shifts. While the plant seems to avoid Zn densities below 20 mg $kg^{-1}$ in the leaf blades and grains, the moment the average plant Zn status raises above 20 mg $kg^{-1}$, stems and roots increase faster in Zn density than leaf blades and grains, while leaf sheaths and panicles tissues other than grain are intermediate (Jiang et al., 2008a). The consequence of this internal Zn balance between organs is that when plant Zn uptake is enhanced through, for instance, fertilization, much of the additional Zn does not end-up in the rice grain (Gao et al., 2006, Wissuwa et al., 2008).

A debate in the literature is whether Zn in rice grains comes from uptake during grain filling or from re-allocation of Zn that was earlier allocated to leaves or other organs. On a Zn balance level several papers have shown that total post-flowering Zn uptake was larger than total grain allocation (e.g., Impa et al., 2013b; Jiang et al., 2008b). Studies with stable isotope-labelled Zn in hydroponics suggest that that the Zn ending up in grains is most probably a mix of Zn taken up and allocated directly to the grains, and re-allocated Zn earlier present in leaves or stems (Stomph et al., 2014; Wu et al., 2010). Another debate is on the efficacy of foliar-applied Zn in rice (see also section 9.2). Phattarakul et al. (2012) found that foliar application increased grain Zn density, and that this effect was much larger than soil application. This seemingly contrasts with data reported by Jiang et al. (2007) and Wu et al. (2010; 2011) that seemed to indicate leaf applied Zn is hardly re-allocated to the grains. These studies using isotopes seem to indicate re-allocation can take place but is mainly to other organs than grains. This apparent contradiction may be explained as follows: (1) foliar application will lead to higher leaf Zn concentrations and through re-allocation to higher concentrations in other organs like stems (2) this will lead to a reduced allocation of Zn taken up via the roots to these leaves and stems allowing an enhanced direct allocation to the grain as suggested earlier by Stomph et al. (2014). An important further observation is that there seem to be genotype differences in allocation and re-allocation of Zn (Mabesa et al., 2013; Wu et al., 2010).

The literature on possible bottlenecks for Zn uptake and Zn allocation to the grain is not conclusive. Palmgren et al. (2008) suggested that in all cereals root to shoot transfer can be assumed to be problematic. Data reported by

Jiang et al. (2008a) in fact suggest root to shoot transfer in rice is not a major problem at least in the tested cultivars. Although cultivar differences may exist, further data on Zn density between organs in rice seem to indicate that the major bottleneck in transport, once plant Zn density is increased above 20 mg $kg^{-1}$ on average, is between stem and reproductive tissue. The bottleneck within the seed between endosperm and surrounding tissues observed in wheat (Stomph et al., 2011) was not observed in rice (Stomph et al., 2014) implying that there are some fundamental differences between wheat and rice that may partially be due to anatomical differences (Stomph et al., 2009).

A recent study indicates that enhancing the plant nicotianamine status directly enhances grain Zn density (Johnson et al., 2011), hinting at options to alleviate the above mentioned bottleneck. As nicotianamine is a precursor of phytosiderophores and a strong chelator of $Zn^{2+}$ in plants (Broadley et al., 2012), a positive effect not only on uptake but also on within plant re-allocation is not illogical. How well this works under Zn limited conditions remains to be tested.

# 7 Zinc in the edible produce

In grains and seeds, most of the Zn is localized in so-called protein bodies in the form of discrete particles, the globoid crystals (Lott and Buttrose, 1978; Welch, 1986). These globoids mainly consist of phytate, i.e., salts of phytic acid. In wheat seeds, similarly high Zn concentrations (600 µg $g^{-1}$ dw) were found in the scutellum (Mazzolini et al., 1985). In barley, though, Zn was found mainly to co-localize with proteins rather than phytate, as opposed to Fe (Persson et al., 2009). Zinc, Fe and proteins are generally co-localized within seed tissues (Cakmak et al., 2010b; Liang et al., 2007) and there is a very high positive correlation between the concentrations of Zn, Fe and protein in seeds across genotypes (Cakmak et al., 2010b; Morgounov et al., 2007; Zhao et al., 2009). These results suggest that Zn and Fe are associated with proteins. Lombi et al. (2009) used synchrotron X-ray fluorescence (XRF) analyses for elemental mapping in rice grains. Zinc was mainly found in the central part of the embryo, which likely corresponds to the plumule. The Zn concentration decreased gradually from the aleurone/pericarp and outer parts of the endosperm to the interior of the endosperm. A cross section of rice grain is shown in Figure 4.

This knowledge may be useful in optimizing various procedures to process rice grains (e.g., dehusking, milling, etc.) described in detail by Liang (2007). Because the seeds of rice are mainly consumed after polishing, the concentrations of Zn in food is lower than in whole grains. The same is observed for white flour of wheat as opposed to whole grain wheat.

The distribution of Zn in the rice grains is genotype dependent, so through genotype selection the extent of Zn losses during processing can be reduced (Gregorio et al., 2000; Prom-u-thai et al., 2007; Vasconcelos et al., 2003). Vasconcelos et al. (2003) showed that the Zn concentration in the endosperm varied between 33 to 56 mg Zn $g^{-1}$.

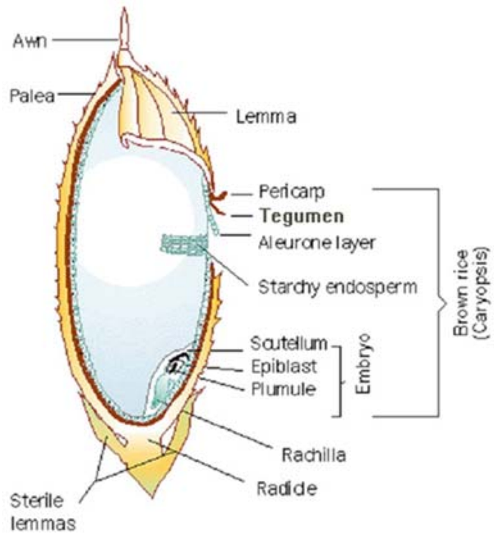


***Figure 4.*** *Cross section of a rice grain (Source: http://www.goldenrice.org/image/why_grain.jpg).*

Not all Zn present in cereal grains is available to the human consumer. Phytate is a potent inhibitor of Zn absorption by the animal consumer. Phytate is the storage form of P in seeds (Raboy, 2001), and usually accounts for 70-80% of total seed P. Phytate forms insoluble complexes with mainly $K^+$ and $Mg^{2+}$. It may, however, also complexate Zn (Frossard et al., 2000), for which it has a relatively high affinity. Plants free these minerals again at germination by releasing phytase. The strong binding of Zn and Fe to phytic acid is of concern to nutritionists as it reduces the bioavailability of these minerals for monogastric animals including humans. During food processing, Zn bound otherwise in the original plant product is exchanged and becomes unavailable in the presence of phytate. This complexation can reduce the bioavailability of Zn to less than 3% of total Zn in the grain (Bosscher et al., 2001).

# 8 Phosphorus-zinc interactions

The phosphorous (P) fertilization and P status of plants plays a significant role in Zn bioavailability to rice (Duffner et al., 2012; Norvell et al., 1987; Zhu et al., 2001). High application rates of P can induce Zn deficiency by changing soil or plant factors (Broadley et al., 2012). In general, increasing P fertilization rates decreases plant Zn content and plant Zn tissue concentration. Balancing P and Zn fertilization therefore deserves needed attention.

Several studies report a negative effect of P fertilization on soil Zn availability. Mandal and Mandal (1990) compared the effect of different P fertilization levels on transformation of native and applied Zn in a rice-growing soil under flooded and non-flooded regimes in a greenhouse. Application of P not only decreased water-soluble and exchangeable Zn, but concomitantly increased bound forms of soil Zn. These effects were more pronounced under the flooded than under the non-flooded regime. Application of P also decreased shoot and root Zn concentration. Saeed and Fox (1979) observed an increase in Zn sorption in tropical soils due to P fertilization. They suggested that in the studied acidic soils sorption of P on the surfaces of Fe and Al oxides increased their negative charges resulting in an increased sorption of Zn.

High P supply suppresses the P deficiency responses that also contribute to Zn mobilization. An increase of soil P supply has a negative effect on AMF colonization. Due to the above described role of AMF, effects of P upon AMF may in turn also have negative consequences on plant Zn nutrition. Ryan et al. (2008) showed that P fertilization reduced grain Zn concentration by 33-39% and root colonization with AMF by 33-75% in wheat. High P supply also suppresses root exudation. Under low P conditions plant roots are able to exude root low molecular weight organic anions such as citrate and malate, which can increase the mobility and availability of both Zn and P (Hoffland et al., 2006). An indication that this might play a role under field conditions is given by Quijano-Guerta and Kirk (2002) who showed that P efficient rice cultivars are also Zn efficient.

Besides the P-Zn interactions in the soils, high P concentrations influence also the plant metabolism including uptake, translocation, and utilization of Zn. A decrease in grain Zn concentration by P fertilization can for instance be related to dilution of Zn due to increased grain yield with P fertilization (Fig. 2). This is one of the reasons why problems of Zn deficiency have been aggravated by the Green Revolution (Nubé and Voortman, 2000), during which P fertilizer use has increased. The decrease in Zn concentration in shoots and an induction of Zn deficiency symptoms by high P supply is the result of enhanced shoot growth and, thus, 'dilution' of Zn in the plants (Loneragan et al., 1979).

Zinc deficiency is frequently reported to induce P toxicity to various crops (Cakmak and Marschner, 1986; Gianquinto et al., 2000; Loneragan et al., 1979). This is understood only since Huang et al. (2000) demonstrated that Zn is essential for the control of P uptake in barley. At low P availability, P uptake is mediated by a high affinity P uptake system. This system is normally down-regulated at high P availability, to protect the plant against P toxicity. Zn is necessary for this down-regulation. So at Zn deficiency this down-regulation is impaired, resulting in P hyperaccumulation and P toxicity. In potato, okra and cotton, low Zn treatments combined with high P supply induced P toxicity by enhancing the rate of P absorption into plant and by causing P to accumulate preferentially in leaves, apparently by depressing the export of P from the leaves (Marschner and Cakmak, 1986). In wheat, Zn deficiency had only a transitory effect in stimulating P absorption and induced P toxicity primarily through preferential accumulation of P in older leaves by depressing P export (Webb and Loneragan, 1990). We are not aware of any publication describing these effects for rice.

This Zn deficiency-induced P toxicity and imbalanced fertilization with P and Zn not only affects crop production, but could potentially also lead to phytate accumulation and further reduction of the Zn bioavailability to the consumers of the rice grains. No reports on Zn deficiency-induced phytate accumulation are known to us, however.

# 9 Zinc management options in rice-based systems

## 9.1 Soil application of zinc fertilizers

There are several factors that influence the effectiveness of Zn fertilizers. These include the concentration of Zn, impurities (e.g., presence of contaminants such as cadmium), water solubility, soil type and method of application. The application methods can be divided into foliar applied and soil supplied Zn fertilization.

There are several varieties of Zn fertilizers available. These include: $ZnSO_4$, ZnO, Zn-oxysulfates, ZnEDTA (a synthetic Zn chelate) and Zn-lignosulfonates (organically complexed Zn source). These Zn fertilizers differ in total Zn, water-soluble Zn concentration and composition (Table 1). Fertilizers with Zn sulfate, especially in its heptahydrate form ($ZnSO_4.7H_2O$), are the most commonly applied Zn fertilizers due to the higher solubility of Zn sulfate (Impa and Johnson-Beebout, 2012; Mortvedt and Gilkes, 1993). Mortvedt and Gilkes (1993) showed the importance of water solubility of Zn in fertilizers. Increasing the percentage of water-soluble Zn in fertilizers with the same amount of total Zn enhanced dry matter production of corn grown in soils with high pH.

***Table 1.*** *Selected Zn fertilizers and their formula, Zn content, water solubility and commonly applied soil types (adapted from Mortvedt and Gilkes [1993])*

| Zinc Source | Formula | Zn Content (%) | Solubility | Soil Type |
|---|---|---|---|---|
| Zn sulfate heptahydrate | $ZnSO_4.7H_2O$ | 22 | High | All soils |
| Zn sulfate monohydrate | $ZnSO_4.H_2O$ | 36 | High | All soils |
| Zn oxysulfate | $xZnSO_4$ $xZnO$* | 20-50 | Variable | Variable |
| Zn oxide | $ZnO$ | 72-80 | Very low | Acidic soils |
| Zn chloride | $ZnCl_2$ | 50 | High | All soils |
| Zn nitrate | $Zn(NO_3)_2.H_2O$ | 23 | High | All soils |
| ZnEDTA | $Na_2ZnEDTA$ | 8-14 | High | All soils |

* This type of fertilizer is sold in different $ZnSO_4/ZnO$ ratios.

The most widely used Zn fertilizer recommendation for rice is a basal application of 5-10 kg Zn $ha^{-1}$ as Zn sulfate (heptahydrate or monohydrate) per growing season. It is usually mixed with NPK fertilizers and incorporated into the soil during land preparation immediately prior to crop establishment or broadcast on the soil surface shortly thereafter (Impa and Johnson-Beebout, 2012).

The recovery fraction of Zn fertilizers (defined as ($Zn_{\text{taken up by fertilized crop}} - Zn_{\text{taken up by unfertilized control}}$) / $Zn_{\text{applied}}$ x 100%) is usually well below 10% in field studies (Naik and Das, 2008; Savithri et al., 1998; Slaton et al., 2005). This is caused by the interaction of fertilizer $Zn^{2+}$ with the soil's solid phase: fertilizer Zn is subject to the chemical equilibria in soils and is converted to forms unavailable to plants. Use of synthetic Zn chelates such as EDTA could partly overcome this problem. The effectiveness of a Zn on chelate depends on (1) its ability to remain in solution, (2) its susceptibility to competition from other metal ions, (3) its ability to deliver Zn to the plant, (4) its capacity to chelate Zn from the soil, either after having delivered a Zn ion to the plant (shuttle effect) or upon initial contact with the soil (cf. Schenkeveld et al., 2007) and (5) its susceptibility to degradation (Schenkeveld et al., 2012). This is a delicate balance: if the Zn-chelate complex is too stable, it may not dissociate at the root surface, inhibiting Zn uptake (Gramlich et al., 2013). But if the complex is too labile, it may be ineffective, and Zn may adsorb to the soil's solid phase. In a field experiment on calcareous soils the major part of the Zn applied as Zn-DTPA was adsorbed to soil organic matter within 7 days after application, after which this fraction declined again in favor of poorly available mineral-bound Zn (Lu et al., 2012). Splitting the fertilizer dose into smaller but more frequent amounts could increase the recovery.

Chemical equilibrium models could be a useful tool to predict the fate and the bioavailability of fertilizer Zn to improve the profitability of Zn application. Duffner et al. (2014) showed that multisurface models are able to accurately predict Zn speciation in soils with low Zn levels. The predicted $Zn^{2+}$ concentration was mainly controlled by adsorption, where organic matter was predicted to be the dominant soil sorbent. The predicted effect of Zn fertilization was very much soil dependent. In acid low Zn soils it could increase Zn in the soil solution by an order of magnitude, whereas in other soils the predicted effect of Zn fertilization was minor or even absent. These kinds of models need a considerable amount of soil input data. For routine use, they have to be simplified.

## 9.2 Foliar application of zinc

Foliar Zn application has the conceptual advantage of avoiding interactions of fertilizer Zn with the soil's solid phase, but also has its own challenges related to achieving leaf adsorption of the sprayed solution before it is washed off by rain. There are no data available regarding farmers' opinions on foliar Zn application, including increases in labor cost for the spraying operation. The best mode for such an approach therefore needs more research (Impa and Johnson-Beebout, 2012).

Zinc can be absorbed by leaf stomata when applied as foliar spray and then transported via the vascular system to where it is needed (Broadley et al., 2012). A number of Zn sources ($ZnSO_4$, $Zn(NO_3)_2$, Zn-EDTA) can be used as foliar fertilizers in a number of crops (Yoshida et al., 1970).

A positive effect of foliar application on grain Zn density in multiple field studies has been reported by Phattarakul et al. (2012). This study reports an average 25% increase in Zn density in brown rice after foliar application of Zn across a number of studies in different soils and with different cultivars and an average increase in polished (white) rice of 13% (against 7 and 1% after soil application in brown and white rice respectively). Nevertheless this seemingly large effect implies in absolute terms only an increase from 19.4 to 25.5 mg $kg^{-1}$ of dry rice. The effect of such an increase for consumers that depend mainly on rice, is still below the recommended level. Interestingly a high density rice genotype from IRRI (IR68144) tested in one of the 15 site x year combinations hardly reacted to fertilization and varied between 31.0 and 35.7 mg $kg^{-1}$. In other words the genetic improvement possible through such germplasm seems more relevant here than foliar application. At the same time these densities are not reported on more difficult soils. Wissuwa et al. (2008) reported that potential for enhanced grain Zn is not expressed on all soils, while also Mabesa et al. (2013) report genotypic differences in response to foliar Zn application. The physiology underlying this genotypic variation in response to foliar Zn application is unknown. There remains therefore the need for a further understanding how genetic potential should be harnessed through appropriate Zn management on different soils.

## 9.3 Seed treatments

Zinc application via seed treatment can in general be divided into seed priming and seed coating (Farooq et al., 2012). Seed priming consists of soaking seeds in solutions of different salts or for a specified time following by re-drying (Farooq et al., 2006; Rehman et al., 2011). In seed coating, a more or less continuous layer of finely ground solids or liquids containing dissolved or suspended solids are covering the seed (Farooq et al., 2012). Seed treatment is a relatively good option compared with soil application because relatively small amounts of Zn are needed (Welch and Graham, 1999). Zn-treated seed rice is available throughout the southern United States rice-growing area (Slaton et al., 2001).

Seed priming with solutions of Zn-EDTA and fritted Zn resulted in higher yield and Zn uptake as compared to broadcast or banded soil application (Kang and Okoro, 1976). A solution with 0.5% (w/v) Zn was suitable for direct-seeded rice. Slaton et al. (2001) reported better dry matter production and higher tissue Zn concentration and grain yields from rice seeds primed with Zn than those fertilized via soil-applied Zn on one location, but not at another. At the second location, soil fertilization and seed priming were equally effective. Rice seed priming, however, with a

4 mM Zn solution (0.03%) increased primed seed Zn content by a factor seven, but there were neither priming nor soil fertilization effects on yield or progeny seed Zn content, indicating that factors other than Zn may have been yield-limiting (Johnson et al., 2005).

Seed coating with Zn had no adverse effect on germination and may therefore provide an effective as well as economic means of preventing Zn deficiency and improving seedling establishment in soils with low Zn availability. Coating rice seeds with low concentrations of $ZnSO_4$ was equally effective as mixing $ZnSO_4$ with soil (Giordano and Mortvedt, 1973).

Hence, seed treatment with Zn is a promising method for aerobic rice to support early vigor and correct Zn deficiency even in calcareous or alkaline soils during crop establishment period. However, an increase in rice grain Zn concentration is rarely found upon seed treatment, especially under field conditions; further research is required to elucidate potential reasons and to clarify whether seed treatment x genotype interactions could play a role (see chapter 6).

## 9.4 Organic matter management

Soil organic matter plays a dual role in Zn bioavailability to crops: on the one hand, solid organic matter is the most important sorbent of Zn (section 4.2), reducing Zn bioavailability to the crop. On the other hand, dissolved organic matter can form complexes with Zn that enhance Zn solubility and Zn transport towards the root, regardless of whether this DOM originates from SOM (section 4.2) or from root exudation (section 5.1).

Although few experiments with proper factorial designs (organic matter x Zn) have been reported, the general picture emerging is that application of chemical Zn fertilizer in combination with organic matter seems[1] to increase the agronomic efficiency of Zn fertilizer. In a pot experiment the recovery fraction of Zn from $ZnSO_4$ increased from 1-2% to 2-5% when combined with compost, depending on the dosage, the increase being bigger with a lower dosage of Zn (Ahmad et al., 2012). Similar but more modest results were obtained when $ZnSO_4$ was combined with cellulose in pots (Mandal et al., 2000) or biosludge in a field experiment (Srivastava et al., 2009). Including green manures in systems where rice is rotated with wheat, for instance, could potentially increase Zn use efficiency. We are not aware, however, of any such type of experiments on low Zn soils (but see Mishra et al., 2006).

Addition of organic matter probably changes the speciation of Zn. Long-term application of compost, farmyard manure and sewage sludge not only increased the total Zn content of a soil, but also the percentage of “labile” Zn, which is supposed to be easily available for plant uptake (Santos et al., 2009), but contrasting effects have been obtained on the short term (Dias et al., 2003). It is not clear which mechanisms are responsible for this effect. Potentially a reduction in pH caused by decomposition of organic matter and Zn-complexating ligands produced by decomposing microorganisms could play a role (Altomare et al., 1999). Duffner et al. (2014) suggested that application of organic matter that does not contain any Zn would reduce plant available Zn because soil organic matter is a major Zn sorbent (section 4.2) but organic matter with a common Zn content of 80 mg Zn $kg^{-1}$ dw would increase bioavailable Zn. Different crop residues may have different effects, as different ligands are released in different proportions into the soil solution with their decomposition (Gramlich, 2013). In selecting organic matter to increase Zn use efficiency, this may have to be taken into account.

[1]In calculating the recovery fraction of Zn, it was assumed that the $ZnSO_4$ application rate was adjusted to the Zn content of the organic matter (compost, biosludge). If this assumption is incorrect, the recovery fraction may not have been increased.

## 9.5 Cropping system adaptations

There are few but inspiring examples of biofortification with Zn and Fe through intercropping (characterized by alternating rows of two or more crop species), reviewed by Zuo and Zhang (2009). Growing peanut plants in a mixture with maize enhanced the shoot concentrations of Zn nearly threefold in peanut (Inal et al., 2007). In this combination maize is thought to mobilize Zn through root exudation of phytosiderophores and/or rhizosphere acidification, from which peanut plants growing next to the maize plants can benefit as well.

Rice/rice intercropping systems do exist and are known to reduce the impact of diseases (Zhu et al., 2000). Potentially, intercropping an aerobic rice genotype that is well adapted to low soil supply, could increase the yield of another genotype that is desirable for another reason. We are not aware of any report on such hypothetical effects of intercropping on rice biofortification and it could be worthwhile to test for potential synergistic effect on Zn grain density of intercropping two genotypes varying in Zn acquisition capacity.

Increasing rooting density could also improve Zn nutrition of rice cultivated on low Zn soils (Rose et al., 2013). Higher rice plant density in a low Zn field has been shown to increase biomass production through improved Zn nutrition (Hoffland et al., 2006). This effect was attributed to a higher and more effective Zn-mobilizing concentration of LMWOA exudates. An alternative explanation could be a higher effectively of recapturing Zn-LMWOA complexes by neighbor plants with higher root density (Rose et al., 2013).

## 9.6 Options to reduce the phytic acid content of rice grains

It is possible to reduce the phytate concentration of seeds and grains by selection and breeding, or by P deficiency. Developing new varieties through plant breeding and genetic manipulation (Raboy, 2009) could contribute to an increase of Zn in the edible part of the grain. But one has also to keep in mind that phytate is the major P storage form in cereal grains and typically accounts for 65-85% of seed total P (Strother, 1980). Therefore a lower phytate concentration of seeds without an increase in other phosphate forms has been associated with various negative effects such as reduced seedling emergence and poor agronomic performance (Oltmans et al., 2005).

One approach to reduce the phytate content of cereal grains is the development of low phytate mutants by using chemical or physical mutation induction. Ren et al. (2007) showed that in a low phytate mutant the phyate P (PA-P) content was about 40% lower than in the parent line, whereas total P levels where not significantly different. Their results showed that the low phytate mutation could not only potentially increase the bioavailability but also the amount of Zn in the edible part of rice grains.

An improved nitrogen management is another option to decrease the phytate content and to increase the Zn concentrations both in the whole grain and the endosperm fraction of wheat (Kutman et al., 2011). Kutman et al. (2011) conducted a pot experiment with different soil applications of N and Zn and with or without foliar Zn spray. They showed that an increased N supply significantly enhanced the Zn concentrations in all grain fractions (~50%), especially in the endosperm (~80%) which is the most widely consumed part in many countries. High N also generally decreased the P/Zn ratios (~30%) in whole grain and endosperm and the phytate concentration. Their results from a greenhouse experiment are in line with the findings of recent field studies with the same objective (Cakmak et al., 2010a; Shi et al., 2010). Shi et al. (2010) showed that high N application increased Zn densities in all three milling fractions (flour, shorts, and bran) of the wheat grain. Bi et al. (2013) conducted a field experiment with six different rice cultivars and concluded that the combination of decreased plant P uptake and dilution effect of increased grain yield by N is proposed as underlying mechanism of the decreased grain P concentration by high N. There is some evidence from a pot study also that an enhanced N nutrition leads to a prolonged Zn uptake during grain filling of rice which might explain the improved grain Zn density (Van der Linden, 2012).

# 10 Sustainability of the production system and impact on the environment

## 10.1 Role of zinc fertilizer in improving soil health and maintaining soil production capacity

In areas where Zn deficiency is both a public health issue and an important soil constraint to crop production, like in India, enrichment of widely applied fertilizers with Zn would be an excellent opportunity for improving grain Zn while contributing to increased crop production (Cakmak, 2009). Recent work in India indicates that the use of Zn-enriched urea in rice may increase both grain Zn concentration by up to two- to threefold and grain production (Cakmak, 2009), comparable to observations made in long-term multi-site experiments on wheat in Anatolia, Turkey, in the 1990s. Cakmak (2008) stated that these results could be extrapolated worldwide and that the contribution to human health through Zn foliar fertilization of staple food crops seems to be a good tool to alleviate Zn deficiency-related problems. However, this statement is not underpinned yet by a quantitative data analysis. A meta-analysis would therefore give more quantitative insights.

## 10.2 Externalities causing threat to the environment and ecosystem functioning

Water scarcity will be one of the major economic, social, and ecological problems in Asia during the next decades (Bouman et al., 2002). In China, measures to save water have been introduced over the past 40 years now. During the same period, paddy rice production increased from 56 to 180 million tons per annum (FAO, 2013). So, despite these measures, the total water demand for rice continued to grow. Now, water scarcity affects large areas, and economic as well as agricultural developments are slowly forcing farmers to move to crops less water demanding than rice. Therefore rice production systems that do not rely on flooded soil are urgently needed. Bindraban et al. (2006) discuss options and opportunities for transforming inundated rice cultivation to comply with changing conditions. The main challenge will be to grow rice as well as any other irrigated cereal crop while maintaining its current production level.

Water-saving rice production methods have been developed in China and in other water-limited environments, for example, in Indonesia and in the Indo-Gangetic rice-wheat belt. In the North China Plain, the most promising options include alternating wetting and drying (AWD), aerobic rice and ground-cover rice production systems (GCRPS), also called “plastic film mulched dryland rice” (Belder et al., 2004; Belder et al., 2007; Liang et al., 1999). Ground-cover rice production systems have been reported to use only 40% of the amount of water usually needed to grow rice under flooded conditions, while grain yields remain at 90% of those of the high-yielding flooded systems (Liang et al., 1999).

The consequences of the shift from flooded to aerobic cultivation for the bioavailability of the Zn was described Gao et al. (2012) and Gao (2007). On calcareous soils with relatively high pH, the cultivation shift from flooded to aerobic conditions increased Zn deficiency problems. Geochemical modeling (Gao et al., 2009) showed that dissolved organic anions, pH and redox effects on other metal ions (potentially Fe, Mn, Ca) can greatly modify the effect of the shift on Zn in the soil solution. Also changes in root growth and rhizosphere conditions are interfering with the physical/chemical effects of aerobicity. The consequence of a cultivation shift for Zn bioavailability is a function of the changes of all these factors, whereas the relative importance of each factor will differ among soils.

# 11 Bioavailability of increased zinc content in rice and significance for human intake and health

Biofortification can target the poorest population segment that is most in need, but yet hardest to reach with other strategies. Biofortified crops, either through breeding programs or by agronomic adaptations, require high adoption by farmers to be effective, and this is an area that still needs further investigation (Bouis et al., 2011; Saltzman, 2012). The best example that biofortification for better nutrient content can be successful is the case of orange fleshed sweet potato with high β-carotene, which has widely been adopted by farmers in eastern and southern Africa (Low et al., 2007).

## 11.1 Food chain approaches

Slingerland et al. (2007; 2009) showed how biofortification can be improved in a food chain approach in West Africa and China, respectively. Such a food chain approach includes agronomic practices and storage, processing and food preparation, and diets. Slingerland et al. (2009) concluded that such a food chain approach is useful in advancing the biofortification approach by providing breeding programs with objectives that address the shifting of rice production to aerobic systems, the current processing methods of rice and the proportion of rice in a mixed diet. In addition, this food chain approach provides options for improving fertilizer application methods, for breeding targets related to Zn loading to grains and for processing for enhanced mineral bioavailability from brown rice grains.

The objective of a remarkable field-to-fork study done in four Bangladesh villages was to 'plug the nutrient leaks' in the rice-based food system at local level using a participatory approach (Mayer et al., 2001; Figure 5). They showed that if locally acceptable and feasible adjustments are made to milling, cooking and the choice of rice varieties, Zn intake can increase. Adjustments included were increasing bioavailable soil Zn, reducing the amount of Zn lost in milling and cooking and choosing rice varieties that have high Zn content (after milling). They demonstrated that unpolished rice Zn content was increased by 11% if the soil Zn is increased from below to above the critical level (0.8 ppm DTPA-Zn). Further increases of 15% and 16% could be realized by adjustments to milling and cooking, respectively. Selecting locally available high Zn varieties resulted in another increase in rice Zn content of 38% or 27%, depending on the village. Altogether, a promising increase in children's total dietary Zn of 64% was concluded feasible.

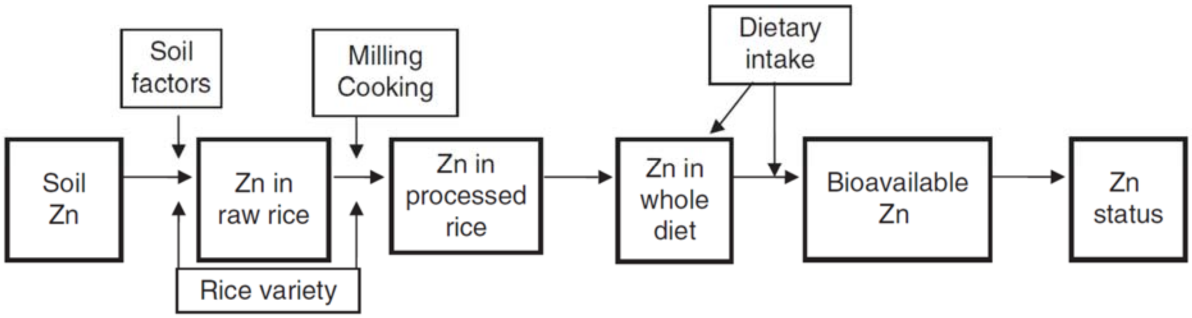


***Figure 5.*** *Schematic depiction of the passage of zinc from soil to humans. Copied from Mayer et al. (2001, p. 255).*

## 11.2 Zinc uptake from biofortified rice

Several studies have shown that Zn from biofortified rice is readily absorbed by humans. Zn absorption from different rice diets was assessed by Islam et al. (2013). They studied the Zn absorption from a diet containing either conventional rice or higher-Zn rice in Bangladeshi children using an isotope dilution technique with external labeling. Doing so, they measured the total absorbed Zn (TAZ) from mixed diets containing high-Zn rice (HZnR), conventional rice (CR), or CR plus Zn added to the grain (CR+Zn). Total dietary Zn was 3.83, 4.83 and 6.03 mg $d^{-1}$ when the children were fed the CR, HZnR and CR+Zn-containing diets, respectively. Mean fractional Zn absorption (FZA) from the CR diet was greater than from the HZnR diet (25.1 vs. 20.1%, $P < 0.001$), and the mean FZA from the CR+Zn diet (18.8%) was less than from both the CR diet ($P < 0.001$) and the HZnR diet ($P = 0.014$). The mean TAZ was 0.96 ± 0.16, 0.97± 0.18 and 1.13± 0.20 mg $d^{-1}$ from the CR, HZnR and CR +Zn diets, respectively. Total absorbed Zn was not different for the CR and HZnR diets ($P = 0.99$) but was significantly greater from the CR+Zn diet compared with the other two diets ($P < 0.001$). They concluded that rice cultivars with yet higher Zn and/or lower phytate content are needed to increase TAZ by young children consuming this amount of rice.

Brnic et al. (2013) compared Zn absorption from intrinsically isotope-labeled rice produced in a hydroponic system at high Zn levels to obtain a higher Zn density, with Zn absorption from the same cultivar but produced at normal Zn levels in the hydroponic system and extrinsically labeled with Zn fortificant (CR+Zn) in young healthy adults in Switzerland. In contrast to the study by Islam et al. (2013), they found the mean fractional Zn absorption of HZnR to be somewhat higher than that of CR+Zn (25.1 vs. 20.8 %, P=0.076), although just not statistically significant. They concluded that Zn from rice biofortified through fertilization is readily absorbed and comparable to Zn absorption from post-harvest Zn fortified rice.

## 11.3 Contribution of zinc-biofortified rice to reduce human zinc deficiency

There is evidence that Zn-biofortified rice has the potential to increase Zn intake in humans significantly. Arsenault et al. (2010) assessed if Zn inadequacy among children and women in rural Bangladesh could be ameliorated by dietary modification of rice by recalculating dietary Zn intake with inclusion of high-Zn rice. The percentage of children and women with total dietary Zn inadequacy decreased by over 50% with a simulated[2] high adoption rate of Zn-biofortified rice. In a comparable study, Qin et al. (2012) evaluated Zn biofortification of rice in China by studying Zn intake with four different dietary patterns: (1) the “traditional” pattern, characterized by high intakes of

[2] Dietary Zn intake was calculated assuming normal rice would be replaced by high Zn rice.

rice and fresh vegetables and low intake of wheat flour; (2) the "macho" pattern, characterized by intake of animal foods and alcohol, i.e., foods commonly eaten by men; (3) the "sweet tooth" pattern, characterized by intake of cake, milk, yoghurt and beverages; and (4) the "healthy" pattern, characterized by intake of whole grain products, fruits, root vegetables and fresh and pickled vegetables. They looked at the potential increase in dietary Zn intake when normal rice would be replaced by biofortified rice at an intermediate level of 27 µg/g of polished rice, or a high level of 38 µg/g of polished rice. They concluded that replacing normal rice with Zn-biofortified rice would reduce the proportion of Chinese adults with insufficient Zn intake from 15% to 6.5% for the intermediate biofortification level and to 4.4% at the high biofortification level, respectively. This effect was more pronounced for persons with a more "traditional" food pattern in whom insufficient intake was reduced to 1.6% and 0.7% at the intermediate and high level, respectively, but less so for subjects with a "sweet tooth" food pattern.

### 11.4 Processing

There have been several attempts to popularize brown rice consumption in Southeast Asia instead of milled rice. Brown rice is richer in fat, vitamins, protein and minerals than milled rice, but also richer in dietary fiber and phytate that may inhibit absorption of minerals such as iron and Zn. Diets with a phytate to Zn molar ratio (calculated as (mg phyate/660)/(mg Zn/65.4) which is greater than 15 inhibit Zn absorption severely and reduce absorption to ~15% from intake, whereas absorption from diets with a phytate to Zn molar ratio <5 is estimated to be ~50% (Hotz and Brown, 2007). The phytate to Zn molar ratio of refined rice is typically in the middle range of 5-15, classifying it as a moderately inhibitory matrix for Zn absorption, while for brown rice this ratio tends to be well above 15.

The influence of milling on Zn bioavailability was shown by Hunt et al. (2002). They studied the bioavailability of Zn from cooked Philippine milled, undermilled and brown rice in rats by using growth, bone Zn and $^{65}Zn$ retention. Milling reduced the phytic acid and mineral content of the rice, resulting in Zn concentrations of 16.5, 19.4 and 27.2 µg per gram of rice, and phytate/Zn molar ratios of 4, 20 and 28 for milled, undermilled and brown rice, respectively. Measured Zn bioavailability was similar whether using growth, bone Zn, or radioisotope retention as criteria, at approximately 92, 86 and 77% of added $ZnSO_4$, for milled, undermilled and brown rice, respectively. However, the higher percent bioavailability of the Zn after milling was insufficient to compensate for the lower Zn content. With respect to Zn, the nutritional value was inversely related to milling, providing approximately 15, 17 and 21 µg bioavailable Zn $g^{-1}$ rice, respectively, for milled, undermilled and brown rice.

## 12 Alternative approaches of resolving zinc deficiency in human nutrition

Zinc deficiency can be resolved through various other manners, such as supplementation, diet diversification or food fortification. In general, food-based approaches can be considered to be safer options as compared to supplementation, since the risk of excessive intake is minimal due to the natural barrier provided by the volume and energy content of food. Moreover, although supplementation is efficacious on the short-term, food-based approaches are more sustainable.

## 12.1 Zinc supplementation

Zinc supplementation has been proven to be effective in enhancing growth of children (Brown et al., 2009; Brown et al., 2002), decreasing morbidity from diarrhea and pneumonia (Bhutta et al., 1999; Brown et al., 2009) and decreasing mortality (Brown et al., 2009; Jones et al., 2003). The World Health Organization recommends supplemental Zn to be given to children presenting with diarrhea in hospitals, in addition to oral rehydration therapy, as a means to enhance recovery (WHO, 2004). This recommendation has been implemented in most countries in Africa and South East Asia but does not reach the general population at large.

Micronutrient powders, or ‘sprinkles,’ containing a vitamin and mineral mixture including Zn that can be added to infant food, are recommended to be used on a daily basis in areas where childhood malnutrition is highly prevalent (WHO, 2011). However, a recent study from Pakistan showed that daily provision of sprinkles, either with or without Zn, increased the incidence and duration of diarrhea episodes (Soofi et al., 2013). Therefore, the safety of this strategy is currently under debate.

Although supplementation is an efficacious strategy to improve nutritional status, the coverage and scale of such programs is often poor (Jones et al., 2003). Effective supplementation programs require a reliable health system, but in practice programs mostly depend heavily on foreign aid.

## 12.2 Dietary diversification

Dietary diversification, particularly inclusion of animal-derived foods (beef and liver) in the daily diet, can potentially improve dietary Zn intake (Gibson and Anderson, 2009). However, accessibility to animal foods is usually low in food insecure areas where Zn deficiency is common. Therefore, this would provide little prospect to improve Zn status, unless drastic changes in the food system can be achieved.

## 12.3 Food fortification

Food fortification can be very efficacious, for example with iodization of salt (Andersson et al., 2012) or addition of iron to flour (Andang’o et al., 2007), although inhibition of absorption by food components such as phytate will be of concern (WHO/FAO, 2006). In contrast to supplementation, food fortification does not rely on the health system, but it requires some form of food processing for fortificant to be added, and people should have the purchasing power to buy such foods. For the very poor, this is rarely an option.

Zinc is not yet routinely incorporated in large scale food fortification programs. The efficacy to improve Zn status, growth, morbidity or mortality has been investigated, but generally showed variable results (Hess and Brown, 2009). Extruded fortified rice grain has been developed as a means to fortify rice by mixing extruded rice grains with normal rice and was shown to improve Zn status of school children in Thailand (Pinkaew et al., 2013). One of the major obstacles for the evaluation of this strategy is the lack of a reliable biomarker to measure Zn status in humans.

## 12.4 Alternative biofortification strategies

Zinc content in crops other than rice can potentially also be increased through breeding and selection processes, or by optimizing agronomic measures, as for example in wheat (Cakmak et al., 2010). After rice, wheat is the cereal mostly consumed in Asia. Recently, also high Zn varieties of pearl millet have been identified by ICRISAT, India, and are currently being evaluated for their potential to contribute to better Zn nutrition in humans (Rai et al., 2013).

# 13 Conclusions and vision

There are ample, promising and acceptable options to eliminate Zn deficiency in rice-based systems. A distinction should be made between the rice *production* systems and the rice-based *food system*. While the former focusses on production alone, the latter includes both the production and the processing and consumption of rice and other food items. Eliminating deficiency in the rice production system implies a combination of breeding for Zn deficiency tolerance at cultivar level and Zn bioavailability enhancing soil management. To alleviate Zn deficiency at the level of the human population in rice-based food systems, additional steps are needed. Cultivars should genetically have the potential to accumulate a high Zn density in the rice endosperm, so it will able to both take-up Zn and to use it efficiently for production and allocate more of plant Zn to the endosperm. Here also foliar fertilization could play an important role.

Our understanding of the underlying genetics and physiology in rice is growing, and there seems room to design accompanying fertilization systems that would make Zn more bioavailable. These should include a decision support tool to assess the agronomic feasibility of Zn fertilization, proper and timely combinations of N and Zn fertilizer to promote prolonged Zn uptake during the grain filling stage and P fertilizer fortification with Zn to avoid imbalanced Zn and P fertilization as this would lead to enhanced levels of phytate beyond what is necessary for crop performance and good for human consumers.

Major contributions to elimination of Zn deficiency in the rice production system can be expected from a combination of:

- Increased efficiency of soil and fertilizer management. Targeting soil fertilizers to fields where they are effectively increasing rice yield and grain quality can be done by combining current knowledge on soil chemistry and a meta-analysis on observed effects in the field into a decision support tool.
- Evaluation of contributions of breeding for high Zn grain and seed Zn and foliar Zn application and their interaction.
- Balancing N, P and Zn fertilization to increase yield and grain quality, possibly through enrichment of N and/or P fertilizers. Results from studies on other grains (wheat, barley) should be validated for rice.

Obviously, there is no “one size fits all” solution. The optimal approach is context-dependent and depends, for instance on the soil (on some soils the crop responds to soil fertilization, on others it does not), access of farmers to fortified or improved seeds (do they buy seeds at all?) or fortified P fertilizers, options to reduce Zn losses during rice processing and food preparations, feasibility of other interventions, etc. In addition to that, there are still too many uncertainties. The current state-of-the-art research, though, provides a strong basis for tangible, tailor-made strategies.

For people depending on local rice-based food systems, optimization is possible by combining early Zn fertilization to have the crop perform at its maximum combined with soil management and (foliar) fertilization strategies and rice genotypes that have next to all other desired traits high grain endosperm loading of Zn. Further quantitative food system simulation and intervention studies will be needed to see how much can be gained by the above-mentioned steps and how much would still be needed by innovating other aspects of the food system. These studies have to be done against a background of an increasing world population, with an increasing demand for water and a consequent need for highly productive water-saving rice cultivation systems.

VFRC could play a role in supporting relevant research on the fertilization strategies and the linkage of such research with the physiology and breeding studies to have an integral improvement of the Zn delivery by rice-based food systems. Farming systems optimization to increase absorbable Zn in the edible rice grain should target genotype x environment x management interactions. The focus of VFRC could be on creating the best conditions for phenotypic expression of desirable crop traits.

# 14 References


Ahmad, H.R., T. Aziz, S. Hussain, M. Akraam, M. Sabir, S.U.R. Khashif and M.M. Hanafi, 2012.
Zinc-enriched farm yard manure improves grain yield and grain zinc concentration in rice grown on a saline-sodic soil. *Int. J. Agric. Biol.* 14:787-792.

Alloway, B.J., 2008.
Zinc in soils and crop nutrition. IZA Publications. IZA, Brussels.

Alloway, B.J., 2009.
Soil factors associated with zinc deficiency in crops and humans. *Environ. Geochem. Health* 31:537-548

Altomare C., W.A. Norvell, T. Björkman and G.E. Harman, 1999.
Solubilization of phosphates and micronutrients by the lant-growth-promoting and biocontrol ungus *Trichoderma harzianum* Rifai 1295-22. Appl. Environ. Microbiol. 65:2926-2933.

Andang'o P.E., S.J. Osendarp, R. Ayah, C.E. West, D.L. Mwaniki, C.A. de Wolf, R. Kraaijenhagen, F.J. Kok and H. Verhoef 2007.
Efficacy of iron-fortified whole maize flour on iron status of schoolchildren in Kenya: a randomised controlled trial. *Lancet* 369 (9575):1799-806.

Andersson, M., V. Karumbunathan and M.B. Zimmermann, 2012.
Global iodine status in 2011 and trends over the past decade. *J Nutr.* 142:744-50.

Arnold, T., G.J.D. Kirk, M. Wissuwa, M. Frei, F.J. Zhao, T.F.D. Mason and D.J. Weiss, 2010.
Evidence for the mechanisms of zinc uptake by rice using isotope fractionation. *Plant Cell Environ.* 33:370-381.

Arsenault, J.E., E.A. Yakes, M.B. Hossain, M.M. Islam, T. Ahmed, C. Hotz, B. Lewis, A.S. Rahman, K.M. Jamil and K.H. Brown, 2010.
The current high prevalence of dietary zinc inadequacy among children and women in rural Bangladesh could be substantially ameliorated by zinc biofortification of rice. *J. Nutr.* 140:1683-1690.

Awad, F. and V. Römheld, 2000.
Mobilization of heavy metals from contaminated calcareous soils by plant born, microbial and synthetic chelators and their uptake by wheat plants. *J. Plant Nutr.* 23:1847-1855.

Belder, P., B.A.M. Bouman, R. Cabangon, L. Guoan, E.J.P. Quilang, L. Yuanhua, J.H.J. Spiertz and T.P. Tuong, 2004.
Effect of water-saving irrigation on rice yield and water use in typical lowland conditions in Asia. *Agr. Water Manage.* 65:193-210.

Belder, P., B.A.M. Bouman and J.H.J. Spiertz, 2007.
Exploring options for water savings in lowland rice using a modelling approach. *Agricult. Sys.* 92:91-114.

Bhutta, Z.A., R.E. Black, K.H. Brown, J. Meeks Gardner, S. Gore, A. Hidayat, F. Khatun, R. Martorell, N.X. Ninb, M.E. Penny, J.L. Rosado, S.K. Roy, M. Ruel, S. Sazawal and A. Shankar, 1999.
Prevention of diarrhea and pneumonia by zinc supplementation in children in developing countries: Pooled analysis of randomized controlled trials. *J. Pediatr.* 135:689-697.

Bi, J., Z. Liu, Z. Lin, M. Alim, M.A. Rehmani, G. Li, Q. Wang, S. Wang and Y. Ding, 2013.
Phosphorus accumulation in grains of japonica rice as affected by nitrogen fertilizer. *Plant Soil* 369:231-240.

Bindraban, P.S., H. Hengsdijk, W. Cao, Q. Shi, T.M. Thiyagarajan, W. Van Der Krogt and I.P. Wardana, 2006.
Transforming inundated rice cultivation. *Int. J. Water Resour. Dev.* 22:87-100.

Black, R.E., L.H. Allen, Z.A. Bhutta, L.E. Caulfield, M. de Onis, M. Ezzati, C. Mathers and J. Rivera, 2008. Maternal and child undernutrition: global and regional exposures and health consequences. *The Lancet* 371:243-260.

Bouman, B.A.M., H. Hengstdijk, B. Hardy, P.S. Bindraban, T.P. Tuong and J.K. Ladha, 2002. *Water-wise rice production*. IRRI, Metro Manila.

Bouman, B.A.M., S. Peng, A.R. Castañeda and R.M. Visperas, 2005. Yield and water use of irrigated tropical aerobic rice systems. *Agr. Water Manage.* 74:87-105.

Brnic, M., R. Wegmueller, A. Melse-Boonstra, C. Zeder, F.M. Tay and R.F. Hurrell, 2013. Zinc absorption from rice is similar when zinc is intrinsically increased through biofortification or extrinsically added as $ZnSO_4$. *Ann. Nutr. Metab.* 63:206-206.

Broadley, M., P. Brown, I. Cakmak, Z. Rengel, F. Zhao and M. Petra, 2012. Function of nutrients: micronutrients. In: P Marschner (ed) *Marschner's Mineral Nutrition of Higher Plants (Third Edition)*, pp. 191-248, Academic Press, San Diego.

Broadley, M.R., P.J. White, J.P. Hammond, I. Zelko and A. Lux, 2007. Zinc in plants: Tansley review. *New Phytol.* 173:677-702.

Brown, K.H., J.M. Peerson, S.K. Baker and S.Y. Hess, 2009. Preventive zinc supplementation among infants, preschoolers, and older prepubertal children. *Food Nutr. Bull.* 30:S12-S40.

Brown, K.H., J.M. Peerson, J. Rivera and.H. Allen, 2002. Effect of supplemental zinc on the growth and serum zinc concentrations of prepubertal children: A meta-analysis of randomized controlled trials 1-3. *Am. J. Clin. Nutr.* 75:1062-1071.

Bouis, H.E., C. Hotz, B. McClafferty, J.V. Meenakshi and W.H. Pfeiffer, 2011. Biofortification: a new tool to reduce micronutrient malnutrition. *Food Nutr Bull*. 1 (Suppl):S31-40.

Cakmak, I., 2008. Enrichment of cereal grains with zinc: Agronomic or genetic biofortification? *Plant Soil* 302:1-17.

Cakmak, I., 2009. Enrichment of fertilizers with zinc: An excellent investment for humanity and crop production in India. *J. Trace Elem. Med. Biol.* 23:281-289.

Cakmak, I., 2012. HarvestPlus zinc fertilizer project: HarvestZinc. *Better Crops* 96:17-19.

Cakmak, I. and H. Marschner, 1986. Mechanism of phosphorus-induced zinc deficiency in cotton. I. Zinc deficiency-enhanced uptake rate of phosphorus. Physiol. Plant. 68:483-490.

Cakmak, I. and H. Marschner, 1987. Mechanism of phosphorus-induced zinc deficiency in cotton. III. Changes in physiological availability of zinc in plants Is mail. *Physiol. Plant.* 70:13-20.

Cakmak, I., N. Sari, H. Marschner, H. Ekiz, M. Kalayci, A. Yilmaz and H.J. Braun, 1996. Phytosiderophore release in bread and durum wheat genotypes differing in zinc efficiency. *Plant Soil* 180:183-189.

Cakmak, I., B. Erenoglu, K.Y. Gülüt, R. Derici and V. Römheld, 1998. Light-mediated release of phytosiderophores in wheat and barley under iron or zinc deficiency. *Plant Soil* 202:309-315.

Cakmak, I., M. Kalayci, Y. Kaya, A.A. Torun, N. Aydin, Y. Wang, Z. Arisoy, H. Erdem, A. Yazici, O. Gokmen, L. Ozturk and W.J. Horst, 2010a. Biofortification and localization of zinc in wheat grain. *J. Agric. Food Chem.* 58:9092-9102.

Cakmak, I., W.H. Pfeiffer and B. McClafferty, 2010b.
Biofortification of durum wheat with zinc and iron. *Cereal Chem.* 87:10-20.

Cavagnaro T.R., 2008.
The role of arbuscular mycorrhizas in improving plant zinc nutrition under low soil zinc concentrations: a review. *Plant Soil* 304:315-325.

Dell'mour, M., G. Koellensperger, J.P. Quirino, P.R. Haddad, C. Stanetty, E. Oburger, M. Puschenreiter and S. Hann, 2010.
Complexation of metals by phytosiderophores revealed by CE-ESI-MS and CE-ICP-MS. *Electrophoresis* 31:1201-1207.

Dias, A.C.B., M.A. Pavan, M. Miyazawa and D.C. Zocoler, 2003.
Plant residues: Short term effect on sulphate, borate, zinc and copper adsorption by an acid oxisol. Brazilian Arch. Biol. Technol. 46:199-202.

Dobermann, A. and T. Fairhurst, 2000.
*Rice: Nutrient disorders and nutrient management*. Potash & Phosphate Institute, Singapore.

Duffner, A., E. Hoffland and E.M. Temminghoff, 2012.
Bioavailability of zinc and phosphorus in calcareous soils as affected by citrate exudation. *Plant Soil* 361:165-175.

Duffner, A., E. Hoffland, L.P. Weng and S.A.T.M. van der Zee, 2013.
Predicting zinc bioavailability to wheat improved by integrating pH dependent nonlinear root surface adsorption. *Plant Soil* 373:919-930.

Duffner, A., E. Hoffland, L.P. Weng and S.A.T.M. van der Zee, 2014.
A multisurface model to predict $Zn^{2+}$ activity and assess management options in low Zn soils. *Environ. Sci. Technol.*: under review.

Emsley, J., 2003.
*Nature's building blocks : an A-Z guide to the elements*. Oxford University Press, Oxford; New York.

Fan, M.S., F.J. Zhao, S.J. Fairweather-Tait, P.R. Poulton, S.J. Dunham and S.P. McGrath, 2008.
Evidence of decreasing mineral density in wheat grain over the last 160 years. J. Trace Elem. Med. Biol. 22:315-324.

FAO, 2013.
FAOStat available online at http://faostat.fao.org/.

Farooq, M., S.A. Barsa and A. Wahid, 2006.
Priming of field-sown rice seed enhances germination, seedling establishment, allometry and yield. *Plant Growth Regul.* 49:285-294.

Farooq, M., A. Wahid and K.H.M. Siddique, 2012.
Micronutrient application through seed treatments: a review. *J. Soil Sci. Plant Nutr.* 12:125-142.

Gamuyao, R., J. H. Chin, J. Pariasca-Tanaka, P. Pesaresi, S. Catausan, C. Dalid, I. Slamet-Loedin, E.M. Tecson-Mendoza, M. Wissuwa and S. Heuer, 2012.
The protein kinase Pstol1 from traditional rice confers tolerance of phosphorus deficiency. *Nature* 488:535-541.

Gao, X.P., C.Q. Zou, F.S. Zhang, S.E.A.T.M. van der Zee and E. Hoffland, 2005.
Tolerance to Zn deficiency in rice in relation with Zn uptake. *Plant Soil* 278:253-262.

Gao, X.P., 2007.
Bioavailability of Zinc to Aerobic Rice. PhD Thesis. Wageningen University.

Gao, X.P., E. Hoffland, T.J. Stomph, C.A. Grant, C. Zou and F. Zhang, 2012.
Improving zinc bioavailability in transition from flooded to aerobic rice. A review. *Agron. Sustain. Dev.* 32:465-478.

Gao, X.P., T.W. Kuyper, C.Q. Zou, F.S. Zhang and E. Hoffland, 2007.
Mycorrhizal responsiveness of aerobic rice genotypes is negatively correlated with their zinc uptake when nonmycorrhizal. *Plant Soil* 290:283-291.

Gao, X.P., T.J. Schröder, E. Hoffland, C.Q. Zou, F.S. Zhang and S.E.A.T.M. van der Zee, 2009.
Geochemical modeling of zinc bioavailability for rice. *Soil Sci. Soc. Am. J.* 74:301-309.

Gao, X.P., F.S. Zhang and E. Hoffland, 2009.
Malate exudation by six aerobic rice genotypes varying in zinc uptake efficiency. *J. Environ. Qual.* 38:2315-2321.

Gao, X.P, C.Q. Zou, X. Fan, F.S. Zhang and E. Hoffland, 2006.
From flooded to aerobic conditions in rice cultivation: Consequences for zinc uptake. *Plant Soil* 280:41-47.

Gavin, D.F, R.M. Welch and J.W. Finley, 2008.
Historical shifts in the seed mineral micronutrient concentration of US hard red winter wheat germplasm. *J. Sci. Food Agric.* 86:2213-2220.

Gianquinto, G., A. Abu-Rayyan, L. Di Tola, D. Piccotino and B. Pezzarossa, 2000.
Interaction effects of phosphorus and zinc on photosynthesis, growth and yield of dwarf bean grown in two environments. *Plant Soil* 220:219-228.

Gerrior, S.A., 2002.
The nutrient and anthropometric status of physically active and inactive older adults. *J. Nutr. Educ. Behav.* 34:S5-S13.

Gibson, R.S. and V.P. Anderson, 2009.
A review of interventions based on dietary diversification or modification strategies with the potential to enhance intakes of total and absorbable zinc. *Food Nutr Bull* 30:S108-S143.

Giordano, P.M. and J.J. Mortvedt, 1973.
Zinc sources and methods of application for rice. *Agron. J.* 65:51-53.

Graham, R.D., J.S. Ascher and S.C. Hynes, 1992.
Selecting zinc-efficient cereal genotypes for soils of low zinc status. *Plant Soil* 146:241-250.

Graham, R.D., R.M. Welch, D.L. Grunes, E.E. Cary and W.A. Norvell, 1987.
Effect of zinc deficiency on the accumulation of boron and other mineral nutrients in barley. *Soil Sci. Soc. Am. J.* 51:652-657.

Graham, R.D., R.M. Welch, D.A. Saunders, I. Ortiz-Monasterio, H.E. Bouis, M. Bonierbale, S. de Haan, G. Burgos, G. Thiele, R. Liria, C.A. Meisner, S.E. Beebe, M.J. Potts, M. Kadian, P.R. Hobbs, R.K. Gupta and S. Twomlow, 2007.
Nutritious subsistence food systems. *Adv. Agron.* 92:1-74.

Gramlich, A., 2013.
The influence of organic ligands on zinc availability to wheat. PhD Thesis. ETH Zürich.

Gramlich, A., S. Tandy, E. Frossard, J. Eikenberg and R. Schulin, 2013.
Availability of zinc and the ligands citrate and histidine to wheat: Does uptake of entire complexes play a role? J. Agric. Food Chem. 61:10409-10417.

Gregorio, G.B., D. Senadhira, H. Htut and R.D. Graham, 2000.
Breeding for trace mineral density in rice. *Food Nutr. Bull.* 21:382-386.

Guerinot, M.L., 2000.
The ZIP family of metal transporters. *Biochim. Biophys. Acta Biomembr.* 1465:190-198.

Hacisalihoglu, G., J.J. Hart and L.V. Kochian, 2001.
High- and low-affinity zinc transport systems and their possible role in zinc efficiency in bread wheat. *Plant Physiol.* 125:456-463.
Hajiboland, R., N. Aliasgharzad and R. Barzeghar, 2009.
Influence of arbuscular mycorrhizal fungi on uptake of Zn and P by two contrasting rice genotypes. *Plant, Soil and Environment* 55:93-100.
Hajiboland, R., X.E. Yang, V. Römheld and G. Neumann, 2005.
Effect of bicarbonate on elongation and distribution of organic acids in root and root zone of Zn-efficient and Zn-inefficient rice (Oryza sativa L.) genotypes. *Environ. Exp. Bot.* 54:163-173.
Hambidge, M., 2000.
Human zinc deficiency. *J. Nutr.* 130:1344-1349.
Hart, J.J., R.M. Welch, W.A. Norvell and L.V. Kochian, 2002.
Transport interactions between cadmium and zinc in roots of bread and durum wheat seedlings. *Physiol. Plant.* 116:73-78.
Hess, S.Y. and K.H. Brown, 2009.
Impact of zinc fortification on zinc nutrition. *Food Nutr. Bull.* 30:S79-S107.
Hetrick, B.A.D., G.W. Wilson and T.S. Cox, 1992.
Mycorrhizal dependence of modern wheat varieties, landraces, and ancestors. *Can. J. Bot.* 70:2032-2040.
Hoffland, E., C. Wei and M. Wissuwa, 2006.
Organic anion exudation by lowland rice (Oryza sativa L.) at zinc and phosphorus deficiency. *Plant Soil* 283:155-162.
Hotz, C. and K.H. Brown, 2007.
Assessment of the risk of zinc deficiency in populations and options for its control. *Food Nutr. Bull.* 25 (suppl 2):S91-204.
Hu, X.Y. and X.Y. Li, 2009.
Bioavailability of Zn as affected by root-induced changes of pakchoi cultivars with different Zn efficiencies. *J. Soils Sed.* 9:188-193.
Huang, C., S.J. Barker, P. Langridge, F.W. Smith and R.D. Graham, 2000.
Zinc deficiency up-regulates expression of high-affinity phosphate transporter genes in both phosphate-sufficient and -deficient barley roots. *Plant Physiol.* 124:415-422.
Hunt, J.R., L.K. Johnson and B.O. Juliano, 2002.
Bioavailability of zinc from cooked philippine milled, undermilled, and brown rice, as assessed in rats by using growth, bone zinc, and zinc-65 retention. *J. Agric. Food Chem.* 50:5229-5235.
Impa, S.M. and S.E. Johnson-Beebout, 2012.
Mitigating zinc deficiency and achieving high grain Zn in rice through integration of soil chemistry and plant physiology research. *Plant Soil* 361:3-41.
Impa, S.M., A. Gramlich, S. Tandy, R. Schulin, E. Frossard and S.E.J. Beebout, 2013a.
Internal Zn allocation influences Zn deficiency tolerance and grain Zn loading in rice (Oryza sativa L.). *Front. Plant Sci.* 4:534 510.
Impa, S.M., M.U. Morete, A.M. Ismail, R. Schulin and S.E. Johnson-Beebout, 2013b.
Zn uptake, translocation and grain Zn loading in rice (Oryza sativa L.) genotypes selected for Zn deficiency tolerance and high grain Zn. *J. Exp. Bot.* 64:2739-2751.

Islam, M.M., L.R. Woodhouse, M.B. Hossain, T. Ahmed, M.N. Huda, T. Ahmed, J.M. Peerson, C. Hotz and K.H. Brown, 2013.
Total zinc absorption from a diet containing either conventional rice or higher-zinc rice does not differ among Bangladeshi preschool children. *J. Nutr.* 143:519-525.

Jansa, J., A. Mozafar and E. Frossard, 2003.
Long-distance transport of P and Zn through the hyphae of an arbuscular mycorrhizal fungus in symbiosis with maize. *Agronomie* 23:481-488.

Jiang, W., P.C. Struik, J. Lingna, H. Van Keulen, Z. Ming and T.J. Stomph, 2007.
Uptake and distribution of root-applied or foliar-applied $^{65}$Zn after flowering in aerobic rice. *Ann. Appl. Biol.* 150:383-391.

Jiang, W., P.C. Struik, H. Van Keulen, M. Zhao, L.N. Jin and T.J. Stomph, 2008a.
Does increased zinc uptake enhance grain zinc mass concentration in rice? *Ann. Appl. Biol.* 153:135-147.

Jiang, W., P.C. Struik, M. Zhao, H. Van Keulen, T.Q. Fan and T.J. Stomph, 2008b.
Indices to screen for grain yield and grain-zinc mass concentrations in aerobic rice at different soil-Zn levels. *NJAS - Wageningen J. Life Sci.* 55:181-197.

Johnson, S.E., J.G. Lauren, R.M. Welch and J.M. Duxbury, 2005.
A comparison of the effects of micronutrient seed priming and soil fertilization on the mineral nutrition of chickpea (*Cicer arietinum*), lentil (*Lens culinaris*), rice (*Oryza sativa*) and wheat (*Triticum aestivum*) in Nepal. *Exp. Agric.* 41:427-448.

Johnson-Beebout, S.E., J.G. Lauren and J.M. Duxbury, 2009.
Immobilization of zinc fertilizer in flooded soils monitored by adapted DTPA soil test. *Commun. Soil Sci. Plant Anal.* 40:1842-1861.

Johnson, A.A.T., B. Kyriacou, D.L. Callahan, L. Carruthers, J. Stangoulis, E. Lombi and M. Tester, 2011.
Constitutive overexpression of the OsNAS gene family reveals single-gene strategies for effective iron- and zinc-biofortification of rice endosperm. *PLoS ONE* 6:e24476.

Jones, G., R.W. Steketee, R.E. Black, Z.A. Bhutta and S.S. Morris, 2003.
How many child deaths can we prevent this year? *Lancet* 362:65-71.

Kabata-Pendias, A. and H. Pendias, 1984.
*Trace elements in soils and plants*. CRC Press, Boca Raton.

Kalis, E.J.J., E.J.M. Temminghoff, A. Visser and W.H. van Riemsdijk, 2007.
Metal uptake by *Lolium perenne* in contaminated soils using a four-step approach. *Environ. Toxicol. Chem.* 26:335-345.

Kang, B.T. and E.G. Okoro, 1976.
Response of flooded rice grown on a vertisol from northern nigeria to zinc sources and methods of application. *Plant Soil* 44:15-25.

Kittrick, J.A., 1976.
Control of $Zn^{2+}$ in the Soil Solution by Sphalerite. *Soil Sci. Soc. Am. J.* 40:314-317.

Kutman, U.B., B. Yildiz and I. Cakmak, 2011.
Improved nitrogen status enhances zinc and iro.n concentrations both in the whole grain and the endosperm fraction of wheat. *J. Cereal Sci.* 53:118-125

Kutman, U.B., B.Y. Kutman, Y. Ceylan, E.A. Ova and I. Cakmak, 2012.
Contribution of root uptake and remobilization to grain zinc accumulation in wheat depending on post-anthesis Zn availability and nitrogen nutrition. *Plant Soil* 361:177-187.

Lambers, H., C. Mougel, B. Jaillard and P. Hinsinger, 2009.
Plant-microbe-soil interactions in the rhizosphere: An evolutionary perspective. *Plant Soil* 321:83-115

Lehmann, A., S.D. Veresoglou, E.F. Leifheit and M.C. Rillig, 2014.
Arbuscular mycorrhizal influence on zinc nutrition in crop plants – A meta-analysis. *Soil Biol. Biochem.* 69:123-131.

Liang, J., 2007.
Iron, zinc and phytic acid in rice from China: wet and dry processing towards improved mineral bioavailability. PhD thesis. Wageningen University.

Liang, J., B.Z. Han, L. Han, M.J.R. Nout and R.J. Hamer, 2007.
Iron, zinc and phytic acid content of selected rice varieties from China. *J. Sci. Food Agric.* 87:504-510

Liang, Y.C., F. Hu, M.C. Yang, X.L. Zhu, G.P. Wang and Y.L. Wang, 1999.
Mechanisms of high yield and irrigation water use efficiency of rice in plastic film mulched dryland. *Sci Agric Sin* 32:26-32.

Lindsay, W.L., 1972.
Zinc in Soils and Plant Nutrition. *Adv. Agron.* 24:147-186.

Lindsay, W.L., 1979.
*Chemical equilibria in soils*. Wiley, New York.

Lindsay, W.L. and W.A. Norvell, 1978.
Development of a DTPA soil test for zinc, iron, manganese, and copper. *Soil Sci. Soc. Am. J.* 42:421-428

Lombi, E., K.G. Scheckel, J. Pallon, A.M. Carey, Y.G. Zhu and A.A. Meharg, 2009.
Speciation and distribution of arsenic and localization of nutrients in rice grains. *New Phytol.* 184:193-201.

Loneragan, J.F., T.S. Grove, A.D. Robson and K. Snowball, 1979.
Phosphorus toxicity as a factor in zinc-phosphorus interactions in plants. *Soil Sci. Soc. Am. J.* 43:966-972.

Lönnerdal, B., 2002.
Phytic acid-trace element (Zn, Cu, Mn) interactions. *Int. J. Food Sci. Technol.* 37:749-758.

Lott, J. and M. Buttrose, 1978.
Globoids in protein bodies of legume seed cotyledons. *Funct. Plant Biol.* 5:89-111.

Low J.W., M. Arimond, N. Osman, B. Cunguara, F. Zano and D. Tschirley, 2007.
Ensuring the supply of and creating demand for a biofortified crop with a visible trait: lessons learned from the introduction of orange-fleshed sweet potato in drought-prone areas of Mozambique. *Food Nutr. Bull.* 28 (2 Suppl):S258-70.

Lu, X., Cui J., X. Tian, J.E. Ogunniyi, W. J. Gale and A. Zhao, 2012.
Effects of zinc fertilization on zinc dynamics in potentially zinc-deficient calcareous soil. Agron. J. 104:963-969.

Nubé, M., R.L. Voortman, 2011.
Human micronutrient deficiencies: Linkages with micronutrient deficiencies in soils, crops and animal nutrition. In: B Thompson, L. Amoroso (eds) Combating Micronutrient Deficiencies: Food-based Approaches, pp. 289-311, CAB International Wallingford and FAO Rome.

Mabesa, R.L., S.M. Impa, D. Grewal and S.E. Johnson-Beebout, 2013.
Contrasting grain-Zn response of biofortification rice (Oryza sativa L.) breeding lines to foliar Zn application. *Field Crop Res.* 149:223-233.

Marschner H., 2002.
Mineral nutrition of higher plants. 2nd Edition. Academic Press London, San Diego. 889 pp.

Mandal, B. and L.N. Mandal, 1990.
Effect of phosphorus application on transformation of zinc fraction in soil and on the zinc nutrition of lowland rice. *Plant Soil* 121:115-123.

Mandal, B., G.C. Hazra and L. N. Mandal, 2000.
Soil management influences on zinc desorption for rice and maize nutrition. *Soil Sci. Soc. Am. J.* 64:1699-1705.

Mayer, A.B., M.C. Latham, J.M. Duxbury, N. Hassan and E.A. Frongillo, 2011.
A food systems approach to increase dietary zinc intake in Bangladesh based on an analysis of diet, rice production and processing. In: B Thompson, L. Amoroso (eds) *Combating Micronutrient Deficiencies: Food-based Approaches*, pp. 254-267, CAB International Wallingford and FAO Rome.

Mazzolini, A.P., C.K. Pallaghy and G.J.F. Legge, 1985.
Quantitative microanalysis of Mn, Zn and other elements in mature wheat seed. *New Phytol.* 100:483-509.

Mishra, B.N., R. Prasad, B. Gangaiah and B. G. Shivakumar, 2006.
Organic manures for increased productivity and sustained supply of micronutrients Zn and Cu in a rice-wheat cropping system. J. Sustain. Agric. 28:55-66.

Morgounov, A., H. Gómez-Becerra, A. Abugalieva, M. Dzhunusova, M. Yessimbekova, H. Muminjanov, Y. Zelenskiy, L. Ozturk and I. Cakmak, 2007.
Iron and zinc grain density in common wheat grown in Central Asia. *Euphytica* 155:193-203.

Morgounov, A.I., I. Belan, Y. Zelenskiy, L. Roseeva, S. Tomoskozi, F. Bekes, A. Abugalieve, I. Cakmak, M. Vargas and J. Crossa, 2013.
Historical changes in grain yield and quality of spring wheat varieties cultivated in Siberia from 1900 to 2010. *Can. J. Plant Sci.* 93:425-433.

Mortvedt, J.J. and R.J. Gilkes, 1993.
Zinc fertilizers. In: AD Robson (ed) *Zinc in Soils and Plants*, pp. 33-44, Springer Netherlands.

Murphy, K.M. P.G. Reeves, S.S. Jones, S.K. Naik and D.K. Das, 2008.
Relationship between yield and mineral nutrient concentrations in historical and modern spring wheat cultivars. *Euphytica* 163:381-390.

Naik, S.K. and D.K. Das, 2008.
Relative performance of chelated zinc and zinc sulphate for lowland rice (Oryza sativa L.). *Nutr Cycl Agroecosyst* 81:219-227.

Norvell, W.A., H. Dabkowska-Naskret and E.E. Cary, 1987.
Effect of phosphorus and zinc fertilization on the solubility of $Zn^{2+}$ in two alkaline soils. *Soil Sci. Soc. Am. J.* 51:584-588.

Nriagu, J., 2011.
Zinc toxicity in humans. In: JO Nriagu (ed) *Encyclopedia of Environmental Health*, pp. 801-807, Elsevier, Burlington.

Oltmans, S.E., W.R. Fehr, G.A. Welke, V. Raboy and K.L. Peterson, 2005.
Agronomic and seed traits of soybean lines with low-phytate phosphorus. *Crop Sci.* 45:593-598.

Palmgren, M.G., S. Clemens, L.E. Williams, U. Kraemer, S. Borg, J.K. Schjoerring and S. Dale, 2008.
Zinc biofortification of cereals: problems and solutions. *Trends Plant Sci.* 13:464-473.

Persson, D.P., T.H. Hansen, K.H. Laursen, J.K. Schjoerring and S. Husted, 2009.
Simultaneous iron, zinc, sulfur and phosphorus speciation analysis of barley grain tissues using SEC-ICP-MS and IP-ICP-MS. *Metallomics* 1:418-426.

Phattarakul, N., B. Rerkasem, L.J. Li, L.H. Wu, C.Q. Zou, H. Ram, V.S. Sohu, B.S. Kang, H. Surek, M. Kalayci, A. Yazici, F.S. Zhang and I. Cakmak, 2012.
Biofortification of rice grain with zinc through zinc fertilization in different countries. *Plant Soil* 361:131-141.

Pinkaew S., P. Winichagoon, R.F. Hurrell and R. Wegmuller, 2013.
Extruded rice grains fortified with zinc, iron, and vitamin A increase zinc status of Thai school children when incorporated into a school lunch program. *J Nutr.* 143:362-368.

Plette, A.C.C., M.M. Nederlof, E.J.M. Temminghoff and W.H. Van Riemsdijk, 1999.
Bioavailability of heavy metals in terrestrial and aquatic systems: A quantitative approach. *Environ. Toxicol. Chem.* 18:1882-1890.

Prom-u-thai, C., S. Fukai, I.D. Godwin and L. Huang, 2007.
Genotypic variation of iron partitioning in rice grain. *J. Sci. Food Agric.* 87:2049-2054.

Purakayastha, T.J. and P.K. Chhonkar, 2001.
Influence of vesicular-arbuscular mycorrhizal fungi (Glomus etunicatum L.) on mobilization of zinc in wetland rice (Oryza sativa L.). *Biol. Fertil. Soils* 33:323-327.

Qin, Y., A. Melse-Boonstra, B. Yuan, X. Pan, Y. Dai, M. Zhou, R. Wegmueller, J. Zhao, F.J. Kok and Z. Shi, 2012
Zinc biofortification of rice in China: A simulation of zinc intake with different dietary patterns. *Nutrients* 4:517-528.

Raboy, V., 2001.
Seeds for a better future: 'Low phytate' grains help to overcome malnutrition and reduce pollution. *Trends Plant Sci.* 6:458-462.

Raboy, V., 2009.
Approaches and challenges to engineering seed phytate and total phosphorus. *Plant Sci.* 177:es 281-296.

Rai, K.N., O.P. Yadav, B.S. Rajpurohit, H.T. Patil, M. Govindaraj, I.S. Khairwal, A.S. Rao, H. Shivade, V.Y. Pawarand and M.P. Kulkarni, 2013.
Breeding pearl millet cultivars for high iron density with zinc density as an associated trait*. J SAT Agric Res* 11:1-7.

Qin, Y., A. Melse-Boonstra, B. Yuan, X. Pan, Y. Dai, M. Zhou, R. Wegmueller, J. Zhao, F.J. Kok and Z. Shi, 2012.
Zinc biofortification of rice in China: a simulation of zinc intake with different dietary patterns. *Nutrients* 4:517-28.

Quijano-Guerta, C., G.J.D. Kirk, A.M. Portugal, V.I. Bartolome and G.C. McLaren, 2002.
Tolerance of rice germplasm to zinc deficiency*. Field Crops Res*. 76:123-130.

Rauch, J.N., 2011.
Global distributions of Fe, Al, Cu, and Zn contained in Earth's derma layers. *J. Geochem. Explor.* 110:193-201.

Rauch, J.N. and J.M. Pacyna, 2009.
Earth's global Ag, Al, Cr, Cu, Fe, Ni, Pb, and Zn cycles. *Global Biochem. Cy.* 23:art. no. GB2001.

Rehman, H., S.M.A. Basra and M. Farooq, 2011.
Field appraisal of seed priming to improve the growth, yield, and quality of direct seeded rice. *Turk. J. Agric. For.* 35:357-367.

Ren, X.-L., Q.-L. Liu, H.-W. Fu, D.-x. Wu and Q.-Y. Shu, 2007.
Density alteration of nutrient elements in rice grains of a low phytate mutant. *Food Chem.* 102:1400-1406.

Römheld, V., 1991.
The role of phytosiderophores in acquisition of iron and other micronutrients in graminaceous species: An ecological approach. *Plant Soil* 130:127-134.

Rose, M.T., T.J. Rose, J. Pariasca-Tanaka, B. Widodo and M. Wissuwa, 2011.
Revisiting the role of organic acids in the bicarbonate tolerance of zinc-efficient rice genotypes. *Funct. Plant Biol.* 38:493-504.

Rose T.J., S.M. Impa, M.T. Rose, J. Pariasca-Tanaka, A. Mori, S. Heuer, S.E. Johnson-Beebout and M. Wissuwa, 2013.
Enhancing phosphorus and zinc acquisition efficiency in rice: a critical review of root traits and their potential utility in rice breeding. *Ann. Bot.* 112:331-345.

Ryan, M.H., J.K. McInerney, I.R. Record and J.F. Angus, 2008.
Zinc bioavailability in wheat grain in relation to phosphorus fertiliser, crop sequence and mycorrhizal fungi. *J. Sci. Food Agric.* 88:1208-1216.

Saeed, M. and R.L. Fox, 1979.
Influence of phosphate fertilization on zinc adsorption by tropical soils. *Soil Sci. Soc. Am. J.* 43:683-686.

Sajwan, K.S. and W.L. Lindsay, 1988.
Effect of redox, zinc fertilization and incubation time on DTPA-extractable zinc, iron and manganese. *Commun. Soil Sci. Plant Anal.* 19:1-11.

Santos S., C.A.E. Costa, A.C. Duarte, H.W. Scherer, R.J. Schneider, V.I. Esteves abd E.B.H. Santos, 2010.
Influence of different organic amendments on the potential availability of metals from soil: A study on metal fractionation and extraction kinetics by EDTA. Chemosphere 78:389-396.

Savithri, P., R. Perumal and R. Nagarajan, 1998.
Soil and crop management technologies for enhancing rice production under micronutrient constraints. *Nutr Cycl Agroecosyst* 53:83-92.

Schenkeveld, W.D.C., A.M. Reichwein, E.J.M. Temminghoff and W.H. van Riemsdijk, 2008.
The behaviour of EDDHA isomers in soils as influenced by soil properties. Plant Soil 290:85-102.

Schenkeveld W.D.C., E. Hoffland, A.M. Reichwein, E.J.M. Temminghoff and W.H. van Riemsdijk, 2012.
The biodegradability of EDDHA chelates under calcareous soil conditions. *Geoderma* 173-174:282-288.

Schlemmer, U., W. Frølich, R.M. Prieto and F. Grases, 2009.
Phytate in foods and significance for humans: Food sources, intake, processing, bioavailability, protective role and analysis. *Mol. Nutr. Food Res.* 53:330-375.

Schröder, T.J., W.H. van Riemsdijk, S.E.A.T.M. van der Zee and J.P.M. Vink, 2008.
Monitoring and modelling of the solid-solution partitioning of metals and As in a river floodplain redox sequence. *Appl. Geochem.* 23:2350-2363.

Shi, R., Y. Zhang, X. Chen, Q. Sun, F. Zhang, V. Römheld and C. Zou, 2010.
Influence of long-term nitrogen fertilization on micronutrient density in grain of winter wheat (Triticum aestivum L.). *J. Cereal Sci.* 51:165-170.

Sillanpää, M., 1990.
*Micronutrient assessment at the country level : an international study*. FAO, Rome.

Sillanpää, M. and P.L.G. Vlek, 1985.
Micronutrients and the agroecology of tropical and Mediterranean regions. *Fert. Res.* 7:151-167.

Singh, V.K., B.S. Dwivedi, R.J. Buresh, M.L. Jat, K.Majumdar, B. Gangwar, V. Govil and S.K. Singh, 2013.
Potassium fertilization in rice-wheat system across Northern India: Crop performance and soil nutrients. *Agron. J.* 105:471-481.

Slaton, N.A., R.J. Norman and C.E. Wilson, 2005.
Effect of zinc source and application time on zinc uptake and grain yield of flood-irrigated rice. *Agron. J.* 97:272-278.

Slaton, N.A., C.E. Wilson, S. Ntamatungiro, R.J. Norman and D.L. Boothe, 2001.
Evaluation of zinc seed treatments for rice. *Agron. J.* 93:152-157.

Slingerland, M., 2007.
Biofortification in a Food Chain Approach in West Africa. In: P Pinstrup-Andersen, F Cheng (eds) *Food Policy for Developing Countries: Case Studies*, pp. 12.
Slingerland, M.A., F.S. Zhang, T.J. Stomph, X.P. Gao, J.F. Liang and W. Jiang, 2009.
Biofortification in a food chain approach for rice in China. In: GS Bañuelos, ZQ Lin (eds) *Development and Uses of Biofortified Agricultural Products*, pp. 181-203, CRC Press, Boca Raton.
Smolders E., L. Versieren, S.F. Dong, N. Mattielli, D. Weiss, I. Petrov and F. Degryse, 2013.
Isotopic fractionation of Zn in tomato plants suggests the role of root exudates on Zn uptake. *Plant Soil* 370:605-613.
Solaiman, M.Z. and H. Hirata, 1997.
Effect of arbuscular mycorrhizal fungi inoculation of rice seedlings at the nursery stage upon performance in the paddy field and greenhouse. *Plant Soil* 191:1-12.
Srivastava, P.C., A.P. Singh, S. Kumar, V. Ramachandran, M. Shrivastava and S. F. D'souza, 2009.
Comparative study of a Zn-enriched post-methanation bio-sludge and Zn sulfate as Zn sources for a rice-wheat crop rotation. Nutr. Cycl. Agroecosyst. 85:195-202.
Stevenson, F.J., 1982.
Humus chemistry; genesis, composition, reactions. pp. John Wiley & Sons, New York.
Stomph, T.J., W. Jiang and P.C. Struik, 2009.
Zinc biofortification of cereals: rice differs from wheat and barley. *Trends Plant Sci.* 14:123-124.
Stomph, T.J., E.Y. Choi and J.C.R. Stangoulis, 2011.
Temporal dynamics in wheat grain zinc distribution: Is sink limitation the key? *Ann. Bot.* 107:927-937.
Stomph, T.J., N. Hoebe, E. Spaans and P.E.L. van der Putten, 2011.
The relative contribution of post-flowering uptake of zinc to rice grain zinc density. 3rd International Zinc Symposium 10-14 October, 2011, Hyderabad, India.
Stomph, T.J., W. Jiang, P.v.d. Putten and P.C. Struik, 2014.
Zinc allocation and re-allocation in rice. *Front. Plant Sci.* 5:8-12.
Strother, S., 1980.
Homeostasis in germinating seeds. *Ann. Bot.* 45:217-218.
Suzuki, M., M. Takahashi, T. Tsukamoto, S. Watanabe, S. Matsuhashi, J. Yazaki, N. Kishimoto, S. Kikuchi, H. Nakanishi, S. Mori and N.K. Nishizawa, 2006.
Biosynthesis and secretion of mugineic acid family phytosiderophores in zinc-deficient barley. *Plant J.* 48:85-97.
Takagi, S.I., 1976.
Naturally occurring iron chelating compounds in oat and rice root washings. 1. activity measurement and preliminary characterization. *Soil Sci. Plant Nutri.* 22:423-433.
Van der Linden, A., 2012.
Nitrogen – zinc interactions in rice (Oryza sativa): can nitrogen increase grain zinc concentrations? MSc thesis. Wageningen University.
Vasconcelos, M., K. Datta, N. Oliva, M. Khalekuzzaman, L. Torrizo, S. Krishnan, M. Oliveira, F. Goto and S.K. Datta, 2003.
Enhanced iron and zinc accumulation in transgenic rice with the ferritin gene. *Plant Sci.* 164:371-378.
Von Wirén, N., H. Marschner and V. Römheld, 1996.
Roots of iron-efficient maize also absorb phytosiderophore-chelated zinc. *Plant Physiol.* 111:1119-1125.

Walter, A., V. Romheld, H. Marschner and S. Mori, 1994.
Is the release of phytosiderophores in zinc-deficient wheat plants a response to impaired iron utilization? *Physiol. Plant.* 92:493-500.

Wang, H.Q., B.A.M. Bouman, Z. Dule, C. Wang and P.E. Moya, 2002.
Aerobic rice in northern China: Opportunities and challenges. In: BAM Bouman et al. (ed.) *Water-wise rice production*, pp. 143-154, IRRI, Los Baños.

Wang, P., D.M. Zhou, X.S. Luo and L.Z. Li, 2009.
Effects of Zn-complexes on zinc uptake by wheat (*Triticum aestivum*) roots: A comprehensive consideration of physical, chemical and biological processes on biouptake. *Plant Soil* 316:177-192.

Watt, R.G., J. Dykes and A. Sheiham, 2001.
Socio-economic determinants of selected dietary indicators in British pre-school children. *Public Health Nutr.* 4:1229-1233.

Webb, M.J. and J.F. Loneragan, 1990.
Zinc translocation to wheat roots and its implications for a phosphorus zinc interaction in wheat plants. *J. Plant Nutr.* 13:1499-1512.

Welch, R.M., 1986.
Effects of nutrient deficiencies on seed production and quality. In: B Tinker, A Läuchli (eds) *Advances in Plant Nutrition*, pp. 205-247, Praeger Scientific, New York.

Welch, R.M. and R.D. Graham, 1999.
A new paradigm for world agriculture: meeting human needs: Productive, sustainable, nutritious. *Field Crop Res.* 60:1-10.

Welch, R.M. and R.D. Graham, 2002.
Breeding crops for enhanced micronutrient content. *Plant Soil* 245:205-214.

Weng, L., E.J.M. Temminghoff, S. Lofts, E. Tipping and W.H. Van Riemsdijk, 2002.
Complexation with dissolved organic matter and solubility control of heavy metals in a sandy soil. *Environ. Sci. Technol.* 36:4804-4810.

White, P.J., 2012.
Ion uptake mechanisms of individual cells and roots: short-distance transport. In: P Marschner (ed) *Marschner's Mineral Nutrition of Higher Plants (Third Edition)*, pp. 7-47, Academic Press, San Diego.

WHO, 2011.
Guideline. Use of multiple micronutrient powders for home fortification of foods consumed by infants and children 6 – 23 months of age. Geneva.

WHO/FAO, 2006.
Guidelines on food fortification with micronutrients. Geneva.

WHO/UNICEF, 2004.
Joint statement. Clinical management of acute diarrhea. Geneva.

Widodo, B., M.R. Broadley, T. Rose, M. Frei, J. Pariasca-Tanaka, T. Yoshihashi, M. Thomson, J.P. Hammond, A. Aprile, T.J. Close, A.M. Ismail and M. Wissuwa, 2010.
Response to zinc deficiency of two rice lines with contrasting tolerance is determined by root growth maintenance and organic acid exudation rates, and not by zinc-transporter activity. *New Phytol.* 186:400-414.

Wissuwa, M., A.M. Ismail and S. Yanagihara, 2006.
Effects of zinc deficiency on rice growth and genetic factors contributing to tolerance. *Plant Physiol.* 142:731-741.

Wissuwa, M., A. Ismail and R. Graham, 2008.
Rice grain zinc concentrations as affected by genotype, native soil-zinc availability, and zinc fertilization. *Plant Soil* 306:37-48.

Wu, C.Y., L.L. Lu, X.E. Yang, Y. Feng, Y.Y. Wei, H.L. Hao, P.J. Stoffella and Z.L. He, 2010.
Uptake, translocation, and remobilization of zinc absorbed at different growth stages by rice genotypes of different Zn densities. *J. Agric. Food Chem.* 58:6767-6773.

Wu, C., Y. Feng, J.I. Shohag, L. Lu, Y. Wei, C. Gao and X. Yang, 2011.
Characterization of Zn uptake, translocation and accumulation into developing grains and young leaves of high Zn-density rice genotype. *J. Zhejiang Univ. Sci-B.* 12:408-418.

Yang, X., R. Hajiboland and V. Römheld, 2003.
Bicarbonate had greater effects than high pH on inhibiting root growth of zinc-inefficient rice genotype. *J. Plant Nutr.* 26:399-415.

Yoshida, S., G.W. McLean, M. Shafi and K.E. Mueller, 1970.
Effects of different methods of zinc application on growth and yields of rice in a calcareous soil, West Pakistan. *Soil Sci. Plant Nutri.* 16:147-149.

Zhang, F.-S., V. Römheld and H. Marschner, 1991.
Diurnal rhythm of release of phytosiderophores and uptake rate of zinc in iron-deficient wheat. *Soil Sci. Plant Nutri.* 37:671-678.

Zhao, F.J., Y.H. Su, S.J. Dunham, M. Rakszegi, Z. Bedo, S.P. McGrath and P.R. Shewry, 2009.
Variation in mineral micronutrient concentrations in grain of wheat lines of diverse origin. *J. Cereal Sci.* 49:290-295.

Zhu, Y.-G., S.E. Smith and F.A. Smith, 2001.
Zinc (Zn)-phosphorus (P) interactions in two cultivars of spring wheat (*Triticum aestivum* L.) differing in P uptake efficiency. *Ann. Bot.* 88:941-945.

Zou, C.Q., Y.Q. Zhang, A. Rashid, H. Ram, E. Savasli, R.Z. Arisoy, I. Ortiz-Monasterio, S. Simunji, Z.H. Wang, V. Sohu, M. Hassan, Y. Kaya, O. Onder, O. Lungu, M.Y. Mujahid, A.K. Joshi, Y. Zelenskiy, F.S. Zhang and I. Cakmak, 2012.
Biofortification of wheat with zinc through zinc fertilization in seven countries. *Plant Soil* 361:119-130.

Zhu, Y.Y., H. Chen, J.H. Fan, Y.Y. Wang, Y. Li, J.B. Chen, J.X. Fan, S.S. Yang, L.P. Hu, H. Leung, T.W. Mew, P. S. Teng, Z.H. Wang and C.C. Mundt, 2000.
Genetic diversity and disease control in rice. Nature 406:718-722.

Zuo, Y. and F. Zhang, 2009.
Iron and zinc biofortification strategies in dicot plants by intercropping with gramineous species: A review. *Agron. Sustain. Dev.* 29:63-71.

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