Role Of Micronutrient Zinc In Facing Drought Stress And Germination Of Chickpea

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Abstract:

According to extensive surveys the zinc (Zn) content of soils, is generally in the range of 10-300 ppm. Certainly Zn, because of its concentration, can be considered as a trace element in soil. It occurs most frequently in the lithosphere as the mineral ZnS (sphalerite). Zn appears to be scattered throughout the mineral fraction of soils. The critical Zn level found in youngest expanded leaves for 90% maximum yield was 16 mg kg^{-1} dry biomass. Zn-efficient Excalibur had greater net Zn uptake rates as compared to Zn-inefficient variety but they were not different at the 6-week harvest. Zinc-deficient plants had greater net uptake rates of Cu, Mn, B, P, and K but a reduced uptake rate of Fe. It is concluded that higher seed Zn content acted similar to a starter-fertilizer effect. Despite the importance of zinc as a micronutrient for plant growth, there have been relatively few studies of the mechanism of zinc uptake. It is clear from a recent review of the literature that there is currently little agreement on how zinc crosses the plasma-lemma of plant cells. The questions which have not been satisfactorily answered are whether zinc enters via ion channels or via a divalent-cation carrier. Zinc functions as an enzyme activator in carbohydrate metabolism and protein formation. Deficiency symptoms usually appear first on relatively young leaves early in growing season. In broadleaf plants, zinc deficiency results in a shortening of internodes and a decrease in leaf size. Snap bean develops a yellowing between the leaf veins. However, it is very difficult to distinguish between zinc and manganese deficiencies in this crop.

Zn in the leaf is associated with low molecular weight complexes, storage metallo-proteins, free ions, and insoluble forms associated with the cell wall. Zn can become inactivated within the cell either by legend formation or by forming different compounds with phosphorous. High amount of zinc concentration in soil have an adverse effect on seed germination. When the concentration of Zn is too high, the permeability of the seed membrane of the seed is destroyed, the conductivity of the seed increases with the increase of zinc concentration, and the electrolyte leakage rate increases with the increase of zinc concentration. Zinc is vital for all life forms and plays a crucial role in plant defense system due to its involvement in key enzymes activity, gene regulation and protein synthesis. It also helps maintain photosynthetic activities, stabilize biological membranes, maintain CO_2 concentration in mesophyll cells and repair the PSII during photo-inhibition. Zinc deficiency increases sensitivity to heat and drought stresses and may result in drastic reductions in grain yield.

Key words: Zn uptake, heavy metal, plants, germination, Chickpea, *Cicer arietinum*

1.1 Introduction

Foods derived from plants are poorer sources of Zn for humans and monogastric animals when compared to those from animals because they can contain substances which interfere with the absorption and /or utilization (*i.e.*, bioavailability) of Zn. Examples of these substances include phytic acid and certain types of fiber, especially fiber from whole cereal grains. However, these substances are also known to play important roles in either the life cycle of higher plants or possibly, in the prevention of several human diseases including heart disease and certain forms of cancer. Therefore, it is not wise to reduce the content of these substances in food crops or in diets without a more thorough understanding of their role(s) in plant growth or human health (Welch, 1993). According to WHO, about 2 billion (33%) of the world population is affected by Zn deficiency (Stein, 2010), which causes 450,000 death of children under 5 years of age every year (Black *et al*., 2008). In developing countries Zn related deficiency is fifth major cause of human diseases and mortality (Hussain *et al*. 2012).

Chickpea (*Cicer arietinum* L.), the third most important legume crop of the world, is grown in nearly 52 countries (Heidarvand and Maali-Amiri 2013). South Asia is the leading producer of chickpea which contributes about to three-quarters of the global chickpea production (Rao *et al.* 2010). This pulse crop forms an important constituent of vegetarian diet in India. There are two main types of cultivated chickpea genotypes, desi and kabuli which can be distinguished by the size, shape and color of the seeds. Kabuli genotypes have larger, rounder and cream-colored seeds that are largely grown in North Africa, West Asia, North America and Europe, whereas desi genotypes have relatively smaller, angular-shaped and dark colored seeds mostly grown in Asia and Africa (Rachwa-Rosiak *et al.* 2015) . Chickpea seeds have good nutritional value—they contain high amounts of unsaturated fatty acids and are an inexpensive source of high quality plant protein for millions of people in developing countries. They are also rich in minerals, dietary fibers and vitamins such as tocopherol (both γ and α), folic acid, riboflavin (B2), pantothenic acid (B5), pyridoxine (B6), and carotenoids such as β-carotene, lutein, cryptoxanthin and zeaxanthin (Jukanti *et al.* 2012). Chickpea plants have a deep taproot system which helps them extract water from deeper soil layers and enhances their capacity to withstand limited water stress. Chickpea is a crop of temperate areas and most of its cultivation is done on the sandy loam soils under low-rainfall conditions. Loam and fertile sandy soil have good internal drainage; therefore they are considered the best mediums for the growth of chickpea plants (Krishnamurthy *et al*. 1998). Arya and Chauhan (1995) reported seed mycoflora of five varieties of chickpea.

Many of the physiological activities associated with the zinc deficiency are disruption of normal enzyme activity, thus inhibition of photosynthesis was coincident with a decrease in activity of key photosynthetic enzymes. Zinc deficiency also increases membrane leakiness by inhibiting the activity of enzymes involved in the detoxification of membrane damaging oxygen radicals. Recent evidence suggests that zinc plays a key role in stabilizing RNA and DNA structure, in maintaining the activity of DNA synthesizing enzymes and controlling the activity of RNA degrading enzymes. Thus, zinc may play a role in controlling gene expression. It is interesting to note that our understanding of the function of zinc has increased greatly in the last thirty years, there are still many facets of zinc metabolism that remain controversial.

1.2 Presence of Zinc in Soil

Zinc ions are held on the surface of clay and organic matter particles. Soil organic matter holds Zn in a chelated form. Chelation is a process by which certain metals are held within the structure of large organic molecules. Because zinc is held on soil particles and by chelating, it does not move through the soil and is not leached under most condition. The primary factors affecting zinc availability are soil texture, soil pH, soil phosphorus, and weather conditions. Mining activities result in extensive soil damage, causing drastic disturbances in landscape, altering the ecological environment of soil microorganisms, thereby disrupting the functional stability of the microbial community. The ultimate goal of mine land reclamation means the reestablishment of productive healthy and sustainable ecosystem for post mining activity. Currently criteria for successful restoration have largely been restricted to soil erosion, physicochemical status and vegetation characteristics. Microbial community can proceed detectable changes in soil physicochemical properties, thereby providing early signs of environmental stress or ecological environment evolution in the mining area.

Most of the zinc (Zn) in soils exists in unavailable forms. This essential micronutrient occurs in plants either as a free ion, or as a complex with a variety of low molecular weight compounds. Zinc may also be incorporated as a component of proteins and other macromolecules. As a component of proteins, zinc acts as a functional, structural, or regulatory cofactor of various enzymes. It plays an important role in auxin formation and in other enzyme systems. Presently, Zn is recognized as an essential component in several dehydrogenases, proteinases, and peptidases. High concentrations of exchangeable zinc may be toxic to many agronomic crops. It is probably held in crystal lattices, by isomorphous substitution and as occluded ions. Since it is a trace element, it is usually surrounded, by many other solid phases. Zn can also be held, by exchange sites, and adsorbed to solid surfaces. Crops differ in their sensitivity to zinc deficiency. Zn deficiencies are frequently found on soils, with restricted root zones. The movement of Zn to plant roots is dependent on the concentration and on the capacity factors (ability to replenish). Increasing the pH decreases the solubility of zinc in soils, and thereby reduces the concentration, the concentration gradient, and, hence, the uptake of Zn to plants.

Nene (1966) was the first to show that a foliar application of zinc sulfate corrects a disorder of the rice plant long known as Khaira disease in North-Central India. In August of 1966, a visit was made to the Nene's experimental fields at the Uttar Pradesh Agricultural University in Uttar Pradesh, India just as his experiments were being completed. Since, at that time, adequate analytical data on zinc status in the rice plant, as well as in the soil, were not available, the authors collected samples of soil and rice plants from the affected field for chemical analysis. The results of the analysis fully support the conclusion that Khaira disease was indeed an example of zinc deficiency (Yoshida and Tanaka, 1969). Usually crops take up less than 0.5 lb/a of zinc, yet when zinc is deficient, crops need external supply of zinc. Crops with high zinc requirements include corn, onion and spinach. Those with medium requirements are barely, beans, beet, canola, cucumber, lettuce, lupine, potato, radish, sorghum-sudan, soybean, tobacco, and tomato. Other crops have low zinc requirements and seldom exhibit zinc deficiency. In Wisconsin, zinc deficiencies have been observed on corn, snap bean and a few other vegetable crops (Schulte, 2004).

Microorganisms differ greatly in their tolerance for pH, oxidized versus reduced environment. The physical and chemical conditions become more restrictive, the diversity of microbial types that can maintain themselves. The ecosystem so developed is dominated by acidophilus sulphur and iron oxidizers. Some prokaryotic acidophilus heterotrophic organisms are present. Due to open cast mining a large area is disturbed and requires reclamation. To suggest suitable plantation a preliminary study was undertaken. Heavy metal stress, contributed by elements like cadmium, manganese or zinc is one of the crucial factors that limit the distribution and Productivity of major healthy crop plants poses severe health hazards to human beings.

Restoration has been attempted on an experimental scale in various parts of the country and its implementation for the whole area has also started at some locations. In some cases the plantation of exotic and horticulture species has also been considered as restoration, while in other cases, *e.g*. Neyveli, the degraded land has been made productive and changed to agricultural land. One possible approach is to make use of the hyper-accumulators plants & the phytoremediation techniques. Furthermore, a general solution to this problem is chelation, which is generally understood as carbon binding to a compound resulting in a neutrally charged complex that can move more freely through a variety of substrates. Several chelators are known to perform this function in soil plants (Rauser, 1999). The farming land is declining gradually and the main reasons include, intensive use of agricultural practices, urbanization, biotic and abiotic stresses etc. Among the abiotic stresses the salinity problem is increasing at an alarming rate throughout the world. Use of Cyanobacterial and other biofertilizer may help to reclaim the soil and reduce the effect of NaCl stress.

1.3 Deficiency of Zn in Plants

The biological role of Zn was first identified in 1869, that common bread mold (*Aspergillus niger*) did not grow in the absence of Zn (Nielson 2012) . Soon thereafter, Zn was identified as an ubiquitous component of both animal and plant tissue. This observation stimulated Zn research in crops, and in 1914 the first demonstration of Zn deficiency in maize plants was made (Maze, 1914). The evidence that the Zn was essential for plants was further known for barley and sunflower in 1926 (Sommer and Lipman, 1926), while the first identification of Zn deficiency in field conditions was reported in 1937 in the deciduous orchards of California by Chandler (1937).

Zinc deficiency is now recognized as one of the most common micronutrient deficiencies and is becoming increasingly significant in crop production. A specific role for Zn in plants was not identified only in the late 1960's. Since then a series of Zn-containing enzymes have been identified and considerable progress has been made in detection of various chemical forms and physiological effects of Zn deficiency in plants. In the last 20 years several reviews have dealt with aspects of Zn in soils and plants. The two most comprehensive studies of these provide a good account of the reactions of Zn in soils (Lindsay, 1972) and forest ecosystems (Boardman and McGuire, 1990). In chapter discusses the chemical forms of Zn in the plant and the role of Zn in plant metabolism and physiology. Particular attention is paid to the physiological and morphological effects of Zn deficiency in higher plants. In India alone, 50% of the soils that groundnut is grown show Zn deficiency, which is causing considerable yield loss. Zinc is required for chlorophyll production, pollen function, fertilization and germination (Cakmak 2008). Zinc plays an important role in biomass production (Kaya *et al.,* 2000). Zinc in normal soil ranges from 10-300 mgkg-1. The concentration of Zn present in Indian soil varies from 30 to 72 mgkg1 depending on the type of soil (Katyal and Sharma 1991; Sharma *et al.* 2013).

In plants, Zn does not undergo valance changes, and its chemistry differs from Mg2+, Ca2+, or Mn2+ in that it forms more stable complexes with a particular affinity for tetrahedral complexes. The majority of Zn in the leaf is associated with low molecular weight complexes, storage metalloproteins, free ions, and insoluble forms associated with the cell wall. Zn can become inactivated within the cell either by legend formation (Leece, 1978) or by complexation with phosphorous. Depending on plant species, anywhere from 58% to 91 % of plant Zn may be soluble (Welch *et al.*, 1993). This water-soluble Zn fraction is usually considered to be the physiologically active fraction and is regarded as a better indicator of Zn status than is total Zn content (Cakmak and Marschner, 1987). Among these soluble Zn forms, low molecular weight complexes are frequently in the greatest abundance and are probably the most significant form of active Zn. Though more than 70 Zn metallo-enzymes have been identified, functional metallo-proteins represent only a small proportion of the total Zn present in the plant.

A relationship between the affinities of cell wall extracts for free Zn, and the relative tolerance of diverse species to excess Zn, suggests a role for the cell wall in controlling free Zn in the cell (Turner, 1970; Turner and Marshall, 1971). As reviewed by Torre *et aI.*, (1991) various cell wall compounds i.e. lignin, cellulose, hemicellulose, etc. possess a high binding affinity to Zn. Accordingly, it is assumed that 90% or more of the total Zn in roots is adsorbed in the apoplast of rhizodermal and cortical cells (Schmid *et al*. 1965). The significance of cell wall binding of Zn, however, remains controversial, particularly as the total binding capacity of the cell wall may be too small to be physiologically significant (Wainright and Woolhouse, 1978).

1.4 Zinc uptake by Plant cells

Zinc functions as an enzyme activator in carbohydrate metabolism and protein formation. Deficiency symptoms usually appear first on relatively young leaves early in growing season. On corn, a broad band of bleached tissue appears on either side of midrib. The deficiency begins at the base of the leaf and usually stays in the lower half of the leaf. In broadleaf plants, zinc deficiency results in a shortening of internodes and a decrease in leaf size. Snap bean develops a yellowing between the leaf veins. However, it is very difficult to distinguish between zinc and manganese deficiencies in this crop. In most crops, the typical leaf Zn concentration required for adequate growth approximates 15-20 mg Zn/kg DW (Singh and Prasad, 2014).

Despite the importance of zinc as a micronutrient for plant growth, there have been relatively few studies of the mechanism of zinc uptake. It is clear from a recent review of the literature by Kochian (1993) that there is currently little agreement on how zinc crosses the plasma-lemma of plant cells. The questions which have not been satisfactorily answered are whether zinc enters via ion channels or via a divalent-cation carrier, the link between uptake and metabolic energy transduction, the existence of an active efflux mechanism, whether fluxes can be described by Michaelis-Menten kinetics with Vm and K and the possible involvement of phytosiderophores. Most studies of zinc uptake have used solution cultured roots and radioactive 65Zn either to measure short-term influxes or to estimate fluxes from compartmental analysis of efflux kinetics of tissues equilibrated in 65Zn (Schmid *et al*. 1965; Chaudhry and Loneragan 1972). However, it is difficult to assess the reliability of the tracer flux measurements because in none of these studies has a clear distinction been made between extracellular binding and actual membrane influx. It was demonstrated that the characteristics of cation binding in cell walls of *Chara* are not fundamentally different from apoplasmic binding in plant roots, and that by adopting the same techniques used to measure Ca^{2+} (Pifieros and Tester,1995; Reid and Smith 1992).

Cell wall material was obtained from wheat roots by boiling 50-mm apical root segments for 20 min in deionized water. Washed and dried cell wall material was incubated in 10 mmol'm-3 65Zn in APW for 2 h then desorbed in a solution containing 1 mol.m-3 LaC13 in APW. The rinse solutions were periodically changed and sampled to determine the 65Zn desorbed in each rinse period. Initial binding and the time-course of desorption were calculated by summing the 65Zn remaining in the tissue after 60 min and the preceding rinses. The zinc concentration in culture solution, cells and cell walls was measured by graphite furnace atomic absorption spectrometry (GFAAS) after extraction in 1000 tool" m-^{3} nitric acid. Zinc speciation in solution was calculated using GEOCHEM-PC. Unless specified otherwise, zinc concentrations given in the text refer to the sum of all (Parker *et al*.1994). Rauser (1999) reported that plants produce a range of legends for cadmium (Cd), copper (Cu), nickel (Ni), and Zn. Cd- and Zn-citrate complexes are prevalent in leaves, even though malate is more abundant. In the xylem sap moving from roots to leaves, citrate and histidine are the principal legends for Cu, Ni, and Zn. Phosphorus-rich globular bodies in young roots are probably Zn-phytate.

1.5 Role of AM Fungi in Metal hyperaccumulator Plants

In nature, some plants hyperaccumulate heavy metals. For example, *Viola calaminaria* and *Thlapsi calaminare* grow over calamine deposits in Aachen, Germany and contain over 1% (dry weight) zinc in their tissues. Also, some *Alyssum* species like *A. bertolinii* grow on serpentine soils in Tuscany, Italy and contain over 1% (dry weight) nickel. These species are respectively called calamine and serpentine flora. *Thlaspi caerulescens* from the Brassicaceae family can also hyperaccumulate both Zn and Cd (Brooks, 1998). Heavy metal complexes in hyper-accumulators plants are usually associated with carboxylic acids like citric, malic and malonic acids. These organic acids help in the storage of heavy metals in leaf vacuoles. Amino acids like cysteine, histidine glutamic acids, and glycine also forms heavy metal complexes in hyper-accumulators (Homer *et al.*, 1991). These complexes are more stable than those with carboxylic acids. They are mostly involved in heavy metal transport through xylem. Moreover, hyper-accumulator plants can increase availability of metals like Fe and also Zn, Cu and Mn by releasing chelating phytosiderophores. This mechanism may thus be related to rhizosphere processes such as to the release of chelating agents (phytosiderosphores and organic acids) and/or to differences in the number or affinity of metal root transporters (Lombi *et al.*, 2001).

Many researchers have shown that mycorrhizal colonization can have an impact on heavy metal assimilation by plants (Gildon and Tinker, 1983a, b). Dehn and Schuepp (1990) have found that mycorrhizal infection enhances heavy metal accumulation in lettuce roots but not in shoots. However, Jamal *et al.* (2002) have shown that mycorrhizae enhance heavy metal accumulation in legume shoots like soybean, alfalfa and lentils, Killham and Firestone (1983) have found similar results with grasses.

1.6 Effect of $ZnSO_4$ on germination on *Cicer*

Germination rate and germination potential are often used to evaluate the commonly used indicators of seed germination, reflecting the seed germination rate and germination uniformity. Zinc sulphate solution of 100ppm 500ppm and 1000ppm concentrations were prepared. Ten chickpea seeds were taken in 10 Petri dishes in each treatment. These seeds were exposed for 6 hour to the different zinc sulphate solutions prepared. Then they were allowed to germinate and readings were taken after every 2 days and observations were recorded in table 1. Seeds in control solution germinated in expected time period. After 4 days 60% seeds germinated in 100 ppm zinc sulphate, 20% seeds germinated in 500 ppm, while no seed germination was observed in 1000ppm $ZnSO_4$ solution. It is evident from the table that it took longest time to germinate a seed in 1000ppm only 10% seed germination was observed after 10 days. After 8 days at 500 ppm concentration of $ZnSO_4$, 30% germination of chickpea was recorded. Higher concentration of Zn salt showed longer germination time and the germination percentage was very poor.

Table 1: Percentage germination of chickpea at different concentrations of $ZnSO_4$

S.No.	Days of Treatment	Percentage seed germination in different con. of $ZnSO_4$			
		Control	100 ppm	500ppm	1000 ppm
1.	02	20	00	00	00
2.	04	60	20	00	00
3.	06	100	60	20	00
4.	08	100	80	30	00
5.	10	100	80	40	10

Note: Data based on 100 chickpea seeds.

Possible roles of zinc in protecting plant cells from damage by reactive oxygen species and its effect on plant metabolism has also been well reviewed (Cakmak 2008, Broadley, *et al.* 2006). The role of Zn in protecting plant cells from damage by reactive oxygen species may be an important response in seed germination and not simply affecting seed water content, dry matter accumulation and changes in antioxidant balance during germination. Zinc is an indispensable micronutrient which regulates abiotic stress responses in plant. Zinc mitigates arsenic toxicity in plant by modulating ROS and antioxidant function in plant (Das *et al.* 2016). Zinc nutrition also helps in mitigation of iron toxicity in rice (Shahid, 2014). Zinc nutrition also modulates drought induced growth and antioxidant dysfunction in plant and mitigates dehydration induced damages and improves post stress rehydration responses in plant (Upadhyaya *et al*. 2013).

The results showed that low concentration of Zn2+ was beneficial to seed germination. High concentration of Zn2+ could inhibit the seed germination. Rengel and Graham (1995) found that seed nutrient reserves may be important for an early establishment of crops on low-fertility soils.

Zn can effectively reduce the oozing of the seeds in the germination, and the permeability of the seeds during germination. The damage of the seeds after low concentration of Zn2+ treatment was lower than that of the control, and the integrity of the membrane was also repaired. When the concentration of Zn2+ is too high, the permeability of the seed membrane of the seed is destroyed, the conductivity of the seed increases with the increase of zinc concentration, and the electrolyte leakage rate increases with the increase of zinc concentration. The results also showed that zinc could inhibit the root length, bud length and chlorophyll content of rhubarb seedlings. The seeds of *Cucumis* germinated in the presence of some moderate and high concentrations of zinc in substrate. The concentrations used for the treatment do not influence negatively the seed germination, but affect the seedlings growth (especially the root elongation), the formation and growth process of the lateral roots and the seedlings vigor. The effect of delay of the growth process is very pronounced in the case of high concentration (Stratu and Costică, 2015).

Seeds of cluster bean treated with low level of zinc (10 and 25 mgl^{-1}) showed a significant increase in germination, seedling growth, fresh and dry weight over the control. This value indicates that zinc at lower level had a significant stimulatory, beneficiary and nutritional effect (Reichman, 2002). A prediction model of water-stressed versus control plants was built by using thirteen significantly important metabolites of kabuli genotype of chickpea. PLS-DA score plots showed a clear separation trend between the plants grown under rain fed, and irrigated control conditions. Sensitivity and specificity were also measured from the constructed model which was found to be 94.11% and 100% respectively, while the overall accuracy of the model was 97.14%.

1.7 Conclusion

Heavy metals in growth media can function as stresses, causing physiological constraints that decrease plant vigor and inhibit plant growth. Numerous studies conducted on the detrimental effects of metallic pollutants have shown that beyond a particular concentration, the metals can prove to be toxic to plants and animals. Heavy metals like zinc are required as structural and catalytic components of protein and enzymes for normal growth and development. But when present in elevated levels in soils, they are toxic and can ultimately cause the death of plants. Hence efforts have been made to establish the toxic level of zinc on seed germination, growth and various biochemical content of cluster bean seedling in the present study. In present experiment the germination of chickpea at 500ppm and 1000ppm was poor. At 1000 ppm not only germination was delayed but it was least (10%) and at different conc. of $ZnSO_4$ the length of radical was much less than control seedlings.

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