"Probing the Physiological and Biochemical reasons for differential uptake and translocation of Zinc in contrast Finger millet [*Eleusine coracana, (*Gaertn.)] genotypes"

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DEPARTMENT OF CROP PHYSIOLOGY
UNIVERSITY OF AGRICULTURAL SCIENCES
BANGALORE
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Affectionately dedicated to
My beloved Parents,
Brother, Sisters, Teachers
and my family

DEPARTMENT OF CROP PHYSIOLOGY
UNIVERSITY OF AGRICULTURAL SCIENCES
BANGALORE

C E R T I F I C A T E

This is to certify that the thesis entitled **"Probing the Physiological and Biochemical reasons for differential uptake and translocation of Zinc in contrast Finger millet [*Eleusine coracana, (*Gaertn.)] genotypes"** submitted in partial fulfillment of **Master of Science** in **Crop Physiology** to the University of Agricultural Sciences, Bangalore is a record of research work carried out by **RAJANNA, H. B.** with **ID No. PAK 5164** under my guidance and supervision and that no part of the thesis has been submitted for the award of any other degree, diploma, associateship, fellowship or any other similar titles.

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Probing the Physiological and Biochemical reasons for differential uptake and translocation of Zinc in contrast Finger millet [*Eleusine coracana, (*Gaertn.)] genotypes

ABSTRACT

Among trace elements iron and zinc in food grains are par below required level for human nutrition. Nearly 49% of global population does not meet their recommended dietary allowance (RDA), making Zn deficiency the most widespread. Globally soils are Zn deficient and most food crops contain less Zn in edible parts. Cereals bring the major staple food crop of India, are the priority crops, which need to be bred for their nutrient enhancement.

One important strategy to increase grain Zn concentrations is by fertilization via soils or foliar applications. An alternative approach is to exploit the genetic variation in grain concentrations of this micronutrient within plant genome. Application of fertilizers is not cost effective and not successful always because of subsoil constraints. Therefore production of crop species with high grain Zn content through selection or other breeding approaches is a sustainable strategy. It is essential to understand the physiological and biochemical reasons for differential uptake and translocation of Zn within and between crop species, so as to exploit the existing genetic variation to improve crop plants to have high Zn in edible parts.

With this preface, this study mainly focuses on selection of finger millet genotypes and selection of contrast (high and low) types to study the uptake and translocation of Zn. Activities of important Zn requiring enzymes like copper-zinc superoxide dismutase and carbonic anhydrase was assessed to know the relevance of Zn content and activity of these enzymes. Seed Zn content directly reflects the biochemical activity, with high seed Zn types showing high copper-zinc superoxide dismutase and carbonic anhydrase activity compared to low seed Zn types. Differences in uptake and translocation of Zn from external source seems to be the main cause for differences in seed Zn content, were in high uptake, but low translocating types were genotypes containing low seed Zn content.

Signature of the student **signature of Major advisor**

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Introduction



I. INTRODUCTION

The world population is expanding rapidly and will likely to be 10 billion by the year 2050. Virtually all of the anticipated increase in world population will occur in Africa, Latin America and Asia. Presently, these regions have already serious problems concerning food production, access to food, water scarcity and nutritional disorders/ malnutrition. According to the projections, food production on presently used land must be doubled in the next two decades to meet food demand of the growing world population.

Presently, in many developing countries, poor soil fertility, low levels of available mineral nutrients in soil, improper nutrient management, along with the lack of plant genotypes having high tolerance to nutrient deficiencies or toxicities are major constraints contributing to food insecurity and malnutrition (i.e., micronutrient deficiencies).

After moisture stress, nutrient deficiency is one of the major stresses limiting crop productivity. Globally also several elements are reported to be below critical level in soil. According to a recent survey, Zinc deficiency is the most wide spread nutritional disorder, next to Iron, Vitamin A and Iodine. Nearly 49 per cent (~3 billion) of the global populations do not meet their daily-recommended intake of 15 mg/day for an adult resulting in egregious societal costs including learning disabilities among children, increased morbidity and mortality rates, lower worker productivity and high healthcare costs, all factors diminishing human potential, felicity and national economic developments. Nutritional deficiencies (mainly Iron, Zinc and Vitamin A) account for almost two-thirds of the childhood death worldwide.

Similarly, most of the Indian soils and more than 70% of the soils in Karnataka are found to be deficient in zinc. Hence most of the food crops contain less amount of zinc in the edible parts, from human nutrition perspective. The existing information in the literature suggests that, there is significant genetic variability to maintain significant growth and yield under Zinc deficiency conditions (Hacisalihoglu *et al.*, 2003). Significant variation

across the crop species and genotypes exists, not only in their ability for zinc uptake, but also in their ability to transport to edible parts.

Zinc is typically the second most abundant transition metal in organisms after iron and the only metal represented in all six-enzyme classes (Becher *et al.*, 2004). Zinc is essential for the normal healthy growth and reproduction of plants and when the supply of plant - available zinc is inadequate, crop yields are reduced and the quality of crop products is frequently impaired. In plants, zinc plays a key role as a structural constituent or regulatory co-factor of a wide range of around 300 different enzymes in many important biochemical pathways and these are mainly concerned with: carbohydrate metabolism, both in photosynthesis and in the conversion of sugars to starch, protein metabolism, auxin metabolism, pollen formation, the maintenance of the integrity of biological membranes, the resistance to infection by certain pathogens. Zinc deficiency results in as much as 50% reduction in crop growth can result without the appearance of visual symptoms and therefore, it is considered as the most important micronutrient in crop production.

There are several well-known zinc requiring enzymes that have been studied in plants. Copper- zinc super oxide dismutase (Cu/ Zn SOD), plays an important role in protecting plants against oxidative damage catalyzed by reactive oxygen species (Marschner and Cakmak, 1989). Because zinc is directly involved in both gene expression and protein synthesis, Cakmak (2000) has speculated that zinc deficiency stress may inhibit the activities of a number of antioxidant enzymes, resulting in extensive oxidative damage to membrane lipids, proteins, chlorophyll and nucleic acids.

A second well-characterized zinc-requiring enzyme is carbonic anhydrase (CA); in fact it has been suggested that CA activity could be used as an indicator for diagnosing zinc deficiency in plants (Bar- Akiva and Lavon, 1969). Zinc deficiency induces a decrease in the activity of CA, especially in zinc inefficient durum wheat genotypes (Rengel, 1995). In a study with rice (*Oryza sativa*), Sasaki *et al* (1998) found that the level of CA mRNA decreased under zinc deficiency.

But the application of zinc fertilizers alone does not completely alleviate zinc deficiency due to factors such as subsoil constraints, topsoil drying or disease interactions (Graham and Rengel, 1993). Because of the wide spread problems of zinc deficiency and difficulties in alleviating it via application of fertilizers, a promising alternative may be the identification of zinc efficient genotypes. Zinc efficiency is defined as the ability of a plant to maintain good growth and yield on zinc limited soils (Graham, 1984). A number of plant species exhibit significant intraspecific variations in zinc efficiency, which appears to be under genetic control (Graham *et al.*, 1996).

Though physiological mechanisms of differential zinc efficiency remain unknown, it is important to understand these mechanisms to develop improved cultivars for low input crop production on low zinc soils in developing countries. Several mechanisms have been proposed to explain zinc efficiency in crop plants, including increased zinc uptake, increased zinc bioavailability in the rhizosphere due to release of root exudates and more efficient internal zinc use (Hacisalihoglu *et al.*, 2001).

Increasing the seed Zinc concentration via traditional approaches like food fortification and daily individual supplementation, face logistic constraints and are often not cost effective. Dietary modifications are promising, but they require behavioral changes that depend on intensive and costly education, communication and social marketing strategies and may be difficult to implement (Ruel and Bouis, 1998). Plant breeding strategies or biotechnological tools hold great promise for making a significantly low cost and sustainable improvement in the intake of bioavailable zinc in populations (Poletti *et al.*, 2004).

As on today, identification and or development of Zn efficient genotypes is the need of hour. And, we propose to understand the genetic variability in Finger millet genotypes. Finger millet accounts for 8 % of the area and 11 % of production of all millets in the world (Baker *et al.*, 2000). Finger millet is grown on over 4 million ha worldwide and it is a primary food for millions in dry lands of East and Central Africa, and Southern India. Finger millet is a predominant crop grown in both irrigated and dry land condition in the southern parts of India. Karnataka alone accounts for 2.6 million hectares, producing

around 3 million tons. This is one of the major staple foods in Karnataka, Tamil Nadu, Andhra Pradesh, Orissa and Maharastra. The crop is productive in a wide range of environments and growing conditions spanning from the Himalayas in Nepal, India, and throughout the middle-elevation areas of Eastern and Southern Africa (Hotz, 2004). The wide adaptability of the crop can be attributed to its C_4 nature.

Finger millet has a better nutritional profile in terms of minerals (e.g. calcium > 5,000%; Iron and Manganese > 350%; and copper) and essential amino acids than maize (National Research Council, 1996). The grain's protein content (7.4 percent) is comparable to that of rice (7.5 percent). The main protein fraction (eleusinin) has high biological value, with good amounts of tryptophan, cystine, methionine, and total aromatic amino acids, which are all crucial to human health and growth and are deficient in most cereals. Despite of their richness in most of the most of the essential minerals, grains of Finger millet have comparatively low Zn content.

With this in view, experiments were carried out to fulfill the following objectives:

1. Reconfirmation of Zinc status in grains and selection of contrast (high and low) Finger millet genotypes.
2. To asses the level of Carbonic Anhydrase (CA) and Copper Zinc Super Oxide Dismutase (Cu/Zn SOD) and study the relevance of Zinc status and activity of these enzymes.
3. To understand the differences in uptake and translocation in these contrast genotypes using ^{65}Zn radiolabel studies

Review of literature



II. REVIEW OF LITERATURE

Intensive cultivation of high yielding cultivars with heavy applications of N, P and K fertilizers lead to the occurrence of micronutrient deficiencies in many countries. Among micronutrient deficiencies reported to occur worldwide, Zn and Fe deficiencies are of greater importance (Chen and Barak, 1982). Worldwide, Zn deficiency occurs on many million hectares of cereal growing areas (Graham *et al.*, 1992) resulting in significant reductions in yield and quality. Since the primary sources of all nutrients for people come from agricultural products, if agricultural system fails to provide products containing adequate quantities of Zinc, results in dysfunctional bad systems that cannot support healthy lives; unfortunately this is the core for many agricultural systems in many developing nations in global foundation (Graham *et al.*, 2001). Based on analysis of 298 soil samples from different countries Zn deficiency has been found to be the most widespread micronutrient deficiency worldwide. According to Graham and Welch (1996), nearly 50% of the soils cultivated for cereal production globally have low levels of plant available Zn.

Zn deficiency, defined as the condition in which insufficient Zn is available for optimal growth, may cause dramatic reductions in crop yield and quality. Zn deficiency has become a serious agricultural problem. It is associated with high-pH calcareous soils and sandy, highly leached soils, which cause reduced Zn availability and low total Zn content, respectively (Tandon, 1995). It is unfortunate that the use of Zn fertilizers does not completely alleviate Zn deficiency due to factors such as subsoil constraints, topsoil drying, or disease interactions (Graham and Rengel, 1993). Because of the widespread problems of Zn deficiency and difficulties in alleviating it via fertilization, a promising alternative may be the identification of Zn efficient genotypes.

As is the case with plants and soils, Fe and Zn deficiencies are also the most widespread micronutrient deficiencies in humans and has an important role in development and immune function (Hambridge, 2000). These deficiencies affect more than 3 billion people worldwide causing serious health and productivity problems for various population groups, especially among resource-poor women, infants and children (Graham

et al., 2001; Welch and Graham, 1999). These deficiencies are particularly widespread in developing countries where diets are rich in cereal-based foods with low concentration of bioavailabable Zn and Fe.

Zinc inputs to soils

The primary input of Zn to soils is from the chemical and physical weathering of parent rocks. The lithosphere typically comprises 70–80 μg Zn g^{-1}, whilst sedimentary rocks contain 10–120 μg Zn g^{-1}. Mean soil Zn concentrations of 50 and 66 μg total Zn g^{-1} soil are typical for mineral and organic soils, respectively, with most agricultural soils containing 10–300 μg Zn g^{-1} (Alloway, 2003). Zinc occurs in rock-forming minerals as a result of the nonspecific replacement of Mg and Fe with Zn. Rocks containing weathered Zn minerals, including Zn sulphide, sulphate, oxide, carbonate, phosphate and silicate minerals, can form 'calamine' soils containing extremely high concentrations of Zn and other metals. Such sites are usually localized to a few hectares, although adjacent soils can also have high soil Zinc through water seepage from ore bodies (Chen, 1982). Secondary natural inputs of Zn to soils arise because of atmospheric (e.g. volcanoes, forest fires, and surface dusts) and biotic (e.g. decomposition, leaching/wash off from leaf surfaces) processes

Human activities have influenced Zn inputs to soils. The activities like mining and smelting and emissions have increased the Zinc concentrations over years Other anthropogenic inputs of Zn to soils include fossil fuel combustion, mine waste, phosphatic fertilizers, limestone, manure, sewage sludge, other agrochemicals, particles from galvanized (Zn-plated) surfaces and rubber mulches (Graham and Rengel, 1993).

In the Indian context, more than 50% of the agricultural soils are zinc-deficient. The causes for occurrence of Zn deficiencies of this magnitude are related to the introduction of high-yielding varieties, neglect of application of bulky organic manures, imbalanced use of inorganic fertilizer and low Zn uptake depends upon the pH, soil organic matter, temperature, light intensity, crop species, etc. Zn deficiency is quite widespread in the Indo- Gangetic plain and other important cereal-growing states like Punjab, Uttar

Pradesh, etc. which account for almost three-fourths of the country food grain production. The total area under Zn deficiency is about 10 Mha in India and approximately 85% of rice–wheat system cropping takes place in the Indo-Gangetic plain, which has calcareous soils with high pH and thus low Zn availability. Improving production from this cereal belt is therefore vital for sustaining grain production in the country. Zinc occurs in soil as sphalerite, olivine, hornblende, augite and biotite; however, availability of Zn from these sources is guided by several factors mentioned above.

Zinc Uptake by Plants

Zinc appears to be absorbed by roots primarily as a divalent cation (Zn^{2+}) from the soil solution and a protein mediates its uptake with a strong affinity for zinc. Once taken up, Zn is neither oxidized nor reduced; thus, the role of Zn in cells is based on its behavior as a divalent cation that has a strong tendency to form tetrahedral complexes. Despite the importance of Zn as an essential micronutrient for plant growth, relatively few studies have examined the mechanisms and regulation of Zn absorption by roots. Currently, there is little agreement on whether Zn enters via ion channels or via a divalent cation carrier and whether there is a link between uptake and metabolic energy transduction (Graham and Welch ,1996),

The transport of zinc across the plasma membrane was towards a large negative electrical potential so that the process is thermodynamically passive. This negative electrical potential of the plasma membrane is the driving force for zinc by means of a divalent cation channel in dicotyledons and monocotyledons other than the Poaceae. In the Poaceae, the non-protein amino acids called 'phytosiderophores' or 'phytometallophores' form a complex with zinc and transport it to the outer face of the root-cell plasma membrane. These phytosiderophores are released from the roots as a result of iron or zinc deficiency. This complex is then transported to the cell via a transport protein. (Kochian, 1993). The plants could take up zinc from dry soil (matrix potential < -1.5 MPa) via excreted mucilage but this uptake was only 40% as effective as uptake from wet soil. Zinc is taken up as Zn^{2+} or as $Zn(OH)_2$ at high pH. As a result of low concentrations of zinc in the soil solution, uptake is mainly by direct root contact and

is metabolically controlled. Extensive interactions take place between the uptake of zinc and other micronutrients, e.g. zinc - copper (Zn - Cu) which mutually inhibits each other indicating that both are absorbed through the same mechanism of carrier sites. Zinc-deficient rice shows increased uptake of cadmium but zinc is translocated to aerial parts to a greater extent than cadmium. Zinc addition to waterlogged soils has been found to increase DTPA extractable manganese but decrease the uptake and translocation of copper, iron and phosphorus. Zinc is transported in the plant either as Zn^{2+} or bound to organic acids. Zinc accumulates in root tissues but is translocated to the shoot when needed. Zinc is partially translocated from old leaves to developing organs. In rice seedlings, translocation of zinc from roots increases with manganese application. The alkaline earth cations inhibited Zn^{2+} absorption by plants non-competitively, in the order: $Mg^{2+} > Ba^{2+} > Sr^{2+}$ Ca^{2+} (Kochian, 1993).

Genotypic variation in uptake efficiency may not only be due to differences in morphology and architecture, but also due to differences in the affinity of the uptake system (*Km*), maximum uptake rate (*Imax*) and threshold concentration (*Cmin*) – minimum concentration at which the root can deplete nutrient in the external solution.

Radiotracer techniques were employed to characterize Zn^{2+} influx into the root symplasm and translocation to the shoot in *Thalaspi caerulescens* (zinc hyper accumulator) and *Thalaspi arvense* (zinc non-accumulator). Concentration-dependent Zn^{2+} influx in both the species yielded non-saturating kinetic curves that could be resolved into linear and saturable components. These saturable components followed Michaelis–Menton kinetics. The ^{65}Zn content and uptake in root, sheath and blade of maize and barley plants increased significantly with increased levels of zinc application. The labeled Zn rapidly accumulated in the roots of wheat plants upon immersion into the isotope solution (Peterson, 2003 . Root uptake and root to shoot transport of Zn and particularly internal utilization of Zn may be an equally important mechanism involved in the expression of Zn efficiency in cereal genotypes (Erenoglu, *et al.*, 1999).

Based on depletion technique that permits establishment of uptake–substrate relation in low concentration range for intact plants, has shown that the net uptake rate of a nutrient

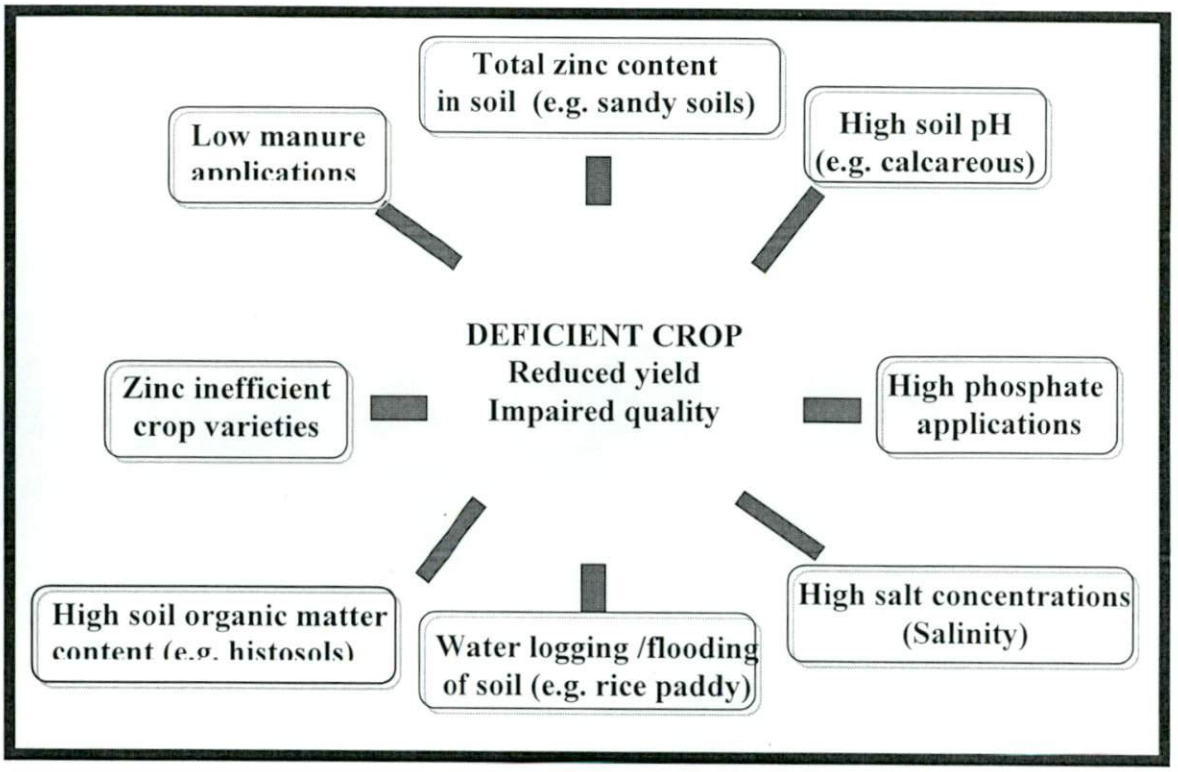
can be related to the external concentration of that nutrient. Kinetic parameters of zinc uptake determined for wheat plants pre-grown at deficient or sufficient zinc supply, showed a saturation response for net zinc uptake with increasing solution zinc concentration. Zinc deficiency caused an increase in *Imax* in zinc-efficient genotypes, but not in zinc-inefficient genotypes. Zinc efficient genotypes possess a better absorption and root to shoot transport, probably due to a more efficient transport system such as ion channel or ion pump, compared with the zinc-inefficient genotypes (Khan, *et.al.*, 2003 . The association of vesicular arbuscular mycorrhizal (VAM) fungi with higher plants has been found to result in higher Zn absorption than their nonmycorrhizal counterparts. This is ascribed to much wider exploration of soil volume by extramatricular hyphae of mycorrhizae and improved uptake of nutrients by hyphae. Higher Zn uptake rate in mycorrhizal roots has been demonstrated in corn. This could be explained on the basis of higher *V*max (maximal uptake rate) in low concentration range and lower km (greater specificity) in the subsequently higher Zn concentration range.

Plant growth under zinc deficiency

It is evident from the available literature that the crop response to zinc deficiency in terms of dry mass production is diverse and there is no unanimity in using root and shoot dry mass production or shoot: root ratio as an indicator for zinc efficiency of cereals under low Zn condition. Although root and shoot growth is distinctly reduced under zinc deficiency (Graham, *et al.*, 1996). Shoot dry weight is depressed to a greater extent than root dry weight. (Rengel, 2001). In some cereal genotypes, root growth was enhanced under Zn deficiency (Cakmak, 2000). Higher sensitivity of durum wheat to Zn deficiency was associated with higher root growth at the expense of shoot growth (Rengel, and Graham, 1995). In nutrient solution experiments, decrease in shoot dry matter production induced by Zn deficiency was more pronounced in durum wheat than in bread wheat (Marschner, 1995; Singh *et.al.*, 2002). Root and shoot weight significantly increased with application of Zn and there was an increase in root density with an increase in root volume. Zn-efficiency based on shoot dry weight and shoot growth showed marked differences among chickpea genotypes for which the shoot dry weight was lower under Zn deficiency compared with the Zn-sufficient condition (Khan, 2003). The root: shoot

ratio in general, increases .As an initial response to Zn deficiency Cakmak *et al.* (1999) observed a decrease in shoot dry matter production of about 16% in rye, 36% in bread and 47% in durum wheat as a result of zinc deficiency. It is observed that Zn content (accumulation) per shoot and not Zn concentration is better correlated with the sensitivity of genotypes to Zn deficiency (Marschner, 1995). In wheat genotypes grown under controlled environmental condition in nutrient solution for 25 days, Zn content in the dry matter was much lower in plants grown without Zn compared to those supplied with Zn (Cakmak, *et.al.*, 1999). Concentration of Zn was significantly higher in plants supplied with Zn than those without Zn supply. Root Zn concentrations were greater than shoot Zn concentrations under Zn-deficient conditions, since under deficient Zn supply the transport of Zn from root to Shoot (Rengel and Graham, 1995) is inhibited. Zn-efficient bread wheat genotypes, in general, contained more Zn in shoots than Zn-inefficient durum wheat genotypes in field (Graham, *et.al.*, 1993), greenhouse and nutrient solution experiments (Rengel, and Graham, 1995). Zn-efficient chickpea was reported to have higher zinc content per plant and higher zinc uptake per gram of root dry weight than those-of-inefficient-genotypes.(Khan,*et.al.*,2003).

Schematic Diagram of the Causes of Zinc Deficiency in Crops



Alloway, 2003

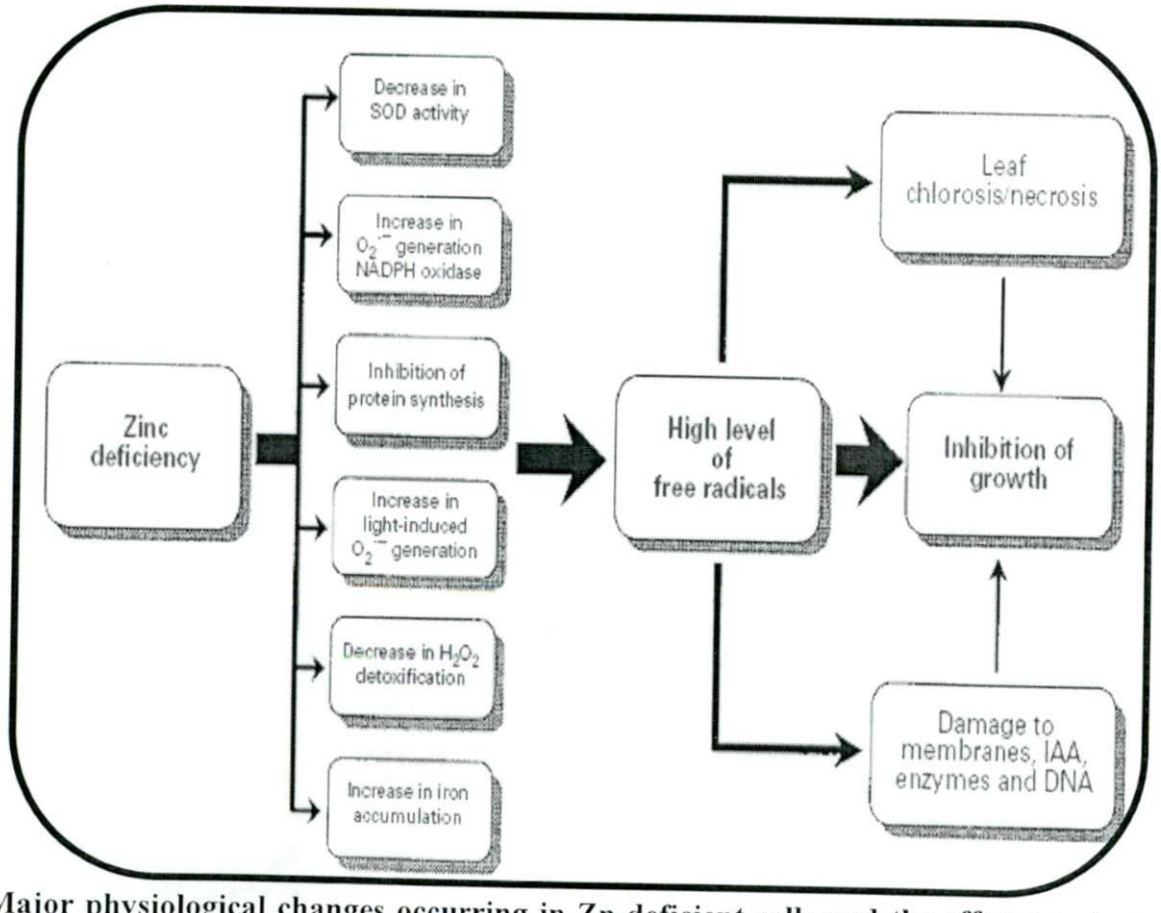
Importance of Zinc in plants

Zinc is an important micronutrient and cofactor of many enzymes and proteins involved in cell division, nucleic acid metabolism and protein synthesis. Plant response to Zn deficiency occurs in terms of decrease in membrane integrity, susceptibility to heat stress, decreased synthesis of carbohydrates, cytochromes, nucleotides, auxin and chlorophyll. Further, Zn-containing enzymes are also inhibited, which include Cu-Zn superoxide dismutase, carbonic anhydrase, alcohol dehydrogenase, alkaline phosphatase, phospholipase, carboxypeptidase, and RNA polymerase. Zinc plays multiple important roles in various physiological and metabolic processes of plants and plants grown in soils where Zn is not available yield less and have a low nutritional quality (Kochian and Garwin, 1999). Zn also offers plant resistance to root diseases.

Even though Zn is mobile element, deficiency symptoms are difficult to diagnose in pasture and legumes, as they may be seen as shortening of internodes, leaves may fail to expand properly and in cereals deficiency is usually seen in the seedling stage with

longitudinal pale green stripes on one or both sides of mid vein in young leaves. The symptoms appear to be worse in cold and wet seasons coupled with low light intensity.

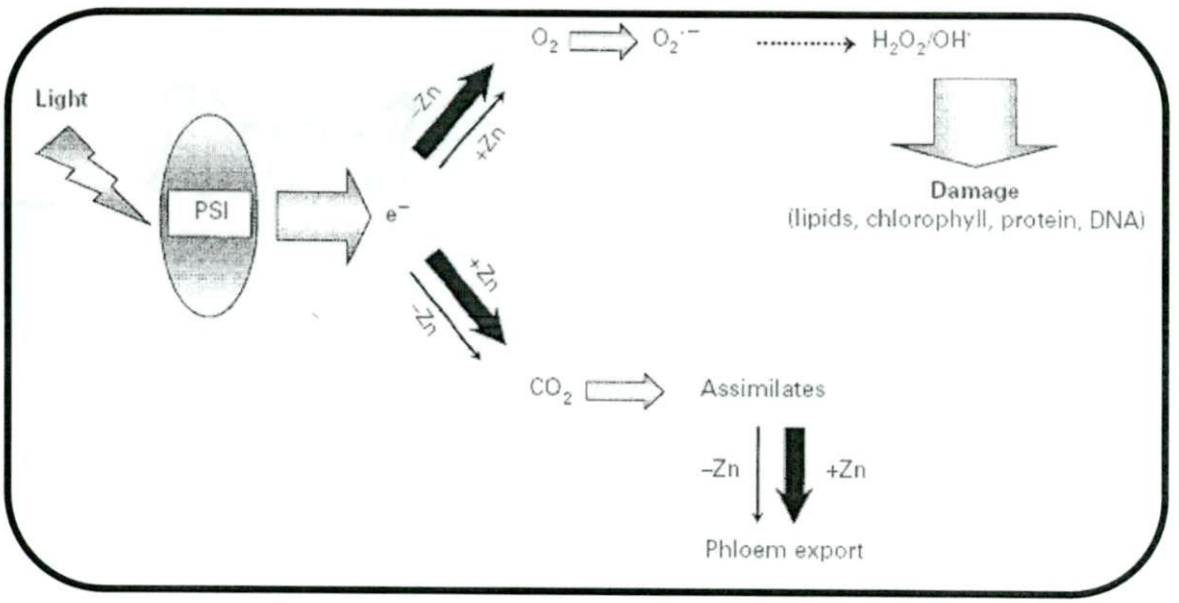
In plants, zinc does not undergo valency changes and its predominant forms in plants are as: low molecular weight complexes, storage metalloproteins, free ions, and insoluble forms associated with the cell walls. Depending on the plant species, between 58% and 91% of the zinc in a plant can be in water - soluble form (low molecular weight complexes and free ions). This water-soluble fraction is widely considered to be the most physiologically active and is regarded as a better indicator of plant zinc status than total zinc contents. The low molecular weight complexes are normally the most abundant soluble form of zinc and are probably the most active form of the metal. (Byrnes *et al.*, 1998).



Major physiological changes occurring in Zn-deficient cells and the effect on plant growth.

Cakmak, 2000

Evidences has indicated that much injury to plants occur due to various environmental stresses associated with oxidative damage through direct or indirect formation of Reactive Oxygen Species (ROS). This ROS includes, super oxide ($O_2^{\cdot -}$), H_2O_2, hydroxyl radicals, singlet oxygen ($^1O_2$) and are irreversible by products of cell metabolism. This ROS attack lipids, proteins and nucleic acids causing lipid peroxidation, protein denaturation and DNA mutation (Colangelo *et al.*, 2006).



Schematic representation of the photosynthetic electron flow to CO_2 and O_2 and formation of toxic oxygen species in chloroplastic stroma.

To prevent such damage to plant cells, an anti oxidant system consisting of (1) low molecular weight antioxidants such as ascorbate, tocopherol, glutathione etc., (2) protective enzymes that scavenge super oxide radicals and product of which H_2O_2 can be detoxified in water - water cycle. Micronutrient deficiency induced changes in activity of SOD has been reported in lupins (Yu and Rengel, 1999). They concluded that micronutrients like Zn, Cu, and Mn deficiencies decrease Zn, Cu and Mn in lupins leaf and decreased plant growth. Cu, Zn or Mn deficiency alters the activity of SOD indicating that oxidative stress is one of the components of micronutrient deficiency stress. The direct evidence of Zn deficiency induced oxidative stress comes from studies of Cakmak and Marschener (1995); they reported in cotton and tomato that Zn deficiency

increases production of $O_2^{\cdot -}$ With an increase in activity of NADPH oxidase. Therefore enhanced $O_2^{\cdot -}$ generation by NADPH oxidase and impaired detoxification of $O_2^{\cdot -}$ and H_2O_2 due to Zn deficiency related inhibition of SOD activity could lead to elevated levels of $O_2^{\cdot -}$ and its derivatives, creating oxidative stress and over expressing SOD protected plants in transgenic tobacco from oxidative stress. Second well characterized Zn requiring enzyme is carbonic anhydrase (CA). Zinc deficiency induces decrease in activity of CA (Rengel, 1995). In a study with rice plants, it was found that the levels of CA mRNA decreases under zinc deficiency and there was an abundance of transcripts encoding CA. Carbonic anhydrase enzyme activity was high in tissue containing high levels of Zn in wheat (Hacisalihoglu *et al.*, 2003).

Zinc fertilization and Water Use Efficiency (WUE)

Applying Zn increased water use and water use efficiency in many crop plants (Khan *et al.*, 2003). Zn nutrition may influence plant growth and water use efficiency under rainfed conditions in a number of ways: (i) by affecting the plant's ability to access soil moisture reserves, (ii) in mediating water loss through the stomata and by influencing plant water status, and (iii) by affecting the plant's sensitivity to plant water deficits. Zn deficiency decreases root growth in chickpea (Khan *et al.*, 2003), which can reduce a plant's ability to exploit soil moisture reserves, particularly as the dependence of subsoil moisture increases. In field grown chickpea, for example, Khan (2003) found that adding Zn increased leaf water potential and stomatal conductance in three chickpea genotypes during pod development, when soil moisture reserves were declining. Similarly, (Colangelo *et al.*, 2006) showed that Zn deficiency induced higher levels of water stress and reduced stomatal conductance in cauliflower.

Apart from its effect on plant water relations, Zn nutrition can affect the susceptibility of plants to drought stress. Activated oxygen species are produced by photoreduction of oxygen during photosynthesis (Lonnerdal, 2000). A reduction in carbon fixation due to water stress can cause an increase in activated oxygen species in the chloroplasts, which can lead to membrane damage and reduced growth. Zinc has a protective role against photo-oxidative stress (Cakmak, 2000), and therefore Zn deficient plants may be more sensitive to drought induced oxidative stress. The relative importance of the different effects of Zn nutrition on water use, growth and yield is likely to be

related to the severity of Zn deficiency and water deficit. Consequently, the effects of Zn deficiency may be manifested in a number of ways: as changes in water use, in water use efficiency and greater sensitivity to drought stress compared to plants supplied with adequate levels of Zn.

Copper/Zinc Superoxide Dismutase

Copper-zinc Superoxide dismutase (Cu/Zn SOD) is one member of a family of antioxidant enzymes present in the cytoplasm, nucleus and to a lesser extent, peroxisomes of all mammalian cells (Ekiz *et al.,* 1998) that provides the cell with a major defense barrier against oxygen toxicity. Copper is essential for the catalytic function of the enzyme, and zinc is thought to play mainly a structural role.

Copper/ zinc superoxide dismutase (SOD1) is a ubiquitously expressed, homodimeric intracellular enzyme essential for antioxidant defense. SOD1 catalyzes the disproportionation of super oxide into oxygen and hydrogen peroxide and this reaction is dependent upon a single bound copper ion present in each monomeric subunit. Among all the three forms of SOD, the copper, zinc form has received much interest, primarily because of its cytosolic location and dual metal requirement. The Mn and Fe SODs are structurally very similar and the electrical properties of Cu/Zn SODs differ greatly from those of Fe and Mn SODs. Because, a major change in the structure of the protein occurred after Cu become a metal cofactor (Baker *et.al.*, 2000).

Properties of Cu/Zn SOD

Cu/ Zn SOD is one of the better-characterized SOD enzymes, is composed of two identical 151-residue subunits with the two active sites facing away from each other. The copper and zinc communicate weakly across the span of two imidazole nitrogen atoms on a common histidine residue. Copper sits on the floor of a deep cleft whereas zinc is completely buried in the protein. Based on relative locations, copper is more easy to displace than zinc and experimental studies performed under careful conditions of pH and temperature tend to show copper the more mobile factor. During catalysis, the copper is reduced with the substrate O_2 to yield first O_2 then (by reoxidation) H_2O_2. Zn^{2+} in the protein is believed to increase the

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redox potential, implying it may also have a role in catalysis. The specificity of zinc is not stringent, but occupation of the zinc site is critical to the native properties of the enzyme

Carbonic Anhydrase (CA)

Carbonic anhydrase (carbonate dehydratase) is a family of metalloenzymes containing one Zinc atom per protein molecule of molecular weight 30,000, catalyzes the interconversion of carbon dioxide to bicarbonate and protons, in solution.

The forward reaction that occurs rather slowly in the absence of a catalyst.

$$CO_2 + H_2O \rightarrow^{\text{Carbonic anhydrase}} HCO_3^- + H^+$$

The reverse reaction is fast and does not require a catalyst.

$$HCO_3^- + H^+ \rightarrow H_2CO_3 \rightarrow CO_2 + H_2O$$

The rate of catalysis, among the fastest known, is pH-dependent; hence it may assist in the regulation of pH in cell compartments (Jacobson *et al.,* 1975). It is a zinc enzyme, which has been shown to have a specific requirement for zinc in both woody and annual plants (Bar-Akiva and Lavon, 1969). It is a second well-characterized Zn-requiring enzyme and its activity could be used as an indicator for diagnosing Zn deficiency in plants (Bar-Akiva and Lavon, 1969). Zn deficiency induces a decrease in the activity of CA (Rengel, 1995).

Zinc in Crop Products and Human Nutrition

Zinc is an essential trace element for animals and humans as well as plants. There are more than 300 enzymes involved in key metabolic processes in humans, which contain zinc and therefore an adequate zinc intake is essential for normal healthy growth and reproduction (FAO/WHO/IAEA, 1996). The International Zinc Nutrition Consultative Group (IZiNCG) has estimated that as much as one third of the world's population is at risk from inadequate zinc intake.

Many food products are derived directly from plants, including staples such as rice, wheat, maize and sorghum, but the zinc content of animal products is also affected by the

soil - plant relationships of zinc since ruminants consume herbage and other animals such as pigs and poultry consume cereals. Zinc deficient plants generally have low tissue Zinc concentrations and therefore, in addition to reduced crop yields, deficiencies of zinc in the field also reduce the nutritional value of the crop with regard to its contribution to the Zinc content of the diet. This can be vitally important in subsistence rural economies where there is often insufficient diversity in the diet to enable low concentrations of Zinc in one component of the diet to be compensated for by a higher Zinc content in another. For example, lean meat, whole grain cereals and pulses generally contain the highest concentrations of zinc (20–50 mg Zn kg^{-1} fresh weight). However, taking meat as an example, many people in developing countries either do not have access to a sufficient amount of meat or their social and religious customs do not permit them to eat it so this significant source of zinc in the diet is often not available to many people.

Cereal - based diets are the major source of nutrients for the majority of the world's population and therefore both the Zinc content of the cereal grains and its bioavailability to the people consuming them are of fundamental importance.

Zinc and Phytic acid

In cereal grains, most of the phosphate is bound in phytic acid (inositol hexaphosphate) that primarily is deposited together with protein and minerals in aleurone storage vacuoles. The phytate, which combines with the zinc and reduces its availability in the human (and other monogastric animals) gastro - intestinal tract hence, it acts as an antinutritional factor for zinc. (Holm *et al.*, 2002)

Phytate, containing large amounts of phosphate, is present in higher concentrations in cereal grains that have been grown on soils with a relatively high phosphorus status. The use of increased amounts of phosphatic fertilizers on crops of modern, high yielding varieties of rice and wheat can increase yields when the zinc supply is adequate but produces grain, which may have a lower percentage of available zinc due to the higher phytate content. However, where the zinc status of the soil is marginal or inadequate, the increased phosphorus supply can induce zinc deficiency in the crop and, if not corrected, result in a reduced yield of grain with a lower total zinc content but also an elevated

phytate content and hence proportionally less zinc available to the consumer (Ekiz *et.al.*, 1998).

Phytate will significantly reduce the availability of zinc where the phytate: zinc molar ratio is greater than 15. Alternatively, calcium x phytate: zinc ratios are also used and where this ratio is greater than150 nmol per 1000 kcal, zinc in the diet will be highly unavailable. The latter value takes account of the antagonistic effect of calcium as well as phytate on the availability of zinc in food products. This is also very important because calcareous soils are widely used for growing wheat in some developing countries and are also an important cause of zinc deficiency in crops. Both the high calcium and low zinc contents in the grain contribute to the low availability of zinc to consumers. Calcareous soils are also a problem with low availability of phosphorus and so require relatively high applications of phosphatic fertilizers, which exacerbates the low dietary availability problem due to both increased phytate concentrations in grains but also increased zinc deficiency, if not corrected (Holm *et al.*, 2002).

Proteins in the diet modify the effect of phytate and animal proteins can improve the absorption of zinc from the diet. This, again, demonstrates the merit of mixed diets with regard to zinc availability but many people in developing countries are unable to have a mixed diet containing both cereals and animal proteins. Phosphorus is a vitally important macronutrient for plants and modern varieties of rice and wheat require an adequate supply of this element in order to realize their potential yield. The adverse effects of high phosphorus in the soil on zinc availability to the plant can be overcome by zinc fertilization but the problem of low dietary availability of the zinc is more difficult to overcome. It is possible that plant breeding may provide a way of getting high yielding cereal crops with adequate levels of bioavailable zinc in their grain. From a review of the literature, Rengel *et al.*,(1995) concluded that soil-applied zinc sulphate could be used to increase the concentration of zinc in the edible portions of crops, such as grains. In many cases, it would also result in increased yields but care would have to be taken to ensure that excess levels of zinc do not accumulate in soils. This field fortification of crops with zinc would need to be accompanied by changes in milling practices to maximize its benefits in terms of dietary availability.

Zinc efficiency

Zinc efficiency is defined as the ability of a plant to grow and yield well under Zn deficiency. Zinc efficiency 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. There are a number of different possible mechanisms by which plants tolerate Zn deficiency (generally expressed as Zn efficiency), such as Zn uptake, translocation to the shoot and physiological efficiency. 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 favouring exploitation of Zn from larger soil volume, higher synthesis and release of Zn-mobilizing phytosiderophore by the roots and uptake of Zn-phytosiderophore complex. Seed Zn content has also been suggested to affect Zinc efficiency. Even there are number of different mechanisms contributing to Zn efficiency, uptake is the major mechanism and the effect of this is modified by the physiological efficiency within the shoot. (Graham, *et al.*, 1993).

Cereal species, as well as genotypes of a given cereal greatly differ in adaptation to Zn-deficient conditions. Among the cereals, rye showed an exceptionally high Zn efficiency. Biomass production and grain yield of rye was not affected under severe Zn-deficient conditions (Cakmak *et al.*, 2000). Genotypic differences in Zn efficiency have been related to various mechanisms operative in the rhizosphere and within a plant system. Considerable progress has been made over the past few years to identify mechanisms that the plant species and genotypes possess for efficient acquisition of Zn from soils low in Zn availability (Rengel, 2001). These include, higher uptake of zinc (Zn^{2+}) by roots, protection against superoxide free radicals, i.e., efficient antioxidative defense mechanism, efficient utilization and (re)-translocation of Zn. The Zn efficiency of cereals is mainly related to difference in acquisition of Zn by the roots (Cakmak *et.al.*, 2002). However, physiological and biochemical processes that control Zn efficiency in general, and Zn acquisition by the roots, in particular, are among the less thoroughly studied aspects of plant Zn-nutrition. More than one mechanism could be responsible for

establishing Zn efficiency in a genotype and it is likely that different genotypes subjected to Zn deficiency under different environmental conditions will respond by one or more different efficiency mechanisms (Graham and Rengel, 1993).

Zn efficient genotypes maintain higher activity of Zn requiring enzymes in conditions of Zn deficiency is correlated with ZE (Hacisalihoglu *et al.*, 2003). They examined Zn requiring enzymes Cu/ Zn SOD and CA in contrasting wheat genotypes, in response to Zn deficiency and found an up regulated transcript levels of Cu/ Zn SOD in ZE genotypes but in case of CA there was no significant increase in transcript levels, but higher CA activity. In these cultivars, the strategy would be posttranscriptional regulation of this enzyme in relation to plant Zn status that leads to significantly lower activity of Cu/ Zn SOD and CA in ZE genotypes. These results were previously shown by Rengel (1995) and Yu and Rengel, (1999). The molecular approach to breeding of mineral deficiency resistance and mineral efficiency is now receiving increasing attention

Approaches to increase Zinc in plants

a) Zinc fertilization

Mineral nutrients are the major contributors to enhance crop production and to maintain soil productivity and preventing soil degradation. Generally improving the nutritional status of plants by applying fertilizers and maintain soil fertility has been the critical step in doubling of food production both in developed and developing countries, since the beginning of green revolution. Large increase in grain yield by Zn application was demonstrated in Australia (Graham *et al.*, 2001) and India (Tandon, 1995).

One important strategy to increase micronutrient concentration in grain is fertilization of plants via soil or foliar application, depending on the plant species. Soil application of Zn can increase Zn concentration of plants by as much as 2 to 3 folds (Rengel, 1995). Under conditions where plant growth is limited by Zn deficiency, adding Zn increase WUE in chick pea (Khan *et al.*, 2003). The factor that will determine the effect of improved Zn

nutrition in WUE and harvest index of genotypes and its ability to respond to higher levels of nutrition.

Cereal species and genotypes of a given cereal species differ greatly in their response to Zn deficiency and Zn fertilization (Ekiz *et al.*, 1998). In wheat this variation is mainly due to poor capacity to synthesize and release mugenic acid from roots (Cakmak *et al.*, 2001). Apparently tremendous release of mugenic acids offer mechanism is also involved in tolerance to Zn deficiency, which is unknown. Even though fertilization increases Zn concentrations in plants by 2-3 folds, even with higher Zn fertilization rates the Zn concentration in wheat grains does not show corresponding increase (Rengel, 1999).

There is also no direct economic motivation for farmers to improve nutritional quality of grains alone by fertilization; by contrast research on enhancing micronutrient concentration in grain and other edible parts of plant is very limited. More research activities are therefore needed aiming at improving bioavailability levels of micronutrients in cereal grains (Cakamk, 2002).

b) Enhance Zn efficiency (ZE) trait

The application of Zn fertilizers is not a totally successful strategy in alleviating Zn deficiency because of agronomic (i.e., subsoil constraints, disease interactions), economic (i.e., unavailability of Zn fertilizers for poor farmers), and environmental (i.e., pollution associated with excessive fertilizer use) factors (Graham and Rengel, 1993; Hacisalihoglu *et al.*, 2003). A more efficient and sustainable solution to Zn deficiency limitations to crop production is the development and use of Zn-efficient plant genotypes that can more effectively function under low soil Zn conditions, which would reduce fertilizer inputs and protect the environment as well. It has been well documented that certain plant species, as well as genotypes within certain species, exhibit a significant genetic-based variation in their tolerance to Zn deficiency. Even though the precise mechanisms underlying ZE are not yet clear but a number of studies have demonstrated differential ZE in several crop species, including bean (Hacisalihoglu *et al.*, 2003), wheat (Graham and Rengel, 1993), rice, and tomato (Bowler *et al.*,1992).A Zn efficient genotype tended

to be less adversely affected by a period of drought than an inefficient genotype, suggesting that ZE may be associated with greater tolerance to drought.

The identification and characterization of the genes conferring ZE should provide important new insights into the molecular and physiological basis of crop plant ZE. Additionally, identification of the genes underlying ZE could provide the molecular tools to facilitate the engineering of more Zn-efficient crops. Another goal of related research could be to engineer food crops that accumulate elevated levels of Zn in the edible portions and thus, have higher nutritional value. This may not only lead to maximal plant and human nutrition with minimal fertilization, but also help to nourish the world population.

c) Plant breeding approaches

The final permanent solution to micronutrient malnutrition in developing countries is a substantial improvement in dietary quality, higher consumption of pulses, fruits, vegetables, fish and animal products that the poor already desire but cannot presently afford. Meanwhile breeding staple foods that are dense in minerals and vitamins provides a low-cost, sustainable strategy for reducing levels of micronutrient malnutrition. Getting plants to do the work of fortification, referred to as "biofortification," can reach relatively remote rural populations that conventional interventions are not now reaching and can even have benefits for increased agricultural productivity. Biofortification, thus, complements conventional interventions. The inherent comparative advantages of biofortification include, it would be cost-effective and sustainable (Milner, 2000).

In principle, once such crops are developed and successfully disseminated, they can be reproduced by farmers and automatically form part of the food chain. Hence, for a largely one-time investment this strategy can produce a stream of future benefits to consumers of these crops. While this reasoning is intuitive, there has been no other attempt to quantify the health benefits of iron-rich and zinc-rich cereals explicitly or to determine the cost-effectiveness of this approach (IIPS, 2000).

d) Exploit genetic variability in Zinc content

An alternate approach to increasing Zn concentration in grains is to exploit genetic variability in concentration of this micronutrient with in plant. Genomes with highly significant genetic differences have been reported for Zn concentration in cereal grains. Numerous studies with a wide range of species have demonstrated genetic variation and breeding program for improved nutritive value like digestibility (Orskov *et al.*, 1990). Certain criteria must be met before new lines of micronutrient enriched staple food crops are distributed. Meeting this condition will ensure the targeted people at risk of developing micronutrient malnutrition will be benefited from such action. The criteria includes (1) productivity (2) the micronutrient enrichment levels achieved must have significant impact on human health, (3) the micronutrient enrichment trait must be relatively stable across various edaphic environment and climatic zones, (4) alternatively the bioavailability of micronutrients in enriched lines must be tested in humans to ensure that they improve the micronutrient status of people preparing and eating them in traditional way within normal household environment, (5) consumer acceptance must be tested; taste and cooking quality (Welch and Graham, 2004).

e) Molecular biology

Molecular biology supports classical cereal breeding to select for particular genes and includes analysis of quantitative trait loci (QTL) and marker-assisted selection (MAS) (Asins, 2002). Wild cereal species can be used to enrich the genetic diversity of breeding populations, for e.g. rice variety contain about 12 ppm Fe and 25 ppm Zn, but some traditional varieties have two fold higher. Despite the important genetic variations among crop genotypes average value for Zn concentration in cereal grains are still lower, when compared to legume grains, which show higher concentration of Zn, a wider range of concentration (Welch and Graham, 1999). There is a weak relationship between grain yield and quality characters of cereal crops and likely these variations are largely depending on soil types and climate.

f) Genetically modified plants

Genetically modified plants for improved trace element nutrition is a complementary approach and genetic modification can be achieved by two fundamentally different approaches first by conventional breeding and selection techniques, the second is genetic engineering techniques can be used to create a novel cultivars with the desired properties e.g. of such approaches are insertion of novel genes, enhancement of expression of genes already present, but at low expression levels and depression of expression of genes or disruption of pathway involved in synthesis of inhibitors of trace element absorption (Bo Lonnerdal, 2003).

g) Insertion of genes for novel metal binding proteins

The expression of human lactoferretin, the major iron binding protein in breast milk was introduced in rice, by using gene gun technology with a strong endosperm-specific promoter from rice and selecting for high-lactoferrin cultivars, very high expression levels could be achieved. This doubled Fe content in dehusked polished rice by 2 folds. Another research group has inserted the ferritin gene from *Phaseolus vulgaris* into rice (Lucca *et al.*, 2001) by agrobacterium mediated transformation, by both the insertion lines were unlikely adequate to substantially increase Fe intake. Gene expression has been stable for more than five generations, and large-scale field trials have yielded harvests at levels of several tons.

h) Overexpression of "native" genes for trace element storage proteins

There are trace element–binding proteins in plants that are expressed either at low levels or in a plant tissue that is normally not eaten. One example of the first category is soybean ferritin, which is present in the soy endosperm, but the level of expression is too low to contribute substantially to the soybean iron content. By either selecting and breeding for high-ferritin varieties or overexpressing the soybean ferritin gene, it should be possible to increase substantially the ferritin content of soy. An example of a trace element–binding protein that is present in the "wrong" tissue of the plant (from the consumer's perspective)

is leghemoglobin (Lehtovaara and Ellfolk, 1975). This protein is present in the root nodules of legumes such as soybeans and accumulates iron in the heme form; this may be a useful form of iron in human nutrition (Hallberg, 1981). Preliminary experiments on rats indicate a high bioavailability of iron from soybean leghemoglobin but further experiments on humans are needed to verify this

i) Expression of transporters that facilitate plant uptake of trace elements

The uptake of trace elements by root system of plants and the accumulation of trace elements by seed or grains is likely to be limited by the capacity of the plant to take up and transport these ions (Welch, 2002). First the mineral ions need to be available at and pass the root soil interface; this must be facilitated to substantially increase the trace element content of plant. Next the absorption mechanisms in root cell plasma membrane needs to be sufficient and adequately specific to allow the trace elements to accumulate once they have entered the apoplast, finally the trace element must be efficiently transported to edible parts of the plant. Plant biologists have identified and characterized the protein and genes involved in these transport process (Grusak *et al.*, 1999). For e.g. the *ZIP* family of metal ions transporters are novel metal transporter family are capable of transporting a variety of cations, including Zn, Fe, Cd and Mn. Information on where in plant each *ZIP* transporters function and how each is controlled in response to nutrient availability may allow the manipulation of plant mineral status with an eye to create food crop with enhanced mineral concentration, developing crop that bio accumulate or exclude toxic metals (Guerinot, 2000). A full length cDNA encoding a putative *ZIP* protein has been isolated from Arabidopsis cDNA library among heterologus DNA probe, which has a leucine zipper and nuclear localization signal domain pointing that leucine zippers might play a role as transcription factors in the regulation of gene expression (Zheng *et al.*, 2002).

IRT1, IRT2 are members of the *Arabidopsis ZIP* metal transporter family that are specifically induced by Fe deprivation in roots, but short term labeling with ^{65}Zn revealed in *irt1* mutant, it responds to Fe and Zn deprivation by altered expression of certain Zn and Fe transporter genes. These data support the conclusion that *IRT1* is an essential

metal transporter required for proper development and regulation of Fe and Zn homeostasis in Arabidopsis (Henrique *et al.*, 2002). The two families of transporters *ZIP* and *CDF* (cation diffusion facilitator) families have been found to play a number of important roles in Zn transport.

j) Decreased inhibitors of trace element absorption

Low phytate varieties

Phytic acid is an inhibitor of Fe and Zn absorption in humans and believed to be a major contributing factor to the worldwide problem of Zn and Fe deficiency (Welch and Graham, 2004). Spontaneous low phytic acid (lpa) mutations have been found in maize, barley and rice and result in seed phytic acid- phosphorous level, that range from 50 to more than 95 per cent of control (Raboy, 2002). It was shown in both animal and human studies that Insertion of phytase gene exogenously or endogenously could reduce the phytate content of the diet and improve trace element bioavailability (Sandberg *et al.*, 1996). Microbial phytase can also be added to feeds and allowed to act during the digestive process to improve mineral utilization in domestic animals (Yi *et al.*, 1996). There was an attempt to insert *Aspergillus hemigahn* phytase into rice (Lucca *et al.*, 2001).

k) Increased synthesis of enhancers of trace element absorption

Various dietary ligands can enhance trace element absorption. One example of this is amino acids, which are largely released from proteins during digestion. Cysteine or Cysteine-rich peptides are shown to have a positive effect on iron absorption (Layrisse *et al.*, 1984; Taylor *et al.*, 1986) and hisstidine is shown to facilitate zinc absorption (Lonnerdal, 2000). Other organic acids such as fumarate, citrate and succinate can also enhance trace element absorption. As an approach to increasing the cysteine content of the diet, Lucca *et al.*, (2001) inserted the gene for a rice metallothionein-like protein. There are reports in the literature that ß-carotene can enhance iron absorption in humans (Lucca *et al.*, 2001).

l) Hyper accumulators

The term hyperaccumulator has come to mean a plant capable of taking up concentrations of trace metals approximately 100 times greater than normal species. Zinc hyperaccumulation has since been defined as the occurrence of > 10 000 μg Zn g^{-1} dry weight in the aerial parts of a plant species when growing in its natural environment (Baker and Brooks, 1989). This property has been recognized for many years; as early as 1855 there were reports of *Thlaspi caerulescens* concentrating Zn to around 17% of the dry plant ash. These plants possess genes that regulate the amount of metals taken up from the soil by roots and deposited at other locations within the plant. There are a number of sites in the plant that could be controlled by different genes contributing to the hyperaccumulation trait.

These hyperaccumulator species have come to dominate the literature, in part because of the desire to transfer the character into crop species for use in phytoremediation, phytomining and biofortification (Baker and Brooks, 1989).

Typically, 10–20 species are reported to be Zn hyper accumulators, with a smaller number of these able to accumulate Cadmium to very high concentrations as well. *Thlaspi caerulescence* is a best-known Zn hyper accumulator (Baker *et al.*, 2000). The genus *Thlaspi* L. is likely to be polyphyletic and its taxonomy is controversial. Seed-coat anatomical and sequence data have thus been used to split the genus into several alternative genera, including *Thlaspi* s.s., *Vania* and a clade containing *Thlaspiceras*, *Noccaea*, *Raparia*, *Microthlaspi* and *Neurotropis* . High $[Zn]_{shoot}$ is probably a general feature of *Noccaea* and its sister clade *Raparia* (Macnair, 2000) but not of *Thlaspiceras*, which nevertheless contains Zn-hypertolerant species (e.g. *Thlaspiceras oxyceras,* Boiss). *Arabidopsis halleri* is the only other Brassicaceae species with an unequivocally high $[Zn]_{shoot}$ character.

Genetics of Zn hyperaccumulation

Thlaspi caerulescens is a short-lived, self-compatible biennial/ perennial species which hyperaccumulates Zn/Cd/Ni. In *T.caerulescens*, Zn hyperaccumulation and hypertolerance are constitutive species-level traits although inter- and intrapopulation

variations in $[Zn]_{shoot}$ and/ or Zn tolerance occur. When *T. caerulescens* are grown under identical experimental conditions, Zn tolerance is generally greater, and $[Zn]_{shoot}$ is lower in high-[Zn]soil populations than in low-[Zn]soil populations.

Hyper metal accumulator plants commonly accumulate more than 1 per cent of their above ground dry biomass in Ni or Zn (Baker and Brooks, 1989). A complex metal homeostasis also exerts for a regulated, specific and targeted supply of micronutrients. Metal cations to metal requiring processes and for the detoxification of excess or non-essential metal cations that have entered the plants (Clemens *et al.*, 2002). Metal hyper accumulators, which are highly metal tolerant are likely to exhibit numerous alterations in their metal homeostasis network throughout the entire plant. In recent years, research is focused on identifying and characterized individual candidate gene implicated in having alteration. The candidates identified in metal hyper accumulators are cation diffusion facilitator *CDF* and Zinc regulated transporter *ZRT*, iron regulated transporter *IRT* like protein (*ZIP*) families of transition metal membrane transport proteins.

In *Arabidopsis thaliana* the expression of *ZIP* families *AtZIP1*, *AtZIP3* and *AtZIP4* is induced under condition of Zn depleted and *AtZIP1* and three have been shown to mediate cellular Zn uptake across the plasma membrane (Grotz *et al.*, 1998). A comparative transcript profiling of metal hyper accumulators and non-accumulator shoot and root (Weber *et al.*, 2004), identified a distinct and tissue specific expression of *ZIP* and *NAS* (Nicotinamine synthase). Metal homeostasis gene involves in Zn hyper accumulator *A. hallari* (Becher *et al.*, 2004). He proposed that the elevated cellular Zn uptake with a prominent role of *ZIP 9* in roots and *ZIP6* in shoot and necessity for low molecular weight chelator biosynthesis to achieve cytoplasmic Zn buffering and intercellular metal mobility with major roles for *NAS2* in root and *NAS3* in shoots. This will provide a starting point for a genetic analogous of metal hyper accumulation in *A. halleri* as well as for the identification of upstream regulator genes, further for transgenics for increasing Zn content in cereals and bio fortification.

Ramesh and group (2004) made an attempt to test the over expression of Zn transporter in cereals affects the plant growth, seed mineral content and Zn transport rates. Known

Zn transporters from Arabidopsis were over expressed in *Hardeum valgare*. They found that short-term uptake rates were higher in the transgenic lines after Zn deprivation. Resupply of Zn after a period of deprivation resulted in the rapid decrease in Zn uptake in transgenic lines in which a Zn transporter gene was constitutively expressed. Similar to processes in yeast and *Arabidopsis*, they hypothesize that this rapid decrease in Zn transport activity may be caused by the degradation of transporters in response to Zn sufficient conditions. Increased seed Zn and iron content by over expression of a Zn transporter provides a new strategy for increasing the micronutrient content of cereals.

In total, homologues of 60 *Arabidopsis thaliana* genes are significantly differentially expressed between hyperaccumulators and nonhyperaccumulators and may have conserved roles in brassicaceous Zn hyperaccumulation. Six of these genes encode proteins with putative roles in Zn transport: three cation diffusion facilitator (CDF) family members (*AtMTP11*), (*AtZAT1/AtMTP1*) and (*AtMTPc3*)); a member of the Zn-Fe permease (*ZIP*) family (*AtIRT3/TcZNT2*)); and a P_{IB}-type heavy-metal-associated domain-containing ATPase (*AtHMA3*)). CDFs appear to mediate vacuolar sequestration of Zn through Zn efflux from the cytoplasm (Persans *et al.*, 2001; Blaudez *et al.*, 2003). Other genes expressed highly in Zn hyperaccumulators include plant defensins (PDFs), which confer Zn tolerance and accumulation in heterologous systems, and may act as blockers of Zn-permeable channels (Mirouze *et al.*, 2006).

Enhance the level of zinc requiring enzymes

The expression and activity of the Zn-requiring enzymes copper (Cu)/Zn superoxide dismutase (SOD) and carbonic anhydrase (CA) did correlate with differences in ZE.

Northern analysis suggested that Cu/Zn SOD gene expression was up regulated in the Zn-efficient but not in inefficient Wheat genotypes. Under Zn deficiency stress, the very Zn-efficient and moderately Zn-efficient genotypes exhibited an increased activity of Cu/Zn SOD and carbonic anhydrase when compared with Zn-inefficient wheat genotype. These results suggest that Zn-efficient genotypes may be able to maintain the functioning of Zn-requiring enzymes under low Zn (Hacisalihoglu *et al.*, 2003).

The expression of genes encoding the Zn-requiring enzymes Cu/ Zn SOD and CA in the contrasting wheat genotypes in response to Zn deficiency was tested. In general, the

abundance of transcripts encoding both enzymes was up regulated with elevated tissue Zn levels. (Hacisalihoglu *et al.*, 2003). This is consistent with previous findings, which reported a decrease in CA expression in Zn-deficient rice plants. In shoot tissue from plants grown under Zn-deficient conditions, the expression of SOD was up regulated in the very Zn-efficient compared with Zn-inefficient wheat genotypes. The significantly lower activity of the Cu/ Zn SOD enzyme in the Zn-inefficient genotype under Zn deficiency conditions suggests that ZE might also be related to activity of this enzyme. Previous reports also showed a positive correlation between Cu/ Zn SOD activity and ZE among and within cereal species (Cakmak *et al.*, 1997; Yu *et al.*, 1999).

A similar pattern was also found with the other Zn containing enzyme studied, CA. Previously, higher CA activity in Zn-efficient bread wheat compared with a Zn-inefficient durum wheat has been reported (Rengel, 1995).

Materials and methods

III. MATERIAL AND METHODS

As is the case with plants and soils, Fe and Zn deficiencies are also the most widespread micronutrient deficiencies in humans. These deficiencies affect more than 3 billion people worldwide causing serious health and productivity problems for various population groups, especially among resource-poor women, infants and children (Graham *et al.,* 2001; Welch and Graham, 1999). These deficiencies are particularly widespread in developing countries where diets are rich in cereal-based foods with low concentration of bioavailable Zn and Fe. The problem with Zn and Fe deficiency has been intensified with the increased cultivation of high-yielding cultivars of the 'Green Revolution' cereals whose grains contain low concentrations of Zn and Fe, that are rich in compounds which limit the bioavailability of Zn and Fe to humans (Welch and Graham, 1999). Increasing the concentrations of Zn and Fe in cereal grains is a high priority research task, and will greatly contribute to the alleviation of micronutrient deficiencies in human populations worldwide. Enhancements in concentration of micronutrients, especially Zn, also result in several positive consequences for crop production, such as improvements in seedling vigor, pathogen resistance, and competition against weeds and finally, an enhanced yield (Rengel,1995).

Depending on the plant species, soil application of Zn to soil can increase Zn concentration of plants by as much as 2–3-fold (Rengel *et al.,* 1999). However, even with very high Zn fertilization rates, the Zn concentration in wheat grain does not show correspondingly high increases from Zn fertilization. Furthermore, the same applies to Fe as well. In most cases, Zn and Fe concentrations in grain do not increase to the desired levels, even by using high rates of fertilizer applications (Rengel *et al.,* 1995). There is also no direct economic motivation for farmers to improve the nutritional quality of grains alone by fertilization.

An alternative approach to increasing Fe and Zn concentrations in grain is to exploit the genetic variation in grain concentrations of these micronutrients within plant genome. Highly significant genetic differences have been reported for both Fe and Zn concentrations in cereal grains. Therefore an attempt was made to assess the genetic variability in grains of finger millet genotypes; uptake and translocation studies were conducted to understand the basis of variation in seed zinc in finger millet genotypes. There are a large number of

enzymes in which zinc is an integral component of the enzyme structure (zinc enzymes) of which, copper zinc super oxide dismutase (Cu/Zn SOD) and carbonic anhydrase (CA) are important zinc-regulated enzymes. Activity of these enzymes has been correlated with zinc availability to the plants. To address these tasks, the following objectives were set.

Objectives

1. Reconfirmation of Zinc status in grains and selection of contrast (high and low) Finger millet genotypes.
2. To asses the level of Carbonic anhydrase (CA) and Copper Zinc Super Oxide Dismutase (Cu/ Zn SOD) and study the relevance of Zinc status and activity of these enzymes.
3. To understand the differences in uptake and translocation in these contrast genotypes using ^{65}Zinc radiolabel studies

The material and methods used to achieve these objectives are described below in this chapter.

3.1. Reconfirmation of Zinc status in grains and selection of contrast (high and low) Finger millet genotypes.

3.1.1 Procurement of seed material and raising of plants

In order to assess the genotypic variation in Finger millet genotypes with regard to zinc content, seeds were procured from All India Coordinated Research Project (AICRP) on millets, GKVK. Seeds of 16 contrast finger millet germplasm lines consisting of locally adopted cultivars and released varieties were procured and grown in in similar environment in the field to reconfirm the selection of these contrast types by adopting RCBD (Randomized Complete Block Design). The plants were maintained in the field till maturity by providing all recommended package of practices. The leaf samples and grains were collected and these were analyzed for zinc content.



Plate No. 1: Field experimental setup for Zn estimation (layout: RCBD)



Plate No. 2: Over view of experimental plots.

3.1.2 Zinc estimation in the leaves and grains of 16 contrast Finger millet germplasm lines

Sample preparation.

Dried grains and leaves were ground with pestle and mortar using liquid nitrogen to make a fine powder. Five ml of concentrated Nitric acid was added to 250 mg of powdered sample and incubated in a digestion hood overnight. The next day, 5 ml of diacid mixture (Nitric: Perchloric acid:: 10:4) was added to it and placed on a sand bath till all the acid fumes evaporated and thick white residue was left out in flask. It was allowed to cool and volume was made up to 25 ml using triple distilled water and further dilutions were made if the concentration of the metal was too high. These diluted solutions were used for Zinc estimation.

Estimation of Zinc

Zinc was estimated in the grain and leaf sample using Polarized Zeeman Atomic Absorption Spectrophotometer (AAS-2-6100).

Standard: 100 ppm standard Zn^{2+} solution was prepared using 1000 ppm Zn^{2+} atomic absorption standard solution (Sisco research laboratories Pvt. Ltd) and appropriate dilutions were made to get standard solutions ranging from 0-0.6 ppm. These standards were fed to AAS as that of sample to get standard curve and a standard graph was fit. To this standard curve, the sample readings were compared and then computed.

Protocol: Suitable dilutions were made from the extractant with deionized double distilled water so as to fit their absorbance with the range of standard curve. The diluted samples were fed to AAS and concentration was recorded in ppm using standard curve. Three replications were maintained and their average was used for calculations. The grain zinc content was calculated using the following formula.

$$\text{Zinc} = \frac{\text{Average ppm} \times \text{Volume of digested sample} \times \text{Volume made up}}{10^6 \times \text{Weight of the sample} \times \text{Aliquot taken}}$$

Zinc content was expressed in mg/100g of sample.

Variation in zinc content was statistically analyzed using one-way ANOVA based on which the genotypes were classified as low and high seed zinc types.

3.1.3 Assessment of genotypic variation in Zinc content in different parts of the plants with external Zinc application

Seedling vigor is an important factor contributing to growth and yield of cereal crops. High seed Zn content can increase seedling vigor when soil available Zn is low. The ability of genotypes to utilize seed Zn reserves more efficiently by greater remobilization of seed Zn into growing tissues may contribute to Zn efficiency. Genotypes were grouped into two categories based on seed Zinc content by previous experiment viz. (a) High Zinc types (b) low Zinc types. In the next season the above experiment was repeated in the field with external application of different levels of zinc.

Sixteen Finger millet [*Eleusine coracana (Gaertn. L)*] genotypes were grown in a zinc deficient red loamy soil under field condition till maturity with three replications of no external Zn supply (control), 20 Kg/ha (T1) and 30 Kg/ha (T2) Zn supply to study genotypic variation in tolerance to Zn deficiency. Plants were maintained till maturity. Leaves and grains were sampled separately and zinc content was estimated. Based on these data genotypes were classified into (a) High zinc types and (b) low zinc types.

3.1.4 Screening for zinc efficiency

The aim of this study was to quantify the relative effects of different physiological mechanisms of Zn efficiency in Finger millet genotypes and for probing the physiological and biochemical reasons for differential uptake and translocation in these genotypes. To select the genotypes for Zinc deficient soils or soils of low Zn content is an important objective of the study. Soil and solution culture techniques were used to characterize Zn uptake, translocation, utilization efficiency and seed Zn remobilization in 16 Finger millet genotypes (includes germplasm lines and present leading cultivars), which have shown consistent differences in Zn efficiency. Three methods are commonly used to screen for Zn efficiency, which are field screening, soil and solution screening techniques under controlled conditions.

(a) Field screening

Relative grain yield in the field is the most relevant parameter to evaluate Zn efficiency because this represents the economic return to the farmer. Sixteen Finger millet [*Eleusine coracana (Gaertn.L)*] genotypes were grown under field condition till maturity with three replications of no external Zn supply (control), 20 Kg/ha (T_1) and 30 Kg/ha (T_2) Zn supply. Plants were maintained till maturity. Leaves and grains were sampled separately and zinc content was estimated.

(b) Soil/ pot screening

The 16 selected Finger millet genotypes were grown in earthen pots of 5 Kg soil capacities. These pots were filled with soil mixture (Soil: sand: FYM:: 50: 40:10). In this pot experiment, there were four replicates of four levels of zinc treatments, viz control (C; no external Zn supply), external zinc supply of 20 Kg/ha (T_1) and 30 Kg/ha (T_2) and30Kg/ha along with 1.5% foliar spray of ZnSo4 (T_3). In each pot three plants were maintained. Plants were watered daily to field capacity and supplied recommended fertilizers and FYM and plants were harvested after maturity. Zinc content in leaves was analyzed collecting leaf samples on 40 days after transplanting and also in grains after maturity was analyzed. Shoots were dried at 70°C and dry weights of plants were determined.

c) Solution screening

Similar experiment was carried out in Hydroponics to estimate zinc efficiency. Plants were grown in thermocole cups filled with sand. The sand was washed with concentrated Sulphuric acid (H_2SO_4) to remove all the traces of soluble salts, organic matter etc. present in the sand. Then it is washed thoroughly with tap water thrice to remove the traces of acid and allowed it for drying under shade. Around 10 holes were made in each cup for the easy penetration of roots. Then 3-4 seeds were sown in each cup and allowed them for germination. After a week the plants were thinned out to maintain one plant per cup. These plants were maintained on sand bed for another12 days for the better penetration of roots.



Plate No. 3: Experimental setup of pot experiment with different levels of Zinc treatment

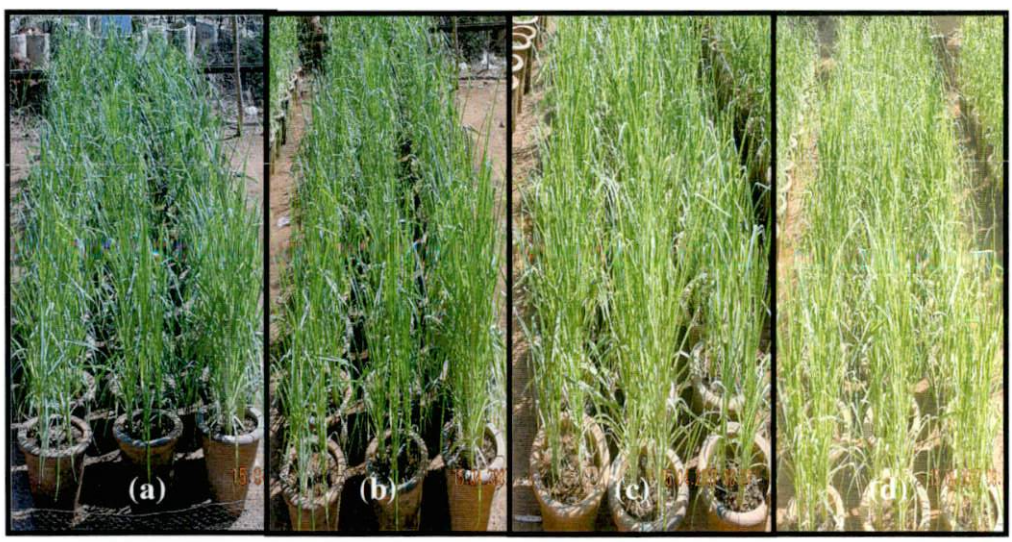


Plate No. 4: Over view of experimental setup

(a) Treatment-3 (T3) : 30 Kg/ ha Zn soil application + 1.5% foliar spray of $ZnSO_4$
(b) Treatment-2 (T2) : 30 Kg/ ha Zn soil application
(c) Treatment-1 (T1) : 20 Kg/ ha Zn soil application
(d) Control (C) : No external Zn application



Plate No. 5: Experimental setup for Hydroponics study

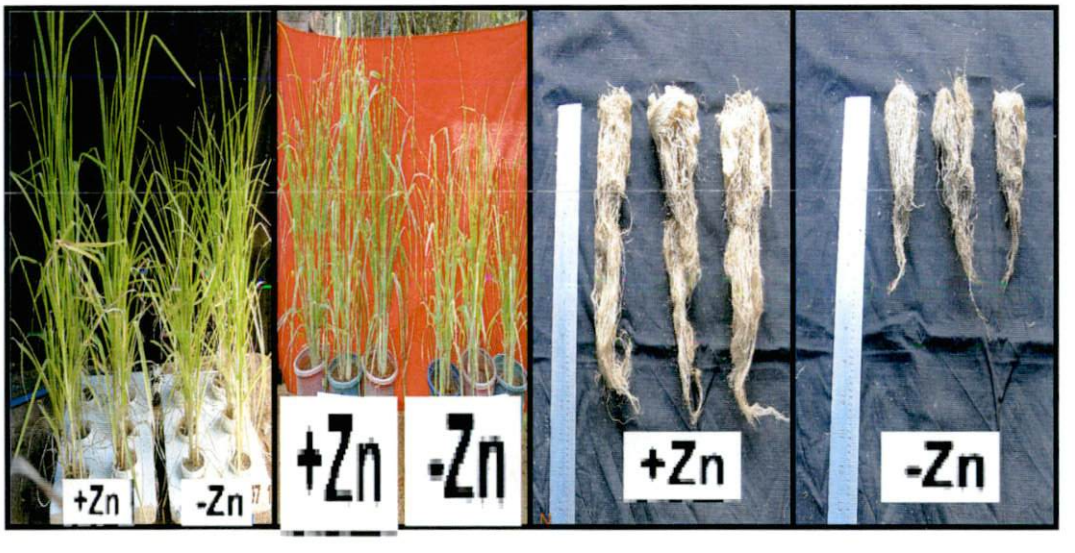


Plate No. 6: Comparison of plants with Zinc (+Zn) and without Zinc (-Zn) in (a) hydroponics and also in (b) pot experiment.
Variation in root length and root volume in (c) Treated (+Zn) and (d) Control plants (-Zn).

After 20 days, these cups were transferred to plastic trays containing half strength Hoagland's solution and maintained in trays till maturity. Meanwhile we have given 1.5% $ZnSO_4$ solution as a foliar spray as treatment for one set of plants and other set was maintained as control without giving any treatment. The objective of this experiment was to determine the extent of seed Zn remobilization and its relation to Zn efficiency in genotypes of known Zn efficiency. For this purpose, a solution culture supplied with all nutrients except for Zn was chosen which allowed us to study remobilization of seed Zn properly as there was no other source of Zn supplied for plant growth.

After maturity the shoot, root and ear head was harvested separately for control and treated plants and dry weight was measured. Although the primary purpose was to study seed Zn remobilization and zinc efficiency, we also recorded data for growth parameters like plant height, number of leaves, root volume, root length and biomass.

In all the above three experiments, plant Zn efficiency was calculated based on the relative shoot growth (Zn efficiency ratio) defined as the ratio of shoot dry weight under Zn deficiency (-DW) to the shoot dry weight under adequate Zn supply (+DW). Based on these data, genotypes were ranked for their tolerance to Zn deficiency as (i) efficient (ii) moderately efficient and (iii) inefficient. Zn efficiency ratio can be used as a reliable plant parameter for screening the Finger millet genotypes for their tolerance to Zn deficiency.

3.1.5 Estimation of plant Zn utilization efficiency

One of the main roles of Zinc in plants is derived from their presence in the active centers of many enzyme molecules. As a result, metalloenzyme activities of Zinc have been used as specific parameters for the appraisal of biologically active Zinc involved in plant metabolic processes. Utilization efficiency in terms of dry matter production per unit of zinc present in the dry matter may be linked to differences in the ability of a genotype to maintain an optimal activity of the important zinc-regulated enzymes, *viz.* copper zinc -super oxide dismutase (Cu/Zn SOD) and carbonic anhydrase (CA). There are also a large number of enzymes in which zinc is an integral component of the enzyme structure (zinc enzymes). Activity of these enzymes has been correlated with zinc availability to the plants. Differences in internal utilization or mobility of Zn have been shown to be involved in expression of Zn efficiency. Therefore plant zinc utilization efficiency is an important parameter, which gives the biologically active zinc in terms of zinc requiring enzymes.
We have determined plant zinc utilization efficiency by using the following formula:

$$\text{Zinc utilization efficiency (ZUE)} = \frac{\text{Amount of dry matter produced}}{\text{Unit amount of zinc present in dry matter}} \text{ X } 100$$

3.3 Zinc uptake studies

Radiotracer is a scientific tool for the determination of the nutrient translocation in plant system. It is possible to delineate beyond doubt, the crop uptake from fertilizer source and from native source employing ^{65}Zn labeled fertilizers. Knowledge on zinc utilization from variable sources will throw light on the ability of genotypes to thrive in soils of low external supply of zinc. Hence ^{65}Zn labeled compounds were employed to bring out the genotypic variations in zinc utilization from soil and fertilizer sources of 16 Finger millet genotypes.

To know the relationship of seed zinc with zinc uptake and translocation in contrasting genotypes, a study was conducted using radio labeled ^{65}Zn.

3.3.1 Raising the plant material.

The plant material was raised in small thermocole cups (10cm long and 5cm diameter). In order to facilitate easy entry of roots through cup, around 5-6 small holes were made at the bottom of the cups. These cups were filled with acid washed sand. They were placed on the sand bed to facilitate easy entry of roots into the sand, and their removal. Five to eight seeds were sown in each cup. When the seedlings were about 10 days old, thinning was done and one plant per cup was maintained. The seedlings were grown under this condition for a period of 21 days and roots were allowed to grow into the bed. Later they were taken out of the sand bed to free the emerged roots. These cups along with the hanging roots were transferred to plastic trays containing 3 liters of half strength Hoagland's solution. The cups were placed such that hanging roots were completely immersed into the solution. It was taken care that none of the roots are damaged. This facilitates absorption of nutrients from the half strength Hoagland's solution through hanging roots. Six such cups for each genotype were placed per tray and two replications were maintained. The seedlings were allowed to acclimatize for a period of 4 days in the new environment. . The set up was maintained in rainout shelter to prevent dilution of nutrient solution due to rain. After 7-8 days of adaptation, half strength Hoagland's solution was decanted and 2.5 liters of half strength Hoagland's solution containing ^{65}Zn was added to each tray.

3.3.2 Preparation of radiolabelled Zinc (^{65}Zn)

1.09 m Ci of ^{65}Zn in HCl solution having specific activity of 2.27 Ci was procured from BRIT (Board of Radiation and Isotope Technology), Mumbai. It was diluted to 100 ml by using distilled water at IIHR (Indian Institute of Horticultural Research), Bangalore. From this 100 ml of stock solution we have taken 11 ml and it was diluted to 22 liters of half strength Hoagland's solution, so that it will have specific activity of 5μCi/ml and 2.0 liters of diluted solution was added to each tray.

Harvesting: The plants were kept in radiolabelled (^{65}Zn) Hoagland's solution for 48 hours. At the end of 48 hours of treatment the plants were harvested. The roots were made free of

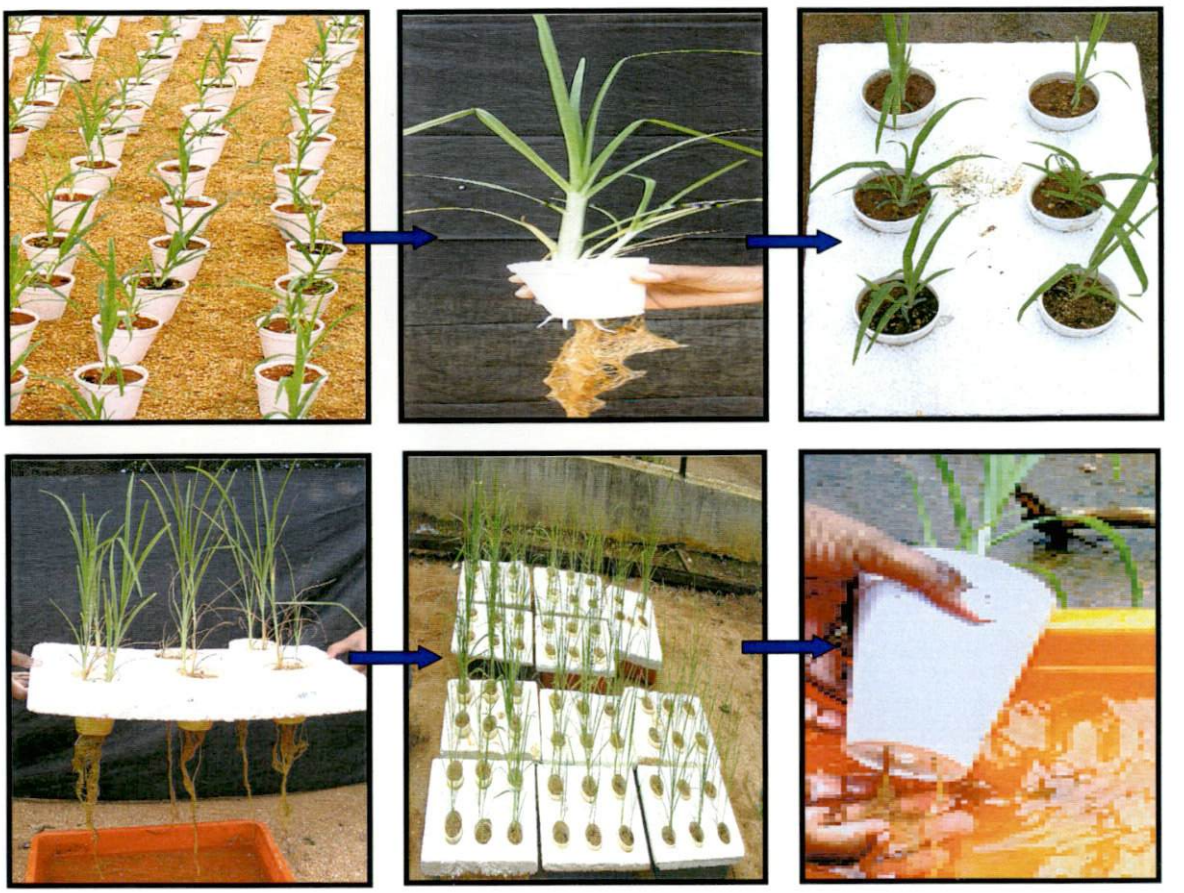


Plate No. 7: Experimental setup for ^{65}Zn uptake study

soil particles without causing any damage. The roots were separated from the shoots, washed in calcium sulphate solution and of ^{65}Zn activity in the fresh samples was measured using Liquid Scintillation Counter (LSC). The root and shoot fresh weights were also recorded.

Estimation of ^{65}Zn

The fresh root and shoot samples were ground to fine powder in a pestle and mortar using liquid nitrogen. A known quantity of sample was taken in a vial containing 4ml of scintillation cocktail (Bray's solution).

Activity of ^{65}Zn was determined using Wallac 1409 liquid scintillation counter (LSC) and counts per minute (cpm) were recorded.

Zn uptake was measured using the formula:

$$\text{Zn activity per weight} = \frac{(\text{Sample cpm} - \text{Blank cpm}) \times \text{Weight of the sample}}{\text{Total weight of the sample}}$$

One-way ANOVA was carried out to assess the significant difference among the high and low zinc types and cpm per organ was calculated, and Z distribution analysis was done.

In order to know the seed Zn content at different levels of Zn the following experiment was conducted. These were grown in earthern pots of 5 kg soil capacity. These pots were filled with soil mixture (Soil: Sand: FYM; 50:40:10). In this experiment three replications of the four levels of Zn treatment, *viz* naturally present zinc in red soil (15kg/ha) was maintained as control indicating low soil Zn status and external zinc supply of 20kg/ha, 30 kg/ha and 30 kg/ha +1.5%foliar spray of zinc sulphate. Three plants per pot were maintained and plants were grown till maturity. The seeds were collected and yield per plant was recorded.

Estimation of Seed and leaf Zn content

The seeds and leaf samples obtained in the above experiment were used for the seed Zn analysis. The seed and leaf Zn content was estimated in all 16 genotypes at 4 levels of Zn treatment.

Sample preparation

The samples were prepared and Zinc content was estimated as described in section 3.1.2 . Two way ANOVA was carried out assess the significant difference among the high and low Zn types for seed yield and seed Zn content, and Z distribution analysis was done.

Measurement of Gas exchange parameters

Photosynthetic rate and Water Use Efficiency (WUE)

There are lots of literatures to show that applying Zn increased water use and water use efficiency of crop plants (Khan *et al.*, 2003). Since zinc is the part of Carbonic Anhydrase enzyme, which accelerates photosynthetic rate in plants and is involved in preventing oxidative damage to cells by being a part of Cu/ Zn SOD enzyme. We presume that the photosynthetic rate and water use efficiency will greatly vary with external zinc application. The photosynthetic rate and water use efficiency (WUE) of 16 genotypes were measured using IRGA (Infra Red Gas Analyzer) also called as portable photosynthetic system (LICOR). We have measured WUE at single leaf level using gas exchange technique. At

the single leaf level, WUE is defined as the ratio of the moles of carbon dioxide fixed (A) to the moles of water transpired (A/T). At a given leaf to air water vapor pressure difference (VPD), the T is determined by the difference in stomatal conductance (g_s). Therefore the ratio of carbon assimilation (A) to g_s (A/g_s) is considered as a reflection of the intrinsic WUE.

The gas exchange parameters namely, assimilation rate (A) and stomatal conductance (g_s) are the two important determinants of the observed differences in WUE. These gas exchange parameters were determined using a portable photosynthetic system). Here, the top fully expanded leaves were clamped to the leaf chamber of the instrument and the observations were recorded when A, g_s, C_i and transpiration rate reached a steady state level. All gas exchange parameters were recorded between 9 AM and 11 AM on bright sunny days. Care was taken to maintain the ambient temperatures and VPD in the leaf chamber by suitably altering the flow of dry air.

Measurement of relative amount of chlorophyll using SPAD (Single Photoelectric Analyzing Diode)

Amongst several leaf characters, leaf thickness (associated with structural and non-structural components) and chlorophyll content determines the leaf transmittance characters. A chlorophyll meter developed by Minolta (SPAD – 502) gives indications of the relative amount of chlorophyll pigments in leaves. Based on this principle, SPAD reading could still be a reflection of leaf thickness or the composition of leaf structural and non-structural constituents other than pigments. We have measured and correlated chlorophyll content of high zinc genotypes and low zinc genotypes. We have estimated chlorophyll content and expressed in terms of SCMR (SPAD Chlorophyll Meter Reading).

Measurement of Specific Leaf Area (SLA)

Specific leaf area (SLA, leaf area per unit dry mass) and leaf dry matter content (LDMC, the ratio of leaf dry mass to fresh mass) are important traits in plant ecology because they are associated with many critical aspects of plant growth and survival (Graham *et. al.1993.* These two traits play an important role in potential relative growth



Plate No. 8: Measurement of Gas exchange parameters using LICOR



Plate No. 9: Measurement of relative amount of chlorophyll using SPAD (Single Photoelectric Analyzing Diode) 502 chlorophyll meter

rate (RGR) and ecological behavior in plants. Since, both SLA and LDMC are involved in the trade-off between rapid biomass production (high SLA, low LDMC species) and efficient conservation of nutrients (low SLA, high LDMC species). Because SLA and LDMC are indicator traits of resource-use strategies, it is important to evaluate these traits for Finger millet genotypes.

The leaf on which SPAD observations were recorded was used for determination of SLA. First we have taken SPAD meter reading of three leaves from each plant, these same three leaves from each plant were collected separately and leaf area was measured and kept them for drying. The dry weights of these leaves were taken and measured SLA using the formula:

$$SLA = \frac{\text{Leaf area } (cm^2)}{\text{Dry weight of the leaf (g)}}$$

This is expressed in terms of leaf area per unit dry mass ($cm^2\ g^{-1}$ or $m^2\ Kg^{-1}$)

Measurement of Specific Leaf Weight (SLW)

Specific leaf weight (SLW, leaf weight per unit area). Specific leaf weight appears to be a good index of previous light environment and possibly of Pn (net photosynthesis) potential (Barden JA, 1977) Significant linear relationships between SLW and Pn have been reported for alfalfa], soybean, and apple. Barden suggested that SLW may be used to estimate the Pn potential of leaves from different zones of a tree. Measuring SLW is less time consuming and requires less expensive equipment than measuring Pn. It is the reciprocal of specific leaf area (SLA).

$$SLW = \frac{\text{Dry weight of the leaf (g)}}{\text{Leaf area } (cm^2)}$$

This is expressed in terms of dry mass per unit leaf area ($g\ cm^{-2}$ or $Kg\ m^{-2}$)

3.2 Measurement of SOD activity

The activity of SOD was assayed by measuring its ability to inhibit the photochemical reduction of NBT (Nitro Blue Tetrazolium) according to the method of Fridovich (1986).

Principle:

Nitro blue tetrazolium (NBT) dye under oxidized state will be in yellow colour and its colour changes to blue when it is reduced. NBT (yellow) in presence of methionine and riboflavin and EDTA raduced to a formazone, a blue coloured complex that is measured at absorbance 570 nM. Extent of inhibition of NBT reduction depends on the activity of SOD.

Protocol for SOD assay

One gram of leaf tissue was homogenized to fine paste in 10 ml chilled extraction buffer containing 50 mM KPO_4 buffer (pH 7.8) containing 0.1 mM Na_2 EDTA

↓

The homogenate was centrifuged at 15,000 g for 15 minutes at 4^0 c

↓

Supernatant was collected and aliquots were drawn into fresh autoclaved eppendorfs

↓

The aliquots were drawn for

Estimation of total protein

Estimation of SOD enzyme

Quantification of total soluble proteins by simple dye binding technique

Quantification of total soluble proteins from the leaf extracts before the enzyme assaying were done by Coomassie Brilliant Blue (CBB) dye binding method using Bovine Serum Albumin (BSA) as a standard (Bradford, 1976).

The method is based on the principle that the Coomassie Brillant Blue (CBB) G-250 binds to proteins and gives a consistent blue chromophore. The protein-dye complex has a high extinction co-efficient thus leading to a great sensitivity in measurement of protein. The binding of dye to the protein is a very rapid process (approx 3 min) and the method is devoid of interference by other soluble components.

Preparation of Bradford reagent

The Bradford reagent was prepared by dissolving CBB G-250 (25 mg) in 12.5 ml of 95 per cent methanol and then adding 25 ml of Orthophosphoric Acid. The volume of the resulting solution was made up to 250 ml with distilled water and the freshly prepared reagents were used every time. It should be stored in brown bottle or bottle should be covered with aluminium foil to avoid contact with light.

Composition of assay buffer:

- ✓ 50 mM potassium phosphate buffer (pH 7.8)
- ✓ 13 mM methionine
- ✓ 75μM NBT
- ✓ 2μM riboflavin
- ✓ 0.1mM EDTA

Composition of reaction mixture:

3ml assay buffer with 0,25 and 50 μl of crude enzyme extract in a separate autoclaved glass test tubes. One set of the reaction mix was exposed to white fluorescent light, subsequently tubes were covered with a black cloth and the absorbance was read at 560nM. The other set of reaction mix was chosen as blank.

The absorbance values were transformed to logarithmic (Log_{10}) values and were plotted as a function of the volume of the enzyme extract used in the reaction mixture (Giannopolitis

and Ries, 1977). From the resulting graph, the volume of the enzyme extract corresponding to 50% inhibition of the reaction was read and was considered as one enzyme unit (Beauchamp and Fridovich, 1971). The SOD activity was expressed as unit of SOD activity mg^{-1} protein min^{-1} as well as activity per gram fresh weight.

One unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of NBT reduction measured at 560 nm.

Measurement of Cu/ Zn SOD activity

Activities of Cu/Zn SOD were calculated by substracting SOD activity in the presence of 3mM Dinitro Phenol (DNP) from total SOD, since DNP inhibits Cu/Zn SOD.

Extraction and assay of Carbonic Anhydrase (CA)

Plant material

Young leaf tissue was taken from 16 Finger millet genotypes growing in pots in the garden soil were used. These plants were grown with recommended FYM and fertilizers and irrigation. We have given four levels of Zinc treatment for plants used for estimation of carbonic anhydrase i.e., (i) control (no external zinc application), (ii) soil application of 20kg/ha Zn (iii) soil application of 30kg/ha Zn and (iv) soil application of 30kg/ha Zn +1.5% foliar spray of $ZnSO_4$.

Extraction of Carbonic anhydrase

Protocol

2g of leaf tissue were homogenized in a pestle and mortar with 2 ml of extraction buffer (0.01M Tris-HCl, pH 8.3, containing 1mM disodium- EDTA and 0.1 M 2-mercaptoethanol) per g of tissue. The extract was filtered through two layers of nylon mesh and the carbonic anhydrase activity of 1 to 50µl was measured in the colorimetric assay of Wilbur and Anderson (1948) as used by Rickli *et al.*, (1964). Activity was expressed in enzyme units on fresh weight of leaf tissue basis and also on units per mg protein basis.

Assay

Method: The electrometric method of Wilbur and Anderson (1948) in which the time required (in seconds) for a saturated CO_2 solution to lower the pH of 0.01 M TrisıHCl buffer from 8.3 to 6.3 at 0°C is determined. The time without enzyme is recorded at T_0; with enzyme, T.

Reagents

- ✓ 0.01 M TrisıHCl buffer, pH 8.0. Store in an ice bath at 0-4°C before and during use.
- ✓ Carbon dioxide saturated water. Bubble CO_2 gas through 200 ml ice cold water for 30 minutes prior to assay. During saturation process, store water at 0-4°C in an ice bath.

Procedure

Blank Determination: Add 6.0 ml of chilled 0.02 M Tris-HCl buffer, pH 8.0 to a 15-20 ml beaker. Maintain temperature at 0-4°C and record pH.

Withdraw in a 5 ml syringe, 4 ml of chilled CO_2 saturated water and add to Tris buffer. Immediately start a stopwatch and record the time required for the pH to drop from 8.3 to 6.3. Record this time as T_0.

Enzyme Determination: Add 6.0 ml of chilled 0.02 M TrisıHCl buffer, pH 8.0 to a 20 ml beaker. Maintain temperature at 0-4°C and record pH. Add 0.1 ml of freshly diluted enzyme. Quickly add 4 ml of CO_2 saturated water and record the time required for the pH to drop from 8.3 to 6.3. Record this time as T.

Calculation:

Units of enzyme activity per mg fresh weight of leaf tissue was measured by using the formula:

$$\text{Units/mg} = \frac{2 \times (T_0 - T)}{T \times \text{mg of enzyme extract in reaction mixture}}$$

Total soluble protein was quantified before the enzyme assaying by Coomassie Brilliant Blue (CBB) dye binding method using Bovine Serum Albumin (BSA) as a standard (Bradford, 1976). The carbonic anhydrase activity was expressed as enzyme unit mg^{-1} protein as well as unit per gram fresh weight basis.


Results

IV. RESULTS

Zinc (Zn) is essential for plant growth and function, and Zn deficiency is one of the most important abiotic stresses limiting crop growth, productivity and quality especially in cereals worldwide. Since most of the global population depends on cereals for their sustenance, zinc deficiency in their diets results in altered physical, cognitive growth and development leads to egregious societal costs including malnutrition in infants, learning disabilities in children, reduced worker productivity and ultimately alters nations economy. Enhancing the zinc content in edible parts of our staple food crops will be the only stable solution to overcome these problems. Unfortunately, application of zinc fertilizers alone will not increase the zinc content in plants due to subsoil constraints and also this is not cost effective and it is unstable. Plant species in general and cereals in particular greatly differ in their ability to acquire Zn from soils or to utilize Zn internally. It has been reported that there is considerable species and varietal variation in cereals in terms of zinc uptake, translocation and accumulation in grains.

In view of such genetic variation selection of genotypes with higher efficiency in Zn acquisition from soils or Zn utilization in plants is a promising approach to enhance the level of seed zinc content. In addition to enhance the yield and quality seeds containing high Zn will have added advantage of high seedling vigor when sown on soils that are poor in zinc and also they are tolerant to pest and disease attack. It may also have important spin-off effects for increasing farm productivity in developing countries in an environmentally beneficial way

Even though the molecular basis of zinc uptake and translocation in plants is not completely elucidated, it is important to understand the physiological and biochemical reasons for differential uptake, translocation and partitioning of zinc to edible parts in cereal crops in both high and low zinc-accumulating types.

Cereal species greatly differ in their zinc efficiency as it 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. Enhancement of Zinc efficiency is an

important approach to enhance the grain productivity and grain Zn content. It is evident from the reports that, there exists a causal relationship between important Zn-containing enzymes, *viz* carbonic anhydrase (CA), copper-zinc superoxide dismutase (Cu/Zn SOD) activities and ZE in many cereal species. Enhanced production and release of these Zn requiring enzymes might be the solution to overcome the problem of zinc deficiency. Increase the activity of these enzymes in plant system will also have an added advantage of increase the photosynthetic rate and protection against oxidative damage to cells.

With this preface the major focus of the study was to probing the physiological and biochemical reasons for differential uptake and translocation of Zinc within and across different Finger millet germplasm lines and also to assess the activity of Zn requiring enzymes under different Zn status in both high and low types.

4.1 Reconfirmation of Zinc status in grains and selection of contrast (high and low) Finger millet genotypes.

As seed Zn content affects many physiological and agronomic parameters it is important to asses the seed Zn content of selected genotypes. To assess the seed zinc content and reconfirm the Zn status in grains of 16 contrast Finger millet genotypes consisting of core germplasm, local adapted cultivars and released varieties were procured from All India Co-ordinated Research Project (AICRP) on millets, UAS, Bangalore. These selected 16 Finger millet genotypes were classified into 7 low seed Zn genotypes and 9 high seed Zn genotypes based on the previous work. These selected genotypes show significant variation in seed Zn content ranging from as low as 0.98 mg/100g in case of GEC- 265 to as high as 10.05mg/100g in case of PES-400. The Zn content of selected low Zn types varies from 0.98 –3.62 mg/100g and of high Zn types varies from 5.10-10.05mg/100g (Table 1). The mean seed Zn content of low Zn types is 1.93 and of high Zn types it is 7.25 mg/ 100g respectively (Figure 1).

These selected lines (7 low and 9 high) were sown under field conditions with naturally available Zn in red soil (15Kg/ ha) to get the seed material of uniform Zn content. These plants were maintained in the field till the maturity with all recommended package of practices with no external Zn application. Meanwhile leaf samples were collected and after

Table1: Finger millet genotypes selected for experiment with accession numbers and seed zinc content as analyzed by Atomic Absorption Spectrophotometer (AAS)

Low Zn seed types		
Sl. No.	Accession No.	Zn (mg/100g)
1	GEC-236	1.63
2	GEC-265	0.98
3	GEC-403	1.63
4	GEC-520	2.06
5	GEC-488	3.62
6	GEC-460	1.43
7	Indaf-5	2.22
	Range	**0.98-3.62**
	Mean	**1.93**
	SD	**0.84**
High seed Zn types		
Sl. No.	Accession No.	Zn (mg/100g)
8	MR-6	8.19
9	PES-400	10.05
10	GEC-45	5.10
11	GEC-108	8.60
12	GEC-94	7.47
13	GEC-271	5.46
14	GEC-21	5.90
15	GPU-45	6.88
16	GPU-28	7.6
	Range	**5.10-10.05**
	Mean	**7.25**
	SD	**1.60**

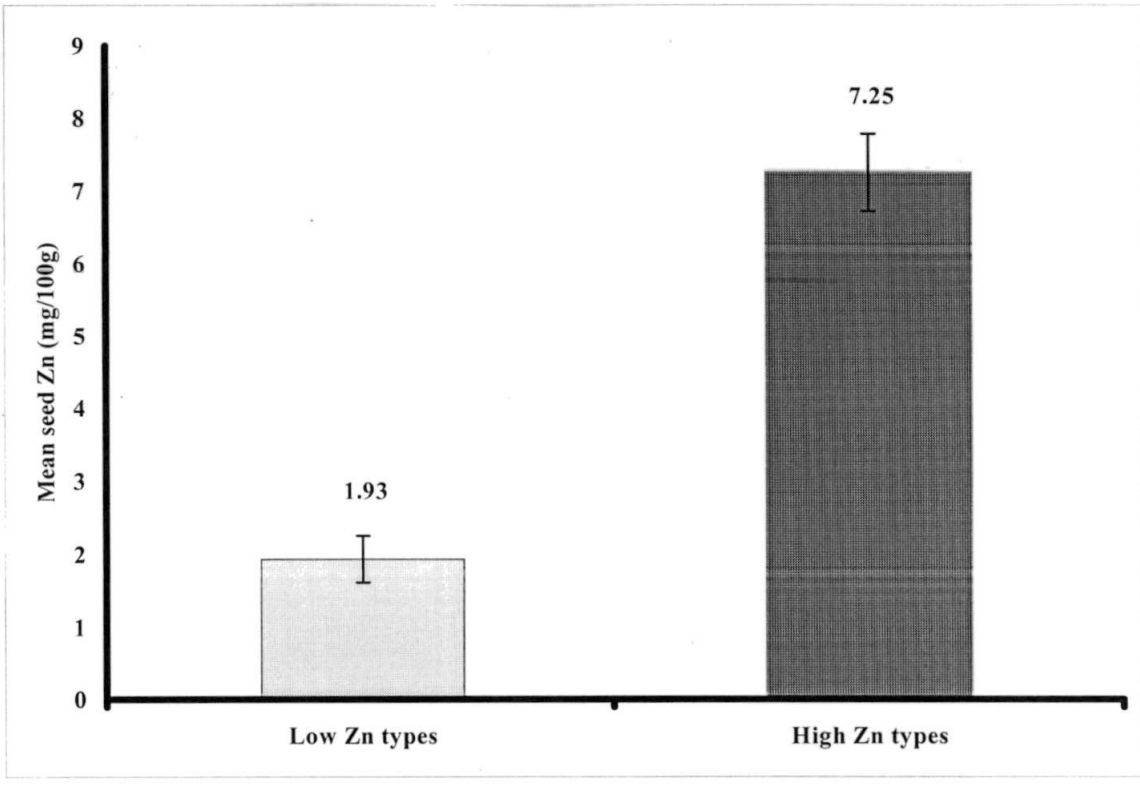


Figure1: Mean Zn content of selected low and high Zn types

maturity grains were harvested and analyzed for Zn content using AAS (Atomic Absorption Spectrometer). These seed material was used for further studies.

To further study the variation in seed and leaf Zn content under different soil zinc status and to study the differential pattern of uptake and translocation with external soil application of Zn in the form of Zn fertilizer, the selected high (9) and low (7) zinc genotypes were sown in the field following RCBD (Randomized Complete Block Design). These were provided with all recommended package of practices including fertilizers and irrigation with three different soil zinc status *viz* naturally present zinc in red soil (15 Kg/ha) was maintained as control (C), external zinc supply of 20kg ha^{-1} as treatment-1 (T1) and external zinc supply of 30kg ha^{-1} as treatment-2 (T2). The same genotypes were sown in earthen pots with four levels of external Zn application *viz* naturally present Zn in red soil was maintained as control (C), external Zn supply of 20kg ha^{-1} as treatment-1 (T1), external zinc supply of 30kg ha^{-1} as treatment-2 (T2) and external zinc supply of 30kg ha^{-1} soil application + 1.5% foliar spray of $ZnSO_4$ as treatment-3 (T3). These were also maintained with all recommended package of practices (Plate No.1,2,3 & 4).

These genotypes were analyzed for various physiological parameters such as chlorophyll content, biomass, photosynthetic rate, stomatal conductance, transpiration rate and water use efficiency under these different levels of external Zn application.

The genotypes were analyzed for relative amount of chlorophyll by using SPAD meter and this was recorded as SCMR (SPAD Chlorophyll Meter Reading) (Plate No.9). There is a significant variation in chlorophyll content among different genotypes and also between treatments. In case of field experiment, the SCMR readings ranging from 30.1 incase of GEC-236 under control to 51.83 under T1 in case of Indaf-5. Even though the mean chlorophyll content is more (44.97 under control, 42.38 under T2) in case of high seed Zn types, the total chlorophyll content increases linearly under both field and pot condition as the external Zn application increases in case of low seed Zn types. In pot experiment the mean chlorophyll content of low Zn types under control condition is 32.07; under T1 it is 36 and 38.28 in case of T2. In the field experiment the mean chlorophyll content of low Zn types under control condition is 39.99; under T1 it is 41.93 and 41.37 in case of T2. In the

pot experiment, in case of low Zn types, the mean chlorophyll content of low Zn types is 31.92 under control, 31.26 under T2 and 34.65, 35.23 under T2 and T3 respectively. But this increasing trend has not observed for high Zn types (i.e., 44.97 under control, 40.72 under T1 and 42.38 under T2) (Table 2).

There exists a significant variation with regard to photosynthetic rate in both low and high Zn types under both pot and field experiments. As increase in external Zn application there is linear increase in mean photosynthetic rate in case of both low and high Zn types but at individual genotypes level the response is more in case of low Zn types. The mean photosynthetic rate of pot experiment in low Zn types under control is 31.87μmol/m^2/s, under T1 it is 32.80 μmol/m^2/s, 29.67 μmol/m^2/s under T2 and 32.22μmol/m^2/s under T3. In case of high Zn types under pot experiment show similar trend of increase in photosynthetic rate with external Zn application. It is 28.91, 30.62, 30.81 and 30.23 μmol/m^2/s under control, T1, T2 and T2 respectively.

Under field condition high Zn types show high mean photosynthetic rate (41.63μmol m^{-2} s^{-1}) even under control condition compared to mean of low Zn types (34.47 μmol m^{-2} s^{-1}) under control. Some low Zn genotypes like Indaf-5, GEC-460, GEC-403, and GEC-265 show higher photosynthetic rate under high external Zn application. The genotypes GEC-403, GEC-265 show highest photosynthetic rate of 70.37μmol m^{-2} s^{-1} and 60μmol m^{-2} s^{-1} under 30kg Zn ha^{-1} (T2) and 20kg ha^{-1} (T3) respectively, which is higher than mean photosynthetic rate of high Zn types (Table 3).

There is no significant variation in case of stomatal conductance under pot experiment in both high and low seed Zn types. Both high and low Zn types show average stomatal conductance of ~ 0.2 mmol m^{-2} s^{-1} under all Zn treatments, but there exists a variation between pot and field experiments. The range of mean stomatal conductance under pot experiment under all treatments varied from 0.15-0.2 mmol m^{-2} s^{-1} but under field condition it is 0.59-0.89 mmol m^{-2} s^{-1}, which indicates the environmental factor and other growth factors, which affects stomatal conductance (Table 4 and Figure 2 & 3).

Table 2: SPAD Chlorophyll meter readings of Finger millet genotypes at different levels of Zinc treatment

Low Zn types								
		Pot experiment				Field experiment		
Sl. No	Genotypes	C	T1	T2	T3	C	T1	T2
1	GEC-236	31.63	39.46	34.06	31.86	30.13	37.73	41.73
2	GEC-265	30.43	34.03	39.26	38.9	44.3	39.4	42.46
3	GEC-403	32.7	32.6	38.53	37.16	41.43	38.73	44.4
4	GEC-520	37.2	35.53	38.26	35.9	40.9	47.26	42.76
5	GEC-488	23.8	35.167	41.26	36.63	37.13	43.86	34.53
6	GEC-460	33.6	37.93	32.03	33.7	40.53	34.73	37.96
7	Indaf-5	35.16	37.3	44.56	38.1	45.56	51.83	45.8
	Range	23.8-37.2	32.6-39.46	32.03-44.56	31.86-38.9	30.13-45.56	37.73-51.83	34.53-45.8
	Mean	32.07	36.00	38.28	36.03	39.99	41.93	41.37
High Zn types								
		Pot experiment				Field experiment		
Sl. No	Genotypes	C	T1	T2	T3	C	T1	T2
8	MR-6	34.9	34.93	38.53	37.63	45.5	44.33	45.3
9	PES-400	30.2	30.8	38.6	37.23	40.56	43.93	39.4
10	GEC-45	29.63	35.73	27.33	37.13	39.1	41.56	37.33
11	GEC-108	29.46	24.76	31.76	36.13	46.8	37.23	37.43
12	GEC-94	27.46	28.96	32.03	34.06	46.76	35.06	43.26
13	GEC-271	36.36	29.33	39.16	29.13	42.93	44.56	46.36
14	GEC-21	32.5	33	34.9	36.5	50.93	38.63	46.36
15	GPU-45	33.26	32.73	36.13	36.36	45.4	41.46	46.2
16	GPU-28	33.53	31.13	33.46	32.96	46.8	39.76	39.83
	Range	29.46-34.9	24.7-35.7	27.33-39.16	29.13-37.63	39.1-50.93	37.23-44.56	37.33-46.36
	Mean	31.92	31.26	34.65	35.23	44.97	40.72	42.38
	CD @0.05	2.50				2.46		

Even though there is no much variation between low and high Zn types in terms of mean water use efficiency (WUE), the low Zn types show linear increase in WUE as increase in external Zn application. Under pot experiment, under control it is 150.71 g/ Kg and it is 141.29 g/ Kg under T1, 157.2g/Kg under T2 and 161.3 g/Kg under T3. In case of high Zn types, there is no much increase in WUE, as increase in external application. Under high Zn types it is 150.48g/Kg under control, 144.69, 148.2 and 146.1 g/ Kg under T1, T2 and T3 respectively. At individual genotype level, some genotypes show significant variation in WUE between treatments in both low and high Zn types. Under low types, the genotypes GEC-403, GEC-520 shows linear increase in WUE as external Zn application increases. Under high Zn types also under pot experiment, some genotypes such as MR-6, PES-400, GEC-45 and GEC-108 show positive trend of increase in WUE as increase in external application of Zn. But under field condition we have not obtained such trend because of unknown reasons. (Table 5).

Both high and low Zn types show significant variation with respect to biomass production. The high Zn types normally produce high biomass simply because of high photosynthetic rate. The low types shows significant increase in biomass as the soil Zn content increases and the biomass also increases in case of high Zn types but not as much as low Zn types. Under field experiment, in case of low Zn types the mean biomass increases linearly as the external Zn application increases. It increases from 271.5 g/plant under control, 376.78 g plant^{-1} under T1 and 474.52 g plant^{-1} under T2. The low Zn types like, GEC-460 and Indaf-5 show highest biomass of 635.98 g plant^{-1} and 546.18 g plant^{-1} respectively under high soil Zn status. The high Zn types also show the similar trend of increase in mean biomass, as there is increase in soil Zn status but not as much as low Zn types. The mean biomass of high types ranges from 291.84 g plant^{-1} under control to 471.7 g plant^{-1} under T2. Some genotypes in both high and low types show two-fold increase in biomass, as there is increase in soil Zn content/ external application of Zn (Table 6).

To study the effect of Zn alone on growth and yield parameters, both high and low Zn types were raised in hydroponics/ Hoagland's solution. One set of plants was maintained as control and to other set 1.5% Zn was applied as foliar spray and maintained till maturity. Meanwhile the observations on various growth parameters were recorded. There is significant variation between high and low Zn types and also within treatments with regards

Table 3: Photosynthetic rate and stomatal conductance of Finger millet genotypes at different levels of Zinc treatment in pot experiment

Low Zn types									
		Photosynthetic rate ($\mu mol/m^2/s$)				Stomatal conductance ($mmol/m^2/s$)			
Sl. No	Genotypes	Control	T1	T2	T3	Control	T1	T2	T3
1	GEC-236	36.92	34.63	35.8	34.9	0.25	0.2	0.2	0.1
2	GEC-265	27.2	34.7	23.6	18.3	0.15	0.2	0.1	0.1
3	GEC-403	24.83	33.8	22.3	37.5	0.18	0.19	0.1	0.3
4	GEC-520	35.26	28.6	27.0	34.3	0.21	0.17	0.18	0.2
5	GEC-488	28.73	28.8	21.8	22.4	0.19	0.21	0.1	0.1
6	GEC-460	32.16	31.7	32.7	43.9	0.22	0.26	0.18	0.2
7	Indaf-5	38.03	37.4	44.5	34.3	0.26	0.39	0.34	0.2
	Range	24.83-38.03	28.6-37.4	21.8-44.5	18.3-43.9	0.15-0.26	0.17-0.39	0.1-0.34	0.1-0.3
	Mean	31.87	32.80	29.67	32.22	0.20	0.23	0.17	0.17
	S.D	5.10	3.26	8.43	8.85	0.03	0.07	0.08	0.07
High Zn types									
		Photosynthetic rate ($\mu mol/m^2/s$)				Stomatal conductance ($mmol/m^2/s$)			
Sl. No	Genotypes	Control	T1	T2	T3	Control	T1	T2	T3
8	MR-6	25.96	25.7	29.5	23.0	0.21	0.20	0.21	0.1
9	PES-400	40.13	39.6	33.4	31.7	0.33	0.29	0.24	0.1
10	GEC-45	27.56	27.7	29.8	30.5	0.22	0.22	0.20	0.2
11	GEC-108	20.16	26.7	27.3	31.9	0.11	0.24	0.16	0.2
12	GEC-94	26.03	28.7	17.9	18.4	0.15	0.15	0.09	0.1
13	GEC-271	19.46	39.4	40.1	31.7	0.10	0.30	0.27	0.1
14	GEC-21	41.4	37.2	33.6	39.0	0.29	0.27	0.22	0.2
15	GPU-45	41.13	30.7	21.4	44.7	0.28	0.22	0.19	0.3
16	GPU-28	18.36	19.9	44.3	21.2	0.10	0.09	0.30	0.1
	Range	18.36-41.4	19.9-39.6	17.9-44.3	18.4-44.7	0.10-0.33	0.09-0.3	0.09-0.30	0.1-0.3
	Mean	28.91	30.62	30.81	30.23	0.19	0.22	0.20	0.15
	CD @ 0.05	1.17				0.23			

Table 4: Photosynthetic rate (µmol m^{-2} s^{-1}) and stomatal conductance (mmol m^{-2} s^{-1}) of Finger millet genotypes at different levels of Zinc treatment in Field experiment

Low Zinc genotypes							
		Photosynthetic rate (µmol/m^2/s)			Stomatal conductance (mmol/m^2/s)		
	Genotypes	C	T1	T2	C	T1	T2
1	GEC-236	51	52.9	43	0.7	1.04	0.74
2	GEC-265	20.8	60	30.33	0.24	1.1	0.5
3	GEC-403	32.6	33.8	70.37	0.47	1.26	0.6
4	GEC-520	44.7	49.2	33.27	0.32	0.57	0.55
5	GEC-488	10.5	46.3	48.9	0.51	1.14	0.77
6	GEC-460	44.8	37.5	45.17	0.91	0.51	0.83
7	Indaf-5	36.9	37.7	14.4	1.01	0.66	0.31
	Range	10.5-51	33.8-60	14.4-70.3	0.24-1.01	0.51-1.26	0.31-0.83
	Mean	34.47	45.34	40.7	0.59	0.89	0.61
High Zinc genotypes							
		Photosynthetic rate (µmol/m^2/s)			Stomatal conductance (mmol/m^2/s)		
	Genotypes	C	T1	T2	C	T1	T2
8	MR-6	42	37.9	24.33	0.63	0.67	0.46
9	PES-400	9	37.7	49.8	0.08	0.61	0.93
10	GEC-45	53	40.9	29	0.6	0.61	0.6
11	GEC-108	34.4	37.8	42.73	0.38	0.56	0.75
12	GEC-94	43.1	42.4	40.37	0.62	0.46	0.76
13	GEC-271	37.7	30.9	60.03	0.8	0.62	0.89
14	GEC-21	53	53.3	27.67	0.83	0.64	0.63
15	GPU-45	48.8	44.3	51.47	0.86	0.78	0.97
16	GPU-28	53.7	44.5	59.8	0.73	0.62	0.76
	Range	9-53.7	30.9-53	24.3-60.0	0.38-0.86	0.46-0.78	0.46-0.97
	Mean	41.63	41.07	42.8	0.61	0.61	0.75
	CD @ 0.05	1.3			0.30		

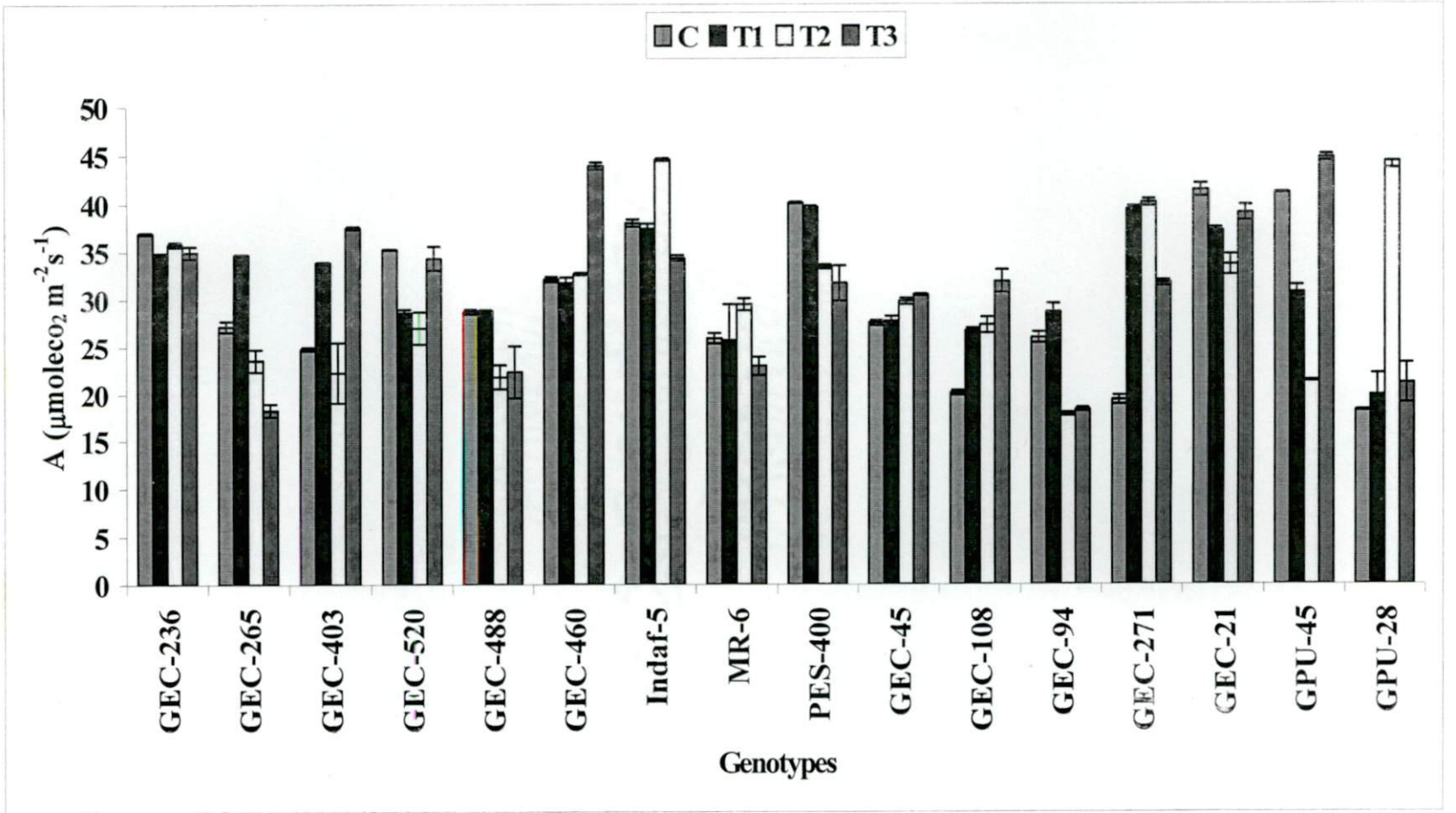


Figure 2: photosynthetic rate of Finger millet genotypes at different levels of external Zn application

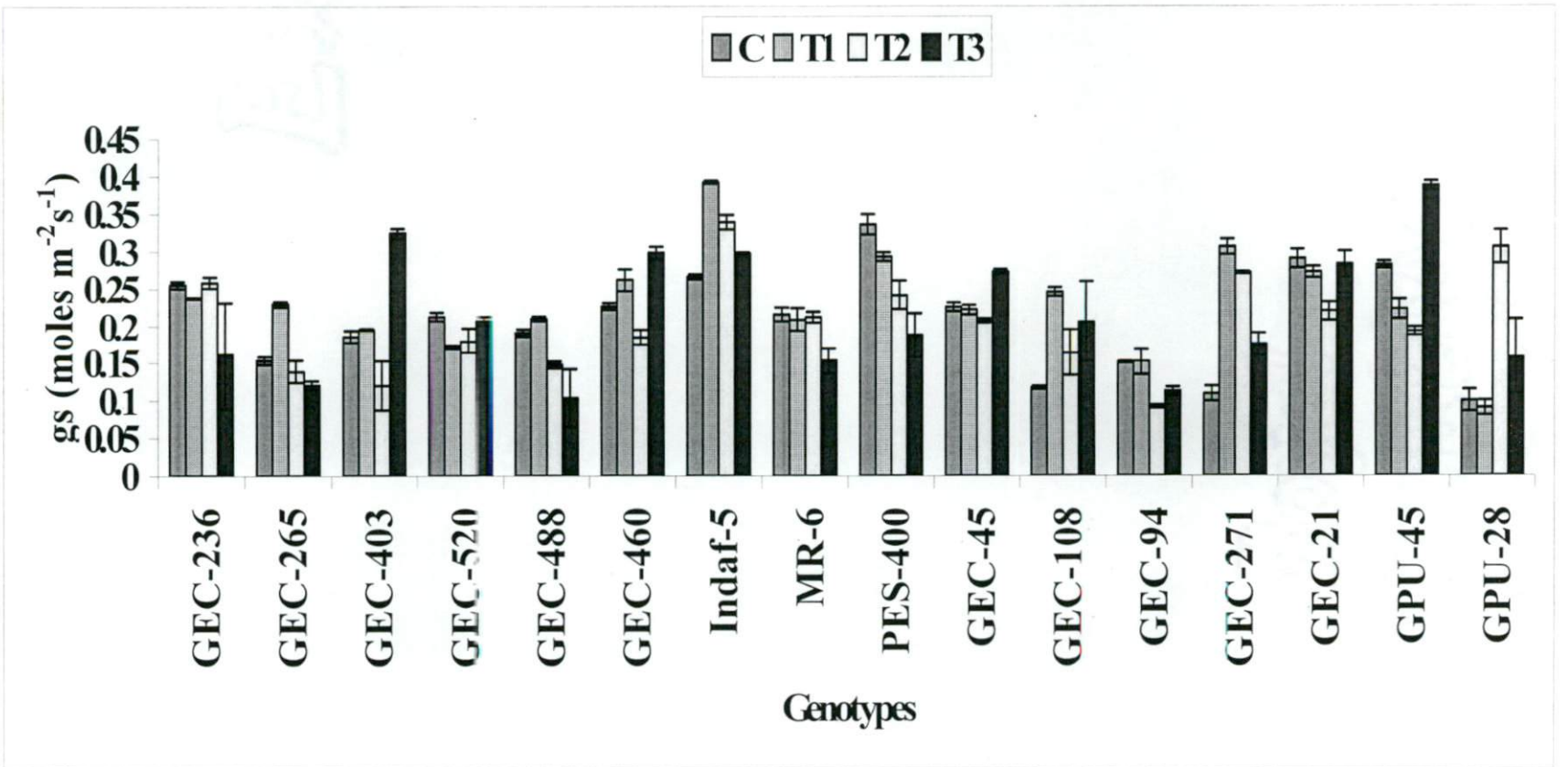


Figure 3: stomatal conductance of Finger millet genotypes at different levels of external Zn application

Table 5: Water use efficiency (WUE) at single leaf level of Finger millet genotypes at different levels of Zinc treatment

Low Zn types								
		Pot experiment				Field experiment		
Sl.No	Genotypes	C	T1	T2	T3	C	T1	T2
1	GEC-236	144.21	145.51	138.2	217.6	72.92	50.80	58.47
2	GEC-265	178.16	150.86	170.4	152.2	86.80	54.35	60.82
3	GEC-403	133.03	173.08	186.1	115.4	69.72	26.78	116.6
4	GEC-520	165.31	166.47	149.9	165.5	141.26	86.81	60.55
5	GEC-488	150.17	136.86	148.7	216.3	20.65	40.73	63.45
6	GEC-460	141.70	120.93	176.3	146.8	49.37	72.89	54.70
7	Indaf-5	142.44	110.33	131.0	115.3	36.42	57.20	46.75
	Range	133.03-178.16	110.33-173.08	131-186.1	115.3-217.6	36.42-141.26	26.78-72.89	46.75-116.6
	Mean	**150.71**	**143.43**	**157.2**	**161.3**	**68.16**	**55.65**	**65.90**
	S.D.	**15.65**	**26.77**	**20.60**	**42.28**	**39.44**	**19.79**	**23.01**
High Zn types								
		Pot experiment				Field experiment		
Sl.No	Genotypes	C	T1	T2	T3	C	T1	T2
8	MR-6	120.03	123.08	138.8	150.2	66.43	56.62	52.51
9	PES-400	119.20	135.07	138.1	168.7	112.07	61.37	53.43
10	GEC-45	121.79	124.70	142.9	111.4	88.43	66.70	48.68
11	GEC-108	171.87	108.51	165.9	154.7	89.59	67.39	56.95
12	GEC-94	172.40	188.81	194.3	163.9	69.49	91.97	53.23
13	GEC-271	177.99	128.44	147.1	180.1	47.32	49.86	67.73
14	GEC-21	142.10	136.21	152.3	137.5	63.52	83.63	44.03
15	GPU-45	145.34	137.55	110.8	115.2	56.95	56.86	52.89
16	GPU-28	183.60	219.85	144.4	133.3	73.14	71.22	79.13
	Range	120.03-183.6	108.51-219.85	138.1-194.3	111.4-180.1	47.32-112.07	47.32-91.97	48.68-79.13
	Mean	**150.48**	**144.69**	**148.2**	**146.1**	**74.10**	**67.29**	**56.50**
	S.D.	**26.49**	**35.79**	**22.60**	**23.64**	**19.65**	**13.47**	**10.63**
	CD @ 0.05	5.03				5.06		

Table 6: Variation in biomass of low and high Zn genotypes as influenced by different levels of external Zn supply

Low Zinc genotypes										
Total biomass (g/ 3plants)										
		Pot experiment					**Field experiment**			
	Genotypes	**C**	**T1**	**T2**	**T3**		**C**	**T1**	**T2**	
1	**GEC-236**	215.4	307	136.1	326.2	NS	155.7	282.6	452.4	NS
2	**GEC-265**	272.6	363.4	121.9	410.4	NS	135.2	211.4	278.0	NS
3	**GEC-403**	237.8	387.5	135.9	349.0	NS	187.7	230.9	262.7	NS
4	**GEC-520**	423.9	388.9	115.8	395.9	**	491.6	601.0	719.4	**
5	**GEC-488**	316.3	306.7	72.9	285.7	NS	210.4	313.7	427.2	NS
6	**GEC-460**	340.8	387.7	178.9	300	NS	369.0	596.6	635.9	**
7	**Indaf-5**	385.5	452.9	155.7	296.1	NS	351.1	401.0	546.1	NS
	Range	215.4-423.9	307-452.9	72.9-178.9	285.7-410.4		135.2-491.6	211.4-601	262.7-719.4	
	Mean	**313.1**	**370.58**	**131.02**	**337.61**		**271.52**	**376.74**	**474.52**	
High Zinc genotypes										
Total biomass (g/ 3plants)										
		Pot experiment					**Field experiment**			
	Genotypes	**C**	**T1**	**T2**	**T3**		**C**	**T1**	**T2**	
8	**MR-6**	457.3	430.7	53.9	341.5	**	281.8	402.2	488.7	**
9	**PES-400**	277.7	354.7	139.1	257.0	NS	210.3	285.2	435.9	NS
10	**GEC-45**	281.3	376.8	118.8	265.2	NS	304.8	402.2	480.7	NS
11	**GEC-108**	253.8	290.7	79.3	237.0	NS	196.9	289.9	368.2	NS
12	**GEC-94**	342.4	348.1	238.4	259.6	**	266.6	403.3	374.3	NS
13	**GEC-271**	223.0	233.6	69.3	275.1	NS	444.2	539.7	701.9	**
14	**GEC-21**	202.1	276.7	74.7	302.6	NS	195.1	378.5	402.3	NS
15	**GPU-45**	177.0	239.9	51.3	162.5	NS	372.3	464.8	567.3	NS
16	**GPU-28**	371.3	221.9	25.9	150.3	NS	354.6	431.3	498.0	**
	Range	177-457.3	221.9-430.7	25.9-238.4	150.3-341.5		195.1-444	285.2-464.	368.2-701.9	
	Mean	**287.32**	**308.12**	**94.522**	**250.08**		**291.84**	**399.67**	**479.7**	
	CD @0.05	4.38					1.71			

NS : Non significant
** :Significant @ 0.05

to growth parameters. Zn treatment of 1.5% was given amazing results with regards to biomass production compared to control. Mean stem length under control in low Zn types is 18.68cm and it is 26.18 in case of treated plants. Root length also increases significantly from 13.9cm to22.5 cm. In case of both high and low Zn types there is increase in biomass and other parameters with external Zn application (Table 7 and Plate No. 5).

By using this biomass and Zn content data, Zinc efficiency of both high and low Zn types were estimated under different levels of external Zn application. As the zinc efficiency is the ability of plants to produce biomass under zinc deficient condition, which gives the information on intrinsic ability (genetic variation) of genotypes to produce biomass under Zn deficiency. Zinc efficiency is a reliable plant parameter to select and screen the Finger millet genotypes for their tolerance to Zn deficiency or for soils of low Zn content.
The genotypes selected for study were also classified into (i) Efficient (E), (ii) Moderately efficient (ME) and (iii) Inefficient (IE) based on their ability to produce biomass under Zn deficient condition. The genotypes which shows Zinc efficiency (%) of >80 were classified as efficient, which includes GEC-403, GEC-520, Indaf-5 under low Zn types and GEC-271, GPU-45 and GPU-28 under high Zn types (totally six genotypes). The genotypes, which show efficiency (%) of 70 and above are classified as moderately efficient, which include MR-6, PES-400 and GEC-45 and all are high Zn types. The genotypes, which show efficiency (%) of < 70 are classified as inefficient, which includes GEC-236, GEC-265, GEC-488, GEC-460 under low Zn types and GEC-108, GEC-94 and GEC-21 under high Zn types (Table 8).

Meanwhile the leaf samples were collected and after maturity the grains were collected from both field and pot experiment and they were analyzed for Zn content using AAS. Both high and low Zn types shows significant variation in uptake and translocation of Zinc under both field and pot experiment under different levels of soil Zn status. There is also a significant variation between leaf and grain Zn content in both types and also between treatments. Most of the low Zn types show gradual increase in leaf Zn content as there is increase in external Zn application. The mean leaf Zn content of low Zn types increases linearly as the soil Zn status increases. Under pot experiment the mean leaf Zn content increases from 2.06 mg/100g under control to 2.83 mg/100g under treatment-1, 3.76 mg/100g under treatment-2

Table 7: Solution culture/ Hydroponics- study the effect of Zn on growth parameters under controlled condition

Low Zinc genotypes													
		Control						Treated					
	Genotypes	Stem length (cm)	Root length (cm)	Root vol. (cm^3)	No. of leaves	Shoot wt. (g)	Root wt. (g)	Stem length (cm)	Root length (cm)	Root vol. (cm^3)	No. of leaves	Shoot wt (g)	Root wt (g)
1	GEC-236	19.1	16	20	16	6.568	8.63	26.16	26.5	52.16	23.83	8.42	10.1
2	GEC-265	18	13.5	16.66	20.66	4.636	9.525	33.5	22.83	18.5	24.16	7.81	10.15
3	GEC-403	17.16	9.5	15.83	24.5	7.876	8.37	19.33	24.5	58.33	32	11.0	8.9
4	GEC-520	16.66	16.5	19.16	20.83	7.054	6.208	33.5	26.5	55.83	30.83	15.8	11.67
5	GEC-488	19	12.83	20.5	19.66	7.69	7.37	24.83	25.33	40.83	24.66	9.7	9.35
6	GEC-460	20	13.5	20	22.16	6.61	6.71	25	16	24.16	24.5	7.52	10.98
7	Indaf-5	20.83	15.83	20.83	21	7.828	6.09	21	16.5	22.5	23.66	9.24	17.43
	Mean	**18.68**	**13.957**	**18.99**	**20.6**	**6.8**	**7.5**	**26.18**	**22.5**	**38.9**	**26.2**	**9.9**	**11.2**
	S.D	**1.50**	**2.437**	**1.96**	**2.5**	**1.1**	**1.3**	**5.53**	**4.5**	**17.0**	**3.5**	**2.8**	**2.8**
High Zinc genotypes													
	Genotypes	Stem length (cm)	Root length (cm)	Root vol. (cm^3)	No. of leaves	Shoot wt. (g)	Root wt. (g)	Stem length (cm)	Root length (cm)	Root vol. (cm^3)	No. of leaves	Shoot wt (g)	Root wt (g)
8	MR-6	22.5	14	20.89	27.5	7.856	9.38	25.83	16.83	24.5	26.5	10.4	10.61
9	PES-400	17.66	14	16.66	19	7.3	9.3	24.16	17.33	26.66	30.16	9.07	10.87
10	GEC-45	27.5	16.66	17	14.16	5.49	8.95	27.66	17.5	17.5	23.83	10.4	9.29
11	GEC-108	23.66	14.66	16.66	12.66	8.37	6.15	25.16	14.97	17	21.66	9.88	17.19
12	GEC-94	24.33	14.83	20.83	19	6.88	11.99	26.33	17.16	22.83	25.33	9.17	15.08
13	GEC-271	23.16	12.83	12.83	18.33	8.556	8.1	24.66	15	19.16	26.16	9.57	11.7
14	GEC-21	18.83	14.5	13.33	17.16	7.85	8.7	26.5	16.1	17.5	18.66	8.93	7.97
15	GPU-45	20	9.66	10.83	18.5	7.76	5.83	21.5	11	20.83	17.66	10.4	6.14
16	GPU-28	21	14.66	13.33	26.83	10.46	6.9	22.83	16.83	22.5	20.33	14.1	6.7
	Mean	**22.07**	**13.97**	**15.81**	**19.2**	**7.8**	**8.3**	**24.95**	**15.8**	**20.9**	**23.3**	**10.2**	**10.6**
	S.D	**3.0**	**1.90**	**3.53**	**5.0**	**1.3**	**1.9**	**1.92**	**2.05**	**3.4**	**4.1**	**1.5**	**3.6**
	CD @0.05	**5.57**						**3.96**					

Control: No external Zn application
Treated: 1.5% foliar spray of Zn

Table 8: classification of Finger millet genotypes based on biomass produced under Zn deficient conditions

Low Zinc genotypes					
Genotypes		Biomass (g/ plant)		Zn efficiency (%)	Zn efficiency rating
		-Zn	+Zn		
1	GEC-236	155.72	282.6	55.10	Inefficient (I)
2	GEC-265	135.22	211.46	63.94	Inefficient (I)
3	GEC-403	187.72	230.96	81.27	Efficient (E)
4	GEC-520	491.63	601.05	81.79	Efficient (E)
5	GEC-488	210.4	313.70	67.06	Inefficient (I)
6	GEC-460	369.04	596.64	61.85	Inefficient (I)
7	Indaf-5	351.19	401.04	87.56	Efficient (E)
High Zinc genotypes					
Genotypes		Biomass (g/ plant)		Zn efficiency (%)	Zn efficiency rating
		-Zn	+Zn		
8	MR-6	281.87	402.27	70.06	Moderately efficient (ME)
9	PES-400	210.39	285.24	73.75	Moderately efficient (ME)
10	GEC-45	304.84	402.28	75.77	Moderately efficient (ME)
11	GEC-108	196.91	289.98	67.90	Inefficient (I)
12	GEC-94	266.67	403.38	66.10	Inefficient (I)
13	GEC-271	444.22	539.74	82.30	Efficient (E)
14	GEC-21	195.19	378.53	51.56	Inefficient (I)
15	GPU-45	372.31	464.8	80.10	Efficient (E)
16	GPU-28	354.68	431.37	82.22	Efficient (E)

and 6.75mg/100g under treatment-3. The genotype GEC-403 can accumulate as high as 10.2 mg/100g Zn in leaves under high soil Zn application of 30kg/ha + 1.5% foliar application of $ZnSO_4$. Some genotypes like GEC-236, Indaf-5 and GEC-265 also accumulate high Zn content of 7.29, 7.69 and 8.24 mg/100g in leaves.

These low Zn types also show significant variation in translocation of Zn from leaves to grains. The mean grain Zn content of low Zn types increases as the level of external Zn application increases as in case of leaf Zn content. It is 3.16mg /100g under control, 3.61mg/100g under T1, 4.08 mg/ 100g under T2 and 5.58 mg/100g under T3. Some genotypes like GEC-460, GEC-365 and Indaf-5 show highest Zn translocation of 6.57, 6.09 and 5.99 mg/100g respectively under high soil Zn status.

The high Zn types also show significant variation in case of uptake and translocation of Zn. As is the case with low Zn types, in case of high Zn types also there is gradual increase in leaf and grain Zn content with increase in soil Zn status. The mean leaf Zn content increases linearly from 2.18mg/100g under control, 3.22mg/100g under T1, 5.25 mg/100g under T2 and 9.39 mg/100g under T3. Most of the genotypes accumulate high Zn in leaves and also in grains under high soil Zn condition. Some genotypes like GEC-21 and GPU-45 can accumulate as high as 7.88 and 9.76 mg/100g Zn in grains respectively under high soil Zn status. In case of both low and high Zn types there is increase in grain Zn content linearly with external application of Zn. Under pot experiment, in case of low Zn types the mean grain Zn content under control is 3.16 mg/100g, 3.61 mg/100g under T1, 4.08 mg/100g under T2 and 5.58 mg/100g under T2. In case of high Zn types it is 3.12, 4.69,4.82 and 6.09 mg/100g under control, T1, T2 and T3 respectively. Under field condition also there is similar trend of increase in grain Zn content with external Zn application (Table 9 and 10; Figure 6 and 7).

By using the data on Zn content and biomass, the Zn utilization efficiency (ZUE) of these genotypes was calculated. As zinc utilization efficiency (ZUE) is the ability of plants to produce amount of biomass per unit amount of Zn present in biomass varies with genotypes. As observed in rice (Rengel, 1998) the ZUE increases with external Zn application up to certain level and further increase in Zn application beyond certain level will not results in increased ZUE. We observed similar trend in Finger millet. The low Zn types are having high ZUE at high Zn application compared to high Zn types. The low Zn types under control

show ZUE of 27.38% and of high Zn types it is 25.92%. It is increased to 27.38% under control to 34.24% under T1 in case of low Zn types and it is increased from 25.92% under control to 26.08% under T1 incase of high Zn types. Under both low and high Zn types, there is no increase in ZUE under both T2 and T3 was observed (Table 11).

4.2. Variation in the activity of Carbonic anhydrase (CA) and Copper zinc super oxide dismutase (Cu/ Zn SOD) and study the relevance of zinc status and activity of these enzymes.

As there is a close relationship between zinc and main zinc requiring enzymes like carbonic anhydrase and copper zinc superoxide dismutase, it is important to asses the level of these enzymes in relation to Zn in all the selected Finger millet genotypes.

Carbonic anhydrase activity was assessed in the young leaves of all selected Finger millet genotypes and activity was expressed in enzyme units on fresh weight of leaf tissue basis and also on units per mg protein basis.

There is significant variation among the genotypes and also between the treatments with respect to carbonic anhydrase activity. As we expected the carbonic anhydrase activity increases linearly with increase in external Zn application. Under low Zn types the genotypes GEC-460, GEC-520, Indaf-5 and GEC-403 shows highest activity of 1200,1116.27, 1071 and1071 units per g fresh weight under control condition. Most of the low Zn types show increase in the activity of enzyme as increase in external application of Zn. The mean activity of enzyme increases linearly from 1027.514 units per g fresh weight under control to 2100.574 units per g fresh weight under T3. The values ranges from 143 U/g fresh weight in case of GEC-403 to 2508.19 U/g fresh weight in case of GEC-265. The mean enzyme activity varies similarly in terms of units per mg protein and this also increases linearly as the application of external Zn increases.

The high Zn types also show the similar trend of increase in the enzyme activity, as there is increase in external Zn application. The mean enzyme activity increases from 1151.35 U/g FW in case of control; 1552.95 U/g FW in case of T1, 1757.61 U/g FW in case of T2 and 2948.18 U/g FW in case of T3. The genotypes GPU-45 and GEC-94 show the highest activity of 3642.85 and 3933.33 U/g FW respectively. The lowest activity of 918.36 U/g

Table 9: Variation in Zinc content in leaves and grains of Finger millet genotypes under different levels of external Zn application in pot experiment

Low Zinc genotypes									
		Leaf				Grain			
	Genotypes	C	T1	T2	T3	C	T1	T2	T3
1	GEC-236	2.09	2.03	2.17	7.29	3.48	3.11	3.87	4.57
2	GEC-265	1.57	4.59	4.62	8.24	3.52	4.03	3.80	6.09
3	GEC-403	2.24	3.05	1.20	10.2	2.46	3.25	5.14	5.60
4	GEC-520	2.34	2.36	6.86	3.51	2.07	4.44	3.92	5.59
5	GEC-488	1.51	2.54	1.75	4.30	2.99	4.38	4.20	4.69
6	GEC-460	2.79	1.76	4.47	6.06	3.91	3.51	4.10	6.57
7	Indaf-5	1.93	3.52	5.26	7.69	3.69	2.59	3.55	5.99
	Range	1.51- .79	1.76-4.59	1.20-5.26	3.51-10.2	2.07-3.91	2.59-4.44	3.55-5.14	4.57-6.57
	Mean	2.06	2.83	3.76	6.75	3.16	3.61	4.08	5.58
	S.D.	0.44	0.97	2.09	2.31	0.68	0.69	0.51	0.73
High Zinc genotypes									
		Leaf				Grain			
	Genotypes	C	T1	T2	T3	C	T1	T2	T3
8	MR-6	1.60	1.71	5.93	5.18	1.78	5.75	4.45	3.72
9	PES-400	2.05	3.08	3.90	8.30	1.62	4.47	4.12	3.79
10	GEC-45	1.64	1.94	5.52	9.41	4.45	4.8	5.30	5.17
11	GEC-108	1.52	1.89	2.58	11.60	2.84	3.51	4.70	6.78
12	GEC-94	2.36	3.78	7.01	13.47	3.88	3.47	5.14	6.36
13	GEC-271	2.27	4.62	4.94	9.87	2.27	6.42	4.31	6.37
14	GEC-21	2.80	4.14	4.16	9.58	5.38	5.10	6.40	7.88
15	GPU-45	2.81	3.96	8.95	10.70	2.79	4.81	4.41	9.76
16	GPU-28	2.60	3.89	4.27	6.44	3.12	3.93	4.63	4.83
	Range	1.52-2.81	1.71-4.62	2.58-7.01	5.18-11.6	1.62-5.38	3.51-6.42	4.12-6.4	3.72-9.76
	Mean	2.18	3.22	5.25	9.39	3.12	4.69	4.82	6.07
	S.D.	0.50	0.003	1.88	2.52	1.24	0.98	0.70	1.95
	CD @ 0.05	1.33				1.21			

Table 10: Variation in Zinc content in leaves and grains of Finger millet genotypes under different levels of external Zn application in field experiment

Low Zinc genotypes							
		Leaf			Grain		
Sl.No.	Genotypes	C	T1	T2	C	T1	T2
1	GEC-236	1.57	6.86	6.58	1.64	5.59	7.58
2	GEC-265	3.13	2.16	5.35	5.15	3.20	6.35
3	GEC-403	3.75	2.27	5.28	5.17	6.28	8.22
4	GEC-520	2.81	4.28	7.98	4.24	3.94	5.28
5	GEC-488	3.52	6.74	5.49	6.49	6.66	7.65
6	GEC-460	2.88	2.91	3.71	3.37	4.71	9.33
7	Indaf-5	2.87	4.08	8.35	2.58	7.08	7.17
	Range	1.57-3.52	2.16-6.86	3.71-8.35	1.64-6.49	3.2-7.08	5.28-9.33
	Mean	2.93	4.18	6.10	4.09	5.35	7.36
	S.D.	0.69	1.96	1.64	1.67	1.45	1.29
High Zinc genotypes							
		Leaf			Grain		
Sl.No.	Genotypes	C	T1	T2	C	T1	T2
8	MR-6	2.72	2.99	4.51	4.83	6.75	5.51
9	PES-400	2.93	3.52	5.11	3.90	4.11	4.25
10	GEC-45	2.49	2.89	4.45	3.13	3.45	7.03
11	GEC-108	1.98	3.28	4.89	2.66	4.40	5.89
12	GEC-94	1.33	2.59	4.43	4.64	5.43	8.38
13	GEC-271	2.08	4.24	5.27	3.35	4.88	6.27
14	GEC-21	3.75	4.87	5.28	3.10	4.87	5.51
15	GPU-45	3.37	6.76	5.77	3.31	4.77	6.00
16	GPU-28	2.70	3.50	4.73	3.62	5.73	6.02
	Range	1.33-3.75	2.59-6.76	4.43-5.77	2.66-4.83	3.45-6.75	4.25-8.38
	Mean	2.59	3.84	4.93	3.61	4.93	6.09
	S.D.	0.73	1.29	0.45	0.72	0.96	1.13
	CD @ 0.05	2.01			1.30		

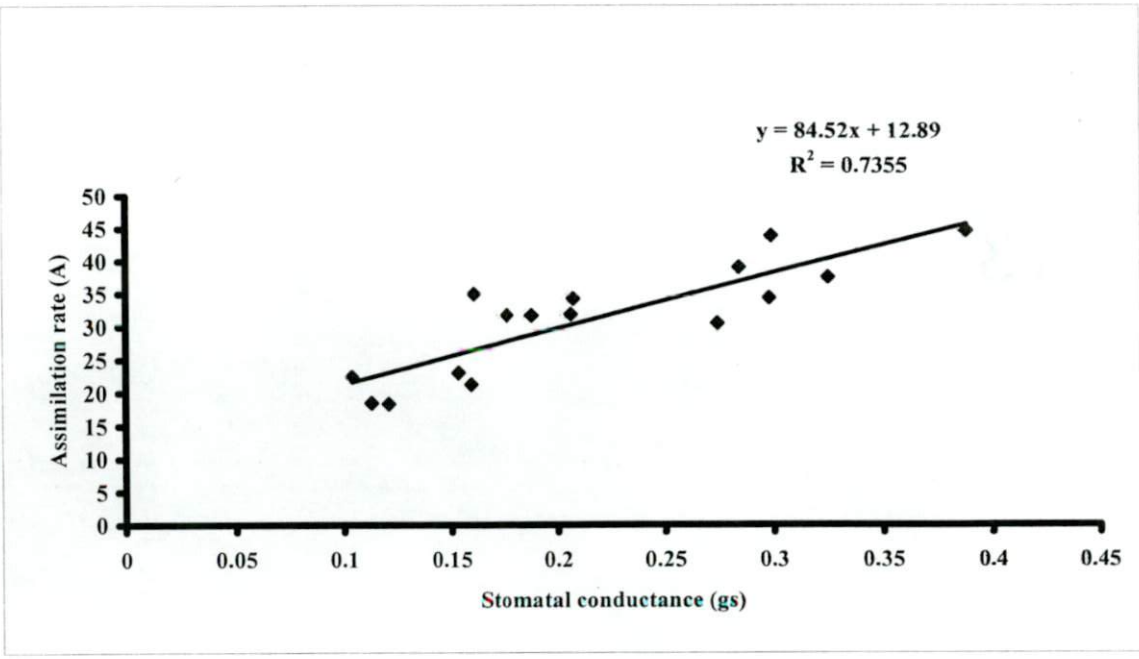


Figure 4: Relationship between assimilation rate (A) and stomatal conductance of Finger millet genotypes treated with Zn

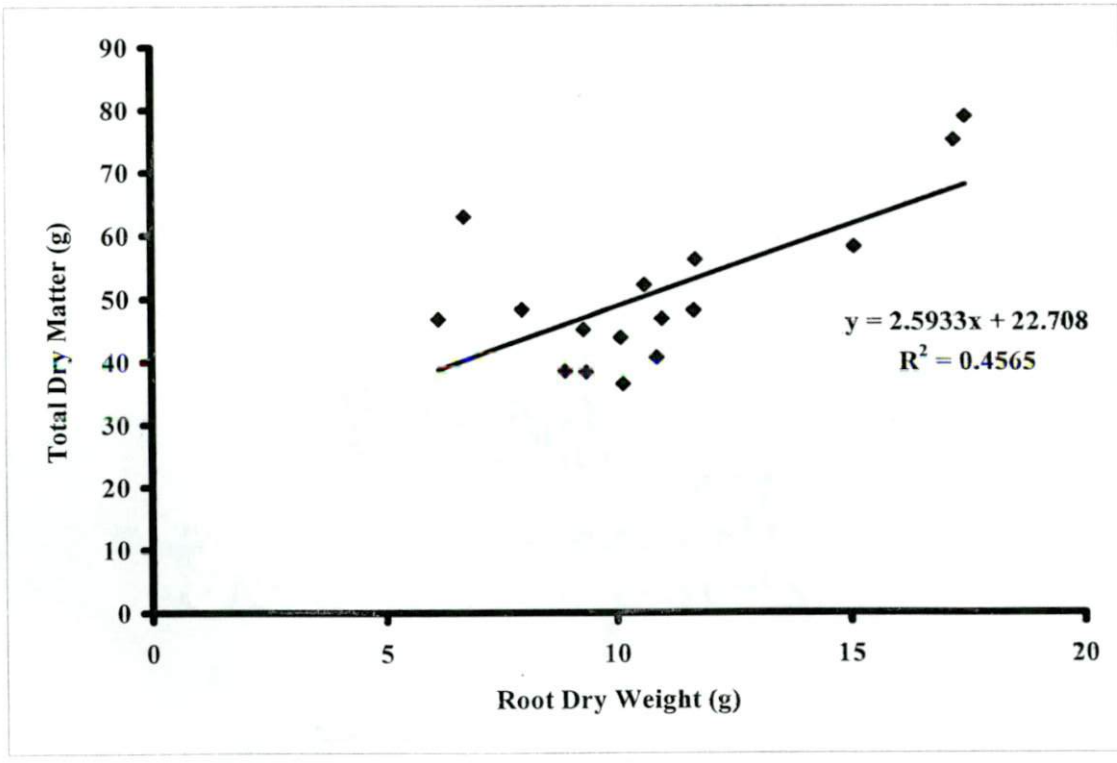


Figure 5: Relationship between total dry matter and root dry weight of Finger millet genotypes treated with Zn

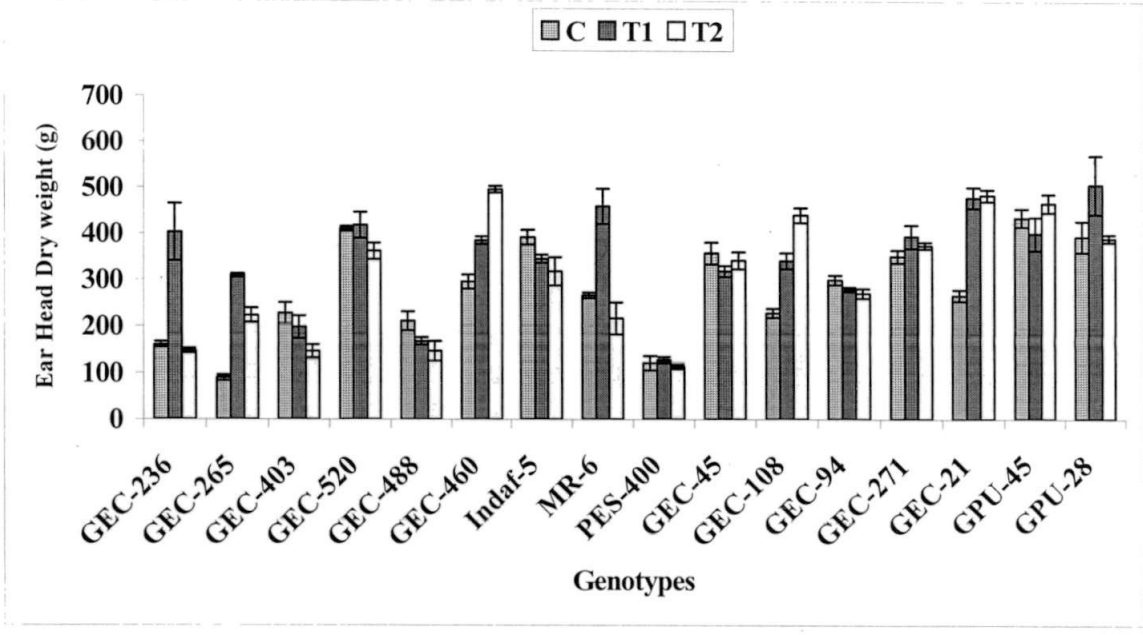


Figure 6: Dry weights of ear heads of Finger millet genotypes at different levels of external Zn application under field

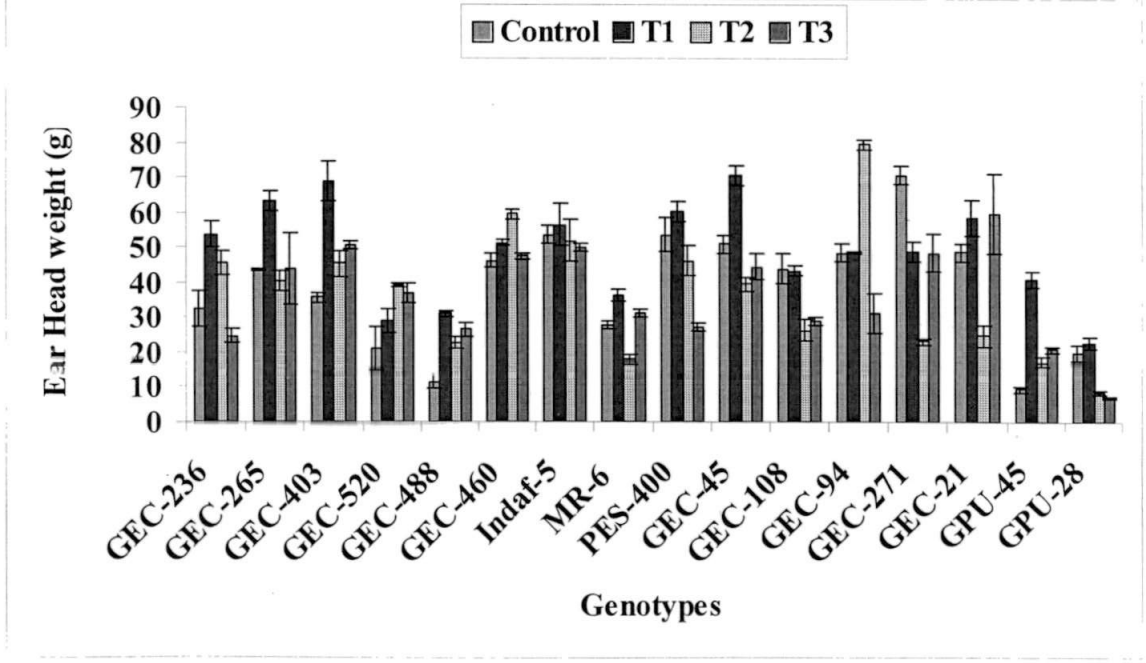
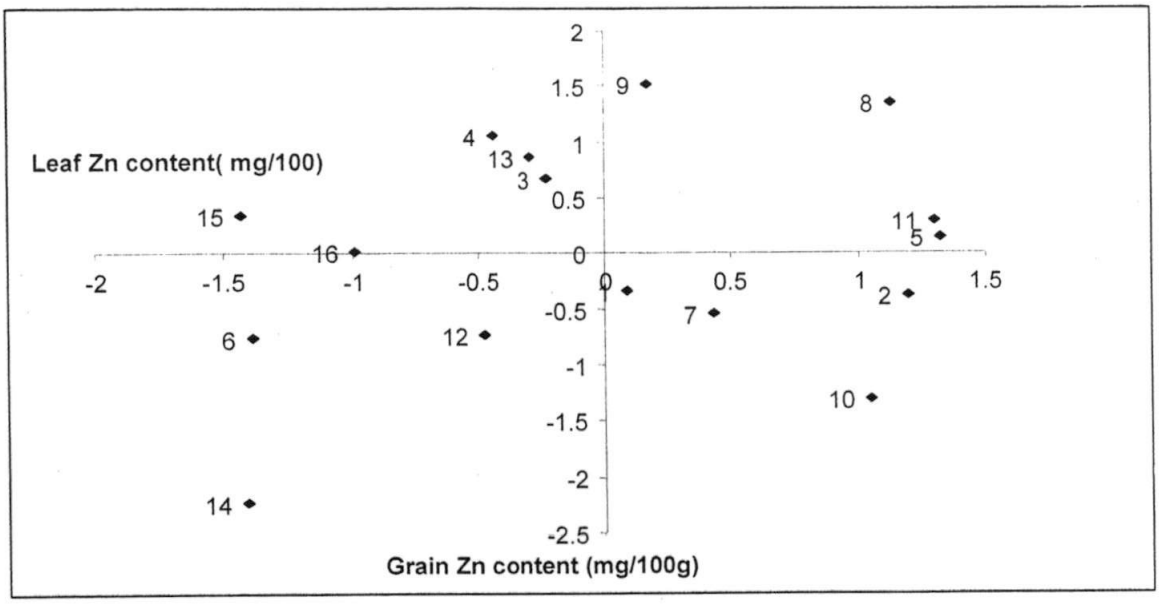
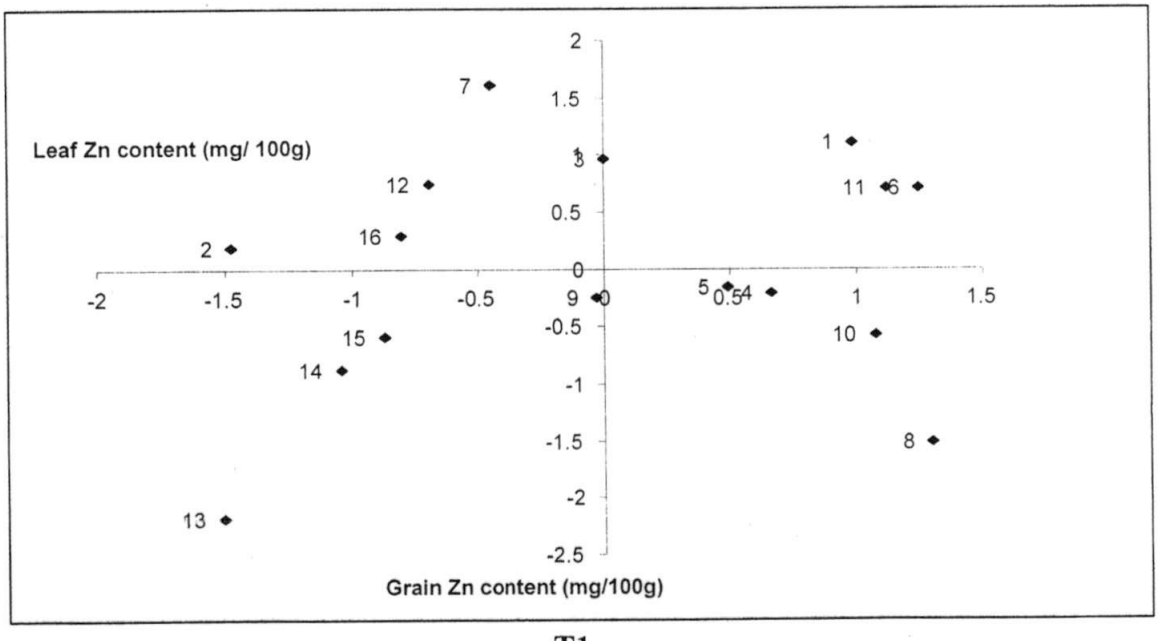


Figure 7: Dry weights of ear heads of Finger millet genotypes at different levels of external Zn application under pot experiment



Control



T1

Figure 8: Z distribution of grain Zn versus leaf Zn content under different external Zn status

Control: Low soil Zn (15 Kg/ha) No external Zn application

T1: 20 Kg Zn /ha

Table: 11 Zinc utilization efficiency (ZUE) of Finger millet genotypes at different levels of external Zinc application

Low Zinc genotypes					
		Zinc Utilization Efficiency (%)			
	Genotypes	C	T1	T2	T3
1	GEC-236	22.95	31.35	27.58	33.31
2	GEC-265	21.88	24.99	19.21	28.22
3	GEC-403	24.82	38.96	32.89	35.09
4	GEC-520	33.07	41.65	47.16	42.41
5	GEC-488	38.29	38.74	51.22	36.09
6	GEC-460	24.94	29.92	31.12	23.14
7	Indaf-5	25.75	34.13	26.29	22.31
	Range	21.88-38.2	24.99-41.65	19.21-51.22	22.31-42.41
	Mean	**27.38**	**34.24**	**33.63**	**31.51**
High Zinc genotypes					
		Zinc Utilization Efficiency (%)			
	Genotypes	C	T1	T2	T3
8	MR-6	38.14	47.60	47.10	37.75
9	PES-400	36.54	37.71	33.95	27.32
10	GEC-45	26.23	30.39	28.04	21.39
11	GEC-108	27.01	27.33	23.13	22.27
12	GEC-94	24.30	25.50	43.78	19.01
13	GEC-271	21.82	12.74	13.73	15.01
14	GEC-21	15.54	15.00	13.93	16.41
15	GPU-45	9.94	16.58	16.04	11.23
16	GPU-28	33.82	21.91	13.66	14.84
	Range	9.94-38.14	12.74-47.6	13.66-47.1	11.23-37.75
	Mean	**25.92**	**26.08**	**25.92**	**20.58**
	CD @ 0.05	**1.37**			

FW was observed in case of PES- 400. As expected the mean enzyme activity of low Zn types is lower than high Zn types (Table 12 and Figure 10, 11).

The activity of copper-Zinc super oxide dismutase also shows significant variation among the high and low Zinc types and also between treatments. As expected, the enzyme activity is higher in high Zn types and low in low Zn types. As the external application of Zn increases, the enzyme activity also increases correspondingly. The lowest enzyme activity of 25.74 units per mg protein was recorded in case of GEC-520 under control condition and the highest activity of 54.38 units per mg protein was recorded in case of GEC-265 under T3 condition. The mean enzyme activity of low Zn types ranges between 33.83 under control to 44.15 units per mg protein under T3.

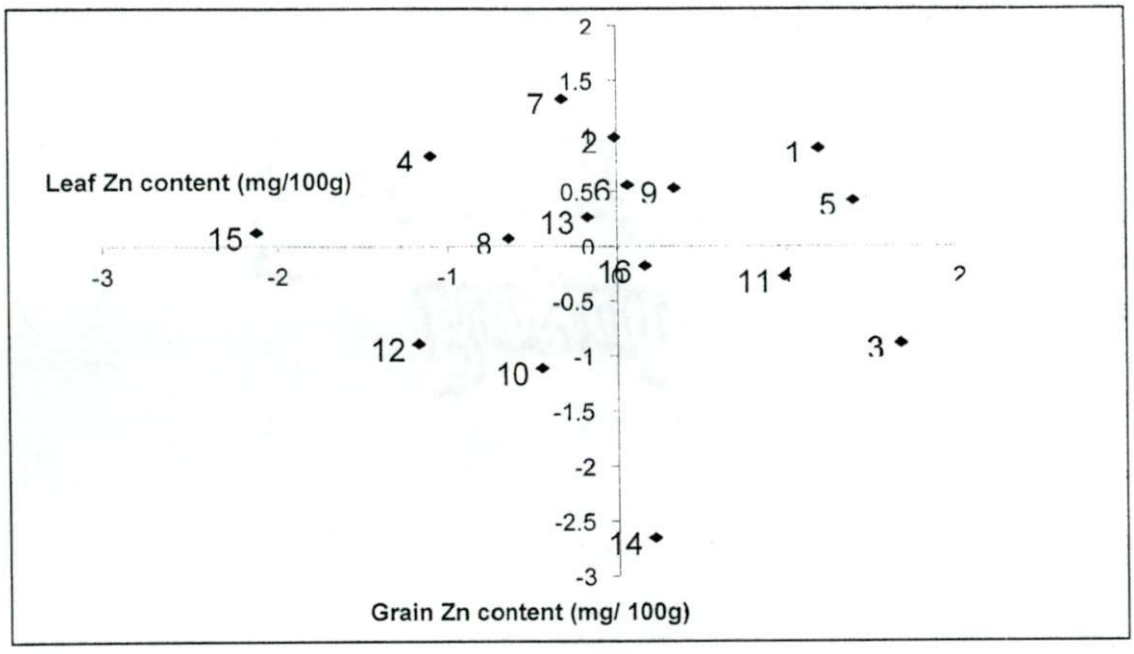
In case of high Zn types also with the increase in external Zn application there is corresponding increase in the enzyme activity. The lowest activity of 25.7 units per mg protein was recorded in case of GEC-21 under control condition and the highest activity of 45.90 units per mg protein was recorded in case of GPU-28. Both high and low Zn types responded well with respect to production of enzyme with external application of Zn and the response is more in case of low Zn types compared to high Zn types (Table 13, Figure 12, 13).

4.3. Radiolabel ^{65}Zn uptake studies to understand the differences in uptake and translocation in the contrast Finger millet genotypes

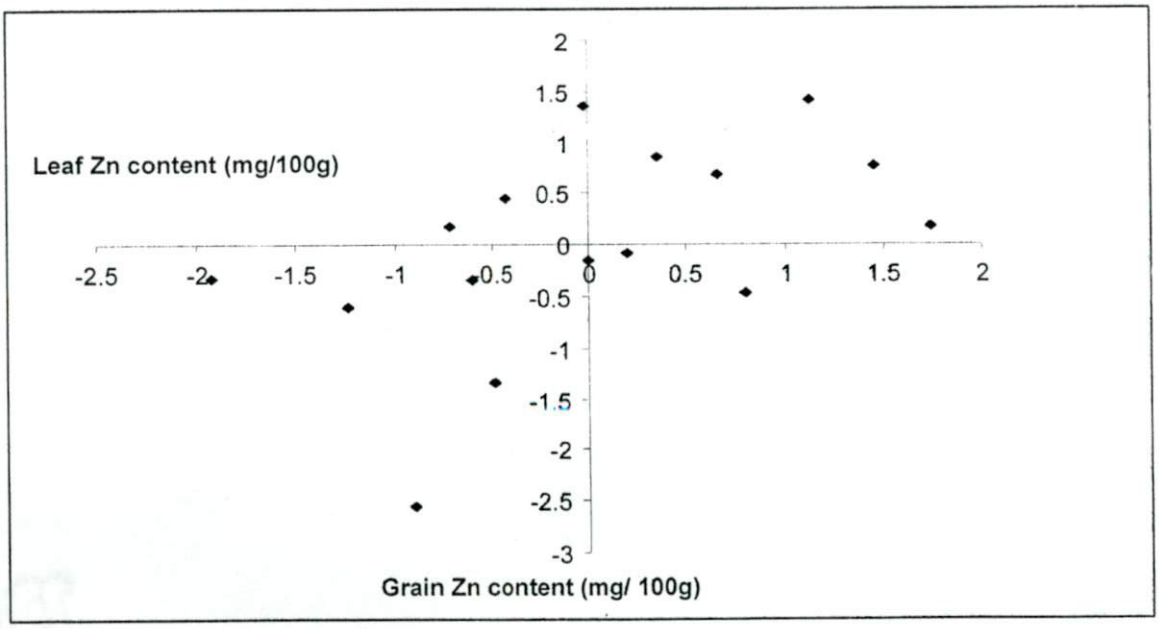
To study whether there is significant differences among the genotypes in uptake and translocation of Zn, conducted an uptake study using radiolabelled ^{65} Zn. Both the high and low Zn types were supplied with radiolabel Zn and after 48 hours the roots and shoots were harvested separately and readings were taken using liquid scintillation counter (LSC) in terms of cpm/ organ and cpm/ gram basis and the uptake and translocation was estimated. The amount of Zn entered into the whole organ (shoot or root) was estimated. The total ^{65}Zn entered into the root and shoot was considered as 100 per cent and the percent age distribution of ^{65}Zn to shoot and root was calculated. There is significant variation among the genotypes both in terms of uptake and translocation among high and low Zn types. Here

Table 12: Carbonic anhydrase activity of Finger millet genotypes at different levels of external Zinc application

Low Zn types									
		Units g^{-1} fresh weight				Units mg^{-1} protein			
Sl.No	Genotypes	Control	T1	T2	T3	Control	T1	T2	T3
1	GEC-236	932.03	888.88	1036.14	2121.21	155.66	120.50	144.31	295.44
2	GEC-265	831.46	1438.73	1757.57	2508.19	158.59	328.95	377.69	538.99
3	GEC-403	1071.42	1219.04	143	3032.25	83.72	88.73	112.29	238.12
4	GEC-520	1116.27	1178.57	1863.63	1491.32	145.01	173.15	335.14	268.18
5	GEC-488	970	1289.61	1289.61	1619.04	73.41	133.08	83.97	105.42
6	GEC-460	1200	1200	1491.32	1708.33	102.17	108.64	156.73	179.53
7	Indaf-5	1071.42	1575.34	1619.04	2223.68	82.93	127.89	148.04	203.32
	Mean	**1027.5**	**1255.7**	**1314.3**	**2100.5**	**114.4**	**154.42**	**194.02**	**261.28**
	S.D	**124.06**	**216.70**	**587.70**	**548.73**	**37.31**	**81.17**	**114.32**	**137.36**
High Zn types									
		Units g^{-1} fresh weight				Units mg^{-1} protein			
Sl.No	Genotypes	Control	T1	T2	T3	Control	T1	T2	T3
8	MR-6	1200	1666.66	1757.57	1575.34	127.56	180.32	228.50	204.81
9	PES-400	918.36	1491.32	1318.18	2780.48	139.48	164.34	167.29	352.87
10	GEC-45	1197.36	1197.36	1913.04	3014.08	66.02	86.23	154.57	243.53
11	GEC-108	747.47	1289.61	1357.14	3032.25	90.01	182.44	172.36	385.10
12	GEC-94	1178.57	1675.67	2060.60	3933.33	82.73	153.41	229.75	438.55
13	GEC-271	1200	1714.28	1642.45	3266.66	180.30	270.58	217.60	432.78
14	GEC-21	1230	1500	1567.56	2780.48	163.48	173.33	235.25	417.27
15	GPU-45	1333.3	1733.33	2202.02	3642.85	105.45	135.39	172.00	284.54
16	GPU-28	1357.14	1708.33	2000	2508.19	90.86	108.37	165.83	207.97
	Mean	**1151.35**	**1552.95**	**1757.61**	**2948.18**	**116.21**	**161.60**	**193.68**	**329.71**
	S.D	**195.77**	**197.82**	**311.26**	**680.11**	**38.88**	**52.52**	**33.05**	**95.93**
	C.D @ 0.01	**13.41**				**4.76**			



T2

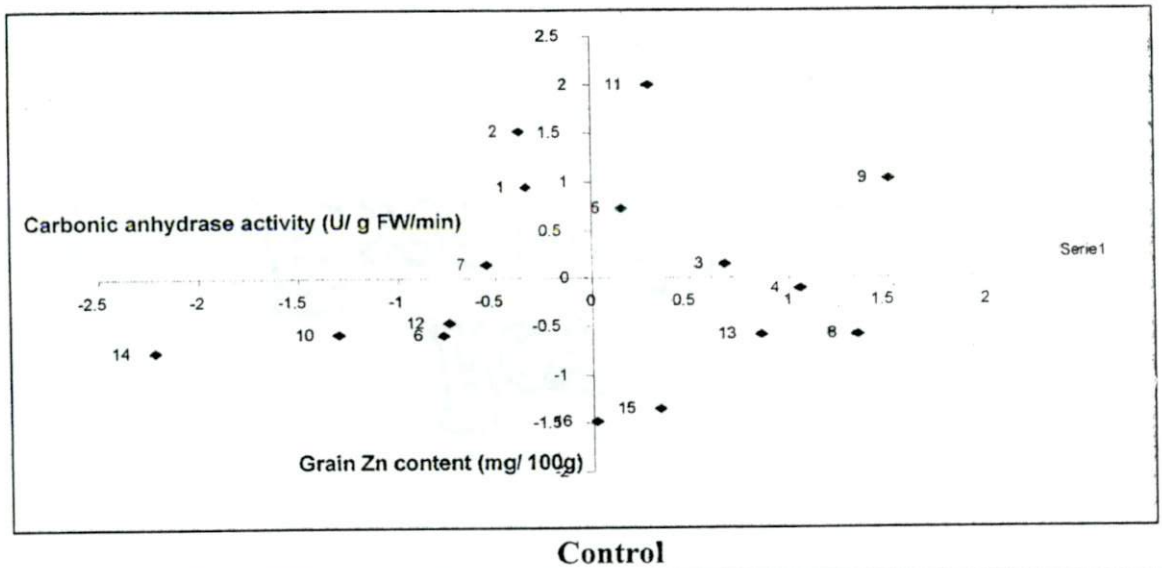


T3

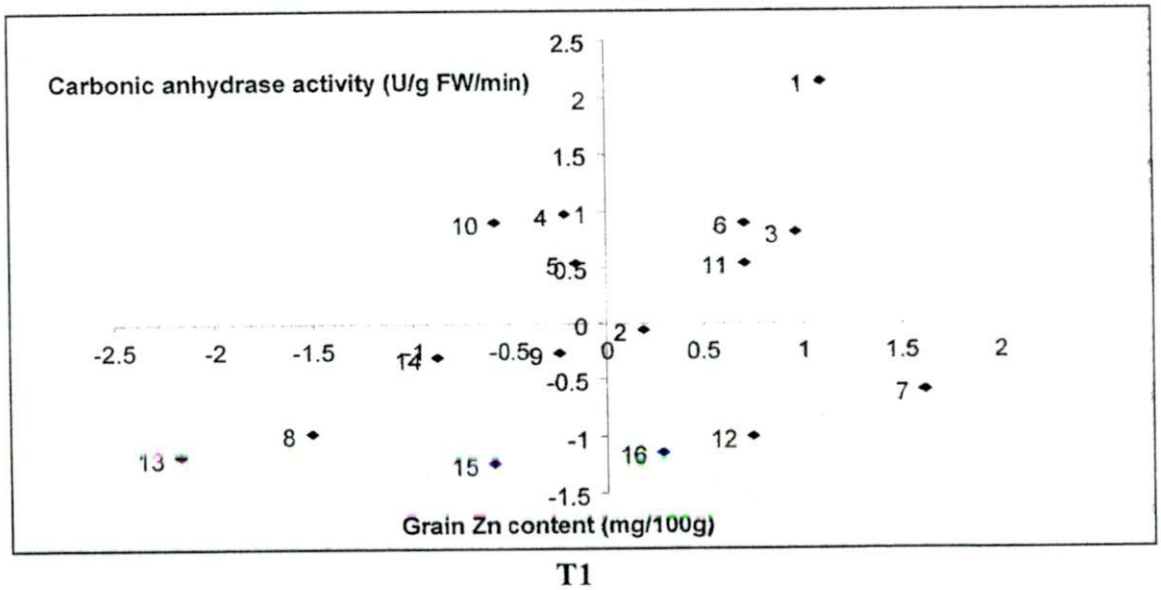
Figure 9: Z distribution of grain Zn versus leaf Zn content under different external Zn status

T2: external Zn application of 30 Kg/ ha

T3: external Zn application of 30 Kg/ ha + 1.5% foliar application of Zn



Control

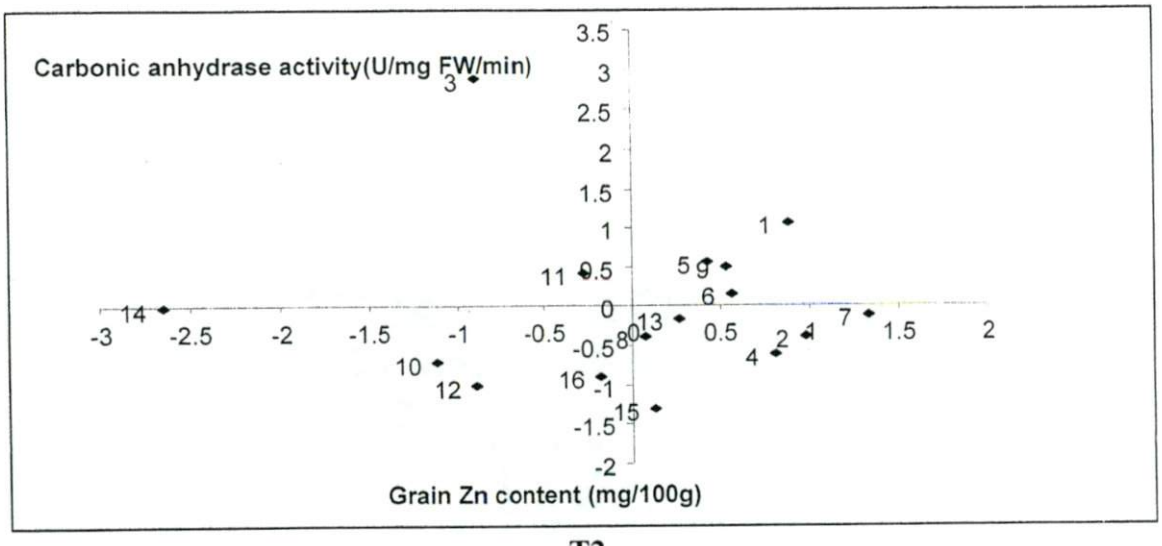


T1

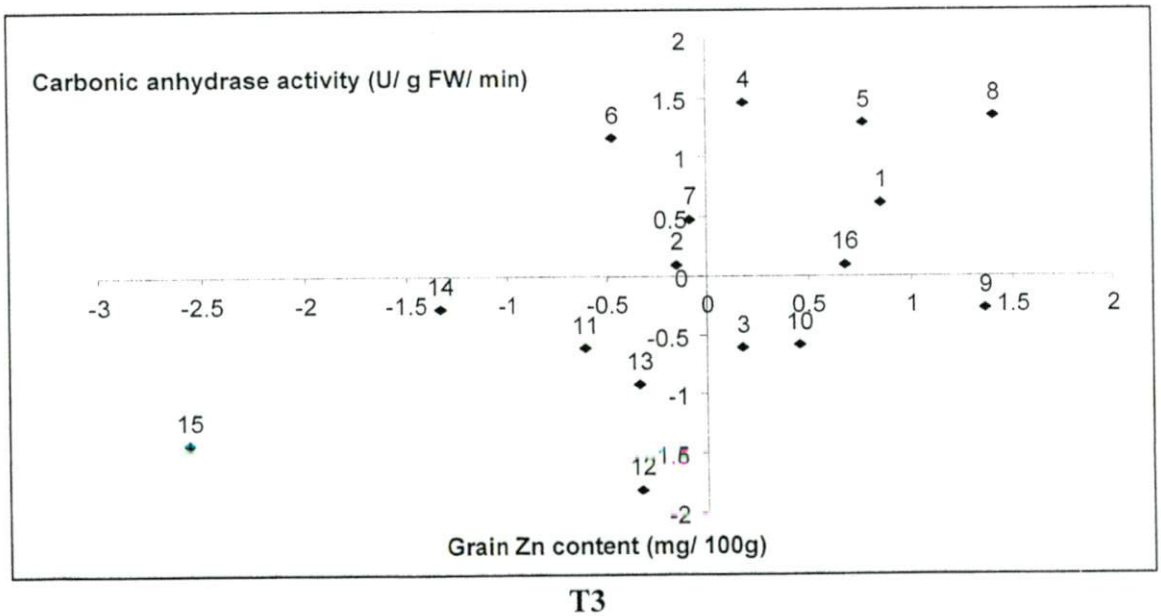
Figure 10: Z distribution of grain Zn versus Carbonic anhydrase activity under different external Zn status

Control: Low soil Zn (15 Kg/ha) No external Zn application

T1: 20 Kg Zn /ha



T2

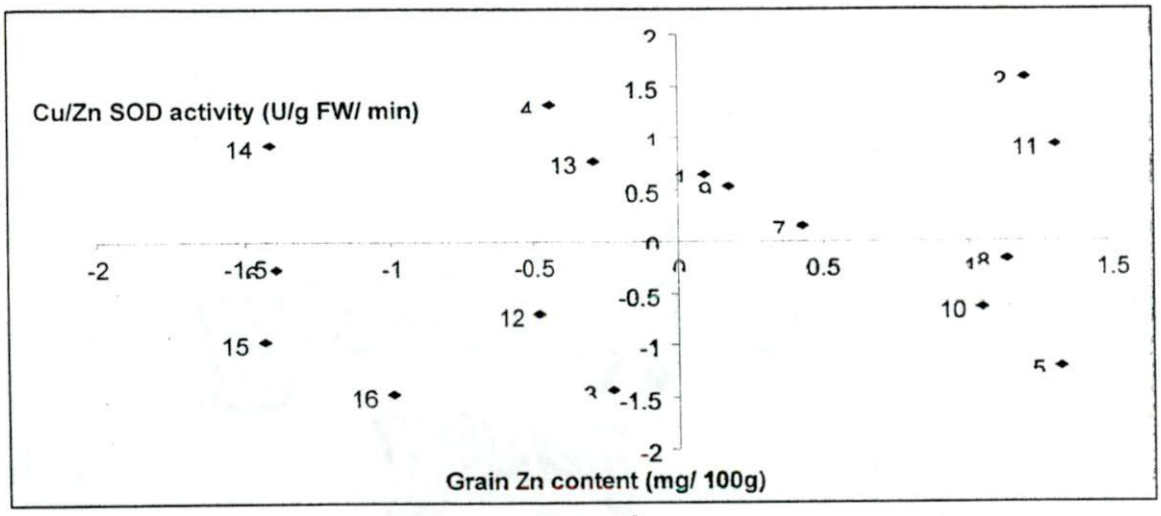


T3

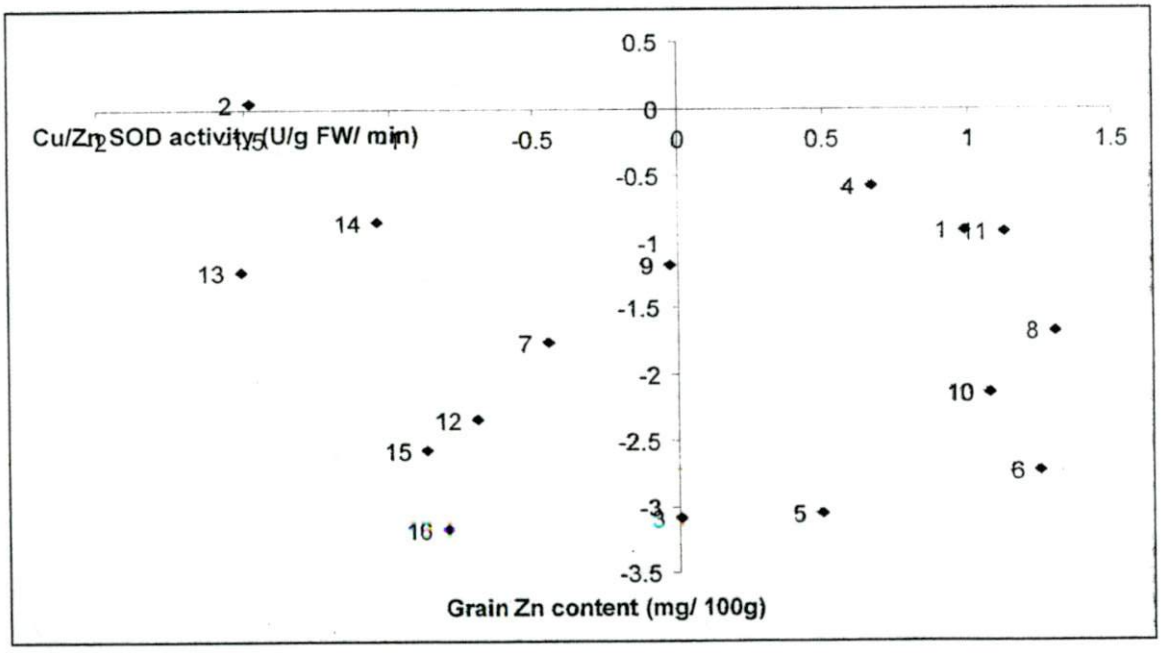
Figure11: Z distribution of grain Zn versus Carbonic anhydrase activity under different external Zn status

T2: external Zn application of 30 Kg/ ha

T3: external Zn application of 30 Kg/ ha + 1.5% foliar application of Zn



Control

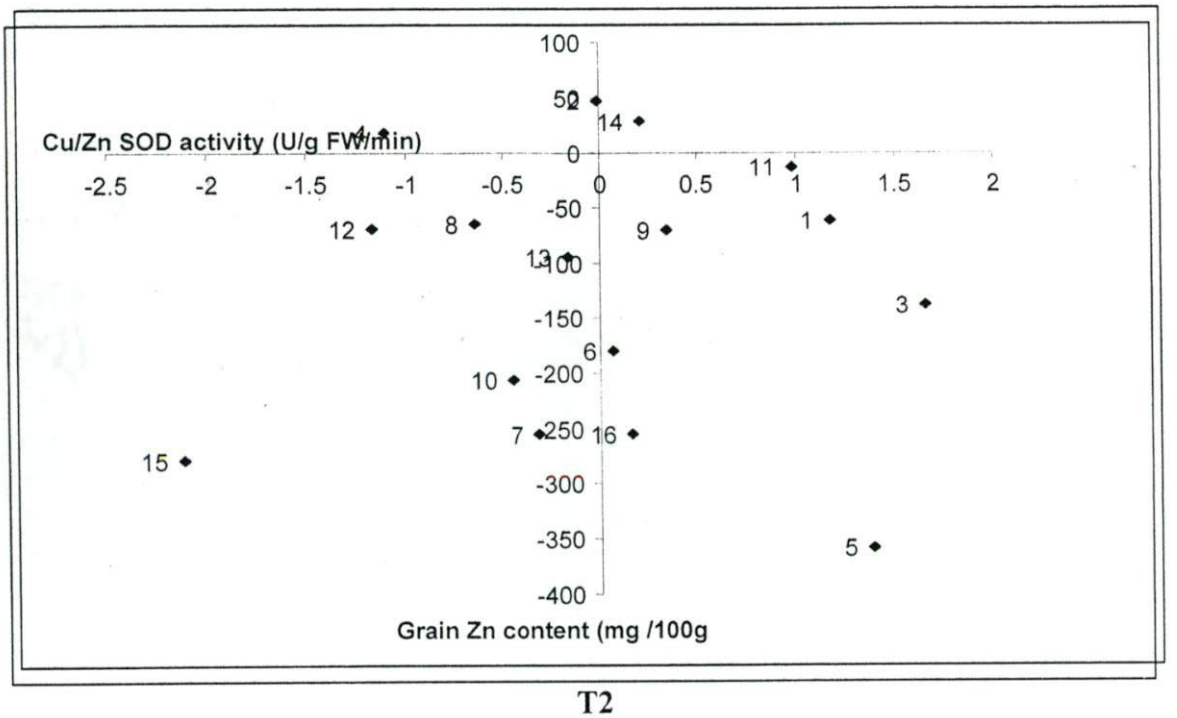


T1

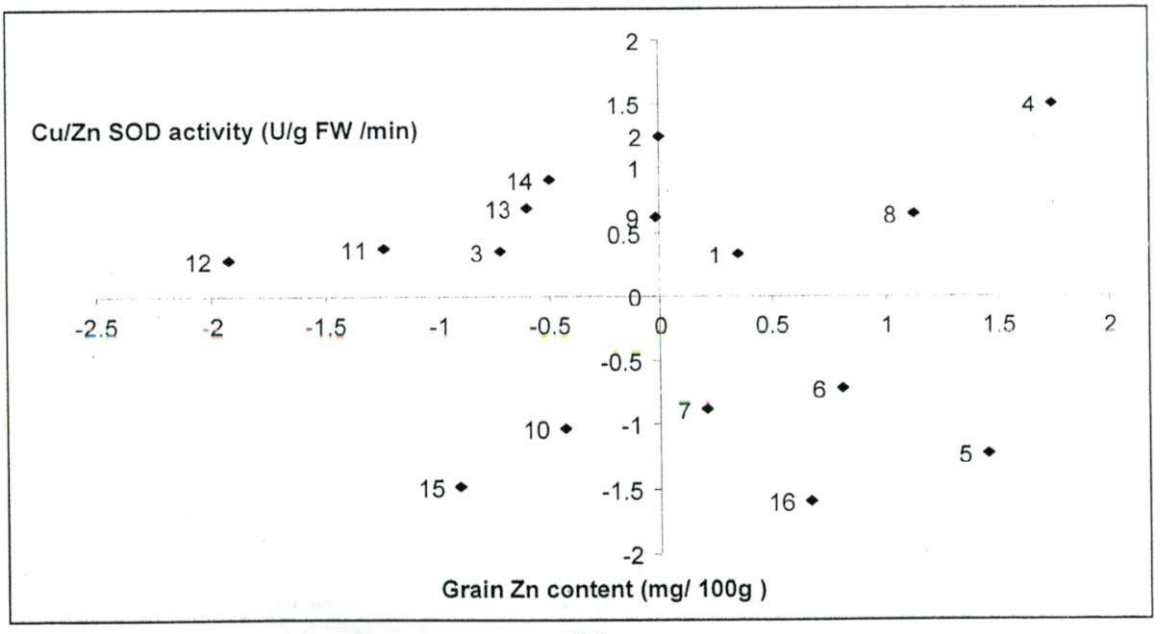
Figure 19: Z distribution of grain Zn versus Cu/ Zn SOD activity under different external Zn status

Control: Low soil Zn (15 Kg/ha) No external Zn application

T1: 20 Kg Zn /ha



T2



T3

Figure 13: Z distribution of grain Zn versus Cu/ Zn SOD activity under different External Zn status

T2: External Zn application of 30 Kg /ha

T3 : External Zn application of 30 Kg /ha + 1.5% foliar application of Zn

Table 13: Cu/Zn SOD activity of Finger millet genotypes at different levels of external Zn application

Low Zn types									
		Cu/Zn SOD (U /g FW/min)				Cu/Zn (U /mg pro/min)			
Sl.No	Genotypes	Control	T1	T2	T3	Control	T1	T2	T3
1	GEC-236	229.47	236.25	290.32	349.33	38.32	39.45	40.43	48.65
2	GEC-265	113.43	116.38	182.48	253.06	34.98	35.89	39.21	54.38
3	GEC-403	483.60	506.83	368.77	347.35	37.78	39.60	42.22	39.77
4	GEC-520	146.68	194.58	212.38	226.07	25.74	34.15	38.19	40.65
5	GEC-488	456.10	501.14	588.87	514.44	34.51	37.92	38.34	33.49
6	GEC-460	339.52	461.97	410.46	460.73	28.91	39.33	43.13	48.42
7	Indaf-5	**289.81**	**341.13**	**485.43**	**478.00**	36.59	43.08	44.38	43.70
	Mean	**294.0**	**336.8**	**362.6**	**375.5**	**33.83**	**38.48**	**40.84**	**44.15**
	S.D	**143.0**	**158.3**	**146.5**	**112.4**	**4.7**	**2.88**	**2.44**	**6.93**
High Zn types									
		Cu/Zn SOD (U /g FW/min)				Cu/Zn SOD (U /mg pro/min)			
Sl.No	Genotypes	Control	T1	T2	T3	Control	T1	T2	T3
8	MR-6	328.29	331.80	293.33	316.42	34.90	35.27	38.13	41.13
9	PES-400	242.48	268.79	298.79	318.45	36.82	40.82	37.92	40.41
10	GEC-45	386.52	388.84	435.74	494.47	31.85	32.04	35.20	39.95
11	GEC-108	193.67	237.86	243.41	344.22	30.72	37.73	30.91	43.71
12	GEC-94	392.64	413.51	297.94	354.48	34.91	36.77	33.21	39.52
13	GEC-271	213.21	273.72	324.12	311.67	32.03	41.12	42.94	41.29
14	GEC-21	193.42	226.85	202.29	289.03	25.70	30.15	30.35	43.37
15	GPU-45	426.21	441.31	507.36	541.77	33.70	34.90	39.63	42.31
16	GPU-28	487.58	515.67	484.72	553.64	32.64	34.52	40.19	45.90
	Mean	**318.2**	**344.2**	**343.0**	**391.5**	**32.58**	**35.92**	**36.49**	**41.95**
	S.D	**110.9**	**100.9**	**107.3**	**106.6**	**3.19**	**3.65**	**4.34**	**2.06**
	CD @ 0.01	**1.33**				**1.38**			

the root ^{65}Zn content denotes the root uptake and the shoot ^{65}Zn content denotes Zn translocation into shoots.

The uptake of ^{65}Zn in leaves of high Zn types is higher compared to low Zn types. The mean activity of ^{65}Zn in shoots of low and high Zn types is 30131.92 cpm/g and56146.91 cpm/g respectively and 87619.29 cpm/ organ and 90314.4 cpm/ organ in shoots of low and high Zn types respectively. The mean activity of ^{65}Zn in roots of low and high Zn types is 133206.2 cpm/g and 95782.66 cpm/g respectively. And the root cpm/ organ is 394202 and 287456 cpm/ organ in low and high Zn types respectively. The mean shoot Zn content in case of high Zn types is higher compared to low Zn types indicating the higher translocation compared to low Zn types. The mean root Zn content of low Zn types is higher (133206.2 cpm/g and 394202.1 cpm/ organ) compared to high Zn types (95782.66 cpm/g and 287456 cpm/ organ). This data indicating that even though the root uptake of low Zn types is higher they are unable to translocate it into the shoot. The highest shoot Zn 204825/ organ and in root 959950 cpm/ organ was recorded for the genotype GPU-28 that was classified as high Zn type. This genotype is showing highest root uptake as well as shoot translocation though the mean root uptake of high Zn types are lower compared to low Zn types (Table 14 and Figure 12).

These calculations were also made based on per gram basis to avoid the variations obtained due to differences in shoot and root weight between genotypes. There was similar trend as was seen, when calculated on per organ basis.

There is significant variation among individual genotypes in terms of Zn uptake and translocation. Among low Zn types the genotype GEC-488 shows low mean stem uptake of 24,482.5 cpm/organ and the genotype GEC-403 shows highest uptake of 1,99,893 cpm/ organ. The genotype GEC- 460 shows low mean root uptake of 98,845 cpm/ organ and the genotype GEC-236 shows highest root uptake of 7,29,335 cpm/organ. Among high Zn types, the genotype GEC-271 shows lowest mean stem uptake of 7130 cpm/ organ and the genotype GPU-28 shows highest uptake of 1,94,420 cpm/ organ. The mean root uptake is lowest in the genotype GPU-45 (1,40,950 cpm /organ) and highest in GPU-28 (9,59,950 cpm /organ).

By comparing shoot to root ratio and root to shoot ratio, estimated the uptake by root and translocation of Zn from root to shoot. The mean root to shoot ratio of low Zn types is 5.14,

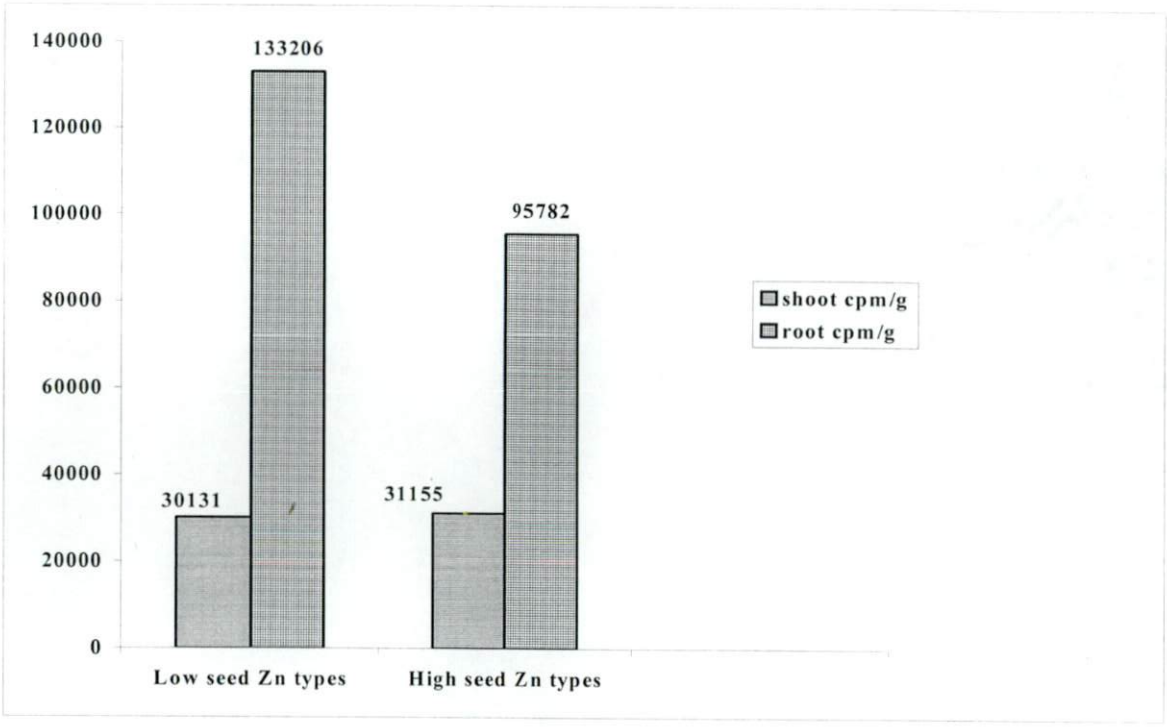


Figure 14 Shoot and root uptake of ^{65} Zn across different Finger millet genotypes differing in their seed Zn content

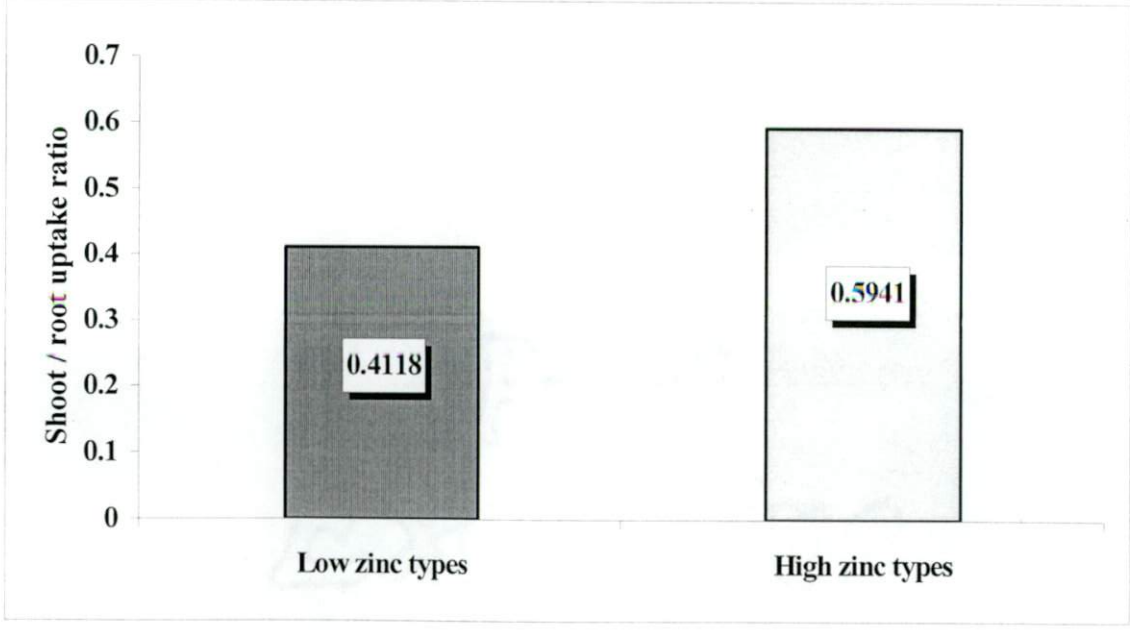


Figure 15 Differences in shoot to root uptake of ^{65} Zn across low and high seed Zn types

Table 14: Variations in shoot and root uptake of ^{65}Zn as influenced by internal Zn content

	High Zn types						
	Genotypes	Shoot wt. (g)	Shoot cpm/g	Shoot cpm/organ	Root wt.	Root cpm/g	Root cpm/ organ
1	GEC-236	1.54	20553.31	57695	1.66	247393.8	729335
2	GEC-265	1.91	34123.35	105655	2	229376.8	663995
3	GEC-403	1.925	51657.64	135160	1.77	153486.4	563510
4	GEC-520	1.94	20286.54	72365	1.92	178348.8	453365
5	GEC-488	1.535	15050.81	47090	1.61	51073.8	130490
6	GEC-460	2.555	34495.05	95755	2.64	35026.16	98845
7	Indaf-5	2.235	34756.72	99615	2.1	37737.63	119875
	Mean	**1.948**	**30131.92**	**87619.29**	**1.957**	**133206.2**	**394202.1**
	Range	1.53-2.55	15050.81-51657	47090-135160	1.61-2.64	35026.1-247393	98845-72933
	Low Zn types						
	Genotypes	Shoot wt.(g)	Shoot cpm/g	Shoot cpm/organ	Root wt.	Root cpm/g	Root cpm/ organ
8	MR-6	1.765	12441.15	27070	1.61	123868.4	441650
9	PES-400	1.885	30595.53	50120	1.92	78193.41	232165
10	GEC-45	1.28	54334.94	78515	1.31	41845.86	144940
11	GEC-108	1.585	70043.59	113790	1.53	46516.95	150180
12	GEC-94	1.68	111437.6	135590	1.63	27292.83	149980
13	GEC-271	1.645	17294.44	45000	1.61	70907.01	165615
14	GEC-21	1.75	47862.1	73335	1.8	75021.79	201645
15	GPU-45	1.775	47089.17	84600	1.84	45095.78	140950
16	GPU-28	2.15	114223.7	204825	2.24	353301.9	959950
	Mean	**1.723**	**56146.91**	**90316.11**	**1.721**	**95782.66**	**287456.1**
	Range	1.28-2.15	12441.1-114223.7	27070-204825	1.31-2.24	27292.8-353301.9	140950-9599

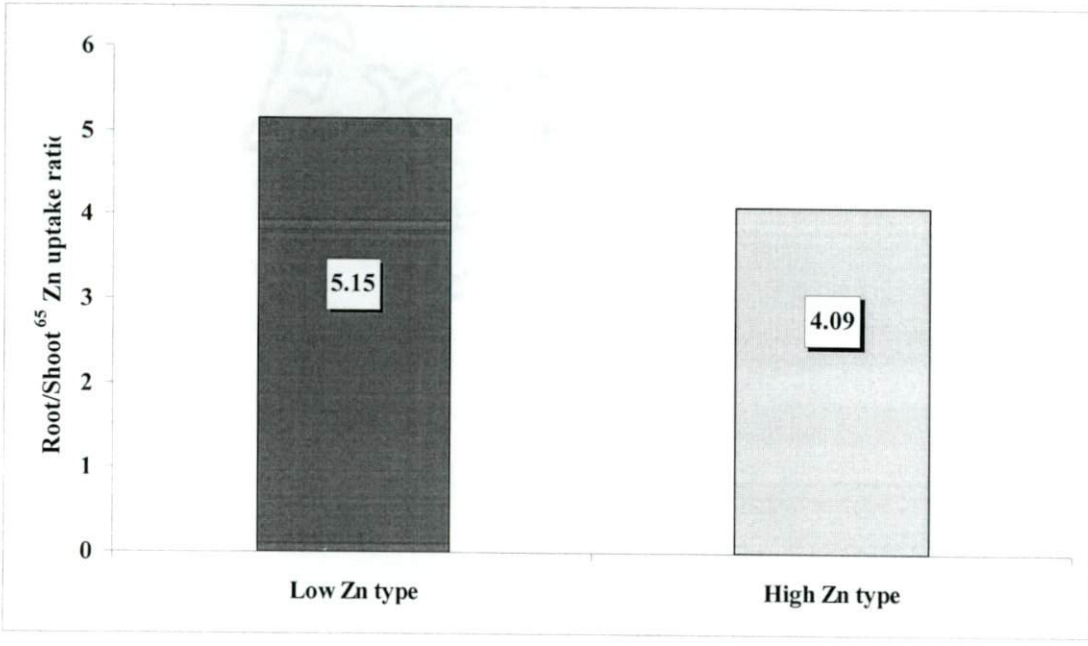


Figure 16: Differences in root to shoot uptake of ^{65}Zn across low and high seed Zn types

which is higher than high Zn types (4.09). The mean shoot to root ratio of low Zn types is 0.41, which is lower than high Zn types (0.59). The results indicate that the low Zn types are efficient in Zn uptake and slightly less efficient in translocation. High Zn types are efficient in Zn uptake as well as efficient in translocation. Among individual genotypes, GEC-236, 265,520 in low Zn types and MR-6, GPU-28 in high Zn types shows high Zn uptake as their root to shoot ratio is comparatively higher than other genotypes. The genotypes GEC-403, 460 and Indaf-5 among low types and the genotypes GEC-45, 108,94,21 and GPU-45 among high types show high translocation (Table 15 and Figure 16).

The amount of Zn entered into the whole organ (Shoot or Root) was estimated. The total ^{65}Zn entered into the root and shoot was considered as 100 per cent and the percentage distribution of ^{65}Zn to individual organ i.e., to shoot and to root was calculated. There was significant variation among the genotypes both in terms of uptake and translocation.

The percentage distribution of ^{65}Zn to roots to shoots shows that, the mean allocation of ^{65}Zn to roots is higher in case of low Zn types (81.80%) compared to high Zn types (76.09%). The mean percentage allocation of ^{65} Zn to shoots in case of low Zn type is lower (18.18%) compared to high Zn types (23.9%). This result reconfirms that the, low Zn types are efficient in Zn uptake compared to high Zn types and high Zn types are efficient in translocation compared to low Zn types.

The percentage allocation of ^{65} Zn to roots and shoots shows that, the roots of most of the genotypes uptakes 5-6 fold higher Zn than shoots and only small portion is translocated to shoot and other aerial parts (Figure 13, 18).

The genotypes which maintained high root zinc both under control and with the external application of Zn are efficient in uptake and the genotypes which maintained high shoot zinc both under control and with the external application of Zn are efficient in translocation. The genotypes with high Zn content both in root and shoot will be the desirable source to study the physiological, biochemical and molecular mechanisms of uptake and translocation of zinc and they are the probable genotypes to be select and released as a variety with high Zinc content in shoot and grains and with high yield.

Table 15: Variations in shoot and root uptake of ^{65}Zn as influenced by internal Zn content

High Zn types					
	Genotypes	Shoot/root ratio	Root/ shoot ratio	% cpm shoot	% cpm root
1	GEC-236	0.083	12.036	7.670	92.329
2	GEC-265	0.148	6.721	12.950	87.049
3	GEC-403	0.336	2.971	25.181	74.818
4	GEC-520	0.113	8.791	10.212	89.787
5	GEC-488	0.294	3.393	22.761	77.238
6	GEC-460	0.984	1.015	49.618	50.381
7	Indaf-5	0.921	1.085	47.944	52.055
	Range	0.083-0.98	1.01-12.03	7.67-49.61	50.38-92.3
	Mean	0.411	5.145	25.191	74.808
	SD	0.353174	4.175393	17.32738	17.32738
Low Zn types					
	Genotypes	Shoot/root ratio	Root/ shoot ratio	% cpm shoot	% cpm root
8	MR-6	0.072	13.881	6.719	93.280
9	PES-400	0.213	4.680	17.603	82.396
10	GEC-45	0.686	1.456	40.714	59.285
11	GEC-108	0.863	1.158	46.329	53.670
12	GEC-94	2.212	0.451	68.875	31.124
13	GEC-271	0.220	4.414	18.468	81.531
14	GEC-21	0.328	3.047	24.706	75.293
15	GPU-45	0.578	1.729	36.636	63.363
16	GPU-28	0.166	6.015	14.253	85.746
		0.072-0.86	0.451-13.88	6.719-68.8	31.12-93.2
	Mean	0.594	4.092	30.478	69.521
	SD	0.662	4.115	19.491	19.491

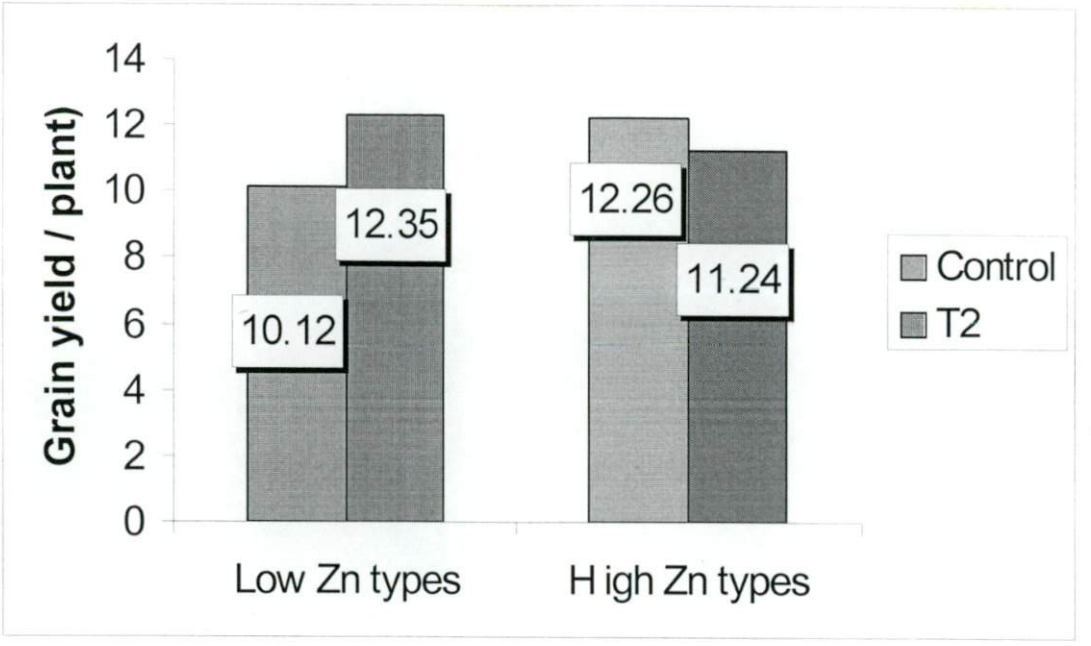


Figure 17: Effect of external Zn on grain yield of low and high Zn types

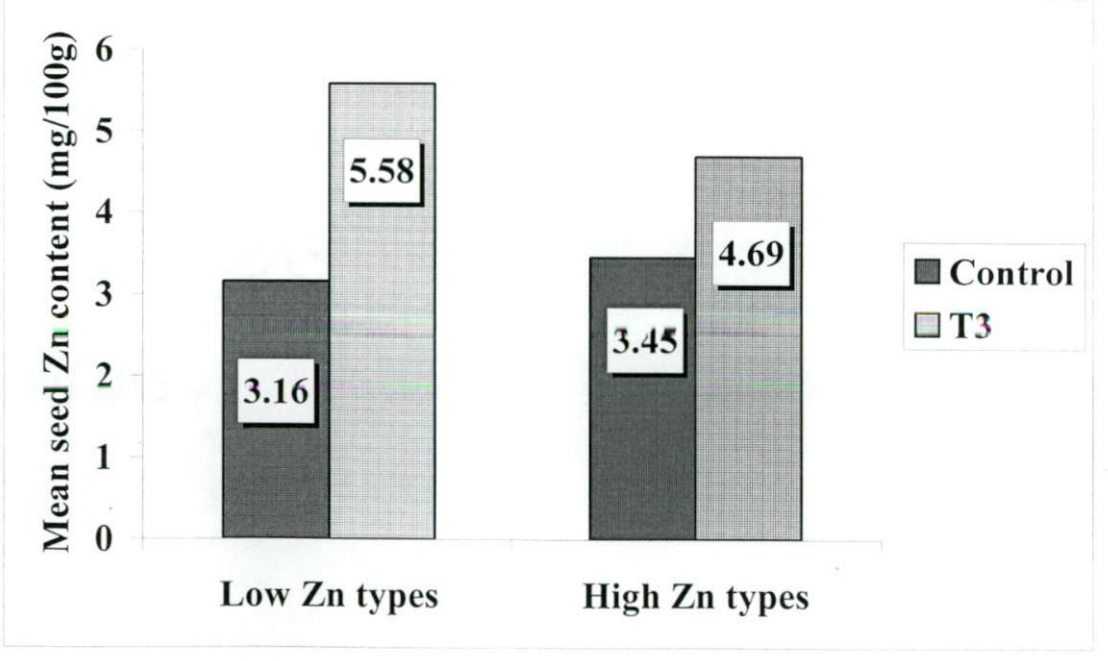
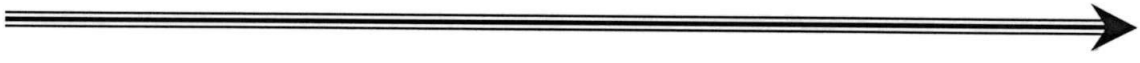


Figure 18: Seed Zinc content of low and high Zn types when grown under high soil Zn status

Discussion



V. DISCUSSION

Zinc deficient soils are common all over the world in both tropical and temperate climates, and Zn deficiency is considered the most widespread micronutrient deficiency (Graham *et al.,* 1992). According to Graham and Welch (1996) about 50% of the soils used for cereal production in the world contain low levels of plant-available Zn, which not only reduces grain yield but also nutritional quality of grains. Cereal crops are most vulnerable to Zn deficiency and leads to severe reduction in yield. A zinc deficiency usually exerts indirect effects by decreasing the parent-plant growth and development, thus resulting in reduced seed vigor and viability. When such seeds are resown on Zn deficient soil, seedling vigor, vegetative growth and yield potential may be affected (Asins, 2002). Zinc deficiency among women, infants and children may be as widespread globally as iron deficiency because the dietary sources of the most bioavailable Fe are also the most important sources of bioavailable Zn in people. A diet, which results in Fe deficiency, therefore, is likely to also predispose an individual to Zn deficiency. Unfortunately, there is no reliable clinical diagnostic criterion available to determine Zn status in humans accurately. For this reason the prevalence of Zn deficiency in the world is still uncertain, but its prevalence is presumed to be very extensive among poor pregnant women, infants and children of many developing countries. When Zn supplements are given to poor children, growth rates increases demonstrating the prevalence of Zn deficiency (Raboy, 2002). Since most of the vulnerable population including resource poor women, children and infants depends on cereals, which are deficient in Zn for their sustenance they are suffering from various disorders. Zn malnutrition not only affects the health, well being and livelihood of all those individuals and families afflicted, but it also adversely impacts programs to control population growth, societal stability and national development efforts. The future challenge is to overcome micronutrient malnutrition by developing crop species that are rich in dietary zinc. The amount of Zn present in soils itself is less and the little amount present in soils is immobilized by conversion of soluble Zn to less soluble form by alkaline or calcareous soils (Welch and Graham, 2004).

Fertilization is not always an option to resolve Zn deficiency because of agronomic and economic factors, such as the relatively high cost of fertilizer (Graham and Rengel, 1993).

Alternatively, exploiting genetic variability to breed staple crops with high Zn efficiency (ZE) could offer a sustainable and cost-effective way to overcome Zn deficiency problems.

Zinc efficient types absorb more Zn from deficient soils; produce more dry matter and grain yield. The physiological basis for micronutrient efficiency in crop plants and the processes controlling the accumulation of micronutrients in edible portions of seeds are not understood with any certainty (Welch and Graham, 2004). There are a number of different possible mechanisms by which plants tolerate Zn deficiency (generally expressed as Zn efficiency), such as Zn uptake, translocation to the shoot and physiological efficiency. However, there have been no direct comparisons of the relative importance of these possible mechanisms of Zn efficiency in a large set of genotypes of contrasting Zn efficiency.

Genotypes of a given species differ greatly in their response to Zn deficiency and Zn fertilization, indicating existence of genetic variability among species. (Cakmak, 2002). There are mechanisms other than release of organic acids from roots that are involved in tolerance to Zn deficiency, such as altering in root uptake, root to shoot transport rates and internal Zn utilization (Cakmak *et al.*, 1998). Since Zn-efficient genotypes that resist the appearance of visible Zn-deficiency symptoms, in several different plant species have been shown to maintain enhanced activity of Zn requiring enzymes such as Cu/ Zn SOD and CA in genotypes. We speculate that biochemical Zn utilization may be one of the underlying mechanisms of Zinc efficiency in plants. There is considerable varietal and species variation in uptake and accumulation of Zn by plants (Holm *et al.*, 2002). Genetic variation in a plant's ability to resist Zn-deficiency termed zinc efficiency has been documented for a number of crop species. Existence of substantial genotypic variation in tolerance to Zn deficiency can be exploited to enhance tolerance, but little information is available on how tolerance to Zn deficiency is inherited.

The uptake of trace elements by roots and the accumulation of trace elements by seed or grain is likely to be limited by the capacity of plant to take up and transport these ions. The physiological basis for these aspects is not yet fully understood (Welch, 2002; Ramesh *et al.*, 2004). Research groups are searching for genes, determining high Zn levels and several candidate genes have been identified in wheat on chromosome 6A and 6B (Cakmak *et al.*,

1999). Therefore integrating molecular biology based research with knowledge provided by physiological basis for plant nutrition is essential to better understand and characterize nutrient uptake and transport to edible parts.

The major emphasis of this study was to probe the physiological and biochemical reasons for differential uptake and translocation of Zn in different Finger millet germplasm lines and identify the high and low Zn types. This study also focuses on determining the grain Zn content, assaying the levels of important Zn requiring enzymes like copper/ zinc superoxide dismutase and carbonic anhydrase and also trace the uptake and translocation of Zn using ^{65}Zn radiolabel isotope.

5.1. Effect of varied Zn status on physiological parameters

Screening of 16 contrasting genotypes showed significant variation in seed Zn content, ranging from as low as 0.98 to10.05mg/100g. The Zn content of selected low Zn types varies from 0.98 –2.22 mg/100g and that of high Zn types varies from 5.10-10.05mg/100g. This indicates the fact that plant species show not only significant interspecific but also intraspecific variation among genotypes for Zn content. It is necessary to understand whether it is genetically controlled or is influenced by environment. The genotypes that have high grain Zn content could be the probable candidates in terms of human nutrition to supply the recommended dietary intake of Zn and are the sources to dissect the molecular basis, for high seed Zn content. This variation among both high and low genotypes offers opportunities for breeding as a tool to resolve Zn deficiency problems in Finger millet genotypes. The genotypes, which have high Zn content, showed high chlorophyll content and high photosynthetic rate may be due to the synthesis of Zn requiring enzymes like carbonic anhydrase, which provides carbon dioxide substrate for photosynthesis. The genotypes with high Zn content will have protection from oxidative damage by maintaining high super oxide dismutase enzyme.

Vast research is going on to understand how some plant genotypes can maintain reasonable growth and yields under low soil Zn, a trait termed zinc efficiency (ZE).

Several studies have shown no correlation between ZE and root Zn uptake, Zn translocation to shoot, or shoot Zn accumulation. Furthermore, it has not been possible to conclusively link differences in leaf sub cellular Zn compartmentation with zinc efficiency. However, biochemical Zn utilization, including the ability to maintain, activity of Zn requiring enzymes in response to Zn deficiency may be a key component of Zinc efficiency. The next logical step in investigations of this trait is to dissect the genetic and molecular basis for ZE, in order to have better understanding of the relationship between Zn utilization and ZE, and to identify the gene(s) controlling ZE. Progress in this research area would facilitate engineering plants to develop Zn efficient varieties, which could help improve, crop production on marginal soils as well as possibly improve the micronutrient density of food crops to help address significant human micronutrient deficiency problems. A more efficient and sustainable solution to Zn deficiency limitations to crop production is the development and use of Zn-efficient plant genotypes that can more effectively function under low soil Zn conditions, which would reduce fertilizer inputs and protect the environment as well. It has been well documented that certain plant species, as well as genotypes within certain species, exhibit a significant genetic-based variation in their tolerance to Zn deficiency. A number of studies have demonstrated differential ZE in several crop species, including bean, wheat, rice, and tomato. The precise mechanisms underlying ZE is not clear yet. Although differential ZE between genotypes of the same plant species has been widely reported, limited information is available on the genetics of zinc efficiency.

The contrasting Finger millet genotypes vary significantly with respect to some physiological parameters like photosynthetic rate and biomass production. The Zn have advantage in terms of enhancing growth parameters. In the present investigation zinc deficiency strongly affected the growth of low Zn types. Relationship between yield and external zinc supply were broadly similar among low and high seed Zn types. Visual symptoms of zinc deficiency in Finger millet are yellowing, reduced internodal length and dwarfing. Though the deficiency symptoms were not so pronounced in Finger millet like other species, it led to severe reduction in yield and the low seed Zn types were more susceptible to Zn deficiency. Some of these effects may be the result of changes in growth rates at the apical meristem. This is likely to be due to reduced IAA concentrations, since

zinc is required for the synthesis of tryptophan. Apart from its role in auxin production zinc plays an integral part in the activity of carbonic anhydrase.

5.2. Relationship between the Zn status and Zn containing enzymes – Carbonic anhydrase and copper/ Zinc superoxide dismutase activity

Zn is an essential component of more than 300 enzymes, thus, the biochemical processes involved in associating cellular Zn with these metalloenzymes could be part of the ZE trait. Despite the large number of Zn requiring enzymes in eukaryotes, only a few of these enzymes have been studied in detail. A second well-characterized Zn-requiring enzyme is carbonic anhydrase (CA); in fact, it has been suggested that CA activity could be used as an indicator for diagnosing Zn deficiency in plants (Bar-Akiva and Lavon, 1969). Zinc deficiency results in decreased activity of CA, especially in Zn-inefficient durum wheat genotypes (Rengel, 1995). In a study with rice (*Oryza sativa*) plants, Bar-Akiva *et al.*, found that the level of CA mRNA decreased under Zn deficiency. The findings presented here indicate that the ability of Zn-efficient genotypes to maintain higher activity of Zn-requiring enzymes in the face of Zn deficiency is correlated with ZE. Carbonic anhydrase is a catalytic Zn requiring metalloenzyme that reversibly catalyses the hydration of CO_2 to yield HCO^{3-}.

The selected Finger millet genotypes show significant variation in the activity of important Zn containing enzymes like copper zinc superoxide dismutase and carbonic anhydrase. In both high and low seed Zn types there is significant increase in the activity of these enzymes as the external application of Zn increases, mainly because of the reason that Zn is the constituent of these enzymes. The high Zn types show significantly high level of activity of these enzymes compared to low Zn types.

The Zn efficient Finger millet genotypes could maintain a somewhat higher CA activity than in Zn inefficient genotypes under Zn deficiency. From these results we can tell that Zn-efficient genotypes could be benefited from higher CA levels that could help maintain higher photosynthesis rates and dry matter production.

Copper/ Zinc superoxide dismutase (Cu/Zn SOD) plays an important role in protecting plants against oxidative damage catalyzed by reactive oxygen species (Marschner and Cakmak, 1989). Because Zn is directly involved in both gene expression and protein

synthesis, Cakmak (2000) has speculated that Zn deficiency may inhibit the activities of a number of antioxidant enzymes, resulting in extensive oxidative damage to membrane lipids, proteins, chlorophyll, and nucleic acids.

The significantly lower activity of the Cu/ Zn SOD enzyme in the Zn-inefficient genotypes under Zn deficiency conditions suggests that Zn might be related to activity of this enzyme. Previous reports showed a positive correlation between Cu/ Zn SOD activity and ZE among and within cereal species (Cakmak et al., 1997). A similar pattern was also found with the other Zn containing enzyme, CA.

5.3. Zinc uptake and translocation among contrasting Finger millet genotypes - ^{65}Zn studies

There is significant variation among high and low seed Zn types in both uptake and translocation of ^{65} Zn. Both under field and pot experimental conditions Zn-efficient genotypes had a higher Zn uptake capacity than Zn-inefficient genotypes. The uptake of ^{65} Zn in leaves of low Zn types is higher compared to high Zn types; this could be because the high Zn types already have high intrinsic Zn. But ^{65}Zn studies revealed that contrastingly Zn efficient plants under adequate Zn supply had much higher uptake rates as revealed by ^{65}Zn studies. However, under Zn-deficient conditions the rate of Zn uptake markedly differed between the cultivars.

Among the cultivated cereal species, rye is known to have an exceptionally high Zn efficiency (Cakmak *et al.*, 1997). The reports suggests that enhancement in the Zn uptake rate under Zn-deficient conditions is an important plant mechanism determining expression of high Zn efficiency in rye. Differences in the Zn uptake rate are also shown within genotypes of cereal species such as rice and sorghum (Cakmak et al., 1997).

The uptake of the relatively immobile Zn^{2+} by plant roots can be determined by root surface area, root-induced chemical rhizosphere changes and the physiological uptake capacity per gram root. An increase in uptake due to increased surface area can be achieved by larger root system. The second statistically significant variable determining ZE is Zn translocation from roots to shoots as Zn absorbed by roots can be rapidly transported to shoots. Higher Zn

translocation was found to be a mechanism to explain the genotypic differences in ZE among chickpea and wheat genotypes (Khan *et al.*, 2003).

Shoot Zn concentration and ZE were found to be positively related in Finger millet, contrary to what was found for wheat (Cakmak et al., 1997), chickpea (Khan et al., 1998) and common bean (Hacisalihoglu *et al.*, 2003). This indicates that low internal Zn requirement was not found to be a mechanism involved in ZE of rice. Shoot Zn concentration is a contributing factor to variation in ZE because it correlates with both Zn uptake and Zn translocations.

The three traits that were examined, uptake, partitioning and Zinc efficiency, varied independently. Under Zn deficiency, Zn uptake and the proportion of Zn translocated to shoot is less, but Zinc efficiency increased, but the magnitude of these changes differed between genotypes.

Salient findings of the study are

1. The genotypes containing high Zn produces high biomass and yield under Zn deficient condition.
2. There exists a genetic variability among high and low Finger millet genotypes for seed Zn content.
3. High and low Finger millet genotypes respond differentially for external Zn application.
4. High Zn types produce high biomass and yield even under Zn deficient condition.
5. There exists a positive correlation between Zn efficiency and important Zn requiring enzymes.
6. External Zn application enhances the activity of both copper zinc superoxide dismutase and carbonic anhydrase enzymes.
7. Both high and low Zn genotypes differ significantly in uptake and translocation of ^{65}Zn.
8. External Zn application enhances Zn uptake in case of low Zn types than high Zn types and translocation will be enhanced in high Zn types compared to low Zn types.

9. Some genotypes could be selected for high Zn uptake and translocation to grains and they may serve as a source material for breeding or for future line of work or could be released as a variety with further detailed analysis.

Summary

VI. SUMMARY

With the world population is expanding rapidly and limited availability of additional arable land and water resources along with declining trends in crop yields, globally make food security a major challenge in the 21st century. According to the projections, food production on presently used land must be doubled in the next two decades to meet the food demand of growing world population. To achieve the required massive increase in food production, large enhancements in application of fertilizers and improvements of soil fertility are indispensable approaches. Presently, in many developing countries, poor soil fertility, low levels of available mineral nutrients in soil, improper nutrient management, along with the lack of plant genotypes having high tolerance to nutrient deficiencies are major constraints contributing to food insecurity, malnutrition (i.e., micronutrient deficiencies) and ecosystem degradation. Plant nutrition research provides invaluable information highly useful in elimination of these constraints, and thus, sustaining food security and well being of humans without harming the environment.

The fact that at least 60% of cultivated soils have growth-limiting problems with mineral-nutrient deficiencies and about 50% of the world population suffers from micronutrient deficiencies, make plant nutrition research a major promising area in meeting the global demand for sufficient food production with enhanced nutritional value in this millennium. Integration of plant nutrition research with plant genetics and molecular biology is indispensable in developing plant genotypes with high genetic ability to adapt to nutrient deficient soil conditions and to allocate more micronutrients into edible plant products such as cereal grains.

Micronutrients, such as Iron and Zinc deficiencies are critical issues in the developing countries, and result in severe impairments of human health and development such as impairments of physical growth, immune system, cognitive development and enhancements in anemia and maternal mortality (Pinstrup-Andersen, 1999; Welch and Graham *et al.*, 2001). Micronutrient deficiencies are also widespread in well-developed industrialized countries. More than 3 billion people globally suffer from Fe and Zn deficiencies alone (Graham *et al.*, 2001). Heavy and monotonous consumption of cereal-based foods with low

concentrations and reduced bioavailability of Fe and Zn has been considered a major reason for the widespread deficiencies of Zn and Fe in developing countries (Graham *et al.*, 2001;Welch and Graham, 1999). According to Graham and Welch (1996), nearly 50% of the soils cultivated for cereal production globally have low levels of plant available Zn.

Increasing the concentrations of Zn and Fe in cereal grains is a high priority research task, and will greatly contribute to the alleviation of micronutrient deficiencies in human population worldwide. Enhancements in concentration of micronutrients, especially Zn, also result in several positive consequences for crop production, such as improvements in seedling vigor, pathogen resistance, competition against weeds and, finally, an enhanced yields (Graham and Rengel, 1993).

One important strategy to increase Zn concentrations in grains is fertilization of plants via soils or foliar applications. An alternative approach to increase Zn concentration in grain is to exploit the genetic variation in grain concentrations of this micronutrient within plant genome. Application of fertilizers is not cost effective and it is not successful always because of subsoil constraints. Therefore the production of crop species with high grain Zn content through selection or other breeding approaches will be a sustainable strategy.

The variations observed in grain Zn content could be due to differences in availability of Zn in soils and uptake differences between crops. Plant species display variation between and within species in their uptake from soil and translocation to aerial parts, there are certain plants native to mineral rich soils, which accumulate high levels of Zn, which otherwise is toxic to normal crop plants.

It is essential to understand the physiological and biochemical reasons for differential uptake and translocation of Zn within and between crop species, so as to exploit the existing genetic variation to improve crop plants to deliver plants with high Zn in edible parts.
With this preface, this study mainly focuses on assessing the grain Zn content in Finger millet genotypes and selection of contrast (high and low) types. The activities of important Zn requiring enzymes like copper-zinc superoxide dismutase and carbonic anhydrase was assessed to know the relevance of Zn content and activity of these enzymes. In addition, the

uptake and translocation of Zn was studied in these genotypes to understand the variation in high and low seed Zn types.

Grain Zinc content across finger millet genotypes

There is significant variation in grain Zn content among high and low Zn types. The seed Zn content of genotypes vary from as low as 2.07mg/ 100g to as high as 9.76 mg/ 100g.
Both high and low types responded differentially to external Zn supply. The genotypes with high seed Zn content will also have significantly high-level of photosynthetic rate and other physiological parameters. These genotypes are again grouped into efficient and inefficient based on Zn efficiency by considering performance under Zn deficient condition. The variation in uptake and translocation in these genotypes also studied. A significant variation between high and low seed Zn types in terms of uptake and translocation of Zn under different soil Zn status was recorded.

Activity of copper-zinc superoxide dismutase and carbonic anhydrase in contrasting seed Zn types

High and low Zn types vary significantly with respect to the activity of this important Zn containing enzymes. The activity of Cu-Zn superoxide dismutase varies from 113.43 enzyme units per gram fresh weight to as high as 588.87 units per gram fresh weight. The activity of carbonic anhydrase enzyme also varies significantly with external application of Zn. The activity ranges from as low as 143 units per gram fresh weight to 3642.85 units per gram fresh weight. The activity of these enzymes increases significantly with external application of Zn. There seems to be relevance of Zn application on the activity of these enzymes.

Tracing uptake and translocation of Zn using ^{65}Zn

The genotypes showed significant variation in uptake and translocation, both in high and low Zn types. Most of the low Zn types had high uptake and less translocation under high

levels of soil Zn, compared to high Zn types showing a similar pattern under high soil Zn levels. The shoot uptake in case of low Zn types is lower compared to high Zn types for unknown reasons. The shoot uptake of ^{65}Zn in case of low Zn types is 46,132.1 cpm/organ and 72,124.4 cpm/organ in case of high Zn types. The uptake of ^{65} Zn in roots is higher in case of low Zn types compared to high Zn types. The mean root uptake of low Zn types is 3,94,202 cpm/ organ whereas it is 2,87,456 cpm/ organ. Majority of the genotypes show high uptake and translocation either at low Zn levels or at high Zn levels and only few genotypes show low uptake and translocation under both high and low external Zn levels.

Future line of work

These genotypes, which show high uptake and translocation of Zn, can be used as a source material for future work of studying uptake and translocation mechanisms or for breeding purposes to develop transgenics. The genotypes that have high grain Zn content could be the probable candidates to dissect the physiological and molecular basis, for high seed Zn content. Some high Zn Finger millet germplasm lines can be released as a variety with further examination under different environmental conditions. Some screened genotypes can serve as a reserve material with Zn dense seeds to alleviate Zn deficiency in crop plants and as well as malnutrition in human beings.

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