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## Some research approaches for augmenting Zn competency in plants: A review

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#### Abstract

Zinc is an essential plant micronutrient and important for production of plant growth hormones and proteins and is also involved in sugar consumption. Root development as well as carbohydrate and chlorophyll formation are also dependent on concentration of zinc in plants. Maintaining adequate zinc levels is important for enabling plants to withstand low air temperatures. Zinc is also involved in the synthesis of auxin, a plant hormone that helps plants determine whether to focus on growing tall or becoming bushy. Ideally, for healthy and productive soil the concentration of zinc should be 1-200 mg/kg. Cereal species greatly differ in their zinc efficiency (ZE), defined in this article as the ability of a plant to grow and yield well under Zn deficiency. ZE has been attributed mainly to the efficiency of acquisition of Zn under conditions of low soil Zn availability rather than to its utilization or (re)-translocation within a plant. A higher Zn acquisition efficiency, further, may be due to either or all of the following: an efficient ionic Zn uptake system, better root architecture, i.e. long and fine roots with architecture favoring exploitation of Zn from larger soil volume, higher synthesis and release of Zn-mobilizing phytosiderophore by the roots and uptake of Zn-phytosiderophore complex. This article attempts to examine critically the scanty and scattered reports available on the status of improving zinc efficiency in plants.

**Keywords:** Augmenting Zn, plant micronutrient, zinc efficiency

#### Introduction

##### Nature and Essentiality of Zn

In plants the chemistry of Zn differs from other metals since it does not undergo valency changes but forms stable tetrahedral complexes. Most Zn in plants occurs as free ions or is associated with low molecular weight complexes, metalloproteins and insoluble forms mounted with the cell wall. Zn can become inactivated within the cell either by ligand formation (Leece, 1978) or by complexation with phosphorous [1]. About 58 to 91% of plant Zn is soluble and it is this water soluble Zn fraction which is considered to be the physiologically active fraction and is regarded as a better indicator of Zn status than the total Zn content (Cakmak and Marschner, 1987) [2]. Among these soluble forms, low molecular weight complexes are most significant forms of active Zn. In solution, Zn exists in the +2 (divalent cation) oxidation state and is redox-stable unlike Fe<sup>2+</sup> and Cu<sup>2+</sup>, under physiological conditions as a result of a complete d-shell of electrons (Auld, 2001. Torre *et al.*, (1991) [3] reported that various cell wall compounds (i.e. lignin, cellulose, hemicellulose, etc.) possess a high binding affinity to Zn [4]. Zn may also be incorporated as a component of proteins and acts as a cofactor of a large number of enzymes. Thus Zn has an important structural, functional and a regulatory role in plant development.

The trace metal Zn is an important mineral constituent of plants. Historically, Raulin (1869), was the first who discovered Zn to be an essential element for the fungus *Aspergillus niger* [5]. On the basis of water culture studies, Maze (1914) [6] demonstrated that Zn, along with manganese, is essential for the growth of maize plants. Sommer and Lipman (1926) [7] established the essentiality of Zn for sunflower and barley. Later, several workers showed the essential role of Zn for a wide variety of crop plants and fruit trees (Scaife and Turner, 1983 [8]. Zinc is now being regarded as the third most important limiting nutrient element in crop production after N and P.

Zinc as an essential micronutrient involved in a wide variety of physiological processes Broadley *et al.*, 2007; Pande, 2010 [9, 10]. The activity of Zn is effective for determining relevant process in the physiological and nutritional homeostasis of the plant, acting as an

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activator or enzyme component, it participates of the photosynthesis in C4 plants, it is necessary for the production of tryptophan and for the maintenance of the biomembrane integrity. Zinc is essential for structural component of large number of proteins such as transcription factors and metallo enzymes (Figueiredo *et al.*, 2012) [11].

### Zinc Efficiency Mechanisms in Plants

Plants differ in Zn efficiency which is defined as the ability of a cultivar to grow and yield well in soils deficient in Zn. Growing Zn-efficient plants on Zn-deficient soils represents the strategy of tailoring the plant to fit the soil in contrast to the older strategy of tailoring the soil to fit the plant. Many crops exhibit differential tolerance to Zn deficiency. Lentil, chickpea and pea are more sensitive to Zn deficiency than oilseeds and cereals [12] (Tiwari and Dwivedi, 1990). Genotypic variation for Zn efficiency in crop plants has been reported from India where nearly half of  $>10^5$  soil samples analysed for available Zn was rated as deficient (Takkar, 1991) [13]. The ability of a genotype to grow and yield well in soils that are too deficient in Zn for standard cultivars is defined as Zn-efficiency. Difference in Zn efficiency have been reported for oats, pearl millet, spinach potato, tomato, sorghum, wheat and rye.

A variety of research approaches involving whole plant and cellular physiology, biochemistry and genetics have been used to study how plant cope with low available Zn. Some studies attributed Zn-efficiency to better internal Zn utilization, while others attributed it to greater Zn uptake by the root Wissua *et al.*, 2006 and Kabir *et al.*, 2014) [14, 15].

Zinc efficient plants appear to employ a plethora of physiological mechanisms that allow them to withstand the Zn deficiency stress better than the Zn in-efficient plants. These mechanisms may operate only in the soil environment (such as root growth), in both soil and plant environments (such as differential uptake, transport and utilization of Zn) and may depend to different extent in genotypic response to nutrient interactions (such as Zn-P and Zn-Fe interactions).

Zinc efficient plants have a greater proportion and longer length of fine roots ( $\leq 0.2 \mu\text{M}$ ) and this plays an important role in differential Zn efficiency among various genotypes (Rengel and Wheal, 1997) [16]. In general, Zn-efficient plants have thinner roots with increased surface area which increase the availability of Zn along with other nutrient due to a more thorough exploration of the soil (Singh *et al.*, 2005) [17]. Genc *et al.*, (2006) [18] in wheat and Chen *et al.*, (2009) [19] in rice showed that Zn efficient genotype developed longer and thinner roots ( $\leq 0.2 \text{ mm}$ ) than a less Zn-efficient genotype. The root cell plasma membrane of the Zn-inefficient genotypes may have a greater requirement for external Zn than those of the Zn-efficient ones, Zinc efficient genotypes may be able to maintain structural and functional stability of their root cell plasma membrane better than Zn-inefficient genotype under Zn deficiency. Increased amount of sulphhydryl group in the root cell plasma membrane of Zn-efficient genotype under Zn deficiency allow better growth and productivity of Zn-efficient genotypes in comparison to the Zn-inefficient ones (Rengel, 1995) [20]. The longer and thinner root systems provide a greater tolerance to bicarbonates at low Zn supply (Yang *et al.*, 2003) [21]. In relation to root ultra structure, it was observed the role of shoot localized process in the mechanism of Zn-efficiency in bread wheat. Genc *et al.*, (2006) [22] in bean and barley observed that root: shoot dry weight ratio of Zn-

efficient genotype was significantly greater than that of Zn-inefficient, indicating that a higher root: shoot ratio will improve Zn-efficiency as also observed by some worker in rice.

Root mediated alteration in rhizosphere chemistry which involves change in rhizosphere pH or the release of organic ligands i.e. phytosiderophores (PS) could increase the availability of Zn to plants Suzuki *et al.*, 2008) [23]. Cakmak *et al.*, (1996) [24] reported that Zn-efficient bread wheat genotypes had higher PS release than Zn-inefficient durum wheat genotypes under Zn deficiency. They suggested that the expression of Zn-efficiency mechanism is related to phytosiderophores mediated enhanced mobilization of Zn from sparingly soluble Zn pool and from adsorption sites, both in the rhizosphere and in plants.

Differential changes in the rhizosphere chemistry and biology in response to the release of greater amount of Zn-chelating phytosiderophores by Zn efficient genotypes help to take up more Zn than by Zn in-efficient genotypes Cakmak *et al.*, (1996) [24] have suggested that enhanced phytosiderophore release under Zn-deficiency (as well as Fe-deficiency) is involved in differences in Zn-efficiency of genotypes. Walter *et al.*, (1994) stated that enhanced release of phytosiderophores in Zn deficient wheat plants was induced primarily by impaired translocation of iron to the shoot.

Cakmak *et al.*, (1996) [24] demonstrated that enhanced synthesis and release of phytosiderophore in wheat at deficient Zn supply is involved in Zn efficiency. Singh (2001) [25] suggested that the expression of Zn efficiency mechanism is related to phytosiderophore-mediated enhanced mobilization of Zn from sparingly soluble Zn pool and from adsorption sites, both in the rhizospheres and in plants. Chen *et al.*, (2009) [19] observed that Zn-efficient 'IR8192' rice cultivar had more biomass production than Zn-inefficient 'Erjiufeng' rice cultivar.

A strong positive Correlation between Zn efficiency, the rate of Zn uptake and dry matter production was observed by Rengel and Graham (1995) [26] and Rengel and Wheal (1997) [27] Rengel and Wheal (1997) [27] observed an increase in maximum uptake rate ( $I_{\text{max}}$ ) resulting in increased net Zn accumulation in Zn-efficient wheat genotypes Excalibur but not in inefficient Durati under Zn deficiency. They attributed this increase in  $I_{\text{max}}$  as one of the mechanism for Zn efficiency of Excalibur.

Under Zn deficiency, the Zn efficient wheat genotypes transported larger amounts of Zn from roots to shoots than the Zn-inefficient genotype (Cakmak *et al.*, 1996) [24]. Jonathan (1998) [28] also suggested that lower root  $\text{Zn}^{2+}$  uptake rate may contribute to reduced Zn efficiency in durum wheat varieties under Zn limiting condition.

Nutrient interactions are important in determining Zn-efficiency. Zinc deficiency-induced P toxicity which is well documented (Loneragan and Webb, 1993) [29], should be taken into account when assessing relative Zn efficiency in crop genotypes. It is observed that the performance of Zn inefficient genotype Excalibur (Rengel and Graham, 1995) [20] increased when grown in a nutrient medium which maintained a low P concentration. Similarly a Zn-Fe interaction is also influential in determining Zn efficiency. As compared to Zn-efficient genotypes, the Zn-inefficient navy bean genotypes had enhanced exudation of reductants which increased the reduction of  $\text{Fe}^{3+}$  and its accumulation in leaves as a result of which Zn uptake and utilization was inhibited. However in Zn-efficient wheat genotypes, Zn deficiency induced Fe

deficiency was expressed resulting in greater uptake of Zn as compared to Zn inefficient genotype. Muhammad *et al.*, (2014) [30] found that the Zn-inefficient cultivars of *Triticum aestivum* L. accumulated higher concentrations of Fe, Cu, Mn and P elements compared to efficient ones. Zinc efficiency of these genotypes under field conditions varied between 57 to 96%, which was quite higher than results obtained in hydroponics studies.

Yang *et al.*, (1994) [31] found that the concentration and total uptake Zn in the shoots of hybrid rice grown in Inceptisols (calcareous soil) were significantly higher than those of the traditional cultivars. In the hybrid rice, a higher ratio of Zn and Fe indicated that the hybrid rice roots avoided absorbing excess Fe from the soils due to its higher oxidizing power and was more efficient in absorbing Zn from the Zn deficient calcareous soil. Rengel and Graham (1995) [26] observed that Zn efficiency of wheat genotypes is at least partly due to a greater ability of the efficient genotypes to accumulate Zn. They suggested that the Zinc-efficient wheat genotype (Warigal) transported less Zn and Fe to shoots and had higher Fe concentrations in roots than the Zinc-inefficient genotype (Durati), supporting the hypothesis that Zn-efficiency may be connected with inefficient transport of Fe from roots to shoots and thus initiation of the Fe-deficiency response resulting in increased release of Zn and Fe binding phytosiderophores.

Zhang *et al.*, (1991) [32], examined the effect of iron plaque on Zn uptake by Fe-deficient rice plants. They found that Fe-deficient plants responded better to Zn treatment than the Fe sufficient plants because the exudates of Fe deficient plants, the phytosiderophores, could enhance Zn uptake in plants with iron plaque. Under Zn deficiency, translocation of Fe from roots to shoots is depressed in the Zn-efficient genotypes resulting in hidden Fe deficiency and increased PS release which increase Zn uptake (Walter *et al.*, 1994; Rengel and Graham, 1995) [33, 27].

Rengel and Romheld, (2000) [34] have shown that greater tolerance to Zn deficiency among wheat genotypes is associated with increased exudation of phytosiderophores, increased Fe uptake and decreased transport of Fe to shoots. The Zn inefficient bean genotypes also show enhanced exudation of reductants, increased reduction of Fe<sup>3+</sup> and increased accumulation of Fe in leaves as compared to Zn efficient genotype when grown under Zn deficiency. Zinc deficiency thus stimulates the reduction and uptake of Fe, which in turn competitively inhibits Zn uptake and utilization leading to enhanced Zn deficiency in Zn inefficient genotypes.

Erenoglu *et al* (1996) [35] did not find the release rate of phytosiderophores to be related with the susceptibility of bread wheat genotypes to Zn deficiency. According to them root uptake and root-to-shoot transport of Zn particularly in terms of utilization of Zn may be more important mechanisms involved in expression of Zn efficiency in bread wheat genotypes than release of phytosiderophores. Cakmak *et al.*, (1997) [36] found a particular role of light intensity in phytosiderophores release from roots under both Zn and Fe deficiency. Hopkins *et al.*, (1998) [37] show that sorghum and wheat plants increased the release of phytosiderophore in response to Zn deficiency as compared to corn and the total amount of phytosiderophore released by the roots was in the order wheat > sorghum > corn. The Zn efficiency of the plants is also affected by the seed size, quality (higher quality expressed as higher seed Zn content) which influences vegetative yield and grain yield of cereals grown under Zn

deficiency. It was concluded from studies that the effects of grain content on Zn-efficient 'Excalibur' and Zn inefficient 'Gatcher'. They concluded that higher grain Zn content acted similar to a "starter" fertilizer by improving early vegetative growth. About 0.2 mg Zn kg<sup>-1</sup> soil was required to achieve 90 percent optimum yield for plants derived from high Zn grain and 0.8 µg per kg soil for plants grown from low Zn grains.

More efficient utilization and compartmentalization of Zn within tissues and organs (Graham and Rengel, 1993) [38] including a greater activity of carbonic anhydrase and anti-oxidative enzymes eg. SOD (Cakmak *et al.*, 1997) [39], maintaining sulfhydryl groups in the root cell plasma membranes in a reduced state and a differential pattern of biosynthesis of the root-cell membrane polypeptides (Rengel and Hawkesford, 1997) [40] are some of the enhanced utilization efficiency mechanism in Zn-efficient genotypes compared to Zn-inefficient ones. In Zn-efficient wheat genotype (Warigal) a 34-kDa polypeptide was increased in the root cell plasma membranes, but not in the Zn-inefficient one (Durati), suggesting that the polypeptide might be connected with Zn-efficiency since both the genotypes had the capacity to synthesize this 34KDa polypeptide during early growth period but the capacity did not appear at later stages in Durati with the onset of Zn deficiency but was present in Warigal (Rengel and Hawkesford, 1997) [40].

Now a days, use of Zn-efficient cultivars has become sustainable solution in response to deficiency limitation to crop production This is because the Zn-efficient plant genotypes can function more effectively under low soil Zn condition and would reduce fertilizer input and protect the environment as well (Rengel, 2001) [41]. A large number of studies have been carried out by different workers in several crops including beans, wheat, rice and tomato.

High Zn efficiency in crops appears to be related to various morphological and physiological traits, such as root surface area, Zn-mobilizing root exudates and better utilization of Zn at the cellular level. However, finding a solution to Zn deficiency requires a comprehensive exploration of potential genetic resources and an in-depth understanding of their role in micronutrient accumulation mechanisms. Zn enrichment traits are present within the genomes of crops that could allow for substantial increases in the Zn concentration of edible parts without negatively impacting yield. The use of Zn dense seeds results in greater seedling vigor and increased crop yields when the seeds were sown in Zn-deficient soils. Field and growth room screening had revealed significant genetic variation for Zn efficiency in cereals, suggesting that selection for improved Zn efficiency is possible. Recent identification of DNA markers that are diagnostic of Zn efficiency can accelerate the development of cultivars that can remain productive even in Zn-deficient soils; these markers can also be used to identify specific genes responsible for differences in the response of genotypes to Zn deficiency, (Sadeghzadeh, 2013) [42].

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