THE EFFECT OF SALINITY ON INORGANIC NUTRITION OF CHICKPEA AND ITS CORRELATION WITH ANATOMICAL AND BIOCHEMICAL CHANGES

by
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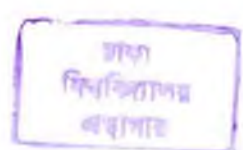
dedicated

to my

FATHER and MOTHER

who always inspired me



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ABSTRACT

Salinity decreased the rate of germination of chickpea seeds. Interaction of 100 mM NaCl salinity and gibberellic acid (GA) or kinetin decreased germination percentage of seeds of chickpea. But interaction of 200 mM NaCl salinity with GA or kinetin both increased germination percentage of seeds. Under salinity, Na^+ and Cl^- accumulation were increased and K^+ accumulation was decreased in the radicle and plumule of germinating seeds of chickpea.

Salinity caused a 4.3- to 18.8-fold increase in Na^+ content in roots and a 7.3- to 7.4-fold of that in the shoot of 7- to 21-day-old chickpea plant. Cl^- content was also increased in the root and the shoot of chickpea seedlings. On the contrary, salinity caused a decrease in K^+ accumulation in the root and in the shoot of chickpea plant.

Accumulation of calcium was decreased in the root and in the shoot under salinity. Salinity increased Mg^{2+} accumulation in the root and also in the shoot of chickpea seedlings. Fe^{2+} accumulation was decreased in the root of chickpea seedlings but that was increased in the shoot under salinity. Phosphate accumulation was decreased in the root but did not show any significant effect in the shoot of chickpea seedlings under salinity.

Salinity decreased amylase activity in germinating cotyledons and radicles of chickpea seeds. But interaction of salinity with GA increased amylase activity in cotyledons of chickpea seeds.

The epidermal cells became thickened in plant grown under saline condition. In the stem of salinity-treated plant, cortex layers was composed of 14-15 rows of parenchyma cell while it was composed of 12-13 rows in control. In the treated plants, cambium layer was thin compared to that of control. In the stem of chickpea, salinity reduced the number of vessels. Metaxylem and protoxylem elements became disorganised and deformed in shape in the stem of salt-stressed chickpea plants while they were spherical and organised in the control plants.

The number of large xylem vessels in the root of NaCl-treated plants were fewer and metaxylem vessels became smaller in size as compared to control plants. The relationship between anatomical structure and ion transport was discussed.

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CONTENTS

LIST OF ABBREVIATIONS

ds/m	Desi Siemens, a unit of conductance
FAA	Formalin aceto alcohol
Fe-EDTA	Ethylene diaminetetraacetic acid ferric monosodium salt
m-equiv.	Milli-equivalent
Net influx	Net transport of ions through plasma membrane
pH	Negative logarithm of hydrogen ion (H^+)
ppm	Parts per million
Transport index	Long distance transport as percentage of net influx
Ψ_{cx}	Flux of ions from cytoplasm of xylem
Ψ_{oc}	Flux of ions from cytoplasm into the vacuole

CHAPTER 1

INTRODUCTION

Salinity stress is a major problem for crop failure all over the world. Vast area of land is becoming nonproductive each year because of salt accumulation. It reduces the yield of almost all crops.

Levitt (1980) broadly grouped environmental stresses under three heads which are physical, chemical and biotic. Salt stress or salinity belonging to the second category (i.e. chemical) of environmental stresses and initiate a serious metabolic irregularities that may be ultimately reflected in the growth activities of the plant. So, soil salinity is one of the most important abiotic factor affecting yield in irrigated areas. Salinity problem in agriculture is usually confined to arid, semiarid and other regions where rainfall is irregular or not sufficient to remove salts from the plant root zone.

In Bangladesh, about 52.8% of the net cultivated area is affected by varying degrees of soil salinity (Karim *et al.*, 1982). The saline areas which are mainly situated in coastal areas are expanding day by day due to the emergence of new islands in the Bay of Bengal in one hand and on the other hand, in some areas of mainland the salinity level is found to increase due to the gradual invasion of saline water from the Bay of Bengal because of very poor discharge of fresh water from some of the major international rivers at the upper stream. It is well known that soil salinity is regulated by a number of factors including tidal

inundation, fresh water discharge of rivers, surface runoff and ground water seepage from adjacent terrestrial areas, runoff and evaporation, soil type and topography, amount and seasonality of rainfall, ground water recharge and depth of impervious subsoil.

The salinity-affected districts of Bangladesh are Bagerhat, Barisal, Bhola, Borguna, Chittagong, Cox's Bazar, Feni, Jhalakathi, Khulna, Lakshmipur, Noakhali, Patuakhali, Pirojpur and Satkhira. Maximum area affected by soil salinity is under greater Khulna district followed by greater Barisal and Patuakhali (Karim *et al.* 1982).

Salinity affects plant growth and development by ion action and by alternating the water potential of the growth media (Bernstein *et al.* 1958).

Plant cell tends to maintain osmotic potential at a reference level under saline stress condition. Inorganic ions play a vital role in bringing about the osmotic adjustment of cell sap which ultimately results in maintenance of osmotic potential at a reference level.

Common salts like NaCl, Na_2CO_3, $NaHCO_3$, $CaCO_3$, $CaCl_2$, $MgCl_2$, etc. are problems in saline soil.

To understand the mechanism of physiological adaptation of plants, the study of effect of salinity on the accumulation, translocation and distribution of ions is important.

1.1 Effects of salinity on seed germination and its relationship with ion transport

Germination is the most sensitive event of plant growth and development under salinity. Salinity affected seed germination as well as it delayed and decreased the final germination percentage (Begum *et al.* 1992).

Charzoulakis (1992) showed in cucumber that germination was delayed by salinity. Germination of pea (*Pisum sativum*) also was delayed but not reduced by salinity (Siddique and Kumar 1986). On the other hand, Ali-Niemi *et al.* (1992) showed that salinity reduced germination in *alfa alfa.* Salinity-induced reduction in germination was also found in *Heroclonium*, *Petunia* (Sharma and Yamadagni, 1989) and groundnut (Navetiyal *et al.*, 1989).

The germination percentage of barley decreased with the increase in NaCl salinity from 0 to 5 and 10 mmhos/cm (Kumar *et al.*, 1988). Begum and coworkers (1996) also observed that the germination percentage of the four cultivars of *Zea mays* was decreased with the increase in salinity stress. Similarly, decreased germination percentage was also found in rice varieties IAC-440 and IAC-162 (Queiroz and Nakagawa, 1992), wheat (Babu and Kumar, 1975) and in groundnut (Navetiyal *et al.*, 1989) due to salinity.

NaCl salinity stress consistently decreased the rate of germination of wheat. On the other hand, gibberellic acid (GA) alone or in combination with kinetin alleviated the inhibitory effect of salinity on germination (Begum *et al.*, 1992). They also showed that the accumulation of Na^+ in plumule and radicle was increased with the increase in NaCl salinity with a consistent inhibition of

germination. On the contrary, salinity stress decreased the amount of endogenous K^+ of plumule and radicle. Alamgir and Ara (1986) showed that under salinity, K^+ was decreased in five rice varieties in the germinating seeds.

Dell'Aquila and Spada (1992) observed that the salt stress-induced inhibition of germination may primarily be due to an osmotic phenomenon which mainly influenced the hydration level in the embryo rather than that in the rest of the seed.

Germination percentage was not affected by salinity up to 100 mM as observed in tomato (Torres-Schumann *et al.*, 1989). Francois and coworkers (1986) reported that lower soil water salinity (4.5 dS/m) had little effect on germination. But according to Dubey and Rani (1990), NaCl salinity increased the germination of salt susceptible rice. cv. Ratna, Jaya and salt-tolerant cultivar CSR-1 and CSR-3-0. Similarly, the rice variety of CSR-1 and Dular germinated well in different NaCl concentrations (Basu and Ghosh, 1991).

1.2 Effects of salinity on the growth of plants and its relationship with ion transport

Salinity affects plant growth activities and nutrient balance (Epstein 1980).

According to Alamgir and Kutube (1997), salinity inhibited the growth of the root and shoot of wheat seedlings. They also showed that the salt stress increased Na^+ content in both root and the shoot whereas K^+ content was decreased with the increase in salinity, but Na^+ and K^+ ratio in both root and shoot was low in the control but it was high in the treatments.

Plant growth was decreased by salinity depending upon the plant species, salinity level and ionic composition of salts (Khavari-Nejad, 1988). Dry weight of the shoot of salt-sensitive variety of bean and salt-tolerant variety of cotton grown in 50 and 150 mM NaCl was decreased (Brugnoli and Lauteri, 1991).

Wignarajah (1990) found that higher salinity reduced shoot growth rather than root growth.

Yasseen and coworkers (1987) found a reduction in growth rate following an increase in salt concentration. However, the duration of growth was not much affected. Salinity stimulated growth of rhodes grass in low concentration of NaCl but growth was inhibited by high salinity (Waisel, 1985). Such type of result was also observed in spinach (Chow *et al.*, 1990).

Alamgir and Akhtar (1992) observed in eleven high-yielding varieties (HYV) of wheat that high salinity decreased the growth of seedlings of all the varieties of wheat except that of aghrani whereas at low salinity level (NaCl) has a general stimulatory effect on growth. But under salinity both Na^+ and K^+ levels in the seedlings were increased in most of the varieties under salinity.

Yeo and Flowers (1986) reported that Na^+ and Cl^- were considered to be toxic and appeared to be responsible for inhibition of growth induced by salinity.

A reduction of shoot length and shoot dry weight of barley and rice were observed when plants were subjected to NaCl salinity stress (Roth, 1989). Brugnoli and Lauteri (1991) also observed a 77 and 91% reduction in shoot dry

weight when a salt-tolerant variety of cotton and salt-sensitive variety of bean were treated with 50 and 150 mM NaCl salinity, respectively.

Hu and Schmidhalter (1997) found that the extreme Na/Ca ratios in plant tissues negatively affected grain yield production at high salinity. They showed that the supplement Ca^{2+}, Mg^{2+}, K^{+} and NO_3^{-} added to 0.2X Hoagland solution increased mineral concentrations in leaves and stems, but did not improve growth or yield in salinized wheat plants except moderately at 100-150 mM NaCl. But in contrast, growth or yield was improved significantly when the concentration of macronutrients was increased from 0.4 to 0.2X Hoagland solution.

Salinity levels (16 mmhos/cm) resulted in a marked growth reduction in root, shoot length, stem and root dry weight (Ai-Yemeny and Basahy, 1997). Hocking (1993) also showed that soil salinity reduced seedling establishment, dry matter and grain yield; they also observed that proportion of Mg, Na and Cl in stems and leaves was reduced more than that of control plants.

The increase in salinity of irrigation water caused significant adverse affects on yield attributes and yield of wheat (Chopra and Chopra, 1997).

NaCl concentration higher than 50 mM significantly inhibited root growth of rice seedlings, but root growth was improved by addition of $CaCl_2$. Mg^{2+} and K^{+} also counteracted the inhibition of root growth induced by NaCl (Lin and Kao 1995).

Lower salinity concentration (425 mM) promoted the growth, but the highest salinity (850 mM) did not have a significant effect in *Cressa cretica* (Khan and

Seemi, 1998). They also showed that plants grew faster and healthier at low salinity treatment, but growth and reproduction of the plants were significantly inhibited by potassium (K) deficiency.

Salinity caused a reduction in total dry weight of lotus plants treated with 140 mM NaCl whereas no significant effects on growth were observed with 70 mM NaCl (Sanchez-Blanco *et al.*, 1998).

Mirza and Tariq (1993) observed that fresh weight of the shoot and the root generally was decreased with increasing salinity levels in *Cicer arietinum*.

The salinity affected the shoot growth more than that of root growth in *Phaseolus vulgaris* (Wignarajah, 1990). But NaCl salinity (85 mM) stimulated the shoot and leaf growth while higher salinity reduced dry weight in *Kostelzkya virginica* (Blits and Gallagher, 1990) and also in sunflower (Khavari-Nejad and Naffafi, 1990).

Salinity affects plant growth and development by ion action and by altering the water potential of the growth media (Bernstein and Jayward 1958).

1.3 Effects of salinity on the transport of monovalent cations and anions

When plants were grown in NaCl or Na_2SO_4 salinities, Na^+ content was increased but K^+ concentration was decreased (Yeo *et al.* 1977). They also observed by using X-ray microanalysis that Na^+ and Cl^- or S were distributed stoichiometrically in the cortex and endodermis in salt-treated roots. They also

showed that in the lumens of the vessels, the concentration of sodium was less than that of anions though it was more relative to either chlorine or sulphur in the adjoining xylem parenchyma cells. They suggested that xylem parenchyma of mature root reabsorbed the sodium from xylem sap and this reabsorption of sodium may minimize the adverse effects of salinity in salt sensitive glycophytes.

John and coworkers (1977) observed that under high NaCl salinity, Na^+ and Cl^- accumulation was increased but K^+ uptake was decreased in both the root and shoot in barley and rice. They also found that, in barley, 100 mM NaCl had no effect on K^+ loss. But rate of efflux of K^+ was increased at 50 mM NaCl or more (Nassery 1975). On the other hand, Shere and coworkers (1974) found that in a highly salt-sensitive soybean, K^+ concentration was reduced under saline condition. Similar result was also found in barley (Wolf and Jaschke 1987).

Aslam and coworkers (1986) observed high concentration of Na^+ at young developing stages of *Atriplex amnicola* under salinity (200 mM + 400 mM NaCl). They found that most of the Na^+ was stored in salt bladder.

The increase in Na^+ concentration was accompanied with a decrease in corresponding K^+ accumulation in *Vicia faba* only when K^+/Na^+ ratio was below 10 (Huq and Larher 1987). They also observed that K^+ was increased in presence of low NaCl concentration in the solution. Ratner and Jacoby (1976) showed that at low pH, sodium efflux was increased in presence of K^+.

Under salinity condition, *Echinocloa crusgalli* maintained a steady state level of K^+ concentration, although Na^+ and Cl^- content were increased with the increase in NaCl concentration (Aslam *et al.* 1987).

Chow and coworkers (1990) suggested that since Na^+ is accumulated predominantly under saline condition, K^+ content in the cytoplasm must be maintained at a critical value for metabolism and this is an essential criterion for salinity condition.

Elrahen and coworkers (1991) reported that K^+ uptake into NaCl adapted cells of tobacco were 1.5-fold greater than unadapted cells and 3.5-fold greater when cells were exposed to 160 mM NaCl. The difference in net K^+ uptake between unadapted and NaCl adapted cells were due primarily to higher rates of entry rather than to reduced K^+ leakage.

Three nonhalophyte wetland macrophytes (*Amphibromus fluitans*, *Potamogeton tricarinatus* and *Tiglochin procera*) exhibited very different patterns of ion accumulation. *Amphibromus fluitans* was unaffected by salinity, it excluded Na^+ and maintained a low concentration of Na^+ in younger leaves and Na^+/K^+ ratios ranged from 2 to 8. On the other hand, Na^+/K^+ ratio was 5-15 in *Potamogeton tricarinatus*, and that in *Tiglochin procera* ranged from 5 in the youngest leaves to 35 in the oldest leaves (Warwick and Bailey 1997).

Venkatesan and coworkers (1997) observed that in *Ipomoea pescapre*, Na^+, K^+ and Cl^- content in tissues was increased following salinity treatment.

The resistant line of *Vigna radiata* accumulated significantly more Na^+ and maintained higher levels of K^+ under NaCl stress than the nonselected line (Gulati and Jaiwal 1995).

Leigh and coworkers (1986) reported that in halophytes, Na^+ replaced the role of K^+ in osmotic adjustment of the vacuole but a relatively higher concentration of K^+ maintained metabolic compartment. Under salinity condition, high Na^+ accumulation interfered with K^+ uptake (Lazof and Cheeseman 1988). But Subbarao and coworkers (1990) observed an increased in K^+ concentration in salt-treated species while decrease of K^+ was observed in salt-sensitive and low salinity stimulated K^+ absorption in *Vicia faba* (Huq *et al.* 1987).

1.4 Effects of salinity on the transport of divalent cations and anions

Divalent cations, such as Ca^{2+} has an important role in maintaining ionic balance under salinity because it maintains permeability characteristics of membrane. Under the salinity stress, Ca^{2+} can prevent the increased cell permeability (Nassery 1975).

Cramer and coworkers (1986) reported that the cell volume was not affected by salinity stress when supplement Ca^{2+} was supplied. Garg and coworkers (1997) found that increasing NaCl concentration (from 0.50 to 150 mM) decreased Ca^{2+} concentration in the shoot of cluster bean, but supplemented calcium significantly ameliorated the adverse effects of NaCl due to enhanced Ca^{2+} uptake. Calcium also alleviated the negative effects of NaCl on activities of N metabolism enzymes.

Ca^{2+} in *Ipomoea pescapre* sweet was increased considerably with increase in salinity up to 200 mM. Fe^{2+} content was also increased but up to optimum salt level (Vankatesan *et al.* 1997). But Okubo and Utsunomiya (1996) showed that NaCl treatment decreased Ca^{2+} in all plant parts of fig (*Ficus carica*) except in the leaves. In annual sweet clover (*Melilotus segetalis*) growing under favourable and saline conditions, immobile Ca^{2+} was accumulated in leaves with age. But Fe^{2+} was diluted in leaves and not affected by salt (Romero and Maranon 1996).

Nakamura *et al.* (1992) reported that decrease of Ca^{2+} level was associated with decrease in K^+ level under salinity stress and it was found that Ca^{2+} is required for maintenance of K^+ level in the root of mungbean. It was also found that K^+ selectivity of the membrane was hampered due to the displacement of Ca^{2+} by Na^+ in the cell membrane (Cramer *et al.* 1987).

Na^+ absorption and translocation were depressed under salinity in presence of Ca^{2+} (Sopandie *et al.* 1990) in *Salicornia virginica* and barley root.

The inhibitory effect of salinity on Ca^{2+} absorption was reported in several plants, e.g. *Artiplex rhagodioidis* (Mahmood and Malik 1987), barley (Lynch and Lauchli 1988, Yasseen *et al.* 1989) and also in sugarbeet, maize, pepper and sunflower (Lessani and Marchner 1978).

Haq and Larher (1983) reported that accumulation of Mg^{2+} was increased with the increase in salinity level and the increase was higher in the shoot than that of the root. But in *Echinochloa crusgalli*, Mg^{2+} concentration was decreased and Ca^{2+} concentration was increased under NaCl salinity stress (Aslam *et al.* 1987).

Yadav *et al.* (1989), however, recorded an increase in Mg^{2+} as well as Ca^{2+} with increase in NaCl salinity stress in chickpea.

Salinity caused a slight increase in Mg^{2+} content in wheat and barley (Yasseen *et al.* 1989) and a decrease in Ca^{2+} content (Kingsburry *et al.* 1984). According to the report of Epstein (1961), Mg^{2+} could not be substituted for Ca^{2+} under salinity stress.

Therios and Mesopolinos (1989) found that an increase in salinity level in apple root stock increased Ca^{2+} and decreased Fe^{2+} accumulation.

1.5 Effects of salinity on the accumulation of phosphate

Accumulation of phosphate was not affected by salinity stress in high phosphate plant, but in low phosphate plant it was decreased (Treeby and Van Steveninck 1988). They observed the uptake and distribution of phosphate in lupin roots under moderate salinity (NaCl) stress, vacuolar inorganic phosphate (Pi) concentration in high phosphate plants were decreased by salt stress, although whole plant root Pi was unaffected. In low phosphate plants, vacuolar Pi was unaffected by salt while whole root Pi was increased. So, phosphate uptake was not alternated by salt in high phosphate plants, but was depressed in low phosphate plants. Martinez and Lauchli (1991) obtained same results in cotton seedlings. But in pepper (*Capsicum annum*), high NaCl salinity decreased P contents of the plant (Gunes *et al.* 1996).

Joshi and Naik (1980) reported that the phosphorus concentration in sugarcane variety (Co-740) was reduced due to the addition of NaCl, Na_2SO_4, $MgCl_2$ and $MgSO_4$ in the irrigation water. Phosphorus contents of wheat leaves showed significant decrease with increasing salinity (Heikal 1977).

Treeby and Van Stevèninck (1988) showed in lupin that increasing supply of phosphate had no effect on the effect of Na^+ accumulation in root cell vacuoles, the epidermis or in the cortex, but the concentration of Cl^- in endodermal vacuoles was lowered under salinity. But in the phloem sap, highest phosphate concentration was found along with the highest concentration of Na^+ and Cl^- in the phloem sap (Munns *et al.* 1988).

Hellbust (1985) observed that phosphate uptake was strongly stimulated by low Na^+ concentration.

1.6 Effects of salinity on the accumulation and activity of enzyme amylase

Amylase showed an increasing activity during germination of seeds because amylase is important for starch turnover (Ching 1972; Prashad and Mathur 1983).

Gill and Shing (1985) showed that under stress condition, the tolerant variety of paddy (Jaya and PR-106) had faster rate of seed germination than that of sensitive varieties (MI-48, Palman and Basmoti-370) due to early induction of amylase activity. Higher rate of water absorption was followed by rapid decrease in starch and greater release of soluble sugars. These are attributable to adaptive mechanisms under salt stress condition.

Amylase activity was decreased in rice (*Oryza sativa* L. var GR-30) under salinity stress (Prokash and Prathapasenan 1988).

Pretreatment of seeds by growth regulator was found to increase stress tolerance in crop plant (Ramachandran and Sakharam 1975, Day *et al.* 1982, Sheoran and Khan 1987). From the work of Alamgir and coworkers (1995), it was apparent that pretreatment of wheat seeds with GA_3 increased amylase activity over control. Seeds treated with and grown under salt stress also increased amylase activity more than that was observed in control plants. GA_3 increased synthesis of amylase during seed germination of barley seeds (Jacobson and Corcoran 1977).

Salinity at higher level inhibited amylase activity but low salinity level stimulated amylase activity (Alamgir *et al.* 1995).

Kumar *et al.* (1981) observed that presowing seed treatment with GA_3 reduced the inhibitory effect of salinity on activities of some enzymes. They also found that seed pretreatment by GA_3 increased amylase activity and reduced the inhibitory effect of salt stress.

1.7 Effects of salinity on the anatomical structures

Salinity (13 dS m^{-1}) caused an increase in leaf thickness, decrease in stem diameter and decrease in the number of flowers. These phenologic changes were accompanied by accumulation of high levels of Na and Cl in leaves but not

in stems (Yermanes *et al.* 1967). Hoffman and coworkers (1989) observed that in plums salt injury becomes apparent only in the second year of exposure to high salinity (8 dS m^{-1}) and in the third year of exposure to medium salinity (6 dS m^{-1}).

Salinity caused different degrees of anatomical disturbances in different organs of *Prosopis cinenaria* (Serrato-Valenti *et al.* 1991).

Gadallah and Ramadan (1997) also showed that salinity induced structural changes in stem, root and leaf tissues of *Carthamus tinctorious*. They also noticed few xylem vessels in salt-stressed plants.

The root stealer diameter was also greater in plants grown with NaCl than that of the control plants of *Suaedo maritima* (Hajibagheri *et al.* 1985).

Ciamprova and Mistrik (1992) reported that in root cells of *Zea mays*, the number of mitochondrial cristae was reduced under salinity indicating that reduction in energy production might have contributed to the decreased growth which is generally accompanied with stress conditions.

Exposure to high salinity (200 mM) induced the formation of an exodermis with casparian bands and suberin lamellae close to the root base and in the transition zone to the hypocotyl (Reinhardt and Rost 1995).

Due to salinity stress, anatomical changes in the roots and also in the number of chloroplasts in the chlorenchyma and bundle sheath cells were observed in *Atriplex semibaccata* (De Villiers *et al.* 1996).

In *Phaseolus vulgaris*, salinity suppressed cell enlargement and cell division proportionately (Nieman 1965), and salinity reduced both the size and the number of epidermal cells without affecting leaf thickness of *Hibiscus cannabinus* (Curtis and Lauchli 1987). It was suggested that cellular expansion processes in the leaf epidermis were more sensitive to salinity than the anticlinal division and elongation of the mesophyll which contribute to leaf thickness.

Serrato and coworkers (1991) recorded an increased number of storage cells in *Prosopis tomarugo* at 200 mM NaCl. Similar increase in number of storage cells in the epidermal and cortical layers in *Prosopis cineraria* was also observed (Serrato *et al.* 1992).

Li and coworkers (1997) showed in *Atriplex prostata* that a facultative halophyte exhibits significant reduction in height of cortex and vascular tissue under salinity condition. But salinity had a stimulatory effect on the differentiation of secondary xylem in *Prosopis cineraria* epicotyl. In 45-day-old seedlings grown in 200 mM NaCl, larger number of xylem was produced than that of the control (Serrato *et al.* 1992).

1.8 Role of endogenous ions in the osmotic adjustments of the cell for survival under salinity

Tolerance of a plant to a stress is the capacity of a plant to survive and grow though subjected to an unfavourable environment. The plant can sustain the effects of stress without suffering from irreparable injury.

Some plants survive under salinity stress by adapting a mechanism to dilute the internal salt concentration developed as a result of the absorption of Na^+ and Cl^-. These plants absorb large quantities of water and then dilute the salt concentration and they themselves become succulent (Rains 1972, Flowers *et al.* 1977).

Salt-tolerant cereal crop avoid salt by pumping out salts using Na^+ and Cl^- extrusion pumps (Greenway 1965, Greenway and Munns 1980, Cheeseman 1988). Bal and Dutt (1986) reported that *Oryza coartata* Roxb, a highly salt-resistant wild rice has some special unicellular salt hairs.

Sopandie and coworkers (1990) observed that in the presence of high concentration of sodium, calcium plays an important role in the maintenance of K^+ transport.

In the work reported here, *Cicer arietinum* var Nabin was chosen as the experimental plant material because reports on the effect of salinity on ion transport and anatomical changes in this species is rare.

The aims of the research work reported in this thesis are as follows:

1) a) To study the

   i) effect of salinity on germination and its correlation with the accumulation of Na^+, K^+ and Cl^-, and

ii) effect of salinity on the accumulation and distribution of

- monovalent ions like Na^+, K^+ and Cl^-,
- divalent cations like Ca^{2+}, Mg^{2+} and Fe^{2+}, and
- anions like PO_4^-.

b) To study the

i) effect of salinity on amylase activity and to establish a correlation between amylase activity and salinity-induced germination, and

ii) effect of salinity on anatomical structures and its relationship with salinity-induced ion transport

2) To correlate the effect of salinity on ion transport to salinity-induced biochemical and anatomical changes and growth.

CHAPTER 2

MATERIAL AND METHODS

2.1 Plant material

Cicer arietinum var Nabin was used as plant material. The seeds were obtained through the courtesy of Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur.

2.2 Solution culture

Plants were grown in half-strength Hoagland solution (Hoagland and Arnon 1950). The composition of modified full-strength Hoagland solution was as follows:

a) **Micronutrients:** 46.25 μM H_3BO_3, 9.15 μM $MnCl_2.4H_2O$, 0.81 μM $ZnCl_2$, 0.29 μM $CaCl_2.2H_2O$ and 0.10 μM $Na_2MoO_4.2H_2O$ were used as micronutrients.

b) **Macronutrients:** 5 μM $Ca(NO_3)_2.4H_2O$, 5 μM KNO_3, 2 μM $MgSO_4.7H_2O$, 1 μM KH_2PO_4 and 1 μM Fe-EDTA were used as macronutrients.

2.3 Preparation of gibberellic acid (GA) and kinetin stock solution

a) Preparation of 10^{-4} M GA solution

10^{-4} M GA stock solution was prepared by dissolving 0.35 mg in 250 ml capacity conical flask which was covered by an aluminium foil to avoid degeneration by light. 120 ml distilled water was taken in the conical flask and 0.35 mg of GA was added and the flask was shaken thoroughly. The content was made up to a final volume of 250 ml with distilled water.

b) Preparation of 2 x 10^{-4} M kinetin stock solution

2 x 10^{-4} M kinetin was prepared by dissolving 0.43 mg kinetin in propylene glycol. Kinetin was dissolved in 1 ml of propylene glycol by stirring with a magnetic stirrer. The solution of kinetin in propylene glycol was taken in a 250 ml volumetric flask. The solution was made up to a final volume of 250 ml with distilled water.

2.4 Methods of application of salinity stress

The plants were subjected to different salinity treatments from the initial state of the experiments. The treatments were as follows:

Control	(half-strength Hoagland solution)
50 mM	(half-strength Hoagland solution + 50 mM NaCl)
75 mM	(half-strength Hoagland solution + 75 mM NaCl)

100 mM (half-strength Hoagland solution + 100 mM NaCl)

150 mM (half-strength Hoagland solution + 150 mM NaCl)

2.5 Culture of plants

2.5.1 Surface sterilization of seeds

In order to avoid fungal infection, surface of seeds were sterilized with 1% sodium hypochlorite (4.7%) solution for one minute. Sodium hypochlorite-treated seeds were washed under tap water for several times (7-12 times). The seeds were finally washed with distilled water for 3 to 4 times. Before sowing, the seeds were soaked in distilled water for 30 minutes and continuously aerated with the help of air-compressor (Model: Compton Com/VAG, Dawson McDonald and Dawson Ltd., Ashbourne, Derbyshire).

2.5.2 Methods of germination of seeds

Surface-sterilized seeds were placed on Whatman filter paper No. 1 (9 cm) contained in a petridish. Three replicates were used for each treatment. Filter papers were soaked in 5 ml of NaCl solution, i.e. 10, 25, 50, 75, 100 and 150 mM NaCl containing 0.1 mM $CaSO_4$. In case of control, filter papers were soaked with 5 ml of 0.1 mM $CaSO_4$. The seeds were allowed to germinate at 27°C. The petridishes containing seeds were covered by black cloth to prevent light. When plumules and radicles could be properly distinguished, the

germination percentage of seed was recorded at 48, 72 and 96 hours from the date of soaking.

2.5.3 Methods of studying the interaction of different concentrations of NaCl and GA and kinetin on the germination of chickpea

Seeds were allowed to germinate in 100 mM NaCl with or without 10^{-6} M, 10^{-5} M GA or kinetin to study the interaction of salinity and GA, salinity and kinetin on germination. Interaction of salinity with GA and kinetin were also studied using same concentrations.

2.5.4 Growing of plants in solution culture

a) Water culture technique

Pyrex beakers (600 ml capacity) were completely filled with half-strength Hoagland solution and different concentrations of NaCl solution. A plastic lid covered with cotton gauze was placed on the beaker. A part of the cotton gauze was sunk into the solution in order to keep the cotton gauze covering the plastic lid moist.

b) Sowing of seeds

Surface-sterilized seeds were soaked in distilled water and aerated for one hour with the help of an air-compressor. Then the seeds were spread uniformly over the cotton gauze placed over plastic lid. The lid was fitted to a Pyrex beaker of 600 ml capacity fitted with nutrient solution. In case of control, the beaker was

filled with only half-strength Hoagland solution in such a way that the solution remained just in touch of the lid so that the seeds were in contact with nutrient solution. Similarly, seeds were placed in a beaker containing 25, 50, 75 and 100 mM NaCl solution with half-strength Hoagland solution.

The outer surface of the beaker was covered with black paper to prevent exposure to light. Then the beaker was kept in dark for 48 hours for germination. The solution was continuously aerated.

The seeds germinated after 48 hours of sowing. The beakers containing the seedlings were transfered to light blank at 22°C/18°C day/night, temperature 10 hours/14 hours day/night length and relative humidity was 65% during day and 75% at night.

2.6 Method of the study of distribution of ions in the intact plant

In order to study the distribution of ions under salinity, the root and the shoot were first separated. Root samples were washed with 0.2 mM $CaSO_4$ (50 ml each) to remove free space ions (Karmoker and Steveninck 1978, 1979). The roots were gently blotted to remove excess water. Both root and shoot were dried in an oven at 80°C for 48 hours. Then dry weight of the samples were recorded with an electric balance (FR-200, Japan).

2.7 Methods of extraction of Na^+, K^+ and Cl^-

The root and shoot samples were put into separate test tubes. Distilled water (10 ml) was added to each test tube and allowed to stand for 30 minutes. The

test tubes with the soaked tissue were boiled for 30 minutes in a boiling water bath. The extract was decanted off in separate test tube. Another 10 ml distilled water was added to the residue and again boiled for 15 minutes. After collection the extract, 5 ml distilled water was added to the residue and boiled for 10 minutes. The extract was decanted off in the test tube. The whole extract was made up to a final volume of 20 ml with distilled water. The extract thus collected were used for measurement of Na^+, K^+ and Cl^-.

2.7.1 Measurement of Na^+ and K^+

Sodium and potassium ions were measured by flame photometer (Gallenkamp, Model FGA 330-C) at a wavelength of 589 and 767 nm respectively.

2.7.2 Measurement of Cl^-

Amount of Cl^- was measured by standard titrametric method by titration with 0.1 N $AgNO_3$ contained in a burette using potassium chromate as an indicator.

2.8 Extraction of Ca^{2+}, Mg^{2+}, Fe^{2+} and phosphate

The root and shoot tissues were taken into separate test tubes. Four ml of a mixture of nitric acid and perchloric acid (4:1) was added to the test tubes containing plant tissues and allowed to stand for an hour in a fumehood. The samples were then heated on a hot sand bath kept in a fumehood and the extracts were dried almost to dryness. The aliquot was made up to a final volume of 20 ml using distilled water.

2.8.1 Measurement of Ca^{2+}, Mg^{2+} and Fe^{2+}

The amount of Ca^{2+}, Mg^{2+} and Fe^{2+} in the extract were measured by atomic absorption spectrophotometer (Model No. 170-1, Model: Perkin-Elmer 3110).

2.8.2 Determination of phosphate

Phosphate was determined by vanadomolybdophosphoric yellow colour method (Jackson 1967). The reagent used was HNO_3 vanadate-molybdate and was prepared as follows:

Preparation of HNO_3 vanadate-molybdate reagent

In order to prepare HNO_3 vanadate-molybdate reagent, three different types of solutions were prepared.

Preparation of solution A: 25 g of ammonium molybdate was taken in 400 ml of distilled water. The solution was heated and cooled at room temperature.

Preparation of solution B: 1.25 g ammonium metavanadate was taken in 300 ml of boiling water and cooled at room temperature. Then 250 ml of concentrated HNO_3 acid was added to it and again cooled at room temperature.

Preparation of solution C: Solution C was prepared just before use. To prepare solution C, solution A, solution B and distilled water were taken at a ratio of 4:5:0.5 and mixed thoroughly.

2.8.3 Measurement of phosphate

Ten ml of extract was taken in a test tube and ten ml of solution C was added to it to develop yellow colour and then allowed to stand for 10 minutes. Finally, absorbance of phosphate was measured at 440 nm wavelength with spectrophotometer (Model: Spectronic Genesys 5, Japan).

2.9 Determination of enzyme (amylase) activity

Amylase activity was measured on fresh germinated radicle and cotyledon after 48, 72 and 96 hours of germination according to the method of Peter Bernfield (1951) with modification. The reducing sugar released by action of amylase was estimated against a standard curve prepared with maltose. Amylase activity was expressed in terms of micrograms of maltose liberated/mg protein/minute.

As the fresh weight of the seeds change during germination, the estimation of protein concentration in extracts from seeds of different ages was very necessary for comparative study. Protein determination was carried out following the method of Lowry and coworkers (1951) using bovine serum albumin (BSA) as standard.

2.9.1 Preparation of reagents for the determination of amylase activity

0.06 M phosphate buffer: 2.1489 g $Na_2HPO_4.12H_2O$ and 0.9078 g KH_2PO_4 were dissolved in 100 ml distilled water separately. To 60 ml of Na_2HPO_4, 40 ml KH_2PO_4 was added followed by addition of 90 mg NaCl.

Substrate: 1 g of soluble starch was dissolved in 100 ml buffer.

Colour reagent: At room temperature, 1 g 3-5-dinitrosalicylic acid was dissolved in 20 ml NaOH and distilled water. Then, 30 g Rochelle salt (K-Na tartrate) was added and made up to 100 ml total volume by adding distilled water.

Standard maltose: 1.44 g maltose was dissolved in 100 ml distilled water.

2.9.2 Preparation of reagents for the determination of protein

2% Na_2CO_3 solution: 10 g Na_2CO_3 was dissolved in distilled water and made up to total volume of 500 ml.

Alkali copper solution: Alkali copper solution is a mixture of 1% $CuSO_4$ (0.5 ml), 2% Na-K tartrate (0.5 ml) and 2% Na_2CO_4 (50 ml).

Folin-ciocalteus-phenol reagent (FCR): FCR solution was was made double-diluted with distilled water.

2.9.3 Procedure and measurement of amylase activity

One gram of fresh germinated tissue (radicle and cotyledon) was taken for the study. At first the tissue was crushed by phosphate buffer solution, then centrifuged for 10 minutes (10,000 rpm). Supernatant (extract) was collected.

For amylase measurement, extract (0.2 ml), buffer (0.4 ml) and substrate (0.5 ml) were mixed for a reaction mixture. Then the mixture was incubated in a water bath at 37°C for 10 minutes. After cooling, colour reagent (1 ml) was

added and then heated on a boiling water bath for 10 minutes. After 3 minutes of cooling, distilled water (10 ml) was added and OD was read at 540 nm wavelength.

For estimation of protein, at first extract (0.5 ml) was mixed with buffer (9.5 ml). Then, from the mixture, 0.1 ml was taken and added to 0.9 ml distilled water and 5 ml alkali solution, and mixed thoroughly. After 20-25 minutes, FCR was added to this mixture and double-diluted. Finally, OD was read at 750 nm wavelength.

The reducing sugar released by action of amylase was estimated against a standard curve prepared with maltose. Amylase activity was expressed in terms of micrograms of maltose liberated/mg protein/minute.

2.10 The method of studying anatomical structure

For studying the anatomical structure, plants were grown in sand culture. Sand was collected from Hyesons Glass Factory, Tongi.

2.10.1 Methods of sand culture technique

Purification of sand: The sand was purified by soaking in 3% HCl for 24 hours, washed with tap water for 24 hours and finally washed with distilled water until the pH was neutral (pH 7.0). The purified sand was dried in the sun (Hewitt 1966).

Surface sterilization of seeds: Seeds were sterilized according to the method described in water culture technique (section 2.4.1).

Preparation of pots: An earthen pot (15 cm in diameter) was lined with plastic sheet to avoid contact between sand and pot, and a hole was made in the plastic sheet corresponding to the hole at the bottom of the pot for drainage of water. The earthen pot was then filled with purified sand.

2.10.1.1 Growing of plants by sand culture technique

Surface sterilized seeds were sown in pots filled with purified sand.

The sand of control pot was soaked with half-strength Hoagland solution and sand of the other pots were soaked with 100 mM NaCl solution for salinity treatment. The seeds germinated within 48 hours of sowing.

Collection of samples: For studying anatomical structure, root and stem segments were taken from 45-day-old seedlings of both control and salinity treated plants. Stems (internodes) were collected from 1 cm above the sand surface while roots were collected from 3 cm below the sand surface.

2.10.1.2 Methods of anatomical studies

Freehand sectioning were done throughout the investigation. Fresh materials were sectioned by hand with the help of ordinary shaving blade. The sections were stained in safranin and fast-green and mounted in glycerine and studied immediately after preparation with the help of compound microscope. Photographs of the sections were taken using a camera (Model: Olympus, Japan 28396).

CHAPTER 3

EFFECTS OF SALINITY ON GERMINATION

3.1 Introduction

Salinity stress affected seed germination as well as it delayed and decreased the final germination percentage (Begum *et al.* 1992). In general, salinity has inhibitory effects on germination of seeds (Jibury *et al.* 1986, Kumar *et al.* 1988, Mondal *et al.* 1988, Aleuan *et al.* 1989, Navetiyal *et al.* 1989, Sharma and Yamdogni 1989, Yasseen *et al.* 1989). Salinity reduced germination in *Heroclonium* and *Petunia* (Sharma and Yamdagni 1989). Ali-Niemi *et al.* (1992) also showed the same result in *alfa alfa*. Chartzoulakis (1992) observed delayed germination in cucumber due to salinity. Under salinity, jojoba plant showed a delay and decrease in germination (Safdal *et al.* 1990). Navetiyal and coworkers (1989) observed that in groundnut, germination percentage was not affected by salinity up to 100 mM NaCl. However, inhibitory effect on germination was observed in tomato under NaCl salinity (Torres and Schumann 1989).

Kabar (1989) observed that gibberellic acid (GA) and kinetin increased the rate of germination of salt-treated barley. GA alone or in combination with kinetin alleviated the inhibitory effect of salinity stress on germination, however, kinetin further decreased the rate of germination of wheat under NaCl salinity stress (Begum *et al.* 1992). But they also showed the interaction of GA, kinetin and

NaCl salinity increased the rate of germination in wheat. They observed that Na^+ accumulation was increased in plumule and radicle with concomitant decrease in K^+ accumulation under NaCl salinity resulting in a consistent inhibition of germination.

3.2 Material and methods

3.2.1 Methods of germination of seeds

The seeds were surface sterilized according to 2.4.1. Fifty surface-sterilized seeds were placed on Whatman filter paper No. 1 contained in a petridish (11 cm). Three replicates were used for each treatment. Filter papers were soaked in 7 ml of NaCl solution, e.g. 25, 50, 100, 200 and 300 mM, each contained 0.1 mM $CaSO_4$. In case of control, the filter paper was soaked in 7 ml of 0.1 mM $CaSO_4$. The seeds were allowed to germinate in dark at $25 \pm 1°C/16 \pm 1°C$ day/night temperature. Seeds were considered to be germinated when radicle and plumule could be clearly distinguished. The germination of seeds were recorded at 48, 72 and 96 hours from the date of sowing.

3.2.2 Methods of studying interaction of different concentrations of NaCl and gibberellic acid and kinetin on germination of chickpea

In order to study the interaction of salinity and gibberellic acid, and salinity and kinetin on germination, seeds were allowed to germinate in 100, 200 and 300 mM NaCl solution with or without 10^{-6} M, 10^{-5} M GA or kinetin.

Interaction of salinity and GA, and kinetin were also studied using the same concentration. Germination technique was the same as described above (section 2.5.3).

3.2.3 Preparation of gibberellic acid and kinetin stock solution

10^{-4} M GA and 2 x 10^{-4} M kinetin stock solutions were prepared according to section 2.3a and 2.3b respectively.

3.2.4 Methods of extraction and analysis of Na^+, K^+ and Cl^- in plumule and radicle

Seeds, which were germinated after 48, 72 and 96 hours of soaking in various concentrations of salinity, were used for analysis of Na^+, K^+ and Cl^- ions in plumule and radicle. Plumules and radicles were separated from cotyledons. The radicle was washed with 0.2 mM $CaSO_4$ to remove free space ions (Karmoker and Van Steveninck 1978). Plumules and radicles were dried as described in section 2.6 and ions were extracted from plumules and radicles as described in section 2.7.

Na^+ and K^+ ions were measured using a flame analyzer (Model: Gellenkamp, FGA 330-C). Cl^- was measured according to section 2.7.2.

3.3 Results

3.3.1 Effects of salinity on germination percentage in chickpea

Germination percentage was not significantly affected by salinity up to 100 mM NaCl at 48 hours of treatment. However, 150-300 mM of NaCl salinity decreased the rate of germination from 32-39% as compared to control (Fig. 1). NaCl solution ranging from 10-300 mM sodium chloride decreased the germination percentage of chickpea by 9.4-36.6% after 72 hours. The rate of germination reduced following 10-300 mM NaCl salinity treatment at 96 hours of treatment (Fig. 1).

3.3.2 Effects of NaCl stress on Na^+, K^+ and Cl^- accumulation in germinating seeds of chickpea

Under 10-200 mM NaCl salinity treatment, Na^+ accumulation in radicle was increased by 27.3% to 6.2-fold at 48 hours of treatment. But accumulation of K^+ was reduced by 17.9, 29.5 and 14.3% at 10, 25 and 200 mM of treatment respectively at 48 hours of treatment (Table 1). Salinity ranging from 10 to 200 mM NaCl resulted in 18.41% to 8.5-fold increase in accumulation of chloride in the radicle at 48 hours of treatment (Table 1). After 72 hours of treatment, Na^+ accumulation in the radicle was increased from 93% to 19.7-fold under salinity (10-200 mM) (Table 2). Similar results were observed in radicle of chickpea under salinity at 72 hours and also at 96 hours of treatment (Table 3).

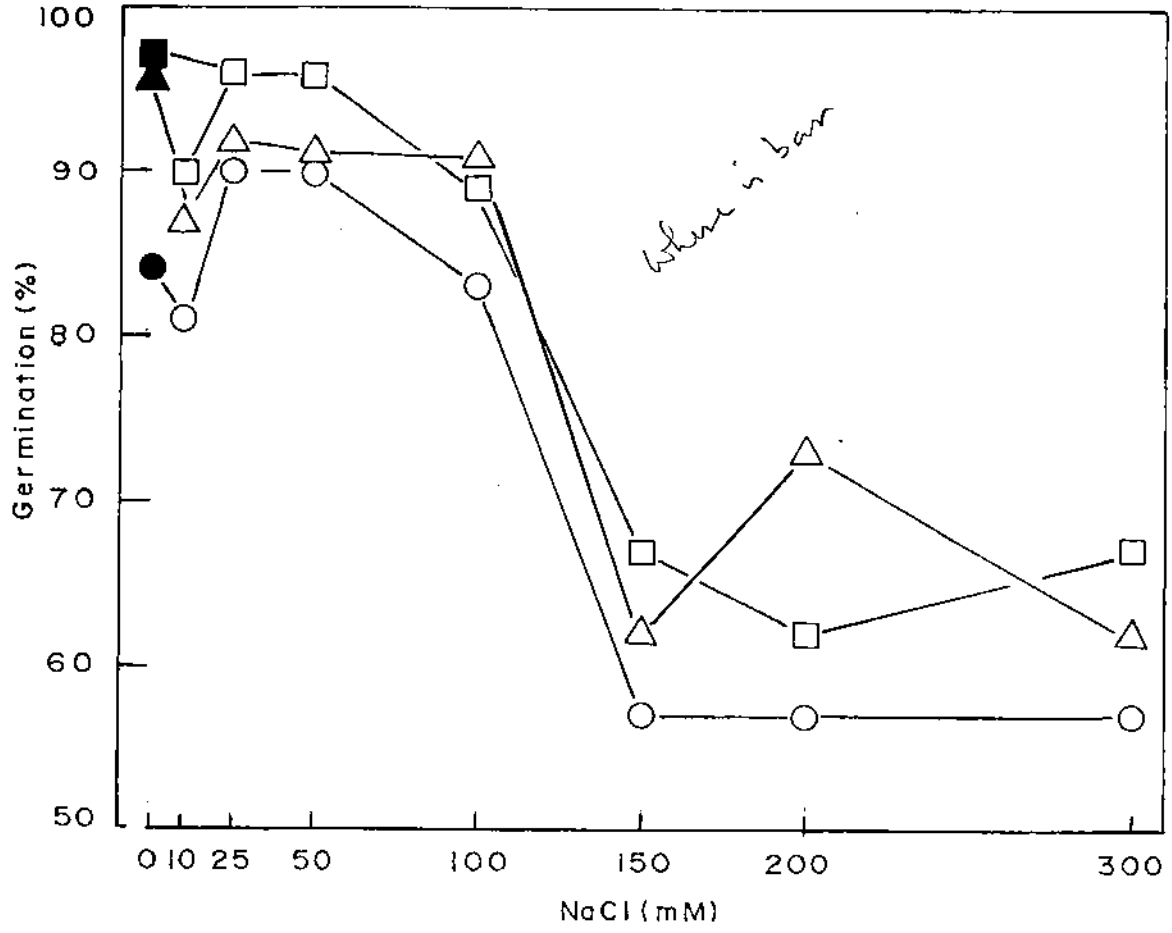

Fig. 1 The effect of NaCl salinity stress on germination of chickpea seed (cv. Nabin). Solid symbols represent control; O = 48 hours, Δ = 72 hours, □ 96 hours. Bars represent ± standard error. Each value is mean of three replicates.

Table 1 The effect of NaCl salinity stress on accumulation of Na^+, Cl^- and K^+ in plumules and radicles of germinating chickpea seeds after 48 hours of treatment. Each value is the mean of three replicates ± standard error.

Treatment	Ion content (m-equiv. g^{-1} dry tissue)					
	Radicles			Plumules		
	Na^+	Cl^-	K^+	Na^+	Cl^-	K^+
0.1 mM $CaSO_4$ (control)	0.11 ±0.030	6.03 ±2.190	1.12 ±0.283	-	-	-
0.1 mM $CaSO_4$ + 10 mM NaCl	0.14 ±0.030	7.14 ±1.590	0.92 ±0.610	-	-	-
" + 25 mM NaCl	0.153 ±0.021	8.73 ±0.935	0.79 ±0.050	-	-	-
" + 50 mM NaCl	0.45 ±0.103	34.92 ±3.900	1.15 ±0.260			
" + 100 mM NaCl	0.36 ±0.070	21.03 ±1.030	1.15 ±0.0088	-	-	-
" + 150 mM NaCl	0.34 ±0.010	31.48 ±7.100	1.15 ±0.170	-	-	-
" + 200 mM NaCl	0.68 ±0.137	57.50 ±2.800	6.96 ±0.060	-	-	-

Table 2 The effect of NaCl salinity stress on accumulation of Na^+, Cl^- and K^+ in plumules and radicles of germinating chickpea seeds after 72 hours of treatment. Otherwise as Table 1.

Treatment	Ion content (m-equiv. g^{-1} dry tissue)					
	Radicles			Plumules		
	Na^+	Cl^-	K^+	Na^+	Cl^-	K^+
0.1 mM $CaSO_4$ (control)	0.10 ±0.007	6.67 ±1.870	0.81 ±0.047	0.22 ±0.029	8.94 ±2.110	1.0 ±0.120
0.1 mM $CaSO_4$ + 10 mM NaCl	0.193 ±0.009	6.24 ±0.830	0.86 ±0.110	0.20 ±0.038	10.73 ±0.860	1.0 ±0.044
" + 25 mM NaCl	0.203 ±0.020	4.46 ±0.715	0.74 ±0.075	0.46 ±0.101	5.73 ±0.744	0.90 ±0.080
" + 50 mM NaCl	0.35 ±0.055	4.02 ±0.481	0.93 ±0.328	0.516 ±0.049	9.45 ±1.810	0.89 ±0.211
" + 100 mM NaCl	0.92 ±0.272	11.92 ±2.390	0.60 ±0.064	-	-	-
" + 150 mM NaCl	0.66 ±0.050	31.98 ±24.080	0.63 ±0.165	-	-	-
" + 200 mM NaCl	0.75 ±0.045	9.08 ±1.230	0.41 ±0.030	-	-	-

Table 3 The effect of NaCl salinity stress on accumulation of Na^+, Cl^- and K^+ in plumules and radicles of germinating chickpea seeds after 96 hours of treatment. Otherwise as Table 1.

Treatment	Ion content (m-equiv. g^{-1} dry tissue)					
	Radicles			Plumules		
	Na^+	Cl^-	K^+	Na^+	Cl^-	K^+
0.1 mM $CaSO_4$ (control)	0.10 ±0.007	4.38 ±0.910	0.73 ±0.056	0.14 ±0.020	8.01 ±0.310	0.91 ±0.080
0.1 mM $CaSO_4$ + 10 mM NaCl	0.203 ±0.046	8.43 ±0.150	0.53 ±0.200	0.21 ±0.030	11.52 ±0.990	0.71 ±0.102
" + 25 mM NaCl	0.230 ±0.054	7.54 ±0.110	0.72 ±0.050	0.233 ±0.060	10.58 ±0.840	0.73 ±0.165
" + 50 mM NaCl	0.88 ±0.049	6.12 ±0.035	0.38 ±0.100	0.27 ±0.120	11.15 ±0.570	0.67 ±0.193
" + 100 mM NaCl	0.61 ±0.040	13.03 ±2.350	0.34 ±0.020	0.69 ±0.078	9.67 ±0.560	0.72 ±0.110
" + 150 mM NaCl	0.31 ±0.090	9.50 ±0.170	0.30 ±0.040	0.62 ±0.080	12.25 ±0.250	0.62 ±0.045
" + 200 mM NaCl	0.55 ±0.090	7.55 ±0.500	0.33 ±0.080	0.75 ±0.235	8.40 ±1.950	0.46 ±0.100

In plumule, Na^+ accumulation was increased by 2.1- and 2.3-fold at 10 and 25 mM of NaCl salinity stress respectively at 72 hours of treatment (Table 2). But K^+ accumulation was decreased in the plumule from 25 to 50 mM of NaCl salinity (Table 2). Cl^- accumulation was also increased in plumule in saline condition at 72 hours of treatment (Table 2). An increase in Na^+ and Cl^- accumulation with a concomitant decrease in K^+ accumulation was also observed at 96 hours of treatment under salinity stress (Table 3).

3.3.3 Interaction of salinity with gibberellic acid, kinetin on germination

Interaction of NaCl salinity and GA (10^{-6} and 10^{-5} M) caused a further inhibition of germination of chickpea as compared to that in 100 mM NaCl (control) (Fig. 2). Application of 10^{-6} and 10^{-5} M kinetin in combination with 100 mM NaCl caused a 25 to 13.34% and 20.183 to 33.55% decrease in germination of seeds respectively from 48 to 96 hours of treatment as compared to control (100 mM NaCl) (Fig. 2). Interaction of 100 mM NaCl, GA and kinetin (10^{-6} and 10^{-5} M) decreased the rate of germination compared to inhibition caused by 100 mM NaCl (Fig. 2).

The rate of germination was increased by 2.2-fold to 65% and 2.1-fold to 85.70% when seeds were treated with 200 mM NaCl in combination with 10^{-5} and 10^{-6} M GA respectively as compared to control (200 mM NaCl) (Fig. 3). Combination of 200 mM NaCl salinity and 10^{-5} M and 10^{-6} M kinetin increased the rate of germination by 2.3-fold to 65.7% and 31.94 to 18.18% at 48 to 96

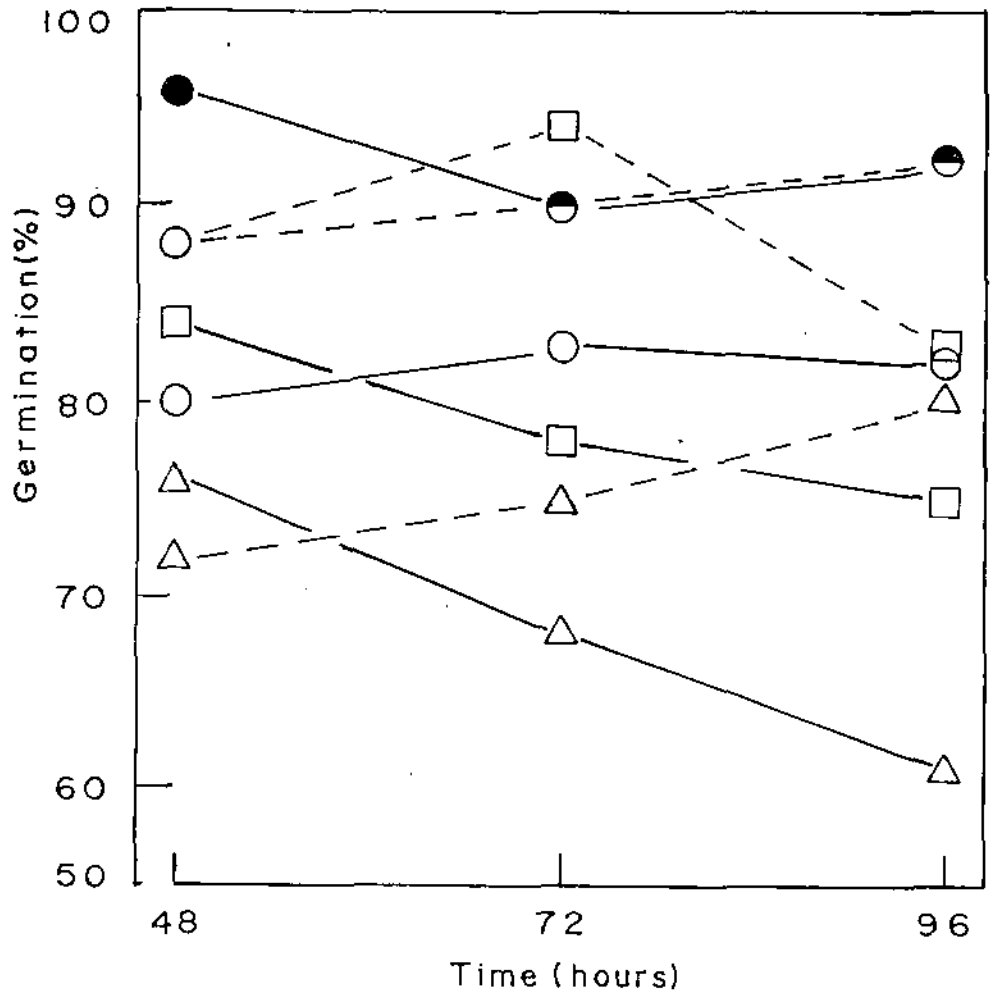

Fig. 2 Interaction of 100 mM NaCl salinity stress and gibberellic acid, kinetin on rate of germination of chickpea seed (cv. Nabin). Control: ● = 100 mM NaCl, O = NaCl + GA, △ 100 mM NaCl + kinetin, □ = 100 mM NaCl + GA + kinetin. Solid line (—) represents 10^{-6} M, broken line (---) represents 10^{-5} M. Each value is mean of three replicates.

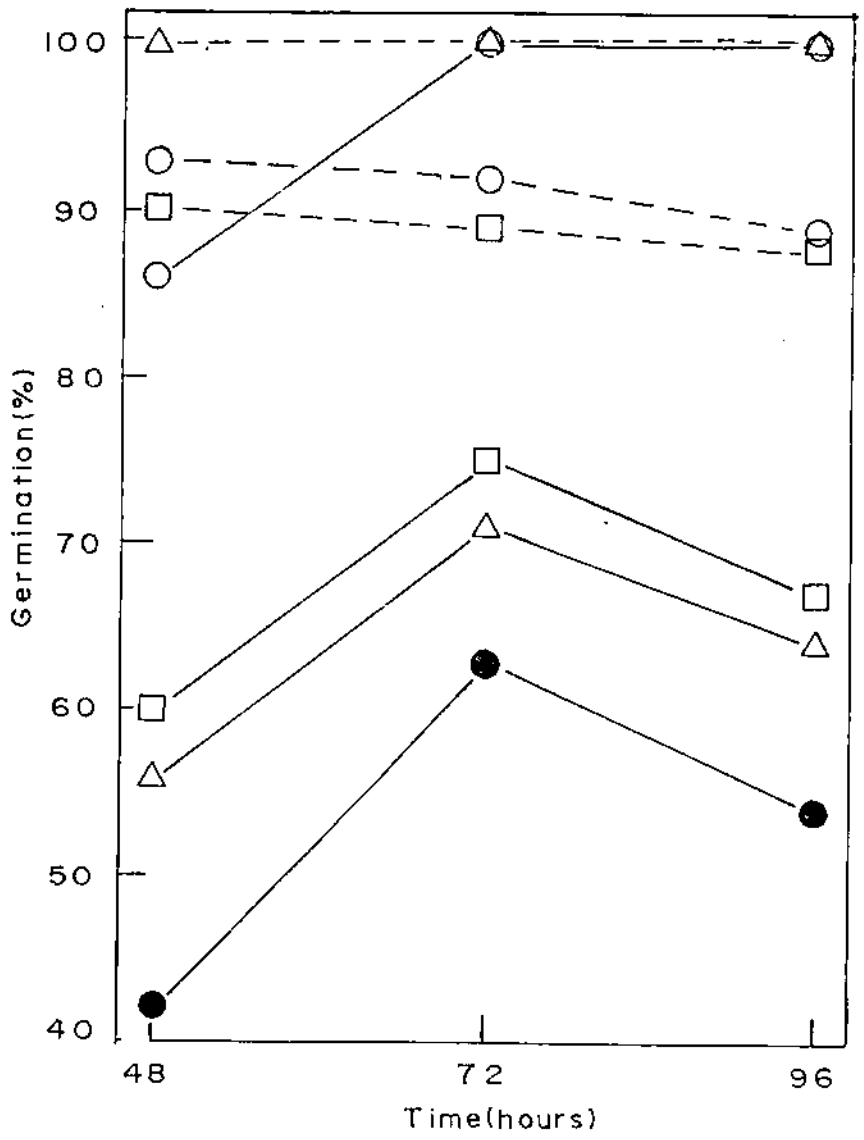

Fig. 3 Interaction of 200 mM NaCl salinity stress and gibberellic acid and kinetin on rate of germination of chickpea seed (cv. Nabin). Control: ● = 200 mM NaCl. Otherwise as Fig. 2.

hours of treatment respectively as compared to that in 200 mM NaCl (control) (Fig. 3). Interaction of 200 mM NaCl and 10^{-5} M GA + 10^{-5} M kinetin caused 2.1-fold to 60.95% increase in germination at 48 to 96 hours of treatment (Fig. 3). The rate of germination was increased by 4.25 to 23.81% following treatment of 200 mM NaCl + 10^{-6} M GA + 10^{-6} M kinetin at 48 to 96 hours of treatment as compared to control (200 mM NaCl) (Fig. 3).

3.4 Discussion

Germination percentage was gradually decreased up to 300 mM NaCl in chickpea at 72 and 96 hours of treatment (Fig. 1). Decrease in germination percentage with the increase in NaCl salinity was also observed in barley (Kumar *et al.* 1988) and *Zea mays* (Begum *et al.* 1998), and also in *alfa alfa* (Ali Niemi *et al.* 1992). But Torres-Schumann (1989) showed that germination percentage was not affected by salinity up to 100 mM NaCl in tomato. Salinity at high level inhibited seed germination but at low salinity level stimulated germination percentage (Alamgir *et al.* 1995). Salt stress-induced inhibition of germination may primarily be due to an osmotic phenomenon which mainly influenced the hydration level in the embryo rather than that in the rest of the seed.

Under salinity, Na^+ and Cl^- accumulation were increased and K^+ accumulation was decreased in the germinating seeds of radicle and plumule of chickpea (Table 1 to 3). Similarly, salinity induced increase in Na^+ and Cl^- with concomitant decrease in K^+ in wheat (Begum *et al.* 1992). Salinity-induced

inhibition of germination (Fig. 1) may be due to salinity induced increase in ABA level (Mizrahi *et al.* 1970, 1971, 1972) in germinating seeds. ABA-induced K^+ efflux was reported by Van Steveninck (1972). So, it may be suggested that decrease in K^+ in plumule and radicle had an adverse effect on the growth of the embryo and further enhanced the inhibition of rate of germination but excessive amount of Na^+ and Cl^- accumulation in germinating seed is responsible for inhibition of germination under salinity condition.

Interaction of 100 mM NaCl salinity with GA or kinetin or with GA and kinetin decreased germination percentage in chickpea (Fig. 2). On the contrary, gibberellic acid alone or in combination with kinetin nullified the inhibitory effect of salinity on germination (Begum *et al.* 1992). But interaction of 200 mM NaCl salinity with GA or kinetin or with GA and kinetin both increased germination percentage of chickpea (Fig. 3). Similarly, Kabar (1987) observed that in NaCl-stressed barley seed, GA and kinetin together increased the percentage of germination. Pretreatment of seeds by growth regulator was found to increase stress tolerance in crop plants (Ramachanndran and Sakharam 1975; Day *et al.* 1982; Sheoran and Khan 1987).

CHAPTER 4

EFFECTS OF SALINITY ON UPTAKE AND DISTRIBUTION OF MONOVALENT CATION AND ANION (K^+, Na^+ and Cl^-) IN INTACT CHICKPEA SEEDLINGS

4.1 Introduction

Salinity stress was found to increase or decrease the accumulation of different ions. Chow and coworkers (1990) suggested that Na^+ was accumulated predominantly under saline condition. K^+ content in the cytoplasm must be maintained at a critical value for metabolism and this is an essential criterion for salinity condition. But salinity increased Na^+ content markedly with concomitant decrease in K^+ content in wheat (Begum *et al.* 1992). Under NaCl or Na_2SO_4 salinity, Na^+ content was increased but K^+ concentration was decreased (Yeo *et al.* 1977).

Leigh and coworkers (1986) also showed in halophytes that Na^+ replaced the role of K^+ in osmotic adjustment of the vacuole but relatively higher concentration of K^+ was maintained in metabolic compartment. But in presence of low NaCl concentration, K^+ was found to be increased (Huq *et al.* 1987). Venkatesan and coworkers (1997) also showed in *Ipomoea pescapre* that Na^+, K^+ and Cl^- content in tissues was increased under salinity. Accumulation of Cl^- was also increased in tomato under salinity (Al-Rawahy *et al.* 1992) and in jojoba (Benzioni *et al.* 1992).

In this chapter, the effect of salinity on uptake and distribution of K^+, and Na^+ and Cl^- in intact chickpea seedlings grown in solution culture are reported.

4.2 Material and methods

Plants were grown in solution culture as described in section 2.5.1 and 2.5.4. Plants were grown up to maturity in solution culture to study ionic relation at different developmental stages. Samples were collected at 7, 14 and 21 days of treatment. Tissues were digested following the method described in section 2.7. Na^+ and K^+ ions were measured according to section 2.7.1 and Cl^- was determined according to section 2.7.2.

4.3 Results

4.3.1 Effects of salinity on accumulation and distribution of Na^+, K^+ and Cl^- ions at different developmental stages of chickpea seedlings grown in solution culture

In order to study the effect of salinity on ion transport at different developmental stages, ion accumulation in the root and shoot was studied at time intervals of 7, 14 and 21 days of treatment. The effect of salinity on ion accumulation in plants of different ages are discussed below.

4.3.1.1 Accumulation and distribution of Na^+, K^+ and Cl^- in the root and shoot of 7-day-old intact chickpea seedling under salinity

With the increase in salinity level from 50 to 150 mM NaCl, Na^+ accumulation was increased by 8.3- to 20-fold in the root (Fig. 4a) and by 14.3- to 58-fold in the shoot of chickpea seedlings at 7 days of treatment (Fig. 4b).

Under salinity treatment, K^+ was decreased in general, and it caused a maximum decrease of 84.71% in the root of chickpea seedlings at 100 mM NaCl treatment (Fig. 4a). In the shoot, K^+ accumulation was also decreased by 28.6 to 39% of salinity treatment (50 to 150 mM) at 7 days of treatment (Fig. 4b).

In the root of chickpea seedlings, accumulation of Cl^- was increased by 19.84% to 2.1-fold over control at 50 to 150 mM NaCl salinity (Fig. 4a). Accumulation of Cl^- was also increased 2.4- to 8.4-fold in the shoot of chickpea seedlings at 7 days of treatment (Fig. 4b).

Net influx (Ψ_{oc}) of Na^+ was increased by 8-, 16- and 8-fold at 50, 75 and 100 mM NaCl respectively (Table 4). Long distance transport (Ψ_{cx}) was also increased by 18-, 22- and 18-fold under 50, 75 and 100 mM NaCl salinity treatment respectively (Table 4). Transport of Na^+ was increased by 76, 53 and 91% at 50, 75 and 100 mM NaCl salinity respectively in chickpea seedlings (Table 4).

Net influx (Ψ_{oc}) of Cl^- was increased by 5-, 2.3- and 2-fold at 50,75 and 100 mM NaCl salinity respectively (Table 5). The long distance transport (Ψ_{cx}) was also increased by 4- to 8.3-fold following 50 to 100 mM NaCl salinity treatment

(Table 5). Transport index was increased by 8- to 26-fold at 50 to 100 mM NaCl salinity treatment (Table 5).

Net influx (Ψ_{oc}) of K^+ accumulation was decreased by 48 to 86.46% at 50 to 100 mM NaCl salinity treatment (Table 6). Long distance transport (Ψ_{cx}) of K^+ was also decreased by 29.53 to 67.65% following 5o to 100 mM NaCl salinity treatment (Table 6). But transport index was increased by 37.6% to 2.3-fold at 50 to 100 mM NaCl salinity treatment (Table 6).

4.3.1.2 Accumulation and distribution of Na^+, K^+ and Cl^- in the root and shoot of 14-day-old intact chickpea seedlings under salinity

In the root, Na^+ accumulation was increased by 9.3 to 22-fold at the concentration range of NaCl used (50 to 150 mM) (Fig. 5a). At the same range of NaCl, a 24- to 60-fold increase in Na^+ accumulation was also found in the shoot of chickpea seedlings (Fig. 5b).

In the root, K^+ content was decreased, but maximum decrease of 84.85 and 70.11% was recorded at 100 and 150 mM of NaCl treatment, respectively (Fig. 5a). K^+ accumulation was decreased from 10.72 to 64.29% in the shoot of chickpea seedlings at 75 to 150 mM of treatment (Fig. 5b).

Accumulation of Cl^- in the root of chickpea was increased by 53.48% and 2.2-fold at 50 and 150 mM of NaCl treatment (Fig. 5a). In the shoot, Cl^- accumulation was also increased by 8.4- and 6.5-fold at 50 and 150 mM of NaCl treatment respectively (Fig. 5b).

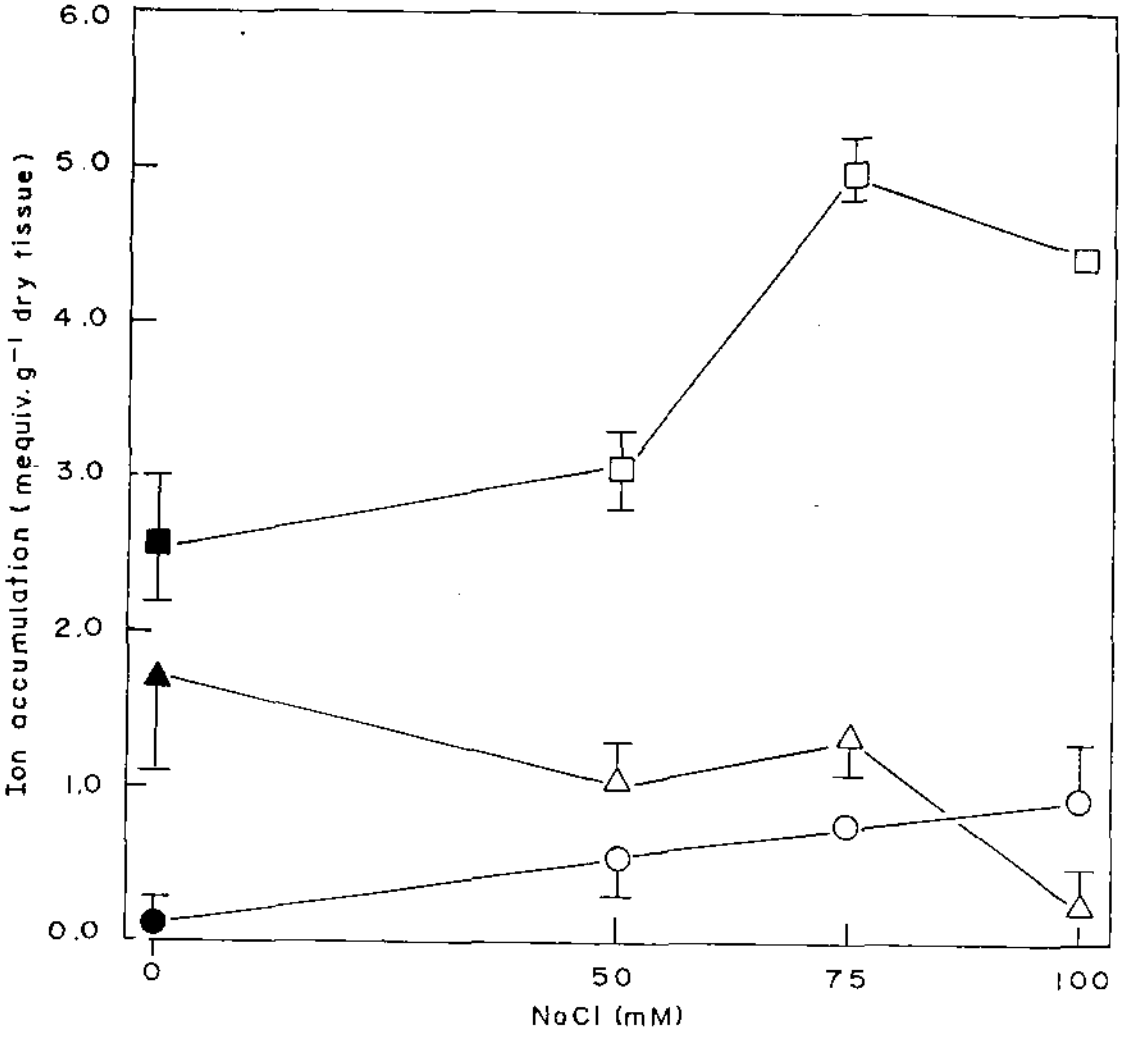

Fig. 4a Effects of salinity on accumulation of Na^+, K^+ and Cl^- in the root of 7-day-old chickpea seedlings (cv. Nabin) grown in solution culture. Solid symbols represent control; O = Na^+, △ = K^+, □ Cl^-. Bars represent ± standard error. Each value is mean of three replicates.

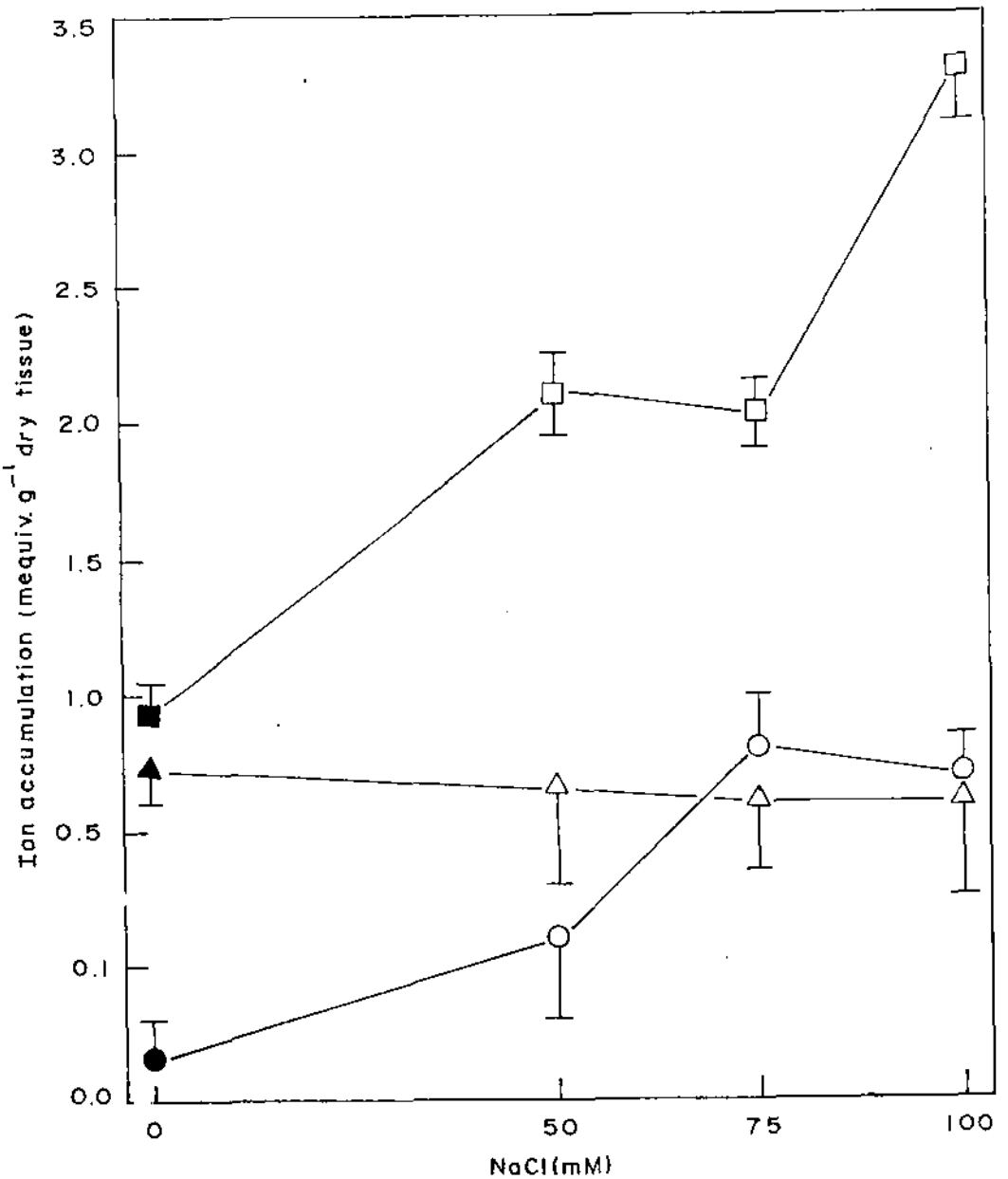

Fig. 4b Effects of salinity on accumulation of Na^+, K^+ and Cl^- in the shoot of 7-day-old chickpea seedlings (cv. Nabin) grown in solution culture. Otherwise as Fig. 4a.

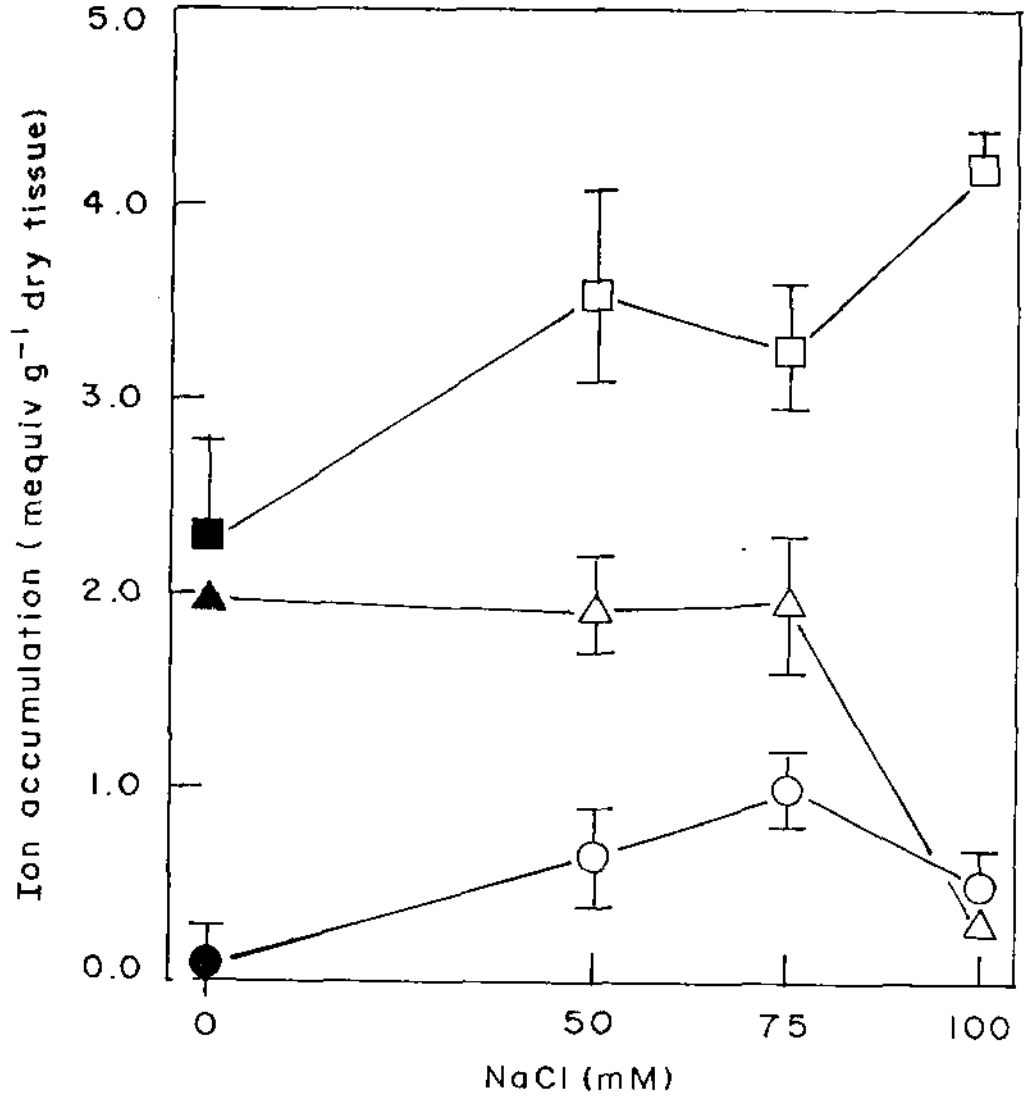

Fig. 5a Effects of salinity on accumulation of Na^+, K^+ and Cl^- in the root of 14-day-old chickpea seedlings (cv. Nabin) grown in solution culture. Otherwise as Fig. 4a.

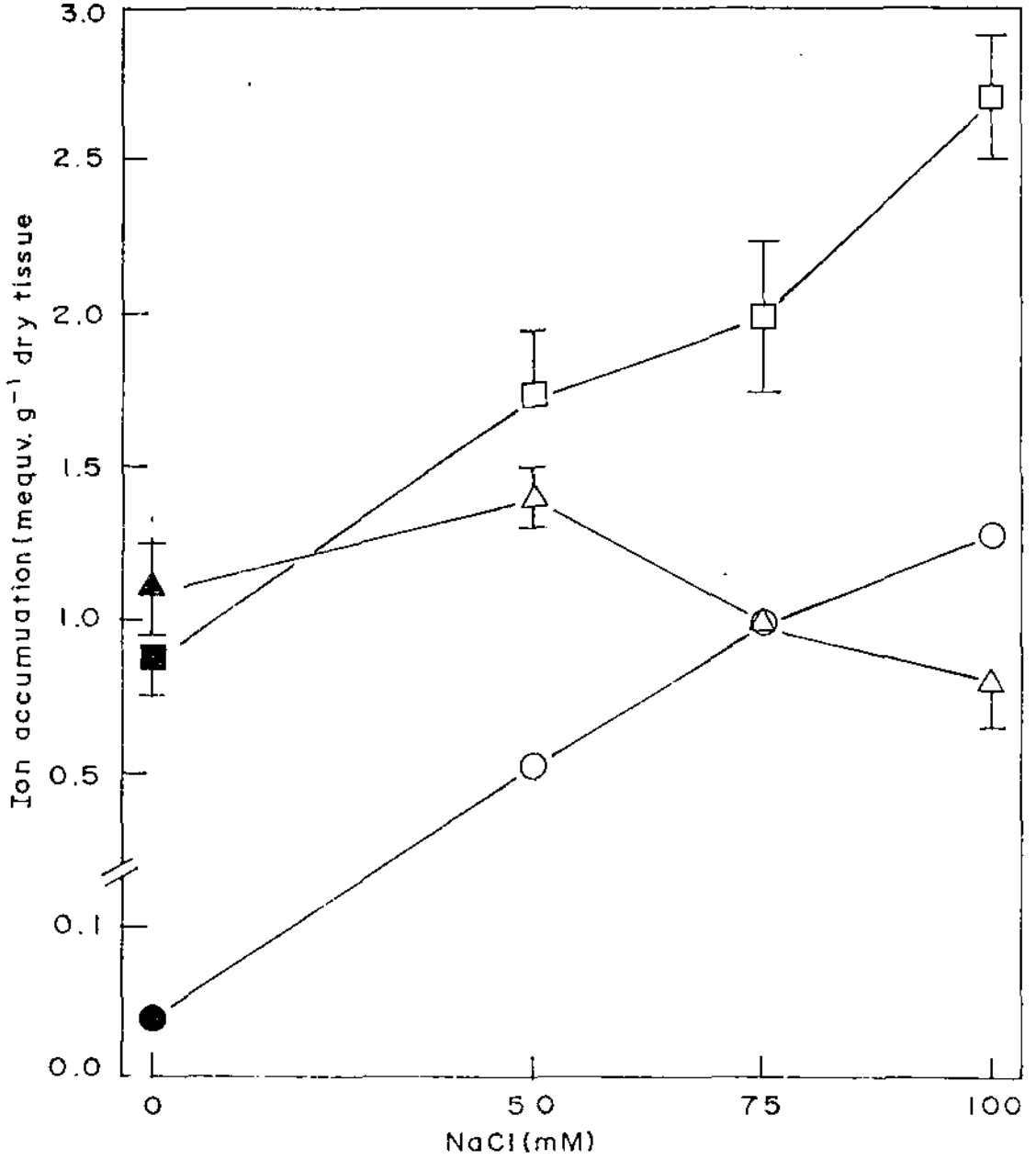

Fig. 5b Effects of salinity on accumulation of Na^+, K^+ and Cl^- in the shoot of 14-day-old chickpea seedlings (cv. Nabin) grown in solution culture. Otherwise as Fig. 4a.

Net influx (Ψ_{oc}) of Na^+ was increased by 5-, 6- and 8-fold at 50, 75 and 100 mM NaCl salinity treatment respectively (Table 4). Long distance transport (Ψ_{cx}) of Na^+ was also increased by 5- to 11-fold following 50 to 100 mM NaCl salinity treatment (Table 4). Transport index of Na^+ was increased by 35% following 100 mM NaCl salinity treatment as compared to control (Table 4) but 50 and 75 mM NaCl salinity treatment did not show any significant effect.

Net influx (Ψ_{oc}) of Cl^- was increased by 2.6-, 2.3- and 2.2-fold at 50, 75 and 100 mM NaCl salinity treatment respectively (Table 5). Long distance transport (Ψ_{cx}) index of Cl^- was also increased at 50 to 100 mM of NaCl salinity stress (Table 5).

But net influx (Ψ_{oc}) of K^+ was decreased by 10-fold and 66% at 75 and 100 mM NaCl salinity treatment respectively (Table 6). Long distance transport (Ψ_{cx}) of K^+ was also decreased.

4.3.1.3 Accumulation and distribution of Na^+, K^+ and Cl^- in the root and shoot of 21-day-old chickpea seedlings under salinity

NaCl salinity stress (50 to 150 mM) caused a 8.6- to 36.8-fold increase in accumulation of Na^+ in the root of chickpea (Fig. 6a). Similarly, it caused a 20.3- to 74-fold increase in Na^+ accumulation in the shoot of chickpea seedlings as compared to control under NaCl salinity (Fig. 6b).

In the root of chickpea seedlings, K^+ accumulation was decreased by 20.57, 29.41 and 52.95% at 50, 100 and 150 mM NaCl treatment respectively

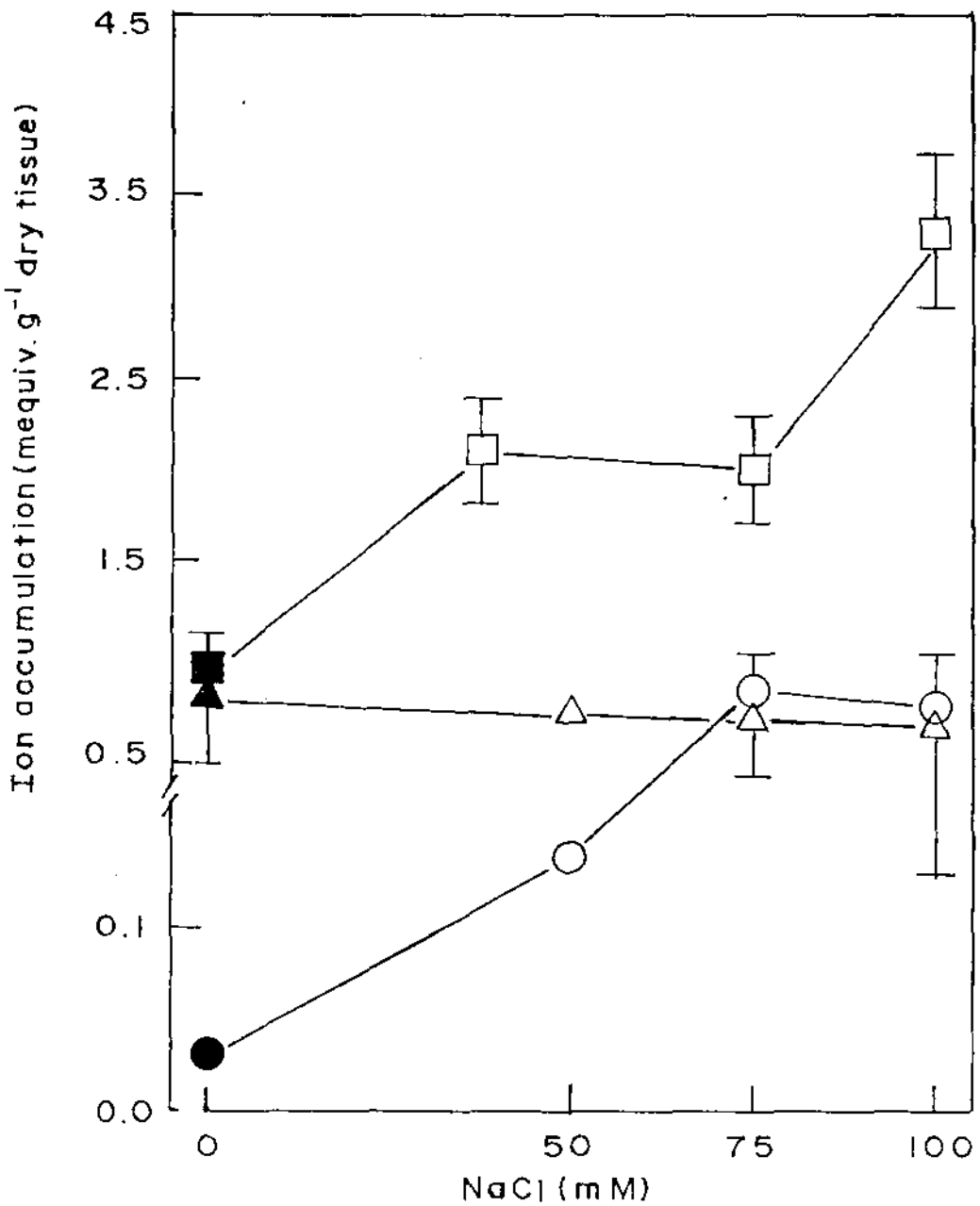

Fig. 6a Effects of salinity on accumulation of Na^+, K^+ and Cl^- in the root of 21-day-old chickpea seedlings (cv. Nabin) grown in solution culture. Otherwise as Fig. 4a.

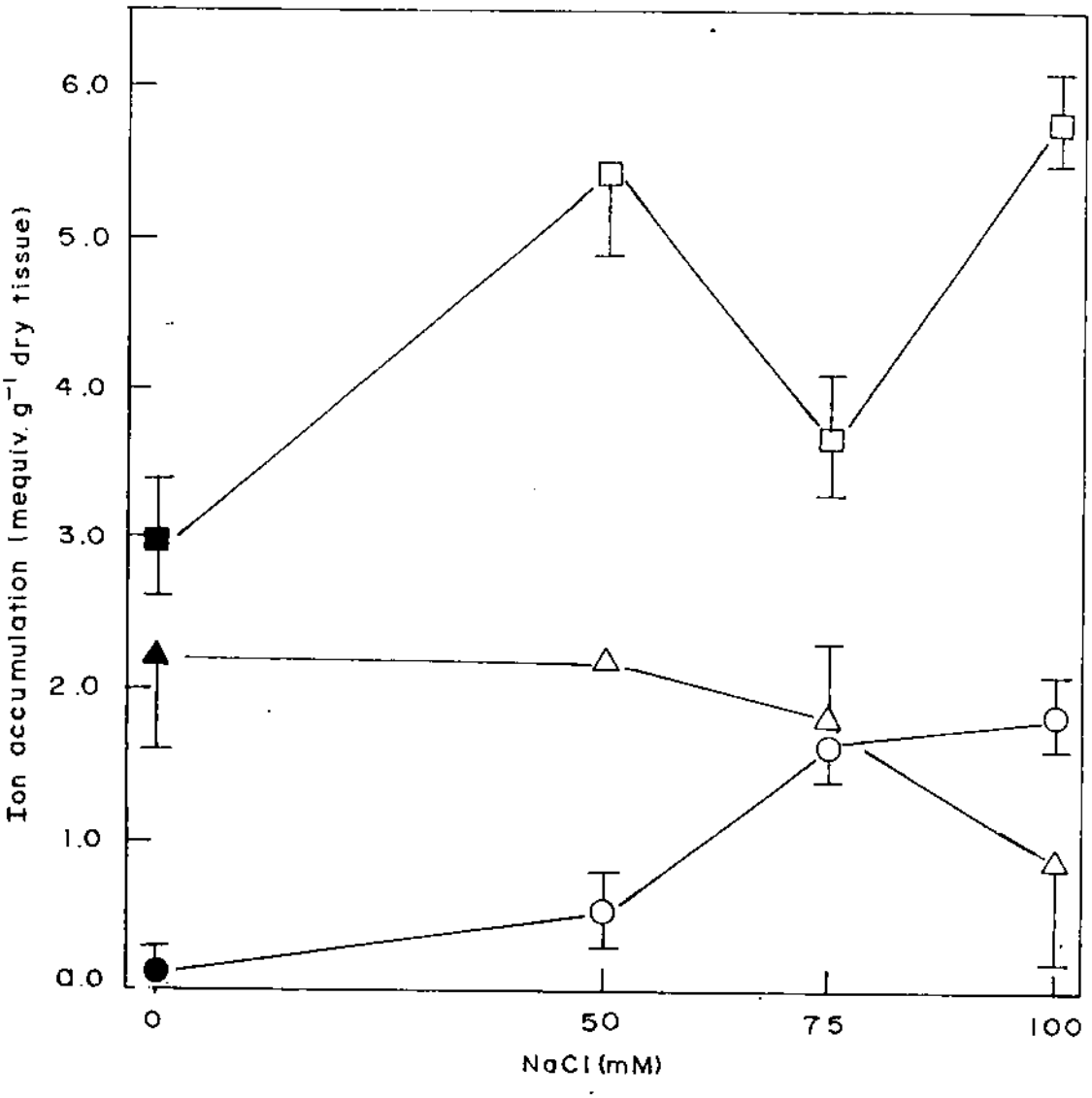

Fig. 6b Effects of salinity on accumulation of Na^+, K^+ and Cl^- in the shoot of 21-day-old chickpea seedlings (cv. Nabin) grown in solution culture. Otherwise as Fig. 4a.

Table 4 The effect of NaCl on long distance transport and net influx of Na^+ in intact chickpea plant grown in half-strength Hoagland solution + NaCl. Each value is a mean of three replicates ± standard error.

Treatments time (days)	Net influx of Na^+ (Ψ_{oc}), (mg/plant)				Transport of Na^+ from root and shoot (Ψ_{ex}), (mg/plant)				Transport index ($[\Psi_{ex} \div \Psi_{oc}] \times 100$			
	NaCl (mM)				NaCl (mM)				NaCl (mM)			
	Control	50	75	100	Control	50	75	100	Control	50	75	100
7	0.02 ±0.001	0.13 ±0.002	0.18 ±0.001	0.12 ±0.002	0.01 ±0.001	0.10 ±0.010	0.12 ±0.002	0.10 ±0.031	43.48	76.92	66.67	83.33
14	0.03 ±0.002	0.33 ±0.031	0.39 ±0.081	0.51 ±0.023	0.02 ±0.002	0.23 ±0.031	0.26 ±0.003	0.46 ±0.002	66.67	69.70	66.67	90.20
21	0.04 ±0.002	0.13 ±0.001	0.46 ±0.002	0.61 ±0.051	0.02 ±0.001	0.03 ±0.002	0.34 ±0.020	0.40 ±0.031	50.00	23.08	73.91	65.57

Table 5 The effect of NaCl on long distance transport and net influx of Cl^- in intact chickpea plant grown in half-strength Hoagland solution + NaCl. Otherwise as Table 4.

Treatments time (days)	Net influx of Cl^- (Υ_{oc}), (mg/plant)				Transport of Cl^- from root and shoot (Υ_{cx}), (mg/plant)				Transport index ($[\Upsilon_{cx} \div \Upsilon_{oc}] \times 100$			
	NaCl (mM)				NaCl (mM)				NaCl (mM)			
	Control	50	75	100	Control	50	75	100	Control	50	75	100
7	0.44 ±0.011	1.32 ±0.050	1.71 ±0.051	1.48 ±0.350	0.20 ±0.020	0.61 ±0.011	0.77 ±0.011	1.67 ±0.180	8.8	46.21	45.03	138.32
14	0.72 ±0.030	1.89 ±0.012	1.66 ±0.021	1.46 ±0.002	0.24 ±0.021	1.05 ±0.022	1.18 ±0.015	2.0 ±0.210	33.33	55.56	71.08	136.99
21	1.14 ±0.018	2.01 ±0.031	2.33 ±0.051	2.45 ±0.151	0.43 ±0.015	1.10 ±0.110	7.30 ±0.120	1.50 ±0.011	37.72	54.73	55.79	61.22

Table 6 The effect of NaCl on long distance transport and net influx of K^+ in intact chickpea plant grown in half-strength Hoagland solution + NaCl. Otherwise as Table 4.

Treatments time (days)	Net influx of K^+ (Ψ_{oc}), (mg/plant)				Transport of K^+ from root and shoot (Ψ_{cx}), (mg/plant)				Transport index ($[\Psi_{cx} \div \Psi_{oc}] \times 100$			
		NaCl (mM)				NaCl (mM)				NaCl (mM)		
	Control	50	75	100	Control	50	75	100	Control	50	75	100
7	0.96 ±0.001	0.50 ±0.015	0.33 ±0.001	0.13 ±0.021	0.34 ±0.030	0.26 ±0.002	0.17 ±0.001	0.11 ±0.002	37.78	52.00	51.52	84.62
14	1.650 ±0.003	1.61 ±0.001	0.67 ±0.003	0.51 ±0.001	0.85 0.015	1.10 ±0.021	0.43 ±0.002	0.49 ±0.001	56.67	68.32	64.18	96.08
21	2.99 ±0.002	2.12 ±0.003	0.95 ±0.001	0.79 ±0.013	2.37 ±0.020	1.23 ±0.022	0.55 ±0.011	0.65 ±0.010	79.26	58.02	57.89	82.28

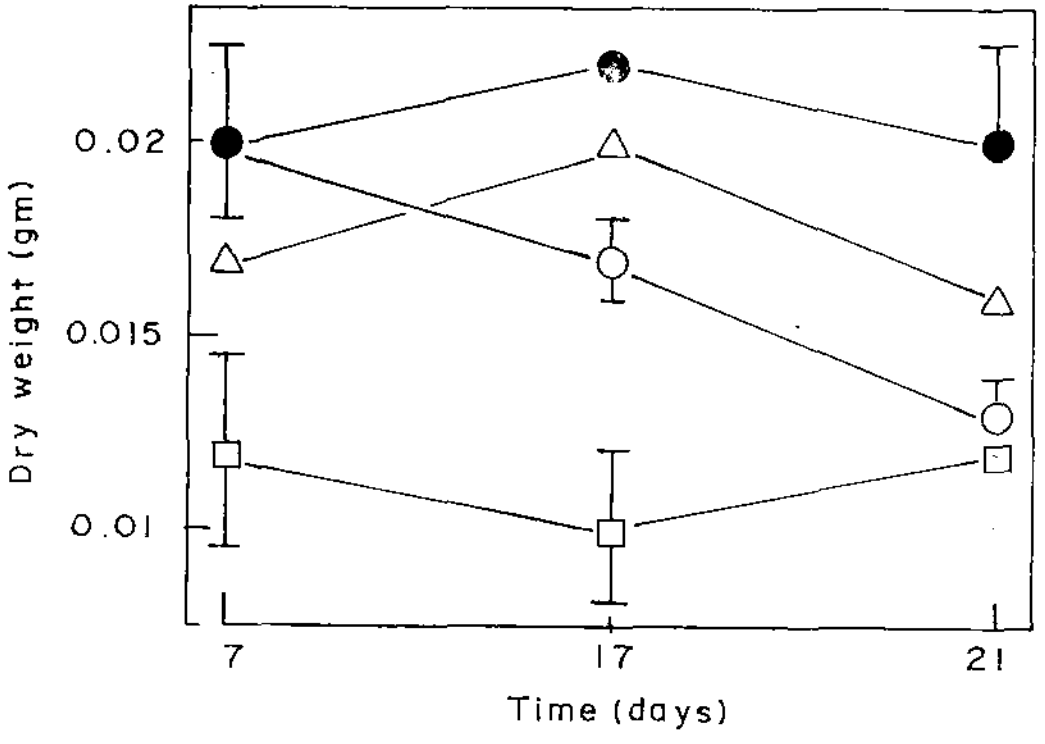

Fig. 7a Effects of salinity on dry weight of the root of chickpea (cv. Nabin) at different developmental stages. Plants were grown in solution culture. Symbols represent: ● = control, O = 50 mM NaCl, △ = 100 mM NaCl, □ = 150 mM NaCl. Bars represent ± standard error. Each value is mean of three replicates.

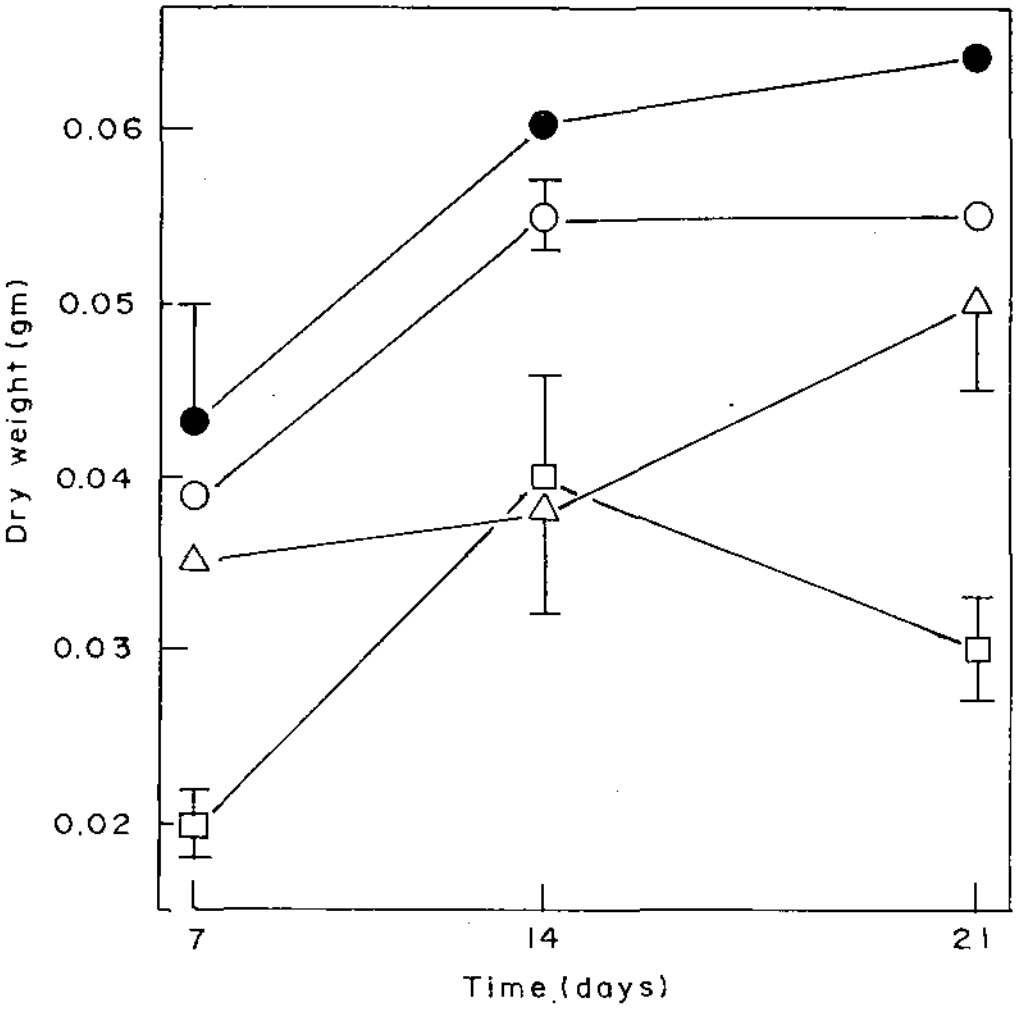

Fig. 7b Effects of salinity on dry weight of the shoot of chickpea (cv. Nabin) at different developmental stages. Otherwise as Fig. 7a.

(Fig. 6a). K^+ accumulation was decreased by 14.07 to 73.47% in the shoot of chickpea seedlings at 50 to 150 mM NaCl salinity (Fig. 6b).

Accumulation of Cl^- was increased by 82.21%, 92.62% and 4.3-fold in the root of chickpea seedlings at 50, 100 and 150 mM NaCl respectively (Fig. 6a). Cl^- content of the shoot of chickpea seedlings was increased by 30.77% to 4.3-fold at 50 to 150 mM NaCl treatment (Fig. 6b).

Net influx (Ψ_{oc}) of Na^+ accumulation was increased by 4.2- to 7-fold at 50 to 100 mM NaCl salinity stress (Table 4). Long distance transport (Ψ_{cx}) index of Na^+ was also increased in chickpea.

Net influx (Ψ_{oc}) of Cl^- was increased by 76% to 2.4-fold at 50 to 100 mM NaCl salinity stress (Table 5). Long distance transport (Ψ_{cx}) of Cl^- was also increased by 2.5- to 4.4-fold at 50 to 100 mM NaCl salinity stress (Table 5). Transport index of Cl^- was also increased in chickpea under NaCl salinity stress (Table 5).

Net influx (Ψ_{oc}) of K^+ was decreased by 29.1 to 73.57% at 50 to 100 mM NaCl salinity stress (Table 6). Transport index of K^+ was also decreased in chickpea seedlings under NaCl salinity stress (Table 6).

4.4 Discussion

In chickpea, salinity caused a dramatic stimulation of Na^+ accumulation but an inhibition of K^+ accumulation (Fig. 4a, 4b, 5a, 5b, 6a, 6b). Yeo and coworkers (1977) observed that when plants were grown in NaCl or Na_2SO_4 salinities, Na^+ content was increased but K^+ content was decreased. Similar results were found

in *Eckinocloa crusgalli* (Aslam *et al.* 1987). Na^+ accumulation was also increased in wide range of plant species including cotton (Cramer *et al.* 1987), barley (Wolf and Jeschke 1987) and in jojoba (Benzioni *et al.* 1992). In the present result, Cl^- accumulation was also increased in the root and shoot of chickpea seedlings (Fig. 4a, 4b, 5a, 5b, 6a, 6b). Similar result was reported by Aslam and coworkers (1987) who found an increase of Cl^- accumulation under NaCl salinity stress in the *Echinocloa crusgalli*. Chow and coworkers (1990) found that Na^+ is accumulated predominantly under saline condition. They suggested that K^+ content in the cytoplasm must be maintained at a reference level for normal metabolism and this is an essential criterion for survival under salinity condition.

NaCl salinity stress decreased the root and shoot growth of chickpea seedlings (Fig. 7a, 7b). Salinity also inhibited the growth of barley (Ball *et al.* 1987), bean, flux and castor beans (Younis *et al.* 1987) and of cucumber (Chartzoulakis 1992). Alamgir and Kutube (1997) suggested that decrease in dry matter of the seeds attached with growing seedlings and dry weight of the root and shoot were also affected under stress condition.

The inhibition of growth caused by salinity may be associated with Cl^- accumulation (Lauter *et al.* 1981). So, it may be suggested that salinity-induced inhibition of growth may be due to toxic effect of large amount of Na^+ and Cl^- accumulation in salt-stressed plant.

Net influx (Ψ_{oc}) and long distance transport (Ψ_{cx}) of Na^+ and Cl^- were increased in chickpea seedlings (Table 4 and 5). Similarly, Begum *et al.* (1994) also showed

that net influx (Ψ_{oc}) and long distance transport (Ψ_{cx}) of Na^+ and Cl^- were increased with the increase of NaCl concentration in wheat. Transport index of Na^+ and Cl^- of chickpea seedlings were also increased (Table 4 and 5). It may be suggested that large amounts of Na^+ and Cl^- were transported to the shoot. So, plant could not avoid the toxic effect of Na^+ and Cl^- hence chickpea seedlings were less salinity tolerant.

Net influx (Ψ_{oc}) and long distance transport (Ψ_{cx}) of K^+ were decreased but transport index was increased at 7 to 14 days of NaCl salinity treatment (Table 6). Increase in transport index of K^+ indicates that comparatively more accumulated K^+ is translocated to the shoot in salinity-stressed plants which may contribute to the demand of K^+ in changing metabolic activity of the shoot under NaCl salinity stress.

CHAPTER 5

THE EFFECT OF SALINITY ON ACCUMULATION AND DISTRIBUTION OF DIVALENT CATIONS (Ca^{2+}, Mg^{2+}, Fe^{2+}) AND PHOSPHATE IN INTACT CHICKPEA SEEDLINGS

5.1 Introduction

Salinity-induced changes in accumulation of essential divalent cations (Ca^{2+}, Mg^{2+}, Fe^{2+}) may change the ionic balance in plants (Epstein 1972). Calcium played an important role in ion transport regulation when the plants were subjected to salt stress (Greenway and Munns 1980, Kamasaki 1983a, b).

Ca^{2+} content was also found to decrease with increase in NaCl salinity (Lessani and Marschner 1978, Mahmood and Malik 1987, Lynch *et al.* 1988, Yasseen *et al.* 1989). Vankatesan and coworkers (1997) found that Ca^{2+} content in *Ipomoea pescapre* sweet was increased considerably with the increase in salinity up to 200 mM. In presence of high concentration of sodium, calcium played an important role in maintenance of K^+ transport (Sopandie *et al.* 1990).

Mg^{2+} accumulation was found to be increased with the increase in salinity level and the accumulation was higher in the shoot than in the root (Huq and Larher 1983). But Subbarao and coworkers (1990) found that Mg^{2+} content was decreased in salt-sensitive species.

In *Ipomoea pescapre*, Fe^{2+} content was also increased but up to optimum level of NaCl (Vankatesan *et al.* 1997). Romero and coworkers (1995) showed Fe^{2+} was diluted in leaves and not affected by salt.

Phosphate is one of the main osmotica in maintaining osmotic potential of the cell sap. Phosphate accumulation was strongly increased by low Na^{+} concentration (Hellbust 1985). He also observed that in chickpea, phosphate uptake was markedly stimulated by salinity. Under salinity, accumulation of phosphate was increased (Gorham *et al.* 1986). Roberts and coworkers (1984) observed that salt stimulated phosphate uptake in maize root tips.

5.2 Material and methods

Plants were grown in solution culture in a controlled environmental condition as described in section 2.5.1 and 2.5.4.

Root and shoot samples were collected at 7, 14 and 21 days after germination. Free-space ions in roots were removed according to section 2.6. Magnesium, iron and calcium ions were extracted by oxidizing dried tissues with 4 ml mixture of nitric acid and perchloric acid as described in section 2.8. Ca^{2+}, Mg^{2+} and Fe^{2+} were determined according to section 2.8.1.

Phosphate was extracted as described in section 2.8.2. Phosphate content was measured by Vanadomolybdophosphoric yellow colour method (Jackson 1967).

5.3 Results

5.3.1 Effects of salinity on accumulation and distribution of Ca^{2+}, Mg^{2+}, Fe^{2+} and phosphate in 7-day-old intact chickpea seedlings

Accumulation of Ca^{2+} in the root of chickpea was increased by 1.8-fold to 42.86% at 75 and 100 mM of treatment respectively as compared to control except in initial decrease at 50 mM treatment of salinity (Fig. 8a). In shoot, Ca^{2+} accumulation was decreased by 14.29 to 42.86% at 50 to 75 mM NaCl treatment. Ca^{2+} accumulation was increased at 100 mM NaCl salinity in the shoot of chickpea seedlings (Fig. 8b).

At all concentration ranges of NaCl (50-100 mM), Mg^{2+} content was increased by 50% in the root of chickpea seedlings (Fig. 8a). In the shoot, Mg^{2+} accumulation was increased by 2-fold at 100 mM NaCl whereas Mg^{2+} content was decreased by 10% at 50 mM NaCl treatment (Fig. 8b).

Accumulation of Fe^{2+} in the root was increased and maximum increase of 2.6-fold occurred at 100 mM NaCl treatment (Fig. 8a). In the shoot, Fe^{2+} accumulation was increased by 33.33% and 2.3-fold at 50 and 100 mM NaCl treatment respectively (Fig. 8b).

Accumulation of phosphate in the root of chickpea seedlings was decreased by 83.34 to 87.5% at 50 to 100 mM NaCl treatment respectively (Fig. 9a). On the contrary, phosphate accumulation in the shoot was increased by 50% at 100 mM NaCl treatment but decreased by 16.67% at 50 mM NaCl treatment (Fig. 9b).

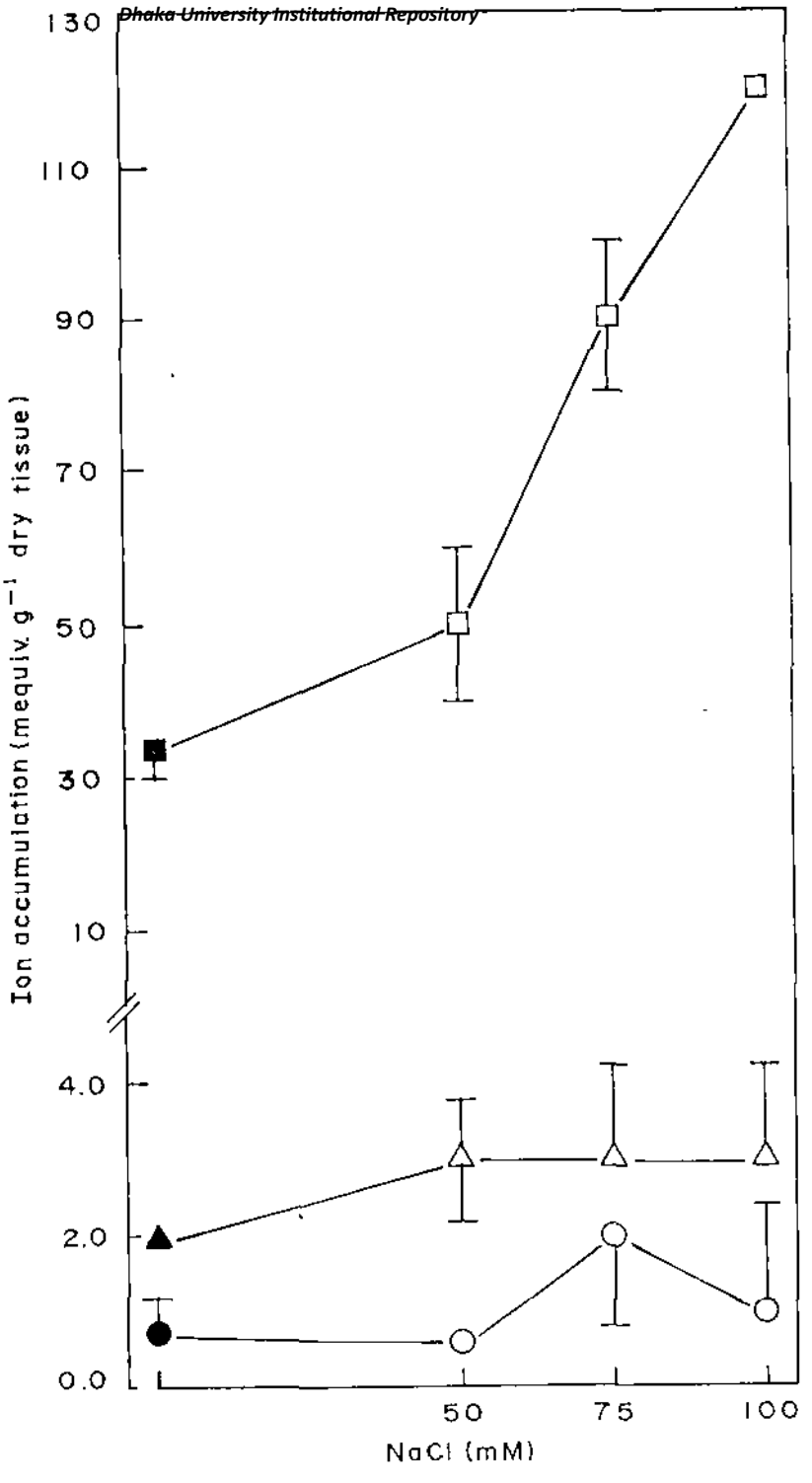

Fig. 8a Effects of salinity on accumulation of Ca^{2+}, Mg^{2+} and Fe^{2+} in the root of 7-day-old chickpea seedlings (cv. Nabin) grown in solution culture. Solid symbols represent control; O = Ca^{2+}, △ = Mg^{2+}, □ Fe^{2+}. Bars represent ± standard error. Each value is mean of three replicates.

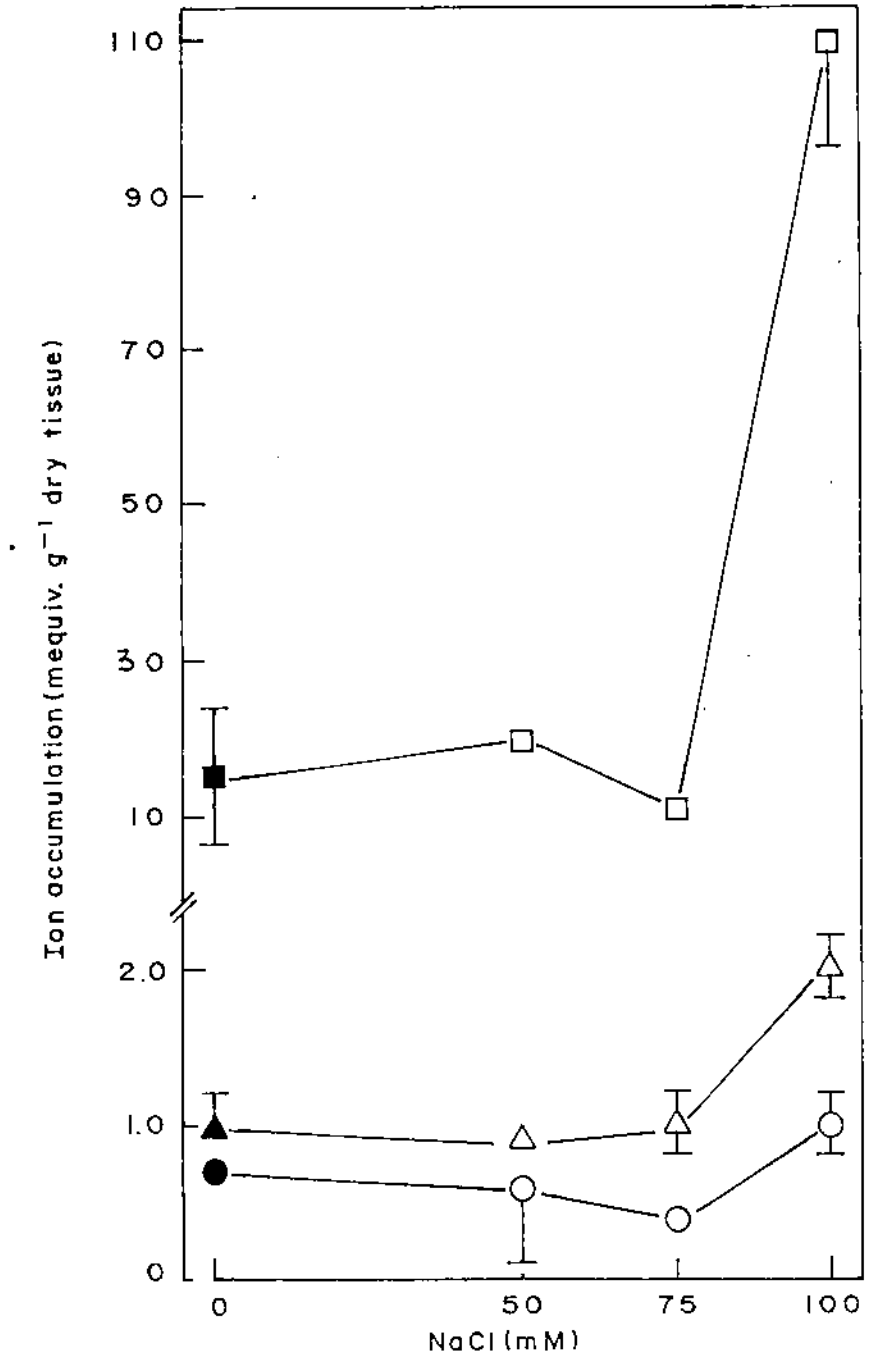

Fig. 8b Effects of salinity on accumulation of Ca^{2+}, Mg^{2+} and Fe^{2+} in the shoot of 7-day-old chickpea seedlings (cv. Nabin) grown in solution culture. Otherwise as Fig. 8a.

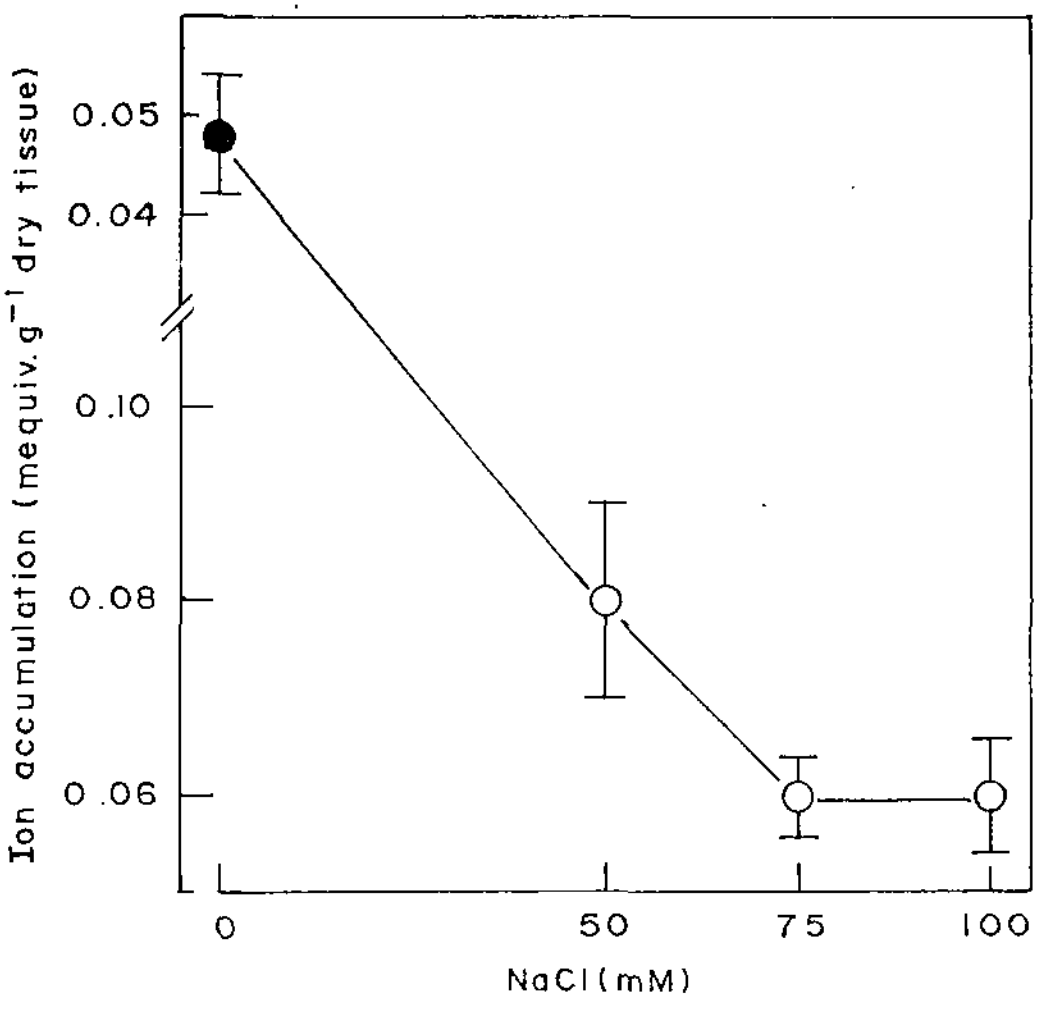

Fig. 9a Effects of salinity on accumulation of phosphate in the root of 7-day-old chickpea seedlings (cv. Nabin) grown in solution culture. Solid symbols represent control. Bars represent ± standard error. Each value is mean of three replicates.

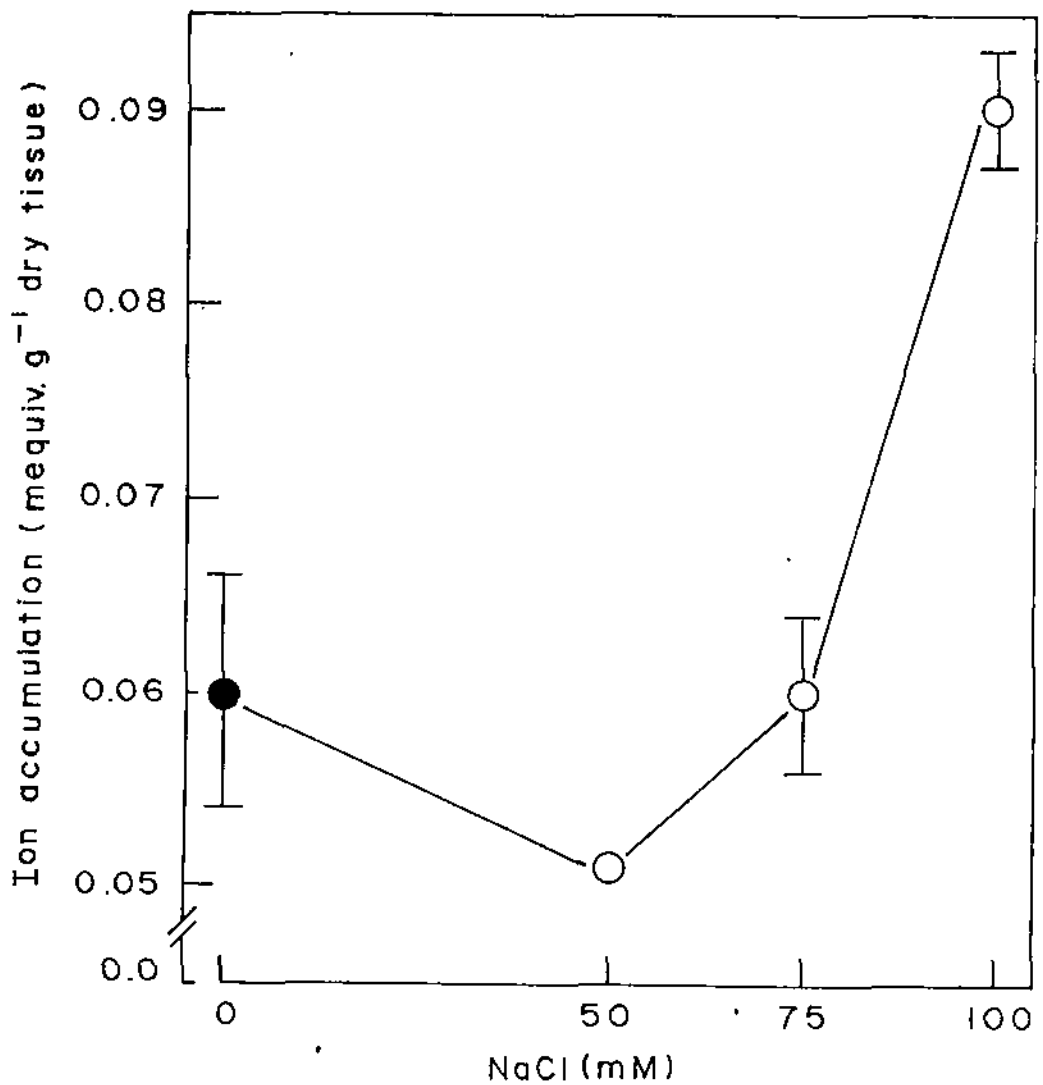

Fig. 9b Effects of salinity on accumulation of phosphate in the shoot of 7-day-old chickpea seedlings (cv. Nabin) grown in solution culture. Otherwise as Fig. 9a.

5.3.2 Effects of NaCl salinity on the accumulation and distribution of Ca^{2+}, Mg^{2+}, Fe^{2+} and phosphate in 14-day-old intact chickpea seedlings

Ca^{2+} accumulation in the root of chickpea seedlings was decreased by 93.29% at 50 mM NaCl but did not show any significant effect at 75 and 100 mM NaCl stress (Fig.10a). In the shoot, Ca^{2+} accumulation was decreased by 20, 30 and 20% at 50, 75 and 100 mM NaCl stress in the chickpea seedlings respectively (Fig.10b).

Salinity stress increased Mg^{2+} accumulation in the root by 50% to 2-fold at 75 to 100 mM NaCl treatment (Fig.10a). Similarly, in the shoot, Mg^{2+} accumulation was also increased by 60% to 2-fold at 50 to 100 mM NaCl treatment (Fig.10b).

In the root of chickpea seedlings, Fe^{2+} accumulation was decreased at 50 mM NaCl treatment but that was increased by 2-fold and 25% at 75 and 100 mM NaCl treatment respectively (Fig.10a). In the shoot, Fe^{2+} accumulation was increased dramatically by 2-fold at 100 mM NaCl treatment after initial decrease at 50 to 75 mM NaCl in the chickpea seedlings (Fig.10b).

In the root, phosphate accumulation was increased by 2.1-fold at 100 mM NaCl treatment, but decreased by 37.5% at 50 mM NaCl treatment (Fig. 11a). Phosphate accumulation was decreased by 25% at 50 mM NaCl salinity but did not show any significant effect at 75 to 150 mM NaCl salinity in the shoot of chickpea seedlings (Fig. 11b).

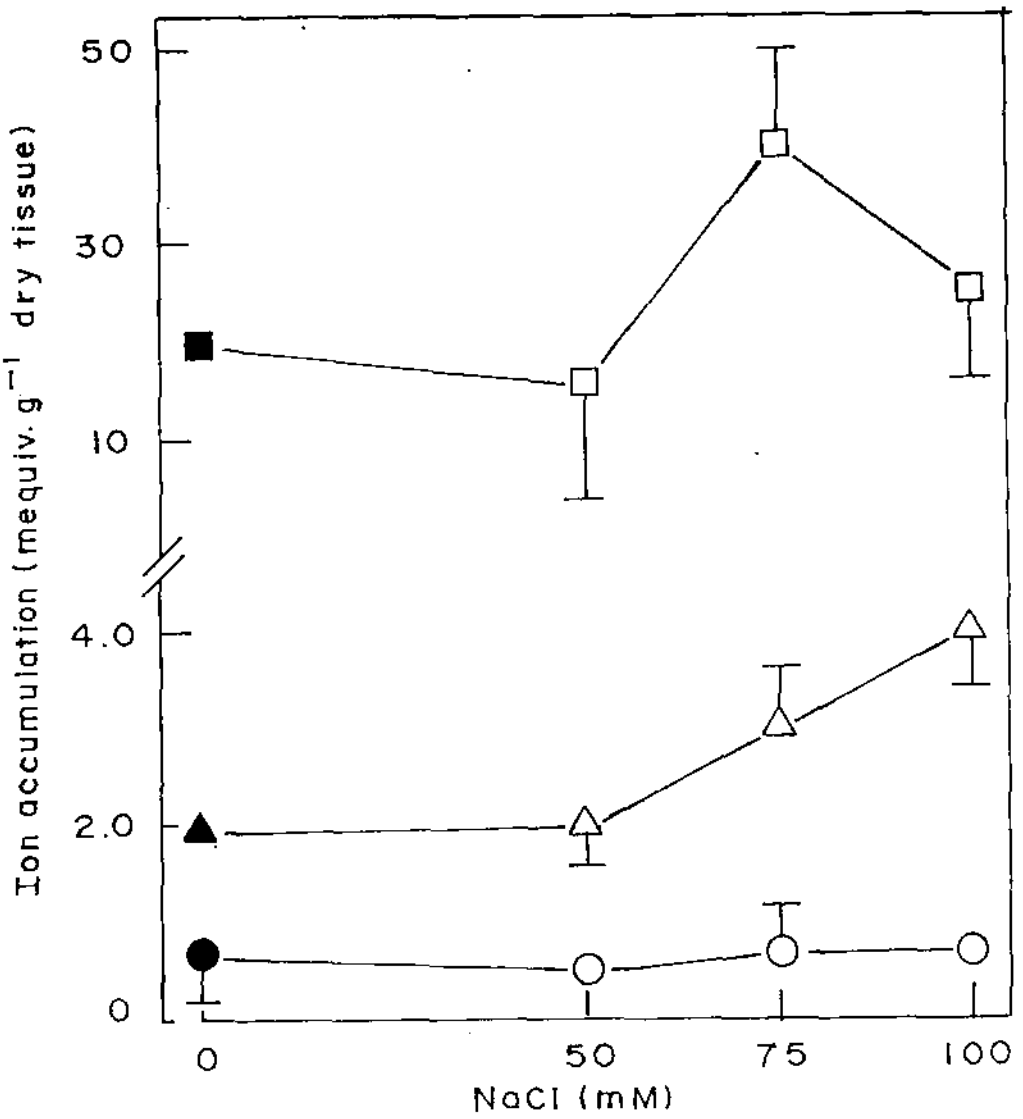

Fig. 10a Effects of salinity on accumulation of Ca^{2+}, Mg^{2+} and Fe^{2+} in the root of 14-day-old chickpea seedlings (cv. Nabin) grown in solution culture. Otherwise as Fig. 8a.

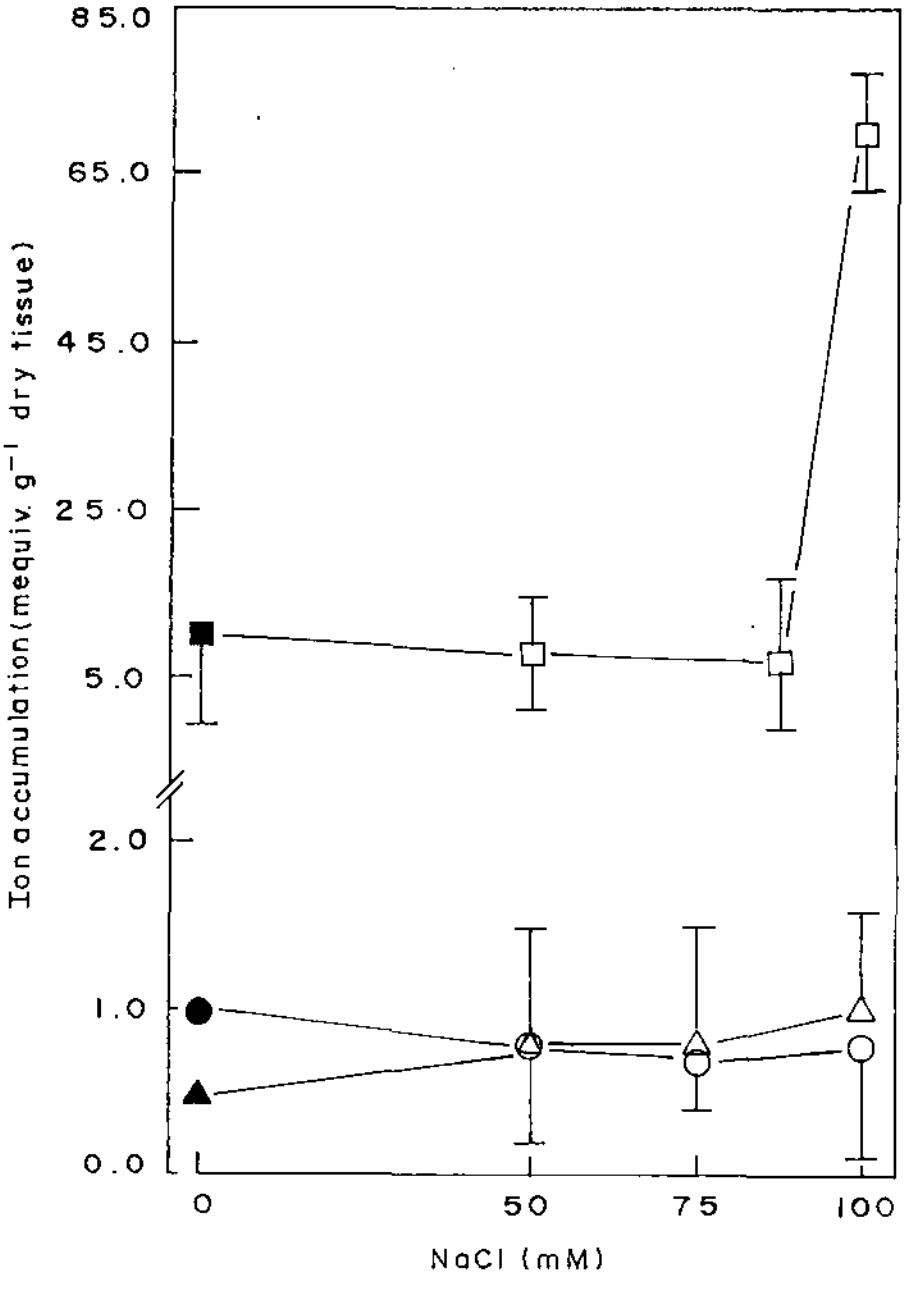

Fig.10b Effects of salinity on accumulation of Ca^{2+}, Mg^{2+} and Fe^{2+} in the shoot of 14-day-old chickpea seedlings (cv. Nabin) grown in solution culture. Otherwise as Fig. 8a.

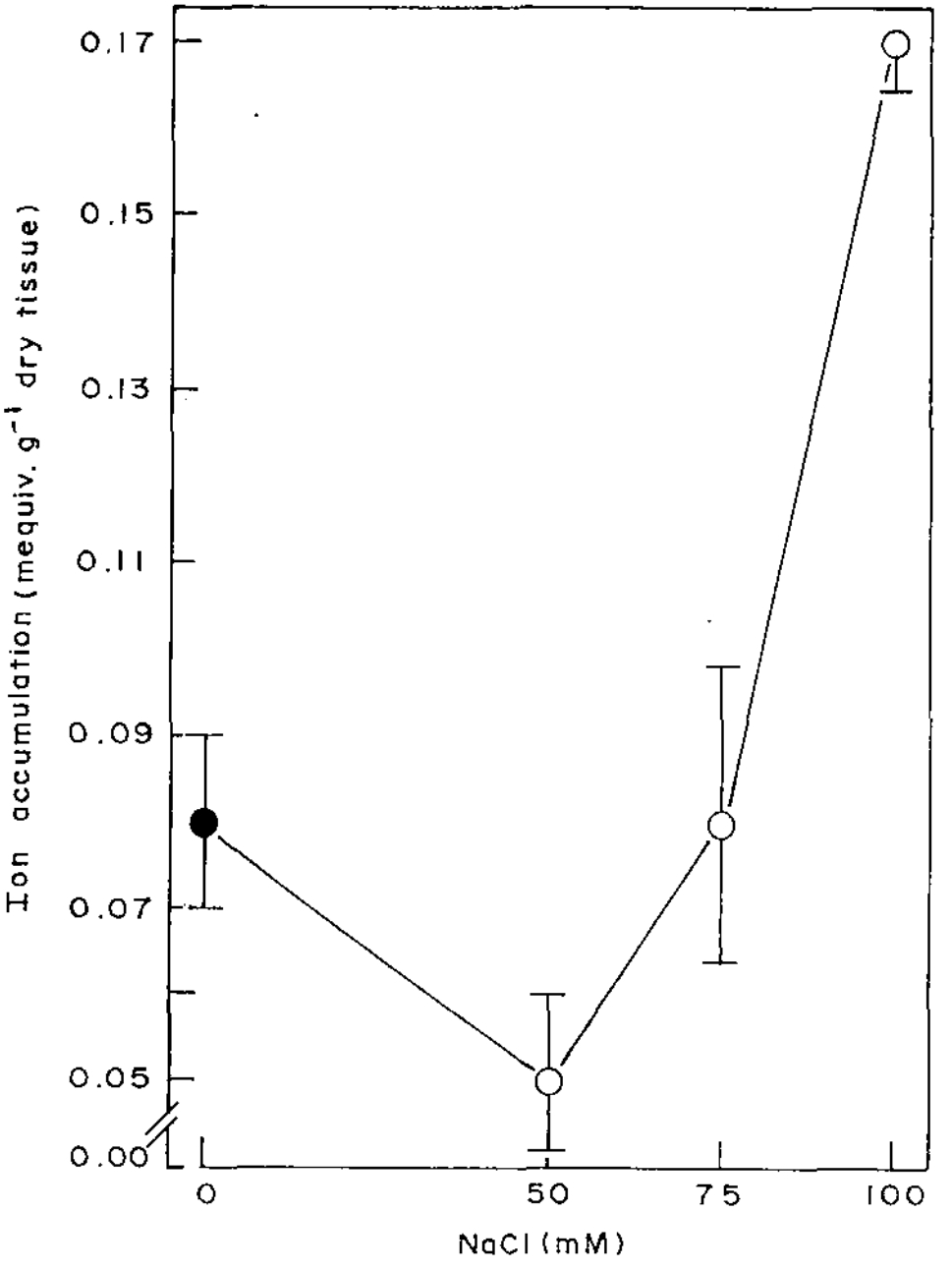

Fig. 11a Effects of salinity on accumulation of phosphate in the root of 14-day-old chickpea seedlings (cv. Nabin) grown in solution culture. Otherwise as Fig. 9a.

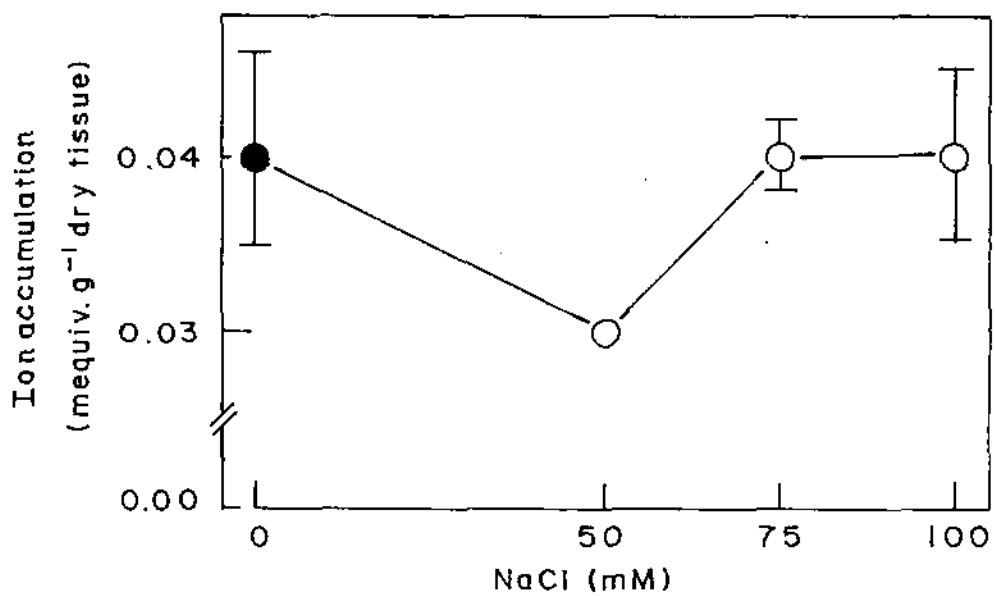

Fig. 11b Effects of salinity on accumulation of phosphate in the shoot of 14-day-old chickpea seedlings (cv. Nabin) grown in solution culture. Otherwise as Fig. 9a.

5.3.3 Effects of NaCl salinity on the accumulation and distribution of Ca^{2+}, Mg^{2+}, Fe^{2+} and phosphate in 21-day-old chickpea seedlings

In the root of chickpea seedlings, Ca^{2+} accumulation was decreased by 30 to 40% at 50 to 75 mM NaCl treatment (Fig.12a). Salinity caused a 52.94% decrease in Ca^{2+} in the shoot of chickpea seedlings at 75 mM NaCl treatment respectively (Fig.12b).

In the root, Mg^{2+} accumulation was increased by 81.81% at 100 mM NaCl but did not show any significant effect at 50 and 75 mM NaCl salinity (Fig.12a). On the contrary, Mg^{2+} accumulation in the shoot was decreased by 33.33% at 75 mM NaCl salinity (Fig12b).

Salinity caused a decrease in Fe^{2+} accumulation in the root of chickpea seedlings by 55.6, 55.6 and 33.33% at 50, 75 and 100 mM NaCl treatment respectively (Fig.12a). Salinity (75 mM NaCl) increased Fe^{2+} accumulation in the shoot by 4.3-fold at 75 mM NaCl salinity but decreased by 39.51 and 33.34% at 50 mM NaCl and 100 mM NaCl respectively (Fig.12b).

Accumulation of phosphate was decreased by 37.5 and 25% at 50 and 75 mM NaCl in the root of chickpea seedlings respectively (Fig. 13a). In the shoot, phosphate content did not show any significant effect on the salinity condition of chickpea seedlings (Fig. 13b).

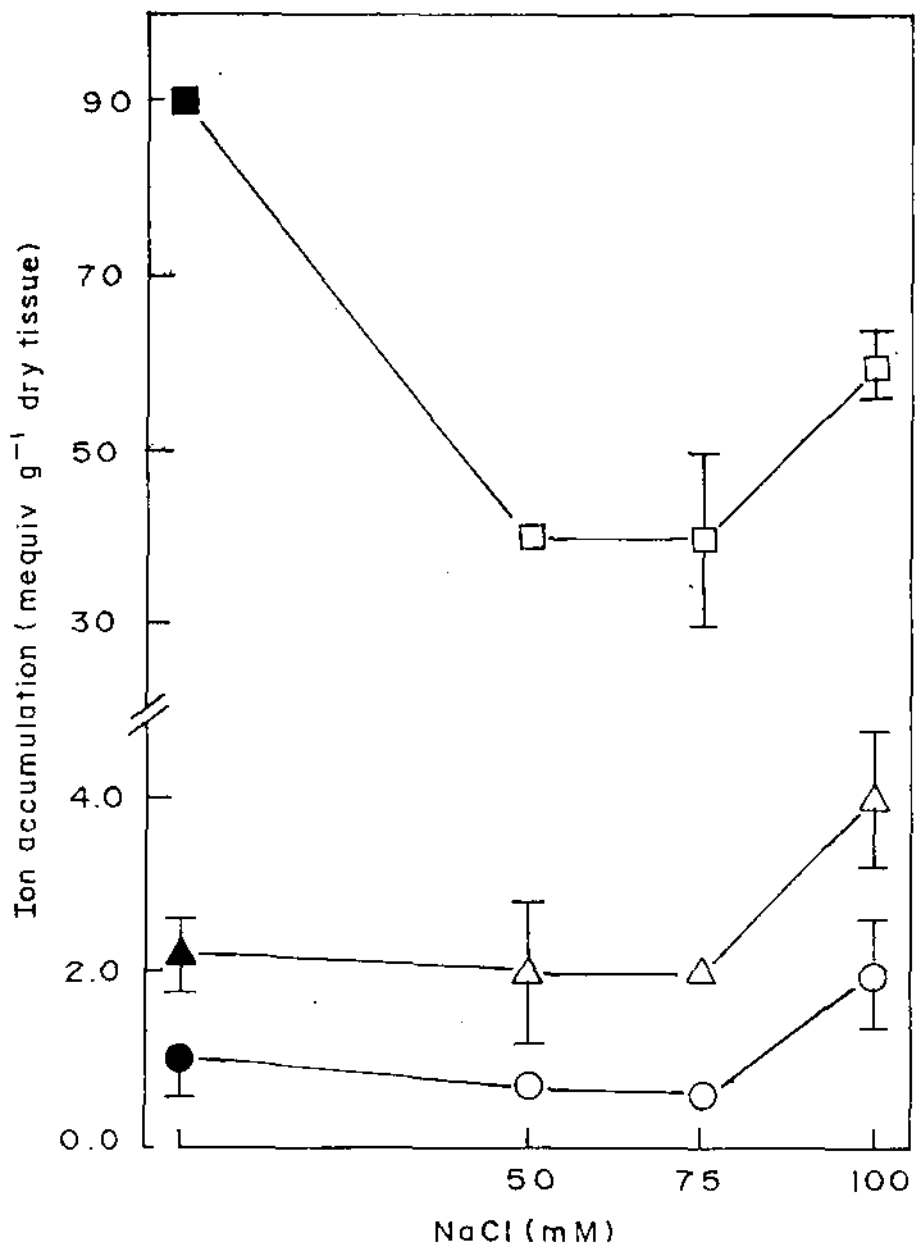

Fig.12a Effects of salinity on accumulation of Ca^{2+}, Mg^{2+} and Fe^{2+} in the root of 21-day-old chickpea seedlings (cv. Nabin) grown in solution culture. Otherwise as Fig. 8a.

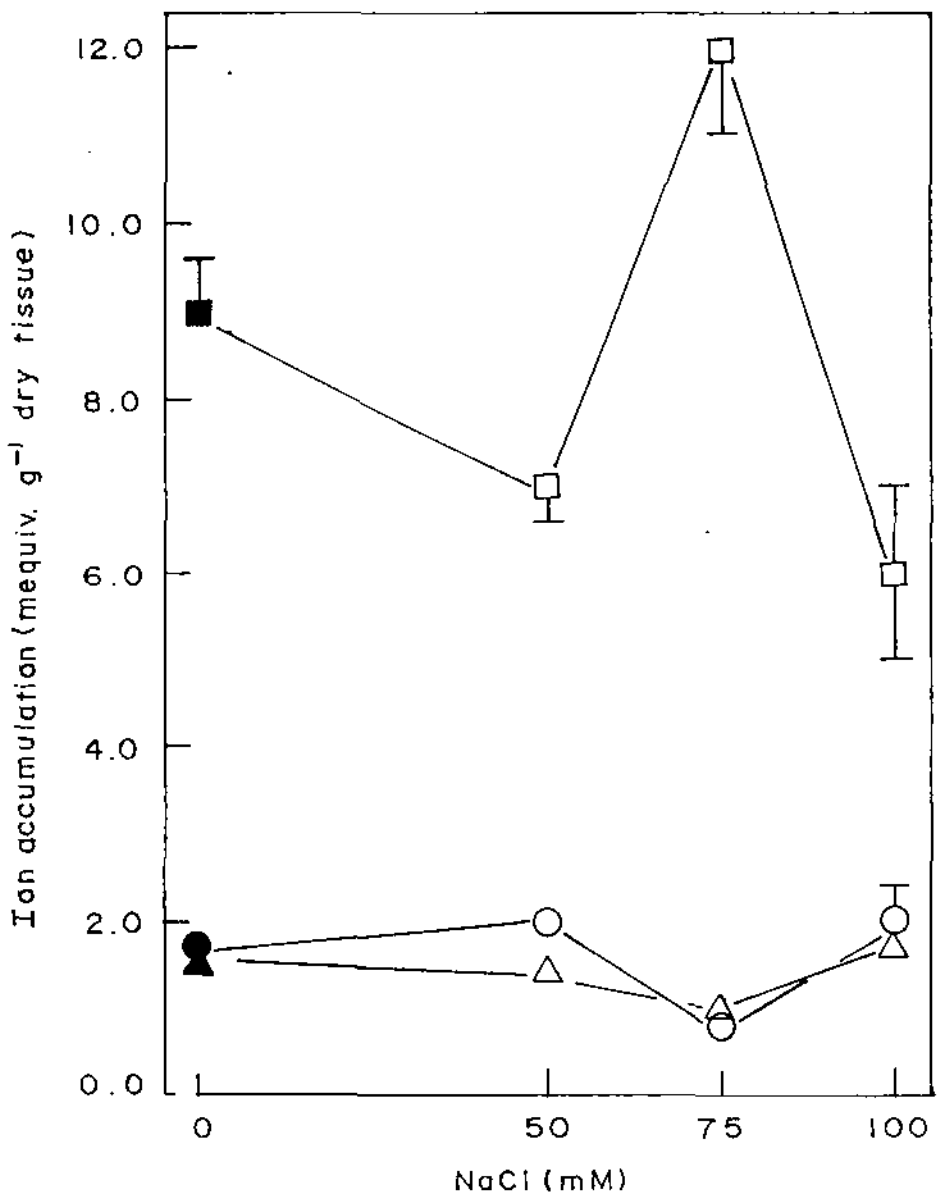

Fig.12b Effects of salinity on accumulation of Ca^{2+}, Mg^{2+} and Fe^{2+} in the shoot of 21-day-old chickpea seedlings (cv. Nabin) grown in solution culture. Otherwise as Fig. 8a.

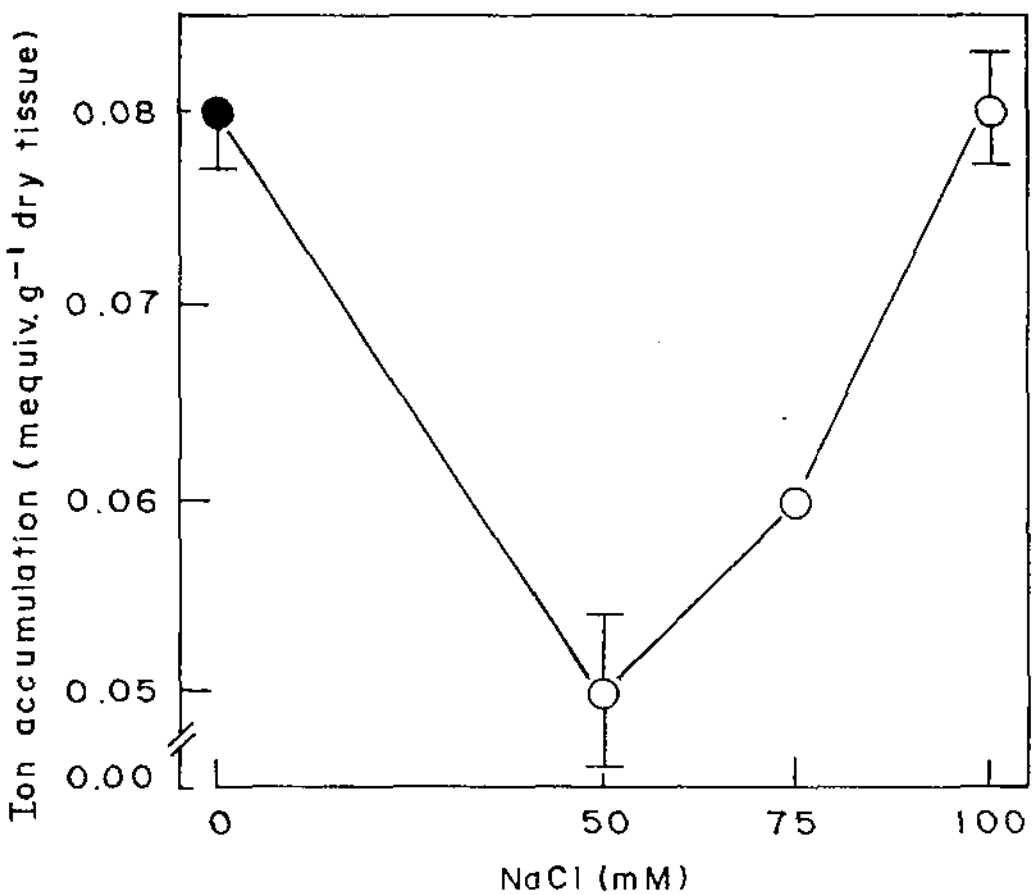

Fig. 13a Effects of salinity on accumulation of phosphate in the root of 21-day-old chickpea seedlings (cv. Nabin) grown in solution culture. Otherwise as Fig. 9a.

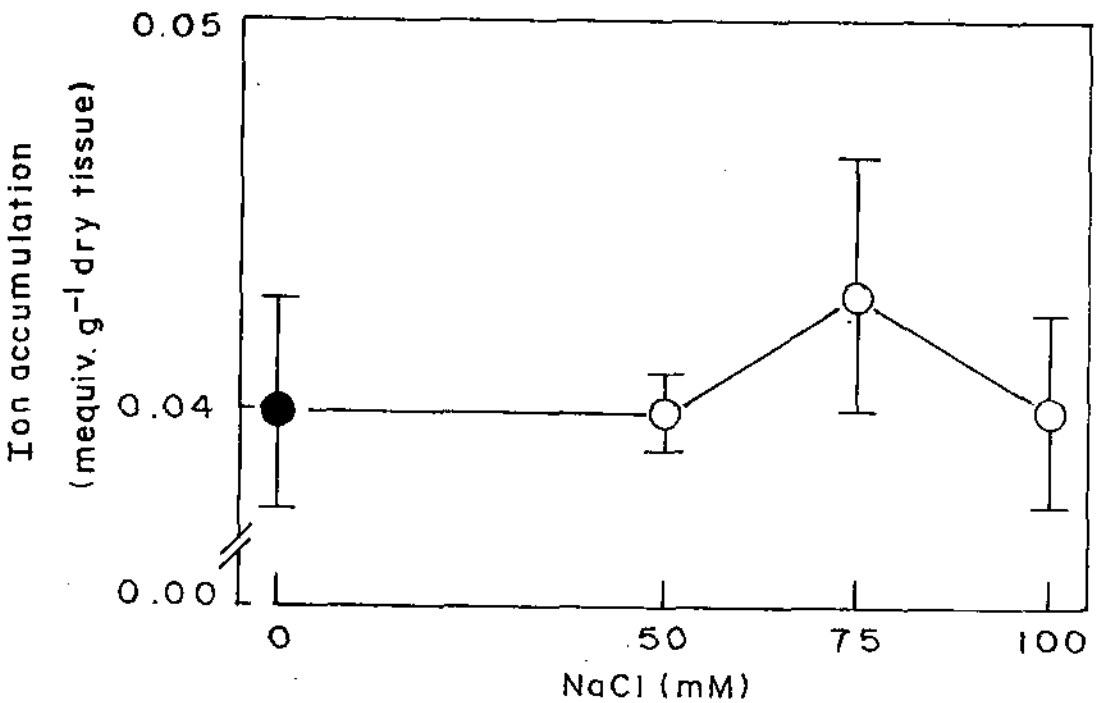

Fig. 13b Effects of salinity on accumulation of phosphate in the shoot of 21-day-old chickpea seedlings (cv. Nabin) grown in solution culture. Otherwise as Fig. 9a.

5.4 Discussion

Ca^{2+} accumulation was decreased both in the root and in the shoot of chickpea seedlings after 14 days of treatment (Fig.10a,10b). Salinity-induced decrease in Ca^{2+} accumulation was also reported by Garg and coworkers (1997). Similar results were found in maize, pepper, safflower and sunflower (Lessani and Marschner 1987), and barley (Lynch and Lauchli 1988; Yasseen *et al.* 1989). Nakamura (1992) observed that Ca^{2+} was required for maintenance of K^+ level in the intact mungbean root under salinity.

Mg^{2+} accumulation was increased in the root and in the shoot of chickpea seedlings after 7 and 14 days salinity treatment (Fig. 8a, 8b,10a,10b). Similarly, Mg^{2+} accumulation was also increased in wheat and barley under salinity stress (Yasseen *et al.* 1989). Same result was also recorded by Yadav (1989) in chickpea who found that Mg^{2+} accumulation was increased with increase in NaCl salinity stress. Basset (1980) suggested that in order to survive under salinity stress, Mg^{2+} may play a role in osmotic adjustment.

In the root of chickpea seedlings, Fe^{2+} accumulation was decreased at 21 days of treatment (Fig. 12a). Therios and Mesopolinos (1989) also found that Fe^{2+} accumulation was decreased in the apple root under salinity condition. But in the shoot of chickpea seedlings, Fe^{2+} accumulation was increased (Fig. 8b,10b) after 7 and 14 days of salinity treatment. Similarly, Vanketesan and coworkers (1997) found that Fe^{2+} accumulation was also increased under optimum salt level in *Ipomoea pescapre* sweet. On the other hand, Romero and Marnon

(1998) observed that Fe^{2+} was diluted in leaves and was not affected by salt in annual sweet clove.

Phosphate accumulation was decreased in the root at 7 days of treatment (Fig. 9a). Gunes and coworkers (1996) also showed that NaCl salinity decreased P contents in the pepper plant. Similar result was also observed by Joshi and Naik 1980) who found that addition of NaCl, Na_2SO_4 and $MgSO_4$ in the irrigation water resulted in the reduction of phosphorous concentration. In the shoot, PO_4 accumulation did not show any significant change under salinity (Fig. 9b, 11b, 13b). Similarly, Treeby and coworkers (1988) showed that accumulation of phosphate was not affected by salinity in high phosphate plant. They suggested that enough phosphate is stored in the shoot of chickpea seedlings and hence phosphate content remained unaltered under salinity.

CHAPTER 6

EFFECTS OF SALINITY ON AMYLASE ACTIVITY IN GERMINATING SEEDS OF CHICKPEA

6.1 Introduction

Salinity at a high concentration inhibited amylase activity but at low concentration of salinity amylase activity was stimulated (Alamgir *et al.* 1995). Prokash and Prathapasenan (1988) showed a decreasing activity of amylase in rice (*Oryza sativa*). But seed pretreatment by GA_3 increased amylase activity and reduced the inhibitory effect of salt stress (Kumar *et al.* 1981). During germination of seeds, amylase showed an increasing activity because amylase is important for starch turnover (Ching 1972; Proshad and Mathur 1983).

6.2 Material and methods

6.2.1 Methods of studying amylase activity in germinating seeds of chickpea

After surface sterilization (section 2.5.1), twenty seeds were placed on Whatman filter paper No. 1 contained in a petri dish (11 cm). Three replicates were used for each treatment. Filter papers were soaked in 7 ml of NaCl solution of 100 and 200 mM NaCl, each containing 0.1 mM $CaSO_4$. In case of control, the filter paper was soaked in 7 ml of 0.1 mM $CaSO_4$ only. The seeds were allowed to germinate in dark at 27°C. Then amylase activity was measured in freshly

germinated radicles and cotyledons after 48, 72 and 96 hours of germination according to the method of Peter Bernfeld (1951) with modification as described in section 2.9.

6.2.2 Methods of studying the effect of salinity and the interaction of different concentrations of NaCl (100 and 200 mM) and GA on amylase activity in germinated seeds

In order to study the effect of salinity and the interaction of salinity and gibberellic acid on amylase activity, seeds were allowed to germinate in 100 and 200 mM NaCl solution with or without 10^{-6} and 10^{-5} M GA. Germination technique was the same as described in section 2.5.3.

6.2.3 Methods of extraction and analysis of amylase activity in radicles and cotyledons

Methods of extraction and determination of amylase activity was same as described in section 2.9.

6.3 Results

6.3.1 Effects of salinity (100 and 200 mM NaCl) on amylase activity in germinating seeds of chickpea

In cotyledons, salinity stress (100 mM NaCl) decreased amylase activity by 57.83, 45.96 and 52.09% at 48, 72 and 96 hours of treatment respectively. 200 mM NaCl also decreased amylase activity by 52.65, 54.44 and 58.06% at 48, 72 and 96 hours of treatment respectively (Fig. 14a).

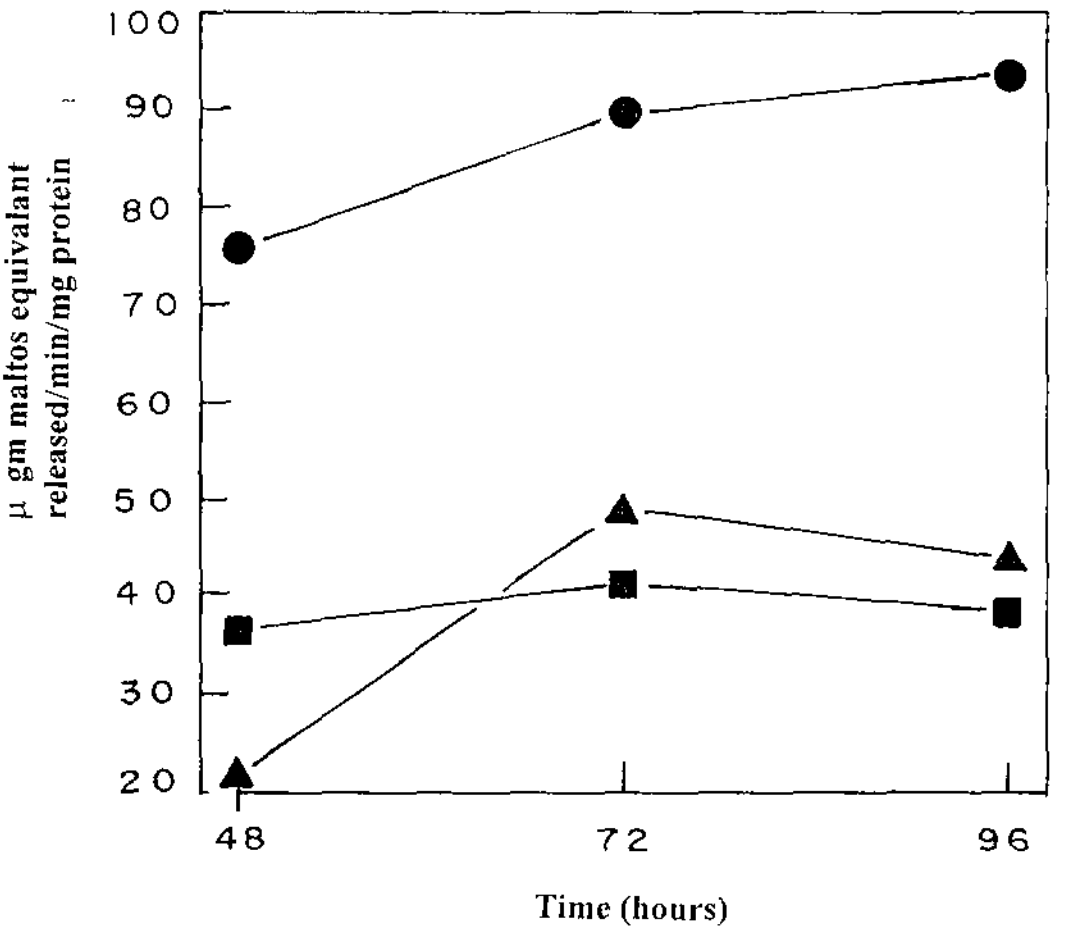

Fig. 14a Effects of salinity on amylase activity on chickpea cotyledon of germinating seeds expressed as percentage of fresh weight basis. ● represents control, ▲ 100 mM NaCl and ■ 200 mM NaCl. Average value of three replicates.

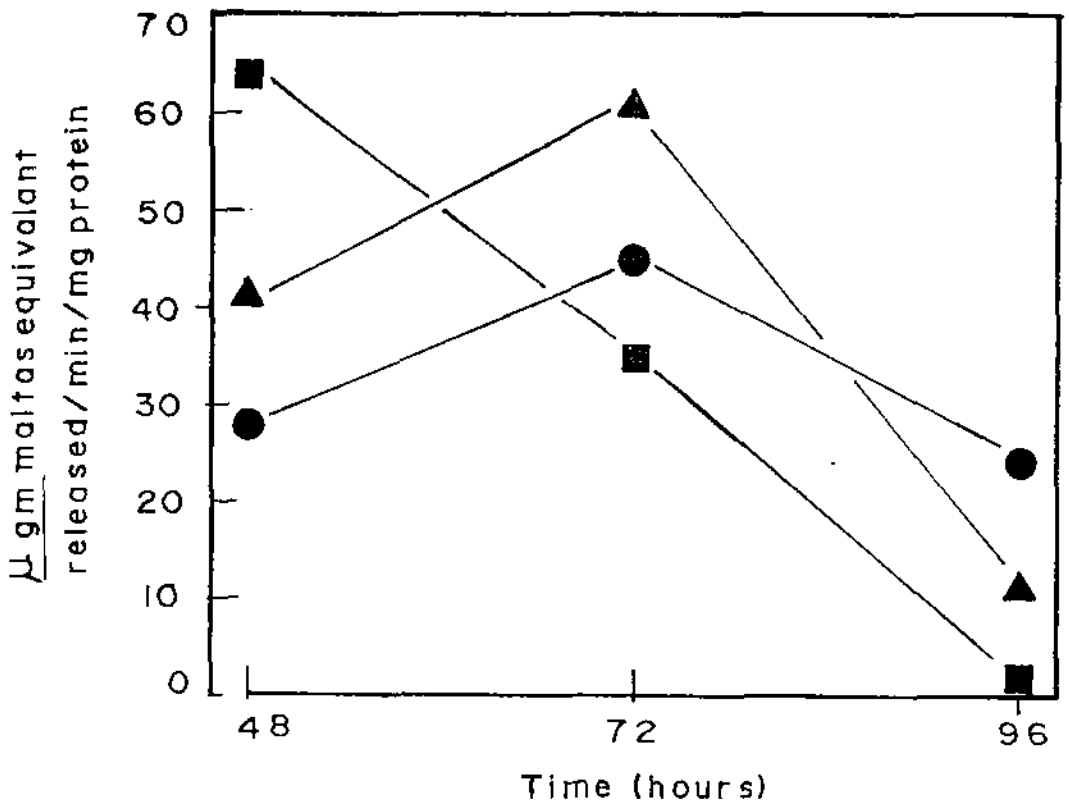

Fig. 14b Effects of salinity on amylase activity of chickpea radicle of germinated seeds. Otherwise as Fig. 14a.

In radicle of chickpea, 100 mM salinity stress increased amylase activity from 46.14 to 37.18% at 48 to 72 hours of treatment (Fig. 14b). Amylase activity was decreased in radicle from 23.18 to 90.67% at 72 to 96 hours of treatment after an initial stimulation at 48 hours of treatment under 200 mM of NaCl salinity stress (Fig. 14b).

6.3.2 Interaction of 100 mM NaCl salinity with GA on amylase activity in radicles and cotyledons of germinating seeds

In cotyledons, amylase activity was increased by 15.71% at 96 hours of treatment but initially it was decreased by 32.80 and 25.87% at 48 and 72 hours following interaction of NaCl and 10^{-6} M GA. But 10^{-5} M GA and salinity increased amylase activity from 8.33 to 11.43% at 72 to 96 hours of treatment after an initial decrease by 73.2% at 48 hours of treatment (Fig. 15a).

In radicle, interaction of salinity and 10^{-6} M GA decreased amylase activity by 21.92, 91.03 and 68.14% at 48, 72 and 96 hours of treatment, respectively. Interaction of 10^{-5} M GA and NaCl decreased amylase activity in radicle by 15.5, 93.38 and 84.8% at 48, 72 and 96 hours of treatment, respectively (Fig. 15b).

6.3.3 Interaction of 200 mM NaCl salinity with GA on amylase activity in radicles and cotyledons of germinating seeds

In cotyledons of chickpea, amylase activity was decreased by 21.43% at 48 hours of treatment but was increased by 12.5 and 1.37% at 72 and 96 hours

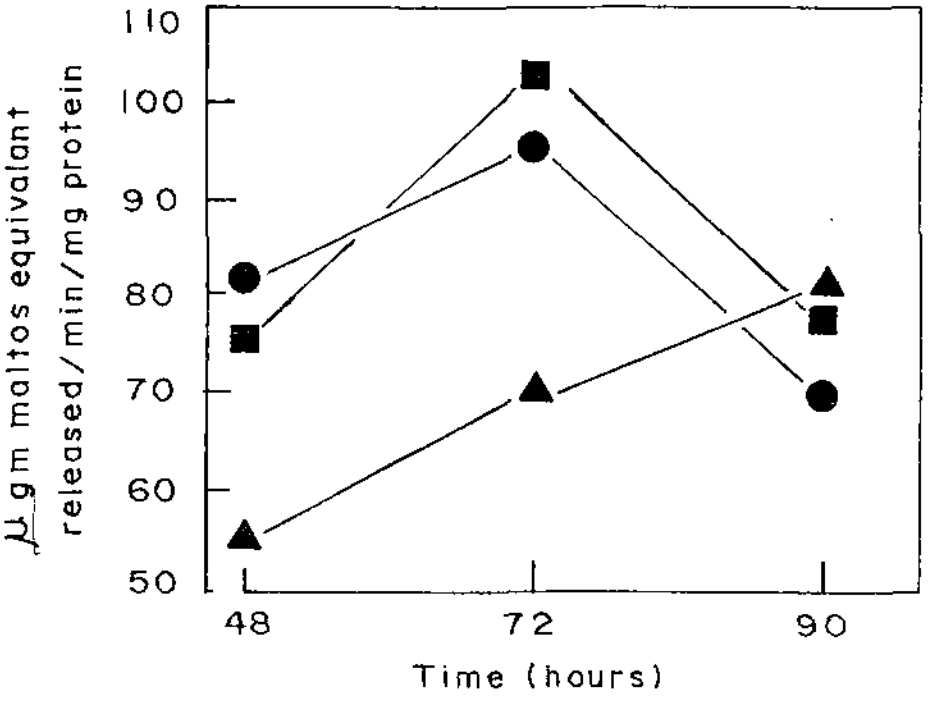

Fig. 15a Effects of salinity (100 mM NaCl) interaction with GA on amylase activity in cotyledon of germinated chickpea. ● represents control (100 mM NaCl), ▲ 10^{-6} M GA and ■ 10^{-5} M GA. Expressed as percentage of fresh weight basis. Average value of three replicates.

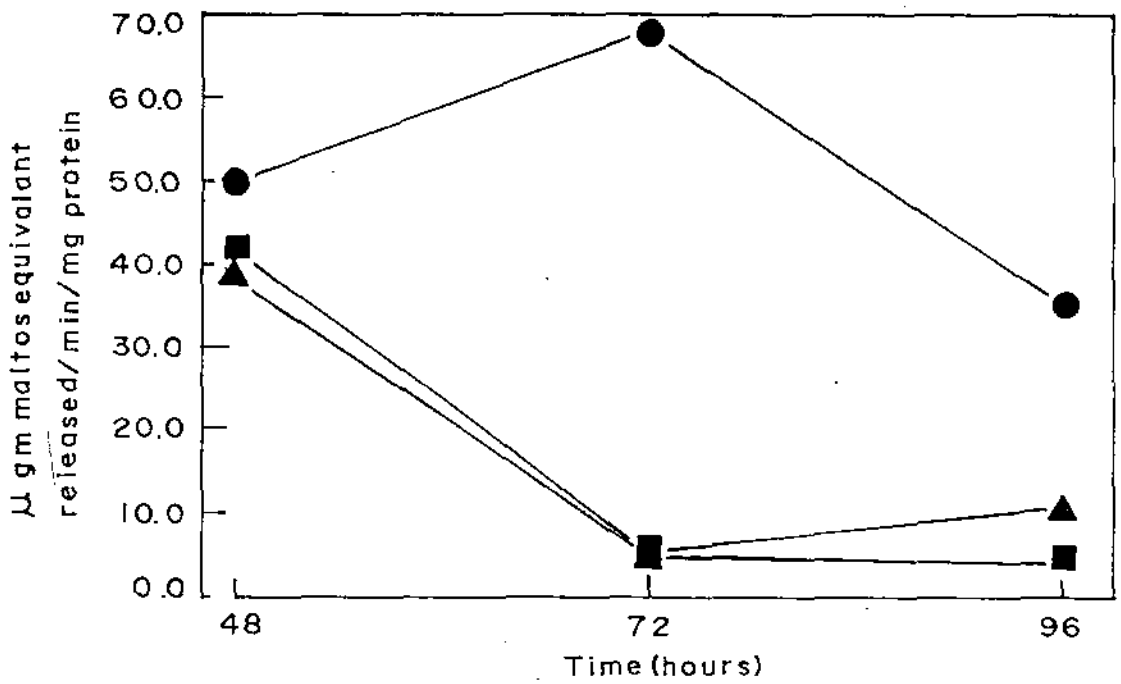

Fig. 15b Effects of salinity (100 mM NaCl) interaction with GA on amylase activity in radicle of germinated chickpea. Otherwise as Fig. 15a.

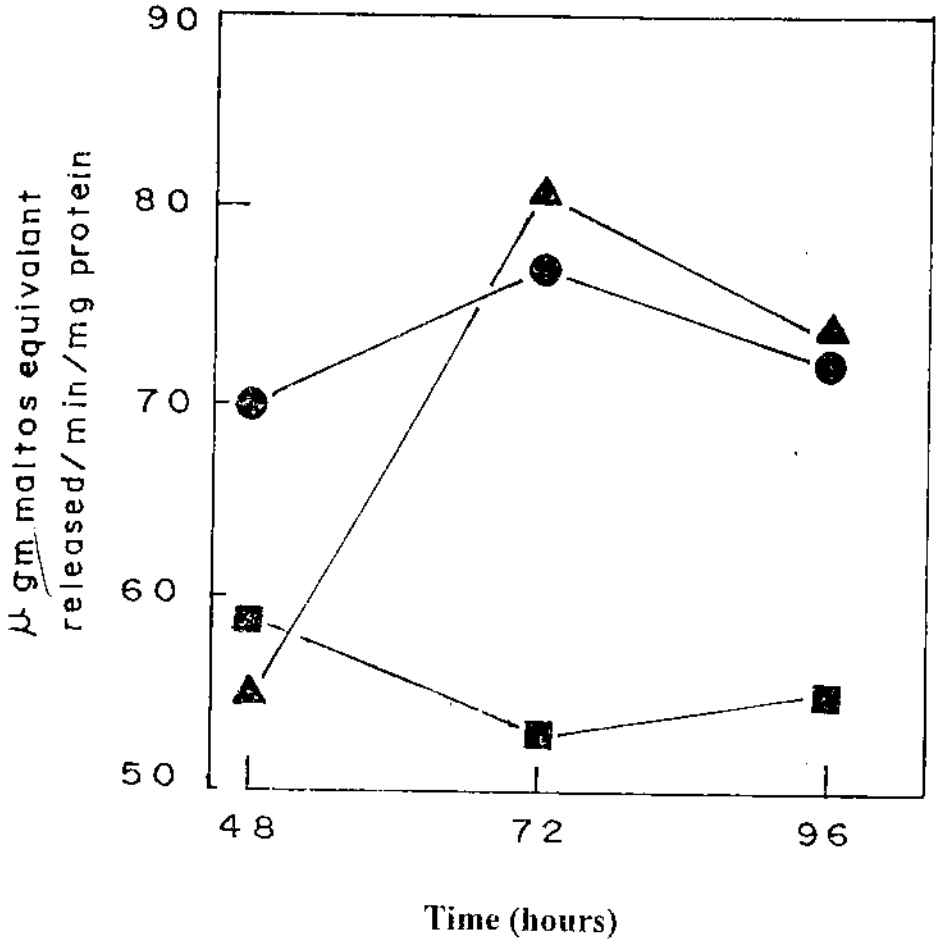

Fig. 16a Effects of salinity (200 mM NaCl) interaction with GA on amylase activity in cotyledon of germinated chickpea. ● represents control (100 mM NaCl), ▲ 10^{-6} M GA and ■ 10^{-5} M GA. Expressed as percentage of fresh weight basis. Average value of three replicates.

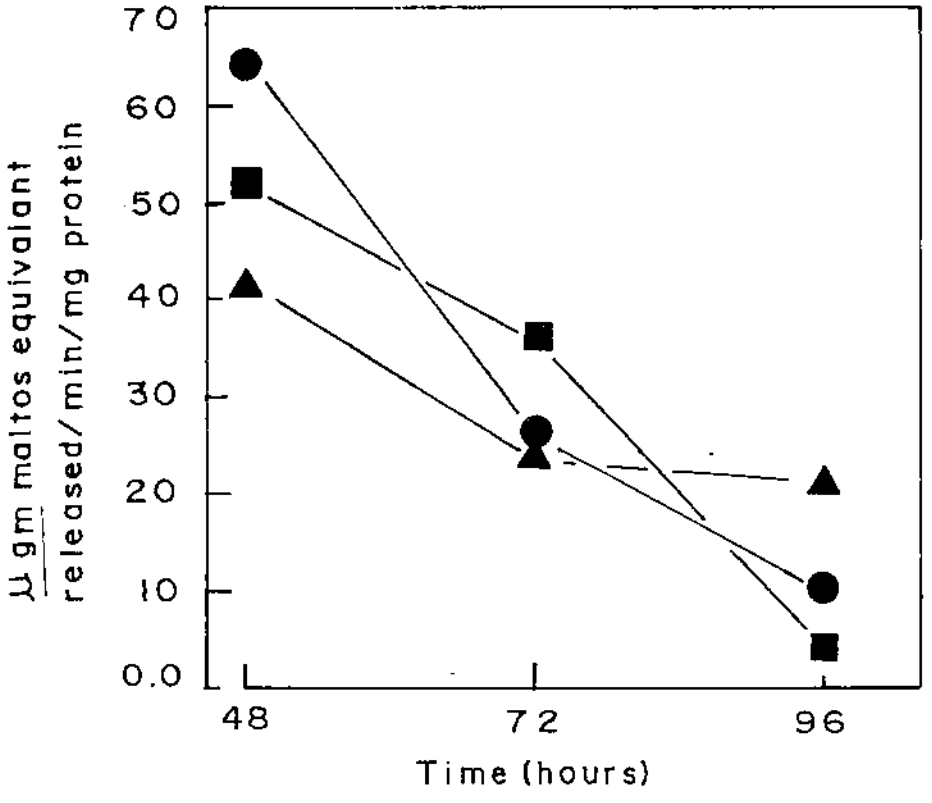

Fig. 16b Effects of salinity (200 mM NaCl) interaction with GA on amylase activity in radicle of germinating chickpea. Otherwise as Fig. 16a.

respectively following the application of 10^{-6} M GA and salinity. 10^{-5} M GA along with salinity decreased amylase activity by 15.71, 26.89 and 22.64% at 48, 72 and 96 hours of treatment, respectively (Fig. 16a).

In radicles, interaction of salinity and 10^{-6} M GA increased amylase activity by 2.2-fold at 96 hours of treatment respectively but decreased by 28.12% at 48 hours of treatment (Fig. 16b). During interaction of 10^{-5} M GA and salinity, amylase activity was decreased by 18.75 and 60% at 48 and 96 hours of treatment, respectively (Fig. 16b).

6.4 Discussion

Salinity stress (100 and 200 mM NaCl) decreased amylase activity at 96 hours of treatment in radicle at 48 to 96 hours of treatment in cotyledons of germinating chickpea seeds (Fig. 14a, 14b). Similar decrease in amylase activity was also recorded in rice following salinity (Prokash and Prathapasenan 1988).

Interaction of 100 mM NaCl with 10^{-6} or with 10^{-5} M GA decreased amylase activity in the radicle of chickpea seeds (Fig. 15b). On the contrary, pretreatment of seeds with GA_3 increased amylase activity more than that was observed under salt stress condition without pretreatment by GA_3 (Alamgir *et al.* 1995).

Interaction of 200 mM NaCl with 10^{-6} M GA increased amylase activity at 96 hours of treatment in radicles and 72 to 96 hours in cotyledon of chickpea seeds (Fig. 16a, 16b) after an initial inhibition. In cotyledons, amylase activity was

increased at 96 hours following interaction of 100 mM NaCl salinity stress and 10^{-6} and 10^{-5} M GA (Fig. 15a). Similarly, Jacobson and Corcoran (1977) showed that GA_3 induced increased synthesis of amylase during seed germination. Prashad and Mathur (1983) also observed an increase in amylase activity when NaCl and GA were used together.

CHAPTER 7

THE EFFECT OF SALINITY ON ANATOMICAL STRUCTURE AND ITS RELATION WITH ION TRANSPORT

7.1 Introduction

Salinity stress was reported to cause marked changes in anatomy and ultrastructure of plants. For example, De Villiers and coworkers (1996) showed that salinity caused anatomical changes, the root diameter and the number of chloroplasts was reduced in the chlorenchyma of *Atriplex semibaccata*. Hajibagheri and coworkers (1993) showed that the root diameter was increased in *Suaedo maritima* under salinity. Reinhardt and coworkers (1995) observed that high salinity (200 mM) resulted in the formation of an exodermis with casparian bands in hypocotyl of cotton. Serrato and coworkers (1991) recorded that 200 mM NaCl reduced the number of storage cells in *Prosopis tamarugo*.

7.2 Material and methods

For studying the anatomical structure, plants were grown in sand culture as described in section 3.1.1 and 3.1.2. After collection of sample, fresh materials were sectioned and studied as described in section 3.1.3.

Plate 1

Transverse section of 45-day-old chickpea stem internode about 1 cm above sand (control). It shows vascular area containing xylem vessels (v), cambium (ca) and pith (p) (82.5X).

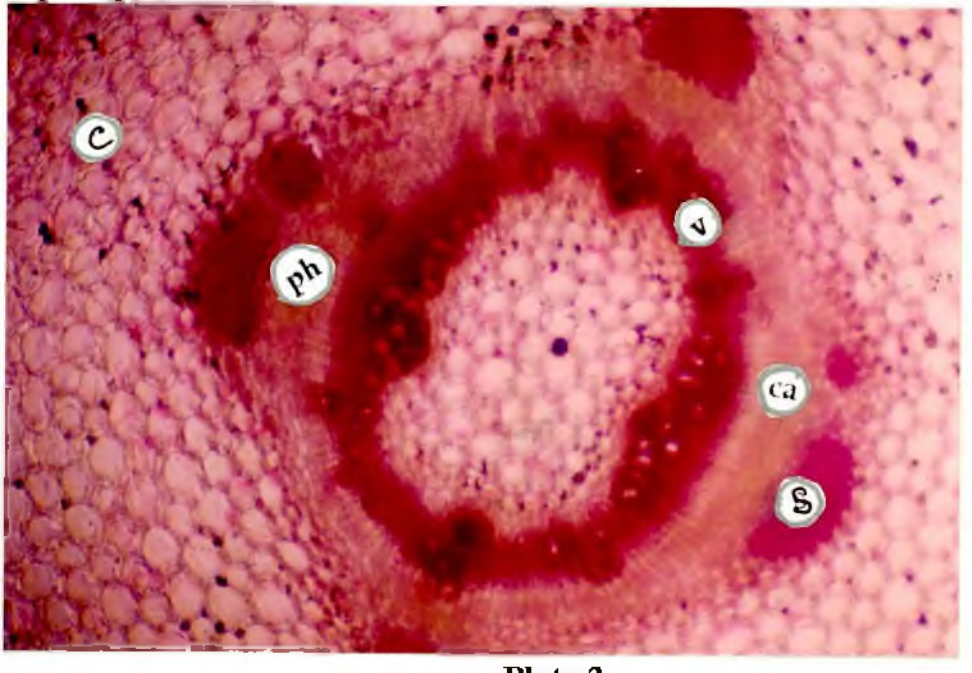

Plate 2

Transverse section of 45-day-old chickpea stem internode about 1 cm above sand surface grown in sand culture (100 mM NaCl treatment). It shows vascular area containing xylem vessel (v), phloem (ph), sclerenchymatous cell (s) and pith (p) (82.5X).

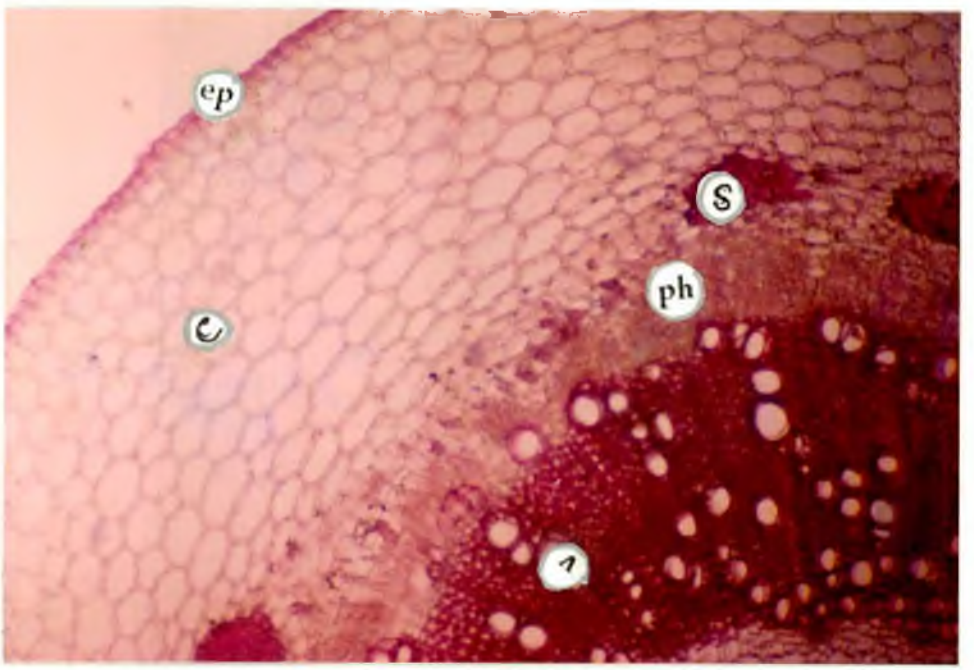

Plate 3

Same as Plate 1, which shows epidermis (ep), cortical cells (c) and sclerenchymatous cell (s) (82.5X).

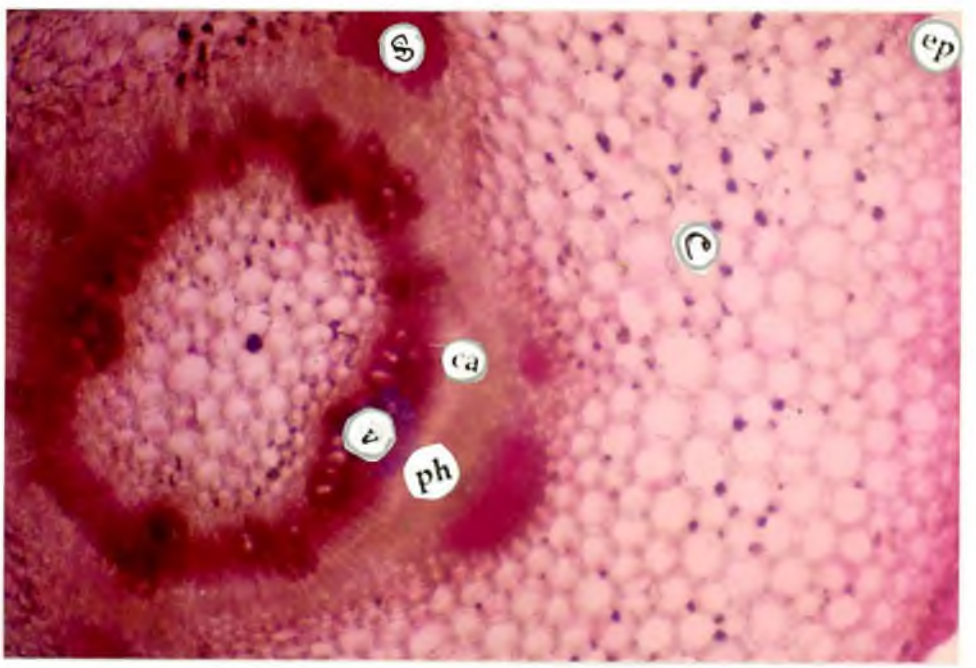

Plate 4

Same as Plate 2, which shows epidermis (ep), cortical cell (c) and sclerenchymatous cell (s) (82.5X).

7.3 Results

7.3.1 The effect of salinity on anatomical structure and its relation with ion transport

7.3.1.1 The effect of salinity on anatomy of stem

The stem of chickpea at about the middle part of the internode about 1 cm above sand surface showed the following structures:

Epidermis: Transverse section of stem of chickpea grown in the absence of NaCl (Plate 3) showed monoseriate epidermis. Under saline condition, seedlings developed thick-walled epidermis (Plate 4) and the epidermal layer was also uniseriate.

Cortex: In control, cortex was composed of 12-13 rows of parenchymatous cells, the cells were thin-walled with large intercellular spaces. Here the cortical cells were more or less flattened (Plate 3). Seedlings grown in 100 mM NaCl (Plate 4) showed cortex layers composed of 14-15 rows of parenchyma cells. The cells were more or less round in shape.

Vascular bundle: Conspicuous groups of sclerenchyma were superimposed upon the phloem (Plate 3 and 4). Vascular bundles were radially arranged. Secondary growth was noticed in both control and treated plant. In treated plant, cambium layer was thin compared to control (Plate 1, 2, 5 and 6). In control plant, more xylem elements in the innerside of the stem was noticed compared to treated plants. In the vascular cylinder, the main differences were

the occurrence of more xylem vessel and large size of vessel cavity in control compared to treated plants.

Pith: No remarkable change was observed in pith.

7.3.1.2 The effect of salinity on anatomy of root

Different tissues of chickpea root are described below which were grown in sand culture with or without salinity:

Epidermis: Epidermis were composed of thin-walled single-layered cells in control plant (Plate 13) while the epidermal cells were thick-walled in seedlings grown under 100 mM NaCl treatment (Plate 14). Here the arrangement of cells are irregular.

Cortex: The parenchyma cells of cortex were large thin-walled and irregular in size both in control and treated plant (Plate 13 and 14).

Endodermis: The inner layer of cortex is endodermis. The endodermal cells are small and thick walled in plant grown under saline condition compared to that of control (Plate 7 and 8).

Pericycle: Pericycle layer was composed of thin-walled parenchyma cells lying just within the endodermis and enclosing the vascular tissue showed differences in shape and size in NaCl-treated seedlings (Plate 8). The cells were smaller and thick-walled than those of the control plants (Plate 7 and 8).

Vascular tissue: Vascular system consists of groups of phloem tissue, cambium, secondary xylem, primary xylem and non-lignified pith. Groups of

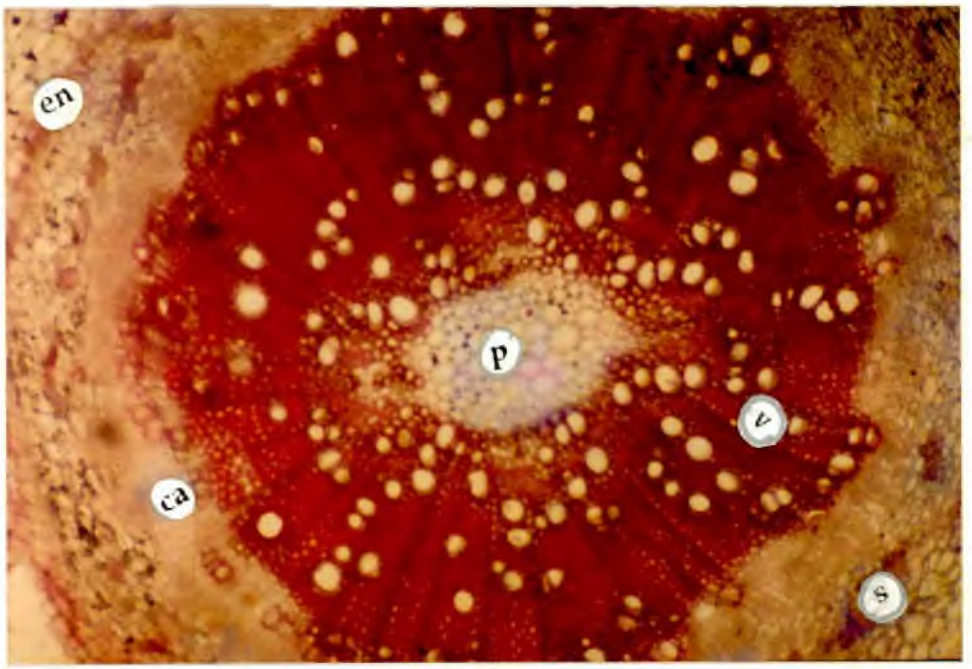

Plate 7

Transverse section of 45-day-old chickpea root grown in sand culture (control). It shows small pith (p), vascular area (v), cambium ring (ca), endodermis (en) and sclerenchymatous cell (s) (82.5X).

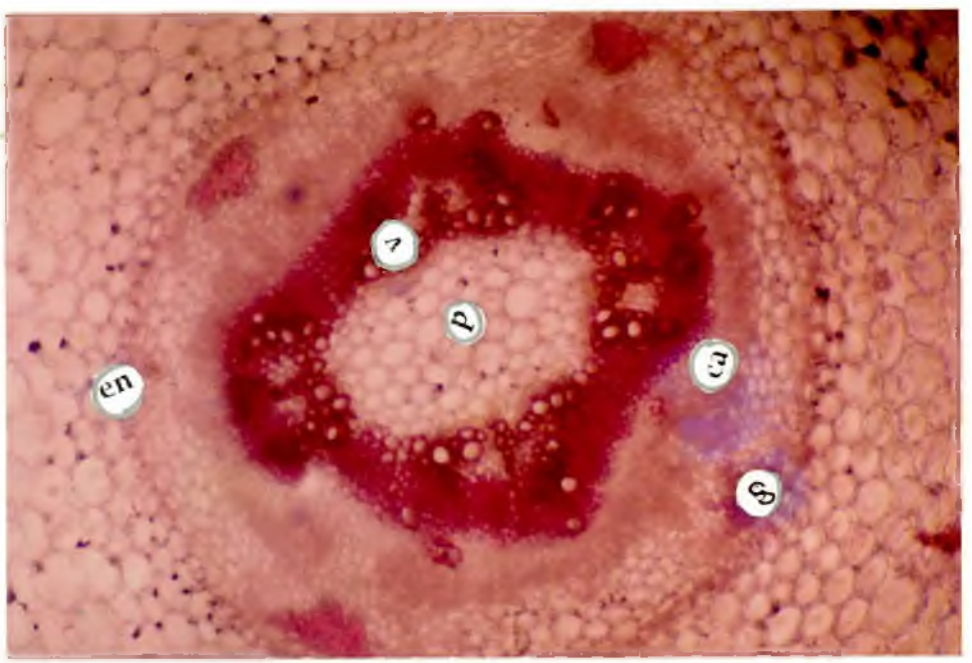

Plate 8

Transverse section of 45-day-old chickpea root grown in sand culture (100 mM NaCl). It shows pith (p), vascular tissue (v), cambium ring (ca), endodermis (en) and sclerenchymatous cell (s) (82.5X).

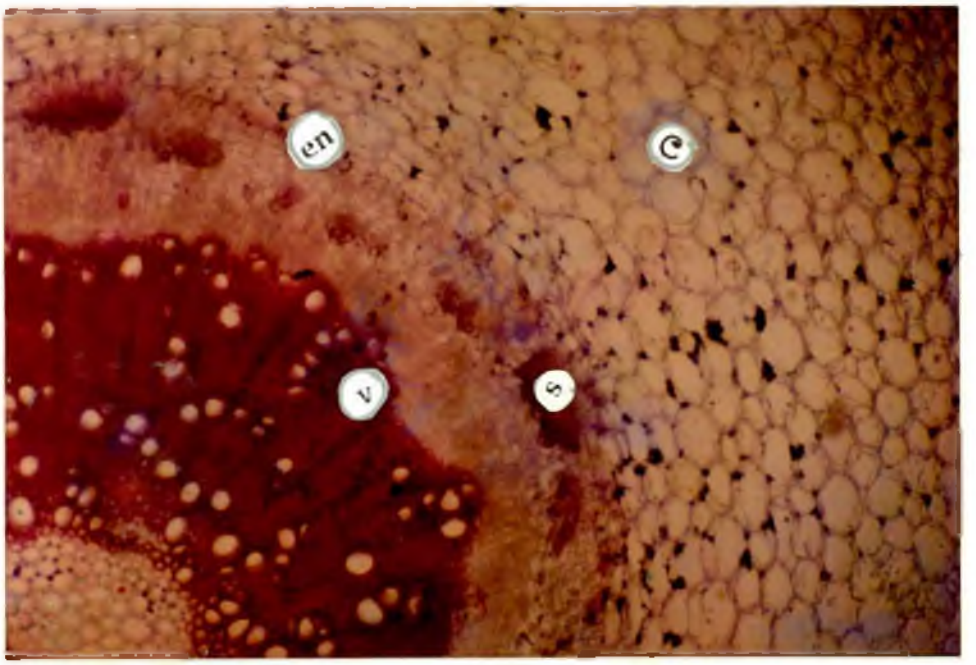

Plate 9

Same as Plate 7, which shows cortical cell (c), endodermis (en), vascular area (v) and pith (82.5X).

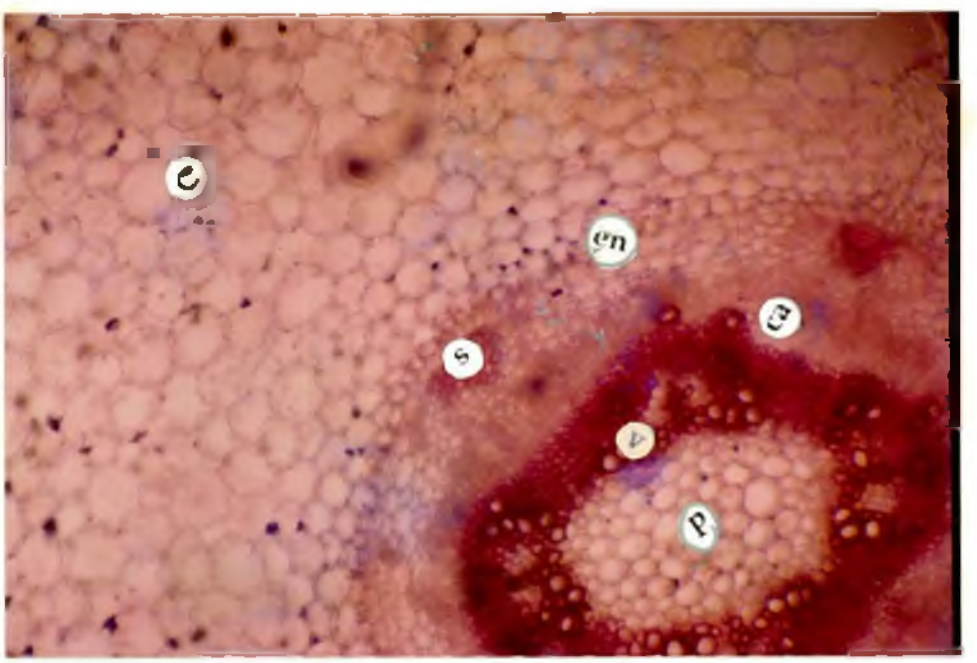

Plate 10

Same as Plate 8, which shows cortical cell (c) endodermis (en), vascular area (v) and pith (82.5X).

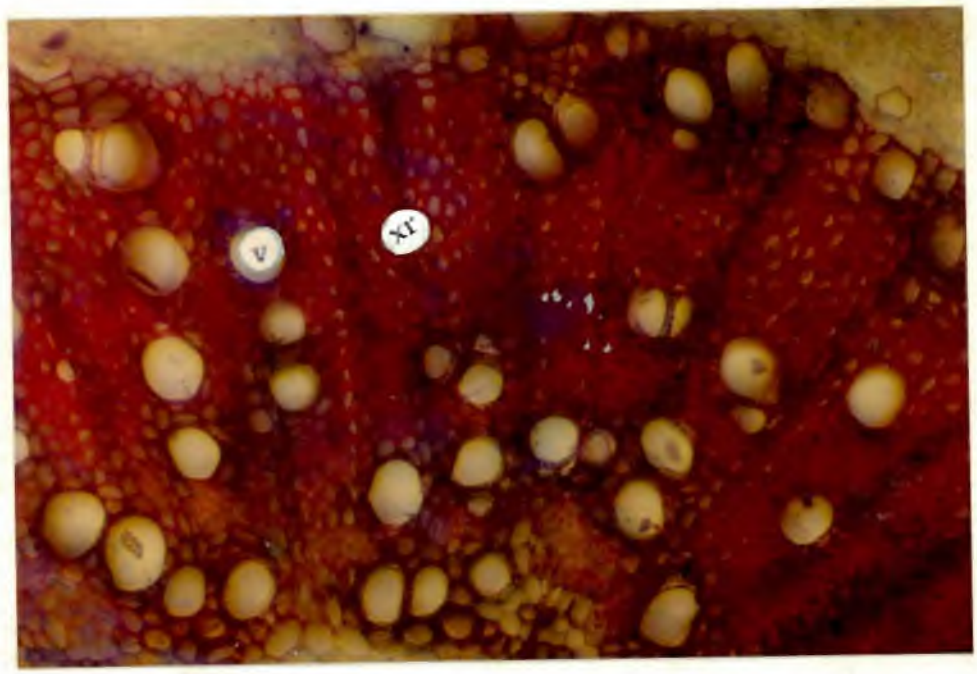

Plate 11

Same as plate 7, but of higher magnification, showing vascular area (v), xylem vessel (v) and xylem ray (xr) (206.25X).

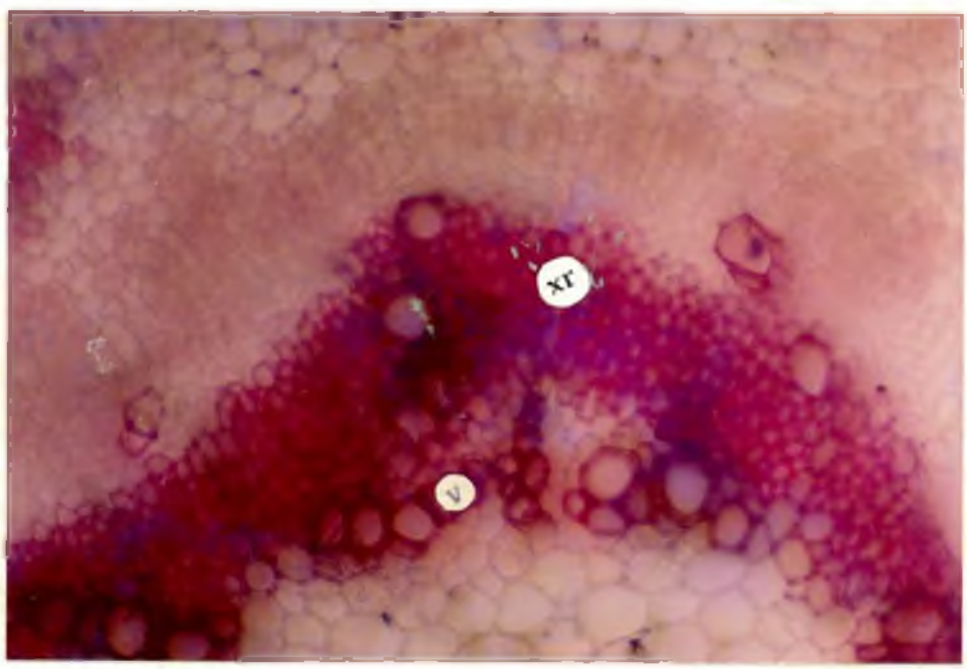

Plate 12

Same as Plate 8, but of higher magnification, showing vascular area (v) and xylem ray (xr) (206.25X).

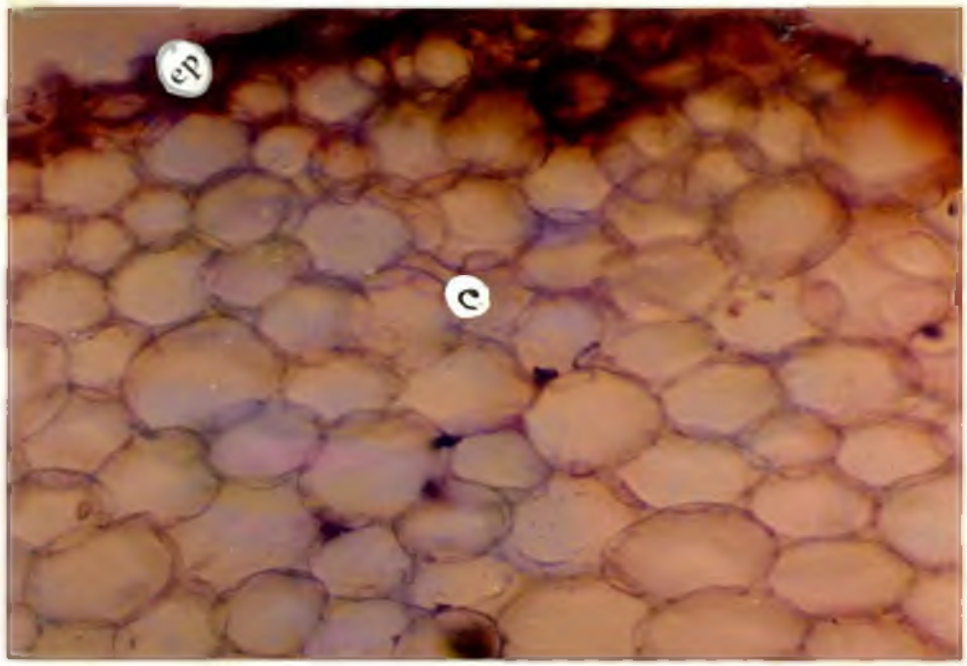

Plate 13

Same as Plate 7, but of higher magnification, showing epidermis and cortical cell (c) (206.25X).

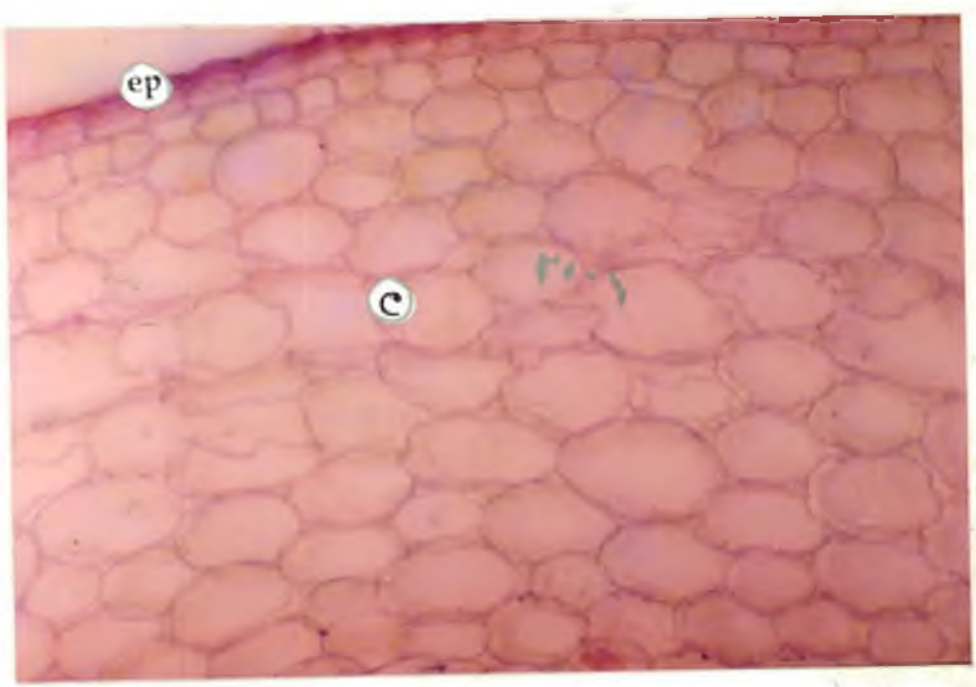

Plate 14

Same as plate 8, but of higher magnification, showing epidermis and cortical cell (c) (206.25X).

sclerenchyma were present upon the phloem. The most significant structural changes induced in root tissue by exposure of seedlings to NaCl occurred in the vascular system (Plate 8, 10 and 12). Secondary growth was noticed in both control and treated roots (Plate 7, 8, 11 and 12). More sclerenchymatous cells and abundance of xylem rays were found in control plant than that of treated root of chickpea seedlings. These sclerenchymatous groups were larger in treated roots (Plate 7 and 8). The seedlings showed a smaller root diameter under salinity as compared to control. The smaller diameter was related with reduction in cell size and also reduction in size of the vascular elements (Plate 7 and 8). Drastic changes in vascular area was noticed in NaCl-treated root. The vascular system in the root showed large prominent metaxylem vessels while the metaxylem vessels were smaller in size in treated plants (Plate 7, 8 11 and 12). In NaCl-treated plant, metaxylem and protoxylem elements of vascular system became disorganized and deformed in shape (Plate 7 and 8).

Pith: No remarkable change was observed in pith.

7.4 Discussion

7.4.1 The effect of salinity on anatomy of stem and root of chickpea seedlings

Thick-walled epidermis was found in the root and stem of chickpea seedlings under salinity treatment (Plate 3, 4, 13 and 14). Epidermal cell was found to be thicker in stem under drought condition in maize (Ali 1992).

In the present work, seedlings showed a smaller root diameter in NaCl-treatment (Plate 8). De Villiers *et al.* (1996) also showed reduced root diameter under

salinity in *Atriplex semibaccta*. In NaCl-treated seedlings, the endodermal and pericycle cells are small and thick-walled (Plate 8 and 10).

Under salinity, the vascular cylinder of the stem of chickpea seedlings showed less number of xylem vessels and smaller size of vessel cavity (Plate 2, 4 and 6). Similarly, decreased number of xylem vessels with smaller area were observed in *Carthanus finctorious* in salinity stress (Gadallah and Ramadan 1997). The vascular system of root also showed smaller sized metaxylem (Plate 8, 10 and 12). Reinhardt and Rost (1995) found that salinity reduced the width and length of metaxylem vessel in cotton seedlings.

7.4.2 Relationship between ion transport and anatomical structure under salinity

Chickpea seedlings showed different degrees of anatomical changes under salinity condition (Plate 2, 4, 6, 8, 10, 12 and 14) which may be related to observed changes in ion transport (Fig. 4to 13, Chapter 4 and 5). For example, under salinity, the epidermal cell of the root of chickpea were thicker (Plate 14) and it may be suggested that the thickening of epidermal cells (Plate 14) may lead to a decrease in accumulation of K^+ in the root (Fig. 4a, 5a and 6a, Chapter 4).

The root of chickpea under salinity showed thick-walled pericycle than that of the control (Plate 7 and 8). Thickened pericycle may lead to the decrease in flux of K^+ into the xylem (Fig 4a, 5a and 6a, Chapter 4). Salinity reduced the number of xylem vessels of vascular system of chickpea and size of xylem cavity

(Plate 2, 4, 6, 8 and 10) and these changes may lead to the decrease in translocation of K^+ (Fig. 4 to 6, Chapter 4). The metaxylem and protoxylem of chickpea root became disorganised and deformed in shape under salinity (Plate 8, 10 and 12) which may also be responsible for decrease in translocation of K^+.

CHAPTER 8

CONCLUSION

In this concluding chapter, the result presented in previous chapters are coordinated to obtain a clear picture about the role of salinity-induced changes in inorganic ion content, metabolic and anatomical changes for physiological adaptation of chickpea plants under salinity.

Salinity decreased rate of germination (Fig. 1, Chapter 3) mainly due to the increase in the accumulation of Na^+ and Cl^- with concomitant decrease in K^+ in plumule and radicle of chickpea (Table 1, 2 and 3, Chapter 3). This view regarding toxic effect of Na^+ and Cl^- on the germination is supported by Begum and coworkers (1992). The detrimental effect of Na^+ and Cl^- on the germination is further aggravated by the decrease in accumulation of extruded K^+ in the plumule and radicle (Table 1, 2 and 3, Chapter 3).

Salinity decreased the amylase activity of cotyledons and radicle of germinating seeds (Fig. 14a and 14b, Chapter 6). Salinity-induced decrease in amylase activity in cotyledons and radicle (Fig. 14a and 14b, Chapter 6) may result in salinity-induced inhibition of germination (Fig. 1, Chapter 3). Interaction of GA and salinity nullified the inhibitory effect of germination leading to a stimulation of the process (Fig. 2 and 3, Chapter 3). This nullification of salinity-induced inhibitory effect on amylase activity may help increase the rate of germination leading to the observed stimulation of germination (Fig. 15 and 16, Chapter 6).

Similarly, salinity-induced inhibition of growth (Fig. 13, Chapter 4) may be due to the accumulation of a large amount of Na^+ and Cl^- with concomitant decrease in K^+ (Fig. 4 to 6, Chapter 4). Under salinity condition Na^+ competes with K^+ (Pitman 1967), as a result, K^+ is replaced by Na^+ and the plant suffers from K^+ deficiency. Thus, salinity-induced K^+ deficiency may contribute to the reduction in growth as K^+ acts as an activator of many metabolic processes like photosynthesis, respiration, etc. A decrease in K^+ content will slow down metabolic activities and thus may lead to a decrease in growth. Cl^- was found to be more toxic than Na^+ with respect to reduction in growth (Lauter 1981, Salim and Pitman 1983).

It was found that a large amount of Na^+ and Cl^- was accumulated in the root and the shoot of chickpea plants (Fig. 4 to 6, Chapter 4). In this situation, plants under salinity is expected to suffer osmotic shock leading to the death of plant because of the toxicity of cytoplasmic Na^+ and Cl^- to metabolic functions. But plants survive the osmotic shock because most of the accumulated Na^+ and Cl^- are stored in the vacuole and thus save the cytoplasm from the toxic effects of Na^+ and Cl^-.

In the present result, Ca^{2+} accumulation was decreased under NaCl salinity stress in chickpea seedlings (Fig. 8b, Chapter 5). A decrease in Ca^{2+} content will result in an inhibition of K^+ accumulation because a decrease in calcium level disrupts membrane permeability leading to a decrease in growth under salinity. This view is supported by Sopandie and coworkers (1990) who showed that Ca^{2+} plays an important role in the maintenance of K^+. Salinity decreased

Fe^{2+} accumulation (Fig. 9b, Chapter 5) as well as phosphate content (Fig. 10a, Chapter 5). Salinity-induced decrease in Fe^{2+} and phosphate content (Fig. 9b and 10a, Chapter 5) may have detrimental effects on energy producing system like respiration and oxidative phosphorylation and photosynthetic phosphorylation which, in turn, may have adverse effect on the survival of plant under salinity.

Salinity-stress-induced thickening of epidermal cells (Plate 13 and 14) and retardation of formation of xylem element of root (Fig. 7 and 8) and the shoot (Plate 5 and 6) may be responsible for observed decrease in absorption and translocation of essential element like K^+ (Fig. 4, 5 and 6), phosphate (Fig. 10a), Fe^{2+} (Fig. 9b) and Ca^{2+} (Fig. 8b), which, in turn, may lead to retardation of growth of chickpea plants (Fig. 13a and 13b).

Further studies on the effect of salinity on abscisic acid level and its correlation with ion transport, organic acid level and on activity of enzymes related to important physiological processes may help understand the mechanism of salinity tolerance.

Finally, it may be concluded based on the light of above overview of ion accumulation and distribution pattern in chickpea under salinity that this variety of chickpea adjust ionic concentration in cells to maintain the osmotic potential at a reference level for survival in saline condition. It may be suggested from GA-induced increase in the germination of seeds that pretreatment of seeds with GA before sowing in saline land may increase germination percentage which is an important step for growing crops in saline areas.

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