ASSESSMENT OF GENETIC VARIABILITY FOR ZINC CONTENT IN GRAINS AND LEAVES OF PIGEON PEA (*Cajanus cajan mills sp.*) AND PHYSIOLOGICAL CHARACTERIZATION

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BENGALURU **OCTOBER, 2010**

Affectionately Dedicated to
My Beloved Parents, Wife
and Daughter

DEPARTMENT OF CROP PHYSIOLOGY
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CERTIFICATE

This is to certify that the thesis entitled **‘‘**Assessment of genetic variability for Zinc content in grains and leaves of pigeon pea (*cajanus cajan mills sp.*) and physiological characterization**’’** submitted in partial fulfillment of the requirement for the degree of **Master of Science (Agriculture)** in **Crop Physiology** to the University of Agricultural Sciences, Bengaluru, is a record of research work carried out by **Mr. Manoa IRANACOLAIVALU, ID No. PAK 8155**, during the period of his study in this University 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.

Bangalore
OCTOBER, 2010

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"Let all those rejoice who put their trust, love, affection in me"

I think it is not easy to effectively put one's cordial feeling into words that too for a person like me. Therefore, whatever I write here is only a reflection of the feelings that is in my heart.

At last the moment has come to look into deeper layers of my heart, which is filled with the feelings of togetherness, loveliness, consolidation, satisfaction, a sign of relief and fulfillment. Some are momentary and some are permanent, but both involve a member of near and dear persons to whom I acknowledge my warm regards and take this opportunity to express my feelings in black and white.

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Bengaluru-65 *Manoa Iranacolaivalu*

Assessment of Genetic Variability for Zinc Content in Grains and Leaves of Pigeon pea (*Cajanus cajan mills sp)* and Physiological Characterization.

Manoa Iranacolaivalu., ID No. PAK 8155

Thesis Abstract

Zinc is an essential micronutrient for both plants and humans, and zinc deficiency is known to cause several health hazards in humans afflicting three billion people globally. In order to overcome this disorder several strategies are employed including supplementation, fortification, diversification and biofortification. Among these strategies, biofortification is a promising solution. Available literature and data suggests that there is significant genetic variation exist both among and within plant species.

With this background an attempt was made to assess the genetic variation in seed zinc content and physiological parameters of 240 germplasm lines of pigeon pea procured from ICRISAT. Contrast zinc types i.e. high and low zinc types were selected and grown under different zinc treatment (foliar and soil application) in pot experiment to study their zinc uptake.

Screening of seed zinc content in 240 pigeon pea genotypes revealed that there is genetic variation exists among of pigeon pea genotypes (1.53mg to 6.95mg/100g). Physiological parameters including shoot and root parameters also showed genetic variation among the genotypes. The result showed a similarity in the seed zinc content of 240 pigeon pea germplasm lines obtained from ICRISAT and the ones which were grown in the root structure facility in UAS Bangalore. Zinc uptake studies of the contrast zinc types with external zinc application, showed positive response to foliar and soil application and exhibited significant increase in zinc content both in leaf and seed. However, high zinc types accumulated more zinc content than low zinc types. Among the various high zinc types screened, the probable genotype with superior genetic character is genotype 14 and 11 (ICP accession 7119 and 2484) with high uptake and translocation efficiency. Genotype 1 (ICP Accession No. 6443) was performing at a consistent nature with increase zinc content in all plant parts, as well as in yield.

The superior types with high zinc content can be used as donor parent for the crosses with agronomically superior genotypes to develop superior lines (with high grain Zn content). These lines will provide high Zn through natural food to humans. They can be used to develop mapping population for Marker Assisted Selection (MAS) breeding.

October 2010

Dr. A. G. Shankar
Chairman – Advisory Committee

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Introduction

I. INTRODUCTION

The World population is expanding rapidly and most likely that to be 10 billion by the year 2050. According to the projections, food production on presently cropped land must be doubled in the next two decades to meet food demand of the growing world population (Cakmak, 2002). In Contemporary agriculture all the scientists are trying to improve the production and neglecting the quality of produce.

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 tolerant to nutrient deficiencies or toxicities are major constraints contributing to food insecurity and malnutrition. (i.e. micronutrient deficiency).

Besides moisture stress, nutrient deficiency is one of the major stresses limiting crop productivity. Globally, several elements are reported to be below critical level in the soil. Based on a recent survey, zinc deficiency is the most wide spread nutritional disorder, next to Iron, Vitamin A and Iodine. Based on analysis of diet compositions and nutritional needs, it has been estimated that 49% of the world's population (equivalent to 3 billion) is at risk of suffering from zinc deficiency (Tauris *et al*., 2009). These are those who do not meet their daily recommended intake of 15mg/day for an adult resulting in egregious social cost including learning disabilities among children, increased morbidity and mortality rates, lower worker productivity and high health care cost, all factors diminishing human potential, felicity and national economic developments.

Similarly, most of the Indian soils are found to be zinc deficient, hence the most of the food crops contain less amount of zinc in the edible parts. Literature information suggests that there is significant genetic variability to maintain significant growth and yield under zinc deficient conditions (Hacisalihoglu *et al*., 2003). Significant variation across crop species and genotypes exists, not only in their ability for zinc uptake, their sequestration but also in their ability to transport to edible parts.

The specific role of zinc in growth and development of plants is not known. However, this nutrient is an important component of various enzymes that are responsible for driving many metabolic reactions in all crops. Growth and development will stop if specific enzymes are not present in plant tissues. Zinc is a co-factor of more than 300 enzymes and proteins involved in cell division, nucleic acid metabolism and protein biosynthesis (Marschner, 1986).

There are several well known zinc requiring enzymes that have been studied in plants and metal deficiencies, particularly zinc (Lopez-Millan *et al*., 2005). Superoxide dismutase as a Zn requiring enzyme can be a good indicator (Chen *et al*., 2009). 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) and Chen *et al*., (2009), speculated that zinc deficiency stress may inhibit the activities of a number of antioxidant enzymes, resulting in extensive damage to membrane lipids, proteins chlorophyll and nucleic acids.

The second well characterized zinc requiring enzyme is carbonic anhydrase (EC 4.2.1.1) which catalyzes the critical first step of C4 photosynthesis, the hydration of atmospheric CO_2 to bicarbonate (HCO_3) the substrate for carboxylation reaction catalyzed by PEP2 carboxylase (Hatch and Burnell 1990). 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 durum wheat genotypes (Rengel, 1995). Sasaki *et al*, (1998) found that the level of CA mRNA in rice (*Oriza sativa*) decreased under zinc deficiency.

It is unfortunate that the use of zinc fertigation 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 fertigation, a promising alternative may be the identification of zinc efficient genotypes. Tolerance of plant genotypes to soils with low plant-available Zn is usually called Zn efficiency and defined as the ability of a plant to grow and yield well in soils deficient in Zn (Graham, 1984; Sadeghzadeh, 2008). A number of plant species exhibit significant intraspecific variations in zinc efficiency, which appears to be under genetic control (Graham, 1992).

Despite the potential agricultural and economic importance of zinc efficient trait, little is known about the physiological mechanisms of differential zinc efficiency. It is important to understand these mechanisms in order to develop improved cultivars for low input crop production on low zinc soils especially in developing countries. Several mechanisms have been proposed to address zinc efficiency in crop plants including increased zinc uptake, increased bioavailability in the rhizosphere due to release of root exudates and more efficient internal zinc use (Hacisalihoglu *et al*., 2001).

Many laboratories are currently pursuing biofortification using breeding and genetic modification, but progress is challenged by technical hurdles and understanding of physiological processes (Poletti *et al.,* 2004). Both food fortification and daily individual supplementation which are the traditional approaches, face logistic constraints and are often cost ineffective. Dietary modifications are promising but they require behavioural changes that depend on costly education, communication and social marketing strategies and may be difficult to implement (Ruel and Bouis, 1998). Plant breeding along with improved agricultural technologies is another possibility that is pursued for the micronutrient-enriching staple foods to fight against hidden hunger (Welch and Graham 2002).

Research is currently well underway to identify the optimal combination of various approaches that will maximize the effect on human zinc nutrition.

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

1. Assessment of Genetic Variability in seed zinc content (240 lines).
2. Physiological Characterization of 240 pigeon pea germplasm lines.
3. Studying the zinc uptake in contrast lines i.e. high and low Zinc types.

Review of Literature

II. REVIEW OF LITERATURE

Micronutrient deficiencies in plants are becoming increasingly important globally. 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 (Cakmak, 2002). In plants, Zn deficiency reduces not only the grain yield (Cakmak *et al.*, 1996c; Graham *et al.*, 1992; McDonald et al., 2001), but also the nutritional quality of grain (Cakmak, 2008) and ultimately nutritional quality of human diet (Sadeghzadeh *et al.*, 2009). Among micronutrient deficiencies reported to occur worldwide, Zn and Fe deficiencies are of greater importance afflicting more than 3 billion and one-third of the population (Cakmak *et al.*, 2004). These resulted in 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 micronutrient deficiencies are particularly widespread in developing countries where diets are rich in cereal-based foods with low concentration of bio-available Zn and Fe.

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 (Sillanpaa, 1990; Sillanpaa and Vlek, 1985). 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.

Zinc inputs to soils

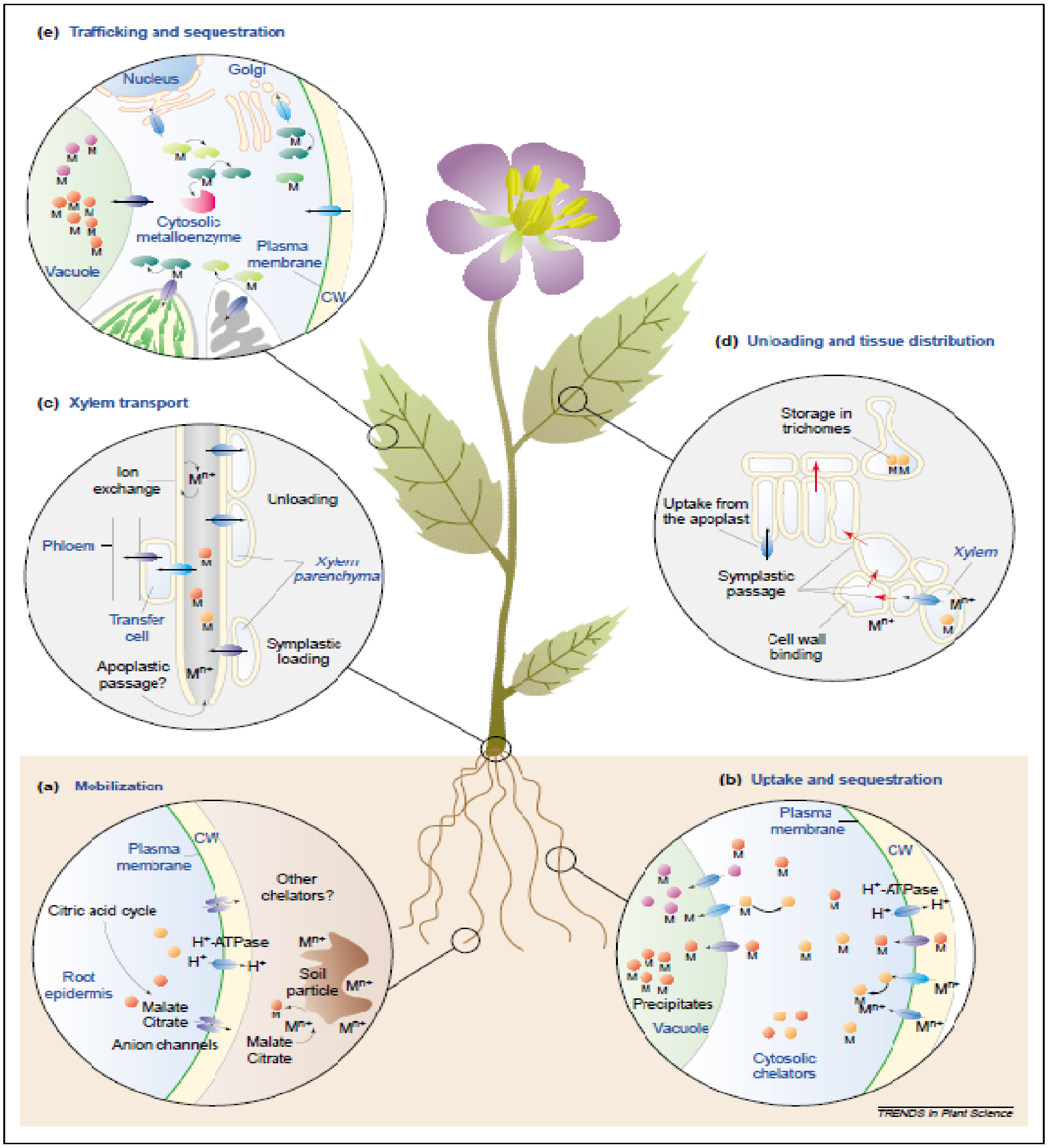
The primary input of Zn to soils is from the chemical and physical weathering of parent rocks. The lithosphere typically comprises 70-80 µg Zn g^{-1}, while sedimentary rocks contain 10-120 µg Zn g^{-1}. Mean soil Zn concentrations of 50 and 66 µg total Zn g^{-1} soil are typical for mineral and organic soils, respectively, with most agricultural soils containing 10-300 /µg Zn g^{-1} (Alloway, 2003). Zinc occurs in rock-foming minerals as a result of the non-specific 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 Homeostasis and Transport to Plant Parts

The primary source of metals for the plant is the soil, thus efficient uptake is essential for life. Accumulation of Zn in grains is controlled by a number of physiological processes (Figure 1) and several barriers have to be overcome to accumulate more zinc in the edible parts of plants (Welch & Graham, 2002) (Figure 2). Even when abundant, metals can be inaccessible in the soil owing to their tendency to be present predominantly in an insoluble form. Zn is primarily insoluble in soils because of adsorption to clay, CaCO3 or organic matter, whereas



Clemens *et al.*, 2002

Figure 1: **Molecular mechanisms involved in transition metal accumulation by plants**

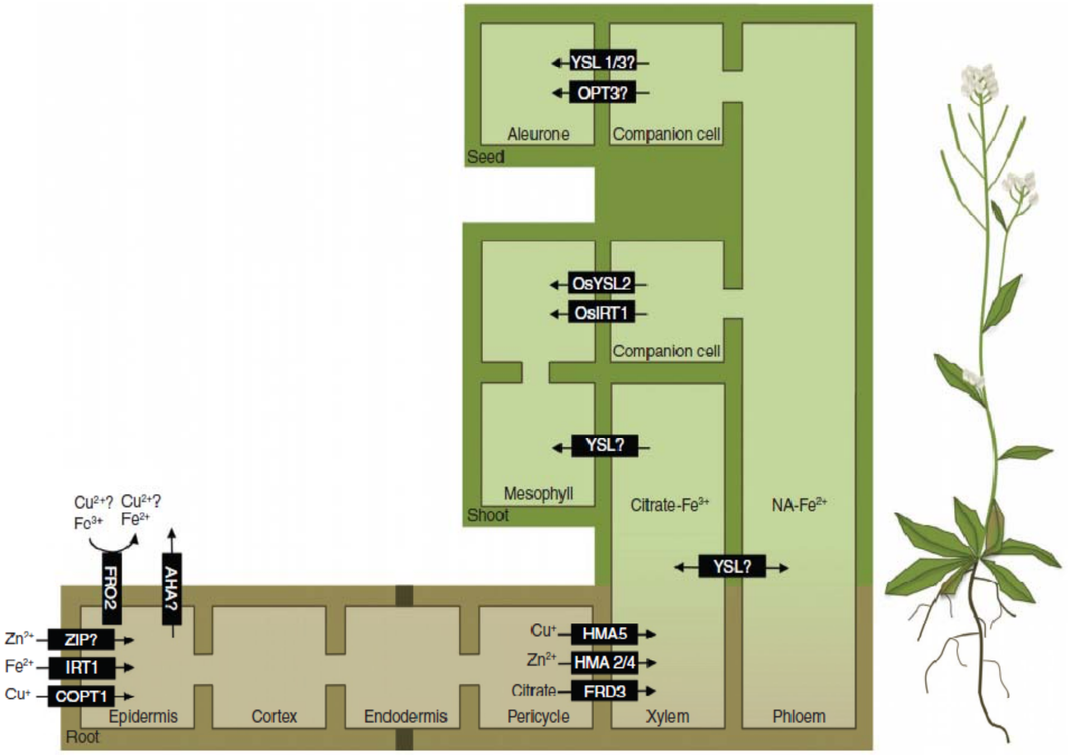
insolubility is particularly pronounced at the higher pH of alkaline soils, which represent approximately 30% of the world’s soils (Palmer and Guerinot, 2009). To deal with the inaccessibility of some metals, non-graminaceous plants rely primarily on a reduction-based strategy of uptake (Figure 2), whereas graminaceous plants (grasses) more commonly use a chelation-based method (Figure 3).

Acidification of the soil

Plant roots contribute to making metal ions more available for uptake by two strategies. First, depending on the nutrient status of the soil, roots acidify the rhizosphere through plasma membrane H+-ATPases, proton pumps in the cell membrane to decrease the pH of the soil under metal-limiting conditions. As the pH decreases, proton concentration in the soil increases results in cation exchange and release of divalent metal ions that are tightly bound to soil particles. ATPase activity also allows for the establishment of a negative membrane potential, along the order of –100 to –250 mV, which serves to drive cation uptake (Palmgren, 2001).

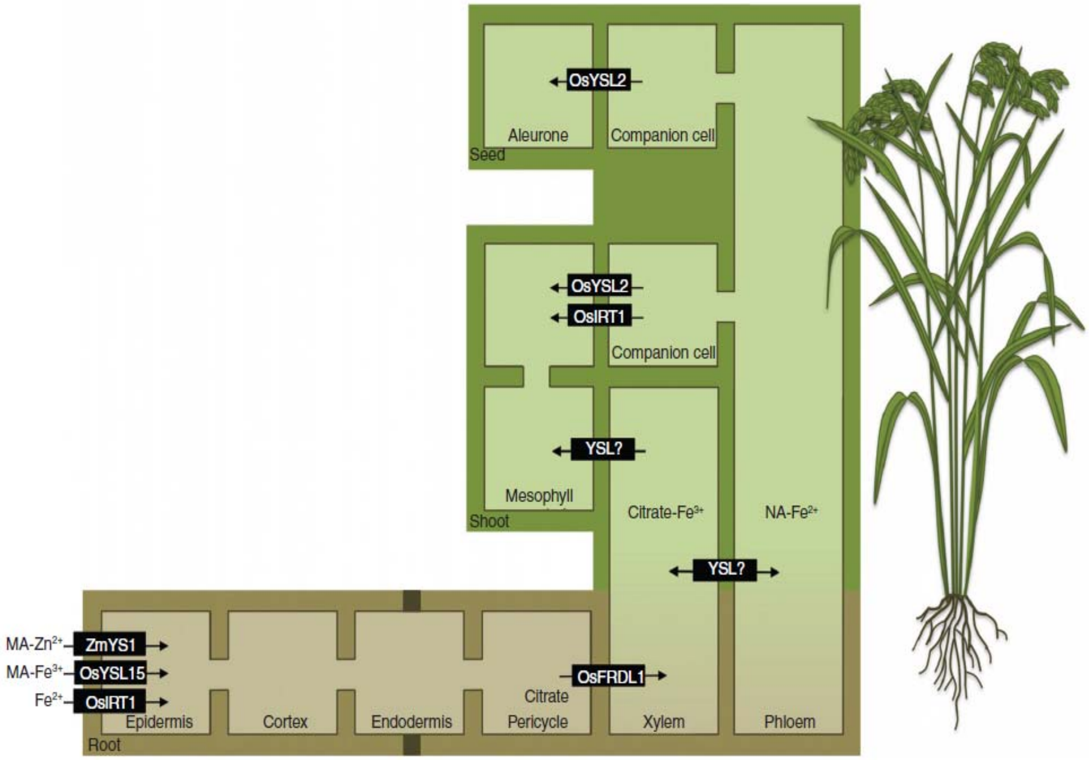
Reduction-based strategy

Once freed from insoluble chelates, the transition metals are more accessible for uptake. However, the transporters in many plants have a specific affinity for a particular oxidation state of each metal. Many plants address this problem by using a reduction-based strategy for metal uptake. Whereas Zn is always found in the +2 oxidation state under physiologically relevant conditions, both Fe (Fe3+ is reduced to Fe2+ by the ferric chelate reductase FRO2) and Cu need to be reduced for uptake by their respective transporters, IRT1 and COPT1 (Puig, *et al*., 2007).



Palmer, 2009

Figure 2: Intercellular metal transport in Dicots



Palmer, 2009

Figure 3: Intercellular metal transport in Monocots

Chelation Based Strategy

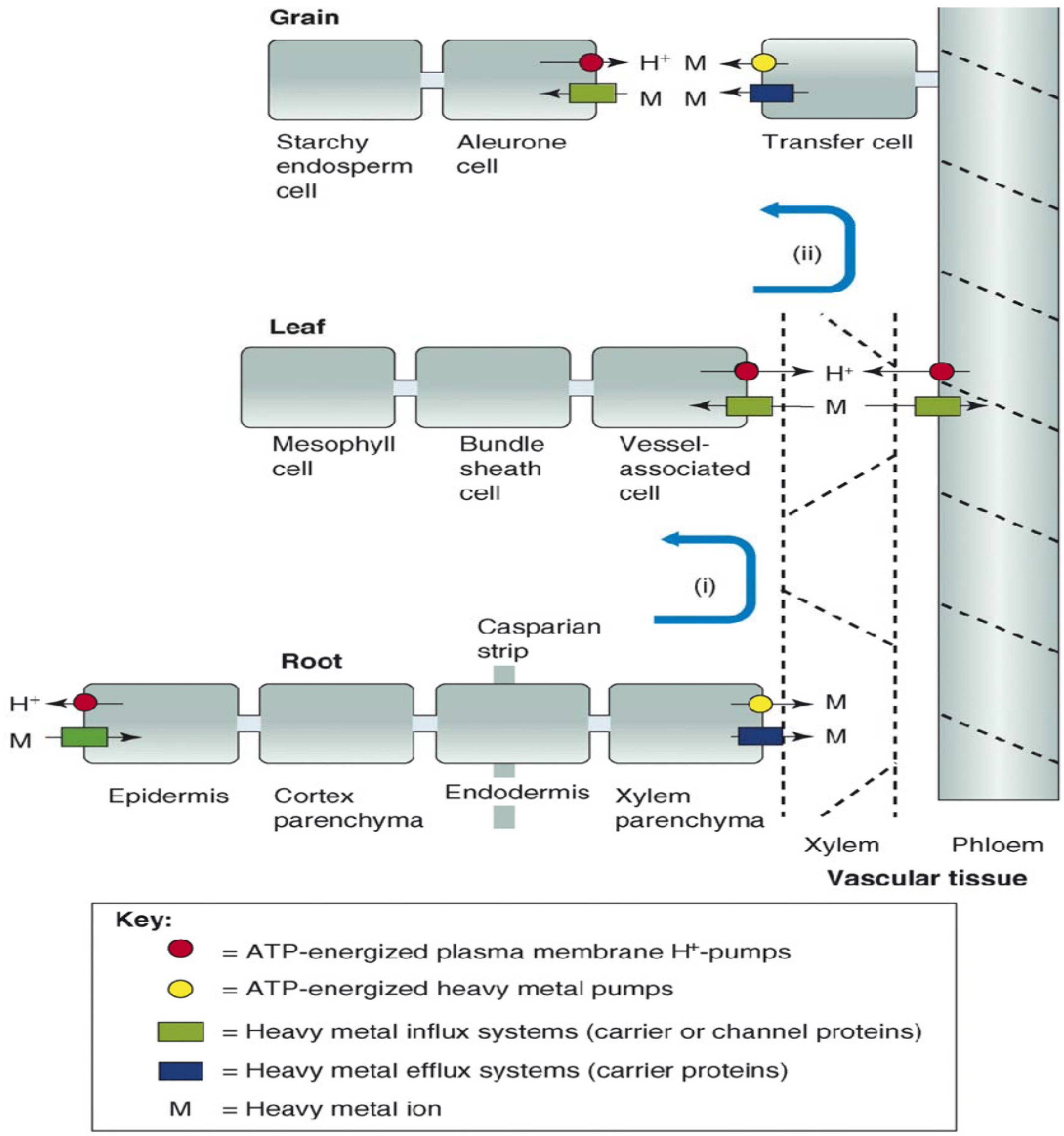
Roots actively secrete low-molecular-weight compounds such as organic, amino acids and phytosiderophores which can change the dissolvability, adsorption and desorption, fractions, and migrations of heavy metals in soils through dissolution, chelation and oxidation/reduction (Clemens *et al.,* 2002; Kuand *et al.*, 2003; Wei-Hong *et al.,* 2007). Phytosiderophores are synthesized from methionine and are usually referred to collectively as belonging to the mugineic acid family (the MAs). In barley, MAs are also thought to play a role in mobilizing Zn in addition to Fe. Roots of Zn-deficient barley plants have increased expression of genes involved in the biosynthesis of MAs, and there is increased secretion of MAs from the roots of these plants (Suzuki, 2006).

Zn uptake by root

To increase Zn uptake by roots, the Zn availability in the rhizosphere must be increased (Welch, 1995), which could be done by enhanced release rates of root-cell H^+, metal chelating compounds and/or reductants, by increasing root absorptive surface area (fine roots and root hairs) and by association with mycorrhizal fungi (Liu *et al*., 2000; Ryan and Angus, 2003; Gao *et al*., 2007) (Figure 4). It is found that when the rice cultivation system was changed from flooded to aerobic conditions, plant Zn uptake decreased, which might be caused by changes in pH, dissolvable organic carbon (DOC) or redox in the rhizosphere, and by the differences in transpiration and diffusion between the two systems (Gao, 2007).

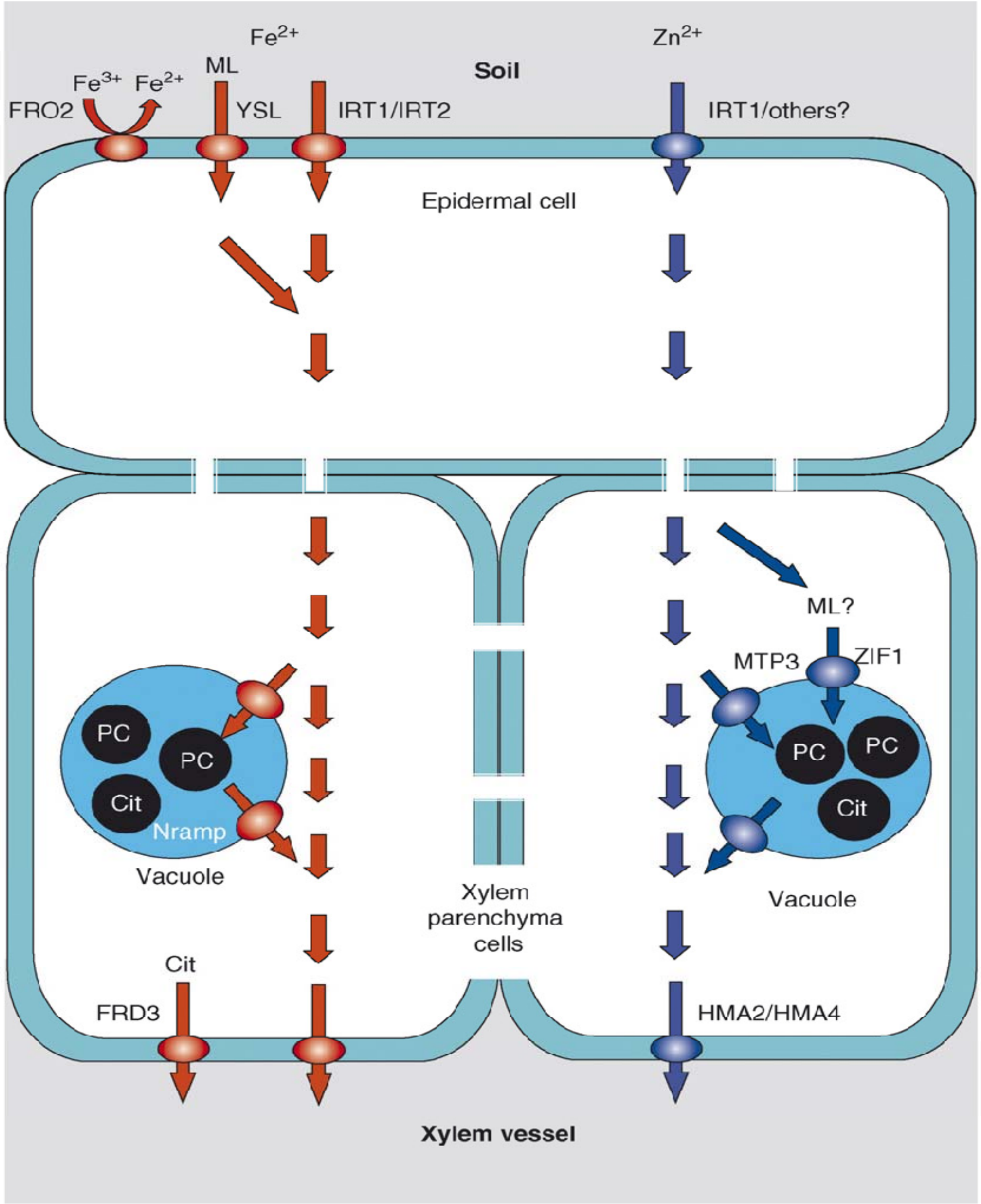
Zinc transport to shoot

Zinc transport in plants takes place both through the xylem and the phloem. Following absorption by the roots, Zn is rapidly transported via the xylem to the shoots (Riceman and Jones, 1958) (Figure 5). Adequate Zn supply leads to a high proportion of Zn located in the shoots, while with toxic levels of Zn supply, a



Palmgren *et al.*, 2007

Figure 4: Overview of the transport pathway of a metal ion from soil to grain



Palmgren *et al*., 2007

Figure 5: Root heavy metal uptake and loading of the vascular tissue leading to aerial parts.

higher proportion of total Zn may accumulate in the roots, like in beans (White *et al.*, 1979). The form in which Zn is actually transported in xylem sap remains unclear. The types and amounts of Zn would be affected by the composition, pH and redox potential of the xylem sap (Welch, 1995; Liao *et al.*, 2000). Computer simulations run on xylem sap composition suggest that Zn is mainly transported in xylem of soybean as Zn-citrate complex and in tomato as Zn-citrate or malate complexes (White *et al.*, 1981).

Zinc remobilization

Zinc appears to be the most mobile of all micronutrients and its remobilization is closely related to leaf senescence (Marschner, 1995; Uauy *et al.*, 2006). Zn can be retranslocated from old leaves of wheat to young leaves and roots via the phloem (Erenoglu *et al.*, 2001). In soybean, it was found that 37.5% of the dose of foliar 65Zn finally accumulated in grain, although plants were grown under sufficient Zn supply (Khan and Weaver, 1989), but the contribution of remobilization to grain Zn is unknown in rice. Transport of metals within phloem is thought to be via the positive hydrostatic pressure gradient developed from the loading of sucrose into the phloem from mature actively photosynthesizing leaves and unloading of sucrose into the sink tissues such as rapidly growing tissues, apical root zones and reproductive organs (MacRobbie, 1971; Hocking, 1980; Welch, 1995).

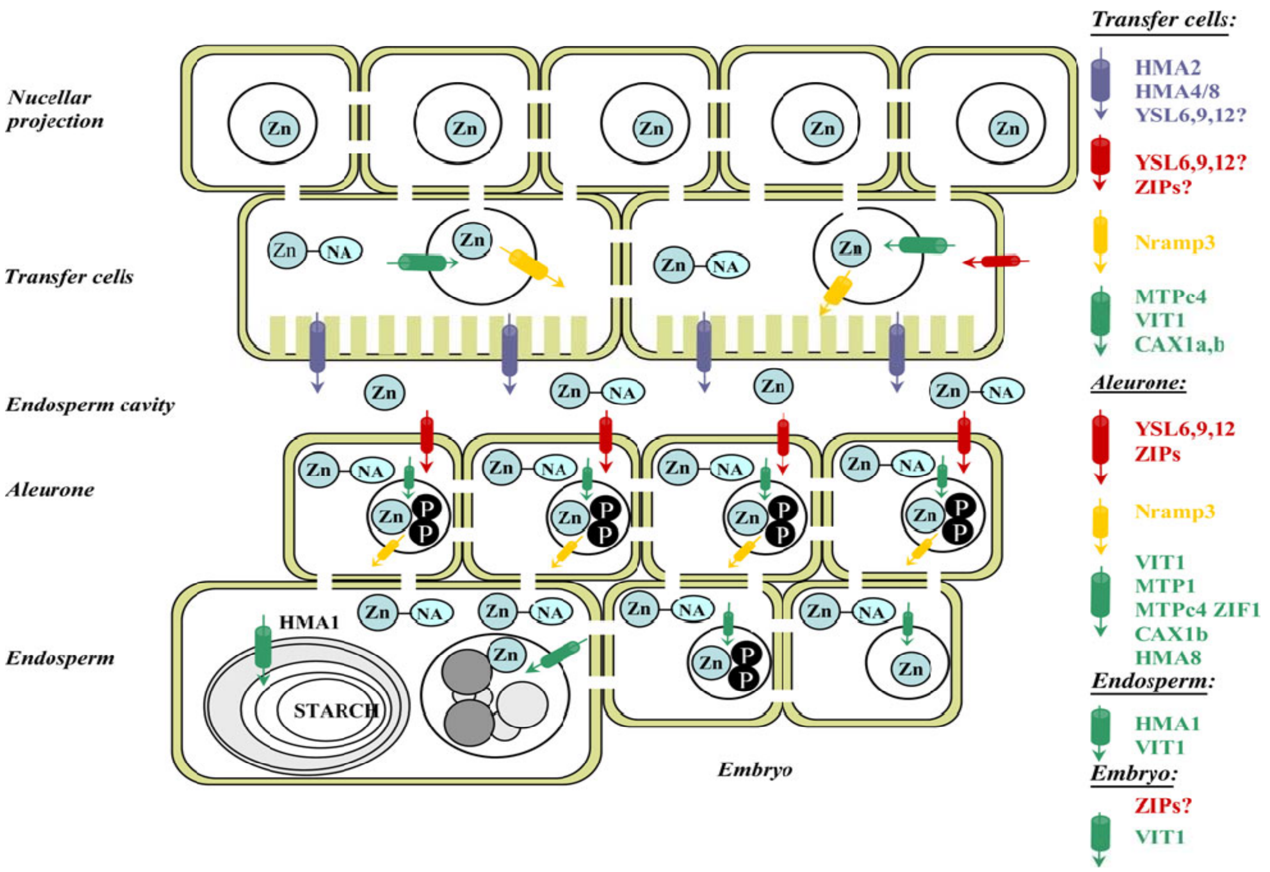
Loading of zinc into grains

Grain Zn accumulation apparently comes from different pools of Zn within the plant (Pearson *et al.*, 1996b). In wheat, Zn reaches the developing wheat grain via the phloem (Herren and Feller, 1994; Pearson and Rengel, 1995b). Before Zn is loaded into the developing grain, the xylem is discontinuous (Zee and O’Brien, 1970) and the xylem-phloem exchange occurs in the rachis and to a lesser extent in the peduncle, lemma and palea (Pearson and Rengel, 1995b). Loading Zn to the phloem may be a rate-limiting step, and the saturation of membrane transporters may reduce the grain loading of Zn (Pearson *et al*., 1996a). In wheat and barley, once Zn enters the grain phloem, Zn is initially mostly transported to the crease and pericarp tissues, but as the grain matures increasingly more to the endosperm and the embryo. In mature wheat grains, the relatively largest amounts of Zn remain stored within the pericarp tissues (Pearson *et al*., 1998). However, in rice, the pathway of Zn loading to the grain is completely different from that in wheat. There is no discontinuity between rachis and grain vascular bundle (Zee, 1971; Krishnan and Dayanandan, 2003). Furthermore, symplastic continuity exists between the cells of the vascular trace, chalaza, nucellar projection and nucellar epidermis (Thorne, 1985; Krishnan & Dayanandan, 2003). The further transport of mineral nutrients from the outer grain tissues to the endosperm is inwards through the apoplast from the nucellar epidermis that completely encircles the endosperm (except near the vascular trace) (Krishnan and Dayanandan, 2003).

Palmgren *et al*., 2008 reported the transport proteins involved in the long distance transport of heavy metals, including zinc, from the soil through the vascular tissues, reaching the shoot and loaded into the grain. Tauris *et al*., 2009 studied the transport proteins involved in zinc trafficking from the phloem to the final sequestering sites in the developing grain (Figure 6).

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,



Blue cylinders - cellular efflux transporters **Red cylinders** - cellular importing transporters

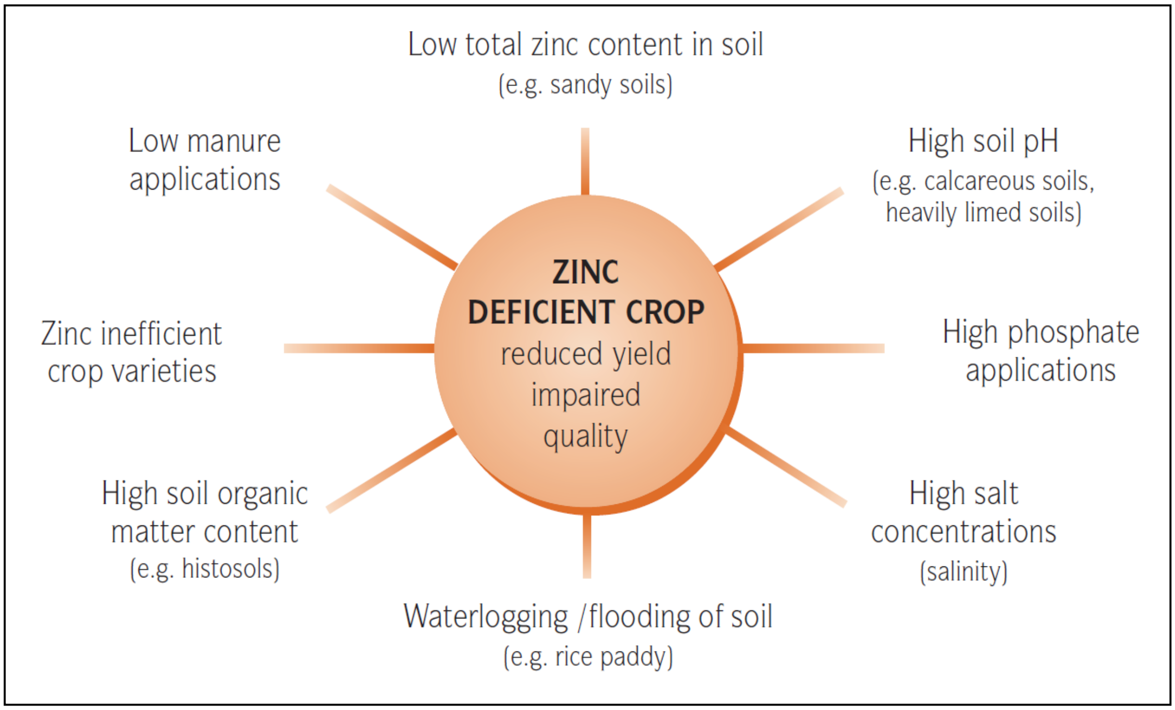
Green cylinders - vacuolar uptake transporters **Yellow cylinders** - vacuolar efflux transporters

Zn – Zinc **NA –** Nicotianamine **P** - Phytate.

Tauris *et al*., 2009

Figure 6: Road map - Zn trafficking from Phloem developing grain

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 that (MarschJler, 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).



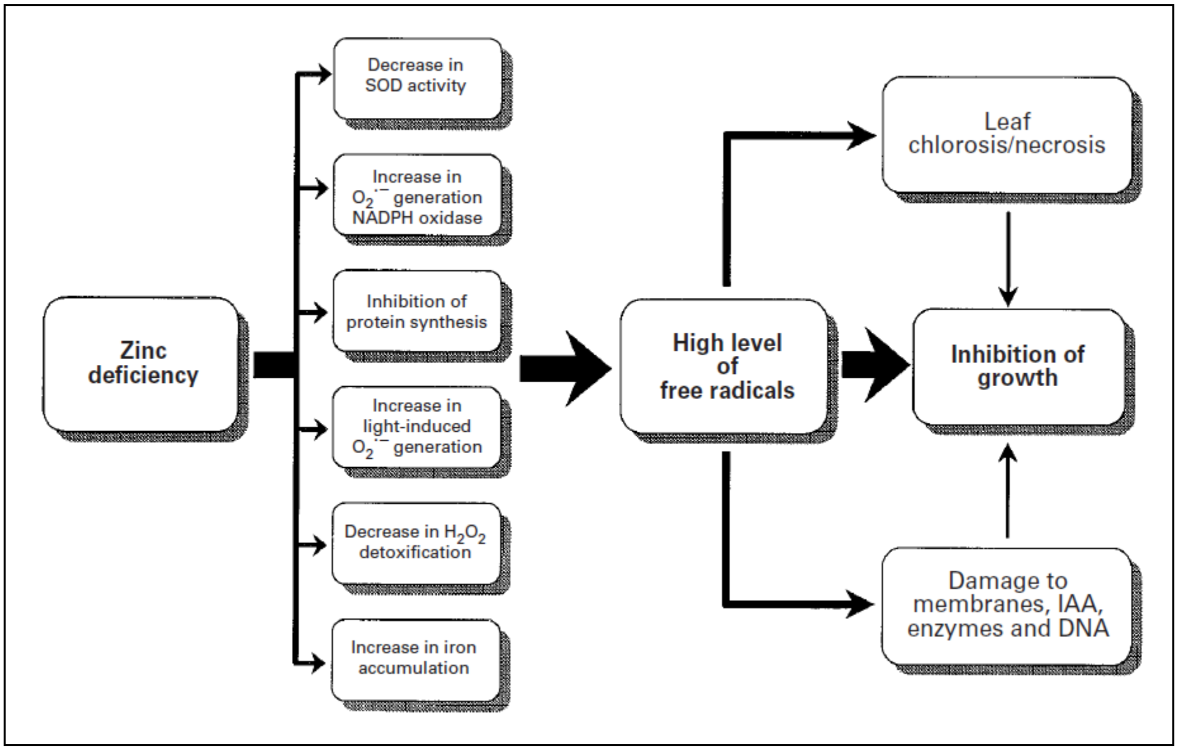
Alloway, 2008

Schematic Diagram of the Causes of Zinc deficiency in Crops

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 Zinc is not available yield less and have a low nutritional quality (Kochian and Garwin, 1999). Zn also offers plant resistance to root diseases.

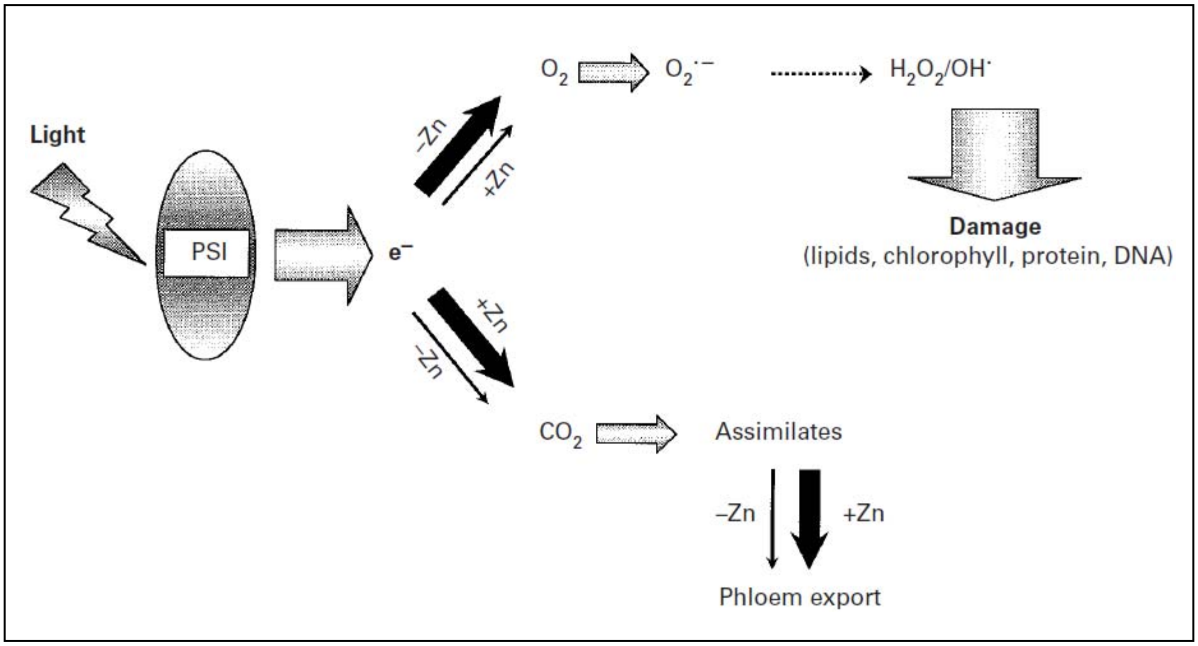
Even though Zn is mobile element, deficiency symptoms are difficult to diagnose in pasture and legumes, as they may be seen as shortening of internodes, leaves may fail to expand properly and in cereals deficiency is usually seen in the seedling stage with longitudinal pale green stripes on one or both 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).



Cakmak, 2000.

Schematic diagram of physiological changes occurring in zinc deficient cells and their effect on plant growth.

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 (0_2'), H_2O_2, hydroxyl radicals, singlet oxygen ($^10_2$) 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).



Cakmak, 2000.

Schematic representation of the photosynthetic electron flow to CO2 and O2 and formation of toxic oxygen species in chloroplastic stroma.

Note: +Zn and **-**Zn refer to adequate and deficient Zn supply, respectively.

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 H202 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 deficient <4 increases production of 02'. Therefore enhanced O_2^- generation by NADPH oxidase and impaired detoxification of O_2^- and H_2O_2 due to Zn deficiency related inhibition of SOD activity could lead to elevated levels of O_2^- 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 \vas 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 grown 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 (SODI) is a ubiquitously expressed, homodimeric intracellular enzyme essential for antioxidant defense. SOD I 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 ISI-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 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.

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

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

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

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.

As for zinc, it has been generally believed together with iron and manganese, was complexed with phytic acid in the protein storage vacuoles of the aleurone and the embryo. In agreement with this, zinc is known to bind strongly to phytic acid (Tauris, *et al.*, 2009) in barley grain. 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 modem, 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) conducted 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 actwity of Zn requiring enzymes in conditions of Zn deficiency is correlated with ZE (Hacisalihoglu *et al.*, 2003). They examined Zn requiring enzymes *Cui* 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 *Cui* 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 micro nutrients 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 is 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)n 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 (l) 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 micro nutrients 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 avtrage 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) Over expression 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 ZlP 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 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 65Zn revealed in irtl mutant, it responds to Fe and Zn deprivation by altered expression of certain Zn and Fe transporter genes. These data support the conclusion that IRTl 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 CD.F (cation diffusion facilitator) families have been found to playa 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 (Ipa) 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 B-carotene can enhance iron absorption in humans (Lucca *et al*., 2001).

l) Hyper accumulators

Hyperaccumulators are plants that can take up and tolerate levels of metals that are toxic to non accumulators (Palmer and Guerinot, 2009) at a concentration approximately 100 times greater than normal species. Zinc hyperaccumulation has since been defined as the occurrence of >10 000 Ilg 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 *Thiaspi caeruiescens* concentrating Zn to around 17% of the dry plant ash. Although naturally hyperaccumulators can tolerate and accumulate high concentrations of toxic metals, they usually have small biomass, grow slowly and cannot be easily cultivated (Tong *et al*., 2004). However, 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. *Thiaspi caeruiescence* 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, lVoccaea, 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 Znhypertolerant 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

Metal hyper accumulators, which are highly metal tolerant are likely to exhibit numerous alterations in their metal homeostasis network throughout the entire plant. A complex metal homeostasis also exerts for a regulated, specific and targeted supply of micronutrients. 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). 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.

So far, 18 Zn accumulators have been reported, and most of them belonged to Thlapi caerulescens. *T. caerulescens* is a short-lived, self-compatible biennial/ perennial species which hyperaccumulates ZnlCd/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 (Broadley, *et al.*, 2007). Its shoot can accumulate up to 43,710μg Zn per gram dry weight. (Wei and Chen, 2001).

In 2007, Wei *et al* reported two genotypes of Ryegrass (*Lolium perenne* L.), which is found to accumulate high Zn in shoot. Ryegrass, known for its high tolerance to zinc toxicity, is an annual monocotyledon which grows rapidly and produces large biomass. The two genotypes, Airs and Tede, accumulated more zinc as soil zinc levels increased, and zinc concentration in the shoot were about

540 µg per gram in Airs and 583.9 µg per gram shoot dry weight in Tede in response to 16mmol Zn per kilogram of soil.

In *Arabidopsis thaliana* the expression of ZIP families AtZIP 1, 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. haleri* 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 vulgare*. 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), (AtZAT /AtMTP1) and (AtMTPc3); a member of the Zn-Fe permease (ZIP) family (AtIRT3/TcZNT2) and a Pm-type heavy-metal-associated domain-containing ATPase (AtH/vi43». 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 hyperaccurnulators 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 CulZn 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 Cui 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 Cui 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).

Material and Methods

III. MATERIAL AND METHOD

Objectives:

1. Assessment of Genetic Variability in seed zinc content (around 240 lines).
2. Physiological Characterization of 240 Pigeon pea germplasm lines.
3. Studying the zinc uptake characterization in contrast lines i.e. high and low types.

1. Assessment of Genetic Variability in seed zinc content

In order to carry out this research work, seeds of pigeon pea germplasm lines (240) were procured from the International Crop Research Institute for the Semi Arid Tropics (ICRISAT) in Hyderabad.

Seed zinc content in the pigeon pea germplasm lines were analyzed following the series of protocol below:

Sample Preparation

About 3g of seeds was grounded to small particles using an electric mixer and then with the pestle and mortar to obtain fine powder. Using an electric weighing balance, 250mg of powdered sample was obtained and placed in the labeled conical flask, after which five milliliter (5ml) of concentrated Nitric Acid was added and the mixture was incubated overnight in the acid digestion hood. On the following day, five milliliters (5ml) of diacid mixture (Nitric acid: Perchloric acid:: 10:4) was added to the incubated sample.

The mixture was then placed on the electric hot plate, allowing the evaporation of acid in the form of white fumes, until 1ml white precipitate was formed at the base of the flask. This was left to cool and the volume was then made up to 50ml i.e. 1:50 using double distilled water.

(A) Atomic Absorption Spectrometer (AAS) facility at Dryland Agriculture, UAS, Bangalore.

(B) ICP-OES facility at plant nutrition lab, Dept of Crop Physiology, UAS, Bangalore

Plate: 1 - AAS and ICP-OES facility at UAS, Bangalore (A + B)

The diluted solution was then filtered with watman filter paper (12microns) before being transferred to a clean, airtight bottle and was kept ready for estimation of zinc content.

Estimation of Zinc

Zinc was estimated in the grain sample using Polarised Zeeman Atomic Absorption Spectrophotometer (AAS-2-6100) [Plate 1(A)].

Atomic absorption spectroscopy (AAS) working principle

Atomic absorption spectroscopy (AAS) determines the presence of metals in liquid samples. Metals include Fe, Cu, Al, Pb, Ca, Zn, Cd and many more. It also measures the concentrations of metals in the samples. Typical concentrations range in the low mg/L range. In their elemental form, metals will absorb ultraviolet light when they are excited by heat. Each metal has a characteristic wavelength that will be absorbed. The AAS instrument looks for a particular metal by focusing a beam of UV light at a specific wavelength through a flame and into a detector. The sample of interest is aspirated into the flame. If that metal is present in the sample, it will absorb some of the light, thus reducing its intensity. The instrument measures the change in intensity. A computer data system converts the change in intensity into an absorbance. As concentration of the element increases (goes up), absorbance also increases (goes up). The researcher can construct a calibration curve by running standards of various concentrations on the AAS and observing the absorbances.

Standard:

100ppm standard Zn^{2+} solution was prepared using 1000ppm Zn^{2+} atomic absorption standard solution and appropriate dilutions was made to get standard solutions ranging from 0.0 to 1.0 ppm. These standards were subjected to AAS as that of sample to get a standard curve. The samples were then analyzed based on the standard curve.

Protocol:

Suitable dilutions were made from the extract with double distilled water so as to fit their absorbance with the range of standard curve. The diluted samples were subjected to AAS and the concentration was recorded in ppm using the standard curve. Two replications were maintained and their average was used for calculation of seed zinc content using the following formula:

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

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

The frequency distribution and the range of the mean zinc content in the seed was computed.

2. Physiological Characterization of contrasting zinc types of pigeon pea.

Plant Culture:

The seeds of 240 pigeon pea germplasm lines were sown in the Root Structure [Plate 2 (A)] at the Crop Physiology research field on the Kharif season (18/08/09) of 2009. Management procedures and agronomic practices were executed based on the ICRISAT pigeon pea planting manual (1992).

The following phenotypic and physiological characteristics were recorded on the genotypes at 85-95 days after sowing (DAS) [Plate 2 (B)]:

i. ***Recording of physiological characters***

The physiological characters, photosynthetic rate, stomatal conductance, transpiration rate, SPAD chlorophyll readings were taken at the active vegetative growth stage of the crop i.e. 80 to 90 days after sowing.

(A) Establishment stage (15DAS) of Pigeon pea Germplasm screening in Root Structure

(B) Pigeon pea Germplasm screening at 85 – 95 Days after Sowing in Root Structure

Plate: 2 – Experimental setup in the root structure

IRGA working principle

The portable photosynthetic device called IRGA (Infra Red Gas Analyzer) works on the principle that heteroatomic molecules in the air absorb infrared radiation such as CO_2, H_2O, SO_2, etc. The wavelength of absorption is specific for each molecule (monoatomic) such as O_2, N_2, and H_2 will not absorb infrared radiation. The quantity of CO_2 assimilated in the leaf in a closed chamber air is directly related to the decreased CO_2concentration in the chamber. The quantity of CO_2 released from the chamber gives the quantity of CO_2 assimilated in the leaf in the presence of visible light.

The two types of IRGA are:

a) *Closed System:* The leaf is kept in a closed chamber, therefore the CO_2 concentration decreases constantly and the measured photosynthetic rate is not accurate.

b) *Open System:* It will not allow the decrease in CO_2 concentration and therefore measures accurate photosynthetic rate.

SPAD working principle

Among several characters, leaf thickness (associated with structural and non structural components) and chlorophyll content determines the leaf transmittance characters. The chlorophyll meter called Soil Plant Analysis and Development has been developed by Minolta Corporation for determining the chlorophyll content which indicates the Nitrogen status of the crop. The devise is a simple and portable diagnostic tool that measures the greenness or the relative chlorophyll content of the leaves.

SPAD (SPAD-502) utilizes two light emitting diodes, 650 and 940nm and a photodiode detector to sequentially measure light transmission through the leaves of Red and Infrared light. Red light transmits at 650nm, the wavelength at which chlorophyll absorbs light and at 940nm where no light absorption occurs. Upon initial calibration, the Minolta-502 processor converts a current produced by the red (650nm) and infrared (940nm) light beam into a voltage and stores the digital values in a unit memory. When the leaf is subsequently measured, the device successively measures the transmission of red and infrared light outputs. A processed value based on the ratio of the measured voltage produced by each wavelength relative to the values stored in the memory.

Since chlorophyll a and b are the dominant pigments absorbing light at 650nm, the ideal SPAD chlorophyll meter reading would be proportional to the chlorophyll concentration in the leaf.

ii. *Plant height and Days to 50% flowering*

The measurement of plant height was also taken on the same day of the 50% flowering record.

iii. *Yield and 100 seed mass*

Harvesting of pods was conducted when 75-80% physiological maturity and at moisture content was around 20-24% i.e. when the testa was dried and turned brown or maroon (for red/maroon colored pods) in color. The harvesting process was conducted consecutively based on the maturity period of the pods of a particular genotype and other genotypes as well. Weight of pods was taken to determine the yield of a particular genotype, then shelling of seeds, counting and weighing of 100 seeds.

iv. *Biomass*

The records of biomass were taken at maturity stage of the plant. Since the plants were grown in the root structure, first the concrete walls were broken and then high water pressure was used to loosen the soil without damaging the roots. The root length and root volume were then taken before packing the whole plant to the paper bags for drying in the oven at 70^0C. Weight of shoot and roots were taken when the samples were fully dried.

3. Studying the zinc uptake in contrast lines i.e. low and high types.

Based on the range of seed zinc content of Objective 1, 15 high zinc content and 15 low zinc content were selected for further field research. Seeds for these contrast zinc types were sown in clay pots holding approximately 10-12kg of soil on the Kharif season (04/10/09) of 2009. The pots were arranged based on Randomized Complete Block Design (RCBD), with 3 treatments and 3 replications [Plate 3].

The 31 genotypes (30 contrast types + 1 check variety i.e. TTB7) were subjected to the three (3) treatments:

a. T1 – Foliar Zinc Application [30ppm Zn/plant]
b. T2 – Soil Zinc Application [60mg Zn/plant]
c. T3 – Control (Without Zinc)

The soil (T1) and foliar (T2) zinc treatments were applied on 60 days after sowing (60DAS).

Zinc Treatment Preparation

Treatment 1 – Foliar Zinc application [30ppm Zn/plant]

Preparation of 1% Zn :

- Molecular weight of Zinc Sulphate ($ZnSO_4.7H_2O$) = 287.84g
- Molecular weight of Zn = 65g in 287.84g of $ZnSO_4$
- H_2O required for foliar spray = 3 liters (3000ml)

T1 – Foliar Zn Application (1% $ZnSO_4$)

T2 – Soil Zn Application (60mg Zn SO_4/pl)

T3 – Control (No Zn application)

Plate:3 Experiment set up of external zinc treatment

- Zn required for foliar spray = 30g
- $ZnSO_4$ required for foliar spray = 132g

Calculation: ***1% Zn solution = 1g Zn in 100ml water***

For 3000ml H_2O, we require 30g of Zn

1g Zn is present in 4.4g of $ZnSO_4$

30g Zn = 30 x 4.4

= **132g** $ZnSO_4$

Therefore, 132g of $ZnSO_4$ is required to prepare 1% Zn in 3 liters of Water.

The required amount of $ZnSO_4$ (132g) was mixed thoroughly in 3 liters of water and sprayed on the leaves of the pigeon pea plants using a knapsack sprayer.

Treatment 2 – Soil Zinc application

The weight of soil in the pots was taken, and these soil weights were used to compute the amount of Zinc Sulphate required for the experiment.

- Average soil weight = 12kg
- Zinc required = 5mg per kg of soil (5mg/kg)
- $ZnSO_4$ required per pot = 60mg
- $ZnSO_4$ required for 91 pots = 5460mg
  (31genotypes X 3 replication)

The required amount (60mg) of zinc sulphate was applied around (basal application) around the plant in every pot and slight irrigation was done to dissolve the compound as well as facilitate the transport of zinc to the vicinity of the root surface.

SPAD Chlorophyll Reading

The SPAD chlorophyll reading was taken on the 8th day after the application of treatments (soil and foliar zinc application).

Pod Harvesting

Harvesting of pods was conducted when 75-80% physiological maturity and at moisture content was around 20-24% i.e. when the testa was dried and turned brown or maroon (for red/maroon colored pods) in color.

Harvesting of plants for Biomass and Zinc Estimation

Harvesting of whole plant was conducted on 66 days after sowing (66 DAS). Roots and shoot were separated, and packed in paper bags before placed in the oven for drying at 75-80^{o}C. When the plants were fully dried, the shoot and root dry weight was taken.

Zinc Estimation of different plant parts

Sample preparation:

Leaf sampling was conducted during the active vegetative stage of the plant i.e. 85 to 95 days after sowing (DAS). Fully expanded leaves, generally this is the third leaf, were collected from the primary branch of the plant. The root, stem, leaf and seed were further separated and grounded using electric mixer and then using pastel and mortar to form fine powder. Samples of 250mg fine powder of the different plant parts were placed in the 50ml, labeled conical flask, after which was subjected to acid digestion following the procedures in Objective 1.

The white precipitate formed was left to cool and the volume was then made up to 50ml i.e. 1:50 using double distilled water (EC = 6.6 ds). The diluted solution was then filtered using cellulose filter paper (4 microns) powered by electric vacuum pump, before being transferred to a clean, airtight bottle and was kept ready for estimation of zinc content.

Estimation of Zinc

Zinc was estimated in the grain, leaf, stem and root sample using Inductively Coupled Argon Plasma – Omission Optical Spectrometer (ICAP-OES) [Plate 1 (B)].

Standard

100ppm standard Zn^{2+} solution was prepared using 1000ppm Zn^{2+} multi element absorption standard solution and appropriate dilutions was made to get standard solutions ranging from 0.0 to 2.0 ppm. These standards were subjected to ICAP-OES as that of sample to get a standard curve. The samples were then analyzed based on the standard curve.

Overview and working principle of ICAP-OES

Thermo Inductively Coupled Argon Plasma – Omission Optical Spectrometer (ICAP-OES) offers extreme performance with increased sensitivity, improved stability and unmatched speed of analysis. Both radial and dual viewing of the plasma is available.

Based on a rugged and well proven optical design, the instruments feature an ultra temperature-stabilized optical tank with new high efficiency components to improve energy throughput. The patented Charge Injection Device (CID) detector has been updated to use an enhanced chip, yielding a detector with unparalleled sensitivity and signal to-noise ratio. Integration times are much reduced resulting in faster analysis, less gas consumption and lower cost of ownership. Instruments are further enhanced with mass flow gas control, optimized sample introduction assembly and high power RF generator, to offer levels of sensitivity and detection limits previously unobtainable.

The high temperature plasma source atomizes the sample and excites the atoms resulting in emission of photons. The atoms of each element in the sample emit specific wavelengths of light. The optical spectrum from the plasma is dispersed by an optical spectrometer, so that the intensities of the individual wavelengths can be measured.

The number of photons emitted is directly proportional to the concentration of the element. The photons may be detected either sequentially or simultaneously. The Thermo patented Charge Injection Device (CID) enables the simultaneous collection of data from all chosen wavelengths. Quantitative analysis is achieved by measuring the intensities of these specific wavelengths and, after performing a calibration, converting to concentration.

ICAP-OES Zn Analysis Protocol:

Suitable dilutions i.e. 1:2.5 (4ml sample :10ml water) were made from the extract with double distilled water so as to fit their absorbance with the range of standard curve. The diluted samples were to ICAP-OES and the concentration was recorded in ppm using the standard curve. Two replications were maintained and their average was used for calculation of zinc content in the sample using the following formula:

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

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

DATA ANALYSIS

The raw data obtained from the pot experiment was computed using MSTAT data analysis software.

Results

IV. RESULTS

Plants play vital role in human nutrition by providing all essential nutrients required for human health. However, they are not in a position to meet the daily requirements of mineral nutrients due to the fact that most of these essential nutrients or elements especially microelements are not present in the required concentrations. Most of our food crops contain lower amounts of zinc in their edible parts. Therefore, it is essential for value addition of our crops in terms of micronutrient fortification. Globally the soils are Zn deficient and plants are not in a position to take up, translocate and accumulate enough zinc in edible parts, enough zinc that is essential to meet the human nutrition requirement. Hence, there is a need to correct this. The prior information on physiological, biochemical and molecular mechanisms that contribute to the uptake, transport and accumulation of zinc in plants is very crucial. If this information is available it can be used in developing strategies to manipulate the crops for their nutritional status.

Major emphasis of most researchers would be to enhance zinc levels in edible portions of staple food crops to overcome this disorder and add value to our cereals and pulses. And also it is reported that seeds containing high Zn will have high seedling vigor when sown on soils that are poor in zinc. Therefore, emphasis should be focused to understand the physiological reasons for low Zn uptake and partitioning to edible parts in low zinc accumulating types.

It has been reported that there is considerable species and varietal variation in Zn uptake and translocation in crop species despite the fact that all food crops have very low zinc content in edible parts with considerable variation across species and varieties. Heavy metal accumulator *Thalaspi caerulscens* is known to accumulate 3% of zinc in shoots without toxicity. However, the molecular basis of zinc uptake and translocation in plants is not completely elucidated.

With this background the major focus of the study was to assess the genetic variability of zinc content in seed of 240 pigeon pea germplasm lines and also to assess if the differences in seed zinc content in pigeon pea genotypes are due to differences in uptake and translocation of zinc under various soil zinc status.

Pigeon pea, relatively a drought resistant crop and a good source of protein (25-30%) and rich in other nutrients was selected for study and analyzed for its seed zinc content in different germplasm lines. An attempt was made to identify genotypes with all these parameters along with high seed Zn, so that this trait could be transferred through conventional or non-conventional breeding approaches to an agronomically superior variety.

4.1 Variation in seed zinc content across pigeon pea genotypes.

Differences in zinc status seem to exist not only between species but also within a species. Therefore, 240 genotypes from pigeon pea germplasm, representing 28 countries of the world, consisting of core germplasm, local adapted cultivars and released varieties were procured from International Crop Research Institute for the Semi Arid Tropics (ICRISAT), Hyderabad, to assess the seed zinc content and for further studies (Table 1).

All the 240 genotypes were analyzed for their seed zinc content using AAS. There was significant variation among the genotypes with zinc content ranging from as low as 1.53 mg/100gm to as high as 6.95 mg/100g seed weight (Table 2). A released pigeon pea genotype, TTB-7, with seed zinc content of 3.51mg/100g was used as the check for the experiments.

Maximum number of genotypes had zinc content in the range of more than 2 to 4mg/100g [3mg/100g (109 genotypes) and more than 3 to 4mg/100g (92 genotypes)], accounting to approximately 84 percent of the germplasm lines. Moderate number of genotypes had seed zinc content of 1 to 2 (19 genotypes) and

Table 1 : Passport data of Pigeon pea genotypes germplasm lines received from ICRISAT on 30/04/10

Series	Code No.	ICP No.	Origin	Remarks	Series	Code No.	ICP No.	Origin	Remarks
<100	1	7	India	Mini core		46	6859	India	Mini core
	2	26	India	Released cultivar		47	6929	Trinidad & Tobago	Mini core
	3	28	India	Released cultivar		48	6971	India	Mini core control
	4	60	India	Released cultivar		49	6972	India	Released cultivar
<1000	5	348	India	Mini core		50	6973	India	Released cultivar
	6	472	India	Released cultivar		51	6974	India	Released cultivar
	7	655	India	Mini core		52	6992	India	Mini core
	8	772	India	Mini core		53	6997	India	Released cultivar
	9	939	India	Mini core	7000	54	7035	India	Released cultivar
	10	995	India	Mini core		55	7057	India	Mini core
1000	11	1071	India	Mini core		56	7076	India	Mini core
	12	1126	India	Mini core		57	7118	India	Released cultivar
	13	1156	India	Mini core		58	7119	India	Released cultivar
	14	1273	India	Mini core		59	7148	Sri Lanka	Mini core
	15	1279	India	Mini core		60	7180	India	Released cultivar
	16	1641	India	Released cultivar		61	7182	India	Released cultivar
2000	17	2577	Myanmar	Mini core		62	7191	India	Released cultivar
	18	2626	India	Released cultivar		63	7196	India	Released cultivar
	19	2698	India	Mini core		64	7197	India	Released cultivar
	20	2746	India	Mini core		65	7198	India	Released cultivar
3000	21	3046	India	Mini core		66	7199	India	Released cultivar
	22	3049	India	Mini core		67	7200	India	Released cultivar
	23	3451	India	Mini core		68	7202	India	Released cultivar
	24	3576	India	Mini core		69	7218	India	Released cultivar
4000	25	4029	India	Mini core		70	7219	India	Released cultivar
	26	4176	India	Mini core		71	7220	India	Released cultivar
	27	4307	India	Mini core		72	7221	India	Mini core control
	28	4317	India	Mini core		73	7222	India	Released cultivar
	29	4392	India	Mini core		74	7223	India	Mini core
	30	4575	India	Mini core		75	7260	India	Mini core
	31	4715	Ghana	Mini core		76	7314	India	Mini core
	32	4903	India	Mini core		77	7366	India	Mini core
5000	33	5142	India	Mini core		78	7375	India	Mini core
	34	5863	India	Mini core		79	7426	India	Mini core
6000	35	6049	India	Mini core		80	7507	India	Mini core
	36	6123	India	Mini core		81	7609	India	Released cultivar
	37	6128	Bangladesh	Mini core		82	7623	India	Released cultivar
	38	6304	India	Released cultivar		83	7647	India	Released cultivar
	39	6344	India	Released cultivar		84	7718	India	Released cultivar
	40	6370	unknown	Mini core		85	7803	India	Mini core
	41	6443	India	Released cultivar		86	7869	India	Mini core
	42	6668	India	Mini core	8000	87	8012	India	Mini core
	43	6739	India	Mini core		88	8152	India	Mini core
	44	6815	India	Mini core		89	8227	India	Mini core
	45	6845	India	Mini core		90	8255	India	Mini core

Series	Code No.	ICP No.	Origin	Remarks	Series	Code No.	ICP No.	Origin	Remarks
	91	8266	India	Mini core		136	11910	India	Mini core
	92	8384	India	Mini core		137	11916	India	Released cultivar
	93	8508	India	Released cultivar		138	11946	India	Mini core
	94	8513	India	Released cultivar	12000	139	12105	Tanzania	Mini core
	95	8516	India	Released cultivar		140	12123	Tanzania	Mini core
	96	8518	India	Released cultivar		141	12142	Tanzania	Mini core
	97	8602	India	Mini core		142	12298	India	Mini core
	98	8700	India	Mini core		143	12410	India	Mini core
	99	8757	India	Mini core		144	12515	India	Mini core
	100	8793	India	Mini core		145	12596	India	Mini core
	101	8840	India	Mini core		146	12654	India	Mini core
	102	8860	ICRISAT	Mini core		147	12680	India	Mini core
	103	8863	ICRISAT	Mini core control	13000	148	13011	India	Mini core
	104	8921	India	Mini core		149	13139	Kenya	Mini core
	105	8949	India	Mini core		150	13167	Kenya	Mini core
9000	106	9045	India	Mini core		151	13191	Australia	Mini core
	107	9145	Kenya	Released cultivar		152	13194	Australia	Released cultivar
	108	9336	India	Mini core		153	13244	Kenya	Mini core
	109	9414	India	Mini core		154	13270	Kenya	Mini core
	110	9655	India	Mini core		155	13304	Italy	Mini core
	111	9691	India	Mini core		156	13359	Malawi	Mini core
	112	9750	India	Mini core		157	13431	Malawi	Mini core
	113	9905	ICRISAT	Released cultivar		158	13571	Nigeria	Mini core
10000	114	10094	India	Mini core		159	13575	Sierra Leone	Mini core
	115	10228	India	Mini core		160	13577	China	Mini core
	116	10397	India	Mini core		161	13579	Philipines	Mini core
	117	10447	India	Mini core		162	13633	Nigeria	Mini core
	118	10503	India	Mini core		163	13662	India	Mini core
	119	10559	India	Mini core		164	13829	Grenada	Released cultivar
	120	10654	India	Mini core		165	13884	Puerto Rico	Mini core
	121	10960	India	Released cultivar	14000	166	14094	Venezuela	Mini core
11000	122	11015	ICRISAT	Mini core		167	14116	Jamica	Mini core
	123	11059	ICRISAT	Mini core		168	14120	Jamica	Mini core
	124	11230	ICRISAT	Mini core		169	14147	Brazil	Mini core
	125	11281	ICRISAT	Mini core		170	14155	United Kingdom	Mini core
	126	11320	Nepal	Mini core		171	14229	Zambia	Mini core
	127	11321	Nepal	Mini core		172	14294	Italy	Mini core
	128	11384	Nepal	Released cultivar		173	14368	Venezuela	Mini core
	129	11477	unknown	Mini core		174	14421	ICRISAT	Released cultivar
	130	11543	ICRISAT	Mini core control		175	14444	ICRISAT	Mini core
	131	11605	ICRISAT	Released cultivar		176	14471	ICRISAT	Mini core
	132	11627	ICRISAT	Mini core		177	14545	India	Mini core
	133	11690	ICRISAT	Mini core		178	14569	Thailand	Mini core
	134	11823	India	Mini core		179	14638	ICRISAT	Mini core
	135	11833	India	Mini core		180	14701	ICRISAT	Mini core

Series	Code No.	ICP No.	Origin	Remarks
	181	14720	ICRISAT	Released cultivar
	182	14722	ICRISAT	Mini core
	183	14770	ICRISAT	Released cultivar
	184	14801	ICRISAT	Mini core
	185	14819	ICRISAT	Mini core
	186	14832	ICRISAT	Mini core
	187	14900	ICRISAT	Mini core
	188	14903	ICRISAT	Mini core
	189	14976	ICRISAT	Mini core
15000	190	15049	ICRISAT	Mini core
	191	15068	ICRISAT	Mini core
	192	15109	Uganda	Mini core
	193	15161	ICRISAT	Mini core
	194	15185	ICRISAT	Mini core
	195	15382	Nigeria	Mini core
	196	15493	Uganda	Mini core
	197	15585	India	Released cultivar
	198	15600	India	Released cultivar
1600	199	16264	ICRISAT	Mini core
	200	16309	ICRISAT	Mini core
	201	1027	India	Medium
	202	2059	India	Medium
	203	2173	India	Medium
	204	2484	India	Medium
	205	3495	India	Medium
	206	3593	India	Medium
	207	7966	India	Medium
	208	9133	Kenya	Long
	209	9293	India	Medium
	210	9328	India	Medium
	211	9457	India	Medium
	212	10225	India	Medium
	213	10905	Australia	Early
	214	10923	Australia	Early
	215	11726	ICRISAT	Medium
	216	11785	India	Long
	217	12637	India	Medium
	218	13118	Kenya	Long
	219	13125	Kenya	Long
	220	13151	Kenya	Long
	221	13400	Malawi	Long
	222	13453	Malawi	Long
	223	13486	Malawi	Long
	224	13496	Malawi	Long
	225	13501	Malawi	Long
	226	13529	Malawi	Long
	227	13601	Kenya	Long
	228	14049	India	Medium
	229	14179	India	Early
	230	14416	ICRISAT	Early
	231	14461	ICRISAT	Early
	232	14828	ICRISAT	Early
	233	14936	ICRISAT	Early
	234	14944	ICRISAT	Early
	235	14946	ICRISAT	Early
	236	14954	ICRISAT	Early
	237	14971	Indonesia	Medium
	238	15066	ICRISAT	Early
	239	16208	ICRISAT	Early
	240	16313	ICRISAT	Early

Table: 2 Variation in Seed Zn content of 240 pigeon pea lines from ICRISAT

Check (Control) : TTB-7

Range (mg/100g)	**1.53 - 6.95**
Mean seed Zn content (mg/100g)	**3.06**
CV (%)	**9.76**
CD: Events	**0.106**
Check (TTB-7) (mg/100g)	**3.51**

Table:3 Selected contrast pigeon pea genotypes for varying seed zinc content

S.l.	**High Zinc Types**		**Medium Zinc Types**		**Low Zinc Types**	
	ICP Accession No.	**Seed zinc content (mg/100g)**	**ICP Accession No.**	**Seed zinc content (mg/100g)**	**ICP Accession No.**	**Seed zinc content (mg/100g)**
1	6443	6.2	10923	3.08	10960	1.91
2	7035	5.66	6668	3.08	10654	1.91
3	7118	5.65	13453	3.10	8602	1.94
4	6974	5.13	1027	3.10	8863	1.95
5	14954	4.96	13431	3.10	8513	1.97
6	6997	4.62	7260	3.11	9336	2.12
7	6992	4.6	14368	3.12	8508	2.15
8	7182	4.56	13575	3.12	11946	2.15
9	7314	4.54	14720	3.12	12410	2.17
10	6370	4.38	14471	3.12	7623	2.18
11	2484	4.35	8384	3.13	9691	2.22
12	2059	4.28	14229	3.13	11543	2.23
13	2626	4.22	10225	3.16	7221	2.24
14	7119	4.21	15600	3.17	8516	2.32
15	14421	4.19	13011	3.18	9655	2.36
	Range	**4.19 - 6.2**		**3.08 - 3.18**		**1.91 - 2.36**
	Mean	**4.77**		**3.13**		**2.12**
	Check (TTB-7)	**3.51**				

more than 4 to 5mg/100g (15 genotypes) respectively accounting for 14 per cent of the germplasm lines. Only 2 per cent of the germplasm lines had seed zinc content more than 5 mg/100g (Figure 7).

Based on the seed zinc content, genotypes with less than 2.5 mg/100 g were classified as low zinc types, more than 2.5mg/100g but less than 3.5mg/100g were classified as medium zinc types and those above 3.5 mg/100g were considered as high zinc types For further study only the lowest and highest 15 seed zinc types in each group were considered as low and high zinc types and used for another experiment. There was significant difference in mean seed zinc content of low and high seed zinc types. High zinc types had more zinc to the extent of 125% compared to low zinc types (2.12mg/100g) (Table 3). When comparing the means of the contrast types against the check variety (TTB-7), there was a significant difference in the mean seed zinc content of high zinc types (4.77mg/100g) over the check which was slightly higher than the mean seed zinc content of medium zinc types (3.12mg/100g) and significantly higher than the low zinc types (2.12mg/100g) (Figure 8).

Since, there was no physiological background information of the pigeon pea germplasm lines obtained from ICRISAT in a common environment, the 240 pigeon pea genotypes were grown in the root structure facility at the Department of crop physiology, UAS, Bangalore, for the assessment of their genetic variability based on physiological parameters and zinc accumulation. Further assessment was conducted to find out the relationship between the physiological parameters observed and the zinc content of seeds and leaves of contrast seed zinc types.

4.2 Variation in Physiological parameters of pigeon pea genotypes grown in root structure

From the 240 pigeon pea genotypes sown in the root structure, only 217 survived for which the physiological assessment was conducted.

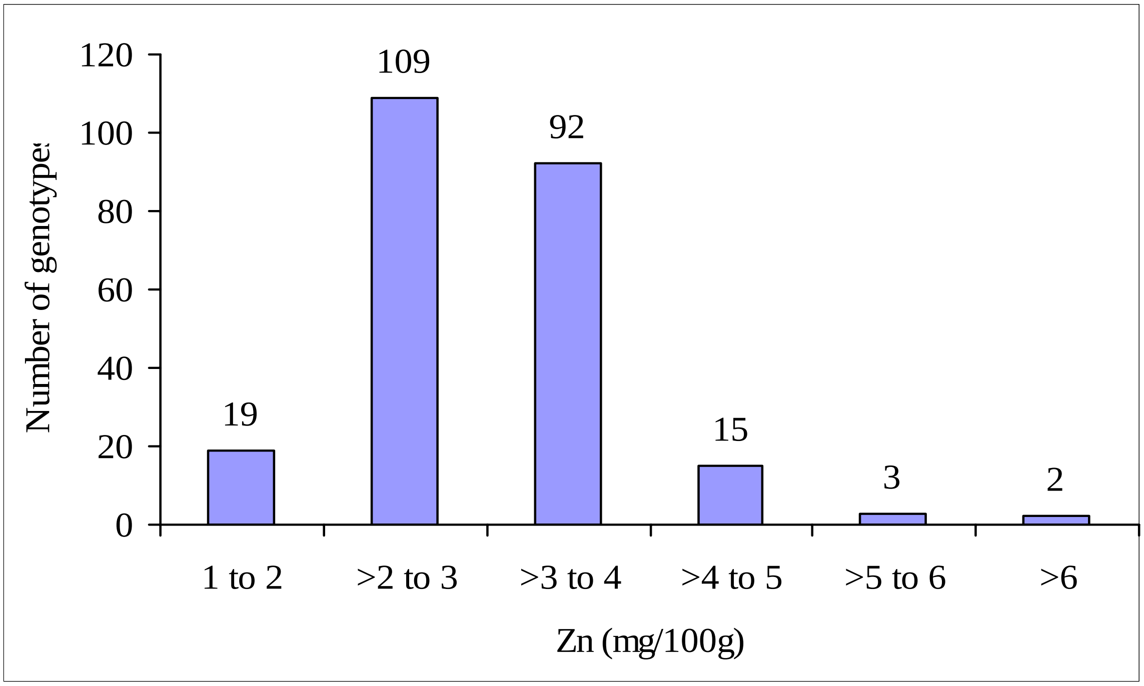


Figure 7: Frequency distribution of 240 pigeon pea genotypes with varying zinc content

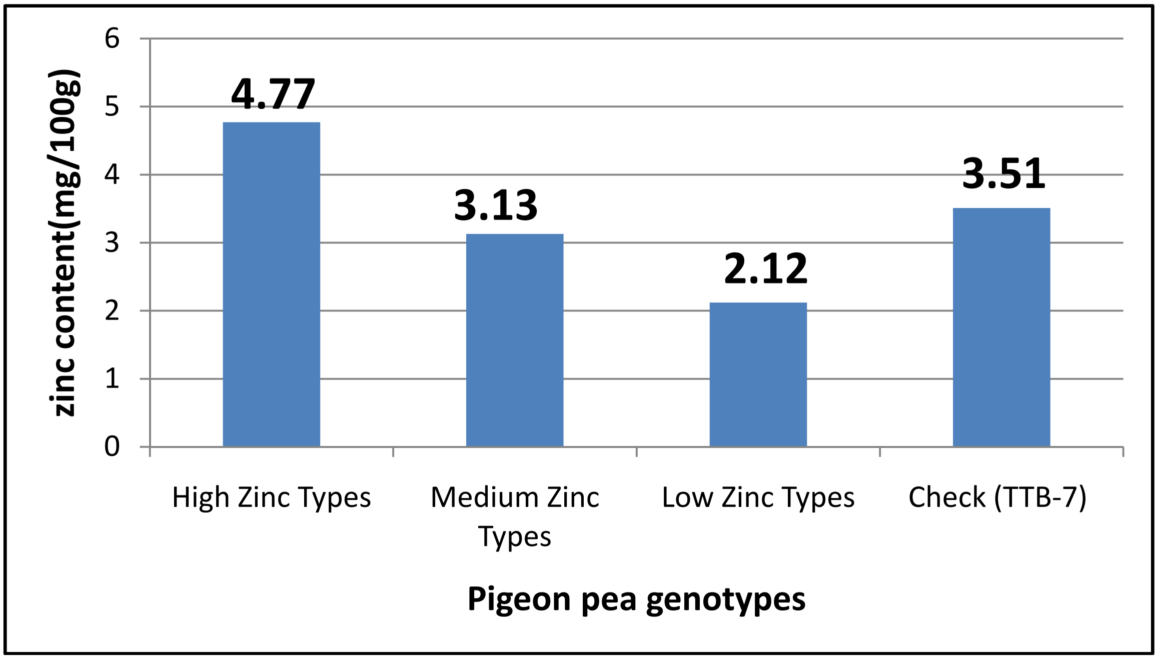


Figure 8: Mean seed zinc content of contrast pigeon pea genotypes

4.2.1 Root parameters

4.2.1.1 Root length

Significant difference was noticed in the root length of the 217 genotypes, with mean of 63cm, ranging from 14.7cm to as high as 204.6cm. There was significant variation between root length and of high zinc types (ranging from 23 to 74.6cm) and root lenghth low zinc types (ranging from 34.8 to 103.6cm). However, there was no significant difference between the mean root length of high zinc types (58.57cm) and low zinc types (60.7cm) (Table 4). TTB-7 (check) variety had root length of 85.20cm higher than high zinc types.

4.2.1.2 Root volume

There was significant variation in root volume among the genotypes grown in root structure, ranged from mean root volume of 6.5cm3 to as high as 257.5cm3, with mean root volume of 6.12cm3. Contrast zinc types showed significant difference with 55cm (ranged from 17.5cm to 217.5cm) for high zinc types and 36.23cm (ranged from 10.87cm to 87.5cm) for low zinc types (Table 4).

4.2.1.3 Root dry weight

Root dry weight showed significant differences between the 217 genotypes ranged from 4.7g to 123.15g, with mean root dry weight of 22.9g. Significant variation in root dry weight existed among the genotypes within the contrast zinc types, as root dry weight of high zinc types ranged from 18.8g to 107.3g and low zinc types ranged between 5.56cm to 42.0cm (Table 4). However, contrast types did not show significant difference for root dry weight.

4.2.2 Shoot Parameters

4.2.2.1 SPAD meter reading

There was significant difference in the SPAD meter reading of the 217 pigeon pea genotypes, with mean of 53.5 and ranged from 42.1 to as high as 66.0.

Table: 4 Root parameters of pigeon pea genotypes grown in root structure

217 genotypes	**Root Length (cm)**		**Root Volume (cm3)**		**Root dry wt (g)**	
Range	14.0-204.6		6.5-257.5		14.7-123.15	
Mean	63		49.5		22.9	
CV (%)	5.69		6.12		14	
CD: Events	7.03		5.13		6.28	
Check (TTB-7)	85.2		27.5		12.0	
Contrast Types	**High**	**Low**	**High**	**Low**	**High**	**Low**
Mean	**58.57**	**60.7**	**55**	**36.23**	**58.6**	**55.0**
Range	**23-74.6**	**34.8-103.6**	**17.5-217.5**	**10-87.5**	**8.8-107.3**	**5.6-42.0**

Table: 5 Shoot parameters of pigeon pea genotypes grown in root structure

217 genotypes	**SPAD Chl. Reading**		**Days to 50% flowering (DAS)**		**Plant height (cm)**		**Shoot weight (g)**		**TDM (g)**	
Range	42.1 - 66.0		65 - 164		47.3 - 248.8		10.94 - 366.85		15.2 - 489.9	
Mean	53.5		94.4		158.7		119.1		142.4	
CV (%)	2.69		2.84		4.8		8.23		5.83	
CD: Events	2.82		5.31		4.94		7.47		8.32	
Check (TTB-7)	52.4		80.5		115.5		76.8		88.8	
Contrast Types	**High**	**Low**	**High**	**Low**	**High**	**Low**	**High**	**Low**	**High**	**Low**
Mean	**55.8**	**54.4**	**95.4**	**92.9**	**149.3**	**156**	**107.0**	**93.4**	**134.7**	**110.8**
Range	**49.7- 63.9**	**49 - 63.7**	**72.5 - 125.5**	**80.5 - 143.5**	**50.8 - 224.8**	**47.3 - 240.7**	**43.96 - 203.97**	**34.59 - 176.29**	**69.2 - 345.6**	**41.3 - 218.3**

However, there was no significant difference in the mean of SPAD meter reading of contrast types, with 55.8 and 54.4 respectively for high zinc and low types (Table 5). It indicates that contrast types did not vary for their chlorophyll content in the root structure experiment.

4.2.2.2 Days to 50% flowering

Significant difference exist in the days taken to 50% flowering of the 217 genotypes, with mean of 80.5 days after sowing (DAS) and with the range of 65 to 164 days after sowing (Table 5). There was no significant difference in the means of days to 50% flowering between the contrast type lines. However there was significant difference among the genotypes within the contrast type (Table5).

4.2.2.3 Plant height

Significant genetic variation exists in the plant height of the 217 genotypes, with mean of 158.7cm, varying from as low as 47.3cm to as high as 366.6cm (Table 5). Mean plant height of low zinc contrast type is slightly higher (156cm) than high zinc types and also had a high range from 47.3 to 240.7cm. High zinc types having mean plant height of 149.3cm with range of 50.8cm to 224.8cm (Table 5).

4.2.2.4 Shoot weight

There was significant difference in the shoot weight of the 217 pigeon pea genotypes, with mean of 119.1g and ranged from 10.94 to as high as 366.85. (Table 5). The high zinc types had higher mean shoot weight (107.34g) ranging from 43.96cm to 203.97cm whereas low zinc types have mean shoot eight of 93.39g and ranged from 34.59g to 176.29g (Table 5).

4.2.2.5 Total dry matter (TDM)

Total dry matter (TDM) showed significant difference between the 217 pigeon pea genotypes grown in the root structure with mean TDM of 142.4, ranging from 15.2g to as high as 489.9g (Table 5). Comparing the contrast types, high zinc types had significantly higher mean TDM (134.7g) and had range of 69.2g to 345.6g, while low zinc types had mean TDM of 110g ranging from 41.3 to 218.3g (Table 5).

4.2.2.6 Physiological parameters of contrast types grown in root structure

There was no significant difference in the mean photosynthetic rate and stomatal conductance between the high and low zinc types. However, there was significant variation in physiological parameters among the genotypes within a particular contrast type. Photosynthetic rate of high zinc types ranged from 20.45 to 35.50umol/m2/sec and low zinc types ranged from 20.85 to 37.85umol/m2/sec. High zinc types stomatal conductance ranging from 0.64 to 0.77umol/m2/sec and low zinc types stomatal conductance ranged from 0.71 to 0.84umol/m2/sec (Table 6).

4.2.2.7 Seed zinc

There was a significant genetic variation in seed zinc content of 217 pigeon pea genotypes with mean seed zinc of 3.0mg/100g seed weight and ranged from 1.16mg/100g to 6.36mg/100g seed weight (Table 7). While comparing the mean seed zinc of 217 genotypes with the check (TTB-7), the check variety showed more seed zinc content (3.9mg/100g) than the 217 genotypes (3.0mg/100g) (Figure 9). However, mean leaf zinc content was more in the 217 (6.41mg/100g) compared to check variety.

Table 6: Photosynthetic characters of pigeon pea contrast types grown in root structure

	High Zinc Types			Low Zinc Types		
S.I.	**ICP Accession No.**	**Photosynthetic rate (µmol/m2/sec)**	**Stomatal conductance) (µmol/m2/sec)**	**ICP Accession No.**	**Photosynthetic rate (µmol/m2/sec)**	**Stomatal conductance) (µmol/m2/sec)**
1	6443	34.3	0.75	10960	36.45	0.79
2	7035	29.3	0.70	10654	37.85	0.74
3	7118	30.7	0.78	8602	31.00	0.72
4	6974	31.9	0.77	8863	33.60	0.8
5	14954	25.1	0.76	8513	35.60	0.74
6	6997	33.1	0.77	9336	27.45	0.77
7	6992	33.0	0.60	8508	29.80	0.71
8	7182	25.4	0.79	11946	20.85	0.74
9	7314	33.0	0.71	12410	22.00	0.75
10	6370	35.5	0.70	7623	30.65	0.57
11	2484	23.4	0.76	9691	33.35	0.67
12	2059	20.5	0.77	11543	23.35	0.73
13	2626	34.7	0.68	7221	24.40	0.74
14	7119	22.5	0.64	8516	34.30	0.77
15	14421	25.2	0.71	9655	32.65	0.84
	Range	**20.45-35.50**	**0.64-0.77**	**Range**	**20.85-37.85**	**0.71-0.84**
	Mean	**29.15**	**0.73**	**Mean**	**30.22**	**0.74**
	CV (%)	**4.42**	**7.81**	**CV (%)**	**5.73**	**9.07**
	CD: Events	**2.76**	**0.14**	**CD: Events**	**3.71**	**0.14**

Table: 7 Shoot parameters of pigeon pea genotypes grown in root structure

217 genotypes	**Seed Zn (mg/100g)**		**Leaf Zn (mg/100g)**		**Pod weight (g)**		**100 seed weight (g)**	
Range	1.16 - 6.36		2.68 - 11.87		13.5 - 295.8		5.25 - 24.45	
Mean	2.99		6.41		69.6		10.51	
CV (%)	0.99		0.48		5.6		6.89	
CD: Events	0.11		0.05		7.8		1.49	
Check (TTB-7)	3.961		5.229		38		12.45	
Contrast Types	**High**	**Low**	**High**	**Low**	**High**	**Low**	**High**	**Low**
Mean	5.48	3.99	4.58	2.09	90.15	80.63	11.18	10.5
Range	4.16-6.71	3.02-5.50	3.75-6.36	1.16-3.11	35.8-150.2	14.5-154.3	8.3-24.5	7.1-16.6

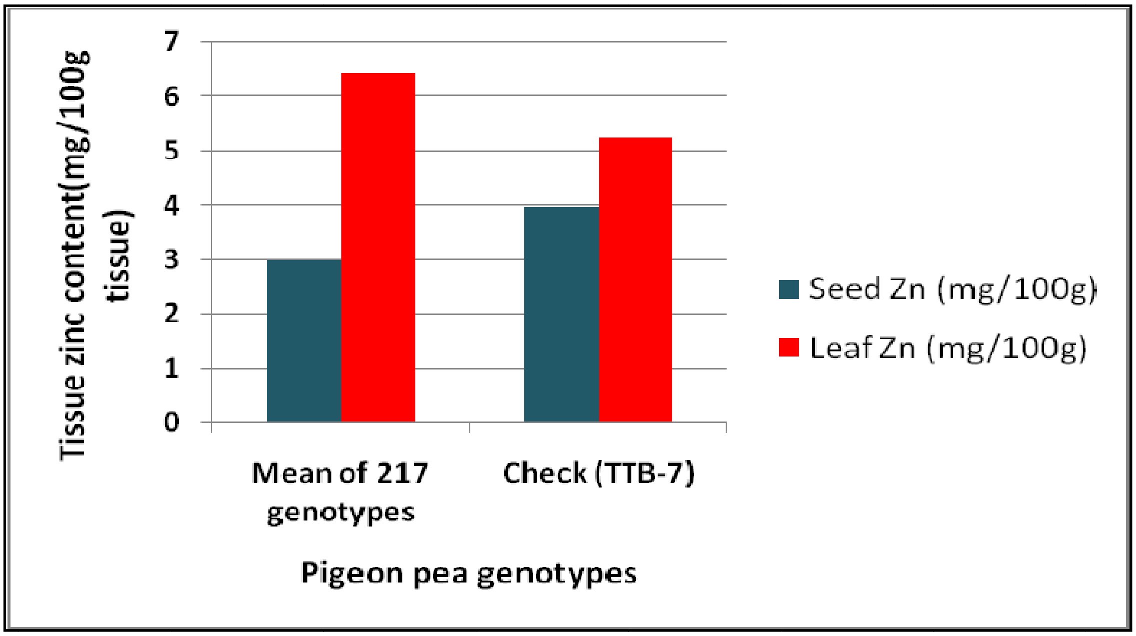


Figure 9: Mean seed zinc and leaf zinc content of 217 germplasm lines Vs check (TTB-7)

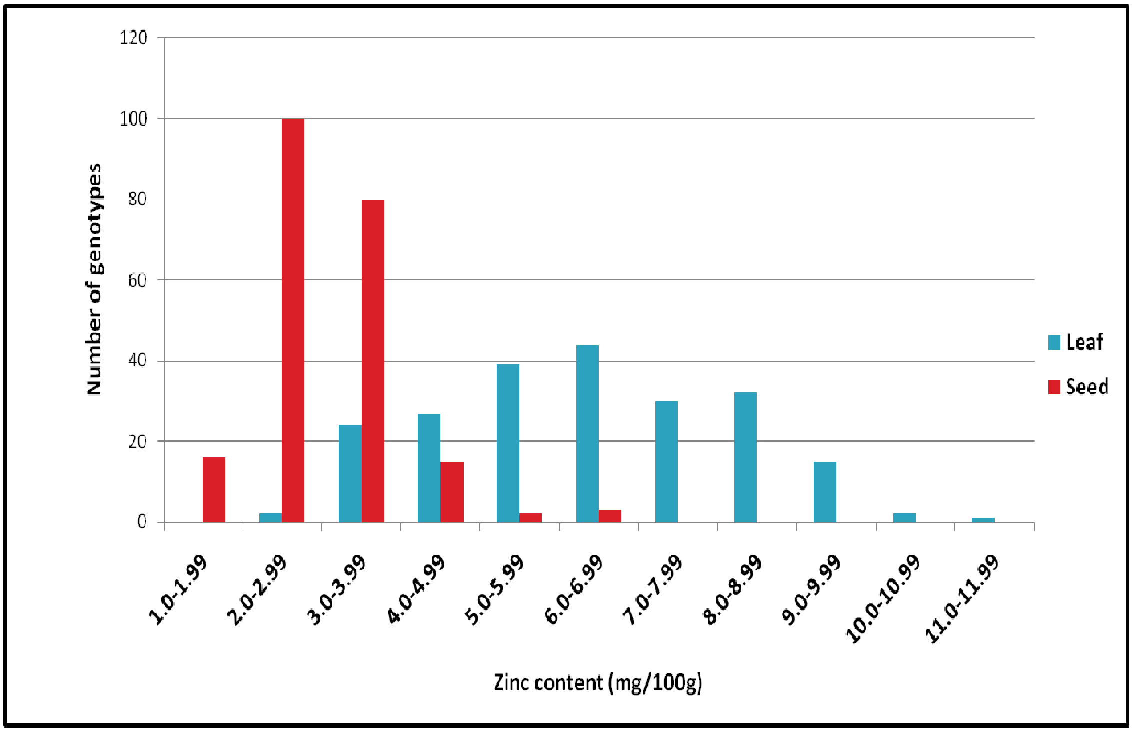


Figure 10: Frequency distribution of seed and leaf zinc content of 217 genotypes analysed by ICP-OES.

4.2.2.8 Leaf zinc content

Significant variation also exists in leaf zinc content which ranged from 2.68mg/100g to 11.87mg/100g and mean leaf zinc of 6.41mg/100g seed weight. While comparing the contrast types, higher zinc types accumulated higher zinc content in seed (4.58mg/100g) compared to low zinc types. Low zinc types accumulated less leaf zinc (2.09mg/100g) ranged from 1.16mg/100g to 3.11mg/100g (Table 6).

4.2.2.8 Frequency distribution of seed zinc and leaf zinc of 217 genotypes

The frequency distribution graph (Figure 10) showed that there is a good genetic variability in seed zinc and leaf zinc content of the 217 genotypes grown in root structure. About 83% of the genotypes had seed zinc content from 2mg/100g to 3.99mg/100g. For leaf zinc content, few genotypes (1%) were below 3mg/100g, majority of the genotypes (68%) had leaf zinc content between 5 mg/100g to 8mg/100g leaf weight and about 24% of genotypes had leaf zinc content between 3mg/100g to 4.99mg/100g and 6% of genotypes were having leaf zinc content between 9mg/100 to 10mg/100g and mere 1% of genotypes were above 10mg/100g leaf weight.

4.2.2.9 Pod weight

Significant genetic variations exist in pod weight of the 217 genotypes, which ranged from 13.5g to 295.8g and had mean pod weight of 69.6g. The high zinc types had significantly higher mean pod weight (90.15g) ranging from 35.8g to 150.17g compared to low zinc types with mean pod weight 80.63g ranging from 14.45g to 154.3g (Table 7).

4.2.2.10 100 seed weight

100 seed weight showed genetic variation between the 217 genotypes, ranging from 5.25g to 24.45g with mean 100 seed weight of 10.51g. However,

there was no significant difference between the mean 100 seed weight of contrast types, i.e. 11.18g for high zinc types and 10.5g for low zinc types respectively (Table 7).

4.2.2.11 Comparison between ICRISAT seeds and seeds grown in GKVK environment

Zinc element analysis of pigeon pea seeds was done twice in this investigation. First, pigeon pea germplasm seeds obtained from ICRISAT were analyzed using Atomic Absorption Spectrometer (AAS) at Dryland agriculture, GKVK. The second time, seeds of 217 pigeon pea genotypes grown in GKVK environment were analyzed using Inductively Coupled Plasma – Optical Emission Spectrometer (ICP-OES). The results showed that there was no significant difference in the frequency distribution of seeds zinc content between the two batches of analysis of pigeon pea seed (Table 8). For ICRISAT seeds, about 84% of genotypes had seed zinc content between 2 to 4mg/100g seeds [Figure 11(A)], whereas the GKVK seeds, approximately 83% fell in the same category, and similar trend was observed in the minor categories of classification [Figure 11(B)].

4.2.3 Correlation between seed zinc and leaf zinc content

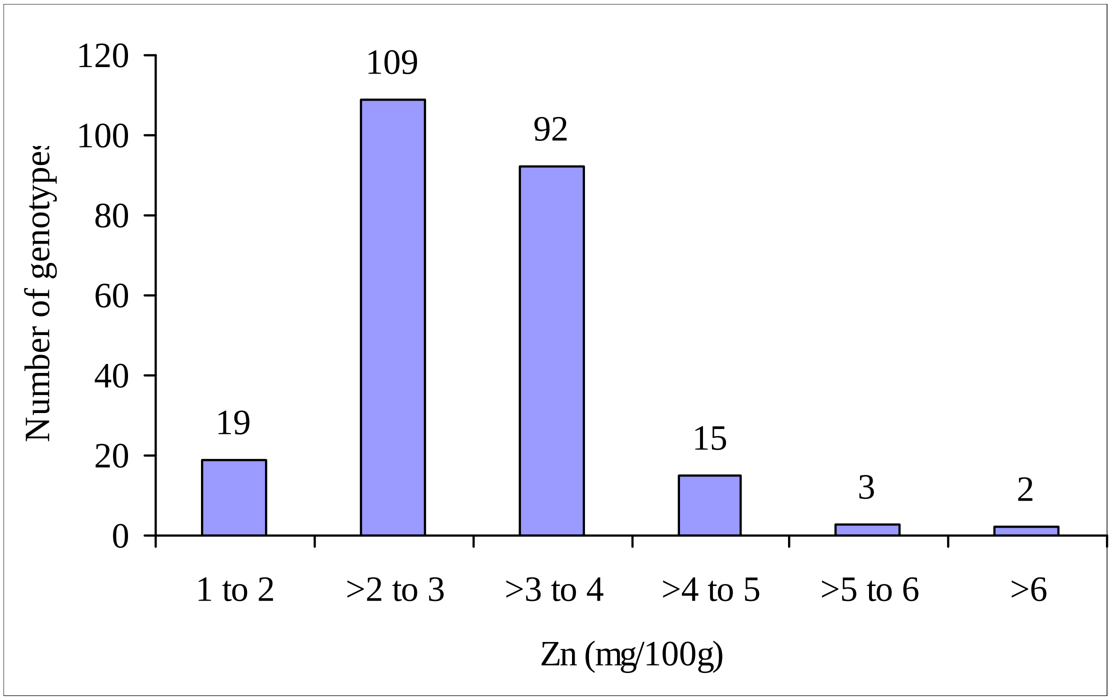
There was no significant relationship between seed zinc and leaf zinc content of the 217 genotypes grown in the root structure (Figure 12).

4.2.4 Correlation between seed zinc and root dry weight

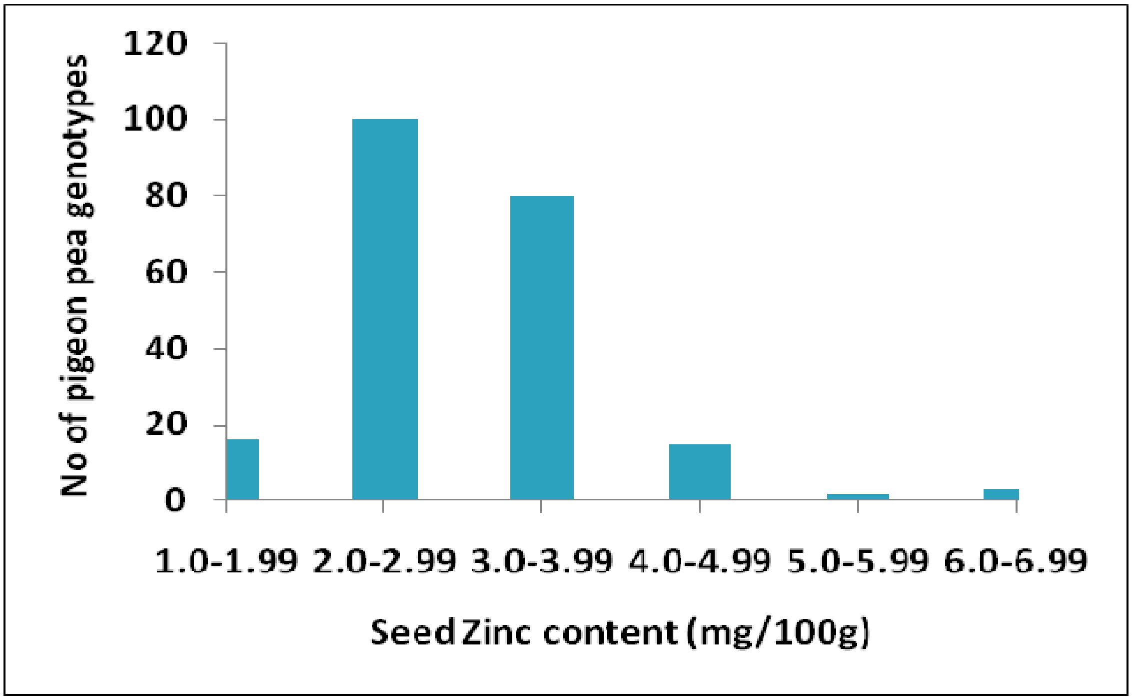
There was no significant relationship between seed zinc and root zinc content of the 217 genotypes grown in the root structure (Figure 13).

4.2.5 Correlation between leaf zinc and root dry weight

There was no significant relationship between seed zinc and leaf zinc content of the 217 genotypes grown in the root structure (Figure 14).



(A) Seed Zinc content of 240 pigeon pea germplasm lines Analyzed By Atomic Absorption Spectrometer (AAS)



(B) Seed Zinc content of 240 pigeon pea germplasm lines Analyzed By Inductively Coupled Plasma- Optical Emission Spectrometer (ICP-OES)

Figure 11: Comparison of frequency distribution of seed zinc content analyzed by AAS (A) and ICP-OES (B)

Table 8: Comparison of mean seed zinc content analyzed by AAS and ICP-OES

	Seed zinc content From AAS	**Seed zinc content From ICP-OES**
Mean	3.06	2.99
Range	1.53-6.95	1.16 - 6.36
CV (%)	9.76	0.99

Table 9: Variation in SPAD meter readings of contrast types under different levels of external zinc supply

	High Zinc Types				**Low Zinc Types**			
SI	**ICP Accession No.**	**Control (g)**	**T1 (g)**	**T2 (g)**	**ICP Accession No.**	**Control (g)**	**T1 (g)**	**T2 (g)**
1	**6443**	49.6	57.2	56.4	**10960**	48.4	53.6	48.5
2	**7035**	48.1	53.5	56.3	**10654**	50	53.1	50.7
3	**7118**	50.2	50.5	54.3	**8602**	48.3	52.8	51.3
4	**6974**	51.7	53	56	**8863**	48.5	52.2	54.7
5	**14954**	51.9	50	50.4	**8513**	50.2	52	50.3
6	**6997**	47.3	49.5	62	**9336**	51.9	51.4	55
7	**6992**	45.6	54.1	47.5	**8508**	49.8	53.2	57.5
8	**7182**	46.6	48.8	52	**11946**	52.1	53.9	53.7
9	**7314**	50.7	50.7	51.1	**12410**	51.9	48.5	50.2
10	**6370**	55	42.2	52.1	**7623**	41.6	51.5	50.9
11	**2484**	49.2	48.6	52.1	**9691**	54.8	53.2	53.3
12	**2059**	53.2	44.9	62.5	**11543**	46.6	63.4	57
13	**2626**	50.2	52.9	56.3	**7221**	53.3	54.7	55.4
14	**7119**	42.4	52.3	49.2	**8516**	57.2	55.5	55
15	**14421**	50.6	50.7	52.3	**9655**	49.9	52	58.8
	Range	**42.4-53.2**	**42.2-57.2**	**47.5-62.5**	**Range**	**46.6-57.2**	**52.0-63.4**	**48.5-58.8**
	Mean	**49.5**	**50.6**	**54**	**Mean**	**50.3**	**53.4**	**53.5**
	CV (%)	**4.48**			**CV (%)**	**4.06**		
	CD: Treatment	**1.2**			**CD: Treatment**	**1.11**		
	CD: Events	**2.68**			**CD: Events**	**2.47**		
	CD: T x E	**4.64**			**CD: T X E**	**4.28**		
Check	**TTB7**	**50.1**	**39.9**	**47.1**				

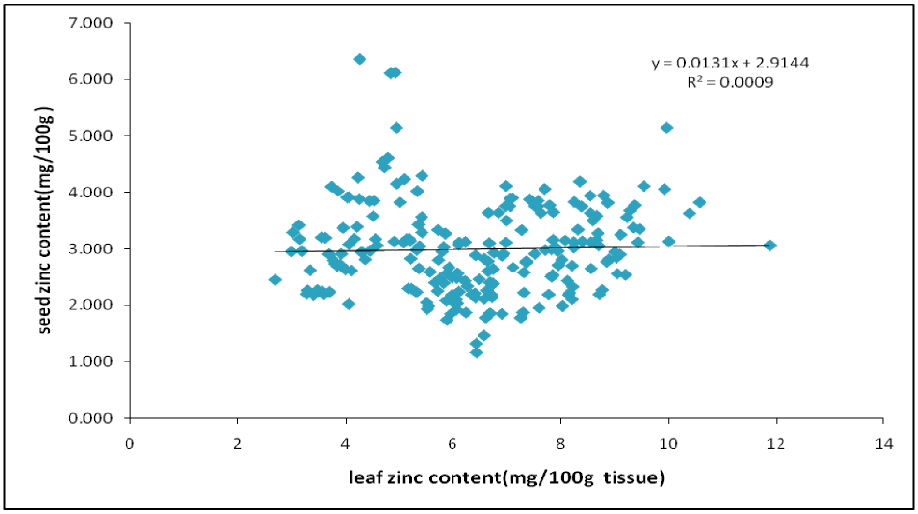


Figure 12: Correlation between seed zinc and leaf zinc of 217 genotypes

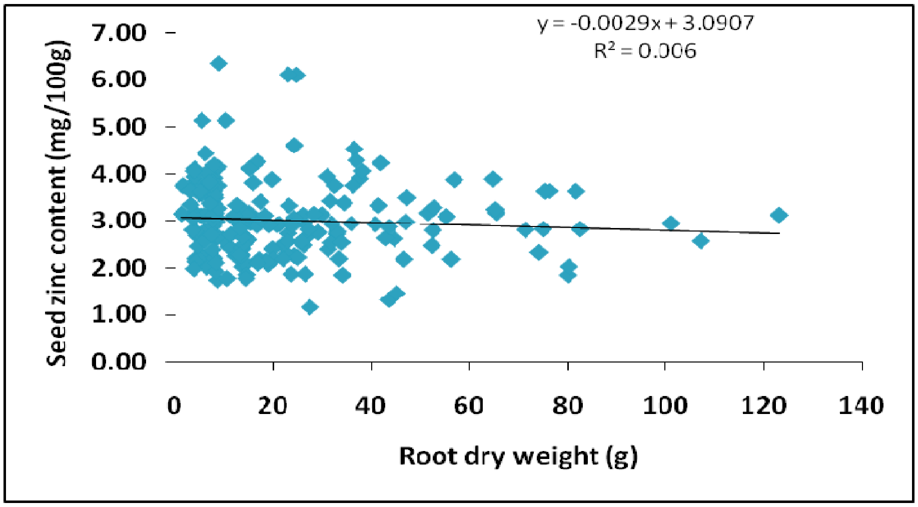


Figure 13: Correlation between seed zinc and root dry weight

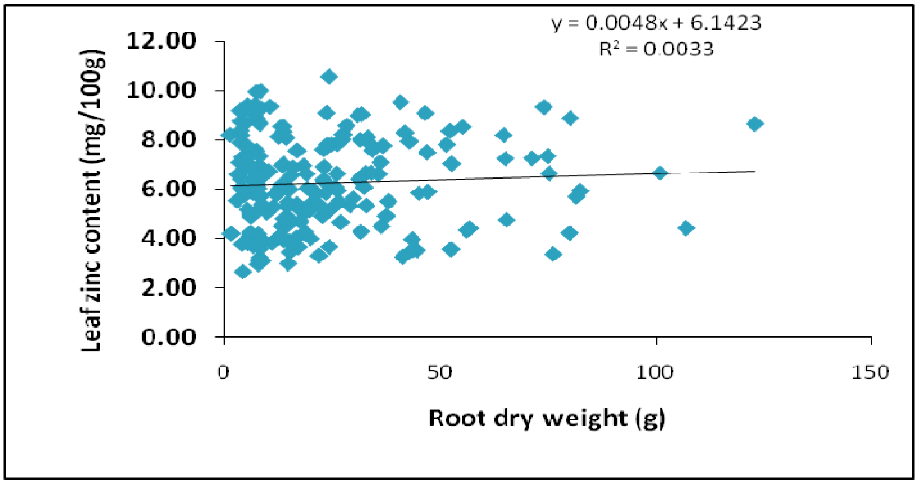


Figure 14: Correlation between leaf zinc and root dry weight

4.3 Studying the zinc uptake in contrast lines i.e. high and low types

The classified low (15) and high (15) seed zinc types were grown under the same soil zinc status in three blocks (treatments). Two blocks were applied with two different zinc treatments, while the third block was maintained as the control. Foliar zinc treatment (1% $ZnSO4$) and soil zinc treatment (60mg/pot) were applied at the same time i.e. 60 DAS, and the experiment was maintained until maturity, to study the differential pattern of uptake and translocation of zinc to seed.

The genotypes were analyzed for their root and shoot biomass and uptake of zinc by roots and translocation of zinc to different plant parts (stem, leaf and seed) when grown under the 3 different Zn levels. The biomass was analyzed in 30 days after treatment (foliar and soil zinc application) and seeds were analyzed at maturity stage after harvest.

4.3.1 Root and shoot biomass

The results indicated that there was a significant variation in root biomass among the contrast genotypes grown in the different zinc treatments compared to the control. Comparatively, low zinc types had significantly higher mean root biomass in both the treatments compared to high zinc types. However there was no significant difference between the mean root biomass under foliar spray and soil application in both the contrast types, high and low zinc types.

Shoot biomass showed a different trend compared to root biomass. High types had significantly higher mean shoot biomass in both the treatments (T1 and T2) compared to low zinc types. However, comparing the two external zinc treatments, foliar zinc application showed higher mean shoot biomass than soil zinc application in both high and low contrast types (Figure 16).

4.3.2 Pod weight

Soil zinc application showed higher mean pod weight in both high and low types, however, low zinc type significantly had higher mean pod weight (14.98g) than higher zinc types. There was no significant difference in mean pod weight of foliar application between the contrast zinc types (Figure 15)

4.3.3 SPAD chlorophyll reading

The results showed that there was no significant difference in mean SPAD meter reading between the treatments and also between the contrast types. However, soil zinc treated genotypes showed slightly higher mean SPAD chlorophyll reading in high (T1 had 50.6 and T2 had 54.0) and low contrast types (T1 had 53.4 and T2 had 53.5) compared to the control (Table 9).

4.3.4 Zinc content in different plant parts

The results indicated that high zinc types accumulated more zinc content in all plant parts (Figure 16) which was analyzed using ICP-OES.

4.3.4.1 Stem zinc content

Stem mean zinc content under different external zinc treatments showed no significant difference in high zinc types, however foliar zinc treatment showed significantly higher mean zinc content (3.32mg/100g) than soil zinc treatment (2.95mg/100g) (Figure 16)

4.3.4.2 Root zinc content

Mean root zinc of high and low zinc types showed a positive response of the genotypes to the soil zinc application. Mean root zinc content of high zinc types was significantly higher (7.12mg/100g) compared to low zinc types (4.11mg/100g) (Figure 16).

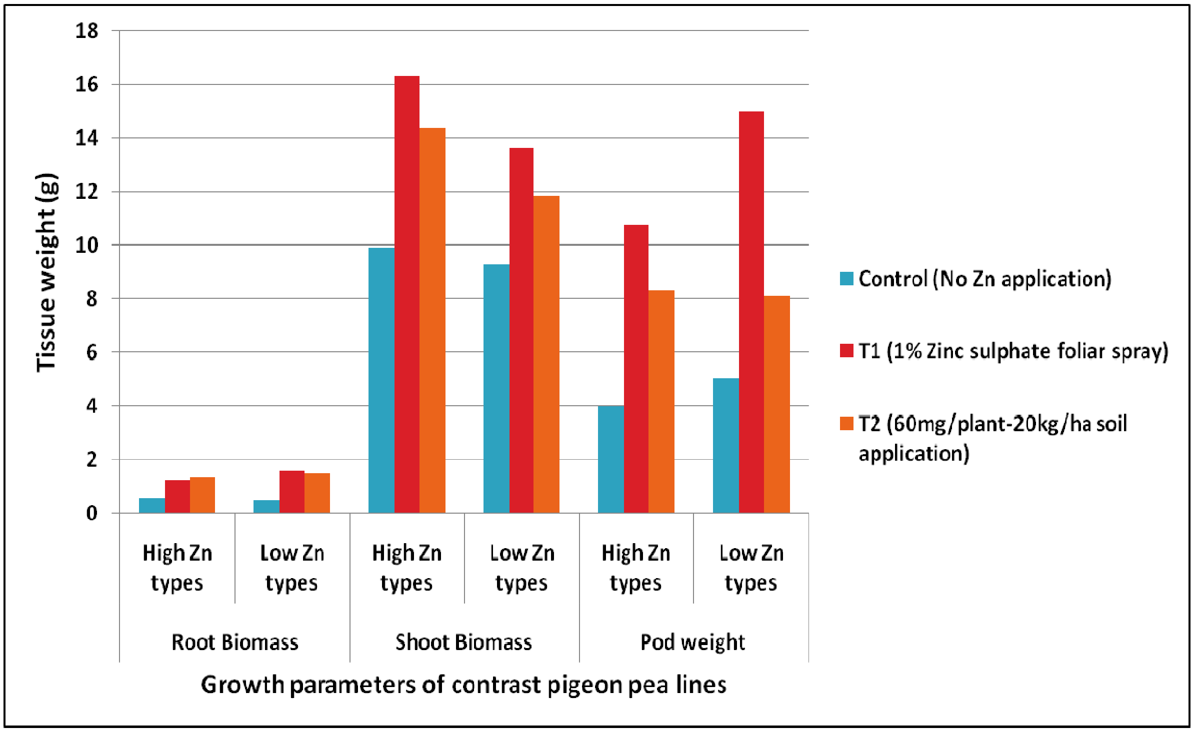


Figure 15: Biomass variation of Contrast types under different Zinc treatments

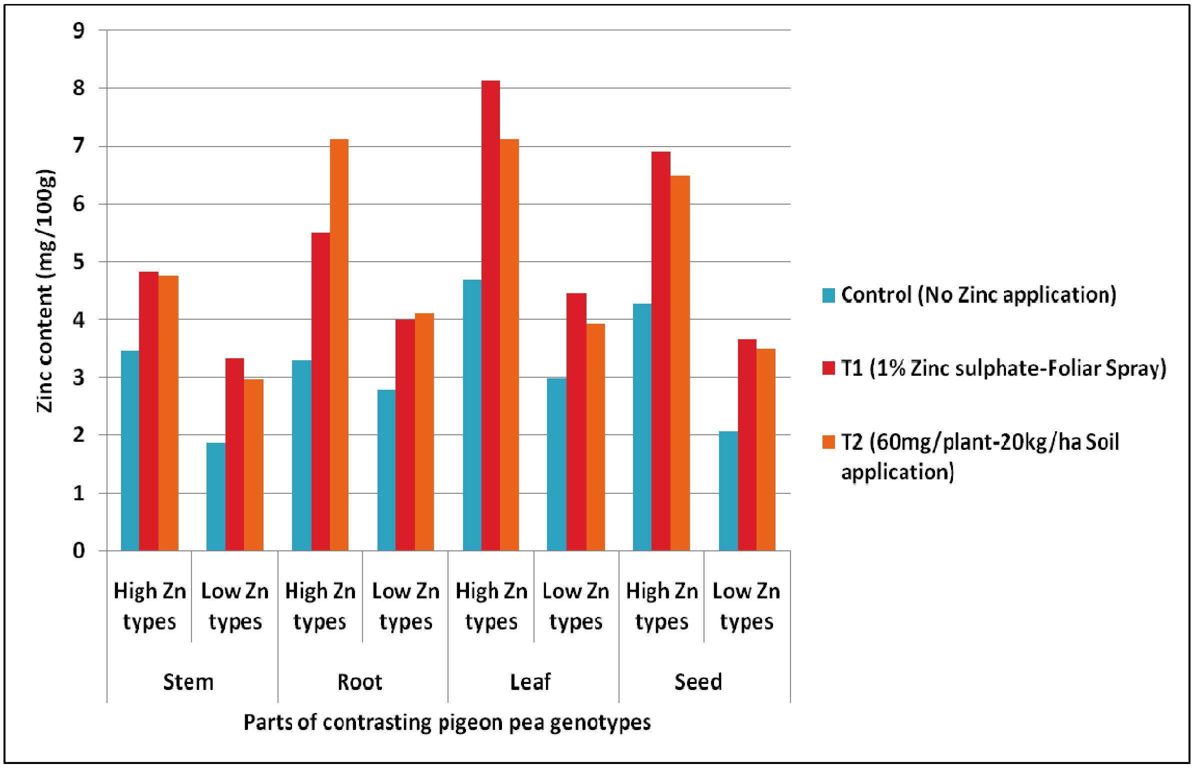


Figure 16: Zinc content in different plant parts of high and low zinc types

4.3.4.3 Leaf zinc content

Foliar zinc application showed a significant increase in leaf zinc content of both contrast types compared to soil zinc application and control. Leaf zinc in high zinc types ranged from 6.87 to 11.38mg/100g, while in soil zinc application leaf zinc ranged from 5.47 to 9.85mg/100g (Figure 16)

4.3.4.4 Seed zinc content

There was no significant difference in the mean seed zinc content of soil and foliar zinc application in both the contrast types. However, foliar zinc treatment showed a slight increase in mean seed content compared to soil zinc treated genotypes (Figure 16).

4.4 Effect of tissue zinc concentration and external zinc supply on seed zinc content

The seed zinc content of 31 genotypes (15 high zinc types and 15 low zinc types + TTB-7) were analyzed using Z-distribution to find out the nature of zinc accumulation in the different plant parts in relation to seed zinc content.

The serial number 1 to 15 depicts high zinc types and 16 to 30 show low zinc types (Figure 17).

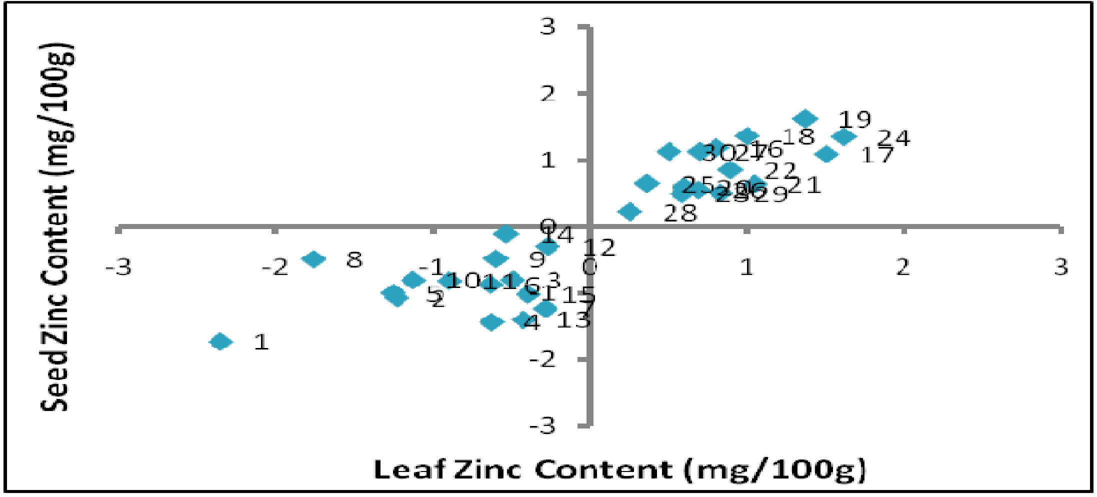
4.4.1 Leaf zinc content vs seed zinc content

The results showed a clear picture that genotypes containing high leaf zinc also contained higher seed zinc. The low zinc contrast genotypes has lower seed zinc despite of external zinc application except for ICP accession 2059 (12) which had lower seed zinc at higher leaf zinc content (Figure 17).

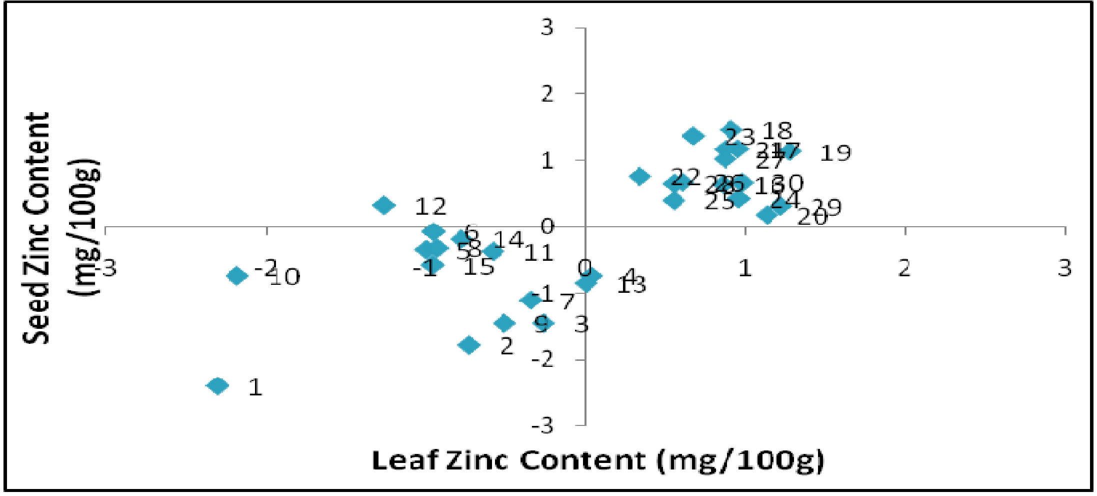
4.4.2 Root zinc content vs seed zinc content

In the foliar zinc treatment high zinc types occupied the lower quadrants, where 8 genotypes (1, 2, 5, 7, 9, 10, 13, and 15) accumulated high zinc content in

T1 - Zinc foliar application



T2 - Soil Zinc application



T3 - Control

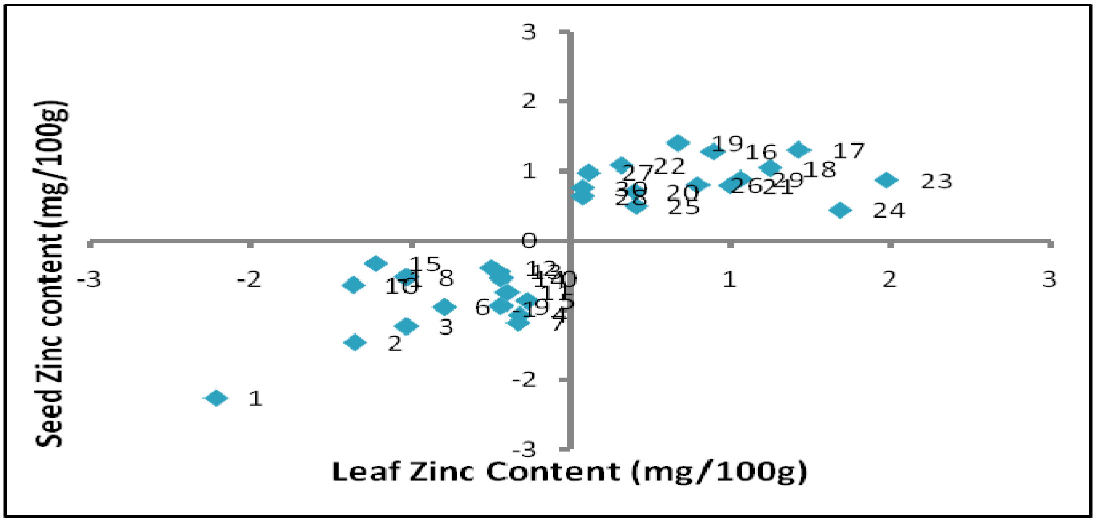


Figure 17: Z-distribution of seed zinc content Vs leaf zinc content under different external zinc levels

both root and seed. Another 7 high zinc genotypes (3, 4, 6, 8, 11, 12, 14) accumulated low root zinc but with high seed zinc. The lower zinc types fell in the two upper quadrants, at which 12 genotypes accumulated low root zinc and low seed zinc, and the other 3 genotypes (22, 26, and 27) had high root zinc and low seed zinc.

Soil zinc application followed a similar trend where all low zinc genotypes accumulated lower root zinc as well as lower seed zinc. For high zinc types, eight genotypes (1, 2, 3, 5, 8, 9, 11 and 13) accumulated high zinc content in the root and seed. Five genotypes (4, 6, 7, 14 and 15) had low root zinc but accumulated higher seed zinc.

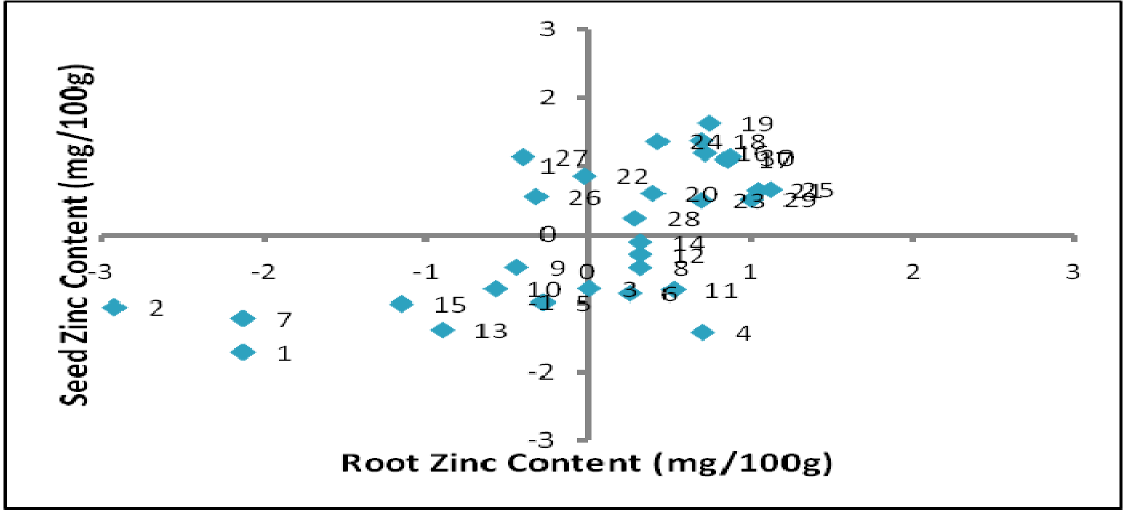
In the control, similar trend was observed as other two treatments and it was evident when all low zinc types fell in the above quadrants, of which nine genotypes (17, 20, 21, 22, 23, 24, 25, 26 and 28) had low root zinc and low seed zinc, whereas six genotypes accumulated higher root zinc but lower seed zinc (Figure 18).

4.4.3 Stem zinc content vs seed zinc content

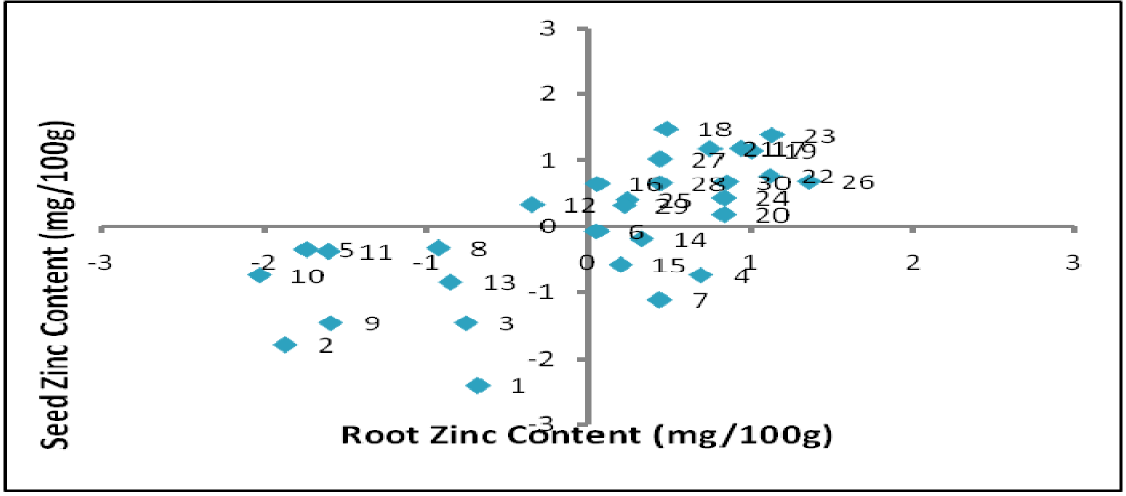
Foliar zinc treatment showed similar trend when compared with the accumulation of zinc in roots. All the low zinc types occupied the two above quadrants, where twelve genotypes had lower stem zinc as well as seed zinc, and three genotypes (22, 26 and 27) accumulated high stem zinc and low seed zinc.

High zinc types occupied the lower quadrants where nine genotypes accumulated high stem zinc and high seed zinc and six (2, 4, 10, 11, 14 and 15) genotypes had lower stem zinc and high seed zinc (Figure 19).

T1 - Zinc foliar Application



T2 - Soil Zn Application



T3 - Control

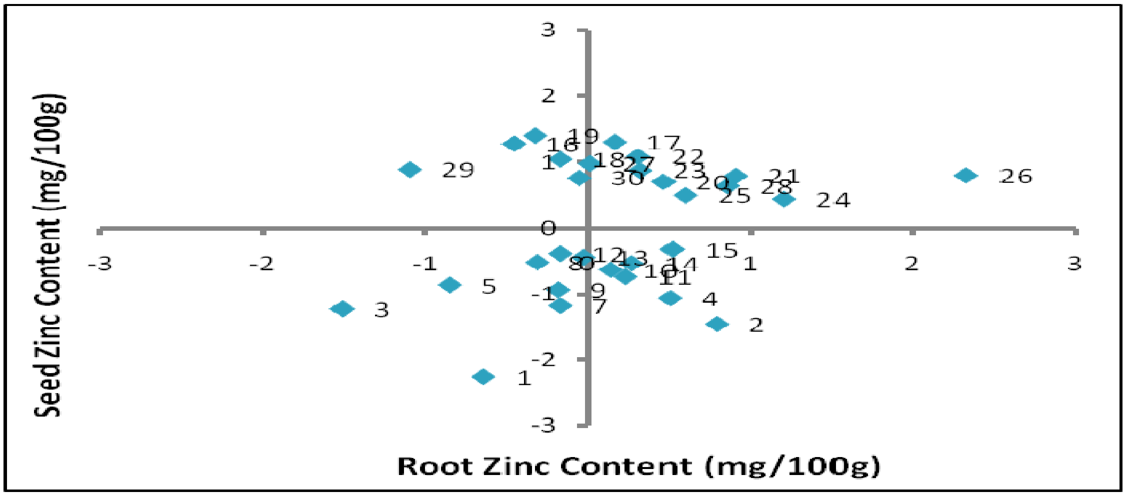
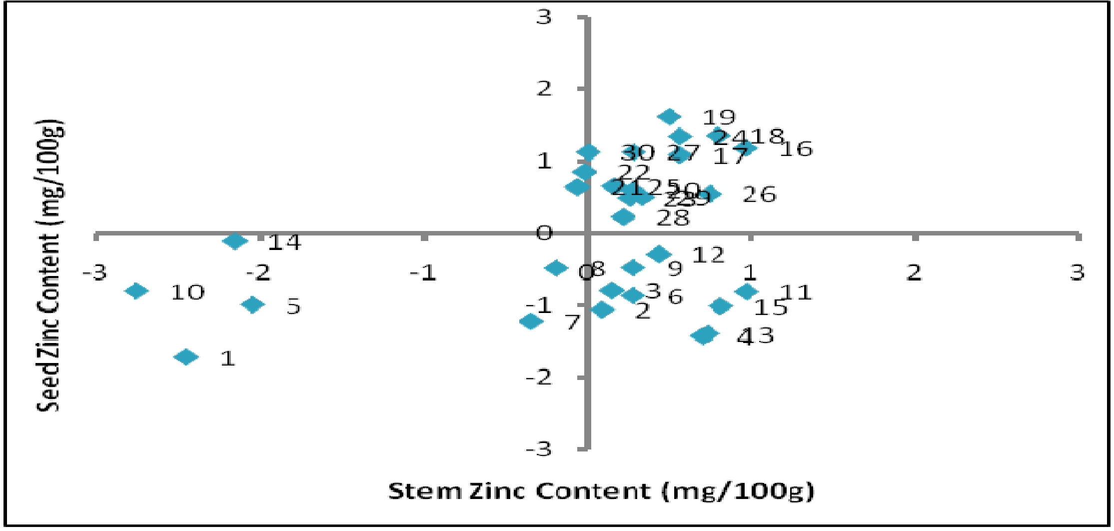
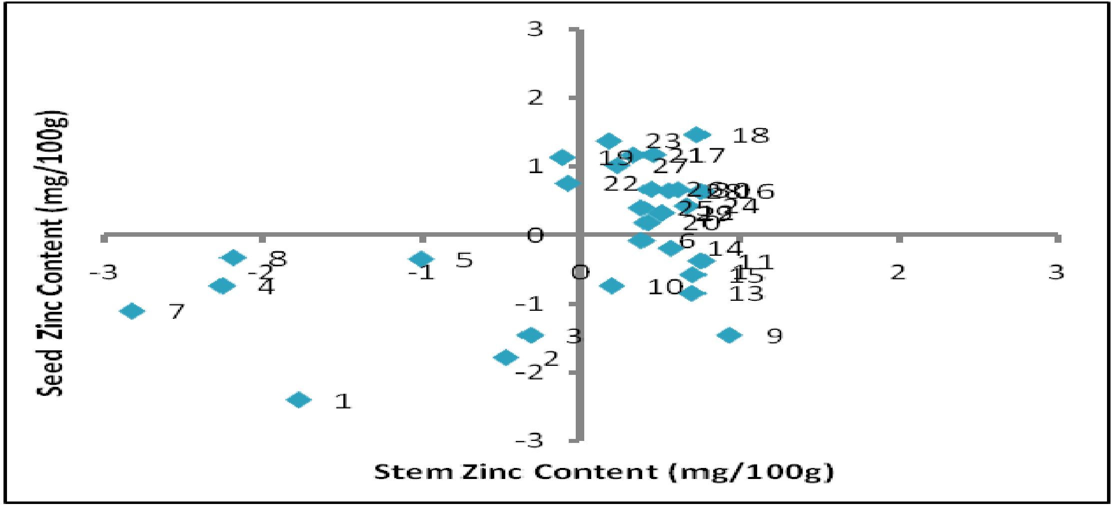


Figure 18: Z-distribution of seed zinc content Vs root zinc content under different external zinc levels

T1 - Zinc foliar application



T2 - Soil Zinc application



T3- Control

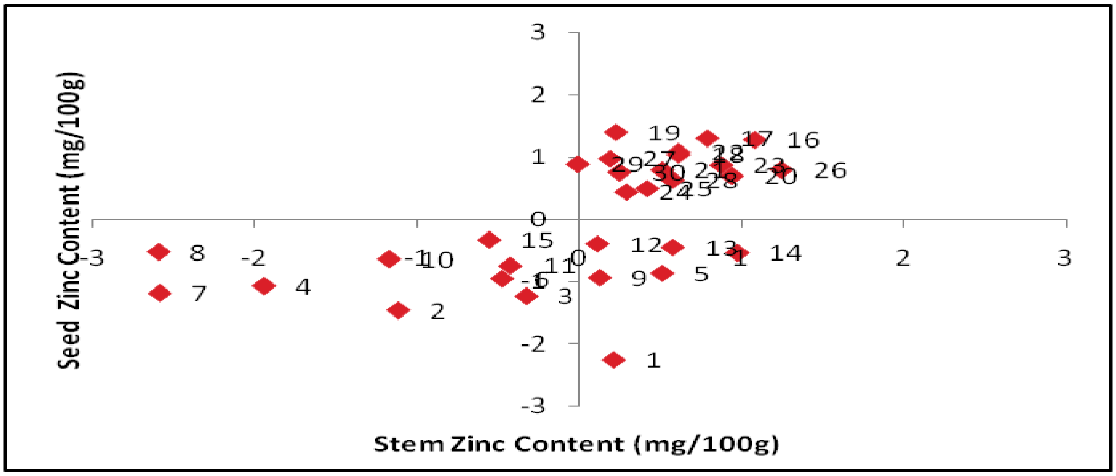


Figure 19: Z-distribution of seed zinc content Vs stem zinc content under different external zinc levels

Discussion

V. DISCUSSION

Soil productivity is decreasing globally due to enhanced soil degradation in the form of erosion, nutrient depletion, water scarcity, acidity, salanization, depletion of organic matter and poor drainage (Scherr, 1999). The enhanced soil degradation along with slow down in crop production will contribute to food insecurity. To feed the huge increasing population and to sustain the well being of humans, a large increase in food production must be achieved and the annual crop production needs to be increased by 40 per cent by 2020 to meet the food demand (Cakmak, 2002). Of this increased demand, 85 per cent will be contributed majorly by developing countries (Dyson, 1999). To achieve this, one of the bottleneck is nutrient stress especially micronutrient deficiencies, particularly Zn and Fe deficiency. They (Zn and Fe) are wide spread throughout the world and occur nearly in all countries, with about 50 per cent of the cultivated soils for cereal production globally are having low levels of plant available zinc. Based on analysis of soil samples from different countries, Zn deficiency was found to be the widest spread micronutrient deficiency (Graham and Welch, 1996). The little amount of zinc present in soils is efficiently immobilized in soils by conversion of soluble Zn to less soluble form by alkaline or calcareous soils (Welch and Graham, 2004).

Zinc efficient types absorb more Zn from deficient soils, produce more dry matter and grain yield, but do not necessarily have high Zn in grains. Despite the fact that Zn is the most mobile of all micronutrients in plants, edible parts of most crop plants contain low Zn. Only a few studies have been examined the mechanism and regulation of Zn absorption by roots, with many physiological processes fine tuning the regulation of uptake and translocation (Hacisalihoglu *et al.*, 2003). 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). The most important probable physiological barriers that might limit Zn uptake are (a) distribution of Zn at the rhizosphere or root – soil interferace, (b) absorption and translocation mechanisms (Transporters at root epidermis and ion channels) and (c) once taken up by root cells, their efficient translocation to and accumulation in edible plant organs (Bo Lonnedral, 2003, Palmer and Gerinot, 2009). Cereal species and genotypes of a given species differ greatly in their response to Zn deficiency and Zn fertilization (Cakmak *et al*., 1998; Cakmak, 2002). There are mechanisms other than release of organic acids from roots that are involved in tolerance to Zn deficiency, such as root uptake and root to shoot transport rates and internal Zn utilization (Cakmak *et al*., 1999; Rengel, 2001).

Plant species and genotypes differ in their zinc requirement (Sadeghhzadeh, 2009) which resulted in considerable variation for Zn uptake and accumulation by plants (Holm *et al*., 2002). Existence of substantial genotypic variation in tolerance to Zn deficiency can be exploited to enhance tolerance, but little information is available on how tolerance is inherited. Based on previous research, significant variations have been demonstrated for grain concentrations of Zn ranging from 0.84 to 5.84 mg/ 100g in rice (Nagarathna, et al., 2010), 2.5 to 5.3 mg/100g in wheat and 1.4 to 2.4 mg/100g in maize (Gregorio *et al*., 2000; Beebe *et al*., 2000). Despite the important genetic variation among crop genotypes, average values for Zn and Fe concentrations in cereal grains are still low, when compared with legumes (5.4 to 6 mg/100g) (Welch and Graham, 2004). The uptake of trace elements by roots and the accumulation of trace elements by seed or grain are 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 the genes determining high Fe and 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.

With this background, major emphasis of the study was to examine the genetic variability for Zn across species and to screen pigeon pea germplasm for variation in grain or seed zinc content and study the differential uptake and translocation pattern of Zn across the genotypes of pigeon pea.

Variation in seed Zinc content across 240 pigeon pea genotypes

Among the 240 genotypes, which were analyzed for their seed Zn content using AAS, approximately 40 per cent of them had 2- 3 mg/ 100g of seed, with about 84 per cent having a range of 2 - 4 mg/ 100g. Though there is variation in seed Zn the number of genotypes with high seed Zn is very less. It is necessary to understand if it is genetically efficient in uptake or is influenced by environment. These genotypes could be probable candidates in terms of human nutrition to supply the recommended daily intake of 15mg and are plant material to dissect the molecular basis, for high seed Zn content. It is also essential to understand the uptake and translocation pattern among these genotypes whether there is any variation in uptake and translocation ultimately leading to variation in seed Zn content. It is therefore important to understand the influence of genetic and environmental effects on distribution of Zn and its translocation to seeds (Khan *et al*., 2003).

Variation of physiological parameters among pigeon pea genotypes

Since, there was no physiological background information of the pigeon pea germplasm lines obtained from ICRISAT, the 240 pigeon pea genotypes were grown in a common environment at the root structure facility for the assessment of their genetic variability based on physiological parameters. Further assessment was conducted to find out the relationship between the physiological parameters taken and the zinc content of seeds and leaves of contrast seed zinc types.

There was significant variation among the genotypes in terms of root and shoot parameters. Root parameters studied include root length, root volume and root biomass. Root length varied significantly from as low as 14.0cm to as high as 204cm with a mean of 63cm. The same trend was observed in root volume, which ranged from a lower 6.6cm^3 to as high as 57.5cm with a mean of 45cm. Root dry weight which had a close relationship with root volume, had a higher variation from 14.7g to 123.5cm.

There was also a great variation noticed in the shoot parameters which may be due to the increase uptake of zinc, which was clearly shown by the zinc content in leaf and seed of contrast types. This trend depicted that the transition metals are more accessible for uptake. However, the transporters in many plants have a specific affinity for a particular oxidation state of each metal. Many plants address this problem by using a reduction-based strategy for metal uptake. Whereas Zn is always found in the +2 oxidation state under physiologically relevant conditions (Palmer, 2009). Graminaceous monocot roots may be able to absorb both Zn and Zn- Phytosiderophore chelates (Hacisalihoglu et al., 2001). The high Zn types performed much better under high soil Zn conditions with few genotypes behaving like low Zn types, indicating that plants would respond to Zn fertigation, if there is over expression of high affinity transporters for maximum uptake of applied zinc. Over expression of high affinity Zinc transporters in Barley showed the increase in short-term uptake after zinc deprivation and seed zinc content. Increased expression of genes encoding Zn transporters can increase plant Zn uptake (Ramesh *et al.*, 2004). The high expression of Zn transporter in *T. caerulescens* has been suggested to be the main reason for the enhanced Zn uptake (Assuncao *et* *al*., 2001). Transgenic Arabidopsis over expressing ZAT gene exhibited enhanced Zn resistance and increased Zn content in roots of the plants grown in higher external Zn environment (Ramesh *et al*., 2004). An increased uptake of Zn might be related to the level of expression of corresponding transporter protein located on the plasma membrane of root cells (Cakmak, 2002). Transporter like MtZIP2 is known to be up regulated in roots by Zn fertilization (Burleigh *et al*., 2003).

The genotypes also differed significantly in translocation of Zn from roots to shoots. Only few low Zn types were able to efficiently translocate Zn to their shoots, with most of them unable to translocate. This could be one of the reasons for cereals having the lowest grain Zn content. When externally higher levels of Zn are supplied, few high Zn types are able to translocate high amounts of Zn to shoots. This intern enhanced the whole plant metabolic process in which zinc was involved as the co-factor of more than 300 enzymes. Under low soil Zn levels there is no variation in root or shoot Zn. Therefore the uptake and translocation seems to vary with soil Zn status, indicating that plants posses different classes of transporters (Low and High affinity), which operates under different Zn concentrations. There are reports of concentration dependent kinetics of Zn influx into roots of wheat cultivars (Hacisalihoglu *et al*., 2001).

These high zinc type genotypes, which are able to maintain high root and shoot Zn would be useful as they might be the ones with high seed or grain Zn content. Long term Zn uptake in these studies can reflect and be influenced by a number of factors including compartmentation in roots as well as translocation and use in shoots (Hacisalihoglu *et al*., 2001).

Effect of external Zinc supply by foliar and soil zinc application on seed Zinc content and grain yield

Both low and high zinc types relatively responded to external Zn, with no effect by high Zn types. Few low Zn types were also Zn efficient, indicating that they were able to translocate high Zn to seeds and had high grain yield. The high Zn types had no positive effect of external Zn on either seed Zn or grain yield, suggesting that agronomic efficiency and grain Zn content are independently regulated. Depending on plant species, soil application of Zn can increase Zn concentration in plants by as much as 2 to 3 folds (Cakmak, 2002). However even with very high Zn fertilization rates, the Zn concentration in wheat grains did not increase correspondingly (Rengel, 1999; Welch and Graham, 2004). Although there is Zn fertilization, under most conditions there appears to be some biological limit that makes it difficult to increase concentration of Zn in grains (Bo Lonnerdal, 2003). However there was substantial increase in grain yield by Zn application in addition to NPK application indicating critical importance of this nutrient in crop production (Cakmak, 2002). Large increase in grain yield is also demonstrated by Graham and group (1992).

Classification of genotypes as high leaf high seed (HLHS), high leaf low seed (HLLS), low leaf high seed (LLHS) and low leaf low seed (LLLS) Zn types, can provide the leads in understanding the basic mechanisms in acquisition and translocation of Zn along with the other factors associated with variability in seed Zn content. The observed variability helps in identifying specific donor genotypes with high Zn efficiency and high Zn content (Nagarathna et al. 2010).

Field tests in Central Anatolia, Turkey, where Zn deficiency is widespread, showed that soil and foliar-applied $ZnSO_4$ significantly enhanced grain Zn concentration in wheat. Under certain conditions, Zn fertilizers were also highly effective in increasing grain yield of wheat. However, irrespective of soil Zn status, foliar Zn applications resulted in significant increases in grain Zn (Cakmak 2010).

There is variation among genotypes in terms of uptake and translocation of Zn to both shoot and seed. Ultimately it is the seed Zn concentration that is important from human nutrition point of view. Therefore it is necessary to identify genotypes that are able to take up and translocate Zn efficiently under low and high soil Zn status, with high grain yield. One of the probable genotype with most of these parameters is genotype 14 and 11 (ICP accession 7119 and 2484) with high uptake and translocation efficiency, high seed Zn content and increased yield under low soil Zn status, also increase in yield under enhanced soil Zn status. Genotype 1 (ICP Accession No.6443), was performing at a consistent nature with increase zinc content in all plant parts, as well as in yield. Despite being classified as low zinc contrast types, two genotypes showed significantly high seed zinc content at low leaf zinc content, i.e. ICP accession 7623 and 7221. These were also reflected in high root and shoot biomass as well as pod weight. They can be selected together with the high zinc genotype 1, 11 and 14 as probable variety to be release after subsequent confirmation by field trails and used to understand the molecular mechanism operating.

Salient findings of the study are

1. Significant genetic variability exists within pigeon pea species.
2. Significant genetic variability exists among pigeon pea genotypes for root and shoot characters.
3. Differential response exists in contrasting pigeon pea genotypes for external Zn application.
4. External application of Zn enhances uptake by roots and translocation to shoots, and it is reflected in enhanced seed Zn content which in turn increase yield per plant.
5. However in both the groups (Low and High), few genotypes respond better to external Zn application by showing enhanced seed Zn content.

6. A close examination is required by selecting a few genotypes (2 or 3) from each group before any conclusive statements are made, with regard to uptake by root, root to shoot translocation and sequestration in seeds. But the findings of this study strongly suggest it is possible to exploit genetic variability to enhance seed Zn content from plant and human nutrition perspective.

Summary

VI. SUMMARY

Food production on presently used land must be doubled in next two decades to meet food demand of the growing world population, for which large enhancement in application of fertilizers and improvement of soil fertility are indispensable. But simultaneously intensive cultivation of high yielding cultivars with heavy application of N, P and K fertilizer leads to occurrence of micronutrient deficiencies in plants. Among the micronutrients, Zn and Fe deficiency are reported to be wide spread (Chen and Barak, 1982; Vose, 1982). Therefore special attention should be paid to the micronutrient status of plants and soils to ensure further increases in crop production and sustained soil fertility. As is the case with plants and soils, Zn and Fe deficiencies are also the most widespread micronutrient deficiencies in humans, affecting more than three billion people (Cakamak, 2010., Graham *et al*., 2001). The problem with Zn and Fe deficiency is intensified with increased cultivation of cereals whose grains contain low concentrations of Zn and Fe, and are rich in compounds, which limits the bioavailability of Fe and Zn to humans (Cakamak, 2008., Welch and Graham, 1999).

Two important strategies to increase micronutrient concentration in grains is by fertigation of plants via soils or foliar application or by exploiting the genetic variability in grain concentration of these micronutrients within plant genomes and further use in plant breeding (Gregorio *et al.,* 2000). Genetic modifications can be achieved by 2 fundamentally different approaches, first by using conventional breeding and selection techniques and secondly by genetic engineering techniques that can be used to create novel cultivars with desired properties (Bo Lonnedral, 2003). The variations observed in grain Zn content could be due to availability of Zn in soils and uptake differences between crops. Plant species have variation in their uptake from soil between and within species, there also certain plants native to mineral rich soils, which accumulate high levels of Zn, otherwise toxic to normal crop plants. First identified Zn transporters were from model plant Arabidopsis, to date 4 transporters *ZIP1, ZIP2, ZIP3* and *ZIP4* have been identified. *ZIP 1* and *ZIP3* are known to be expressed under Zn deficient conditions and *ZIP4* is predicted to play a role in transport of Zn from roots to shoots (Grotz *et al*., 1998; Assuncao *et al*., 2001).

It is essential to understand the physiological and molecular mechanisms operating in high Zn accumulators, so as to improve crop plants with high Zn in edible parts, before manipulating the genes. With this background, this study was conducted to assess the genetic variation in Zn content in seeds of pigeon pea germplasam lines. The uptake and translocation was studied to have an understanding of the basis of differences in seed Zn content.

Screening of 240 pigeon pea genotypes revealed there is variation not only between species, but variation also exists within species among genotypes. The germplasm had genotypes with as low as approximately 1.53mg/100g to as high as 6.95mg/100g and approximately 92 per cent were having moderate seed Zn of 1-4 mg/ 100g and only 2 per cent of the germplasm has seed Zn more than 5mg/ 100g. Whether the difference is due to differences in uptake and translocation was also analyzed.

There was significant variation in physiological parameters of the 217 pigeon pea genotypes along with the check (TTB-7) grown at the root structure facility. Root parameters include root length, root volume translocation pattern among the low and high Zn types as well as between low and high Zn types. Most of the low Zn types had high uptake and translocation under medium levels of soil Zn, compared to high Zn types showing a similar pattern under high soil Zn levels. Majority of the genotypes had either high uptake or translocation, but only one or two from the high zinc contrast genotypes displayed both high uptake and translocation, i.e. 14 and 11 (ICP accession 7119 and 2484) showing considerable consistency in performance. Genotype 1 (ICP Accession No.6443), was performing at a consistent nature with increase zinc content in all plant parts, as well as in yield.

Despite being classified as low zinc contrast types, two genotypes showed significantly high seed zinc content at low leaf zinc content, i.e. ICP accession 7623 and 7221. These were also reflected in high root and shoot biomass as well as pod weight. High seed Zn types had internsic ability to maintain high seed Zn content, when grown under low soil Zn status, but there was no effect of external Zn on increasing seed Zn. Low Zn types (ICP Accession 7623 and 7221) were also unaffected by external supply of Zn, but showed slight increase in grain yield almost on par with high Zn types.

Among the various high zinc types screened, the probable genotype with most of these parameters is genotype 14 and 11 (ICP accession 7119 and 2484) with high uptake and translocation efficiency, high seed Zn content and increased yield under low soil Zn status, also increase in yield under enhanced soil Zn status. Genotype 1 (ICP Accession No.6443), was performing at a consistent nature with increase zinc content in all plant parts, as well as in yield.

Future line of work

The contrast germplasm lines having high zinc content can be used as donor parent and crosses can be affected using these donor parent and Agronomically superior genotypes to develop superior lines (with high grain Zn content). These superior types will provide high Zn through natural food to humans and when sown in deficient soils vigorous seedlings will be established. There is a need for molecular and biochemical characterization of contrast types which could be used in molecular breeding program of abiotic stress tolerance genotypes. The contrast types identified can be used to develop mapping population for Marker Assisted Selection (MAS) breeding.

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Appendices

Appendix 1: Seed zinc content of 240 Pigeon pea germplasm lines obtained from ICRISAT.

SI	ICP Accession No.	mg/100g	SI	ICP Accession No.	mg/100g
1	11230	1.53	**52**	11321	2.45
2	12515	1.73	**53**	10228	2.45
3	8700	1.73	**54**	9905	2.46
4	12123	1.74	**55**	3593	2.47
5	8266	1.76	**56**	8793	2.48
6	12298	1.80	**57**	11605	2.49
7	11281	1.83	**58**	2746	2.49
8	8860	1.86	**59**	9750	2.50
9	11015	1.87	**60**	1273	2.51
10	10960	1.91	**61**	14801	2.52
11	10654	1.91	**62**	9414	2.53
12	13139	1.91	**63**	7869	2.53
13	10094	1.93	**64**	10503	2.54
14	7375	1.93	**65**	6304	2.54
15	4317	1.93	**66**	14819	2.54
16	8602	1.94	**67**	7609	2.55
17	8863	1.95	**68**	15585	2.55
18	8513	1.97	**69**	4176	2.55
19	9045	1.97	**70**	6049	2.55
20	7507	2.06	**71**	11690	2.55
21	15382	2.08	**72**	3046	2.56
22	13633	2.08	**73**	10447	2.58
23	8757	2.09	**74**	8518	2.59
24	9145	2.09	**75**	13167	2.60
25	8921	2.11	**76**	8012	2.60
26	9336	2.12	**77**	7199	2.62
27	8508	2.15	**78**	7803	2.62
28	8949	2.15	**79**	3576	2.64
29	11946	2.15	**80**	11627	2.64
30	12410	2.17	**81**	11384	2.65
31	7426	2.17	**82**	8840	2.65
32	7623	2.18	**83**	14094	2.66
33	15109	2.19	**84**	995	2.67
34	11320	2.19	**85**	8255	2.67
35	9691	2.22	**86**	10559	2.67
36	11543	2.23	**87**	13579	2.68
37	16264	2.23	**88**	7647	2.69
38	7221	2.24	**89**	15068	2.69
39	8227	2.29	**90**	13601	2.69
40	8516	2.32	**91**	13359	2.69
41	9655	2.36	**92**	6344	2.72
42	8152	2.36	**93**	12142	2.73
43	10397	2.37	**94**	7202	2.74
44	11477	2.38	**95**	7366	2.74
45	12596	2.39	**96**	7196	2.77
46	2698	2.41	**97**	16309	2.78
47	13884	2.41	**98**	4715	2.79
48	12105	2.42	**99**	1071	2.80
49	15185	2.42	**100**	939	2.80
50	2577	2.43	**101**	11785	2.81
51	11910	2.44	**102**	13571	2.81

SI	ICP Accession No.	mg/100g	SI	ICP Accession No.	mg/100g
103	12637	2.81	**155**	14471	3.12
104	13304	2.81	**156**	8384	3.13
105	7200	2.82	**157**	14229	3.13
106	13244	2.82	**158**	10225	3.16
107	1156	2.84	**159**	15600	3.17
108	11059	2.84	**160**	13011	3.18
109	3451	2.85	**161**	9133	3.18
110	655	2.85	**162**	7220	3.19
111	13400	2.86	**163**	4575	3.20
112	772	2.86	**164**	7057	3.21
113	15049	2.86	**165**	13829	3.22
114	14701	2.88	**166**	14976	3.25
115	11916	2.88	**167**	6845	3.26
116	14770	2.88	**168**	7222	3.26
117	5863	2.90	**169**	13529	3.27
118	12654	2.91	**170**	14179	3.27
119	14116	2.91	**171**	9293	3.27
120	7198	2.91	**172**	14638	3.27
121	15161	2.92	**173**	26	3.29
122	13496	2.93	**174**	7718	3.30
123	6859	2.94	**175**	7223	3.30
124	15493	2.94	**176**	4903	3.30
125	13125	2.95	**177**	7218	3.32
126	14120	2.96	**178**	4029	3.34
127	13501	2.97	**179**	6929	3.35
128	14903	2.98	**180**	3495	3.36
129	12680	3.01	**181**	7219	3.37
130	14294	3.02	**182**	13270	3.37
131	6739	3.02	**183**	11833	3.38
132	13662	3.02	**184**	9457	3.41
133	1126	3.03	**185**	14832	3.42
134	9328	3.04	**186**	348	3.42
135	472	3.04	**187**	15066	3.43
136	13194	3.05	**188**	14971	3.45
137	1641	3.05	**189**	6123	3.47
138	14722	3.05	**190**	6815	3.47
139	7	3.05	**191**	14155	3.47
140	14147	3.06	**192**	14444	3.48
141	4392	3.06	**193**	7966	3.49
142	7191	3.06	**194**	14049	3.51
143	10905	3.07	**195**	6973	3.51
144	13486	3.07	**196**	1279	3.52
145	14900	3.07	**197**	13118	3.52
146	10923	3.08	**198**	13151	3.52
147	6668	3.08	**199**	16313	3.53
148	13453	3.10	**200**	2173	3.54
149	1027	3.10	**201**	60	3.55
150	13431	3.10	**202**	16208	3.55
151	7260	3.11	**203**	14828	3.55
152	14368	3.12	**204**	6972	3.61
153	13575	3.12	**205**	4307	3.63
154	14720	3.12	**206**	5142	3.64

SI	ICP Accession No.	mg/100g
207	14944	3.64
208	14545	3.65
209	13191	3.66
210	14569	3.68
211	3049	3.70
212	13577	3.70
213	7180	3.73
214	14416	3.78
215	6128	3.87
216	7148	3.90
217	11726	3.91
218	7197	3.95
219	28	3.97
220	14936	3.99
221	11823	4.02
222	6971	4.05
223	14461	4.06
224	14946	4.17
225	14421	4.19
226	7119	4.21
227	2626	4.22
228	2059	4.28
229	2484	4.35
230	6370	4.38
231	7314	4.54
232	7182	4.56
233	6992	4.60
234	6997	4.62
235	14954	4.96
236	6974	5.13
237	7118	5.65
238	7035	5.66
239	6443	6.20
240	7076	6.95
	Mean	**3.06**
	CV (%)	**9.76**
	CD: Events	**0.106**
	Range	**1.53-6.95**
Check	**TTB-7**	**3.51**

Appendix 2: Growth parameters of 217 pigeon pea germplasm lines grown in Root structure

Sl	ICP Accession No.	Plant Height (cm)	SPAD chl. Reading	Days - 50% flowering (DAS)	Shoot weight (g)	TDM (g)
1	7	130.4	58.2	77.0	76.9	88.1
2	26	137.3	50.7	76.5	173.8	197.3
3	28	84.1	64.4	78.0	55.2	63.5
4	60	143.0	48.0	98.0	84.4	101.9
5	348	156.5	51.8	73.5	85.8	101.0
6	472	151.1	57.1	153.5	164.3	179.8
7	655	158.1	55.1	72.5	54.0	58.2
8	772	174.1	49.2	96.0	128.8	153.2
9	939	222.0	49.2	92.0	126.6	171.4
10	995	178.6	47.2	78.0	124.1	141.3
11	1071	156.1	49.6	104.5	183.3	197.0
12	1126	169.2	50.2	103.0	52.2	60.1
13	1156	149.2	47.4	87.0	25.5	31.3
14	1273	123.6	49.6	92.0	82.0	90.1
15	1279	182.5	56.1	81.5	69.7	73.7
16	1641	153.0	46.6	164.0	144.6	161.3
17	2577	155.5	48.7	87.0	60.1	65.6
18	2626	145.2	52.9	99.0	86.2	94.9
19	2698	154.3	50.6	90.0	57.7	63.8
20	2746	151.8	52.0	84.5	189.7	212.8
21	3046	153.0	42.1	76.5	46.8	51.2
22	3049	150.1	50.9	84.5	65.2	72.3
23	3451	225.9	50.0	132.5	50.7	83.6
24	3576	165.8	50.4	99.5	97.5	111.3
25	4029	170.0	50.5	85.0	60.1	68.3
26	4176	175.3	50.7	154.5	306.5	349.5
27	4307	223.6	51.3	158.0	91.3	99.5
28	4317	132.2	49.7	110.0	33.8	40.4
29	4392	202.6	50.2	113.0	71.2	78.7
30	4575	124.8	58.1	85.0	123.4	132.1
31	4715	197.7	50.4	100.5	282.3	292.2
32	6123	114.6	50.6	91.0	246.7	312.2
33	6128	171.5	51.8	87.5	61.0	95.5
34	6304	210.8	50.4	76.5	127.6	144.5
35	6370	169.9	49.7	112.0	83.1	102.9
36	6443	224.8	56.7	108.5	147.5	156.5

Sl	ICP Accession No.	Plant Height (cm)	SPAD chl. Reading	Days - 50% flowering (DAS)	Shoot weight (g)	TDM (g)
37	6668	186.2	50.8	102.0	140.0	175.9
38	6739	189.1	51.4	99.0	260.5	281.7
39	6815	159.0	48.0	111.0	116.4	187.8
40	6845	165.0	50.0	107.0	65.4	91.7
41	6859	133.5	49.3	110.5	31.5	36.6
42	6929	154.7	52.5	91.5	142.4	146.8
43	6971	164.3	49.3	105.5	35.0	43.3
44	6972	104.4	65.5	76.5	68.5	80.6
45	6973	111.3	51.4	87.0	78.0	114.6
46	6974	182.4	56.9	75.5	134.3	140.5
47	6992	101.2	62.5	79.5	145.6	170.0
48	6997	129.8	60.1	88.5	56.8	81.6
49	7035	114.6	62.1	84.5	91.0	117.1
50	7057	115.4	49.6	106.0	39.8	62.9
51	7076	164.0	47.0	148.0	176.3	186.8
52	7118	159.7	54.8	105.0	114.0	122.9
53	7119	154.0	51.7	103.5	172.9	178.3
54	7148	54.5	55.1	66.0	135.6	177.6
55	7180	101.6	55.7	76.0	299.5	308.0
56	7182	175.3	50.6	125.5	46.0	68.8
57	7191	148.4	61.9	87.0	95.9	147.5
58	7196	172.5	52.3	80.0	46.1	76.0
59	7197	150.9	48.9	95.0	24.6	107.2
60	7198	224.8	51.9	145.5	89.5	103.0
61	7199	190.4	52.8	134.5	65.1	78.5
62	7200	159.6	49.1	96.0	252.7	299.8
63	7202	83.2	49.0	86.0	156.3	163.0
64	7218	160.3	57.5	87.0	55.9	87.6
65	7219	77.0	49.9	87.0	236.5	250.9
66	7220	71.0	49.9	85.5	231.4	246.1
67	7221	76.4	58.8	101.0	99.3	106.1
68	7222	157.3	57.1	83.0	52.8	105.7
69	7223	133.0	53.0	104.0	103.1	140.1
70	7260	175.8	57.2	85.5	51.8	66.6
71	7314	168.8	50.5	84.5	204.0	217.8
72	7375	161.8	47.8	89.0	288.6	301.1
73	7426	143.7	53.9	93.5	265.4	307.0

Sl	ICP Accession No.	Plant Height (cm)	SPAD chl. Reading	Days - 50% flowering (DAS)	Shoot weight (g)	TDM (g)
74	7507	161.4	53.0	101.0	99.6	152.3
75	7609	76.9	58.3	77.0	50.3	102.8
76	7623	106.0	57.4	79.5	49.4	150.6
77	7647	133.1	63.6	73.0	46.5	77.7
78	7718	202.9	53.8	99.0	33.9	47.6
79	7803	200.4	53.6	106.0	47.0	66.1
80	7869	145.0	52.6	96.0	257.3	266.1
81	8012	240.0	47.7	127.5	55.1	162.4
82	8227	171.7	51.0	113.0	34.0	60.9
83	8255	194.8	54.2	99.5	34.0	114.1
84	8384	129.6	59.0	91.5	59.8	116.1
85	8508	143.0	59.2	80.0	56.1	74.7
86	8513	156.2	52.2	100.5	78.5	100.4
87	8516	214.5	51.3	143.5	151.6	165.4
88	8518	155.6	53.9	92.0	140.7	155.1
89	8602	226.6	50.1	90.5	144.5	153.0
90	8757	239.6	51.8	116.5	122.3	148.6
91	8793	177.0	51.5	81.5	28.6	108.9
92	8840	164.0	53.7	88.0	292.4	315.4
93	8860	172.7	49.8	105.5	355.1	368.6
94	8863	169.6	57.1	87.0	38.4	53.6
95	8921	150.7	47.1	90.5	72.8	86.6
96	8949	186.0	47.9	144.5	10.9	17.0
97	9045	238.8	46.9	101.0	104.9	128.6
98	9145	110.2	58.1	102.0	293.8	298.8
99	9336	165.5	51.2	94.5	293.1	301.2
100	9414	173.5	52.6	89.0	185.3	189.5
101	9655	168.0	57.5	90.5	160.6	168.5
102	9691	167.8	50.2	86.0	122.9	166.5
103	9750	134.1	52.1	92.0	71.2	98.8
104	9905	167.1	51.0	102.0	50.9	55.5
105	10228	198.1	54.2	90.0	86.4	131.6
106	10397	157.1	53.5	90.0	34.3	109.4
107	10447	216.0	60.0	82.0	41.4	49.9
108	10503	132.5	54.2	82.5	32.9	43.6
109	10559	209.3	47.8	120.0	117.1	121.4
110	10654	240.7	51.0	96.0	53.7	68.6

Sl	ICP Accession No.	Plant Height (cm)	SPAD chl. Reading	Days - 50% flowering (DAS)	Shoot weight (g)	TDM (g)
111	10960	178.3	56.5	95.0	344.8	421.1
112	11059	96.0	49.8	75.0	54.1	109.4
113	11281	170.0	48.4	138.0	148.1	162.7
114	11384	242.6	52.9	111.5	124.8	144.8
115	11477	154.0	60.2	83.5	200.4	220.5
116	11543	47.3	63.7	80.5	122.0	140.8
117	11605	59.9	65.5	103.0	130.5	139.0
118	11627	67.9	63.6	67.0	31.1	38.9
119	11690	161.0	48.0	120.0	251.2	292.2
120	11823	226.2	50.4	144.0	231.4	239.6
121	11916	150.5	49.3	104.0	62.1	96.3
122	11946	146.7	49.0	99.0	95.1	138.9
123	12105	184.1	51.0	105.5	99.5	120.4
124	12142	152.2	50.1	105.0	113.9	118.7
125	12410	133.8	51.2	86.0	181.7	196.5
126	12596	134.5	54.3	99.0	32.0	47.3
127	12654	98.2	47.1	105.0	162.8	210.1
128	12680	165.0	55.5	85.5	152.6	217.5
129	13011	109.0	61.7	76.5	20.0	52.5
130	13139	173.4	47.5	103.5	35.6	73.3
131	13167	164.0	57.1	102.0	203.0	211.8
132	13191	129.0	55.7	72.5	298.1	312.7
133	13194	75.5	63.3	79.5	165.2	188.5
134	13244	181.5	52.2	80.0	70.8	97.3
135	13270	171.4	61.7	91.0	250.8	275.8
136	13359	181.0	62.0	103.5	100.4	106.5
137	13431	155.8	53.1	103.0	84.6	113.7
138	13575	157.7	53.9	101.5	72.6	129.6
139	13577	153.3	57.4	114.0	161.0	180.2
140	13579	126.0	48.1	111.0	61.8	98.1
141	13633	161.3	51.8	109.5	42.0	43.7
142	13662	176.2	49.3	107.5	132.9	137.3
143	13829	187.5	53.1	112.0	37.6	119.3
144	14094	196.0	51.5	103.5	238.7	276.9
145	14116	153.0	54.7	100.5	187.2	200.1
146	14120	184.0	51.6	94.0	100.5	122.6
147	14147	178.0	52.9	101.5	83.9	91.7

Sl	ICP Accession No.	Plant Height (cm)	SPAD chl. Reading	Days - 50% flowering (DAS)	Shoot weight (g)	TDM (g)
148	14155	232.5	54.4	89.0	71.3	79.9
149	14229	232.5	49.9	94.0	17.1	31.2
150	14368	205.5	50.2	90.5	84.1	115.9
151	14421	50.8	55.4	72.5	191.1	194.2
152	14444	61.0	64.5	74.5	206.6	224.3
153	14471	102.7	60.7	75.0	114.2	125.7
154	14545	172.5	47.6	85.5	180.5	184.1
155	14569	193.3	49.8	98.5	235.2	239.1
156	14638	161.0	52.5	99.0	121.5	149.9
157	14701	247.1	50.5	85.5	179.5	226.2
158	14720	157.3	50.8	80.0	213.1	230.0
159	14722	172.0	46.7	95.5	140.4	148.0
160	14770	148.2	51.2	95.5	130.7	204.9
161	14801	211.5	49.5	79.5	149.6	157.4
162	14832	171.0	54.9	77.0	351.6	363.8
163	14900	139.5	54.7	74.0	182.6	190.2
164	14903	169.5	61.4	96.0	83.4	96.1
165	14976	185.7	55.1	94.5	41.3	49.3
166	15049	195.7	49.3	99.5	127.9	136.9
167	15068	62.6	65.4	73.0	34.6	42.5
168	15109	84.7	45.0	94.5	31.2	35.2
169	15161	183.3	47.6	102.5	41.3	116.7
170	15185	139.5	53.8	80.0	31.7	50.1
171	15382	185.4	49.4	82.0	33.2	47.2
172	15493	191.2	44.7	85.5	366.8	375.2
173	15585	212.0	53.1	82.5	42.0	47.5
174	15600	149.9	61.6	85.0	59.1	60.6
175	16264	220.2	48.1	98.0	50.2	59.2
176	16309	169.8	48.0	86.0	185.3	209.6
177	1027	153.0	49.4	103.0	160.6	194.0
178	2059	167.3	54.6	107.5	122.9	136.1
179	2173	149.5	51.3	104.0	71.2	102.3
180	2484	212.2	55.2	87.5	50.9	83.9
181	3495	178.7	54.9	88.5	86.4	102.4
182	3593	144.5	47.2	124.5	34.3	61.6
183	7966	138.8	55.5	86.5	41.4	73.4
184	9133	221.0	55.8	113.5	32.9	46.2

Sl	ICP Accession No.	Plant Height (cm)	SPAD chl. Reading	Days - 50% flowering (DAS)	Shoot weight (g)	TDM (g)
185	9293	189.5	51.2	90.0	117.1	142.9
186	9328	151.1	48.0	93.0	53.7	77.6
187	9457	136.5	49.9	86.0	344.8	410.0
188	10225	122.5	56.0	98.5	54.1	88.0
189	10905	97.3	63.4	72.5	148.1	156.4
190	10923	48.5	66.0	68.0	124.8	130.9
191	11726	200.0	49.6	90.0	200.4	207.6
192	11785	183.5	51.6	91.5	122.0	129.5
193	12637	131.2	53.3	86.0	130.5	134.4
194	13118	180.1	50.7	99.5	31.1	34.4
195	13125	209.0	54.6	111.5	251.2	255.2
196	13151	201.5	51.1	111.0	231.4	235.3
197	13400	236.0	54.2	93.5	62.1	67.7
198	13453	193.5	54.2	112.0	95.1	218.3
199	13486	214.6	52.8	114.0	99.5	104.8
200	13496	228.5	50.6	112.0	113.9	121.0
201	13501	211.9	54.0	111.0	144.5	148.3
202	13529	194.8	55.4	90.0	113.9	118.7
203	13601	233.9	52.8	89.5	181.7	196.5
204	14049	166.5	46.5	92.5	32.0	47.3
205	14179	106.6	62.8	74.5	162.8	210.1
206	14416	140.8	60.6	77.5	152.6	217.5
207	14461	133.2	63.2	70.5	20.0	52.5
208	14828	123.2	62.9	72.0	35.6	73.3
209	14936	106.4	59.7	74.5	62.1	67.7
210	14944	92.1	53.8	74.0	52.2	60.1
211	14946	69.9	65.1	68.0	25.5	31.3
212	14954	84.2	63.9	97.5	82.0	90.1
213	14971	248.8	53.1	104.0	69.7	73.7
214	15066	118.7	65.2	65.0	144.6	161.3
215	16208	110.7	62.7	72.0	60.1	65.6
216	16313	136.0	65.2	75.0	86.2	94.9
	Mean	**158.7**	**53.5**	**94.4**	119.4	142.3
	CV (%)	**4.8**	**2.69**	**2.84**	**8.23**	**5.83**
	CD: Events	**4.94**	**2.82**	**5.31**	**7.47**	**8.32**
	Range	**47.3-248.8**	**42.1-66.0**	**65-164**	**10.94 - 366.85**	**15.2 - 489.9**
Check	**TTB-7**	**115.5**	**52.4**	**80.5**		

Appendix 3: Zinc content and yield parameters of 217 pigeon pea germplasm lines grown in Root structure.

Sl	ICP Accession No.	Seed Zn (mg/100g)	Leaf Zn (mg/100g)	Pod Weight (g)	100 Seed Weight (g)
1	7	2.45	3.85	129.8	7.8
2	26	2.95	5.87	143.2	10.3
3	28	3.29	3.26	45.7	10.8
4	60	3.42	5.20	79.7	9.8
5	348	3.17	3.91	85.7	12.3
6	472	2.96	6.57	75.6	12.6
7	655	2.20	3.78	102.5	9.3
8	772	2.27	3.68	61.6	8.8
9	939	2.62	3.54	144.3	11.8
10	995	2.18	3.67	147.1	10.7
11	1071	2.27	4.58	94.2	8.5
12	1126	3.20	2.98	111.6	14.5
13	1156	2.24	4.10	13.5	9.7
14	1273	2.19	3.76	124.0	8.5
15	1279	3.19	6.10	230.5	9.5
16	1641	2.90	4.05	50.1	8.2
17	2577	2.24	3.83	68.2	12.5
18	2626	4.10	5.75	104.8	11.3
19	2698	2.79	5.00	80.8	10.7
20	2746	2.73	6.40	141.9	8.3
21	3046	2.69	2.68	40.4	8.3
22	3049	4.02	6.73	110.3	9.3
23	3451	2.72	6.67	123.0	8.8
24	3576	2.91	7.05	90.1	10.0
25	4029	3.37	8.71	36.4	10.0
26	4176	2.63	7.95	77.6	11.2
27	4307	3.91	6.97	61.0	9.8
28	4317	2.02	6.39	126.4	5.5
29	4392	3.91	7.57	26.8	8.5
30	4575	3.08	6.73	160.9	9.8
31	4715	2.61	5.07	78.4	11.7
32	6123	3.18	4.78	54.8	9.3
33	6128	3.39	7.59	81.8	9.5
34	6304	4.26	5.41	295.8	13.1
35	6370	3.88	5.41	150.2	11.1
36	6443	6.36	6.71	70.0	11.8

Sl	ICP Accession No.	Seed Zn (mg/100g)	Leaf Zn (mg/100g)	Pod Weight (g)	100 Seed Weight (g)
37	6668	2.94	6.63	104.0	11.8
38	6739	2.93	5.81	140.1	14.3
39	6815	2.81	7.27	30.0	6.9
40	6845	3.84	5.97	75.7	11.4
41	6859	2.96	6.83	54.8	11.9
42	6929	3.58	9.22	55.5	16.6
43	6971	3.85	5.70	34.0	8.8
44	6972	3.17	5.81	23.3	10.1
45	6973	3.06	4.52	64.4	10.2
46	6974	4.54	4.92	135.6	8.9
47	6992	4.44	5.16	55.7	8.4
48	6997	4.61	5.22	83.6	12.2
49	7035	6.11	6.65	86.9	24.5
50	7057	3.13	6.92	38.4	8.0
51	7076	6.12	6.74	42.1	9.4
52	7118	5.140	6.598	50.7	9.8
53	7119	4.150	5.193	90.4	12.0
54	7148	3.822	8.314	77.7	11.1
55	7180	3.109	9.999	17.7	13.2
56	7182	4.230	4.94	123.6	11.7
57	7191	3.169	7.824	31.7	13.2
58	7196	2.299	5.659	31.5	12.4
59	7197	3.174	5.949	67.9	11.3
60	7198	3.139	4.509	84.2	11.1
61	7199	2.824	6.209	149.8	9.3
62	7200	2.294	7.514	29.8	8.4
63	7202	2.234	5.884	25.6	11.7
64	7218	2.980	8.024	36.1	11.5
65	7219	4.021	6.074	27.7	7.9
66	7220	3.429	4.554	25.7	7.8
67	7221	2.650	3.723	27.9	11.1
68	7222	3.044	7.054	61.0	12.5
69	7223	3.564	7.774	36.5	10.4
70	7260	3.288	4.895	123.8	11.9
71	7314	4.290	8.351	98.6	10.2
72	7375	2.046	4.05	64.1	8.4
73	7426	1.933	3.278	17.5	7.2
74	7507	1.983	3.587	25.5	7.6

Sl	ICP Accession No.	Seed Zn (mg/100g)	Leaf Zn (mg/100g)	Pod Weight (g)	100 Seed Weight (g)
75	7609	2.590	8.379	23.0	11.3
76	7623	2.400	6.674	44.1	10.7
77	7647	2.254	6.419	16.2	10.6
78	7718	3.334	8.554	89.4	12.0
79	7803	2.799	6.639	52.1	13.9
80	7869	2.474	6.894	132.0	16.0
81	8012	2.949	4.449	69.5	8.8
82	8227	2.395	4.685	33.6	9.5
83	8255	3.030	4.249	121.4	11.3
84	8384	3.269	4.359	18.3	8.2
85	8508	2.080	5.088	74.8	9.0
86	8513	1.740	3.33	135.1	8.3
87	8516	2.570	4.83	87.3	9.3
88	8518	2.669	8.074	109.0	10.3
89	8602	1.850	3.608	106.0	8.3
90	8757	2.184	7.854	82.0	10.4
91	8793	2.144	8.889	79.9	12.3
92	8840	2.489	7.629	152.1	11.8
93	8860	2.029	6.979	68.8	12.3
94	8863	2.100	3.465	128.8	11.3
95	8921	1.924	3.944	19.8	12.2
96	8949	2.484	6.964	22.4	6.9
97	9045	2.029	9.104	104.6	8.9
98	9145	2.564	8.699	16.0	6.4
99	9336	2.240	3.135	52.7	11.0
100	9414	3.074	7.314	45.2	8.3
101	9655	3.110	4.205	109.7	13.0
102	9691	2.430	3.988	72.1	7.5
103	9750	3.120	8.239	31.2	15.1
104	9905	1.872	3.889	47.7	10.7
105	10228	2.339	5.864	59.9	10.1
106	10397	2.189	7.359	89.6	9.7
107	10447	2.209	3.174	31.4	9.8
108	10503	2.134	9.364	31.2	8.9
109	10559	2.884	6.059	154.3	9.5
110	10654	1.320	3.015	93.6	13.8
111	10960	1.160	3.393	22.5	9.5
112	11059	2.459	8.539	24.6	9.4

SI	ICP Accession No.	Seed Zn (mg/100g)	Leaf Zn (mg/100g)	Pod Weight (g)	100 Seed Weight (g)
113	11281	1.464	5.314	163.9	11.7
114	11384	2.824	5.179	66.7	8.7
115	11477	2.110	4.019	14.5	7.1
116	11543	1.780	4.285	72.3	8.7
117	11605	2.264	4.054	20.6	6.9
118	11627	2.779	8.864	49.7	11.3
119	11690	2.604	9.539	87.1	9.6
120	11823	3.639	6.404	101.1	19.2
121	11916	3.099	6.689	42.7	16.6
122	11946	1.860	3.558	259.8	18.3
123	12105	2.404	5.964	55.6	9.9
124	12142	2.189	5.784	66.0	14.1
125	12410	2.140	5.498	67.6	9.3
126	12596	2.639	6.014	35.5	7.7
127	12654	2.384	5.914	168.9	10.3
128	12680	2.930	8.209	31.9	11.4
129	13011	3.640	6.099	46.0	8.2
130	13139	1.849	4.939	60.6	7.8
131	13167	2.874	5.409	48.7	10.0
132	13191	2.914	5.325	54.9	9.8
133	13194	3.754	6.329	19.3	8.2
134	13244	3.111	5.509	51.8	13.9
135	13270	4.112	7.844	20.6	9.7
136	13359	3.499	4.259	46.0	6.5
137	13431	3.890	5.349	45.7	8.4
138	13575	3.754	4.439	69.9	8.9
139	13577	3.899	6.054	46.9	10.5
140	13579	2.669	7.109	17.8	6.1
141	13633	1.780	4.224	13.7	9.0
142	13662	3.330	5.969	183.6	13.8
143	13829	1.874	5.719	18.0	9.1
144	14094	2.224	5.529	15.7	10.9
145	14116	2.580	6.044	32.0	9.0
146	14120	2.754	5.364	31.6	13.1
147	14147	2.779	6.474	50.1	10.4
148	14155	3.873	9.279	89.9	12.5
149	14229	2.910	8.159	122.9	11.7
150	14368	3.754	4.304	35.8	8.9

Sl	ICP Accession No.	Seed Zn (mg/100g)	Leaf Zn (mg/100g)	Pod Weight (g)	100 Seed Weight (g)
151	14421	3.750	5.563	15.6	11.8
152	14444	3.850	4.721	56.3	11.5
153	14471	1.953	5.309	50.9	8.7
154	14545	3.634	7.104	57.0	11.1
155	14569	4.059	8.764	31.5	12.0
156	14638	2.979	8.589	59.3	15.0
157	14701	2.189	9.094	59.9	11.7
158	14720	3.769	7.579	55.5	14.2
159	14722	2.989	9.959	91.3	10.1
160	14770	2.504	9.344	39.4	9.3
161	14801	2.534	6.214	30.5	9.2
162	14832	3.649	5.844	45.7	11.5
163	14900	3.157	6.254	29.9	8.4
164	14903	3.024	8.139	53.5	13.9
165	14976	2.959	6.064	132.5	11.9
166	15049	2.709	6.564	136.2	20.7
167	15068	2.804	6.569	89.0	11.3
168	15109	1.984	6.424	41.9	7.4
169	15161	3.149	6.659	76.4	21.0
170	15185	2.434	6.969	96.4	11.7
171	15382	2.184	6.224	40.8	7.3
172	15493	2.219	7.359	75.3	11.3
173	15585	2.102	9.424	92.4	12.0
174	15600	2.699	8.219	55.6	11.1
175	16264	2.329	3.119	60.1	11.5
176	16309	3.020	10.574	87.6	10.3
177	1027	3.119	8.119	76.3	10.4
178	2059	3.830	4.155	34.7	10.8
179	2173	3.339	8.999	139.3	8.3
180	2484	4.190	5.353	62.1	9.0
181	3495	3.750	6.899	27.3	7.6
182	3593	3.130	8.014	70.3	11.2
183	7966	3.939	9.044	95.5	11.0
184	9133	3.634	8.554	84.2	9.5
185	9293	3.120	6.104	79.5	9.8
186	9328	2.649	7.829	40.6	9.5
187	9457	3.499	7.259	114.7	9.2
188	10225	3.584	7.854	21.9	6.4

Sl	**ICP Accession No.**	**Seed Zn (mg/100g)**	**Leaf Zn (mg/100g)**	**Pod Weight (g)**	**100 Seed Weight (g)**
189	10905	3.154	8.664	35.2	6.4
190	10923	3.274	7.519	81.0	11.8
191	11726	3.059	9.454	16.2	7.2
192	11785	2.193	5.129	35.9	10.7
193	12637	2.274	7.899	66.9	13.4
194	13118	3.944	6.619	59.5	13.5
195	13125	2.769	8.209	19.9	7.2
196	13151	3.814	8.394	26.7	5.3
197	13400	2.814	7.314	25.4	5.7
198	13453	2.944	8.664	24.9	7.1
199	13486	2.839	7.789	16.5	7.1
200	13496	2.559	9.029	36.1	9.5
201	13501	2.899	9.194	44.0	10.4
202	13529	3.249	8.689	48.4	10.3
203	13601	2.539	7.699	36.4	13.5
204	14049	3.559	8.849	53.9	9.5
205	14179	3.679	10.384	88.0	11.3
206	14416	3.374	11.874	78.7	13.3
207	14461	3.770	8.789	76.4	21.0
208	14828	3.114	7.419	63.3	10.8
209	14936	3.349	9.919	50.8	8.4
210	14944	4.109	8.239	53.7	11.7
211	14946	4.056	8.249	41.4	11.2
212	14954	5.140	5.71	51.1	8.7
213	14971	3.130	7.714	165.9	9.0
214	15066	3.629	7.299	87.2	12.5
215	16208	3.820	8.544	93.2	13.1
216	16313	3.060	7.934	95.0	12.8
	Mean	**2.99**	**6.41**	**69.6**	**10.51**
	CV (%)	**0.99**	**0.48**	**5.6**	**6.89**
	CD: Events	**0.11**	**0.05**	**7.8**	**1.49**
	Range	**1.16-6.36**	**2.68-11.87**	**13.5-295.8**	**5.25-24.45**
Check	**TTB7**	**3.961**	**5.229**	**38**	**12.45**

Appendix 4: Root parameters of 217 pigeon pea germplasm lines grown in Root structure.

Sl	ICP Accession No.	Root Length (cm)	Root Volume (cm3)	Root dry wt (g)	Sl	ICP Accession No.	Root Length (cm)	Root Volume (cm3)	Root dry wt (g)
1	7	70.8	28.5	11.1	**44**	6972	52.0	28.5	12.1
2	26	71.6	47.5	23.5	**45**	6973	65.5	77	36.6
3	28	60.8	23.5	8.4	**46**	6974	23.0	13.5	6.3
4	60	51.8	38	17.5	**47**	6992	51.0	48	24.4
5	348	47.5	34.5	15.3	**48**	6997	69.5	51.5	24.8
6	472	56.0	33.5	15.5	**49**	7035	60.6	57.5	26.1
7	655	74.5	10	4.2	**50**	7057	54.3	48	23.1
8	772	72.3	57.5	24.4	**51**	7076	50.1	22.5	10.5
9	939	100.4	96.5	44.8	**52**	7118	46.9	19	8.9
10	995	57.8	38.5	17.2	**53**	7119	16.8	11.5	5.5
11	1071	54.0	28	13.7	**54**	7148	58.8	87.5	42.0
12	1126	60.0	18.5	7.9	**55**	7180	49.4	18	8.5
13	1156	31.2	14	5.8	**56**	7182	51.5	48	22.8
14	1273	49.5	19	8.1	**57**	7191	77.8	107	51.6
15	1279	49.8	10	4.1	**58**	7196	56.4	62.5	29.9
16	1641	71.1	37.5	16.8	**59**	7197	133.0	172.5	82.6
17	2577	40.0	13.5	5.6	**60**	7198	45.7	28.5	13.5
18	2626	70.6	20	8.6	**61**	7199	26.5	28	13.4
19	2698	31.0	13.5	6.1	**62**	7200	82.5	97.5	47.1
20	2746	71.6	48.5	23.1	**63**	7202	45.3	13.5	6.7
21	3046	32.9	11	4.4	**64**	7218	54.1	68	31.6
22	3049	44.5	16	7.2	**65**	7219	42.4	22.5	14.4
23	3451	41.3	68	32.9	**66**	7220	59.2	30.5	14.7
24	3576	65.3	28.5	13.8	**67**	7221	53.9	13.5	6.8
25	4029	43.4	18	8.2	**68**	7222	68.5	107.5	52.8
26	4176	68.1	87.5	43.0	**69**	7223	73.6	77.5	37.0
27	4307	42.7	21	8.2	**70**	7260	43.2	28	14.8
28	4317	55.6	14	6.6	**71**	7314	34.8	27.5	13.8
29	4392	41.9	18.5	7.5	**72**	7375	49.5	24.5	12.5
30	4575	56.9	21.5	8.7	**73**	7426	81.0	87.5	41.5
31	4715	45.0	23.5	10.0	**74**	7507	90.7	107.5	52.7
32	6123	85.4	132.5	65.5	**75**	7609	85.2	108.5	52.5
33	6128	67.9	77.5	34.5	**76**	7623	142.8	202.5	101.2
34	6304	70.2	33.5	16.9	**77**	7647	60.0	67.5	31.2
35	6370	49.2	43	19.8	**78**	7718	82.2	28	13.7
36	6443	74.6	24	9.0	**79**	7803	70.0	37.5	19.1
37	6668	76.5	77	35.9	**80**	7869	55.0	18.5	8.8
38	6739	71.4	48	21.3	**81**	8012	103.6	217.5	107.3
39	6815	56.8	147.5	71.5	**82**	8227	106.2	57.5	27.0
40	6845	57.5	58.5	26.3	**83**	8255	95.3	167.5	80.2
41	6859	46.4	10	5.0	**84**	8384	57.7	117.5	56.3
42	6929	47.6	11.5	4.4	**85**	8508	49.8	38	18.6
43	6971	33.5	19	8.3	**86**	8513	60.2	47.5	22.0

Sl	ICP Accession No.	Root Length (cm)	Root Volume (cm3)	Root dry wt (g)	Sl	ICP Accession No.	Root Length (cm)	Root Volume (cm3)	Root dry wt (g)
87	8516	58.9	28.5	13.8	**132**	13191	66.2	30	14.6
88	8518	60.5	27.5	14.4	**133**	13194	36.4	48.5	23.3
89	8602	35.7	18	8.5	**134**	13244	85.0	57.5	26.6
90	8757	45.5	57.5	26.3	**135**	13270	71.8	67.5	25.0
91	8793	91.9	162.5	80.3	**136**	13359	64.3	38.5	6.2
92	8840	69.5	48.5	23.0	**137**	13431	65.8	77.5	29.1
93	8860	40.6	28	13.5	**138**	13575	64.0	197.5	57.0
94	8863	43.3	32.5	15.2	**139**	13577	68.4	67.5	19.3
95	8921	49.5	17.5	13.7	**140**	13579	108.9	77.5	36.3
96	8949	46.0	12.5	6.1	**141**	13633	72.0	10	1.7
97	9045	48.0	47.5	23.7	**142**	13662	51.0	10	4.4
98	9145	21.5	10	5.1	**143**	13829	115.9	167.5	81.7
99	9336	91.2	17.5	8.1	**144**	14094	176.2	77.5	38.2
100	9414	31.7	10	4.2	**145**	14116	82.3	28.5	12.9
101	9655	40.7	17.5	7.9	**146**	14120	53.2	47.5	22.1
102	9691	81.2	87.5	43.7	**147**	14147	85.6	17.5	7.8
103	9750	52.0	58	27.5	**148**	14155	51.4	20	8.6
104	9905	17.0	10	4.7	**149**	14229	65.6	33.5	14.1
105	10228	80.7	92.5	45.2	**150**	14368	55.8	72.5	31.8
106	10397	60.5	157.5	75.1	**151**	14421	48.1	7.5	3.1
107	10447	38.5	17.5	8.5	**152**	14444	91.4	39	17.7
108	10503	45.1	22.5	10.7	**153**	14471	89.9	27.5	11.5
109	10559	14.0	9	4.3	**154**	14545	41.2	8.5	3.6
110	10654	49.0	30	14.9	**155**	14569	47.5	10	3.9
111	10960	94.0	157.5	76.4	**156**	14638	56.3	62.5	28.4
112	11059	136.1	117.5	55.4	**157**	14701	93.5	107.5	46.7
113	11281	49.9	28	14.6	**158**	14720	86.1	37.5	16.9
114	11384	65.5	42.5	20.0	**159**	14722	77.5	17.5	7.6
115	11477	75.0	42.5	20.1	**160**	14770	86.5	167.5	74.2
116	11543	57.6	32.5	18.8	**161**	14801	62.8	18.5	7.8
117	11605	60.7	20	8.5	**162**	14832	72.6	27.5	12.3
118	11627	51.5	17.5	7.8	**163**	14900	48.3	16.5	7.6
119	11690	69.9	87.5	41.0	**164**	14903	55.7	27.5	12.6
120	11823	53.7	17.5	8.2	**165**	14976	63.0	20	8.0
121	11916	69.0	67.5	34.1	**166**	15049	46.5	23	9.0
122	11946	55.0	87.5	43.7	**167**	15068	61.0	17.5	7.9
123	12105	59.5	47.5	20.8	**168**	15109	48.8	10	4.0
124	12142	46.0	10	4.8	**169**	15161	118.6	167.5	75.4
125	12410	54.5	30	14.8	**170**	15185	56.8	42.5	18.4
126	12596	53.5	38	15.3	**171**	15382	67.5	32.5	13.9
127	12654	33.6	107.5	47.3	**172**	15493	77.2	18	8.4
128	12680	58.0	127.5	64.9	**173**	15585	58.8	12.5	5.6
129	13011	54.3	67.5	32.5	**174**	15600	64.5	6.5	1.5
130	13139	62.5	77.5	37.7	**175**	16264	61.3	17.5	9.0
131	13167	52.5	20	8.9	**176**	16309	59.7	57.5	24.3

Sl	ICP Accession No.	Root Length (cm)	Root Volume (cm3)	Root dry wt (g)	Sl	ICP Accession No.	Root Length (cm)	Root Volume (cm3)	Root dry wt (g)
177	1027	77.4	67.5	33.4	**200**	13496	49.4	18.5	7.2
178	2059	70.0	33.5	13.2	**201**	13501	59.0	7.5	3.7
179	2173	121.8	68	31.0	**202**	13529	56.3	62.5	28.4
180	2484	204.6	67.5	33.1	**203**	13601	93.5	107.5	46.7
181	3495	67.9	37.5	16.0	**204**	14049	86.1	37.5	16.9
182	3593	53.3	58	27.3	**205**	14179	77.5	17.5	7.6
183	7966	78.9	67.5	32.0	**206**	14416	86.5	167.5	74.2
184	9133	61.0	30	13.3	**207**	14461	45.1	22.5	10.7
185	9293	49.0	60	25.8	**208**	14828	14.0	9	4.3
186	9328	68.3	48.5	23.9	**209**	14936	49.0	30	14.9
187	9457	59.5	157.5	65.3	**210**	14944	94.0	157.5	76.4
188	10225	118.1	67.5	33.9	**211**	14946	136.1	117.5	55.4
189	10905	43.7	18.5	8.3	**212**	14954	26.5	28	13.4
190	10923	54.1	13.5	6.0	**213**	14971	82.5	97.5	47.1
191	11726	51.6	17.5	7.3	**214**	15066	45.3	13.5	6.7
192	11785	50.3	18	7.5	**215**	16208	54.1	68	31.6
193	12637	32.6	10	3.9	**216**	16313	42.4	22.5	14.4
194	13118	48.8	9	3.2		**Mean**	**62.99**	**49.5**	**22.87**
195	13125	58.2	10	4.0		**CV (%)**	**5.69**	**6.12**	**14.01**
196	13151	43.3	10	3.9		**CD: Events**	**7.03**	**5.13**	**6.28**
197	13400	62.7	13.5	5.6		**Range**	**14.0-204.6**	**6.5-257.5**	**1.47-123.15**
198	13453	105.1	257.5	123.2	**Check**	**TTB7**	**85.2**	**27.5**	**12.0**
199	13486	44.8	12.5	5.2					