



**RESPONSES OF CHICKPEA (*CICER ARIETINUM* L.) TO
ZINC SUPPLY AND WATER DEFICITS**

by

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I dedicate this thesis to the memory of my father

Hafiz Muhammad Ramzan Khan

1918-1997



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Declaration

This thesis contains no material which has been accepted for the reward of any other degree or diploma in any other university, and to the best of my knowledge and belief, contains no material previously published or written by any other person, except where due reference is made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying.

Habib ur Rahman Khan

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Summary

Chickpea is a major grain legume traditionally grown on the Indo-Pakistan subcontinent and in the Mediterranean basin, and is an important source of dietary protein and vital mineral nutrients for human nutrition. In Australia, chickpea is a relatively new but rapidly expanding, grain legume crop that has received considerable attention in the cereal belt of southern Australia. However, productivity of chickpea like that of other grain legumes, has been low and variable in the past. Widespread deficiencies of mineral nutrients in the soils along with limited moisture supply are considered major environmental stresses leading toward yield loss in chickpea.

In the Mediterranean-type climate, most of the rainfall occurs during winter and declines in spring. Lack of moisture becomes a major constraint for production, particularly during the reproductive phase when evapotranspiration demand is high. In the Indo-Pakistan subcontinent, chickpea is sown in the post-rainy season and largely dependent on moisture stored in the soil during the preceding monsoon rains. The intensity of water deficits progressively increases with advancing growth and the crop suffers from drought stress.

Many areas of the world suitable for chickpea have widespread zinc deficiency. Zinc is important for the function of a large number of enzymes in plants and is recognized for its pivotal role in the integrity and maintenance of cell membrane. Zinc deficiency affects plant water relations, induces stomatal closure and decreases transpiration in plants. Chickpea is generally considered sensitive to zinc deficiency compared to various crop species. Therefore, an extensive cultivation of chickpea in regions having zinc-deficient soils could result in its low productivity. Despite its importance as a major pulse crop worldwide, there is little information on the zinc nutrition of chickpea particularly on the importance of zinc in drought-prone environments. Therefore, the present study was conducted to determine the zinc requirement of chickpea and to establish a critical zinc concentration. Differential response of chickpea genotypes having diverse genetic background were investigated and their zinc efficiencies were estimated. The effect of zinc fertilization on grain yield and on distribution of zinc in plant parts, particularly loading of zinc in the seed, was examined. Effects of zinc application on water usage, plant water relations, stomatal

conductance and degree of osmotic adjustment were investigated. The highlights of the results are as follow:

- Zinc fertilization increased tissue zinc concentration and accumulation of shoot dry matter in two widely grown chickpea cultivars Tyson and Dooen. Zinc deficiency suppressed vegetative growth and visual symptoms of zinc deficiency, such as pale foliage colour, chlorosis of younger leaves, twisting of growing buds, abnormal increase in leaf thickness in older leaves and necrosis of leaf margins followed by abscission, were observed. The minimum nutrient concentration in the tissue associated with 90% of maximum growth was determined from curves that were drawn free-hand to fit the respective X and Y values in the graph. The critical zinc concentration was estimated as 20-21 mg/kg in the whole shoot. The results of a second experiment showed that high and low moisture regimes had no effect on critical zinc concentration and value remained almost the same in all genotypes, suggesting that critical zinc concentration could be used over a range of environments for diagnosis of zinc status of chickpea.
- Sensitivity of 28 chickpea genotypes (24 desi and 4 kabuli type) were evaluated at two zinc levels (no added Zn and Zn 2.5 mg/kg soil) in three pot experiments under controlled conditions. The genotypes differed in sensitivity to zinc deficiency and advanced breeding lines ICC-4958, T-1587, CTS-11308, NEC-138-2 × CM-72 and cultivar Punjab-91 were relatively zinc efficient compared to other Australian and Pakistani genotypes. The cultivars Tyson, CM-88 and Piadar-91 were more sensitive to zinc deficiency. Total zinc accumulation (content) was closely related to zinc efficiency and an efficient zinc absorption coupled with a better root to shoot transport could a characteristic for zinc efficiency in chickpea.
- Zinc fertilization studies under field conditions both in Australia (Lameroo, Murray Mallee area) and Pakistan (Attock, Potowar region) showed that application of zinc increased grain yield in all chickpea genotypes. Differential response of chickpea genotypes to zinc deficiency was generally consistent ^{with} ~~to~~ pot assay results. The cultivar Punjab-91 was an outstanding genotype as it produced high yield without zinc application and responded well to zinc fertilization. Fe-chlorosis sensitive cultivars C-44 and CM-88 produced poor grain yield in the Potowar region and exhibited low zinc efficiency. At Lameroo, zinc efficiency ranking was apparently influenced by climatic factors such as temperature and drought. Under conditions of terminal drought associated with high temperature, early maturing genotypes

Amethyst and CTS-11308 had an advantage of escaping drought and produced higher yields compared to other genotypes.

- Zinc fertilization studies in pots at two moisture regimes (12% and 4% W/W) showed that application of 2.5 mg zinc per kg soil at high moisture availability increased grain yield mainly by increasing number of pods per plant. However, high zinc at 12% moisture promoted vegetative growth during the reproductive phase that reduced translocation of assimilates into developing seed, decreasing individual seed mass. Plants grown under zinc deficiency could not exploit available soil moisture and water use and water use efficiency was reduced. Among plant organs, seed accumulated the highest amount of zinc which is regarded as a desirable quality factor for human consumption and seeds having high zinc content may improve seedling vigour due to the carry-over effect of zinc from seeds to seedlings.
- The study relating to the effect of zinc nutrition on plant water relations provided no evidence of zinc deficiency induced water stress at adequate moisture supply as the values of water potential and relative water content (RWC) were either similar or even higher in zinc deficient plants compared to zinc adequate plants. However, low zinc availability induced stomatal closure which reduced transpiration and apparently resulted into a water build up. Osmotic potential was the most-affected component of plant water relations under zinc deficiency and lowering of osmotic potential was associated with accumulation of solutes, which was presumably either due to free amino acids and amides resulted from impairment of protein synthesis or due to elevated concentration of other mineral nutrients in zinc starved leaves. The relationship between shoot dry matter and carbon isotope discrimination showed a strong negative correlation in zinc adequate plants subjected to water stress, but no significant correlation was observed in zinc deficient plants. Finally, the study showed that the magnitude of osmotic adjustment was significantly higher in plants supplied with high zinc compared to zinc deficient plants and zinc deficiency apparently restricted the capacity of drought induced osmotic adjustment in chickpea. It was concluded that high zinc availability may enhance the ability of plants to endure periods of drought by promoting osmotic adjustment.

Publications and Conference Proceedings

The following publications and conference proceedings were produced during the Ph.D candidature from present study:

Khan, H. R., McDonald, G. K. and Rengel, Z. (1998). Assessment of the zinc status of chickpea by plant analysis. *Plant and Soil* **198**, 1-9.

Khan, H. R., McDonald, G. K. and Rengel, Z. (1998). Chickpea genotypes differ in their sensitivity to zinc deficiency. *Plant and Soil* **198**, 11-18.

(One of the photographs of the study was printed on the front cover of the issue of *Plant and Soil*).

Khan, H. R., McDonald, G. K. and Rengel, Z. (1998). Response of chickpea to zinc nutrition and water stress. pp. 94. International food legume Conference III, Adelaide, Australia.



CHAPTER 1

General Introduction

Chickpea (*Cicer arietinum* L.), ranks among the world's three most important food legumes with an annual production of about 9 Mt from about 12 Mha (FAO, 1996). It is grown in more than 35 countries in different parts of the world, but the main producing regions are the Indo-Pakistan subcontinent and the Mediterranean basin. On the subcontinent, the crop is the principal pulse and provides a major source of protein and vital mineral nutrients in the diet of the low-income and predominantly vegetarian population. However, in the recent past the yields have declined as chickpea has been displaced from its traditional areas of production to marginal lands because of the rapid expansion of irrigated areas and the introduction of high yielding cultivars of wheat.

In Australia, the production of chickpea has increased substantially over the past decade because of the profitability of the crop and the growing awareness of the value of grain legumes. In higher rainfall areas, farming has intensified and the increase in continuous cropping in southern Australia has partly been based on the availability of a number of grain legumes including chickpea. Chickpea is a comparatively recent introduction in Australia, but it offers promise as an alternative legume crop and is well suited into broadacre cropping systems. ^(Siddique and Sedgley, 1986) Breeding work in chickpea has been carried out and a number of cultivars suitable for the Mediterranean-type climate of southern Australia have been released, but the nutrient requirements including zinc for high seed yield have not been satisfactorily defined.

The agronomic problems associated with chickpea crops differ little between geographical areas; drought and nutrient deficiencies appear to be major limiting factors. The area and production of chickpea show pronounced seasonal fluctuations, world wide, which ^{are} ~~is~~ partly associated with the amount and distribution of rainfall. Often, the yields attained by the farmers are considerably less than its potential yields. The low and

variable yields in many countries ^{are} ~~is~~ due in part to the relegation of chickpeas to marginal lands and to adverse effects of environmental stresses on growth and development. Limited availability of water and deficiencies of mineral nutrients both contribute to poor and unstable yields, and it is important to find ways to improve yield stability.

Zinc deficiency is considered the most widespread of the micronutrient deficiencies, with zinc-deficient soils being common in both tropical and temperate climates. In the agricultural zones of Australia, there are reports of extensive areas of zinc-deficient soils. Riceman (1948) reported severe zinc deficiency in the Ninety Mile Desert on the border of Victoria and South Australia and in several million hectares of clover-grass pastures in western and southern Australia. Zinc deficiency also has been observed in field crops grown on vertisol soils in Queensland and northern New South Wales (Weir and Holland, 1980).

In India, zinc deficiency is recognized as one of the most common micronutrient deficiencies, and Katyal (1985) has classified about 50% of the Indian soils as zinc deficient. Similarly, most of the arable land in Pakistan contains inadequate levels of available soil zinc; zinc deficiency is the most common disorder in alkaline and calcareous soils. ^(Smithson *et al.*, 1985) Therefore, the occurrence of large areas of zinc-deficient soils in the regions where chickpea is extensively cultivated could affect its productivity.

Worldwide, most of the chickpea crop is cultivated either in the arid and semi-arid tropics or in Mediterranean-type climates where crops are exposed to drought at some stage during the growing season. In the Indo-Pakistan subcontinent, chickpea is sown in the post-rainy season and largely depends on moisture stored in the soil during the preceding monsoon rains because winter rainfall in the production region is low and erratic. The growing season (winter/spring) is succeeded by a hot dry summer and the intensity of water deficits progressively increases with advancing growth. Consequently many crops suffer from drought stress. Under the Mediterranean type climate of southern Australia, most of the rainfall occurs during winter, with declining rainfall in

spring. Limited availability of water in spring becomes a major constraint for crop production in the region, particularly during the reproductive phase when evaporative demands of plants are high.

The unfortunate concurrence of zinc deficiency and drought in many areas of the world important for chickpea production suggests that the crop may suffer from zinc deficiency and water stress simultaneously. Zinc has a specific role in the integrity and functioning of the cell membrane and zinc nutrition may affect the water relations of plants (Sharma *et al.*, 1982) and plants that suffered from zinc deficiency appeared to be more sensitive to water deficits. Field observations in wheat indicated that application of zinc increased drought tolerance in the South Australia (King, 1994). Therefore, adequate zinc nutrition may be important for maintaining productivity under drought stress.

Despite its importance as a major pulse crop worldwide, there is little information on the zinc nutrition of chickpea and particularly on the importance of zinc in drought-prone environments. Therefore, the present study was designed to determine the zinc requirement of chickpea and to establish critical zinc concentration for diagnosing the zinc status of plants. In addition, one of the aims of this research programme was to evaluate the differential response to zinc of chickpea genotypes having diverse genetic background, to estimate zinc efficiency and to suggest the possible physiological mechanisms related to zinc efficiency in chickpea. It was also part of the study to examine the effect of zinc fertilization on grain yield, distribution of zinc in plant parts and loading of zinc in the seed. The studies relating to the effect of zinc application on water usage, water use efficiency, plant water relations, stomatal conductance and on degree of osmotic adjustment were of particular interest to ascertain the physiological role of zinc in drought tolerance.

CHAPTER 2

Literature Review

2.1. Introduction

The coincidence of drought and zinc deficiency is common in most of the chickpea-growing areas worldwide and is considered to be responsible for large yield and production losses in chickpea. This review first presents background information on the morphology and phenology of chickpea. The research identifying widespread zinc deficiency in chickpea-growing regions is discussed and followed by the role of zinc in plants. Studies relating to the effect of zinc nutrition on plant water relations are also reviewed. Finally, features of drought stress occurring in the Mediterranean type environment of southern Australia and in the arid and semi-arid tropics of the Indo-Pakistan subcontinent and the effects of drought stress on chickpea growth and development are highlighted.

2.2. Chickpea (*Cicer arietinum* L.)

2.2.1. Morphology

Chickpea is a short herbaceous annual, having hypogeal germination. Its development starts from the elongation of the main shoot. The imparipinnate leaves arise alternatively, generally from the third node. The number of leaflets range from eleven to eighteen per leaf and are sub-sessile, opposite or sub opposite, and ovate with serrate margins. Strongly acidic solutions of malic and oxalic acid are secreted by the foliage, particularly by the leaves (Ladizinsky and Adler, 1976).

Plant habit varies from the prostrate to erect depending on the angle of the branches. The branches on a chickpea plant are classified as primary, secondary or tertiary, but all arise from the base of the plant. The main stem produces a variable (0-7)

number of primary branches. Smithson *et al.* (1985) classified branches as basal or upper on the basis of their agronomic importance.

Flowering in chickpea is indeterminate (Knights, 1991) and flowers are borne singly on pedicels subtended by a single peduncle in the axil of the leaves. Normally there is one flower on each peduncle but genotypes with double flowers are also common. The corolla is generally pink in desi and white in kabuli types. However, various shades of pink and blue have been reported (Rao *et al.*, 1980). The first flowers produced by chickpea are pseudo-flowers and abort after some time. The number of such flowers varies among genotypes and is affected by temperature, daylength and water supply (Smithson *et al.*, 1985).

Chickpea is a self-pollinated crop and chances for out-crossing are negligible (Muehlbauer and Singh, 1987). The fruit of chickpea is an inflated pod having two to four ovules, but usually only one or two develop into a seed. Seed size varies and seed weight ranges from 40 to 600 mg per grain (Smithson *et al.*, 1985). Testa colour ranges from black through various shades of brown to orange and yellow to white and even green. Seed shape varies from irregular to rounded, and texture of the testa can be smooth or rough.

Chickpea has strong roots which grow deep in the soil, as required by a plant growing in water-limited environments. In adult roots peripheral tissues are replaced by thin-walled cork and secondary cortex which are produced by the pericambium (Cubero, 1987). Roots can bear *Rhizobium* nodules which are initially spherical to cylindrical but later form a multi-lobed structures (Rupela and Saxena, 1987).

2.2.2. Distribution

Chickpea is a traditional pulse crop in many parts of the world; in particular, it is an important part of the farming systems in many developing countries. Total world production of chickpea is around 9 million tonnes, produced on 12 million hectares of land (FAO, 1996). Chickpea occupies 15% of the total area allocated to food legumes

world-wide, which indicates that it takes a prominent position within the pulses. Saxena (1993) divided chickpea growing areas into four major eco-graphic regions, namely, the Indo-Pakistan subcontinent, west Asia and north Africa, east Africa and the Americas.

Chickpea is grown in about 35 countries of the world, but the major production area is located in the countries of the arid tropical regions. India is the single largest producer, with 67 % of the world's production area (Figure 2.1). Pakistan, Mexico, Turkey, Ethiopia, Burma and Australia are other important chickpea-producing countries. Global survey of grain legumes indicate that chickpea is adapted to regions with a Mediterranean-type climate having heavier-textured soil types of neutral to alkaline pH (Saxena, 1979).

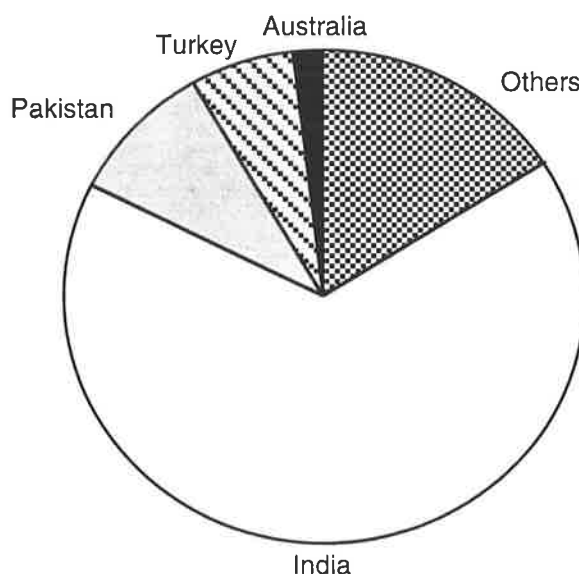


Figure 2.1: Distribution of area grown to chickpea during 1996 (FAO, 1996).

Chickpea production in the developing countries came under increasing pressure during the 'Green Revolution' of the 1960s, because cereals displaced ^{chickpea} ~~it~~ from its traditional areas of production (Smithson *et al.*, 1985). This resulted in a reduction of chickpea area or a shift to relatively marginal lands and low rainfall areas. Consequently, there was a reduction in the yield and production of chickpea (Sharma and Jodh, 1984). In many parts of the world, chickpea is now grown in areas prone to drought stress, and often on soils of low fertility.

2.2.2.1. Chickpea in Pakistan

Pakistan is the second largest chickpea producing country after India (Figure 2.1) accounting for about 7% of global production from 10% of the area devoted to the crop. (FAO, 1996). The major chickpea production area in Pakistan is known as 'Thal'. This region is located between latitudes 29° 58' N and 32° 35' N and longitudes 70° 43' E and 72° 18' E, and is roughly triangular in shape with the base to the north and apex to the south. The climate is very hot in summer but mild in winter. Annual rainfall is relatively higher in the north-east part and ranges between 261 to 285 mm but is less than 170 mm in the south. The annual rainfall follows a bi-modal pattern, with nearly 70% falling in summer (June to September). The soils of Thal are formed from mixed calcareous alluvium, deposited by the river Indus. Almost the entire area consists of sand dunes and inter-dunal valleys. Soils on the dunes are sandy with low water-holding capacity and poor fertility, but the soil of the valleys is loamy in texture, particularly in the north-eastern sector (Khan *et al.*, 1991).

Chickpea is cultivated as a post-rainy, cool-season crop and mostly depends on stored soil moisture for its growth. The common rotation followed by the farmers is chickpea-fallow-chickpea. However, wheat and clusterbean (*Cymopsis tetragonoloba*) are other important crops planted once in a four or five year rotation. Average chickpea yield in Thal is low ranging between 410 and 520 kg/ha. Low and erratic seasonal rainfall makes its cultivation precarious, so area and production fluctuates widely. Chickpeas crops are seldom fertilized and limited availability of water along with nutrients deficiencies are the possible causes of low productivity (Malik and Tufail, 1981; Tufail, 1989).

2.2.2.2. Chickpea in Australia

The temperate region of Australia lies approximately between latitudes 27° to 37° S and is famous for its cereal production. The climate of these zones varies from Mediterranean in south-western Western Australia and South Australia to humid

mesothermal climates with almost evenly distributed rainfall throughout the year in Victoria, New South Wales and southern Queensland. Corbin (1975) identified the suitability of this temperate cropping area for chickpea cultivation.

Chickpea was introduced in Australia in the 1890s and initial experiments were conducted in New South Wales (Valder, 1893; 1896). Further research was not conducted until 1972, when a new research phase commenced at the Agricultural Research Institute, Wagga Wagga, New South Wales. On the basis of plant type, grain yield and suitability for mechanical harvesting, 25 elite lines were selected from an initial collection of 142 cultivars. During 1977-78 these selected line were tested at 20 different sites in Queensland. The accession CPI-53007 was selected and finally released for commercial production by the Division of Tropical Crops and Pastures, CSIRO, as cultivar Tyson in 1978 (Beech and Brinsmead, 1980).

Tyson was originally released as cultivar C-235 in India in 1962. It was selected from the cross IP 58 x C 1234 made at the Indian Agricultural Research Institute on the basis of high yield and *Ascochyta* blight resistance (Bedi and Athwal, 1962). C-235 has been commercially grown in India and performed very well in the north Indian agro-ecological zones, which shows some similarities to southern Australia (Siddique and Khan, 1993).

The first commercial crop of chickpea in Australia was planted in 1979, and there has been a substantial increase in its area and production in Australia since then. In 1991, chickpea was planted on more than 250,000 ha, which produced 220,000 tonnes of grains. The intervention of chickpea in traditional cropping system was so popular that Australia became the fifth largest producer of chickpea in only one decade. Most of the chickpea cultivation was confined initially to the eastern states (New South Wales, Queensland and Victoria) but recent trends shows that chickpea is becoming an important commercial grain legume in South Australia and also in Western Australia. (Siddique and Sykes, 1997). Siddique and Sedgley (1986) recognised the potential of chickpea as a commercial grain legume in Western Australia because it is well adapted to the heavy textured-soils in the low to

medium rainfall zone of south-western cereal belt.

2.3. Zinc

Zinc (Zn) is classified as an essential micronutrient for plant growth and development (Marschner, 1995). The importance of zinc was first identified in the last century when Raulin in 1869 showed that zinc is required for growth of certain fungi in culture. Later, Javillier (1914, cited in Thorne, 1957) confirmed that *Aspergillus niger* did not grow when zinc was not included in the culture medium.

Among crop plants, increased growth of wheat, oats, maize, lupins and peas due to zinc application was first reported as early as in 1914 by Javillier (cited in Thorne, 1957). The essential nature of zinc as a fertilizer was accepted when Sommer and Lipman (1926, cited in Brown *et al.*, 1993) reported increased yields of barley, sunflower, buckwheat, beans and vetch due to zinc application. It appears that depletion due to intensive cultivation may have increased zinc deficiency because it is now recognized as one of the most widespread micronutrient deficiencies and zinc nutrition is becoming significant for crop production and quality as well.

2.3.1. The chemistry of zinc

Zinc is a metallic element with atomic number 30 and average atomic mass 65.38. It is found in numerous minerals of different types, for example sulfides (ZnS, sphalerite and wurtzite), sulfate (ZnSO₄, zincosite; ZnSO₄.2H₂O, goslarite), oxides (ZnO, zincite; ZnFe₂O₄, franklinite; ZnAl₂O₄, gahnite), carbonates (smithsonite, ZnCO₃), phosphates (Zn₃(PO₄)₂.4H₂O, hopeite) and silicates (Zn₂SiO₄, willemite; Zn₄Si₂O₇(OH)₂.H₂O, hemimorphite). The stability of aqueous zinc complexes has been measured for hydroxide, chloride, carbonate, sulfate, sulfide, phosphate, simple organic acids, amino acids, and synthetic chelates (Barak and Helmke, 1993).

The composition of the parent material and soil mineralogy are important for the total zinc concentration in the soil, but the concentration of quartz, which can dilute most

elements, is of particular significance. However, only a small fraction of the total zinc is soluble or exchangeable (Barak and Helmke, 1993). The analytical determination of zinc concentration is mostly made by spectrometric techniques such as atomic absorption spectrophotometry, inductively coupled plasma (ICP) atomic emission spectroscopy and inductively coupled plasma (ICP) mass spectroscopy (Jarvis *et al.*, 1992).

2.3.2. Zinc in soil

The average concentration of total zinc in the upper continental crust is approximately 57 mg/kg (Brehler and Wedepohl, 1978), but the concentration of total zinc varies greatly among soils. Swaine (1955) for example reported that the zinc concentration in soils from 18 countries ranged from 10 to 300 mg/kg. Zinc in the solid phase of the soil was associated with clay minerals, hydrous oxide, and organic matter and only 4 - 6% of the total zinc in soils was exchangeable (Tiller *et al.*, 1972). The concentration of total dissolved zinc in a water saturation extract of the soil is usually very small (in a micromolar range). About one-half of the dissolved zinc occurs as a free cation (Zn^{2+}) and this free cation concentration usually depends on the free cation activity of calcium and magnesium in solution, indicating that calcium and magnesium can displace zinc from adsorption sites (Barak and Helmke, 1993).

Zinc also forms complexes with many organic acids, like citric, malic and oxalic, but these are not usually analysed in soil solution. Similarly, zinc forms complexes with humic substances, for example fulvic acid, humic acid and dissolved organic carbon (Stevenson, 1982).

2.3.2.1. Soil factors related to zinc deficiency

The availability of micronutrients, including zinc, to plants is affected by mineral solubility, precipitation of secondary compounds, and reactions at the solid-solution interface. Generally, deficiencies occur when the micronutrient concentration in the soil solution is not maintained at a level sufficient to meet plant needs (Harter, 1991).

Zinc is divalent in the soil system and is hydrolysed in aqueous solution with pK_a of 9.0; the hydrated form of the ions is unlikely to exist in an aqueous system with pH below 6.0 (Harter, 1983). Zinc sorption by soil clay is common, and montmorillonite-dominated soils have higher zinc sorption capacity than other clay types (Krishnasamy *et al.*, 1985). However, variation in mineralogy and differences in surface conditions also affect zinc retention (Harter, 1991).

Zinc deficiency can occur in a range of soil types. Sandy soils are inherently low in total zinc content because of the large amount of quartz, which generally has a low zinc content (Lindsay, 1972). Calcareous soils having high pH (> 7.4) are also low in available zinc (Kausar *et al.*, 1976) because the solubility of Zn^{2+} is pH dependent and decreases with increased pH. Hence, a greater incidence of zinc deficiency would be expected in calcareous soils than in acid to neutral soils (Lindsay, 1972).

Zinc mobility is strongly dependent on pH (Lins and Fox, 1988). The availability of zinc to plants decreases as soil pH increases partly due to increased adsorption by soil constituents. Transfer of zinc from soil solution to roots, which occurs mainly by diffusion, is reduced at high pH and deficiencies are more common on soils having high pH levels (Moraghan and Mascagni, 1991). Norvell and Lindsay (1970) found that Zn^{2+} activity decreased 100-fold for each unit increase in pH. The pH changes accompanying the use of acidifying nitrogen fertilisers have a great effect on zinc solubility; nitrogen sources that reduced soil pH increased zinc availability (Boawn *et al.*, 1960).

At high pH, zinc is adsorbed by hydrous oxides of minerals. These hydrous oxides play a role in controlling zinc concentration in soil solution. For example, zinc is retained by hydrous iron oxides (Ghanem and Mikkelsen, 1988), hydrous manganese oxides (Loganathan *et al.*, 1977) and hydrous aluminium oxides (Barrow, 1986).

High levels of organic matter are important in keeping zinc availability high in the soil, and chemically-extractable or available zinc has a high correlation with organic

matter (Lindsay, 1972). Zinc deficiency is frequently found in soils with low organic matter or in areas where surface soil has been removed, and application of organic manures helps in correcting zinc deficiency. Apparently sufficient organic matter in the upper horizon of soil enhances zinc availability to plants (Lindsay, 1972).

Soil moisture certainly affects general growth and nutrient requirements and probably has a direct role in zinc deficiency in flooded field crops (Moraghan and Mascagni, 1991). Zinc moves to plant roots in soils mainly by diffusion. Hence, a decrease in the soil water status causes disruption in the diffusion process (Warncke and Barber, 1972). The distribution of total zinc in soil profiles is variable, but extractable or available zinc accumulates in the topsoil or upper soil horizons. Therefore, removal of top soil through erosion can increase the likelihood of zinc deficiency in crops (Moraghan and Mascagni, 1991). Moreover, topsoil drying in low rainfall areas decreases zinc availability to plants, inducing zinc deficiency (Cakmak *et al.*, 1996c), probably due to deactivation of roots in the nutrient zone (Graham *et al.*, 1992).

2.3.2.2. Zinc-deficient soils

Zinc deficiency is arguably the most widespread micronutrient deficiency worldwide. It is common in both tropical and temperate environments and occurs in many of the major agricultural regions of the world.

Zinc deficiency in Australia

Early reports showed the occurrence of extensive zinc-deficient soils in Australia. For example, Riceman (1948) found severe zinc deficiency in the Ninety Mile Desert on the border of Victoria and South Australia. Several million hectares of clover-grass pastures, grown on calcareous or siliceous sands and loams in western and southern Australia, experienced zinc deficiency (Riceman, 1948). In Queensland and northern New South Wales, zinc deficiency has been observed on cereals on vertisols (Duncan, 1967; Weir and Holland, 1980). Similarly, extensive zinc deficiency on almost 8 million ha in the south-west of Western Australia has been identified (Gartrell, 1974; Donald and

Prescott, 1975).

Zinc deficiency is recognised as one of the most common micronutrient deficiencies and is widespread in most of the agricultural zones of South Australia. The deficiency has been observed in the pastures and crops grown on acid sandy soils of the Coonalpyn Downs area, acid to neutral red brown earth (sandy loam to loam textures) and on a wide variety of alkaline calcareous soils. Most of the soils in the South East, Murray Mallee, southern parts of the Yorke Peninsula and vast areas of the Eyre Peninsula regions have inadequate zinc levels (Reuter *et al.*, 1988).

Zinc deficiency in India

Zinc deficiency in India was first noticed as a problem of rice crops on alkaline soils by Nene (1966). Since then, zinc deficiency in India has been found over wide areas (Takkar, 1991). Analysis of more than 11,000 soil and 20,000 plant samples indicated that 45% of soils were deficient in zinc. The proportion of soils considered deficient ranged from 60-70% in the states of Haryana and Madhya Pradesh; 50-59% in Andhra Pradesh and Punjab and 30-49% in Kerala, Bihar and Tamil Nadu (Takkar *et al.*, 1989). Although occurring over a range of soils, zinc deficiency was more severe in calcareous, coarse-texture sands with low organic matter (Takkar and Walker, 1993). It is important to note that most of the chickpea in India is grown in the states of Madhya Pradesh, Uttar Pradesh, Rajasthan and Haryana (Singh and Das, 1987), which are also reported to have large areas of the zinc-deficient soils.

Zinc deficiency in Pakistan

Reports on micronutrient surveys reveal that most of the arable land in Pakistan has inadequate levels of available soil zinc. Zinc deficiency is the most common disorder in alkaline and calcareous soils (Kausar *et al.*, 1979; Sillanpää, 1982; Khattak and Perveen, 1986; Rashid *et al.*, 1987). Rashid *et al.* (1990) reported that most of the soils in the Potowar region of northern Punjab and Hazara division (North Western Frontier Province) are zinc-deficient. Severe zinc deficiency was recorded in the areas of

Attock and Haripur districts, followed by Chakwal and Jhelum districts. Perveen *et al.* (1993) assessed the micronutrient status of the important soil series of the North Western Frontier Province and found that DTPA-extractable zinc ranged from 0.36 to 1.84 mg/kg of soil. The results showed that zinc is quite deficient in four soil series and marginally deficient in another sixteen. Unfortunately, data regarding zinc status of soils and its availability to plants in the major production zone of chickpea (Thal) is not available. However, the calcareous nature of the soils combined with sandy texture and high soil pH, suggest that zinc availability may not be sufficient for plant growth and development (Section 2.3.2.1) and it might be a contributing cause for average chickpea yields in the region being so low (< 500 kg/ha).

Zinc deficiency in Turkey

Zinc deficiency is common in the Mediterranean basin and particularly in Turkey. Analysis of the soil and plant samples showed that most of the soils in central and eastern Anatolia have very low zinc status (Sillanpää and Vlek, 1985). It is noteworthy that chickpea is an important grain legume crop grown in the area, and Turkey is the largest exporter of chickpea in the world (Açikgoz *et al.*, 1994). It seems fair to assume therefore that in Turkey chickpea may suffer from zinc deficiency similar to cereals (Cakmak *et al.*, 1996b), and zinc deficiency could be a limiting factor for its low productivity.

2.3.3. Role of zinc in plants

Zinc is taken up by plants predominantly as Zn^{2+} and it mainly functions as a divalent cation by coupling enzymes with corresponding substrates and forming tetrahedral chelates with different organic compounds such as peptides (Brown *et al.*, 1993). Zinc acts as a functional, structural, or regulatory co-factor of a large number of enzymes in plants. The important zinc-containing enzymes in higher plants are alcohol dehydrogenase, Cu-Zn-superoxide dismutase (SOD), carbonic anhydrase (Ohki, 1976; 1978), carboxypeptidase and RNA polymerase (Römheld and Marschner, 1991). In

SOD, zinc is associated with Cu and represents the structural metal component of the enzyme (Marschner, 1995). The decrease in SOD activity under zinc deficiency corresponds to higher level of free oxygen radicals and oxidants, which may result in increased membrane permeability (Cakmak and Marschner, 1988). Zinc deficiency in plants causes complex changes that include changes in metabolism of carbohydrates, proteins, auxins and impaired membrane integrity (Marschner, 1995). The role of zinc in DNA and RNA metabolism was known earlier, but recently zinc-dependent protein molecules have been identified which regulate genes expression as they are involved in DNA replication and translation (Vallee and Falchuk, 1993).

2.3.3.1. Photosynthesis

Zinc deficiency results in impaired photosynthesis in all plant species (Brown *et al.*, 1993). Deficiency of zinc induces a sharp decline in the activity of carbonic anhydrase (Ohki, 1976; 1978), which is localised in cytoplasm and chloroplast. Carbonic anhydrase appears to facilitate the transfer of CO_2/HCO_3 for photosynthetic CO_2 fixation (Römheld and Marschner, 1991). However, photosynthesis is presumably inhibited due to disrupted chloroplast structure, which affects photosynthetic electron transfer (Sharma *et al.*, 1982), rather than a reduction in carbonic anhydrase activity (Römheld and Marschner, 1991; Brown, *et al.*, 1993). The carbonic anhydrase from dicotyledons is composed of six subunits with a higher molecular weight while the enzyme in monocotyledons is much smaller and contains less zinc (Brown *et al.*, 1993), but the relationship between high molecular weight and zinc requirements of dicotyledons is not clear.

The reduction in photosynthesis that results from zinc deficiency may be related to inhibition in the activity of ribulose 1,5-biphosphate carboxylase (RuBPC), which catalyzes photosynthetic CO_2 fixation. The reduction in RuBPC was observed in zinc-deficient navy bean (Jyung *et al.*, 1972), in barley (Stiborova *et al.*, 1987) and in rice and pearl millet (Seethambaram and Das, 1985). Stomatal response to carbon fixation was also affected under zinc deficiency in pecan leaves. Chlorophyll concentration increased

with an increase in leaf zinc concentration and photosynthesis was sensitively regulated by zinc supply in pecan when it was in the deficiency range (Hu and Sparks, 1991).

2.3.3.2. Membrane integrity

Membrane damage has been considered to be a primary biochemical effect of zinc deficiency as zinc is essential for maintenance of the integrity of biomembranes (Bettger and O'Dell, 1981). Welch *et al.* (1982) reported loss of membrane integrity in root cells from zinc deficiency, which resulted in the accumulation of nutrients to toxic levels in plant leaves. The internal zinc contents of the plants grown without zinc during early growth, was not enough for maintenance of membrane integrity. Hence, root cell membranes require the presence of zinc in their external environment for maintenance of the ion transport and to prevent leakiness due to deficiency (Welch *et al.*, 1982).

Zinc appears to have some role in loading of mineral elements into the vascular system of the plant and this function might be related to the effect of zinc on cell membrane integrity (Graham *et al.*, 1987). Absence of zinc from the rhizosphere may modify the pH of the rhizosphere soil which could be due to changes in root membrane properties of wheat (Loneragan *et al.*, 1987). Marschner and Cakmak (1986) reported that a higher uptake rate of phosphorus in zinc-deficient plants is related to functions of zinc in cell membranes as zinc deficiency increased the membrane permeability of root cells. On the basis of rapid responses in repression of uptake and translocation of phosphorus after re-supply of zinc, it was suggested that zinc directly affects membrane functioning (Marschner and Cakmak, 1986).

Zinc deficiency results in peroxidative damage to biomembranes by toxic oxygen radicals, which enhance leakage of organic and inorganic solutes from root cells. Besides its role as a structural component in biomembranes, zinc decreases the generation of free oxygen radicals which can cause damage to membrane lipids and sulfhydryl groups and also play a role in detoxification (Brown *et al.*, 1993). The amount of reactive sulfhydryl groups in cell membrane of wheat were related to zinc supply; zinc deficiency

Welch and Norvell, 1993

decreased the amount of reactive -SH groups (Rengel, 1995b). It appears that zinc maintains integrity of membranes by keeping sulfhydryl groups in the reduced state.

2.3.4. Distribution and mobilization of zinc in plants

Zinc is a nutrient with intermediate mobility and its distribution in plants is affected by the level of zinc supply (Longnecker and Robson, 1993). Plant species and varieties differ widely in their ability to accumulate zinc even when grown under similar conditions and these differences are important in determining the sensitivity of various crops to zinc deficiency (Lindsay, 1972). Pearson and Rengel (1994) suggested that zinc is phloem mobile but has a complex translocation system. Zinc concentration is generally higher in growing tissues compared to mature tissues when plant have adequate zinc availability and distribution of zinc between root and shoot is dependent on zinc supply (Longnecker and Robson, 1993). McGrath and Robson (1984) reported that zinc was readily mobilized from seed reserves to seedlings in pine and when grown with deficient zinc supply, ^{zinc} seed content of the seedlings corresponded to the seed zinc status.

Roots often contain much higher contents of zinc than shoots, particularly when plants are supplied with high levels of zinc, but at low zinc supply zinc readily moves from roots to the shoots (Riceman and Jones, 1958). Experiments using a split root system have shown that zinc was transported from root zones supplied with zinc to root zones with no external zinc supply (Loneragan *et al.*, 1987). Lindsay (1972) reported that in plants grown under zinc deficiency much of the zinc was redistributed and utilized by the top.

The pattern of zinc accumulation in leaves depends on the availability of zinc. The mobilization of zinc from old leaves to new growth was greater in plants supplied with adequate zinc (McGrath and Robson, 1984). Riceman and Jones (1958) found that total zinc in leaves and petioles decreased as zinc accumulated in inflorescence and seeds of subterranean clover. Reproductive organs generally contain higher zinc concentrations compared to vegetative parts (Ramani and Kannan, 1985; Hocking and Pate, 1977), but

species and even genotypes within species differ widely in their ability to load zinc into their seeds (Graham *et al.*, 1992). Information relating to transport and distribution of zinc in chickpea plant is not available.

2.3.5. Zinc deficiency and water stress

Under rainfed conditions, the productivity of crops is frequently limited by water deficit. The availability of moisture can affect the availability and uptake of zinc (Section 2.3.2.1), but there is also evidence that the level of zinc nutrition can influence the ability of plants to adapt to water deficits either by increasing root growth and water uptake (Nable and Webb, 1993) or by playing some physiological role. Therefore, it seems important to evaluate the interaction of micronutrient deficiency, like zinc, with water stress in the context of drought that occurs in nature.

2.3.5.1. Plant water relations

Relatively less attention has been paid to water relations in nutrient-deficient plants compared to plants experiencing nutrient toxicities. Consequently, there is little information available in relation to the effect of zinc deficiency on plant water relations. The water status of a plant is the function of three interdependent processes: water uptake, water transport and water loss. The movement of water in a healthy plant, under adequate water supply is controlled by stomatal resistance (Barcelo and Poschenieder, 1990). One of the few studies on the effect of zinc on plant water relations was undertaken by Sharma *et al.* (1982) with cabbage. They found that a large proportion of the stomata in leaves of zinc-deficient plants remained closed throughout the day and as a consequence, zinc-deficient plants were less prone to midday wilting than zinc-sufficient plants. However, water potential and water saturation deficit values indicated a high level of water stress in zinc-deficient plants compared to zinc-sufficient plants. Zinc-deficient leaves were thick and leathery, contained larger amounts of water per unit leaf area, had a higher water saturation deficit and took a longer time to reach full saturation than leaves of zinc-sufficient plants. Measurements on detached leaves showed a marked

decrease in water loss under zinc deficiency, indicating a decrease in the hydrosensitivity of the stomata. Sharma *et al.* (1984) also discovered a yellowish to light brown thick viscous substance, possibly an alkaloid or a glucosinolate derivative, in xylem vessels of the roots of zinc-deficient cabbage plants, which apparently impeded water transport from root to plant tops inducing water stress.

Zinc deficiency caused an increase in stomatal diffusive resistance in leaves of zinc-deficient cabbage plants which probably led to a decrease in rates of transpiration besides increasing water saturation deficit (Sharma *et al.*, 1994). It appears that a higher stomatal resistance contributes significantly towards a decrease in photosynthetic rates in plants grown under low zinc status. In a study on zinc requirement for stomatal opening in cauliflower, Sharma *et al.* (1995) reported a drastic decrease in the K^+ content of guard cells, which could be a reason for smaller stomatal opening in the zinc-deficient leaves. The decrease in the K^+ accumulation in the guard cells of zinc deficient leaves may be attributed to enhanced efflux of K^+ , relative to its influx through the leaky membranes (Welch *et al.*, 1982). The possible involvement of zinc in stomatal regulation could therefore be due to its role in maintaining the membrane integrity and K^+ uptake (Sharma *et al.* 1995).

The importance of zinc in water-limited environments has not been frequently reported in field crops. However, field observations during a drought-affected year from the Wudinna area of South Australia showed that wheat in the headland areas which did not receive zinc died, whereas the rest of the crop which received foliar application of zinc matured normally (King, 1994). Zinc deficiency was widespread early in the season (1994), and drought tipping was common in zinc deficient sites. It is therefore speculated that adequate zinc nutrition helps in drought tolerance (King, 1994). However, it requires more experimental work on water relations and growth and development under water stress and zinc-deficient conditions to identify the possible physiological role that may enhance the performance of plants simultaneously exposed to drought and zinc deficiency stress.

2.3.6. Interactions with mineral nutrients

The concentrations of other mineral nutrients affect the availability, uptake and distribution of zinc (Loneragan and Webb, 1993). Conversely, zinc deficiency also affects uptake of other nutrients.

2.3.6.1. Interactions with macronutrients

The uptake and translocation of phosphorus from root to shoot is generally increased in zinc-deficient plants and ^{may} result in accumulation of phosphorus to toxic concentrations (Loneragan *et al.*, 1982). Welch *et al.* (1982) found excessive phosphorus uptake at inadequate zinc supply in different plant species and suggested that zinc has some role in uptake and translocation of phosphorus in plants.

Zinc-phosphorus interaction in tomato grown in chelate-buffered nutrient solutions showed a dramatic increase in tissue concentration of water-soluble phosphorus with a decrease in zinc supply (Parker *et al.*, 1992). This uncontrolled increase in phosphorus uptake by zinc-deficient plants was probably due to interference of zinc deficiency with phosphorus metabolism. Apparently, zinc deficiency leads to a unique loss of the plant's ability to regulate phosphorus uptake, and impaired shoot-to-root translocation of phosphorus may be partly responsible for phosphorus accumulation (Parker *et al.*, 1992).

The application of phosphorus in the absence of added zinc caused water-soaked appearance in leaves of navy bean, followed by necrosis, symptoms indicative of zinc deficiency (Ibrikci and Moraghan, 1993). However, concurrent application of phosphorus and zinc eliminated these abnormal symptoms (Moraghan, 1984). Cakmak and Marschner (1986) studied zinc-deficiency-induced phosphorus toxicity in cotton and found that elevated phosphorus levels in shoots of zinc-deficient plants decreased the physiological availability of zinc in the plant. It shows that at sub-optimal zinc supply, a high phosphorus availability may affect the zinc availability and accentuate zinc deficiency in plants.

Apart from phosphorus uptake, zinc deficiency interferes with regulation of other ions and generally increases net uptake rates of potassium and sulfur by plants (Rengel and Graham, 1995a). The results showed that wheat grown at low levels of zinc, and reliant on zinc reserve in seeds, produced seeds with higher concentrations of Ca and K, indicating interactions between zinc and uptake of certain macronutrients (Rengel and Graham 1995b).

The macronutrient cations calcium, magnesium and potassium inhibited the absorption of zinc by plants from solution culture (Loneragan and Webb, 1993). Calcium levels in solution culture affected zinc concentration in shoots of six grain legumes; concentration of zinc was highest at lowest calcium level and decreased with increasing calcium concentration in solution (Bell *et al.*, 1989). Calcium compounds in soil also affected zinc availability in soil by changing soil pH and hence the amount of zinc in soil solution. Application of CaSO_4 (gypsum) increased zinc concentration in plants presumably by lowering pH and increasing zinc uptake, while CaCO_3 increased pH and consequently decreased zinc uptake. (Wear, 1956).

Zinc appears to play a role in regulating uptake of several mineral nutrients in crop plants. MacNaoidhe and Fleming (1990) studied the effect of zinc and magnesium on winter and spring barley crops grown on Irish soils. The crop fertilised with magnesium sulphate showed no response in terms of grain yield probably due to low exchangeable Mg concentration, a characteristic of soils with low zinc. However, the application of zinc sulphate along with Mg sulphate increased the barley grain yield, indicating a relationship between low zinc and low magnesium in the soil.

Sulfur availability influenced zinc status in soybean. A higher sulfur concentration in solution culture increased the amount of peptides capable of binding zinc, which resulted in an elevation of zinc-ion complex^{es} in soybean leaves (Fontes and Cox, 1993). There was a significant interaction between zinc and sulfur concentration in barley. Concentration of sulfur increased in shoots when no zinc was applied but it was less affected at higher zinc supply (Graham *et al.*, 1987).

2.3.6.2. Interactions with micronutrients

Zinc deficiency results in several physiological changes in higher plants, causing disturbances in absorption and translocation of nutrients (Römheld and Marschner, 1991), which consequently changes the ionic balance of plants (Romero, 1996).

The occurrence of both low available soil zinc and high levels of soluble boron is a common problem in some alkaline soils of semi-arid regions. This may result in a complex nutritional disorder with symptoms expressed as boron toxicity (Graham *et al.*, 1987). Swietlik (1995) studied the interaction between zinc deficiency and boron toxicity on sour orange seedlings and found reduced growth together with higher boron accumulation in leaves under zinc deficiency. The results also revealed that zinc-deficient seedlings were more sensitive to boron toxicity than zinc-sufficient ones.

Graham *et al.* (1987) found highly significant decrease in the rate of boron uptake by barley plants supplied with adequate levels of zinc. The zinc effect was apparently the result of the presence of zinc in the solution bathing the roots and the root cells membranes, which supported the hypothesis that zinc deficiency affects transport of ions, including boron by damaging root cell membrane integrity. Singh *et al.* (1990) working with wheat, noted an increase in the concentration and uptake of boron in the absence of applied zinc. They suggested that application of zinc provided a protective mechanism in the root cell environment against the excessive uptake of boron.

A strong copper-zinc interaction has been observed in field crops growing on soils deficient in both copper and zinc (Chaudhry and Loneragan, 1970). There was usually a positive ^{response to} ~~interaction between~~ increasing supply of copper and zinc when both were limiting, but an abnormal depression of grain yield was noted with zinc fertilisation in the absence of copper. This showed that zinc application intensified copper deficiency in plants probably by depressing copper uptake (Loneragan and Webb, 1993).

Zinc and copper increase each other's solubility in soil solution by decreasing their fixation on soil components, which may increase their absorption by plants.

However, the effect of zinc $\times$ copper interaction on their uptake by plants, can vary with the nature of soils and plant species (Kausar *et al.*, 1976). Zinc and copper interactions are more important for fertiliser practice on marginal and deficient soils where they can accentuate zinc or copper deficiency, which may lead to grain yield loss (Kausar *et al.*, 1976). Copper deficiency also delayed senescence of the old leaves in crop plants, which may affect redistribution of zinc and several other nutrients in plants (Hill *et al.*, 1979).

The interaction between zinc and iron is complex and can produce conflicting results due to differences in plant species, ionic state and complexation of iron (Loneragan and Webb, 1993). However, unusually high concentration of Fe^{2+} in flooded soils exacerbate zinc deficiency by suppressing zinc absorption (Forno *et al.*, 1975).

An increase in Zn^{2+} activity significantly decreased shoot concentration of manganese in barley. Similarly, roots of zinc deficient barley seedlings accumulated higher concentration of manganese and iron than those supplied with higher zinc. These differences in accumulation of manganese and iron were the result of altered absorption of cations by root cells (Norvell and Welch, 1993). The classic antagonism between iron and manganese was also affected by severe zinc deficiency in maize plant (Romero, 1996), which suggested that zinc can affect the relationship between iron and manganese by altering the uptake processes of both iron and manganese.

Deficiency of zinc may intensify the problem of cadmium toxicity by enhancing accumulation of cadmium to a toxic level (Choudhary *et al.*, 1994). Therefore, zinc and cadmium interactions are important and have been studied in various crops. Smilde *et al.* (1992) working with lettuce, spinach, spring wheat and maize reported that interactive effects of native and soil-applied zinc on cadmium levels in the plants were obvious. The pattern between zinc and cadmium was antagonistic; applied zinc strongly decreased the plant cadmium, especially in sandy soils, while applied cadmium suppressed plant zinc to

a lesser extent (Smilde *et al.*, 1992). It appears that zinc fertilisation can reduce the cadmium contamination in food crops to some extent.

2.3.7. Diagnosis of zinc deficiency

2.3.7.1. Symptoms of zinc deficiency

The symptoms of zinc deficiency in plants are not easy to identify and can be confused with deficiency or toxicity of other nutrients, diseases or with frost damage (Schmidt *et al.*, 1972; Webb and Loneragan, 1988). However, the most common symptoms of acute deficiency are stunted growth, shortening of internodes ^{and} petioles, and small malformed leaves, which result in 'rosette' symptoms in the growing shoot of dicotyledons (Snowball and Robson, 1986). Grundon (1987) described zinc deficiency symptoms in soybean and navy bean as suppressed crop growth, lack of vigour and slow maturity. Zinc-deficient plants were stunted, with thin, short stems and pale green, bronzed foliage. Branching was reduced and almost ceased in case of severe deficiency. Zinc-deficient plants produced fewer flowers and few pods were set, resulting in low yields. In navy bean, symptoms first appear on older leaves, but if deficiency persists, young leaves are also affected. Usually, symptoms begin as pale green to yellow-green interveinal chlorosis. Eventually, veins turn brown, leaflets die and fall off while the youngest leaflets often remain small and brittle, showing interveinal chlorosis.

A 'fan shaped stem' in monocotyledons is a common symptom of zinc deficiency (Grundon, 1987). With careful observation, symptoms can normally be seen first in younger leaves. These leaves remain small, develop interveinal chlorosis and necrotic spots that appear adjacent to the midrib on the upper leaf surface. The necrosis is often more noticeable on middle age leaves (Brennan *et al.*, 1993). Zinc deficiency in older wheat plants often results in delayed heading and anthesis, accompanied by a reduction in the capacity of the anther to produce pollen (Sharma *et al.*, 1979).

Little has been reported about zinc deficiency symptoms in chickpea. The only report available, described stunted growth and chlorosis of the younger leaves. A red

brown pigmentation on the margins of upper surface of the leaflets and on lower portions of the stem was visible under zinc deficiency. Chlorosis of stipules of younger leaves is also common in zinc deficient chickpea plants (Ahlawat, 1990).

2.3.7.2. Soil analysis

Soil analysis is a useful analytical method to predict zinc status of soils and its availability to crop plants. Soil analysis before sowing has the advantage in predicting likelihood of zinc deficiency because it allows for zinc application at sowing to prevent yield loss (Armour *et al.*, 1990).

The amount of soil zinc extracted from soils by different chemical extract^{ants} varies with the nature of extractant, pH of solution, kinetics of reaction, and the mode and time of extraction (Bansal *et al.*, 1980). However, the relative efficiency of chemical extractants for estimating available zinc under calcareous alkaline soils suggests that DTPA (diethylene triamine penta-acetic acid) is more reliable than EDTA-amonium acetate, EDTA, triethanol amine and deionized water (Takkar *et al.*, 1976; Bansal *et al.*, 1980). DTPA-extractable zinc from 39 different alkaline calcareous test soils in India, ranged between 0.22 and 2.06 mg per kg. Wheat crops growing in soils which contained 0.60 mg zinc/kg or less were more responsive to zinc application than those having higher zinc concentration, which suggest 0.60 mg/kg DTPA-extractable zinc as a critical limit for wheat below which economic responses are more likely to occur (Bansal *et al.*, 1990). However, the results of zinc response experiments indicate that the critical level varies with soil types and may differ for different species.

Despite this, DTPA-extractable zinc has been considered a reliable soil test for determining critical concentrations for various crops (Brennan, 1992). The DTPA soil test procedure successfully classified a wide range of soils in Colorado (USA) according to their response to zinc application in maize; a DTPA-extractable zinc level of 0.8 mg zinc per kg soil separated the responsive and non-responsive soils (Norvell and Lindsay, 1978). The response of chickpea to zinc fertilization, examined in a range of soils from

semi-arid tropics, showed a significant correlation with DTPA-extractable zinc and grain yield. The critical value for available zinc, below which yield response to zinc fertilization may be expected, was 0.48 mg per kg soil (Singh and Gupta, 1986).

The critical level of DTPA-extractable zinc for wheat in soils of south-western Australian varied from 0.12 to 0.27 mg zinc per kg soil, depending on the soil type. ^(Smith 1986) The critical DTPA-extractable zinc required for maximum wheat production can be determined by using a model, if pH, clay (%) and organic carbon are known for each soil (Brennan, 1992). This suggested that interactions between soil zinc and certain soil properties are important, because the zinc critical level in soil increases with an increase in soil pH due to decreased diffusion and solubility of zinc at higher pH (Lins and Cox, 1988). Thus, a combination of physical and chemical properties of soil with DTPA extraction may enhance the reliability of soil analysis (Dang *et al.*, 1993a).

2.3.7.3. Plant tissue analysis

Plant analysis is an alternate means of diagnosing zinc deficiency, ~~as soil tests are more soil specific.~~ Tissue analysis is being successfully used as a tool to determine plant nutrient status, and the critical concentration is a useful reference point for its interpretation. However, several variables usually act on the results which are generally complex and difficult to interpret. Among these are variety, season, soil, crop management and fertiliser treatments (Melsted *et al.*, 1969).

The critical deficiency concentration value was defined by Ulrich and Hills (1973) as the nutrient concentration in the tissue which relates to a 10% reduction in dry matter yield due to nutrient deficiency (Figure 2.2). The critical concentration represents the nutrient level in the plant below which a growth stress may be expected to occur. The critical deficiency concentration is obtained from the relationship between shoot dry matter and zinc concentration in the tissue.

Interpretation of plant tissue analysis depends on a calibration relating to growth (usually plant dry matter) and nutrient concentration in specific plant tissues for each

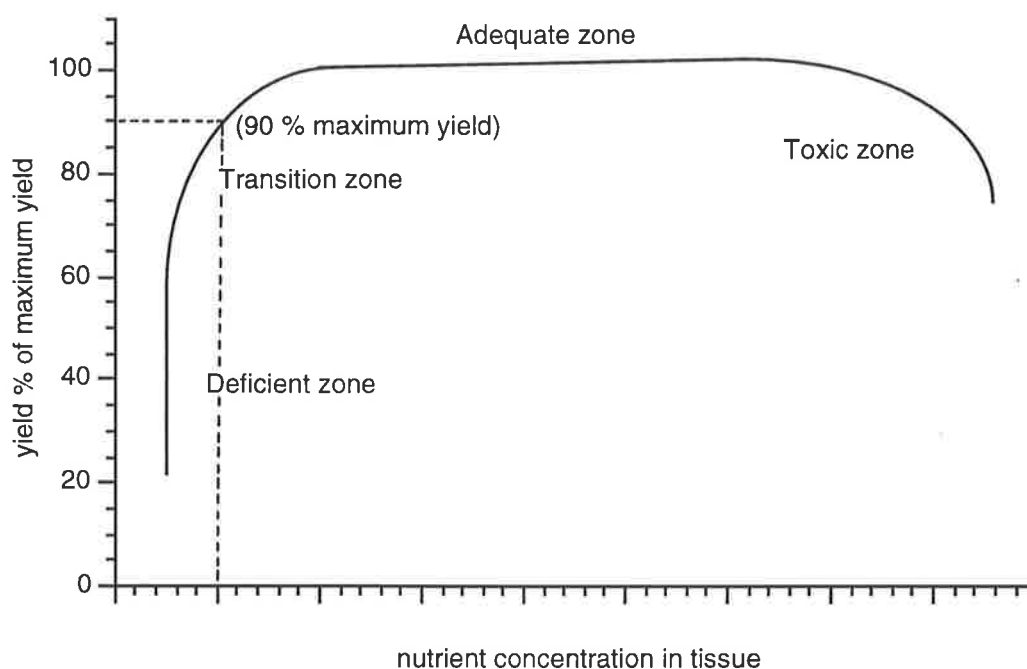


Figure 2.2: Derivation of critical nutrient concentration (adopted from Smith, 1986).

essential nutrient. The minimal nutrient concentrations in the tissue associated with maximum growth have been determined from response curves that were drawn free-hand to best fit the nutrient concentration vs. growth relationship. Ware *et al.* (1982) used the Mitscherlich plant growth model to establish the critical zinc concentration mathematically, and to reduce the arbitrariness of the graphical procedure. A modified Mitscherlich response equation to quantify critical deficiency concentration was developed by characterising plant growth as a function of tissue nutrient concentration. The model presented is $y = \beta (1 - \gamma e^{-\alpha x})$ where y is plant yield at tissue concentration x , and α , β and γ are parameters to be estimated.

Values for critical deficiency concentration vary with the plant parts and age of plant part. The use of a single plant part of a defined physiological age was favoured over samples of composite plant parts for diagnosis of zinc deficiency (Reuter *et al.*, 1982). The effect of plant part was seen in cowpea where the critical concentration was found to be 13, 20, 30 and 50 mg/kg for upper petioles, upper leaves, bottom petioles

and bottom leaves, respectively (Marsh and Waters, 1985). The petioles of recently matured leaf blades were preferred as the tissue that should be sampled for zinc, while recently matured leaf blade tissue had the most sensitive transition zone between deficient and normal zinc concentrations. In navy bean, Armour *et al.* (1990) determined the critical zinc concentrations to be 21 mg/kg for the young leaf, 11 mg/kg for the youngest fully expanded leaf and 13 mg/kg for the whole top in navy bean. The youngest fully expanded leaf was selected as an appropriate diagnostic tissue because it is readily identifiable in the field and had the best correlation with fresh weight. Similarly, in wheat the youngest fully emerged blades (YEBs) were chosen as the most appropriate tissue for zinc deficiency diagnosis because plant growth was more sensitive to decreasing zinc concentration in YEBs than in the whole plant and it can also be easily identified in young wheat plants (Wilhelm *et al.*, 1993).

The critical zinc concentration in chickpea has not been documented, so, it is important that a critical zinc concentration be determined by plant tissue analysis to assist with management of zinc nutrition of chickpea.

2.3.8. Sensitivity of crops species to zinc deficiency

It is well known that the zinc requirement of different crops varies greatly and that plant species differ in their response to applied zinc (Veit *et al.*, 1954; Tiwari and Pathak, 1982, Singh *et al.*, 1983). Among cereals, rice, sorghum and maize are reported to be sensitive to zinc deficiency, whereas barley, wheat, oat and rye are considered as less sensitive species (Clark, 1990). Tiwari and Pathak (1976) reported that rice tends to be more responsive to applied zinc than wheat. The All India Coordinated Scheme on Micronutrients suggested that summer crops, especially maize, rice and sorghum, were more responsive to zinc than wheat and chickpea, which are grown in the cool season (Katyal, 1985). However, variation in these responses may not have been due to crop characteristics alone because climatic and edaphic factors also affect response to zinc application (Lindsay, 1972).

Crops generally show variation in response to zinc fertilization when grown under identical soil conditions due to difference in their requirements and ability to absorb zinc from the soil (Singh *et al.*, 1983). Moraghan (1984) reported that corn, flax, navy bean and soybean responded to zinc fertiliser, while wheat did not. Among the species examined, flax and navy bean showed the largest response to added zinc. The suppression in growth of navy bean under zinc deficiency was presumably associated with accumulation of phosphorus to a toxic level. The sensitivity of wheat, chickpea, mustard and Egyptian clover to zinc deficiency was examined by Tiwari and Pathak (1982). Among these species, chickpea and Egyptian clover were more sensitive to zinc deficiency than wheat, while mustard was relatively tolerant. The response of eight winter crops to zinc fertiliser were evaluated on a Typic Ustochrept soil (Tiwari and Dwivedi, 1990). Application of zinc resulted in a significant increase in seed and straw yield of all crops, but the magnitude of response differed among species. The largest increase in seed yield due to zinc application occurred in lentil (28%), and chickpea (27%) and then pea (24%), wheat (18%), linseed (16%), mustard (13%), barley (8%) and oats (6%).

A comparison of legume crops revealed that navy bean (cv. Seafarer) was more sensitive to zinc deficiency than soybean (cv. McCall) when grown on a calcareous soil (Ibrikci and Moraghan, 1993). The difference in susceptibility of navy bean to zinc deficiency apparently resulted from restricted translocation of zinc from roots to shoots. However, it is difficult to generalise about the greater susceptibility of navy bean to zinc deficiency on the basis of a single cultivar comparison.

The effect of zinc on the growth and chemical composition of eight tropical and sub-tropical pasture and forage legumes showed significant differences among legume species (Andrew *et al.*, 1981). The species were ranked in response to applied zinc in the order *Desmodium intortum* > *Centrosema pubescens* > *Macroptilium atropurpureum* > *Neonotonia wightii* > *Vigna sinensis* > *Stylosanthes humilis* > *Lotononis bainsii* > *Medicago sativa*. Fawazi *et al.* (1993) reported a greater response of pea (cv. Perfection)

to applied micronutrients including zinc than cowpea (cv. Creama).

This review indicates that differences in sensitivity to zinc deficiency exist among crop plant species. Grain legumes are generally more sensitive to zinc deficiency than other field crops and chickpea seems to be a sensitive grain legume. However, before any final conclusion is drawn, genotypic variability in each species needs to be investigated.

2.3.8.1. Genotypic variation in response to zinc deficiency

Genotypes of crops vary widely in their response to zinc deficiency. Some are relatively more tolerant to deficient soils and maintain adequate zinc uptake and utilisation even under unfavourable conditions (Graham and Rengel, 1993). Tolerance to zinc-deficient soils is a genetic trait and is often described as zinc efficiency, which is defined as the ability of a cultivar to grow and yield well in soils too deficient in zinc for a standard cultivar (Graham, 1984). It is quantified by comparing the growth of plants under limiting and non-limiting supplies of zinc. Zinc efficiency has agronomic significance and can be determined in a simple field experiment because it does not imply a mechanism.

Despite the availability of chemical fertilisers, genetic efficiency for nutrient uptake is the best control method to avoid yield losses (Graham *et al.*, 1992). Correcting micronutrient deficiencies through application of micronutrients in fertilisers is not always an effective technique. Therefore, the selection and development of cultivars tolerant to nutrient deficiencies is an excellent approach for increasing production on deficient soils (Takkar, 1993). Growing zinc-efficient genotypes of crop plants on zinc-deficient soils is an environmentally friendly approach which could reduce land degradation by minimising use of chemical fertilisers (Graham and Rengel, 1993). An enhanced zinc efficiency in crops can decrease fertiliser requirements, overcome yield losses from nutrient deficiencies and enhance yield as well as quality of product. Zinc efficient genotypes may also show greater tolerance to drought tolerance because of their

ability to utilise stored soil moisture at depth from infertile sub soils (Graham *et al.*, 1992).

Useful, genetically-controlled zinc efficiency factors exist in wheat, oats and barley (Graham *et al.*, 1992). However, their exploitation in breeding is limited due to ^{and seasonal} spatial variability of zinc ^{deficiency} in field sites. Sorghum genotypes screened for susceptibility to zinc deficiency exhibited differences in the magnitude of expression of deficiency symptoms, which were reflected in nutrient absorption and translocation, which were genetically controlled (Shulka *et al.*, 1973). The benefits of greater zinc efficiency may occur across years if enhanced uptake improves seed zinc content. Genotypic differences relating to zinc efficiency in barley revealed that the zinc-efficient advanced breeding lines WI 2597, WA 73S276 and the cultivar Forrest had high seed zinc concentrations, which can contribute to the seedling vigour when planted in the zinc-deficient soils (Rengel and Graham, 1995a).

Genotypic differences in zinc nutrition have been reported in legumes. Differences among cowpea genotypes in concentration of zinc in leaves were considerable, which may be due to differential compartmentation into active and inactive pools of zinc taken up by plants (Ghildiyal *et al.*, 1978). Ambler and Brown (1969) suggested that cowpea genotypes differ in utilisation and requirement for zinc for growth and development. Almost similar differences in zinc uptake and translocation have been obtained in soybean (Demeterio *et al.*, 1972; Hartwig *et al.* (1991). This review suggests that the mechanism of zinc efficiency in field crops is probably related to a better zinc absorption together with an efficient transport system under conditions of limited zinc availability.

Differential response of chickpea genotypes to ion uptake (Ashraf and Waheed, 1993) and differences in sensitivity to Fe deficiency have been reported (Ohwaki and Sugahara, 1993). Takkar (1993), reviewing the requirement and response of Indian crop cultivars to micronutrients, mentioned differences in chickpea genotypes for sensitivity to zinc deficiency. However, there is no information available regarding responsiveness of

Australian and Pakistani chickpea cultivars to inadequate zinc supply.

2.4. Water stress

Any environmental factor capable of inducing a potentially injurious strain in living organisms is termed a biological stress (Levitt, 1980). As stress due to lack of water is more common than due to excess of water, water deficit is generally referred to as water stress and assessed in terms of water status of plants. In an agronomic sense, drought stress is defined as a reduction in grain yield attributable to plant water deficit (Subbarao *et al.*, 1995).

Water stress occurs due to an inability of the crop plant to extract soil moisture at a rate sufficient to meet its evaporative demand and to carry out metabolic activities. Physiologically, water deficits develop as a result of water loss from leaves as the stomata open to allow the uptake of carbon dioxide from the atmosphere for photosynthesis. The water lost by transpiration from the leaves is replaced by water uptake from the soil and transport via xylem of root, stem, and leaf (Passioura, 1982; Turner and Burch, 1983). The magnitude of plant water stress depends on water availability in the soil and plant characteristics such as root growth and the rate of evapotranspiration. Interaction of numerous factors like weather, topography and soil can affect the seasonal pattern of water availability and plant water status (Blum, 1988).

Generally, reduced growth is the most obvious effect of water stress, but in addition to growth inhibition, water stress greatly modifies plant development and morphology. For example, large increases can occur in root-shoot ratio during water stress due to the differential sensitivity of roots and shoots (Jones, 1983). Water deficit also affects the reproductive development and often advances flowering in crops (Morgan, 1980).

2.4.1. Water stress in chickpea

Drought is a major constraint for crop production in semi-arid environments. Most of the world's production of chickpea is from rain-fed agriculture, mostly under conditions of limited water supply (Smithson *et al.*, 1985). Consequently, chickpea crops suffer from moisture deficits during some stage of the growth cycle. However, the timing of stress and its severity varies from region to region and largely reflects the distribution of rainfall (Subbarao *et al.*, 1995). In the sub-tropical and warm temperature climates, chickpea is grown during the winter season and growth mostly depends on the water conserved from the preceding monsoon rains. Winter rains in the production region are erratic and moisture stress is great enough to reduce productivity (Papendick *et al.*, 1988).

In the Mediterranean basin, which have a cold, wet winter and warm, dry summer, chickpea is sown either in winter or in spring and suffers from water stress during reproductive phase. Although chickpea is considered relatively drought-tolerant ~~species~~ compared to other cool season grain legumes (Sharma *et al.*, 1974), grain yield is affected by drought stress during the post flowering period because water availability generally declines during reproductive development (Silim and Saxena, 1993b).

2.4.1.1. Germination and seedling vigour

Germination and seedling establishment are important components for maintaining plant stand in water-limited environments. Gupta *et al.* (1991) observed genotypic differences among chickpea cultivars in germination when grown under moisture stress induced by polyethylene-glycol. Interestingly, the cultivars adapted to irrigated conditions were adversely affected by water deficit. Saxena (1987) reported the inability of chickpea seedlings to emerge at a soil moisture below 20% (w/w) in a vertisol. The field capacity of these soils is around 34% and a permanent wilting point almost 19%.

Drought can influence crop growth directly and also through its interactions with other environment factors. For example, seedlings that may be growing slowly due to water deficit in drought environments were found to be more vulnerable to pests and diseases (Hall *et al.*, 1979). A preliminary survey conducted by ICRISAT in peninsular India revealed that plant stands of chickpea were poor due to limiting moisture while plant population was good where rains occurred soon after sowing (Saxena, 1987).

2.4.1.2. Vegetative growth

In Mediterranean type environments rapid early growth and canopy development can reduce soil surface evaporation and increase moisture availability for transpiration (Silim and Saxena, 1993a), but the gradual depletion of stored soil moisture and coincident increase in potential evapotranspiration generally leads to the progressive development of drought. However, a ^{relatively slow}~~optimum~~ or balanced early growth is better, when the crop is raised entirely on stored soil moisture, because it is important to ensure that adequate reserves of soil moisture are left for the critical reproductive development. In this regard, some physiologists propose that water use by crops growing on stored soil moisture should be budgeted in a way that sufficient water will be available at flowering and grain filling stage (Subbarao *et al.*, 1995).

Severe drought at early growth stages may drastically reduce leaf area in chickpea (Wilson *et al.*, 1976). Sheldrake and Saxena (1979) found a decline in leaf area index (LAI) as an initial symptom of senescence and maturation of plants, induced by progressive depletion of soil moisture. The decline in LAI started much earlier in the reproductive phase of late maturing cultivars. Thomas and Fukai (1995) reported that photosynthetically active radiation (PAR) interception was severely reduced in chickpea beyond 60 days after sowing due to reduced leaf area induced by moisture deficit. Saxena (1987) found that chickpea may suffer from water deficits early in the season in the Indo-Pakistan subcontinent and the magnitude of water stress increases with advancement of growth when evaporative demands of plants are high.

Water deficit adversely affected canopy development in chickpea. Although it was decreased during all growth phases, reduction in the foliage growth was greatest when the crop was stressed at late vegetative stage prior to flowering (Singh, 1991). It appears that water deficit suppresses early growth in chickpea which consequently results in decline in LAI.

2.4.1.3. Root development

Root growth and root function in relation to water uptake is very important in dryland farming. An optimum root growth is essential for securing water supplies and nutrients for sustaining growth. Gregory (1988) found that a certain minimal amount of active roots reaching the wet soil layers is sufficient for satisfying water requirements of crop plants. Thus, deep root penetration under water-limited environment is an effective component of drought tolerance (Mambani and Lal, 1983; Myers *et al.*, 1984).

The pattern of root distribution in the soil differs between well watered and moisture-stress environments (Blum, 1988). Gregory (1988) reported a sigmoidal pattern of root growth in winter, and spring-sown chickpea crops in northern Syria. A rapid increase in total root length was noted until the start of pod filling; root growth was greater in winter-sown chickpea as compared to the spring-sown crop.

Chickpea is considered a deeper rooted legume compared to lentil, pea and faba bean (Gregory, 1988). While maximum rooting depth is determined genetically, many environmental factors may affect full expression of its genetic potential. Studies conducted in Syria showed that root growth in chickpea varied from 75 cm at the low rainfall site, to 120 cm at relatively high rainfall (Keatinge and Cooper, 1983). The rooting depth probably depends on the depth of soil profiles recharged during winter rains as well as on soil moisture characteristics. The results indicated that root growth at low rainfall sites is more affected by the depth of soil profiles recharged during the season and moisture level of the soil than by genotypic differences. When adequate water was available in upper horizons, chickpea roots grew and extracted moisture from

only upper 10 cm. Sheldrake and Saxena (1979) found restricted root development in the soil surface zones after drying, but in the deeper zones where soil moisture was available, root growth continued even during reproductive phase. Studies showed that chickpea produced a relatively smaller root system in water-limited environments as compared to cereals but its roots had a greater ability to extract moisture than wheat and barley (Keatinge and Cooper, 1983; Thomas *et al.*, 1995).

Differences between chickpea genotypes in root growth and distribution pattern have been little studied. However, this information may be very useful in selection for drought tolerance. Singh *et al.* (1980) reported wide variation in root growth among chickpea genotypes and also in their pattern of root distribution. The chickpea genotype ICC-4958 has been identified as a drought tolerant entry out of 1500 germplasm lines as it produced 20% more grain yield compared to other entries in a water deficit environment (Saxena *et al.*, 1993). An important characteristics of ICC 4958 was its extensive root development under water-limited conditions and it produced 30% more root growth compared to standard cultivar Annigeri. Silim and Saxena (1993a) reported that genotypes ILC-1919, ILC-1930, ICC-4958 and Annigeri possessed deep root system that enabled them to extract water from deeper profiles. Genotype ILC-1930 produced more roots under both drought and irrigated conditions, but its roots were deeper under drought conditions. The chickpea cultivars with deep root systems often produce higher grain yield when subjected to drought stress, which indicates that deep rooting is advantageous particularly where crops depend on residual moisture and experience water stress.

2.4.1.4. Reproductive development

The rainfed crop of chickpea is subjected to intermittent drought during its growth in both semi-arid tropics and the Mediterranean-type environments. Terminal drought stress is common but more critical for crops growing during the post-rainy season and relying on stored soil moisture (Subbarao *et al.*, 1995). The crops often suffer from water stress during reproductive growth because rainfall declines and evapotranspiration

increases. Naturally, plants depend on reserve soil moisture for sustaining growth and development during the critical reproductive stage (Singh and Sri Rama, 1989).

Lack of moisture usually results in earlier senescence and forced maturity in chickpea. Singh (1991) reported 10 days earlier flowering in chickpea grown under water stress than in the well-watered conditions. The duration of flowering and physiological maturity also decreased; reduction in the reproductive phase was nearly three weeks. Hastening of crop development due to water stress has also been observed in wheat (Angus and Moncur, 1977) and in sorghum (Done *et al.*, 1984).

Total grain yield of chickpea is determined by the yield components, number of pods and seed weight per plant. Flower and immature pod abortion along with empty pods therefore can reduce grain yield. Moisture stress decreased total number of pods with an increased number of empty pods, which resulted in low grain yield (Keatinge and Cooper, 1983).

Reports of large responses of chickpea in grain yield to supplemental irrigation at flowering stage have been documented (Sheldrake and Saxena, 1979; Saxena *et al.*, 1990b), which indicate that water deficit at flowering is a major constraint for achieving potential yields. When compared with mustard and safflower in northern India, the seed yield of chickpea was highly correlated with rainfall received during the season. It is, therefore, suggested that productivity of chickpea can be assessed from stored soil moisture at sowing plus anticipated winter rains (Singh and Das, 1987).

2.5. Adaptation to water deficits

The modification in structure and function that increases the chances of a crop to survive and reproduce in a stress-environment is termed adaptation (Kramer, 1980). The adaptive mechanisms that are related to the ability of crops to improve drought resistance, can be classified as drought escape and drought tolerance. Levitt (1980) proposed that drought resistance is a sum of drought avoidance and drought tolerance. Avoidance is to drought escape or related to the maintenance of high tissue water potential and consists of mechanism that

reduces water loss from plants and also maintains water uptake. Drought tolerance refers to the ability of the plants to withstand low tissue water potential.

The effect of internal water status on plant functions may provide clues concerning mechanisms of drought tolerance. Plant responses to water deficit are often complex; it is possible that drought tolerance may be due to a plant's ability to maintain a balanced response using various mechanisms (Hall *et al.*, 1979). Clarke and Townley-Smith (1984) suggested that a combination of both avoidance and tolerance mechanisms is required. The best drought-avoiding genotypes require ^{some} tolerance because ~~some~~ reduction in plant water potential is unavoidable during drought. It appears that drought avoidance is operative during vegetative growth, while tolerance plays a key role during reproductive phase in crop plants (Clarke and Townley-Smith, 1984).

2.5.1. Drought escape

Drought escape is one of the simplest mechanisms by which plants deal with drought. In the regions where the growing season is short and terminal drought stress predominates, drought escape is an important strategy of matching phenological development with the periods of soil moisture availability to minimise the impact of drought stress (Turner, 1986). Well adapted local cultivars to such environments often coincide flowering with the cessation of the rainy season, which enables them to complete reproductive growth when seasonal soil moisture is available (Hall *et al.*, 1979).

Breeding varieties for shorter ^{growth} duration is required to match crop phenology to the season length. In the regions with predominant terminal drought, the success of increasing crop production in drier regions has been largely due to the development of short season varieties that enable the crop to escape severe soil water deficits (Turner, 1986). However, various penalties are associated with the reduction in crop duration. Earliness usually reduces potential yield of the crop by reducing dry matter at anthesis (Subbarao *et al.*, 1995). The use of early maturity as an escape strategy for chickpea has

a limited scope particularly in Mediterranean type environments where early flowering can expose crop to low temperature and frost damage, which will subsequently reduce the grain yield (Siddique and Sedgley, 1986).

2.5.2. Drought avoidance

Crop species have developed several mechanisms to maintain plant water status for metabolic functioning under limited water supply. Such plants either maximise water uptake through improving the capacity of root system to acquire water or reduce water loss beside optimising the use of absorbed water for dry matter production. An extensive root system of plants helps in maintaining water uptake. It is commonly observed that cultivars having mechanisms for efficient water uptake, for storing water or for minimising water loss have the ability to avoid drought (Hall *et al.* 1979).

The root systems have a fundamental importance in drought avoidance of plants. The genotypic differences in crop species in root system and its relevance to drought avoidance has been discussed by Passioura (1987). The depth of rooting and the ability to sustain an uninterrupted supply of water are important factors in water-limited environment. Maintenance of water uptake depends on the root development deep into water-containing soil horizons. Cultivars with deep root systems have the ability to utilise stored soil moisture from depth and have a yield advantage over shallow-rooted cultivars (Clark and Townley-Smith, 1984).

Reduction of water loss is another important avoidance mechanism which depends on stomatal control of transpiration. Crop species have considerable variation in sensitivity of stomata to water stress. Stomatal closure is the strategy developed by plants for reducing water loss (Turner, 1986).

2.5.3. Drought tolerance

Maintenance of turgor pressure in crop species is an important characteristic for adaptation to drought. Drought tolerance at low water potential in crop plants is

associated with their capacity for osmotic adjustment as it helps to maintain turgor pressure and hence cell volume (Muchow, 1985a; Morgan, 1992). Osmotic adjustment (OA) is defined as the active accumulation of solutes within the plant tissue in response to a lowering of soil water potential (Morgan, 1984). The reduction in osmotic potential in drought-stressed plants develops due to increased cell solute concentration (Begg and Turner, 1976).

Osmotic adjustment can help to maintain photosynthesis during water stress. Drought-tolerant lines of wheat have higher photosynthetic rate at low leaf water potential than less drought tolerant lines (Clark and Townley-Smith, 1984). Discussing the role of osmotic adjustment as a component of drought tolerance in barley, Blum (1989) stated that osmotic adjustment under water stress may be an important component of drought resistance in barley growth and development. Morgan (1992) studied the mechanism of turgor maintenance in three summer grain legumes viz soybean (*Glycine max*), cowpea (*Vigna unguiculata*) and blackgram (*Vigna mungo*) and suggested two pathways for the maintenance of turgor pressure: the modulation of leaf water potential, and changes in the osmotic potential due to solute accumulation. The turgor pressure was maintained in the upper expanded leaves despite variations in soil and atmosphere water deficit, indicating the importance of turgor pressure for sustaining leaf function. Greater variations in water potential due to diurnal changes in radiation and water vapour pressure occurred in soybean than in cowpea and blackgram.

Differences in osmotic adjustment in chickpea genotypes were investigated by Morgan *et al.* (1991). They suggested two groups of cultivars/breeding lines based on their potential to drought-induced osmotic adjustment. Chickpea variety Tyson showed a higher degree of osmotic adjustment, while ^{the} magnitude of osmotic adjustment was low in Dooen. Results showed a positive relationship between osmotic adjustment of the leaves and seed yield in water-limited environment. It was concluded that grain yield in the field was related more to the genetically-controlled osmotic adjustment than osmotic adjustment differences based on the environment. Recently, Turner *et al.* (1996)

reported that chickpea (breeding line T-1587) osmotically adjusted to a greater degree with the decrease in leaf water potential compared to pea and faba bean.

Drought resistance in crop plants can be examined by measuring proline and non-structural carbohydrates, which are considered to act as osmoregulators (Clark and Townley-Smith, 1984). Accumulation of proline has been observed in water stressed chickpea plants and it was positively correlated to water saturation deficit (WSD), but proline accumulation showed no clear pattern between drought resistant and susceptible chickpea cultivars (Singh and Ria, 1981). The magnitude of drought induced osmotic adjustment in different chickpea genotypes and its relevance to active growth and physiological activities need further experimentation.

2.6. Conclusion

Chickpea is an important pulse crop which is grown in environments where water stress and zinc deficiency may occur coincidentally. It is most likely that chickpea crops suffer from water stress and zinc deficiency simultaneously. So far, there has been lack of information about zinc requirement of chickpea and the response of chickpea genotypes to zinc deficiency has not been documented as well. Research reports indicated that zinc deficiency damaged membrane integrity, influenced plant water relations and probably zinc application enhanced drought tolerance. The present study was carried out to describe the zinc nutrition of chickpea particularly under water-limited environments. A range of experiments was conducted with following objectives:

- to determine zinc requirement of chickpea and to establish critical zinc concentration for diagnosing the zinc status of chickpea.
- to evaluate responsiveness of chickpea genotypes and to quantify their zinc efficiency.
- to examine the effect of zinc fertilization on grain yield, distribution of zinc in plant parts and loading of zinc in the seed.
- to investigate the effect of zinc fertilization on plant water relations and the degree of drought-induced osmotic adjustment in chickpea.

CHAPTER 3

General Materials and Methods

3.1. Introduction

During the present study, nine pot experiments were conducted in the glasshouse or growth chamber between 1994 and 1998. In addition, one field experiment was carried out on a zinc-deficient soil at Lameroo in the Murray Mallee area of South Australia in 1997, and two field experiments were sown in District Attock, the Potowar region of Pakistan, during the 1995-96 growing season. The objectives of the initial pot experiments were to examine the effect of zinc application on plant growth and to determine the sensitivity of different chickpea genotypes to zinc deficiency. The effect of zinc fertilisation under moisture deficit was studied in a number of experiments using genotypes selected from the initial experiments. The field studies were conducted to verify the results obtained from the pot experiments, carried out under controlled environmental condition. This chapter presents the general materials and methods ^{for the} ~~particularly used in~~ pot experiments reported in the thesis. Further details and specific materials and methods used in each experiment are described in the relevant chapters.

3.2. Soil preparation and sowing

Zinc-deficient siliceous sandy soil, having a DTPA-extractable zinc concentration 0.06 - 0.07 mg/kg, was collected from a virgin scrub near Tintinara, in the south-east of South Australia (Sparrow and Graham, 1988). The top layer of the soil was removed to reduce the amount of organic matter and zinc. The soil was air-dried, passed through a 2-mm stainless steel sieve, washed three times with deionised water to remove excess of boron and root debris, and again air-dried in a glasshouse. Calcium carbonate powder, 0.3 % (w/w), was added and mixed thoroughly with the dry soil. The following nutrients were then separately applied in solution (in mg/kg dry soil): NH_4NO_3 , 350;

K_2PO_4 , 150; K_2SO_4 , 120; $MgSO_4 \cdot 7H_2O$, 90; $CuSO_4 \cdot 5H_2O$, 10; $MnSO_4 \cdot 4H_2O$, 7; H_3BO_3 , 1; $CoSO_4 \cdot 7H_2O$, 1; $FeSO_4 \cdot 7H_2O$, 0.7; $H_2MoO_4 \cdot H_2O$, 0.5; and $NiSO_4 \cdot 7H_2O$, 0.15. The soil was divided into separate lots and a solution of $ZnSO_4 \cdot 7H_2O$ was added to each lot of soil to correspond to the zinc treatments selected for each experiment. Double-deionised water (DD water) with 18 MOhms resistivity was used for watering the plants in all experiments.

The soil was weighed into polythene-lined pots and DD water was added in amounts sufficient to bring the soil water content to field capacity (12% w/w). Seeds having similar size within each variety were selected, surface-sterilised by soaking in 70 % (v/v) ethanol for 1 minute and in sodium hypochlorite (3% active chlorine, v/v) for 6 minutes, and thoroughly rinsed in DD water. Prior to sowing, seeds were germinated on a filter paper (Whatman No. 42, ashless), pre-soaked with DD water, in an incubator at 20 °C for 48 hours, after which the uniformly germinated seeds were sown 2-cm deep in each pot. A few drops of DD water were added to aid soil setting around the seeds. A layer of black polypropylene beads approximately 1 cm deep was placed over the soil surface in the pot as a mulch to reduce evaporation.

3.3. Measurements of plant water status

3.3.1. Leaf water potential

Leaf water potential (ψ) was measured ^{the} on ^{on} top portion of the main stem, ^{es} or [^] one of the basal branch that was fully exposed to light, using a pressure chamber (Scholander *et al.*, 1965). [^] Stems were ~~The branch was~~ selected because the leaf petiole in chickpea is very short, making it difficult to insert into the rubber seal (Sivakumar and Virmani, 1979).

The branch was enclosed in a plastic bag to avoid water loss (Turner, 1981), cut from the plant just beneath ^{the} 4th to 5th leaf from ^{the} top with a sharp razor blade, and placed in a pressure chamber with the cut end of the branch protruding from the chamber through a rubber seal. The top of the chamber was quickly sealed and pressure was gradually

increased (0.005 MPa/s) by compressed air (Turner, 1981). The cut surface was carefully monitored through a low-power lens; the pressure at which the meniscus of the xylem sap just appeared at the severed end of the branch was read on the gauge. The pressure was released and sample was removed. Measurements of water potential were done between 12:00 and 14:00 h.

3.3.2. Relative water content

The upper-most fully expanded leaf (4th-5th) from ^{the} top of the main stem or one of the basal branches was used for relative water content (RWC) measurement (Morgan *et al.*, 1991). It was difficult to punch leaf discs in chickpea due to their small leaflet size, especially when leaf growth was reduced by zinc deficiency and water stress, and a modification of the method was developed. Ten leaflets were collected into a tared glass vial which was immediately closed. After the fresh weight was determined, the leaflets were cut transversely into halves to enhance re-hydration and floated for about 20 hours at room temperature on double deionised water in a covered petri dish at light near the compensation point (10-12 $\mu\text{E m}^{-2} \text{s}^{-1}$). The technique was modified on the basis of results obtained from a preliminary experiment that showed that cut-leaflets were better re-hydrated than the whole leaflets. After rehydration, the leaflets were surface-dried using tissue paper, transferred into a glass vial and turgid weight was obtained. The final dry weight was determined after drying in a pre-heated oven at 80 °C for 20 hours. The RWC was calculated using the following equation (Turner, 1981).

$$\text{RWC} = \frac{\text{Fresh Weight} - \text{Oven Dry Weight}}{\text{Turgid Weight} - \text{Oven Dry Weight}} \times 100$$

3.4. Tissue analysis for nutrients

The plant material was rinsed in deionised water, dried at 80 °C in an oven for 48 hours. Dry tissue was ground in an electric grinder or chopped into small pieces using stainless steel scissors. A homogeneous sub-sample was weighed into 15-mL or 25-mL glass tubes, depending on the sample weight, pre-digested in 6 mL or 10 mL of 70%

(v/v) HNO_3 overnight at room temperature, vortexed and then heated at 126°C until complete digestion occurred. The volume of acid was reduced to about 0.5 mL or 1 mL. After cooling, digests were made up to 15 mL or 25 mL with 1% (v/v) HNO_3 , vortexed and left overnight to settle. The digested samples were decanted into polystyrene tubes and analysed by an Inductively Coupled Plasma (ICP) Emission Spectrometer (model ARL 3580, Analytical Research Laboratories, Switzerland). A set of standard plant materials of known concentration of zinc and other elements was digested and analysed in the same manner as the experimental samples to ensure the procedure was accurate (Zarcinas *et al.*, 1987).

3.5. Statistical analysis

Data obtained from the experiments were statistically analysed by analysis of variance using routines of the Super Anova computer program. Data for some variables were log-transformed, where necessary, to overcome the problem of non-homogeneity of variance, before being analysed by analysis of variance. Pairwise comparisons of means were done using least significant differences (LSD) at 5% level of probability ($P=0.05$).

CHAPTER 4

Zinc requirement of chickpea and assessment of critical zinc concentration using plant analysis

4.1. Introduction

Chickpea is a major grain legume traditionally grown in the Indo-Pakistan subcontinent, which accounts for > 76% of total world's chickpea area (FAO, 1996). The crop has traditionally been used as an important source of dietary protein. In contrast, in Australia, chickpea is a relatively new crop; extensive cultivation has only occurred since the 1980s, but it has increased greatly since then because of its adaptation in the cereal-based cropping systems and high economic benefits (Siddique and Khan, 1993). However, yields of chickpea in both regions are variable and low compared to its potential yields. One of the possible constraints causing low productivity is micronutrient deficiency.

Zinc deficiency is considered the most widespread micronutrient deficiency in both tropical and temperate climates (Sillanpää and Vlek, 1985). Several reports reveal occurrence of extensive areas of zinc-deficient soils in the major cropping areas of western and southern Australia (Riceman, 1948; Duncan, 1967; Reuter *et al.*, 1988; Graham *et al.*, 1992). In the Indo-Pakistan subcontinent, zinc deficiency is also recognized as one of the most common micronutrient deficiencies (Kausar *et al.*, 1979; Katyal, 1985; Khattak and Perveen, 1986; Takkar, 1993). A comparison of several crop species has shown that chickpea is more sensitive to low soil zinc than wheat and other cereals (Tiwari and Pathak, 1982). Therefore, the prevalence of zinc deficient soils in regions growing chickpea could be a reason for its low and variable productivity. The ability to diagnose zinc deficiency would therefore be a useful tool in growing chickpea.

Plant tissue analysis is a reliable method of diagnosing nutrient deficiencies, and the critical concentration is used as a reference point for its interpretation (Melsted *et al.*,

1969). The critical concentration is defined as the maximum nutrient concentration in the tissue associated with a 10% growth reduction due to deficiency (Ulrich and Hills, 1973). Despite its importance as a pulse crop worldwide, there is little information on the zinc requirements of chickpea, and the critical zinc concentration in chickpea has not been documented so far. Therefore, a number of experiments were set up to investigate the zinc requirement of chickpea, and to determine the critical zinc concentration for assessment of zinc status in chickpea. A number of genotypes and moisture treatments were used to examine the reliability of the estimate over a range of conditions.

4.2. Materials and methods

4.2.1. Experiment 1. Effect of zinc application on chickpea growth and critical zinc concentration

Zinc-deficient siliceous sandy soil having DTPA-extractable zinc concentration 0.07 mg/kg was prepared; basal nutrient solutions and calcium carbonate were applied as described earlier in Chapter 3 (Section 3.2). The soil was then divided into five separate lots and a solution of $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ was added to four lots of soil to correspond to 0.04, 0.2, 1.0 and 5.0 mg zinc per kg of soil. These zinc treatments were designated as $\text{Zn}_{0.04}$, $\text{Zn}_{0.2}$, $\text{Zn}_{1.0}$ and $\text{Zn}_{5.0}$, respectively. No zinc was applied to the control (Zn_0). Double-deionized (DD) water having 18 MOhms resistivity was used.

Two kg of the prepared soil was weighed into polythene-lined 150-mm diameter plastic pots and DD water was added in amounts sufficient to bring the soil water content to field capacity (12% w/w). Seed of two widely-grown cultivars of desi chickpea (Dooen and Tyson) was sown in the experiment. The zinc concentrations of the seed of Dooen and Tyson were 34 ± 1 and 38 ± 1 mg per kg, equivalent to zinc contents of 5.75 ± 0.4 and 4.75 ± 0.3 $\mu\text{g}/\text{seed}$, respectively. Seeds having similar size within each variety (Dooen 169 ± 4 mg and Tyson 125 ± 3 mg) were selected, surface-sterilised and germinated on a filter paper (Whatman No. 42, ashless) at 20 °C in an incubator. After

48 hours three uniformly germinated seeds were sown in each pot. A few drops of DD water were added to aid soil setting around the seeds.

Plants were grown in a glasshouse at the Waite Agricultural Research Institute (Adelaide, South Australia) for 12 weeks. Temperature was 25 ± 2 °C during the day and 15 ± 2 °C at night. Pots were watered with DD water to weight (12% moisture w/w) on alternate days. Measurements of plant height ^(main stem) and leaf numbers ^{per plant} were taken weekly. A single plant from each pot was harvested 4 weeks after planting (10-12 leaf stage) and again 6 weeks after planting (18-20 leaf stage). After harvest, the plants were rinsed in deionized water, dried at 80 °C for 48 hours and weighed for dry matter. A final harvest (the third plant) was made 12 weeks after sowing when plants were beginning to flower. At this harvest, soil was washed off the roots under running tap water. Both roots and shoots were rinsed in deionized water and dipped into DD water. Plants were separated into roots and shoots, wrapped in a special weighing paper, before drying and being weighed for dry weight. Dry plant material was prepared according to the procedure described in Chapter 3 (Section 3.4) and analysed by an Inductively Coupled Plasma Emission Spectrometer.

The experiment was set up in a completely randomised factorial design (2 genotypes $\times$ 5 Zn levels) with four replicates. Results were statistically analysed as reported in Chapter 3 (Section 3.5). To overcome the problem of non-homogeneity of variances, the data for tissue zinc concentration and content were log-transformed before being analysed by analysis of variance.

The Mitscherlich plant growth model was used to determine the critical concentration of zinc mathematically (Ware *et al.*, 1982). A modified Mitscherlich response equation assumed that when nutrient concentration (x) = 0, there will be some yield y_0 , which results in to following model written as

$$y = \beta (1 - \gamma e^{-\alpha x}) \quad [1]$$

where $\gamma = (\beta - y_0)/\beta$, α is the constant of proportionality and β denotes the asymptotic maximum yield as x approaches infinity. The parameters α , β and γ of the equation are estimated from observed data. The solution of equation [1] for critical nutrient level (x), yields:

$$x = -\ln(0.1/\gamma)/\alpha \quad [2]$$

4.2.2. Experiment 2. Response of chickpea to zinc fertilization grown at two soil moisture levels

The experiment was designed as a completely randomized factorial design having three replicates. Soil preparation and basal nutrient solutions were the same as described in Chapter 3 (Section 3.2). Three zinc levels, no applied zinc (severely deficient), 0.5 mg zinc per kg of soil (marginally deficient) and 2.5 mg zinc per kg of soil (adequate) were selected based on the results of experiment 1 and designated as Zn_0 , $Zn_{0.5}$ and $Zn_{2.5}$, respectively. The soil moisture was kept at field capacity (12% w/w) in all pots by adding DD water on alternate days until the induction of the two water regimes three weeks after planting, then water was applied daily. Water stress treatment was induced gradually in half of the pots (W_1) by decreasing water application by 1% (w/w) per day to bring the moisture level from field capacity to 4% (w/w). Pots were weighed daily and if water use was $> 1\%$ (w/w), water was applied to control the rate of development of the stress to avoid rapid water deficits occurring, and to keep the development of soil water deficits by plants of different sizes, due to zinc fertilization, as similar as possible. The remaining pots (W_2 treatment) continued to receive water at field capacity (12% w/w). Seed of two genotypes, Tyson and T-1587, having similar seed weight within each variety (150 ± 4 mg/seed for Tyson and 204 ± 3 mg/seed for T-1587) were selected and planted as described in Chapter 3 (Section 3.2). Seed zinc contents were 4.85 ± 0.4 and 5.00 ± 0.2 $\mu\text{g}/\text{seed}$ for Tyson and T-1587, respectively. The experiment was terminated at the same phenological stage (flowering) as Experiment 1, and plants were separated into shoot and root. Dry weight of shoot and root was determined after drying

in an oven at 80 °C for 48 hours. Plant material was prepared and analysed for nutrients concentration by an Inductively Coupled Plasma Emission Spectrometer as described in Chapter 3 (Section 3.5).

4.3. Results

4.3.1. Experiment 1

4.3.1.1. Development of visual symptoms

Symptoms of zinc deficiency first appeared in Zn_0 and $Zn_{0.04}$ treatments as slightly pale foliage on the youngest leaves about 6 weeks after planting. These symptoms progressively worsened, and a severe reduction in shoot growth and in the size of younger leaves was observed. At 7 weeks after planting, zinc deficiency symptoms similar to that described by Ahlawat (1990) were obvious: chlorosis of young leaves and stipules, followed by necrosis of leaf margins. Older leaves of zinc-deficient plants were thick, lacking in succulence, dull in appearance and prematurely abscised.

4.3.1.2. Plant growth and development

Plant height and the number of leaves were both significantly reduced by zinc deficiency. Differences among zinc treatments started after 6 weeks of growth (Figure 4.1a and b) but there was no difference between cultivars. Plant height and number of leaves increased with an increase in the zinc level and were highest in $Zn_{5.0}$. Growth and development at Zn_0 to $Zn_{0.2}$ were reduced and symptoms of zinc deficiency become more severe by week 12. The $Zn_{1.0}$ plants showed no visible symptoms of zinc deficiency, but growth was slightly reduced.

At 4 weeks after sowing there was no difference in shoot dry matter production between zinc treatments (Figure 4.2a), and no visible symptoms of zinc deficiency. Differences in growth among treatments were just apparent at 6 weeks after sowing when growth was reduced at Zn_0 and $Zn_{0.04}$ (Figure 4.2b). By 12 weeks after sowing, zinc

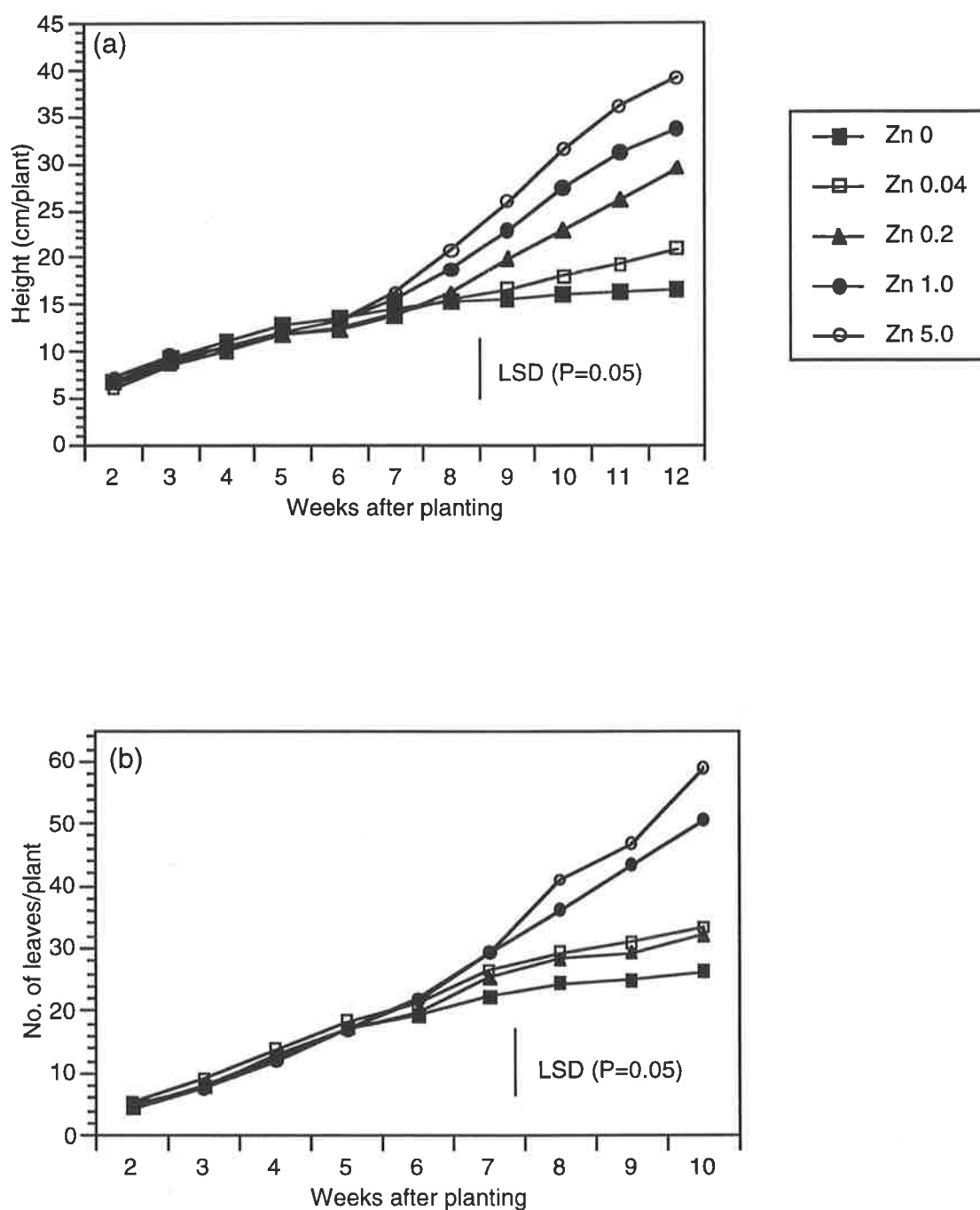


Figure 4.1: Effect of zinc nutrition on height of plants (a) and on number of leaves per plant (b). The data were averaged over two genotypes because no interaction involving the genotype effect was significant. The vertical bars represent LSD ($P=0.05$) values for the zinc fertilization main effect.

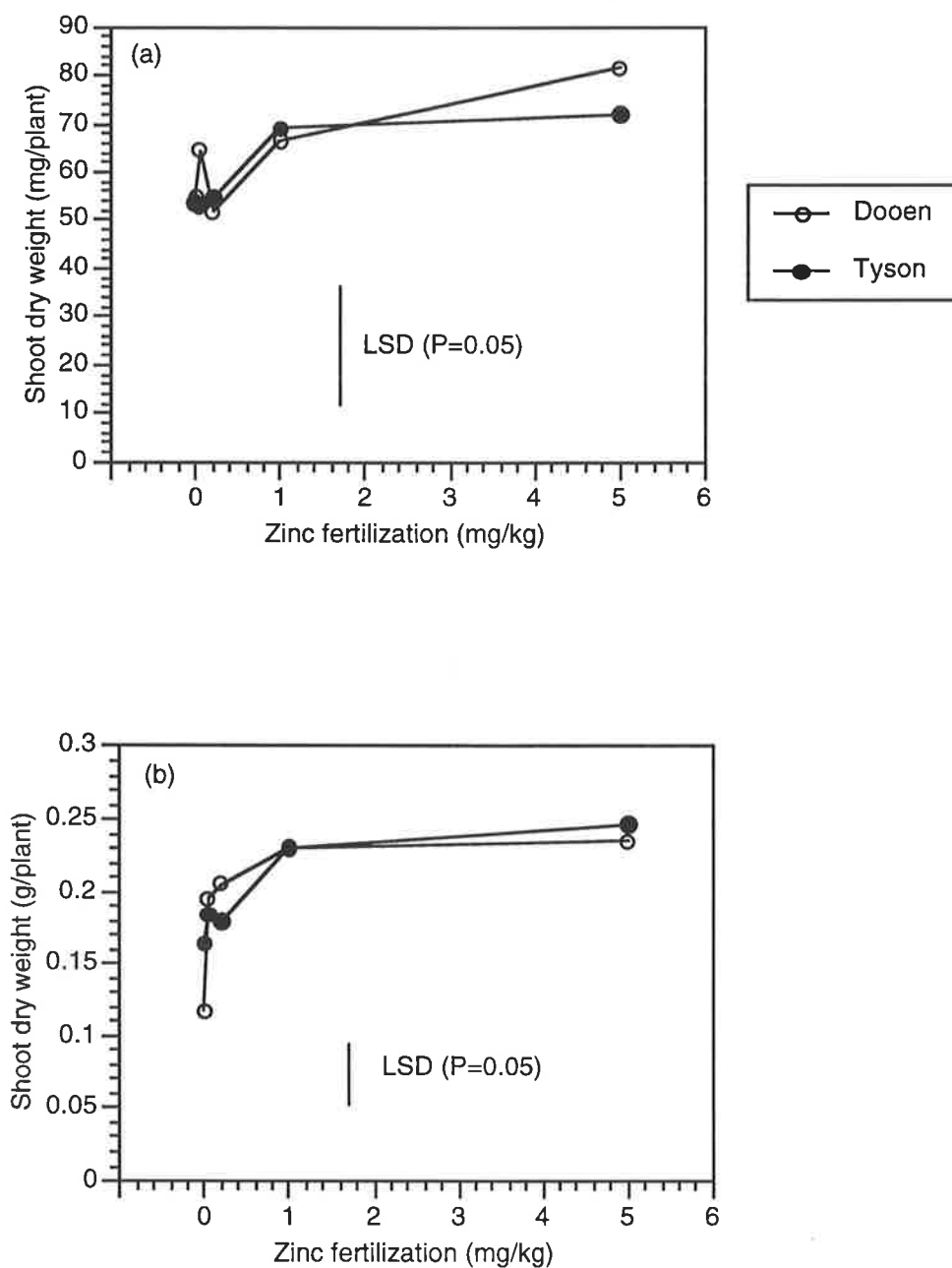


Figure 4.2: Effects of zinc fertilization on shoot dry matter accumulation after 4 weeks (a) after 6 weeks (b) of growth of two chickpea genotypes. The vertical bars represent the LSD ($P=0.05$) values for the interaction genotype $\times$ zinc fertilisation.

supply had a marked effect on shoot dry matter in both cultivars, which responded similarly (Figure 4.3a). Averaged over both genotypes, shoot dry weight at Zn_0 was only 24% of that produced in the $Zn_{5.0}$ treatment.

Root dry weight measured at 12 weeks was significantly reduced by low zinc in both cultivars (Figure 4.3b), but the reduction was less compared to that of shoot growth. Root growth increased from 0.52 g/plant when no zinc was applied to 1.04 g/plant in the treatment with 0.2 mg Zn per kg of soil and did not respond to further increases in zinc fertilization in Dooen but in Tyson root growth increased up to 1.0 mg zinc per kg soil (Figure 4.3b). Total plant dry matter was influenced by zinc fertilisation and showed an increasing trend with an increase in zinc supply to both cultivars. The highest total dry matter was produced in the $Zn_{5.0}$ treatment (Figure 4.3c).

4.3.1.3. Tissue zinc concentration and content

Zinc concentrations in shoots increased significantly with applied zinc (Table 4.1). The zinc concentration in the whole shoot was 6.3 mg per kg when the only source of zinc for plant growth was the seed zinc reserve and native soil zinc. However, it showed a significant increase when zinc rates were increased, particularly at $Zn_{1.0}$ and $Zn_{5.0}$ (Table 4.1). Roots had equivalent or higher zinc concentrations than the shoot. Adding zinc increased the zinc concentration in the root but it did not increase significantly until zinc was applied at the rate of 1 mg/kg soil or higher (Table 4.1). Dooen and Tyson both showed a similar trend in root and shoot zinc concentrations with respect to zinc supply, and there were no significant differences between the cultivars in tissue zinc concentration.

There was a sharp increase in zinc content in the shoot with an increase in zinc fertilization (Table 4.1). Zinc content of roots was similar to or lower than the shoot under zinc-deficient conditions but higher than that of shoots at 5 mg/kg soil zinc fertilization, indicating preferential accumulation of zinc in roots at sufficient zinc supply.

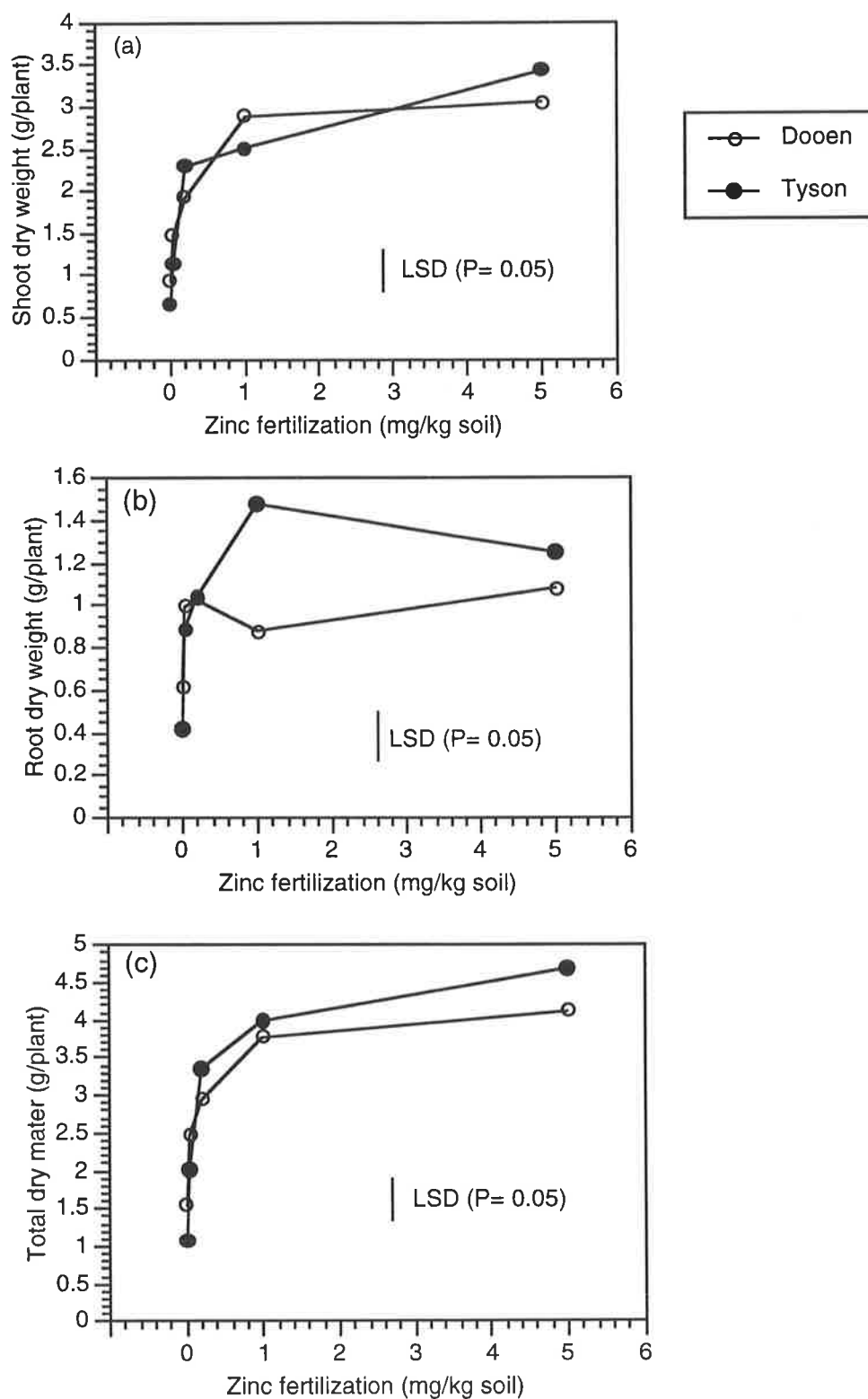


Figure 4.3: Effects of zinc fertilization on shoot (a), root (b) and total dry matter (c) by two chickpea genotypes after 12 weeks of growth. The vertical bars represent the LSD ($P=0.05$) values for the interaction genotype $\times$ zinc fertilization.

Table 4.1: Effects of zinc fertilization (mg/kg soil) on zinc concentration (mg/kg dry weight) and content (mg/plant) in shoot and root of chickpea at the final harvest (12 weeks).

Zinc fertilization	Zinc concentration		Zinc content	
	Shoot	Root	Shoot	Root
0	6.3 (1.80) ^a	10.5 (2.35)	5.1 (1.53)	5.4 (1.66)
0.04	7.1 (1.90)	8.3 (2.09)	9.6 (2.08)	7.9 (1.98)
0.2	9.9 (2.19)	9.5 (2.23)	23.7 (2.90)	9.8 (2.24)
1.00	20.0 (2.99)	23.7 (3.15)	54.5 (3.97)	27.4 (3.27)
5.00	58.8 (4.07)	322.4 (5.75)	191.5 (5.23)	360.0 (5.87)
LSD (P=0.05)	(0.31) ^b	(0.20)	(0.50)	(0.30)
<u>Varieties</u>				
Dooen	21.0 (2.64)	81.1 (3.12)	56.5 (3.24)	84.2 (3.00)
Tyson	19.9 (2.54)	68.5 (3.11)	57.3 (3.04)	80.0 (3.01)
LSD (P=0.05)	ns	ns	ns	ns

^a Numbers in the parentheses refer to averages obtained from the analysis of variance of log-transformed data.

^b The LSD values in parentheses are applicable to the log-transformed data.

ns: non significant

4.3.1.4. Concentration of other nutrients

Zinc supply had a significant influence on concentration of other mineral nutrients in shoot. Among the micronutrients the concentration of manganese (Mn), boron (B) and molybdenum (Mo) was influenced by zinc fertilization (Figure 4.4a). Generally the concentration was higher under zinc-deficient conditions than in the Zn_{1.0} and Zn_{5.0} treatments. Varieties showed differences for their Fe concentration: Tyson had a lower concentration in shoot than Dooen (Figure 4.4b). Zinc application also reduced the concentration of Ca, P, S and Mg in the shoot tissue (Figure 4.5a and b). However, the concentration of K in chickpea shoots increased when the soil zinc was increased.

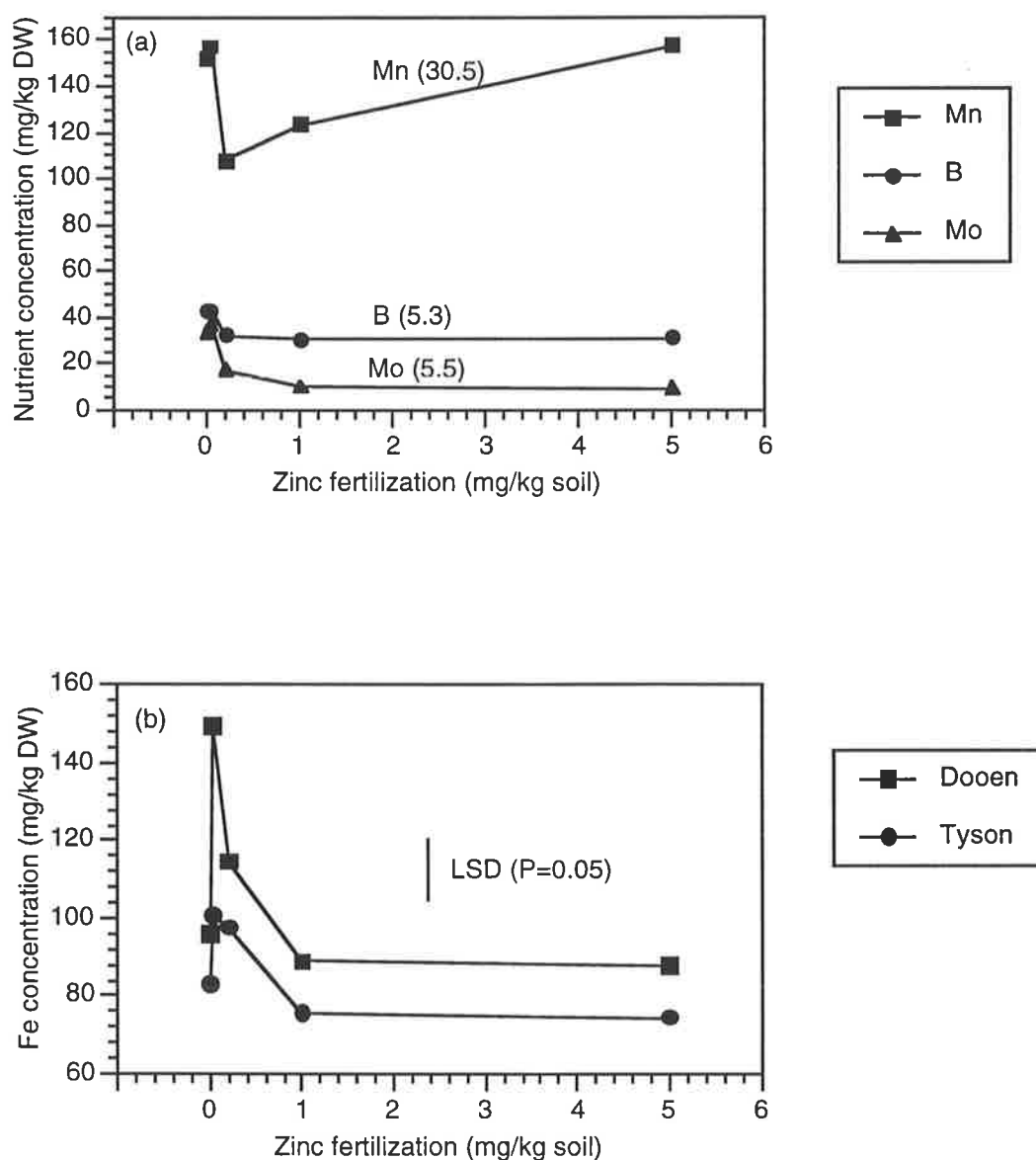


Figure 4.4: (a) The concentration of Mn, B and Mo in the shoots of chickpea 12 weeks after sowing as influenced by zinc fertilization. The data were averaged for the two genotypes because zinc $\times$ genotype interaction was non significant. Values in parentheses represent the LSD ($P=0.05$) values for the zinc fertilization effect. (b) Effect of zinc fertilisation on iron concentration in Dooen and Tyson. The vertical bar represents the LSD ($P=0.05$) value for zinc $\times$ genotype interaction.

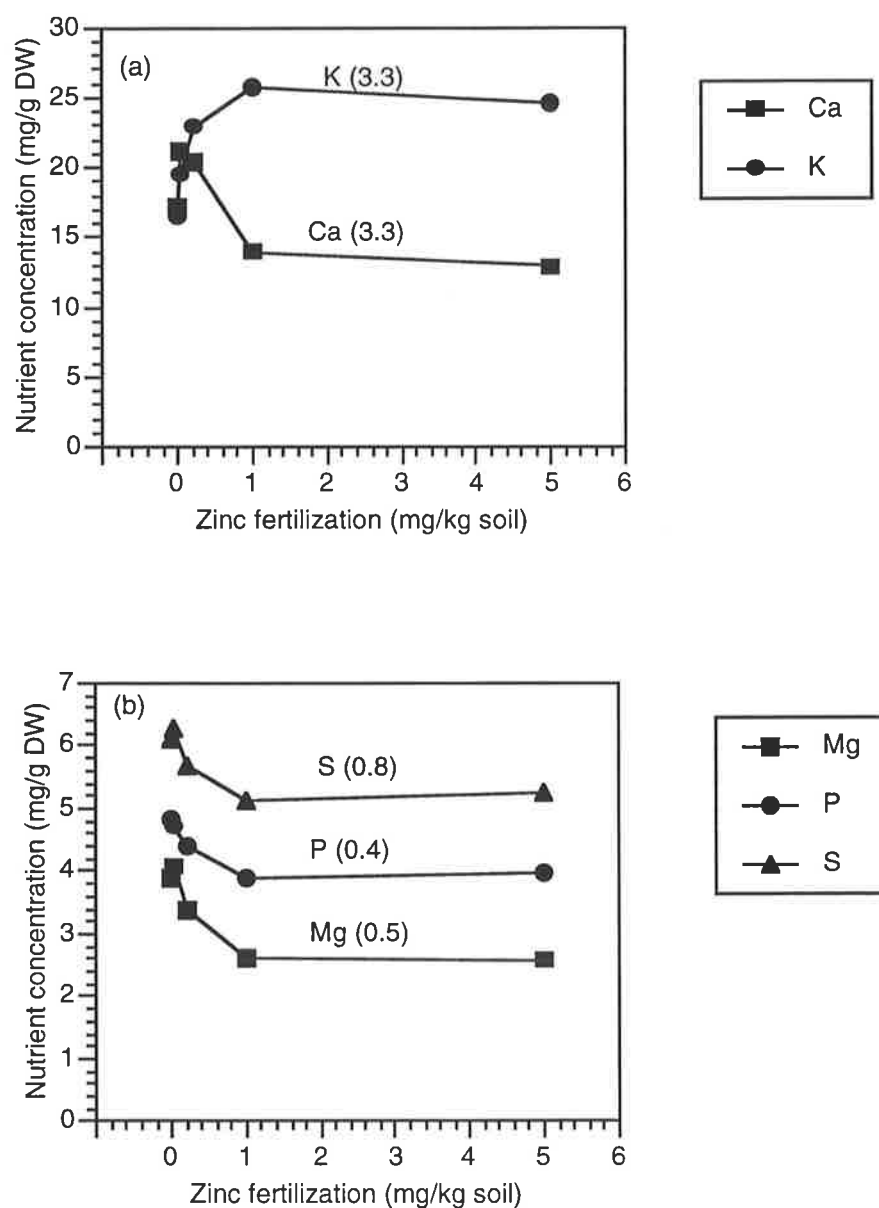


Figure 4.5: The concentration of (a) potassium and calcium, and (b) magnesium, sulphur and phosphorus as influenced by zinc fertilisation. The data were averaged for the two genotypes because zinc $\times$ genotype interaction was non significant. Values in parentheses represent the LSD ($P=0.05$) for the zinc fertilization.

4.3.1.5. Critical zinc concentration

The minimum nutrient concentration in the tissue associated with 90% of maximum growth was determined from curves that were drawn free-hand. The critical zinc concentration was determined as 20 mg per kg in the whole shoot (Figure 4.6). The critical value required for a response equal to 90% of the maximum growth, calculated mathematically using the Mitscherlich plant growth model was 31 mg per kg shoot dry matter (Table 4.2).

4.3.2. Experiment 2

4.3.2.1. Shoot dry matter

Zinc nutrition and moisture stress both significantly affected shoot dry matter. Growth increased with an increase in zinc fertilization; dry weight at the Zn_0 was 31% of that recorded in the $Zn_{2.5}$ treatment (Figure 4.7). Varietal differences were significant, and T-1587 produced higher shoot dry matter than Tyson at the high moisture level when no zinc was applied. The effect of water stress was more obvious in $Zn_{0.5}$ and $Zn_{2.5}$, where shoot dry weight was reduced 52 and 46%, respectively, than in Zn_0 where the reduction in shoot dry weight was 24%. This shows that the plants in $Zn_{0.5}$ and $Zn_{2.5}$ suffered more due to induced moisture stress as compared to severely zinc-deficient plants. Although dry matter was reduced by water stress, the response to zinc application and adequate zinc level were similar as observed in previous experiment.

4.3.2.2. Zinc in shoot tissue and critical zinc concentration

Zinc concentration in the shoot tissue increased with an increase in zinc application (Table 4.3). Under severe zinc deficiency (Zn_0), the water-stressed plants of T-1587 had a higher zinc concentration than the well-watered plants, while Tyson had similar concentration of zinc at both moisture regimes when no zinc was applied. T-1587 also had higher zinc concentration than Tyson in the Zn_0 treatment at the low moisture level. In $Zn_{0.5}$ and $Zn_{2.5}$ treatments, zinc concentration increased in T-1587 at high water

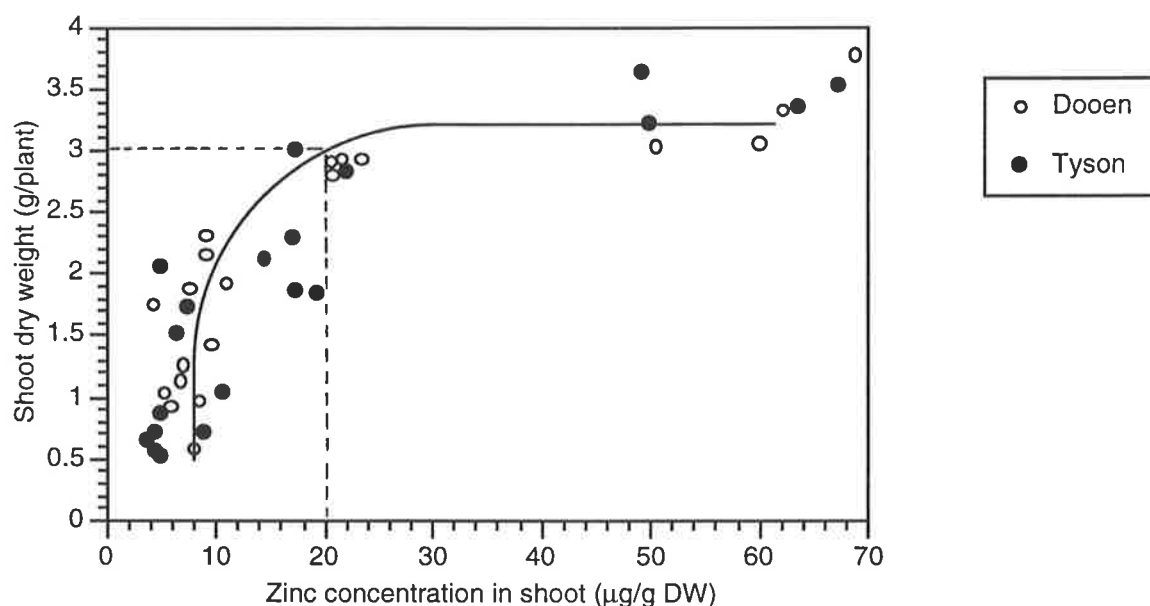


Figure 4.6: Zinc calibration curve relating zinc concentration in whole shoot of chickpea to dry weight of shoots. Curve was fitted by eye and 90% of the mean maximum shoot dry weight (3.44 g/plant) was used for calculation of critical concentration value.

Table 4.2: Estimated parameters and critical deficiency levels for the modified Mitscherlich model used to characterize the relationship between tissue zinc concentration and shoot dry weight in experiment 1.

<u>Parameters</u>	<u>Values</u>
α	0.074
β	3.439
γ	1.071
Adjusted R^2	87.3 ^a
<u>Critical deficiency levels</u>	
Model	31
Graphical (Figure 4.6)	20

^a Adjusted R^2 is defined as 100 minus the ratio of residual mean squares to the total mean squares.

The equation is $y = 3.44 (1 - 1.07 e^{-0.07 x})$.

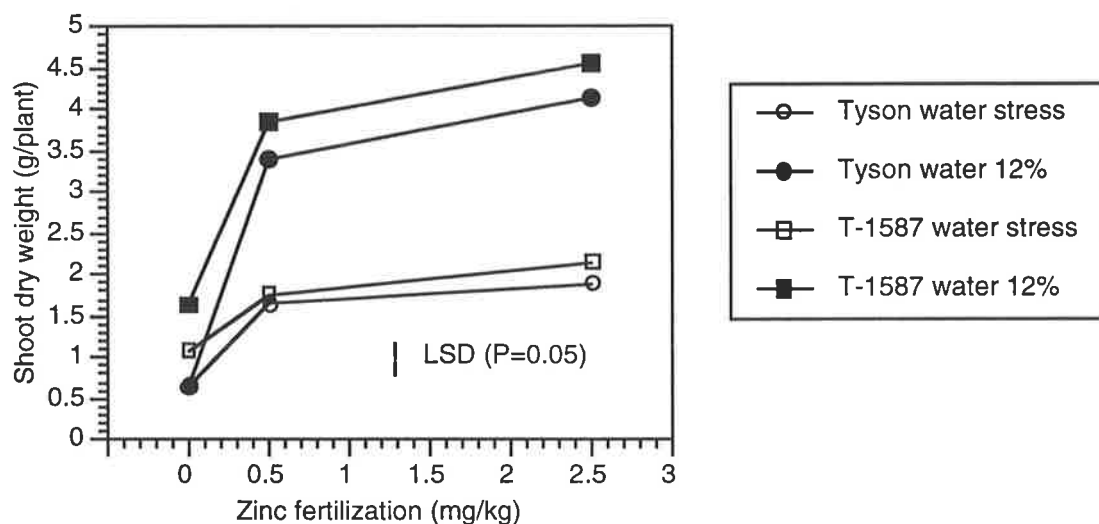


Figure 4.7: Effect of zinc nutrition and water stress on shoot dry weight. Plants were harvested at the flowering stage. The vertical bar represents the LSD ($P=0.05$) value.

availability. Water supply had no effect on zinc concentration in Tyson in the $Zn_{0.5}$ treatment, but at adequate zinc supply in Tyson zinc concentration increased in water-stressed plants. The critical zinc concentration for shoot dry weight, derived by a hand-fitted curve, was 21 mg per kg in the whole shoot harvested at the flowering stage (Figures 4.8a). The value of the critical zinc concentration calculated mathematically using the Mitscherlich plant growth model was found to be 20 mg/kg in same tissue (Table 4.4).

Table 4.3: Effects of zinc fertilization (mg/kg soil) on zinc concentration (mg/kg dry weight) and content (mg/plant) in shoot and root of chickpea at the final harvest.

Genotypes	Water	Zinc fertilization (mg/kg soil)		
		0	0.5	2.5
Tyson	4 %	4.9 (0.69) ^a	14.2 (1.15)	45.4 (1.66)
	12 %	4.5 (0.65)	14.5 (1.16)	41.0 (1.61)
T-1587	4 %	6.4 (0.80)	13.9 (1.14)	32.9 (1.52)
	12 %	4.5 (0.66)	15.9 (1.20)	39.7 (1.60)
LSD (P=0.05)				
Gen × Zinc × Water		(0.04)		

^a Number in the parenthesis refer to averages obtained from the analysis of variance of log-transformed data.

^b The LSD values in parentheses are applicable to the log-transformed data.

Table 4.4: Estimated parameters and critical zinc concentration for the modified Mitscherlich model used to characterize the relationship between tissue zinc concentration and shoot dry weight in experiment 2.

<u>Parameters</u>	<u>Values</u>
α	1.327
β	0.309
γ	1.329
Adjusted R ²	49.0 ^a
<u>Critical deficiency levels</u>	
Model	20
Graphical (Figure 4.8b)	21

^a Adjusted R² is defined as 100 minus the ratio of residual mean squares to the total mean squares.

The equation is $y = 0.309 (1 - 1.327 e^{-1.329 x})$.

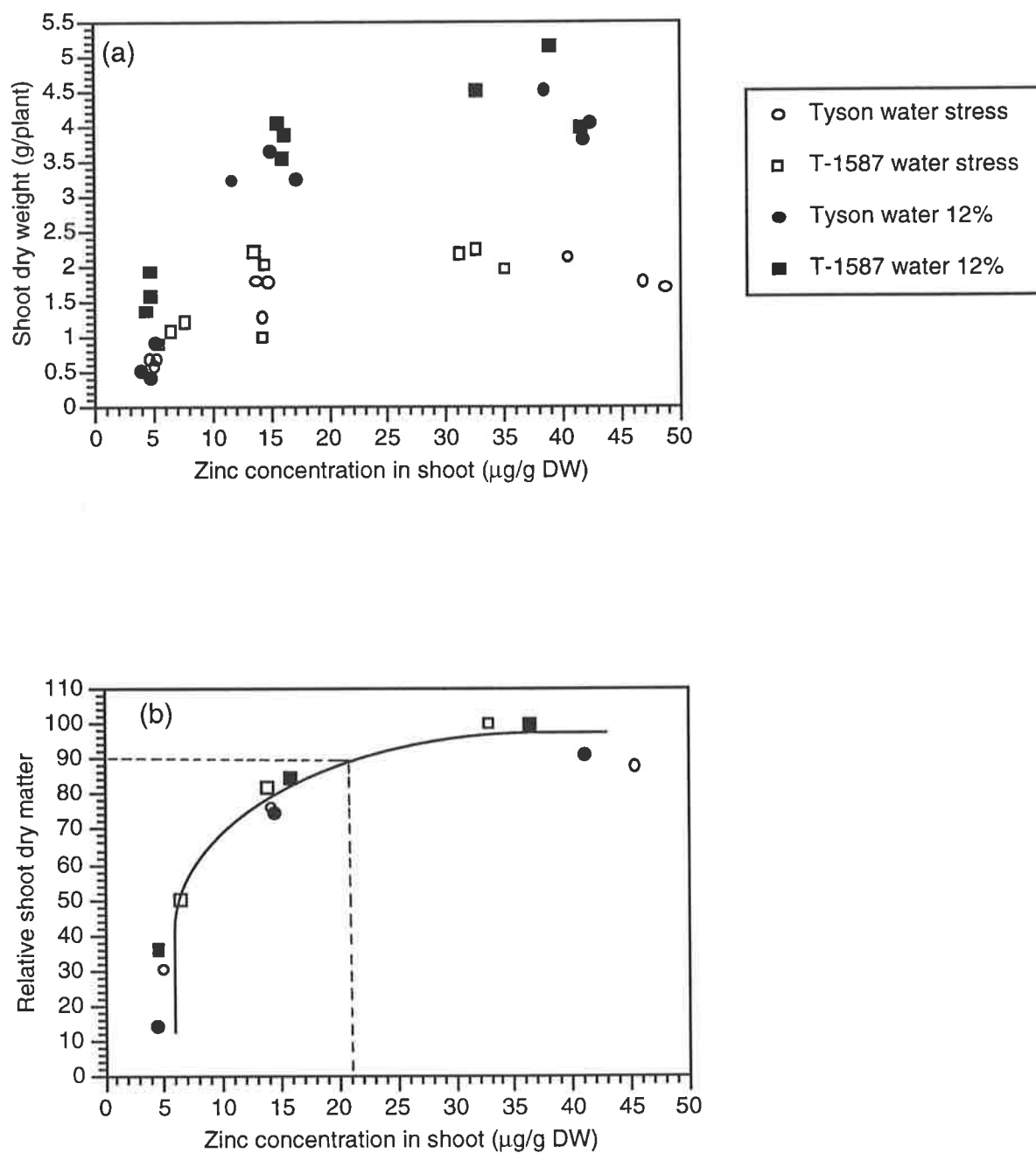


Figure 4.8: Effects of zinc concentration and water stress on shoot dry matter accumulation (a) and the zinc calibration curve relating zinc concentration in whole shoot to the relative dry weight of shoots at two water regimes (b).

4.4. Discussion

Zinc deficiency depressed dry matter production in chickpea and led to a cessation of growth under severely zinc-deficient conditions after about 8 weeks. Similar results are reported from the study of Cakmak *et al.* (1989) where zinc deficiency severely depressed shoot growth in beans (*Phaseolus vulgaris* L.). Zinc plays a key role in photosynthesis, influencing the activity of enzymes such as carbonic anhydrase (Ohki, 1976; 1978; Rengel, 1995a), as well as affecting chlorophyll concentration and stomatal conductance (Hu and Sparks, 1991). The present data regarding plant height and number of leaves showed that growth was depressed at rates up to 0.2 mg/kg of soil zinc, which can be considered as deficient in Laffer sand. Plant growth increased with increasing the zinc fertilization up to 5 mg zinc per kg of soil, which was the highest rate in experiment 1, and the plants were free from any symptoms of zinc toxicity. In contrast, Rengel and Graham (1995a) using Laffer sand observed zinc-induced toxicity in wheat at a relatively low zinc level (3.2 mg zinc per kg soil), which indicates that the zinc requirement of chickpea may be higher than that of wheat.

It is well known that the zinc requirement of different crop species varies greatly and that they show differential response to zinc fertilization (Singh *et al.*, 1983). For example, in a study comparing four crop species, Tiwari and Pathak (1982) reported that chickpea and Egyptian clover were more sensitive to zinc deficiency than wheat and mustard. Similarly, Tiwari and Dwivedi (1990) evaluated the response of eight winter crops including chickpea to application of zinc. Chickpea responded well with a 27% increase in seed yield. The findings from the present experiments also suggest that the zinc requirement of chickpea may be higher than that of cereals and that chickpea may suffer more when planted under zinc-deficient conditions. Therefore, it is likely that the use of chickpea in cereal-based cropping systems where soils are zinc-deficient for cereals will result into substantial yield loss. However, before any final conclusion is reached, it would be necessary to test a greater number of genotypes than examined in the present study.

Zinc deficiency affects the absorption of other nutrients. The results of Experiment 1 showed that Mn and Fe concentrations were significantly higher under zinc-deficient conditions, which may have been due to the similarity in uptake mechanisms with zinc, to greater availability of these nutrients, or to reduced growth. The concentrations of phosphorus, sulphur and boron concentrations were also higher in the zinc-deficient treatments and decreased with an increase in zinc fertilization. Zinc and phosphorus interaction and accumulation of phosphorus under zinc deficient conditions have been reported previously by Loneragan *et al.* (1982); an imbalance in boron uptake is often caused by zinc deficiency (Singh *et al.*, 1990). It is possible that abnormal increase in the concentration of other mineral nutrients under zinc deficiency conditions may affect metabolic activities of chickpea plants.

A critical concentration of 20-21 mg per kg in the whole shoots of chickpea was determined using the graphical method in the present study and the values appeared to be unaffected by genotype or watering treatment. In earlier studies, Ohki (1977) found that the critical zinc concentration in the blade 5 of soybean was 21 mg per kg, and Reuter *et al.* (1982) determined the critical zinc concentration in the youngest open leaf blade of subterranean clover (*Trifolium subterraneum* L.) as 18 mg per kg using the graphical method. Critical zinc concentrations reported by Wade and Bath (1985) and Leggett and Westermann (1986) for navy beans ranged from 17-20 mg per kg. Marsh and Waters (1985) investigated the response to zinc in cowpea and found the critical concentration to be 20 mg per kg for upper leaves. Thus, critical concentration determined in the present study using the graphical method is very close to the values reported for other legumes.

The critical concentration calculated using the Mitscherlich model was 31 mg/kg in the first experiment, higher than graphical method (20 mg/kg), however, it was 20 mg/kg of shoot dry matter in Experiment 2. A similar discrepancy has been previously reported by Ware *et al.* (1982); the values for critical zinc concentration calculated using the Mitscherlich model were significantly higher than those derived from the graphical method. Bell *et al.* (1990) also reported that the Mitscherlich model gave more variable

values of critical concentrations for peanut plant tissue. Reuter *et al.* (1983) compared the graphical method with several mathematical models, and found that the hand-fitted curve best described the relationship between the copper concentration in youngest open leaves (YOL) and shoot dry matter of subterranean clover. Wilhelm *et al.* (1993) also preferred a hand-fitted curve over the Mitscherlich model when determining the critical zinc concentration in wheat, as it was more useful for plant test criteria as well as for biological interpretation of the data. In view of this evidence, a graphical procedure is preferred to using the Mitscherlich model.

4.5. Conclusion

In conclusion, these results indicate that the zinc status of chickpea plant can be assessed using critical zinc concentration of 20-21 mg/kg shoot dry matter. The study showed that critical concentration remained the same in all three cultivars even when grown under different soil moisture conditions. It appears that low and high moisture regimes had no significant effect on critical zinc concentration, which suggests that the value determined here may be useful in a range of environments and also in wet as well as dry seasons for diagnosing the zinc status of chickpea crops.

CHAPTER 5

Sensitivity of chickpea genotypes to zinc deficiency and characteristics related to zinc efficiency in chickpea

5.1. Introduction

Genotypes of crops vary widely in their response to zinc deficiency. Some are relatively tolerant to deficient soils and maintain adequate zinc uptake and utilization when available zinc is low (Graham and Rengel, 1993; Graham *et al.*, 1993; Takkar, 1993). Tolerance to zinc deficiency is considered a genetic trait, termed zinc efficiency, which can be defined as the ability of a genotype to grow and yield well in soils too deficient in zinc for a standard cultivar (Graham *et al.*, 1992). Growing zinc-efficient genotypes on zinc-deficient soils is an environmentally benign approach which could reduce land degradation by maintaining more vigorous ground cover and minimizing the use of chemical fertilizers (Graham and Rengel, 1993).

Even though chickpea is frequently grown on soils prone to zinc deficiency both in Australia and overseas, little work has been done to examine the variation in zinc sensitivity among chickpea genotypes. The aim of the present study which comprised three pot experiments, was to assess the responsiveness to zinc deficiency among a range of chickpea genotypes and to determine their zinc efficiency based on vegetative growth. An attempt was also made to identify some plant characteristics that could be related to zinc efficiency in chickpea.

5.2. Experiment 1. Sensitivity of Australian chickpea genotypes and advanced breeding lines to zinc deficiency

Chickpea breeding programs in Australia have been successful in developing a number of varieties which are considered high yielding and disease resistant, but their

sensitivity to zinc deficiency has not been evaluated so far. In this experiment, 13 chickpea genotypes including eight widely grown cultivars were tested for their tolerance to zinc deficiency and their level of tolerance was quantified by determining zinc efficiency. The seed of genotypes was provided by K. H. M. Siddique, Centre for Legumes in Mediterranean Agriculture (CLIMA), Perth and Wayne Hawthorne, Department of Primary Industries South Australia (PISA), Struan.

5.2.1. Materials and methods

Zinc-deficient siliceous sandy soil having DTPA-extractable zinc concentration of 0.06 mg/kg was prepared, and basal nutrient solutions were added as described in Chapter 3 (Section 3.2). The soil was then divided into two separate lots and a solution of $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ was added to one lot of soil to correspond to 2.5 mg zinc per kg soil, designated as 'zinc+'. This level of zinc was found adequate for plant growth in the previous experiment using Laffer sand (Chapter 4). No zinc was applied to the second lot, and double-deionized (DD) water having 18 MOhms resistivity was used as a control; this was designated as 'zinc-'.

The soil (1.5 kg) was weighed into 125-mm-diameter plastic pots, and DD water was added in amounts sufficient to bring the soil water content to field capacity (12% w/w). Seed of 13 genotypes (10 desi and three kabuli types), including eight widely-grown Australian cultivars, were sown in the experiment (Appendix 1). Similar seed size within each variety was selected, and a sample was analysed for zinc concentration to allow the zinc content for each genotype to be assessed (Table 5.1). Seeds were surface-sterilised and germinated on a filter paper (Whatman No. 42, ashless) at 20 °C in an incubator. After 48 h three uniformly-germinated seeds were sown two cm deep in each pot.

Plants were grown in a glasshouse at the Waite Agricultural Research Institute (Adelaide, South Australia) for 6 weeks from mid December 1995 until the end of January 1996. Temperature within the glasshouse was 25 ± 2 °C during the day and 18

± 2 °C during the night. Plants were grown under natural daylength and water was applied on alternate days to return the soil to field capacity using DD water. One plant from each pot was harvested 4 weeks after planting (12-15 leaf stage). The second and final harvest was made at the start of flowering, 6 weeks after sowing. At the second harvest, soil was washed from roots under running tap water. Plants were then rinsed in deionized water followed by a final dipping into DD water. After gentle blotting, plants were separated into roots and shoots, dried at 80 °C for 48 hours before being weighed for dry matter.

Table 5.1: Seed weight (mg/seed), zinc concentration (mg/kg dry weight) and seed zinc content ($\mu\text{g}/\text{seed}$) of different genotypes used in the present study. Data are presented as mean $\pm$ standard error of three replications.

Cultivar/genotype	Type	Seed weight	Zinc concentration	Seed zinc content
Dooen	Desi	195 $\pm$ 3	22 $\pm$ 1	4.3 $\pm$ 0.3
Tyson	Desi	178 $\pm$ 4	22 $\pm$ 2	3.9 $\pm$ 0.4
Semsen	Desi	240 $\pm$ 1	23 $\pm$ 1	5.5 $\pm$ 0.2
Amethyst	Desi	192 $\pm$ 4	24 $\pm$ 3	4.5 $\pm$ 0.5
Barwon	Desi	201 $\pm$ 3	20 $\pm$ 2	4.0 $\pm$ 0.3
Kaniva	Kabuli	425 $\pm$ 6	19 $\pm$ 1	8.0 $\pm$ 0.3
Garnet	Kabuli	394 $\pm$ 5	23 $\pm$ 3	9.0 $\pm$ 1.0
Narayan	Kabuli	236 $\pm$ 3	20 $\pm$ 3	4.6 $\pm$ 0.8
940-26	Desi	262 $\pm$ 3	21 $\pm$ 2	5.6 $\pm$ 0.5
PI-13768 (Desavie)	Desi	239 $\pm$ 2	25 $\pm$ 2	5.9 $\pm$ 0.4
CTS-11308	Desi	217 $\pm$ 2	26 $\pm$ 1	5.7 $\pm$ 0.2
CTS-60543	Desi	218 $\pm$ 2	30 $\pm$ 3	6.4 $\pm$ 0.7
T-1587	Desi	214 $\pm$ 3	25 $\pm$ 1	5.2 $\pm$ 0.1

Oven-dried plant material was analysed using an Inductively Coupled Plasma Emission Spectrometer as described in Chapter 3 (Section 3.4). Zinc content of shoots and roots was calculated by multiplying shoot and root zinc concentration with shoot and root dry weights. Total zinc accumulation per plant was calculated from the sum of shoot and root zinc contents. Zinc uptake per gram root dry matter was calculated by dividing plant zinc content with root dry weight. Relative zinc transport (%) to the shoot was estimated by dividing the shoot zinc content by the total plant zinc content.

The experiment was set up in a completely randomised factorial design (13 genotypes $\times$ two zinc levels) with three replicates. Results were analysed as described in Chapter 3 (Section 3.5). Data for shoot and root zinc concentration as well as for shoot Zn, Fe and Mn content were log-transformed to overcome the problem of non-homogeneity of variance, before being analysed by analysis of variance; reported LSD values refer to the comparison between log-transformed data.

5.2.2. Results

5.2.2.1. Symptoms development

The development of visual zinc deficiency symptoms, such as reduction in growth with slightly pale foliage appeared 3-4 weeks after planting (Figure 5.1). These symptoms were most apparent in cultivars Tyson, Amethyst and Dooen and least apparent in Kaniva and T-1587. Symptoms progressively worsened during the experiment. A severe reduction in the shoot growth as well as in the size of younger leaves was observed 6 weeks after planting. Chlorosis of younger leaves and stipules was followed by necrosis of leaf margins. The older leaves of zinc-starved plants in sensitive genotypes were abnormally thick, lacking in succulence, were dull in appearance and shed prematurely. These differences in the sensitivity of genotypes to zinc deficiency based on the visual symptoms were not related to their seed zinc contents (Table 5.1).

5.2.2.2. Dry matter production

At the first harvest, shoot dry weight was lower under zinc deficiency compared with the zinc+ treatment in all genotypes. However, shoot growth was most depressed in Dooen, Garnet and Tyson in the zinc- treatment compared to plants supplied with sufficient zinc (Figure 5.2). There were differences in early seedling vigour when adequate zinc was applied, with Dooen and Garnet showing the greatest vigour. Kaniva (kabuli) and Barwon (desi) showed good early growth under zinc deficient conditions during the first four weeks. By the second harvest, the advanced breeding

desi lines T-1587 and CTS-11308 and the kabuli cultivar Kaniva produced significantly more shoot dry matter than Tyson, Dooen, Amethyst and Barwon under zinc deficiency stress (Figure 5.3).

Significant differences among genotypes in root growth under inadequate zinc nutrition were measured at 6 weeks (Table 5.2). In terms of root growth, Tyson produced the lowest root dry weight under low soil zinc. Garnet and PI-13768 showed greater root growth than other genotypes under deficient conditions but there was a marked reduction in shoot growth of these genotypes (Figure 5.3).

5.2.2.3. Zinc concentration and contents

Root zinc concentration ranged from 5.5 mg per kg in breeding lines 940-26 and PI-13768 to 8.6 mg per kg in CTS-60543 when no zinc was applied (Table 5.2). The addition of zinc resulted in a very large increase in root zinc with the highest concentration measured in cultivar Narayen and the lowest in T-1587 and Barwon.

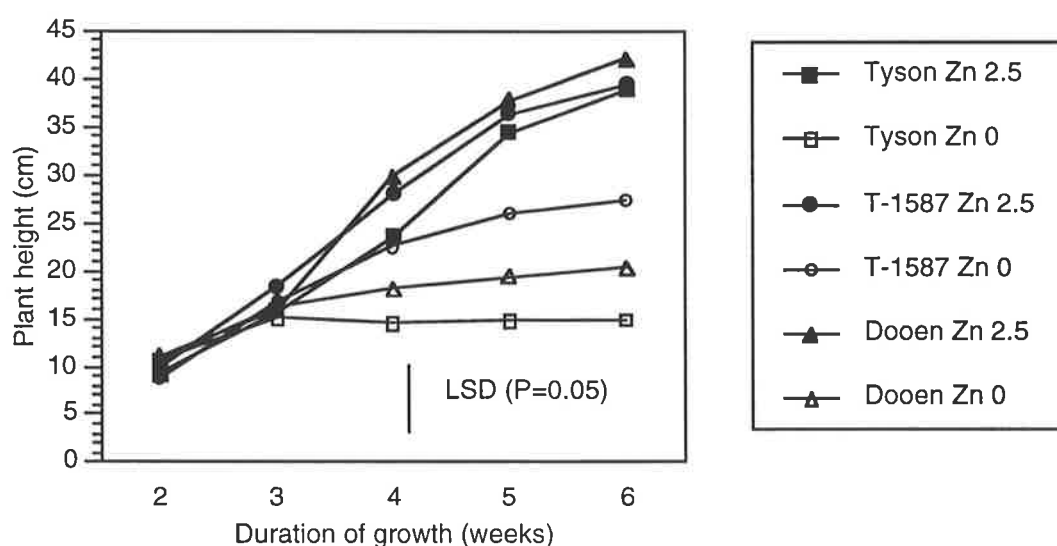


Figure 5.1: Comparison of plant height between zinc-sensitive cultivars (Tyson and Dooen) and zinc-tolerant breeding line T-1587. The vertical bar represents LSD ($P=0.05$) value for the interaction genotypes $\times$ zinc fertilization.

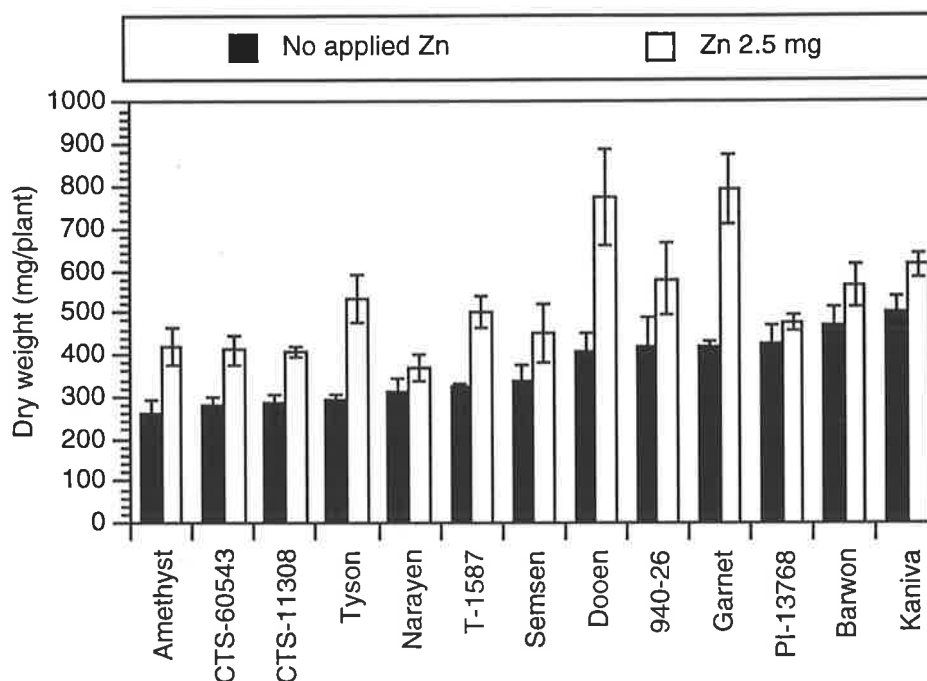


Figure 5.2: Effect of zinc nutrition on shoot dry matter of 13 chickpea genotypes, harvested 4 weeks after planting. The vertical bars represent $\pm$ standard errors based on three replicates.

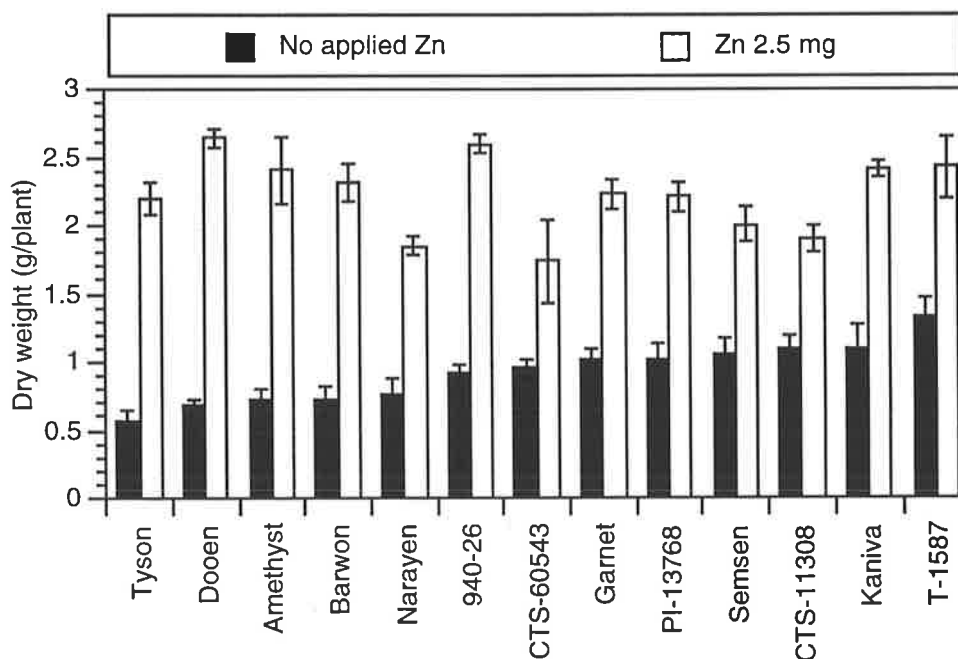


Figure 5.3: Effect of zinc nutrition on shoot dry matter yields of 13 chickpea genotypes. Plants were harvested six-weeks-old, at flowering stage. The vertical bars represent $\pm$ standard errors based on three replicates.

Table 5.2: Root dry matter (g/plant) and root zinc concentration (mg/kg dry weight) as influenced by zinc nutrition. Plants were 6-weeks-old at the flowering stage.

Genotypes	Root dry weight		Root zinc concentration	
	zinc-	zinc+	zinc-	zinc+
Dooen	0.48	1.38	7.1 (1.96) ^a	102.7 (4.63)
Tyson	0.31	0.99	6.6 (1.88)	118.1 (4.77)
Sensen	0.68	0.83	7.8 (2.02)	131.4 (4.88)
Amethyst	0.47	0.70	7.5 (2.00)	127.6 (4.85)
Barwon	0.54	1.29	7.9 (2.04)	88.3 (4.48)
Kaniva	0.74	0.88	7.3 (1.96)	97.2 (4.58)
Garnet	1.13	1.63	6.7 (1.91)	112.7 (4.72)
Narayan	0.52	0.63	7.8 (2.05)	148.3 (5.00)
940-26	0.72	0.78	5.5 (1.68)	143.4 (4.97)
PI-13768	0.95	1.42	5.5 (1.71)	91.4 (4.51)
CTS-11308	0.39	0.58	7.7 (2.03)	143.9 (4.97)
CTS-60543	0.43	0.63	8.6 (2.15)	143.4 (4.96)
T-1587	0.58	1.53	6.7 (1.90)	85.5 (4.45)
LSD (P=0.05)				
Gen. × Zinc	0.36		(0.23) ^b	

^a Numbers in parentheses refer to averages obtained from the analysis of variance of log-transformed data.

^b The LSD value is applicable to the log-transformed data (in parentheses).

At both harvests, zinc concentrations in shoot tissue were much higher in plants supplied with zinc than those without zinc supply. The variation in the concentration was greater at the first harvest in the zinc- treatment, where Kaniva and Garnet contained the highest zinc concentrations (Table 5.3), which may be due to their larger seed size and higher seed zinc content (Table 5.1). By the second harvest, the zinc concentration decreased slightly in most of the genotypes grown under zinc deficient conditions. With sufficient zinc fertilization, T-1587 had the lowest and cultivar Narayan the highest zinc concentration in shoot tissues.

For shoot zinc content, the genotype × zinc interaction was significant. The lines T-1587 and CTS-11308 tended to have a higher zinc content in their shoot under severe zinc deficiency than the cultivar Tyson (Table 5.4). The highest zinc uptake from soil not fertilized with zinc was found in T-1587, followed by Garnet, Kaniva and

Table 5.3: Effects of zinc fertilization on zinc concentration (mg/kg dry weight) in the whole shoot, harvested 4 and 6 weeks after planting.

Genotypes	4 weeks		6 weeks	
	Zinc-	Zinc+	Zinc-	Zinc+
Dooen	7.6 (2.01) ^a	23.2 (3.14)	8.2 (2.09) ^a	41.7 (3.73)
Tyson	9.2 (2.21)	31.6 (3.43)	7.1 (1.96)	41.6 (3.73)
Semsen	6.5 (1.86)	43.3 (3.77)	6.5 (1.86)	36.2 (3.59)
Amethyst	7.6 (2.02)	49.6 (3.90)	7.5 (1.98)	33.8 (3.52)
Barwon	6.0 (1.79)	51.3 (3.94)	8.0 (2.07)	42.8 (3.75)
Kaniva	13.1 (2.56)	38.8 (3.65)	8.2 (2.09)	43.9 (3.77)
Garnet	14.4 (2.66)	29.8 (3.39)	6.7 (1.90)	29.3 (3.38)
Narayan	7.5 (1.98)	53.3 (3.98)	7.2 (1.97)	54.2 (3.99)
940-26	8.1 (2.08)	32.4 (3.48)	7.9 (2.05)	33.0 (3.49)
PI-13768	7.7 (2.01)	33.8 (3.52)	5.4 (1.68)	31.0 (3.43)
CTS-11308	14.3 (2.65)	56.8 (4.04)	8.9 (2.14)	43.4 (3.74)
CTS-60543	11.6 (2.44)	47.6 (3.85)	6.0 (1.70)	39.1 (3.65)
T-1587	11.4 (2.43)	42.6 (3.75)	8.6 (2.12)	27.9 (3.31)
LSD (P=0.05)				
Var. × Zinc	(0.23) ^b		(0.37) ^b	

^a Numbers in parentheses refer to averages obtained from the analysis of variance of log-transformed data.

^b The LSD values are applicable to the log-transformed data (in parentheses).

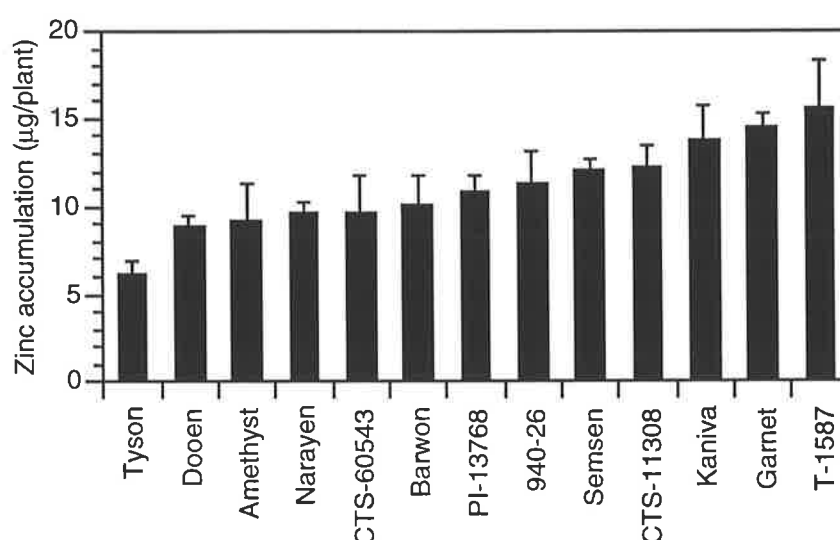


Figure 5.4: Differential response of chickpea genotypes to total zinc accumulation in the zinc- treatment. Total zinc accumulation represents shoot zinc content plus root zinc content. The vertical bars represent standard errors based on three replicates.

Table 5.4: Shoot zinc content ($\mu\text{g/plant}$) as affected by zinc fertilization at 2nd harvest when plants were six week old.

Genotypes	Shoot zinc content	
	zinc-	zinc+
Dooen	5.6 (1.70) ^a	110.1 (4.70)
Tyson	4.0 (1.37)	91.3 (4.51)
Semsen	6.8 (1.91)	72.6 (4.28)
Amethyst	5.6 (1.64)	81.3 (4.39)
Barwon	5.9 (1.73)	98.4 (4.58)
Kaniva	8.4 (2.09)	106.4 (4.65)
Garnet	6.8 (1.91)	65.2 (4.18)
Narayan	5.6 (1.70)	100.1 (4.60)
940-26	7.4 (1.97)	85.5 (4.44)
PI-13768	5.6 (1.69)	68.3 (4.22)
CTS-11308	9.4 (2.22)	81.6 (4.38)
CTS-60543	6.0 (1.66)	67.0 (4.17)
T-1587	11.7 (2.39)	68.7 (4.19)
LSD (P=0.05)		
Gen. $\times$ Zinc	(0.46) ^b	

^a Numbers in parentheses refer to averages obtained from the analysis of variance of log-transformed data.

^b The LSD value is applicable to the log-transformed data (in parentheses).

CTS-11308 (Figure 5.4). On the other hand, Tyson appears to be inefficient in zinc uptake, having zinc content about 3-fold smaller than T-1587 in the zinc- treatment (Table 5.4).

5.2.2.4. Root : shoot ratio and zinc transport

Zinc nutrition altered the root:shoot ratio; in most genotypes the root:shoot ratio was higher in zinc-deficient than in zinc-sufficient plants (Table 5.5). However, in CTS-11308, CTS-60543 and T-1587 the root:shoot ratio under zinc-deficient conditions was lower than many of the other genotypes. The high root:shoot ratio in Garnet indicates that its shoot growth was more affected than root growth. Total zinc accumulation (Figure 5.4) showed that T-1587 was efficient in extracting zinc from severely deficient soil. In contrast, the root

system of Tyson seems to be inefficient, as the zinc uptake in Tyson was about 40% of that recorded in T-1587.

The partitioning of zinc between root and shoot was affected by zinc fertilization. Proportionately more zinc was transported to the shoot from the roots when the soil was severely deficient (Table 5.5), and under adequate zinc supply, roots retained more zinc and less was transported to shoots. The lines T-1587 and CTS-11308 were relatively more efficient in zinc transport as more than 70% of the zinc was transported to shoots in the treatment with severely deficient soil. It was noted that T-1587 at adequate zinc supply transported 34% of the total absorbed zinc from roots to shoots, whereas Kaniva supplied more zinc to the shoot (55%) under similar conditions. Zinc deficiency increased the root:shoot dry matter ratio, but also resulted proportionately more zinc being transported to the shoot (Table 5.5).

Table 5.5: Effects of zinc fertilization on the root:shoot ratio of dry matter and relative zinc transport [(shoot zinc content/total zinc content) $\times$ 100]. Plants were 6-weeks-old at the flowering stage.

Genotypes	Root:shoot ratio		Relative zinc transport (%)	
	zinc-	zinc+	zinc-	zinc+
Dooen	0.71	0.52	62	44
Tyson	0.56	0.45	67	44
Sensen	0.65	0.41	57	41
Amethyst	0.66	0.30	60	47
Barwon	0.76	0.56	58	47
Kaniva	0.74	0.37	61	55
Garnet	1.13	0.74	47	27
Narayan	0.70	0.34	57	52
940-26	0.77	0.30	65	43
PI-13768	0.96	0.65	51	35
CTS-11308	0.36	0.31	76	49
CTS-60543	0.44	0.37	59	42
T-1587	0.45	0.63	73	34
LSD (P=0.05)				
Gen. $\times$ Zinc	0.19		12	

5.2.2.5. Zinc efficiency

The ratio of shoot growth at low and high zinc levels, which is described as zinc efficiency, ranged from 25% to 60%, with most Australian cultivars showing poor efficiency (Figure 5.5). Zinc efficiency of Tyson and Dooen was half of that recorded in CTS-60543, CTS-11308 and T-1587. According to this efficiency ranking, the desi cultivars Dooen, Tyson, Amethyst and Barwon can be categorised as inefficient, while Semsen seems to be moderately efficient. All the three kabuli cultivars (Garnet, Kaniva and Narayen) included in the present study were intermediate in their zinc efficiency.

5.2.2.6. Shoot Fe content

The Fe accumulation in chickpea genotypes was examined to find out any possible relationship between zinc efficiency and Fe uptake because chickpea genotypes differ in sensitivity to Fe-chlorosis (Saxena *et al.*, 1990a). Zinc fertilization affected accumulation of Fe in the shoot tissues; zinc-sensitive varieties Tyson, Dooen and Barwon had lower Fe content compared with the tolerant line T-1587 when no

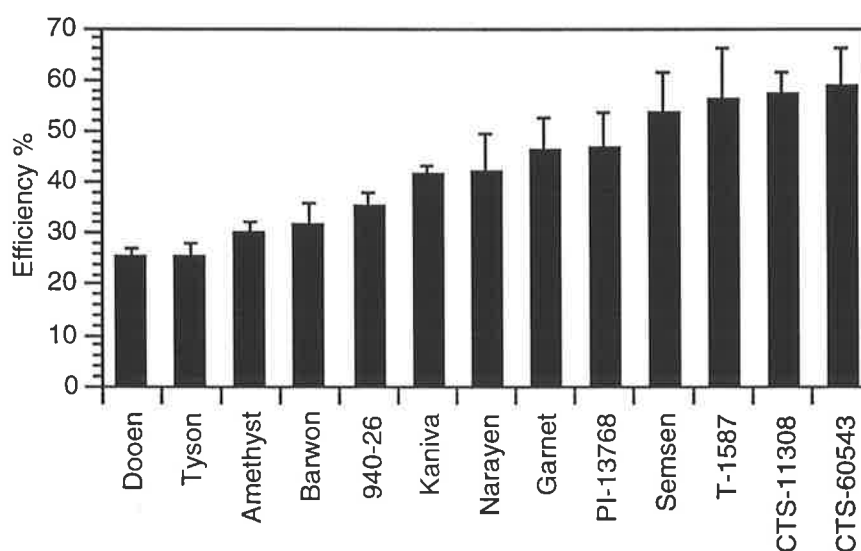


Figure 5.5: Zinc efficiency $[(\text{zinc-}/\text{zinc+}) \times 100]$ based on shoot growth of different chickpea genotypes grown in zinc-deficient Laffer sand in pots. The vertical bars represent standard errors based on three replicates.

additional zinc was applied (Table 5.6). High accumulation of Fe was also observed in Garnet, Semsen and 940-26 under zinc deficiency. Genotypic response at adequate zinc supply showed that Dooen, Kaniva, 940-26 and Amethyst tended to have highest Fe content compared to T-1587 and CTS-11308.

Table 5.6: Effects of zinc fertilization on Fe ($\mu\text{g/plant}$) in shoot. Plants were 6-week-old at the flowering stage.

Genotypes	Shoot Fe content	
	zinc-	zinc+
Dooen	33 (3.50) ^a	139 (4.93)
Tyson	18 (2.89)	103 (4.64)
Semsen	57 (4.04)	98 (4.58)
Amethyst	36 (3.58)	121 (4.79)
Barwon	34 (3.51)	103 (4.63)
Kaniva	45 (3.80)	124 (4.82)
Garnet	70 (4.24)	112 (4.71)
Narayan	43 (3.74)	98 (4.58)
940-26	57 (4.04)	123 (4.80)
PI-13768	45 (3.81)	90 (4.50)
CTS-11308	49 (3.87)	92 (4.51)
CTS-60543	44 (3.77)	85 (4.41)
T-1587	59 (4.06)	90 (4.48)
LSD (P=0.05)		
Gen. $\times$ Zinc	(0.28) ^b	

^a Numbers in parentheses refer to averages obtained from the analysis of variance of log-transformed data.

^b The LSD value is applicable to the log-transformed data (in parentheses).

5.3. Experiment 2. Responsiveness of Australian chickpea genotypes and advanced breeding lines to zinc deficiency

In the previous experiment most of the Australian chickpea cultivars studied were found to be sensitive to zinc deficiency, although some of the advanced breeding lines were relatively tolerant. To find out the response of more Australian chickpea cultivars and to verify the performance of breeding lines tested before, a second experiment was set up. The most sensitive chickpea cultivar, Tyson was used as a check and the two relatively tolerant breeding lines, T-1587 and CTS-11308, were also

included in the study. Seed of chickpea cultivars and advanced breeding lines were provided by Wayne Hawthorne, PISA, Struan (SA) and E. J. Knights, New South Wales Department of Agriculture, Tamworth (NSW).

5.3.1. Materials and methods

Zinc-deficient siliceous sandy soil from the same lot as used in the previous experiment was prepared, and basal nutrient solutions were added as described in Chapter 3 (Section 3.2). The zinc treatments were imposed in exactly the same manner as in Experiment 1 (Section 5.2).

Seed of eight desi genotypes of diverse genetic background, including three widely-grown Australian cultivars, were sown in experiment (Appendix 1). Seeds of similar size within each variety were hand-sorted, and a sample was analysed for zinc concentration to allow the zinc content for each genotype to be assessed (Table 5.7). Seeds were surface-sterilised and germinated on a filter paper (Whatman No. 42, ashless) at 20 °C in an incubator. After 48 hours three uniformly germinated seeds were sown at a uniform depth (2 cm) in each pot.

Plants were grown in a glasshouse at the Waite Agricultural Research Institute (Adelaide, South Australia) for 6 weeks from mid June to early August 1996. Temperature within the glasshouse was 23 ± 2 °C during the day and 15 ± 2 °C during the night. Plants were grown under natural daylength. DD water was applied to the soil on alternate days to bring it to the field capacity. The plants were harvested just before the start of flowering, 6 weeks after sowing. Plant material was washed and dried as described earlier (Section 5.2.1) and weighed for dry matter.

Plant material was prepared and analysed using an Inductively Coupled Plasma Emission Spectrometer as described in Chapter 3 (Section 3.4). Zinc content of shoots and roots was calculated by multiplying shoot and root zinc concentration with shoot and root dry weights. Total zinc accumulation per plant was calculated from the sum of shoot and root zinc contents.

Table 5.7. Seed weight (mg/seed), zinc concentration (mg/kg dry weight) and seed zinc content ($\mu\text{g}/\text{seed}$) of genotypes used in experiment.

Cultivar/genotypes	Type	Seed weight	Zinc concentration	Seed zinc content
Tyson	Desi	152 $\pm$ 2.3	32 $\pm$ 1.5	4.9 $\pm$ 0.3
Desavic	Desi	222 $\pm$ 2.2	26 $\pm$ 1.0	5.7 $\pm$ 0.3
Norwin	Desi	172 $\pm$ 2.9	32 $\pm$ 1.3	5.5 $\pm$ 0.3
T-1587	Desi	182 $\pm$ 0.4	25 $\pm$ 1.7	4.5 $\pm$ 0.3
ICC-4958	Desi	300 $\pm$ 0.3	18 $\pm$ 1.2	5.3 $\pm$ 0.4
ICC-14307	Desi	297 $\pm$ 0.7	19 $\pm$ 0.8	5.6 $\pm$ 0.2
CPI-56288	Desi	172 $\pm$ 1.5	30 $\pm$ 1.8	5.1 $\pm$ 0.3
CTS-11308	Desi	180 $\pm$ 1.2	21 $\pm$ 1.3	3.8 $\pm$ 0.3

The experiment was set up as a completely randomised factorial design (eight genotypes $\times$ two zinc levels) with three replicates. Results were analysed statistically as described in Chapter 3 (Section 3.5). Data for shoot and root zinc concentration as well as for zinc content were log-transformed to overcome the problem of non-homogeneity of variance, before being analysed by analysis of variance; reported LSD values refer to the comparison between log-transformed data.

5.3.2. Results

5.3.2.1. Visible deficiency symptoms

The appearance of zinc deficiency symptoms started in plants grown without additional zinc about 4 weeks after planting. The leaves turned chlorotic, and a marked reduction in growth, together with twisting of growing tips, was apparent. These symptoms were first observed in Tyson and CPI-56288 and were more severe when compared with other genotypes such as ICC-4958, T-1587 and CTS-11308. Chlorosis of young growing leaves was followed by necrosis. The older leaves of zinc-deficient plants were looking abnormal as they were thick, with brittle lamina and dull in appearance. Similar symptoms were observed in the previous experiment and were not well related to the seed zinc content of the genotypes (Table 5.7).

5.3.2.2. Dry matter production

Plant growth in the experiment was slower compared with the previous experiment, which was probably due to the lower temperatures in the glasshouse. Consequently, shoot dry matter in 4-week-old plants showed little response to zinc treatment, although visual signs of zinc stress were apparent. Genotypic differences were also obvious (Figure 5.6). Seedling growth was faster in ICC-4958 and ICC-14307 compared to Tyson and Norwin, probably due to the larger seed size of the former two genotypes. A reduction in shoot dry matter due to zinc deficiency was noted only in Tyson 4 weeks after sowing.

Differences in shoot dry matter due to zinc supply were much greater at 6 weeks after planting (Figure 5.7). In the zinc deficient treatment, shoot dry matter produced by CPI-56288, Tyson and Desavic was significantly less than recorded in the breeding lines ICC-4958, T-1587, ICC-14307 and CTS-11308. The results of shoot dry matter indicate that Tyson and CPI-56288 are more sensitive to zinc deficiency than rest of the genotypes tested.

Zinc deficiency also reduced root growth in all genotypes. The lowest root dry matter was recorded in Tyson, followed by CPI-56288 in the zinc- treatment (Figure 5.8). Breeding line ICC-4958 exhibited the highest root growth both under high and low levels of zinc. The relative reduction in root growth due to severe zinc deficiency was high in CPI-56288 (36%) and in Tyson (27%). The breeding lines T-1587 and ICC-4958 maintained root growth in soil without zinc supply as their root dry matter was 94% and 92%, respectively of that produced at adequate zinc supply.

5.3.2.3. Zinc concentration and zinc content

Zinc concentration in shoot tissue significantly increased with increasing zinc application. Zinc-deficient plants of ICC-14307 had a significantly lower zinc concentration than Norwin, which could be due to dilution effect resulted from increased growth in ICC-14307. At adequate zinc supply, Tyson and CPI-56288

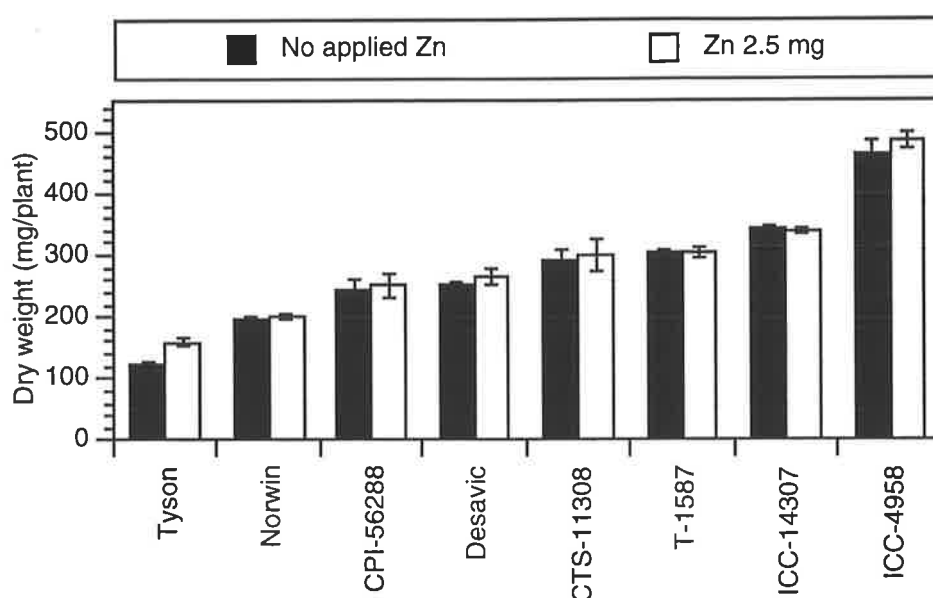


Figure 5.6: Effect of zinc fertilization on shoot dry matter yield of chickpea genotypes. Plants were 4-week-old when harvested. The vertical bars represent standard errors based on three replicates.

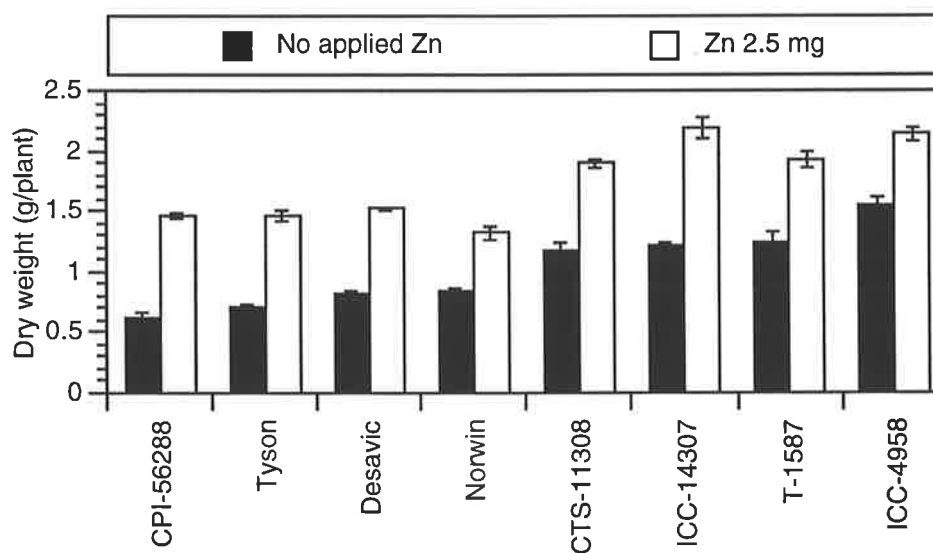


Figure 5.7: Effect of zinc nutrition on shoot dry matter of chickpea genotypes, harvested 6 weeks after planting at the start of flowering. The vertical bars represent standard errors based on three replicates.

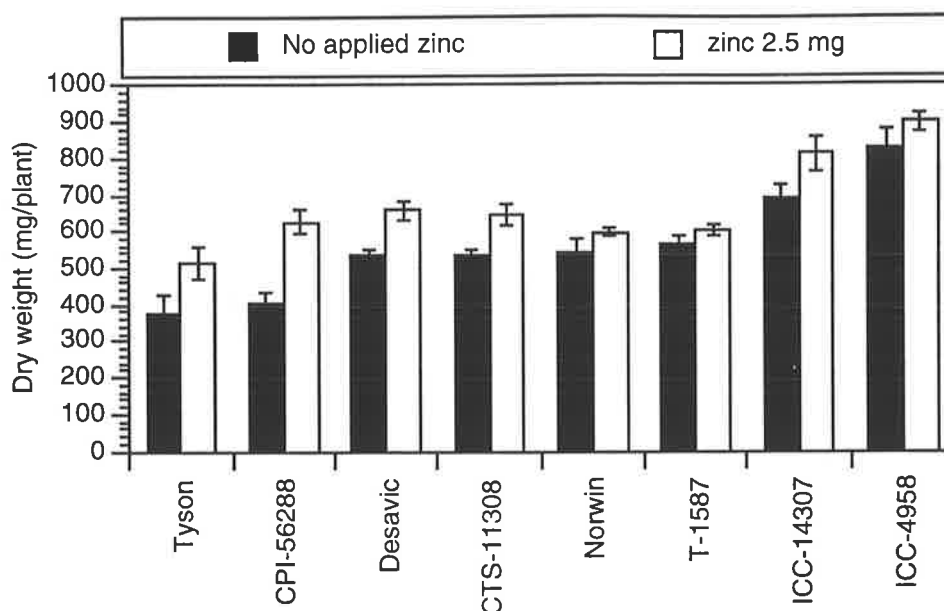


Figure 5.8: Effect of zinc fertilization on root growth of chickpea genotypes. Plants were 6-week-old when harvested. The vertical bars represent standard errors based on three replicates.

tended to have the highest zinc concentrations and Desavic, ICC-4958 and ICC-14307 the lowest (Table 5.8). Shoot zinc content varied among genotypes and was also affected by zinc nutrition. Under severe zinc deficiency, genotypes ICC-4958 and T-1587 had higher zinc contents than CPI-56288 and Tyson (Table 5.8).

Zinc fertilization significantly increased zinc concentration in root tissues of all genotypes. Norwin had the highest root zinc concentration when zinc was not applied, while at adequate zinc supply, Desavic had a lower zinc concentration than Tyson and CPI-56288 (Table 5.9). Total zinc accumulation per plant was highest in ICC-4958, followed by Norwin and T-1587 grown under zinc deficient conditions, while Tyson and CPI-56288 accumulated less than half the zinc measured in ICC-4958 (Figure 5.9).

5.3.2.4. Root:shoot ratio

The root:shoot ratio generally increased under zinc deficiency, which indicates that shoot growth was more affected than root growth when plants grew under zinc-deficient conditions (Table 5.10). However, genotypic differences were significant, and an increase in root:shoot ratio was relatively higher in genotypes CPI-56288,

Table 5.8: Effects of zinc fertilization on zinc concentration (mg/kg dry weight) in the whole shoot, and shoot zinc content ($\mu\text{g/plant}$) harvested 6 weeks after planting.

Genotypes	Shoot zinc concentration		Shoot zinc content	
	Zinc-	Zinc+	Zinc-	Zinc+
Tyson	4.4 (0.64) ^a	40.0 (1.60)	3.1 (0.49) ^a	58.8 (1.77)
Desavic	4.8 (0.68)	28.0 (1.45)	3.9 (0.59)	42.5 (1.63)
Norwin	5.9 (0.77)	34.7 (1.54)	5.0 (0.69)	45.4 (1.66)
T-1587	4.4 (0.64)	38.4 (1.58)	5.4 (0.74)	74.3 (1.87)
ICC-4958	4.2 (0.62)	32.5 (1.51)	6.6 (0.81)	69.9 (1.84)
ICC-14307	3.7 (0.57)	32.5 (1.51)	4.6 (0.66)	71.9 (1.85)
CPI-56288	4.9 (0.69)	40.7 (1.61)	3.0 (0.48)	59.9 (1.78)
CTS-11308	4.0 (0.60)	39.7 (1.60)	4.7 (0.67)	75.4 (1.88)
LSD (P=0.05)				
Gen. $\times$ zinc	(0.06) ^b		(0.07) ^b	

^a Numbers in parentheses refer to averages obtained from the analysis of variance of log-transformed data.

^b The LSD values are applicable to the log-transformed data (in parentheses).

Table 5.9: Root zinc concentration (mg/kg dry weight) as influenced by zinc nutrition. Plants were 6-week-old at the start of flowering.

Genotypes	Root zinc concentration	
	Zinc-	Zinc+
Tyson	8.4 (0.92) ^a	162.3 (2.21)
Desavic	7.3 (0.86)	110.1 (2.04)
Norwin	11.3 (1.05)	129.2 (2.11)
T-1587	7.0 (0.84)	152.5 (2.18)
ICC-4958	7.0 (0.84)	126.7 (2.10)
ICC-14307	5.2 (0.72)	148.8 (2.17)
CPI-56288	5.9 (0.77)	162.7 (2.21)
CTS-11308	5.9 (0.77)	139.7 (2.14)
LSD (P=0.05)		
Gen. $\times$ Zinc	(0.07) ^b	

^a Numbers in parentheses refer to averages obtained from the analysis of variance of log-transformed data.

^b The LSD values are applicable to the log-transformed data (in parenthesis).

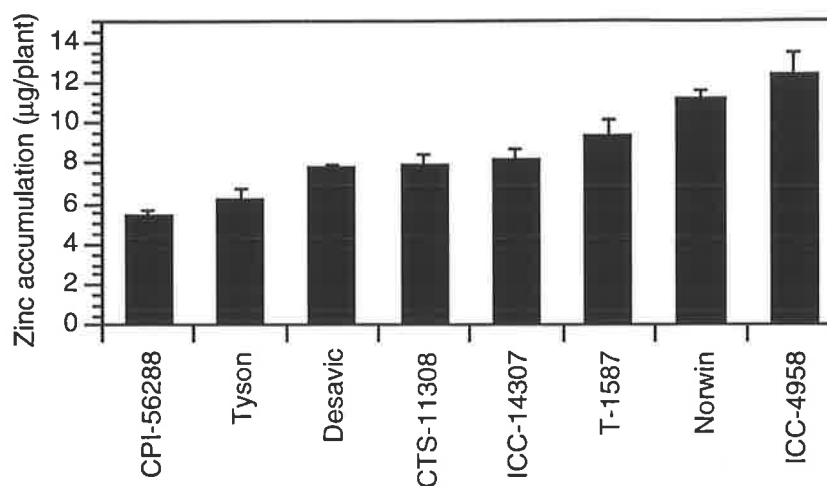


Figure 5.9: Differential response of chickpea genotypes to total zinc accumulation (shoot zinc content + root zinc content) in plants grown in the zinc-deficient treatment. The vertical bars represents standard errors based on three replicates.

Desavic and Tyson than the breeding lines ICC-4958, CTS-11308 and T-1587. Based on these findings, it appears that CPI-56288, Desavic and Tyson were unable to maintain a balance between root and shoot growth when experiencing severe zinc deficiency.

5.3.2.5. Shoot Fe content

The application of zinc generally increased accumulation of Fe in the shoot tissues, but genotypes exhibited differences in Fe content in both zinc treatments (Table 5.11). Tyson and CPI-56288 accumulated the least amount of Fe under zinc-deficient conditions compared to ICC-4958, T-1587 and ICC-14307. At adequate zinc availability, Tyson, Desavic and Norwin had lower Fe content than that in ICC-4958 and ICC-14307. The results suggested that Tyson was relatively inefficient in Fe uptake compared to other chickpea genotypes.

Table 5.10: Effects of zinc fertilization on the root:shoot ratio. Plants were harvested 6 weeks after sowing at the start of flowering.

Genotypes	zinc-	zinc+	Mean (variety)
Tyson	0.54	0.35	0.44
Desavic	0.65	0.43	0.54
Norwin	0.65	0.46	0.55
T-1587	0.46	0.31	0.38
ICC-4958	0.53	0.42	0.47
ICC-14307	0.56	0.37	0.46
CPI-56288	0.65	0.43	0.54
CTS-11308	0.46	0.34	0.40
Mean (zinc)	0.56	0.39	
LSD (P=0.05)			
Genotype		0.05	
Zinc		0.01	
Gen. × Zinc		ns	

ns: non significant

Table 5.11: Effects of zinc fertilization on Fe content ($\mu\text{g}/\text{plant}$) in shoot. Plants were 6-week-old at the flowering stage.

Genotypes	Shoot Fe content	
	zinc-	zinc+
Tyson	33 (1.51) ^a	68 (1.83)
Desavic	40 (1.60)	73 (1.86)
Norwin	53 (1.73)	71 (1.85)
T-1587	71 (1.85)	88 (1.94)
ICC-4958	91 (1.95)	120 (2.08)
ICC-14307	61 (1.79)	110 (2.04)
CPI-56288	35 (1.55)	80 (1.90)
CTS-11308	57 (1.76)	94 (1.97)
LSD (P=0.05)		
Gen. × Zinc		(0.08) ^b

^a Numbers in parentheses refer to averages obtained from the analysis of variance of log-transformed data.

^b The LSD value is applicable to the log-transformed data (in parentheses).

5.3.2.6. Zinc efficiency

Zinc efficiency differed considerably between genotypes (Figure 5.10). The breeding line ICC-4958 had the highest zinc efficiency (74%), followed by T-1587 (65%), Norwin (64%) and CTS-11308 (62%). The genotype CPI-56288 and cultivar Tyson both showed poor performance in terms of zinc efficiency as the efficiency values were 43% and 48% for CPI-56288 and Tyson, respectively.

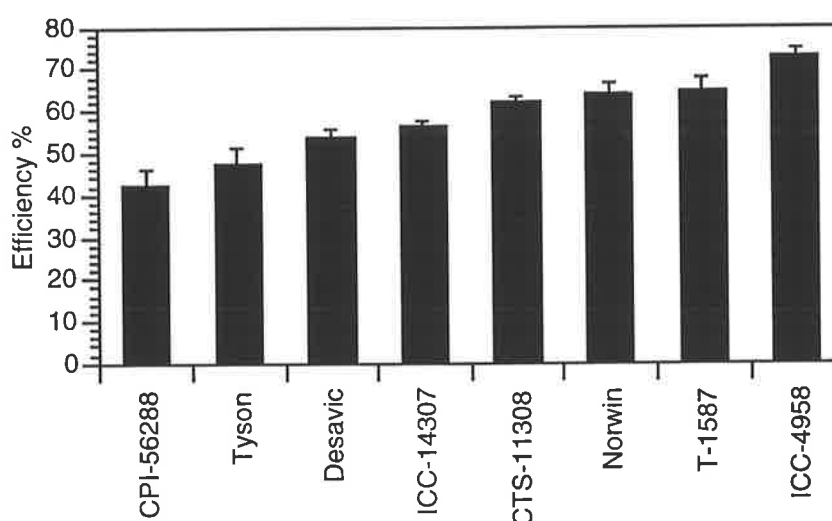


Figure 5.10: Zinc efficiency $[(\text{zinc}/\text{zinc}+) \times 100]$ based on shoot growth of different chickpea genotypes grown in zinc-deficient Laffer sand in pots. The vertical bars represent standard errors based on three replicates.

5.4. Experiment 3. Sensitivity of Pakistani chickpea genotypes and advanced breeding lines to zinc deficiency

Chickpea is the most widely grown grain legume in Pakistan (Ali *et al.*, 1988; Jamil *et al.*, 1993). Breeding work in chickpea started in early 1930s when a chickpea breeding station was set up at Campbellpore (Attock) in the northern Pakistan. Since then various breeding programs have been established in the country which have developed a number of high-yielding cultivars (Tufail and Yousaf, 1992). There are large areas of zinc-deficient soil in Pakistan, yet the response of locally-based cultivars and breeding lines to zinc deficiency has not been studied. Evaluation of breeding lines in Australia shows that chickpeas bred in the Indo-Pakistan subcontinent are well

adapted to the Mediterranean-type environment of Australia (Siddique and Khan, 1993) and since a number of the current Australian genotypes appear to be sensitive to zinc deficiency, exotic material may be a useful source of zinc efficiency. Therefore, an experiment was conducted to study the response of Pakistani chickpea genotypes to zinc deficiency and to determine their zinc efficiency based on vegetative growth.

5.4.1. Materials and methods

Chickpea lines were grown at two levels of zinc, 0 and 2.5 mg zinc per kg soil. The preparation of the soil and application of nutrients were exactly the same as described in Chapter 3 (Section 3.2).

The soil (1.0 kg) was weighed into polythene-lined PVC pots measuring 70 mm diameter $\times$ 250 mm deep. DD water was added in amounts sufficient to bring the soil water content to field capacity (12% w/w). Seed of nine Pakistani genotypes (eight desi and one kabuli), including seven widely-grown cultivars were sown in the experiment and cultivar Tyson was used as check in the experiment (Appendix 1). Seeds of similar size within each variety were selected and a sample was analysed for zinc concentration to allow the zinc content for each genotype to be assessed (Table 5.12). Seeds were surface-sterilised and sown as described earlier (Section 5.2.1).

The experiment was set up as a completely randomised factorial design with three replicates. Plants were grown in a growth chamber at the Waite Agricultural Research Institute (Adelaide, South Australia) for 10 weeks. Photoperiod was 10 hours light and 14 hours dark, and temperature was 20 °C during day and 10 °C during night. Photosynthetically active radiation (PAR) was 440 $\mu\text{mol s}^{-1} \text{ m}^{-2}$ at canopy level supplied by halide lamps. DD water was applied to return the soil to field capacity on alternate days. The plants were harvested just before the start of flowering, 10 weeks after sowing. Harvested plants were washed and dried in the same manner as described earlier (Section 5.2.1).

Table 5.12: Seed weight (mg/seed), zinc concentration (mg/kg dry weight) and seed zinc content ($\mu\text{g}/\text{seed}$) of different genotypes used in the experiment.

Cultivar/genotype	Type	Seed weight	zinc concentration	Seed zinc content
C-44	Desi	233 $\pm$ 5	73 $\pm$ 1	16.9 $\pm$ 0.55
CM-72	Desi	164 $\pm$ 3	59 $\pm$ 1	9.7 $\pm$ 0.02
Piadar-91	Desi	128 $\pm$ 2	82 $\pm$ 1	10.45 $\pm$ 0.02
Punjab-91	Desi	211 $\pm$ 3	69 $\pm$ 2	14.65 $\pm$ 0.29
NIFA-88	Desi	170 $\pm$ 1	57 $\pm$ 3	9.59 $\pm$ 0.50
CM-88	Desi	148 $\pm$ 4	78 $\pm$ 2	11.53 $\pm$ 0.01
Noor-91	Kabuli	256 $\pm$ 3	67 $\pm$ 1	17.12 $\pm$ 0.13
Cross-1 ^a	Desi	165 $\pm$ 5	69 $\pm$ 3	11.42 $\pm$ 0.74
Cross-2 ^b	Desi	197 $\pm$ 3	66 $\pm$ 1	12.94 $\pm$ 0.12
Tyson	Desi	152 $\pm$ 2	36 $\pm$ 2	5.52 $\pm$ 0.23

^a ICC-11514 $\times$ ILC-482^b NEC-138-2 $\times$ CM-72

Root and shoot tissues were analysed for nutrients using an Inductively Coupled Plasma Emission Spectrometer as described in Chapter 3 (Section 3.4). The zinc contents of shoots and roots were calculated by multiplying shoot and root zinc concentration with shoot and root dry weights. Total zinc accumulation per plant was calculated from the sum of shoot and root zinc contents.

Results were statistically analysed as reported earlier in Chapter 3 (Section 3.5). Data for shoot and root zinc concentration as well as for zinc content were log-transformed, before being analysed by analysis of variance; reported LSD values refer to the comparison between log-transformed data.

5.4.2. Results

5.4.2.1. Deficiency symptoms

The development of zinc deficiency symptoms was similar to that described earlier (Sections 5.2.2.1 and 5.3.2.1): reduction in growth, chlorotic foliage, twisting of growing meristem was followed by senescence. These symptoms first appeared in the

cultivars Tyson, CM-72 and CM-88 about 4-5 weeks after sowing and were more pronounced than in the other genotypes.

5.4.2.2. Dry matter production

After 10 weeks of growth, shoot dry matter was lower in the zinc-deficient treatment compared with the adequate zinc level (Figure 5.11). Zinc deficiency decreased shoot dry matter in all genotypes, although Cross-2 (NEC-138-2 $\times$ CM-72), Punjab-91 and Noor-91 produced relatively more dry matter than other genotypes. Reduction in shoot growth caused by zinc deficiency was greatest in cultivars Tyson, CM-88 and Piadar-91, while it was less in Cross-2 (NEC-138-2 $\times$ CM-72), Punjab-91 and Noor-91. Under zinc deficiency, shoot dry matter production of Tyson was only 23% that of Punjab-91, while dry matter yields in cultivars CM-72 and CM-88 were 53% and 56% less when compared with Punjab-91.

Root dry matter produced in the treatment without zinc fertilization was significantly lower than ⁱⁿ those plants grown at adequate zinc supply (Table 5.13). Among genotypes, Tyson had the lowest root dry matter (0.56 g/plant). Genotype $\times$ zinc interaction was significant; root dry matter production was less depressed in NIFA-88, Noor-91, Cross-1 (ICC-11514 $\times$ ILC-482) and C-44 due to zinc deficiency because the root dry matter of these genotypes was more than 80% of that produced at adequate zinc supply.

5.4.2.3. Zinc concentration and zinc content

The zinc concentration of the whole shoot was much higher in plants fertilized with zinc than those without additional zinc supply. Cultivars CM-72 and NIFA-88 had higher zinc concentrations compared with Tyson, C-44 and CM-88 (Table 5.14). The zinc content in shoot as well as in roots differed significantly among genotypes under zinc deficiency. Shoot zinc content was higher in Cross-2 (NEC-138-2 $\times$ CM-72), Noor-91, Punjab-91 and NIFA-88 than the cultivars Tyson, CM-88 and Piadar-91 when plants were grown in the zinc- treatment (Table 5.14).

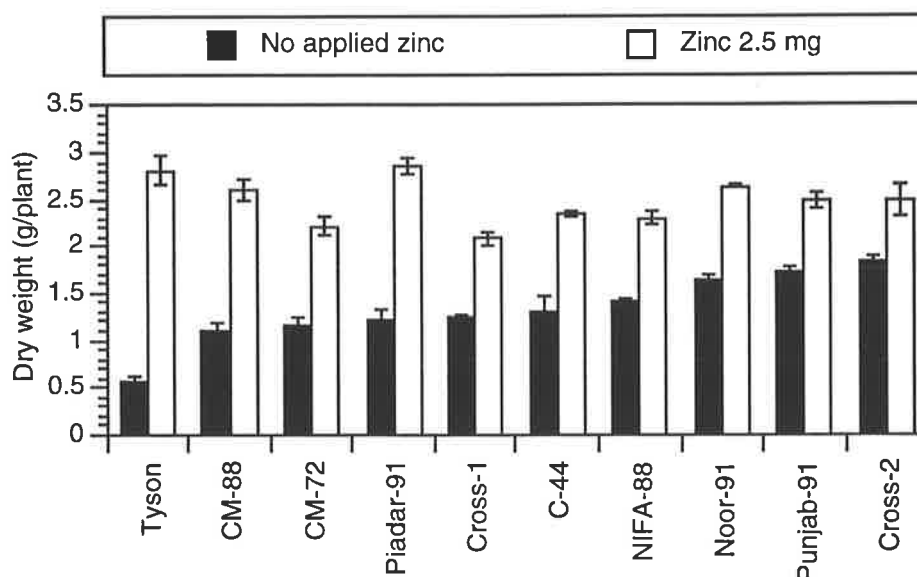


Figure 5.11: Effect of zinc fertilization on shoot dry matter yield of chickpea genotypes. Plants were 10-weeks-old when harvested. The vertical bars represent standard errors based on three replicates.

Table 5.13: Root dry matter (g/plant) and root zinc content ($\mu\text{g/plant}$) as influenced by zinc nutrition. Plants were 10-week-old at the start of flowering.

Genotypes	Root dry weight		Root zinc content	
	Zinc-	Zinc+	Zinc-	Zinc+
C-44	2.04	2.53	8.2 (0.91)	148.8 (2.17)
CM-72	1.36	2.08	6.8 (0.82)	134.9 (2.13)
Piadar-91	1.45	2.43	7.7 (0.87)	142.6 (2.15)
Punjab-91	1.66	2.42	8.0 (0.90)	162.7 (2.21)
NIFA-88	1.75	2.05	7.9 (0.89)	134.7 (2.13)
CM-88	1.34	1.97	6.0 (0.77)	122.7 (2.09)
Noor-91	1.85	2.25	10.2 (0.97)	126.0 (2.10)
Cross-1	1.37	1.66	6.0 (0.77)	113.6 (2.06)
Cross-2	1.57	2.01	7.2 (0.86)	128.1 (2.10)
Tyson	0.56	2.90	2.2 (0.34)	164.9 (2.22)
LSD 0.05				
Gen. $\times$ zinc	(0.33)		(0.05)	

^a Numbers in parentheses refer to averages obtained from the analysis of variance of log-transformed data.

^b The LSD values are applicable to the log-transformed data (in parentheses).

Under zinc deficiency, genotypes showed differences in root zinc content. The highest contents were recorded in Noor-91, C-44 and Punjab-91 (Table 5.13). Total zinc accumulation in plants showed the same trend as for shoot zinc content (Figure 5.12). The cultivars Punjab-91, Noor-91, NIFA-88 and the advanced breeding line Cross-2 (NEC-138-2 $\times$ CM-72) accumulated zinc approximately three times as much in root and shoot as the cultivar Tyson. It was noted that among Pakistani genotypes, zinc accumulation in CM-88 and Piadar-91 was also low, and these genotypes appeared to be more susceptible to zinc deficiency.

5.4.2.4. Root: shoot ratio

As noted in the two previous experiments the root:shoot ratio generally increased under zinc deficiency. However, the increase in root:shoot ratio under zinc deficiency showed considerable differences among genotypes (Table 5.15). Punjab-91 and Cross-2 (NEC-138-2 $\times$ CM-72) maintained their root:shoot ratio, even under low zinc availability, while the ratio was significantly increased in other genotypes. For example, in C-44 and CM-88 increases in root:shoot ratio were 32% and 39%, respectively. Both cultivars produced low dry matter in the zinc- treatment and seem sensitive to zinc deficiency. The relatively low root:shoot ratio of Tyson in the zinc-treatment was probably a result of acute zinc deficiency that severely depressed shoot as well as root growth.

5.4.2.5. Zinc efficiency

The highest zinc efficiency (74%) was measured in Cross-2 (NEC-138-2 $\times$ CM-72) while cultivars Punjab-91, Noor-91 and NIFA-88 were also relatively less sensitive to zinc deficiency, having values above 60% (Figure 5.13). Tyson was once again found to be the least efficient cultivar having about 20% zinc efficiency. The cultivars CM-88 and Piadar-91 were less efficient (42% zinc efficiency) as compared with the rest of the Pakistani chickpea genotypes examined in the study.

Table 5.14: Effects of zinc fertilization on zinc concentration (mg/kg dry weight) in the whole shoot, and shoot zinc content ($\mu\text{g/plant}$) harvested 10 weeks after planting.

Genotypes	Shoot zinc concentration		Shoot zinc content	
	Zinc -	Zinc +	Zinc -	Zinc +
C-44	4.2 (0.62)	25.9 (1.41)	5.4 (0.72)	60.7 (1.78)
CM-72	5.4 (0.72)	26.6 (1.42)	6.3 (0.78)	59.1 (1.77)
Piadar-91	4.4 (0.64)	22.5 (1.35)	5.3 (0.72)	64.1 (1.80)
Punjab-91	4.2 (0.62)	26.7 (1.43)	7.1 (0.85)	66.0 (1.82)
NIFA-88	5.1 (0.70)	26.7 (1.43)	7.1 (0.85)	61.3 (1.79)
CM-88	4.3 (0.63)	25.3 (1.40)	4.6 (0.66)	66.0 (1.82)
Noor-91	4.4 (0.64)	23.9 (1.38)	7.2 (0.86)	63.2 (1.80)
Cross-1	4.6 (0.66)	26.6 (1.42)	5.7 (0.75)	55.7 (1.74)
Cross-2	4.7 (0.67)	25.9 (1.41)	8.7 (0.94)	64.7 (1.81)
Tyson	3.5 (0.54)	27.0 (1.43)	2.0 (0.29)	75.6 (1.88)
LSD (P= 0.05)				
Gen. $\times$ Zinc		(0.06)		(0.10)

^a Numbers in parentheses refer to averages obtained from the analysis of variance of log-transformed data.

^b The LSD values are applicable to the log-transformed data (in parentheses).

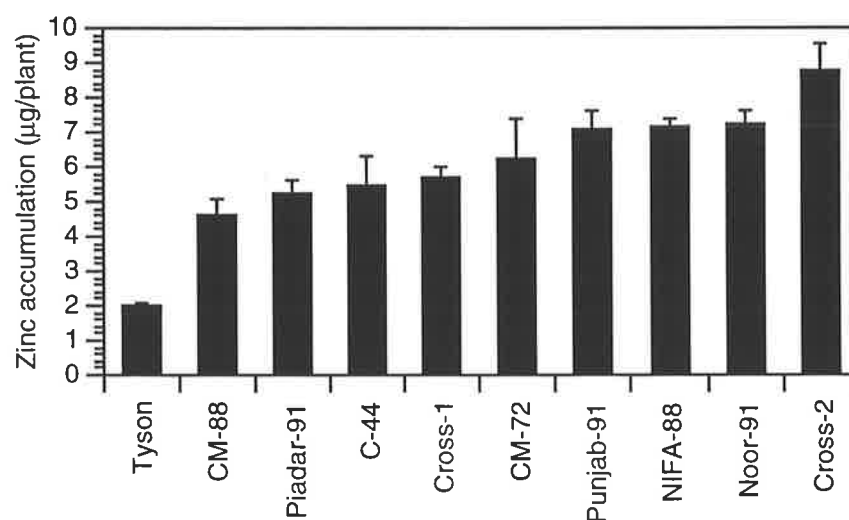
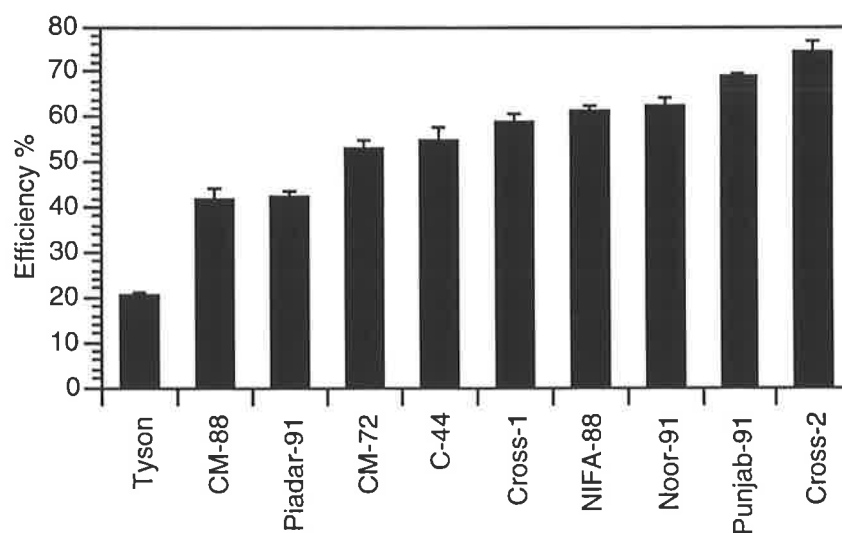


Figure 5.12: Differential response of chickpea genotypes to total zinc accumulation (shoot zinc content + root zinc content) in plants grown in the zinc-deficient treatment. The vertical bars represents standard errors based on three replicates.

Table 5.15: Effects of zinc fertilization on the root:shoot ratio. Plants were 10-week-old at the start of flowering.

Genotypes	Root:shoot ratio	
	Zinc -	Zinc +
C-44	1.59	1.08
CM-72	1.17	0.94
Piadar-91	1.20	0.86
Punjab-91	0.97	0.97
NIFA-88	1.24	0.90
CM-88	1.23	0.75
Noor-91	1.12	0.85
Cross-1	1.12	0.79
Cross-2	0.85	0.80
Tyson	0.99	1.04
LSD (P= 0.05)		
Gen. × Zinc	0.17	

**Figure 5.13:** Zinc efficiency $[(\text{zinc}/\text{zinc}+) \times 100]$ based on shoot growth of different chickpea genotypes grown in zinc-deficient Laffer sand in pots. The vertical bars represent standard errors based on three replicates.

5.5. Discussion

There were considerable differences among Australian as well Pakistani genotypes in the development of visible zinc deficiency symptoms. In some cultivars, like Tyson, CPI-56288, Piadar-91 and CM-88, zinc deficiency symptoms similar to those described earlier in Chapter 4 (Section 4.2.2.1) were observed relatively early and were more pronounced than in other genotypes. Based on visual symptoms, Tyson, CPI-56288, Piadar-91 and CM-88 appeared to be the most sensitive cultivars. The zinc concentration in shoot tissue of Dooen, Barwon and Narayen at four weeks after sowing was lower than in other genotypes, which was probably related to their inability to effectively extract zinc when the soil was severely zinc deficient, even though a distribution from root to shoot could have contributed to such an observation. Similar observations were made in soybean genotypes which have differential sensitivity to zinc deficiency; a sensitive line, D82-3298, had 50% lower zinc concentration in its fully-developed trifoliolate leaves compared to a tolerant line D77-6056, suggesting a poor absorption of zinc in the sensitive line (Hartwig *et al.*, 1991). In contrast, wheat genotypes differentially affected by zinc deficiency in growth, did not differ in their zinc concentration (Dong *et al.*, 1995), which could be due to differential zinc utilization or compartmentation in leaf cells as one of the mechanisms of zinc efficiency in cereals (Graham and Rengel, 1993).

The sensitivity of chickpea genotypes to zinc deficiency (Figures 5.2, 5.6 and 5.11) was not fully related to their zinc concentration in the seeds (Tables 5.1, 5.7 and 5.12). However, higher zinc content in the large-seeded kabuli cultivars Kaniva and Garnet resulted in higher zinc concentration compared with small-seeded genotypes, but differences disappeared with advancing growth. Similar observations in wheat were made by Cakmak *et al.* (1994; 1996a). Graham and Rengel (1993) suggested that high seed zinc content is not the only mechanism responsible for good growth in zinc-deficient environment as they found that the zinc-inefficient durum wheat cultivar Durati showed poorer growth than other genotypes despite having 30% greater seed

zinc content. The results of the current experiments indicate that the differences in seed size or seed zinc content may not be important in screening experiments of chickpea grown for 6-10 weeks. Such reports will be useful in future work because it is difficult in crops like chickpea, with considerable natural variation in seed size among genotypes, to use similar seed size and the same seed zinc content for a number of genotypes.

Shoot dry matter was significantly depressed when zinc was omitted from the soil. However, genotypes ICC-4958, T-1587, CTS-11308, Cross-2 (NEC138-2 $\times$ CM-72), Punjab-91 and Noor-91 produced significantly higher shoot dry matter under severe zinc-deficient conditions as compared with Tyson, CPI-56288, CM-88, CM-72. The results suggest differential sensitivity of chickpea genotypes to zinc deficiency and an ability of some genotypes to grow better than others under zinc-deficient environment. A better relative shoot growth at low zinc availability has been considered as an expression of zinc efficiency among wheat genotypes (Rengel and Graham, 1995c) and is also evident in chickpea.

The chickpea genotypes showed differential responses in shoot and root growth under zinc-deficient conditions. Greater root dry weight in Garnet, PI-13768 and C-44 as well as lower shoot growth under zinc deficient conditions indicates an imbalance in shoot and root growth, or that root growth may occur at the expense of shoot growth. A similar disorder was reported by Cakmak *et al.* (1996) in wheat. An extensive root growth with a high proportion of thin roots without adverse effects on shoot growth has been suggested as a desirable characteristic of zinc-efficient wheat genotypes under zinc deficiency because of increased surface to volume ratio, enabling wheat to acquire more zinc from a soil with limited availability (Dong *et al.*, 1995). Among the chickpea genotypes evaluated in the study presented here, the advanced breeding line ICC-4958 was identified as a zinc-efficient genotype. One of its desirable characteristics is its large root system which may also be beneficial for yield under dryland conditions: ICC-4958 proved to be the most drought-tolerant genotype at the

International Crop Research Institute for the Semi-Arid Tropics (ICRISAT) as well as at the International Centre of Agriculture for Dry Areas (ICARDA) (Saxena *et al.*, 1993). It is quite likely that zinc efficiency in ICC-4958 is related to its ability to produce an extensive root system in zinc-deficient soil and, in turn, extract more zinc from the soil. Graham *et al.* (1992) suggested that breeding varieties with a large root system which have better capacity to mobilise strongly bound zinc from soil particles in the rhizosphere is a sustainable approach to enhancing the productivity of crops in the areas prone to zinc deficiency.

The root:shoot ratio generally increases under zinc deficiency as an initial response to zinc deficiency (Loneragan *et al.*, 1987). The change in the partitioning of dry matter resulted in depressed shoot growth and enhanced root growth in wheat, possibly enabling proportionally greater nutrient uptake (Cumbus, 1985). The results from the present experiments support this hypothesis: the root:shoot ratio increased in most genotypes when grown under zinc deficiency and the increase was most apparent in inefficient genotypes. A reduction in shoot growth may decrease the metabolic demands of shoots and increase relative root surface area for ion absorption. However, Cakmak *et al.* (1996) did not consider it an adaptive response of zinc-starved wheat plants, but rather described it as a consequence of zinc deficiency-induced photo-oxidative damage in shoots, which leads to a lower shoot growth. The relatively zinc-efficient chickpea genotypes ICC-4958, T-1587, Punjab-91 and Cross-2 (NEC138-2 $\times$ CM-72) maintained a low root:shoot ratio under severe zinc deficiency as their greater root growth apparently enhanced shoot growth. In contrast, the root:shoot ratio was significantly increased in the sensitive genotypes grown under zinc deficiency. These findings agree with the results of experiments with wheat where the root:shoot ratio of zinc inefficient cultivar, Durati, grown at low zinc supply increased, while remaining constant in zinc-efficient cultivar Warigal at similar zinc availability (Rengel and Graham, 1995c).

Genotypic differences in sensitivity to zinc deficiency were closely related to the zinc content per plant but not to tissue zinc concentration. Zinc accumulation was significantly higher in T-1587, ICC-4958, CTS-11308, Punjab-91, Noor-91 and Cross-2 (NEC138-2 $\times$ CM-72) than Tyson, CPI-56288, CM-88 and Piadar-91. Relative zinc transport to shoots was higher in T-1587 and CTS-11308, probably enabling these genotypes to maintain a higher rate of zinc accumulation and shoot growth compared with sensitive genotypes. Such an observation appears to be consistent with reports of a greater ability of the zinc-efficient wheat cultivar Excalibur to acquire zinc from a deficient soil (Nable and Webb, 1993), as well as with a higher rate of zinc uptake in the zinc-efficient wheat genotype Warigal (Rengel and Graham, 1996) and a poor zinc transport mechanism in the zinc-inefficient durum wheat Durati (Grewal *et al.*, 1996).

The differential susceptibility to Fe deficiency in chickpea genotypes has been well documented (Saxena *et al.*, 1990; Ohwaki and Sugahara, 1993); it has been established that Fe-chlorosis resistance in chickpea is a genetic trait. Although the mechanism is not fully understood, it is assumed that Fe-efficient genotypes may have the ability to acidify the rhizosphere (Marschner and Römheld, 1983), which enables them to acquire more nutrients from an inhospitable environment. The study presented here also suggests that chickpea genotypes differ in their ability to extract Fe, particularly under limited zinc supply.

In the present study the most sensitive cultivar to zinc deficiency was Tyson, which was released in 1979 as the first commercial variety of chickpea for general cultivation in Australia (Beech and Brinsmead, 1980). Originally, it was an Indian cultivar C-235, which was re-selected to minimize its sensitivity to Fe deficiency. Data on Fe accumulation in the present study revealed Tyson having the lowest Fe accumulation of all the genotypes tested under severe zinc deficiency (Tables 5.6 and 5.11). Rashid and Din (1992), working with chickpea in Pakistan, identified cultivars CM-88 and C-44 as susceptible to Fe-chlorosis when grown on a calcareous soil having high pH. The result of the study presented here showed that both cultivars CM-88 and

C-44, together with Tyson, tended to have lower zinc concentration in the shoot tissue as compared with CM-72 and NIFA-88 (Table 5.14). The cultivars Tyson and CM-88 appear to be more sensitive to zinc deficiency and ranked as zinc inefficient while C-44 was intermediate in zinc efficiency. Walter *et al.* (1994) reported a reduction in Fe transport due to zinc deficiency in wheat. Fe uptake was significantly reduced in zinc inefficient durum wheat variety Durati, but increased in zinc efficient wheat variety Warigal at deficient zinc supply (Rengel and Graham, 1995d). These results suggest there might be a correlation between iron and zinc efficiencies in chickpea but further investigation is required to elucidate the response of Fe-sensitive chickpea genotypes to zinc deficiency.

5.6. Conclusion

In conclusion, chickpea genotypes differ in their sensitivity to zinc deficiency. Advanced breeding lines ICC-4958, T-1587, CTS-11308, Cross-2 (NEC138-2 $\times$ CM-72), Punjab-91 and Noor-91 were relatively more zinc-efficient compared with other desi as well as kabuli cultivars of Australia and Pakistan origin, while genotypes Tyson, CPI-56288, CM-88, Piadar-91 were more sensitive to zinc deficiency. The symptoms of zinc deficiency appeared first in the zinc inefficient genotypes such as Tyson, and were more pronounced. Total zinc accumulation but not tissue zinc concentration was well related to zinc efficiency. As more than one mechanism is considered to be responsible for zinc efficiency (Graham and Rengel, 1993), an efficient zinc absorption coupled with a better root-to-shoot transport could be a characteristic of zinc efficiency in chickpea genotypes.

CHAPTER 6

Response of chickpea genotypes to zinc fertilization under field conditions

6.1. Introduction

The results obtained from the pot experiments described in Chapters 4 and 5 demonstrated that in a severely zinc-deficient Laffer sand under controlled conditions, zinc fertilization up to 5.0 mg/kg increased shoot growth in chickpea, and that there is genotypic variation in sensitivity to zinc deficiency. These results need to be verified under field conditions. Accordingly, the growth of chickpea under zinc-deficient field conditions and their response to zinc fertilization in terms of vegetative growth as well as in grain yield, was examined in three field experiments: one in the Murray Mallee area of South Australia and two in the Potowar region of Pakistan.

The Murray Mallee region has some similarities with the major chickpea production zone of Pakistan known as Thal (Khan *et al.*, 1991). The predominant soil of the region is sandy textured top soil with high pH, and it receives relatively low seasonal rainfall (Reuter *et al.*, 1988). A nutritional survey of the Murray Mallee in 1986 revealed that >96 % of the wheat crops surveyed were diagnosed as zinc deficient (< 20 mg/kg in the youngest emerged leaf blades (YEBs), Graham *et al.*, 1989). Since this survey, the application of zinc fertilizers by some farmers has increased but zinc deficiency is still commonly diagnosed in the area. Although not a major crop in the region at present, there is still considerable interest by farmers in chickpea as an alternate crop. The area under chickpea is increasing fast in southern Australia and there are excellent prospects for further expansion (Siddique and Sykes, 1997). Consequently, it is likely that cultivation of chickpea on soils having low available zinc will increase, and zinc deficiency may cause a considerable loss of yield.

The Potowar region in northern Punjab (Pakistan) is an important zone where chickpeas are predominantly grown during winter. The major soils of the region are alkaline and calcareous (Chapter 2); reports on the nutritional status of the soils in the region suggests that zinc deficiency is a common disorder (Rashid and Rafique, 1989; Rashid *et al.*, 1994). This could be one of causes for low chickpea yields in the area (Singh, 1990).

In this chapter, three field experiments are reported. Out of these, one field experiment was conducted near Lameroo in the Murray Mallee (South Australia) during 1997 and two experiments were established near Attock in the Potowar region (Pakistan) during 1995-96.

6.2. Materials and methods

6.2.1. Lameroo (South Australia)

A field experiment was sown during 1997 at Lameroo, 220 km southeast of Adelaide. The soil at the site was a sandy loam over permeable medium clay. DTPA-extractable soil zinc was 0.27 mg/kg, and soil pH in water was 8.0. The experiment consisted of 10 chickpea genotypes $\times$ $\pm$ zinc $\times$ three replications. The genotypes tested in the experiment were all desi types and were Desavic, Amethyst, Dooen, Barwon, Semsan, Tyson, T-1587, ICC-4958, CTS-11308 and CTS-60543. Subplots consisted of six rows, 0.15 m apart and 6.0 m long. Border plots were also sown on both sides of the experimental plots. The seed rate was 80 seeds per m² and seeds were inoculated with *Rhizobium* using Nitrogerm 100 (Legume inoculant, group N chickpea) a day before sowing. The experiment was sown on 16 June 1997 in a randomized complete block, split-plot design, keeping genotypes in main plots and zinc treatments in subplots. Basal nutrients 26 kg N, 30 kg P, 5.1 kg Mn, 2.2 kg S, 2.0 kg Cu, 0.25 kg Co and 0.25 kg Mo per ha were applied at sowing using a commercial fertilizer. Zinc was applied to the zinc+ treatments as granules of zinc oxysulphate, at a rate of 6 kg zinc per ha at sowing in zinc+ treatments. These plots were also supplemented with foliar spray of



Zincsol ® (2 litre/ha) three months after sowing, prior to flowering. The total application to the zinc+ treatments was 6.5 kg zinc/ha.

A sample of plants was taken 85 days after ~~planting~~^{sowing} and at 112 days after sowing, at the post flowering stage: at each harvest five plants/plot were taken from the middle rows, dried in an oven at 80 °C for 48 hours, weighed and analysed for nutrients by spectrometry. At physiological maturity, five plants per plot were sampled, oven-dried at 40 °C for 4 days. Plants were weighed for shoot dry matter, then pods were separated from the plants, counted and threshed for seeds. The plots were reaped by a small plot harvester. Seed was cleaned before being weighed for grain yield per plot. For nutrient analysis, shoot tissue and seeds were oven-dried at 80°C prior to grinding, digested in nitric acid for analysis by ICP spectrometry (Chapter 3). The results were statistically analysed as discussed in Chapter 3 (Section 3.5).

6.2.2. Attock, Potowar region (Pakistan)

Experiments were conducted during the post-rainy season of 1995-96 at Barani Research Station (BRS), Fatehjang and Altaf Farm, Kamalpore Sherjang in the Potowar region of Punjab in District Attock. The soils were sandy loam (Typic Ustorthents) having a surface pH 7.8 to 8.0 and a DTPA-extractable zinc of 0.30 mg/kg at BRS, Fatehjang, and 0.24 mg/kg at Kamalpore Sherjang. At Fatehjang, the experimental field remained fallow during the summer season, while at Kamalpore Sherjang, sorghum was grown in the rainy season, and the chickpea trial followed the sorghum crop. Experiments consisted of 10 chickpea genotypes $\times$ $\pm$ zinc $\times$ three replications, laid out in a randomized complete block, split-plot design having zinc treatment in main plots and genotypes in subplots. The genotypes used in the trials were C-44, CM-72, NIFA-88, Punjab-91, Noor-91 (kabuli), Piadar-91, CM-88, ICC-11514 $\times$ ILC-3279 (Cross-1), ICC-11514 $\times$ ILC-482 (Cross-2) and Tyson (check). Subplots consisted of six rows, 0.3 m apart and 4.0 m long. Crop density was 33 plants per m². The trials were sown manually using single row hand-drawn drill from 31 October to 1 November, 1995.

Basal nutrients (25 kg N and 60 kg P per ha) were applied before sowing as urea and diammonium phosphate (DAP). Zinc was applied at a rate of 6 kg per ha in zinc+ plots as commercial-grade zinc sulphate at sowing and later supplemented with a foliar spray of zinc sulphate (0.5 kg zinc/ha) at the start of flowering.

At the start of podding, five plants per plot were harvested, dried in a hot-air oven and sent to Adelaide. There was some soil contamination on older leaves and stems, therefore only the youngest five to seven leaves and associated stem material (including floral parts and young pods) were used for nutrient analysis as described in Chapter 3 (Section 3.4). Plots were harvested manually, sun-dried and threshed using a small chickpea thresher, and seed yield was recorded.

6.3. Results

6.3.1. Lameroo

6.3.1.1. Weather

Monthly rainfall data recorded at Lameroo showed that the 1997 growing season was drier than average (Table 6.1). Winter rainfall was low and therefore there was little accumulation of water in the soil for use during spring. The growing season (April–October) rainfall in 1997, which is closely related to crop yields under South Australian conditions (French and Schultz, 1984), was much lower than the average rainfall. In addition, low temperatures may have reduced early seedling growth (Table 6.1). Rainfall showed a decreasing trend later in the season (from October onwards) when the crop was flowering and filling pods and subsequently the evaporative demands on the plants were high. Low night temperature together with frost during reproductive stage (August–October) may also have affected pod bearing and seed filling.

Table 6.1: Rainfall and temperature data of Lameroo (South Australia).

Month	Rainfall		Temperature (1997)			
	1997	Average ^a	Monthly mean		Extreme values	
			Max(Mean)	Min.(Mean)	Max.(High)	Min.(Low)
January	27	19	31.6	15.2	42	8
February	24	22	34.5	17.5	43	9
March	11	19	25.3	9.8	33	5
April	1	28	24.3	8.8	31	3
May	23	41	18.2	7.5	26	1
June	8	42	16.1	5.0	22	0
July	11	42	15.0	3.6	19	-1
August	51	45	16.3	4.8	22	-1
September	55	43	18.7	6.9	23	3
October	38	38	24.2	8.1	35	1
November	20	27	28.4	12.2	43	5
December	15	27	29.5	12.2	37	8
April-Oct ^b	187	279				
Total Rain	284	389				

^a Average rainfall of last 96 years.^b Seasonal rainfall (April-October)

Source: Monthly Weather Review (South Australia), Bureau of Meteorology, Australia.

6.3.1.2. Zinc concentration and content

Zinc fertilization increased zinc concentration in plants harvested 85 days after sowing (Table 6.2). The average zinc concentration in plants grown with no additional zinc was nearly half (15 mg/kg) of that in zinc-fertilized plants (29 mg/kg).

The genotype $\times$ zinc interaction for zinc content was significant. The advanced breeding line CTS-60543 had higher zinc content (13 $\mu\text{g/plant}$) than cultivars Tyson,

Amethyst and Barwon when zinc was not applied. Genotypes also showed differences in zinc accumulation in zinc+ treatment; ICC-4958 had highest zinc content among all genotypes, whereas there was relatively low zinc accumulation in Tyson, Amethyst and Desavic when zinc was applied.

By the second harvest, 112 days after sowing, after the plants had started to flower, the zinc concentration in shoot tissue had increased in both zinc treatments, but the increase was more pronounced in zinc- plants: zinc concentration increased from 15 mg/kg (85 days after sowing) to 26 mg/kg, whereas when zinc fertilizer was applied the increase was from 29 mg/kg to 33 mg/kg (Table 6.3). In addition, greater growth and higher zinc concentration following the addition of zinc fertilizer enhanced zinc accumulation per plant at this stage (Table 6.3). The genotype $\times$ zinc interaction for zinc content was significant. Genotypes Barwon and T-1587 had higher shoot zinc content than Tyson, CTS-11308 and Amethyst under zinc- conditions. In zinc+ treatment cultivar Barwon and breeding line CTS-60543 tended to have higher zinc content than Tyson, Amethyst, Desavic and Dooen.

6.3.1.3. Boron concentration

Boron concentration was assessed because soils in Lameroo area contain high levels of boron. Boron concentration, determined in the shoot tissues, indicated high boron accumulation in all genotypes up to toxic levels (Table 6.4). However, differences among genotypes were significant, with Tyson having highest boron concentration (81 mg/kg). The concentration of boron was relatively low in shoot tissues of Barwon and Dooen (59 mg/kg), which might be related to their higher vegetative growth and a corresponding dilution effect (Table 6.6). Zinc treatment had no ^{significant} effect on boron concentration in the shoot tissue.

6.3.1.4. Flowering

Number of flowering nodes on the main branch, counted on October 7 (112 days after sowing), showed significant differences among genotypes (Table 6.5). In contrast, zinc application had no significant effect on the number of flowering nodes. Genotypes ICC-4958, T-1587, CTS-60543, CTS-11308 and Amethyst were early flowering when compared with Barwon and Dooen while Desavic, Tyson and Semsan were intermediate in flowering.

Table 6.2: Effect of zinc application on shoot dry matter (g/plant), zinc concentration (mg/kg) and zinc content ($\mu\text{g/plant}$) of the shoots at 85 days after sowing.

Genotypes	Shoot dry weight		Zinc concentration		Zinc content	
	Zinc-	Zinc+	Zinc-	Zinc+	Zinc-	Zinc+
Desavic	0.68	0.67	14.2	26.9	9.7	14.9
Amethyst	0.58	0.59	14.2	28.3	7.5	15.9
Dooen	0.61	0.64	17.9	40.2	10.1	21.7
Barwon	0.64	0.79	16.3	24.9	10.3	19.3
Semsen	0.76	0.79	14.3	28.5	10.2	19.0
Tyson	0.56	0.56	13.8	22.3	6.8	10.7
T-1587	0.80	0.71	15.1	28.8	10.0	18.7
ICC-4958	0.71	0.72	15.5	42.2	10.1	26.0
CTS-11308	0.78	0.69	14.3	25.6	10.3	17.1
CTS-60543	0.82	0.77	16.1	21.8	13.1	16.8
Mean (Zinc)	0.70	0.69	15.2	28.9		
LSD (P=0.05)						
Genotype	ns		ns		3.9	
Zinc	ns		2.9		1.1	
Gen. $\times$ Zinc	ns		ns		3.3	

ns: non-significant

Table 6.3: Effect of zinc fertilization on shoot dry matter (g/plant), zinc concentration (mg/kg) and zinc content ($\mu\text{g/plant}$) of the shoots, 112 days after sowing.

Genotypes	Shoot dry matter		Zinc concentration			Zinc content	
	Zinc-	Zinc+	Zinc-	Zinc+	Mean (Var)	Zinc-	Zinc+
Desavic	3.3	3.4	27.1	29.5	28.3	90.3	100.6
Amethyst	2.8	2.9	26.6	30.3	28.4	74.5	91.6
Dooen	3.1	2.9	29.0	34.6	31.8	89.6	102.0
Barwon	3.4	3.7	27.4	42.5	34.9	99.5	154.9
Semsen	3.1	3.9	25.1	35.6	30.4	78.4	139.3
Tyson	2.5	2.8	26.9	32.1	29.5	66.7	91.4
T-1587	3.4	3.4	27.8	35.7	31.7	96.4	124.8
ICC-4958	4.0	4.2	22.1	25.8	24.0	86.7	110.0
CTS-11308	3.2	4.1	22.2	34.6	28.4	72.9	142.0
CTS-60543	3.5	3.7	24.5	30.1	27.3	93.0	114.2
Mean (Zinc)	3.2	3.5	25.9	33.1			
LSD (P=0.05)							
Genotype	ns		3.7				
Zinc	0.2		1.9				
Gen. $\times$ Zinc	ns		ns			24.7	

ns: non-significant

6.3.1.5. Dry matter production

Shoot dry matter harvested at the early vegetative stage (85 days after sowing) showed no significant difference among zinc treatments (Table 6.2), but zinc fertilization increased shoot dry matter accumulation by 9% at the flowering stage (112 days after sowing, Table 6.3).

Above ground total dry matter produced at maturity showed significant differences among zinc treatments as well as among genotypes (Table 6.6). Zinc fertilization enhanced dry matter production by more than 13% on average. Among genotypes, Barwon produced the highest total dry matter (8.2 g/plant), followed by ICC-4958 (7.9 g/plant), while the lowest dry matter was recorded in Tyson (4.3 g/plant). Zinc efficiency based on total dry matter showed that cultivars Tyson, Dooen and Desavic were more sensitive to zinc deficiency when compared with Barwon, ICC-4958, CTS-11308 and CTS-60543 (Table 6.6).

6.3.1.6. Grain yield and zinc efficiency

Grain yields obtained from Lameroo were low probably due to frost during flowering and to drought at the seed development stage. Grain yield produced in zinc-fertilized treatment was 7% higher than in plants grown without zinc application (Table 6.7) and there were significant differences in grain yield between genotypes. Cultivar Amethyst and breeding line CTS-11308 outyielded other genotypes, and Barwon and Dooen produced the lowest yields. Zinc efficiency, based on grain yield, showed that most of the genotypes had a zinc efficiency > 90%, with T-1587 showing highest zinc efficiency. The high yielding genotypes Amethyst and CTS-11308 had an intermediate zinc efficiency ranking. However, the ranking of genotypes was not consistent when compared with zinc efficiency based on total dry matter, probably due to differences in the harvest index (Figure 6.1), which is related to maturity.

Table 6.4: Boron concentration (mg/kg) in shoot tissue of chickpea. Plants were sampled at the post-flowering stage, 112 days after sowing.

Genotypes	Boron concentration (mg/kg)
Desavic	61.8
Amethyst	70.6
Dooen	58.9
Barwon	59.4
Semsen	60.2
Tyson	80.7
T-1587	73.4
ICC-4958	70.5
CTS-11308	60.9
CTS-60543	65.0
LSD (P= 0.05)	
Genotypes	3.8

Table 6.5: The number of flowering nodes per plant on main branch, counted 112 days after sowing in different chickpea genotypes.

Genotypes	flowering nodes/plant
Desavic	3.9
Amethyst	5.0
Dooen	2.3
Barwon	1.9
Semsen	4.9
Tyson	4.9
T-1587	5.2
ICC-4958	5.5
CTS-11308	5.0
CTS-60543	5.2
LSD (P=0.05)	
Genotype	1.3

Data on yield components indicate that zinc application slightly (5%) increased pod bearing (Table 6.8) and also increased individual seed weight (3%) (Table 6.9), but differences were not significant at $P= 0.05$. However, a significant increase in grain yield was probably attributed to the additive effect of increased pod numbers coupled with seed mass in zinc+ treatment.

Table 6.6: Effect of zinc application on above ground total dry matter (g/plant) at maturity and the zinc efficiency of genotypes. Zinc efficiency was calculated using the formula [(total dry matter in zinc-/ total dry matter in zinc+) $\times$ 100].

Genotypes	Zinc-	Zinc+	Mean (Gen.)	Zinc efficiency (%)
Desavic	5.45	6.74	6.10	80.9
Amethyst	5.80	6.71	6.26	86.4
Dooen	5.42	7.24	6.33	74.9
Barwon	8.10	8.33	8.22	97.2
Semsen	5.09	5.49	5.29	92.7
Tyson	3.55	4.94	4.25	71.9
T-1587	4.82	5.20	5.01	92.7
ICC-4958	7.68	8.02	7.85	95.8
CTS-11308	5.09	5.32	5.21	95.7
CTS-60543	4.86	5.13	5.00	94.7
Mean (Zinc)	5.59	6.31		
LSD (P=0.05)				
Genotype	1.71			4.6
Zinc	0.50			
Gen. $\times$ Zinc	ns			

ns: non-significant

Table 6.7: Grain yield (kg/ha) as affected by zinc fertilization among chickpea genotypes grown at Lameroo. Zinc efficiency was calculated using the formula [(grain yield in zinc-/ grain yield in zinc+) $\times$ 100].

Genotypes	Zinc-	Zinc+	Mean (Gen.)	Zinc efficiency (%)
Desavic	384	403	394	95.3
Amethyst	421	451	436	93.3
Dooen	308	323	316	95.4
Barwon	226	234	230	96.6
Semsen	279	332	306	84.0
Tyson	349	379	364	92.1
T-1587	403	385	394	104.7
ICC-4958	337	364	351	92.6
CTS-11308	415	454	435	91.4
CTS-60543	332	354	343	93.8
Mean (Zinc)	345	368		
LSD (P=0.05)				
Genotype	79			4.5
Zinc	13			
Gen. $\times$ Zinc	ns			

ns: non-significant

Table 6.8: Effect of zinc fertilization on the number of filled pods per plant in different chickpea genotypes at maturity at Lameroo.

Genotypes	Zinc-	Zinc+	Mean (Gen.)
Desavic	11.1	11.9	11.5
Amethyst	12.3	13.1	12.7
Dooen	11.7	10.6	11.1
Barwon	9.2	11.5	10.3
Semsen	10.5	11.5	11.0
Tyson	10.9	12.1	11.5
T-1587	12.7	12.9	12.8
ICC-4958	8.5	8.7	8.6
CTS-11308	11.4	11.7	11.6
CTS-60543	11.3	11.3	10.3
Mean (Zinc)	11.0	11.5	
LSD (P= 0.05)			
Genotype		3.7	
Zinc		ns	
Gen. × Zinc		ns	

ns: non-significant

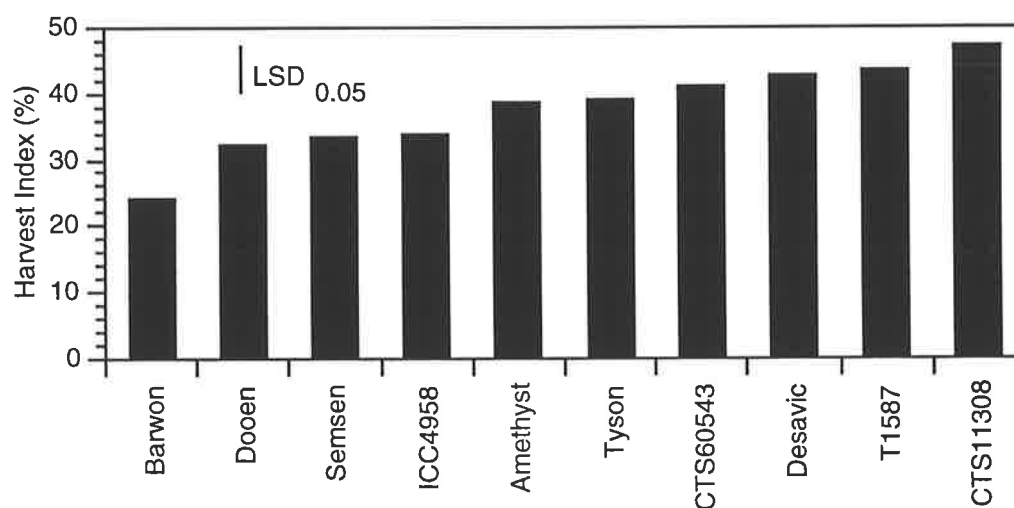
**Figure 6.1.** Variation in the harvest index [(grain yield/ total dry matter) × 100] among chickpea genotypes grown at Lameroo. Zinc treatments has no effect on the harvest index.

Table 6.9. Seed weight (mg/seed) as affected by zinc fertilization and genotypes of chickpea grown at Lameroo.

Genotypes	Zinc-	Zinc+	Mean (Var)
Desavic	152	195	174
Amethyst	142	155	149
Dooen	155	150	153
Barwon	186	180	183
Semsen	153	156	155
Tyson	128	122	125
T-1587	155	155	155
ICC-4958	322	337	330
CTS-11308	174	181	178
CTS-60543	169	165	167
Mean (Zinc)	174	180	
LSD (P= 0.05)			
Genotype		41	
Zinc		ns	
Gen. × Zinc		ns	

ns: non-significant

6.3.1.7. Seed zinc concentration

Zinc application enhanced zinc concentration by 27% in chickpea seeds (Table 6.10). Varietal differences in zinc concentration were also significant. Among high yielding genotypes, T-1587 loaded more zinc into the seed but low-yielding cultivars Dooen and Barwon also had higher seed zinc concentration than ICC-4958, Semsen and CTS-11308.

Table 6.10. Effect of zinc fertilization on zinc concentration (mg/kg) in seed produced at Lameroo.

Genotypes	Zinc-	Zinc+	Mean (Gen.)
Desavic	27.97	33.17	30.57
Amethyst	23.58	34.61	29.10
Dooen	28.04	40.70	34.37
Barwon	29.59	38.86	34.22
Semsen	22.90	31.78	27.34
Tyson	27.79	35.25	31.52
T-1587	28.88	40.09	34.49
ICC-4958	22.98	32.38	27.68
CTS-11308	23.63	33.18	28.41
CTS-60543	23.27	35.96	29.62
Mean (Zinc)	25.86	35.60	
LSD (P= 0.05)			
Genotype		4.9	
Zinc		1.7	
Gen. × Zinc		ns	

ns: non-significant

6.3.2. Barani Research Station (BRS), Fatehjang

6.3.2.1. Weather

Monthly rainfall recorded at BRS, Fatehjang, and Adaptive Research Farm (ARF), Attock, which was close to Kamalpore Sherjang, showed that the 1995-96 growing season was a wet season (Table 6.11). Fatehjang received relatively higher pre-season rainfall (625 mm) during the monsoon than did Attock (492 mm). However, winter rainfall, which is important for plant growth and development, remained above average at both sites, which suggest that moisture availability to crop was adequate during the growing season, particularly during the reproductive stage.

6.3.2.2. Zinc concentration

Zinc application significantly increased zinc concentration in shoot tissue determined at the flowering stage (Table 6.12). The genotype × zinc interaction for zinc concentration was significant. In the zinc- treatment, genotype (ICC 11514 × ILC 482)

Table 6.11. Rainfall (mm) received during the growing season 1995-96 at Barani Agricultural Research Station, Fatehjang (Pakistan) and Adaptive Research Farm, Attock (Pakistan) .

Month	Fatehjang	Attock
July	329	324
August	280	157
September	16	11
Pre-season total	625	492
October	9	42
November	22	18
December	10	55
January	39	69
February	105	152
March	111	97
April	14	30
Seasonal total	310	463

had lower zinc concentration (23 mg/kg) than that of Punjab-91 (33 mg/kg) and CM-88 (31 mg/kg). CM-88 also contained highest zinc concentration in zinc-fertilized plants.

6.3.2.3. Grain yield and zinc efficiency

Application of zinc significantly increased grain yield of chickpea by 10% on average (Table 6.13). Genotypic differences for grain yield were also significant; the line (ICC 11514 $\times$ ILC 482) and cultivar Punjab-91 produced higher yields than the other genotypes. Cultivars C-44 and CM-88 showed poor adaptation as their grain yield was low while Tyson and Piadar-91 were intermediate for grain production.

Zinc efficiencies were all > 85% but the high-yielding genotypes Punjab-91 and (ICC-11514 $\times$ ILC-482) tended to be more zinc-efficient than the other genotypes (Table 6.13). Cultivars CM-88, C-44 and Tyson appeared to be the most sensitive to zinc deficiency as zinc efficiency determined for these varieties was relatively low (85 to 88%).

Table 6.12. Effect of zinc fertilization on zinc concentration (mg/kg) in shoot tissue (youngest five to seven leaves and associated stem material) of 10 chickpea genotypes, determined at the flowering stage at BARS, Fatehjang (Pakistan).

Genotypes	Zinc-	Zinc+	Mean (Gen.)
C 44	25.8	46.1	36.0
CM 72	28.4	42.7	35.6
NIFA 88	28.9	39.6	34.3
Punjab 91	32.5	40.8	36.7
Noor 91	26.8	42.6	34.7
Piadar 91	24.5	40.0	32.3
CM 88	30.7	53.4	42.1
ICC 11514 x ILC 3279	26.2	40.2	33.2
ICC 11514 x ILC 482	22.7	42.5	32.6
Tyson	26.7	38.3	32.5
Mean (Zinc)	27.3	42.6	
LSD (P= 0.05)			
Genotype		3.5	
Zinc		4.0	
Gen. x Zinc		5.0	

Table 6.13. Effect of zinc fertilization on chickpea grain yield (kg/ha) of 10 genotypes at Fatehjang (Pakistan) and genotypic difference in their zinc efficiency.

Genotypes	Zinc-	Zinc+	Mean (Gen)	Zinc efficiency (%)
C 44	1727	1976	1852	87.4
CM 72	2178	2430	2304	89.6
NIFA 88	2399	2541	2470	94.4
Punjab 91	2560	2675	2618	95.7
Noor 91	2157	2335	2246	92.4
Piadar 91	2260	2532	2396	89.3
CM 88	1831	2158	1995	84.8
ICC 11514 x ILC 3279	2340	2502	2421	93.5
ICC 11514 x ILC 482	2668	2796	2732	95.4
Tyson	2199	2510	2355	87.6
Mean (Zinc)	2232	2445		
LSD (P= 0.05)				
Genotype		123		4.1
Zinc		48		
Gen. x Zinc		ns		

ns: non-significant

6.3.3. Altaf Farm, Kamalpore Sherjang

6.3.3.1. Zinc concentration

Zinc concentration in shoot tissue, determined at the flowering stage, significantly increased when zinc was applied (Table 6.14). However, zinc concentration in the zinc-treatment was lower (19 mg/kg) at this site as compared with Fatehjang (27 mg/kg), suggesting that zinc availability was relatively less at Kamalpore Sherjang. Among genotypes, (ICC 11514 × ILC 482), Punjab-91 and C-44 contained higher zinc concentration than (ICC 11514 × ILC 3279), Noor-91 and Piadar-91 when the source of zinc was native soil zinc. In the zinc+ treatment, Punjab-91 had highest zinc concentration (79 mg/kg), while Piadar-91 and Tyson tended to have low zinc concentration.

Table 6.14. Effect of zinc fertilization on zinc concentration (mg/kg) in shoot tissue (youngest five to seven leaves and associated stem material) of chickpea plants grown at Kamalpore Sherjang, Attock (Pakistan).

Genotypes	Zinc-	Zinc+	Mean (Gen)
C 44	20.0	67.4	43.7
CM 72	19.9	76.1	48.0
NIFA 88	18.4	46.4	32.4
Punjab 91	20.0	79.2	49.6
Noor 91	17.4	66.0	41.7
Piadar 91	17.0	55.0	36.0
CM 88	19.5	72.1	45.8
ICC 11514 x ILC 3279	15.9	67.3	41.6
ICC 11514 x ILC 482	22.2	58.6	40.4
Tyson	17.4	48.4	32.9
Mean (Zinc)	18.8	63.7	
LSD (P= 0.05)			
Genotype		2.3	
Zinc		5.1	
Gen. x Zinc		7.1	

6.3.3.2. Grain yield and zinc efficiency

Zinc fertilization resulted in 19% higher grain yield (Table 6.15). The breeding line (ICC 11514 × ILC 3279), Punjab-91 and NIFA-88 yielded more than the other genotypes. Lack of adaptation was obvious in C-44, which produced 46% less yield than the (ICC 11514 × ILC 3279).

The breeding lines (ICC 11514 × ILC 3279), (ICC 11514 × ILC 482) and cultivar Punjab-91 showed higher zinc efficiency (above 90%) than Tyson (72%), CM 88 (74%), and C 44 (76%). The zinc efficiency ranking based on relative grain yield at Kamalpore Sherjang was similar to that recorded at Fatehjang.

Table 6.15. Effect of zinc fertilization on grain yield (kg/ha) at Kamalpore Sherjang, Attock (Pakistan).

Genotypes	Zinc-	Zinc+	Mean (Gen)	Zinc efficiency (%)
C 44	805	1055	930	76.3
CM 72	1153	1393	1273	82.8
NIFA 88	1496	1695	1596	88.3
Punjab 91	1556	1704	1630	91.3
Noor 91	1185	1472	1329	80.5
Piadar 91	1375	1690	1533	81.4
CM 88	975	1320	1148	73.9
ICC 11514 x ILC 3279	1663	1750	1707	95.0
ICC 11514 x ILC 482	1375	1468	1422	93.7
Tyson	1076	1487	1282	72.4
Mean (Zinc)	1266	1504		
LSD (P= 0.05)				
Genotype		158		4.4
Zinc		106		
Gen. x Zinc		ns		

ns: non-significant

6.4. Discussion

Results of the study showed that application of zinc increased zinc concentration as well as zinc content in tissue harvested during the growing season in all genotypes. However, compared to pot assays using Laffer sand, the severity of zinc deficiency was considerably less under field conditions. A similar difference regarding the severity of zinc deficiency in pots compared to zinc-deficient soil of Lameroo has been observed in canola (Grewal *et al.*, 1997). Although plant material used for nutrient analysis differed slightly, the results indicated that zinc availability was higher at Fatehjang compared to Kamalpore Sherjang and Lameroo. At all sites, chickpea genotypes were looking healthy and were free of visible zinc deficiency symptoms but responded to zinc fertilization. The zinc status of the soils, determined through DTPA-extractable zinc (considered available soil zinc), ranged from 0.24 mg/kg at Kamalpore Sherjang to 0.30 mg/kg at Fatehjang while Lameroo soil contained 0.27 mg/kg DTPA-extractable zinc. Singh and Gupta (1986) studied a response of chickpea to zinc fertilization over a range of soils from semi-arid tropics in India and found 0.48 mg/kg DTPA-extractable zinc as critical level for chickpea below which response to zinc fertilization in terms of grain yield is expected. The results of the present study showed that chickpea grown on soils containing DTPA-extractable zinc lower than the critical value, was responsive to zinc fertilization.

At Lameroo, tissue zinc concentration recorded at 85 days after sowing ranged from 14-18 mg/kg without added zinc, but at flowering it was 22-29 mg/kg, which is within the deficiency range (Chapter 4). No differences among varieties were found in zinc concentration. However, genotypes differed for zinc accumulation in both zinc treatments; in the zinc- treatment, the cold-tolerant advanced breeding line CTS-60543 had the highest zinc accumulation in above ground biomass while cultivar Tyson had the lowest zinc content. These results are in agreement with the pot experiment (Chapter 5), as they verified the sensitivity of Tyson to zinc deficiency and the superiority of CTS-60543 in zinc absorption and translocation under field conditions. At this early growth

stage, zinc-fertilized plants of ICC-4958 tended to accumulate more zinc, which might be due to the more extensive root growth of this genotype (Saxena *et al.*, 1993; Silim and Saxena, 1993a). Studies on zinc in wheat have demonstrated that efficient genotypes produce large root systems comprised of fine, thin roots that increases the surface-to-volume ratio to explore more soil zinc (Graham *et al.*, 1992; Dong *et al.*, 1995).

Analysis of shoot tissue at the reproductive stage 112 days after sowing showed an increase in zinc concentration compared to the early growth stage, grown either with or without zinc application at Lameroo. Similarly, Reuter *et al.* (1982) reported a doubling in the zinc concentration in the apical leaf blades of subterranean clover between 21 and 42 days after sowing. In contrast, it is commonly observed in cereals that the zinc concentration in shoot tissue declines with an increase in growth and plant age, which is attributed to a dilution effect (Cakmak *et al.*, 1996; Yilmaz *et al.*, 1997). However, there is no information available about effects of zinc supply and plant age on concentration of zinc among plant parts in chickpea. One possible explanation regarding lower zinc concentration at early growth stage is associated with slow or restricted root growth early in the season when temperatures remain low. Thomas and Fukai (1995) compared early growth of chickpea with barley and found that chickpea is more sensitive to low temperature and has extremely slow growth in winter. Brennan *et al.* (1993) noted that zinc deficiency symptoms in annual crops in Australia are more pronounced during the cool winter months; symptoms often disappear with warmer conditions in spring. They suggested that severity of zinc deficiency is due to low soil temperatures that restrict root development of young plants. Translocation of zinc to actively growing reproductive parts is another possibility which may have increased zinc concentration in the shoot tissues sampled for nutrient analysis. Studies with navy bean support this hypothesis as higher zinc concentration was found in tops and seeds compared to other parts (Moraghan, 1996).

The Lameroo area is considered a relatively dry site with erratic seasonal rainfall (Graham *et al.*, 1992). Weather data suggest that the year 1997 was a dry season, and

crop experienced drought at the reproductive stage. In the Mediterranean-type environment of southern Australia, the use of early flowering cultivars is important to escape drought (Richards, 1991). Chickpea has an indeterminate growth habit, but in most of the drier areas of the world, the duration of the crop is cut short by terminal drought (Hay, 1995). Graham *et al.* (1992) have pointed out that seasonal interactions with zinc fertilization are common and may influence the overall performance of genotypes grown in drought-prone environments. Field experiments at Lameroo with wheat and canola clearly indicate that the early maturing wheat variety Aroona (Graham *et al.*, 1992) and early maturing mustard variety Narendra (Grewal *et al.*, 1997) produced outstanding yields under zinc-deficient conditions, particularly in drier seasons, showing higher zinc efficiency compared to other genotypes. The data on flowering (Table 6.5) showed differences in time of flowering among chickpea genotypes. The cultivar Amethyst flowered earlier than Dooen and Barwon and produced highest grain yield. Similarly, advanced breeding lines T-1587 and CTS-11308 were early flowering and produced the high yield. This showed that early flowering genotypes were less affected due to the dry season and had an advantage to escape drought. However, zinc application had no effect on flowering and maturity at Lameroo.

The harvest index values suggest that most of the early-flowering genotypes were efficient in partitioning of dry matter into grain, showing the high harvest index. Silim and Saxena (1993b), working with chickpea suggested that the high harvest index was clearly associated with drought escape, particularly under conditions of terminal drought in the Mediterranean type environment ^{of northern Syria}. At high levels of drought stress, partitioning seems to be a major factor contributing to high grain yield. Cultivars Barwon and Dooen produced high biomass, but their poor partitioning resulted in low grain yield at Lameroo. High temperatures late in the season undoubtedly reduce the duration of reproductive phase by hastening maturity. For chickpea, maximum temperature ^{combined with lack of soil moisture} exceeding 30-32 °C _Λ can impose a limit on yield potential due to forced-maturity (Harris, 1979). Thus, high temperature stress at Lameroo in November/ December (summer in

southern hemisphere) between flowering and grain filling stage (depending on the genotypes), could be responsible for yield loss particularly in late-maturing genotypes.

Boron toxicity appears to be another constraint on yield in chickpea at Lameroo. Most of the soils in South Australia which are comprised of calcareous sands with free carbonates and high pH contain high levels of boron (Cartwright *et al.*, 1986). In certain alkaline soils high concentrations of soluble boron and low levels of available zinc may occur simultaneously. Under these conditions when boron levels in the rooting zone is marginally high, it may accumulate to a toxic level in plants (Graham *et al.*, 1987). Moreover, in dry years higher boron concentrations increase the risk of yields loss because in seasons with high rainfall boron concentrations are expected to remain low in the rooting zone due to leaching (Spouncer *et al.*, 1990). Tissue analysis of chickpea plants grown at Lameroo indicated that boron concentration ranged from 59 mg/kg in Dooen to 81 mg/kg in Tyson but difference among zinc treatments was not significant, which could be due to marginal zinc deficiency. However, the concentration of boron was not high in chickpea plants grown in Potowar region (Pakistan). Reuter and Robinson (1997) recently published 34 mg/kg boron as critical concentration for boron toxicity in chickpea. Similarly, Bagheri (1993) working with legumes, identified chickpea as more sensitive to boron toxicity than pea and lentil. However, sensitivity to boron toxicity varied among desi and kabuli types of chickpea and slightly among genotypes within each type.

Zinc fertilization increased grain yield 7%, 10% and 19% at Lameroo, Fatehjang and Kamalpore Sherjang, respectively. The increase in grain yield was generally related to the native soil zinc as discussed earlier and zinc concentration in plant tissues. The site at Kamalpore Sherjang was the most responsive to zinc application as yield increase was higher compared to the other sites. It should be noted that the soil at Kamalpore Sherjang also had the lowest DTPA-extractable zinc, and plant tissue contained lower zinc concentration (17 mg/kg) than that of Fatehjang and Lameroo. The lower increase in grain yield recorded at Fatehjang (although yields were higher) was related to more soil

zinc, which was also reflected in higher tissue zinc concentrations. The results obtained by Kausar *et al.* (1976) revealed that lowland rice responded to zinc fertilization even on those Pakistani soils which contain relatively high zinc content and were generally considered not deficient for most of the crops. The increase in grain yield in chickpea in zinc fertilized plants at Lameroo was due to an additive effect of a slightly increased number of pods per plant as well as an increase in individual seed mass, although none of the single yield component differed significantly at the $P = 0.05$ level among zinc treatments. Similarly, studies with cowpea (Ghildiyal *et al.*, 1978) and navy bean (Moraghan, 1996) showed that zinc application increased pod ^{number} ~~bearing~~ and consequently grain yield.

The comparison of grain yield between Fatehjang and Kamalpore Sherjang sites indicated that the genotypic ranking remained almost ^{the} same across the sites. However, grain yields were higher at Fatehjang than that of Kamalpore Sherjang, because the field at BRS, Fatehjang, remained fallow during the summer season and received higher rainfall in the monsoon than that of other site. The paddock at Kamalpore Sherjang (a farmer's field) was used for a sorghum crop during summer and had, therefore, less stored soil moisture when chickpea was ^{sown} ~~drilled~~. It was also noted that the Fe-chlorosis sensitive cultivars C-44 and CM-88 (Ali *et al.*, 1989), produced lowest grain yields at both sites. The Australian cultivar Tyson was intermediate in ranking based on grain yield at both sites and produced higher grain yield than that of C-44 and CM-88, probably due to its wider adaptability over a range of environments. At Fatehjang its performance was similar to the Pakistani small-seeded cultivar Piadar-91 that is a derivative from a cross C 235 (Tyson) $\times$ ILC 191, selected for its disease resistance and the erect-growing habit (Tufail and Yousaf, 1992). Among the Pakistani genotypes, Punjab-91 proved to be an outstanding cultivar by accumulating high shoot dry matter, having high zinc efficiency in pot experiments (Chapter 5) and finally producing high grain yield across two sites in the Potowar region.

6.5. Conclusion

Zinc fertilization increased grain yield at all sites and in all chickpea genotypes but relative increase was higher at the most zinc deficient site, Kamalpore Sherjang. A differential response of chickpea genotypes to zinc deficiency was consistent with the pot assay results, particularly in Pakistani genotypes. Punjab-91 was an outstanding cultivar as it produced high yield without zinc application and responded significantly to zinc fertilization. Fe-chlorosis sensitive cultivars C-44 and CM-88 were poorly adapted in Potowar region and produced lowest grain yield.

At Lameroo, the zinc efficiency ranking was slightly different, compared to pot experiments probably due to an interaction of climatic factors, such as temperatures and drought, and soil factors like boron toxicity. However, it appears that under conditions of terminal drought associated with high temperatures, early-maturing genotypes like Amethyst and CTS-11308 had an advantage to escape drought, which may effect the ultimate zinc efficiency ranking of genotypes.

CHAPTER 7

Effect of zinc supply and moisture stress on water use, grain yield and zinc distribution in chickpea

7.1. Introduction

Drought is considered a major constraint in almost all the chickpea growing regions of the world, but particularly in the tropical and subtropical environments of the Indo-Pakistan subcontinent (Saxena, 1987; Singh, 1993), the Mediterranean basin (Saxena *et al.*, 1990b) and Mediterranean-type environment of southern Australia (Siddique and Sedgley, 1986). In these areas the rainfall is highly seasonal, and consequently the chickpea crop relies on stored soil moisture for growth during part of the growing season. Severe moisture ^{and high temperature} stress during flowering and pod development is a common occurrence.

Zinc deficiency is also widespread in the major chickpea growing areas of the world (Section 2.3.2.2). Saxena (1993) reported the appearance of visual zinc deficiency symptoms on chickpea plants grown on sandy soils of the Indian subcontinent. Thakur *et al.* (1989) observed an increase in chickpea grain yield in central India when zinc was included in the fertilizer. The results discussed in Chapter 6, were consistent with these reports and showed that zinc fertilization can increase grain yield in chickpea when grown under zinc-deficient field conditions in South Australia and Pakistan. However, it was noted that drought at the reproductive stage affected the performance of chickpea genotypes at Lameroo, South Australia. At this site, early-maturing genotypes produced higher grain yields than late-maturing genotypes. It appears, therefore, that the chickpea crop often suffers from water deficits and zinc deficiency coincidentally during its growth and development. The effect of this may be two-fold: firstly, zinc deficient plants may be more sensitive to water deficit than zinc

adequate plants which exacerbates the effect of drought, and secondly, drying of soil may reduce uptake of zinc, increasing the severity of zinc deficiency.

The importance of zinc supply in drought prone environments has not been studied in field crops, including chickpea. In a dry season in the Wudinna area in South Australia it was observed that the part of the wheat crop which received a foliar application of zinc matured normally whilst the crop in headland areas which did not receive zinc died prematurely (King, 1994). Riceman and Jones (1958) in an earlier study with subterranean clover noted the similarity of severe zinc deficiency symptoms, such as folding of the young leaves during warmest periods of the day, with the visible symptoms commonly seen in water-stressed plants. These observation led to the speculation that zinc might have some physiological role in crop plants which could help in drought tolerance or that the severity of water stress intensifies under zinc-deficient conditions.

Soil moisture content affects zinc uptake. Zinc nutrition studies carried out by Graham *et al.* (1992) suggested that drying of top soil during spring in the Mediterranean type environment of southern Australia may suppress zinc uptake by deactivating roots in cereals. Cakmak *et al.* (1996b) observed that low annual precipitation exacerbated zinc deficiency stress in central Anatolia (Turkey), probably by affecting zinc uptake and transport because zinc moves to plant roots by diffusion; hence a lack of soil moisture increases tortuosity in the diffusion path.

Micronutrients differ in their distribution and transport within a plant (Marschner, 1995). Studies with zinc in subterranean clover demonstrated that plant parts vary widely in their zinc concentration; factors like zinc supply and growth stage had an influence on distribution of zinc among plant organs (Reuter *et al.*, 1982). However, the distribution of zinc in chickpea plants and the impact of soil moisture status on uptake and translocation of zinc have not been documented so far. Therefore, a study was conducted to investigate the effect of zinc supply on grain yield in chickpea subjected to

water stress and to determine the zinc distribution in different plant organs, with an emphasis on seed zinc concentration.

The chapter presents the findings of two pot experiments. The first experiment is related to the effect of two levels of zinc fertilization on grain yield and yield components at two moisture regimes, whereas the second experiment deals with the distribution of zinc in different plant parts at the start of reproductive stage and at maturity. In both experiments genotypes with different zinc efficiencies were used.

7.2. Materials and methods

7.2.1. Experiment 1. Effect of zinc fertilization on grain yield and yield components in plants grown at two moisture regimes.

The experiment was designed as a completely randomized factorial design having four replicates. Soil preparation and basal nutrient solutions were the same as described in Chapter 3 (Section 3.2). Two zinc levels, 0.1 and 2.5 mg zinc per kg soil, were selected and designated as $Zn_{0.1}$ and $Zn_{2.5}$, respectively. The soil (3.0 kg) was weighed into polythene-lined PVC pots measuring 110 mm diameter $\times$ 300 mm deep. DD water was added in amounts sufficient to bring the soil water content to field capacity (12% w/w). A layer of black polypropylene beads approximately 1 cm deep was placed over the soil surface in the pots as a mulch to reduce evaporation. The soil moisture was kept at field capacity (12% w/w) in all pots by adding DD water on alternate days during the initial stage of growth and then daily until the induction of the water stress treatment at the start of flowering. Water stress was induced gradually in half of the pots (W_1) by decreasing water application by 1% (w/w) per day to bring the moisture level down from 12% (w/w) to 4% (w/w). Pots were weighed daily and if water use was $> 1\%$ (w/w), water was added. The aim was to keep the development of soil water deficits by plants of different sizes (due to zinc treatments) as similar as possible, so that the stress treatments were not confounded by the different rates of development of stress. The remaining pots (W_2) were maintained at field capacity (12% w/w) by regular watering.

Seed of three genotypes which differed in sensitivity to zinc deficiency viz, Tyson, T-1587 and ICC 4958 (Chapter 5), having similar seed weight within each variety (150 mg/seed for Tyson, 204 mg/seed for T-1587 and 268 mg/seed for ICC 4958) were selected and planted as described in Chapter 3. Seed zinc contents were 4.85, 5.00 and 5.3 $\mu\text{g}/\text{seed}$ for Tyson, T-1587 and ICC 4958, respectively.

Plants were grown in a glasshouse at the Waite Agricultural Research Institute (Adelaide, South Australia) from mid September until the end of December, 1996. Temperature within the glasshouse was $25 \pm 2^\circ\text{C}$ during the day and $18 \pm 2^\circ\text{C}$ during the night. Plants were grown under natural daylength. Initially, five seeds were sown in each pot and these were later thinned to three. One plant per pot was harvested at the start of flowering (45 days after sowing) prior to the induction of water stress. Shoot dry weight was determined after drying in an oven at 80°C for 48 hours, and tissues were analysed for nutrient by ICP spectrometry (Chapter 3, Section 3.4). The remaining two plants per pot were harvested at physiological maturity and dried in a hot-air oven at 40°C for 4 days. The plants were weighed for shoot dry matter; pods were then separated from the plants, counted and threshed for seeds. For nutrient analysis, shoot tissue and seeds were oven dried at 80°C prior to grinding and digested in nitric acid for analysis by ICP spectrometry (Chapter 3, Section 3.4). The data for zinc concentration and zinc content were log-transformed to overcome the problem of non-homogeneity of variance. Results were statistically analysed as described in Chapter 3 (Section 3.5).

7.2.2. Experiment 2. Effect of zinc supply and water stress on grain yield and distribution of zinc.

The experiment was established as a completely randomized factorial design having four replicates. Soil preparation and basal nutrient solutions were the same as described in Chapter 3 (Section 3.2). In Experiment 1, 0.1 mg zinc was found to be marginally deficient. Therefore, three zinc levels, 0.05 mg, 0.1 mg and 2.5 mg zinc per kg soil were selected and designated as $\text{Zn}_{0.05}$, $\text{Zn}_{0.1}$ and $\text{Zn}_{2.5}$, respectively. The soil

(3.0 kg) was weighed into polythene-lined PVC pots measuring 110 mm diameter $\times$ 300 mm deep. DD water was added in amounts sufficient to bring the soil water content to field capacity (12% w/w). A layer of black polypropylene beads approximately 1 cm deep was placed over the soil surface in the pots as a mulch to reduce evaporation. The water treatments in the experiment were same as described in Section 7.2.1.

Two genotypes, Tyson and ICC 4958, which are considered drought tolerant (Morgan *et al.*, 1991; Saxena *et al.*, 1993) but differ in their sensitivity to zinc deficiency (Chapter 5) were selected. Seeds were chosen from the same lot used in Experiment 1 and planted as described in Chapter 3 (Section 3.2).

Plants were grown in a glasshouse at the Waite Agricultural Research Institute (Adelaide, South Australia) from end of September until the end of December, 1997. Temperature within the glasshouse was 25 ± 2 °C during the day and 18 ± 2 °C during the night. Plants were grown under natural daylength. One plant per pot was harvested at the start of pod formation prior to the induction of water stress. Plants were separated into stem, leaves and floral parts/young pods; dry weight of each plant part was determined after drying in an oven at 80 °C for 48 hours. Tissues were analysed for nutrient by ICP spectrometry (Chapter 3, Section 3.4). The remaining two plants per pot were harvested at physiological maturity, separated into stem, leaves and pods, and dried in a hot-air oven at 40 °C for 4 days. Dry weight for each part was determined, pods were counted and threshed for seeds. It was difficult to retrieve all the senesced leaves because they were shed during the reproductive phase, so zinc was determined on a sample of leaves as they dropped. For nutrient analysis, each plant part was ground, dried in an oven at 80 °C for 48 hours, and digested in nitric acid for analysis by ICP spectrometry (Chapter 3, Section 3.4). The zinc harvest index was calculated as the ratio of seed zinc content to total zinc content of the above ground dry matter. The data for zinc concentration and zinc content were log-transformed to overcome the problem of non-homogeneity of variance. Results were statistically analysed as described in Chapter 3 (Section 3.5).

7.3. Results

7.3.1. Experiment 1

7.3.1.1. Shoot zinc concentration and content

The zinc concentration of the shoot tissue determined at flowering (45 days after sowing) was increased 5-6 times by the application of 2.5 mg zinc per kg soil (Table 7.1). The genotype $\times$ zinc interaction was significant; ICC 4958 had higher zinc concentration in the $Zn_{0.1}$ treatment than Tyson and T-1587 but there was no significant difference between genotypes at $Zn_{2.5}$. The shoot zinc concentration values obtained in the $Zn_{0.1}$ treatment ^{were} ~~was~~ in the zinc deficiency range (Chapter 4).

ICC 4958 accumulated more zinc in the shoot than Tyson when the zinc supply was 0.1 mg/kg soil (Table 7.1). However, at $Zn_{2.5}$ relatively more zinc was accumulated by T-1587. The results suggest that the response of genotypes to zinc accumulation varies with variation in the zinc supplies.

7.3.1.2. Shoot dry matter

The shoot dry matter increased considerably when zinc fertilization was increased from 0.1 to 2.5 mg/kg soil (Figure 7.1). At the higher zinc supply, T-1587 produced significantly more shoot dry matter than Tyson and ICC 4958, and its plants were significantly larger in size when water stress was induced at the flowering stage. Vegetative growth in Tyson was suppressed more than ICC 4958 and T-1587 in the $Zn_{0.1}$ treatment.

Table 7.1: Effect of zinc fertilization on shoot zinc concentration (mg/kg) and shoot zinc content ($\mu\text{g}/\text{plant}$). Plants were harvested before the commencement of water stress, 45 days after sowing.

Genotypes	Zinc concentration		Zinc content	
	Zinc 0.1	Zinc 2.5	Zinc 0.1	Zinc 2.5
Tyson	5.5 (0.738) ^a	37.7 (1.574)	9.5 (0.976)	88.9 (1.944)
T-1587	5.5 (0.741)	37.5 (1.572)	10.9 (1.037)	109.4 (2.036)
ICC 4958	6.8 (0.830)	33.7 (1.526)	13.0 (1.110)	79.3 (1.899)
LSD (P=0.05)				
Genotype	ns		(0.057)	
Zinc	(0.038) ^b		(0.046)	
Gen. $\times$ zinc	(0.066)		(0.081)	

^a Numbers in parentheses refer to averages obtained from the analysis of variance of log-transformed data.

^b The LSD values in parentheses are applicable to the log-transformed data.

ns: non significant

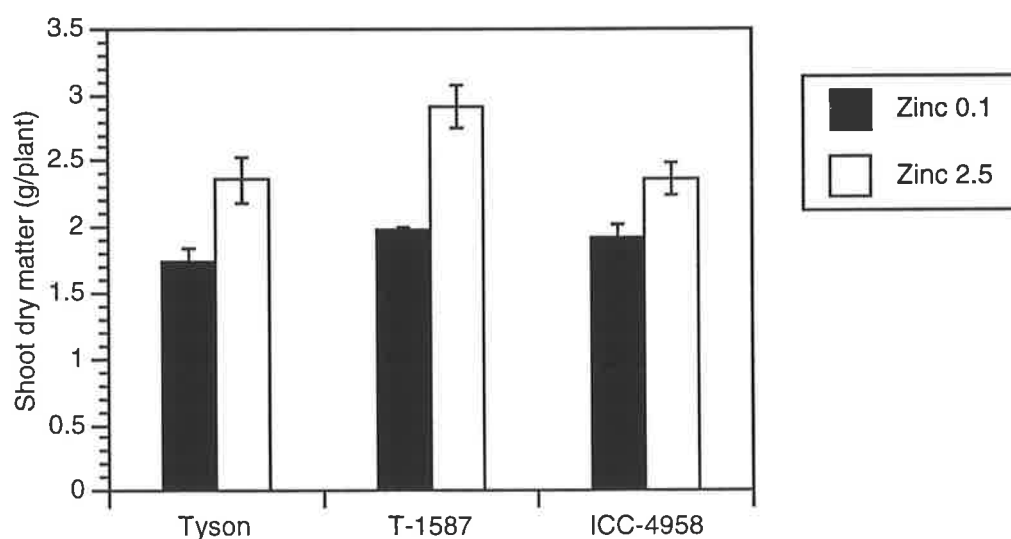


Figure 7.1: Effect of zinc fertilization on shoot dry matter of three chickpea genotypes. Plants were harvested 45 days after sowing before inducing water stress. The vertical bars represent standard errors based on four replicates.

7.3.1.3. Water use and water use efficiency

Application of 2.5 mg zinc per kg soil significantly increased water usage by plants prior to flowering. Evapotranspiration was relatively higher in T-1587, probably due to its large size (Figure 7.2). Water use declined when zinc supply was 0.1 mg/kg soil. There was no difference in total water consumption among genotypes in the $Zn_{0.1}$ treatment, before imposition of water stress.

The data on total water use (Figure 7.3) showed that plants supplied with 0.1 mg zinc per kg soil consumed significantly less water than those grown at adequate zinc supply when soil moisture was maintained at field capacity (12%) irrespective of genotype. The water use in Tyson in the $Zn_{2.5}$ treatment was relatively higher than in other genotypes when water content was 12%, which could be due to its continued vegetative growth during the reproductive phase. Under conditions of water deficit (4% water) genotypes responded similarly, and genotypic difference within zinc treatments in water consumption was non-significant. However, plants fertilized with 0.1 mg zinc per kg soil and subjected to water stress used significantly less water than water-stressed plants grown at adequate zinc supply ($Zn_{2.5}$).

Water use efficiency based on shoot dry matter, determined at the start of flowering before inducing water stress, showed significant difference among zinc treatments (Figure 7.4a). Water use efficiency was significantly higher in plants fertilized with 2.5 mg zinc per kg soil compared with those supplied with low zinc (0.1 mg/kg soil). The values of water use efficiency estimated on grain yield basis were generally higher in water-stressed plants compared to well-watered plants (Figure 7.4b). At 2.5 mg/kg soil zinc supply in water stress treatment ICC-4958 had greater water use efficiency than Tyson and T-1587. Under well-watered condition, genotypes had non significant differences in the $Zn_{0.1}$ treatment, but at adequate zinc supply (2.5 mg/kg) water use efficiency was significantly higher in ICC-4958 compared with T-1587 and Tyson.

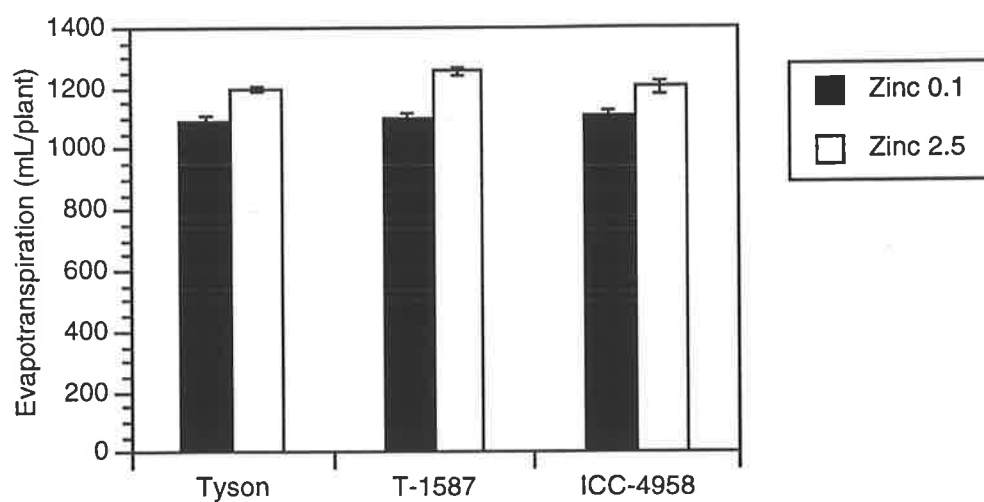


Figure 7.2: Effect of zinc fertilization on water use during first 45 days after sowing, before imposing water stress on three chickpea genotypes. The vertical bars represent standard errors based on four replicates.

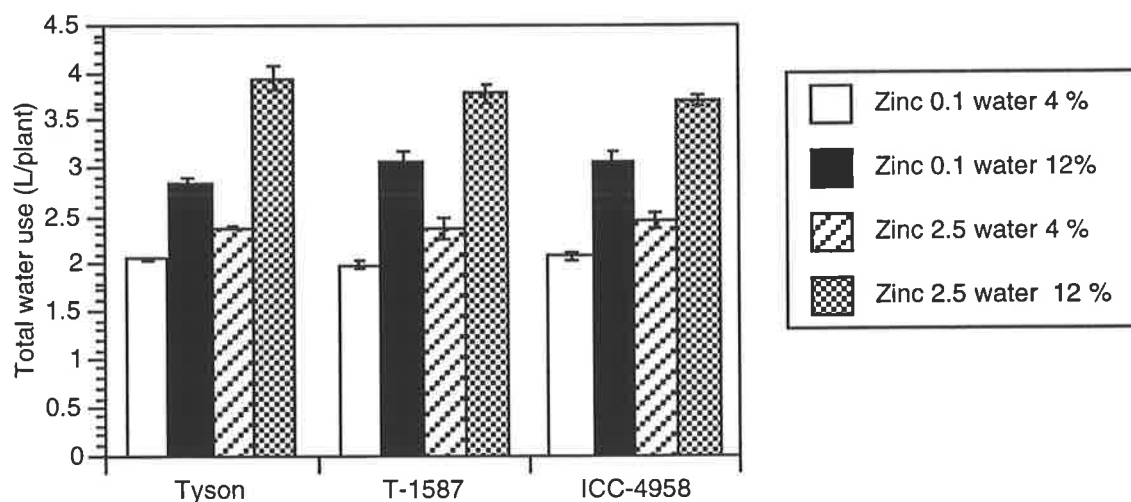


Figure 7.3: Effect of zinc fertilization and water stress on total water use (L/plant) for the three chickpea genotypes till maturity. The vertical bars represent standard errors based on four replicates.

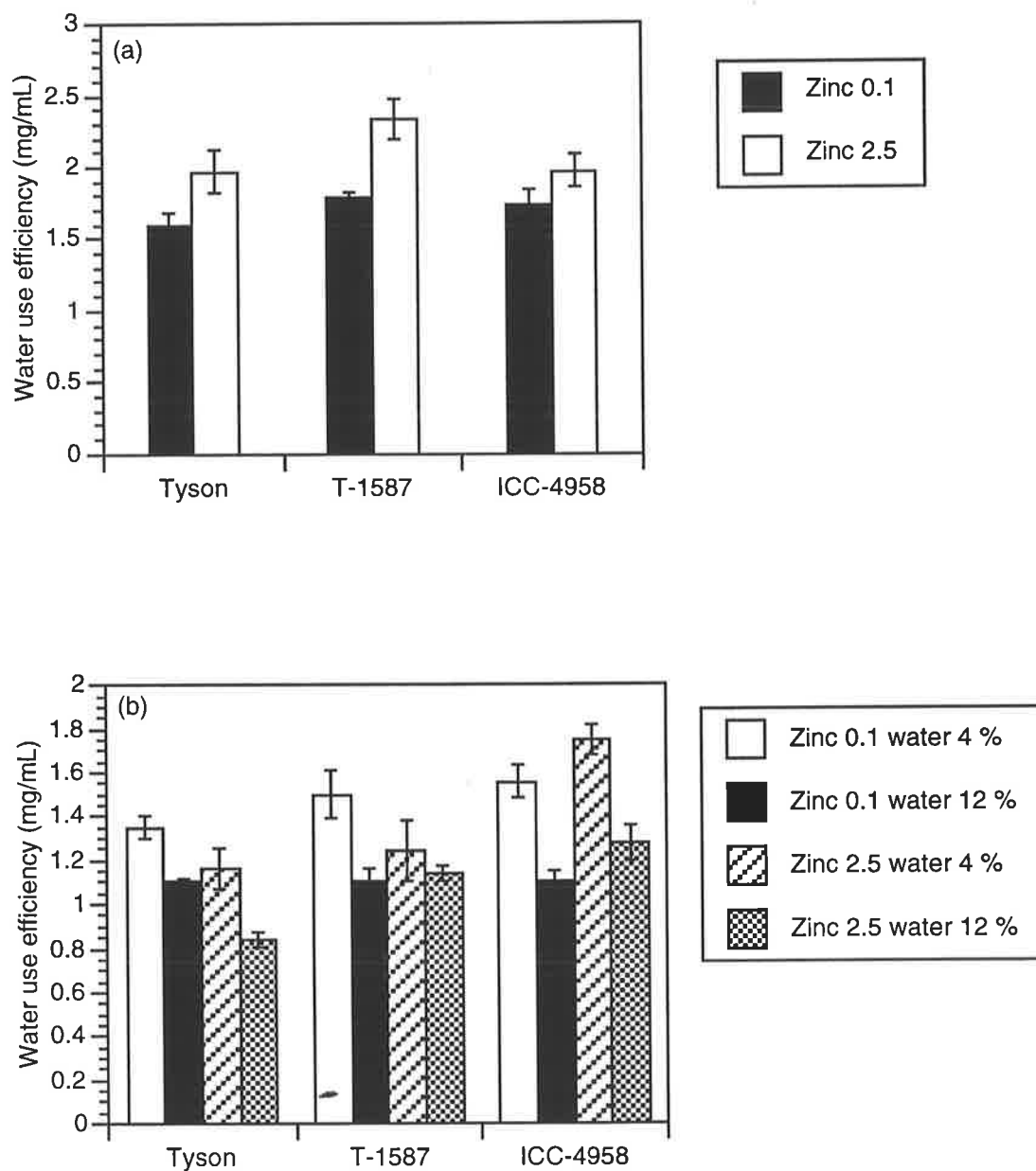


Figure 7.4: Effect of zinc fertilization on water use efficiency (based on shoot dry matter) during first 45 days after sowing before inducing water stress (a) water use efficiency (based on grain yield) as influenced by zinc application and water stress in three chickpea genotypes (b).

7.3.1.4. Grain yield and yield components

Zinc fertilization at high moisture availability increased grain yield of T-1587 and ICC-4958 but there was no significant increase in the grain yield of Tyson (Figure 7.5). The reduction in grain yield due to water stress was greater in plants fertilized with 2.5 mg zinc per kg soil than in plants grown at low zinc. The sensitivity to water stress at higher zinc fertilization was in the order T-1587 (33% yield reduction) > Tyson (16% reduction) > ICC-4958 (10% yield reduction).

At low zinc supply and under well-watered conditions, T-1587 and ICC 4958 produced higher grain yield than Tyson (Figure 7.5). Under water deficit in the $Zn_{0.1}$ treatment ICC 4958 produced 17% higher grain yield than Tyson. The results showed that plants fertilized with 0.1 mg zinc per kg soil could not take full advantage of a greater water availability, probably due to limited zinc supply, but at adequate zinc supply ($Zn_{2.5}$) water stress appears to be more detrimental for grain yield than for plants grown under marginally zinc deficient conditions.

The data on yield components revealed that a higher number of pods per plant and consequently, seeds/plant were the main causes of yield variation (Table 7.2). Pod number in ICC 4958 was least affected by water stress, although ICC 4958 produced fewer pods compared with Tyson and T-1587. The effect of water stress was more severe at $Zn_{2.5}$ as a 29% reduction in pod numbers was observed compared with 24% reduction in the $Zn_{0.1}$ treatment. The numbers of pods/plant and seeds/plant were increased with increasing zinc fertilization and water supply. T-1587 produced 45% fewer seeds under water stress in the $Zn_{2.5}$ treatment compared with 31% in Tyson and 11% in ICC 4958. Although interactions for individual seed mass were not significant, the main effect of the treatments showed that water stress increased seed weight on an average by 10% (224 mg *c.f.* 246 mg), which compensated, to some extent, for reduction in the number of seeds per plant. High water availability during pod-fill apparently

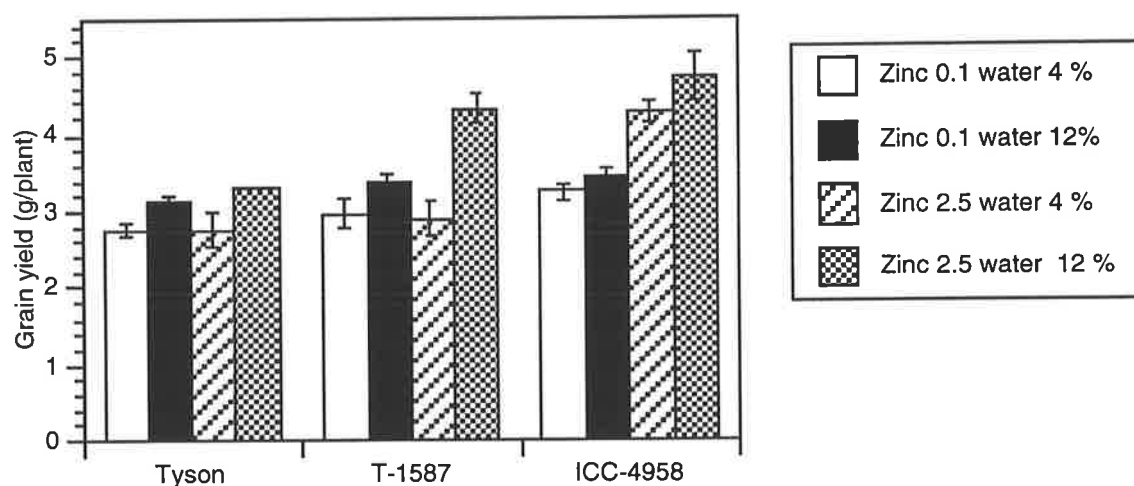


Figure 7.5: Effect of zinc application and water availability on grain yield (g/plant) of three chickpea genotypes. The vertical bars represent standard errors based on four replicates.

affected seed development, increasing the number of shrivelled or under-developed seeds.

7.3.1.5. Harvest Index

Zinc fertilization and moisture availability influenced the harvest index (Figure 7.6). The values of the harvest index were higher in plants fertilized with low zinc and experiencing water stress compared with the $Zn_{2.5}$ and 12% water treatment. Among genotypes, Tyson was least efficient in partitioning of assimilates under high input conditions and had a lower harvest index than ICC 4958 and T-1587. The data suggest that zinc deficiency affected leaf and stem growth more than pod set increasing harvest index; alternatively adequate zinc and high water (12% w/w) availability promoted vegetative growth but yield was not increased proportionately.

Table 7.2: Effect of zinc fertilization and water stress on yield components in three chickpea genotypes.

Genotypes	Treatments		Pods/plant	Seeds/plant	Seed weight (mg)	Shrivelled seed/plant
	Zinc	Water %				
Tyson	0.1	4	12.0	16.5	169	0.8
	0.1	12	16.0	24.8	127	2.1
	2.5	4	16.1	19.3	143	2.8
	2.5	12	24.6	28.0	115	5.4
T-1587	0.1	4	12.4	13.5	222	0.3
	0.1	12	16.9	16.9	196	1.4
	2.5	4	17.5	13.2	172	0.8
	2.5	12	23.4	23.8	170	3.3
ICC 4958	0.1	4	8.6	8.3	394	0.1
	0.1	12	10.5	9.5	366	0.5
	2.5	4	11.6	11.5	376	0.4
	2.5	12	15.6	12.9	367	1.8
LSD (P = 0.05)						
Gen × Zn			ns	ns	ns	0.6
Gen × water			1.97	2.0	ns	0.6
Zn × water			1.61	1.7	ns	0.5
Gen × Zn × wat			ns	2.9	ns	ns

ns: non significant

7.3.1.6. Seed zinc concentration

Seed zinc concentration was significantly lower in the $Zn_{0.1}$ than in the $Zn_{2.5}$ treatment. At high zinc availability, ICC 4958 had a lower seed zinc concentration compared with Tyson and T-1587, both of which were similar (Figure 7.7). However, at lower zinc supply, there was no difference in seed zinc concentration among genotypes. Water stress had no overall effect on seed zinc concentration.

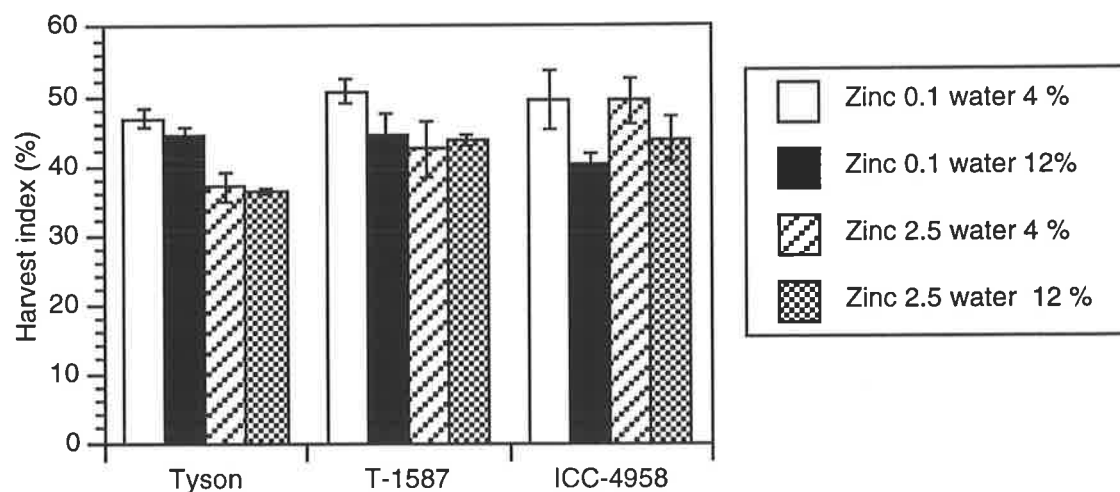


Figure 7.6: Effect of zinc fertilization and water stress on the harvest index [(grain yield/biological yield) $\times$ 100] in three chickpea genotypes. The vertical bars represent standard errors based on four replicates.

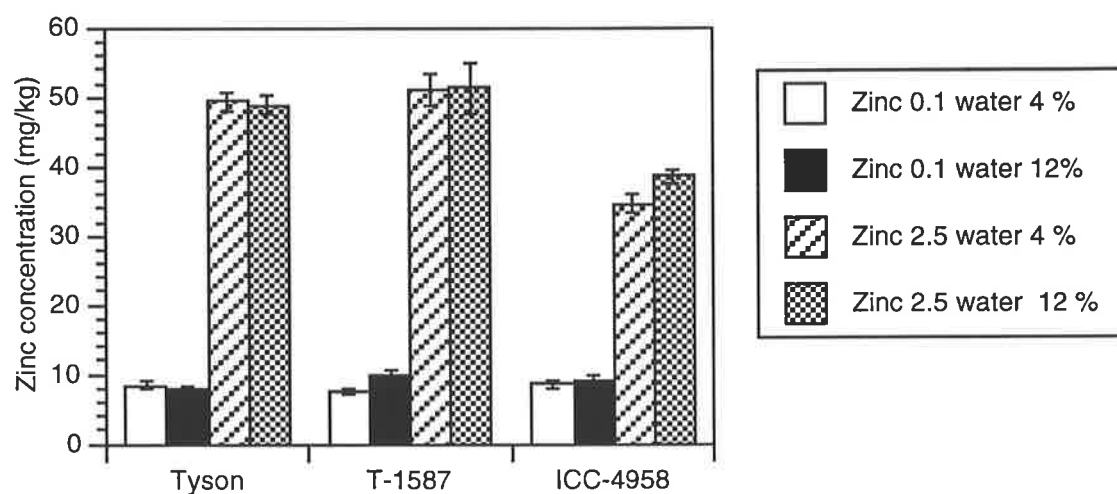


Figure 7.7: Effect of zinc fertilization on seed zinc concentration produced by three chickpea genotypes under two moisture regimes. The vertical bars represent standard errors based on four replicates.

7.3.2. Experiment 2

7.3.2.1. Shoot dry matter

Zinc fertilization significantly increased shoot dry matter in Tyson and plants fertilized with 2.5 mg zinc per kg soil produced highest dry matter compared with the $Zn_{0.05}$ and $Zn_{0.1}$ treatments (Figure 7.8). In ICC-4958, shoot dry matter accumulation increased when plants were fertilized with 2.5 mg/kg soil zinc, while difference between the $Zn_{0.05}$ and $Zn_{0.1}$ treatments was not significant. At low zinc supply (0.05 mg/kg) ICC-4958 accumulated significantly higher shoot dry matter at the start of pod formation compared with Tyson.

7.3.2.2. Zinc concentration at the podding stage

Zinc concentration determined before imposing the water stress treatments showed striking differences in distribution of zinc among plant parts (Table 7.3). The stem and leaves generally had lower zinc concentration at all levels of applied zinc compared with floral parts and young pods. In Tyson, increasing zinc supply from 0.05 to 0.1 mg/kg soil had no influence on zinc concentration in the above ground parts; however, zinc concentrations increased significantly when plants were fertilized with 2.5 mg zinc per kg soil. The breeding line ICC 4958 was more responsive to an increase in zinc fertilization from 0.05 to 0.1 mg/kg soil; zinc concentration increased at all levels of zinc fertilization, particularly in stem and reproductive parts. Like Tyson, the zinc concentration in the young pods and floral parts of ICC 4958 (48 mg/kg) was much higher than in the other parts.

7.3.2.3. Zinc content at podding stage

Zinc supply affected accumulation of zinc in different plant organs by influencing plant growth as well as zinc concentration. In all zinc treatments, both genotypes had lower zinc contents in their reproductive parts due to their lower dry matter compared with leaves and stems, in spite of having much higher zinc concentration in the floral

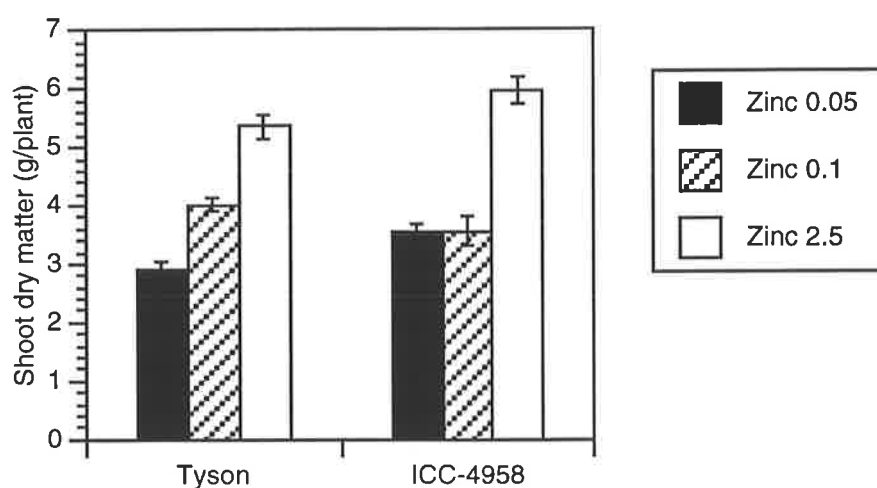


Figure 7.8: Effect of zinc fertilization on shoot dry matter of two chickpea genotypes. Plants were harvested at the start of pod formation before inducing water stress. The vertical bars represent standard error based on four replicates.

Table 7.3: Effect of zinc application on zinc concentration (mg/kg) in different plant parts of two chickpea genotypes. Plants were harvested at the podding stage before inducing water stress.

Genotype	Zinc treatments (mg/kg soil)	Stem	Leaves	Floral parts/pod
Tyson	0.05	7.1 (0.849) ^a	6.0 (0.775)	16.2 (1.205)
	0.1	5.9 (0.768)	5.0 (0.691)	17.6 (1.243)
	2.5	16.1 (1.204)	30.4 (1.481)	53.4 (1.725)
ICC 4958	0.05	5.4 (0.727)	5.1 (0.702)	17.1 (1.230)
	0.1	12.9 (1.100)	7.3 (0.840)	25.6 (1.404)
	2.5	20.6 (1.313)	22.0 (1.341)	48.1 (1.682)
LSD (P=0.05)				
Gen × Zn		(0.090) ^b	(0.119)	(0.078)

^a Numbers in parentheses refer to averages obtained from the analysis of variance of log-transformed data.

^b The LSD values in parentheses are applicable to the log-transformed data.

Table 7.4: Effect of zinc application on zinc content (μg) in different plant parts of two chickpea genotypes. Plants were harvested at the podding stage before inducing water stress.

Genotype	Zinc treatments (mg/kg soil)	Stem	Leaves	Floral parts/pod
Tyson	0.05	8.5 (0.921) ^a	8.7 (0.937)	3.8 (0.569)
	0.1	8.6 (0.929)	11.1 (1.036)	6.1 (0.777)
	2.5	35.0 (1.533)	82.0 (1.909)	26.7 (1.424)
ICC 4958	0.05	7.8 (0.884)	10.0 (1.997)	1.7 (0.207)
	0.1	16.0 (1.177)	15.5 (1.170)	3.6 (0.542)
	2.5	41.2 (1.613)	82.0 (1.910)	11.3 (1.041)
LSD 0.05				
Gen $\times$ Zn		(0.143) ^b	(0.119)	(0.143)

^a Number in parentheses refer to averages obtained from the analysis of variance of log-transformed data.

^b The LSD values in parentheses are applicable to the log-transformed data.

parts and young pods (Table 7.4). The plants fertilized with 2.5 mg zinc per kg soil accumulated more zinc in stems, leaves and floral parts/young pods than those grown at 0.05 or 0.1 zinc levels. The $\text{Zn}_{0.1}$ treatment only increased the zinc content in floral parts and young pods in Tyson than $\text{Zn}_{0.05}$ treatment, but ICC 4958 showed a considerable increase in zinc accumulation in all above-ground parts at $\text{Zn}_{0.1}$ compared with $\text{Zn}_{0.05}$. The comparison of zinc content in different plant parts indicated that as the zinc supply increased zinc accumulation was relatively higher in leaves than in the stem and reproductive parts, due to a combined effect of both higher zinc concentration and higher dry matter.

7.3.2.4. Zinc concentration at maturity

At maturity, the stem, old senesced leaves and pod walls had a relatively lower concentration of zinc in their tissues compared to the younger leaves, which were not abscised, and seeds in all zinc treatment (Table 7.5). Moisture availability had no significant effect on zinc concentration in the $\text{Zn}_{0.05}$ and $\text{Zn}_{0.1}$ treatments, but water stress in ICC 4958 slightly increased zinc concentration in stem and leaves when fertilized with 2.5 mg zinc per kg soil. At the higher zinc supply ($\text{Zn}_{2.5}$), the genotypes differed in their

zinc concentration in various parts. Tyson had higher zinc concentrations in young attached leaves and seeds but lower zinc concentration in its pod wall and roots compared to ICC 4958. It was noted that zinc concentration in various plant parts at maturity was greater than that recorded at the start of the reproductive stage, suggesting a continued accumulation of zinc by the shoot during the podding phase.

7.3.2.5. Zinc content at maturity

Zinc accumulation in plant organs generally increased with increasing zinc supply. At maturity the largest proportion of zinc was found in the seed in both genotypes. At higher zinc supply ($Zn_{2.5}$), leaves and stems of Tyson accumulated more zinc than those of ICC 4958 (Table 7.6), but the root zinc content of Tyson was significantly lower than that of ICC 4958. The plants grown at 12% soil moisture and fertilized with 2.5 mg zinc per kg soil accumulated larger amounts of zinc in their seeds than water-stressed plants of both genotypes. However, water deficit at the reproductive stage had no significant influence on zinc accumulation in other plant parts.

7.3.2.6. Zinc harvest index

The zinc harvest indices (the ratio of seed zinc content to the total zinc content of above-ground dry matter) ranged from 37% to 58% among treatments which indicated that nearly half of the total zinc (depending on the conditions) taken up by plants were retained in its structural parts at maturity (Figure 7.9). The zinc harvest index was 23% higher, on an average, in ICC 4958 than in Tyson. However, zinc $\times$ water interaction revealed that at lower levels of zinc supply ($Zn_{0.05}$ and $Zn_{0.1}$) soil moisture had no significant effect on the zinc harvest index, but water stress significantly reduced the partitioning of zinc to the seed in plants fertilized with 2.5 mg zinc per kg of soil. Under well-watered conditions an increase in zinc supply from 0.05 to 0.1 mg per kg soil slightly improved the zinc harvest index.

Table 7.5: Effect of zinc fertilization and water stress on zinc concentration (mg/kg) in different plant parts of two chickpea genotypes at maturity.

Genotypes	Treatments		Stem	Attached leaves	Abscised leaves	Pod wall	Seed	Root
	Zinc (mg/kg)	Water %						
Tyson	0.05	4	11.6 (1.06)	23.6 (1.37)	8.9 (0.94)	8.9 (0.94)	10.6 (1.02)	5.4 (0.73)
	0.05	12	7.6 (0.88)	17.5 (1.24)	7.0 (0.83)	6.2 (0.78)	8.1 (0.91)	5.0 (0.68)
	0.1	4	9.6 (0.98)	24.8 (1.39)	9.6 (0.98)	6.5 (0.81)	12.8 (1.10)	6.1 (0.77)
	0.1	12	8.7 (0.94)	21.3 (1.33)	8.5 (0.93)	6.2 (0.79)	12.1 (1.08)	4.5 (0.63)
	2.5	4	35.7 (1.55)	101.6 (2.01)	33.1 (1.52)	19.7 (1.29)	68.4 (1.83)	51.2 (1.71)
	2.5	12	34.1 (1.53)	102.9 (2.01)	33.6 (1.52)	18.2 (1.26)	73.4 (1.87)	61.3 (1.79)
ICC 4958	0.05	4	6.5 (0.81)	12.2 (1.08)	5.7 (0.75)	9.8 (0.99)	9.5 (0.98)	4.9 (0.68)
	0.05	12	6.8 (0.83)	11.8 (1.07)	5.0 (0.69)	7.0 (0.84)	10.0 (1.00)	5.5 (0.72)
	0.1	4	7.2 (0.85)	14.2 (1.15)	8.5 (0.93)	12.2 (1.08)	13.4 (1.13)	6.1 (0.77)
	0.1	12	6.9 (0.84)	14.7 (1.16)	9.9 (0.99)	7.8 (0.88)	12.1 (1.08)	5.5 (0.73)
	2.5	4	29.0 (1.46)	64.7 (1.81)	38.1 (1.58)	20.7 (1.31)	47.8 (1.68)	115.5 (2.06)
	2.5	12	22.0 (1.34)	44.4 (1.65)	28.8 (1.46)	26.1 (1.42)	56.6 (1.75)	97.9 (1.99)
LSD (P= 0.05)								
Gen × Zn × Wat.			(0.08)	(0.07)	(0.09)	(0.10)	(0.05)	(0.14)

^a Number in parentheses refer to averages obtained from the analysis of variance of log-transformed data.

^b The LSD values in parentheses are applicable to the log-transformed data.

ns: non significant

Table 7.6: Effect of zinc fertilization and water stress on zinc content ($\mu\text{g/plant}$) in different plant parts of two chickpea genotypes at maturity.

Genotypes	Treatments		Stem	Leaves	Pod wall	Seed	Root
	Zinc (mg/kg)	Water %					
Tyson	0.05	4	19.5 (1.29)	15.3 (1.18)	5.7 (0.75)	24.1 (1.38)	10.5 (1.01)
	0.05	12	13.1 (1.11)	15.8 (1.19)	4.6 (0.65)	21.9 (1.34)	9.5 (0.95)
	0.1	4	19.4 (1.28)	16.5 (1.21)	4.9 (0.69)	28.3 (1.45)	12.0 (1.07)
	0.1	12	19.9 (1.30)	19.9 (1.30)	6.7 (0.82)	36.8 (1.57)	10.5 (1.00)
	2.5	4	117.4 (2.07)	122.0 (2.08)	20.1 (1.30)	160.2 (2.20)	154.8 (2.19)
	2.5	12	119.8 (2.07)	102.7 (2.01)	29.9 (1.46)	248.0 (2.39)	187.6 (2.27)
ICC 4958	0.05	4	14.2 (1.15)	17.9 (1.25)	7.2 (0.85)	35.1 (1.54)	9.9 (0.98)
	0.05	12	15.8 (1.19)	16.7 (1.21)	6.6 (0.81)	36.9 (1.56)	12.8 (1.09)
	0.1	4	13.1 (1.12)	22.1 (1.33)	10.0 (0.99)	51.8 (1.71)	11.6 (1.05)
	0.1	12	13.9 (1.14)	22.7 (1.35)	10.8 (1.02)	53.2 (1.72)	12.6 (1.09)
	2.5	4	71.2 (1.84)	107.0 (2.03)	22.8 (1.35)	174.0 (2.24)	238.4 (2.38)
	2.5	12	69.6 (1.84)	89.1 (1.95)	42.7 (1.63)	284.2 (2.45)	261.5 (2.41)
LSD (P= 0.05)							
Gen $\times$ Zn			(0.10)	(0.10)	ns	(0.05)	ns
Gen $\times$ Wat.			ns	ns	ns	ns	ns
Gen $\times$ Wat.			ns	ns	(0.10)	(0.05)	ns
Gen $\times$ Zn $\times$ Wat.			ns	ns	ns	ns	ns

^a Number in parentheses refer to averages obtained from the analysis of variance of log-transformed data.

^b The LSD values in parentheses are applicable to the log-transformed data.

ns: non significant

7.3.2.7. Water use and water use efficiency

Zinc fertilization significantly increased water use by plants during vegetative growth in both varieties (Figure 7.10). In Tyson, plants fertilized with 0.05 and 0.01 mg zinc consumed less water compared to the corresponding zinc treatments in ICC-4958. However, at adequate zinc supply, there was no difference in water use between these genotypes.

Total water use declined in the water stress treatments because of induction of water stress at reproductive stage, but water stressed plants of Tyson in the $Zn_{2.5}$ treatment consumed relatively more water than the other water stressed plants probably due to continued vegetative growth (Figure 7.11). At adequate water supply, increasing zinc application enhanced total water use (from sowing till physiological maturity) in Tyson, and plants fertilized with 2.5 mg zinc used highest amount of water. In ICC 4958, water use at adequate zinc supply (2.5 mg) was significantly higher compared to the $Zn_{0.05}$ and $Zn_{0.1}$ treatments which use similar amount of water.

Water use efficiency, determined at the start of pod formation, increased significantly with increasing zinc fertilization in Tyson (Figure 7.12). In ICC-4958, water use efficiency was significantly higher in the $Zn_{2.5}$ treatment compared with the $Zn_{0.05}$ and $Zn_{0.1}$ treatments, while the difference between the $Zn_{0.05}$ and $Zn_{0.1}$ treatments was not significant.

7.3.2.8. Grain yield

Zinc fertilization and water stress both influenced grain yield in chickpea genotypes (Figure 7.13). The breeding line ICC 4958 produced the highest grain yield under adequate moisture and high zinc, while the response of Tyson to zinc and water supplies was relatively poor. Grain yield in Tyson increased slightly with increasing zinc supply when soil moisture was maintained at 12%, whereas plants experiencing water stress showed no increase in grain yield even at 2.5 mg zinc supply. Under zinc

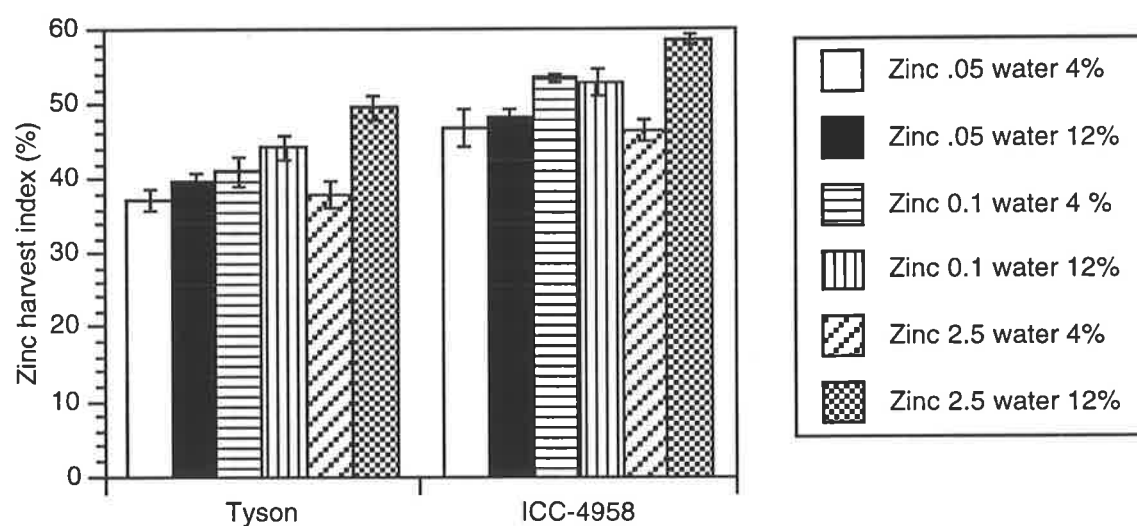


Figure 7.9: Effect of zinc fertilization and moisture regimes on the zinc harvest index [(zinc content in seed/zinc content in the above-ground dry matter) $\times$ 100] of two chickpea genotypes. The vertical bars represent standard errors based on four replicates.

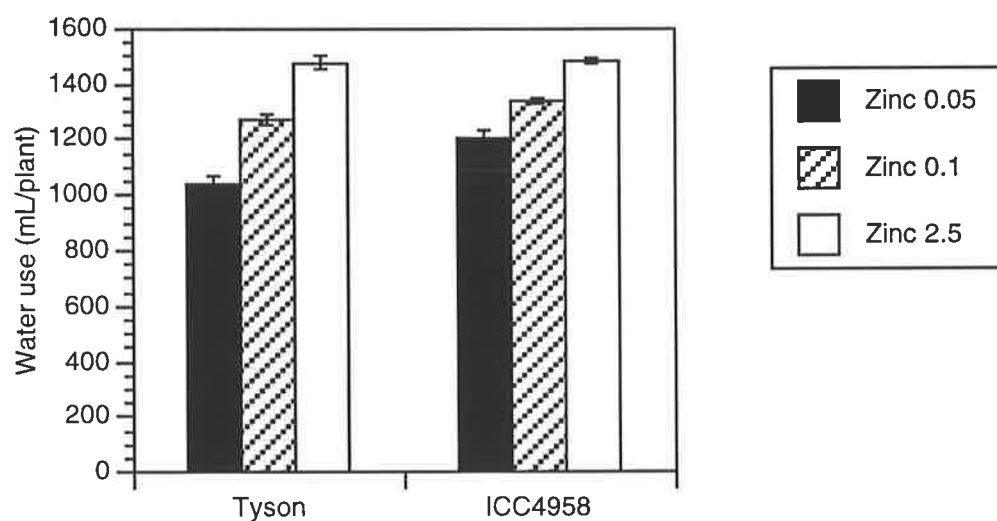


Figure 7.10: Effect of zinc fertilization on water use (mL/plant) from sowing until the start of pod formation when water stress was induced. The vertical bars represent standard errors based on four replicates.

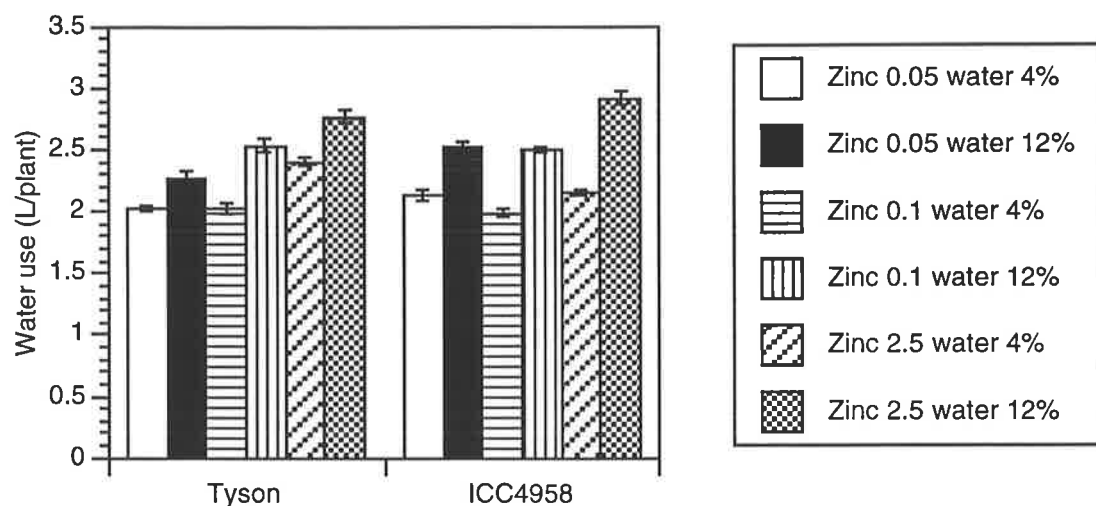


Figure 7.11: Effect of zinc fertilization on total water use (mL/plant) from sowing until physiological maturity in two chickpea genotypes. The vertical bars represent standard errors based on four replicates.

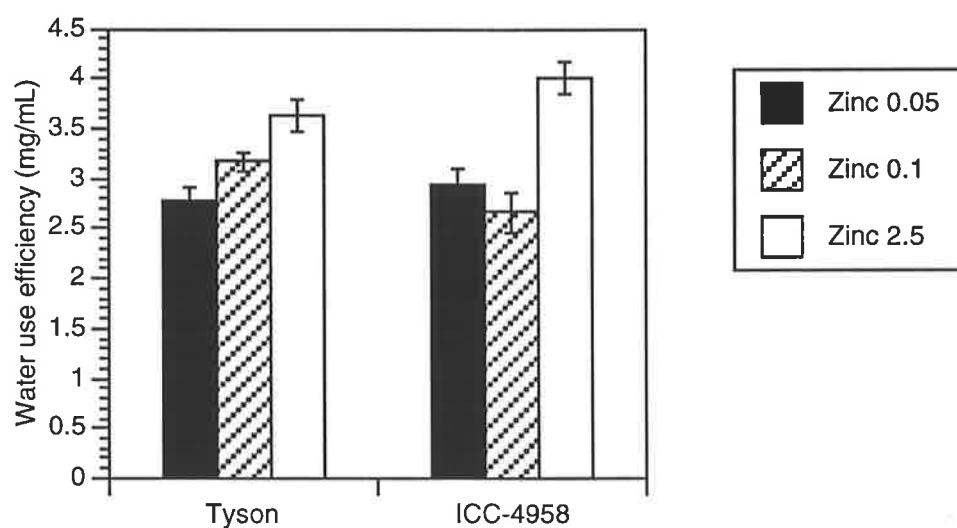


Figure 7.12: Effect of zinc fertilization on water use efficiency in two chickpea genotypes. Plants were harvested at the start of pod formation before inducing water stress. The vertical bars represent standard errors based on four replicates.

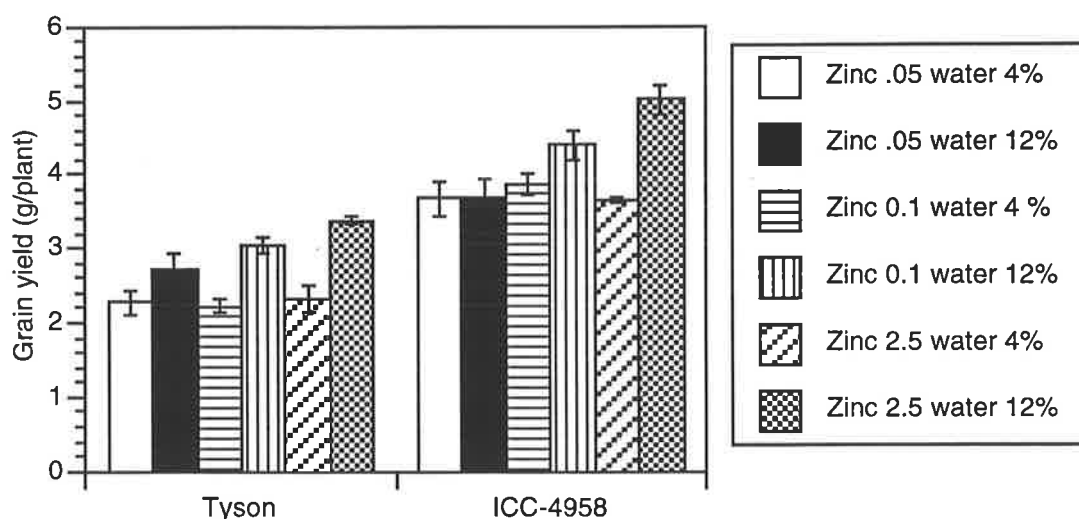


Figure 7.13: Effect of zinc fertilization and water stress on grain yield (g/plant) of two chickpea genotypes. The vertical bars represent standard errors based on four replicates.

deficient conditions, at both moisture regimes ICC 4958 produced significantly higher grain yield than that of Tyson. Based on the relative reductions in grain yield, the results suggested that the effect of water stress was severe when plants grown at higher zinc availability were subjected to water deficit.

7.4. Discussion

Results of the study showed that application of zinc enhanced vegetative growth in all genotypes. The response of genotypes in shoot dry matter, at low level of zinc ($Zn_{0.1}$) was in agreement with the results presented in Chapter 5; dry matter produced in Tyson was significantly lower than T-1587 and ICC 4958. Visual symptoms of zinc deficiency, such as slightly pale foliage and necrosis of leaf margins in older leaves, only appeared in plants grown at 0.05 mg zinc per kg soil in Experiment 2, nearly 4 weeks after sowing. However, these symptoms were not severe and almost disappeared at a later stage, although plants remained stunted. The treatment supplying 0.1 mg zinc per kg soil showed no symptoms of zinc deficiency on the foliage in both experiments, except a reduction in growth and slightly delayed maturity. Reuter *et al.* (1982), working with subterranean clover, detected hidden zinc deficiency in the absence of visible

symptoms, which presumably appear only in case of severe zinc deficiency. It appears, therefore, that chickpea plants were suffering in marginal zinc deficiency when supplied with 0.1 mg zinc per kg soil (Chapter 4).

Evapotranspiration in plants increased with increasing the level of zinc fertilization. The low water use by plants grown at low zinc supply could be attributed to their smaller size, having limited leaf area. Nable and Webb (1993) in a study using wheat, observed that withholding zinc from the lower root zone decreased water use by 12% in a zinc-sensitive variety Gatcher. The results of the study presented here suggested that zinc fertilization also increased water use efficiency and plants supplied with adequate zinc (2.5 mg/kg soil) had highest water use efficiency. It is also possible that zinc may have some physiological role in stomatal regulation, and zinc deficiency may have impaired the transpiration process by causing stomatal closure in plants. In pecan leaves, Hu and Sparks (1991) concluded that zinc deficiency reduced stomatal responsiveness and allowed gas exchange to occur over a range of conditions. Similarly, Sharma *et al.* (1995) working with cauliflower, suggested there is a specific requirement for zinc for stomatal opening, possibly because zinc is a constituent of certain enzymes. However, further experimentation is needed to examine the effect of zinc nutrition in stomatal conductance and plant water relations.

At adequate water supply and when soil zinc was deficient, Tyson produced significantly lower grain yield than T-1587 and ICC 4958 (Figures 7.4 & 7.8), confirming its sensitivity to zinc deficiency (Chapter 6). However, when zinc fertilization was increased and water availability was maintained at 12%, Tyson produced more vegetative growth but with little increase in the grain yield, whereas T-1587 and ICC 4958 produced considerably higher grain yield. In chickpea, high moisture availability during the later stages of growth can cause excessive vegetative growth which may restrict grain yield (Knights, 1991).

Chickpea's indeterminate growth habit results in the main stem and branches continuing to grow throughout the reproductive phase (Khanna-chopra and Sinha, 1987). This simultaneous growth of structural material and grain often creates a competition in distribution of assimilates between vegetative parts and developing seeds under high-input conditions (Singh and Pandey, 1980). Bonfil and Pinthus (1995) reported a considerably slower growth rate of chickpea seed compared to wheat grain, which was due to smaller allocation of assimilates to seeds of chickpea than to the grain of wheat during grain filling. The results of the present study showed that at adequate zinc supply and well-watered conditions, individual seed weight decreased with an increase in the number of shrivelled or under-develop seeds, particularly in Tyson. It indicates that assimilate supply could be a limiting factor during seed development, but genotypes may differ in allocation of assimilates. Almost similar observations was recorded by Saxena *et al.* (1990) who recorded a decline in individual seed weight in chickpea when supplementary irrigation was applied during reproductive growth. However, the irrigation ultimately increased yield by increasing the number of seeds per unit area. Sandhu *et al.* (1978) reported excessive vegetative growth in the chickpea cultivar C 235 (Tyson) in response to irrigations during reproductive stage in north India and recorded no advantage of high soil moisture in terms of grain yield as grain-to-straw ratio (the harvest index) decreased. These observations are consistent with the results pertaining to the harvest index in Experiment 1 (Figure 7.5) that 12% soil moisture during the reproductive phase caused an appreciable decrease in partitioning of dry matter towards developing seeds, particularly when zinc supply was adequate.

Another possible reason for poor performance of chickpea cultivars under high-input conditions is that most of the improvement of chickpea crop has been confined to selection for varieties and types to suit low-rainfall environments (Saxena, 1987) and to give some yield even in the most extreme conditions (Silim and Saxena, 1993b). The present study indicated that under favourable conditions partitioning of assimilates in chickpea was generally inadequate for seed development that resulted in the production of

shrivelled seed. This emphasizes the need for increasing the magnitude of the harvest index at increased biomass yield.

It is evident from the yield component data that the increase in grain yield in chickpea was mainly governed by the number of pods and consequently the number of seeds per plant. The results revealed that zinc application coupled with high soil moisture enhanced pod bearing capacity of the plants in all chickpea genotypes. Earlier studies with a number of legumes have shown that zinc fertilization increased the number of pods in subterranean clover (Riceman and Jones, 1958), cowpea (Ghildiyal *et al.*, 1978), chickpea (Thakur *et al.*, 1989) and navy bean (Moraghan, 1996). However, it is difficult to conclude from the available data whether an elevated number of pods per plant at adequate zinc supply resulted from higher number of flowers produced, or whether zinc availability increased realisation of flowers into pods by reducing flower abortion.

The reproductive phase is considered the most sensitive stage to drought stress in chickpea (Saxena, 1993). The results showed that water stress at the podding stage adversely affected grain yield in all genotypes at all levels of zinc fertilization. However, in water-stressed plants the relative reduction in grain yield was increased by increasing zinc supply. In fact, high zinc nutrition promoted plant growth which resulted in higher rates of water use by the plants. Although water stress was induced gradually by regular watering and decreasing soil moisture 1% (w/w) per day, the rate of development and severity of water stress is likely to have varied with the size of plants. It appears, therefore, that the plants fertilized with 2.5 mg zinc per kg soil have experienced more stress and consequently suffered higher yield losses compared with low zinc treatments. The limitations of pot assays, such as restricted root growth and rapid development of water deficit, which rarely occur under field conditions, did not allow the role of zinc nutrition in drought tolerance to be clearly defined and requires further investigation in relation to physiological characteristics. This view is also supported by Moraghan (1996) that prediction of field performance based on the results of glasshouse studies is not always possible because of the differences in rooting characteristics, water regimes

and soil profiles. It is likely that the extensive root systems of zinc-fertilized plants (Chapter 4) may penetrate into deeper soil profiles and acquire more water during dry seasons.

The distribution of zinc, as reflected in the tissue zinc concentration, varied widely within the plant. The comparison of zinc concentrations among stem, leaves and reproductive organs clearly indicated preferential accumulation of zinc in the floral and young pods at all levels of zinc fertilization. However, higher zinc supply ($Zn_{2.5}$) increased zinc concentration in the reproductive parts, relative to 0.1 and 0.05 mg zinc per kg soil, showing dependence of zinc concentration on zinc availability. The increase in zinc concentration in the floral parts and the young pods could be due to increase in demand of seed development for zinc. In beans (*Phaseolus vulgaris*) actively growing organs had a greater capacity to accumulate zinc (Ramani and Kannan, 1985), and Pate *et al.* (1977) have highlighted the significance of the legume pod, which acts as a temporary reservoir of seed-bound nutrients during seed development.

The results on zinc concentration also showed that zinc tended to accumulate in advance of dry matter in the young pods. This could be due to a precocious start to zinc accumulation during the early stage of reproductive growth, as found in lupin pods (Hocking and Pate, 1977). The retention of zinc in the young pod appeared to be temporary and it seems to be readily transferred to seed, probably through phloem transport. However, as a result of continued vegetative growth during final stages of reproductive development, dry matter in stem and leaves increased, which made it difficult to precisely estimate the material transported into the developing seeds. The amount of dry matter which was produced during the seed development appears to be sufficient to compensate for the transport of metabolites into the pods and the seeds.

The distribution of zinc in chickpea plant appears to have some relationship with the growth stage. The tissue analysis at maturity clearly showed a higher zinc concentration in all plant parts except the pod walls compared with zinc concentration at

the start of reproductive stage, which suggested that rate of zinc uptake was faster than the rate of dry matter accumulation. It also appears that zinc transport from root to the above-ground parts rapidly increased during seed development. These observations are also in agreement with the results of the field experiment (Chapter 6) where higher zinc concentration was recorded at the podding stage compared with the early vegetative stage. Pearson and Rengel (1994) reported that in wheat supplied with adequate zinc, roots acted as the largest pool of zinc; this pool was remobilized quickly to the head and developing grains during the first 14 days after anthesis. In the study presented here, zinc concentration in the root tissue at maturity was lower, particularly in $Zn_{0.05}$ and $Zn_{0.1}$ treatments compared with other plant parts, whereas at $Zn_{2.5}$ it was higher. This could be due to the translocation of zinc from senesced roots to the actively growing vegetative parts and seeds. Gregory (1988) observed that in chickpea the exponential period of root growth coincides with the period of rapid shoot growth; by the start of reproductive phase some of the early roots begin to senesce. It is probable that an elevated demand for zinc during seed filling in chickpea plants supplied with limited zinc may have induced early root senescence to enhance remobilization of stored zinc. Water stress significantly affected root zinc concentration only in the $Zn_{2.5}$ treatment and genotypes responded differently.

The senescence of older leaves during reproductive growth is common in chickpea. The results indicated that zinc concentration of the older abscised leaves was less than half compared to young attached leaves in most of the treatments. Similarly, pod walls contained significantly less zinc after seed maturity than that the young pods at the start of seed formation. These observations support the view that a considerable amount of zinc was remobilized from older leaves and pod walls to younger leaves and developing seed. The remobilization seems to be in the phloem and may have followed the same pathway as found in other assimilates, which are translocated from leaves to seeds (Singh and Pandey, 1980; Singh, 1991).

The profound effect of water stress on the zinc harvest index was noted in plants fertilized with 2.5 mg zinc per kg soil. Water stress significantly reduced the zinc harvest index in both genotypes, either by reducing remobilization of zinc or by affecting its uptake. Warncke and Barber (1972) indicated that the rate of zinc diffusion in soil increased with increasing soil moisture. An increase in soil moisture can increase the thickness of water films around soil particles which may reduce the chemical interaction between ion and soil. It was also inferred from the present results of the zinc harvest index that a considerable amount of zinc, particularly in low zinc treatments, was retained by the structural parts or lost through leaf abscission. This effect has important consequences for zinc recycling in the field. Plant parts having an appreciable zinc content, when incorporated in soil, may have a considerable residual effect on the following crop. Takkar *et al.* (1975) demonstrated in a wheat-groundnut rotation in India that a higher application of zinc (22 kg/ha) to the wheat crop provided adequate residual zinc to at least two additional crops. The residual effect of zinc application in chickpea can be predicted to be greater even as compared to cereals which retain their structural parts (leaves) during maturity.

Micronutrient malnutrition is common both in the developing world and the developed world. The extent of widespread deficiency suggests that almost 2 billion people are zinc deficient (Graham *et al.*, 1997). Chickpea is the most popular food legume and an important source of proteins and vital mineral nutrients for poor societies worldwide, particularly for vegetarian population groups in the Indo-Pakistan subcontinent (Pushpamma and Geervani, 1987). An elevated zinc concentration in chickpea seed is undoubtedly an impressive outcome of the zinc fertilization, because it produced seed with high zinc density. It is most likely that a crop produced on zinc deficient soils, without supplemental zinc application, will produce seeds having low zinc concentration. The present results showed that zinc concentration in chickpea seeds ranged from 8 to 13 mg/kg when zinc supply was low (0.05 or 0.1 mg/kg soil). Working with navy bean, Moraghan (1996) found that zinc application of 4 and 8 mg

zinc per kg soil increased seed zinc concentration by 60 and 68%, respectively, compared with the zero applied zinc treatment. Zinc concentration in the different types of beans in America, as published by the Human Nutrition Information Service, USA (1986), ranged from 29 to 41 mg/kg, which suggested that zinc concentration in the chickpea seed, produced in zinc-deficient treatments in the present study was low. Therefore, an improvement in the seed zinc concentration through soil application of zinc should be regarded as a desirable quality factor for human nutrition. In addition, seeds having high zinc content may improve seedling vigour due to the carry-over effect of zinc from seeds to seedlings (Rengel and Graham, 1995a) and may also increase resistance to soil-borne pathogens (Graham *et al.*, 1992) that pose a threat to chickpea production in many parts of the world (Nene and Reddy, 1987).

7.5. Conclusion

The application of 2.5 mg zinc per kg soil at high moisture availability increased grain yield mainly by increasing the number of pods per plant. High soil zinc also promoted vegetative growth at 12% soil moisture, which influenced translocation of assimilates into developing seeds; however, there was also decreased individual seed mass and consequently the increase in grain yield due to zinc application was relatively low. Plants grown under zinc-deficient conditions obviously could not exploit available soil moisture; water use was therefore suppressed in zinc-deficient plants.

A higher accumulation of zinc in floral parts and young pods at the start of the reproductive stage reflects precocious accumulation of zinc to be used by the developing seeds. The loss of zinc from old senesced leaves and pod walls was most probably due to translocation of zinc toward young leaves and seeds. Water stress apparently had no effect on distribution of zinc within the plants, but decreased seed zinc content in plants supplied with 2.5 mg zinc per kg soil. Among plant organs, seed accumulated the highest amount of zinc, which is considered a beneficial nutritional trait for human nutrition.

CHAPTER 8

Effect of zinc fertilization and water stress on plant water relations, stomatal conductance and osmotic adjustment in chickpea

8.1. Introduction

Drought accompanied with mineral nutrient deficiencies is a common limiting factor responsible for low productivity of chickpea in the major chickpea growing areas worldwide (Johansen *et al.*, 1994; Saxena, 1993; Singh and Das, 1987). The crop, whether grown on stored soil moisture in the Indo-Pakistan subcontinent (Saxena, 1987) or sown in the Mediterranean-type environments (Saxena *et al.*, 1990b), often suffers from moisture deficits during its growth and development. Many of the soils on which chickpea is grown in these regions have low available zinc and hence are categorised as zinc-deficient (Section 2.3.2.2).

Zinc deficiency affected plant water relations by lowering osmotic potential, increasing water saturation deficit and inducing stomatal closure in cabbage (Sharma *et al.*, 1984). Low zinc availability also increased diffusive resistance in cauliflower plants and consequently decreased transpiration rate (Sharma and Sharma, 1987). Studies with pecan (Hu and Sparks, 1991) and cauliflower (Sharma *et al.*, 1995) revealed that zinc played a specific role in stomatal regulation, and its deficiency caused closure of stomata.

The effect of zinc on stomatal regulation may have an important effect on plant water use efficiency. Over the past decade, the C isotopic composition of plants has been used to study genotypic and environmental control of water use efficiency (Hubick *et al.*, 1986; Condon *et al.*, 1987), and it would be valuable to use this method to examine the effects of zinc nutrition on water use efficiency. During photosynthesis in C₃ plants there is discrimination against the naturally occurring stable isotope ¹³C which affects the

isotopic composition of plant material (Farquhar *et al.*, 1982). Carbon isotope discrimination (Δ) is largely affected by the ratio of the intercellular (p_i) and atmospheric (p_a) partial pressures of CO_2 (p_i/p_a); the lower the ratio, the lower the value of carbon isotope discrimination. Since stomatal aperture affects p_i/p_a , if zinc deficiency alters stomatal regulation, there may be corresponding changes in Δ . Variation in Δ also shows a strong correlation with genotypic variation in water use efficiency (Hubick *et al.*, 1986; Condon *et al.*, 1987). The results pertaining to water use efficiency (Chapter 7) indicated that plants supplied with low zinc had significantly lower water use efficiency compared to zinc-fertilized plants, despite being smaller and with a lower rate of water use. Measurements of Δ may therefore indicate whether zinc has a physiological ~~role in water use efficiency~~ ^{effect on transpiration through its influence on stomatal aperture}, as well as enabling genotypic variation in ^{transpiration} ~~water use efficiency~~ to be studied.

Plants have evolved various physiological mechanisms to endure periods of water stress (Levitt, 1980). Many drought-tolerant plants are known to rely on osmotic adjustment as it helps to maintain turgor during periods of water deficit and hence sustain metabolic activity (Ludlow *et al.*, 1985). Osmotic adjustment is defined as the active accumulation of solutes within the plant tissue in response to dehydration (Jones and Turner, 1978; Morgan, 1984). Among the cool-season grain legumes, chickpea is considered a drought-tolerant species, and there is evidence of osmotic adjustment in chickpea during periods of moisture stress (Morgan *et al.*, 1991; Turner *et al.*, 1996; Amede and Schubert, 1997). As zinc deficiency impairs various physiological processes, it was hypothesized that zinc deficiency may weaken the ability of plants to tolerate drought by affecting plant water relations and its ability for osmotic adjustment. The present study was designed to investigate the effect of zinc nutrition on plant water relations and especially on the degree of osmotic adjustment.

In this chapter two pot experiments are presented. The first experiment deals with the response of four chickpea genotypes differing in sensitivity to zinc deficiency (Chapter 5) to water stress, grown with and without soil applied zinc. In the second

experiment different components of plant water relations were determined and the level of osmotic adjustment was estimated in zinc-deficient and zinc-sufficient plants of a zinc sensitive chickpea cultivar Tyson.

8.2. Materials and methods

8.2.1. Experiment 1

The experiment was designed as a completely randomized factorial design having three replicates. Soil preparation and basal nutrient solutions were the same as described in Chapter 3 (Section 3.2). Two zinc levels, no applied zinc (zinc-deficient) and 2.5 mg zinc per kg soil (zinc-adequate), were selected and designated as zinc- and zinc+, respectively. The soil (3.0 kg) was weighed into polythene-lined PVC pots measuring 110 mm diameter $\times$ 300 mm deep. DD water was added in amounts sufficient to bring the soil water content to field capacity (12% w/w). A layer of black polypropylene beads approximately 1 cm deep was placed over the soil surface in the pots as a mulch to reduce evaporation. The soil moisture was kept at field capacity (12% w/w) in all pots by adding DD water on alternate days during the initial stage of growth and then daily until the induction of the water stress, 53 days after sowing. Water stress was induced in all pots gradually by decreasing water application by 1% (w/w) per day to bring the moisture level down from field capacity to moisture stress. ^(c. 2% w/w) _^ Pots were weighed daily and if water use was $> 1\%$ (w/w), water was added. The aim was to control the rate of development of the stress to avoid rapid water deficits occurring and to keep the development of soil water deficits by plants of different sizes (due to zinc fertilization) as similar as possible, so that the stress treatments were not confounded by different rates of development of stress.

Seed of four genotypes which differed in sensitivity to zinc deficiency viz, Tyson, ICC 4958, T-1587 and NIFA 88 (Chapter 5), having similar seed weight within each variety (153 mg/seed for Tyson, 270 mg/seed for ICC 4958, 205 mg/seed for T-1587 and 172 mg/seed for NIFA 88), were selected and planted as described in Chapter

3 (Section 3.2). Seed zinc contents were 4.9, 5.4, 5.0, and 5.5 $\mu\text{g}/\text{seed}$ for Tyson, ICC 4958, T-1587 and NIFA 88, respectively. Initially, five seeds were sown in each pot and these were later thinned to three plants per pot.

Plants were grown in a growth chamber at the Waite Agricultural Research Institute (Adelaide, South Australia) for 65 days. Photoperiod was adjusted to 10 hours light and 14 hours dark, and temperature was 18 and 10 $^{\circ}\text{C}$ during the day and night, respectively. Photosynthetically active radiation (PAR), supplied with halide lamps was 440 $\mu\text{mol/s per m}^2$ at the canopy level. Leaf water potential (ψ) was measured using a pressure chamber (Scholander *et al.*, 1965). Relative water content (RWC) was determined on 10 leaflets (cut in half) from the upper-most fully expanded leaf (4th - 5th from top) of the main stem or one of the basal branches (Morgan *et al.*, 1991) on days 0, 2, 5, 7, 9 and 12 after induction of water stress (for techniques see Section 3.3). Stomatal conductance was determined on both abaxial and adaxial surfaces of the terminal leaflet of the upper-most fully expanded leaf of the main stem or one of the basal branches 10 days after induction of water stress. Water relations measurements were taken between 12:00 and 14:00 h. Plant material used for these measurements was saved in the labelled bags and later included in the total shoot dry matter. For estimation of carbon isotope discrimination, oven-dried leaf material was ground to pass a 100- μm sieve using a ball mill (SPEX Industries, Edison, USA, Model 8000-230). The stable carbon isotope composition in leaf tissue was determined by a mass spectrometer using techniques and equipment described by Condon *et al.* (1987). Carbon isotope discrimination was calculated using the method of Hubick *et al.* (1986), assuming an isotopic composition for the air (δ_{air}) of -8‰ (Farquhar and Richards, 1984) relative to the international standard:

$$\Delta = (\delta_{\text{air}} - \delta_{\text{leaf}}) / (1 + \delta_{\text{leaf}})$$

where Δ = carbon isotope discrimination; δ_{air} = carbon isotope composition in the air and δ_{leaf} = carbon isotope composition in leaf tissue.

Ethanol-soluble sugars in leaf tissues of 65-day-old plants were determined by boiling the samples in 80% ethanol/water (v/v) and assessing soluble carbohydrates using the method described by Dubois *et al.* (1956). For extraction, 25 mg of finely ground leaf material was weighed into 10 mL centrifuge tubes, then 5 mL 80% ethanol was added, and samples were boiled for 30 min in a water bath at 80 °C. After cooling the samples to room temperature, 5 mL of 80% ethanol was added to the each tube. The samples were centrifuged for 10 min at 10,000 r.p.m., and the supernatant was transferred into a centrifuge tube, and volume was made up to 10 mL with 80% ethanol. A portion (0.1 mL) of ethanol extract was pipetted into 150 × 18 mm glass tubes and 1 mL of aqueous phenol was added. A jet of concentrated sulphuric acid (5 mL) was pumped into the sample and the contents were vortexed when hot. After cooling the samples to room temperature, optical density was measured at 490 nm using a spectrophotometer. The assay was calibrated with a standard curve of sucrose (0 to 100 µg per tube), and results were expressed as sucrose equivalents to per g tissue dry weight.

For nutrient analysis, leaf material was dried in an oven at 80 °C for 48 hours and analysed by ICP spectrometry (Chapter 3, Section 3.4). The data recorded during the course of experiment were statistically analysed as described in Chapter 3 (Section 3.5).

8.2.2. Experiment 2

The experiment was established as a completely randomized factorial design having two zinc treatments, two moisture levels (well-watered and stressed) with four replicates. Soil preparation and basal nutrient solutions were the same as described in Chapter 3 (Section 3.2). Two zinc levels, no added zinc (zinc-deficient) and 2.5 mg zinc per kg of soil (zinc-adequate), were selected and designated as zinc- and zinc+, respectively. The soil (3.0 kg) was weighed into polythene-lined PVC pots measuring 110 mm diameter × 300 mm deep. DD water was added in amounts sufficient to bring the soil water content to field capacity (12% w/w). A layer of black polypropylene beads

approximately 1 cm deep was placed over the soil surface in the pots as a mulch to reduce evaporation. The water stress treatment was induced relatively earlier (40 days after sowing) compared to Experiment 1 in half of the pots (W_1) before zinc deficiency reduced growth substantially, in order to minimize the effect of plant size due to zinc fertilization. Water stress was imposed gradually as described earlier (Section 8.2.1) to allow plants to adapt to moisture deficits. The remaining half (W_2) continued to be watered daily to field capacity throughout the course of the experiment.

A zinc-sensitive chickpea cultivar Tyson (Chapter 5) was selected. Seeds were hand-sorted from the same lot used in the Experiment 1 and planted as described in Chapter 3 (Section 3.2). Plants were grown in a growth chamber at the Waite Agricultural Research Institute (Adelaide, South Australia) for 63 days. Photoperiod, temperature and photosynthetically active radiation (PAR) were similar to that described for Experiment 1 (Section 8.2.1). Relative water content (RWC), water potential (ψ) and osmotic potential (ψ_π) were determined on days 0, 5, 9, 14, 18 in all pots, and on day 23 only in zinc-deficient plants, after induction of water stress. To measure osmotic potential (ψ_π), 10 leaflets from the same leaves used for RWC were excised directly into a plastic microcentrifuge tube and immediately frozen in liquid nitrogen. The tubes were later transferred to -80°C and kept frozen until measurements. The frozen leaf material was left at room temperature for thawing for 30 min and sap was extracted using a 2.5-mL plastic syringe. A filter paper disc was immersed into the sap and allowed to fully saturate. The saturated disc was placed in the sampling chamber of an osmometer (model 5100B; Wescor, USA) to measure osmotic potential (Qian and Fry, 1997). Turgor potential (ψ_p) was calculated from the difference of ψ and ψ_π . Stomatal conductance was determined, on both abaxial and adaxial surfaces of the terminal leaflet of upper-most fully expanded of the main stem or one of the basal branches, 15 days after induction of the water stress treatment. Relative water content, water potential and stomatal conductance were measured between 12:00 and 14:00 h. Plant material used for these measurements was saved and included in the estimates of total shoot dry matter.

To assess osmotic adjustment, the method of Chapman and Augé (1994) was used. The main stem or one of the basal branches having 6-7 leaves of both droughted and well-watered plants were excised after the development of moisture stress at similar soil water content (approximately 1% w/w) on day 14 and day 21 in zinc+ and zinc- plants, respectively. Shoots were brought to full hydration by placing the cut end in DD water for 4 hours in the dark at room temperature (20 °C). After rehydration, the osmotic potential at full turgor (ψ_{π}^{100}) was estimated: ten leaflets from the upper-most fully expanded leaf were excised and placed into a microcentrifuge tube, frozen in liquid nitrogen and osmotic potential was recorded as described above. Osmotic potential at full turgor in water-stressed and well-watered plants was designated as $(\psi_{\pi}^{100})^a$ and $(\psi_{\pi}^{100})^b$, respectively. Leaf osmotic adjustment was calculated using the following formula:

$$OA = (\psi_{\pi}^{100})^b - (\psi_{\pi}^{100})^a$$

For nutrient analysis, leaf material was dried in an oven at 80 °C for 48 hours and analysed by ICP spectrometry (Section 3.4). All the data recorded in the experiment were statistically analysed as described in Chapter 3 (Section 3.5).

8.3. Results

8.3.1. Experiment 1

8.3.1.1. Shoot dry matter and leaf tissue zinc concentration

Zinc fertilization increased plant growth, which resulted in higher shoot dry matter in plants supplied with zinc compared to zinc-deficient plants (Figure 8.1). However, genotypes varied in their sensitivity to zinc deficiency, with ICC 4958 producing highest shoot dry matter and Tyson being consistently the most sensitive genotype.

Zinc concentration in the leaf tissue was much higher (> 30 mg/kg) in zinc-fertilized plants than plants grown without zinc application (Figure 8.2) and that

depended on their seed zinc reserve and on native soil zinc for their zinc supply. The average zinc concentration in the leaves of zinc- plants was 13 mg/kg dry weight which showed that plants were suffering from zinc deficiency. At adequate zinc supply, NIFA-88 had significantly higher zinc concentration than the other genotypes, which may be partly associated with its relatively slow growth.

8.3.1.2. Water use and water use efficiency

The application of zinc increased water use in plants; zinc-deficient plants used significantly less water during their growth (Figure 8.3). Under zinc-deficient conditions, genotypes differed in total water usage and in zinc-starved plants of Tyson, water use was reduced by 61% compared with the zinc-adequate plants. However, water consumption was relatively less affected by zinc deficiency in ICC 4958 as reduction was only 36% compared to zinc-sufficient plants.

Zinc fertilization significantly increased water use efficiency in Tyson, T-1587 and NIFA-88. In ICC-4958 water use efficiency was slightly higher in zinc-fertilized plants compared with zinc-deficient plants, but the difference was not significant. Under severely zinc deficient conditions, ICC 4958 had the highest and NIFA-88 the lowest water use efficiencies while Tyson and T-1587 were intermediate (Figure 8.4).

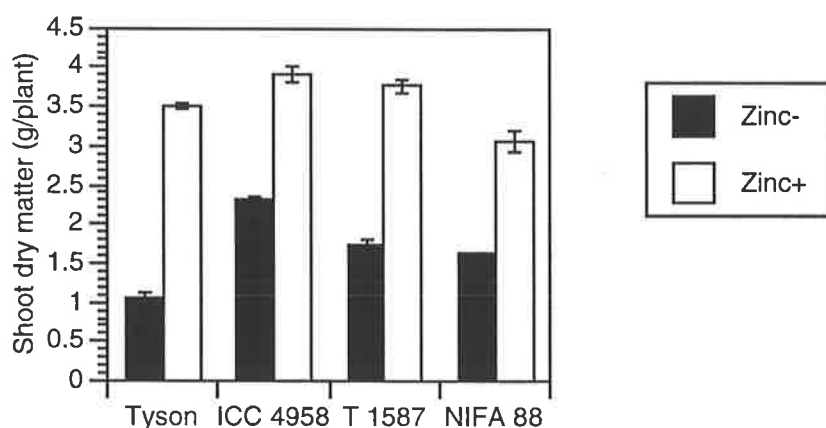


Figure 8.1: Effect of zinc fertilization on shoot dry matter (g/plant). Plants of four chickpea genotypes were subjected to water stress for 12 days before final harvest (65 days after sowing). The vertical bars represent standard errors based on four replicates.

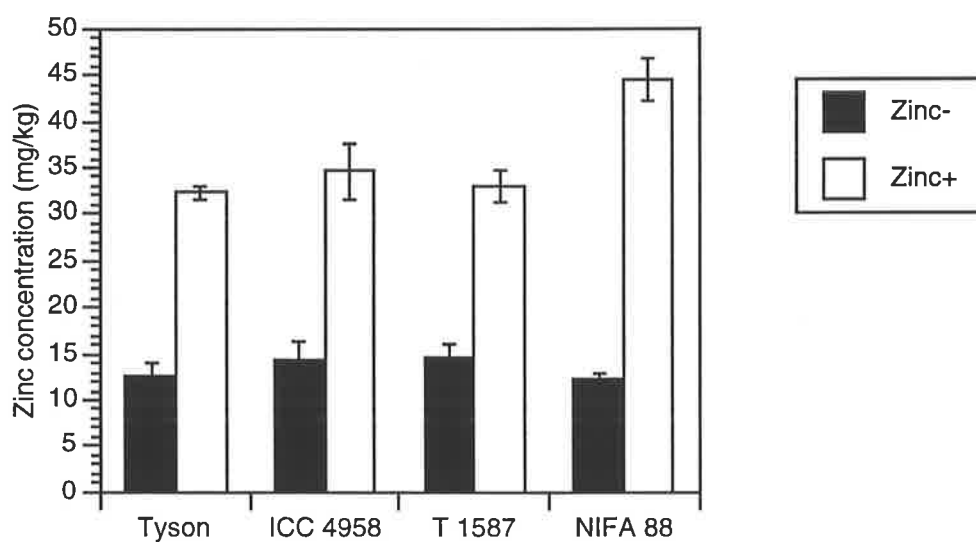


Figure 8.2: Effect of zinc fertilization on zinc concentration in the leaf tissue. Plants of four chickpea genotypes were subjected to water stress for 12 days before final harvest (65 days after sowing). The vertical bars represent standard errors based on four replicates.

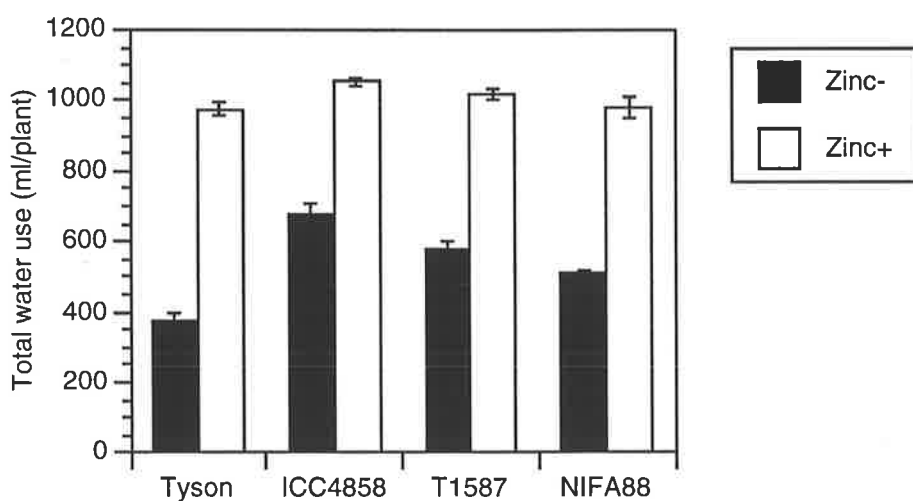


Figure 8.3: Effect of zinc fertilization on total water use (mL/plant). Plants of four chickpea genotypes were subjected to water stress for 12 days before final harvest (65 days after sowing). The vertical bars represent standard errors based on four replicates.

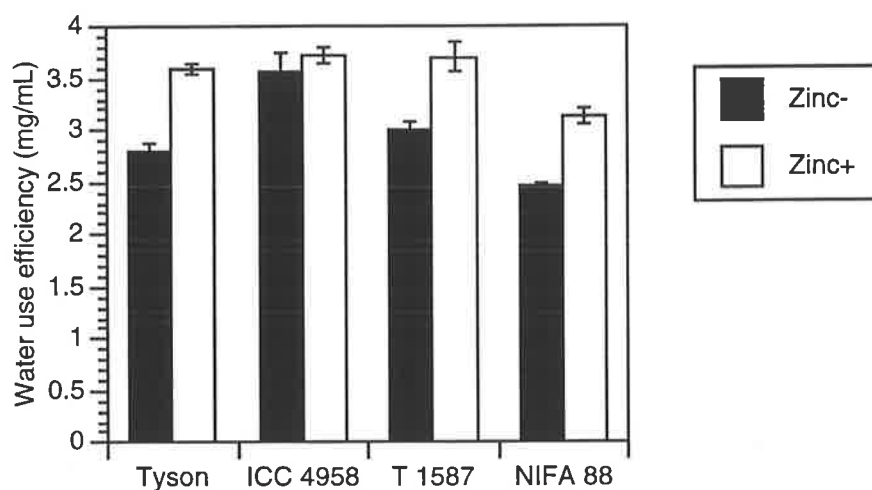


Figure 8.4: Effect of zinc fertilization on water use efficiency (mg/mL). Plants of four chickpea genotypes were subjected to water stress for 12 days before final harvest (65 days after sowing). The vertical bars represent standard errors based on four replicates.

8.3.1.3. Plant water relations

At the start of the water stress treatment, when water supply was adequate, leaf water potential in zinc-deficient plants of Tyson and T-1587 was slightly but significantly higher than that of zinc adequate plants. A similar trend was evident in ICC 4958 and NIFA 88 but the differences in water potential were non-significant. Plants supplied with sufficient zinc maintained leaf water potential (-0.5 MPa) for almost 7 days after the initiation of water stress but after that showed a sharp decline (Figure 8.5). However, the development of water stress was relatively slow under zinc-deficient conditions, as water potential fell nearly 10 days after imposition of water stress in all genotypes except in Tyson, which showed no decline in leaf water potential. In Tyson severe zinc deficiency decreased water use by greatly reducing growth and this was associated with plants having a higher water potential. At the end of the water stress period, 12 days after the induction of water stress, the water potential was relatively higher in zinc-deficient plants (-0.8 MPa) compared to the plants supplied with adequate zinc (< -1.2 MPa).

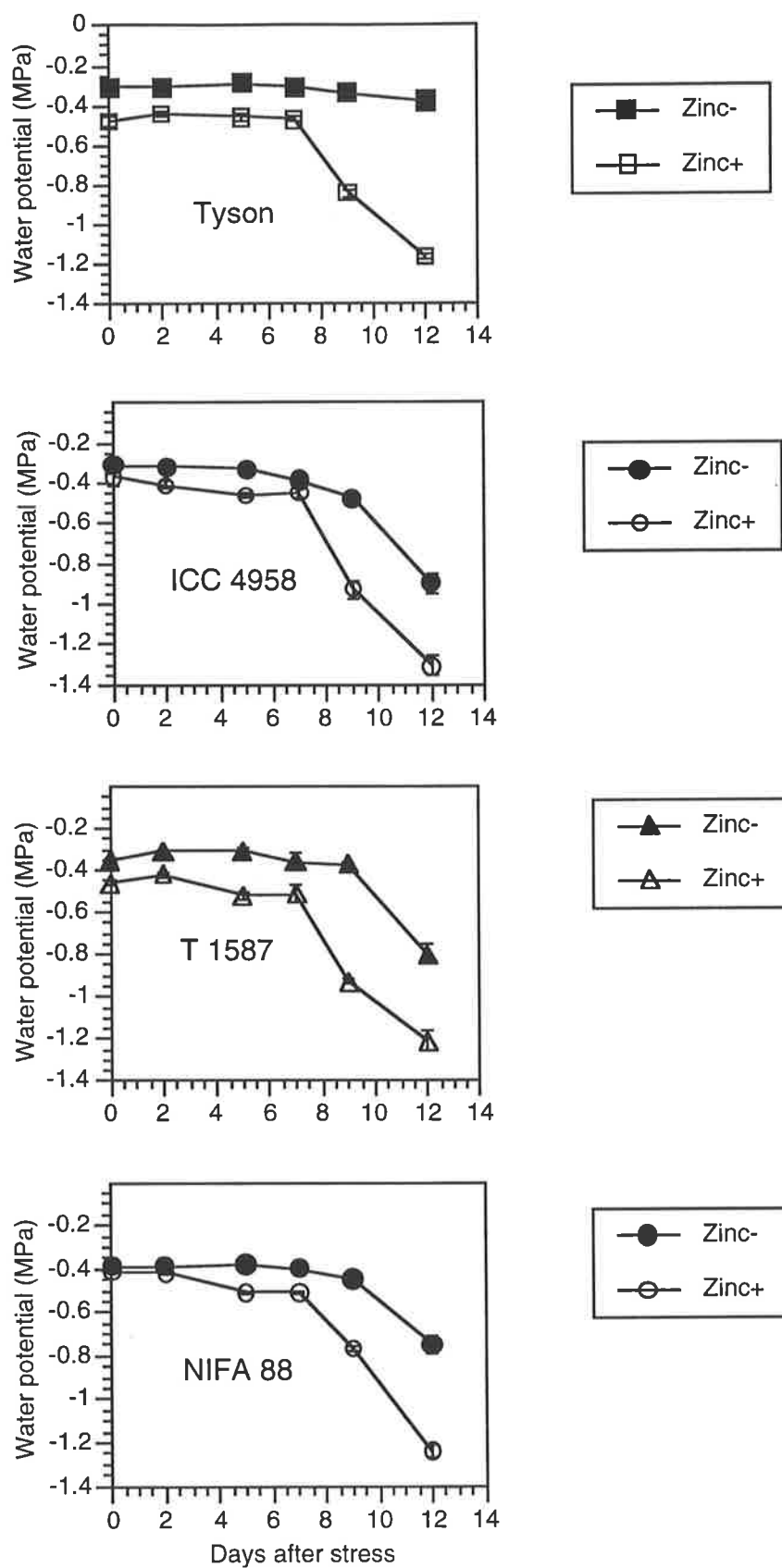


Figure 8.5: Effect of zinc fertilization and water stress on leaf water potential (ψ). Plants were 53-day-old when water stress was induced. The vertical bars represent standard errors based on four replicates.

Relative water content in zinc-deficient plants also remained high and showed little decline in Tyson, T-1587 and NIFA-88 during water stress (Figure 8.6). However, in ICC 4958 relative water content fell considerably 12 days after the initiation of water stress. At adequate zinc supply, plants maintained water content initially for almost 7 days but after that relative water content dropped sharply, particularly in ICC 4958 as water stress developed.

8.3.1.4. Stomatal conductance

Stomatal conductance prior to the induction of water stress at adequate water supply was significantly less in plants grown without zinc fertilization in all genotypes compared to zinc-sufficient plants (Table 8.1); among genotypes, Tyson had the lowest stomatal conductance in the zinc- treatments. However, at higher zinc availability, Tyson exhibited significantly higher stomatal conductance than the other genotypes, which suggests that low stomatal conductance in Tyson at low zinc supply was probably associated with its higher sensitivity to zinc deficiency

Stomatal conductance decreased significantly when plants were exposed to water stress. Under restricted water supply, the relative reduction in stomatal conductance was higher in zinc-sufficient plants ⁱⁿ than [^] those grown without soil-applied zinc. The water-stressed plants of Tyson grown without zinc application had higher stomatal conductance than other genotypes, probably due to their smaller size which depleted less water and consequently experienced less severe soil moisture deficits.

8.3.1.5. Ethanol-soluble sugars

The concentration of ethanol-soluble sugars in the leaf tissue of water-stressed plants showed no difference among zinc treatments (Table 8.2). However, genotypes exhibited significant differences, with ICC-4958 having the highest concentration (196 mg/kg) of ethanol-soluble carbohydrates in its leaf tissue followed by Tyson (164 mg/kg)

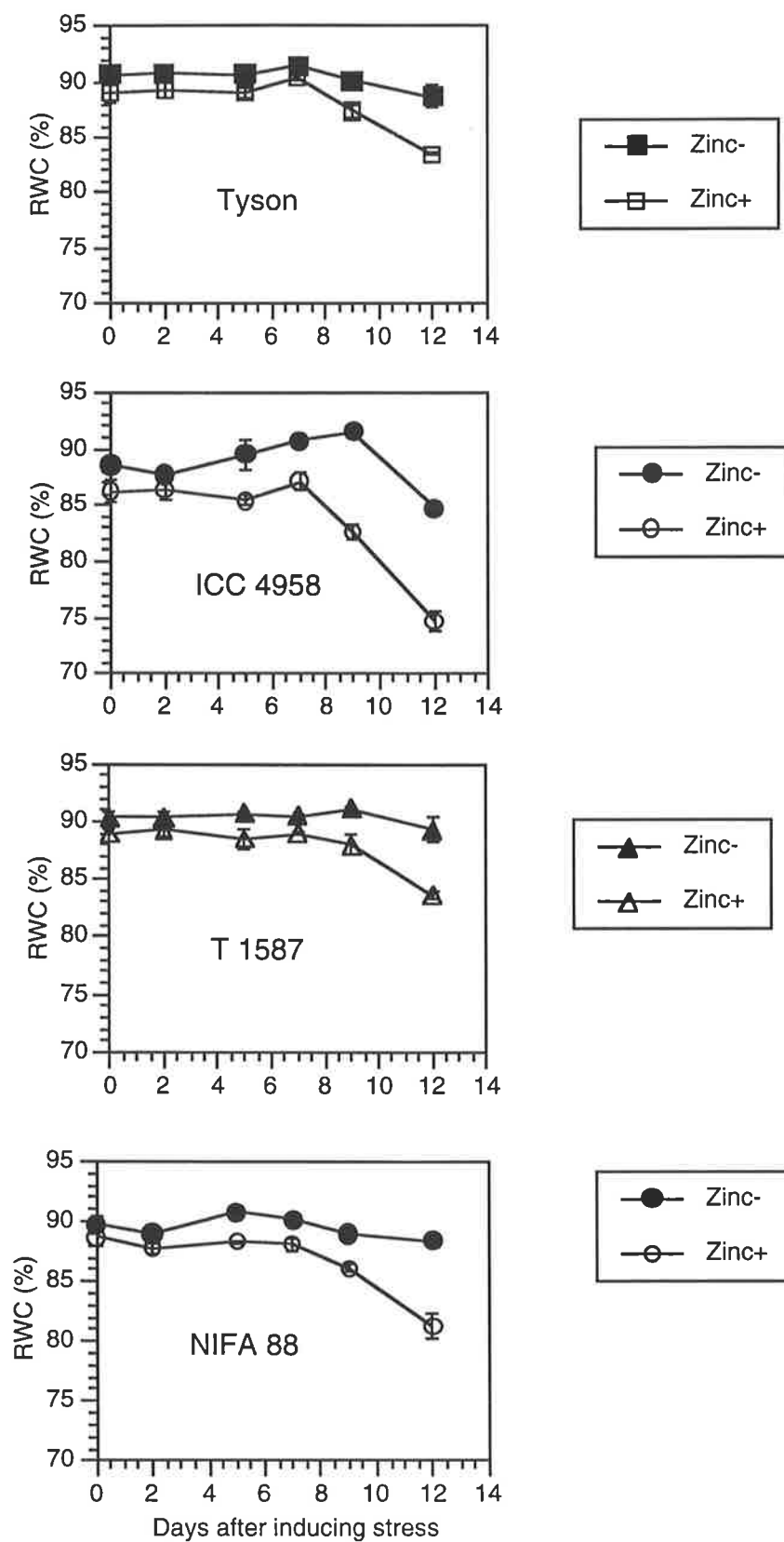


Figure 8.6: Effect of zinc fertilization and water stress on relative water content (RWC) in chickpea leaves. Plants were 53-day-old when water stress was induced. The vertical bars represent standard errors based on four replicates.

Table 8.1: Effect of zinc nutrition on stomatal conductance ($\text{m mol/m}^2/\text{s}$) in leaves of four chickpea genotypes before and after induction of water stress. Water stress was induced 53 days after sowing.

Genotypes	Stomatal conductance ($\text{m mol/m}^2/\text{s}$)					
	Before water stress			10 days after water stress		
	zinc-	zinc+	Mean (Gen)	zinc-	zinc+	Mean (Gen)
Tyson	353	886	620	116	54	85
ICC 4958	500	677	589	93	56	75
T 1587	435	668	552	94	51	73
NIFA 88	426	694	560	91	73	82
Mean (zinc)	429	731		98	59	
LSD (P = 0.05)						
Genotype		ns			ns	
Zinc		69			9	
Gen $\times$ Zinc		138			18	

ns: non significant

Table 8.2: Effect of zinc nutrition on ethanol-soluble carbohydrates (mg/kg) in water-stressed chickpea leaves. Water stress was induced 53 days after sowing and plants were harvested 12 days after induction of water stress.

Genotypes	Ethanol soluble sugars (mg/kg dry weight)		
	zinc-	zinc+	Mean (Gen)
Tyson	163	167	164
ICC 4958	199	193	196
T 1587	149	169	159
NIFA 88	139	127	133
Mean (Zinc)	162	164	
LSD (P = 0.05)			
Genotype		22	
Zinc		ns	
Gen $\times$ Zinc		ns	

ns: non significant

and T-1587 (159 mg/kg). The genotype NIFA 88 tended to have significantly lower ethanol soluble sugars (133 mg/kg) compared to other genotypes.

8.3.1.6. Concentration of other nutrients

Zinc deficiency increased the concentration of various micro- as well as macro-nutrients in the leaf tissue (Table 8.3). Among micronutrients, the concentration of Fe, Mn, B, Cu and Mo was significantly higher when plants were grown under severe zinc deficiency. Similarly, the concentration of macronutrients such as Ca, Mg, P and S also increased considerably in the leaf tissue of zinc-deficient plants. The elevated concentration of nutrients could be either due to impairment of certain metabolic activities, or under severely zinc-deficient conditions uptake of various nutrients may have been enhanced due to nonselectivity of ions or related to similarities in uptake mechanisms of zinc.

8.3.1.7. Carbon isotope discrimination

Carbon isotope discrimination (Δ) varied significantly among chickpea genotypes and was affected by the zinc treatments, while the genotype $\times$ zinc interaction was not significant (Table 8.4). Among genotypes, the range of isotope discrimination was 2.57×10^{-3} and NIFA-88 had highest value of Δ (21.77×10^{-3}). Carbon isotope discrimination was relatively low in ICC-4958 (19.20×10^{-3}) compared to other genotypes. The difference between zinc treatment was significant, indicating that zinc status of plants influenced stomatal opening or enzyme activity. The relationship between carbon isotope discrimination and dry matter showed a strong negative correlation in zinc-sufficient plants, while no relationship was observed in zinc-deficient plants (Figure 8.7). Transpiration efficiency was negatively correlated with Δ in both zinc treatments, but the slope was more negative and the goodness of fit greater in high zinc plants compared to zinc-deficient plants (Figure 8.8).

Table 8.3: Effect of zinc fertilization on concentration of other nutrients in the leaves of four chickpea genotypes. Plants were subjected to water stressed for 12 days before final harvest (65 days after sowing). Data represent the average of all genotypes.

Nutrients	Zinc-	Zinc+	LSD (P = 0.05)
<u>Micronutrients (mg/kg)</u>			
Fe	158.4	65.2	11.6
Mn	141.7	133.7	7.4
B	57.3	32.4	3.4
Cu	19.4	15.3	1.7
Mo	45.0	18.4	2.9
<u>Macronutrients (g/kg)</u>			
Ca	25.0	18.1	1.5
Mg	2.8	1.7	0.2
P	5.5	3.8	0.2
S	4.8	4.0	0.3

Table 8.4: Effect of zinc nutrition and water stress on carbon isotope discrimination ($\Delta \times 10^{-3}$) in leaves of four chickpea genotypes. Water stress was induced 53 days after sowing and plants were harvested 12 days after induction of water stress.

Genotypes	Carbon isotope discrimination ($\Delta \times 10^{-3}$)		
	zinc-	zinc+	Mean (Gen)
Tyson	18.77	20.62	19.70
ICC 4958	18.47	19.93	19.20
T 1587	19.57	20.28	19.93
NIFA 88	21.79	21.74	21.77
Mean (Zinc)	19.65	20.64	
LSD (P = 0.05)			
Genotypes		0.70	
Zinc		0.50	
Gen $\times$ Zinc		ns	

ns: non significant

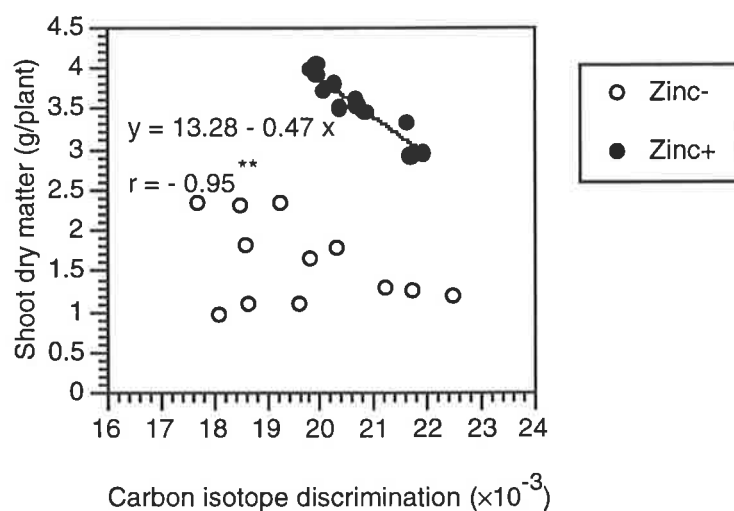


Figure 8.7: Relationship between carbon isotope discrimination and shoot dry matter in water-stressed plants as influenced by zinc fertilization. Plants were subjected to water stress for 12 days before harvest (65 days after sowing).

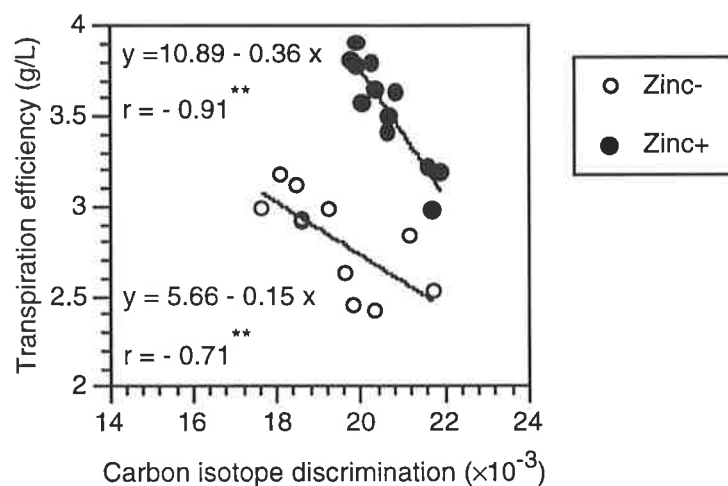


Figure 8.8: Relationship between carbon isotope discrimination and transpiration efficiency in water-stressed plants as influenced by zinc fertilization. Plants were subjected to water stress for 12 days before harvest (65 days after sowing).

8.3.2. Experiment 2

8.3.2.1. Shoot dry matter and shoot tissue zinc concentration

The restricted water supply suppressed plant growth 53% at adequate zinc availability and had no effect under severely zinc-deficient conditions (Figure 8.9). Shoot dry matter produced by zinc-deficient plants was 26% and 53% of that recorded at sufficient zinc supply in well-watered and water-stressed plants, respectively.

The application of zinc increased shoot tissue zinc concentration several-fold in zinc-sufficient plants compared to those grown without application of zinc (Figure 8.10). The values of zinc concentration clearly show that plants in the zinc- treatment were suffering from zinc deficiency while plants supplied with 2.5 mg zinc per kg soil had adequate shoot zinc concentration.

8.3.2.2. Water use and water use efficiency

Total water use was mainly affected by the zinc treatments, with the application of zinc significantly increasing water use presumably by promoting growth (Figure 8.11). The differences in water usage among zinc-deficient plants were not significant at both moisture regimes, which showed that under severely zinc-deficient conditions plants were unable to utilize available soil moisture.

Water use efficiency was significantly higher in the plants supplied with 2.5 mg zinc per kg soil compared with severely zinc deficient plants at both moisture regimes, showing that zinc fertilization improved water use efficiency (Figure 8.12). The induction of water stress also influenced water use efficiency and water-stressed plants had greater water use efficiency than the well-watered plants, but the relative increase was more in zinc-fertilized plants when compared with zinc-deficient plants.

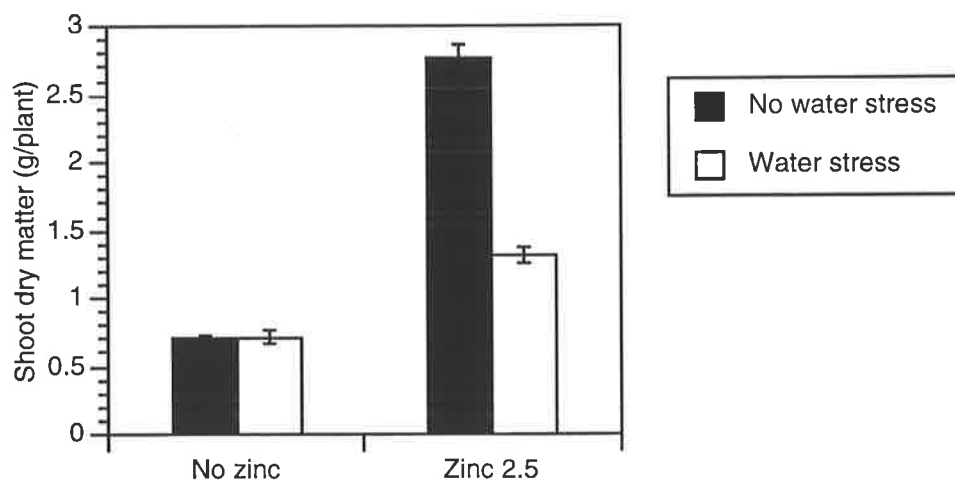


Figure 8.9: Effect of zinc fertilization and water stress on shoot dry matter of Tyson. Plants were 40-day-old when water stress was induced; they were harvested 23 days after stress. The vertical bars represent standard errors based on four replicates.

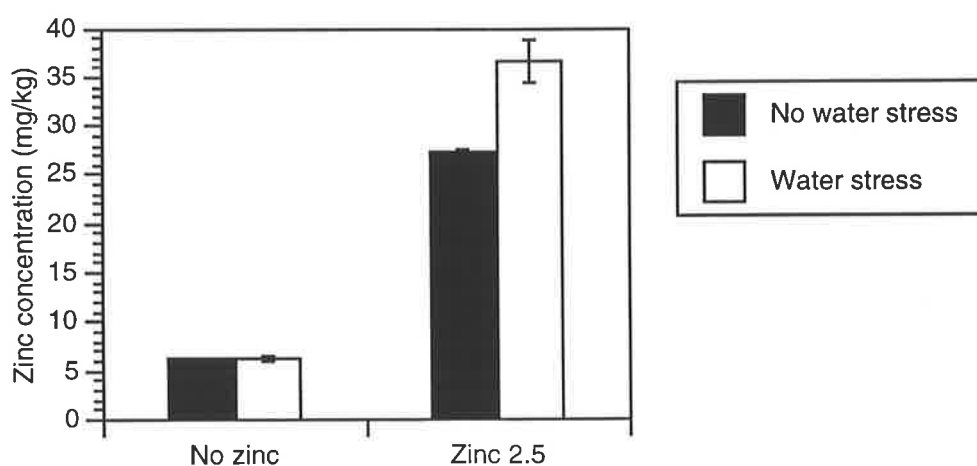


Figure 8.10: Effect of zinc fertilization and water stress on zinc concentration in shoot tissue. Plants were 40-day-old when water stress was induced; they were harvested 23 days after stress. The vertical bars represent standard errors based on four replicates.

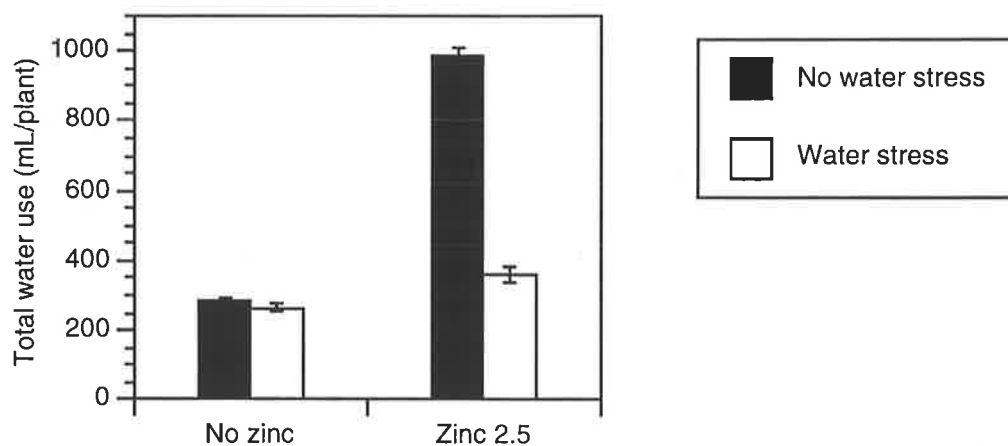


Figure 8.11: Effect of zinc fertilization and water stress on total water use (mL/plant) by Tyson. Plants were 40 days old when water stress was induced; they were harvested 23 days after stress. The vertical bars represent standard errors based on four replicates.

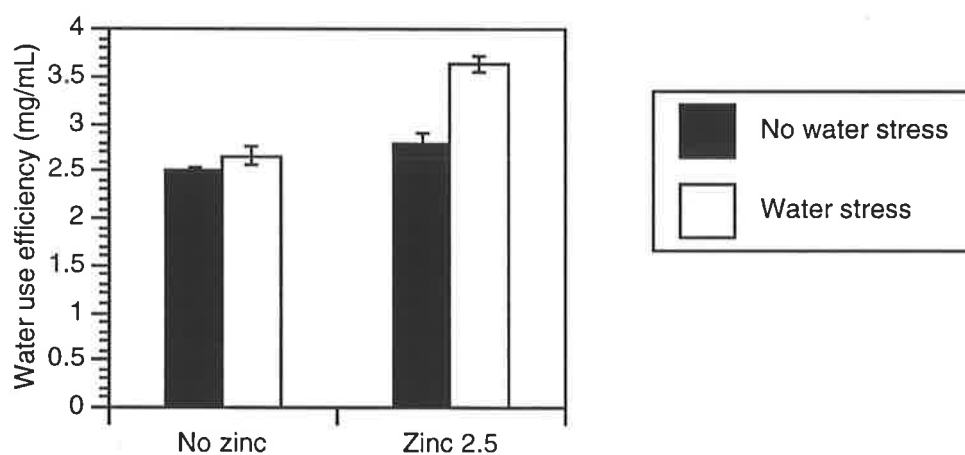


Figure 8.12: Effect of zinc fertilization and water stress on water use efficiency (mg/mL) in Tyson. Plants were 40 days old when water stress was induced; they were harvested 23 days after stress. The vertical bars represent standard errors based on four replicates.

8.3.2.3. Plant water relations

At adequate water supply, no difference in total water potential was observed between zinc-deficient and zinc-sufficient plants; it remained approximately -0.4 MPa during the course of the experiment (Figure 8.13). However, after the initiation of water stress, water potential started to decline relatively earlier (after 6 days) in zinc-sufficient plants and signs of water stress started to appear 12 days after the water stress was induced when the leaf water potential was about -1.3 MPa (Figure 8.13). The lowering of water potential in zinc-deficient plants started later, almost 15 days after the onset of water stress, with water potential declining to -1.3 MPa after 23 days of drying (Figure 8.13).

The trend in relative water content of the leaves was similar to that observed with water potential, but RWC in plants grown without zinc application was slightly higher on days 5 and 10 compared to zinc-adequate plants (Figure 8.14). The values of RWC also showed an earlier decline due to depletion of water when plants were grown with higher zinc supply and then fell rapidly as the moisture stress progressed. In zinc-deficient plants, the RWC was maintained during the initial two weeks of water stress, but then declined rapidly.

Zinc nutrition significantly influenced osmotic potential as it was significantly lower in zinc-deficient plants even at adequate soil moisture (Figure 8.15). Water stress also caused osmotic potential to decline; it showed a sharp decline as moisture stress developed in zinc-sufficient plants, however, there was no reduction in response to water stress in zinc deficient plants. Turgor pressure was higher in the zinc-deficient plants and remained high during the start of water stress. However, after day 12 turgor fell sharply as moisture was depleted further and plants suffered from water stress (Figure 8.16).

The relationship between osmotic potential and RWC was altered by the zinc nutrition of plants. At adequate zinc supply RWC decreased with the development of water stress and a reduction in osmotic potential was evident (Figure 8.17). Severely

zinc-deficient plants maintained relatively high RWC over a range of osmotic potentials during the initial days of water stress when soil moisture content was high but as the severity of water stress increased, plants lost water rapidly without decreasing their osmotic potential (Figure 8.18).

8.3.2.4. Stomatal conductance

Zinc deficiency and water stress caused reductions in stomatal conductance (Figure 8.19). The plants supplied with high zinc and grown at adequate moisture supply had greater stomatal conductance ($657 \text{ m mol/m}^2/\text{s}$), compared to low zinc plants ($246 \text{ m mol/m}^2/\text{s}$), indicating a zinc deficiency induced stomatal closure in plants. The stomatal conductance could not be measured in water-stressed plants at high zinc availability due to wilting of small leaflets, but the comparison of zinc-deficient plants at low and high moisture availability showed appreciable difference in stomatal conductance caused by differential water supply.

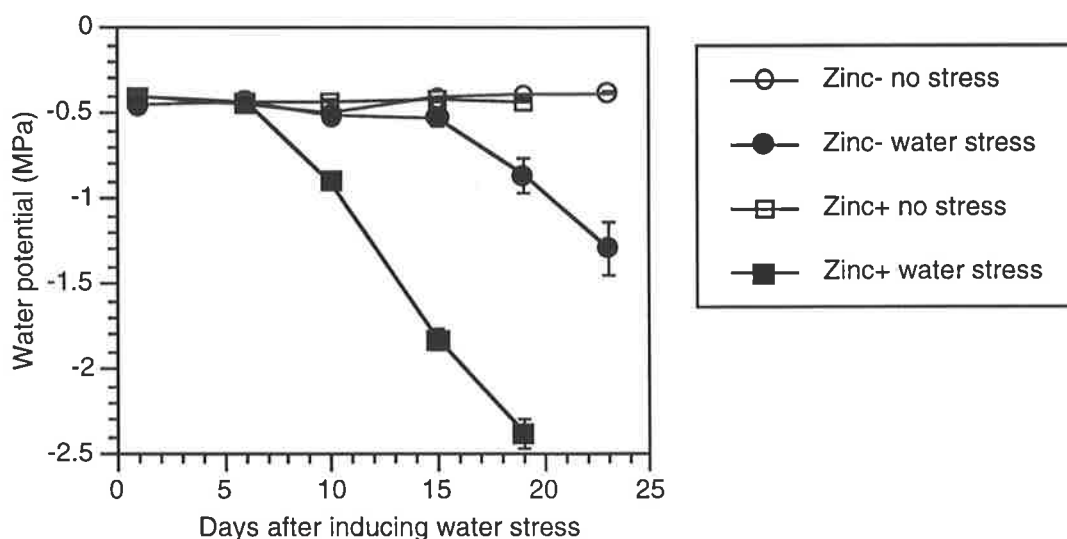


Figure 8.13: Effect of zinc nutrition and water stress on leaf water potential (ψ). Plants were 40-day-old when water stress was induced. The vertical bars represent $\pm$ standard errors based on four replicates.

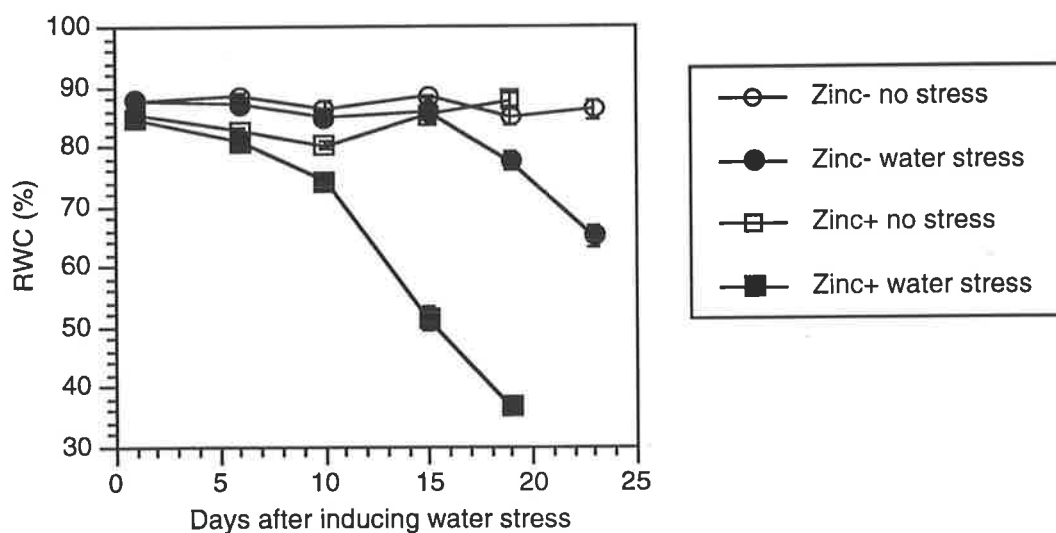


Figure 8.14: Effect of zinc fertilization and water stress on relative water content (RWC). Plants were 40-day-old when water stress was induced. The vertical bars represent $\pm$ standard errors based on four replicates.

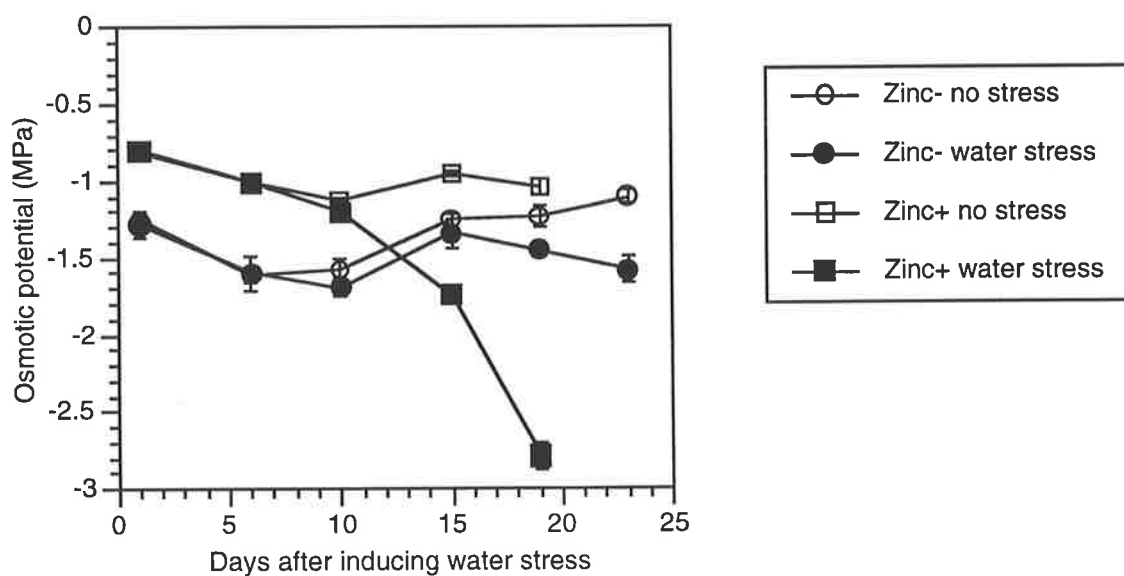


Figure 8.15: Effect of zinc fertilization and water stress on osmotic potential (ψ_{π}). Plants were 40-day-old when water stress was induced. The vertical bars represent $\pm$ standard errors based on four replicates.

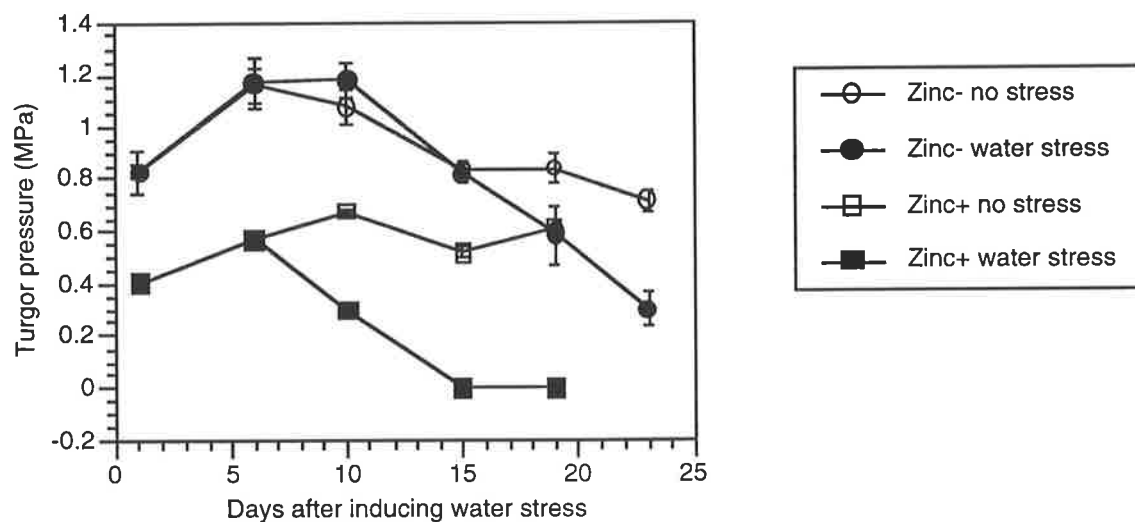


Figure 8.16: Effect of zinc fertilization and water stress on turgor pressure (ψ_p). Plants were 40-day-old when water stress was induced. The vertical bars represent $\pm$ standard errors based on four replicates.

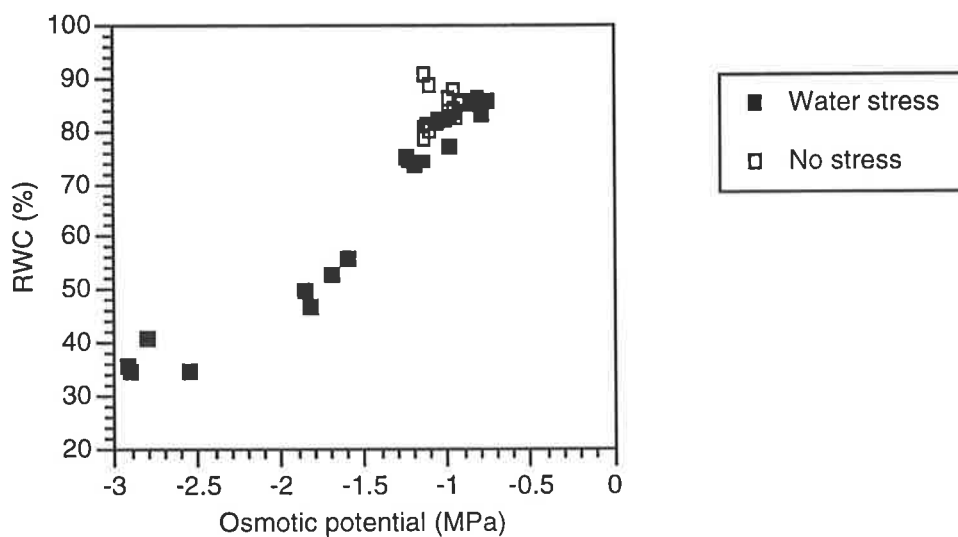


Figure 8.17: Relationship between osmotic potential (ψ_π) and relative water content (RWC) in leaves of zinc-sufficient plants of Tyson. Plants were 40-day-old when water stress was induced.

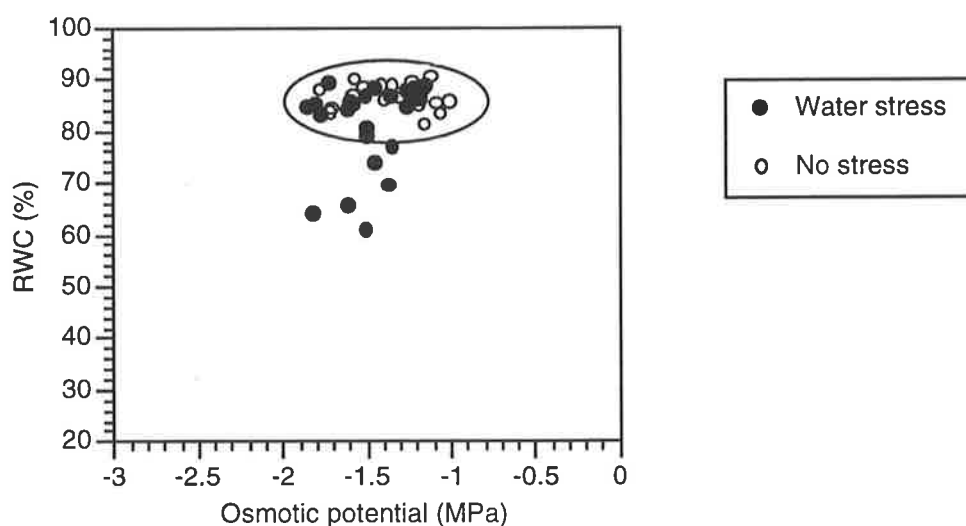


Figure 8.18: Relationship between osmotic potential (ψ_{π}) and relative water content (RWC) in leaves of zinc-deficient plants of Tyson. Values in the circle represent measurements before development of water stress. Plants were 40-day-old when water stress was induced.

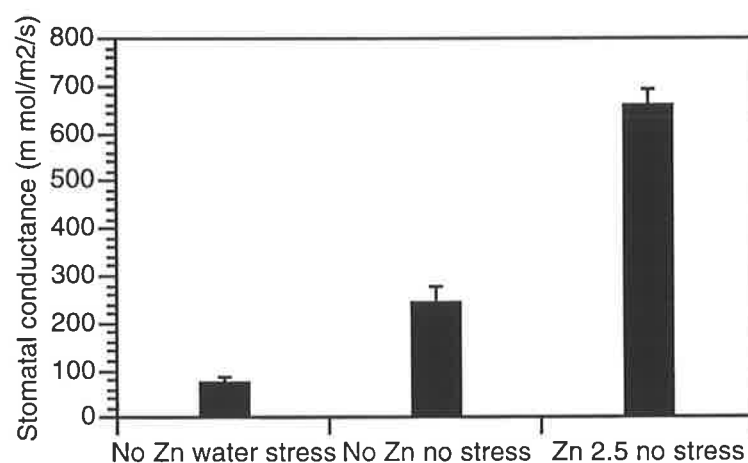


Figure 8.19: Effect of zinc fertilization and water stress on stomatal conductance ($\text{m mol/m}^2/\text{s}$) in leaves of Tyson. Plants were 40-day-old when water stress was induced; stomatal conductance was determined 16 days after induction of water stress.

8.3.2.5. Concentration of other nutrients

The comparison of zinc-deficient and zinc-sufficient plants, when supplied with adequate water, showed a significant increase in the concentration of other nutrients in leaves of zinc-deficient plants (Table 8.5). Among micronutrients, the concentration of Fe, Mn, B, Cu and Mo was higher in zinc-deficient plants. Similarly, the concentration of macronutrients such as Ca, Mg, K, P and S increased when zinc was omitted from soil. The increase in the concentrations was not due to lack of water as all the plants were supplied with adequate soil moisture (12% w/w).

8.3.2.6. Osmotic adjustment

The assessment of osmotic adjustment was made using the difference in the osmotic potential at full turgor in water-stressed and well-watered plants at both low and high zinc supply (Table 8.6). The data indicate that zinc deficiency reduced the ability of plants to adjust osmotically to stress as the value of osmotic adjustment was significantly lower in severely zinc deficient plants (0.23 MPa) compared to those grown at adequate zinc supply (0.40 MPa). Similarly, comparative osmotic adjustment in zinc-sufficient plants was almost twice that recorded in plants suffering from zinc deficiency (Table 8.6). Although osmotic potential at full turgor in zinc-deficient plants grown at adequate moisture availability was relatively low, the decrease in the osmotic potential induced by water deficit was considerably lower in zinc-deficient plants, which resulted in low osmotic adjustment.

8.4. Discussion

The results of this study showed that fertilization with zinc enhanced shoot dry matter accumulation by promoting vegetative growth in all chickpea genotypes tested. When zinc was not added to soil and the only source of zinc for plant requirements was seed reserve and native soil zinc, plant growth was significantly suppressed. However, genotypes exhibited differences in shoot dry matter which were related to their

Table 8.5: Effect of zinc fertilization on concentration of other nutrients in the leaves of Tyson in the well-watered treatment. Plants were 65 days old when harvested.

Nutrients	zinc-	zinc+	LSD (P = 0.05)
<u>Micronutrients (mg/kg)</u>			
Fe	89.0	69.5	16.2
Mn	242.5	202.3	31.7
B	84.5	79.0	6.8
Cu	11.1	5.9	1.3
Mo	110.0	46.8	12.7
<u>Macronutrients (g/kg)</u>			
Ca	26.8	22.8	2.1
Mg	4.2	2.0	0.2
K	22.5	17.9	1.9
P	10.0	3.9	0.7
S	4.4	3.3	0.5

Table 8.6: Effect of zinc fertilization on magnitude of osmotic adjustment (OA) and on comparative osmotic adjustment in water-stressed Tyson plants. Comparative osmotic adjustment % was calculated as $[OA / (\psi_{\pi}^{100})^b \times 100]$. Plants were 45-day-old when water stress was induced and osmotic adjustment was estimated at about 1% (w/w) soil moisture.

Zinc levels	Osmotic potential		OA (MPa) $(\psi_{\pi}^{100})^b - (\psi_{\pi}^{100})^a$	Comp. OA%
	Well-watered $(\psi_{\pi}^{100})^b$	Water-stressed $(\psi_{\pi}^{100})^a$		
No added zinc	-0.98 ± .01	-1.21 ± .03	0.23	24
Zinc 2.5 mg	-0.89 ± .02	-1.29 ± .03	0.40	45

$(\psi_{\pi}^{100})^a$: Leaf osmotic potential in water stressed plants.

$(\psi_{\pi}^{100})^b$: Leaf osmotic potential in well watered plants.

differential sensitivity to zinc deficiency and were consistent with previous findings (Chapter 5). The tissue zinc concentration improved significantly due to soil-applied zinc showing zinc adequacy in the zinc+ treatment while plants grown without zinc fertilization had low shoot zinc concentration, indicating zinc deficiency. Plants grown without soil-applied zinc showed a substantial reduction in growth, and visible symptoms of zinc deficiency, such as slightly pale foliage, necrosis of leaf margins in the older leaves and twisting of the growing buds which later died, appeared about 4 weeks after planting and intensified further during the course of study.

Water usage in plants increased significantly when zinc was applied, largely due to increased leaf area and vegetative growth. In addition, water use efficiency was also greater in zinc-fertilized plants compared with zinc-deficient plants. The growth in zinc-deficient plants was so poor that they were unable to utilize water even at field capacity. The differences in total water use and water use efficiency due to zinc supply were also noted in previous experiments (Chapter 7) and are in agreement with the findings of Nable and Webb (1993) in wheat. The restricted plant growth under zinc-deficient conditions reduced water usage, but measurement of stomatal conductance in zinc-deficient leaves indicated greater stomatal resistance during daytime, which might have limited the rate of transpiration. Partial stomatal closure from water stress frequently results in increased water use efficiency as reduction in transpiration is greater than movement of CO₂ into the leaf. However, in zinc-deficient plants, stomatal conductance as well as water use efficiency decreased, indicating a physiological role of zinc in chickpea. Hu and Sparks (1991) found that inadequate supply of zinc influenced stomatal regulation in pecan. Working with cabbage, Sharma *et al.* (1984) observed that a large number of stomata remained closed in zinc-deficient leaves, which consequently decreased transpiration. The comparison of water loss between normal and zinc-deficient cabbage leaves showed that zinc-deficient leaves had a higher water content, indicating the loss of hydrosensitivity of stomata under zinc deficiency (Sharma *et al.*, 1984). In a subsequent study with cauliflower, Sharma and Sharma (1987) reported an increase in

diffusive resistance in the zinc-deficient plants and suggested involvement of zinc in stomatal opening, possibly as a constituent of certain enzymes or as a factor responsible for maintenance of membrane integrity (Sharma *et al.*, 1995). The results of the present study also indicate that differences in water usage and water use efficiency may be due to physiological role of zinc in stomatal regulation and CO₂ assimilation.

In C₃ crop plants such as wheat and peanut, genotypes showed substantial differences in carbon isotope discrimination under drought stress (Hubick *et al.*, 1986; Condon *et al.*, 1987). Chickpea is a C₃ plant (Khanna-chopra and Sinha, 1987), and results of the present study showed significant differences in Δ among chickpea genotypes subjected to water stress. ICC 4958, which is both a zinc efficient (Chapter 5) and drought-tolerant genotype (Saxena *et al.*, 1993) had the lowest value of Δ and produced highest shoot dry matter among genotypes tested.

The relationship between shoot dry matter and Δ in chickpea showed a strong negative correlation in zinc-sufficient plants subjected to water stress, but no significant correlation was observed in zinc-deficient plants. Transpiration efficiency also exhibited a negative correlation with Δ but the trend differed among zinc treatments, and it was relatively more negative in zinc adequate plants. The results pertaining to the negative correlation of shoot dry matter and transpiration efficiency with Δ are in agreement with those reported in groundnut (Hubick *et al.*, 1986) and in wheat (Condon *et al.*, 1990). The mean value of Δ was slightly higher in zinc-sufficient chickpea plants subjected to water stress, which may be associated with their greater stomatal conductance prior to the development of severe water stress (Table 8.1). Condon *et al.* (1987) demonstrated that in wheat the potential source of variation in Δ was differences in stomatal conductance. However, Hu and Sparks (1993) suggested that in zinc-deficient pecan plant, CO₂ availability was not restricted by stomatal aperture as usually happens in water-stressed plants, but that zinc deficiency impaired CO₂ fixation. It appears that Δ is sensitive to stomatal conductance, soil moisture contents and is controlled genetically, therefore,

further experimentation is required to elucidate the relationship between zinc nutrition and Δ in chickpea.

The measurement of water potential is widely accepted as a fundamental indicator of plant water status (Hall *et al.*, 1979; Turner, 1981). The values of water potential generally showed no effect of zinc nutrition at adequate water supply. However, water potential started to decline after the induction of water stress and fell to - 2.4 MPa as the water stress intensified. The reduced growth and rate of water use of the zinc-deficient plants caused the development of moisture stress to be relatively slow and hence, initially they maintained higher water potential values. The RWC values were equal or even higher in plants suffering from zinc deficiency and declined slowly compared to zinc-fertilized plants. The values of RWC together with water potential suggested a water build-up in zinc-deficient plants, which may have resulted from closure of stomata and restricted transpiration. This is consistent with the observation that the leaflets of zinc-deficient plants were very turgid and stiff. However, the measurements of water potential and RWC failed to prove any zinc-deficiency-induced water stress in chickpea as reported in cauliflower (Sharma and Sharma, 1987; Sharma *et al.*, 1994; Sharma *et al.*, 1995) and cabbage (Sharma *et al.*, 1984). The water potential value recorded in zinc-deficient plants of cabbage was -0.48 MPa (Sharma *et al.*, 1982), which was obviously lower compared to high zinc plants (- 0.37 MPa), but transpiring plants at such a level of water potential are seldom considered water-stressed provided the turgor is maintained. However, plant species may vary in their leaf water potential. The water potential measurements in cabbage and cauliflower were made hygrometrically using a microvoltmeter, which can be subjected to different sources of errors such as high cuticular resistance in leaf samples and an equilibration error caused by the diffusive resistance of the plant sample (Nulsen, 1980). There is possibility that differences in diffusive resistance and rate of transpiration among zinc-deficient and zinc-adequate plants may have confounded water potential measurement by the hygrometer in these studies. In the study presented here, water potential of chickpea leaves was determined

using the pressure chamber technique and the values obtained were comparable to those reported for chickpea at different moisture regimes in other studies (Sivakumar and Virmani, 1979; Turner *et al.*, 1996).

Among the parameters of water relations evaluated in chickpea in the present study, osmotic potential was significantly influenced by zinc fertilization. In zinc-deficient plants, at adequate soil moisture, osmotic potential was considerably lower compared to that in plants supplied with sufficient zinc. The lower values of osmotic potential indicated accumulation of solutes under zinc deficiency. This was also reflected in the appearance of leaf laminae of zinc-deficient plants which looked abnormally thick and stiff. An almost similar disorder in the leaf morphology of zinc-deficient cauliflower plants was observed by Sharma *et al.* (1994). Earlier, Riceman and Jones (1958), working with subterranean clover, reported that older leaves became exceptionally heavy in zinc-deficient plants, and the onset of zinc deficiency caused considerable accumulation of metabolites. The concentration of free amino acids and amides increased several-fold in bean plants supplied with low zinc, which was considered to be due to impairment of protein synthesis (Cakmak *et al.*, 1989), whereas Marschner and Cakmak (1989) found that in zinc-deficient bean plants the concentrations of sugars and starch in the trifoliate leaves were similar to those of zinc-sufficient plants. The results of the present study were in agreement with these latter findings as difference in the concentration of ethanol-soluble sugars among zinc treatment was non-significant. This suggests that increase in solute concentration was presumably either due to free amino acids and amides resulting from impairment of protein synthesis or due to elevated concentration of other mineral nutrients.

The study presented here showed that zinc deficiency increased the concentration of other micro- and macronutrients in the leaf tissue. These results are in accordance with published results on increased accumulation of P (Loneragan *et al.*, 1982), B (Graham *et al.*, 1987; Singh *et al.*, 1990), Mn (Rengel and Graham, 1995a), Ca and Mg (Norvell and Welch, 1993). Zinc is known to play a protective role in structural and

functional integrity of root cell plasma membrane (Welch *et al.*, 1982) and zinc deficiency can induce damage to plasma membrane and disturb selectivity and regulation of ion uptake (Norvell and Welch, 1993). In zinc-deficient plants, it is quite possible that increased concentration of certain nutrients in the leaf tissue have an osmotic effect which may have resulted in lowering of osmotic potential.

An important objective of this study was to determine the effect of zinc nutritional status of plants on their potential for osmotic adjustment. The assessment of the degree of osmotic adjustment was made using the difference in osmotic potential at full turgor between previously water-stressed and adequately-watered plants of the zinc- and zinc+ treatments. This technique provides firm evidence of osmotic adjustment (Muchow, 1980), with an advantage of simplicity of measurements (Morgan, 1980); it has been used successfully for assessment of drought-induced osmotic adjustment (Jones and Turner, 1978; Blum, 1989; Chapman and Augé, 1994; Wang *et al.*, 1995; Pomper and Breen, 1997; Qian and Fry, 1997). Muchow (1980) reported that direct measurement of osmotic potential is more useful than the pressure-volume technique, particularly with a large number of treatments or genotypes for determining the level of osmotic adjustment. With the pressure-volume technique, crushing of soft plant tissue with a tight rubber seal in the pressure chamber is a common problem, and it is also difficult to quantitatively recover the small amount of expressed sap exuded from plant material.

The results showed that the magnitude of osmotic adjustment was significantly higher (0.4 MPa) in plants supplied with sufficient zinc. Previous studies suggest occurrence of osmotic adjustment in response to drought stress in chickpea (Morgan *et al.*, 1991; Turner *et al.*, 1996; Amede and Schubert, 1997). However, the degree of osmotic adjustment, reported by Turner *et al.* (1996), was relatively higher (0.6 - 0.7 MPa) under field conditions in the advanced breeding line T-1587 compared to results obtained in the present study, which could be due to either a slower development of drought in the field, or genotypic differences (Morgan *et al.*, 1991). Despite the zinc deficient plants having a lower value of osmotic potential at adequate water supply,

which was probably a concentration effect, active accumulation of solutes due to water deficits was negligible. As the rate of water stress development was comparatively slow in zinc-deficient plants, one would have expected a higher degree of osmotic adjustment in these plants because generally the faster the development of water stress, the smaller the amount of osmotic adjustment (Jones and Rawson, 1979). The results therefore indicate that zinc deficiency restricted the capacity of drought-induced osmotic adjustment in chickpea, and hence a lower osmotic adjustment may affect the ability of plants to endure periods of drought.

Osmotic adjustment in the leaves can be evaluated by the relationship between osmotic potential and RWC in water-stressed plants (Morgan *et al.*, 1991). The results showed that zinc treatments altered the relationship between osmotic potential and RWC in plants subjected to water stress. In zinc-deficient plants, prior to development of severe water stress, RWC in the leaves remained higher over a range of osmotic potentials, but as the soil moisture was depleted further, leaves lost water quickly without showing any substantial decrease in their osmotic potential. Morgan (1980) suggested that osmotic potential usually has two components; one due to active accumulation of solute in the cell and the other due to a concentration effect. Working with barley, Grumet *et al.* (1987) observed differences among genotypes in osmotic potential at full turgor without a previous exposure to water stress, apparently due to a concentration effect, and termed it constitutive osmotic adjustment. Blum (1989) differentiated between drought-induced active osmotic adjustment and constitutive osmotic adjustment, ~~denying~~ ^{rejecting} the role of the latter as a component of drought tolerance. The changes in the relative rates of metabolic assimilation may increase solute concentration; a higher concentration of solute can not always ^{confirm} ~~verify~~ the occurrence of effective osmotic adjustment (Munns, 1988). It appears that maintenance of high RWC at the initial stage of water stress was simply a concentration effect and not a drought induced osmotic adjustment. Therefore, the relationship between osmotic potential and RWC supports the

hypothesis that zinc deficiency may have weakened the ability of the plants to adjust osmotically in response to water stress.

The composition of the osmotic pool of the water-stressed plants grown at sufficient zinc supply ~~could~~^{was} not determined in the present study. However, Ford (1984) identified the accumulation of fructose, inositol, lactone and proline in drought-stressed chickpea plants, and suggested that these compounds possibly act as osmoregulators. Recently, Amede and Schubert (1997), working with chickpea, found that 55% of the osmotic pool in the leaves of water-stressed plants consisted of water-soluble sugars and inositol, while 19% and 18% was contributed by Ca^{2+} and amino acids, respectively. The possibility of zinc as a constituent of chickpea osmoregulators cannot be ruled out at this stage and will be interesting to further investigate the physio-chemical role of zinc in the process of osmotic adjustment.

8.5. Conclusion

This study showed no evidence of zinc-deficiency-induced water stress at adequate moisture supply as the values of water potential and RWC were either similar or even higher in zinc-deficient plants compared to zinc-sufficient plants. However, low zinc availability induced stomatal closure, which reduced transpiration and probably resulted in an increase in tissue water content. Osmotic potential was the most affected component of plant water relations under zinc deficiency; lowering of osmotic potential was apparently associated with accumulation of solute in zinc-starved leaves. Finally, the present study provided unequivocal evidence of osmotic adjustment in zinc-sufficient chickpea plants, which may enhance drought tolerance, but the specific physio-chemical role of zinc as an osmoregulator remains to be elucidated.

CHAPTER 9

General Discussion

Zinc deficiency is a widespread micronutrient deficiency in the traditional chickpea growing areas of the world such as the Indo-Pakistan subcontinent (Katyal, 1985; Takkar, 1991) and the Mediterranean basin (Sillanpää and Vlek, 1985; Cakmak *et al.*, 1996b). There are also extensive areas of zinc-deficient soils in southern Australia (Reuter *et al.*, 1988; Graham *et al.*, 1992), where chickpea cultivation is rapidly expanding. One of the causes of variable and low yields of chickpea world-wide, is likely to be deficiency of zinc. Therefore, a series of experiments was conducted to determine the zinc requirements of chickpea and to establish a critical zinc concentration for diagnosis of zinc deficiency. Experiments were also conducted to evaluate the response of a range of chickpea genotypes to zinc to quantify genotypic variation in zinc efficiency and to suggest possible physiological mechanisms related to zinc efficiency in chickpea. The effect of zinc fertilization on grain yield, on distribution of zinc in plant parts and loading of zinc in the seed were also examined. Studies on the effect of zinc nutrition on water use, water use efficiency, plant water relations, stomatal conductance and on capacity of osmotic adjustment were conducted to ascertain the physiological role of zinc in drought tolerance.

Chickpea was shown to be sensitive to zinc deficiency as most of the leaves abscised prematurely and the growing meristem died under severe deficiency. Plant growth as well as tissue zinc concentration increased with increasing fertilization up to 5 mg zinc/kg soil (the highest level used in a pot experiment; Chapter 4) in Laffer sand, although the relative increase in shoot growth from fertilization of 1 to 5 mg/kg zinc was less compared to low zinc treatments. Based on the response curve, zinc fertilization of 2.5 mg/kg soil was selected as the adequate zinc level in Laffer sand and used in the following pot experiments. By comparison, an application of 3.2 mg zinc per kg soil under comparable conditions induced zinc toxicity in wheat growing in Laffer sand

(Rengel and Graham, 1995a). The results support the findings of previous studies which indicated that chickpea is more sensitive to low soil zinc compared to wheat and other cereals (Tiwari and Pathak, 1982; Tiwari and Dwivedi, 1990) and suggests that the zinc requirement of chickpea is relatively higher than that of cereals. Thus the occurrence of zinc deficiency in chickpea on soils marginally zinc deficient is likely to be greater than that for cereals.

Tissue analysis is successfully used to assess the nutrient status of plants with values for critical concentration being important in interpreting the results of tissue analyses (Melsted *et al.*, 1969). Ulrich and Hill (1973) defined the critical concentration as the nutrient concentration in the tissue at which a 10% reduction in dry matter yield occurs. Critical concentrations have been established in soybean (Ohki, 1977), subterranean clover (Reuter *et al.*, 1982), cowpea (Marsh and Waters, 1985) and wheat (Dang *et al.*, 1993b; Wilhelm *et al.*, 1993), but there was no report available on the critical concentration of zinc in chickpea. The ^{adequate}~~critical~~ concentration values reported by Reuter and Robinson (1986) ranged from 12 to 500 mg/kg and hence were not reliable for routine use. Therefore, it was an important outcome of the present study that a critical zinc concentration of 20-21 mg/kg in whole shoot of chickpea was determined and used subsequently to assess of the zinc status of plants. The critical value showed no significant difference among genotypes nor was it affected by soil moisture deficits (Chapter 4). This enhanced the applicability of the critical concentration values to a range of environments.

Genotypes of crops vary widely in their response to zinc deficiency, and tolerant genotypes maintain adequate zinc uptake even under unfavourable conditions (Graham and Rengel, 1993). Tolerance to zinc deficiency is genetically controlled and described as zinc efficiency (Graham, 1984). The selection and development of cultivars tolerant to nutrient deficiencies is an excellent approach for increasing production on deficient soils (Takkar, 1993). The present results revealed genotypic differences among chickpea genotypes in their responsiveness to zinc when grown under controlled environmental

conditions. Based on vegetative growth in pot assays, the advanced breeding lines ICC-4958, T-1587, CTS-11308, NEC-138-2×CM-72 and Pakistani cultivars Punjab-91 and Noor-91 exhibited higher zinc efficiency compared to Tyson, CPI-56288, CM-88 and Piadar-91 (Chapter 5). It was also noted that most of the Australian as well as Pakistani cultivars examined showed poor zinc efficiency when grown under severe zinc deficiency. Interestingly, most of the zinc-inefficient genotypes have been already identified as Fe-chlorosis sensitive (Beech and Brinsmead, 1980; Rashid and Din, 1992). Walter *et al.* (1994) observed a reduction in Fe transport due to zinc deficiency in wheat and a positive relationship between Fe uptake and zinc efficiency has been reported in a study with wheat (Rengel and Graham, 1995d), but further research is needed to establish any connection between Fe-chlorosis tolerance and zinc efficiency in chickpea.

The genotypic differences in zinc efficiency in the pot studies appeared to be related to the sensitivity of root growth to zinc supply. In general the root:shoot ratio increased under zinc deficiency, but the increase was much less in the zinc-efficient genotypes ICC-4958, T-1587, Punjab-91 and NEC-138-2×CM-72 compared with inefficient genotypes. The greater root growth apparently enhanced shoot growth in zinc-efficient genotypes maintaining the root:shoot ratio while the root:shoot ratio was considerably increased in zinc-sensitive genotypes due to enhanced root growth and suppressed shoot growth. Genotypic differences in zinc efficiency were closely related to the zinc content per plant and accumulation of zinc was significantly higher in the zinc-efficient genotypes compared to inefficient genotypes when grown in zinc-deficient soil without additional zinc. This indicates a greater ability of zinc-efficient genotypes to acquire zinc from deficient soil and a better zinc transport from root to shoot (Chapter 5). Almost similar mechanisms of zinc efficiency have been suggested in cereals (Nable and Webb, 1993; Rengel and Graham, 1995c; Rengel and Graham, 1996; Grewal *et al.*, 1996) and in canola (Grewal *et al.*, 1997).

Field experiments conducted at Lameroo (Murray Mallee region, South Australia) and Fatehjang and Kamalpore Sherjang (Potowar area, Pakistan) showed that zinc

fertilization increased grain yield by between 7 to 19% depending on the site. The responsiveness of the different sites appeared to be associated with the amount of DTPA-extractable soil zinc. The response to zinc application was highest at Kamalpore Sherjang, which contained the lowest DTPA-extractable soil zinc (0.24 mg/kg). By comparison the DTPA-extractable zinc at Lameroo was 0.27 mg/kg and Fatehjang 0.30 mg/kg. Singh and Gupta (1986) considered 0.48 mg/kg DTPA-extractable zinc as a critical zinc level for chickpea in soils from semi-arid tropics in India. However, critical zinc level varies with soil type and is also influenced by physical and chemical properties of the soil (Brennan, 1992). This shows that DTPA-extractable soil zinc would indicate likely responsive sites, however, the reliable diagnosis of zinc deficiency depends on the tissue test.

The Lameroo area generally receives low seasonal rainfall, and the growing season (April-October) rainfall in 1997, which is closely related to crop yields under South Australian conditions (French and Schultz, 1984), was much lower than the average rainfall (Chapter 6). Consequently the chickpea crop experienced drought during the reproductive stage, which is a common characteristic of the Mediterranean type environment (Richards, 1991), and maturity influenced final yield and zinc responsiveness. The early-maturing genotypes Amethyst, T-1587 and CTS-11308 produced higher grain yield compared to the late-maturing cultivars Dooen and Barwon (Chapter 6). Although the advanced breeding lines T-1587 and CTS-11308 displayed high zinc efficiency, which was consistent with results obtained from the pot experiments, the ranking of other genotypes for zinc efficiency was influenced by their maturity. Such interactions are common and may affect performance of genotypes in drought-prone environments (Graham *et al.*, 1992). It was also noted that early-flowering genotypes were more efficient in partitioning of dry matter into grain and therefore exhibited a high harvest index. Earlier studies with chickpea suggested that under conditions of terminal drought in the Mediterranean type environment, the high harvest index was associated with drought escape (Silim and Saxena, 1993b). This

suggests that climatic factors, particularly terminal drought, through their influence on grain yield can affect the responsiveness of genotypes to zinc and hence the ranking of zinc efficiency. Therefore, results obtained from field experiments, which are subjected to seasonal variation, need to be treated with caution and the effect of environmental factors needs to be considered in evaluation of genotypes. It would be wise if observations on maturity were made routinely in field experiments and that these experiments ~~are~~ ^{were} backed up with pot experiments to distinguish zinc response and environmental effects.

In contrast to the results at Lameroo, the response of Pakistani genotypes to zinc deficiency was closely related to their performance under controlled conditions (Table 9.1). The genotypic ranking remained almost consistent across the sites. Moreover, the cultivars Punjab-91 and NIFA-88 were consistently zinc-efficient both in pot experiments and under field conditions, while Tyson and CM-88 were consistently classified as zinc-inefficient. The only kabuli cultivar of Pakistan origin evaluated in the study showed slight variation in zinc efficiency ranking, with intermediate performance under field conditions that might be related to its lack of adaptation in the Potowar region. In general, the results from both the Lameroo and Pakistan field trials indicated an appreciable agreement between pot assay screening and genotypic performance under field conditions, suggesting that the pot assay can be used successfully for evaluation of genotypes for zinc efficiency. However, in environments characterized by terminal drought of unpredictable severity, crop maturity also needs to be considered.

Besides widespread zinc deficiency, drought is another constraint in chickpea growing regions of the world (Saxena *et al.*, 1990b; Subbarao *et al.*, 1995), often causing crops to suffer from water deficit and zinc deficiency simultaneously. Prompted by field observations that zinc nutrition enhanced the ability of a wheat crop to tolerate drought (King, 1994), pot experiments were designed to investigate the effect of zinc fertilization on grain yield at adequate and low moisture regimes. The results showed

Table 9.1: Comparison of zinc efficiency ranking in Pakistani chickpea cultivars and advanced breeding lines in pot assay and field experiments. Zinc efficiency in pot experiments based on vegetative growth while in field experiments the efficiency was calculated using grain yield.

Genotype	Type	Pot Assay	Field Experiments	
			Fatehjang	K. Sherjang
C-44*	Desi	6	9	8
CM-72	Desi	7	6	5
NIFA-88	Desi	4	3	4
Punjab-91	Desi	2	1	3
Noor-91	Kabuli	3	5	7
Piadar-91	Desi	8	7	6
CM-88*	Desi	9	10	9
ICC-11514× ILC-3279	Desi	-	4	1
ICC-11514× ILC-482	Desi	5	2	2
NEC-138-2× CM-72	Desi	1	-	-
Tyson*	Desi	10	8	10

*: Fe-chlorosis sensitive

increase in the grain yield from adequate zinc supply at high moisture availability compared with low zinc and restricted water supply, which was mainly due to a larger number of pods per plant (Chapter 7). This result is consistent with other studies with legumes: zinc fertilization promoted pod bearing in cow pea (Ghildiyal *et al.*, 1978), chickpea (Thakur, 1989) and navy bean (Moraghan, 1996). However, with adequate zinc and under well-watered conditions there was continued vegetative growth during the reproductive phase and an associated decline in mean seed weight and harvest index. The simultaneous growth of structural material ^{appears to have} created competition in the distribution of assimilates between vegetative parts and the developing seeds, which resulted in low individual seed weight with an increase in the number of underdeveloped seeds. Similar findings in chickpea have been documented by Sandhu *et al.* (1978), Saxena *et al.* (1990b), Singh and Pandey (1980) and Bonfil and Pinthus (1995). This emphasizes the

need to breed varieties that have a high harvest index when grown under high-input conditions to get the maximum benefit from favourable conditions.

Water stress imposed at podding suppressed grain yield at all levels of zinc supply, but the reduction in grain yield was greatest at the highest level of zinc fertilization (Chapter 7). It appears that the increased growth that occurred with the correction of zinc deficiency increased transpiration of plants by promoting vegetative growth. Although water stress was induced gradually and attempts were made to keep the degree of water stress similar among zinc treatments by regular watering, the extraction of soil water and the subsequent development of water stress was still relatively more rapid in plants fertilized with 2.5 mg zinc/kg soil. The limitations of pot assays compared to field conditions in terms of restricted root growth (Moraghan, 1996) and rapid development of water stress (Passioura, 1980) probably did not allow the role of zinc in drought tolerance to be clearly elucidated. The ~~closed~~ pot system used in these experiments did not allow the importance of factors such as enhanced root growth to improved drought tolerance to be evaluated. This has been found to be an important factor in wheat (Nable and Webb, 1993).

The limitation of pot experiments was reinforced by trends observed at Lameroo in 1997 which indicated that zinc fertilization may improve a crop's ability to cope with dry conditions. Leaf water potentials and stomatal conductances of plants given zinc fertilizers were slightly higher than those of plants grown without additional zinc (Figures 9.1 & 9.2), despite an increase in plant dry matter. These results infer that zinc improved plant growth as well as reduced stress suggesting either better root growth and/or better soil water extraction by roots. The differences between the zinc treatments were not statistically significant and so some caution needs to be taken when interpreting the results. The measurements were taken late in the season when the level of stress was quite high which may have narrowed the differences between treatments. However, the data do suggest that zinc nutrition may enhance a crop's ability to grow under increasing water deficits. Among the three genotypes, ICC-4958 exhibited higher stomatal

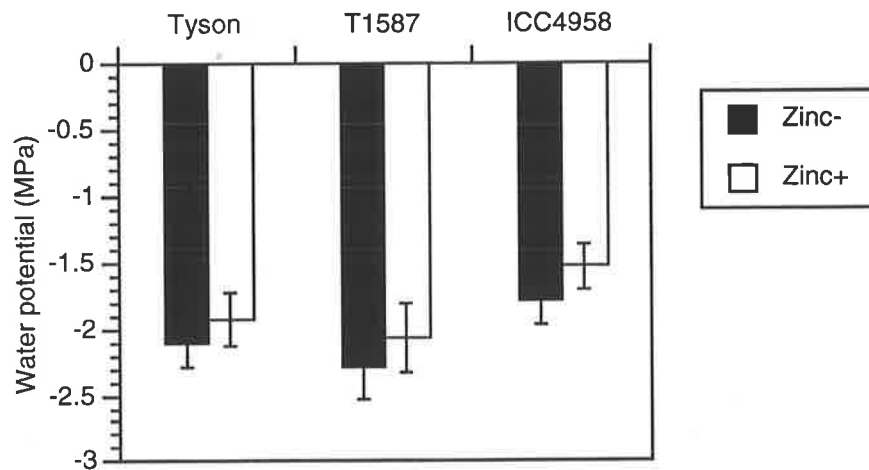


Figure 9.1: Effect of zinc fertilization on water potential in chickpea plants grown under field conditions at Lameroo during 1997. Plants were at the podding stage when measurements were taken using a pressure chamber technique. The vertical bars represent standard errors based on three replications.

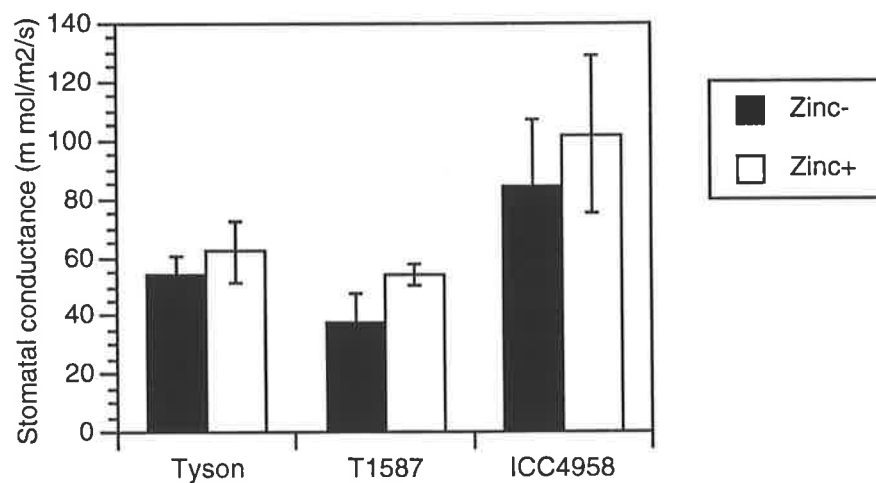


Figure 9.2: Effect of zinc fertilization on stomatal conductance (m mol/m²/s) in chickpea plants grown under field conditions at Lameroo during 1997. Plants were at the podding stage when measurements were taken. The vertical bars represent standard errors based on three replications.

conductance together with higher water potentials compared to the other genotypes. As discussed earlier (Chapter 5), ICC-4958 has been identified as a drought-tolerant genotype due to its extensive root system that enabled it to maintain water uptake under water-limited conditions (Saxena *et al.*, 1993; Silim and Saxena, 1993a). The results obtained from pot experiments (Chapters 4 and 5) showed an appreciable increase in root growth when zinc supply was adequate. It is ^{possible} ~~likely~~ that extensive root system of zinc-fertilized plants may penetrate into deeper soil profiles and extract more water during dry seasons, enabling plants to tolerate drought. Further studies in this area are warranted.

Poorer root growth under zinc deficiency may adversely affect soil water extraction, thereby inducing water stress earlier, but studies with cabbage have also shown that zinc deficient plants maintained a lower leaf water potential even under well watered conditions (Sharma *et al.*, 1982; 1984). These results suggested that the level of zinc nutrition may have a direct effect on leaf water relations and therefore two experiments were conducted to examine this effect. In contrast to the earlier work of Sharma and colleagues, both experiments showed that leaf water potential and RWC were equal or even higher in plants suffering from zinc deficiency compared to zinc-fertilized plants at adequate water supply (Chapter 8). Both results suggested an accumulation of water in leaves of zinc-deficient plants rather than the zinc-induced water stress as reported by Sharma *et al.* (1984). Data pertaining to stomatal conductance also supported these findings as zinc deficiency induced stomatal closure in chickpea, thus restricting transpiration. The discrepancy about the effect of zinc deficiency on water potential between cabbage (Sharma *et al.*, 1984) and chickpea may have resulted from the different techniques used for water potential measurements or it could be related to differences in species. In the present experiments, water potential was measured using the pressure chamber technique and values obtained were comparable with previous studies with chickpea (Sivakumar and Virmani, 1979; Turner *et al.*, 1996). Based on the present results, there was no evidence to suggest that zinc deficiency induces water stress in chickpea.

However, zinc deficiency significantly influenced osmotic potential with values being considerably lower in zinc-deficient plants compared to those supplied with adequate zinc (Chapter 8). Such a reduction in osmotic potential in zinc-deficient plants at adequate moisture supply indicated accumulation of solutes. The concentration of ethanol-soluble sugars was not affected among zinc treatments, but Cakmak *et al.* (1989) noted an increase in the concentration of free amino acids and amides in bean plants supplied with low zinc, which resulted from impairment in protein synthesis. It is possible that zinc deficiency in chickpea may have affected protein synthesis and other metabolic activities causing accumulation of solutes, which was reflected in low osmotic potential values.

The effect of zinc fertilization on the degree of osmotic adjustment in water-stressed chickpea plants was studied to establish the role of zinc nutrition in drought tolerance because osmotic adjustment helps in sustaining metabolic activities during periods of drought (Ludlow *et al.*, 1985). Zinc deficiency evidently weakened the ability of the plants to adjust osmotically as the value of osmotic adjustment was significantly lower in zinc-deficient plants compared to zinc-fertilized plants (Chapter 8). Despite a lower osmotic potential in zinc-deficient plants at adequate water supply, which can simply be attributed to a concentration effect (Morgan, 1980; Grumet, 1987; Munns, 1988; Blum, 1989; Amede and Schubert, 1997), drought-induced accumulation of solutes was negligible. In zinc-deficient plants, water stress developed relatively slowly because of their reduced growth compared to plants supplied with 2.5 mg zinc/kg soil; a higher degree of osmotic adjustment was expected in zinc deficient plants because, in general, the faster the rate of water stress development, the smaller the magnitude of osmotic adjustment (Jones and Rawson, 1979). The results indicate that zinc deficiency hampered active accumulation of solutes in plants ~~when~~ subjected to drought. Therefore, the results of these experiments indicate that zinc deficiency leads to lower water use and a slower rate of stress development; although initially, there is an accumulation of

solutes leading to low osmotic potential, once stress develops the zinc-deficient plants have less capacity to adjust osmotically.

The relationship between osmotic potential and RWC was also used to assess the level of osmotic adjustment (Morgan *et al.*, 1991). This showed a considerable difference in the relationship between osmotic potential and RWC before and after the development of water stress, particularly in zinc-deficient plants. Prior to the development of water stress, RWC in the leaves of zinc-stressed plants remained higher over a range of osmotic potentials, but with depletion of soil moisture, leaves lost water rapidly without lowering osmotic potential (Chapter 8). This supported the previous results that at adequate water supply, higher RWC and lower osmotic potential in zinc-deficient compared to zinc-fertilized plants was a concentration effect rather than a drought-induced active accumulation of solutes. It was concluded, therefore, that zinc deficiency restricted the capacity of drought induced osmotic adjustment and that zinc fertilization enhanced a degree of osmotic adjustment in chickpea, which may raise the ability of plants to endure periods of drought.

The other aspect of the ability to improve productivity under rainfed conditions is water use efficiency. Consistently, the pot studies showed enhanced water use efficiency under adequate zinc nutrition. The poorer water use efficiency of zinc deficient plants could be associated with impaired stomatal functioning and/or the effects on the biochemical reactions of photosynthesis.

Zinc fertilization promoted root growth and zinc fertilized plants had slightly higher water potential along with greater stomatal conductance compared with marginally zinc-deficient plants grown under water-limited field conditions. In addition, zinc adequate plants had higher water use efficiency and showed higher degree of osmotic adjustment compared to zinc-deficient plants. These observations lead to the conclusion that adequate zinc nutrition may enhance drought tolerance in chickpea.

There are some limitations associated with pot assays in inducing a uniform water stress particularly when plant size differs due to differences in zinc fertilization. Admittedly, the rate of water stress development is often more rapid and more severe in pots than in field-grown plants. In addition, the plants grown in Laffer sand which has a low water holding capacity and dries out quickly, were severely zinc deficient. It would be worth-while for future research to elucidate the role of zinc nutrition in drought tolerance in chickpea under water-limited and marginally zinc-deficient field conditions.

Micronutrient malnutrition is common worldwide and the extent of widespread deficiency suggests that about 2 billion people are zinc deficient (Graham *et al.*, 1997). Chickpea is the principal pulse and provides a major source of protein and vital mineral nutrients in the diet of the poor and predominantly vegetarian population (Smithson *et al.*, 1985; Pushpamma and Geervani, 1987). The results of the field trial at Lameroo (Chapter 6) and pot experiments (Chapter 7) showed that zinc fertilization significantly enhanced seed zinc concentration. It appears that crops produced on zinc deficient soils, without supplemental zinc application, will produce seeds having low zinc concentration. Therefore, the improvement in seed zinc concentration in chickpea through soil application of zinc should be regarded as a desirable quality factor for human nutrition. Moreover, seeds having high zinc reserves may improve seedling vigour due to the carry-over effect of zinc from seed to seedlings (Rengel and Graham 1995a) and may also enhance resistance to soil borne pathogens (Graham *et al.*, 1992) that are responsible for severe yield losses in chickpea (Nene and Reddy, 1987).

In conclusion, the highlights of the results of study presented here are as follow:

- The critical zinc concentration was estimated as 20-21 mg/kg in the whole shoot and the critical value showed no significant difference among genotypes nor was it affected by soil moisture deficits, suggesting that critical concentration could be used over a range of environments.

- Chickpea genotypes differed in sensitivity to zinc deficiency and advanced breeding lines ICC-4858, T-1587, CTS-11308, NEC-138-2 × CM-72 and cultivar Punjab-91 were relatively zinc efficient compared to other Australian and Pakistani genotypes. Maintenance of root:shoot ratio, total zinc accumulation (content), and better root to shoot transport ~~was~~ ^{were} closely related to zinc efficiency.
- Zinc fertilization increased grain yield under field conditions both in Australia (Lameroo, Murray Mallee area) and Pakistan (Attock, Potowar region). The cultivar Punjab-91 was outstanding as it produced high yield without additional zinc and responded well to zinc fertilization. At Lameroo, under conditions of terminal drought, the early maturing genotypes Amethyst and CTS-11308 had an advantage of escaping drought.
- Low zinc availability induced partial stomatal closure, reduced water use and lowered water use efficiency, suggesting that zinc has a physiological role in plant water relations.
- The magnitude of osmotic adjustment was significantly higher in plants supplied with high zinc compared to zinc deficient plants, which indicated that severe zinc deficiency restricted the capacity of drought-induced osmotic adjustment in chickpea.
- The distribution of zinc in plant parts showed that application of zinc enhanced accumulation of zinc in seed which is a desirable quality factor for human consumption and also for seedling vigour.

Appendix 1: Type and origin of chickpea genotypes used in the study.

Genotypes	Type	Origin
Tyson	desi	India
Dooen	desi	USSR
Amethyst	desi	Australia
Semsen	desi	Australia
Barwon	desi	Australia
Desavic	desi	~~Australia~~ India
Norwin	desi	Australia
C-44	desi	Pakistan
CM-72	desi	Pakistan
CM-88	desi	Pakistan
NIFA-88	desi	Pakistan
Punjab-91	desi	Pakistan
Piadar-91	desi	Pakistan
Garnet	kabuli	Turkey
Kaniva	kabuli	Spain
Narayen	kabuli	USSR
Noor-91	kabuli	Pakistan
940-26	desi	Australia
T-1587	desi	Iran
CPI-56288	desi	Iran
CTS-11308	desi	ICRISAT (India)
CTS-60543	desi	ICRISAT (India)
ICC-4958	desi	ICRISAT (India)
ICC-14307	desi	ICRISAT (India)
ICC-11514 × ILC-482	desi	Pakistan
ICC-11514 × ILC-3279	desi	Pakistan
NEC-138-2 × CM-72	desi	Pakistan
PI-13768	desi	-

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