EFFECTS OF SALINITY ON ION TRANSPORT AND DISTRIBUTION IN SALT SENSITIVE AND SALT RESISTANT CULTIVAR OF RICE

by
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This is to certify that the research work embodying this thesis entitled "Effects of salinity on ion transport and distribution in salt sensitive and salt resistant cultivar of rice" has been carried out under my supervision in the Plant Physiology Laboratory, Department of Botany, University of Dhaka. This thesis contains no material published elsewhere or extracted from a thesis presented by the candidate for another degree or diploma. No other person's work has been used without due acknowledgement. This thesis has not been submitted for the award of any other degree or diploma in any other university.

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Sultana Razia

Sultana Razia

dedicated

to my

MOTHER

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ABSTRACT

Germination percentage of cultivar BR16 and variety Pokkali was consistently decreased with the increase in NaCl salinity. Gibberellic acid alone or in combination with kinetin partially nullified the inhibitory effect of salinity on germination. However, interaction of salinity and kinetin decreased the rate of germination. NaCl salinity increased accumulation of Na^+ and Cl^- while it decreased K^+ accumulation in germinating seeds. Gibberellic acid caused a decrease in Cl^- accumulation and an increase in K^+ accumulation in the germinating seeds while kinetin increased Na^+ and Cl^- accumulation under salinity stressed plants.

In cv. BR16, salinity caused a 2.6- to 6.0-fold increase in Na^+ content in the root and 77% to 48-fold of that in the shoot from 7 to 21 days of treatment. In roots of var. Pokkali, Na^+ content was increased by 2.2- to 16.7-fold and in the shoot 10.5- to 36.5-fold from 7 to 21 days of application of salinity.

Salinity also increased Cl^- accumulation in the root and the shoot of both cv. BR16 and var. Pokkali but decreased K^+ accumulation. An increase in Na^+ and Cl^- content with concomitant decrease with K^+ content was responsible for the inhibitory effect of salinity on growth.

Calcium content was decreased following NaCl treatment in the root and the shoot of cv. BR16 and var. Pokkali. A decrease in Ca^{2+} level may be related to a decrease in K^+ content by the root because Ca^{2+} plays an important role to

maintain the permeability characteristics of cell membrane. Mg^{2+} accumulation was increased in the root and the shoot of both cv. BR16 and var. Pokkali.

Salinity increased Fe^{2+} content in the root and the shoot of cv. BR16 and var. Pokkali. This salinity-induced increase of Fe^{2+} may help enhance respiration.

Phosphate content was increased in the root and the shoot of both cv. BR16 and var. Pokkali under NaCl salinity. This stimulatory effect of phosphate also helped rice plants in sustaining metabolic activities under salt stress.

Salinity caused a decrease in NO_3^- accumulation in the root and the shoot of cv. BR16 and var. Pokkali.

Salinity caused a dramatic increase in proline level in the root and the shoot of both cv. BR16 and var. Pokkali.

Finally, regulation of transport of Na^+, Cl^-, K^+, Ca^{2+}, Mg^{2+}, Fe^{2+}, PO_4^{-3} and NO_3^- in rice under salinity with respect to intracellular osmotic adjustment necessary for survival of plants will be discussed.

CONTENTS

LIST OF ABBREVIATIONS

ABA	Abscisic acid
ds/m	Desi Siemens per meter s = Siemens, a unit of conductance
Ec	Electrical conductivity
Fe-EDTA	Ethylene diamine tetraacetic acid monoferrous salt
GA	Gibberellic acid
Kn	Kinetin
m-equiv.	Milliequivalent
Net influx	Net transport of ions through the plasma membrane
ngm	Nanogram
ppm	Parts per million
rpm	Revolution per minute
Transport index	Long distance transport as percentage of net influx
Ψ_{cx}	Flux of ions from cytoplasm into the xylem
Ψ_{oc}	Flux of ions from outside solution into the cytoplasm
μmole	Micromole

CHAPTER 1

GENERAL INTRODUCTION

Salinity is a serious problem in agriculture in many parts of the world (Epstein 1980, Norlyn 1980, Staples and Teonnissen 1984). Salinity problems in agriculture are usually confined to arid and semi-arid regions where rainfall is not sufficient to transport salts away from the plant root zone. Irrigation is one of the main causes of salinization of cultivated land. Soil becomes saline around the coastal areas due to inundation by sea water.

Reeve and Fireman (1967) reported that about 40 million hectares of the world's irrigated land became saline in nature. In Bangladesh also, salinity is a major problem in coastal areas. About one-fifth of the total area of Bangladesh is affected by various degrees of salinity (Karim *et al.* 1982). Flood and irrigation are the main causes of the salinization in Bangladesh. The severity of saline problem in Bangladesh increases with the desiccation of the soil and due to increased withdrawal of water in upstream regions which results in saline water movement along the main rivers and tributaries. In recent years, saline water from the sea is encroaching towards the mainland at about 90.965 sq.km. Within an interval of two decades, salinity has increased 21% in the coastal regions in comparison with the previous years. As a result, lacs of acres of land lost fertility. It is also reported that cultivars of rice is facing extinction in these areas.

As a vast saline area is becoming uncultivable in this country, efforts are being made to bring this area under cultivation by selecting salinity tolerant crops. The main cereal crop in Bangladesh is rice. Expansion of rice cultivation in the saline belt of Bangladesh may contribute to increase rice production.

1.1 The effect of salinity on germination and its relation with ion transport

Salinity in general has an inhibitory effects on germination. Yasseen and coworkers (1989) found that germination percentage was decreased with the increase in NaCl concentration (0-400 mM) in barley. The germination percentage of most crops is largely decreased at soil having Ec extract value of 8 dS m^{-1}. Other than maize, the germination percentage of most field crops is reduced to 50% when irrigated with saline water having an Ec water value of 12 dS m^{-1}. In *Crotalaria striata* germination was completely inhibited at 0.8% NaCl (Chandrashekar and Sandhyarani 1995).

Muthukumarasamy and coworkers (1997) observed that the germination percentage was decreased under salinity in chickpea seedlings. Al-Yemeny and Basahy (1997) also found that the germination percentage was reduced by 44% due to increase in salinity levels from 4 to 16 mmhos/cm in *Cyanopsis tetragonoloba* (L). At lower salinity (0 to 0.6 M Pa) the germination percentage was 92 to 85% but at higher salinity (0.6 to 1.5 M Pa) it was reduced to 85 to 26% in *Agropyron desertorum* (Johnson 1990). Germination percentage was not affected by salinity upto 100 mM NaCl in tomato (Torres-Schumann *et al.* 1989).

Salinity caused a reduction of percentage and speed of germination of two salt-tolerance cultivars of rice (Queiroz and Nakagawa 1992). Raeber and Lee (1991) observed that imbibition of seed in 24.2 or 68.4 mM NaCl reduced the germination percentage of *Penstemon parryi* seed.

Cucci and coworkers (1994) found that in some vegetable crops, viz chicory, tomato, *Phaseolus vulgaris*, aubergine, spinach and cowpea, the germination was significantly reduced due to salinity. Within the salinity range from 6 to 16 dS/m, bean and aubergine were the most sensitive and chicory and spinach were the most tolerant.

It was reported that salinity caused a 50% reduction in seed germination of *Sorghum bicolar* (Macharia *et al.* 1995). The germination percentage was reduced by 20 to 43.3% at 10.5 to 20.0 mmhos/cm NaCl respectively as compared to control (0.7 mmhos/cm) (Alwan *et al.* 1989). A delay and decrease in germination was observed in different cultivars of rice (Mondal *et al.* 1988). The germination percentage of barley decrease with the increase in NaCl salinity from 0 to 5 and 10 mmhos/cm (Kumar *et al.* 1988).

The germination percentage declined as salinity increased in seeds of *Pachycereus pringlei*, a valued ornamental cactus (Nolasco *et al.* 1996). Tort (1996) observed in *Gossypium hirsutum* that the germination was gradually decreased with the increase in salinity from 0 to 300 mM NaCl. The lower soil water salinity (4.5 dS/m) had little effect on germination. But germination was delayed and decreased significantly under salinity (8.8 dS/m) (Francois *et al.* 1986).

Mahmood and coworkers (1996) found that seven plant species - *Suaeda fruticosa*, *Kochia indica*, *Atriplex crassifolia*, *Sporobolus arabicus*, *Cynodon dactylon*, *Polypogon monspeliensis* and *Desmostachya bipinnata* varied greatly in their seed germination to soil salinity. Increase in salinity resulted in a gradual decrease in seed germination of each species. It was reported that in rice, the rate of germination was decreased with the increase in salinity (Alamgir and Ara 1986). They also found that at 40.92 mmhos/cm, germination was totally inhibited in all the varieties of rice. At low salinity level, germination rate was almost unaffected.

1.2 The effect of salinity on transport of Na^+, K^+ and Cl^-

Salinity was found to increase or decrease the accumulation of different ions. For example, an increase in Na^+ and Cl^- accumulation and a decrease in K^+ uptake in both the root and shoot were observed when barley and rice were exposed to high NaCl salinity (John *et al.* 1977). Similar results were also found in wheat and rice (Roth 1989, Sharma 1989, Begum 1994), apple root (Therios and Misopolinos 1989), *Atriplex rhagodioides* (Mahmood and Malik 1987), *Lupinus albus* (Jeschke *et al.* 1986, 1992) and in tomatoes (Al-Rawahy *et al.* 1992). Sharma (1997) also found that plant growth was declined with the increase in levels of salinity while concentration of Na^+ and Cl^- was increased and that of K^+ decreased in chickpea. Accumulation of Na^+ and Cl^- increased in *Ipomoea pescaprae* under extreme salinity (Venkatesan *et al.* 1997).

Shere and coworkers (1974) observed that in a highly salt-sensitive soybean, K^+ concentration was reduced under saline condition. In barley, 10 mM NaCl had no effect on K^+ loss, but rate of efflux of K^+ was increased at 50 mM NaCl or more (Nassery 1975). Similar results were also observed in barley (Wolf and Jeschke 1987), Jojoba (Tal *et al.* 1979), *Sueda maritima* (Clipson 1987), *Melilotus segetalis* (Romero and Maranon 1996) and *Atriplex prostata* (Li-Wen *et al.* 1997).

Chaudhuri and Chaudhuri (1998) observed that short-term salinity stress caused a greater accumulation of Na^+ and Cl^- and a lower ratio of K^+/Na^+ in the root and shoot of two varieties of jute. On the other hand, in the salt-tolerant line of sugarcane, Na^+ and Cl^- accumulation were decreased while that of K^+ was increased (Wahid *et al.* 1997). Huang and Yuan (1989) observed that Cl^- at low concentration of NaCl (0.143 and 1.43 mM) stimulated K^+ uptake while at higher concentration (14.3 mM) it decreased K^+ absorption.

Under salinity condition, high Na^+ accumulation interfered with K^+ uptake (Lazof and Cheeseman 1988). Jeschke (1972) found that Na^+ transport was inhibited by K^+, consequently inhibition and efflux of Na^+ from the symplasm through the root cortex was increased. Aslam and coworkers (1987) reported that in *Echinochloa crussgalli*, an increase in NaCl concentration resulted in a steady state level of K^+ concentration, although Na^+ and Cl^- accumulation was increased. Since Na^+ is accumulated predominantly under saline condition, the K^+ content in the cytoplasm must be maintained at a critical value for metabolism and this is an essential criterion for salinity condition (Chow *et al.* 1990).

In halophytes, under saline condition Na^+ replaced the role of K^+ in osmotic adjustment of the vacuole but a relatively higher concentration of K^+ maintained metabolic compartment (Leigh *et al.* 1986). Low salinity stimulated K^+ absorption in *Vicia faba* (Huq *et al.* 1987). An increase in K^+ concentration in salt-tolerant species of pigeon pea while a decrease in K^+ content was observed in salt-sensitive one (Subbarao *et al.* 1990a).

Alamgir and Aktar (1992) found that in most of the wheat varieties, both Na^+ and K^+ levels in the seedlings were increased under NaCl salinity condition. The highest Na^+/K^+ ratio was in the root of Tanori and in the shoot of Ananda and the lowest was in the root of cv. Balaka, Doel, Inia-66 and in the shoot of Aghrani of wheat. It was reported that the higher K^+/Na^+ ratio in leaves of the tolerant variety of rice indicated its greater capacity to adopt under salinity. Excess Na^+ is the major cause of injury to the rice plant under salt treatment (Zongli *et al.* 1989). Alamgir *et al.* (1992) reported that in four high-yielding varieties (HYV) of wheat, the Na^+/K^+ ratio was low under low salt stress and in plants exposed to high salt stress at advanced growth stage. Among the varieties considered, Sonalika had comparatively low Na^+/K^+ ratio at all growth stages and showed more tolerant to salt stress than the rest. In two varieties (salt-sensitive cv. IKP and salt-tolerant cv. Aiwu) of rice under salinity condition, Na^+ and Cl^- accumulation was increased in roots and younger leaves of cv. IKP, coupled with the highest decrease in K^+ content. On the other hand, accumulation of Na^+ and Cl^- were restricted to older leaves in cv. Aiwu under saline condition (Lutts and Guerrier 1995). It was reported that salt-tolerant rice

excluded more Na^+ and absorbed more K^+ to maintain a optimum Na^+/K^+ balance in the shoot (Gregorio and Senadhira 1992).

Jeschke and coworkers (1992) observed that K^+ transport in the phloem was even more severely restricted under saline conditions. On the other hand, Na^+ and Cl^- accumulation in the phloem were substantially increased in presence of NaCl. Under salinity, there were massive flow of Na^+ in the phloem in white lupin (*Lupinus albus*). Salt-stressed plants were smaller and the products of leaf biomass was comparatively less. It was reported that Na^+ was accumulated in roots of young plants and excluded from leaves and fruits, whereas K^+ was depleted from roots and accumulated in leaves and fruits (Romero and Maranon 1996). Under saline condition, increased Na^+ accumulation and that of K^+ was decreased in rice (Alamgir *et al.* 1989). Similar result was found in wheat (Alamgir and Kutube 1997).

Salinity (150 mM NaCl) caused the highest accumulation of Na^+ and Cl^- in calli, roots and younger leaves with a concomitant decrease in K^+ content. Similarly, 30 mM NaCl increased Na^+ and Cl^- accumulation in the rice (cv. IKP) (Lutts and Guerrier 1995). On the other hand, accumulation of Na^+ and Cl^- were restricted to older leaves in rice cv. Aiwu. It was reported that salt-stressed plants increased Na^+ and Cl^- accumulation and decreased K^+ accumulation in the leaf tissue (Prakash and Prathapasenan 1990). Basu and coworkers (1988) reported that in rice (cv. Rupsail), influx of Na^+ and Cl^- and efflux of K^+ in coleoptiles and roots are directly proportional to the concentration of NaCl applied. Interaction of gibberellic acid and NaCl salinity increased K^+ content

and reduced Na^+ content of both root and shoot in wheat seedlings (Alamgir and Kutube 2000).

1.3 The effect of NaCl salinity on uptake and distribution of divalent cation (Ca^{2+}, Mg^{2+} and Fe^{2+})

Calcium is an important ion for plants because it maintains permeability characteristic of membrane. Calcium is also important in maintaining ionic balance under salinity. Calcium can prevent the increased cell permeability (Nassery 1975), and it counteracted the adverse effect due to salinity stress (Epstein 1961, Lauchli and Epstein 1970, Cramer *et al.* 1986). Cramer and coworkers (1986) also reported that cell volume was not affected by salinity stress when supplement Ca^{2+} was supplied.

Ca^{2+} content was found to decrease with increasing salinity in *Vigna radiata*, *Helianthus annuus*, *Phaseolus vulgaris*, *Lycopersicon esculentum* and *Atriplex spongiosa* (Salim 1989). High concentration of salts, Ca^{2+} plays an important role in the maintenance of K^+ transport (Sopandie *et al.* 1990). Ca^{2+} content decreased with increase in salinity level in salt-treated grass roots (Elzam and Epstein 1969) and in chickpea (Mumurkar and Chavan 1986).

It was reported that calcium had a beneficial effect on the salt tolerance of some plants of family Tiliaceae (Wyne Jones *et al.* 1977), in *Phaseolus aureus* (Huq and Larhar 1984). Sodium ion displaced Ca^{2+} ions present in cell membrane (Cramer *et al.* 1985) and K^+ selectivity was hampered due to the displacement of Ca^{2+} by Na^+ (Cramer *et al.* 1987).

Presence of Ca^{2+} in saline solution is essential for K^+ selectivity (Epstein 1961). He also reported that Mg^{2+} could not be substituted for Ca^{2+} under salinity condition. Yadav *et al.* (1989) found that an increase in Ca^{2+} and Mg^{2+} occurs with the increase in NaCl salinity stress in chickpea. On the contrary, Kingsbury *et al.* (1984) observed a decrease in Ca^{2+} and a slight increase in Mg^{2+} under salinity condition. Aslam and coworkers (1987) reported that an increase in Ca^{2+} and a decrease in Mg^{2+} content was observed in *Echinochloa crussgalli*.

Grattan and Grieve (1992) suggested that micronutrient concentration may increase, decrease or may not be affected by salinity depending upon the plant species, tissue, salt type and environmental condition. It was reported that with low concentration of NaCl, Fe^{2+} absorption was enhanced in bean seedlings (Pandey and Kannan 1979). Venkatesan and coworkers (1997) found that Fe^{2+} was increased in *Ipomoea pescaprae* upto 200 mM salt level.

Padole (1991) observed that high saline content significantly decreased the uptake of Fe^{2+} in wheat (var. HDM1553). On the other hand, Alam and Naqvi (1991) reported that salinity caused an increase in Fe^{2+} content in pearl millet.

An increase in Ca^{2+} and a decrease in Fe^{2+} was observed in salinity level in apple root stock (Therios and Misopolinos 1989). Fe^{2+} tended to be diluted in leaves and concentration of Fe^{2+} in root of *Melilotus segetalis* was not much affected by salinity (Romero and Maranon 1996). It was reported by Curtin *et al.* (1993) that barley and kochia tended to have higher selectivity for nutrient cations like Ca^{2+} and Mg^{2+} over Na^+ when the plants were grown on the Cl^-

salt system. Shannon and coworkers (1998) found that at the highest salinity, in leaf concentration of K^+ was decreased by about 40% by salinity, but tissue levels of Ca^{2+} and Mg^{2+} were unaffected.

1.4 The effect of NaCl salinity on accumulation of phosphate and nitrate

In order to survive under salinity stress, plant accumulate phosphate. Phosphate is one of the main osmotica in maintaining osmotic potential of the cell sap (Munns *et al.* 1988). Hellebust (1985) reported that phosphate uptake is strongly stimulated by low Na^+ concentration. Similar findings were also reported (Gorham *et al.* 1986, Mumurkar and Chavan 1986) in salt-treated plant.

Phosphate concentration was not affected by salinity in wheat (Hu and Schmidhalter 1997). It was reported that in case of high phosphate plant, phosphate uptake was not affected by salt but in case of low phosphate plant, it was depressed under salinity (Treeby and Van Steveninck 1988). Phosphate accumulation was increased in *Zea mays* following NaCl salinity treatment (Roberts *et al.* 1984). Similar increase in phosphate was found in tomato roots (Award *et al.* 1990), older shoot tissue of cotton (Martinez and Lauchli 1991), wheat (Astaraei and Chauhan 1994), pearl millet (Alam and Naqvi 1991) and in chickpea (Hellebust 1985). Marked reduction in the content of phosphate was observed in response to increase in salinity in wood apple (*Aegle marmelos* C.) leaves (Shukla and Singh 1996). Similar findings were reported in wheat (Padole 1991, Kumar and Singh 1996). Al-Karaki (1997) found that increased NaCl levels reduced shoot phosphate concentration of many plants.

Accumulation of nitrate nitrogen was observed under salinity condition (Langdale *et al.* 1973). Gorham *et al.* (1986) found that in leaves of some perennial triticeae, concentration of NO_3^- was decreased under saline condition. Hu and Schmidhalter (1997) also reported that NO_3^- decreased significantly with an increase in salinity in leaves and stems of wheat. Salinity decreased the net uptake rate of nitrate in wheat (Abdul-Kadir and Paulsen 1982, Botella *et al.* 1997).

Ohta and coworkers (1988) observed that nitrate fluxes into the roots of *Amaranthus tricolor* was increased by 2.1-fold when the plants were transferred to 0.5 mM NaCl culture solution. Khan and coworkers (1998) reported that NO_3^- content was decreased by salinity in *Medicago sativa*. An increase in nitrate accumulation was recorded in roots of *Phaseolus aureus* with an increase in salinity. On the other hand, NO_3^- accumulation in the shoot was decreased with the increase in NaCl salinity (Huq *et al.* 1987).

Venkatesan and coworkers (1997) found that in *Ipomoea pescaprae* NO_3^- contents in tissues increased considerably with the increase in salinity upto optimum salt level. Romero and Maranon (1996) reported that salinity reduced NO_3^- accumulation probably by Cl^- salts impeding NO_3^- uptake. In contrast, nitrate concentrations in leaves, stem and reproductive structures of *Melilotus segetalis* was not affected by salinity. Moreover, NO_3^- accumulated more in roots of salt-stressed plants than that of control.

1.5 The effect of NaCl salinity on the accumulation and distribution of proline

Proline content was increased with the increase in salinity stress in rice seedling (Dubey and Rani 1989). Proline is considered as an indicator of environmental stress (Hansen and Tully 1979). Stewart and Lee (1974) recorded an increase in proline level with the increase in salinity level. They suggested that proline acts as a solute and plays an important role for intracellular osmotic adjustment under saline conditions. Jain and coworkers (1991) also observed that in all the genotypes of *Brassica juncea*, proline was increased with the increase in NaCl stress in the medium or irrigated solution.

Veeranjaneylu and Kumari (1989) observed that proline accumulation was increased in roots and leaves of mulberry under stress conditions. The proline level was higher in roots than that in leaves. They suggested that under stress condition, proline dehydrogenase and proline oxidase enzymes were inhibited. Similar result was also found in *Capsicum annum* L. (Palfi and Juhasz 1970).

An increase in proline content with the increase in NaCl concentration was observed in white clover seedling (Turner 1990), *Nicotiana sylvestris* (Dix and Pearce 1981), sorghum (Yang *et al.* 1990b), rice plant (Thomas *et al.* 1992), barley (Yasseen *et al.* 1989), the leaves of pigeon pea and in some of its wild relatives (Subbarao *et al.* 1990a) and in two varieties of Gerick 79 and Bezoslaya 1 of wheat (Tipirdamaz and Cakirlar 1990). Quayyum and coworkers (1991) observed that in two rice varieties Pokkali and MI48, external stress induced proline accumulation within the plant more so when the stress resulted

from salinity, but the amount of proline accumulated was unrelated to the degree of salt tolerance of the two varieties.

Kishore and coworkers (1986) observed an initial decrease in proline content in salt-stressed grape vines. But they found an increase in accumulation of proline content in all the vines that survived under salinity stress. Chu and coworkers (1976a, b) observed an accumulation of proline in salt-stressed barley plants. Heyser and coworkers (1989) reported that cell suspensions of *Distichlis spicata* which adapted to 260 mM NaCl showed greater metabolism of C^{13} glutamic acid to proline as compared to that in control (cells without NaCl).

Lutts and Guerrier (1995) found that under salinity, the highest accumulation of proline occurred in calli, roots and younger leaves of cv. IKP. Treichel (1975) found that halophytes, like *Salicornia fructosa*, *Aster tripolium* and *Mesembryanthemum nodiflorum* responded to high osmotic potential in culture medium by increasing the concentration of proline. It was also reported that the increase in free proline concentration is strongly related to the concentration of Na^+ and Cl^- in the plant sap and thus proline might be involved in the process of osmotic adjustment. Lutts and coworkers (1996) also observed that salt-resistant rice cultivars Nona Bokra and IR 4630 exposed at the seedling stage during one or two weeks to 0, 20, 30, 40 or 50 mM NaCl accumulated less proline in root and shoot leaves than that of salt-sensitive I Kong Poa (IKP) and IR 31785, Aiwa-a moderately resistant genotype. They suggested that proline did not appear to be involved in osmotic adjustment in salt-stressed rice plants.

1.6 The effect of NaCl salinity on growth of plants and its relation with ion transport

The growth of plant is mainly controlled by the area and activities of the photosynthetic tissue (Kriedemann 1986). Plant growth is generally reduced by salinity (Greenway and Munns 1980, Lauter *et al.* 1981, 1986, Stiborova *et al.* 1987). Grattan and Grieve (1992) reported that salinity stress caused an imbalance in the availability of mineral nutrients, their uptake and distribution within the plant and increased requirements for essential elements.

Sharma (1997) found that the concentration of Na^+ and Cl^- ions was increased the plant growth and rate of photosynthesis declined with the increase in levels of salinity in chickpea. Yeo and Flowers (1986) suggested that Na^+ and Cl^- was considered to be toxic and appeared to be responsible for inhibition of growth induced by salinity. Lin and Kao (1995) reported that root growth of rice seedlings was significantly decreased by higher concentration of NaCl (above 50 mM). On the other hand, Wignarajah (1990) found that in *Phaseolus vulgaris* higher salinity inhibited the shoot growth rather than the root growth. Ai-Yemeny and Basahy (1997) also reported that increase in salinity caused a reduction in growth of roots, shoot length and stem of *Cyanopsis tetragonoloba*. Alamgir and Kutube (1997) reported that in wheat salinity inhibited the growth of the root and shoot. A reduction in 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 reported that salinity caused a decrease in shoot dry weight when a salt-tolerant variety of cotton and salt-sensitive bean were treated with salinity. They observed that 50 mM and

150 mM NaCl salinity caused a reduction in shoot dry weight of beans. Firmansyah and coworkers (1989) reported that salinity of irrigation water reduced straw weight, root weight, plant height and tiller number of rice plants.

Increase in NaCl concentration progressively led to a decrease in the growth of barley (Leonova 1987), chickpea (Elsheike and Wood 1990), Rhodes grass (Waisel 1985), *Opuntia ficus-indica* (Nerd *et al.* 1991), *Ficus carica* (Okubo and Utsunomiya 1996), *Capsicum annum* (Gunes and Alpaslan 1996) and in cluster bean (Garg *et al.* 1997). Muthukumarasamy and Panneerselvam (1997) found that NaCl salinity at 50 mM level did not have an adverse effect on the plant growth of green gram. An adverse effect in growth was observed in root, stem and leaf at 100 and 150 mM NaCl concentration. In *Lolium multiflorum*, shoot and root dry weight was inhibited at 166 and 148 mM NaCl treatment respectively (Kangsoo *et al.* 1995).

Alamgir and coworkers (1989) found that in rice, low level salinity at early growth phase stimulated plant growth, in some cases, but high salinity level at all growth phases inhibited it. Similar result was found in two rice varieties IR42 and IR64 (Faijaroenmongkol 1987). Venkatesan and coworkers (1997) observed that in *Ipomoea pescaprae* upto 200 mM NaCl alone promoted its growth, increased fresh weight and leaf area. Faustino and coworkers (1996) reported the sensitivity thresholds for some physiological process against NaCl-salinity mapped in both salt-tolerant IR 9884-54-3-1 and salt-sensitive IR 29. They found that at 50 mM NaCl salinity, plant dry weight was decreased by 30% in salt-sensitive IR 29 whereas the salt-tolerant IR 9884 by 25%. The

generalized assumption is that root growth is almost always less affected than shoot growth by salinity (Munns and Termaat 1986, Lauchli 1990).

Huq and Larhar (1983a) observed that growth of *Phaseolus aureus* L. was affected due to application of NaCl at the external medium (0 to 150 mM). Excess amount of internal Na^+ became toxic to the plant and inhibited the transport of other inorganic ions particularly the accumulation of K^+. It was reported that K^+ in salt-treated (150 mM) plants constituted 10% and in control 55% of the total mineral ions. They also found that Cl^- was the only encounter of Na^+. From these observations, they suggested that the excess of Na^+ ions was the cause of reduced growth.

Yasseen *et al.* (1987) reported that salinity decreased leaf area by reducing number and volume of cells. He also recorded a reduction in cell number due to salinity (Yasseen *et al.* 1988). In *Cressa cretica*, a lower salinity concentration (425 mM) promoted the growth but the highest salinity (850 mM) did not have a significant effect on growth. They suggested that growth of these plants was significantly decreased by K^+ deficiency (Khan and Aziz 1998). Papps and coworkers (1983) observed a linear decline in the initial rate of leaf extension and also final leaf length with the increase in NaCl concentration. Kefu *et al.* (1991) who worked on the inhibitory effect of NaCl on the growth of cotton seedling reported that salt-excluding ability of the cotton seedling was low and he suggested that salt injury was due to excess amount of ions in tissue. Alamgir and Kutube (2000) found that in presence of gibberellic acid under salinity stress growth was increased in wheat.

Khavari-Nejad (1988) reported that plant growth was decreased by salinity depending upon the plant species, salinity level and ionic composition of salts.

In the work reported here, *Oryza sativa* L. was chosen as the experimental plant because this species is the main cereal crop in Bangladesh. Salinity is a serious problem in rice cultivation in Bangladesh. As a vast saline area is becoming uncultivable in this country, efforts are being made to bring this area under cultivation by selecting salt-tolerant crops. For this purpose, rice cultivation is being expanded in the saline belt of Bangladesh. Rice production in the coastal areas can, however, be greatly increased through the development of high yielding salt-tolerant varieties. In doing so, we must have to understand the mechanism of salinity tolerance i.e. to ascertain whether the plant is a salt-avoider or a salt-tolerant one. Hence, the study on the effect of salinity on the accumulation, translocation and distribution of ions was undertaken in order to understand the mechanism of physiological adaptation of rice plant.

In view of the above background, the objectives of the present investigation were as follows:

a) To study the effect of salinity on germination and its correlation with the accumulation of Na^+, K^+ and Cl^-.

b) To study the effect of salinity on the accumulation and distribution of important

   i) monovalent ions like Na^+, K^+, Cl^- and NO_3^-,

ii) essential divalent cations like Ca^{2+}, Mg^{2+} and Fe^{2+} and

iii) polyvalent ions like phosphate (PO_4^{-3}) in intact plants.

c) To study the effect of salinity on accumulation of proline.

d) To correlate the effect of salinity on growth and ion accumulation in rice.

CHAPTER 2

MATERIAL AND METHODS

2.1 Plant material

Oryza sativa L. cv. BR16 and var. Pokkali were used as plant material. The seeds were obtained through the courtesy of Bangladesh Rice Research Institute (BRRI), Joydebpur, Gazipur.

2.2 Solution culture technique

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 $CuCl_2$, $2H_2O$ and 0.10 μM $Na_2MoO_4.2H_2O$ were used as micronutrients.

b) Macronutrients

5.0 mM $Ca(NO_3)_2.4H_2O$, 5.0 mM KNO_3, 2.0 mM $MgSO_4.7H_2O$, 0.5 mM KH_2PO_4 and 100 μM Fe-EDTA were used as macronutrients.

2.3.1 Preparation of stock solution of gibberellic acid (GA)

10^{-4} M stock solution of gibberellic acid (GA) was made by dissolving 8.7 mg GA in distilled water which was then made upto a final volume of 250 ml with distilled water.

2.3.2 Preparation of stock solution of kinetin

1.075 mg kinetin was taken in a test tube. One ml of propylene glycol was added to it and stirred with a magnetic stirrer until kinetin was dissolved. The dissolved kinetin was then taken in a 250 ml volumetric flask and made upto a final volume of 250 ml with distilled water. Thus, 2×10^{-5} M stock solution of kinetin was prepared.

2.4 Culture of plants

2.4.1 Surface sterilization of seeds

In order to avoid fungal infection, surface of seeds were sterilized with sodium hypochlorite (4-7%) solution for two minutes. Sodium hypochlorite-treated seeds were washed under tap water for several times (8-12 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 by passing air with the help of an air compressor (Model: Compton Com/VAG pump, Type D/351 VM, Dawson McDonald, Derbyshire).

2.4.2 Method of application of salinity stress

Plants were subjected to different salinity treatments from the initial stage of the experiment. The treatments were as follows: Control (half-strength Hoagland solution) and 25, 50, 75 and 100 mM NaCl made in half-strength Hoagland solution.

2.4.3 Methods of studying the effect of NaCl salinity stress on germination of rice

Fifty surface sterilized seeds (section 2.4.1) were placed on Whatman filter paper No. 1 contained in a petridish (11 cm). Each filter paper contained in a petridish was soaked in 7 ml of 10, 25, 50, 100, 150, 200 and 300 mM NaCl solution. 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 25-20°C temperature. Seeds were considered to be germinated when radicle and plumule could be clearly distinguished. The germination of seeds was recorded at 24, 48, 72, 96, 120 and 144 hours from the date of sowing.

2.4.4 Methods of studying interaction of different concentrations of NaCl and gibberellic acid (GA) and kinetin on germination of rice

In order to study the interaction of salinity and gibberellic acid, seeds were allowed to germinate in 100, 200 and 300 mM NaCl solution with or without 10^{-6}, 10^{-5} M GA. Interaction of salinity and kinetin were also studied using the same concentration. Germination technique was the same as described above.

2.4.5 Growing of plants in solution culture

Surface sterilized seeds were spread on cotton gauze on a perforated plastic cover fitted to a Pyrex glass beaker of 500 ml capacity filled with half-strength Hoagland solution (control) and different concentrations (25, 50, 75 and 100 mM) of NaCl solutions made in half-strength Hoagland solution. The beaker was painted black to prevent the roots being exposed to light. The solution was taken in a beaker to such a level that the cotton gauze was just in contact with the solution. Two pieces of cotton gauze were inserted into the solution through the holes of the plastic bid, so that the cotton gauze remained wet all the time to keep the germinating seeds wet.

Beaker with the sown seeds was kept in dark during germination at 25°C. Seeds were germinated within 48 hours of sowing. The beaker with the seedlings was transferred to a light bank.

Growth condition of the light-bank was as follows: Day temperature was 30 ± 1°C and night temperature was 25 ± 1°C. Day and night lengths were 14 and 10 hours respectively. Relative humidity was 80% by day and 90% by night.

The solution of the beaker was continuously aerated with the help of an air compressor.

2.5 Methods of the study of distribution of ions in intact plant

In order to study the distribution of ions under salinity the root and the shoot were first separated. Root samples were soaked in two five minutes changes for 0.2 mM $CaSO_4$ (50 ml each) to remove free space ions (Karmoker and Van Steveninck 1978, 1979). The roots were gently blotted to remove excess water. Root and shoot samples were dried in an oven at 80°C for 72 hours. Then the dry weight of samples were recorded with an electronic balance (FK-200, Japan).

2.6 Methods of extraction and measurement of ions in plant tissue

2.6.1 Extraction of K^+, Na^+ and Cl^- from plumule and radicle

The radicle and plumule samples were taken separately in test tubes after recording the dry weight. Five ml distilled water was added to each test tube and allowed to stand for 30 minutes. The test tube with soaked tissue was boiled for 30 minutes in a boiling water bath. The extract was collected in another test tube. Again, 2.5 ml distilled water was added to the residue and boiled for another 15 minutes. The extract was collected. Finally, 2.5 ml distilled water was added to the residue and boiled for another 10 minutes and the extract was collected. The combined extract was made upto a final volume of 10 ml.

2.6.2 Extraction of K^+, Na^+ and Cl^- from root and shoot tissues of intact plant

After recording dry weight, the root and shoot samples were taken in test tube separately. Samples were soaked in 10 ml of distilled water for 30 minutes and then boiled in a water bath for 30 minutes. The extract was collected in another test tube. Another 5 ml of distilled water was added to the residue and boiled for 15 minutes and extract was collected. Again, 5 ml of distilled water was added to the residue and boiled for another 10 minutes and extract was collected. Finally, the combined extract was made upto a final volume of 20 ml.

2.6.3 Extraction of nitrate (NO_3^-)

In order to extract total amount of nitrate (NO_3^-), the root and the shoot samples were taken separately in test tube after recording dry weight. Five ml distilled water was added to each test tube and allowed to stand for 30 minutes. The test tubes with soaked tissue were boiled for 30 minutes in a boiling water bath. The extract was collected in another test tube. Again, 2.5 ml of distilled water was added to the residue and boiled for 15 minutes. The extract was collected. Finally, 2.5 ml distilled water was added to the residue and boiled for 5 minutes. The extract was collected and finally the combined extract was made upto a final volume of 10 ml with distilled water.

2.6.4 Extraction of Ca^{2+}, Mg^{2+}, Fe^{2+} and PO_4^{-3}

The root and shoot tissue were taken into separate test tubes. Four ml of a mixture of concentrated nitric acid (HNO_3) and perchloric acid (4:1) were added

to the test tubes containing plant tissues and allowed to stand for 30 minutes. The test tubes with the samples and acid mixture were heated in a sand bath in order to digest the tissue. The extract was evaporated almost to dryness. The extract was then diluted to a final volume of 20 ml with distilled water.

2.6.5 Measurement of K^+ and Na^+

The amount of K^+ and Na^+ by plant tissue was measured using a flame photometer (Model: Jenway: PEP-7, UK) at wavelength of 767 and 589 nm respectively. Concentration of K^+ and Na^+ in the extract (ppm) was determined with the help of a standard curve as shown in Fig. 1 and Fig. 2 respectively.

The amount of K^+ and Na^+ were calculated using the following formula:

$$\text{Ion content} = \frac{\text{Concentration of ion in the extract (ppm)} \times \text{Final volume of the plant extract (ml)}}{1000 \times \text{Dry weight of the tissue (g)} \times \text{Atomic weight of the respective ion}}$$

$$= X \text{ m-equivalent } g^{-1} \text{ dry tissue}$$

2.6.6 Measurement of Cl^-

Amount of Cl^- in plant tissue was measured by titrametric method. Five ml of extract was titrated with 0.1 N $AgNO_3$ using potassium chromate (K_2CrO_4) as an indicator. The concentration of Cl^- in the extract was determined using a standard curve for Cl^- (Fig. 3).

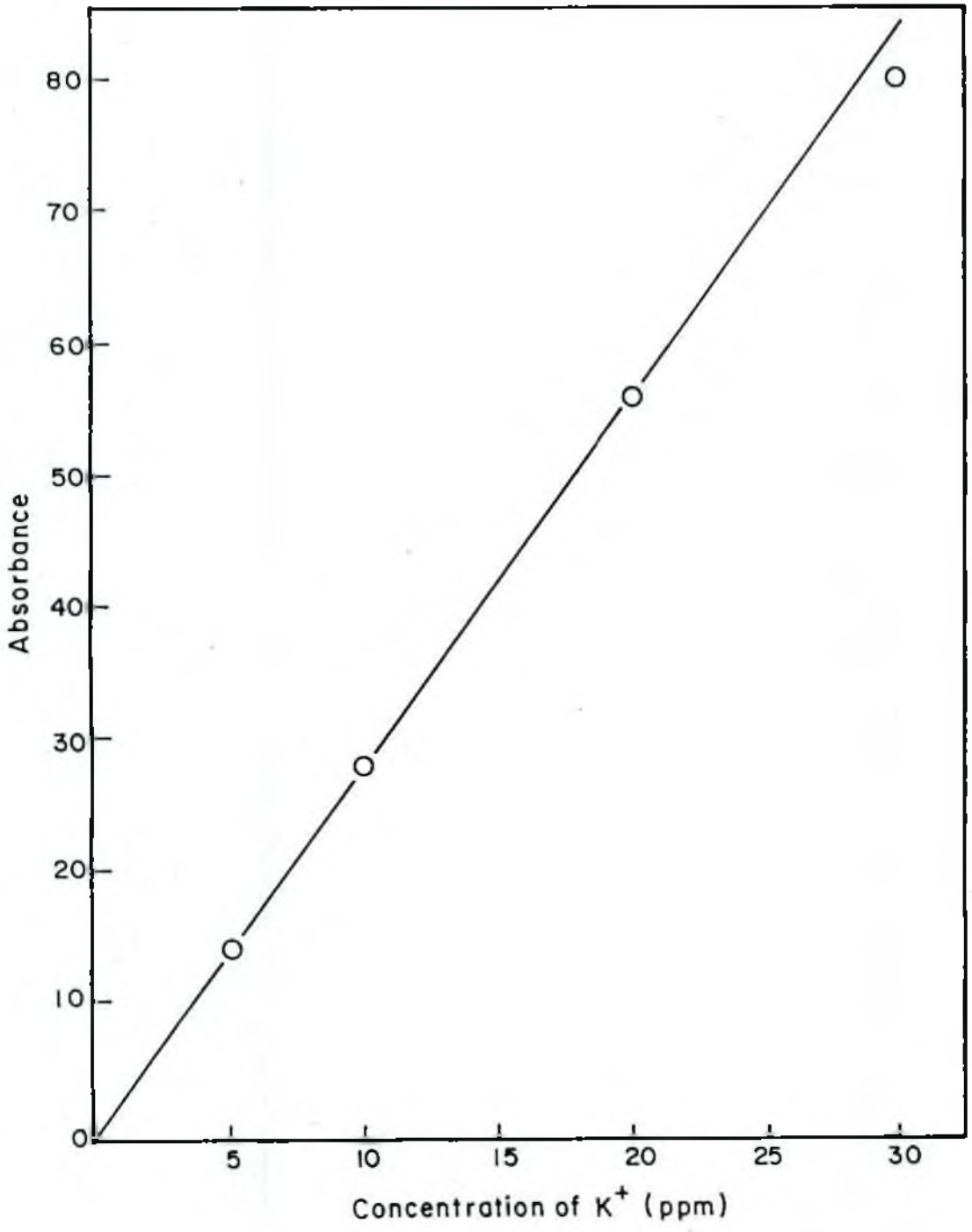

Fig. 1 Standard curve for potassium.

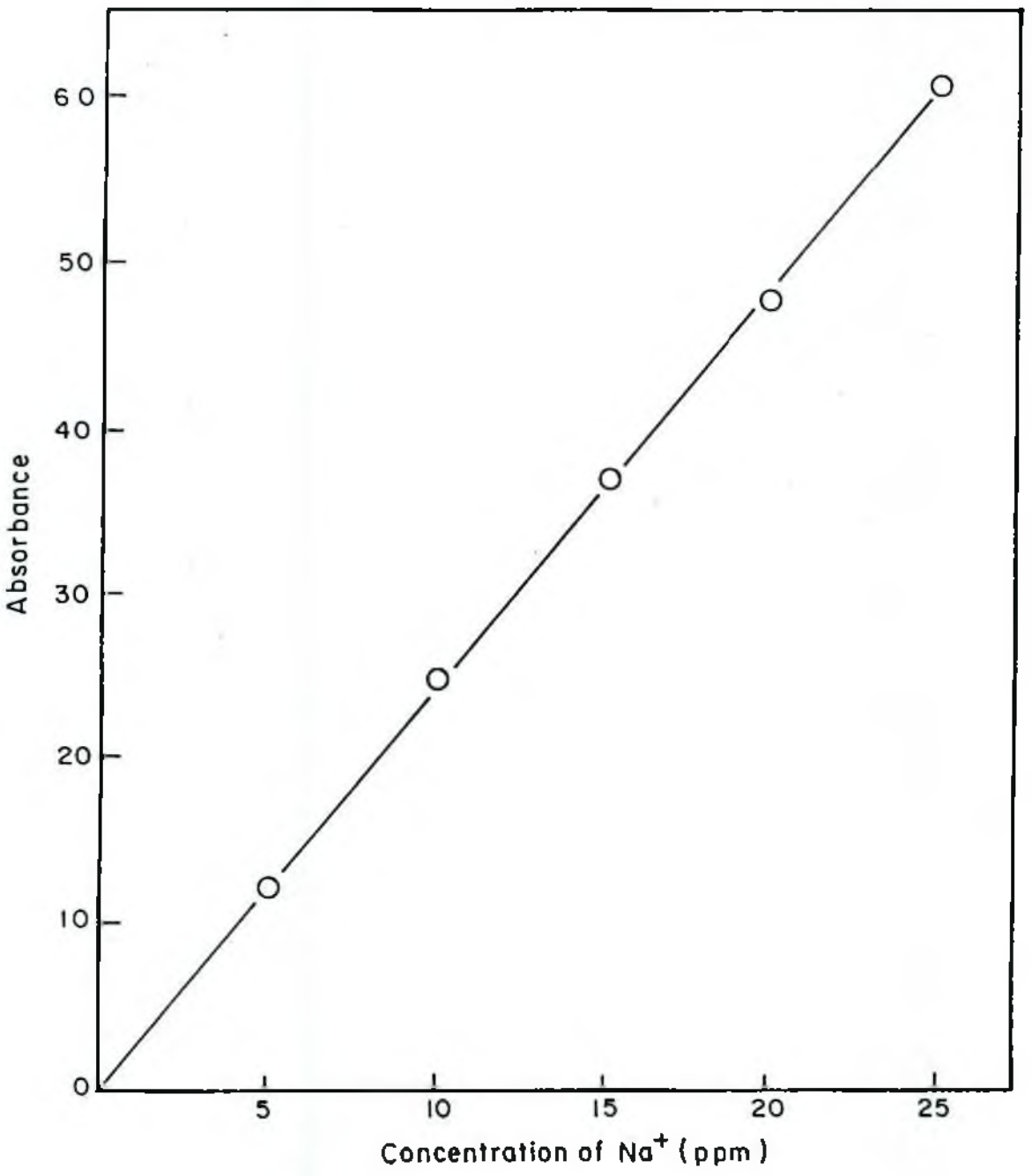

Fig. 2 Standard curve for sodium.

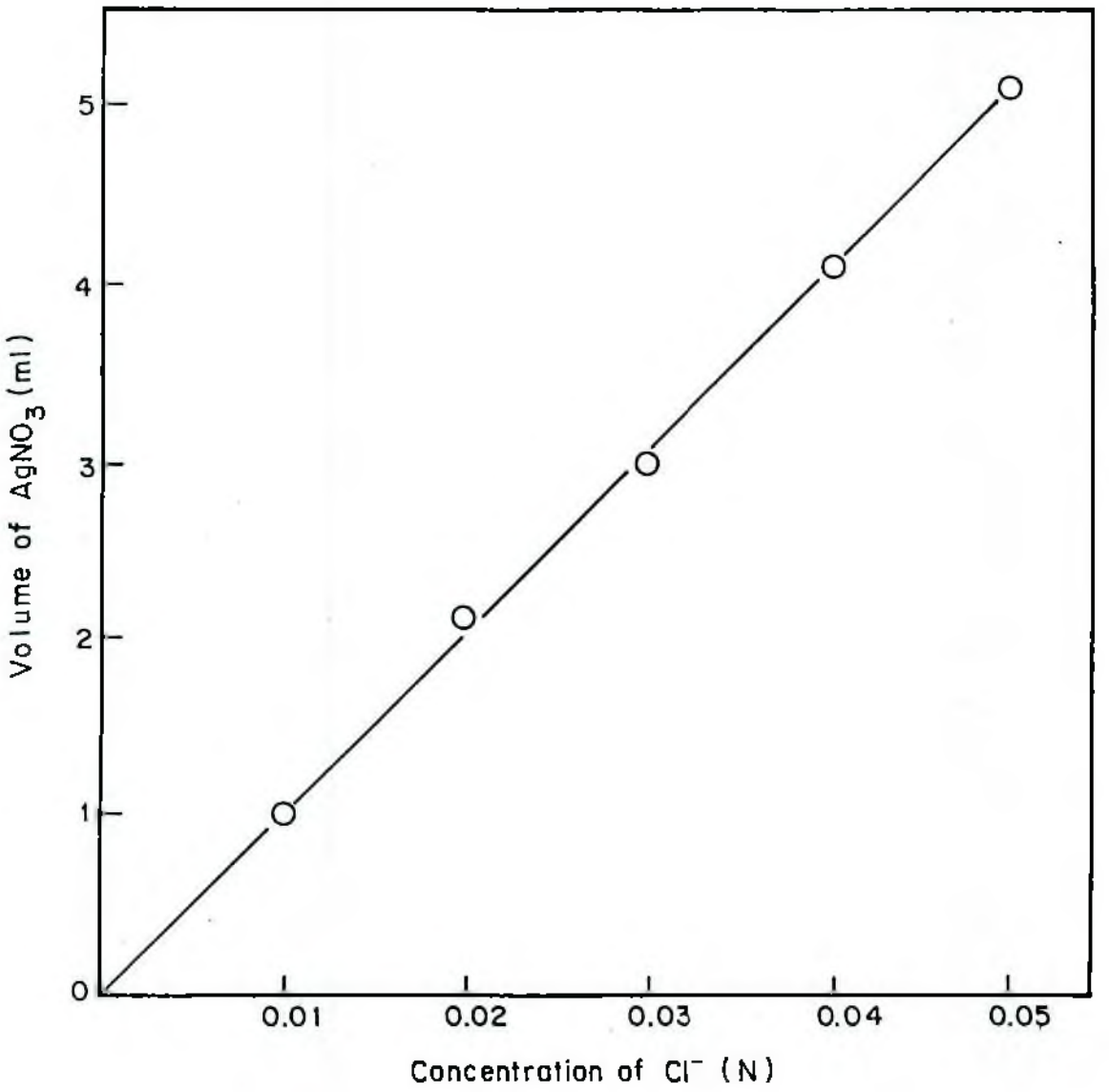

Fig. 3 Standard curve for chloride.

The amount of Cl^- in the tissue of plant was calculated using the following formula:

$$Cl^- \text{ content} = \frac{\text{Concentration of } Cl^- \text{ in plant extract (ppm)} \times \text{Final volume of the plant extract (ml)}}{1000 \times \text{Dry weight of the tissue (g)} \times \text{Atomic weight of chloride}}$$

$$= X \text{ m-equivalent } g^{-1} \text{ dry tissue}$$

2.6.7 Measurement of nitrate (NO_3^-)

Nitrate (NO_3^-) was measured by salicylic acid method (Cataldo *et al*. 1975).

2.6.7.1 Preparation of 5% salicylic acid

Five gram of salicylic acid (SA) was taken in 50 ml (approximately) of H_2SO_4 in a conical flask of 100 ml capacity. After dissolving salicylic acid, the content was made upto a final volume of 100 ml with H_2SO_4. Thus, 5% (w/v) salicylic acid was prepared. The SA-H_2SO_4 reagent was made just prior to use.

2.6.7.2 Preparation of 2.5 N NaOH solution

One hundred gram of NaOH was taken in 500-600 ml distilled water and was shaken well. After dissolving NaOH the content was made upto a final volume of 1000 ml with distilled water. Thus, 2.5 N NaOH was prepared.

2.6.7.3 Determination of nitrate

In order to determine NO_3^- in plant tissue, 0.2 ml of extract was taken in test tube and 0.8 ml of 5% SA-H_2SO_4 was added to it. The content of the test tube was mixed thoroughly and allowed to stand for 20 minutes at room temperature. 19 ml of 2.5 N NaOH was added slowly to raise the pH above 12.0. Samples were cooled at room temperature.

0.2 ml of reagent extract was taken and similarly above reagent was mixed to it. A blank was also run where 0.2 ml of distilled water was taken.

Finally, absorbance was measured at 410 nm wavelength with the help of a spectrophotometer (Model: UV-120-02, Japan).

The concentration of NO_3^- (ppm) in the extract was determined using a standard curve for NO_3^- (Fig. 4).

The amount of NO_3^- in the plant tissue was calculated using the following formula:

$$NO_3^- \text{ content} = \frac{\text{Concentration of } NO_3^- \text{ in the extract (ppm)} \times \text{Final volume of the plant extract (ml)}}{1000 \times \text{Dry weight of the tissue (g)} \times \text{Atomic weight of } NO_3^-}$$

$$= X \text{ m-equivalent } g^{-1} \text{ dry tissue}$$

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

The amount of Ca^{2+}, Mg^{2+} and Fe^{2+} was measured using an atomic absorption spectrophotometer (Perkin-Elmer, Model: 3110) at wavelengths of 285.2, 422.7

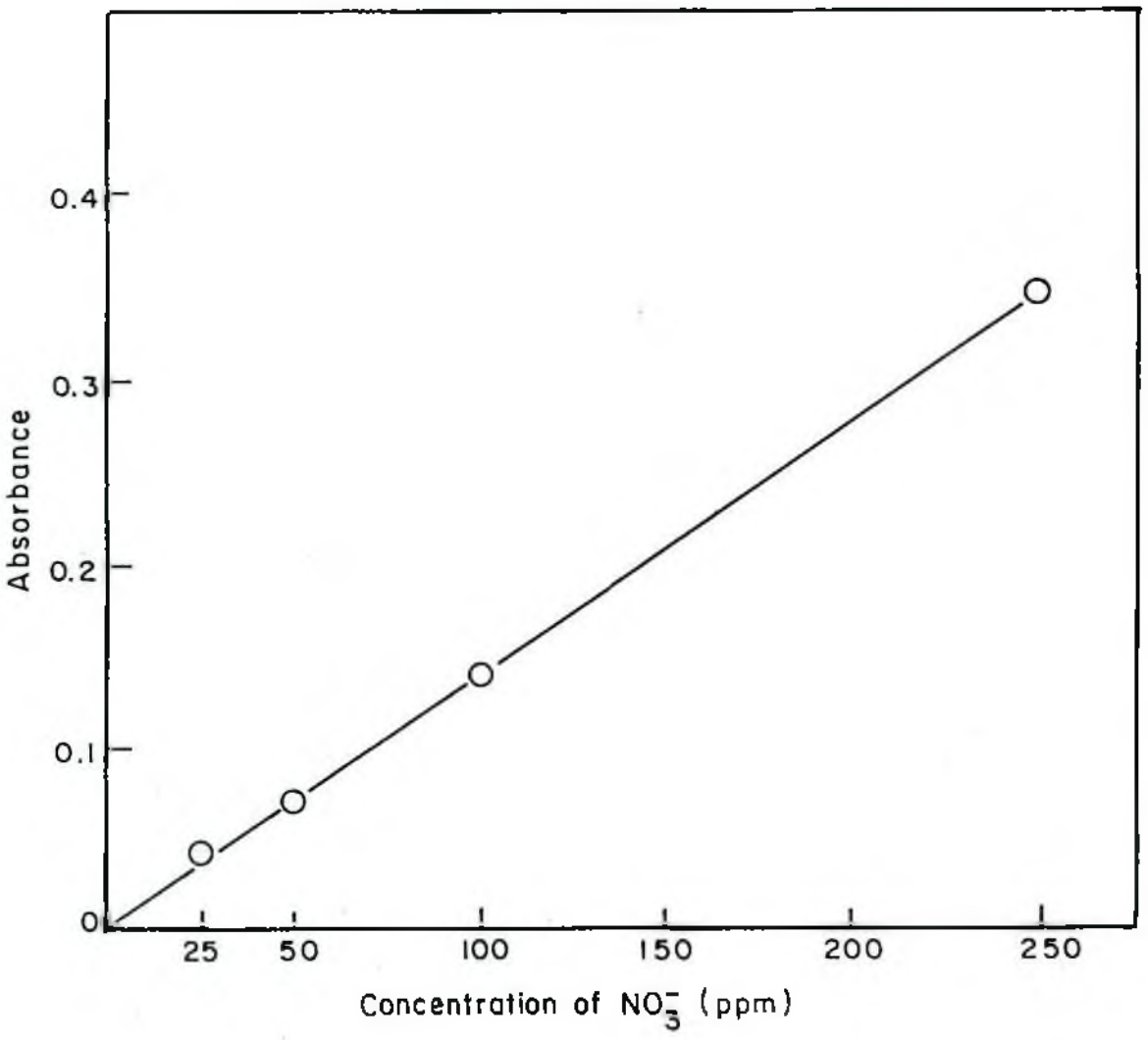

Fig. 4 Standard curve for nitrate.

and 248.3 respectively. The concentration of sample was directly obtained from the spectrophotometer. Therefore, no standard curve was required.

The amount of Mg^{2+}, Ca^{2+} and Fe^{2+} were calculated using the following formula:

$$\text{Ion content} = \frac{\text{Concentration of ion in the extract (ppm)} \times \text{Final volume of the plant extract (ml)}}{1000 \times \text{Dry weight of the tissue (g)} \times \text{Atomic weight of respective ion}}$$

$$= \text{X m-equivalent } g^{-1} \text{ dry tissue}$$

2.6.9 Measurement of phosphate

Phosphate was measured by ammonium molybdate-ascorbic acid blue colour method (Petersen 1997). In order to measure phosphate, the following reagents were prepared:

2.6.9.1 Preparation of reagent A

Ammonium molybdate solution: 24.0 g of ammonium molybdate ($(NH_4)_6Mo_7O_{24}.4H_2O$) was dissolved in 500 ml distilled water in a beaker. Then, 0.5816 g of potassium antimony tartarate ($C_4H_4O_7Ksb$) was dissolved in 100 ml distilled water in another beaker.

Two litre distilled water was taken into a 5 litre volumetric flask, 300 ml concentrated H_2SO_4 was added slowly. After the addition of sulphuric acid (H_2SO_4) was completed, the solution was allowed to be cooled at room

temperature. Solutions of ammonium molybdate and potassium antimony tartrate was transferred to the flask and made upto a final volume of 5 litre with distilled water and mixed thoroughly. Then it was stored in dark in a refrigerator.

2.6.9.2 Preparation of reagent B

Ammonium molybdate-ascorbic acid solution: 4.1 g of ascorbic acid was dissolved in one litre of reagent A and mixed thoroughly. Thus, reagent B was prepared as required just before when it was needed.

2.6.9.3 Determination of phosphate

Ten ml of reagent B was added to 5 ml of extract in a 50 ml volumetric flask and made upto a final volume of 50 ml adding distilled water. A blue colour appeared and the solution was then allowed to stand for 15 minutes. The method was calibrated using a standard phosphate solution. A blank was prepared with distilled water (45 ml) and 5 ml of reagent B.

The absorbance of the aliquot was measured at a wavelength of 890 nm with spectrophotometer (Model: Perkin-Elmer UV/VIS Spectrometer Lambda 11/BIO [2.2]).

The amount of phosphate in the plant tissue was calculated using the following formula:

$$\text{Phosphate content} = \frac{\text{Concentration of phosphate in the extract (ppm)} \times \text{Total volume of the content (ml)} \times \text{Total volume of the extract (ml)}}{1000 \times \text{Amount of extract taken (ml)} \times \text{Dry weight of the tissue (g)} \times \text{Atomic weight of phosphate}}$$

$$= X \text{ m-equivalent } g^{-1} \text{ dry tissue}$$

2.6.10 Extraction of proline

Proline was extracted from dried powdered plant material in 4 ml of 90% ethanol. The aliquot was then centrifuged at 5000 rpm using a centrifuge (Model: OSK-13698, Ogawa Seiki and Co. Ltd., Japan), and the clear solution containing proline was separated and evaporated almost to dryness and diluted to 2 ml with distilled water (Huq 1984).

2.6.10.1 Determination of proline

Proline content was measured in 0.5 ml sample of extract by Troll and Lindsley method (Troll and Lindsley 1954).

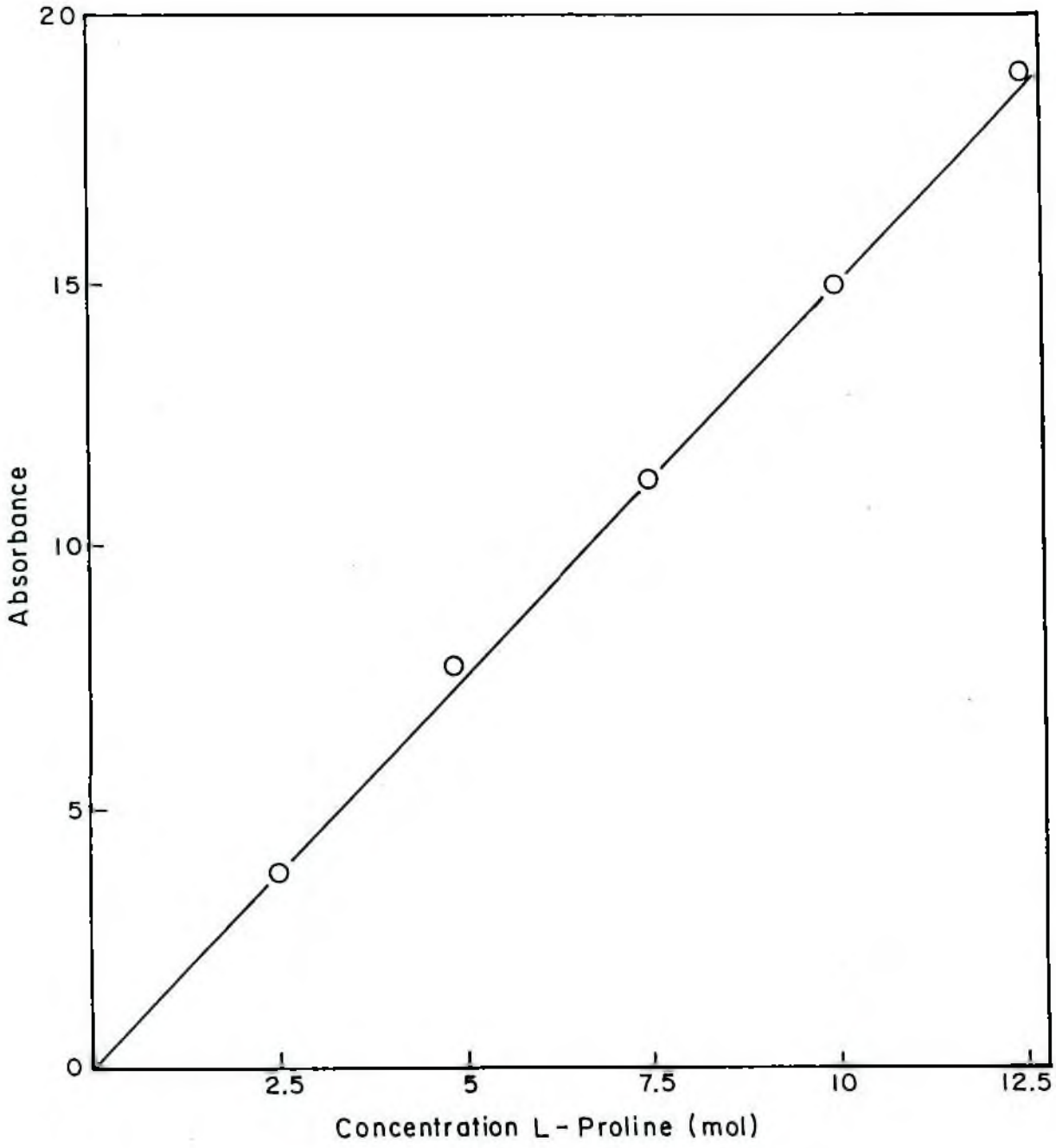

Fig. 5 Standard curve for L-proline.

Plate 1 Growth of rice cv. BR16 in solution culture (control: half-strength Hoagland solution; salinity: 25 to 100 mM NaCl) for 7 days.

Plate 2 Growth of rice var. Pokkali in solution culture (control: half-strength Hoagland solution; salinity: 25 to 100 mM NaCl) for 7 days.

CHAPTER 3

THE EFFECT OF SALINITY ON GERMINATION

3.1 Introduction

Germination of most crops seeds are affected by salinity. In general, salinity has inhibitory effects on germination of seeds (Jibury *et al.* 1986, Kumar *et al.* 1988, Mondal *et al.* 1988, Alwan *et al.* 1989, Navetiyal *et al.* 1989, Yasseen *et al.* 1989, Sharma and Yamdagni 1989, Begum *et al.* 1992). The inhibitory effects of salinity on germination is a major hindrance in cultivation of crops in saline areas.

A reduction of percentage and speed of germination under different levels of salinity in two salt-tolerant cultivars of rice was observed (Queiroz and Nakagawa 1992).

The germination percentage was decreased under salinity in chickpea (Muthukumarasamy *et al.* 1997), wheat (Begum *et al.* 1992, Kumar and Singh 1996) and in seeds of *Penstemon parryi* (Raeber and Lee 1991).

Begum and coworkers (1992) found that gibberellic acid alone or in combination with kinetin alleviated the inhibitory effect of salinity on germination and kinetin decreased the rate of germination of wheat under NaCl salinity stress. Alamgir and coworkers (1995) also observed that pretreatment of wheat seeds by GA_3 caused a stimulation on the germination percentage. GA and kinetin stimulated the rate of germination of salt (1.75% NaCl) treated barley (Kabar 1987).

Begum and coworkers (1992) reported that NaCl salinity increased accumulation of Na^+ and Cl^- while it decreased K^+ accumulation during germination of seeds. Quayyum and coworkers (1991) also observed that Pokkali and MI48 varieties of rice absorbed large quantities of Na^+ and Cl^- from NaCl-treated solution. Salinity stress decreased K^+ content in germinated seeds of rice (Alamgir and Ara 1986).

In this chapter, the correlation of the effect of salinity on K^+, Na^+ and Cl^- in developing embryo with salinity-induced effect on germination is reported. The effect of NaCl salinity and the interaction of GA, kinetin with NaCl salinity on germination and ionic balance will be discussed.

3.2 Material and Methods

3.2.1 Methods of germination of seeds

Seeds were surface-sterilized according to 2.4.1. The germination of seeds were done according to the method described in 2.4.3.

3.2.2 Methods of studying interaction of different concentrations of NaCl and gibberellic acid (GA) and kinetin on on germination of rice

The interaction of different concentrations of NaCl and phytohormones on germination of rice was studied according to the method described in 2.4.4.

3.2.3 Preparation of stock solution of GA and kinetin

Stock solution of GA and kinetin was prepared according to the method described in 2.3.1 and 2.3.2.

10^{-4} M GA and 2 x 10^{-5} M stock solutions of kinetin were prepared.

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

Seeds (which were germinated after 72 hours of soaking in 10-200 mM NaCl, and 100-200 mM NaCl with or without GA and/or kinetin) were used for analysis of K^+, Na^+ and Cl^- ions in the plumule and radicle. The plumule and radicle were separated from cotyledons. Free space ions of radicles were removed by washing in two, three minutes changes of 0.2 mM $CaSO_4$. The plumules were washed with distilled water, excess water was removed from the tissue of the sample (radicles and plumules) by blotting with a blotting paper. The plumules and radicles were than dried in an oven at 70°C for 48 hours, and the dry weight was recorded. Ions were extracted from plumules and radicles according to the method described in 2.6.1.

Na^+ and K^+ ions were measured according to 2.6.5 and Cl^- was measured according to the method described in 2.6.6.

3.3 Results

3.3.1 The effect of salinity on germination percentage

Germination was not affected when seeds of cv. BR16 was treated with 50 mM NaCl solution. The germination percentage declined with the increase in salinity level from 100 to 300 mM NaCl. NaCl solution ranging from 100 to 300 mM decreased the germination percentage of cv. BR16 by 2.2 to 78.79% (Fig. 6). Rate of germination in var. Pokkali was reduced by 26.67, 46 and 67% at 100, 150 and 200 mM NaCl solution respectively as compared to control. Seeds failed to germinate at 300 mM NaCl solution (Fig. 6).

3.3.2 The effect of salinity and/or its interaction with GA, kinetin on germination and NaCl salinity stress

NaCl salinity stress (100-300 mM) decreased the rate of germination of rice. Application of GA (10^{-6} M, 10^{-5} M) along with NaCl (100 mM) nullified the inhibitory effect of salinity on germination in both cv. BR16 and var. Pokkali (Fig. 7 and 8). The rate of germination was decreased by 55 to 21% and 65 to 30% when seeds were treated with 10^{-6} M and 10^{-5} M kinetin respectively along with 100 mM NaCl in cv. BR16 (Fig. 7). On the other hand, in var. Pokkali, the rate of germination was initially decreased by 15 and 22% when seeds were treated with 10^{-6} M and 10^{-5} M kinetin respectively along with 100 mM NaCl. But 10^{-6} M kinetin increased the rate of germination as compared to that in control (Fig. 8). Interaction of 100 mM NaCl and 10^{-6} M (GA + kinetin) and 10^{-5} M (GA + kinetin) caused an increase in the rate of germination by 38 to

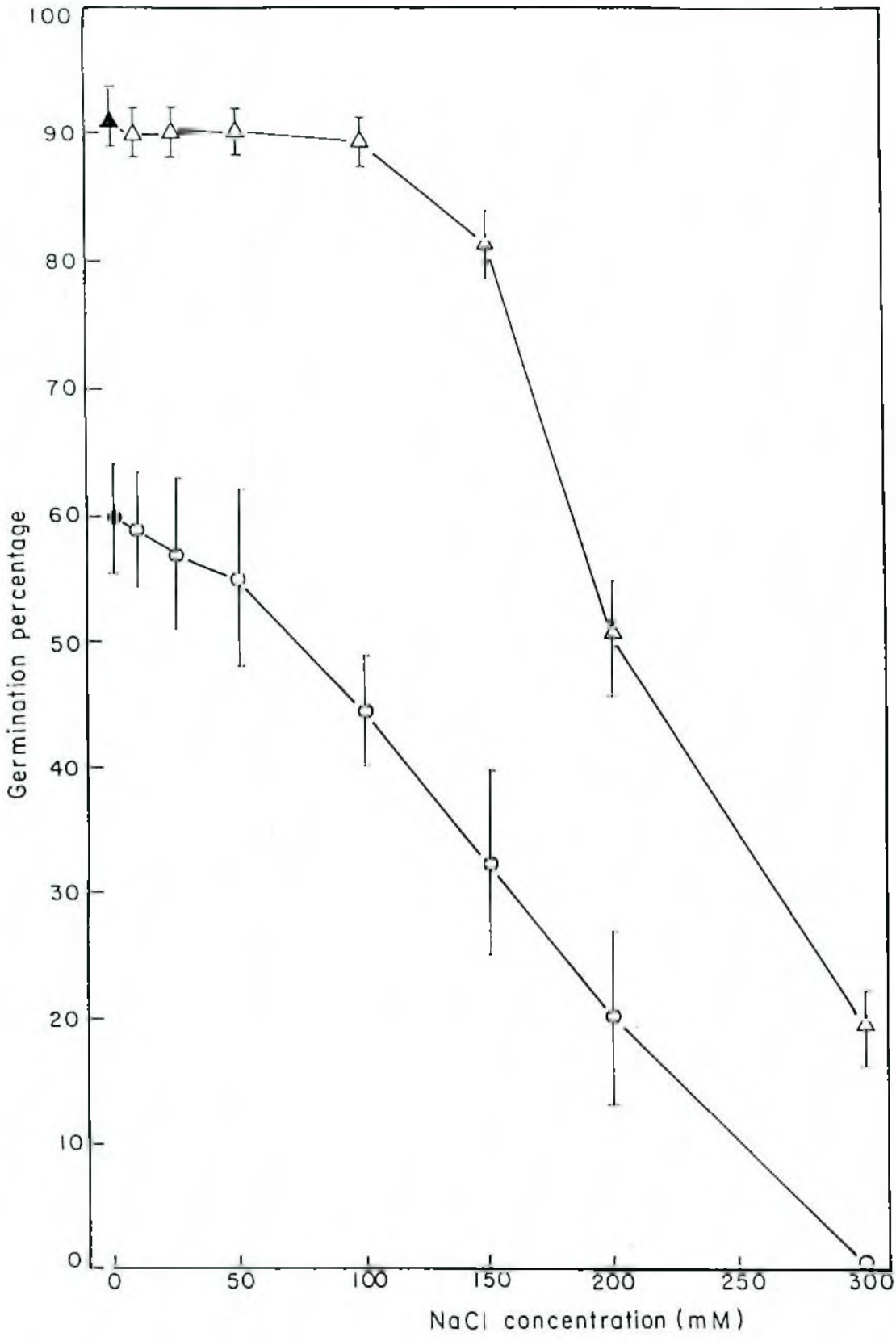

Fig. 6 The effect of NaCl salinity stress on germination of rice seed. △ represents cv. BR16; O represents var. Pokkali. Solid symbols represent control. Bars represent ± standard error. Each value is a mean of three replicates.

17% and 57 to 13% respectively (Fig. 7). On the other hand, interaction of 100 mM NaCl and 10^{6} M (GA + kinetin) and 10^{-5} M (GA + kinetin) had no significant effect on germination of var. Pokkali, but the germination rate was increased by 24% at 96 hours of treatment (Fig. 8).

When applied together with 200 mM NaCl and 10^{-6} M GA, the rate of germination was decreased by 22-8% in cv. BR16. 10^{-5} M GA caused a 41% increase in germination of seed of cv. BR16 (Fig. 9). In var. Pokkali, a combination of 200 mM NaCl and GA (10^{-6}, 10^{-5} M) nullified the inhibitory effect of salinity (Fig. 10). Application of 200 mM NaCl along with 10^{-6} M and 10^{-5} M kinetin caused an inhibition in the rate of germination of seeds of cv. BR16 by 30 to 61% and 53 to 72% respectively (Fig. 9). On the other hand, 10^{-5} M kinetin caused a decrease in the rate of germination of seed of var. Pokkali by 41 to 39% as compared to that in control (Fig. 10). 10^{-6} M kinetin with 200 mM NaCl increased the rate of germination by 18 to 10% in var. Pokkali (Fig. 10). In cv. BR16, the rate of germination was increased by 40 to 18% and 12 to 6% when seeds were treated with 200 mM NaCl + 10^{-6} M (GA + kinetin) and 200 mM NaCl + 10^{-5} M (GA + kinetin) respectively (Fig. 9). In contrast, in Pokkali 200 mM NaCl + 10^{-6} M (GA + kinetin) initially inhibited the rate of germination by 12 to 8% whereas the germination rate was increased by 9% at 144 hours of treatment (Fig. 10). When seeds were treated with 200 mM NaCl + 10^{-5} M (GA + kinetin), the germination rate of seed of var. Pokkali was increased by 18 to 19% (Fig. 10).

A combination of 300 mM NaCl and GA (10^{-6} M, 10^{-5} M) alleviated the rate of germination by 21 to 54% and 52 to 71% respectively as compared to control in

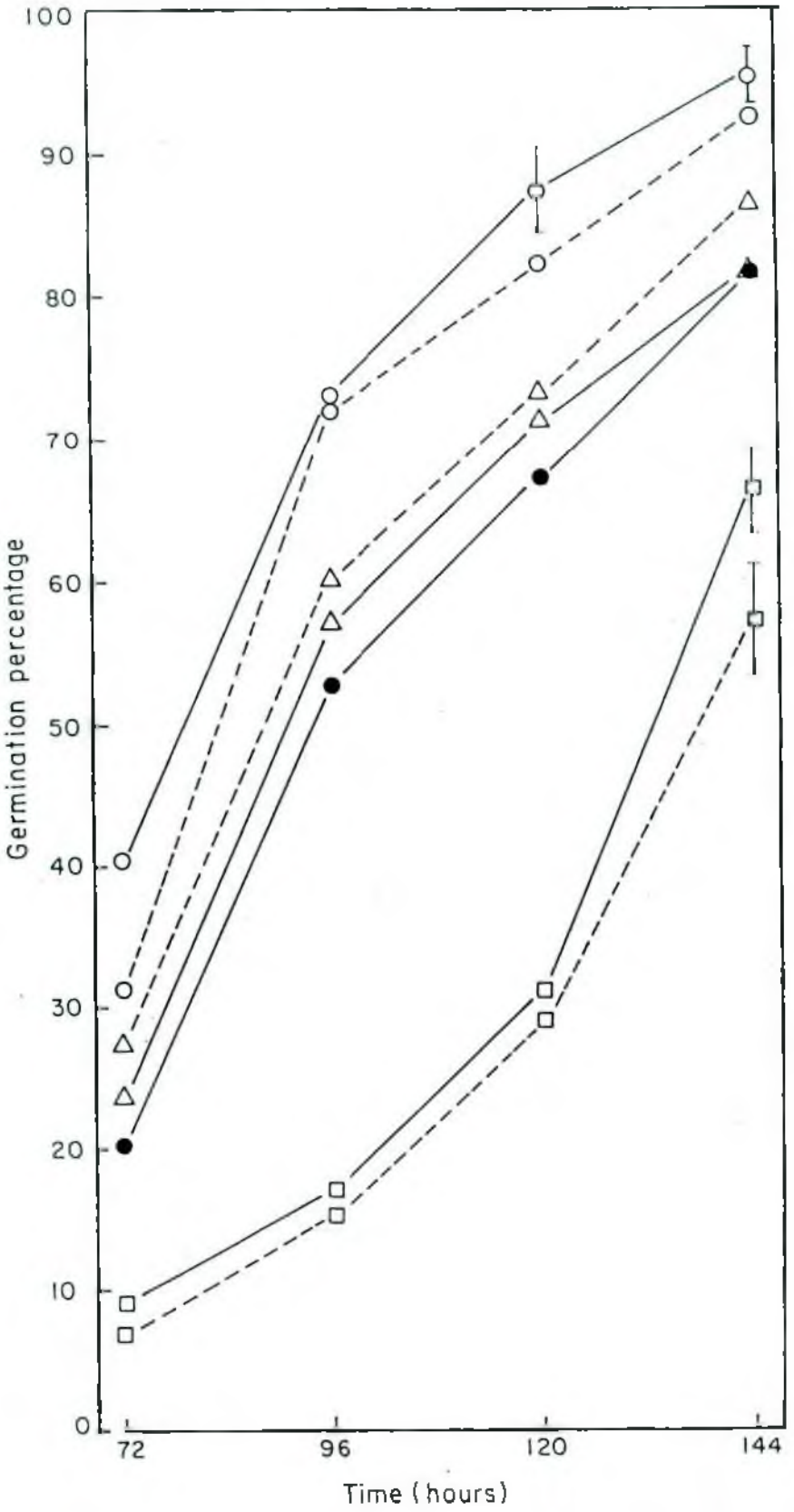

Fig. 7 Interaction of 100 mM NaCl salinity stress and gibberellic acid, kinetin on rate of germination of rice seed (cv. BR16) ● control (100 mM NaCl); △100 mM NaCl + GA; □100 mM NaCl + Kn; O 100 mM NaCl + GA + kinetin. Solid lines (—) represents 10^{-6} M, broken line (---) represents 10^{-5} M. Bars represent ± standard error.

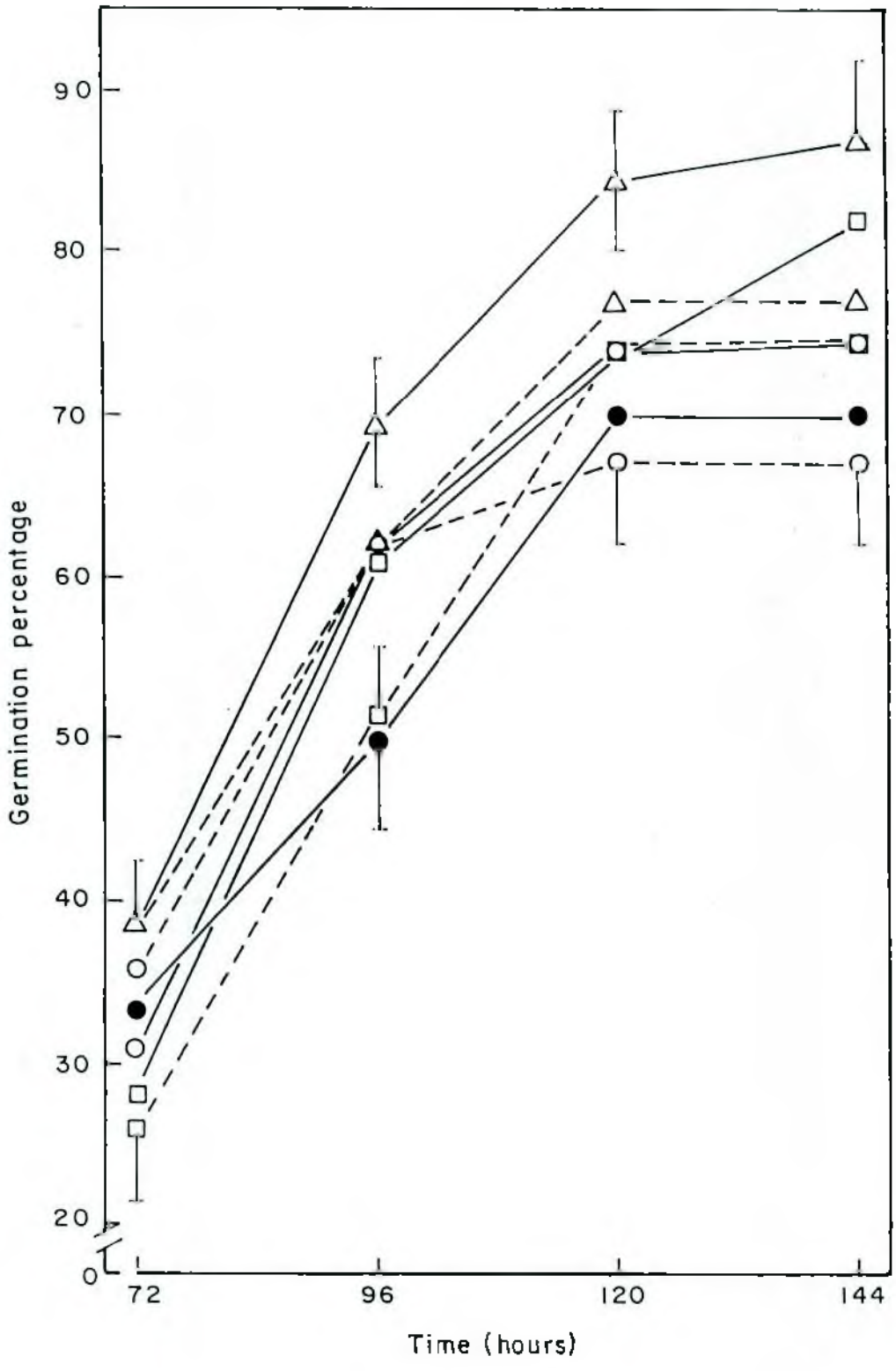

Fig. 8 Interaction of 100 mM NaCl salinity stress and gibberellic acid, kinetin on rate of germination of rice seed (var. Pokkali). Otherwise as Fig. 7.

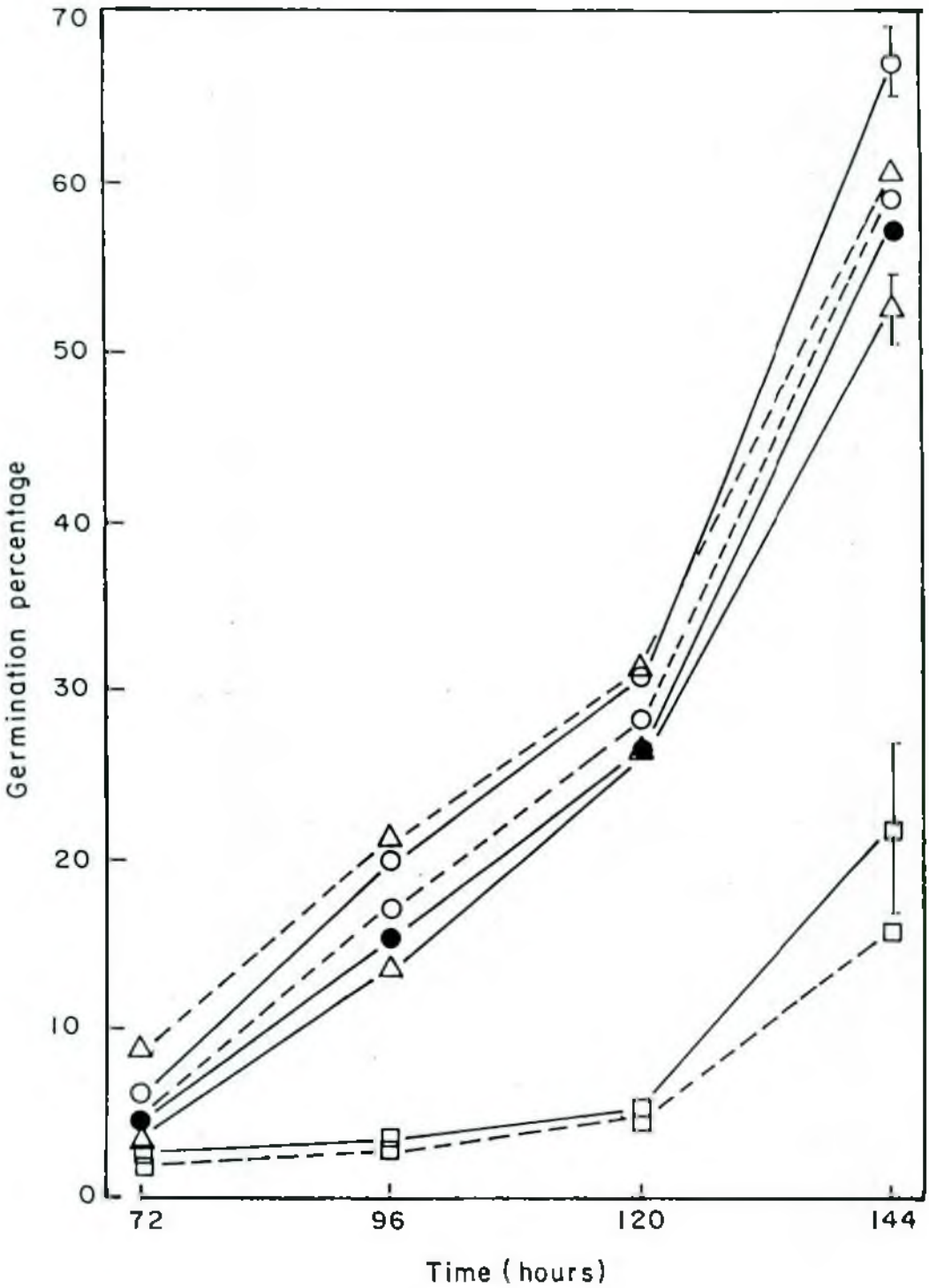

Fig. 9 Interaction of 200 mM NaCl salinity stress and gibberellic acid, kinetin on rate of germination of rice seed (cv. BR16) ● control (200 mM NaCl); △200 mM NaCl + GA; □200 mM NaCl + Kn; O 200 mM NaCl + GA + kinetin. Solid lines (—) represents 10^{-6} M, broken line (---) represents 10^{-5} M. Bars represent ± standard error.

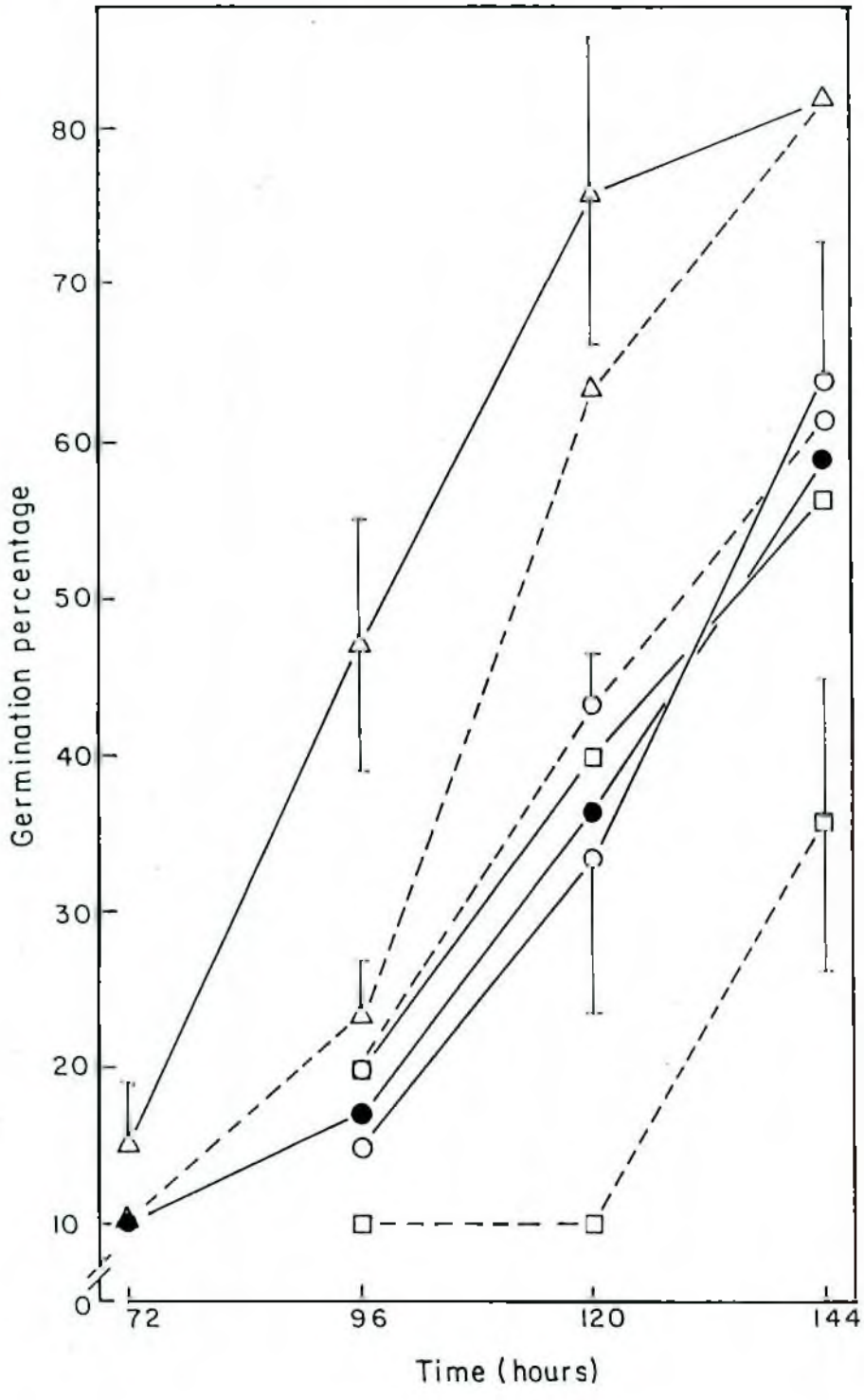

Fig. 10 Interaction of 200 mM NaCl salinity stress and gibberellic acid, kinetin on rate of germination of rice seed (var. Pokkali). Otherwise as Fig. 9.

cv. BR16 of rice (Fig. 11). Similar result was observed in var. Pokkali (Fig. 12). In cv. BR16, application of 10^{-6} M and 10^{-5} M kinetin along with 300 mM NaCl decreased the rate of germination by 54 to 49% and 70 to 57% respectively (Fig. 11). In var. Pokkali, the rate of germination was increased by 50 to 33% when seeds were treated with 10^{-6} M kinetin along with 300 mM NaCl. On the other hand, 10^{-5} M kinetin together with NaCl (300 mM) inhibited the rate of germination of var. Pokkali seeds by 22% (Fig. 12). Interaction of 300 mM NaCl and 10^{-6} M (GA + kinetin) and 10^{-5} M (GA + kinetin) alleviated the inhibitory effect of salinity on germination in both of rice cultivar BR16 and var. Pokkali (Fig. 11 and 12).

3.3.3 The effect of NaCl stress and interaction of NaCl stress and GA/kinetin on Na^+, K^+ and Cl^- accumulation in germinating seeds

NaCl stress (100 mM) increased Na^+ and Cl^- accumulation by 5% and 4.5-fold respectively and decreased K^+ accumulation by 11.0% in plumules and radicles of both cv. BR16 and var. Pokkali (Table 1). Similarly, salinity (200 mM) caused a 47% and 45% increase in Na^+ and Cl^- accumulation respectively and 43% decrease in K^+ accumulation in plumule and radicles of germinating rice seeds (cv. BR16) (Table 1). Similar result was observed in var. Pokkali under 200 mM NaCl stress (Table 1).

Interaction of 100 mM NaCl with GA (10^{-6} M and 10^{-5} M) decreased Cl^- accumulation by 40 to 31% and increased K^+ accumulation by 10% as compared to control (100 mM NaCl) in radicles and plumules of germination seeds of cv.

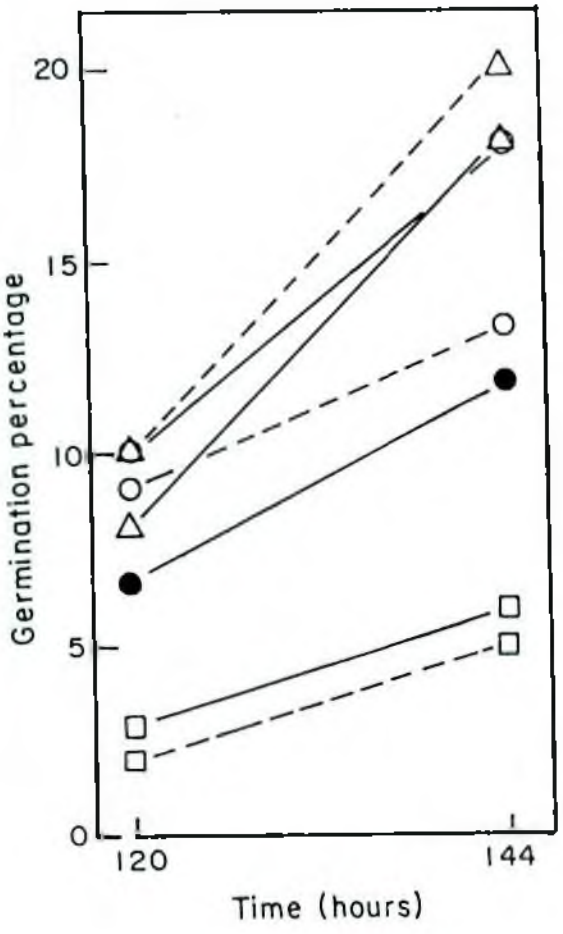

Fig. 11 Interaction of 300 mM NaCl salinity stress and gibberellic acid, kinetin on rate of germination of rice seed (cv. BR16) ● control (300 mM NaCl); △300 mM NaCl + GA; □300 mM NaCl + Kn; O 300 mM NaCl + GA + kinetin. Solid lines (—) represents 10^{-6} M, broken line (---) represents 10^{-5} M. Bars represent ± standard error.

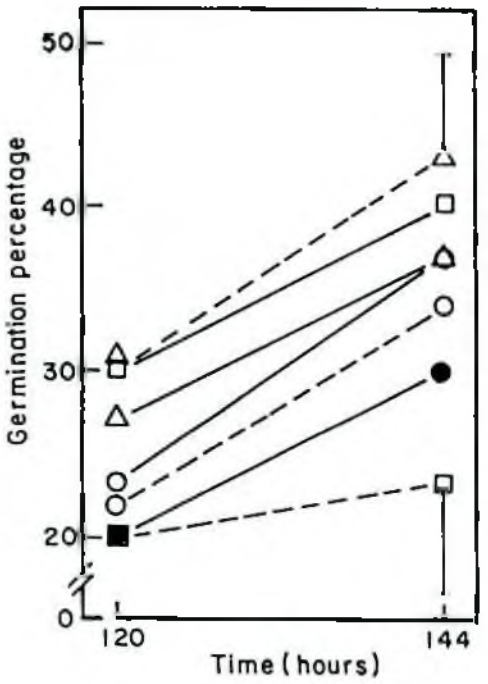

Fig. 12 Interaction of 300 mM NaCl salinity stress and gibberellic acid, kinetin on rate of germination of rice seed (var. Pokkali). Otherwise as Fig. 11.

BR16 (Table 2). Similarly, in var. Pokkali 100 mM NaCl + GA (10^{-6} M and 10^{-5} M) decreased Cl^- accumulation by 9% and increased K^+ accumulation by 6 to 14% as compared to control (100 mM NaCl) in radicles and plumules of germinating seeds (Table 3). In cv. BR16, 200 mM NaCl along with GA (10^{-6} M and 10^{-5} M) inhibited Cl^- accumulation by 36 to 17% and stimulated K^+ accumulation by 13% (Table 4). NaCl (100 mM and 200 mM) along with GA did not have significant effect on Na^+ accumulation in cv. BR16 of rice (Table 2 and 4). On the other hand, 200 mM NaCl along with 10^{-6} M and 10^{-5} M GA inhibited Na^+ and Cl^- accumulation by 15 to 28% and 21% respectively in var. Pokkali (Table 5). 200 mM NaCl with GA have no significant effect on K^+ accumulation in var. Pokkali of rice (Table 5).

Application of 10^{-6} M and 10^{-5} M kinetin along with 100 mM NaCl resulted in an increase in Na^+ and Cl^- accumulation by 71 to 33% and 50% respectively as compared to control (100 mM NaCl) in radicles and plumules of germinating seeds of rice (cv. BR16) (Table 2). 100 mM NaCl with kinetin (10^{-6} M and 10^{-5} M) had no significant effect on K^+ accumulation in cv. BR16 (Table 2). Similar results were found in var. Pokkali (Table 3). But, 100 mM NaCl with kinetin (10^{-6} M and 10^{-5} M) decreased K^+ accumulation by 18 to 6% in radicles and plumules of germinating seeds of var. Pokkali (Table 3). Similar effects were also observed when 200 mM NaCl along with kinetin (10^{-6} M and 10^{-5} M) were used (Table 4 and 5).

Accumulation of Cl^- was also decreased by 50 to 37% when 100 mM NaCl in combination with 10^{-6} M (GA + kinetin) and 10^{-5} M (GA + kinetin) were used (Table 2). Similarly, accumulation of Na^+ was decreased by 38.4 to 17%

Table 1. The effect of NaCl salinity stress on accumulation of K^+, Na^+ and Cl^- in plumule and radicle of germinating rice seeds (cv. BR16 and var. Pokkali). Each value is the mean of nine replicates ± standard error.

Treatments	cv. BR16				var. Pokkali			
	Ion accumulation (m-equiv. g^{-1} dry tissue)				Ion accumulation (m-equiv. g^{-1} dry tissue)			
	Na^+	K^+	Cl^-	Germination percentage	Na^+	K^+	Cl^-	Germination percentage
0.1 mM $CaSO_4$	0.154 ±0.026	0.802 ±0.105	0.515 ±0.083	91.000 ±0.000	0.611 ±0.139	0.513 ±0.050	0.656 ±0.107	60.000 ±5.210
0.1 mM $CaSO_4$ + 100 mM NaCl	1.620 ±0.668	0.717 ±0.162	1.820 ±0.547	89.000 ±1.500	1.352 ±0.069	0.456 ±0.044	0.640 ±0.085	44.300 ±4.440
0.1 mM $CaSO_4$ + 200 mM NaCl	2.279 ±0.469	0.455 ±0.063	1.483 ±0.343	50.300 ±4.850	1.312 ±0.225	0.402 ±0.044	0.762 ±0.121	20.000 ±6.67

Table 2. The effect of NaCl salinity stress and interaction of 100 mM salinity and gibberellic acid (GA) and kinetin (Kn) on accumulation of K^+, Na^+ and Cl^- in plumule and radicle of germinating rice seeds (cv. BR16). Otherwise as Table 1.

Treatments	Ion accumulation (m-equiv. g^{-1} dry tissue)								
	Plumule			Radicle			Total		
	Na^+	K^+	Cl^-	Na^+	K^+	Cl^-	Na^+	K^+	Cl^-
100 mM NaCl									
+ 0.1 mM $CaSO_4$	1.257 ±0.370	0.693 ±0.139	1.454 ±0.535	3.162 ±1.141	0.858 ±0.423	2.774 ±0.751	1.620 ±0.668	0.717 ±0.167	1.820 ±0.547
" + 10^{-6} M GA	1.212 ±0.792	0.729 ±0.886	0.794 ±0.095	2.191 ±1.002	0.844 ±0.155	1.844 ±0.377	1.666 ±0.573	0.786 ±0.074	1.098 ±0.127
" + 10^{-5} M GA	1.442 ±0.240	0.748 ±0.078	0.909 ±0.193	2.781 ±0.709	0.809 ±0.343	2.011 ±0.492	1.605 ±0.253	0.789 ±0.134	1.255 ±0.213
" + 10^{-6} M Kn	2.331 ±0.531	0.886 ±0.248	1.618 ±0.454	3.699 ±1.147	0.543 ±0.207	2.386 ±0.608	2.770 ±0.648	0.713 ±0.156	1.801 ±0.319
" + 10^{-5} M Kn	3.847 ±0.985	1.078 ±0.181	2.820 ±0.824	3.673 ±0.814	0.516 ±0.229	2.761 ±0.452	3.774 ±0.957	0.745 ±0.135	2.735 ±0.638
" + 10^{-6} M GA + Kn	0.896 ±0.129	0.716 ±0.065	0.721 ±0.131	2.068 ±0.599	0.813 ±0.095	1.535 ±0.321	0.998 ±0.268	0.790 ±0.049	0.908 ±0.139
" + 10^{-5} M GA + Kn	1.021 ±0.392	0.792 ±0.043	0.689 ±0.107	2.689 ±0.388	0.696 ±0.087	3.000 ±0.681	1.347 ±0.318	0.767 ±0.040	1.147 ±0.164

Table 3. The effect of NaCl salinity stress and interaction of 100 mM salinity and gibberellic acid (GA) and kinetin (Kn) on accumulation of K^+, Na^+ and Cl^- in plumule and radicle of germinating rice seeds (var. Pokkali). Otherwise as Table 1.

Treatments	Ion accumulation (m-equiv. g^{-1} dry tissue)								
	Plumule			Radicle			Total		
	Na^+	K^+	Cl^-	Na^+	K^+	Cl^-	Na^+	K^+	Cl^-
100 mM NaCl									
+ 0.1 mM $CaSO_4$	1.104 ±0.086	0.509 ±0.069	0.541 ±0.125	2.087 ±0.328	0.379 ±0.088	1.043 ±0.126	1.352 ±0.069	0.456 ±0 044	0.637 ±0.085
" + 10^{-6} M GA	0.921 ±0.073	0.501 ±0.064	0.501 ±0.108	2.255 ±0.130	0.320 ±0 048	1.433 ±0.382	1.310 ±0.063	0 482 ±0.048	0.580 ±0.148
" + 10^{-5} M GA	1.001 ±0.146	0.565 ±0.090	0.479 ±0.133	2.032 ±0.272	0.425 ±0.186	1.046 ±0.136	1.360 ±0.126	0.518 ±0.073	0.632 ±0.108
" + 10^{-6} M Kn	1.321 ±0.227	0.504 ±0.108	0.484 ±0.074	2.684 ±0.433	0.405 ±0.060	1.131 ±0.251	1.802 ±0.255	0.385 ±0.070	0.703 ±0.102
" + 10^{-5} M Kn	1.282 ±0.406	0.488 ±0.040	0.379 ±0.072	4.530 ±2.479	0.286 ±0.081	1.331 ±0.354	1.970 ±0.711	0.429 ±0.024	0.618 ±0.111
" + 10^{-6} M GA + Kn	1.400 ±0.489	0.501 ±0.028	0.388 ±0.068	3.042 ±1.616	0 410 ±0.037	1.041 ±0.193	1.275 ±0.513	0.471 ±0.027	0.548 ±0.067
" + 10^{-5} M GA + Kn	1.005 ±0.557	0 432 ±0.033	0 535 ±0.042	1.978 ±0.581	0.194 ±0.044	1.040 ±0.206	1.140 ±0.504	0.465 ±0.025	0.570 ±0.034

Table 4. The effect of NaCl salinity stress and interaction of 200 mM salinity and gibberellic acid (GA) and kinetin (Kn) on accumulation of K^+, Na^+ and Cl^- in plumule and radicle of germinating rice seeds (cv. BR16). Otherwise as Table 1.

Treatments	Ion accumulation (m-equiv. g^{-1} dry tissue)								
	Plumule			Radicle			Total		
	Na^+	K^+	Cl^-	Na^+	K^+	Cl^-	Na^+	K^+	Cl^-
200 mM NaCl + 0.1 mM $CaSO_4$	2.091 ±0.447	0.512 ±0.092	1.089 ±0.312	2.925 ±0.823	0.356 ±0.106	2.875 ±0.756	2.279 ±0.469	0.455 ±0.063	1.483 ±0.343
" + 10^{-6} M GA	1.721 ±0.475	0.512 ±0.087	0.666 ±0.095	2.611 ±1.036	1.054 ±0.721	1.695 ±0.261	2.177 ±0.566	0.513 ±0.094	0.952 ±0.127
" + 10^{-5} M GA	1.661 ±0.094	0.502 ±0.071	0.834 ±0.145	6.537 ±1.932	0.652 ±0.232	5.193 ±1.118	2.192 ±0.146	0.514 ±0.072	1.227 ±0.127
" + 10^{-6} M Kn	2.486 ±0.539	0.413 ±0.187	1.078 ±0.297	3.925 ±1.043	0.483 ±0.135	5.325 ±1.057	2.710 ±0.578	0.444 ±0.131	1.685 ±0.352
" + 10^{-5} M Kn	2.427 ±0.334	0.359 ±0.086	0.755 ±0.089	5.784 ±1.146	0.676 ±0.216	3.615 ±0.399	3.123 ±0.479	0.384 ±0.070	1.511 ±0.191
" + 10^{-6} M GA + Kn	1.178 ±0.146	0.537 ±0.072	0.874 ±0.231	2.945 ±0.592	0.421 ±0.102	4.043 ±1.206	1.277 ±0.231	0.461 ±0.101	1.304 ±0.246
" + 10^{-5} M GA + Kn	1.441 ±0.268	0.502 ±0.439	0.653 ±0.128	2.165 ±1.020	0.321 ±0.091	2.740 ±0.845	1.551 ±0.228	0.431 ±0.038	0.969 ±0.132

Table 5. The effect of NaCl salinity stress and interaction of 2oo mM salinity and gibberellic acid (GA) and kinetin (Kn) on accumulation of K^+, Na^+ and Cl^- in plumule and radicle of germinating rice seeds (var. Pokkali) Otherwise as Table 1.

Treatments	Inn accumulation (m-equiv. g^{-1} dry tissue)								
	Plumule			Radicle			Tbtal		
	Na^+	K^+	Cl^-	Na^+	K^+	Cl^-	Na^+	K^+	Cl^-
200 mM NaCl + 0.1 mM $CaSO_4$	1.212 ±0.173	0.410 ±0.034	0 534 ±0.128	2.375 ±0.427	0.402 ±0.141	1.933 ±0.367	1.312 ±0.225	0.402 ±0.044	0.812 ±0.121
" + 10^{-6} M GA	0.921 ±0.149	0.409 ±0.020	0.394 ±0.032	2.086 ±0.219	0.272 ±0.039	1.972 ±0.283	1.115 ±0.104	0.403 ±0.014	0.639 ±0 059
" + 10^{-5} M GA	0.800 ±0.141	0.396 ±0.054	0.589 ±0.203	1.489 ±0.421	0.313 ±0.071	1.759 ±0.406	0.951 ±0.169	0.402 ±0.033	0.818 ±0.264
" + 10^{-6} M Kn	1.185 ±0.349	0.400 ±0.066	0.532 ±0.142	2.367 ±0.837	0.414 ±0.094	1.300 ±0.406	1.527 ±0.451	0.387 ±0.362	0.800 ±0.201
" + 10^{-5} M Kn	2.196 ±0.701	0.479 ±0.083	1.555 ±0.368	4.831 ±2.109	0.491 ±0.244	3.944 ±0.520	2.364 ±0.529	0.370 ±0.082	2.121 ±0.418
" + 10^{-6} M GA + Kn	1.236 ±0.386	0.375 ±0.112	0.726 ±0.116	2.282 ±0.703	0.413 ±0.072	3.408 ±0.818	1.234 ±0.375	0.424 ±0.075	0.751 ±0.186
" + 10^{-5} M GA + Kn	1.367 ±0.378	0.582 ±0.127	0.794 ±0.165	2.414 ±1.275	0.725 ±0.583	3.271 ±0.584	1.008 ±0 413	0.549 ±0.095	0.713 ±0.176

respectively when 10^{-6} M (GA + kinetin) and 10^{-5} M (GA + kinetin) and 100 mM NaCl were used together in cv. BR16 (Table 2). In radicles and plumules of germinating seeds of cv. BR16, K^+ was increased by 10 to 7% respectively when 10^{-6} M (GA + kinetin) and 10^{-5} M (GA + kinetin) and 100 mM NaCl was used as compared to control (Table 2). Similar effects were also observed in var. Pokkali (Table 3). But 100 mM NaCl with 10^{-6} M (GA + kinetin) and 10^{-5} M (GA + kinetin) have no significant effect on K^+ accumulation in var. Pokkali (Table 3). 200 mM NaCl along with 10^{-6} M (GA + kinetin) and 10^{-5} M (GA + kinetin) decreased Na^+ accumulation by 44 to 32% and Cl^- accumulation 12 to 35% respectively (Table 4). K^+ accumulation was decreased by 5% when 10^{-5} M GA, kinetin and 200 mM NaCl were used together (Table 4). Similar effects were also observed in var. Pokkali in case of accumulation of Na^+ and Cl^- (Table 5). But K^+ accumulation was increased by 5.4 to 37% when seeds were treated with 200 mM NaCl along with 10^{-6} M (GA + kinetin) and 10^{-5} M (GA + kinetin) (Table 5).

3.4 Discussion

Fifty to 300 mM NaCl consistently decreased the rate of germination of rice cv. BR16 and 10 to 40 mM NaCl had no significant effect on germination (Fig. 6). In var. Pokkali, 10 to 300 mM NaCl consistently decreased the rate of germination (Fig. 6). This result was supported by Narele *et al.* (1969) who found that salinity at 8.9 mmhos m^{-1} decreased the rate of germination. It was also reported that salinity decreased the rate of germination in barley (Kabar 1987), chickpea (Muthukumarasamy *et al.* 1997), *Crotalaria striata*

(Chandrashekar and Sandhyarani 1995), *Cyanopsis tetragonoloba* (Ai-Yemeny and Basahy 1997), *Gossypium hirsutum* (Tort 1996) and in wheat (Babu and Kumar 1975, Begum *et al.* 1992).

Na^+ and Cl^- accumulation in plumule and radicles was increased with the increase in NaCl salinity with inhibition of germination in both the cultivar and variety of rice (Table 1). On the other hand, salinity stress decreased the amount of endogenous K^+ of plumule and radicle (Table 1). This suggests that in this case, the accumulation of excessive amount of Na^+ and Cl^- in germinating seed is responsible for inhibition of germination under salinity conditions and the shortage amount of K^+ in plumule and radicle which had an adverse effect on the growth of the embryo and thus further enhanced the inhibition of rate on germination (Begum *et al.* 1992).

Coccuci and Cocuccin (1977) found that abscisic acid (ABA) inhibited seed germination. It was also reported that salinity increased ABA level in tobacco (Mizrahi *et al.* 1970, 1971, 1972). Hence, it is suggested that salinity-induced inhibition of germination may be due to an increase in salinity-induced ABA level in germinating seeds. Accumulation of K^+ in plumules and radicles was decreased by salinity stress (Table 1) which may be due to an increase in K^+ efflux as a result of an increase in ABA level in salinity-stressed plant (Mizrahi *et al.* 1970, 1971, 1972). This view is supported by the work of Van Steveninck (1972) who showed that ABA altered K^+/Na^+ selectivity in beet root disc.

Interaction of salinity and GA increased the amount of K^+ and a decrease in Cl^- accumulation in plumule and radicle of rice (Table 2 and 3). Begum and

coworkers (1992) also observed that GA caused an increase in K^+ accumulation and decreased Cl^- accumulation in germinating seeds of wheat. This suggests that GA may cause activation of the uptake of extruded K^+ in place of Na^+ influx. This view is supported by the work of Ezekiel and coworkers (1978) who found that GA caused an increase in K^+ uptake in excised cucumber cotyledons. The alleviation of germination by GA in salinity condition (Fig. 7 to 12) may be due to GA-induced decrease in Cl^- and Na^+ accumulation in germinating seeds (Table 2 to 5).

Application of kinetin along with 100 to 300 mM NaCl had an inhibitory effect on germination in cv. BR16 (Fig. 7, 9 and 11). This result is in agreement with the findings of Babu and Kumar (1975) who found that at 3.5% soil salt concentration, kinetin decreased seed germination by 6.8%. However, at 3% soil salinity kinetin increased germination. Begum *et al.* (1992) also found that kinetin decreased the rate of germination under NaCl-salinity stress. On the other hand, in var. Pokkali 10^{-6} M kinetin along with NaCl (100-300 mM) increased the rate of germination (Fig. 8, 10 and 12). This may be due to varietal difference between BR16 and Pokkali.

Kinetin stimulated the uptake of Na^+ and Cl^- in germinating salt-treated seeds of cv. BR16 and var. Pokkali (Table 2 to 5). This result suggests that salinity-induced inhibition of germination may be due to the accumulation of excess amount of Na^+ and Cl^- in the developing young embryo of the seeds of cv. BR16 and var. Pokkali. On the contrary, kinetin inhibited the uptake of K^+ in germinating salt-treated seeds of var. Pokkali (Table 3 and 5). Excess Na^+ may cause an inhibition of K^+ uptake by the rice plants (Quayyum *et al.* 1991).

Interaction of NaCl salinity, GA and kinetin eliminated the inhibitory effect of salinity on germination of rice seeds of cv. BR16 and var. Pokkali (Fig. 7 to 12). Such an stimulatory effect of interaction of GA, kinetin and NaCl salinity on the percentage of germination was also found in wheat (Begum *et al.* 1992) and barley seed (Kabar 1987).

Interaction of GA and kinetin with NaCl stress decreased the accumulation of Na^+ and Cl^- but increased K^+ accumulation in radicle and plumule of rice seedlings in cv. BR16 and var. Pokkali (Table 2 to 5). NaCl salinity along with GA and kinetin decreased the accumulation of Cl^- in radicle and plumule in wheat (Begum *et al.* 1992).

It is suggested that salinity-induced inhibition of germination in both cultivar and variety of rice (BR16 and Pokkali) may be due to accumulation of Na^+ and Cl^- in the developing embryo with concomitant reduction of K^+ accumulation (Table 1). The partial nullification of inhibitory effect of salinity following GA treatment (Fig. 7 to 12) may be due to the decrease in accumulation of Na^+ and Cl^- in germinating seed along with the increase in uptake of extruded K^+ (Table 2 to 5).

CHAPTER 4

THE EFFECT OF SALINITY ON UPTAKE AND DISTRIBUTION OF Na^+, Cl^- and K^+ IN INTACT RICE SEEDLINGS IN DIFFERENT DEVELOPMENTAL STAGES

4.1 Introduction

Excess of salt in the external medium caused marked nutrient imbalances. For example, in NaCl salt-stressed plant *Melilotus segetalis*, the concentration of sodium in stem was seven times higher than in controls whereas salinity reduced the concentration of K^+ in root and shoot (Romero and Maranon 1996). Under short-term salinity stress, greater accumulation of Na^+ and Cl^- and a lower ratio of K^+/Na^+ in the root and shoot of two varieties of jute species were observed by Chaudhuri and Chaudhuri (1998).

Li-Wen and coworkers (1997) observed that there was significant accumulation of Na^+ in plant organs of *Atriplex prostata* when plants were grown in saline conditions where the concentration of K^+ has decreased. K^+ transport in salt-stressed barley (100 mM NaCl) was greatly reduced as compared to control (Wolf and Jeschke 1987).

Chow and coworkers (1990) found that lower salinity stimulated growth while higher salinity reduced growth and also K^+ uptake in the leaves of spinach was decreased. They suggested that higher K^+ content was required for shoot growth under higher salinity. On the other hand, higher concentration of Na^+ is

required to maintain turgor pressure under salinity, but it could not substitute K^+ requirement. Geetha and Rao (1997) reported that tolerant callus line of *Vigna mungo* maintained lower Na^+ and higher K^+ levels than in sensitive line.

Decreased Na^+ and Cl^- and increased K^+ were also observed by Wahid *et al.* (1997) in the salt-tolerant line of sugarcane. Sharma (1997) observed that plant growth declined with the increase in levels of salinity while concentration of Na^+ and Cl^- was increased and that of K^+ was decreased in chickpea. Similar results were also observed in wheat (Begum 1994, Astaraei and Chauhan 1994, Maliwal 1997), cluster bean (Garg *et al.* 1997), *Capsicum annum* (Gunes *et al.* 1996), *Ficus carica* (Okubo and Utsunomiya 1996) and *Tiglochin maritima* (Warwick and Bailey 1997).

In this chapter, the effect of salinity on uptake and distribution of Na^+, Cl^- and K^+ in intact rice seedlings grown in solution culture is reported. A correlation between growth and ion transport under salinity will be discussed.

4.2 Material and methods

Plants were grown in solution culture as described in 2.4.5. Plants were grown upto day 21 in solution culture to study ionic relation at different developmental stages. Samples were collected for analysis of Na^+, Cl^- and K^+ according to 2.5. Tissue was digested following the method described in 2.6.2. Na^+ and K^+ were measured by flame photometer and Cl^- were determined by titrimetric method.

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.

4.3 Results

4.3.1 The effect of salinity on accumulation and distribution of Na^+, Cl^- and K^+ in the root and shoot of 7-day-old intact rice seedlings

With the increase in salinity level from 25 to 100 mM NaCl, Na^+ accumulation was increased by 2.6- to 4.5-fold in the root and by 77 to 89% in the shoot of cv. BR16 of rice (Fig. 13a, b).

In var. Pokkali of rice, an increase in Na^+ accumulation in the root ranging from 2.2- to 12.7-fold was observed following NaCl (25 to 100 mM) treatment (Fig. 14a). A 10- to 32-fold stimulation of Na^+ was observed in the shoot of var. Pokkali having the same treatment (Fig. 14b).

Accumulation of Cl^- in the root of cv. BR16 was increased by 88 to 81% at 25 to 100 mM NaCl salinity (Fig. 13a). Accumulation of Cl^- in the shoot was increased by salinity treatment in general and it caused a maximum increase of 4.5-fold in cv. BR16 at 100 mM NaCl treatment (Fig. 13b).

In var. Pokkali, NaCl salinity (25 to 100 mM) caused an increase in Cl^- accumulation 20% to 2.8-fold in the root (Fig. 14a). It caused a maximum increase in Cl^- accumulation of 4-fold in the shoot of var. Pokkali at 75 mM NaCl treatment (Fig. 14b).

Accumulation of K^+ was decreased by 15 to 25% in the root of cv. BR16 (Fig. 13a) and 26 to 69% in the shoot of cv. BR16 following NaCl treatment (Fig. 13b).

Potassium content was decreased by 62 to 31% in the root of var. Pokkali (Fig. 14a) and 18 to 22% in the shoot of var. Pokkali (Fig. 14b) over control following salinity treatment.

4.3.2 The effect of salinity on accumulation and distribution of Na^+, Cl^- and K^+ in the root and shoot of 14-day-old intact rice seedlings

Sodium content was increased by a 6.4- to 8.0-fold and 2.7- to 6.3-fold in the root of cv. BR16 and var. Pokkali respectively following NaCl (25 to 100 mM) treatment (Fig. 15a and 16a). Accumulation of Na^+ in the shoot of cv. BR16 dramatically increased from 30- to 60-fold at the range of NaCl concentration used (Fig. 15b). In var. Pokkali, Na^+ accumulation was also increased from 12- to 18-fold at the same concentration (Fig. 16b).

In roots of cv. BR16, chloride content increased by 17 to 99% (Fig. 15a) at 25 to 100 mM NaCl treatment. Accumulation of Cl^- in the shoot of cv. BR16 increased from 24 to 54% at the range of NaCl concentration used (Fig. 15b).

In var. Pokkali, Cl^- content was increased from 32 to 27% in the root following NaCl (25 to 100 mM) treatments (Fig. 16a). In the shoot of var. Pokkali, accumulation of Cl^- was increased from 47 to 39% at the range of NaCl concentration used (Fig. 16b).

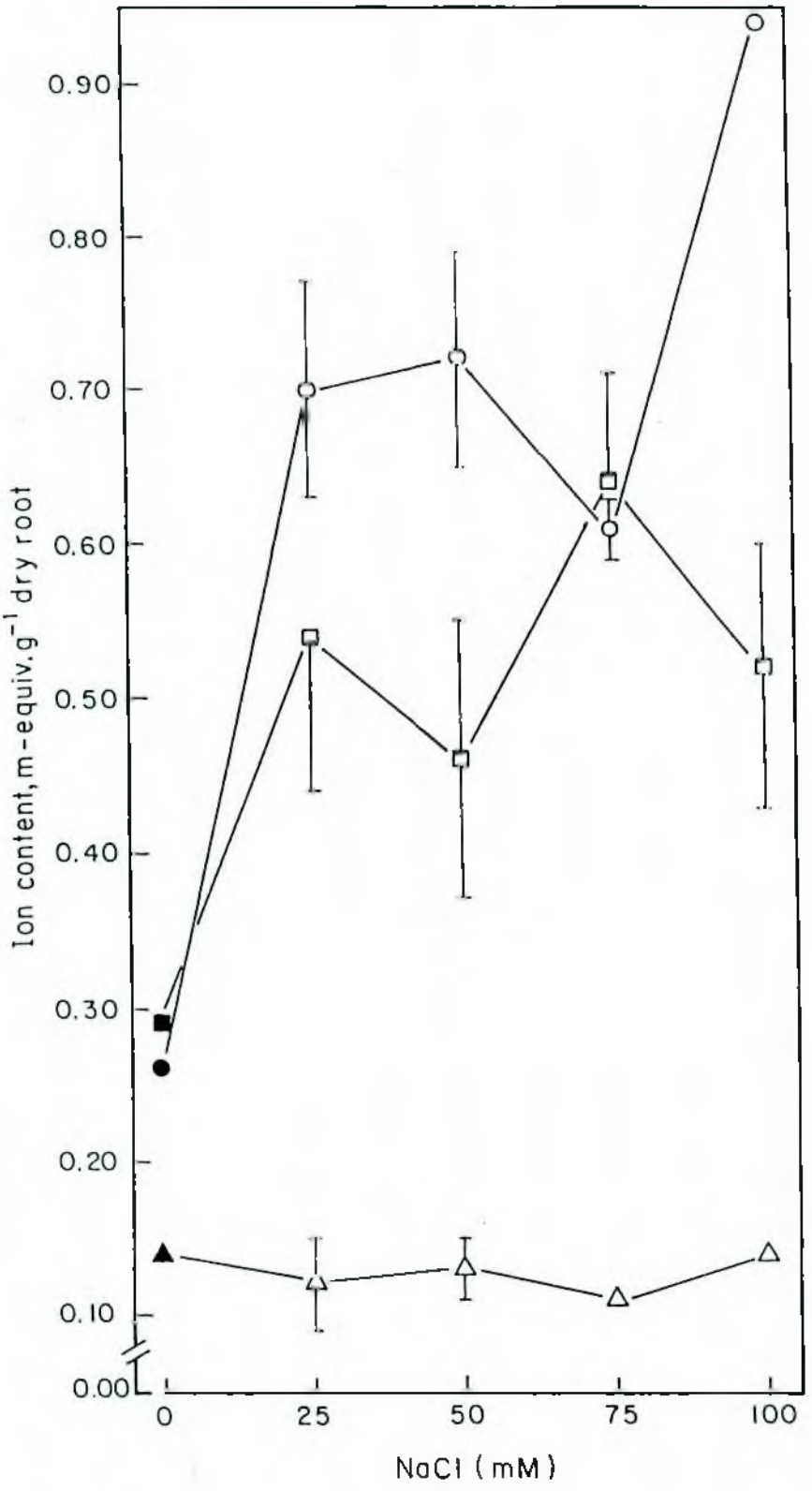

Fig. 13a The effect of salinity on accumulation of Na^+, Cl^- and K^+ in the root of 7-day-old intact seedlings of rice cv. BR16. O Na^+; □Cl^-; △K^+. Solid symbols represent control. Bars represent ± standard error. Each value is a mean of three replicates.

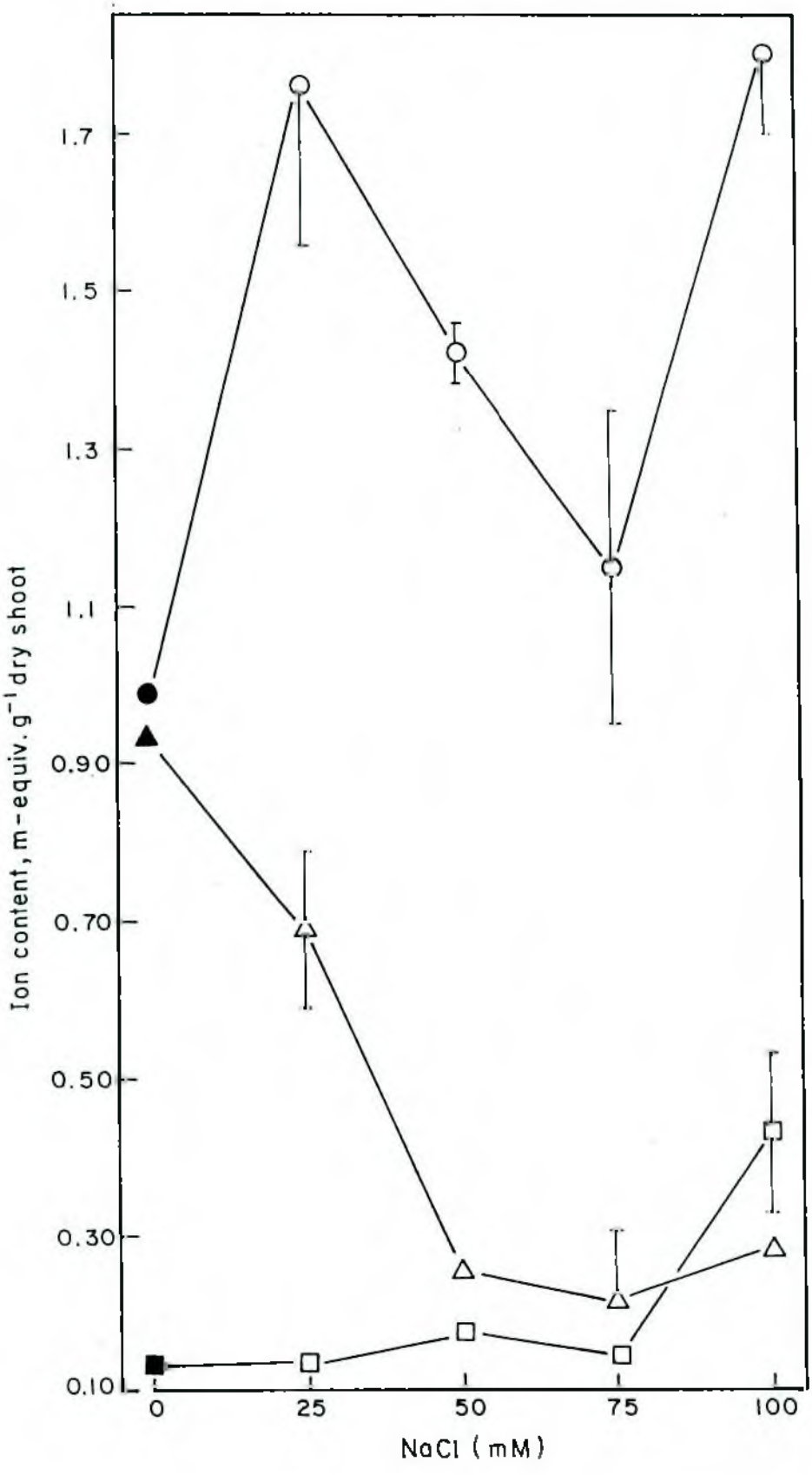

Fig. 13b The effect of salinity on accumulation of Na^+, Cl^- and K^+ in the shoot of 7-day-old intact seedlings of rice cv. BR16. Otherwise as Fig. 13a.

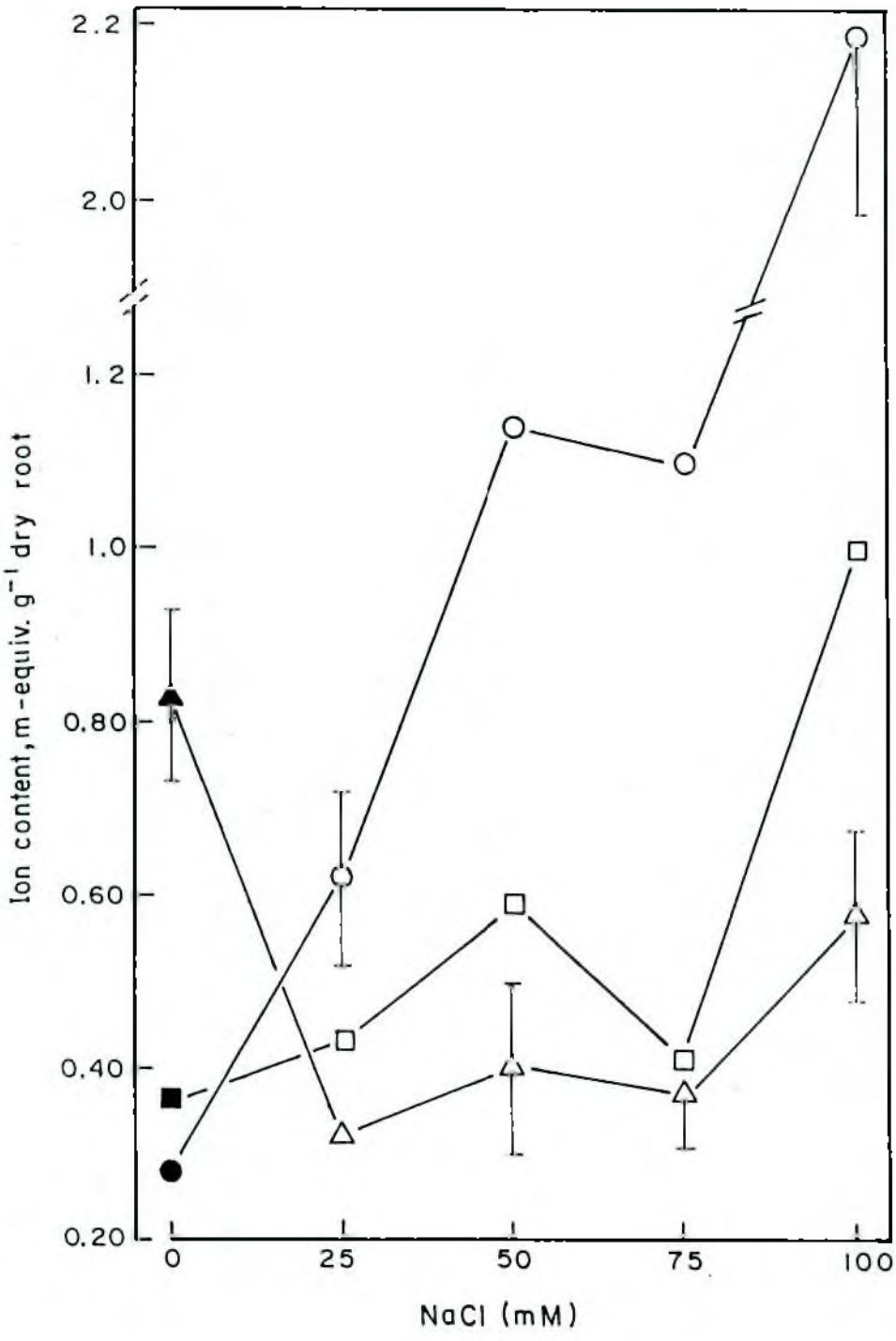

Fig. 14a The effect of salinity on accumulation of Na^+, Cl^- and K^+ in the root of 7-day-old intact seedlings of rice var. Pokkali. Otherwise as Fig. 13a.

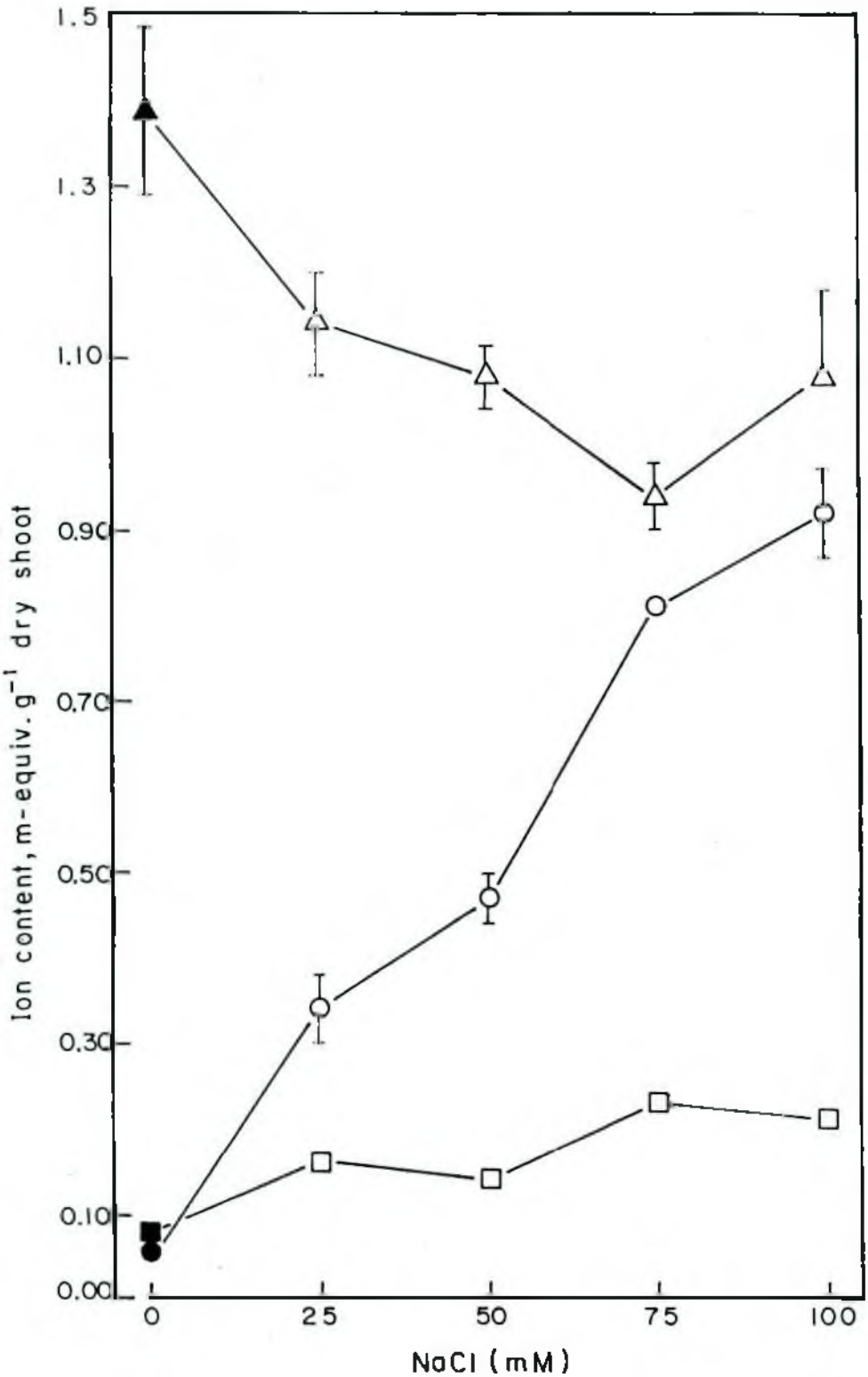

Fig. 14b The effect of salinity on accumulation of Na^+, Cl^- and K^+ in the shoot of 7-day-old intact seedlings of rice var. Pokkali. Otherwise as Fig. 13a.

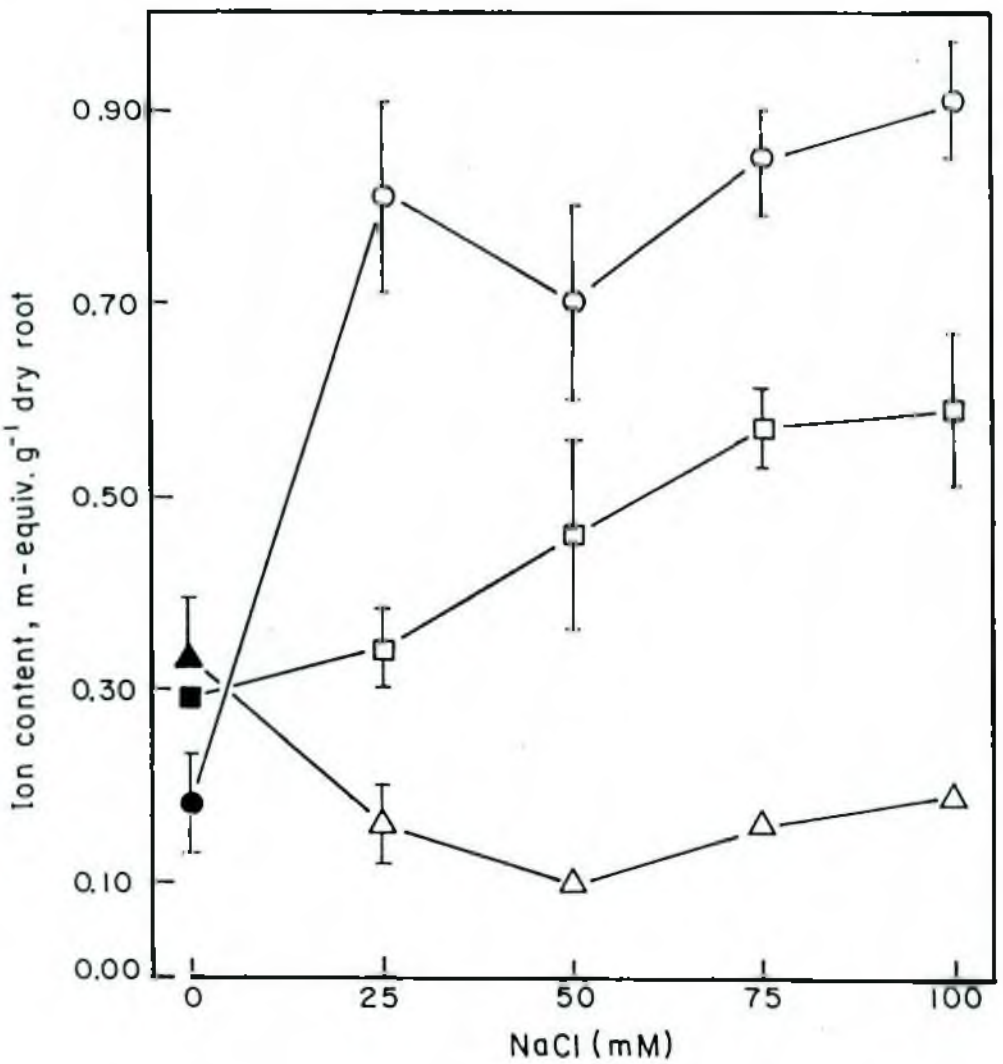

Fig. 15a The effect of salinity on accumulation of Na^+, Cl^- and K^+ in the root of 14-day-old intact seedlings of rice cv. BR16. Otherwise as Fig. 13a.

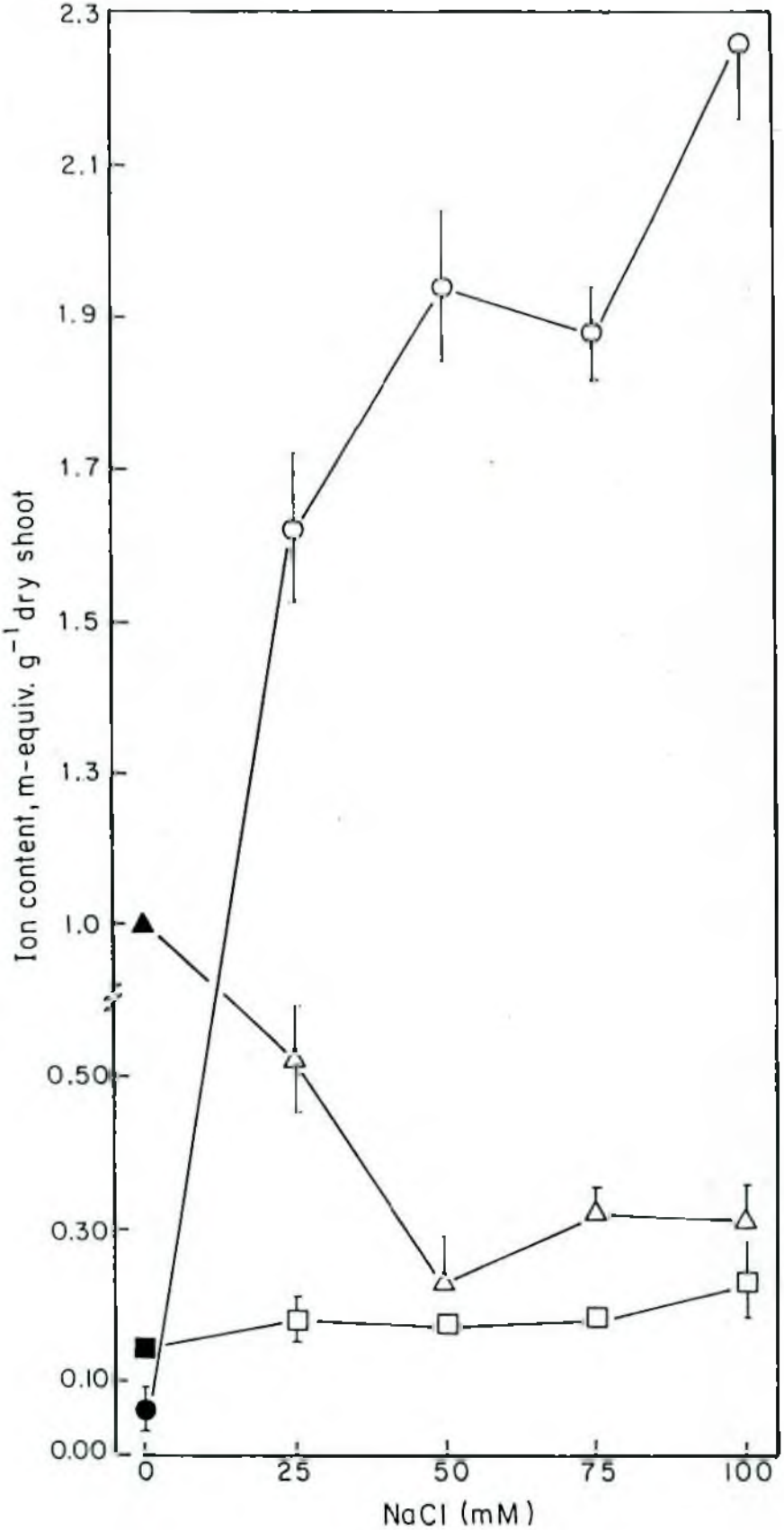

Fig. 15b The effect of salinity on accumulation of Na^+, Cl^- and K^+ in the shoot of 14-day-old intact seedlings of rice cv. BR16. Otherwise as Fig. 13a.

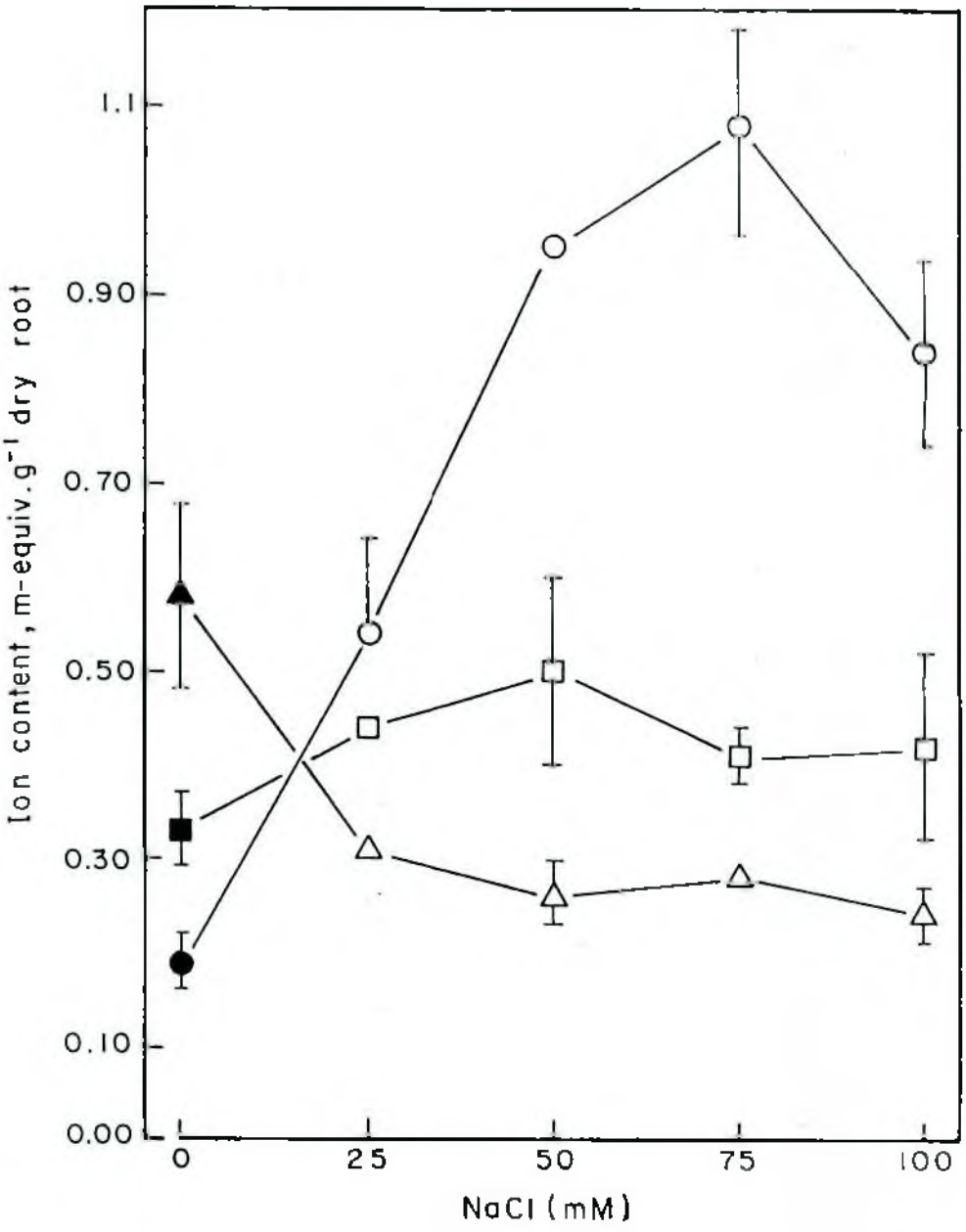

Fig. 16a The effect of salinity on accumulation of Na^+, Cl^- and K^+ in the root of 14-day-old intact seedlings of rice var. Pokkali. Otherwise as Fig. 13a.

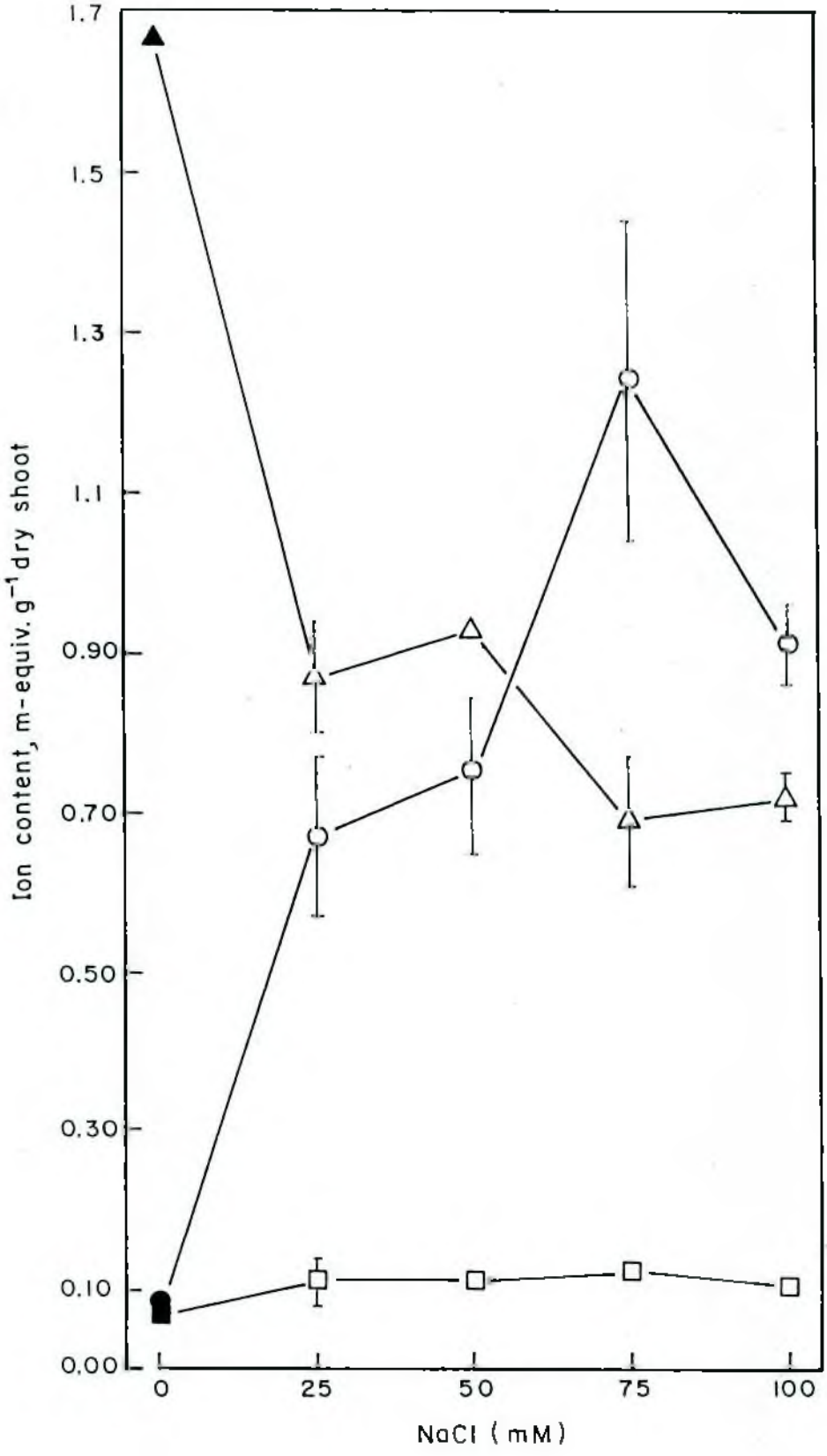

Fig. 16b The effect of salinity on accumulation of Na^+, Cl^- and K^+ in the shoot of 14-day-old intact seedlings of rice var. Pokkali. Otherwise as Fig. 13a.

Potassium content in the root of cv. BR16 and var. Pokkali was decreased by 53 to 40% and 46 to 59% respectively following NaCl (25 to 100 mM) treatment (Fig. 15a and 16a). K^+ content was decreased by 49 to 69% and 48 to 57% in the shoot of cv. BR16 and var. Pokkali respectively (Fig. 15b and 16b).

4.3.3 The effect of salinity on accumulation and distribution of Na^+, Cl^- and K^+ in the root and shoot of 21-day-old rice seedlings

Sodium chloride stress (25 to 100 mM) caused a 10- to 6-fold increase in accumulation of Na^+ in the root of cv. BR16 (Fig. 17a). Accumulation of Na^+ in the shoot of cv. BR16 dramatically increased from 2.6 to 40-fold at the range of NaCl concentration used (Fig. 17b).

Na^+ content of the root of var. Pokkali was increased by 8.4- to 16.0-fold as compared to control from 25 to 100 mM NaCl salinity (Fig. 18a). A 22- to 36-fold increase in Na^+ accumulation in the shoot of var. Pokkali was observed at the range of NaCl concentration used (Fig. 18b).

Chloride content was increased by 30 to 61% in the root of both cv. BR16 and var. Pokkali (Fig. 17a and 18a). Accumulation of Cl^- was increased by 21 to 64% and 24 to 5% in the shoot of both cv. BR16 and var. Pokkali respectively (Fig. 17b and 18b).

K^+ accumulation was decreased by 52 to 65% in roots of cv. BR16 at 25 to 100 mM NaCl salinity (Fig. 17a). In the shoot of cv. BR16, K^+ accumulation was decreased from 41 to 74% (Fig. 17b).

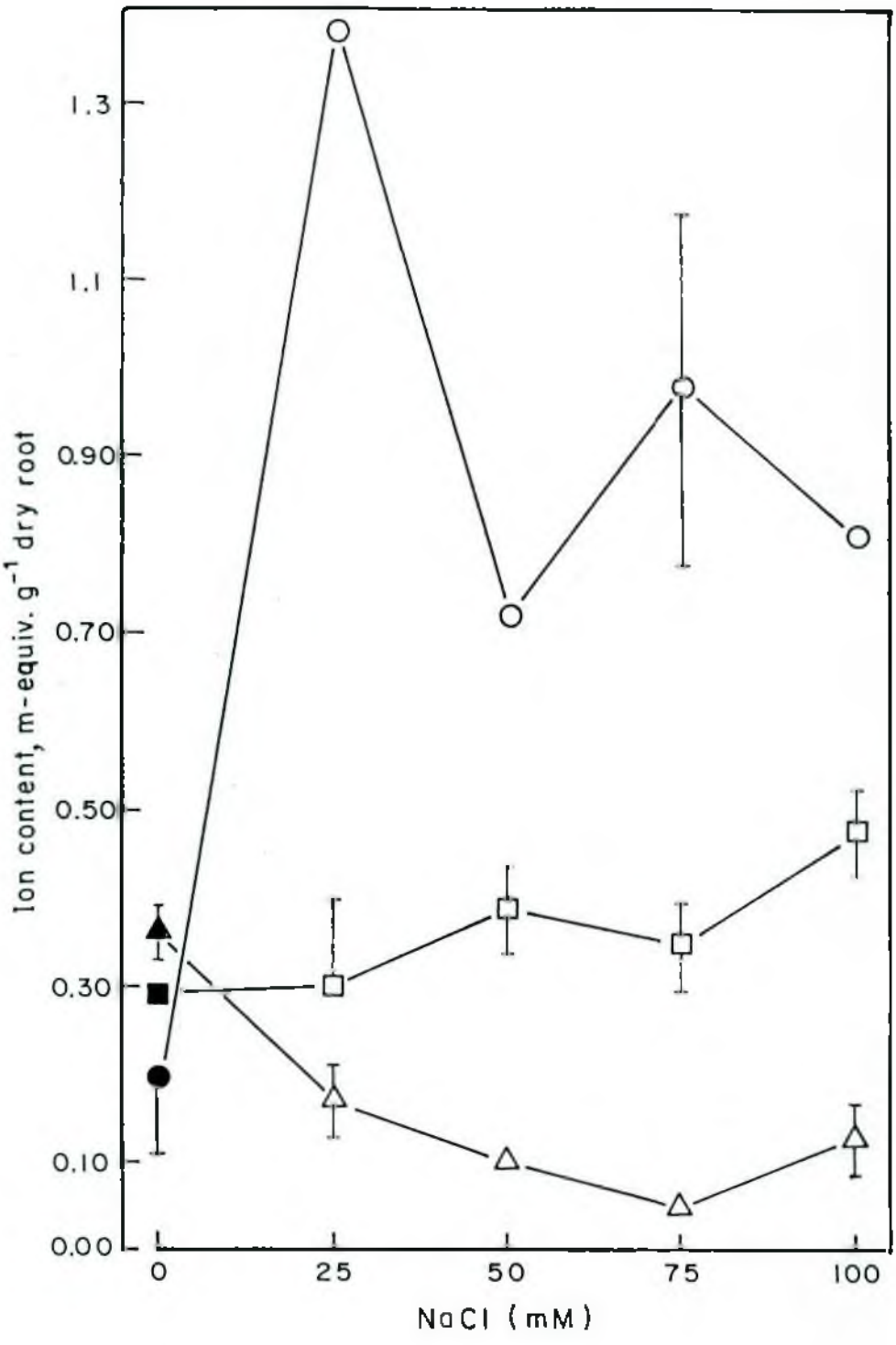

Fig. 17a The effect of salinity on accumulation of Na^+, Cl^- and K^+ in the root of 21-day-old intact seedlings of rice cv. BR16. Otherwise as Fig. 13a.

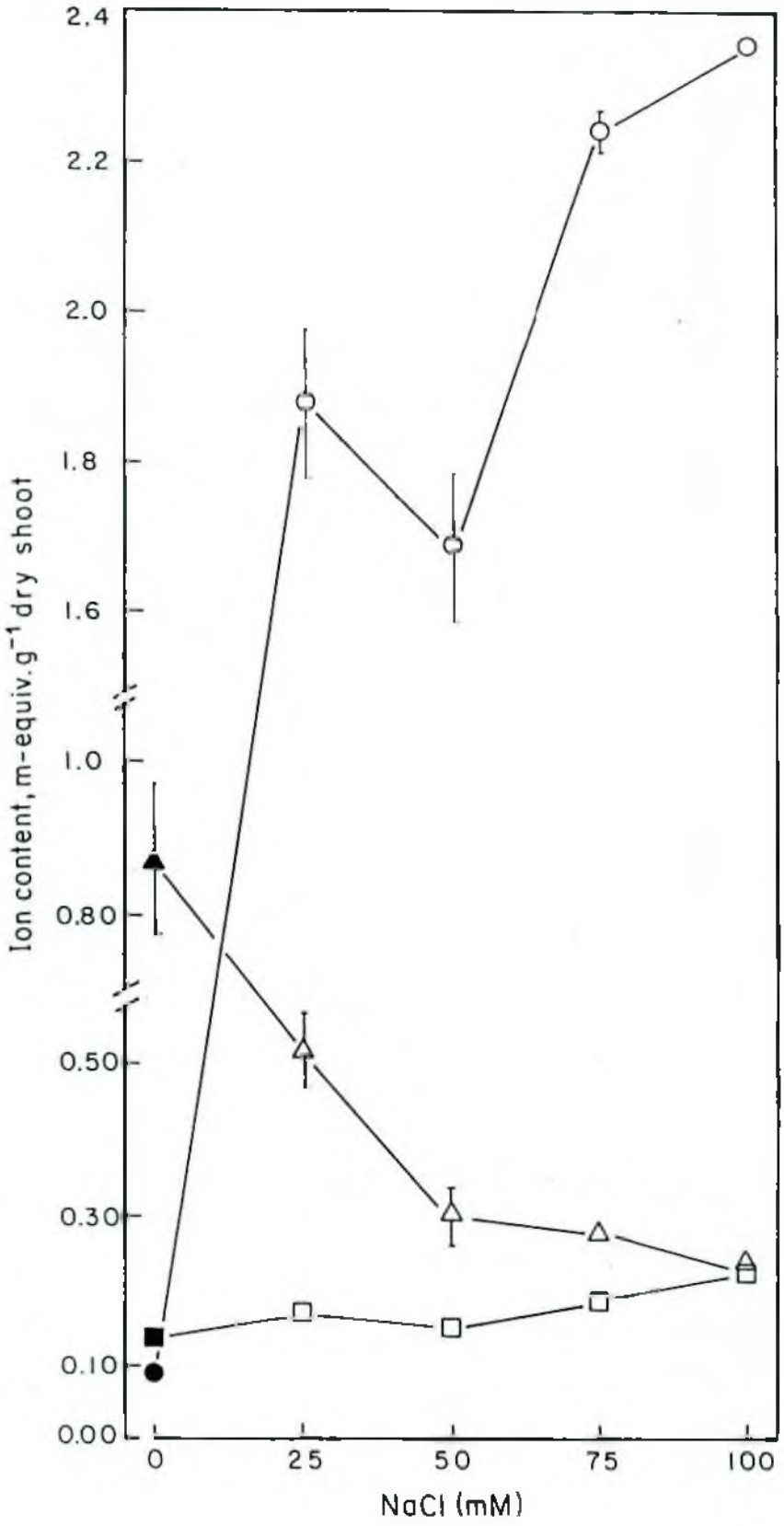

Fig. 17b The effect of salinity on accumulation of Na^+, Cl^- and K^+ in the shoot of 21-day-old intact seedlings of rice cv. BR16. Otherwise as Fig. 13a.

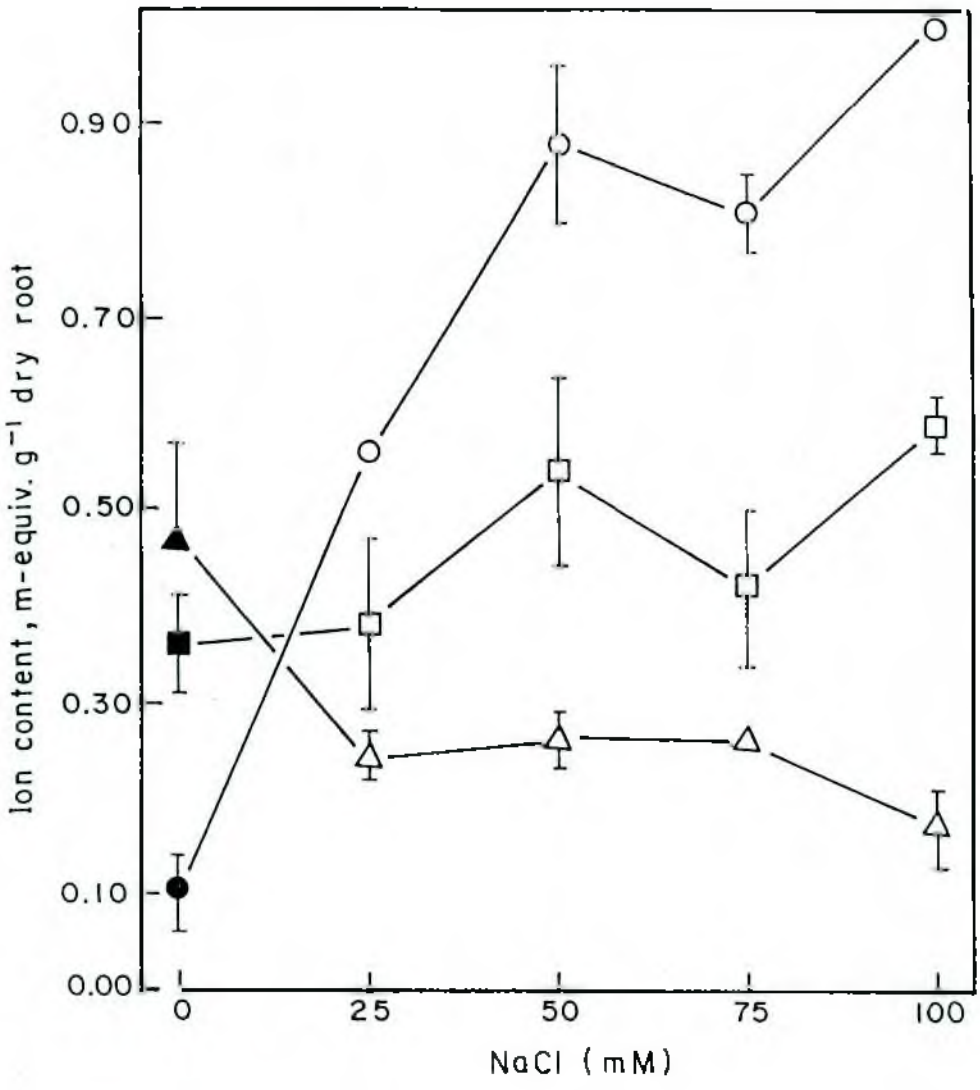

Fig. 18a The effect of salinity on accumulation of Na^+, Cl^- and K^+ in the root of 21-day-old intact seedlings of rice var. Pokkali. Otherwise as Fig. 13a.

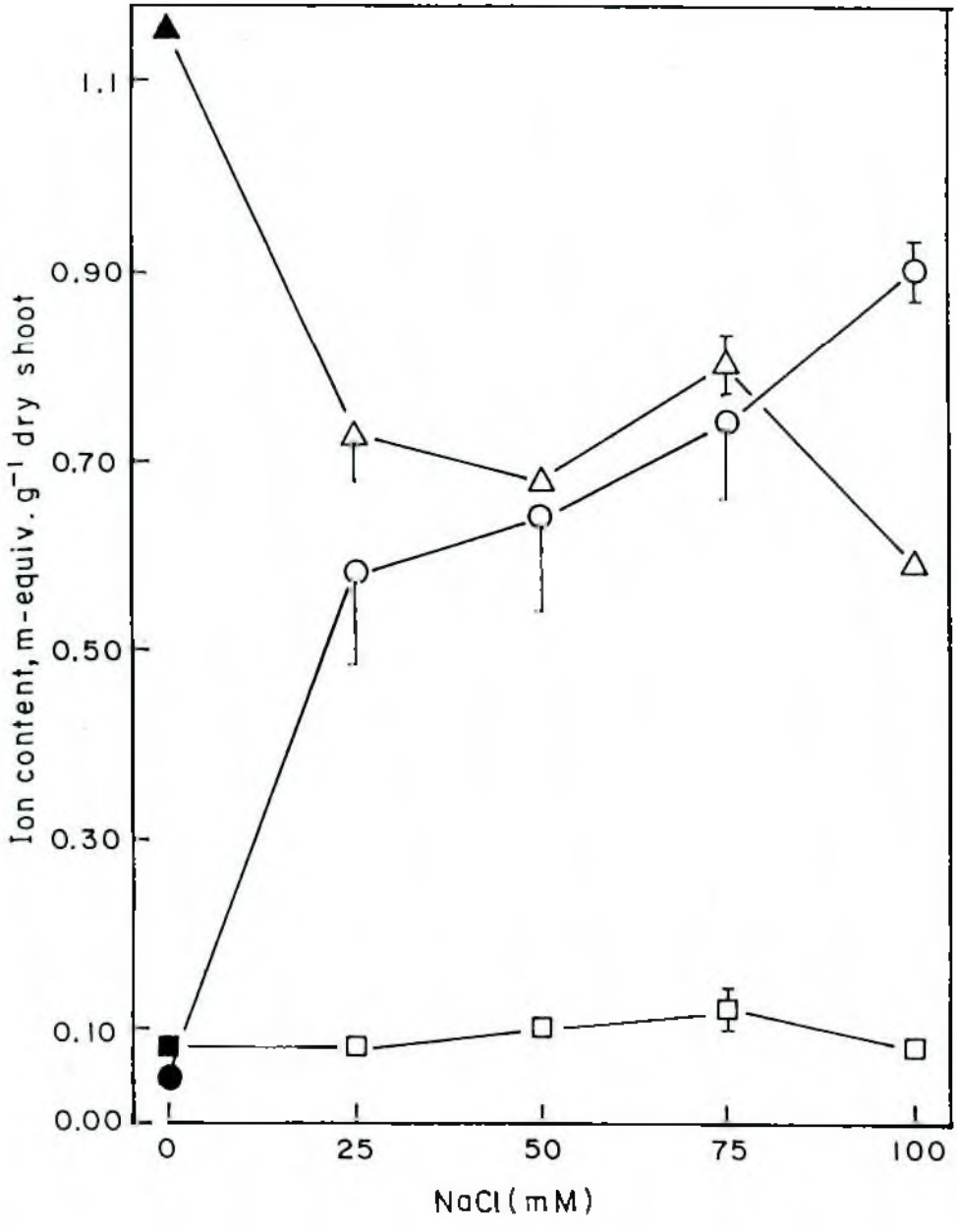

Fig. 18b The effect of salinity on accumulation of Na^+, Cl^- and K^+ in the shoot of 21-day-old intact seedlings of rice var. Pokkali. Otherwise as Fig. 13a.

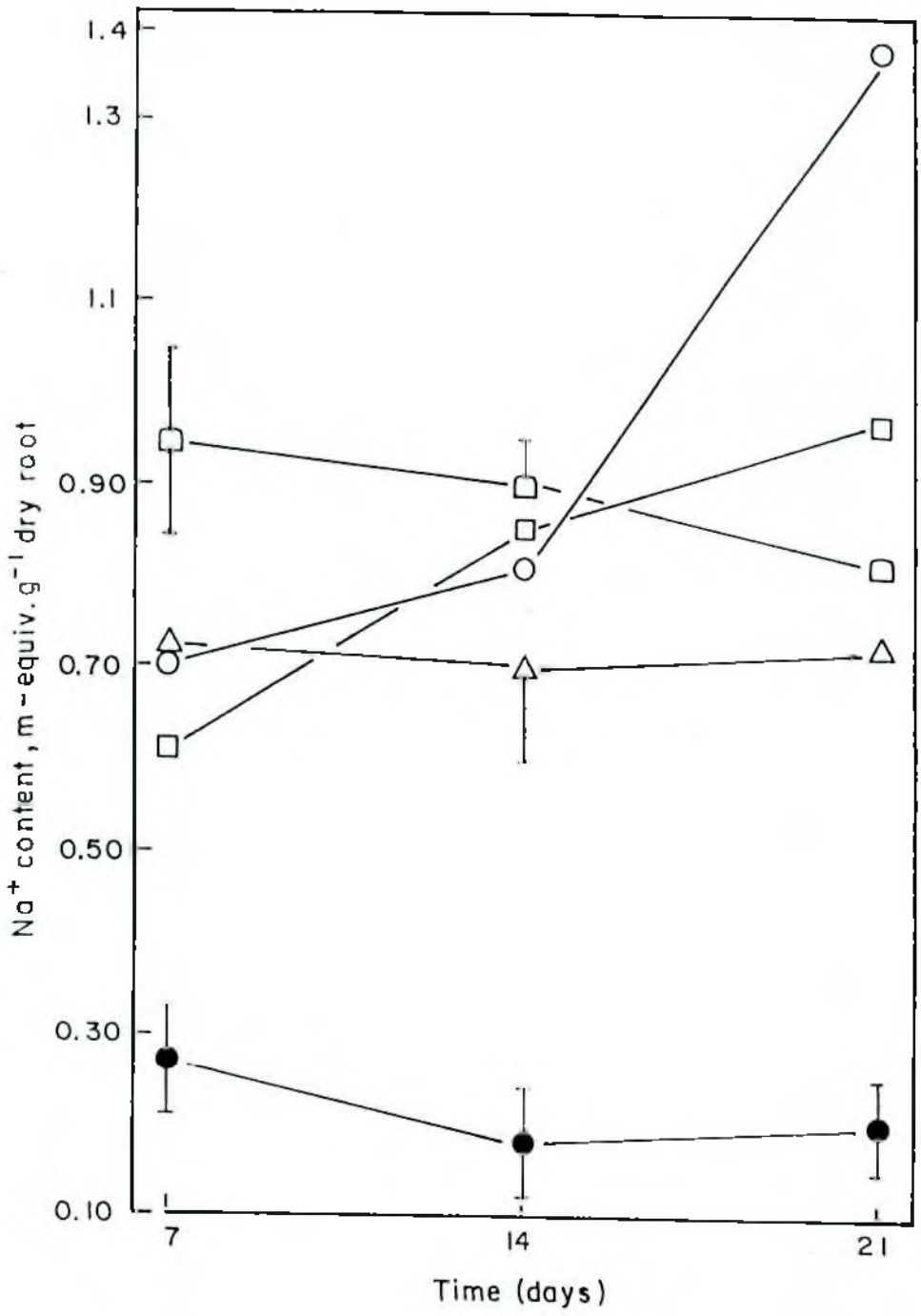

Fig. 19a The effect of salinity on accumulation of Na^+ in the root of rice cv. BR16 at different developmental stages. ● control; O 25 mM NaCl; △50 mM NaCl; □75 mM NaCl; □100 mM NaCl. Bars represent ± standard error. Each value is a mean of three replicates.

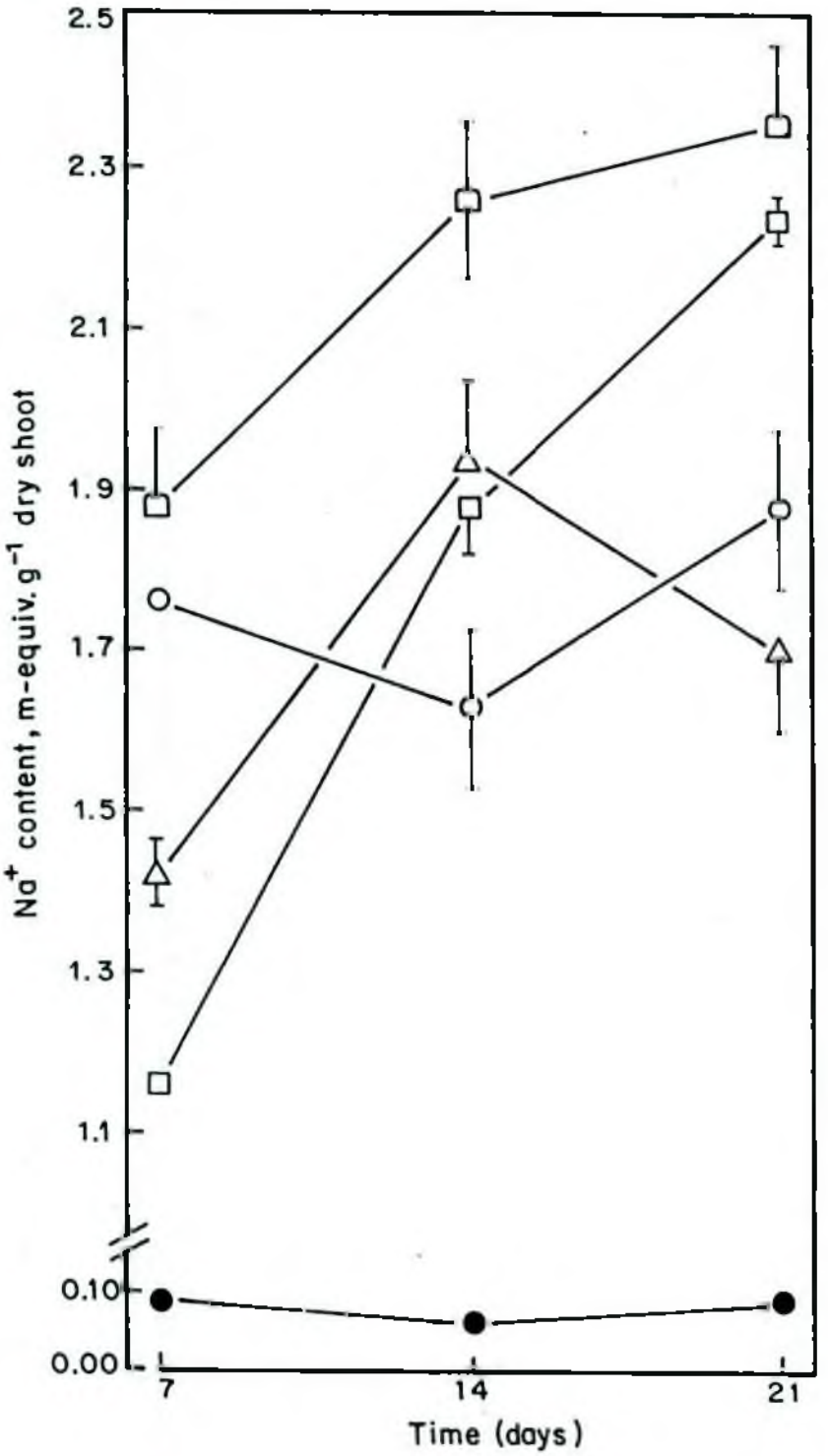

Fig. 19b The effect of salinity on accumulation of Na^+ in the shoot of rice cv. BR16 at different developmental stages. Otherwise as Fig. 19a.

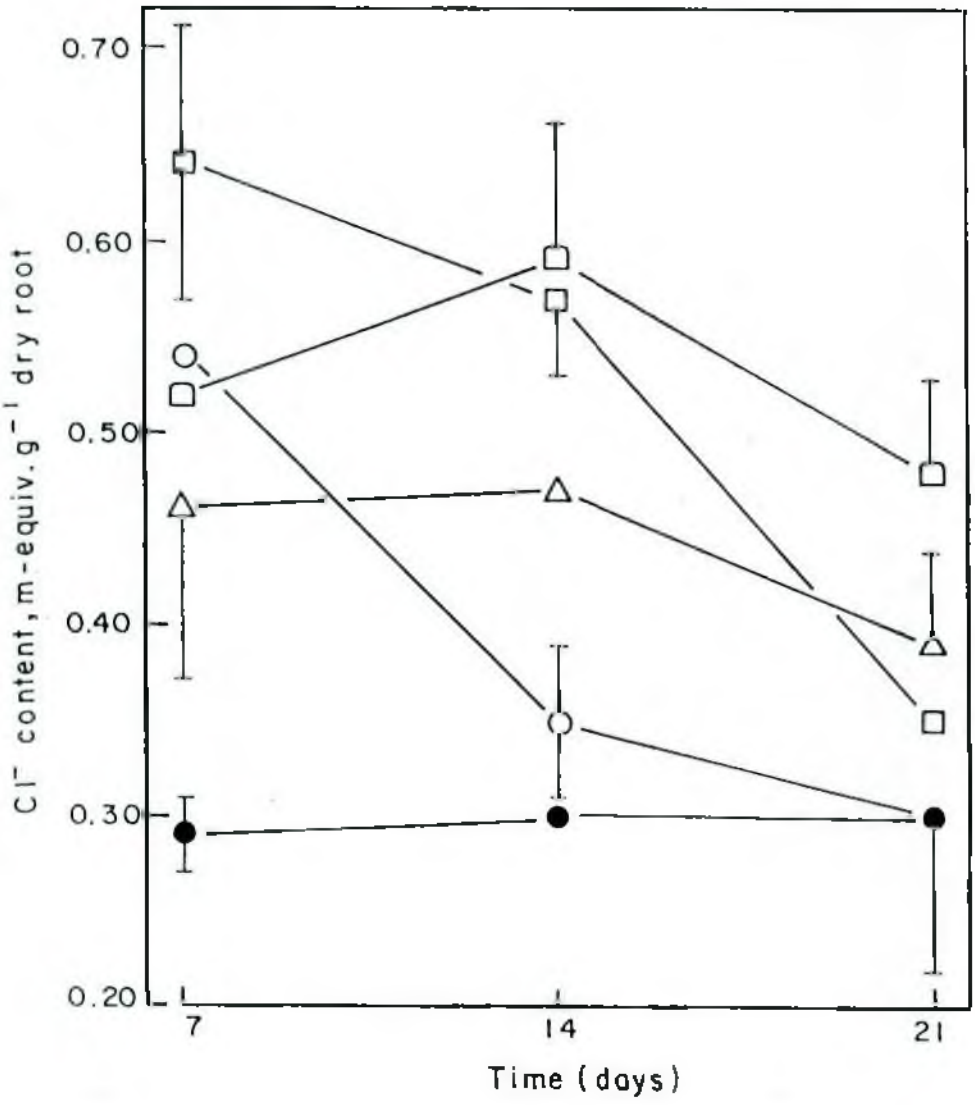

Fig. 20a The effect of salinity on accumulation of Cl^- in the root of rice cv. BR16 at different developmental stages. Otherwise Fig. 19a.

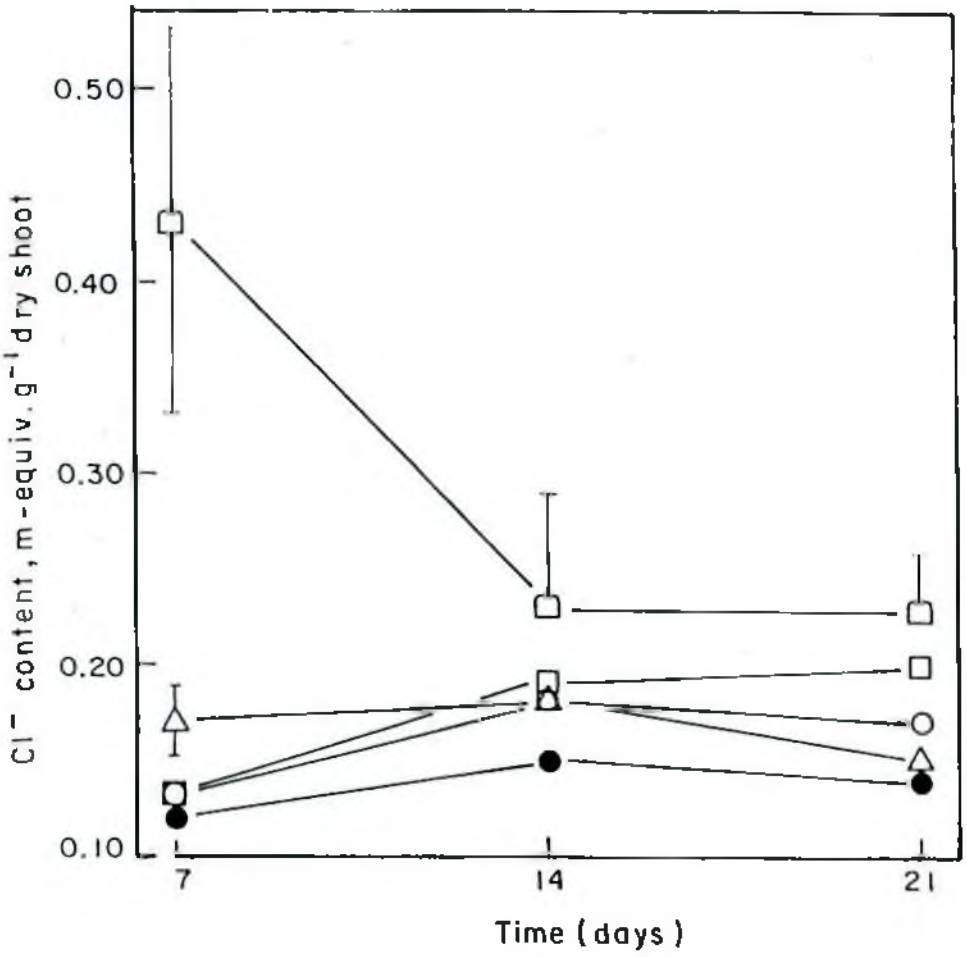

Fig. 20b The effect of salinity on accumulation of Cl^- in the shoot of rice cv. BR16 at different developmental stages. Otherwise as Fig. 19a.

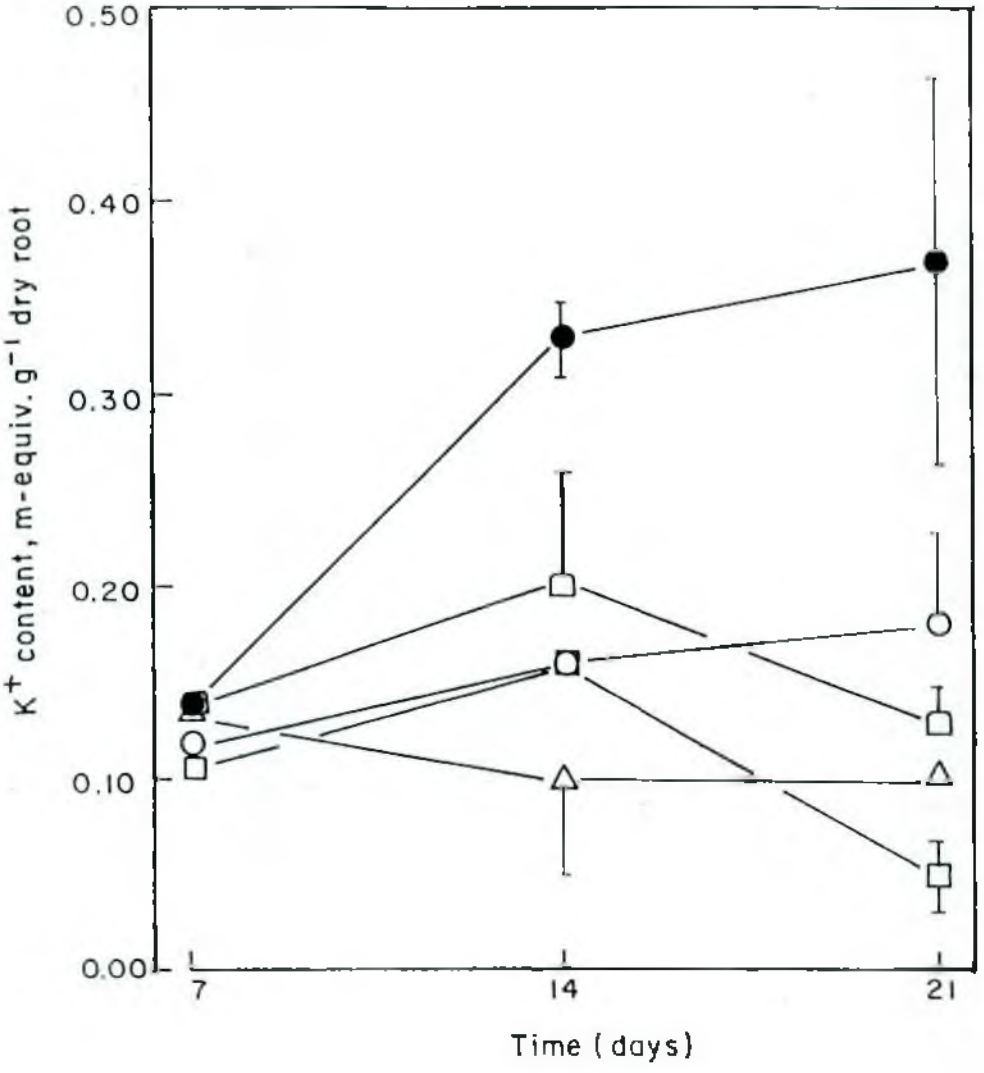

Fig. 21a The effect of salinity on accumulation of K^+ in the root of rice cv. BR16 at different developmental stages. Otherwise as Fig. 19a.

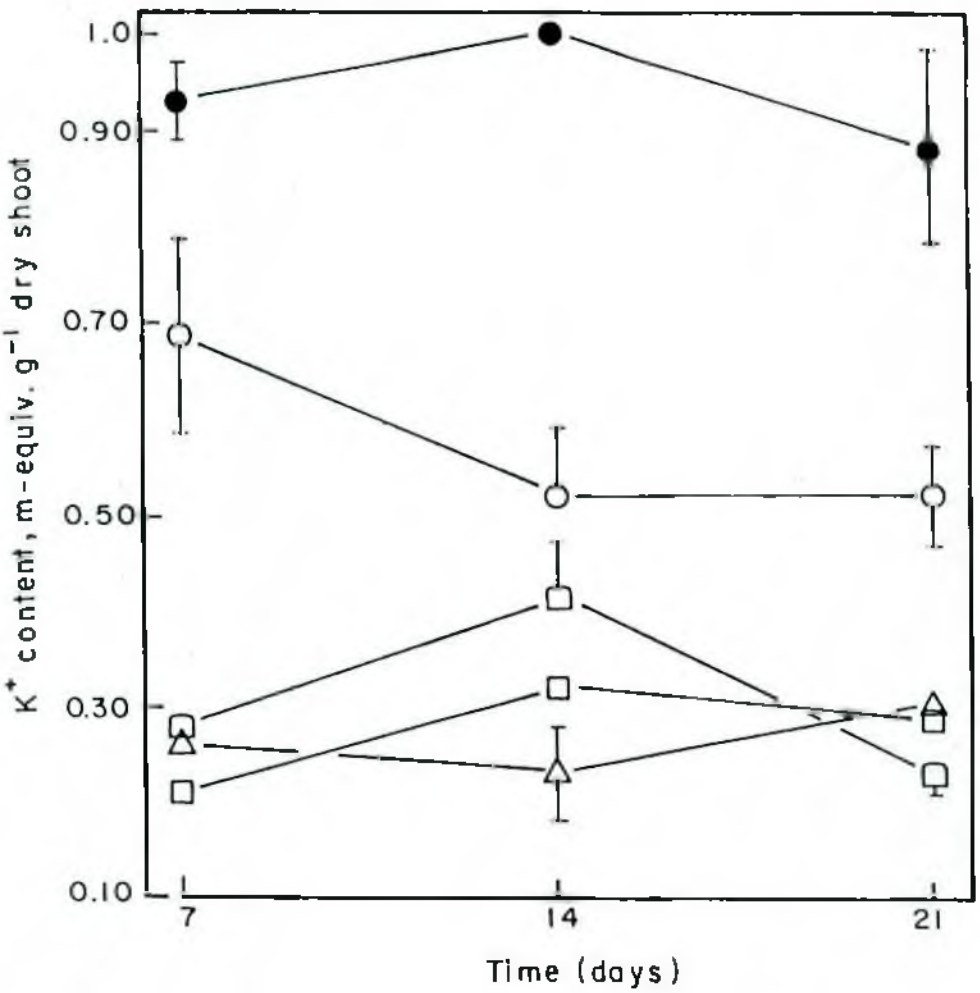

Fig. 21b The effect of salinity on accumulation of K^+ in the shoot of rice cv. BR16 at different developmental stages. Otherwise as Fig. 19a.

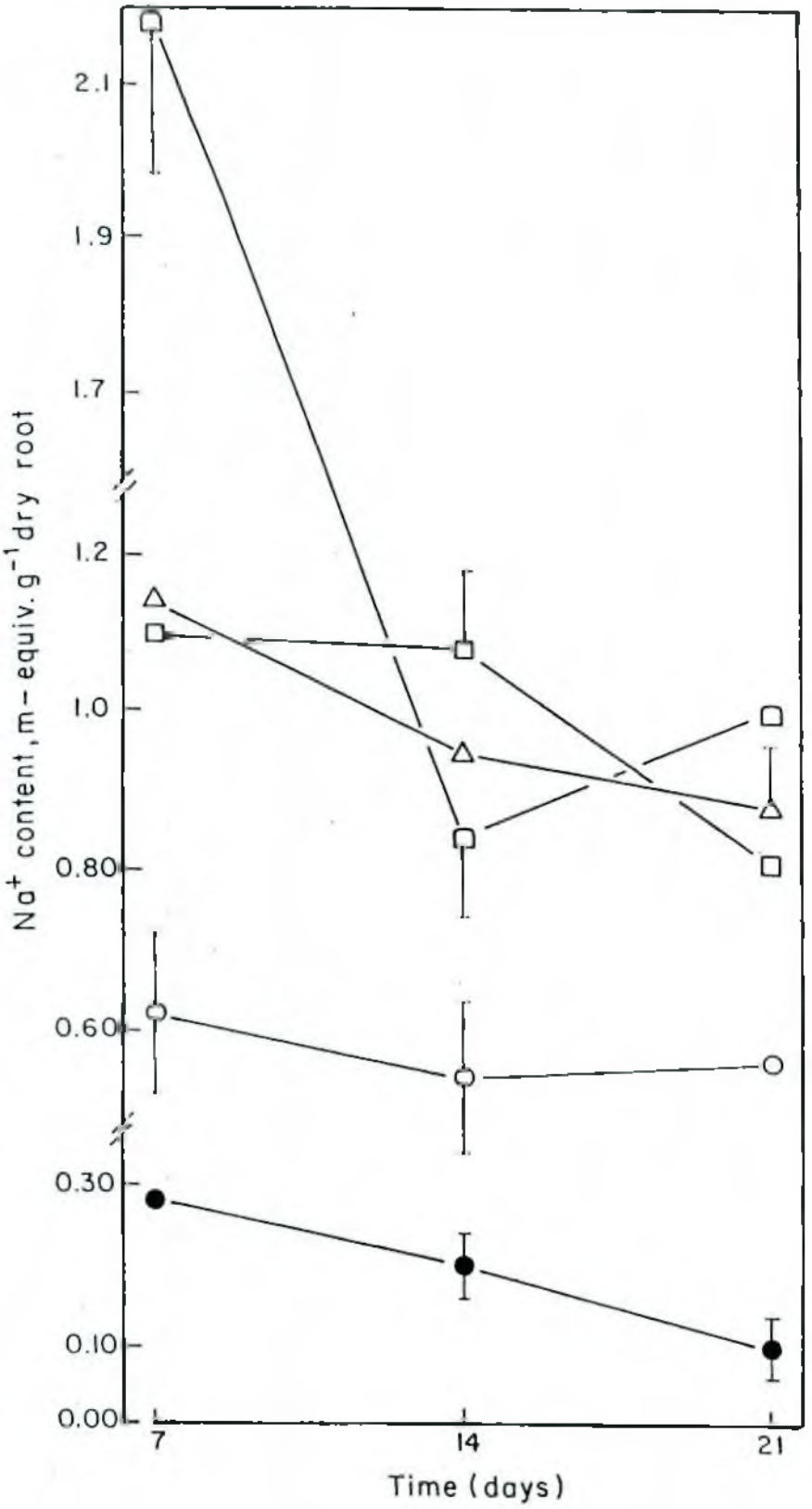

Fig. 22a The effect of salinity on accumulation of Na^+ in the root of rice var. Pokkali at different developmental stages. Otherwise as Fig. 19a.

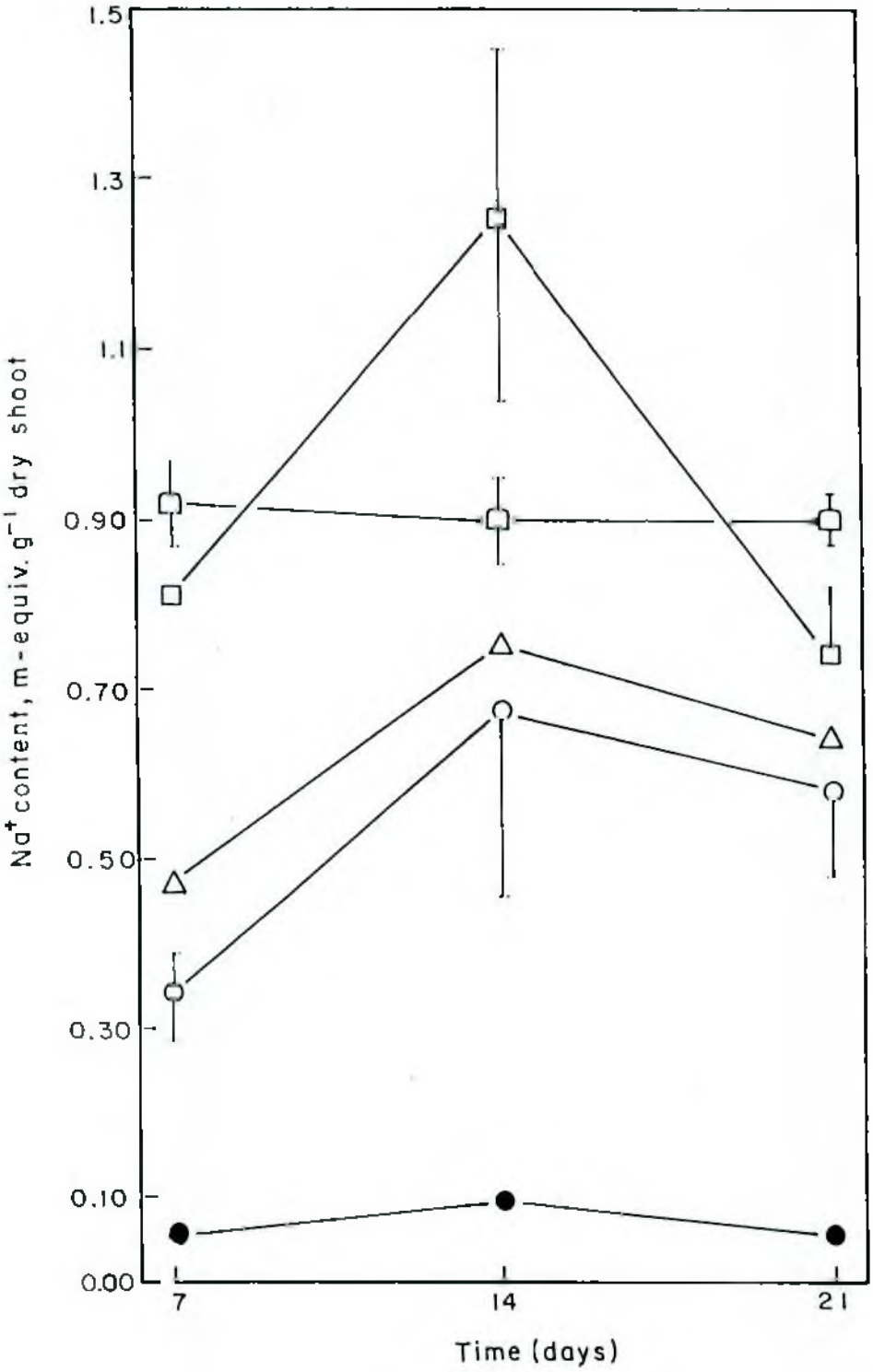

Fig. 22b The effect of salinity on accumulation of Na^+ in the shoot of rice var. Pokkali at different developmental stages. Otherwise as Fig. 19a.

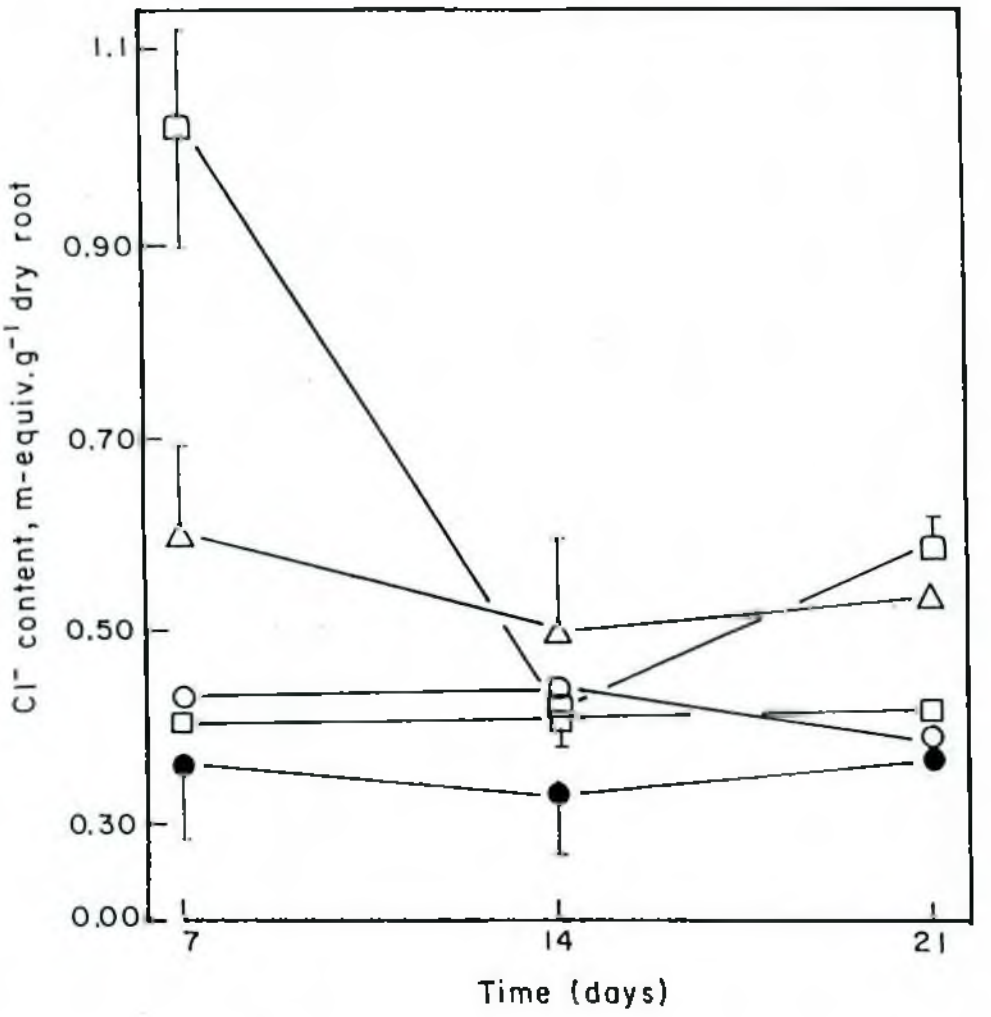

Fig. 23a The effect of salinity on accumulation of Cl^- in the root of rice var. Pokkali at different developmental stages. Otherwise Fig. 19a.

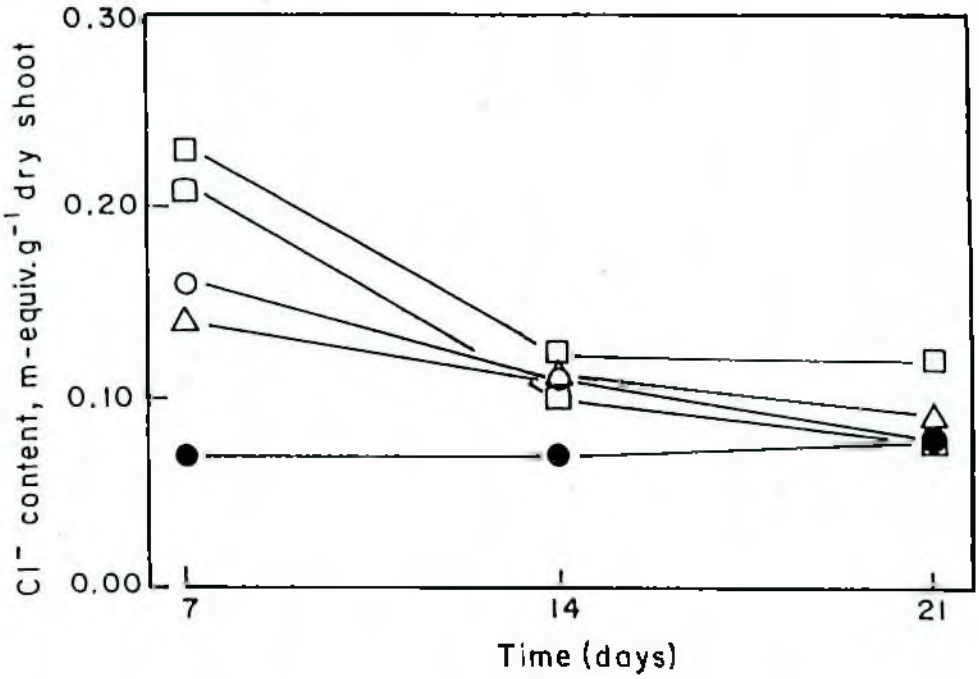

Fig. 23b The effect of salinity on accumulation of Cl^- in the shoot of rice var. Pokkali at different developmental stages. Otherwise as Fig. 19a.

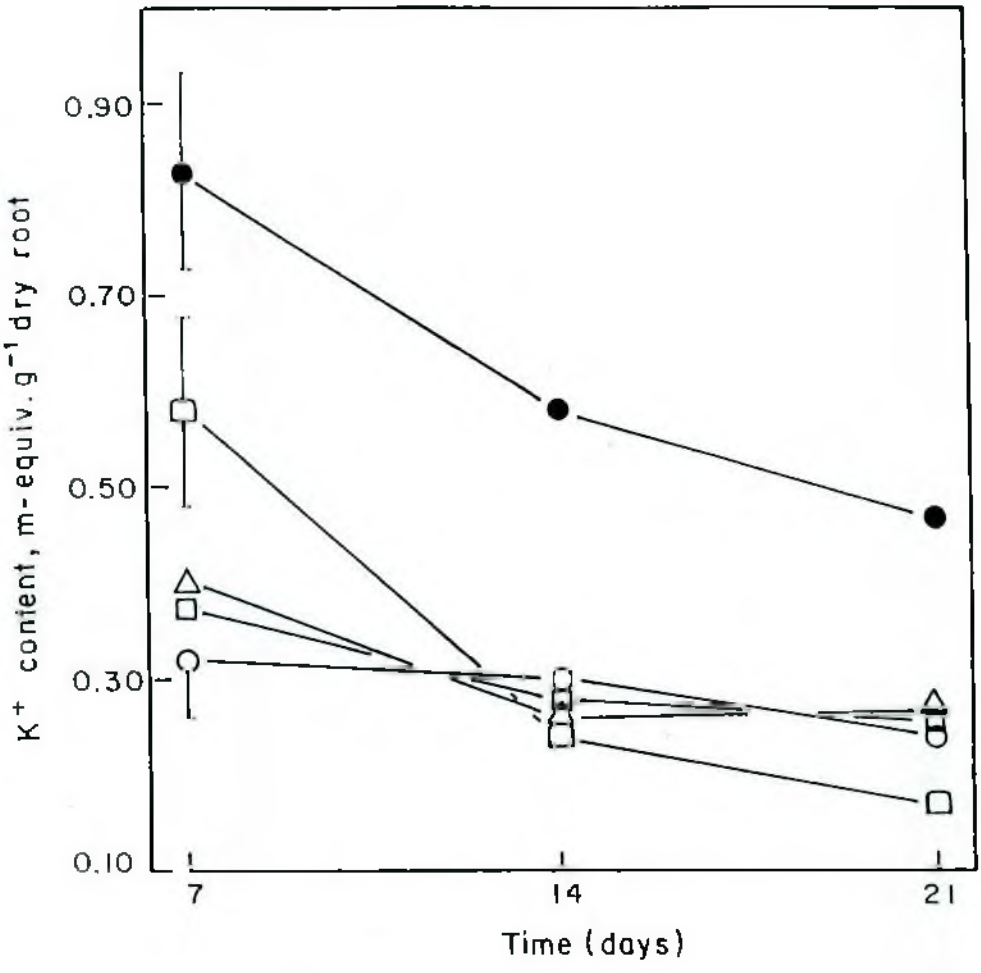

Fig. 24a The effect of salinity on accumulation of K^+ in the root of rice var. Pokkali at different developmental stages. Otherwise as Fig. 19a.

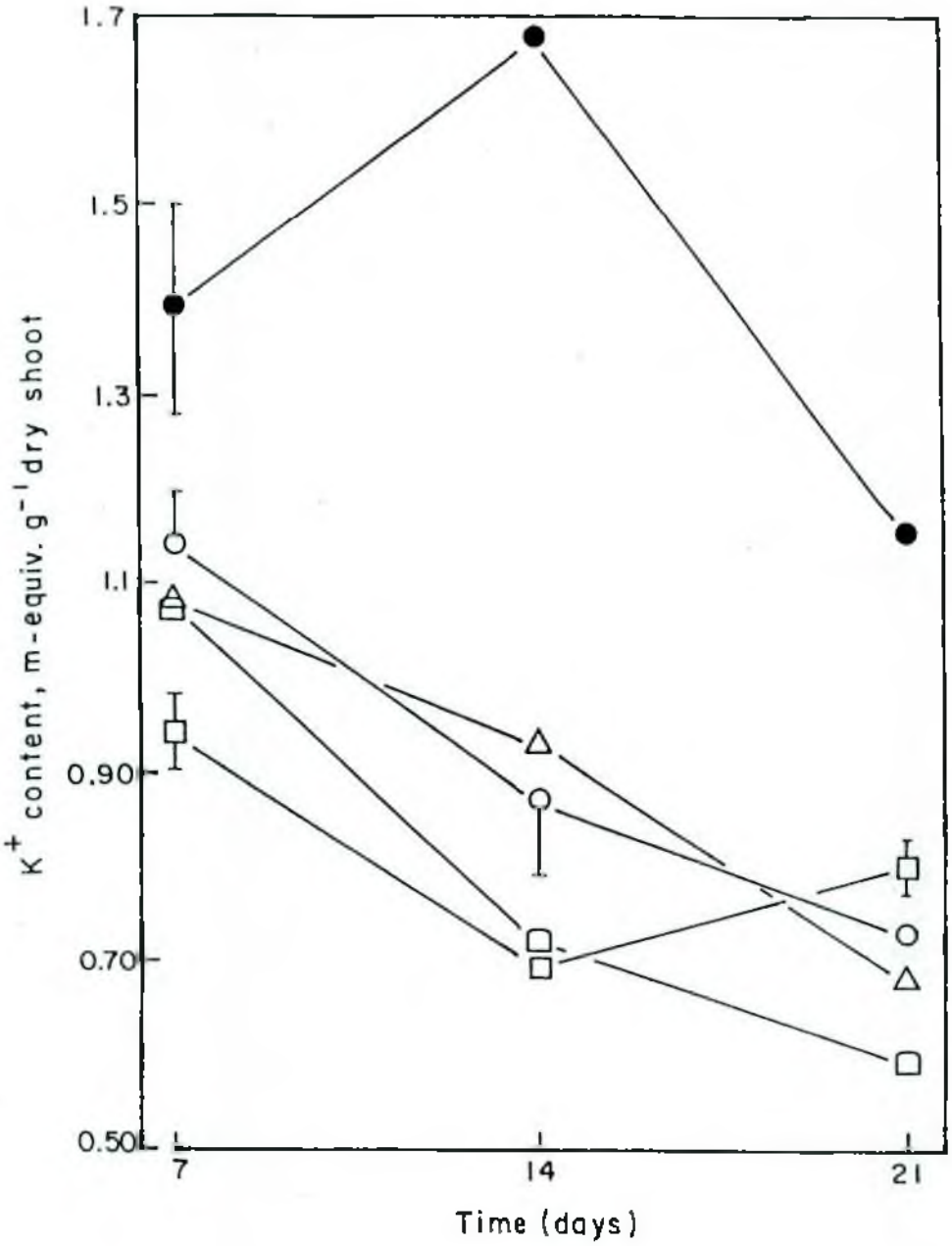

Fig. 24b The effect of salinity on accumulation of K^+ in the shoot of rice var. Pokkali at different developmental stages. Otherwise as Fig. 19a.

Transport index of Na^+ increased by 59 to 62% at 7-day, 59 to 64% at 14-day and 56 to 83% at 21-day in cv. BR16 under NaCl stress (Table 6).

In var. Pokkali of rice, net influx of (Ψ_{oc}) of Na^+ was increased by 8.5- to 14.5-fold at 7-day, 8.6- to 14.0-fold at 14-day and 12.9- to 22.6-fold at 21-day when treated with 25, 50, 75 and 100 mM NaCl treatment respectively (Table 9).

In intact seedlings of var. Pokkali, long distance transport (Ψ_{cx}) of Na^+ was increased by 10.5- to 20.0-fold at 7-day, 12- to 19-fold at 14-day and 26- to 42-fold at 21-day following 25 to 100 mM NaCl salinity (Table 9).

Transport index of Na^+ was increased by 18 to 33% at 7-day, 24 to 30% at 14-day and 79 to 74% at 21-day under NaCl salinity stress as compared to control (Table 9).

4.3.4b The effect of NaCl salinity on net influx and long distance transport of Cl^- in intact rice seedlings

In cv. BR16 of rice, net influx (Ψ_{oc}) of Cl^- was increased by 18 to 26% at 7-day, 16 to 19% at 14-day and 45 to 28% at 21-day following 25 to 100 mM NaCl salinity (Table 7).

Long distance transport (Ψ_{cx}) of Cl^- was increased by 7 to 57% at 7-day, 17 to 10% at 14-day and 75.0% to 2.1-fold at 21-day when treated with NaCl salinity treatment (25 to 100 mM) (Table 7).

Transport index of Cl^- was increased by 12 to 25% at 7-day and 21 to 59% at 21-day. On the other hand, there was no significant effect on transport index of Cl^- at 14-day-old plant when treated with 25 to 100 mM NaCl salinity (Table 7).

In var. Pokkali, net influx (Ψ_{oc}) of Cl^- was increased by 28 to 83% at 7-day, 24 to 8% at 14-day and 19 to 61% at 21-day following 25 to 100 mM NaCl salinity as compared to control (Table 10).

In var. Pokkali of rice, long distance transport (Ψ_{cx}) of Cl^- was increased by 2-fold to 60% at 7-day, 25.0% at 14-day and 11 to 15% at 21-day from 25 to 100 mM NaCl salinity treatment (Table 10).

Transport index of Cl^- was decreased by 12 to 13% at 7-day and 7 to 28% at 21-day. On the other hand, transport index of Cl^- was increased by 12 to 16% at 14-day at 75 and 100 mM NaCl salinity treatment as compared to control (Table 10).

4.3.4c The effect of NaCl salinity on net influx and long distance transport of K^+ in intact rice seedlings

Net influx (Ψ_{oc}) of K^+ was decreased by 17 to 72% at 7-day, 46 to 76% at 14-day and 33 to 74% at 21-day with 25, 50, 75 and 100 mM NaCl salinity treatment respectively (Table 8) in rice cv. BR16.

In cv. BR16, long distance transport (Ψ_{cx}) of K^+ was decreased by 21 to 74% at 7-day, by 46 to 78% at 14-day and 33 to 73% at 21-day from 25 to 100 mM NaCl salinity treatment (Table 8).

Table 6. The effect of NaCl on long distance transport and net influx of Na^+ in intact rice plant cv. BR16 grown in half-strength Hoagland solution + NaCl. Each value is the mean of three replicates; ± standard error.

Treatments time (days)	Net influx of Na^+ (Y_{in}), mg/plant					Transport of Na^+ from root to shoot (Y_{ex}), mg/plant					Transport index ((Y_{ex}/Y_{in}) x 100)				
		NaCl (mM)					NaCl (mM)					NaCl (mM)			
	Control	25	50	75	100	Control	25	50	75	100	Control	25	50	75	100
7	0.029 ±0.002	0.363 ±0.087	0.246 ±0.025	0.201 ±0.027	0.306 ±0.027	0.016 ±0.001	0.319 ±0.021	0.214 ±0.198	0.181 ±0.027	0.274 ±0.027	55.17	87.87	86.99	90.00	89.54
14	0.024 ±0.004	0.407 ±0.020	0.406 ±0.044	0.432 ±0.010	0.385 ±0.025	0.013 ±0.006	0.350 ±0.025	0.370 ±0.036	0.391 ±0.028	0.342 ±0.030	54.16	86.00	91.13	90.50	88.83
21	0.026 ±0.002	0.417 ±0.045	0.356 ±0.022	0.440 ±0.013	0.405 ±0.024	0.013 ±0.002	0.326 ±0.039	0.321 ±0.028	0.380 ±0.020	0.370 ±0.019	50.00	78.17	90.16	86.36	91.35

Table 7. The effect of NaCl on long distance transport and net influx of Cl^- in intact rice plant cv. BR16 grown in half-strength Hoagland solution + NaCl. Otherwise as Table 6.

Treatments time (days)	Net influx of Cl^- (Y_{ni}), mg/plant					Transport of Cl^- from root to shoot (Y_{ts}), mg/plant					Transport index ((Y_{ts}/Y_{ni}) x 100)				
		NaCl (mM)					NaCl (mM)					NaCl (mM)			
	Control	25	50	75	100	Control	25	50	75	100	Control	25	50	75	100
7	0.450 ±0.076	0.533 ±0.072	0.550 ±0.028	0.525 ±0.028	0.566 ±0.033	0.233 ±0.060	0.250 ±0.050	0.320 ±0.033	0.266 ±0.033	0.366 ±0.016	51.77	46.90	58.18	50.66	64.66
14	0.616 ±0.016	0.716 ±0.072	0.716 ±0.072	0.633 ±0.044	0.733 ±0.088	0.383 ±0.016	0.450 ±0.076	0.400 ±0.028	0.300 ±0.028	0.420 ±0.000	62.17	62.84	55.86	47.39	57.29
21	0.483 ±0.044	0.700 ±0.076	0.583 ±0.044	0.683 ±0.016	0.618 ±0.135	0.200 ±0.000	0.350 ±0.028	0.350 ±0.028	0.400 ±0.028	0.433 ±0.044	41.40	50.00	60.00	58.56	70.00

Table 8. The effect of NaCl on long distance transport and net influx of K^+ in intact rice plant cv. BR16 grown in half-strength Hoagland solution + NaCl. Otherwise as Table 6.

Treatments time (days)	Net influx of K^+ (Y_{in}), mg/plant					Transport of K^+ from root to shoot (Y_{ex}), mg/plant					Transport index ((Y_{ex}/Y_{in}) x 100)				
	NaCl (mM)					NaCl (mM)					NaCl (mM)				
	Control	25	50	75	100	Control	25	50	75	100	Control	25	50	75	100
7	0.285 ±0.015	0.238 ±0.055	0.075 ±0.007	0.061 ±0.015	0.079 ±0.009	0.273 ±0.014	0.215 ±0.052	0.065 ±0.007	0.056 ±0.015	0.071 ±0.008	95.78	90.33	87.33	91.88	89.87
14	0.384 ±0.026	0.208 ±0.030	0.108 ±0.004	0.126 ±0.012	0.094 ±0.007	0.384 ±0.022	0.189 ±0.027	0.100 ±0.005	0.113 ±0.012	0.078 ±0.007	90.62	90.86	92.59	89.68	82.97
21	0.252 ±0.048	0.168 ±0.013	0.105 ±0.012	0.086 ±0.006	0.067 ±0.006	0.222 ±0.046	0.149 ±0.010	0.095 ±0.011	0.081 ±0.007	0.060 ±0.005	88.09	88.69	90.47	94.18	89.55

Table 9. The effect of NaCl on long distance transport and net influx of Na^+ in intact rice plant var. Pokkali grown in half-strength Hoagland solution + NaCl. Otherwise as Table 6.

Treatments time (days)	Net influx of Na^+ (γ_{in}), mg/plant					Transport of Na^+ from root to shoot (γ_{rs}), mg/plant					Transport index ((γ_{rs}/γ_{in}) x 100)				
		NaCl (mM)					NaCl (mM)					NaCl (mM)			
	Control	25	50	75	100	Control	25	50	75	100	Control	25	50	75	100
7	0.026 ±0.002	0.144 ±0.020	0.172 ±0.008	0.244 ±0.013	0.223 ±0.049	0.013 ±0.001	0.085 ±0.012	0.099 ±0.005	0.157 ±0.021	0.148 ±0.044	50.00	59.00	57.55	64.34	66.36
14	0.042 ±0.003	0.237 ±0.060	0.263 ±0.060	0.430 ±0.055	0.346 ±0.042	0.028 ±0.004	0.196 ±0.074	0.208 ±0.048	0.362 ±0.057	0.300 ±0.032	66.66	82.70	79.08	84.18	86.70
21	0.032 ±0.008	0.253 ±0.056	0.320 ±0.090	0.295 ±0.035	0.405 ±0.045	0.015 ±0.001	0.213 ±0.061	0.253 ±0.078	0.244 ±0.039	0.331 ±0.026	46.87	84.19	79.06	82.71	81.72

Table 10. The effect of NaCl on long distance transport and net influx of Cl^- in intact rice plant var. Pokkali grown in half-strength Hoagland solution + NaCl. Otherwise as Table 6.

Treatments time (days)	Net influx of Cl (γ), mg/plant					Transport of Cl^- from root to shoot (γ), mg/plant					Transport index ((γ /γ x 100)				
	NaCl (mM)					NaCl (mM)					NaCl (mM)				
	Control	25	50	75	100	Control	25	50	75	100	Control	25	50	75	100
7	0.450 ±0.000	1.025 ±0.025	0.750 ±0.050	0.950 ±0.000	0.825 ±0.125	0.250 ±0.050	0.500 ±0.050	0.350 ±0.050	0.550 ±0.050	0.400 ±0.100	55.55	48.78	46.66	57.89	48.48
14	0.625 ±0.125	0.775 ±0.175	0.675 ±0.075	0.700 ±0.050	0.675 ±0.075	0.300 ±0.000	0.375 ±0.125	0.300 ±0.000	0.375 ±0.067	0.375 ±0.025	48.00	48.38	44.44	53.57	55.55
21	0.575 ±0.075	0.685 ±0.015	0.900 ±0.050	0.825 ±0.125	0.925 ±0.025	0.325 ±0.025	0.360 ±0.010	0.475 ±0.075	0.487 ±0.053	0.375 ±0.025	56.52	52.55	52.77	59.00	40.54

Table 11. The effect of NaCl on long distance transport and net influx of K^+ in intact rice plant var. Pokkali grown in half-strength Hoagland solution + NaCl. Otherwise as Table 6.

Treatments time (days)	Net influx of K^+ (Y_{ug}), mg/plant					Transport of K^+ from root to shoot (Y_{cs}), mg/plant					Transport index ((Y_{cs}/Y_{ug}) x 100)				
		NaCl (mM)					NaCl (mM)					NaCl (mM)			
	Control	25	50	75	100	Control	25	50	75	100	Control	25	50	75	100
7	0.667 ±0.002	0.536 ±0.014	0.414 ±0.011	0.354 ±0.034	0.503 ±0.229	0.602 ±0.012	0.482 ±0.006	0.385 ±0.010	0.304 ±0.029	0.281 ±0.031	90.25	89.92	93.00	85.87	55.86
14	0.718 ±0.024	0.461 ±0.018	0.474 ±0.026	0.379 ±0.068	0.424 ±0.006	0.647 ±0.033	0.421 ±0.013	0.446 ±0.024	0.349 ±0.063	0.400 ±0.004	90.11	91.32	94.09	92.08	94.34
21	0.699 ±0.025	0.474 ±0.010	0.488 ±0.009	0.474 ±0.001	0.393 ±0.028	0.655 ±0.017	0.445 ±0.009	0.445 ±0.011	0.447 ±0.001	0.371 ±0.026	93.70	93.88	91.18	94.30	94.40

Transport index of K^+ was decreased by 6 to 9% at 7-day and 9% at 14-day. On the other hand, transport index of K^+ was slightly increased at 21-day following 25 to 100 mM NaCl salinity (Table 8).

In var. Pokkali of rice seedlings, net influx (Ψ_{oc}) of K^+ was decreased by 20 to 25% at 7-day, 36 to 41% at 14-day and 32 to 44% at 21-day from 25 to 100 mM NaCl salinity as compared to control (Table 11).

Long distance transport (Ψ_{cx}) of K^+ was decreased by 20 to 53% at 7-day, 35 to 38% at 14-day and 32 to 43% at 21-day in var. Pokkali when treated with 25 to 100 mM NaCl salinity treatment (Table 11).

Transport index of K^+ was decreased by 38% at 7-day. However, there was no significant effect on transport index of K^+ at 14-day and 21-day following 25 to 100 mM NaCl salinity as compared to control (Table 11).

4.3.5 The effect of salinity on growth of rice

In solution culture, the growth and development of rice cv. BR16 and var. Pokkali was recorded upto 21-day after germination because plants did not survive beyond that period in solution culture.

The root and shoot growth in terms of dry matter production in rice cv. BR16 was decreased with the increase in salinity (Fig. 25a, b). In intact seedlings, growing in 25 to 100 mM NaCl, dry weight of the root was decreased by 30 to 48% at 7-day, 13 to 36% at 14-day and 9 to 28% at 21-day of treatment respectively as compared to control (Fig. 25a). Dry matter of the shoot was

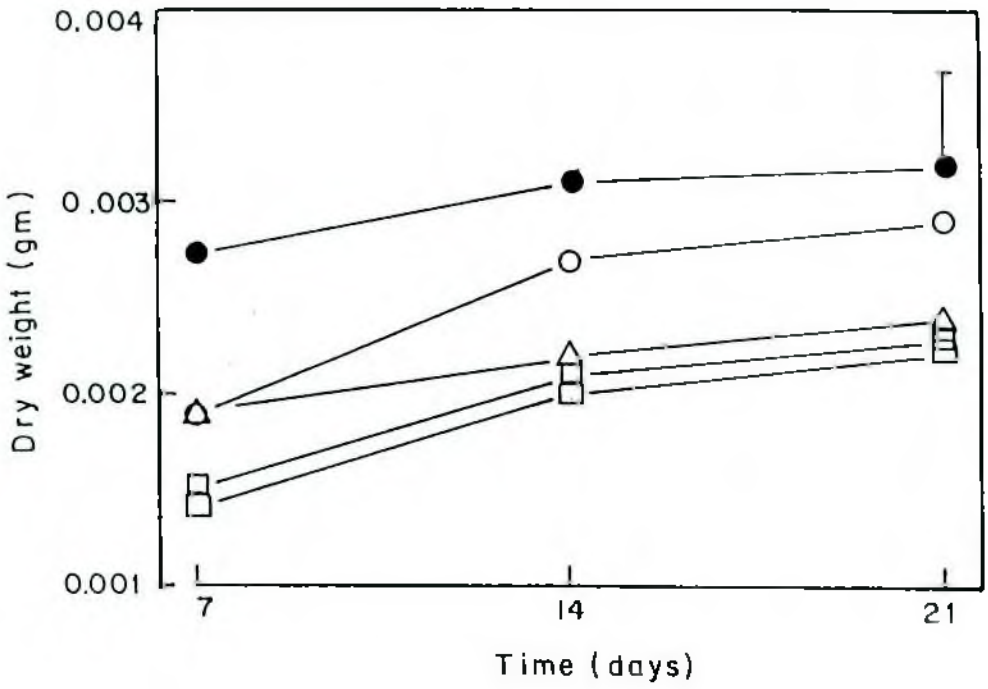

Fig. 25a The effect of salinity on dry weight of the root of rice cv. BR16 at different developmental stages. ● control; O 25 mM NaCl; △ 50 mM NaCl; □75 mM NaCl; □100 mM NaCl. Bars represent ± standard error. Each value is a mean of three replicates.

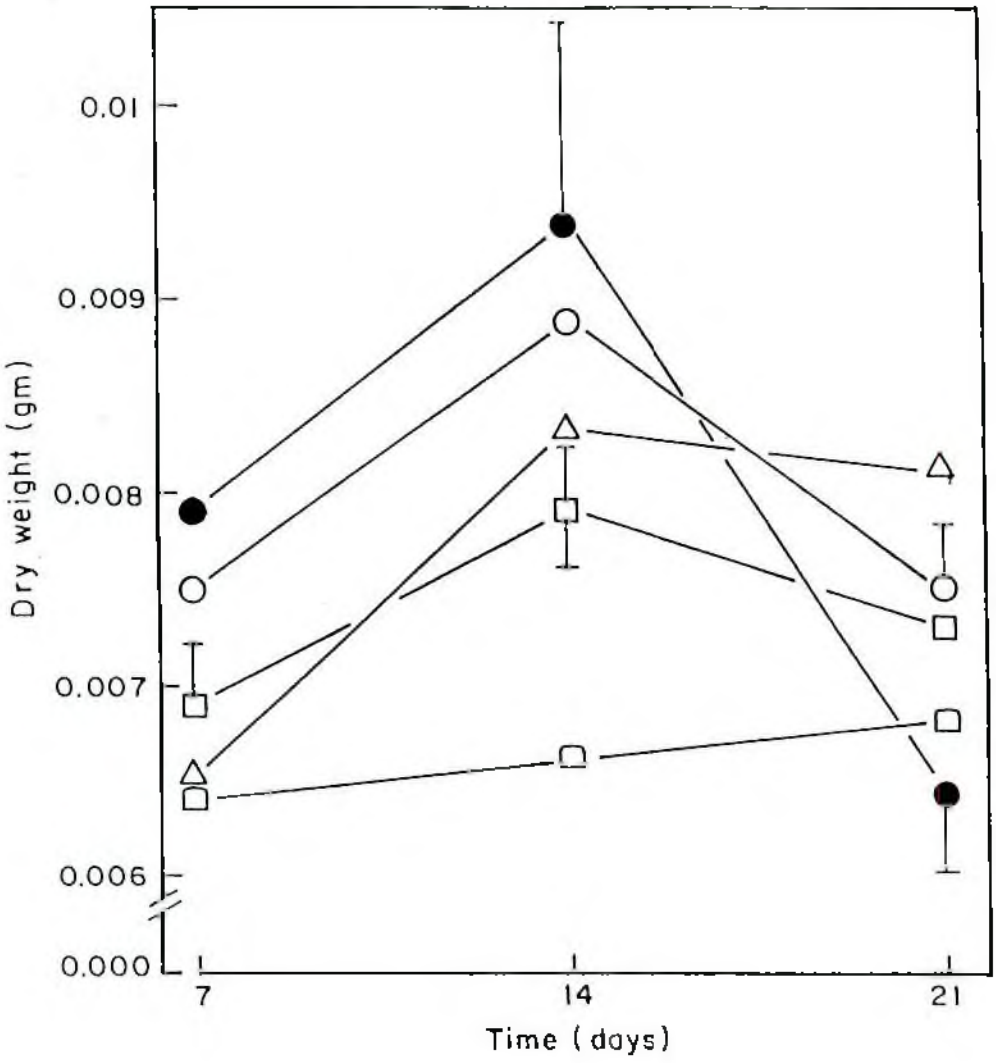

Fig. 25b The effect of salinity on dry weight of the shoot of rice cv. BR16 at different developmental stages. Otherwise as Fig. 25a.

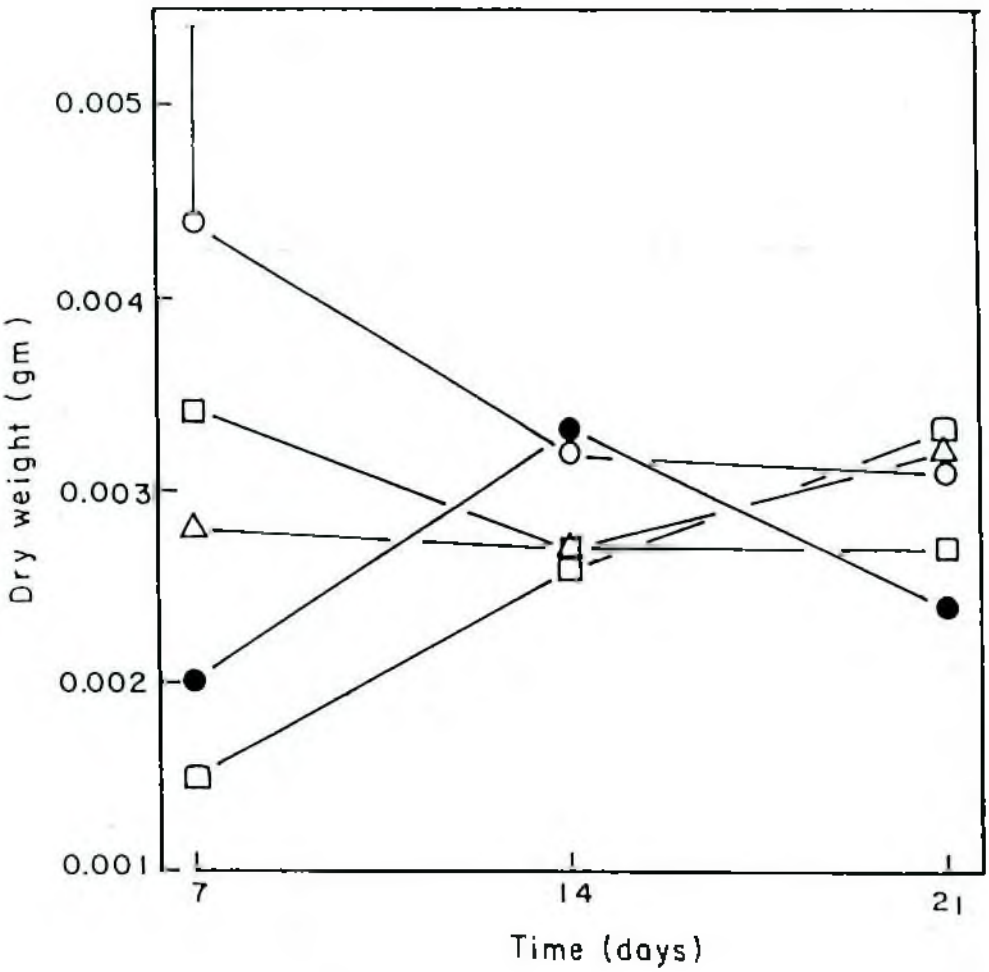

Fig. 26a The effect of salinity on dry weight of the root of rice var. Pokkali at different developmental stages. Otherwise as Fig. 25a.

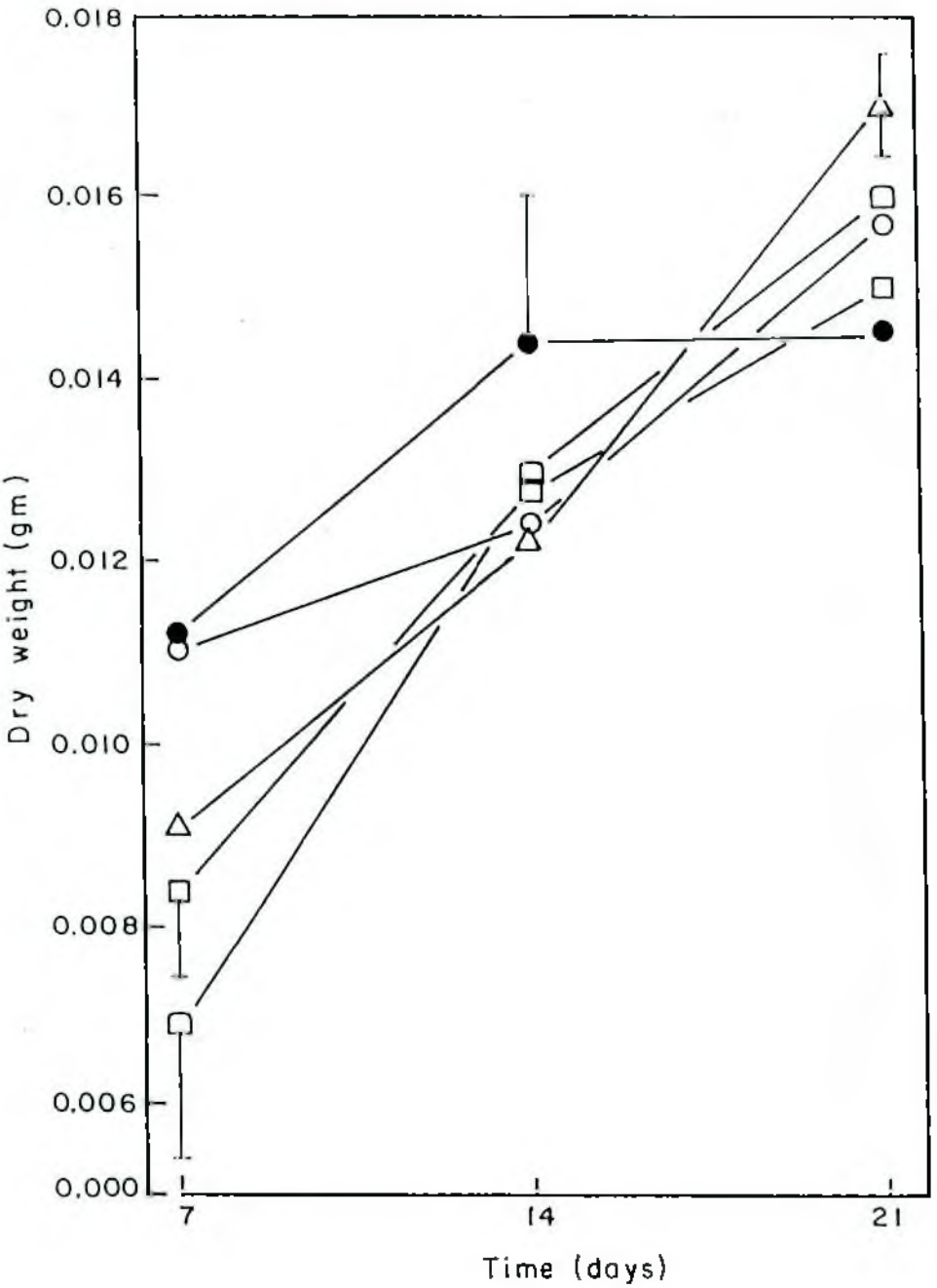

Fig. 26b The effect of salinity on dry weight of the shoot of rice var. Pokkali at different developmental stages. Otherwise as Fig. 25a.

decreased by 5 to 19% at 7-day, 5 to 30% at 14-day (Fig. 25b). On the other hand, dry matter production was increased by 17 to 6% at 21-day in cv. BR16 (Fig. 25b).

On the contrary, in var. Pokkali, NaCl salinity increased root growth at 7 and 21-day (Fig. 26a). On the other hand, shoot growth was decreased by 17 to 38% at 7-day, 14 to 13% at 14-day and shoot growth was increased by 8 to 10% at 21-day of treatment respectively as compared to control (Fig. 26b).

4.6 Discussion

The present result on rice shows that salinity caused a dramatic stimulation of Na^+ and Cl^- accumulation with a concomitant inhibition of the accumulation of K^+ both in cv. BR16 (Fig. 19, 20 and 21a, b) and also in var. Pokkali (Fig. 22, 23 and 24a, b). Similar result was found in two rice cultivars (salt-sensitive cv. IKP and salt-tolerant cv. Aiwu) by Lutts and Guerrier (1995). Shannon *et al.* (1998) also observed that rice plant from saline basins had significantly higher concentration of leaf Na^+ and Cl^- than those from less saline basins and leaf concentration of K^+ was decreased. Prakash and Prathapasenan (1990) showed that salt-stressed rice plants showed higher concentration of Na^+ and Cl^- and lower concentration of K^+ in the leaf tissue.

Accumulation of Na^+ and Cl^- increased in *Ipomoea pescaprae* under extreme salinity (Venkatesan *et al.* 1997). Wahid *et al.* (1997) found that in the salt-tolerant line of sugarcane, Na^+ and Cl^- accumulation were decreased while that of K^+ was increased.

It is apparent from the results that salinity had adverse effect on the plant growth. The root and shoot in terms of dry matter production in rice was decreased with the increase in salinity (Fig. 25a, b and 26a, b). Similar inhibition of salinity on growth was recorded in chickpea (Sharma 1997), *Vicia faba* (Huq *et al.* 1987), *Cyanopsis tetragonoloba* (Ai-Yemeny and Basahy 1997), wheat (Begum 1994, Alamgir and Kutube 1997), rice (Roth 1989), cucumber (Chartzoulakis 1992), jojoba (Benzioni *et al.* 1992), *Cicer arietinum* (Mirza and Tariq 1993), *Sorghum bicolor* (Yang *et al.* 1990a) and in rice (Lin and Kao 1995). Sanchez-Blanco *et al.* (1998) found that salinity caused a reduction in total dry weight of lotus plants treated with 140 mM NaCl whereas no significant effect on growth was observed with 70 mM NaCl. Salinity stress caused an imbalance in the availability of mineral nutrients, their uptake and distribution within the plant and increased requirements for essential elements (Grattan and Grieve 1992).

On the other hand, 25 to 75 mM NaCl stress stimulated the root growth of rice in terms of dry matter production at 7-day (Fig. 26a) in var. Pokkali following a decrease in growth at 100 mM NaCl treatment. This result is in consistent with the work of Waisel and Brekle (1987) who found that at 50 mM NaCl, growth of radish root was not affected while it was affected at higher concentration of NaCl. Similar results were also observed in *Kosteletzkiya virginica* (Blits and Gallagher 1990), *Vicia faba* (Huq *et al.* 1987) and in rice (Lin and Kao 1995). In intact rice (var. Pokkali) seedlings, it is apparent that root growth was increased while the shoot growth was decreased (Fig. 26a, b). On the other

hand, Romero and Maranon (1994) reported that in *Melilotus segetalis* root growth was more severely decreased than shoot growth resulting in a decreased root to shoot ratio that was marked with age. Similarly, Macharia *et al.* (1995) observed root extension to be more sensitive to salinity stress than shoot extension in three cultivars of shorghum. Wignarajah (1990) found that in *Phaseolus vulgaris*, higher salinity reduced shoot growth rather than root growth. It was also observed that increase in salinity levels resulted in a marked growth reduction upto 40% in root, 73% in shoot length, 54% in stem, 58% in root dry weight of *Cyanopsis tetragonoloba* (Ai-Yemeny and Basahy 1997).

It is suggested that salinity-induced inhibition growth may be due to the toxic effect of huge amount of Na^+ and Cl^- accumulation in salt-stressed plant (Fig. 13a, b to 26a, b). Yeo and Flowers (1986) suggested that Na^+ and Cl^- was considered to be toxic and appeared to be responsible for inhibition on growth induced by salinity.

Sharma (1997) also observed that plant growth was declined with the increase in levels of salinity while concentration of Na^+ and Cl^- was increased and that of K^+ decreased in chickpea.

Based on these observations, it was suggested that negative influence of NaCl on growth was caused by a detrimental effect of sodium on metabolic processes. Our result shows that rice plant accumulate large amount of Na^+ and Cl^- and decreased K^+ (Fig. 13a, b to 24a, b). Hence, it is suggested that salinity-induced inhibition of growth may be due to toxic effect of large among of Na^+ and Cl^- accumulation in salt-stress plant.

Net influx (Ψ_{oc}), long distance transport (Ψ_{cx}) and transport index of Na^+ was increased with the increase in NaCl concentration from 25 to 100 mM NaCl in both cv. BR16 and var. Pokkali (Table 6 and 9). Similarly, NaCl salinity increased net influx and long distance transport of Cl^- (Table 7 and 10). However, transport index of Cl^- was decreased in intact rice seedlings of cv. BR16 and var. Pokkali under NaCl treatment (Table 7 and 10). Similar result was found in wheat (Begum 1994, Yasmin 1999). Decrease in transport index of Cl^- indicated that less amount of Cl^- was transported to the shoot as compared to large increase in the accumulation of Cl^- in the root. It is suggested that Cl^- was accumulated in the vacuole and less amount of Cl^- was transported to the shoot to help the plant overcome the toxic effect of this ion. NaCl salinity decreased net influx, long distance transport of K^+ in both cv. BR16 and var. Pokkali (Table 8 and 11). Similar result was also observed in barley (Zeb-un-Nessa 1992) and wheat (Yasmin 1999). But, there was no significant effect on transport index of K^+ in both cv. BR16 and var. Pokkali (Table 8 and 11). This indicates that K^+ is mobile in the plant under salinity. Increase in transportation to the shoot can meet the demand of K^+ in the changing metabolic activity of the shoot under salinity condition. Begum (1994) observed that the K^+ influx was not affected by the increase in concentration of salinity. Maintenance of K^+ concentration at the steady state despite and increase in Na^+ and Cl^- accumulation helps the plant survive under salinity condition.

CHAPTER 5

THE EFFECT OF SALINITY ON ACCUMULATION AND DISTRIBUTION OF DIVALENT CATIONS IN INTACT RICE SEEDLINGS

5.1 Introduction

Salinity-induced changes in accumulation of divalent cations may change the ionic balance in plants. This, in turn, may have some effect on the adaptation of plants under salinity conditions. Therefore, the effect of salinity on the accumulation of divalent cations like Ca^{2+}, Mg^{2+} and Fe^{2+} were studied.

Divalent cations like Ca^{2+}, Mg^{2+} and Fe^{2+} are major essential elements necessary for plant growth and development (Epstein 1972). Calcium plays a role in maintaining permeability characteristics of membrane. Calcium is also important in maintaining ionic balance under salinity. Calcium can prevent the adverse effect due to salinity stress (Epstein 1961, Lauchli and Epstein 1970, Cramer *et al.* 1986). In presence of Ca^{2+}, Na^{+} absorption and translocation was depressed under salinity (Sopandie *et al.* 1990). It was reported that by lowering Na/Ca ratio, K^{+} uptake was increased and Na^{+} uptake was decreased (Subbarao *et al.* 1990b). They also reported that shoot dry weight increased with the decrease in Na/Ca ratio. Epstein (1961) observed that Na^{+} uptake was remarkably reduced when Ca^{2+} was added to the medium in excised barley roots. Sodium ion displaced Ca^{2+} ions present in cell membrane (Cramer *et al.*

1985) and K^+ selectivity was hampered due to the displacement of Ca^{2+} by Na^+ (Cramer *et al.* 1987).

It was found that the increase in NaCl salinity resulted in a decrease the Ca^{2+} content (Lessani and Marschner 1978; Mahmood and Malik 1987, Lynch and Lauchli 1988, Yasseen *et al.* 1989). Wyne Jones *et al.* (1977) observed that calcium had a beneficial effect on the salt tolerance of some plants of family *Tiliaceae*, Ca^{2+} content decreased with increase in salinity level in salt-treated grass roots (Elzam and Epstein 1969), chickpea (Mumurkar and Chavan 1986) and in *Vigna radiata* L. (Salim and Pitman 1983).

Kingsbury *et al.* (1984) found that a decrease in Ca^{2+} and a slight increase in Mg^{2+} under salinity condition. On the other hand, an increase in Ca^{2+} and Mg^{2+} with the increase in NaCl salinity stress in chickpea observed by Yadav and coworkers (1989). Aslam *et al.* (1987) observed that in *Echinochloa crussgalli*, a decrease in Mg^{2+} and an increase in Ca^{2+} content. However, Yasseen *et al.* (1989) observed a decrease in Ca^{2+} accumulation in barley leaves under salinity condition. It was reported by Curtin and coworkers (1993) that barley and kochia tended to have higher selectivity for nutrient cations like Ca^{2+} and Mg^{2+} over Na^+ when the plants were grown under NaCl salinity.

Shannon and coworkers (1998) found that at the highest salinity, concentration of K^+ in leaf was decreased by about 40% by salinity, but tissue levels of Ca^{2+} and Mg^{2+} were unaffected.

Therios and Misopolinos (1989) observed that an increase in Ca^{2+} and a decrease in Fe^{2+} was observed with the increase in salinity level in apple root stock. Padole (1991) observed that high saline content significantly decreased the uptake of Fe^{2+} in wheat (var. HDM1553). On the other hand, Alam and Naqvi (1991) reported that salinity caused an increase in Fe^{2+} content in pearl millet. Romero and Maranon (1996) observed that Fe^{2+} tended to be diluted in leaves and concentrations in roots of *Melilotus sagetalis* and Fe^{2+} concentration was not much affected by salinity.

It was reported that with low concentration of NaCl, Fe^{2+} absorption was enhanced in bean seedlings (Pandey and Kannan 1979). Fe^{2+} was increased in *Ipomoea pescaprae* under salinity level of 200 mM (Venkatesan *et al.* 1997). It was suggested that micronutrient concentration can increase, decrease or may not be affected by salinity depending upon the plant species, tissue, salt type and environmental conditions (Grattan and Grieve 1992).

5.2 Material and methods

Plants were grown in solution culture as described in 2.4.5. Root and shoot samples were collected at 7, 14 and 21 days after germination. Free space ions in roots were removed according to 2.5. Calcium, magnesium and iron were extracted by oxidizing dried tissue with 4 ml mixture nitric acid-perchloric acid as described in 2.6.4. Calcium, magnesium and iron were determined according to 2.6.8.

5.3 Results

5.3.1 The effect of salinity on accumulation and distribution of Ca^{2+}, Mg^{2+} and Fe^{2+} in 7-day-old intact rice seedlings

Accumulation of Ca^{2+} in the root of cv. BR16 was decreased by 13 to 10% with the increase in salinity from 25 to 50 mM NaCl (Fig. 27a). In the shoot of cv. BR16, Ca^{2+} accumulation was decreased by 31 to 30% under NaCl salinity (25-100 mM) (Fig. 27b).

In the root of var. Pokkali, Ca^{2+} content was decreased by 4 to 23% following the NaCl treatment ranging from 50 to 100 mM (Fig. 28a). The accumulation of Ca^{2+} was decreased by 16% at 25 mM NaCl treatment and this trend was maintained upto 100 mM NaCl treatment (Fig. 28b).

Magnesium content was increased by 38 to 54% over control in the root of cv. BR16 at 25 to 100 mM NaCl (Fig. 27a). In the shoot of cv. BR16, Mg^{2+} accumulation was decreased by 20 to 30% at 25 to 100 mM NaCl treatment (Fig. 27b).

On the other hand, accumulation of Mg^{2+} was increased by 16 to 41% in the root and 24 to 34% in the shoot in var. Pokkali following the NaCl treatment ranging from 50 to 100 mM (Fig. 28a, b).

Accumulation of Fe^{2+} in the root of cv. BR16 was increased by 14.2- to 4.2-fold with the increase in salinity level (Fig. 27a). In the shoot of cv. BR16, Fe^{2+} accumulation was increased by 64% at 50 mM NaCl treatment, and this trend was maintained upto 100 mM NaCl treatment (Fig. 27b).

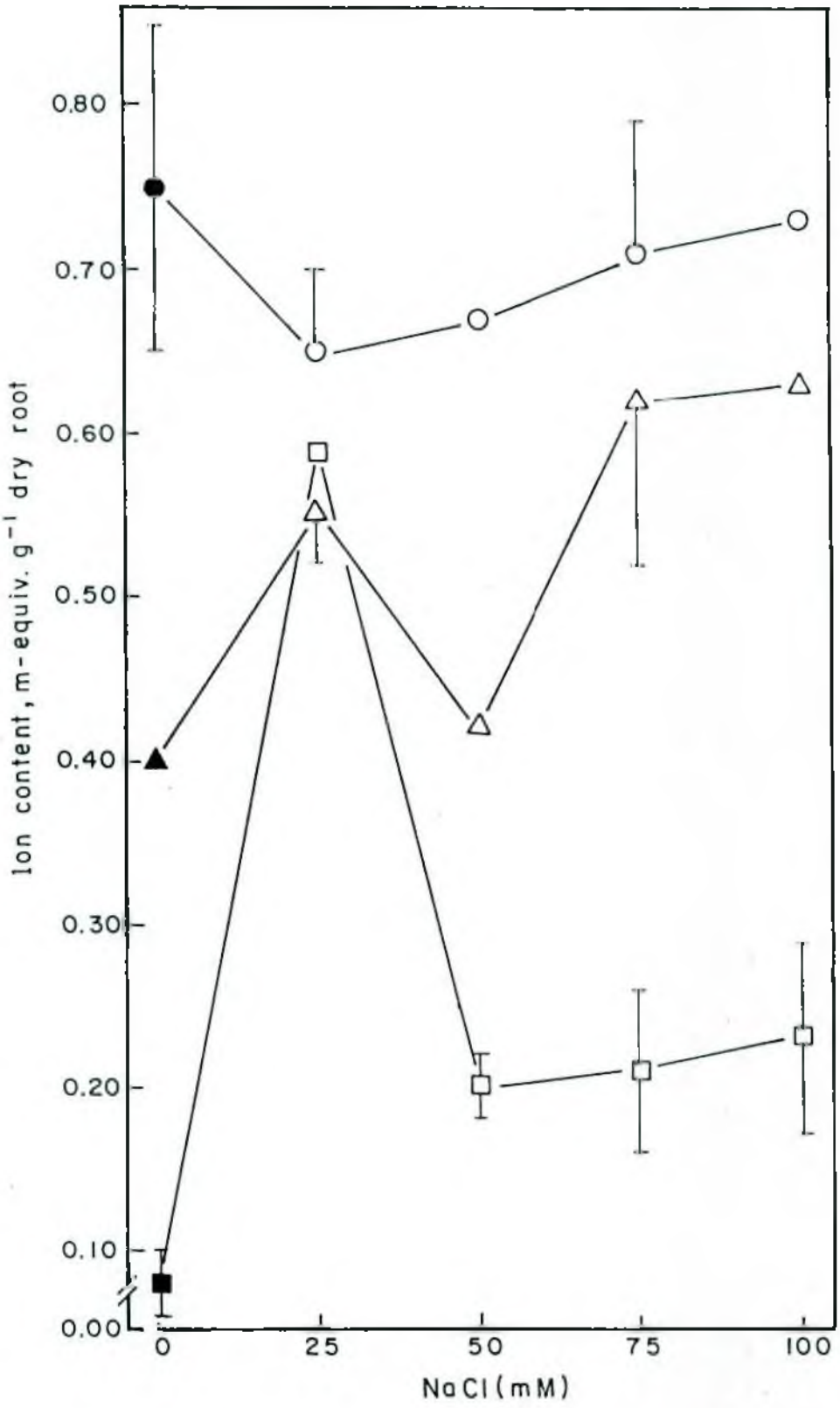

Fig. 27a The effect of salinity on accumulation of Ca^{2+}, Mg^{2+} and Fe^{2+} in the root of 7-day-old intact seedlings of rice cv. BR16. O Ca^{2+}, △ Mg^{2+}, □ Fe^{2+}. Solid symbols represent control. Bars represent ± standard error. Each value is mean of three replicates.

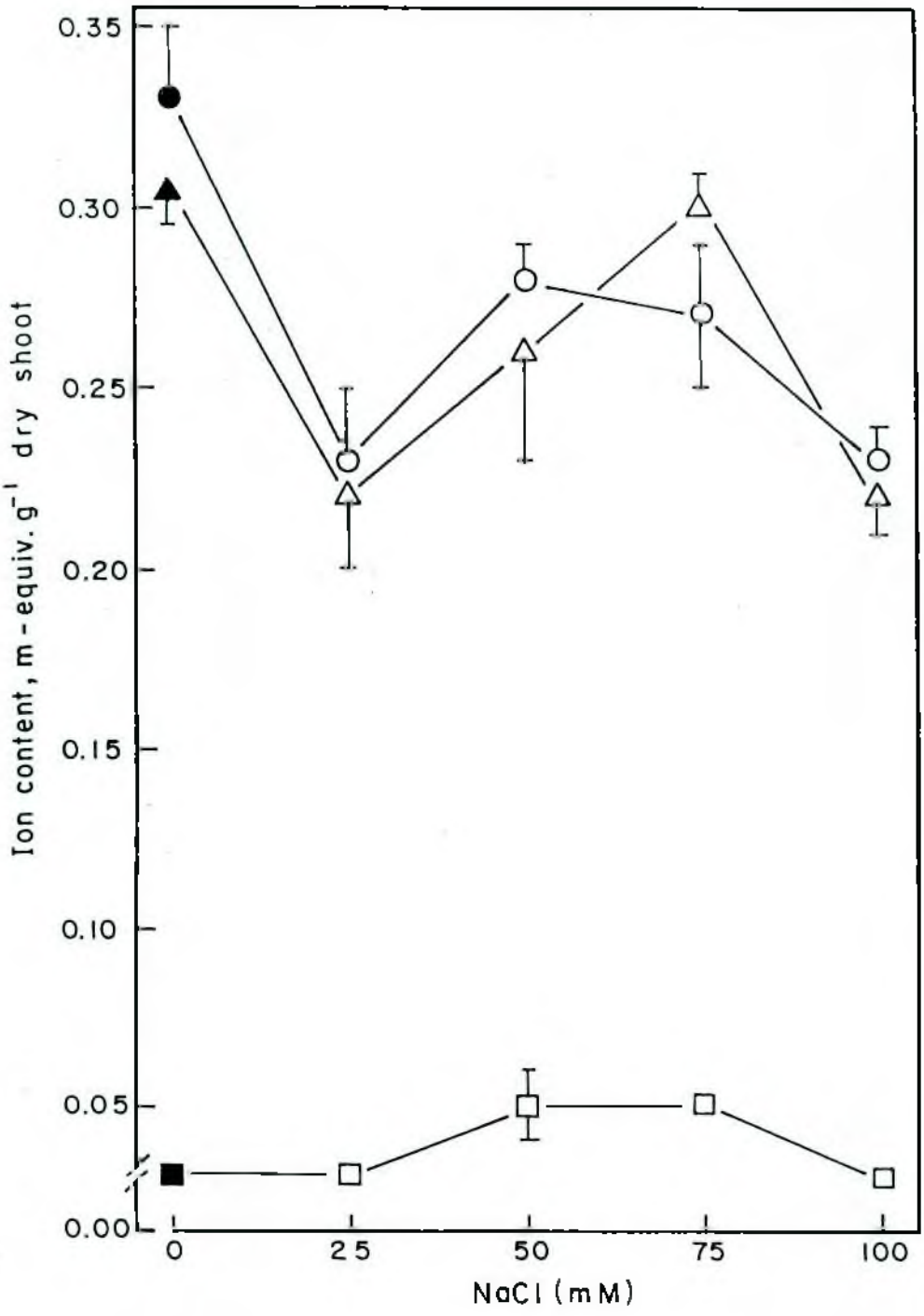

Fig. 27b The effect of salinity on accumulation of Ca^{2+}, Mg^{2+} and Fe^{2+} in the shoot of 7-day-old intact seedlings of rice cv. BR16. Otherwise as Fig. 27a.

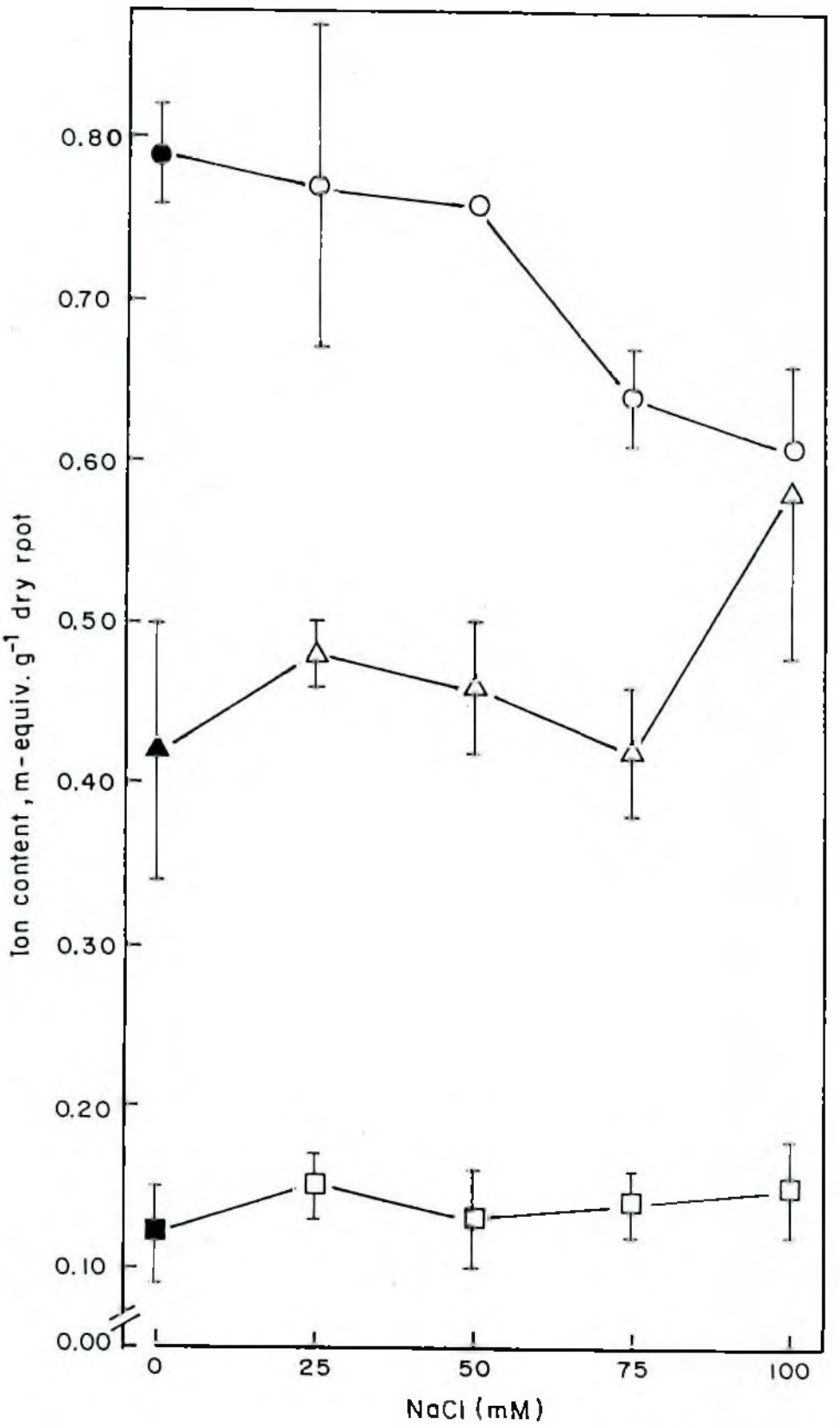

Fig. 28a The effect of salinity on accumulation of Ca^{2+}, Mg^{2+} and Fe^{2+} in the root of 7-day-old intact seedlings of rice var. Pokkali. Otherwise as Fig. 27a.

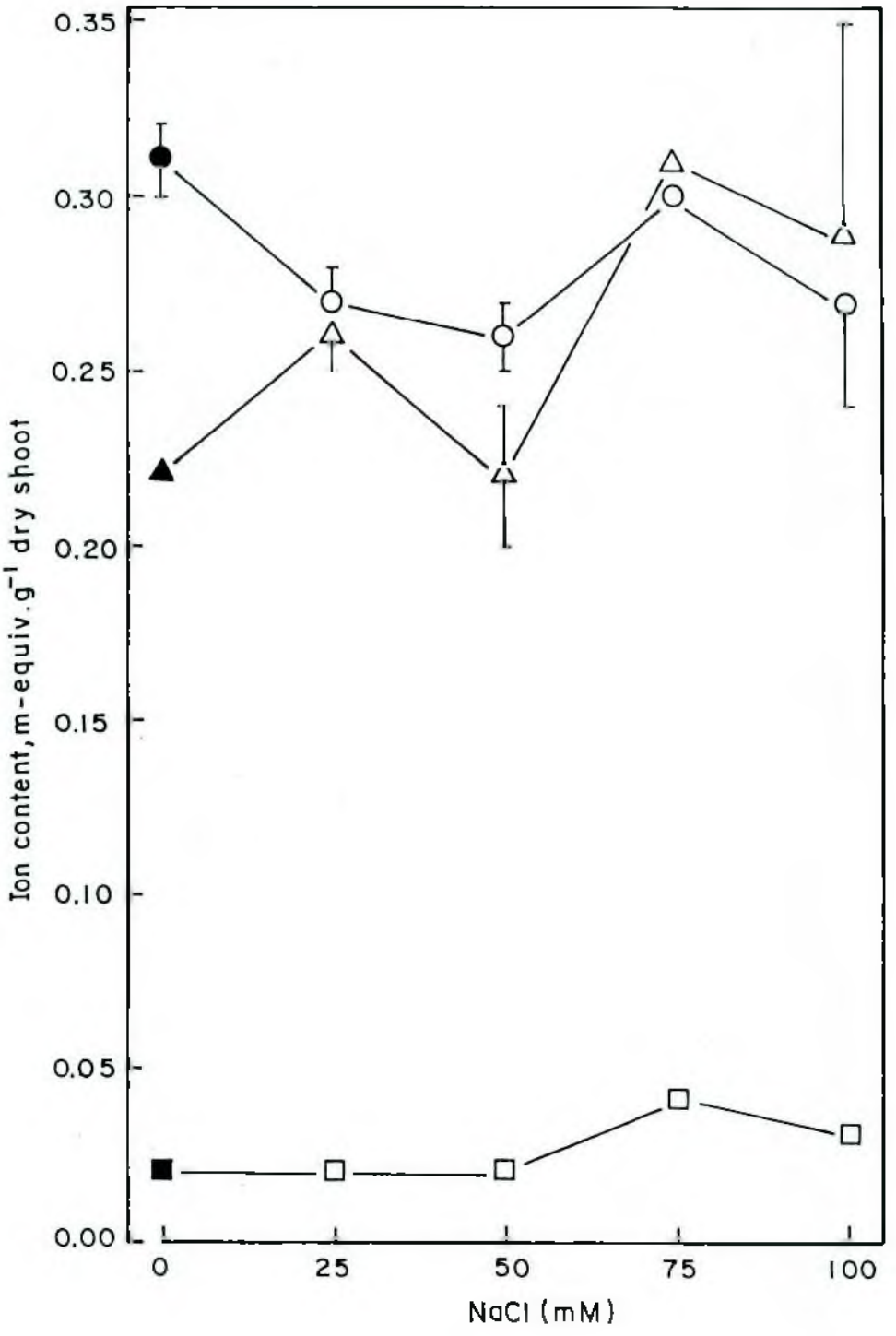

Fig. 28b The effect of salinity on accumulation of Ca^{2+}, Mg^{2+} and Fe^{2+} in the shoot of 7-day-old intact seedlings of rice var. Pokkali. Otherwise as Fig. 27a.

However, accumulation of Fe^{2+} by the root of var. Pokkali was increased by 21 to 20% at 25 to 100 mM NaCl respectively (Fig. 28a). A 17 to 50% increase in Fe^{2+} accumulation by the shoot of var. Pokkali was observed at 25 to 100 mM NaCl (Fig. 28b).

5.3.2 The effect of salinity on accumulation and distribution of Ca^{2+}, Mg^{2+} and Fe^{2+} in 14-day-old intact rice seedlings

Ca^{2+} content in the root of cv. BR16 was decreased by 43 to 31% at 25 to 75 mM NaCl salinity (Fig. 29a). A 7 to 19% decrease in Ca^{2+} content was observed in the shoot of cv. BR16 at 25 to 100 mM NaCl as compared to control (Fig. 29b).

In the root of var. Pokkali, Ca^{2+} content was decreased by 32, 48, 38 and 43% at 25, 50, 75 and 100 mM NaCl respectively over control (Fig. 30a). Similar result was found in the shoot of var. Pokkali (Fig. 30b).

Salinity stress increased the accumulation of Mg^{2+} in the root cv. BR16 by 87% to 2-fold following 25 to 100 mM NaCl treatment (Fig. 29a). The accumulation of Mg^{2+} in the shoot of cv. BR16 was increased by 91 to 51% at 25 to 100 mM NaCl (Fig. 29b).

In the root of var. Pokkali, Mg^{2+} content was increased by 13 to 43% at 50 to 100 mM NaCl treatment (Fig. 30a). In the shoot of var. Pokkali, Mg^{2+} content was increased by 18 to 15% over control (Fig. 30b).

Salinity stress (25 to 100 mM) increased Fe^{2+} content in the root of cv. BR16 by 15.0 to 4.5-fold over control (Fig. 29a). Low salinity (25 mM NaCl) decreased Fe^{2+} content in the shoot of cv. BR16 by 13% and the inhibitory effect gradually declined upto 100 mM NaCl treatment (Fig. 29b).

However, salinity caused an increase in Fe^{2+} accumulation in the root and the shoot from 4-fold to 76% and 20 to 76% respectively following salinity treatment (Fig. 30a, b).

5.3.3 The effect of salinity on accumulation and distribution of Ca^{2+}, Mg^{2+} and Fe^{2+} in 21-day-old intact rice seedlings

In cv. BR16, Ca^{2+} accumulation was decreased by 39 to 8% in the root following NaCl salinity treatment (50 to 100 mM) (Fig. 31a). Salinity caused a 24 to 56% decrease in Ca^{2+} in the shoot of cv. BR16 following 50 to 100 mM NaCl treatment (Fig. 31b).

Salinity caused a 36 to 57% decrease in Ca^{2+} content in the root of var. Pokkali following 25 to 100 mM NaCl treatment (Fig. 32a). Calcium accumulation was decreased by 22 to 17% at 25 to 100 mM NaCl treatment as compared to control in the shoot of var. Pokkali (Fig. 32b).

In cv. BR16, Mg^{2+} content in the root was increased by 16 to 10% over control at 25 to 100 mM NaCl treatment (Fig. 31a). In the shoot of cv. BR16, accumulation of Mg^{2+} was increased by 2-fold to 55% at 25 to 100 mM NaCl treatment respectively (Fig. 31b).

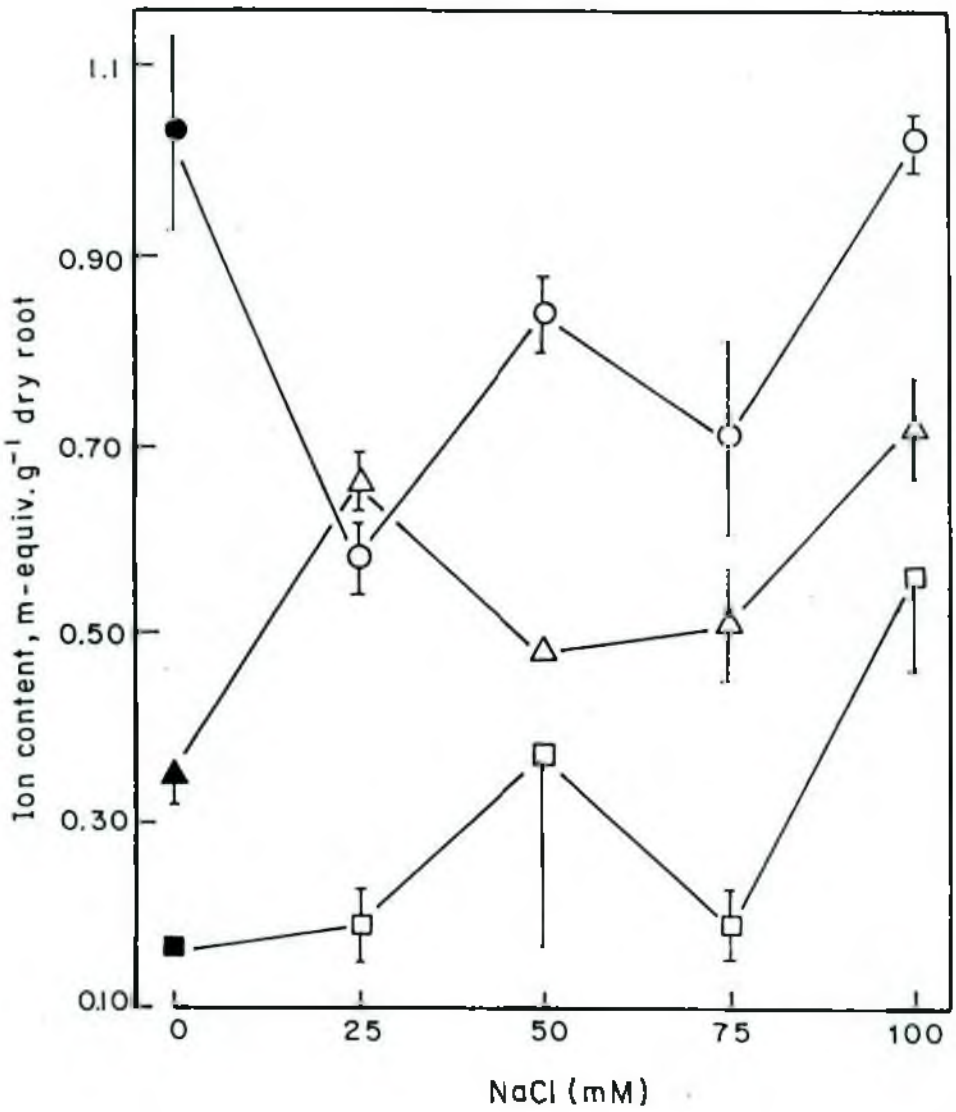

Fig. 29a The effect of salinity on accumulation of Ca^{2+}, Mg^{2+} and Fe^{2+} in the root of 14-day-old intact seedlings of rice cv. BR16. Otherwise as Fig. 27a.

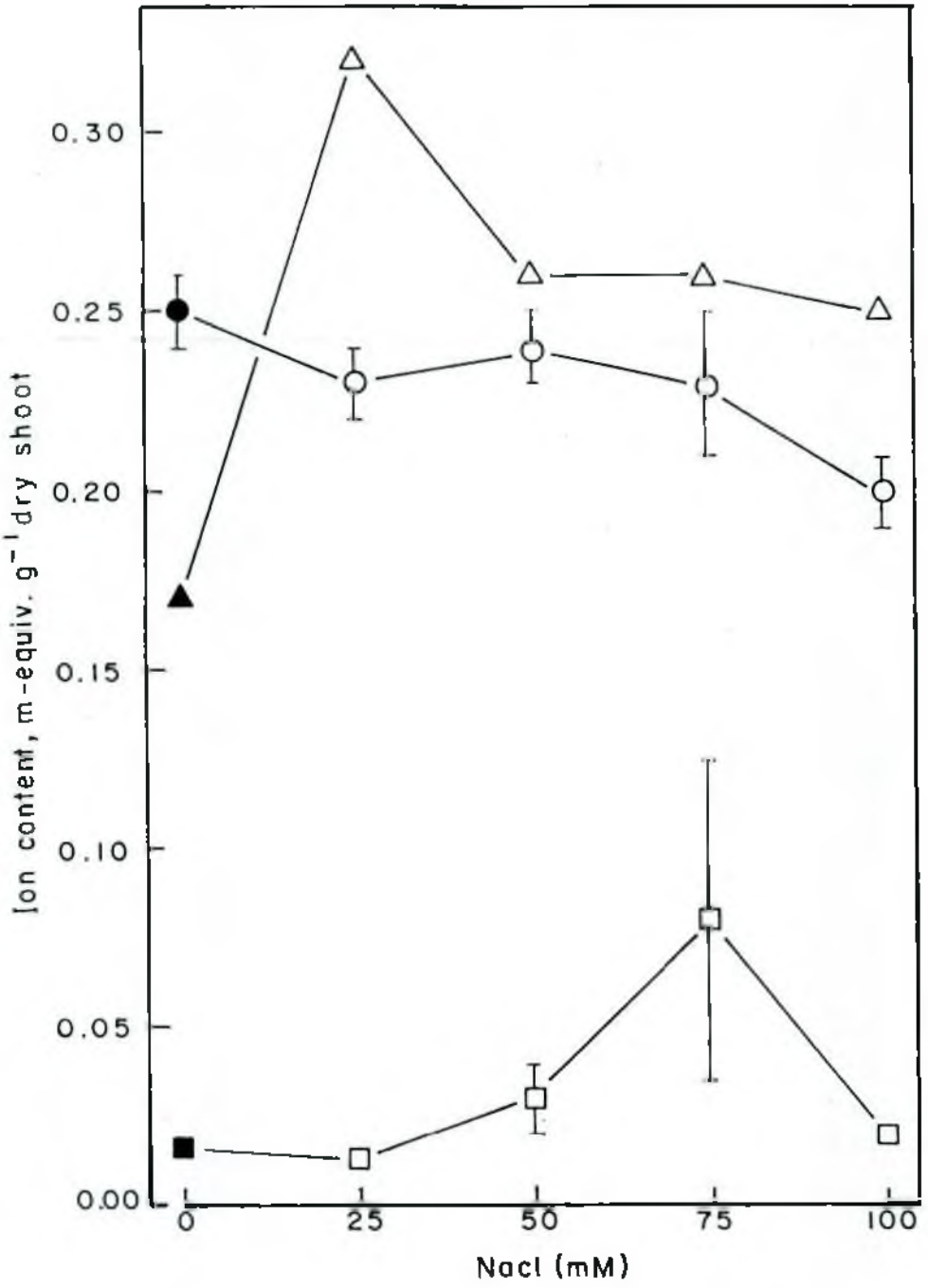

Fig. 29b The effect of salinity on accumulation of Ca^{2+}, Mg^{2+} and Fe^{2+} in the shoot of 14-day-old intact seedlings of rice cv. BR16. Otherwise as Fig. 27a.

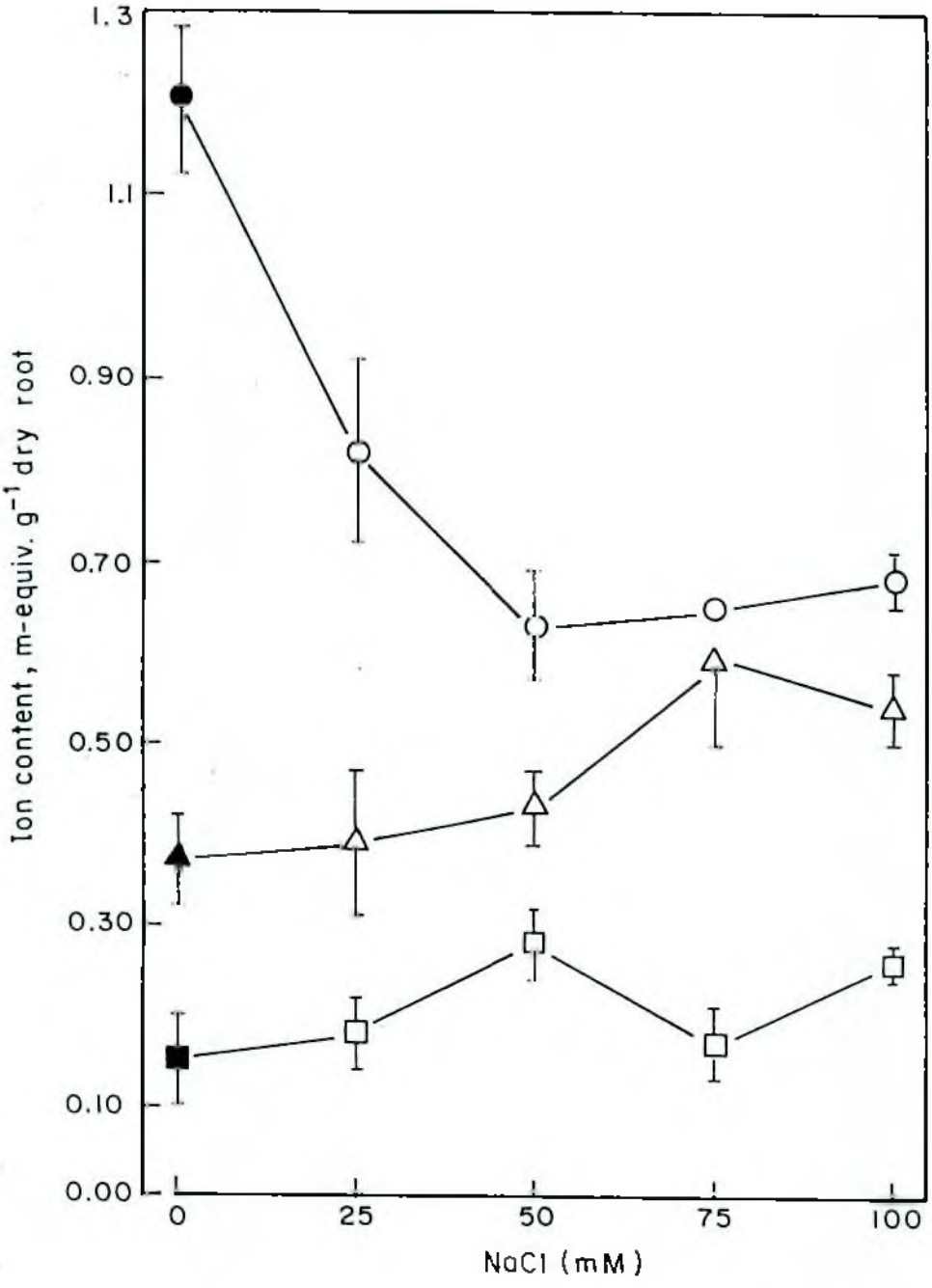

Fig. 30a The effect of salinity on accumulation of Ca^{2+}, Mg^{2+} and Fe^{2+} in the root of 14-day-old intact seedlings of rice var. Pokkali. Otherwise as Fig. 27a.

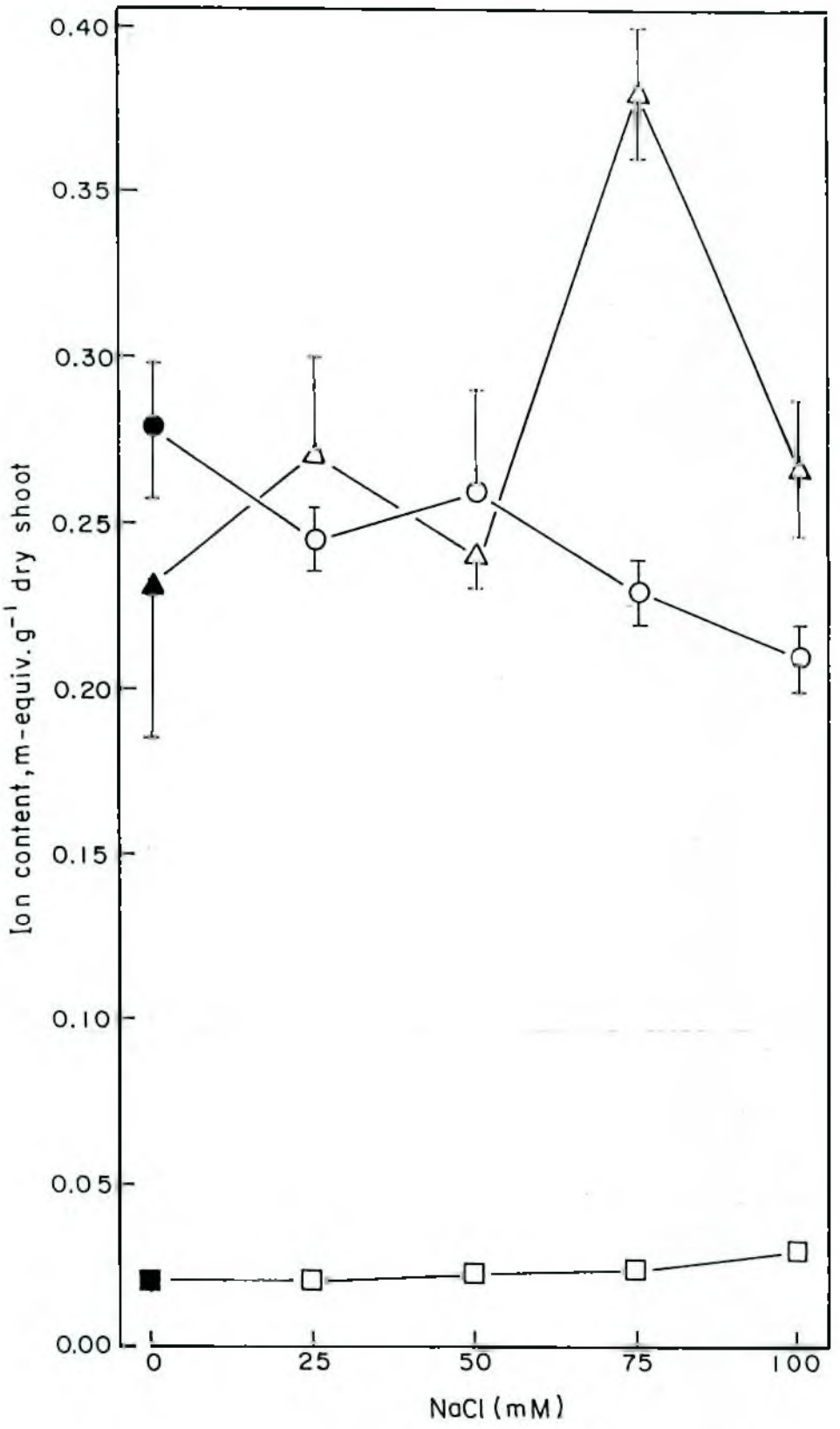

Fig. 30b The effect of salinity on accumulation of Ca^{2+}, Mg^{2+} and Fe^{2+} in the shoot of 14-day-old intact seedlings of rice var. Pokkali. Otherwise as Fig. 27a.

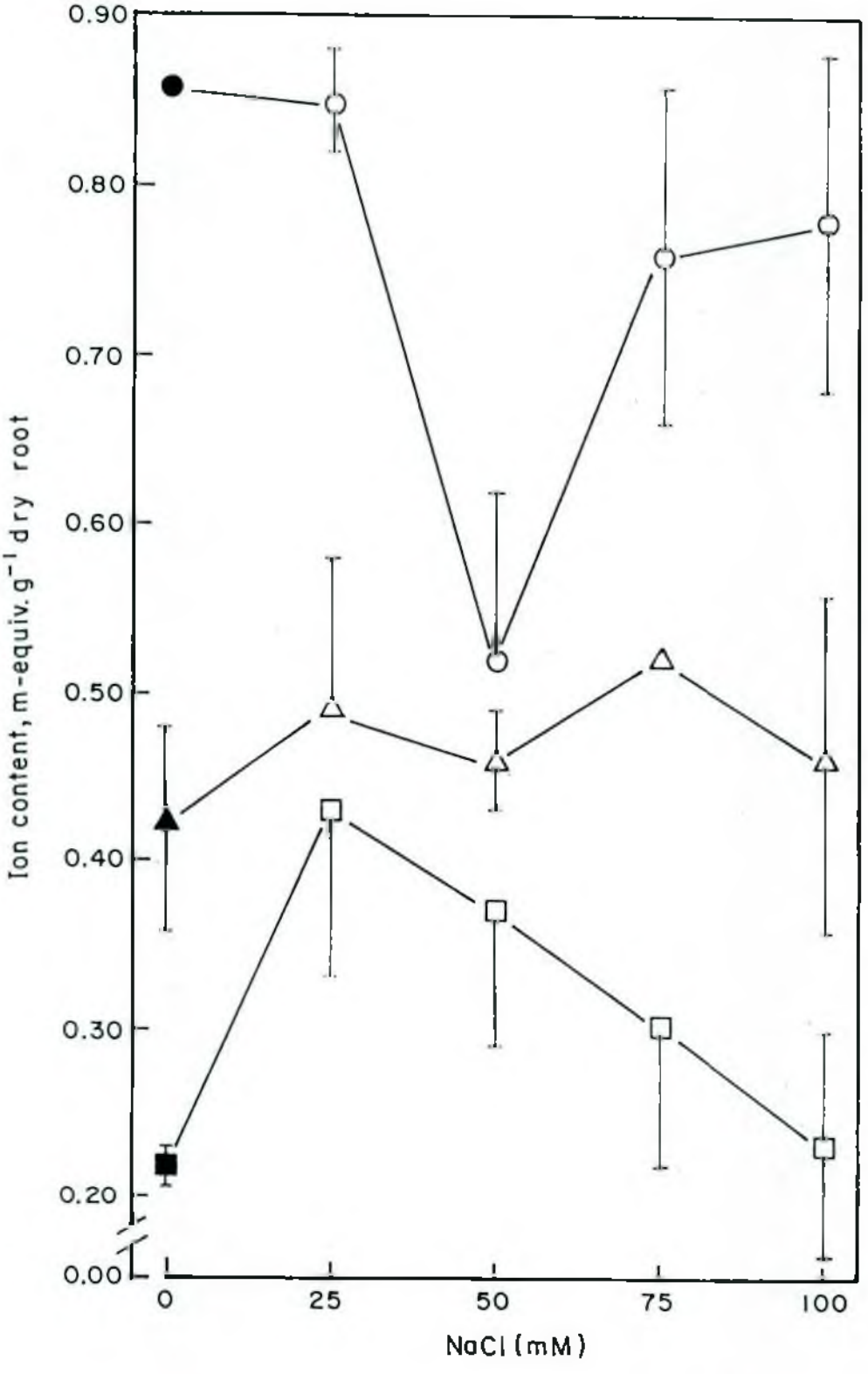

Fig. 31a The effect of salinity on accumulation of Ca^{2+}, Mg^{2+} and Fe^{2+} in the root of 21-day-old intact seedlings of rice cv. BR16. Otherwise as Fig. 27a.

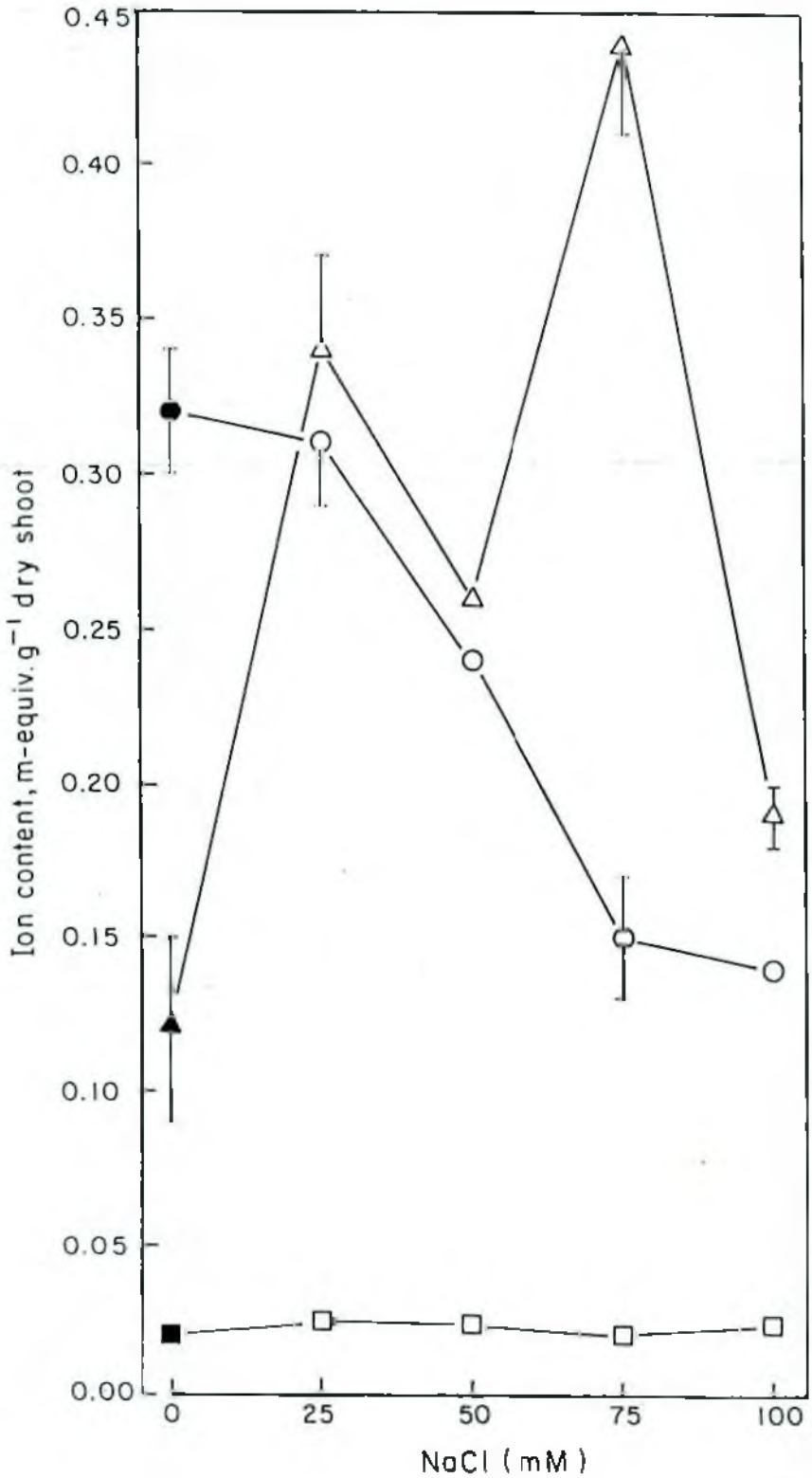

Fig. 31b The effect of salinity on accumulation of Ca^{2+}, Mg^{2+} and Fe^{2+} in the shoot of 21-day-old intact seedlings of rice cv. BR16. Otherwise as Fig. 27a.

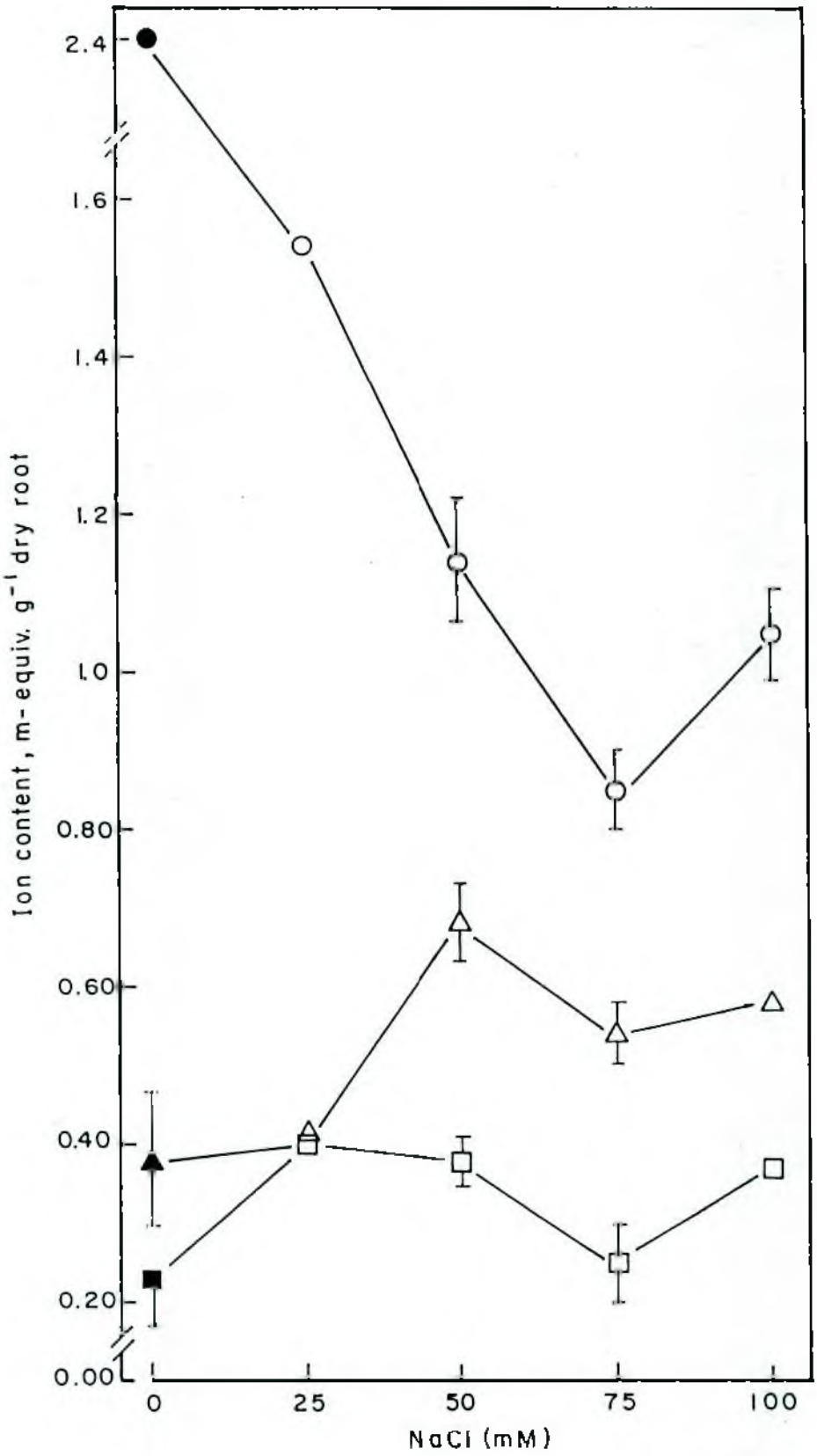

Fig. 32a The effect of salinity on accumulation of Ca^{2+}, Mg^{2+} and Fe^{2+} in the root of 21-day-old intact seedlings of rice var. Pokkali. Otherwise as Fig. 27a.

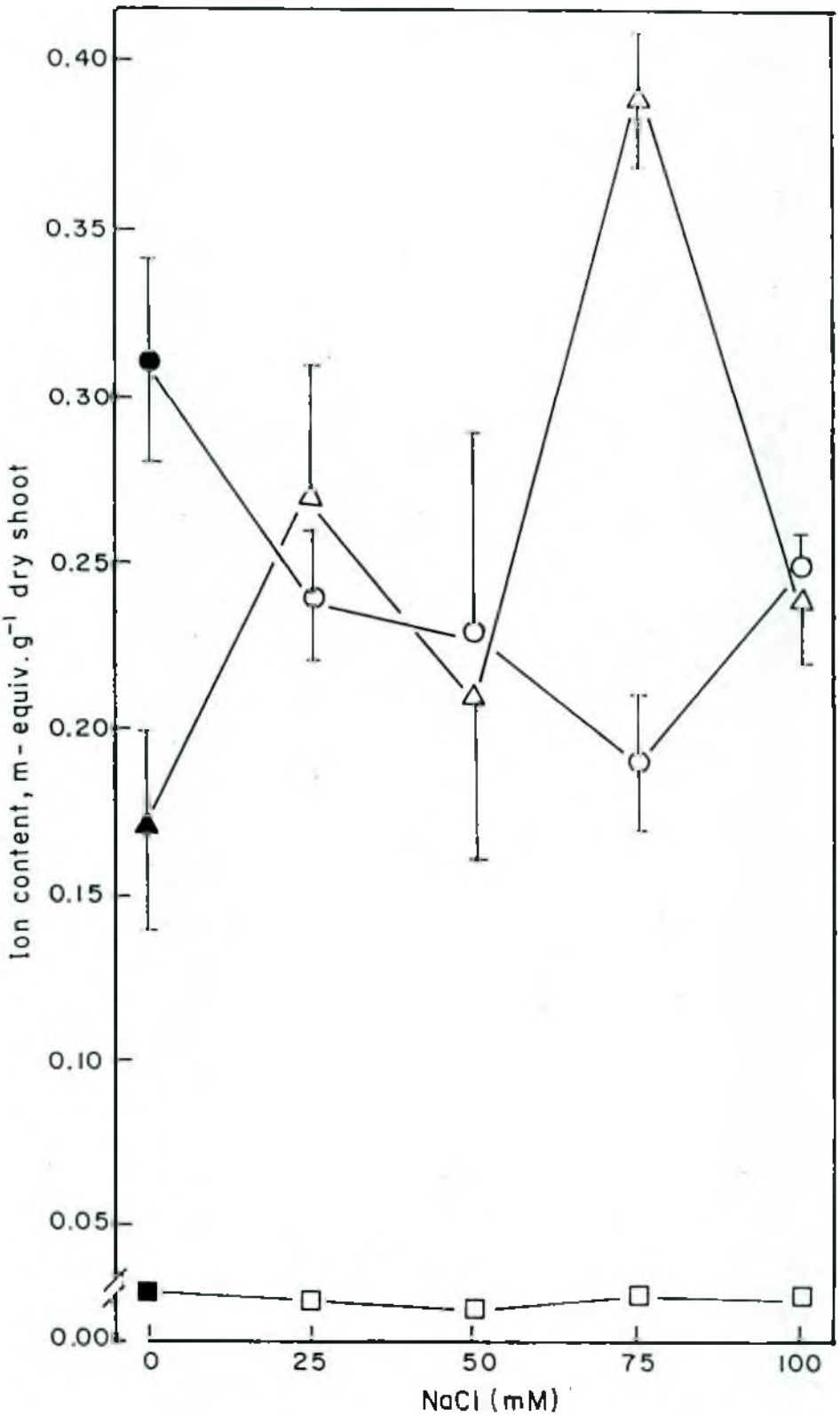

Fig. 32b The effect of salinity on accumulation of Ca^{2+}, Mg^{2+} and Fe^{2+} in the shoot of 21-day-old intact seedlings of rice var. Pokkali. Otherwise as Fig. 27a.

In the root of var. Pokkali, Mg^{2+} content was increased by 5 to 52% at 25 to 100 mM NaCl treatment (Fig. 32a). Similarly, in the shoot of var. Pokkali, Mg^{2+} content was increased by 59% at 25 mM NaCl treatment and this trend was maintained upto 100 mM NaCl treatment (Fig. 32b).

Accumulation of Fe^{2+} in the root of cv. BR16 was increased by 91 to 5% over control at 25 to 100 mM NaCl treatment (Fig. 31a). A 10% increase in the shoot of cv. BR16 was observed at 25 to 100 mM NaCl treatment (Fig. 31b).

In the root of var. Pokkali, Fe^{2+} accumulation was increased by 75 to 60% at 25 to 100 mM NaCl respectively (Fig. 32a). However, in the shoot of var. Pokkali there was no significant effect on accumulation of Fe^{2+} at 25 to 100 mM NaCl treatment as compared to control (Fig. 32b).

5.4 Discussion

The results presented herein show that NaCl salinity decreased Ca^{2+} accumulation in the root and the shoot both of cv. BR16 and var. Pokkali (Fig. 27a, b to 32a, b). Similar result was found in *Atriplex prostata* (Li-wen *et al.* 1997), chickpea (Mumurkar and Chavan 1986), *Vigna radiata* L., *Helianthus annuus* L., *Phaseolus vulgaris* L., *Lycopersicon esculentum* L. and *Atriplex spongiosa* L. (Salim 1989). In wheat, concentration of Ca^{2+} was decreased or not affected by salinity except at high salinity (Hu and Schmidhalter 1997). Astaraei and Chauhan (1994) also found that Ca^{2+} content was decreased with the increase in salinity in wheat. On the other hand, *Ipomoea pescaprae* survived upto 600 mM NaCl. Ca^{2+} content in tissue was increased considerably

with the increase in salinity upto 200 mM NaCl (Venkatesan *et al.* 1997). Salinity-induced inhibition of Ca^{2+} content was also observed in sugar beat, maize, pepper, safflower and sunflower (Lessani and Marschner 1978) and in barley (Yasseen *et al.* 1989) and in guar (Khan *et al.* 1989). On the other hand, Ca^{2+} accumulation was found to increase in *Eichinochloa crussgalli* under salinity (Aslam *et al.* 1987). The decrease in Ca^{2+} content may be due to displacement of Ca^{2+} by Na^{+} in the cell membrane (Cramer *et al.* 1985, 1987).

A decrease in Ca^{2+} level may be associated with the decrease in K^{+} level under salinity stress. This view is supported by Nakamura *et al.* (1992) who found that Ca^{2+} required for maintenance of K^{+} level in the intact mungbean root. It was also reported that in presence of Ca^{2+}, K^{+} ion was increased and Na^{+} was decreased (Jacobson *et al.* 1961) and thus the inhibitory effect of NaCl stress was partially nullified.

Magnesium content was increased in the root and the shoot of both cv. BR16 and var. Pokkali under NaCl salinity (Fig. 27a, b to 32a, b). Similarly, Mg^{2+} content was increased in wheat (Astaraei and Chauhan 1994), *Cyanopsis tetragonoloba* (Khan *et al.* 1989) and in *Amaranthus tricolor* (Matoh *et al.* 1986). On the contrary, Aslam and coworkers (1987) reported that salinity decreased Mg^{2+} content in *Echinochloa crussgalli*. Shannon and coworkers (1998) found that at the highest salinity, in leaf, concentration of K^{+} was decreased by about 40% by salinity, but tissue levels of Ca^{2+} and Mg^{2+} were unaffected. On the contrary, Mg^{2+} accumulation was decreased in sensitive rice variety IR2B observed by Bohra and Kdoerffling (1993). Khan and coworkers

(1992) also observed a decrease in concentration of Mg^{2+} under NaCl salinity stress. Mg^{2+} plays a role in osmotic adjustment to survive under salinity stress (Basset 1980). Magnesium is a constituent of chlorophyll which helps in photosynthesis. Hence, an increase in magnesium in cv. BR16 and var. Pokkali may help the plant to survive under salinity by increasing chlorophyll content.

An increase in Fe^{2+} content was observed in cv. BR16 and var. Pokkali under NaCl treatment (Fig. 27a, b to 32a, b). Venkatesan and coworkers (1997) also observed that Fe^{2+} was increased in *Ipomoea pescaprae* upto salt level (200 mM). It was also reported that salinity caused an increase in Fe^{2+} content in pearl millet (Alam and Naqvi 1991) and wheat (Begum 1994). On the other hand, Padole (1991) observed that high saline content significantly decreased the uptake of Fe^{2+} in wheat (var. HDM1553). Fe^{2+} tended to be diluted in leaves and concentration of Fe^{2+} in root of *Melilotus sagetalis* was not much affected by salinity (Romero and Maranon 1996).

CHAPTER 6

THE EFFECT OF SALINITY ON ACCUMULATION AND DISTRIBUTION OF PHOSPHATE AND NITRATE IN INTACT RICE SEEDLINGS

6.1 Introduction

Phosphate and nitrate are two of the main osmotica in maintaining osmotic potential of the cell sap. Phosphate concentration was not affected by salinity in wheat (Hu and Schmidhalter 1997). Al-Karaki (1997) observed that increased NaCl levels reduced shoot phosphate concentration of many plants. NaCl salt stress induced the maintenance of high phosphate concentration in roots and leaves with age, without significantly diminishing phosphate concentration in developing leaves and reproductive structures of annual sweet clover (Romero and Maranon 1996). It was reported that phosphate accumulation was increased in roots of tomato (Award *et al*. 1990) and in wheat (Padole 1991, Astaraei and Chauhan 1994) under salinity.

Under salt treatment, relatively more nitrate was reduced in the root as compared with nontreated plants of *Ricinus communis* (Peuke *et al*. 1996). Salinity decreased the net uptake rate of NO_3^- in wheat (Botella *et al*. 1997, Hu and Schmidhalter 1997). Khan and coworkers (1998) reported that NO_3^- content was decreased by salinity in *Medicago sativa*. In *Capsicum annum*, total nitrogen contents of the plants were decreased under high salinity condition (Gunes and

Alpaslan 1996). On the other hand, Sagi and coworkers (1997) observed that salinity increased the concentration of NO_3^- and Cl^-. An increase in nitrate accumulation was recorded in roots of *Phaseolus aureus* with an increase in salinity (Huq *et al.* 1987). Venkatesan and coworkers (1997) found that in *Ipomoea pescaprae* NO_3^- contents in tissues increased considerably with the increase in salinity upto optimum salt level.

In this chapter, the effect of salinity on the accumulation and distribution of phosphate and nitrate in intact rice seedlings is reported.

6.2 Material and methods

Plants were grown in solution culture as described in 2.4.5. Samples were collected at 7-day interval upto 21-day. Roots were collected according to the method as described in 2.5. The root and the shoot tissues were dried in an oven for 72 hours as in 2.5.

Nitrate and phosphate ions were extracted as described in 2.6.3 and 2.6.4 respectively. Nitrate content was measured by salicylic acid method (Cataldo *et al.* 1975) as described in 2.6.7 and phosphate content was measured by ammonium molybdate-ascorbic acid blue colour method (Petersen 1997) as described in 2.6.9.

6.3 Results

6.3.1 The effect of NaCl stress on accumulation and distribution of phosphate in 7-day-old intact rice seedlings

Accumulation of phosphate in the root of cv. BR16 was increased by 2.1-fold at 25 mM NaCl treatment and this stimulatory effect was maintained upto 100 mM NaCl treatment (Fig. 33). In the shoot of cv. BR16, a 13 to 17% increase in phosphate content was observed at salinity ranging from 25 to 100 mM NaCl (Fig. 33).

In var. Pokkali, phosphate content in the root was increased by 2-fold at 25 mM NaCl treatment and this stimulatory effect was maintained upto 100 mM NaCl treatment (Fig. 34). On the contrary, phosphate accumulation in the shoot was increased by 36 to 37% following 25 to 100 mM NaCl treatment as compared control (Fig. 34).

6.3.2 The effect of NaCl stress on accumulation and distribution of phosphate in 14-day-old intact rice seedlings

Accumulation of phosphate in the root of cv. BR16 was increased by 71% to 2.9-fold from 25 to 100 mM NaCl treatment (Fig. 35). A 16 to 61% increase in phosphate level was observed in the shoot of cv. BR16 due to salinity treatment (25 to 100 mM) (Fig. 35).

In the root of var. Pokkali, phosphate content was increased by 81% to 25 mM NaCl treatment and this stimulatory effect was maintained upto 100 mM NaCl treatment (Fig. 36). In the shoot of var. Pokkali, phosphate content was

increased by 68 to 59% at salinity ranging from 25 to 100 mM NaCl treatment (Fig. 36).

6.3.3 The effect of NaCl stress on accumulation and distribution of phosphate in 21-day-old intact rice seedlings

Accumulation of phosphate was increased by 28% at 25 mM NaCl treatment and this stimulatory effect was maintained upto 100 mM NaCl treatment (Fig. 37). In the shoot of cv. BR16, salinity stress increased phosphate content from 11 to 9% from 25 to 100 mM NaCl treatment (Fig. 37).

In the root of var. Pokkali, phosphate accumulation was increased by 77 to 59% under NaCl (25 to 100 mM) treatment (Fig. 38). Accumulation of phosphate in the shoot of var. Pokkali was increased by 70 to 93% following 25 to 100 mM NaCl treatment (Fig. 38).

6.3.4 The effect of NaCl stress on accumulation and distribution of nitrate in 7-day-old intact rice seedlings

In the root of cv. BR16, nitrate accumulation was decreased by 30 to 37% at 50 to 100 mM NaCl over control (Fig. 39). Nitrate accumulation in the shoot of cv. BR16 was decreased by 6 to 61% under salinity (25 to 100 mM NaCl) (Fig. 39).

In the root of var. Pokkali, nitrate accumulation was decreased by 22% at 25 mM NaCl treatment and this inhibitory effect was sustained upto 100 mM NaCl treatment (Fig. 40). In the shoot of var. Pokkali, nitrate content was decreased by 31 to 41% under salinity treatment (25 to 100 mM NaCl) (Fig. 40).

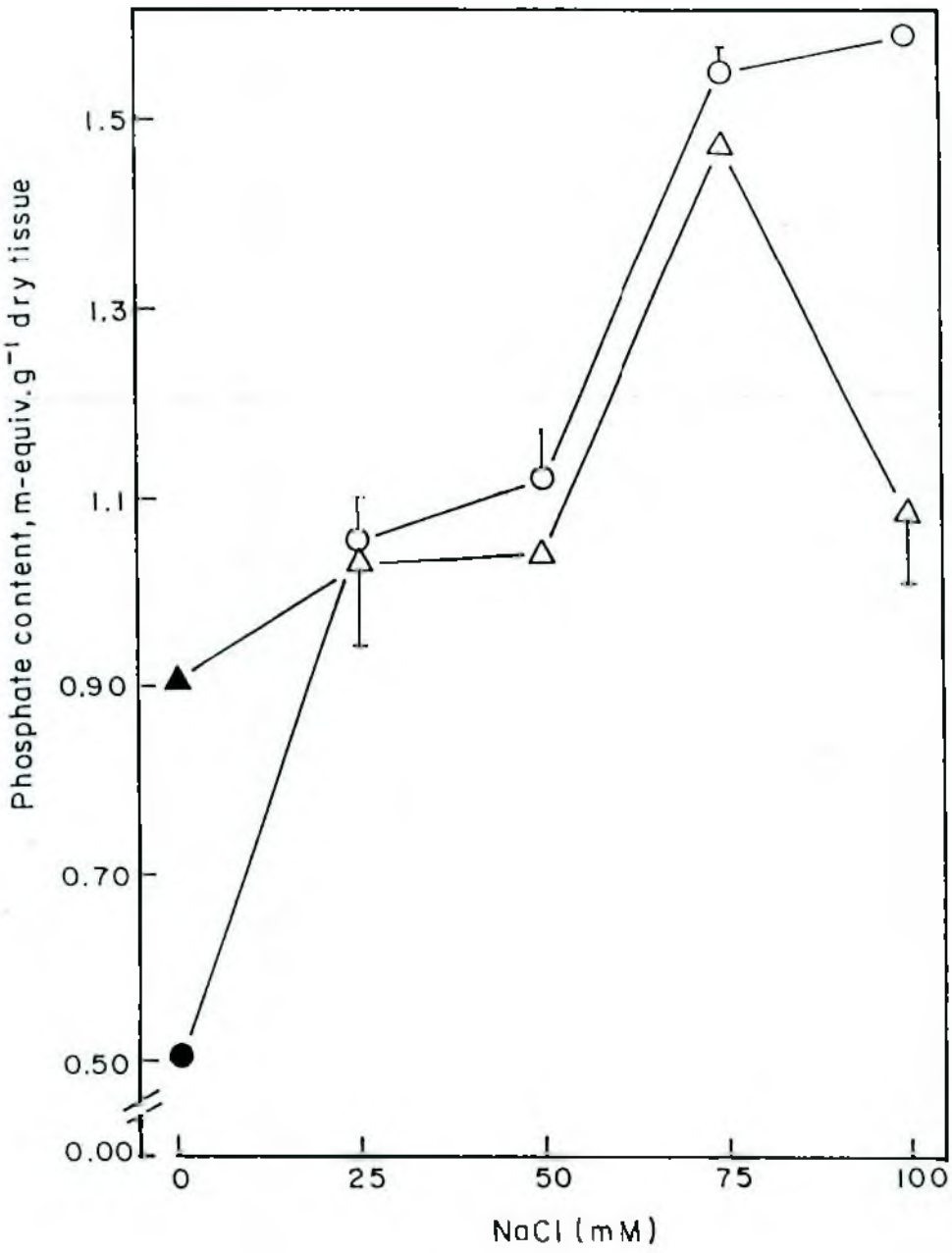

Fig. 33 The effect of salinity on accumulation and distribution of phosphate in the root and shoot of 7-day-old intact seedlings of rice cv. BR16. O root; △ shoot. Solid symbols represent control. Bars represent ± standard error. Each value is a mean of three replicates.

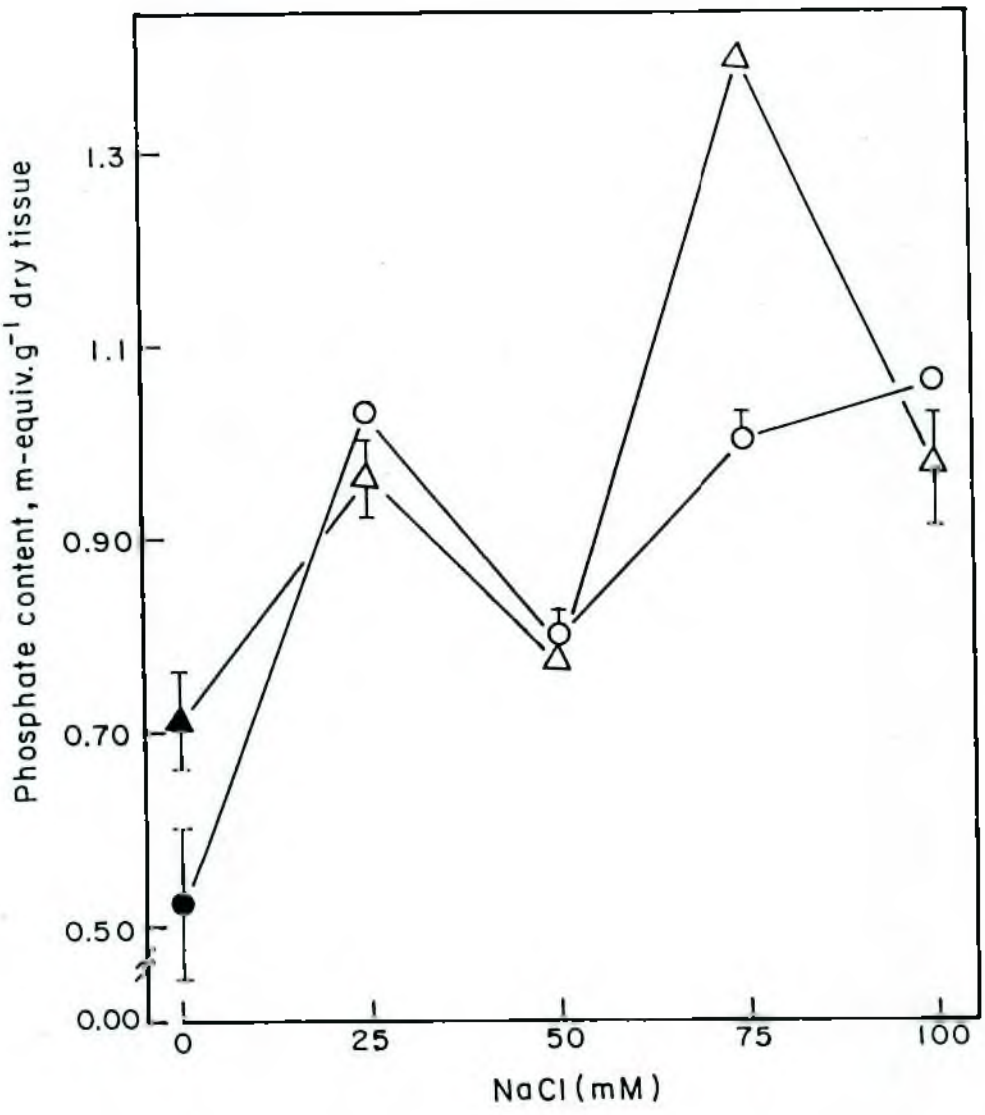

Fig. 34 The effect of salinity on accumulation and distribution of phosphate in the root and shoot of 7-day-old intact seedlings of rice var. Pokkali. Otherwise as Fig. 33.

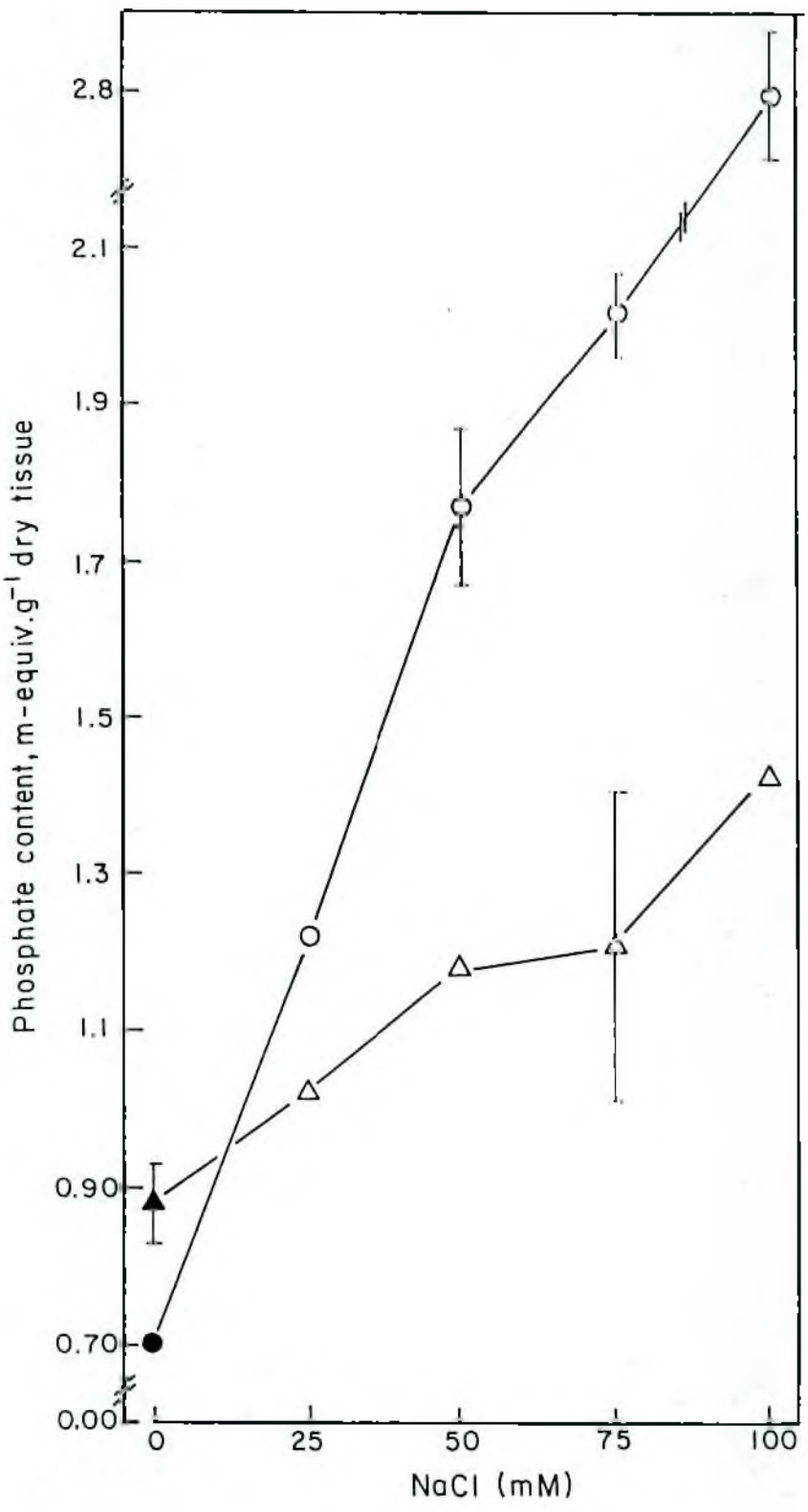

Fig. 35 The effect of salinity on accumulation and distribution of phosphate in the root and shoot of 14-day-old intact seedlings of rice cv. BR16. Otherwise as Fig. 33.

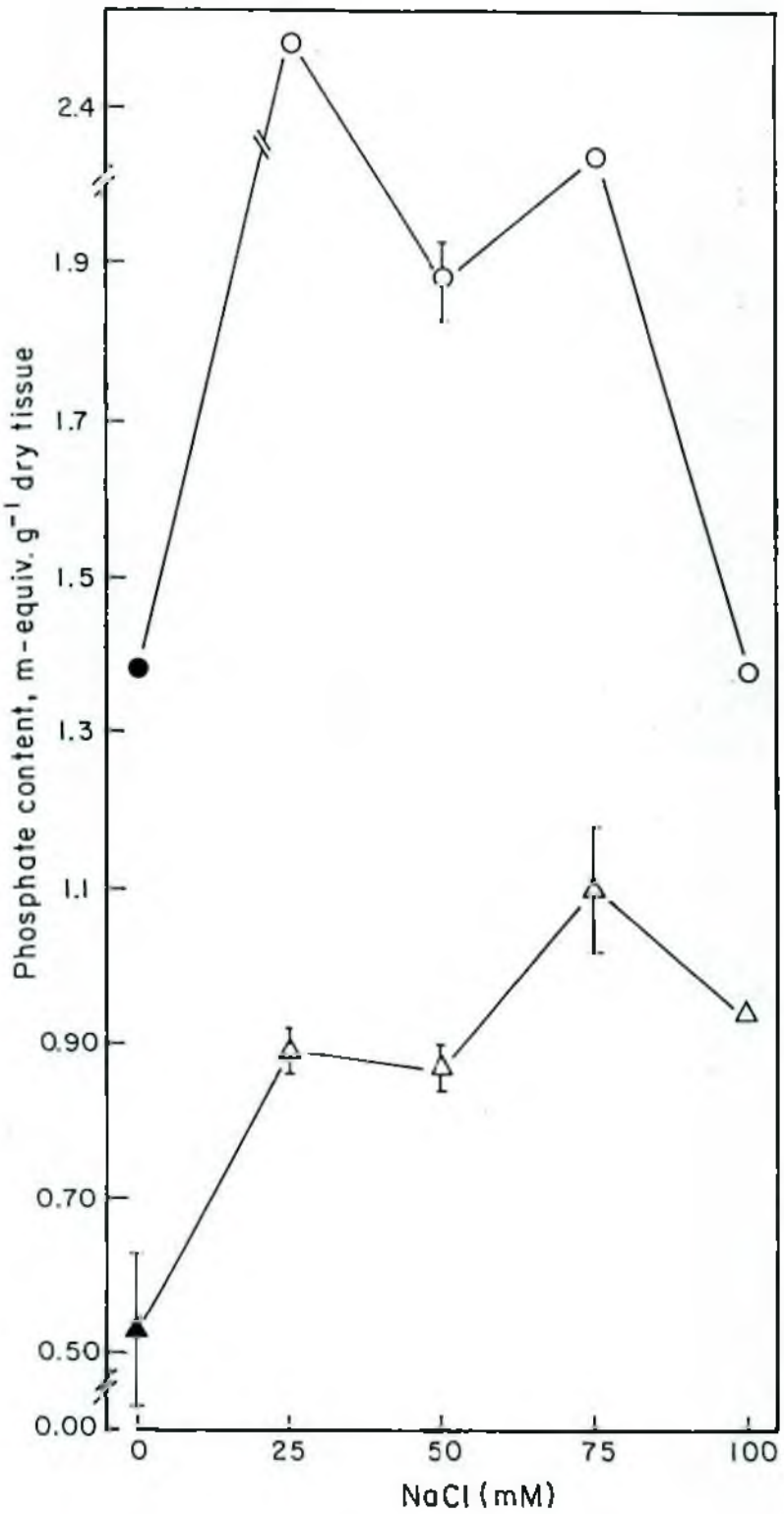

Fig. 36 The effect of salinity on accumulation and distribution of phosphate in the root and shoot of 14-day-old intact seedlings of rice var. Pokkali. Otherwise as Fig. 33.

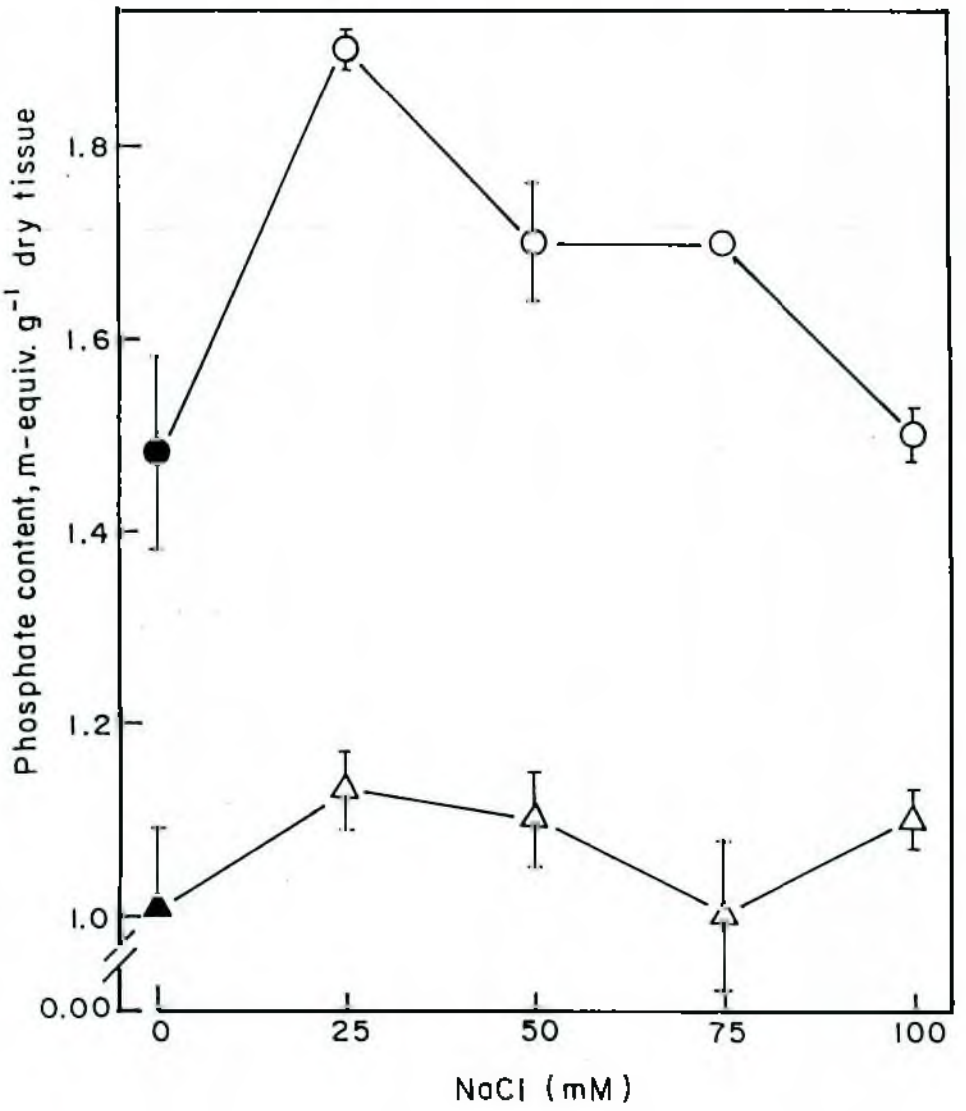

Fig. 37 The effect of salinity on accumulation and distribution of phosphate in the root and shoot of 21-day-old intact seedlings of rice cv. BR16. Otherwise as Fig. 33.

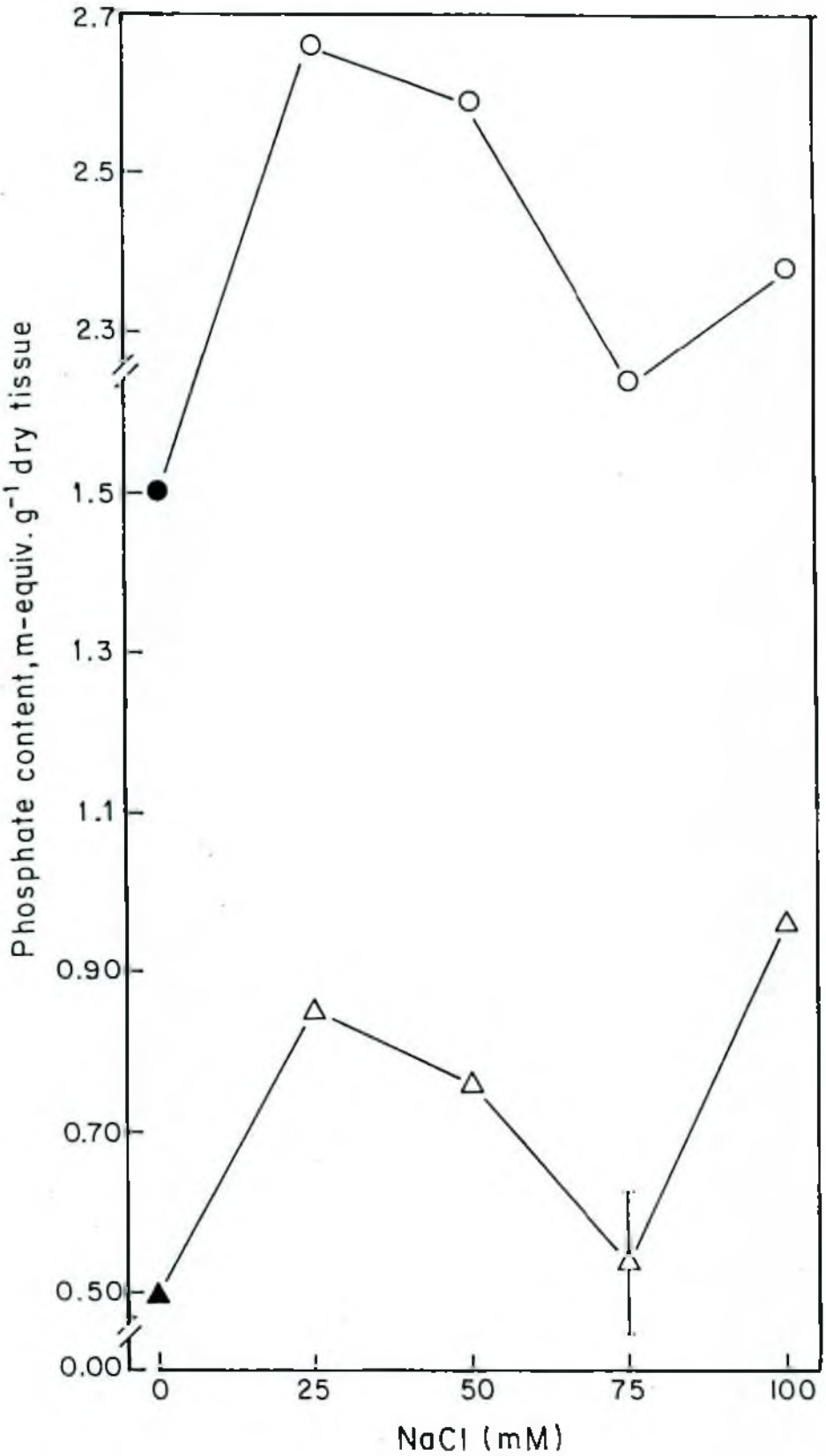

Fig. 38 The effect of salinity on accumulation and distribution of phosphate in the root and shoot of 21-day-old intact seedlings of rice var. Pokkali. Otherwise as Fig. 33.

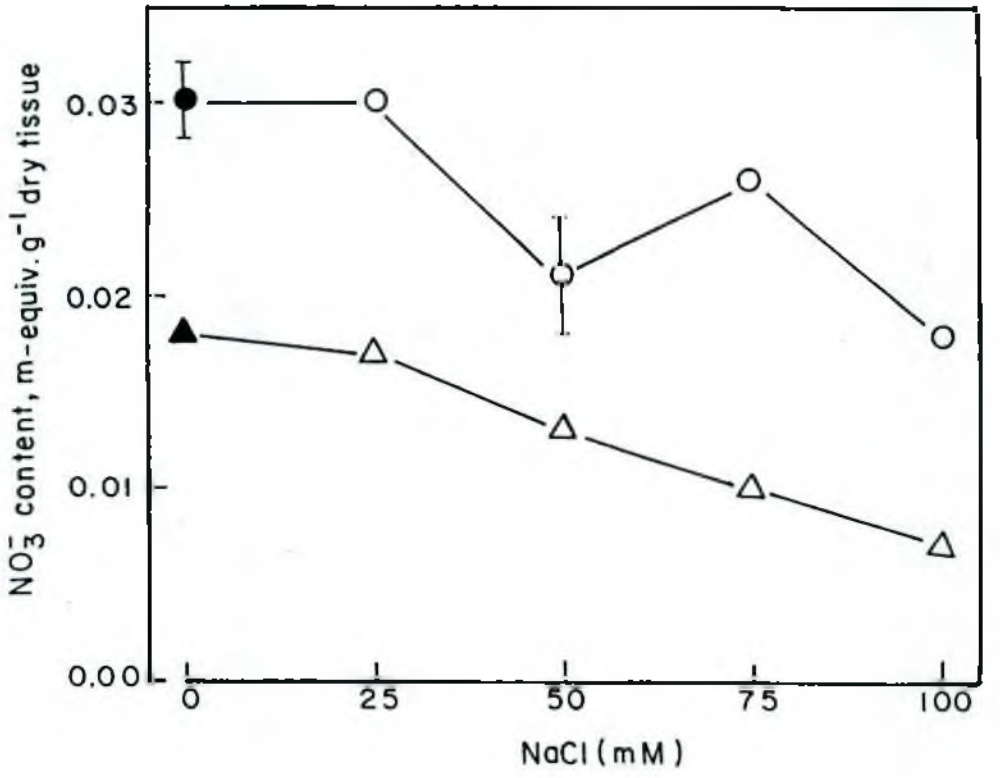

Fig. 39 The effect of salinity on accumulation and distribution of nitrate in the root and shoot of 7-day-old intact seedlings of rice cv. BR16. Otherwise as Fig. 33.

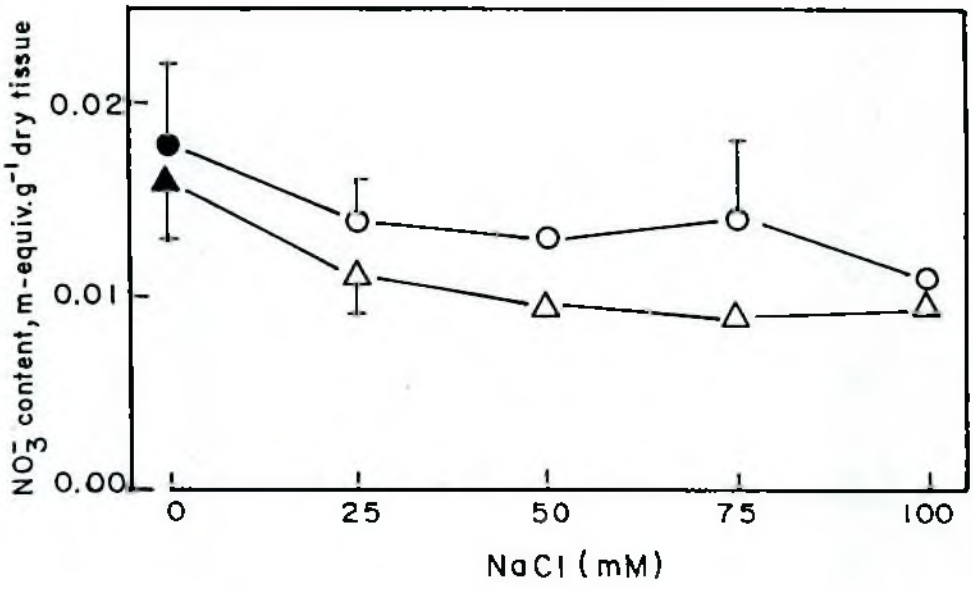

Fig. 40 The effect of salinity on accumulation and distribution of nitrate in the root and shoot of 7-day-old intact seedlings of rice var. Pokkali. Otherwise as Fig. 33.

6.3.5 The effect of NaCl stress on accumulation and distribution of nitrate in 14-day-old intact rice seedlings

In the root of cv. BR16, accumulation of nitrate was decreased by 15% at 25 mM NaCl treatment and this inhibitory effect was observed upto 100 mM NaCl treatment (Fig. 41). A 16 to 63% decrease in the nitrate accumulation was observed in the shoot of cv. BR16 under salinity treatment (25 to 150 mM NaCl) (Fig. 41).

A 24% decrease in the nitrate accumulation was observed in the root of var. Pokkali at 25 mM NaCl and this inhibitory effect was sustained upto 100 mM NaCl (Fig. 42). In the shoot of var. Pokkali, a 23 to 46% decrease in nitrate content was observed at salinity ranging from 25 to 150 mM NaCl (Fig. 42).

6.3.6 The effect of NaCl stress on accumulation and distribution of nitrate in 21-day-old intact rice seedlings

Accumulation of nitrate in the root of cv. BR16 was decreased by 12 to 6% under 25 to 100 mM NaCl treatment (Fig. 43). In the shoot of cv. BR16, accumulation of nitrate was decreased by 29% at 25 mM NaCl and this inhibitory effect of NaCl on nitrate accumulation was maintained upto 100 mM NaCl (Fig. 43).

In the root of var. Pokkali, accumulation of nitrate was decreased by 51 to 63% at salinity ranging from 25 to 100 mM NaCl (Fig. 44). A 29 to 58% decrease in nitrate content was observed in the shoot of var. Pokkali (Fig. 44).

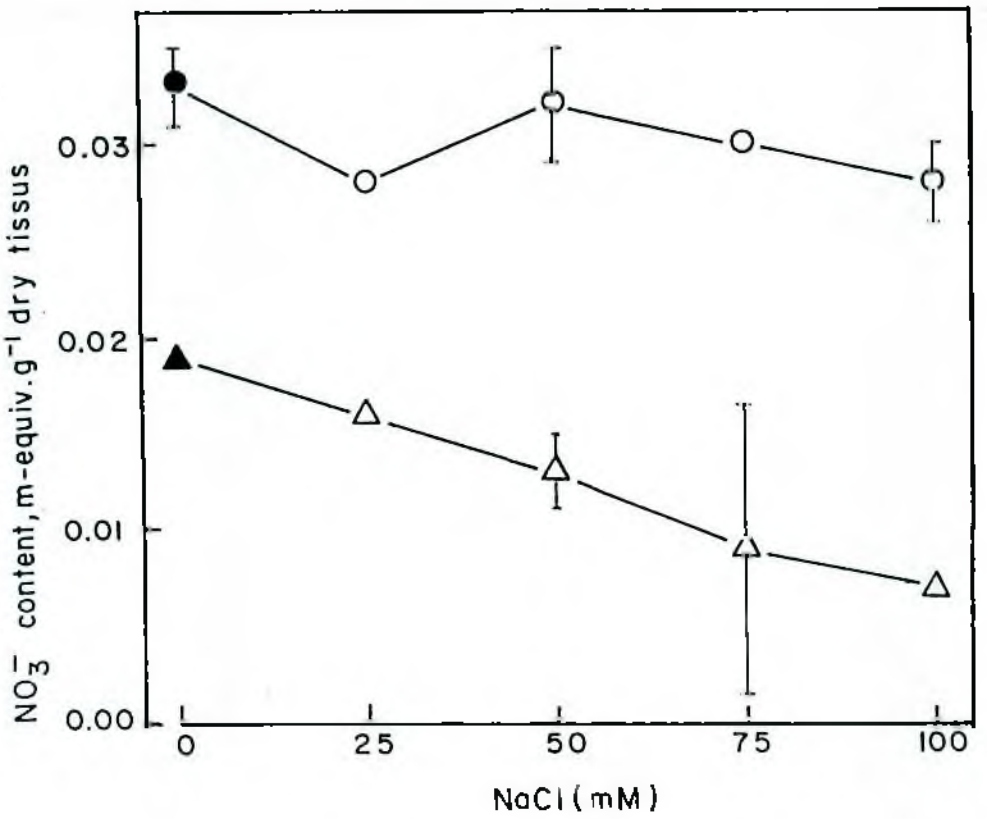

Fig. 41 The effect of salinity on accumulation and distribution of nitrate in the root and shoot of 14-day-old intact seedlings of rice cv. BR16. Otherwise as Fig. 33.

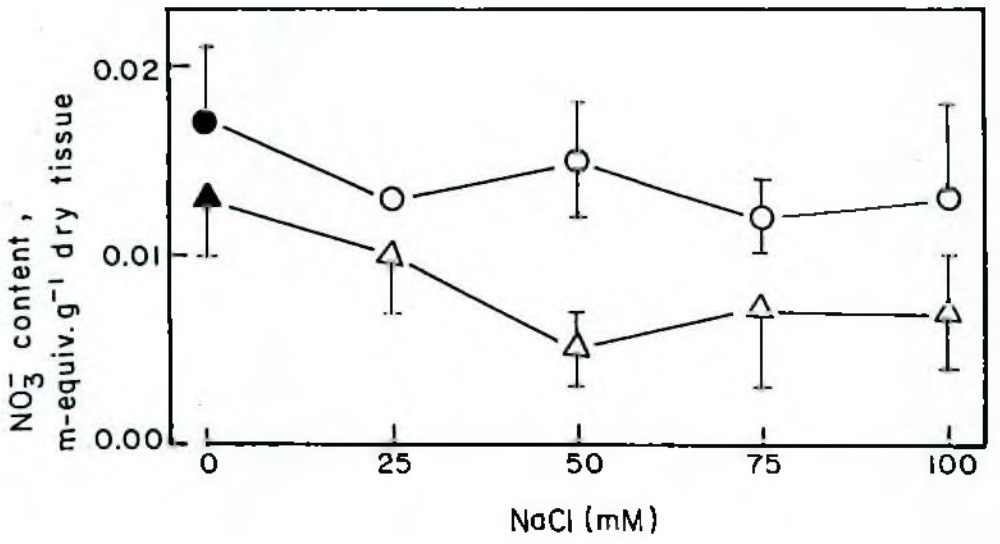

Fig. 42 The effect of salinity on accumulation and distribution of nitrate in the root and shoot of 14-day-old intact seedlings of rice var. Pokkali. Otherwise as Fig. 33.

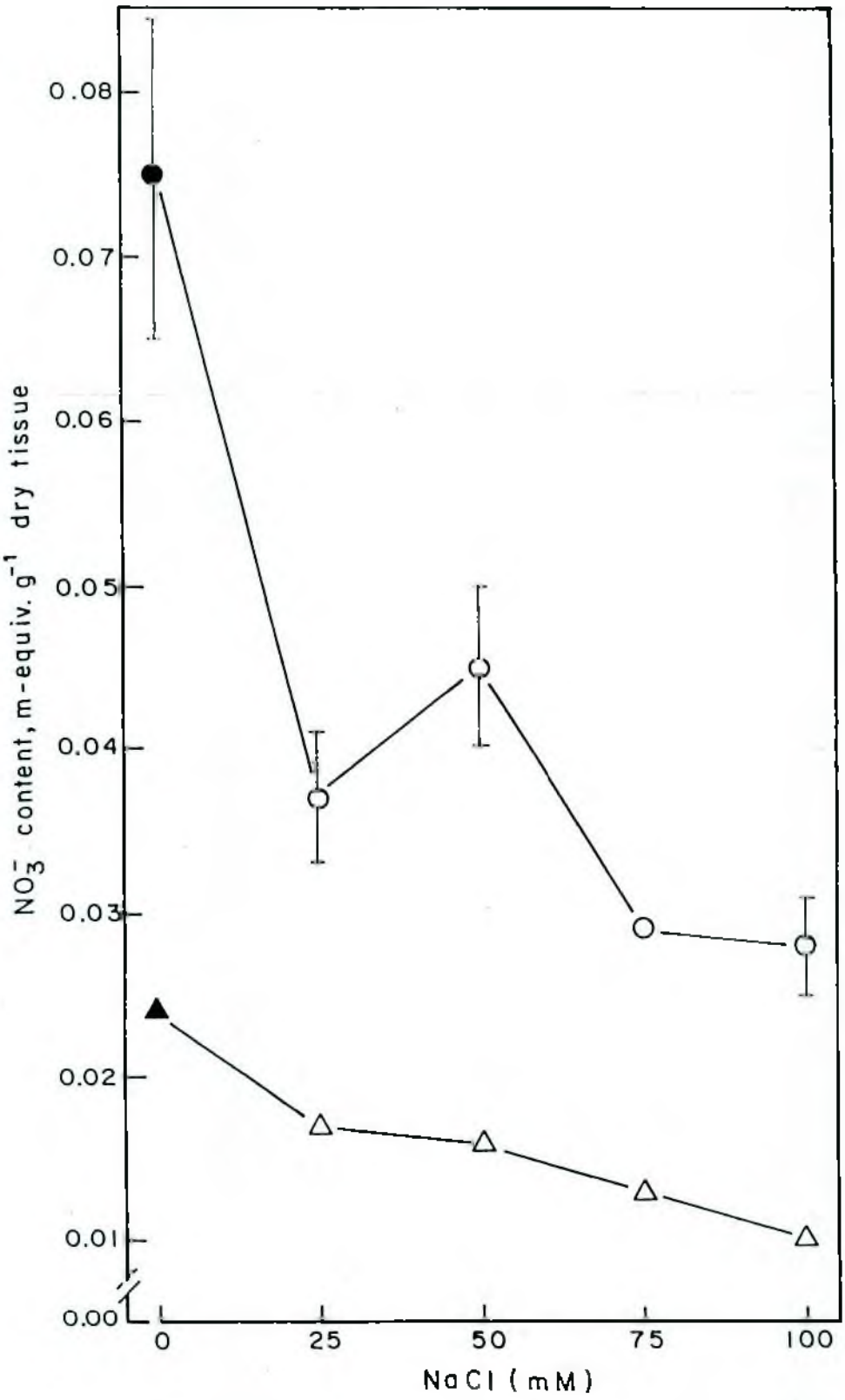

Fig. 43 The effect of salinity on accumulation and distribution of nitrate in the root and shoot of 21-day-old intact seedlings of rice cv. BR16. Otherwise as Fig. 33.

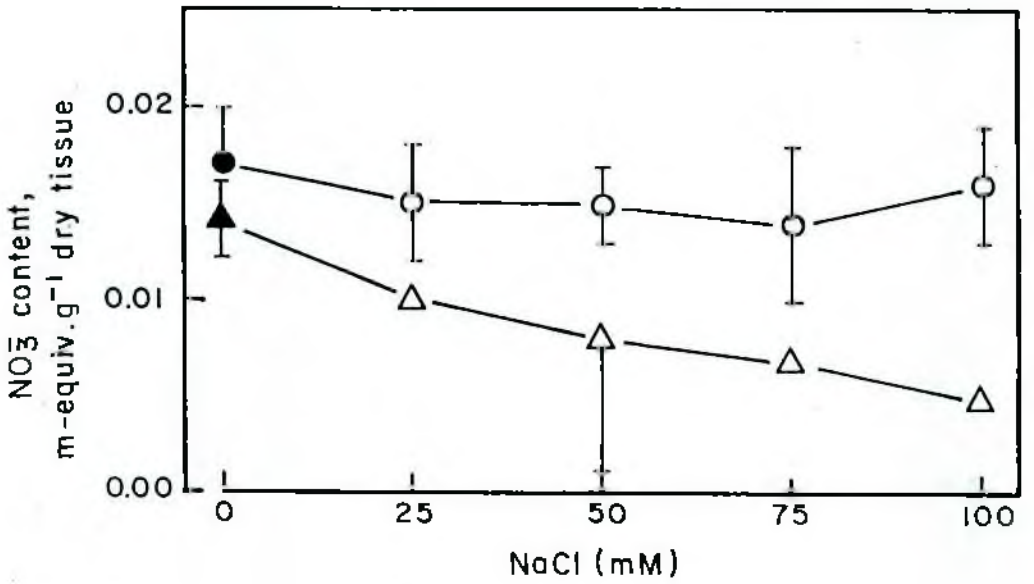

Fig. 44 The effect of salinity on accumulation and distribution of nitrate in the root and shoot of 21-day-old intact seedlings of rice var. Pokkali. Otherwise as Fig. 33.

6.4 Discussion

Accumulation of phosphate was increased with the increase in NaCl salinity both in the root and shoot of cv. BR16 and var. Pokkali of rice (Fig. 33 to 38). This result was supported by Begum (1994) who found an increase in accumulation of phosphate in wheat under salinity stress. Similarly, salinity increased phosphate accumulation in wheat (Astaraei and Chauhan 1994, Yasmin 1999), tomato roots (Award *et al.* 1990) and in barley (Zeb-un-Nessa 1992). Aslam *et al.* (1996) also found that salt sensitive cultivar of rice (IR 1561) highly accumulated phosphate in the shoot. On the other hand, it was reported that increased NaCl levels reduced shoot phosphate concentration of many plants (Al-Karaki 1997). In case of high phosphate plant, phosphate uptake was not affected by salt, but in case of low phosphate plant it was depressed (Treeby and Van Steveninck 1988). Increase in salinity resulted in a decrease of phosphate content in wood apple (*Aegle marmelos* C.) leaves (Shukla and Singh 1996). Similar findings were reported in wheat (Kumar and Singh 1996, Padole 1991).

It is suggested that salinity-induced increase in accumulation of phosphate in cv. BR16 and var. Pokkali (Fig. 33 to 38) may play an important role for adaptation of rice under salinity.

Nitrate content in the root and shoot of rice was decreased with the increase in NaCl salinity (Fig. 39 to 44). Similarly, Botella and coworkers (1997) found that salinity decreased the net uptake rate of nitrate in wheat. Khan and coworkers (1998) also reported that NO_3^- content was decreased by salinity in

Medicago sativa. Salinity reduced NO_3^- accumulation in *Melilotus segetalis* (Romero and Maranon 1996). They found that NO_3^- accumulation was higher in roots of salt-stressed plants than that of control. Salinity-induced decrease in nitrate accumulation was also reported in wheat (Abdul-Kadir and Paulsen 1982, Hu and Schmidhalter 1997, Yasmin 1999) and in leaves of some perennial triticeae (Gorham *et al.* 1986). On the other hand, Begum (1994) found that nitrate content increased in the root of wheat under salinity condition. Venkatesan and coworkers (1997) also reported that NO_3^- content increased with the increase in salinity upto optimum salt level in *Ipomoea pescaprae*. Under NaCl salinity, NO_3^- content was increased in the root of *Phaseolus aureus* but decreased in the shoot. They suggested that the decrease in the nitrate content in the shoot was due to reduced nitrate translocation from root to the shoot (Huq *et al.* 1987).

CHAPTER 7

THE EFFECT OF SALINITY STRESS ON ACCUMULATION AND DISTRIBUTION OF PROLINE IN INTACT RICE SEEDLINGS

7.1 Introduction

Proline is considered as an indicator of stress damage (Hansen and Tully 1979). Proline content was increased with the increase in salinity stress in rice seedling (Dubey and Rani 1989). Similar result were found in white clover seedling (Turner 1990), sorghum (Yang *et al.* 1990b), rice plant (Thomas *et al.* 1992), the leaves of pigeon pea and in some of its wild relatives (Subbarao *et al.* 1990a) and in two varieties Gerick 79 and Bezoslaya 1 of wheat (Tipirdamaz and Cakirlar 1990). Jain and coworkers (1991) also observed that in all the genotypes of *Brassica juncea* proline was increased with the increase in NaCl stress in the medium or irrigated solution.

Lutts and Guerrier (1995) found that under salinity the highest accumulation of proline occurred in calli, roots and younger leaves of cv. IKP of rice. Lutts and coworkers (1996) also observed in salt-resistant rice cultivar Nona Bokra 20 to 50 mM NaCl stress, less proline was accumulated in root and shoot than that of salt-sensitive I Kong Poa.

Kishore and coworkers (1986) observed an initial decrease in proline content in salt-stressed grape vines. But they found an increase in accumulation of proline

content in all the vines that survived under salinity. Salinity increased proline concentration in root, stem and leaf in green gram (Muthukumarasamy and Panneerselvam 1997). Chandrashekar and Sandhyarani (1995) also found that in *Crotalaria striata* DC seeds, proline content increased with increasing salinity levels. Gunes and Alpaslan (1996) observed that in pepper (*Capsicum annum* L.) plants, salinity markedly increased proline contents. Gulati and coworkers (1995) found that under NaCl stress, proline accumulation was greater in the non-selected line than in the resistant one. Veeranjaneylu and Kumari (1989) found that proline content was increased roots and leaves of mulberry under salinity stress and the proline level was higher in roots than that in leaves.

In the present study, the proline content in the root and the shoot of cv. BR16 and var. Pokkali were measured in order to ascertain the relationship between proline level and salinity tolerance of cv. BR16 and var. Pokkali.

7.2 Material and methods

Plants were grown in half-strength Hoagland solution containing 25, 50, 75 and 100 mM NaCl for 21 days. Plants grown in half-strength Hoagland solution were used as control. Growth condition was the same as described in 2.4.5. Proline was extracted in 90% ethyl alcohol (section 2.6.10) and determined by the method used by Troll and Lindsley (1954).

7.3 Results

Proline accumulation was increased by 89% to 30-fold in root of cv. BR16 at 25 to 100 mM NaCl treatment as compared to control (Fig. 45). In var. Pokkali,

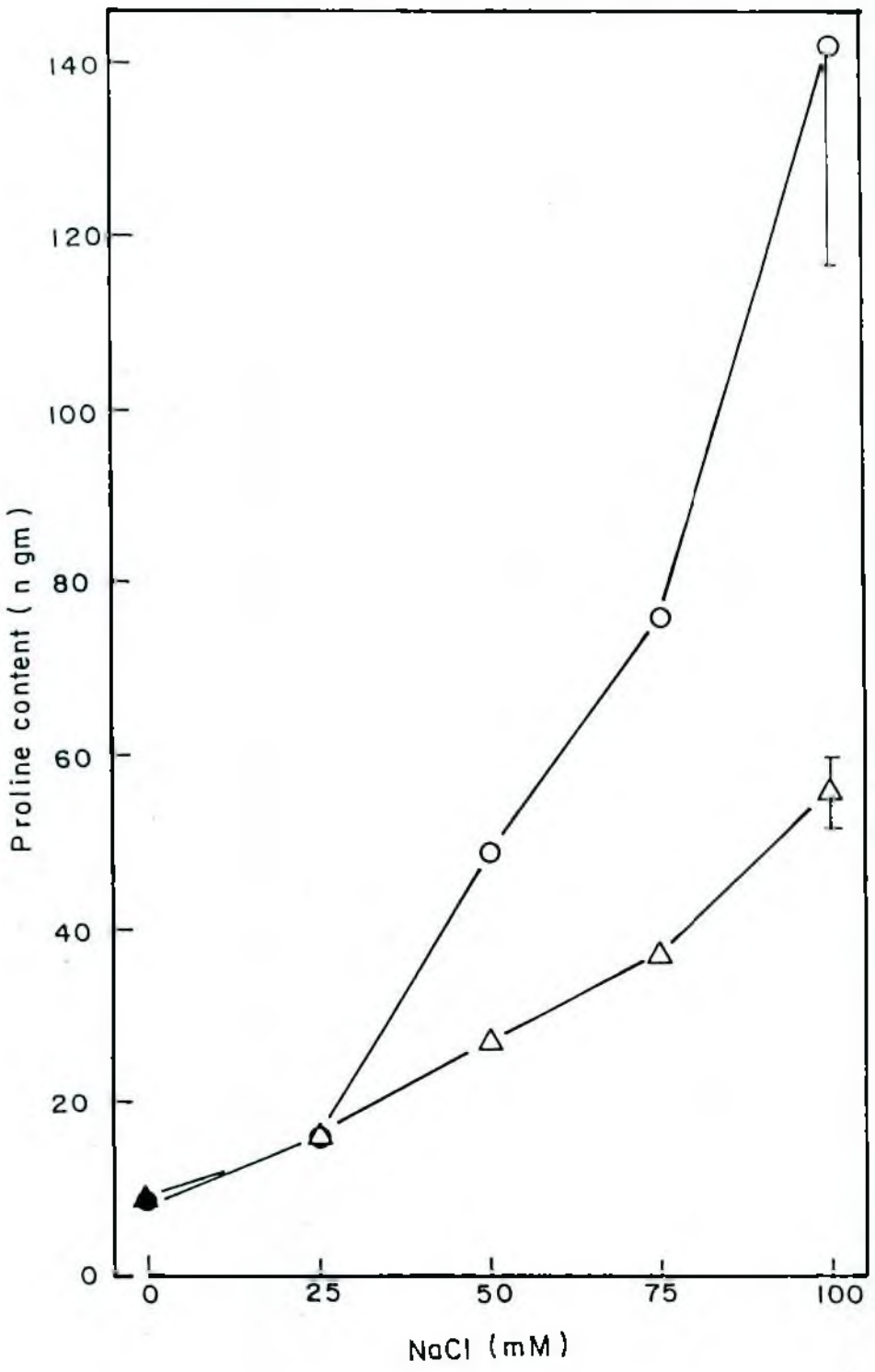

Fig. 45 The effect of salinity on accumulation of proline in the root and shoot of 21-day-old intact seedlings of rice cv. BR16. O root; △ shoot. Solid symbols represent control. Bars represent ± standard error. Each value is a mean of four replicates.

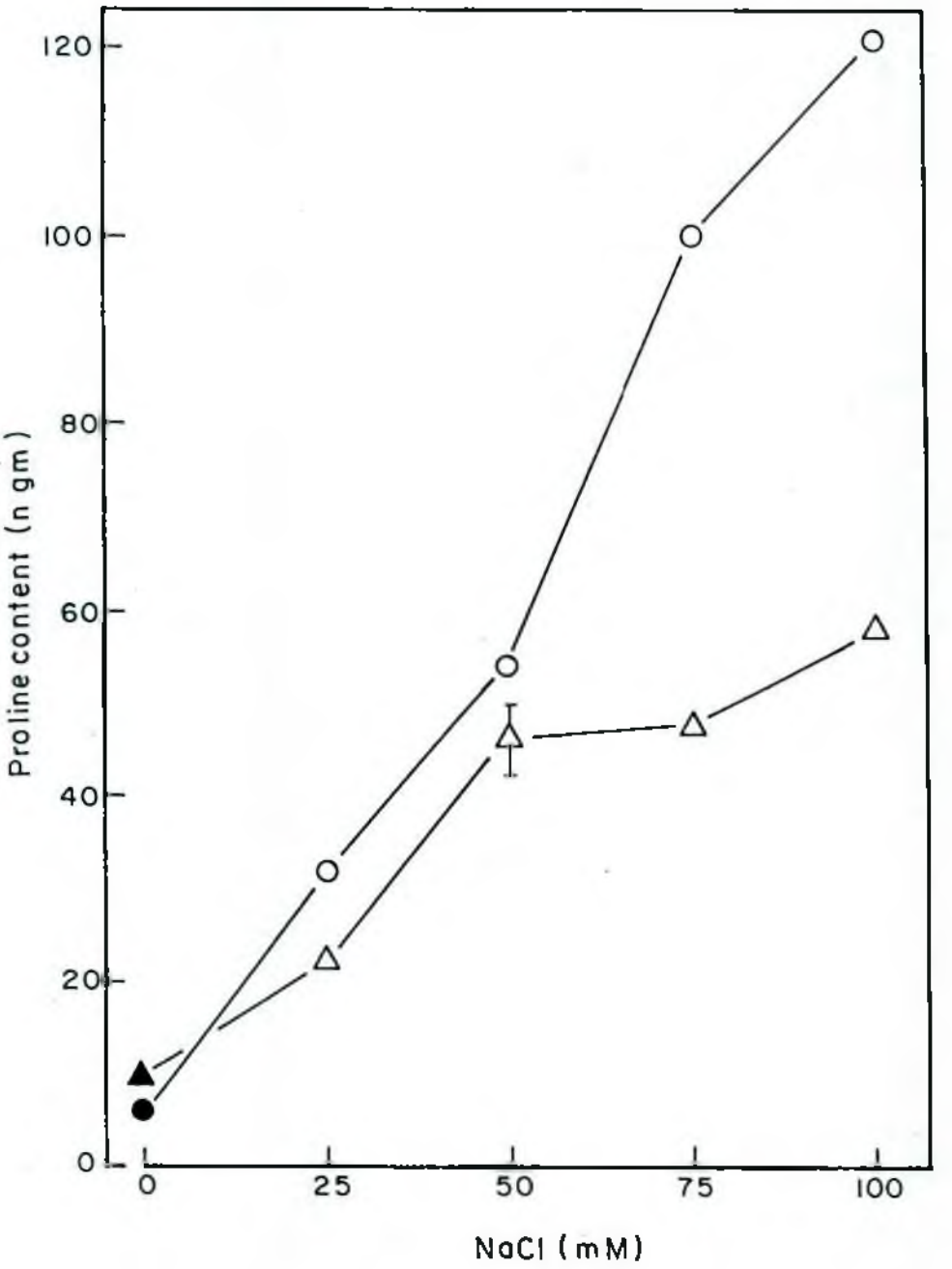

Fig. 46 The effect of salinity on accumulation of proline in the root and shoot of 21-day-old intact seedlings of rice var. Pokkali. Otherwise as Fig. 45.

the accumulation of proline in roots was increased by 8- to 36-fold at salinity ranging from 25 to 100 mM NaCl treatment (Fig. 46).

At 25 mM NaCl stress, proline content in the shoot was increased by 91% and 2.3-fold in cv. BR16 and var. Pokkali respectively (Fig. 45 and 46). Both 50 and 75 mM NaCl treatment caused a 4.1- and 6.3-fold increase in proline level in the shoot cv. BR16 respectively (Fig. 45). Similarly, salinity caused a 6.8-, 18- and 10-fold increase in proline content in the shoot of var. Pokkali at 50, 75 and 100 mM NaCl respectively (Fig. 46).

7.4 Discussion

Salinity caused a dramatic increase in proline level in the root and shoot of both cv. BR16 and var. Pokkali rice (Fig. 45 and 46). The increase in accumulation of proline under salinity in rice (Fig. 45 and 46) plays an important role in maintaining osmotic potential of cell which is necessary for survival of plant under salinity. These results were supported by Quayyum and coworkers (1991) who found that proline content was increased in two rice varieties Pokkali and MI 48. They also found that the amount of proline accumulation was unrelated to the degree of salt tolerance of the two varieties. Begum and Karmoker (1999) also found that salinity caused a dramatic increase in proline level in the root and the shoot of wheat under salinity.

Proline content was increased in wheat under NaCl salinity stress (Tipirdamaz and Cakirlar 1990). Salinity also increased proline content in dicotyledonous plants like *Brassica juncea* (Jain *et al.* 1991), green gram (Muthukumarasamy

and Panneerselvam 1997) *Crotalaria striata* (Chandrashekhar and Sandhyarani 1995) and in *Vigna radiata* (Gulati *et al.* 1995).

Kishore and coworkers (1986) observed that an initial decrease in proline content in salt-stressed grape vines. But they found an increase in accumulation of proline content in all the vines that survived under salinity stress.

In cv. BR16 and var. Pokkali, salinity-induced increase in proline content in the root was more than that in the shoot (Fig. 45 and 46). This result suggested that proline is synthesized in the shoot under salinity and transported to the root. Increased amount of proline in the root may play a vital role in maintaining osmotic potential of the root cells.

Accumulation of proline in the roots and the shoots of rice under salinity stress may be attributed to an increase in abscisic acid because salinity is known to increase both ABA and proline level. For example, abscisic acid level was found to be increased by salinity stress (Roeb *et al.* 1982, Zeevart and Creelman 1988, Baker and Lachno 1987, Kefu *et al.* 1991). Stewart (1980) observed increased accumulation of proline in barley plants treated by ABA. Similarly, an increase in abscisic acid-induced proline accumulation under salinity stress was also observed in barley (Pesci and Beffagna 1986).

CHAPTER 8

CONCLUSION

In this concluding chapter, an overview of the effect of salinity on ion accumulation and distribution and metabolism is made in order to throw some light on the mechanism of physiological adaptation of rice plants under salinity.

Previous chapters reveals that in rice, the germination rate was decreased with the increase in NaCl salinity (Fig. 6, Chapter 3). The inhibitory effect of germination was closely related to the accumulation of large amount of Na^+ and Cl^- in the plumule and radicle with concomitant decrease in K^+ accumulation under NaCl salinity condition. It is reported that the accumulation of large amount of Na^+ and Cl^- in germinating seed is responsible for inhibition of germination under salinity and a decrease in K^+ content in plumule and radicle which had an adverse effect on the growth of the embryo and thus further enhanced the inhibition rate of germination (Begum *et al.* 1992).

Salinity-induced inhibitory effect on germination was alleviated by the interaction of gibberellic acid and salinity (Fig. 7 to 12, Chapter 3). Interaction of gibberellic acid and NaCl salinity decreased Na^+ and Cl^- content of plumule and radicle with a concomitant increase in K^+ content (Table 2 to 5, Chapter 3). The stimulatory effect of germination of gibberellic acid in salinity condition may be due to gibberellic acid-induced decrease in Cl^- and Na^+ content in germinating seeds. Begum and coworkers (1992) suggested that gibberellic acid may cause activation of the uptake of extruded K^+ in place of Na^+ influx.

Salinity increased Na^+ and Cl^- content and decreased K^+ content (Fig. 13a, b to 24a, b; Chapter 4) and thereby decreased the growth of intact rice plant (Fig. 25a, b and 26a, b, Chapter 4). It is suggested that salinity-induced inhibition of growth may be due to the toxic effect of huge amount of Na^+ and Cl^- accumulation and decrease in K^+ accumulation. It is reported that salinity stress caused an imbalance in the availability of mineral nutrients, their uptake and distribution within the plant (Grattan and Grieve 1992). Decrease in K^+ content in saline condition is also a reason for the reduction of growth because K^+ plays an important role in plant metabolism. For example, K^+ acts as an activator for many enzymes involved in metabolic activity such as - photosynthesis, protein synthesis and respiration (Clarkson 1974). It is suggested that higher K^+ content was required for growth under higher salinity. On the other hand, higher concentration of Na^- is required to maintain turgor pressure under salinity, but it could not substitute K^+ requirement (Chow *et al.* 1990). Yeo and Flowers (1986) also suggested that Na^+ and Cl^- were considered to be toxic and appeared to be responsible for inhibition of growth induced by salinity.

Calcium content was decreased at 25 to 100 mM NaCl treatment (Fig. 27a, b to 32a, b, Chapter 5). A decrease in Ca^{2+} level may be associated with the decrease in K^+ level under salinity stress which may cause an inhibition of growth (Chapter 4). This view is supported by Nakamura *et al.* (1992) who found that Ca^{2+} was required for maintenance of K^+ level in mungbean. It was reported that in presence of Ca^{2+}, K^+ content was increased and Na^+ was

decreased (Jacobson *et al.* 1961) and thus the inhibitory effect of NaCl stress was partially nullified. Because it is well established that Ca^{2+} plays an important role in the maintenance of K^+ transport (Sopandie *et al.* 1990). However, divalent cation like Ca^{2+} is an essential element for plant growth and development (Epstein 1972). Calcium is important for permeability characteristics of cell membrane and maintaining ionic balance under salinity stress. So, calcium can prevent the adverse effect due to salinity stress (Epstein 1961, Lauchli and Epstein 1970, Cramer *et al.* 1986). The external supply of Ca^{2+} caused an increase in K^+ content with a concomitant decrease in Na^+ (Subbarao *et al.* 1990a, Sopandie *et al.* 1990). It may be suggested that under saline condition plants may survive if the accumulation of Ca^{2+} content is increased through K^+ influx.

Under salinity condition, Mg^{2+} content was increased both in cv. BR16 and var. Pokkali (Fig. 27a, b to 32a, b, Chapter 5). It is well known that magnesium is a constituent of chlorophyll which helps the plant in photosynthesis. Furthermore, Mg^{2+} plays an important role in osmotic adjustment in salinity stress (Basset 1980). Hence, stimulation of Mg^{2+} content may help the plant survive under saline condition. Similarly, salinity increased Fe^{2+} content in cv. BR16 and var. Pokkali (Fig. 27a, b to 32a, b, Chapter 5). Epstein (1972) reported that Fe^{2+} is a constituent of respiratory enzyme, i.e. cytochrome. Thus, an increase in Fe^{2+} content may increase the respiratory rate of plant which may indirectly help the plant survive under salinity condition by increasing the production and turnover of cytochrome.

It was observed from the previous result that phosphate content was increased in the root and the shoot under NaCl treatment (Fig. 33 to 38, Chapter 6). Phosphate is one of the osmotica which maintain osmotic potential of cell sap. For this reason, phosphate accumulation may also play an important role for adaptation of rice under salinity.

Salinity inhibited nitrate accumulation in rice (Fig. 39 to 44, Chapter 6). Decrease in nitrate content may lead to the reduced growth of plants under salinity treatment because it is well established that nitrate is one of the principal osmotica in cells (Huq and Larhar 1983b, Blom-Zandstra and Lampe 1983, Veen and Kleinendorst 1985). Romero and Maranon (1996) reported that salinity reduced NO_3^- accumulation probably by chloride impeding NO_3^- uptake.

Salinity increased proline level both in cv. BR16 and var. Pokkali (Fig. 45 and 46, Chapter 7). Increased accumulation of proline under NaCl salinity may help the plant in maintaining the intracellular osmotic potential of cells and thus plants may survive under the salinity condition. Proline acts as a balancing osmoticum across the tonoplast because cations are located in the vacuoles and proline in the cytoplasm (Weimberg *et al.* 1982).

Further studies on the effect of salinity stress on abscisic acid level and its correlation with ion transport, organic acid level and different enzymes related to metabolic activities of plants may help in understanding the mechanism of salinity tolerance.

Finally, it is apparent from the ion accumulation pattern in rice plants under salinity condition that both the varieties of rice adjust the concentration of various ions in cells in order to establish an intracellular osmotic adjustment for survival. Increase in proline level also plays a vital role in this respect.

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