ZINC METALLOENZYMES IN PLANTS

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SUMMARY

Zinc is an essential plant micronutrient and soil availability is of great importance in many crops. In plants, zinc is neither oxidized nor reduced; instead, the significance of zinc stems from its physiochemical properties as a divalent cation. Many enzymes include zinc as a cofactor, like the alcohol dehydrogenase (EC 1.1.1.1), superoxide dismutase (EC 1.15.1.1), carbonic anhydrase (EC 4.2.1.1) and RNA polymerase (EC 2.7.7.6). In these cases, it is indirectly evident that zinc deficiency inhibits protein synthesis. Plants also require zinc for the synthesis of tryptophan, a key amino acid in the synthesis of the auxin indoleacetic acid. Therefore, zinc also operates in the control of plant development through its indirect action on auxins. Zinc deficiency affects the catalytic activity of all the above enzymes and, thus, the metabolic pathways in which they are involved. The aim of this paper is to analyze the function of Zn in some of metalloproteins involved in plant metabolism.

In contrast to Fe, Mn, Cu and Mo, Zn is a transition element that is not subject to valence change and, thus, it is present in plants only as Zn (II). The element functions mainly as a divalent cation in metalloenzymes and among these functions is the linkage of these enzymes to their corresponding substrates. In other cases, Zn forms tetrahydric complexes with N and O and, particularly, is linked to S in a variety of organic compounds (Samreen *et al*., 2017). Thus, Zn can give support and stability to an enzyme by activating it. Higher plants have a number of enzymes containing Zn, including alcohol dehydrogenase, carbonic anhydrase and RNA polymerase (Eide, 2011). However, Zn activates many other enzymes. Even though the changes caused by Zn deficiency in the growth and development of plants are highly complex, there are some changes that are typical and related to the functions of this micronutrient in particular reactions or in specific steps in metabolic pathways (Hong *et al.,* 2007). Changes in plant metabolism induced by Zn deficiency include effects on carbohydrates, proteins, auxins and damage to membrane integrity. Many Zn dependent enzymes are involved in carbohydrate metabolism, especially in leaves (Xing *et al.,* 2016). When Zn deficiency occurs, carbonic anhydrase activity decreases markedly (Escudero-Almanza *et al.,* 2012). In addition, Zn also activates tryptophan synthetase, enzyme responsible for synthesis of tryptophan in indoleacetic acid (IAA) biosynthesis, which is a hete-

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roauxine (Escudero-Almanza *et al*., 2012; Hafeez *et al*., 2013). As already noted, Zn is involved in a number of physiological processes in plants. To properly understand these processes, it is necessary to consider the Zn-dependent enzymes of plants. The objective of this paper is to analyze the function of Zn in some of metalloproteins involved in plant metabolism.

Generalities and Classification of Metalloproteins

Enzymes that depend on metal ions as cofactors are of two sorts: enzymes activated by metals and metalloenzymes. As the name implies, in enzymes activated by metals, catalysis is triggered by the presence of a mono- or divalent metal ion on the outside of the protein. The metal can activate the substrate (for instance, Mg^{2+} with adenosine triphosphate (ATP)) to fit the enzyme directly, or to enter in balance with the enzyme by potentiating its ionic charge to produce a stronger, more favorable union with the substrate or a better catalytic environment (Eide, 2011).

Metalloproteins (MT) were discovered by Margoshes and Valle (1957), who isolated them from the renal cortex of horses and identified them as proteins able to combine with cadmium (Cd). At first, it was thought that the presence of these proteins was related to a detoxification mechanism for Cd accumulation in the renal tissues of these animals. Since then, MT have been reported in all animal phyla examined, and in certain fungi, plants and cyanobacteria. The MT are a set of combined proteins with low molecular mass, able to bind with a wide variety of metals at a cellular level. The MT are mainly located in the cytoplasm but some research has shown their presence in lysosomes and nuclei. In plants, MT concentrations vary considerably, depending on a range of factors such as the kind of organism, tissue, age, development stage, nutritional state, metal exposure and on other unidentified factors (Bray *et al*., 2000; Eide, 2011).

Biochemistry and Physiology of Zinc

The importance of Zn in plants was first demonstrated in corn, and later in barley and dwarf sunflower (Hafeez *et al*., 2013). The first reports of symptoms of severe Zn deficiency included defective elongation in tomato branches (Hafeez *et al*., 2013). The deficiency was reflected in reduced protein and starch synthesis, but sugar content was not affected. Reduced activity of some respiratory enzymes, accumulation of quinones, and changes in the levels of amino acids and proteins have also been reported in Zn deficiency (Broadley *et al*., 2007). In most crops, the typical foliar concentration of Zn required for normal growth lies between 15 and 20 mg kg^{-1} of dry matter (Broadley *et al*., 2007). This metal is a component of numerous enzymes such as anhydrases, oxidases and peroxidases and it performs a critical role in regulating N metabolism, cell proliferation, photosynthesis, and auxin synthesis. One of the main functions of Zn is the expression and regulation of genes, as Zn finger transcription factors have been implicated in the regulation of biological processes such as flowering, photo morphogenesis and pathogen responses (Hafeez *et al*., 2013).

Zinc is absorbed by the plant as a chelate, either via leaves or roots. In long distance transport in the xylem, it is linked with organic acids or exists as a free, divalent cation. In floematic sap it is present in high concentrations, linked to low molecular weight organic solutes. It can be complexed with phospholipids and sulfhydryl groups, where it protects the lipids and proteins of membranes against oxidative damage (Broadley *et al*., 2012).

The low levels of IAA in plants with Zn deficiency are a result of the oxidative degradation of super oxide dismutase enzymes (SOD) and catalase, thus diminishing their activity (Broadley *et al*., 2012). The characteristic visual symptoms of Zn deficiency in plants are short internodes and decreased foliar expansion, related to a blockage in IAA metabolism (Kirkby and Römheld, 2004). Transport of Zn from cortical and epidermal cells of the root to the xylem can occur in a simplified form, with Zn being pumped to the apoplast of the stele. Zinc can also be released extracellularly to the apoplast of the stele in regions where the Casparian strip is completely formed. Apoplastic flows are governed by cationic interchange in the cell wall, Casparian strips and water flow (Broadley *et al*., 2012).

The dynamics of Zn in the soil are similar to those of Cu and Fe; if pH <5 its availability is higher, but is lost by increased leaching as its solubility increases (Hafeez *et al*., 2013). Zinc is available in soils with pH 5-6. At higher pH, it forms sparingly soluble compounds. In alkaline soils, Zn has very limited mobility because it reacts with calcium carbonate, which reduces its availability (Ojeda-Barrios *et al*., 2012, 2014). On the other hand, a number of factors are involved in Zn absorption by plants, because micronutrient availability is usually a function of the form of the nutrient in the soil, which determines its mobility to the roots (Ojeda-Barrios *et al*., 2014). In alkaline soils (pH 7-8.6) the insoluble $ZnCO_3$ is formed. Hence, Zn applications to calcareous soils are not effective and are thus limited to non-calcareous ones (Perea-Portillo *et al*., 2010). Zinc deficiency is widespread in production systems worldwide. The best diagnosis of Zn deficiency comes from the observation of deficiency symptoms, combined with foliar and soil analysis. Putative values for Zn extraction from the soil by removal of the crop at harvest are used to calculate the values of residual Zn in the soil, both soluble and interchangeable (Broadley *et al*., 2012). From this information, it is possible to calculate the extent to which the system has been impoverished at the end of a cropping cycle. If the available fraction of Zn is lower than the extraction requirement of the following crop then, a deficiency will occur during the next cycle (Ratto and Miguez, 2006).

Zinc as Metallic Cofactor

An enzymatic cofactor is a non-enzymatic component that promotes the catalytic value of an enzyme. This definition emphasizes its function rather than its structure. Almost a third of all enzymes require ionic metals for their catalytic function; hence, ionic metals represent a substantial proportion of all cofactors. Most trace nutrient metals share as a common characteristic, a close involvement with enzymes. Many are active site components that link the enzyme to the substrate. In this role, they accept electrons, stabilize tertiary and quaternary structures and can even regulate the speed of metabolic pathways (Eide, 2011).

Zinc is an ubiquitous and versatile of all the metallic cofactors, with more than 300 enzymes having a Zn cofactor. Proteins linked to Zn attach to deoxyribonucleic acid (DNA). The finger proteins of Zn are clear evidence of the versatility of Zn in biological systems. Approximately 3% of the mammalian genome codes for Zn finger proteins (Auld, 2009). As a cofactor, Zn can act either structurally or catalytically. There are a number of examples: in carbonic anhydrase, Zn works in a coordinated way with the CO_2 substrate; in carboxipeptidase, it takes an active role in the breakage of the peptide union; in multisub union enzymes such as aspartate transcarbamylate, Zn plays a structural role by coordinating the positions of the

regulator and catalytic subunits; in another structural role, the Cu, Zn-superoxide-dismutase requires Zn to position the Cu atom in the channel accessed by the substrate; in Zn finger proteins, the Zn^{2+} helps to stabilize the structure of curls, which contact the major and minor grooves of the DNA (Auld, 2009). These examples illustrate why Zn is such an important cofactor for enzymes and proteins (Patel *et al.,* 2007).

Zinc is considered an adapted metal because it acts as a divalent cation without special geometric preference. This smoothness is perhaps the characteristic that allows Zn to adapt to so many enzymatic environments. Zinc exists as Zn^{2+}, so it does not have redox properties. The electronic configuration of Zn^{2+} is [Ar] $3d^{10}$ where, d sub-shell is full (Auld and Bergman, 2008). For this reason, Zn complexes lack color and Zn behaves mainly as a cation (Zn^{2+}) which it is a good electron acceptor (Lewis acid) and can enter in a coordinated union arrangement that polarizes the groups to which it attaches. This property allows Zn to increase the susceptibility of a chemical union and to make a nucleophilic attack. For example, Zn^{2+} polarizes water, making it behave more like a hydroxyl ion, facilitating the nucleophilic attack of CO_2 to form HCO_{3-} in the reaction catalyzed by carbonic anhydrase. Another example of the use of Zn is to polarize ester or amino groups, promoting in this way a nucleophilic attack of water, as in the catalyzed reactions of carboxipeptidase and aminopeptidase (Auld, 2005; Harris, 2013).

Zinc Site Classification

Zinc site classification is based in its coordinating properties. There are a great number of enzymes in which Zn is an essential component and four different types of union sites of Zn have been identified: catalytic, structural, co-catalytic and protein interface. In ligands, the most common amino acids are glutamine (Glu), asparagine (Asp) and cysteine (Cys). In enzymes in which Zn has a catalytic function (e.g. carbonic anhydrase and carboxipeptidases) the Zn is coordinated to four ligands, three of which are amino acids. Of these, histidine (His) is the most frequent, followed by Glu and Asp, a water molecule is the fourth ligand in all the catalytic centers. The structural sites of Zn are united to four proteins in metal attached to water (Auld, 2005; Patel *et al.,* 2007).

In enzymes in which Zn has a structural function (for instance alcohol dehydrogenase and proteins that are involved in DNA replication and in gene expression), Zn atoms are coordinated to sulfhydryl groups of four residues. Cysteine is the most frequent ligand in these sites, although there may also be a combination of amino acids (Cys, His, Glu and Asp) that can form these types of Zn sites. Twelve combinations of the 22 permutations of these four ligands have so far been reported. The site role on structural Zn is to maintain the stability of the tertiary structure of the protein, which can also influence the folding and function of the protein by providing residues that participate in catalysis and emerge from inside the spacer branches which form a high-stability tertiary structure. Most Zn enzymes have only one atom of Zn per molecule, alcohol dehydrogenase being the exception. Catalytic sites of Zn are found in enzymes that contain one or more metals of Zn that are very close between them and that function together as a catalytic unit. The distance between metals depends on the amino acid (Asp, Glu, His, Lys or a carboxylate acid) that joins the two metals. Sometimes, a water molecule can form a bridge between metal atoms in a co-catalytic site. Asparagine is one of the most frequent ligands in this type of site (Auld, 2005). Metals, such as Zn are important for their catalytic function, besides having an effect on the quaternary structure of a protein. They are made of amino acid ligands that lie in the union surface of Zn sites between two sub-units of the protein or proteins that interact, and generally, they have catalytic or structural properties of coordination (Maret and Li, 2009). There are many enzymes involved in a range of metabolic processes in plants where Zn plays a fundamental role either as a cofactor to activate the enzyme, or in the enzyme structure. A review of the characteristics and metabolic processes of the main Zn metaloenzymes of plants follows.

Alcohol Dehydrogenase and Soil Waterlogging

Alcohol dehydrogenase (ADH, EC 1.1.1.1) catalyzes the reversible oxidation of a wide variety of primary, secondary and cyclic alcohols to their corresponding aldehydes and ketones. Oxidation reactions occur with the concomitant reduction of the coenzyme, generally NAD^+. Alcohol dehydrogenase is an enzyme system located in the cytoplasm and widely distributed in many phyla that include organisms belonging to all kinds of classification of living things. They are dimers made of subunits of around 374 amino acid residues and ~40kDa. Each subunit has two domains, a catalytic domain and a domain of union with the enzyme; both are separated with a hydrophobic slit that forms the unity pocket to the substrate. This enzyme has two Zn atoms per molecule, one with a catalytic function and the other with a structural function (Auld and Bergman, 2008).

ADH metabolizes the alcohol produced under anoxic conditions, such as due to water logging. Water logging reduces the oxygen in the soil and alters many physical, chemical and biological processes. It can result in the production of toxic substances, elevated levels of a number of natural substances including organic acids, carbon dioxide, sulfides and gaseous hydrocarbons (including ethylene, a natural plant hormone) and reduced levels of a number of other substances (including soluble N compounds and imbalances in the availability of mineral nutrients such as Fe).

Many of these negative-going changes are due to microbial activity under anaerobic conditions and others to pH change. In a waterlogged soil, a number of biological, chemical and physical factors control ethylene accumulation (Pardos, 2004). Metabolic rate reduction limits the use of reserve carbohydrates (starch and sucrose) during waterlogging events. With limited lipid and protein storage, plants must struggle to survive in flooded soils. The substitution of glycolysis via the alternative pathway of pentose phosphate produces less toxic substances that can be found in plants tolerant to flooding. In this case, the lack of malic enzyme increases malate accumulation and diminishes the formation of pyruvate and of ethanol, which is highly toxic to plants. Said accumulation can also be prevented by ADH action that catalyzes the reduction of acetaldehyde to ethanol (Pardos, 2004). Ethanol formation takes place in higher plants under aerobic conditions. This occurs mainly in meristematic tissues, such as at the root apexes. Under Zn deficiency, ADH activity diminishes. In rice, flooding doubles the activity of ADH in the roots when Zn is sufficient but not under Zn deficiency. A deficit in the activity of ADH is associated with impaired root function in flooded rice fields (Arshad and Frankenberger, 2002).

Carbonic Anhydrase and Photosynthetic Activity

Carbonic anhydrase (AC) (EC: 4.2.1.1) catalyzes the rapid conversion of carbon dioxide and water into bicarbonate ion (HCO_3^-) and a proton:

$CO_2 + H_2O \rightleftharpoons HCO_3^- + H^+$

Carbonic anhydrase can be found in prokaryotes and in four families of higher plants being βAC. Carbonic anhydrase is a metalloenzyme that requires Zn as a cofactor; it participates in a number of processes such as pH regulation, CO_2 transfer, ion exchange, respiration, photosynthetic CO_2 fixation, and stomatal closure. Carbonic anhydrase catalysis is performed by the metal ion, where Zn acts as a cofactor by its union to water to activate the catalytic site of the enzyme. There are many evidences that suggest that Zn deficiency reduces the content of AC in some plants (Escudero-Almanza *et al.,* 2012). In dicots, AC consists of six subunits, has a molecular weight of 180kDa and has six atoms of Zn per molecule (Figure 1). The enzyme is located in the chloroplasts and in the cytoplasm.

Carbonic anhydrase is involved in a range of physiological processes; any change in AC activity directly affects the photosynthetic fixation of CO_2 under CO_2-limiting conditions. Among the enzymes participating in photosynthetic metabolism in a number of plant species, AC is the only one whose activity varies with atmospheric CO_2 concentration. Carbonic anhydrase functions in the fixation of photosynthetic CO_2: 1) In C3 plants, β-CA helps to elevate CO_2 concentration in the chloroplast, which is a substrate for the RuBisCO enzyme. This raises carboxylation so it can participate in C_2 incorporation into carbohydrates during photosynthesis where only CO_2 can be used as a carbon source. 2) CO_2 hydration to HCO_3^- is a substrate for carboxylase phosphoenolpyruvate (PEPC) in C4 and CAM plants. 3) Carbonic anhydrase enables CO_2 distribution through the plasma membrane and the chloroplast 4) Carbonic anhydrase participates in the active transport of CO_2 through the plasma membrane by CO_2 conversion to HCO_3^- (Tiwari *et al.,* 2005). In the absence of a light, other energy sources are used to fix CO_2 while respiration continues and CO_2 concentration rises, increasing the substrate for AC and producing more $HCO_3^- + H^+$. This lowers pH and inactivates amylase, so glucose and starch are no longer hydrolyzed. This, in turn, reduces the osmotic gradient causing the occlusive cells to lose water and the inflammation closes the stomata (Casson and Gray, 2008). Despite the similar overall AC activities of C_3 and C_4, in C_4 plants, only 1% is located in the beam's sheath but 20-60% is associated with the plasmatic membrane. There is evidence that in C_4 plants, the *in vivo* AC activity can be sufficient to prevent the conversion of CO_2 to HCO_3, thus limiting photosynthesis. As a result, Zn deficiency can have more effects on photosynthesis in C_4 plants than in C_3.

Carbonic anhydrase is an adaptable enzyme; its synthesis is rapidly increased under low-CO_2 conditions but its activity falls after a few days of darkness or under N deficiency. Changes in AC activity are always similar to those of carboxylase PEP, indicating that the expression of the genes that code for these two proteins may be related (Marschner, 2011; Escudero-Almanza *et al.,* 2012).

Tryptophan Synthetase and Indoleacetic Acid Synthesis

Zinc activates tryptophan synthetase, which regulates the synthesis of tryptophan, involved in the biosynthesis of indoleacetic acid (IAA); hence, its main effect is on the meristematic areas involved in foliar expansion and shoot growth. Under conditions of Zn deficiency, IAA is reduced since tryptophan is not synthesized, as it requires the action of an enzyme activated by Zn (the one that unites the serine and the indole ring). Beyond this, the mechanism(s) through which Zn deficiency influences auxin metabolism are unknown. The most obvious symptoms of Zn deficiency (very slow growth and small leaves) are likely related to disturbances in auxin metabolism, IAA in particular (Hanafy-Ahmed *et al*., 2012). The low levels of IAA in Zn deficient plants could be the result either of inhibited IAA synthesis or of enhanced degradation. The tryptophan effect is more likely the primary influence on IAA biosynthesis (Wood *et al*., 2007). There is conflicting evidence for the Zn requirement of tryptophan synthesis. An increase in the tryptophan content of dry matter in rice grains following Zn fertilization of plants cultivated in calcareous soil (Broadley *et al*., 2012). strengthens this assumption. However, such growth could be an expression of a general rise in grain protein content, so the result is unclear in terms of the Zn requirements for tryptophan synthesis (Marschnner, 2011). In plants re-supplied with Zn, leaf tryptophan is increased, instead of diminished. Most likely, this is due to similar increases in the levels of other amino acids, while the low IAA content of Zn deficient leaves is more probably due to increased oxidative degradation of IAA (Hanafy-Ahmed *et al*., 2012).

Superoxide Dismutase and Membrane Integrity

Superoxide dismutase (SOD) catalyzes the dismutation of superoxide and hydrogen peroxide. It is thus

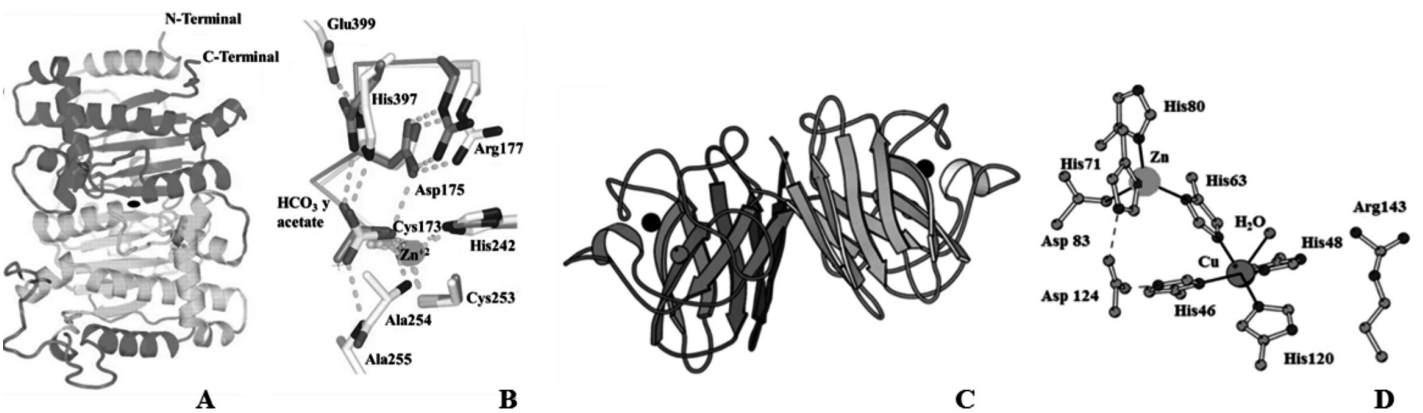

Figure 1. Structure of β-CA tetramere (A) and characterization of the active site metal cofactor Zn^{2+} (B) (Sawaya *et al*. 2006). The metalloenzyme superoxide dismutase (SOD) is firmly attached to two metal cofactors: in C is shown the outlining of the structure in sheet B of the SOD, and in D an imidazole bridge can be appreciated in the immediacies of the active site of the SOD (Hu and Ribbe, 2012; Miller, 2004).

an important anti-oxidant defense in most plant cells exposed to oxygen. In the SOD present in plants, Zn is associated with copper (CuZnSOD) at the active site of the enzyme. The location and role of Cu in SOD The location and role of Cu in SOD has already been discussed by Cakmak (2000). The most probable explanation is that the Cu atom represents the metallic-catalytic component, while Zn represents the structural one (Figure 1). Zinc controls the generation of toxic O_2 radicals as it interferes in NADPH oxidation, as well as in removal of O_2 radicals, for its role in the enzyme CuZn-SOD. When suffering a Zn deficiency, O_2 generation usually increases and it is typical to record a rise in the permeability of the plasma membrane as the toxic O_2 free radicals break the double chains in polyunsaturated fatty acids and phospholipids of membranes. Increased permeability leads to losses in sugars, amino acids and potassium. The increase in lipid oxidation in leaves leads to chlorophyll destruction, necrosis and stunted growth as a product of IAA oxidation, particularly at high radiation intensity (Cakmak, 2000).

With Zn deficiency, SOD activity is considerably lower, but it can be restored *in vitro* by resupplying Zn in the test medium (Marschner,1995). This indicates the Zn atom is a structural component that is essential to the normal functioning of CuZnSOD. The reduction in SOD activity that occurs with Zn deficiency is particularly critical because a simultaneous increase in O_2^- generation rate also occurs. Under Zn deficiency, the level of toxic O_2^- radicals is far greater, as also is that of oxidants related to the principal factors related to lipid peroxidation of the membrane and the increment in membrane permeability. Zinc is required to maintain membrane integrity because it can link with membrane phospholipid and sulfhydryl groups and form complex tetrahedral groups with cysteine residues of polypeptide chains. These further protect the proteins and lipids of the membrane against oxidative damage (Broadley *et al*., 2012). Zinc can also control the generation of toxic oxygen radicals when it interferes with the oxidation of NADPH as well as detoxifying O_2^- in its function as a metallic component CuZn-SOD. Under Zn deficient conditions, there is for this reason a typical increment in plasma membrane permeability. For example, in roots this indicates solute leakage of low molecular weight moieties, a decrease in the phospholipid content and a decrease in the degree of unsaturation of membrane lipids. After 12h of Zn resupply, some restoration of membrane integrity can be observed. Plasma membrane vesicles isolated from roots suffering Zn deficiency, also exhibit greater passive permeability than Zn sufficient roots. The increased membrane permeability of Zn deficient plants is caused by greater generation rates of O_2^- as a result of elevated activity by an oxygen generator dependent on NADPH. A major activity of this oxidase is a consequence of the direct role of Zn in the regulation of its activity, or the indirect result of structural alterations in membrane composition (Kirkby and Römheld, 2004).

Very likely, many of the more obvious symptoms of Zn deficiency, such as chlorosis and foliar necrosis, inhibited stem elongation and increased membrane permeability, are expressions of oxidative stress caused by a greater generation of O_2^- and a simultaneous deterioration of the detox system in Zn deficient plants. On the other hand, there is increasing evidence that Zn not only maintains membrane structure and integrity and controls permeability, but it also protects the plant against various pathogens. In Zn deficient plants, the increased membrane permeability results in increased release of carbohydrates and amino acids. These attract pathogens insects either to the new roots or to the new shoots (Broadley *et al*., 2012; Kirkby and Römheld, 2004).

Aldolase and Carbohydrate Metabolism

Fructose 1,6-bisphosphate aldolase (EC 4.1.2.13), usually known as aldolase, is an enzyme that participates in glycolysis. It catalyzes the excision of fructose 1,6-bisphosphate into two trioses, dihydroxyacetone phosphate and glyceraldehyde 3-phosphate. It also catalyzes the reversible rupture of fructose 1-phosphate into glyceraldehyde and phosphate dihydroxyacetone. In higher plants, two isozymes of aldolase are known (aldolase A or class I, and aldolase B or class II). Different genes that express in different forms during development code them. Small differences in the structures of these two isozymes result in different activities over the two substrates: fructose 1,6-bisphosphate and fructose 1-phosphate. Aldolase B does not exhibit preference over substrates so it catalyzes both reactions, while aldolase A acts on fructose 1,6-bisphosphate. Class I is characterized by a tetrahedral protein structure of 160kD and it is located inside plastids. This enzyme's mechanism implies the formation of a Schiff base intermediary between the substrate and a lysine residue in the active center of the enzyme. On the other hand, class II has a protein structure of an 80kD dimer and is found in the cytosol; unlike class I, it requires divalent cations such as Zn^{+2}, Fe^{+2} or Ca^{+2} as cofactors to stabilize the carbon ion intermediary formed during the reaction (Uematsu *et al*., 2012).

When aldolase activity is low, in most cases sugars and starches accumulate in Zn deficient plants. Meanwhile, 24h after Zn is restored, sugar levels and Hill reaction activity are reestablished (Kirkby and Römheld, 2004; Barker and Eaton, 2015). Aldolase is one of the six enzymes of the Calvin cycle that does not have post-transitional regulation. Moreover, the mathematic model of the metabolic pathway (Zhu *et al*., 2007) suggests that non-regulated enzymes of the Calvin cycle, such as aldolase, have the potential to control photosynthetic carbon flow through the cycle. In addition, it is assumed that aldolase activity accelerates RuBP regeneration and helps raise photosynthetic capacity, growth rate and biomass efficiency (Uematsu *et al*., 2012).

Role of Zinc in Protein Synthesis

Zinc deficit is closely related to nitrgen metabolism. When Zn levels are lowered, the concentration of proteins decreases, while that of amino acids increases. Upon Zn restoration, protein synthesis is rapidly induced. The inhibition of protein synthesis under Zn deficiency is mainly the result of a reduction in ribonucleic acid (RNA). This is attributed to the lower activity of Zn polymerase, to a reduction in the structural integrity of ribosomes and to greater degradation of RNA. The marked decrease in growth rate, due to inhibited protein formation under Zn deficiency results in lowered carbohydrate consumption, and this to reduced photosynthesis, which favors greater production of free oxygen radicals; when these are not removed, the Zn deficiency symptoms are more pronounced, especially under high radiation intensity (Kirkby and Römheld, 2004). The rate of protein synthesis and the protein content are dramatically reduced in plants with Zn deficiency, while amino acids tend to accumulate. When Zn is given to deficient plants, protein synthesis restarts very rapidly (Patel *et al.,* 2007).

In addition to the functions of Zn previously described, at least two other functions of Zn are responsible for these changes in protein metabolism. Zinc is a structural component of ribosomes and is essential for their structural integrity. The Zn content of ribosomal RNA in the unicellular alga *Euglena* is in the range of 650-1280μg·g^{-1} RNA, while that in higher-plant cells suffering Zn deficiency is in the range 300-380μg·g^{-1}

RNA. If Zn is absent ribosomes disintegrate, but if the Zn supply is restored they rebuild (Mendoza-Cózatl *et al*., 2006). In cauline meristems of rice, ribosome disintegration 80S (soluble fraction in cytoplasm) occurs when the Zn content falls under 100µg·g^{-1} dry weight. In contrast to this very high requirement for Zn to maintain ribosomal integrity, protein content starts to fall only if Zn contents fall considerably less. In tissue cultured tobacco cells, the corresponding contents were 70µg of Zn for diminishing the content of ribosomes 80S, and 50µg Zn for reducing protein content (Nava-Guillén *et al*., 2008).

A high specific requirement for Zn has been shown in centers of protein synthesis in pollen tubes (Elsby and Roberts, 2008), where Zn content during the growth peak is near 150µg·g^{-1} dry weight compared to about 50µg in more basal regions. In the steam meristems and presumably in other meristem tissues, a content of Zn of at least 100µg·g^{-1} dry weight is essential for maintaining protein synthesis. This is nearly 5 to 10 times greater than the content considered appropriate for mature leaves. For other mineral nutrients, this difference is usually less marked. Meanwhile, for other minerals the difference can be the other way around, depending on nutritional state. To satisfy the high demand for Zn in caulinar meristems, most Zn provided to the root is preferentially translocated to the caulinar meristem, mediated by xylem-phloem transfer in the stem. Low protein contents and high amino acid contents in plants suffering Zn deficiency are not only the result of a reduced transcription and translation but also of enhanced degradation of RNA. A high activity of ribonuclease (RNase) is a typical characteristic of Zn deficiency. There is a marked inverse relation between Zn supply and RNase activity and between RNase activity and protein content (Elsby and Roberts 2008). An increase in RNase activity is frequently observed even before the symptoms of Zn deficiency appear as slowed growth and changes in foliar anatomy. The polypeptide chain forms a curl or 'finger' usually of 11-13 amino acid residues that links the specific sequences of DNA (Zn finger motif). In these metalloproteinases that link DNA, Zn is directly involved in the passing of gene expression translation and in the activation or repression of DNA elements (Kirkby and Römheld, 2004).

Conclusions

Zinc metalloenzymes take part in the control of plant development through its indirect action on auxin metabolism, in maintaining membrane integrity, in protein synthesis, in carbohydrate metabolism and in photosynthesis regulation. On the other hand, it has other indirect effects in the control of stomatal opening and closing and ROS detoxification. Because of these, Zn deficiency may affect the catalytic function of a number of enzymes and thus the metabolic pathways in which they are involved. Finally, it is suggested that future studies be directed to the regulation of the genetic expression of metalloproteins and their relation in the adaptation of plants to low Zn, in order to pave the way for the development of new strategies to improve the tolerance of plants to Zn deficiency.

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METALOENZIMAS DE ZINC EN PLANTAS

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RESUMEN

El zinc es un micronutriente esencial para las plantas y su disponibilidad en el suelo es de gran importancia en muchos cultivos. En los vegetales, el zinc no se oxida ni se reduce; en lugar de ello, la importancia del zinc proviene de sus propiedades fisicoquímicas como un catión divalente. Muchas enzimas incluyen al zinc como cofactor, tales como la alcohol deshidrogenasa (EC 1.1.1.1), la superóxido dismutasa (EC 1.15.1.1), la anhidrasa carbónica (EC 4.2.1.1)) y la ARN polimerasa (EC 2.7.7.6). En estos casos, es indirectamente evidente que la deficiencia de zinc inhibe la síntesis de proteínas. Las plantas también requieren del zinc para la síntesis de triptófano, un aminoácido clave en la síntesis de la auxina ácido indolacético. Por lo tanto, el zinc también opera en el control del desarrollo de las plantas a través de su acción indirecta sobre las auxinas. De aquí que la deficiencia de zinc afecte la actividad catalítica de las enzimas mencionadas y las vías metabólicas en las que están implicadas. El objetivo del presente trabajo es analizar la función del Zn en algunas metaloproteínas implicadas en el metabolismo vegetal.

METALOENZIMAS DE ZINCO EM PLANTAS

Jorge Castillo-González, Dámaris Ojeda-Barrios, Adriana Hernández-Rodríguez, Ana Cecilia González-Franco, Loreto Robles-Hernández e Gustavo Rogelio López-Ochoa

RESUMO

O zinco é um micronutriente essencial para as plantas e sua disponibilidade no solo é de grande importância em muitos cultivos. Nos vegetais, o zinco não se oxida nem se reduz, por isto a sua importância que provém de suas propriedades físico-químicas como um cátion divalente. Muitas enzimas incluem o zinco como cofator, tais como a álcool desidrogenase (EC 1.1.1.1), a superóxido dismutase (EC 1.15.1.1), a anidrase carbônica (EC 4.2.1.1)) e a ARN polimerase (EC 2.7.7.6). Nestes casos, é indiretamente evidente que a deficiência de zinco inibe a sínteses de proteínas. As plantas também requerem do zinco para a síntese de triptófano, um aminoácido chave na síntese da auxina ácido indolacético. Portanto, o zinco também opera no controle do desenvolvimento das plantas através de sua ação indireta sobre as auxinas. Dai que a deficiência de zinco afete a atividade catalítica das enzimas mencionadas e as vias metabólicas em que estão implicadas. O objetivo do presente trabalho é analisar a função do Zn em algumas metaloproteínas implicadas no metabolismo vegetal.