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MINI REVIEW

Strategies to increase zinc deficiency tolerance and homeostasis in plants

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ABSTRACT

Zinc deficiency is a global problem of considerable importance for agriculture and human health. Under zinc deficiency conditions, many essential zinc-dependent physiological functions are unable to operate normally, and the cellular homeostasis is adversely affected. This paper described the potential damages that low-zinc bioavailability in soil can have for plants, humans, and animals. In addition, current knowledge on physiological and molecular aspects of zinc homeostasis in plants and strategies used to increase zinc deficiency tolerance were discussed.

Keywords: biofortification, mineral nutrition, plant abiotic stress, plant nutrition.

INTRODUCTION

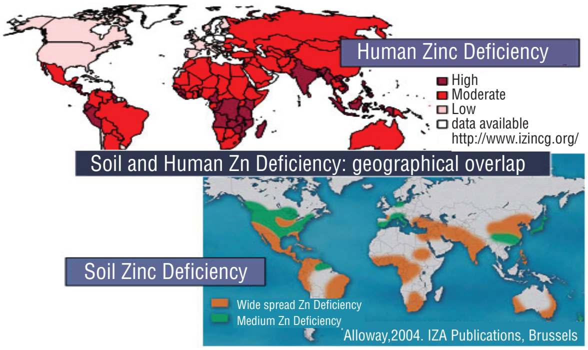
Plants are autotrophic organisms that have the ability to use solar energy to synthesize vital components from carbon dioxide, water, and nutrients. Minerals, macro and micronutrients are essential to plant growth and development. Their absence prevents plants from completing their life cycle (Ramesh et al., 2004), due to their essential role in physiological and metabolic processes in the plant. Zinc is a vital micronutrient for all organisms. This element is involved in many reactions of the cellular metabolism, including biological processes, such as antioxidative defense, protein synthesis, carbohydrate metabolism, auxin metabolism, and stability of genetic materials (Clemens, 2006; Broadley et al., 2007). Thus, zinc deficiency stress in plants, normally due to low-zinc bioavailability in soil, causes significant decreases in the productivity and nutritional quality of food.

When facing zinc shortage, plants undergo a range of physiological and molecular adjustment in order to maintain cellular homeostasis and to avoid abrupt changes in the dynamic and complex process of development (Grusak, 2002). As the natural response mechanism to overcome a low zinc level in the cell, plants increase the expression of several genes encoding zinc transporters and metal chelator biosynthesis enzymes involved in zinc uptake from the soil (van de Mortel et al., 2006). Since there appears to be genetic variation for this ability, plant breeding and genetic engineering approaches can be used to develop new high-zinc content crop genotypes. They are expected to increase the crop production in areas with low-zinc bioavailability and alleviate human malnutrition problems due to zinc deficiency (Cakmak et al., 2010; Gómez-Galera et al., 2010).

This manuscript aimed at providing an overview about zinc deficiency problems in the world, with emphasis on soil-plant-human health interactions. We also described the current strategies that have been used to mitigate such problem.

ZINC DEFICIENCY, A GLOBAL PROBLEM

Zinc deficiency in soils is a serious global problem that affects many agricultural soils. It is estimated that about half of the cultivated soils in the world contains reduced amounts of bioavailable zinc (Figure 1). This problem is aggravated mainly in arid and semi-arid regions, due to low organic matter and soil moisture as well as high levels of pH and $CaCO_3$ (Cakmak, 2008; Gonçalves Junior, 2010). The low availability of this metal in the soil limits zinc uptake by plants, resulting in significant decreases in both productivity and nutritional quality of food.



Source: Doctor Ismail Cakmak.

Figure 1. Overlap between geographical regions with soil zinc deficiency (Cakmak, 2011).

Reduction in both growth and in yield happens at low-zinc concentrations in the shoot (Cakmak, 2011). In it, there is a range from 30 and 100 µg zinc g^{-1} dry weight (DW) to be sufficient to support adequate plant growth, reaching a critical point when the Zn concentration is below 100 μg $g^{-1}$of dry weight in shoot meristems of rice, due to the disintegration of the 80S ribosomes. In addition to that, the same concentration, or most likely even a higher one, is required to maintain protein synthesis in other meristematic tissues. This required amount is five to ten-fold higher when compared to mature leaves, which shows the importance of the nutrient to keep right growth (Marschner, 1995), and even for optimal growth, plants need to keep a tight control over zinc homeostasis.

Zinc is vital for the functionality of more than 300 enzymes. It can act as a functional, structural, or regulatory cofactor of a large number of enzymes. Some enzymes, such as alcohol dehydrogenase, Cu-Zn superoxide dismutase, and carbonic anhydrase, have zinc in their structure. Other enzymes, such as aldolases, enolase, isomerases, peptidases, transfosforylases and RNA and DNA polymerases, require zinc for enzymatic activity (Guerinot and Eide, 1999; Buchanan et al., 2000). The structural and functional integrity of cell membranes is also influenced by zinc, which acts in stabilization of biomembranes by interaction with phospholipids and sulphydril groups of membrane proteins. Zinc deficiency in plants causes biochemical changes in membranes, which modify the permeability and architecture of biological membranes. The protection against the peroxidation of membrane lipids and proteins has been shown to be the major role of zinc in membranes (Marschner, 1995; Cakmak, 2011).

Delayed and reduced growth, small and malformed leaves, short internodes and yellowing effects are common symptoms of plants growing on low-zinc supply, all largely attributed to the disturbance of auxin metabolism. However, the way in which zinc deficiency affects indole-3-acetic acid (IAA) metabolism has not been clear yet. It is believed that zinc is involved in both IAA biosynthesis and in protecting IAA from oxidative degradation by reactive oxygen species – ROS (Robson, 1994; Cakmak, 2011).

As a result of its vital importance to plants, zinc deficiency affects the plant as a whole, causing serious problems in the metabolism of carbohydrates, mainly by the severe decline in photosynthesis and in sugar metabolism, and the synthesis of proteins, attributed to a sharp reduction in transcription, deformation, and reduction of ribosomes (Marenco and Lopes, 2007). The main damage of low-zinc supply on protein metabolism acts through gene expression by affecting RNA and DNA structure and the stability and function of zinc finger proteins, which are required for the expression and regulation of gene expression (Marschner, 1995).

The regions with zinc-deficient soils are strongly correlated with the ones with high incidence of human Zn deficiency. The cultivation of food crops on Zn-deficient is problematic, leading to low yields, low zinc content in food, and low income to farmers, preventing them to invest in zinc fertilizers. Especially when the crops in question constitute the main food and feed sources for the local population, dietary zinc deficiency will be inevitable in both humans and domestic animals (Erenoglu et al., 2010). Cereals-based foods are the most important source of calories in most developing countries. As an example, wheat cultivation on Zn-deficient soils in Turkey lead to the production of wheat (*Triticum aestivum*, L.) grains with approximately 50% reduction in zinc content compared to wheat grown with sufficient zinc supply (Cakmak, 2011).

Zinc deficiency in humans is a nutritional problem worldwide. It is estimated that one-third of the world population (around 2 billion people) suffers from mild zinc deficiency and over 450,000 children die each year due such deficiency (Welch and Grahan, 2004; Cakmak et al., 2010). According to the World Health Organization (2003), zinc deficiency in humans can result in several undesirable consequences, including diminished learning ability, impaired immune response, dysfunction of reproductive system, and reduced growth rates on infants. Thus, ensuring adequate dietary intake of zinc is essential to reduce illness and to decrease child mortality in developing countries.

STRATEGIES USED TO INCREASE ZINC DEFICIENCY TOLERANCE

The impacts caused by zinc deficiency in agriculture and in human health have been studied for decades. Many physiological mechanisms, such as root uptake and translocation, zinc sequestration in leaves, biochemical utilization of zinc and, more recently, identification and manipulation of candidate genes for proteins zinc transporters and chelator enzymes have been investigated (Hacisalihoglu and Kochian, 2003), and these findings have contributed to unraveling the molecular system of zinc homeostasis in plants.

A fast and easy solution to prevent zinc deficiency plants is Zn supplementation by applying fertilizer (Cakmak, 2008). However, there are costs associated with the application, and even though the benefits are wider, this poses a threshold for application. In addition, zinc fertilizer application effect can be impaired by physical and chemical characteristics of soil, which reduce the availability of Zn to plants, leading to a disappointing experience for farmers. An promising and cost-effective alternative strategy is improving plant zinc use efficiency and grain zinc content by plant breeding or genetic engineering. Such biofortification approach is one of the aims of the international Harvest Plus Consortium, which supports the development of new high-zinc content genotypes for increased zinc content in food and crops (Cakmak et al., 2010; Gómez-Galera et al., 2010). Although biotechnological techniques advances and significant progress in understanding structures involved in metal homeostasis have driven research in this field, still little is known about the regulators of zinc homeostasis network in plants.

ZINC HOMEOSTASIS

Zinc is a transition metal essential for terrestrial life, as already described. However, it is only required at low concentrations, making it function as a micronutrient. Concentrations between 30 and 100 μg zinc g^{-1} DW are enough to support adequate plant growth, whereas zinc toxicity symptoms are observed in concentrations above 300 μg g^{-1} DW for species that are not adapted to high-zinc exposure (Marschner, 1995; van de Mortel et al., 2006). Therefore, for optimal growth, plants need to keep tight control over zinc homeostasis.

Zinc homeostasis requires a complex network of cellular or tissue specific functions to control metal uptake, accumulation, trafficking, and detoxification. The ability to take up Zn of higher plants depends much more on its bioavailability from soil than on the absolute soil concentrations. Zinc bioavailability is modulated by various physical and chemical soil factors. Zinc solubility in soil decreases due to high levels of calcium carbonate, metal oxides, and pH and low levels of organic matter and soil moisture as well as high amounts of phosphate (Robson, 1994; Cakmak, 2011). When available in the soil solution, zinc is absorbed and transported in the divalent ion form (Zn^{+2}) from roots to shoots through the xylem, being easily retranslocated by phloem (Clemens, 2001). This transport of ions and molecules from epidermal and cortical cell to xylem can occur through the symplastic or apoplastic route.

Following the apoplastic route, zinc and other minerals (essential and nonessential) traverse with water through cell walls and intercellular spaces outside the cell membrane to the endodermis, where Casparian strips force all solutes that need to be transported through xylem to enter endodermis cells by crossing the plasma membrane. The symplastic route consists of a continuous system of cytoplasmatic transport interconnecting cells by plasmodesmata. Upon entering the stele of the plant root, ions are transported symplastically until they reach the xylem parenchyma cells, which act in loading minerals into the xylem (Taiz and Zeiger, 2004; Broadley et al., 2007).

Regardless of the chosen path, the solutes that reach the xylem parenchyma cells are transferred to the xylem elements in a tightly-controlled process mediating membrane transport. Via the xylem sap, the minerals are released in the apoplast of the leaves and are subsequently distributed intracellularly (Clemens et al., 2002).

Zinc distribution, transport, and accumulation are affected by the level of zinc supply to the plant, as well as by *mycorrhiza fungi*, reducing the distance across which nutrients have to be transported in the soil (Marschner, 1995). At low or adequate zinc supply, roots, vegetative shoots, and reproductive tissues have higher zinc concentrations in young growing tissues than in mature ones. When exposed to toxic zinc levels, tolerant plants can accumulate zinc in the root cortex cells and in leaves, specifically in the cell wall or vacuoles (Robson, 1994).

GENES INVOLVED IN ZINC HOMEOSTASIS IN PLANTS

Accumulation of zinc or other metallic minerals depends on the uptake capacity and on intracellular binding sites. The metal accumulation rates are affected by the concentration and affinities of chelating molecules and by the presence and selectivity of transport activities (Clemens et al., 2002). Metal transporters are required for metal uptake and efflux or intracellular metal transport in the plant, and metal chelators contribute to metal detoxification by buffering free cytosolic metal concentrations. Therefore, both play a major role in metal homeostasis. Several studies have focused on the identification and manipulation of genes for zinc transporter proteins and chelator biosynthesizing enzymes.

The natural resistance associated macrophage protein (NRAMP) is a family of proteins whose function is to take up and transport metal. Research in *Arabidopsis thaliana* (L.) Heynh (Arabidopsis) showed that NRAMP transporters have limited metal specificity. AtNRAMP3 and AtNRAMP4 are localized to the vacuolar membrane and are involved in the intracellular iron transport (Thomine et al., 2000). These genes show similar expression levels in most tissues (Grotz and Guerinot, 2006), and only double mutants show a mutant phenotype, i.e., they are functionally redundantly (Palmer and Guerinot, 2003). Thomine et al. (2003) reported that AtNRAMP3 controls the accumulation of zinc and manganese in roots upon iron starvation.

Another family of transporters involved in zinc efflux is the P_{1B}-ATPase. Arabidopsis has eight genes encoding P_{1B}-ATPases that differ in their structure, function, and regulation (Eren and Argüello, 2004). Among these, HMA1, HMA2, HMA3, and HMA4 are involved in zinc transport (Hussain et al., 2004). AtHMA1 localizes in the chloroplast envelop and can contribute to Zn detoxification under excess zinc conditions (Kim et al, 2009). The AtHMA2 gene encodes a Zn^{+2}-ATPase located in the plasma membrane. Expression is induced by cadmium and zinc (Eren and Argüello, 2004). The AtHMA3 protein possibly mediates zinc hyperaccumulation, since zinc hyperaccumulator species show higher expression of HMA3 than the non-hyperaccumulator ones, such as Arabidopsis (Becher et al., 2004; van de Mortel et al., 2006; Hassan and Aarts, 2011).

Recently, HMA3 has been cloned from *Thlaspi carulescens* Alpine Penny-cress (Ueno et al., 2011), and rice (Ueno et al., 2010), therefore it is a vacuolar influx transporter, important for cadmium tolerance in both species. This suggests that different homologs of HMA3 may have different metal-substrate specificity. AtHMA4, similar to AtHMA2 and acting alike, plays an important role in translocation of zinc, specifically in loading of zinc into the xylem (van de Mortel et al., 2006; Waters and Sankaran, 2011). Hussain et al. (2004) reported that both HMA2 and HMA4 are essential to zinc homeostasis and they show a functional redundancy.

Members of the cation diffusion facilitator (CDF) family play an important role in living organisms, as they control cation concentrations in cells through sequestration into internal compartments and efflux from cell (Gustin et al., 2011). MTP1 and MTP3 seem to be involved in the sequestration of zinc in root vacuoles and can act to limit its translocation to the shoot (Arrivault et al., 2006). In addition, when MTP1 is overexpressed in Arabidopsis, an increased resistance to zinc and higher zinc content in roots are observed (Kobae et al., 2004). MTP2 is also involved in zinc homeostasis. Under zinc deficiency, MTP2 expression increases suggesting it to play a specific function in counteracting the effect of zinc deficiency (van de Mortel et al., 2006). MTP8 is another member of the CDF family, which in addition to mediating manganese transport may also function in zinc uptake (van de Mortel et al., 2006).

The zinc regulated transporter (ZRT), iron-regulated transporter (IRT)-like protein (ZIP) family contains many members thought to be involved in transporting zinc into the cytosol across the plasma membrane, which is an important process for plant zinc uptake (Palmer and Guerinot, 2009; Song et al., 2010). ZIP transporters have eight transmembrane domains and a histidine-rich variable loop between transmembrane domains III and IV that appears to be conserved among all family members (Colangelo and Guerinot, 2004). In Arabidopsis, there are 15 ZIP gene members, ZIP1-12 and IRT1-3, of which at least ten members (ZIP1, 2, 3, 4, 5, 9, 10, 11, 12 and IRT3) appear to play a role in plant zinc uptake. Approximately half of the ZIP genes (ZIP1, 3, 4, 5, 9, 10, and IRT3) is induced in response to zinc deficiency (Wintz et al., 2003; van de Mortel et al., 2006; Assunção et al., 2010).

Metal chelators are also important for metal homeostasis. Nicotianamine (NA) is a metal-chelating compound, made by the action of NA synthase that binds zinc, as well as iron, copper, and nickel (Curie et al., 2009). NA is thought to be involved in long-distance transport, perhaps also playing a role in the entry of metals into the phloem or xylem through metal-NA chelate transporters of the Yellow Stripe-like (YSL) family (Gendre et al., 2007). NA synthase is encoded by four genes in Arabidopsis, AtNAS1-AtNAS4, which act functionally redundant, although they show different expression patterns, suggesting that each NAS gene may have a specialized function (Klatte et al., 2009). As an example, only AtNAS2 and AtNAS4 are highly expressed in roots under zinc deficiency (van de Mortel et al., 2006).

ZINC HOMEOSTASIS REGULATION

The growth and development of all organisms depend on adequate gene expression regulation. Transcription factors play an essential role in modulating gene expression by controlling transcription initiation rates. Thus, strategies that seek to modify the transcription expressions factors have shown a more efficient and promising approach than the modification of a single structural gene in Genetic Engineering (Yang et al., 2009). Currently, there are few groups focusing on this approach, however they obtained very promising results with regards to engineering environmental stress tolerance. In Arabidopsis, overexpression of the transcription factor DREB1A, driven either by a constitutive (CaMV 35S) or conditional promoter (rd29A), increases tolerance to drought, cold, and salt stress (Kasuga et al., 2004). Overexpression of an ERF transcription factor TSRF1 enhances the osmotic and drought tolerances (Quan et al., 2010).

Regarding micronutrient response, experiments carried out on rice showed that OsIRO2 overexpression, a transcription factor involved in the regulation of iron homeostasis genes in rice (*Oriza sativa* L.), increases iron deficiency tolerance by improving growth and yield of rice plants (Ogo et al., 2011). Recently, two transcription factors, bZIP23 and bZIP19, which are involved in the regulation of zinc deficiency response in Arabidopsis, were identified. These transcription factors recognize 8 to 10 bp palindromic motifs called zinc deficiency response elements, found in tandem in promoters of several zinc homeostasis genes, activation of which constitutes the primary response to zinc deficiency (Assunção et al., 2010). Modification of these transcription factors to control zinc deficiency tolerance and accumulation is in progress (Henriques and Aarts, unpublished results). The simultaneous expression of a set of genes implicated in the same process is a promising strategy to enhance productivity and stress tolerance in plants, when activation of multiple genes at the same time is necessary to obtain an effective stress response. Still, although the listed findings offer interesting options to try and modify plant micronutrient deficiency response, the control of post-transcriptional and -translational regulation also deserves attention.

CONCLUSIONS

In terms of understanding the roles of genes involved in uptake and translocation of zinc in plants, a lot has been achieved, but information on where in the plant each transporter functions and how each one is controlled in response to nutrient availability remains still unclear. The identification of transcription factors involved in the control of zinc deficiency response offers interesting opportunities to modulate zinc deficiency responsive gene expression to make plants less sensitive to zinc deficiency or to confer a constitutive zinc deficiency response, which can induce plants to over-accumulate metals. Therefore, understanding the regulator identity of the zinc homeostasis network in plants should provide new insights for the development of crops in areas suffering from low zinc bioavailability and for biofortification strategies. Furthermore, understanding how zinc interacts to other metals, such as cadmium and lead, during its absorption is important to be known, avoiding undesirable accumulation of such heavy metals in plants.

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