# Zinc in Soil Environment for Plant Health and Management Strategy

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**Abstract** Zinc is an essential element for photosynthesis and its deficiency may play a role in the reduction of activity of rubisco and hence decreased rate of photosynthesis in higher plants. Zinc deficiency sometimes resembles Fe/Mn deficiencies. Under severe zinc deficiency the shoot apices die ('die-back') as is widely distributed. Zinc toxicity leads to chlorosis in young leaves. Induced manganese deficiency might also be of importance as high zinc supply strongly decreases the manganese content of plants. Most preventing measures for zinc deficiency are selection of Zn efficient variety that is tolerant to high level of bicarbonate as well as low zinc in soil. Curative measure for correcting are application of 20-25 kg/ha ZnSo4 in acid soil, 22 kg Zn/ha initially followed by 5-10 kg Zn in the later years or 50% gypsum + 10 t GM + 22 kg Zn once in 2-3 years in sodic soils, 1.0-1.5 kg/ha Zn as foliar spray at tillering stage and 2 times latter is very helpful for correct this deficiency. Zinc is very tightly bound to phytic acid and the formation of protein-zinc-phytic acid complexes increases the resistance to hydrolysis that leads to decrease in bioavailability of Zn to human and animals. Enrichment of cereal grains with Zn through both genetic (e.g., breeding) and agronomic (e.g., application of Zn fertilizers) biofortification is a high priority area of research, and will contribute to minimizing Zn-deficiency-related health problems in human populations.

**Keywords** Zinc, Deficiency, Toxicity, Chlorosis, Bicarbonate, Bioavailability

## 1. Introduction

Due to increase availability of Ca, Mg, Cu, Fe, Mn and P under prolonged submergence Zn availability and uptake hampered by the crops.Zinc is an important plant micronutrient for crop growth and higher production. Zinc (Zn) deficiency is the most widespread micronutrient disorder in rice (often combined with P deficiency), but efforts to develop cultivars with improved tolerance have been hampered by insufficient understanding of genetic factors contributing to tolerance [1]. Zinc is essential for several biochemical processes such as cytochrome and nucleotide synthesis, enzyme activation, chlorophyll production, maintenance of membrane activity, increase rate of seed and stalk maturation. Nearly 50% of the cereal-grown areas in the world have soils with low plant availability of Zn to support either high crop yields or provide grains of high nutritional quality [2]. The regions with Zn-deficient soils are also the regions where Zn deficiency in human beings is widespread. Zinc deficiency in soils and plants is a global micronutrient deficiency problem reported in many countries. Low solubility of Zn in soils rather than low total amount of Zn is the major reason for the widespread occurrence of Zn deficiency problem in crop plants. Zinc is an essential element for photosynthesis and its deficiency may play a role in the reduction of activity of rubisco and hence decreased rate of photosynthesis in higher plants [3]. There are a large number of enzymes in which Zn is an integral component. In these enzymes Zn has three functions: catalytic, cocatalytic, or structural. Enrichment of cereal grains with Zn through both genetic (e.g., breeding) and agronomic (e.g., application of Zn fertilizers) biofortification is, therefore, a high priority area of research, and will contribute to minimizing Zn-deficiency-related health problems in human populations.

## 2. Zinc in Plant Enzyme

In enzymes zinc has three functions; catalytic, cocatalytic (coactive), or structural. In enzymes with catalytic zinc functions (e.g., carbonic anhydrase and carboxypeptidase) the zinc atom is coordinated to four ligands, there of which are amino acids, with histidine (His) being the most frequent, followed by glutamine (Glu) and

asparagine (Asp); a water molecule is the fourth ligand at all catalytical sites [4].In enzymes with structural zinc functions (*e.g.,* alcohol dehydrogenase, and the proteins involved in DNA replication and gene expression) the structural zinc atoms are coordinated to the S-groups of four cysteine residues forming a tertiary structure of high stability [5]. Most of the zinc-enzymes have only one zinc atom per molecule, the alcohol dehydrogenase being an exception.

## 2.1. Protein Synthesis

The zinc content of ribosomal RNA in zinc-sufficient cells of Euglena is in the range 650-1280 $\mu g\ g^{-1}$ RNA, whereas that in zinc deficient cells is 300-380 $\mu g\ g^{-1}$ RNA. A clear inverse relationship exists between zinc supply and RNase activity and also between RNase activity and protein content. In the absence of zinc, ribosome disintegrates, but reconstitution takes place after resumption of zinc supply [6]. Zinc is a structural component of the ribosomes and essential for their structural integrity. Low protein contents and high amino acid contents in zinc-deficient plants are not only the result of reduced transcription and translation but also of enhanced rates of RNA degradation [7]. Higher rates of RNase activity are a typical feature of zinc deficiency. In shoot meristems of rice, disintegration of the 80s ribosomes (soluble fraction in the cytoplasm) takes place when the zinc content drops below 100 $\mu g\ g^{-1}$ dry weight [8]. In contrast to this rather high zinc content required for ribosome integrity, protein content starts to decrease at considerably lower zinc content.

## 2.2. Carbonic Anhydrase

This enzyme contains a single zinc atom which catalyzes the hydration of $CO^2$.

$$CO_2 + H_2O \rightleftharpoons HCO_3^- + H^+$$
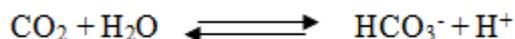
Carbonic anhydrase (CA) from dicotyledons consists of six subunits and has a molecular weight of 180 k Da, and six zinc atoms per molecule. The enzyme is localized both in the chloroplast and in the cytoplasm.

## 2.3. Alcohol Dehydrogenase

In zinc-deficient plants, alcohol dehydrogenase activity decreases, but the consequences for plant metabolism are not known. The situation is different in plants grown in waterlogged or submerged soils. This enzyme contains two zinc atoms per molecules, one with catalytic and the other with structural functions [9]. The enzyme catalyzes the reduction of acetaldehyde to ethanol. In higher plants under aerobic conditions, ethanol formation takes place mainly in meristematic tissues, such as root apices. In lowland rice flooding stimulates the activity of root alcohol dehydrogenase twice as much in zinc-sufficient compared with zinc-deficient plants, and the lower activity of this key enzyme in anaerobic metabolism might impair root functions of submerged rice considerably [10].

## 2.4. CuZn-superoxide Dismutase

In this enzyme zinc is associated with copper (CuZn SOD). Most likely the copper atoms represent the catalytic metal component and zinc the structural. With zinc deficiency SOD activity is much lower but can be restored in vitro by resupplying zinc to the assay medium, indicating that the zinc atom is an essential structural component for the normal functioning of CuZn SOD [11]. Zn-deficiency caused the increase in $O_2\cdot-$ production and higher contents of MDA in leaves. The SOD activity showed a decrease in Zn0 leaves, indicating that lowered capacity to scavenge the free radicals may be related to the over-accumulation of reactive oxygen species (ROS), which enhanced the rate of membrane peroxidation, *i.e.* MDA content in Zn0 leaves [12].

# 3. Zinc on Photosynthesis

Zinc deficiency depresses plant leaf's photosynthetic capacity. In cauliflower, reduction in photosynthesis induced by Zn deficiency is associated with a decrease in intercellular $CO_2$ concentration and stomatal conductance [13]. The accumulation of saccharides can be observed in Zn deficient plants, especially in phloem sap source leaves, possibly resulting from either impaired phloem export of saccharides or decrease of sink demand. Reported a significant role of Zn in the regulation of the stomatal aperture is accounted for possible role of Zn in maintaining a high K content in guard cells [14]. A decrease in carbonic anhydrase activity due to Zn deficiency may also contribute to the reduced net photosynthetic rate, $P$N. A higher $P$N in Zn-deficiency resistant wheat cultivars than in a sensitive cultivar was related to higher carbonic anhydrase activity, because irrespective of Zn supply the resistant cultivar had an inherently higher carbonic anhydrase activity than the sensitive cultivar [15]. Additionally, the accumulation of saccharides in leaves may be related to decreases in photosynthetic $CO_2$ fixation under low Zn. Zn-deficiency strongly reduced the photosynthetic performance in maize leaves: Net photosynthetic rate ($P$N), transpiration rate ($E$), and stomatal conductance ($g$s) were decreased by 80, 62, and 69 %, respectively, in comparison to $Zn_5$ (Zinc @ 5.0 mg $kg^{-1}$ soil). The water use efficiency, as assessed by $P$N/$E$, was enhanced markedly by Zn application [16].

# 4. Zinc Deficiency on Carboxylating Enzymes (CE) and Carbonic Anhydrase (CA)

Carbonic anhydrases is a ubiquitous enzyme among

living organisms which catalyses the reversible interconversion of $CO_2$ and $HCO_3^-$. Zinc has a catalytic role in plant and animal CA, being coordinated to the imidazole rings of three histidines close to the active site [17]. Since CA activity is affected by Zn deficiency and it also plays a very vital role in maintaining the inorganic carbon levels, this enzyme is used as a biochemical marker to diagnose Zn deficiency. RuBPC is the main carboxylating enzyme in $C_3$ plants [18]. It may account for 50 % or more of the total soluble leaf protein, making it the most common protein in nature. The activity of the enzyme decreased in leaves of maize and chick pea plants grown without Zn [19]. The decrease in RuBPC activity was almost 22% of the control values in maize plants, and about 34 % for the chickpea plants. In $C_4$ plants the initial carboxylation reaction occurs by PEPC. In higher plants, CA represents 1 to 2 % of total soluble protein. The majority of CA activity in leaves of $C_3$ plants resides within the stroma of the chloroplasts, thus showing a similar distribution pattern as RuBPC. Experimental evidence showed that Zn is tightly bound to the enzyme and it is essential for its activity [20]. It has been reported that CA activity is affected by Zn deficiency and Zn is an important regulator of the enzyme activity.

# 5. Plant Growth and Zinc Interaction

In plants as in other biological systems, zinc exists only as Zn(II), and does not take part in oxidoreduction reactions. The metabolic functions of zinc are based on its strong tendency to form tetrahedral complexes with N-, O-, and particularly S-ligands and it thereby plays both a functional (catalytic) and structural role in enzyme reactions [21]. In the last decade the role of zinc in protein molecules involved in DNA replication and in the regulation of gene expression has attracted much interest. Changes in metabolism brought about by zinc deficiency are quite complex. Nevertheless, some of the changes are typical and can be rather well explained by the function of zinc in specific enzyme reactions or steps in particular metabolic pathways [22].

# 6. Zinc in Soil Environment

## 6.1. Deficiency

Zinc deficiencies mainly occur when soil pH is high, high organic matter in soil, calcareous soils with high bicarbonate content, intensively cropped soils. Symptoms are common on younger or middle aged leaves [23], brown to dusty brown spots on younger leaves in red soils, yellowing of leaves/ midrib bleaching. Symptoms are prolonged during early growth stages due to immobilization of zinc. Symptoms of zinc deficiency sometimes resemble Fe/Mn deficiencies. Zinc deficiency

has also been associated with high bicarbonate content, a Mg:Ca ratio in soils>1, intensive cropping, use of high yielding cultivars, and irrigation with alkaline water [24]. The most characteristic visible symptoms of zinc deficiency in dicotyledons are stunted growth due to shortening of internodes ('rosetting') and a drastic decrease in leaf size ('little leaf'). Under severe zinc deficiency the shoot apices die ('die-back') as is widely distributed, for example, in forest plantations in South Australia. Quite often these symptoms are combined with chlorosis, which is either highly contrasting or diffusive ('mottle leaf'). These symptoms are usually more severe in high light intensities than in partial shade [25]. In cereals such as sorghum, chlorotic bands along the midrib and red, spotlike discoloration (caused by anthocyanins) on the leaves often occur. In leaves, the critical deficiency laves are below 15-20 µg Zn $g^{-1}$ dry weight.

## 6.2. Toxicity

Zinc toxicity leads to chlorosis in young leaves. This may be an induced deficiency, for example, of magnesium or iron, because of the similar ion radius of $Zn^{2+}$ and $Fe^{2+}$ and $Zn^{2+}$ and $Mg^{2+}$. When the zinc supply is large, zinc toxicity can readily be induced in nontolerant plants, inhibition of root elongation being a very sensitive parameter. Induced manganese deficiency might also be of importance as high zinc supply strongly decreases the manganese content of plants [26]. In bean plants zinc toxicity inhibits photosynthesis at various steps and through different mechanisms. Depressed RuBP carboxylase activity is presumably caused by competition with magnesium and inhibited PS II activity by replacement of manganese in the thylakoid membranes [27]. The critical toxicity levels in leaves in leaves of crop plants are from as low as 100 µg Zn $g^{-1}$ dry weight to more than 300 µg Zn $g^{-1}$ dry weight, the latter value being more typical.

## 6.3. Management Practices

Most preventing measures for zinc deficiency are selection of Zn efficient variety that is tolerant to high level of bicarbonate as well as low zinc in soil [28]. Application of $ZnSo_4$ in nursery beds, drain the field, seedling root dipping in 2-4% ZnO suspension, mid-season correction by spraying 0.5% $ZnSO_4$ thrice at weekly intervals between 3-6 WAT etc. Curative measure for correcting are application of 20-25 kg/ha $ZnSO_4$ in acid soil, 22 kg Zn/ha initially followed by 5-10 kg Zn in the later years or 50% gypsum + 10 t GM + 22 kg Zn once in 2-3 years in sodic soils, 1.0-1.5 kg/ha Zn as foliar spray at tillering stage and 2 times latter is very helpful for correct this deficiency [29]. Plant Zn uptake from low Zn soils can be increased by Zn mobilizing chemical rhizosphere processes.

## 7. Zinc Tolerance in Crop

Fairly close positive correlations in tolerant genotypes occur between accumulation of organic acids such as malate and citrate, and accumulation of zinc, indicating that complexation of zinc with organic acids in the vacuoles is in many cases an important mechanism of zinc tolerance [30].Tolerance is achieved mainly through sequestering zinc in the vacuoles. Whereas in the nontolerant clone receiving an abundant supply of zinc there is a preferential accumulation of zinc in the cytoplasm, in the tolerant clone the zinc concentration in the cytoplasm remains low; instead, zinc is sequestered in the vacuoles.

## 8. Strategies to Control Zinc Deficiency

Nutrition improvement with food fortification and medical supplements achieved in the developed world were unfortunately not successfully transferred to many developing countries even today. Most widely traditional strategies are supplementation with pharmaceutical preparations, disease reduction, food fortification and dietary diversification through consumption of a broad variety of foods, preferably from home gardens and small livestock production [29,30]. Success of fortification programmes and of micronutrient distribution in form of tablets to the population living in remote areas, to certain cultural groups and to the poor in growing towns is not obvious. Therefore, those who mostly need micronutrient supplementation are not reached. None of the current intervention strategies have been very successful in reducing the prevalence of micronutrient deficiency in developing countries, where malnutrition is still a major health problem [30,31]. On the opposite, genetically improved food, by breeding or gene transfer, may be a powerful intervention strategy targeting the most vulnerable population. Genetically improved plants can reach the rural areas and represent a sustainable system, with very low recurrent costs. So, considering the limitations associated with the traditional interventions and the strength of the food bio-fortification techniques, recently the research efforts have shifted towards bio-fortification of micronutrients in the edible tissues of staple food crop [31].

## 9. Bioavailability and Zinc Binding Forms

In wheat seeds, similarly high zinc contents were found in protein bodies in the scutellum. In the protein bodies of maize germ the phytate is mainly a mixed K-Mg-salt [32]. In grains and seeds, most of the Zn and other mineral elements, are localized in so-called 'protein bodies' in the form of discrete particles, the globoid crystals [33]. These globoids consisting mainly of 'phytate' *i.e.,* salts of phytic acid. However, relatively large portions of the micronutrients zinc, manganese and iron, but not copper, are also bound as phytate. Zinc is very tightly bound to phytic acid and the formation of protein-zinc-phytic acid complexes increases the resistance to hydrolysis that leads to decrease in bioavailability of Zn to human and animals [34]. If phytic acid: Zn ratio >15, bioavailability of Zn decreased.

## 10. Zinc in Bio-Fortification

The regions with Zn-deficient soils are also the regions where Zn deficiency in human beings is widespread. There are several examples demonstrating that applying Zn fertilizers to cereal crops improve not only productivity, but also grain Zn concentration of plants [33,34]. Agronomic measures for micronutrients bio-fortification includes adequate fertilization, proper source, method and timing of fertilizer application, FYM application (INM), crop rotation, intercropping and all other agronomic practices that ensure proper availability of micronutrients in soil. Fertilizer strategy could be a rapid solution to the problem of micronutrient deficiency. Depending on the soil conditions and application form, Zn fertilizers can increase grain Zn concentration up to fourfold under field conditions [35,36]. Zinc has been sprayed on foliar in form of $ZnSO_4$ at the rate of 0.5% (w/v). Zinc applications have been realized at the following growth stages: (1) stem elongation and booting stages, (2) milk and dough stages, (3) booting and milk stages and (4) booting, milk and dough stages. Once the micronutrient content of the seed is improved by any bio-fortification technique, say agronomic bio-fortification, it has several agronomic advantages besides improving human nutrition [35,36,37]. Fertilizer studies focusing specifically on increasing Zn concentration of grain (or other edible parts) are, however, very rare, although a large number of studies are available on the role of soil and foliar applied Zn fertilizers in correction of Zn deficiency and increasing plant growth and yield. The highest increase in grain yield was obtained with soil, soil + foliar and seed + foliar applications. When a high concentration of grain Zn is aimed in addition to a high grain yield, combined soil and foliar application is recommended [37]. Alternatively, using seeds with high Zn concentrations at sowing together with foliar application of Zn is also an effective way to improve both grain yield and grain Zn concentration. Timing of foliar Zn application is an important factor determining the effectiveness of the foliarly applied Zn fertilizers in increasing grain Zn concentration. It is expected that large increases in loading of Zn into grain can be achieved when foliar Zn fertilizers are applied to plants at a late growth stage [38-39]. Ozturk *et al.* studied changes in grain

concentration of Zn in wheat during the reproductive stage and found that the highest concentration of Zn in grain occurs during the milk stage of the grain development.

## 11. Conclusions

Zinc is an essential element for photosynthesis and its deficiency may play a role in the reduction of activity of Rubisco and hence rate of photosynthesis in higher plants. Zinc deficiency is responsible for many severe health complications, including impairments of physical growth, immune system and learning ability, combined with increased risk of infections, DNA damage and cancer development. Status of Zn deficiencies should be assessed and the results should be used for planning interventions aimed at improving the Zn status of the population. Enrichment of cereal grains with Zn through both genetic (e.g., breeding) and agronomic (e.g., application of Zn fertilizers) biofortification is a high priority area of research, and will contribute to minimizing Zn-deficiency-related health problems in human populations.

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