THE POTENTIAL FOR IMPROVING SALT TOLERANCE IN MINOR MILLETS, PENNISETUM AMERICANUM (L.) LEEKE (PEARL MILLET) AND ELEUSINE CORACANA (L.) GAERTN (FINGER MILLET), AND ERAGROSTIS TEF (ZUCC.) TROTTER (TEF)

by

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ABSTRACT

The response to increasing NaCl concentration of seedlings of 25 accessions of Ethiopian land races of each of *Pennisetum americanum* (L.) Leeke (pearl millet) and *Eleusine coracana* (L.) Gaertn (finger millet), and 15 accessions of *Eragrostis tef* (Zucc.) Trotter (tef), was examined after two week's growth in NaCl solution culture. Increasing NaCl concentration significantly reduced seedling root lengths, and there was considerable variation within, and between accession means within each species.

Analysis based upon non-linear least square inversion method, using root length data, revealed significant differences in accessions of *P. americanum* and *E. tef* on the basis of the estimated salinity threshold, C_t, the NaCl concentrations at which root length begins to decrease. C_t did not differ significantly between *E. coracana* accessions. Estimates of C_{50} and C_0, minimum concentrations causing a 50% decrease in root length, and zero root growth respectively, revealed differences between and within accessions for all three species. Overall, finger millet was more tolerant than tef, which was more tolerant than pearl millet.

The sensitivity of accessions of pearl millet to salinity was assessed in a sand culture experiment. Sensitivity varied with plant age. Plant height and percentage live leaves were least sensitive during growth stage 1 (the vegetative stage) and most sensitive during growth stage 3 (the maturation stage). Adult plants of pearl millet accession 221726 were shown to be moderately tolerant based on measurements of plant height, percentage live leaves, and root, stem, leaf sheath and leaf blade dry weights. Combining seedling and adult plant responses, this accession would seem to have potential for enhancement of salt tolerance in pearl millet. In contrast accessions 203659 and 203662 were the most salt-sensitive accessions, based on overall growth parameter measured.

The genetic basis of salinity tolerance in *P. americanum* was investigated following the diallel crossing procedure and analysis. Tolerance at both 75 and 175 mM NaCl was due to both additive and dominance genetic effects with some indication of overdominance. At both salinity levels dominance effects were predominant, and towards increased tolerance. The tolerant accessions Kitui Local and 93611, and the sensitive accessions 203659 and 203662 had respectively the highest and lowest gca (general combining ability) estimates, whilst at 175 mM accession 221726 had the highest gca estimate.

Patterns of accumulation of metabolites in tolerant and sensitive accessions were compared in an attempt to identify a physiological marker associated with salt tolerance in pearl millet. Amounts of amino acids, proline, polyols, and water soluble carbohydrates in roots and shoots did not differ between tolerant and sensitive accesions. Na^+ content only was significantly greater in shoots of

sensitive accessions grown in NaCl. In NaCl+$CaCl_2$ (1:1 by weight) significantly higher chloride content was found only in roots of a sensitive accession.

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GENERAL INTRODUCTION

One of the major barriers to the productivity in many environments of the world is the harmful effects of salt stress to plant life. Yet natural saline areas, salt marshes of temperate latitudes, mangrove swamps of subtropics and tropics, interior salt marshes, salt deserts, and smaller areas around salt springs, all support some form of life (Chapman, 1975). Strikingly there exists a great diversity of plants in these seemingly unfit environments which are potentially rich for industrial applications (Zaborsky, 1980).

Arid and semiarid land of the world account for 36% of the available land, equivalent to 134,600,000 km^2 (Zaborsky, 1980). These zones are subject to distinct climatic features. They are hot and dry, with varying rainfall, arid areas receiving 100 - 200 mm per annum, and semi-arid areas receiving 250 - 600 mm with evaporation exceeding precipitation (Kernick, 1986). Kernick added that in these areas rainfall is extremely variable and fluctuates markedly from year to year; extreme temperatures occur in summer and winter; evapotranspiration is high. Thus it is almost inevitable that irrigation is vital for sustainable agricultural production in these areas. However irrigation water almost invariably contains a low level of soluble salts originating from chemical weathering of underlying dissolving rocks (Epstein *et al.*, 1980). With high rates of evaporation and transpiration and/or because the water table may be near the surface, soil water is drawn to the surface by capillarity bringing with it dissolved salts which are left behind as the moisture evaporates. Annual precipitation is insufficient to leach these salts down to the free water, and such a process can rapidly lead to excessive salt accumulation at or near the soil surface.

Nearly all aspects of agricultural technology in those large areas of the world that depend on irrigation seem to work against the maintenance of systems unencumbered by excess salt: evaporation of water from canals, reservoirs, and fields increases salt concentration, soil amendments and fertilisers add to it, and cultural practices may compact the soil and impede the downward percolating of water, causing retention of salts in the root zone (Kingsbury and Epstein, 1984).

Salinity is the presence of an excessive concentration of soluble salts that suppresses plant growth (US Salinity Laboratory Staff, 1954) with a threshold beyond which growth is retarded. The threshold varies with species and between cultivars within species (Maas and Hoffman, 1977). Saline soils are dominated by NaCl, but may contain a variety of other salts, Na_2SO_4, $MgSO_4$, $CaSO_4$, $MgCl_2$, KCl and Na_2CO_3 (Flowers, 1972). Single salt salinity or alkalinity rarely occurs in nature, and salts, if present as a mixture, interact strongly with each other. Salinity however can damage the plant through its osmotic effect, which is equivalent to decrease in water activity, through specific effects of ions, and by disturbing the uptake of essential nutrients, altogether causing stunted growth, rolling of leaves, white leaf tips, white blotches in the laminae, drying of the older leaves, and poor root growth, and ultimately limiting yield or resulting in total plant death.

Soil salinity may be quantified from assessment of the total amount of exchangeable cations that a soil can retain, designated the cation exchange capacity. It is often convenient to express the relative amounts of various exchangeable cations present in a soil as a percentage of the cation exchange capacity. The soluble cations which give saline soils their characteristics are calcium, magnesium, sodium and potassium. The predominant anions are bicarbonate, carbonate, sulphate and chloride. Depending on which of these factors is/are present, soils can be divided broadly in to saline and sodic as follows (Fitzpatrick, 1980). Saline: Electrical Conductivity (EC) of a saturated extract > 4 mmhos cm^{-1}, exchangeable sodium < 15%, and pH < 8.5. Sodic soils have an EC of a saturated extract < 4 mmhos cm^{-1}, exchangeable sodium > 15%, and pH > 8.5. Nevertheless, some workers, for example Milijikovic (1965), as tabulated by Fitzpatrick (1980), have provided a separate scale for salinity and alkalinity as follows:

a) Degree of salinity

Slightly saline EC 2 - 4 mmhos cm^{-1}

Moderately saline EC 4 - 8 mmhos cm^{-1}

Strongly saline EC 8 - 15 mmhos cm^{-1}

Very strongly saline EC > 15 mmhos cm^{-1}

b) Degree of alkalinity

Slightly alkaline < 20% exchangeable sodium

Moderately alkaline 20 - 50% exchangeable sodium

Strongly alkaline > 50% exchangeable sodium

FAO-UNESCO (1974) mapped soils of the world and the distribution of saline lands was extrapolated accordingly by Ponnamperuma (1984). He however combined several factors in characterising saline soils. His variables include salt source; nature and content of salts; lateral, vertical, and seasonal distribution of salts; soil pH; nature and content of clay; relief; temperature and soil toxicities. Some of these characteristics are shown in Table 1.1. Estimates of world saline land areas are given in Table 1.2. Of the total irrigated land about one-third had by 1980 deteriorated because of salinisation according to Epstein *et al.* (1980).

Salinity has been one of the most serious environmental constraints associated with arid-zone agricultural systems since ancient time (Rains, 1979; Downton, 1984). One area examined was Mesopotamia, the once fertile alluvial plain between the Tigris and Euphrates rivers. Irrigated agriculture was the foundation upon which the Mesopotamian empire was built and sustained. Major devastating salt accumulation occurred when canal irrigation from the river Tigris increased flooding, seepage, and over-irrigation, all of which raise ground water levels (Downton, 1984). These led to the breakdown of the Sumerian civilisation in the basin of the Tigris - Euphrates rivers of ancient Mesopotamia, fertile soils becoming so laden with salts that crops withered and died as did the culture which they supported.

Thus the problem of secondary salinisation is more serious especially in arid and semi-arid regions. When salts from irrigation water build up in soil it eventually kills crop plants and renders farm land useless. In the Punjab, India, 21% of 51,000 km^2 agricultural land had been seriously affected by salinity up to 1960 while some 10% had been lost from agriculture and land was going out of use at the rate of 400 km^2 per year (Flowers *et al.*, 1977). Such losses are due primarily to irrigation, the

Table 1.1. Some characteristic of saline soils (after Ponnamperuma, 1984)

Characteristic	Range
Texture	Sandy to clayey
pH	2.5 to 11
E.C. (dS m^{-1})	4 to > 30
Salt (%)	0.1 to 5
Organic C (%)	< 1 to > 30
Fertility	Very low to moderately high
Clay mineralogy	2:1 types to hydrous oxides

Table 1.2. Distribution of saline lands (FAO/UNESCO, 1974; Ponnamperuma, 1984)

Region	Area (million ha)		
	Strongly saline	Moderately saline	Total
Africa	16.5	37.0	53.5
Australia	16.6	0.79	17.4
Mexico and Central America	0.24	1.72	1.96
North America	0	6.2	6.2
South America	10.5	58.9	69.4
North and Central Asia	22.5	69.2	91.7
South Asia	47.2	36.1	83.3
Southeast Asia	0	20.0	20.0
Total	113.5	230.0	343.5

scale of which can be judged from its current consumption of four-fifths of the world's total water during the farming season (Flowers *et al.*, 1977). Also there is a great increase in salinity problems in other regions of the world under irrigation agriculture associated with river systems. The basin regions of the western San Joaquin Valley of California suffer from salinity as a result of intensive irrigation (Epstein, 1980; Norlyn, 1980). An estimated 186,300 ha are already affected by saline water tables (San Joaquin Valley Interagency Programme, 1979; Johnston *et al.*, 1982). Poor irrigation procedures are expected to cause loss of a further 28,350 ha due to soil salinity by the year 2010 (California Department of Water Resources, 1984). Yields have already fallen by 10% or more, a figure that can be put in perspective when it is realised that a loss of 1% in California's irrigated crop production is equivalent to a loss of $80 million annually (Witt, 1985). Similarly, loss of crops in Pakistan attributable to salinity is equivalent to £300 million every year, where approximately 67% of all irrigated land is affected by salinity at least to some degree (Wyn Jones and Gorham, 1986). Similar problems are documented in places such as in the head-waters of the Mekong river system in North-East Thailand, for the Huang and associated rivers in the North China Plain, for the Colorado in South-West USA, the Nile in Egypt, and the Murray-Darling Catchment in Australia (McWilliam, 1986). Hence the long-term viability of agriculture and welfare of millions of people in those areas are being seriously threatened.

The success of the Green Revolution largely depended on exploitation of irrigated lands, but because of the degradation of irrigated lands due to salt accumulation, gains are becoming losses, re-imposing a major danger to the welfare of millions of people (Wyn Jones and Gorham, 1986). Although in general the deleterious effect of soil salinity is explicable in terms of loss of crop yield, the extent of loss differs from farm to farm because of differences in regional aquifer properties and water management practices. The most effective procedure for the long-term mitigation of the saline soil problem involves managing irrigation and the quality of water to satisfy crop needs on the one hand, and on the other, leaching of salts deep in to the soil profile using large volumes of water (Gates and Grismer, 1989). In addition to these requirements an appropriate drainage system must be provided to remove saline leaching water to minimise any return of salts to the root zone by capillary rise. An alternative option is the use of chemicals such as gypsum which helps to speed up the

mobility of salts and water in the soil. These methods, although essential and often effective, tend to be costly and may be inappropriate where only poor quality brackish water is available for irrigation. Furthermore, in many areas there is an increasing shortage of high quality water and a potential conflict between urban and agricultural demands (Wyn Jones and Gorhman, 1986).

Many large and costly schemes have been undertaken for irrigating new lands or for the drainage and reclamation of older but degenerating projects. Although this physical approach has proved to be successful in reclaiming small areas of salt deserts, due to escalating cost of labour and energy the establishment and continued running of these projects does not appear economically feasible in developing countries (Shannon, 1984). At the same time as increasing amounts of agricultural land go out of use, the world population continues to grow and the needs for food, energy, fuels, chemicals, fertilisers, structural materials, fibres and medicinal compounds increase concurrently.

The possibility of modifying the soil environment to alleviate the salinity problem using chemical and physical treatment is extremely limited and quite often impossible because it is very expensive and is therefore unlikely to be used extensively in the near future. The genetic modification of crops by exploiting genetic variability both within and between crop species to provide salt-tolerant plants capable of growing under saline conditions remains the most viable solution to the soil salinity problem (Epstein *et al*., 1980; Shannon, 1984). The need for salt-tolerant crops increases each year as a growing world population seeks to feed itself with dwindling fresh-water supplies.

In order to be able to produce conventional crop plants that can adapt and give reasonable yields in saline conditions, genetic variability for salinity tolerance that is potentially useful in breeding must be available. Sources having the greatest potential for genetic diversity include primitive land races, wild relatives of domesticated crop species, and wild/weedy species that often contain genes for characters of adaptation to various adverse conditions. Other sources of genetic diversity include obsolete cultivars, varieties or cultivars in current use, breeding lines, and special genetic stocks. The available base of genetic variability for salt tolerance in the world germplasm collections is almost certainly not properly explored. However, buoyed by the success of Lyon as early as 1941 who, after working on tomato species, suggested the

possibility of developing salt tolerant conventional crops through selection and breeding, the stage was set in the early 1970's for a considerable expansion of the previously sporadic activities dealing with the introduction of genetics into applied research on salinity. Resistance to salinity has become a desirable attribute for many crops that are raised in warm, arid and semi-arid climates, and has over the years been the subject of a number of conventional breeding programmes.

Because of enormous genetic diversity between and within the local land races of particular crops evolved over long periods in diverse areas with traditional farming systems, these populations of land races constitute a most valuable source of genetic diversity for plant improvement (Frankel, 1977). Too little attention has been given to the enormous diversity of gene complexes determining adaptation and productivity, assembled and incorporated over centuries of cultivation in differing environments (Frankel and Benett, 1970), and coadapted gene complexes of fundamental importance in the adaptation of individual populations to their particular environments (Dobzhansky, 1970). Some of this genetic variability has been incorporated in those cultivars selected from land races. However there must be much more variability than that exploited, some of which confers adaptations to extreme conditions, such as salt tolerance, which as discussed above is a character of great significance in the world. Moreover as emphasised by Vavilov (1926), the combination of parents adapted to widely different environments may provide opportunities for major advances presumably through the combination of different adaptive complexes. Likewise, Moeljopawiro and Ikehashi (1981) suggested that the success of breeding for salt tolerance depends on the cumulative tolerance derived from combining of genetic material from different sources.

In recent years a great deal of information on the evolutionary relations of crops and their wild and weedy relatives has been generated from a spectrum of sources, including archaeology, anthropology and ethnology, plant geography, climatology, ecology, and cytogenetics, and experimental evolution, although there remain many gaps in our knowledge of the ancestry of our crops. Improved understanding of the evolution of crops should heighten the appreciation of wild progenitors and other related species as potentially valuable sources for the improvement and even radical restructuring of crop species (Frankel, 1977). It has been shown that some wild

progenitors of some cultivated plants constitute large gene pools for salinity tolerance. For example, in a number of instances there are indications that salt tolerance is associated with more efficient Na^+ or Cl^- exclusion, which in *Aegilops squarrosa* (the putative source of the D genome of *Triticum aestivum*), is a common character (Wyn Jones *et al*., 1984). Similarly the highly salt-tolerant wild rice, *Oryza coarctata*, can survive up to 30 to 40 dS m^{-1} salinity and this species may be used as a parent for developing better and truly salt-tolerant rice varieties (Bal and Dutt, 1986). Rush and Epstein (1981) made a successful interspecific cross in tomatoes between the wild *Lycopersicon cheesmanii* and the cultivated *L. esculentum* to transfer salt tolerance from the wild into the cultivated species. After many generations of back crossing, selected salt-tolerant lines completed their life cycle when grown in sandy soil irrigated with 70% sea water.

Selection and breeding for resistance to any environmental stress depends on two factors; genetic variability with respect to the particular stress involved, and selection following exposure of genetically variable material to the stress, thereby allowing identification of individuals approaching or possessing the desired phenotypes.

A reliable means of quantifying variability within a species for the stress resistant characters in question is also desirable. Previous studies provide ample evidence about the occurrence of variation in salt tolerance between a considerable number of plant species (Epstein and Norlyn, 1977; Norlyn, 1980; Norlyn and Epstein, 1984; Verma and Yadava, 1986). Variation in salt tolerance has also been found between different wild populations within the same species where this occurs naturally in saline and non-saline habitats. For instance, Hannon and Bradshaw (1968) found significant differences in salt tolerance between different populations of both *Agrostis stolonifera* and *Festuca rubra*.

The amount of evidence about the genetic basis of salinity tolerance is not great, but evidence from four grass species (Ashraf *et al*., 1986a), lucerne (Noble *et al*., 1984; Ashraf *et al*., 1987; Al-Khatib *et al*., 1993), and sorghum (Azhar and McNeilly, 1989) suggest that both additive and non-additive genetic effects are involved. For effective and accelerated improvement through selection and breeding, it has been

suggested that a thorough understanding about the genetic architecture of salinity tolerance in crop species in which tolerance is to be improved, is imperative. Information about the genetic base of salt tolerance would assist the breeder in two ways. Firstly, it helps in the development of appropriate selection protocols for screening tolerant plants, and testing their progenies. Secondly, it provides estimates of heritability for the character, which may be used to predict progress through selection.

Salinity has also been shown to affect plants at all stages of development, and the sensitivity of different crops varies from one growth stage to another (Shannon, 1984). Rice, for example, is tolerant during germination, but becomes very sensitive during early seedling stage, is tolerant during vegetative growth, again becomes sensitive during pollination and fertilisation, and then becomes increasingly more tolerant at maturity. Two wheat varieties were found to be less tolerant at germination than they were after the three leaf stage of growth (Francois *et al*., 1986); two squash cultivars (Scallop and Zucchini) proved to be more salt-tolerant during germination than during vegetative, flowering, or fruiting stages of growth (Francois, 1985). It is thus clear from these examples that plant response and, consequently, its effective salt tolerance are, influenced by its ontogenic stage at the time of the stress (Shannon, 1985). This suggests that the ability of plants to respond to salt stress depends upon genes that are functioning at the particular stage of development during which the stress occurs. Thus, for varietal improvement in salinity tolerance to be effective, information about the effects of salinity on all phases of plant growth is essential and it would be equally valuable to identify the life stage most susceptible/sensitive to the effect of salinity in order to maximise selection efficiency (Azhar and McNeilly, 1989). Moreover, such information, would be of significant value in devising possible management practices for using brackish water for irrigation during certain growth phases in areas where shortage of fresh water is acute, an approach which has been adopted, and recommended by Pasternack *et al*. (1984, 1985).

Plants respond to salinity stress in different ways. Plants may avoid salt stress by maintaining their internal salt concentration unchanged (Levitt, 1978) and this is achieved in several ways. Excess salt may be secreted by salt glands in some species; e.g. *Spartina townsendii* (Skelding and Winterbottom, 1939) and *Limonium* spp. (Ziegler and Lüttge, 1967). Excess salt is sequestered in special hairs in several

Atriplex spp. (Osmond *et al.*, 1969). In *Oryza coarctata*, the adaxial surface of the leaf possesses some specialised unicellular salt hairs (trichomes) which function only for short periods, when the optimum concentration of salt is reached within these cells (salt hairs) they burst and eliminate the salts (Bat and Dutt, 1986). In some other species such as *Allevnrolfia*, *Holoinemum*, and *Salicornia*, excess salt is removed by the physiological loss of salt saturated organs (Chapman 1968). Some species show reduced uptake of salt by their root system; e.g. *Atriplex hastata* (Black, 1956).

Under saline conditions, the continued cellular function of plants depends upon osmotic adjustment by both uptake and accumulation of ions (inorganic solutes) and synthesis of organic solutes. Osmoregulation must satisfy both the growth requirement for sufficient turgor pressure in most plant cells, as well as the physiological demands that the solutes used for osmoregulation do not interfere with the efficient operation of metabolic reactions (Hellebust, 1985). Accumulation of solutes may lead to increased solute concentration in plant tissues as part of osmotic adjustment, but this same accumulation may go beyond the limits of regulation of cytoplasmic content with associated impairment of growth (Pitman, 1984). Increased salt concentration in the rooting medium may cause water potential to decrease to the extent that water supply to the plant is severely impaired. The resulting differential potential developed between plant and salinised medium may cause water to flow from the plant into its root zone, and ultimately, loss of water results in dehydration, adversely affecting physiological plant activities (Steponkus, 1980). It has been suggested (Jeschke, 1984) that compatible solutes or cytosolutes such as glycine and betaine might regulate intracellular Na^+ distribution under salt stress, and could induce increased vacuolar salts in roots, enabling the plant to maintain soil water balance and thus grow under saline conditions. In halophytes, the demand for NaCl for osmotic adjustment in the leaves matches or perhaps exceeds NaCl supply from the root (Flowers and Yeo, 1986). In some salt-tolerant species, K^+ dependent Na^+ extrusion has also been observed (Jeschke, 1984). Similarly, Rush and Epstein (1976, 1981) have reported that salt tolerance differences between the wild halophytic *Lycopersicon cheesmanii* and the salt-sensitive cultivated species *Lycopersicon esculentum* are due to the former's ability to accumulate Na^+ and selectively absorb K^+. The accumulation of salt reduces the

requirement for increased wall extensibility, leaf thickness, and water permeability that might otherwise be required to maintain positive growth and turgor at low soil water potential.

On the basis of the mechanism and degree of salinity tolerance, plants can be grouped into two physiological groups. Plants which are able to tolerate relatively a low level of salt are called glycophytes. Plants that live and reproduce in oceans, sea shores, estuaries, deltas, salt marshes, and saline deserts are generally called halophytes (Epstein *et al.*, 1980), and/or plants which tolerate high levels of salt are referred as halophytic (Wainwright, 1980). Halophytes have been classified into different types in terms of their mechanisms of dealing with the salt problem (Waisel, 1972); this classification was tabulated by Jafari (1990) and is given in Table 1.3.

The major osmotic components of glycophytes (plants intolerant to high salt concentrations) are proline, sugar, glycine-betaine (Shannon, 1984; Hellebust, 1985). However glycophyte tolerance to salinity is commonly correlated with ability to restrict the entry of ions to shoots or to an ability to exclude entry of excess amounts of specific ions (Greenway and Munns, 1980). But still certain glycophytes with halophilic tendencies such as the members of *Chenopodiaceae* accumulate sodium (Na^+ accumulators) and they tolerate higher concentrations of NaCl than other plants which exclude sodium (Na^+ excluder).

In halophytes large quantities of ions, particularly sodium and chloride, are transported to plant tissues to maintain shoot water potentials more negative than those in the external medium, maintaining the movement of water into the plant during growth (Clipson *et al.*, 1988), and at the same time to prevent inhibition of cell metabolism by excessive salt concentrations in cytoplasm (Hellebust, 1985). The cellular basis of salt tolerance in halophytes depends upon the compartmentation of ions necessary for osmoregulation in vacuoles and upon osmotic adjustment of the cytoplasm by compatible solutes (Flowers, 1985).

Some information about the mechanism of salt tolerance in plants may be obtained from examination of physiological parameters suggested as being related to tolerance mechanisms in plant material of the same species shown, from simple

Table 1.3. Classification of plants of saline habitats

Euhalophytes					Pseudohalophytes
Salt requiring halophytes		Salt resistance halophytes			Salt avoiding halophytes
Obligatory	Preferential	Salt enduring (Salt-tolerant)	Salt excluding	Salt evading	
A	B	C	D	E	F

A = plants dependent upon salt for their survival, e.g. *Salicornia* spp. and various bacteria and algae

B = plants whose growth development is improved in the presence of salts

C = plants enduring high protoplasmic salt content

D = plants accumulating salt in special hairs, plants secreting salts from their roots, plants re-transporting salt from shoot into root

E = plants evading salt uptake, plants evading salt transport into the leaves

F = Ephermes, Niche plants

laboratory screening, to be tolerant and non tolerant of salinity. Selected tolerant and unselected material from within an accession has the potential to provide more useful data.

It is becoming self evident that the exploitation of unconventional and unpromising conditions imposed by salinity depends either on the successful development of salt-tolerant varieties of the existing limited range of crop plants or the domestication of natural halophytes (Epstein *et al*., 1980; Jones and Gorham, 1986). The latter, which is being initiated in many laboratories around the world aims to develop naturally tolerant wild species that have evolved high salt tolerance in their native habitats as new crops. This is however generally considered a slow process.

Pearl millet (*Pennisetum americanum*), is the most important crop in the Sahel zone adjacent to the Sahara in tropical Africa namely Senegal, Mali, Upper Volta and other countries bordering the southern Sahara (Purseglove, 1976). From its area of origin in West Africa, pearl millet was carried to East Africa, India, and Pakistan where it is currently cultivated extensively (Poehlman, 1987). The greatest merit of pearl millet is that it can be grown in low rainfall areas on poor, sandy soils where it will give economic (although low) yields on soils too poor and too worn out to support most other cereals. For example in Northern Ethiopia, because of the droughts of the past few decades, land races of the more important cereals such as *Triticum* spp., *Hordeum* spp., *Eleusine coracana*, *Zea mays*, *Vicia faba*, *Lens culinaris*, *Medicago* spp., *Cicer arietinum*, *Linum usitatissimum* and *Sesamum indicum* have been replaced by pearl millet (Kebebew and Adhanom, 1986). Finger millet (*Eleusine coracana*) extends in Africa from Nigeria eastwards to Ethiopia and southwards to southwest Africa and Natal and is a potential staple food in parts of eastern and central Africa (Purseglove, 1976). Tef (*Eragrostis tef*) is the staple cereal grain of Ethiopia, and has been in cultivation since ancient times. Ethiopia still is the only country in the world where the grain is used as a cereal. According to Vavilov (1951), Ethiopia is considered the centre of origin for tef. The best kind of 'injera' a flat, circular and very soft bread is made from tef flour. Milling tef gives a return in flour of 99% in comparison with 60 to 80% for wheat (Ebba, 1969). This clearly indicates that tef is an economical crop because so little of the grain is wasted. Tef grain can also be stored for many years without being damaged by insect pests. The fine straw is used as a valuable animal feed and building

material, indicating high utilisation of the byproducts.

Previous studies on salt tolerance in pearl millet have suggested that the variability observed in root lengths has a genetic basis and based upon realised heritabilities the character is under polygenic control (Ashraf and McNeilly, 1992). Similar studies in finger millet and tef are not available yet.

Ethiopia is a land of great physical diversity, with altitudes ranging from 116 meters below sea level in the Dankil Depression, to 4620 m above sea level at the summit of Ras Dashen, Africa's fourth highest peak. In the north, there are approximately 25 mountains rising over 4000 m. The central Ethiopian highland plateaux vary in height between 2000 and 3000 m. Desert areas occupy about one third of the total area, highlands and plateaux another third, and intermediate land the remainder. A massive gash bisects the surface of the plateaux from the Red Sea to the Kenyan border. This is the Ethiopian Rift Valley, part of the Great Rift Valley system that extends 6000 kilometres from Syria to Mozambique. Ethiopia lies between 3^{o} to 18^{o} North latitude and 33^{o} to 48^{o} longitude. Proximity to the equator, combined with a great altitudinal range results in a climate varying from cold continental to temperate sub-tropical. For example the plateaux have an annual temperature of $16^{o}C$, whereas in the low lands the average is $30^{o}C$. It has a total area of 1,222,000 km^2 and a population of 50,000,000 which was growing at the rate of 2.5% per annum (Ethiopian Statistical Abstract, 1980). About 16% of the total area is salt affected, land which supports about 9% of the human population. Salt affected land mainly occurs in the arid and semi-arid regions of the country (i.e. altitude < 500 m and mean annual temperature > $27.5^{o}C$). Salt affected soils comprise four groups, namely, solonchaks, moderately saline soils, moderately sodic soils, and those that are both moderately saline and sodic (Sissay, 1986). More specifically salt areas are found in the Rift Valley, the Dankil Depression and the South East of the country, on the Eritrean seashore, the southern border, and the Red Sea coast (Sissay, 1986).

Crop losses due to salinisation of soils are considerable and in some cases agriculture on salt affected land has been abandoned. High population and livestock densities, and increased cultivation and intensive use of forest have resulted in a decline

of the native vegetation over exploited as sources of fuel and fodder, and in accelerated soil erosion and salinisation. Over the last 50 years, forest has been reduced from 34% to 5% of the total land area; woodland has been reduced from 20% to 8% of the total land area and bare land and salt flats have increased from 6% to 16% of the total land area (Ministry of Agriculture of Socialist Ethiopia, 1983). The introduction of salt-tolerant plants in to salt affected agricultural lands of the country has been initiated by the Ministry of Agriculture, although no significant success is being achieved (Sissay, 1986).

It is clear that in terms of maintaining agricultural productivity on a world scale in general and in Ethiopia in particular, the development of new crops or lines within existing crops that will grow and yield on saline soils must be a high priority activity to meet the demands of an over growing population. Considerable resources of plant variability are found in gene banks around the world, only a small proportion of which is assessed for its potential value outside the major cereal crops, wheat, rice, and maize.

The material examined for salinity tolerance variation in this project are mainly from the Plant Genetic Resources Centre/Ethiopia (PGRC/E). The germplasm accessions of minor millets and tef maintained by PGRC/E are land races which have evolved under local conditions in the farmers' fields over many generations. Such gene pools provide reservoirs of variation which may become the raw material for crop salt tolerance improvement. Of fundamental importance in the management of the resources maintained in gene banks is the determination of the variation they represent. The goal of the present study is to add basic information to the knowledge of salinity tolerance in minor millets and tef. This entails the following steps which form the basis of the project undertakings:

1. Assessing variation in the response of 25 accessions of pearl millet, 25 finger millet and 15 tef to NaCl in early seedling growth
2. Assessing the genetic basis of that variation
3. Studying the response of accessions to NaCl during ontogeny of the whole plant
4. An examination of physiological mechanisms associated with/involved in salt tolerance
5. Investigating responses of accessions to mixed salt ($NaCl + CaCl_2$) treatments

VARIATION IN RESPONSE OF ACCESSIONS OF MINOR MILLETS, PENNISETUM AMERICANUM (L.) LEEKE (PEARL MILLET) AND ELEUSINE CORACANA (L.) GAERTN (FINGER MILLET), AND ERAGROSTIS TEF (ZUCC.) TROTTER (TEF) TO SALINITY IN EARLY SEEDLING GROWTH

CHAPTER TWO

CHAPTER 2

VARIATION IN RESPONSE OF ACCESSIONS OF MINOR MILLETS, PENNISETUM AMERICANUM (L.) LEEKE (PEARL MILLET) AND ELEUSINE CORACANA (L.) GAERTN (FINGER MILLET), AND ERAGROSTIS TEF (ZUCC.) TROTTER (TEF) TO SALINITY IN EARLY SEEDLING GROWTH

2.1. Introduction

Crop species growing on salt affected soils are subjected to toxic effects of sodium and chloride ions (Flowers, 1985) and the dehydrating impact of salt, (Steponkus 1980), the combined effects of which adversely affect the physiological activities of the plant to such an extent that plant growth becomes severely restricted, or impossible. The result is at least considerable loss, but frequently a total loss of yield. Since estimates put the area of salt affected soils at some 9.5 million square kilometres on a world scale (Szabolcs, 1989), much of it due to inadequate irrigation practices in arid and semi-arid regions, the loss of agricultural production is enormous.

Whilst engineering procedures are capable of ameliorating the problem, they are very expensive. It has been argued therefore that the most promising means of solving the problem is to exploit the products of past evolution by attempting the domestication of wild halophyte species, or alternatively by the exploitation of variation in tolerance to soil salinity which may occur in existing crop species or varieties within crops (Epstein *et al.*, 1980; Shannon, 1984). Such an approach necessitates assessment of the potential for domestication of wild halophytes, or of the extent of variability in salinity tolerance in the germplasm of existing crops, and the genetic architecture of that variability.

Tef (*Eragrostis tef*) is the staple cereal grain of Ethiopia, the only country in the world where tef grain is used as a cereal, and where it is believed to have originated (Vavilov, 1951). Finger millet (*E. coracana*) is another staple food in Ethiopia and is grown extensively in the northern region of the country. Pearl millet (*P. americanum*) on the other hand, is well adapted to light, sandy, low nutrient soils and will generally produce some yield every year in areas where other cereals might periodically fail, and a number of cultivars mature more rapidly than the land races of the important cereals, so it can be grown with less rainfall.

The unrestricted use of vegetation as source of fuel and fodder is resulting in extensive land degradation, deterioration of the vegetation, soil erosion, and accelerated soil salinisation. Some agricultural lands have been abandoned from production due to salinisation of soils, the scale of which can be judged from the current human and livestock populations inhabiting these areas. In typical salt affected areas the average population per km^2 is 11.2 persons whereas it is 25 for the nation as a whole, while the standard animal unit per km^2 averages 0.26 compared with 30 overall (Sissay, 1986). These losses of agricultural land are accompanied by loss of those genetic resources of crop species which were previously grown on them. However collections by PGRC workers have included many of these sites prior to their abandonment.

Collections of land races of crop species from within the country constitute the majority of the germplasm accessions maintained by the Plant Genetic Resources Centre, Addis Ababa, Ethiopia. Since the values of any conserved germplasm will be greatly determined by the information available about it, high priority is being given to systematic characterisation of these accessions. Since loss of soils through salinisation is a growing problem in arid and semi-arid areas of the world, development of crop varieties of any species that can be grown on these soils is clearly of value in maintaining at least some level of productivity from such soils. Assessment of germplasm from such areas would therefore seem to be a useful beginning in the development of tolerant material.

Salt-tolerant plants have been introduced on an experimental basis into arid and semi-arid parts of the country where saline soils land predominate. However, little attention is being given to most of the salt affected land (Sissay, 1986). Nonetheless with a markedly increasing population and loss of agricultural land from production, it is essential that attention is paid to the development of means for exploiting these salt affected areas. The use of, or development of, salinity tolerant crop species/varieties seems to provide the least expensive procedure to follow.

Whilst there is a limited amount of information about salinity tolerance in pearl millet, there is as yet no such information about finger millet and tef.

The work described in this chapter examines patterns of variability in response to salinity of two-week-old seedlings of 25 accessions of each of pearl and finger

millets, and 15 accessions of tef, all of them originating in Ethiopia, and records of collection sites are available from PGRC, Addis Ababa. This preliminary examination was accomplished by using a solution culture technique which has previously been shown to provide acceptable estimates of adult plant reaction to salinity (Azhar and McNeilly, 1988; Ashraf and McNeilly, 1992; Al-Khatib *et al.*, 1993).

2.2. Materials and methods

2.2.1. Plant material

Twenty-five accessions of each of *Pennisetum americanum* (L.) Leeke (pearl millet), and *Eleusine coracana* (L.) Gaertn (finger millet), and fifteen accessions of *Eragrostis tef* (Zucc.) Trotter (tef), consisting of land races collected in Ethiopia were used in this experiment. Material of the former two species were kindly supplied by the Plant Genetic Resources Centre in Addis Ababa, Ethiopia, whilst material of the latter species was kindly provided by the United States Department of Agriculture (USDA).

2.2.2. Tolerance testing

The responses of two-week-old seedlings to increasing salinity were evaluated using the solution culture technique described by Ashraf *et al.* (1986a). Two sets of NaCl concentrations were used. For pearl and finger millet, six NaCl concentrations, namely 0 (control), 50, 75, 100, 150, and 200 mM, were used, whilst for tef nine NaCl concentrations were used, 0 (control), 25, 50, 75, 100, 125, 150, 175, and 200 mM, all prepared in 1/10 strength nutrient solution (see Ashraf *et al.*, opp. cit.).

Twenty seeds of each accession were sown on rafts of black alkathene beads, three layers deep, floating on nutrient solution containing each of the respective NaCl concentrations in 300 cm^3 plastic beakers. The experiment was replicated three times. Before planting, seeds were surface sterilised in 2% sodium hypochlorite solution for 10 minutes. The experiment was set up as a completely randomised design in a growth room at 25 +/- 1^oC, relative humidity 80%, and day length of 16 hours at an intensity of 27 W m^{-2}. The pots were enclosed within perspex chambers to reduce solution evaporation.

After 14 days, ten randomly chosen seedlings from each of the three replicates

of each accession were measured for length of longest root. Data for root length were subjected to analysis of variance.

The set of concentrations used for testing tef was designed to provide a wider range of NaCl concentrations to generate better data sets for data analyses using a computer programme available for analysis of salt tolerance data (van Genuchten and Hoffman, 1984), obtained subsequent to completion of the pearl and finger millet testing, and described in this Chapter.

2.2.3. Data analysis

Root length value of 30 seedlings of each accession of finger millet and tef in each NaCl concentration were subjected to analysis of variance. Since finger millet and tef are strictly self-pollinated species, between accessions variances comprised both genetic and environmental components whereas within accession variance comprised a predominantly environmental component. Genotypic and phenotypic variances were estimated following Prasad *et al.* (1981) and were used to compute broad sense heritability (Falconer, 1981) where broad sense heritability (h^2_B)

$$= \frac{\text{Between accessions variance}}{\text{Between accessions variance + within accession variance}}$$

Within accession variability in absolute root length at 100 mM NaCl was compared between accessions using the method proposed by Lewontin (1966) using the variance of logarithms (to base 10) of each measurement.

The pattern of variability in the salinity responses of accessions of the three species was also examined on the basis of the frequency distribution of relative tolerance of individual seedling within each accession at 100 mM NaCl. Relative tolerance was calculated using the formula below:

$$\text{Relative tolerance} = \frac{\text{Individual seedling root length in saline solution}}{\text{Individual seedling root length in control solution}} \times 100$$

For an obligate self pollinator this would not necessarily seem to be a very

useful piece of data at first glance, but is in fact suggestive of variability within the crop because of its land race origin which could constitute a possibly large-number of genotypes.

The solution culture experiments carried out on *P. americanum* and *E. coracana* were completed before the acquisition of the computer programme, it was not possible to use a wider range of NaCl concentrations in data analysis for them, nor was it possible to repeat the experiments due to shortage of seeds.

A non-linear least square method was used to fit the observed salinity response data to the following response models, following van Genuchten & Hoffman (1984).

The two models chosen were NOPT 5 and NOPT 12, used respectively to establish the salinity response functions of the accessions at the seedling stage.

(i) NOPT 5, the absolute yield curve is given by:

$$Y = \begin{cases} Y_m & 0 \le C \le C_t \\ Y_m - Y_m\, s\, (C - C_t) & C_t < C \le C_0 \\ 0 & C > C_0 \end{cases}$$

where Y = absolute yield;

Y_m = absolute yield under non-saline conditions;

s = absolute value of the slope of the response function between C_t and C_0;

C = average root zone salinity during the growing season;

C_t = threshold concentration at which yield starts to decrease;

C_0 = concentration at which yield equals zero.

(ii) NOPT 12, a sigmoid-form function given by:

$$Y = \frac{Y_m}{1 + (C/C_{50})^p}$$

where C_{50} = salinity at which yield decreases by 50%;

p = an empirical constant that specifies the steepness of the curve

The computer programme, 'SALT', (van Genuchten, 1983) was used to carry out these computations. This programme, applied to the root length of 14-day-old seedlings, provides estimates for C_t, C_0 and s (NOPT 5), and C_{50} and p (NOPT 12), as well as the fitted response curve.

The C_t values generated from the replicates were subjected to analysis of variance.

2.3. Results

The results of analysis of variance using absolute values for root are presented in Tables 2.1, 2.10 and 2.12, the latter two ANOVA separate within and between variance for the accessions across NaCl concentrations.

The data showed that there were significant differences due to salt concentrations and accessions within the three species (Table 2.1). The interaction between accessions and different salt concentrations was also highly significant ($p<0.001$) indicating that increased salinity adversely affected root length to a different degree in different accessions (Table 2.1).

The results of analysis of variance for *E. coracana* (Table 2.10), and *E. tef* (Table 2.12) across all salinity levels showed that accessions differed in their response to different salinity levels.

Analysis of variance using threshold values (C_t) derived from absolute root length data were carried out separately for the three species. C_t estimates differed significantly for accessions within *P. americanum* and *E. tef*, but not for accessions within *E. coracana* (Table 2.2).

Estimates of C_t, C_0, derived from NOPT 5, and C_{50} from NOPT 12 for 25 accessions of each of *P. americanum* and *E. coracana*, and 15 accessions of *E. tef* are presented in Tables 2.3 - 5.

In order to simplify data presentation, a subsample of 9 accessions, was chosen to cover the ranges of response recorded for each species. The absolute root length data of the remaining sixteen accessions of each species of the two minor millets at the six

different NaCl concentrations, and six accessions of tef at the nine different NaCl concentrations are provided in Appendices 2.2 - 4. Relationships of salt tolerance functions based upon root length values for 14-day-old seedlings for nine accessions in each species and solution salinity are presented in Figures 2.1, 2.2, and 2.3 for *E. coracana*, *P. americanum* and *E. tef* respectively.

Examination of the data presented in Tables 2.3 - 5, and Figures 2.1 - 3 clearly show considerable differences in accessions response to salinity. However their response to increasing salinity varies with the character used to assess that response. Thus if the threshold salinity (C_t) is taken as an estimate of tolerance (Martinez-Cob *et al.*, 1987), some *P. americanum* accessions such as 215637, 219569, and 220222, and *E. coracana* accessions 100007, 100010, 100021, 100022, 100024, 100025, and 100030, and *E. tef* accessions 494188, 494213, 494216, and 524436 show relatively enhanced tolerance. However if C_{50} estimates are taken as estimates of tolerance, *P. americanum* accessions 215637, 215663, 219975, 219979, 220134, 221726, and *E. coracana* accessions 100021, 100022, 100024, 100025, 100030, and 100031, and *E. tef* accessions 494188, 494213, 524436, and 524445 are the most tolerant. Finally the predicted salinity level at which growth would be zero (C_0) suggests that for *P. americanum* accessions 203654, 215663, 219975, 219979, 219984, 220134, and 221726, and for *E. coracana* accessions 100001, 100012, 100014, 100016, 100017, 100019, 100021 - 24, 100030 - 31, and 33 - 34, and for *E. tef* accessions 494188, 524436, 524437, and 524445 are superior on the basis of these estimates.

To facilitate data interpretation, accessions have been ranked on arbitrary scales of I to III for tolerance for each of the three estimates of tolerance in Tables 2.3 - 5. This reveals that in *E. coracana* accessions 100021, 100022, 100024, and 100030, and in *E. tef* accessions 494188, and 524436 consistently rank I for all three parameters. In *P. americanum* no accession ranks I across parameters, although four, 215663, 219975, 220134, and 221726 rank I for C_0 and C_{50}, whilst their C_t rankings are 31.85, 5.27, 2.72, and 29.41 respectively, the middle two values being amongst the lowest recorded, whilst 215637 ranks I for C_t and C_{50}, and II for C_0.

Significances of differences between means C_t, C_0, and C_{50} of 25 accessions of each of *E. coracana* and *P. americanum*, and 15 accessions of *E. tef*, based upon t-test are presented in Table 2.6. Whilst difference in mean C_t values between *P.*

amercanum and *E. tef* was non significant ($p>0.05$), all other differences, in mean C_t (threshold salinity beyond which root growth starts to decrease), C_0 (salinity level at which root growth is zero), and C_{50} (salinity level at which root growth decreases by 50%) estimates between each pair of the species were highly significant.

The frequency distributions of relative tolerance of root length in 30 seedlings subsamples at 100 mM NaCl from 9 accessions of each species in class intervals of 5% (Figures 2.4 - 86 suggest the presence of variation within the accession studied. Comparison of variance estimates was made following Lewontin (1966). This test of Lewontin is one whereby one can statistically test for significant differences between accession variances. Thus the results provided in Tables 2.7 - 9 suggest that the extent of within accession variances differed significantly in tef (Table 2.9) indicating that the tef accessions have different intrinsic variation, whilst accessions 100010, 100024, 100030, and 100031 in finger millet (Table 2.7), and accessions 215632, 215633, 219975, 219979, 219984, 219985, 220164, and 221726 in pearl millet (Table 2.8) are significantly different from most of the rest.

Broad sense heritability was estimated from analysis of variance over all salinity levels. Estimated broad sense heritability values for salt tolerance in *E. coracana* (Table 2.11), and *E. tef* (Table 2.13) under different salinity concentrations suggested that a great proportion of the differences in response to salinity of the accessions are genetically determined.

Table 2.1. Mean squares and significances from the analysis of variance of absolute root data of 25 accessions of *Pennisetum americanum* and 25 accessions of *Eleusine coracana* grown in six NaCl concentrations, and 15 accessions of *Eragrostis tef* seedlings grown in nine NaCl concentrations in solution culture for 14 days

Item	Df	*P. americanum* Root	Df	*E. coracana* Root	Df	*E. tef* Root
Blocks	2	0.218^{NS}	2	6.423*	2	0.12^{NS}
Accessions (Acc)	24	7.09***	24	47.35***	14	11.30***
NaCl conc (Sol)	5	657.7***	5	1037.8***	8	191.30***
Acc x Sol	120	6.62**	120	7.03**	112	2.05***
Residual	298	0.442	298	1.943	268	0.31

Table 2.2. Mean squares and significances from the analysis of variance of C_t of 25 accessions of *Pennisetum americanum* and 25 accessions of *Eleusine coracana* seedlings grown in six NaCl concentrations, and 15 accessions of *Eragrostis tef* seedlings grown in nine NaCl concentrations in solution culture for 14 days

Item	Df	*P. americanum*	Df	*E. coracana*	Df	*E. tef*
Blocks	2	413.67^{NS}	2	232.20^{NS}	2	316.90^{NS}
Accessions	24	434.23**	24	788.05^{NS}	14	986.30*
Residual	48	188.35	48	784.98	28	452.40

*, **, ***, indicate differences significant at $P < 0.05$, 0.01, 0.001 respectively, whilst NS denotes differences are not significant. This convention is followed throughout this thesis.

Table 2.3. Calculated values of C_t and C_0 (NOPT 5), and C_{50} (NOPT 12) for 25 accessions of *E. coracana* with tolerance rankings

Accession	C_t	Ranking	C_0	Ranking	C_{50}	Ranking
100001	6.59	III	211.30	I	111.52	II
100002	68.01	II	167.97	III	114.61	II
100004	30.19	III	168.92	III	99.76	III
100005	26.65	III	164.91	III	97.25	III
100006	56.03	II	160.91	III	101.57	III
100007	71.30	I	159.73	III	111.01	II
100008	29.26	III	179.47	II	107.90	III
100009	60.72	II	179.17	II	115.94	II
100010	81.64	I	159.74	III	116.10	II
100012	22.06	III	202.15	I	112.22	II
100014	35.61	III	210.40	I	117.68	II
100015	34.93	III	178.32	II	108.56	III
100016	57.61	II	213.16	I	118.73	II
100017	34.77	III	203.54	I	113.65	II
100018	34.01	III	186.69	II	107.15	III
100019	3.14	III	212.64	I	101.05	III
100021	81.50	I	203.97	I	134.00	I
100022	94.73	I	210.44	I	147.66	I
100024	91.93	I	203.40	I	142.46	I
100025	102.16	I	161.49	II	131.97	I
100030	90.21	I	208.56	I	142.81	I
100031	57.61	II	240.67	I	142.95	I
100032	36.71	III	177.44	II	102.76	III
100033	53.03	II	207.37	I	122.85	II
100034	50.59	II	201.96	I	122.42	II
Mean	**52.44**		**190.97**		**117.78**	

Table 2.4. Calculated values of C_t and C_0 (NOPT 5), and C_{50} (NOPT 12) for 25 accessions of *P. americanum* with tolerance rankings

Accession	C_t	Ranking	C_0	Ranking	C_{50}	Ranking
203654	5.56	III	183.20	I	85.64	II
203656	18.40	III	123.50	III	85.64	III
203657	27.61	III	110.95	III	65.61	III
203658	15.69	III	116.21	III	63.71	III
203659	9.59	III	118.84	III	61.99	III
203661	29.33	II	107.70	III	65.15	III
203662	1.12	III	123.72	III	60.71	III
215631	10.29	III	157.12	II	75.45	II
215632	25.38	III	162.13	II	80.60	II
215633	37.06	II	163.52	II	86.80	II
215634	37.06	II	160.77	II	87.30	II
215637	39.82	I	162.53	II	90.74	I
215663	31.85	II	169.69	I	101.93	I
219336	30.88	II	151.72	II	79.69	II
219569	42.67	I	115.60	III	76.19	II
219975	5.27	III	190.70	I	90.66	I
219979	9.80	III	179.62	I	89.15	I
219984	6.98	III	173.17	I	82.40	II
219985	4.65	III	160.24	II	77.18	II
220134	2.72	III	201.11	I	90.89	I
220139	35.31	II	113.64	III	71.83	II
220164	17.59	III	164.79	II	83.55	II
220220	34.56	II	154.22	II	85.62	II
220222	42.27	I	113.46	III	73.12	III
221726	29.41	II	173.80	I	95.68	I
Mean	**22.03**		**150.04**		**80.29**	

Table 2.5. Calculated (C_t) and C_0 (NOPT 5), and C_{50} (NOPT 12) for 15 accessions of *E. tef* with tolerance rankings

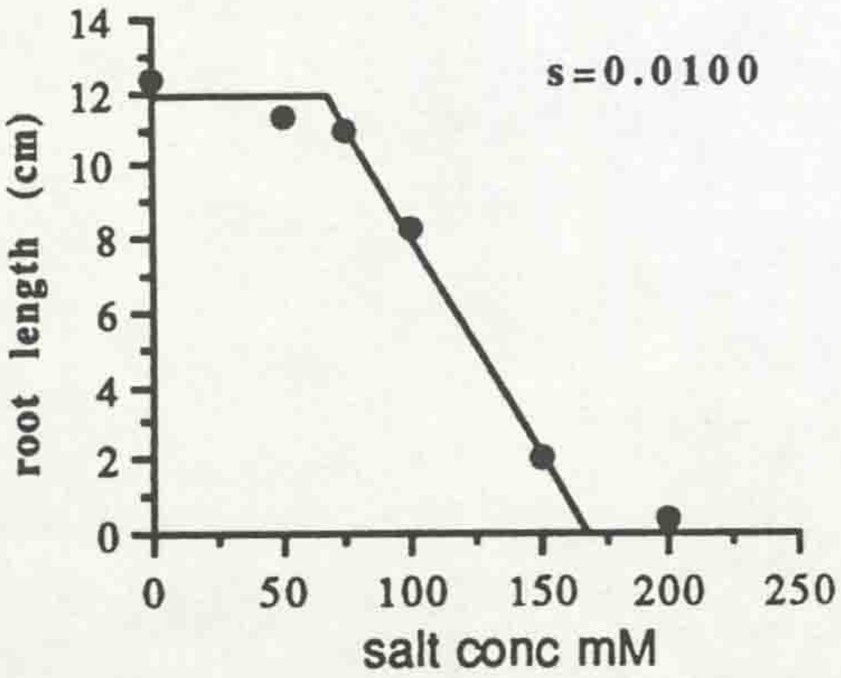
Accession	C_t	Ranking	C_0	Ranking	C_{50}	Ranking
343932	16.50	III	114.98	III	62.66	III
494188	38.97	I	188.38	I	112.21	I
494197	7.81	III	152.41	III	82.66	II
494205	0.00	III	166.22	II	77.83	III
494213	42.48	I	163.64	II	101.18	I
494215	17.84	III	162.94	II	91.83	II
494216	50.00	I	133.58	III	88.82	II
524433	11.20	III	158.51	II	66.42	III
524436	48.10	I	177.82	I	112.39	I
524437	10.47	III	179.65	I	98.62	II
524438	0.00	III	152.06	III	68.06	III
524439	20.05	II	157.81	II	72.04	III
524440	35.83	II	154.01	III	91.53	II
524441	25.00	II	134.91	III	72.82	III
524445	28.91	II	184.20	I	100.82	I
Mean	**23.53**		**158.74**		**86.66**	

Table 2.6. Differences between means C_t and C_0 (NOPT 5), and C_{50} (NOPT 12) of 25 accessions of each of *E. coracana* (see Table 2.3 for means) and *P. americanum* (see Table 2.4 for means), and 15 accessions of *E. tef* (see Table 2.5 for means), based upon t-test

Item	C_t	C_0	C_{50}
Between a and b	***	***	***
Between a and c	***	***	***
Between b and c	NS	**	***

a = *E. coracana* b = *P. americanum* c = *E. tef*

Figure 2.1. Response functions between salt solutions (mM) and root length (cm) of 14-day-old seedlings of *E. coracana* with slope, s (see NOPT 5 in materials and methods) and empirical constant, p (see NOPT 12 in materials and methods)



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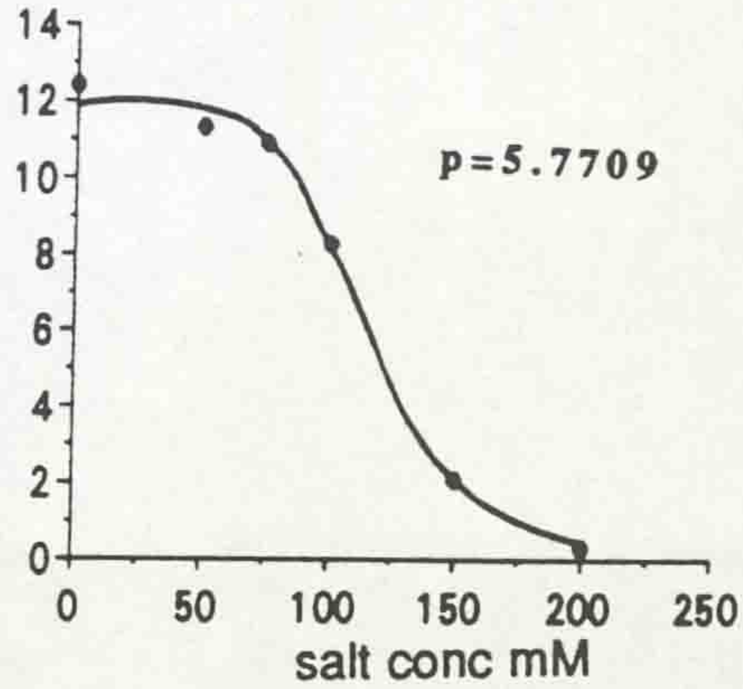

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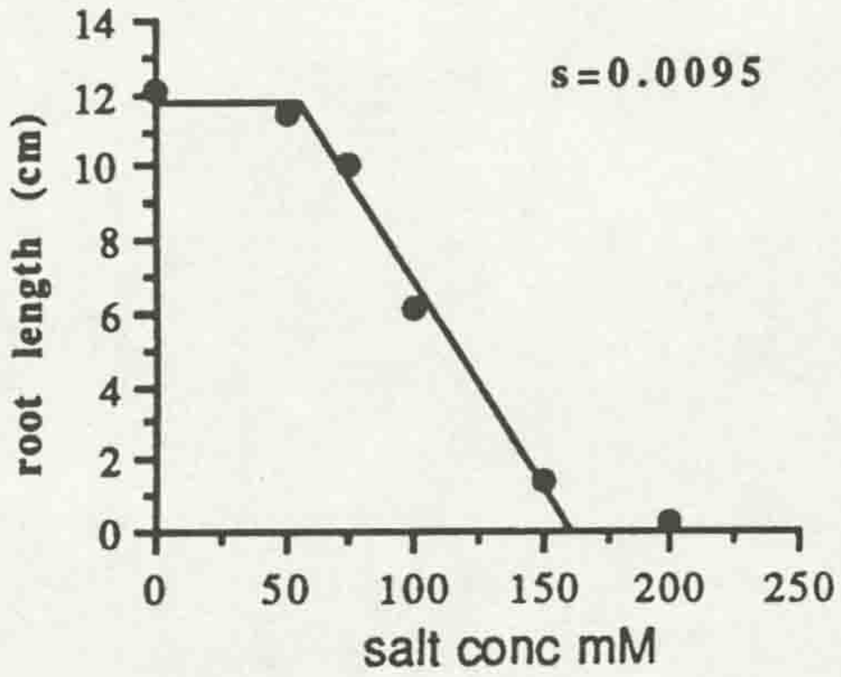

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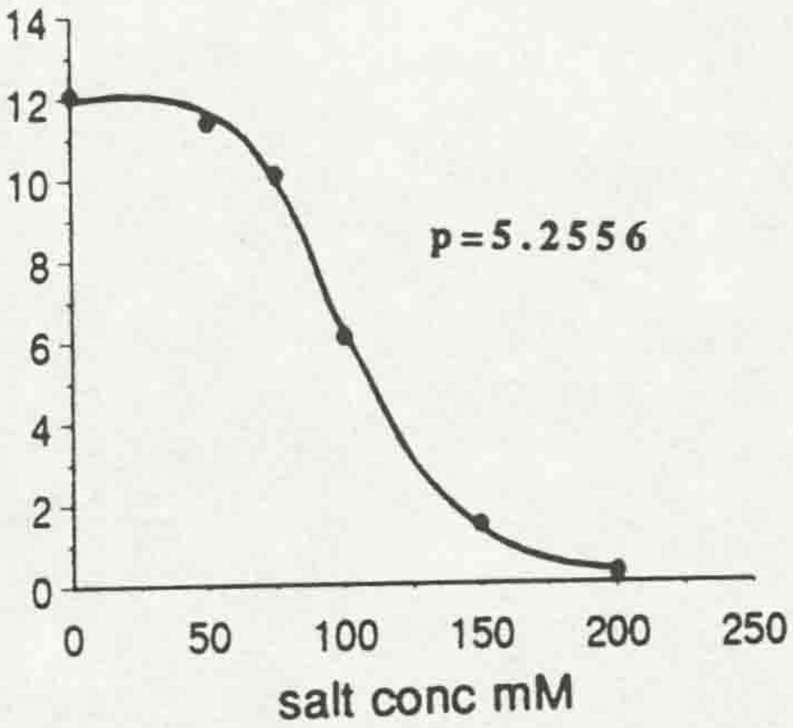

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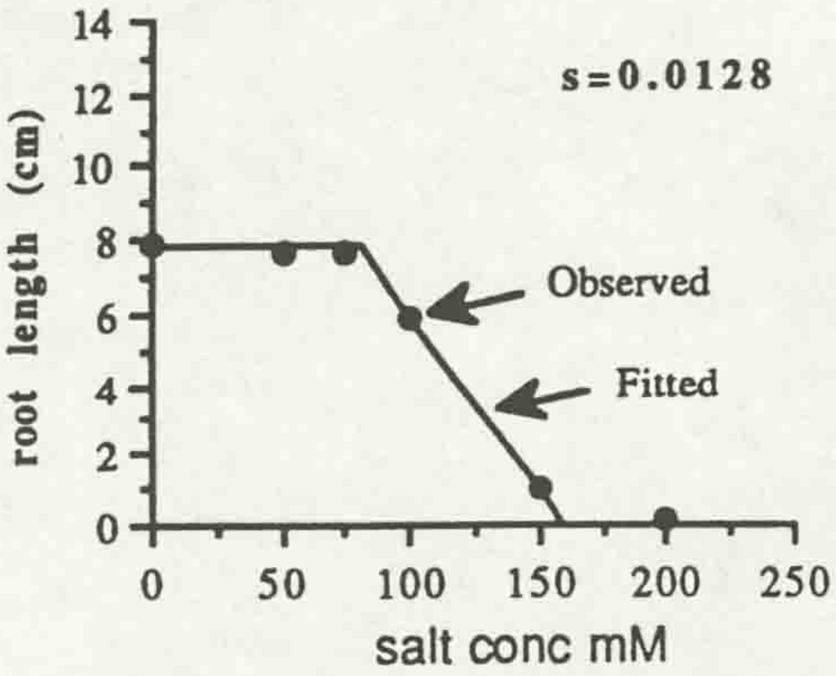

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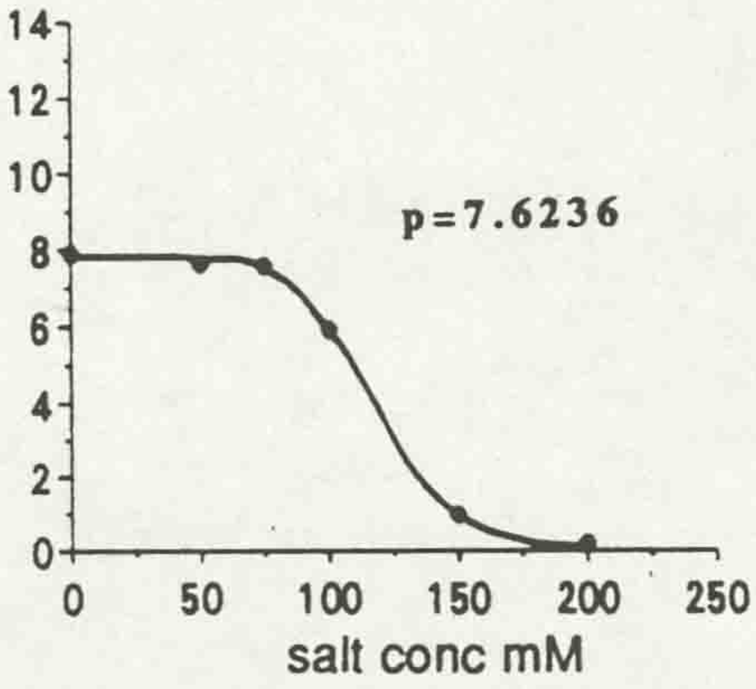

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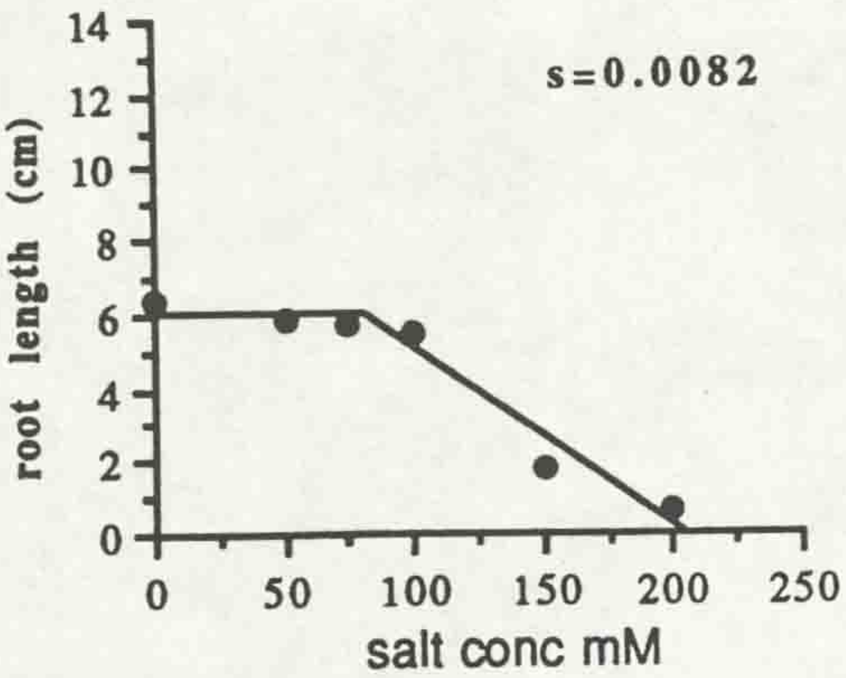
(Figure 2.1 continued)



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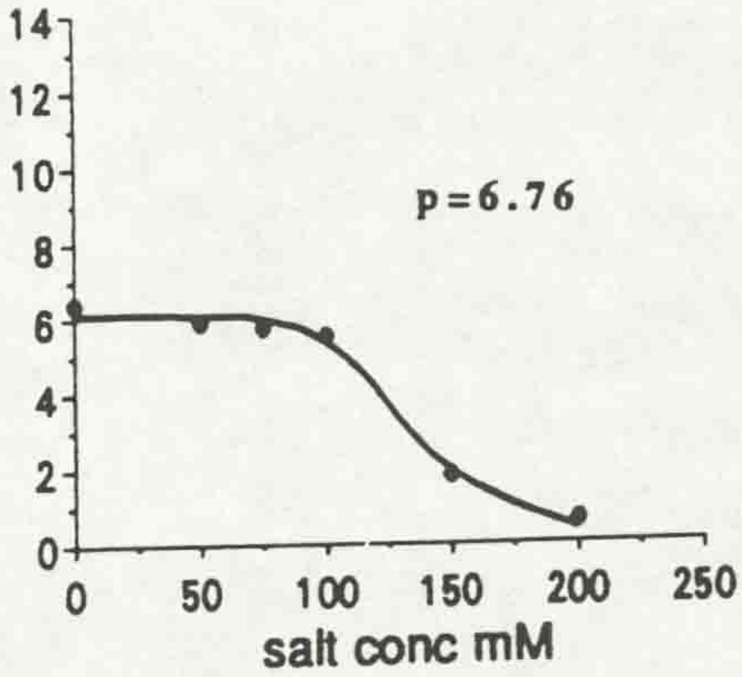

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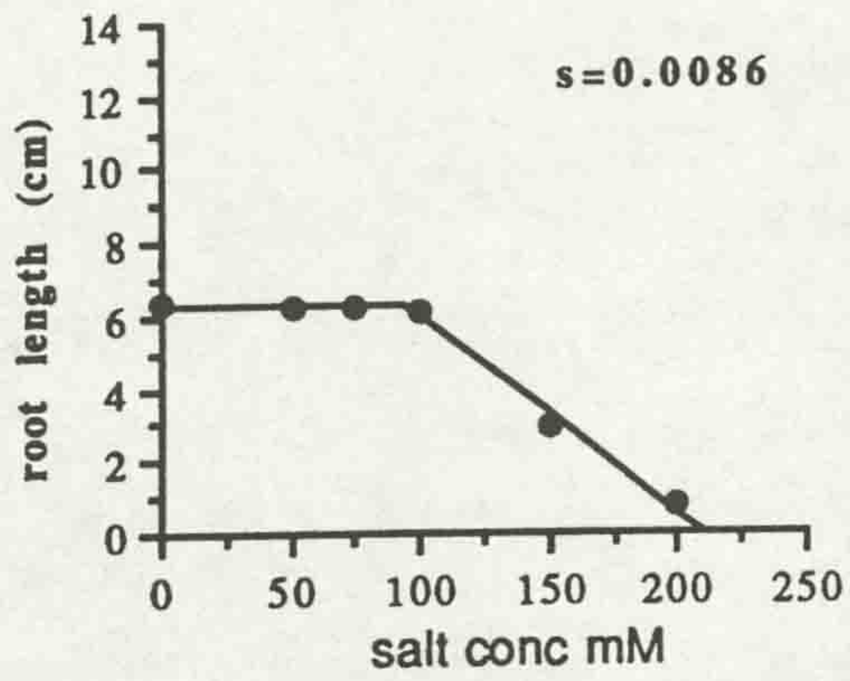

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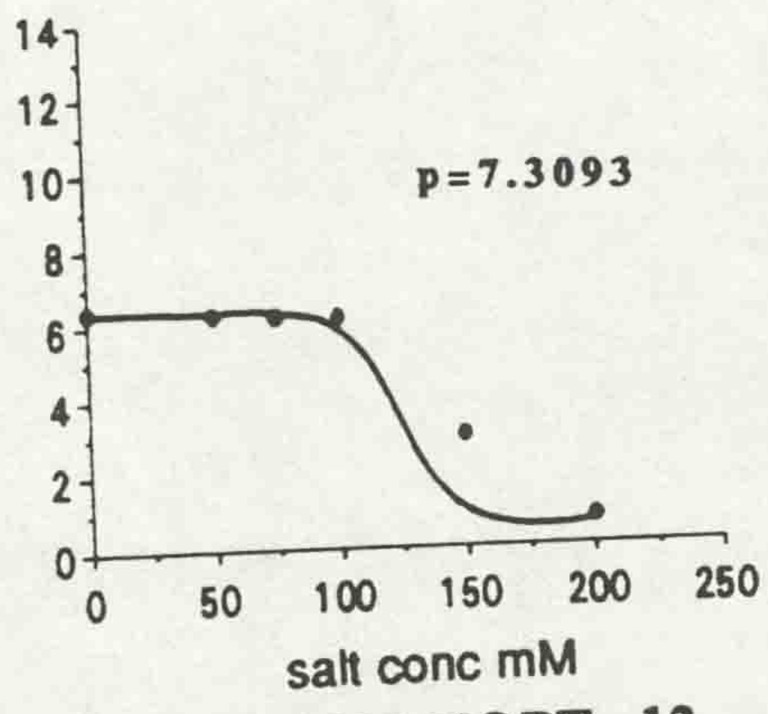

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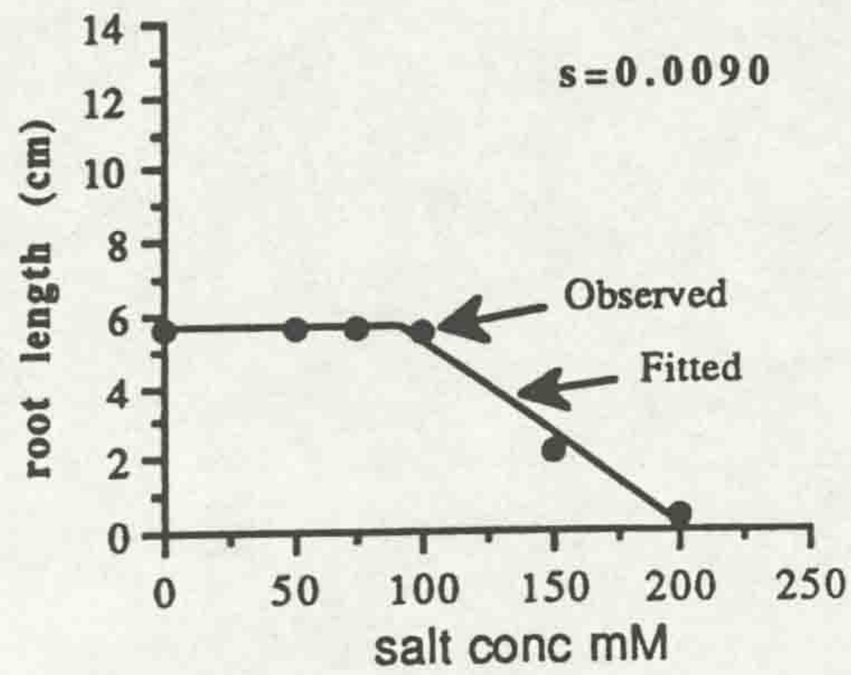

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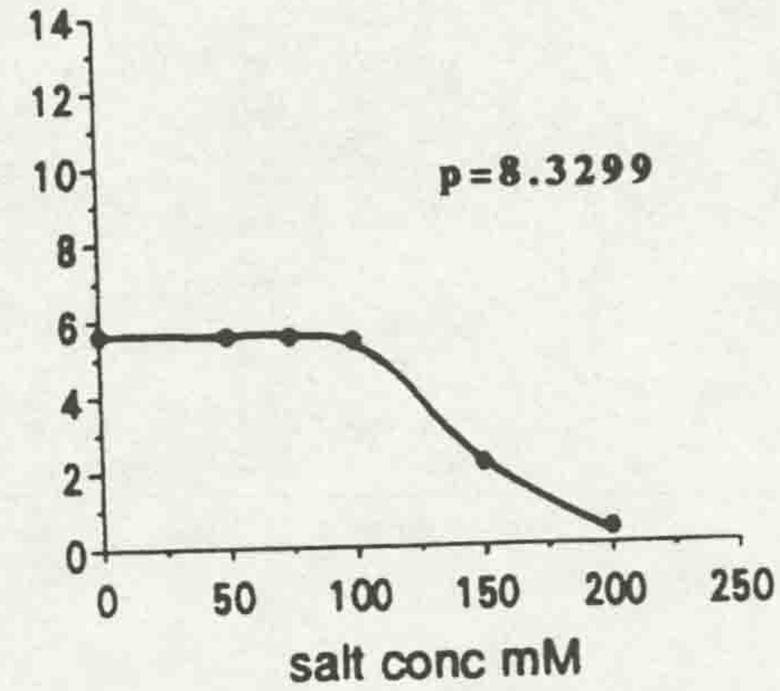

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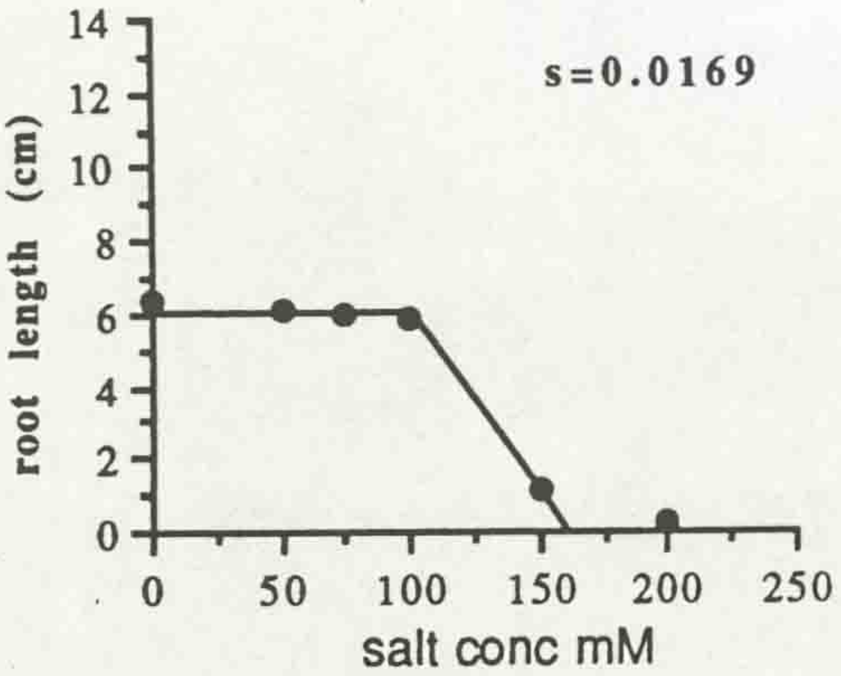

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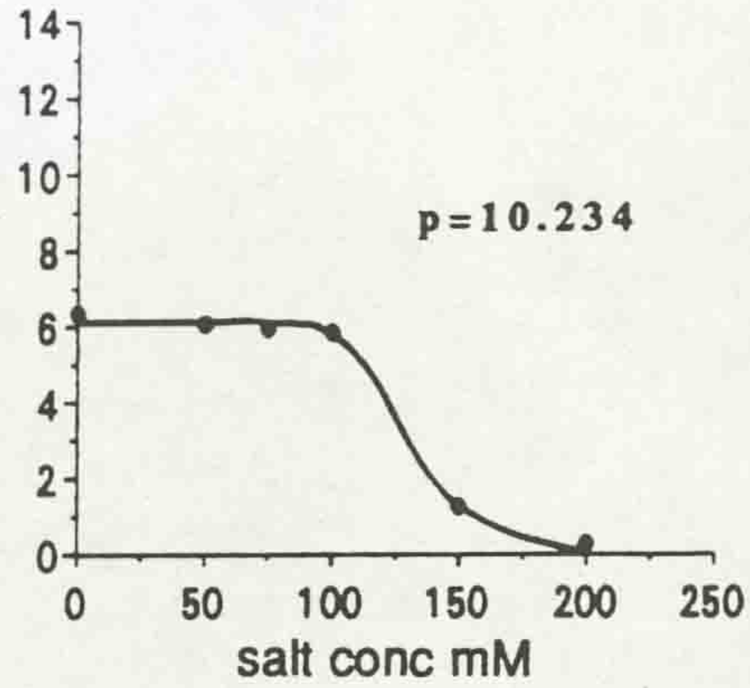

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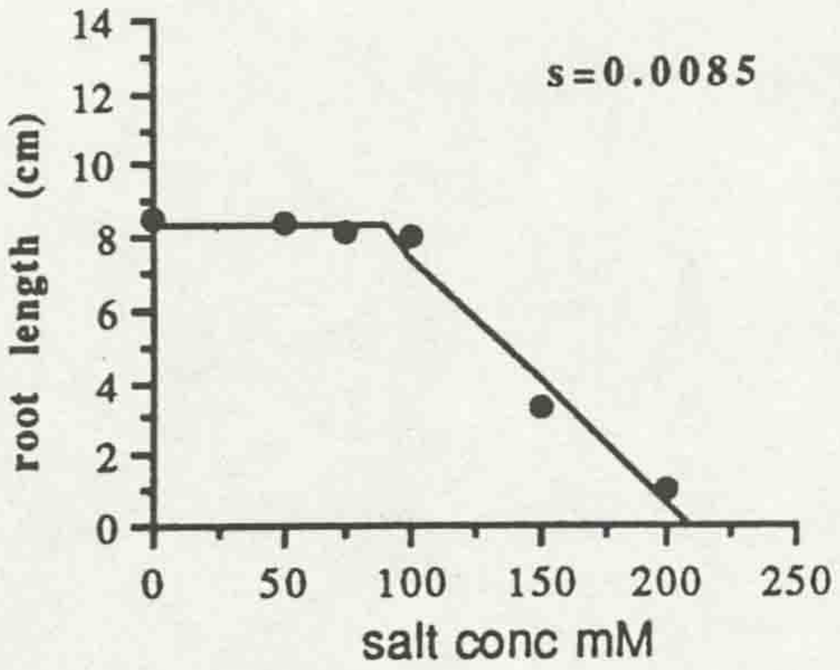

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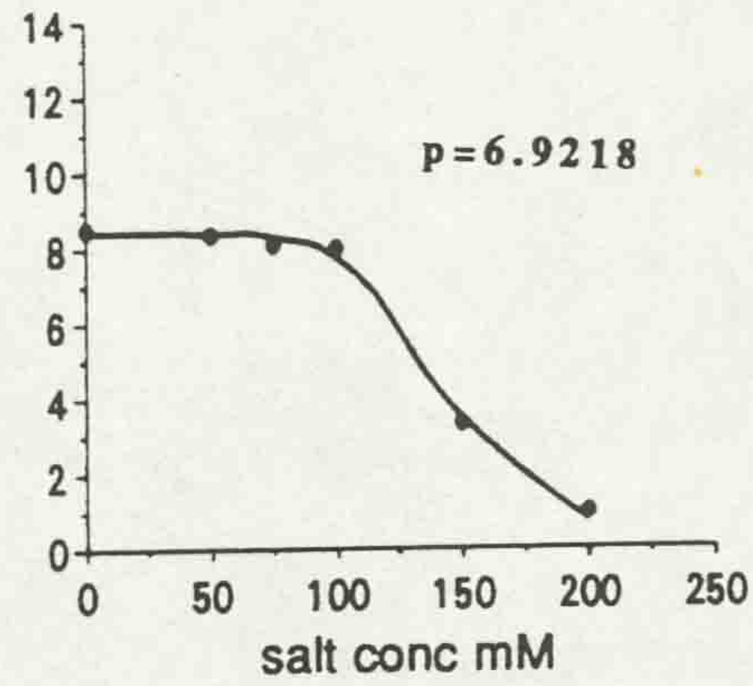

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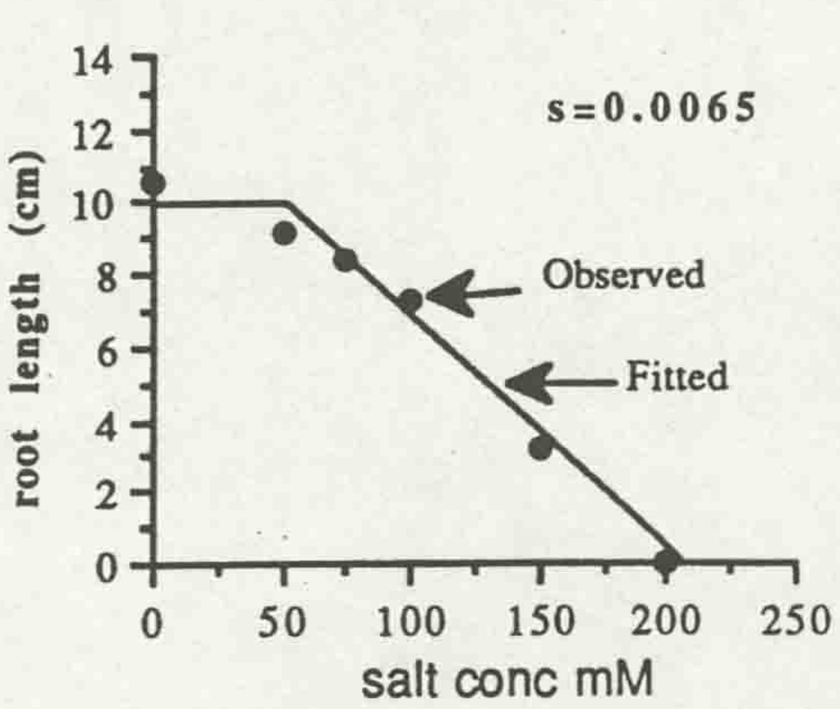

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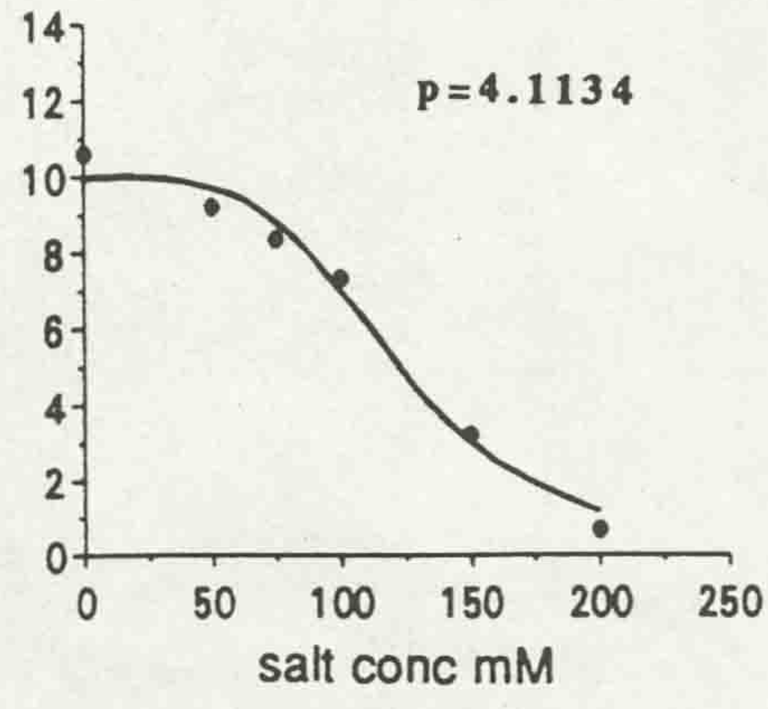
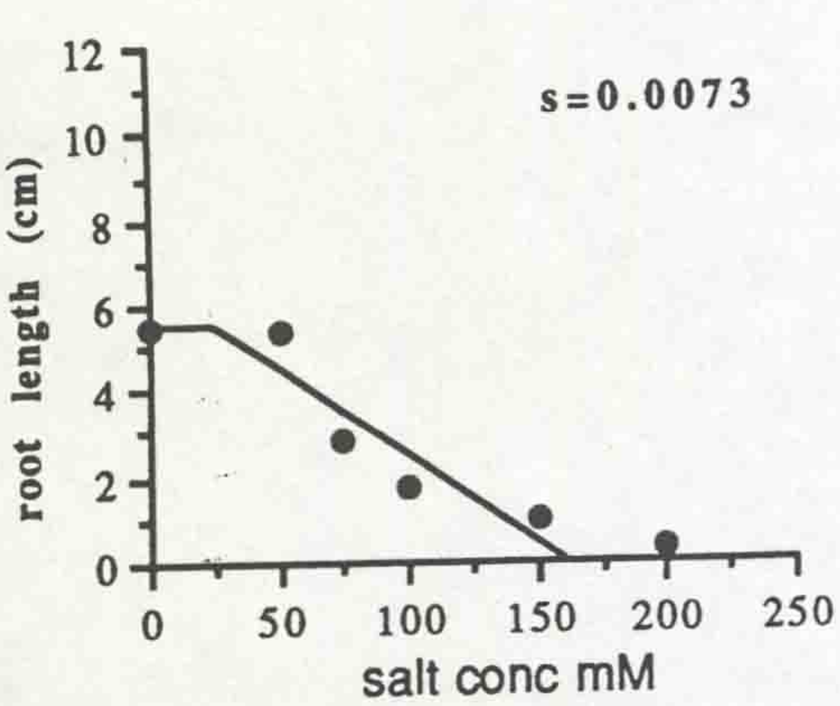
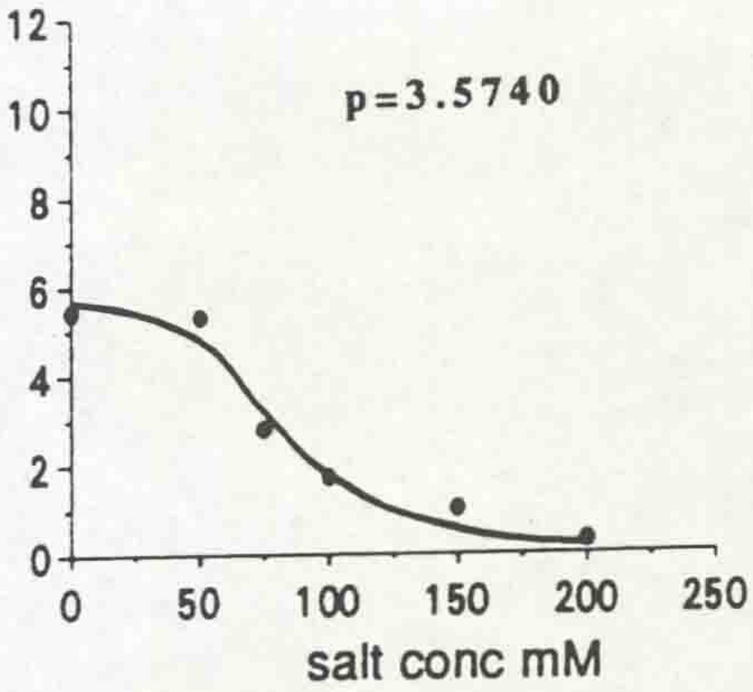


acc.100033-NOPT 12

Figure 2.2. Response functions between salt solution (mM) and root length (cm) of 14-day-old seedlings of *P. americanum* with slope, s (see NOPT 5 in materials and methods) and empirical constant, p (see NOPT 12 in materials and methods)



acc.215632-NOPT 5



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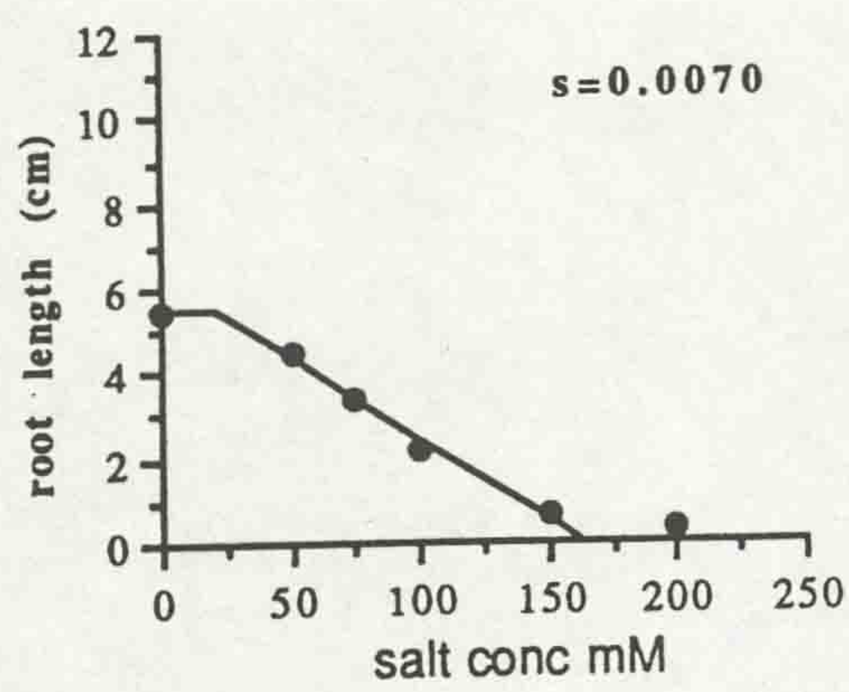

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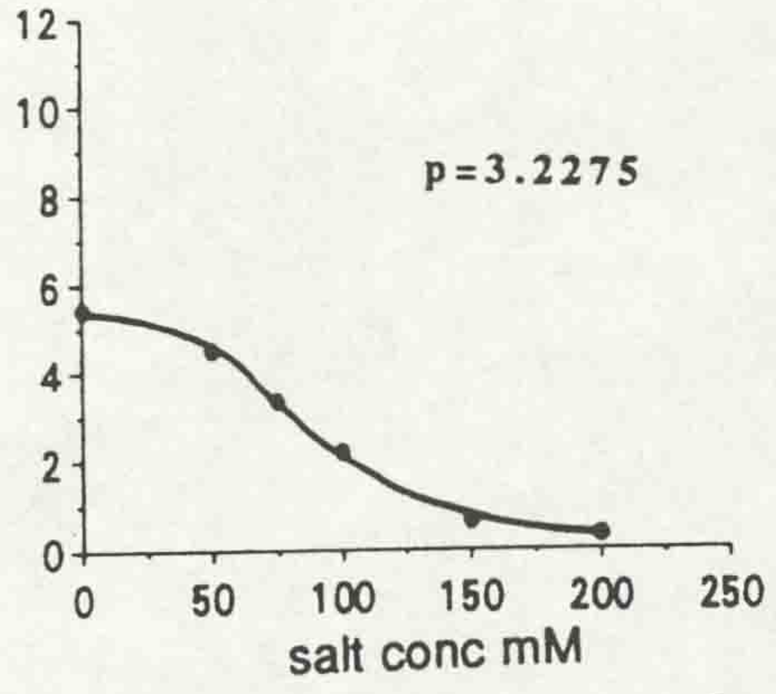

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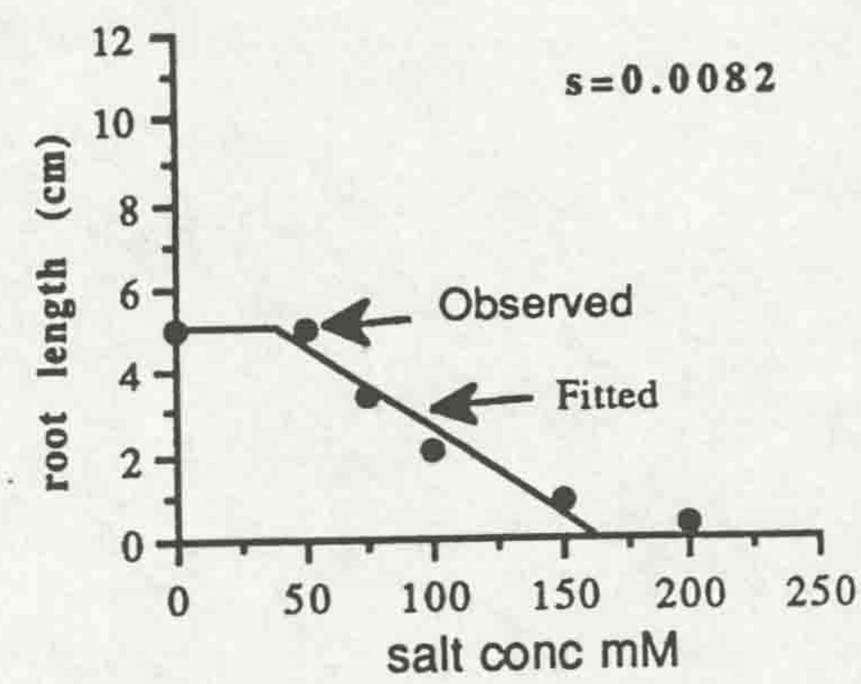

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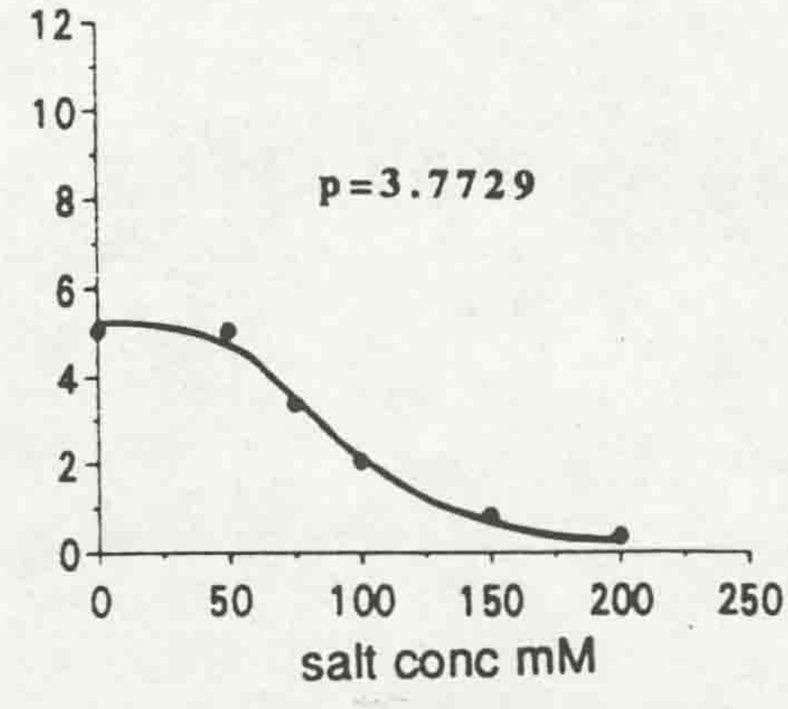

acc.215637-NOPT 12

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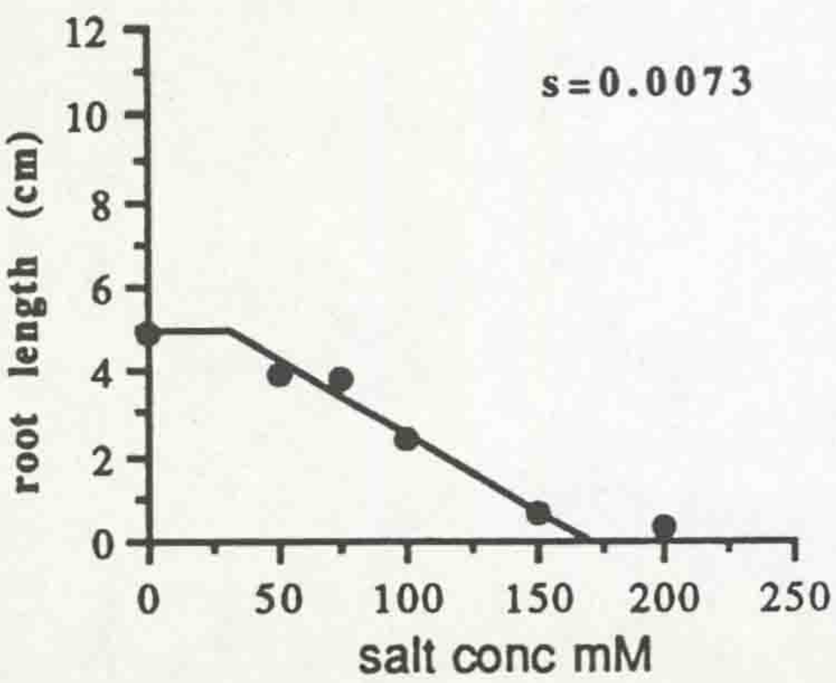

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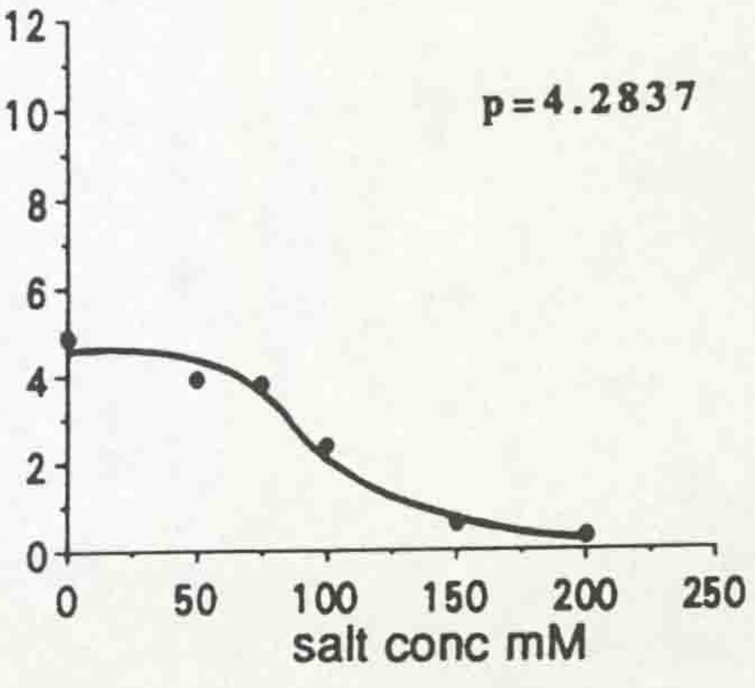

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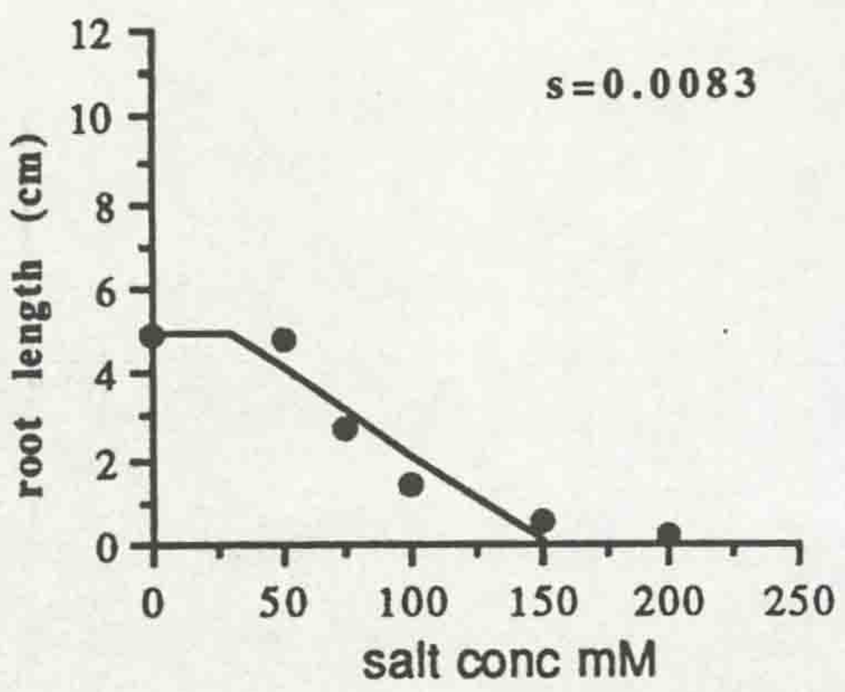

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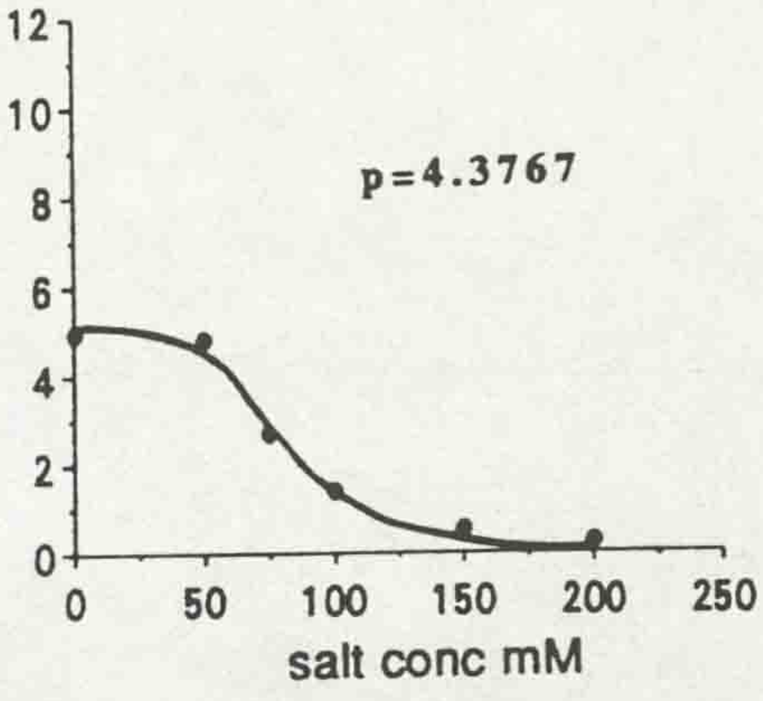

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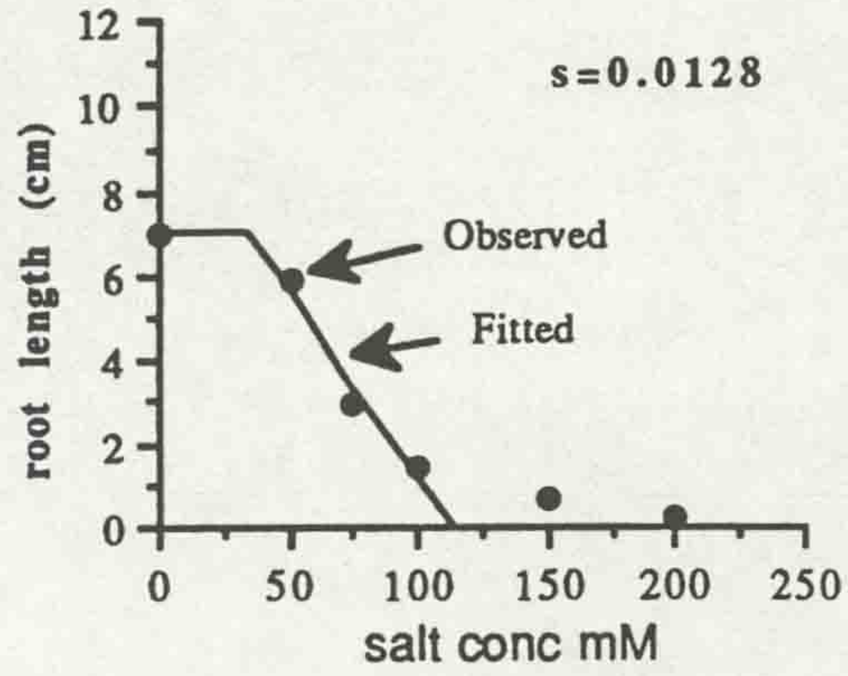
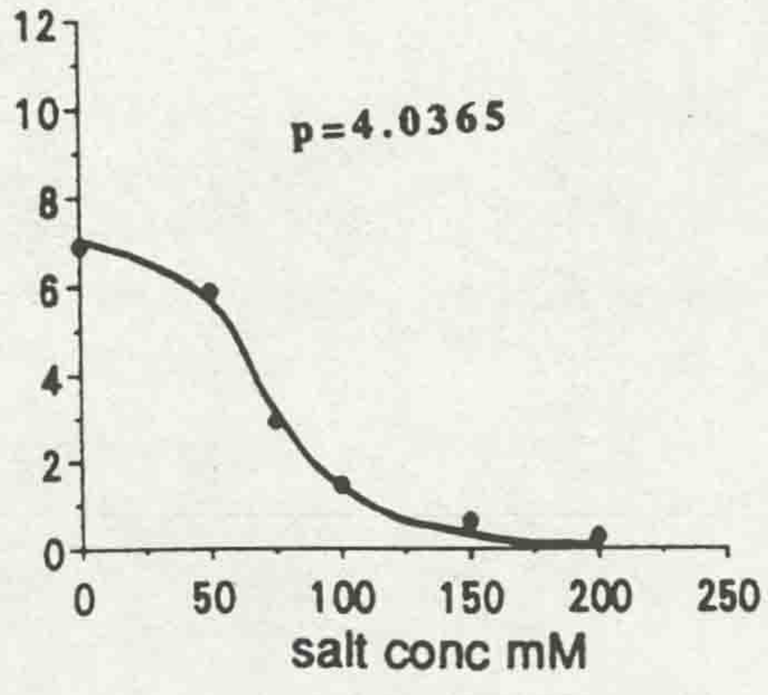
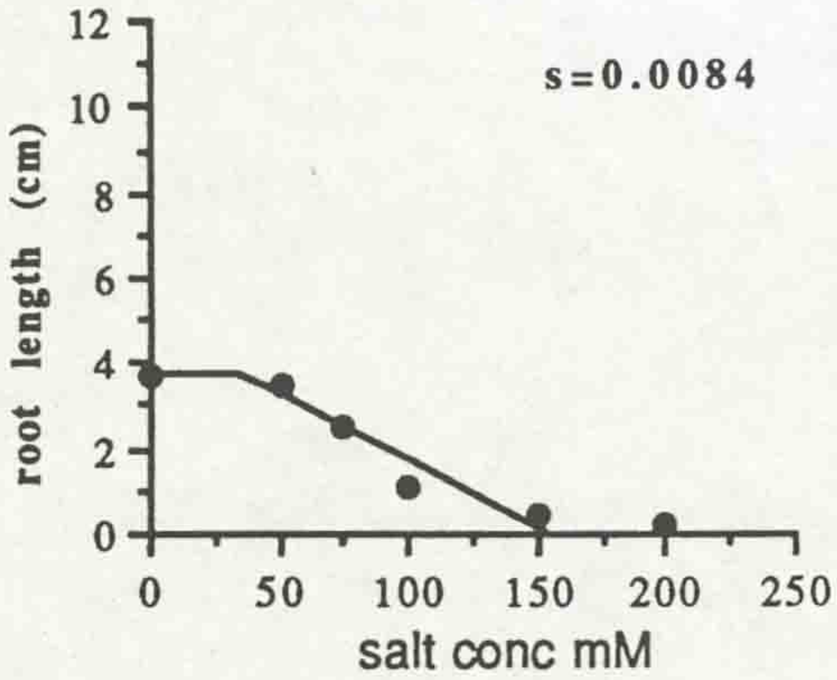


acc.220139-NOPT 5

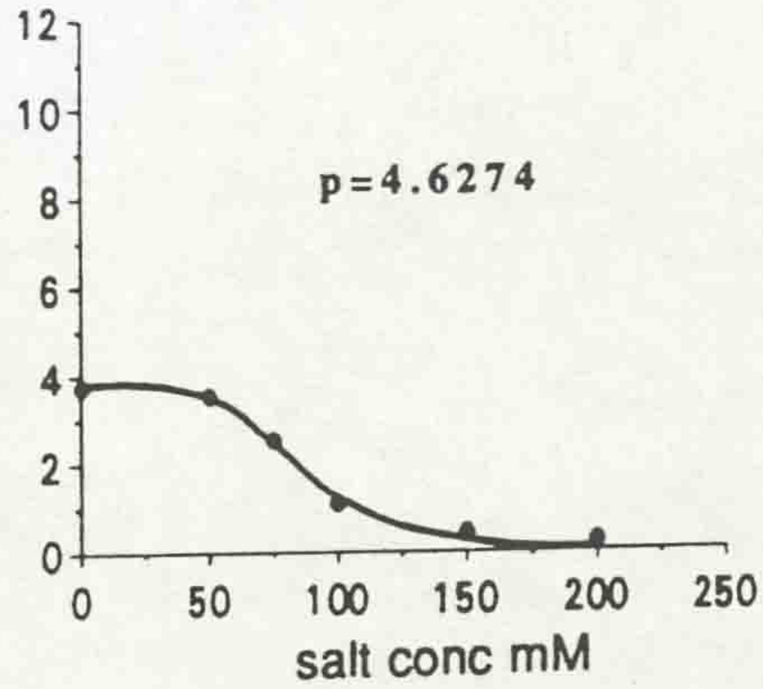


acc.220139-NOPT 12

(Figure 2.2 continued)



acc.220220-NOPT 5



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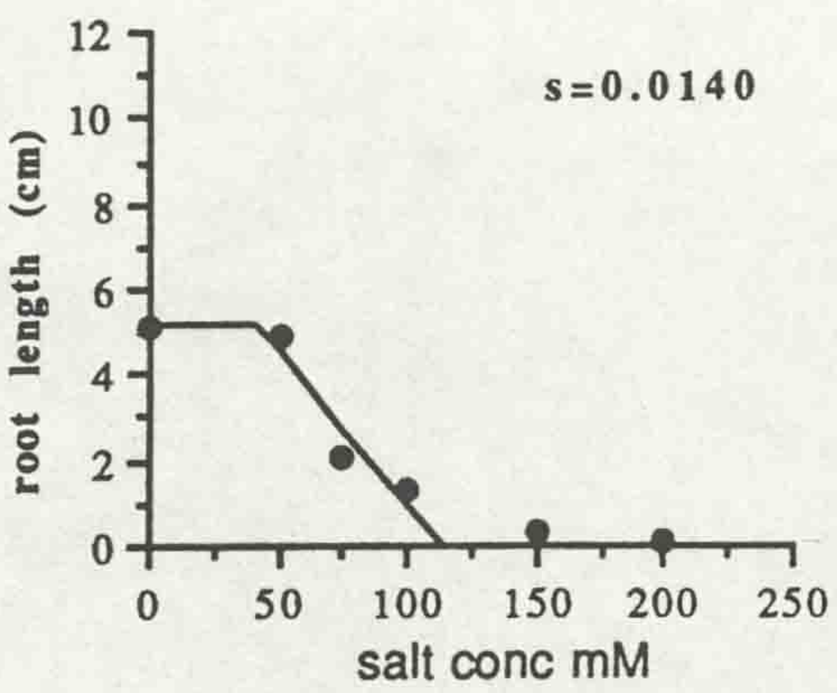

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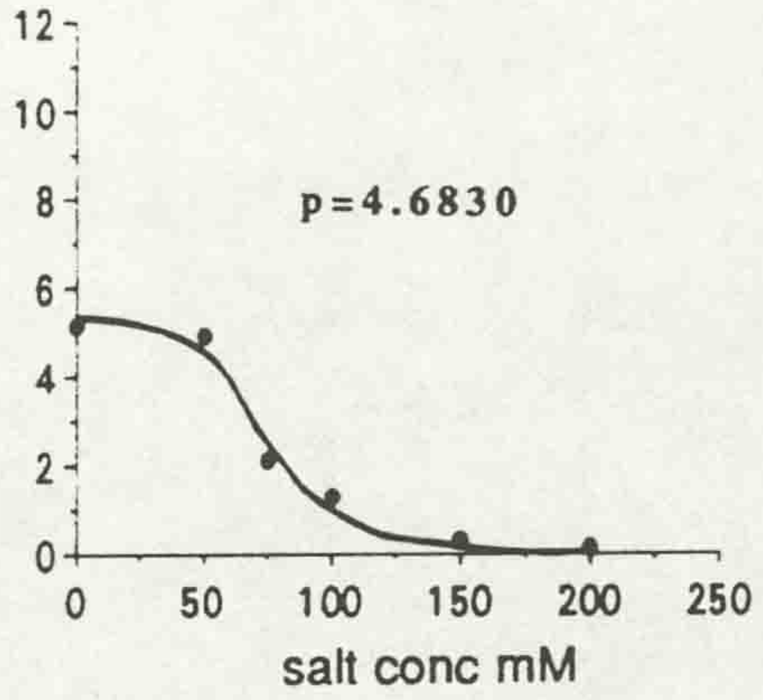

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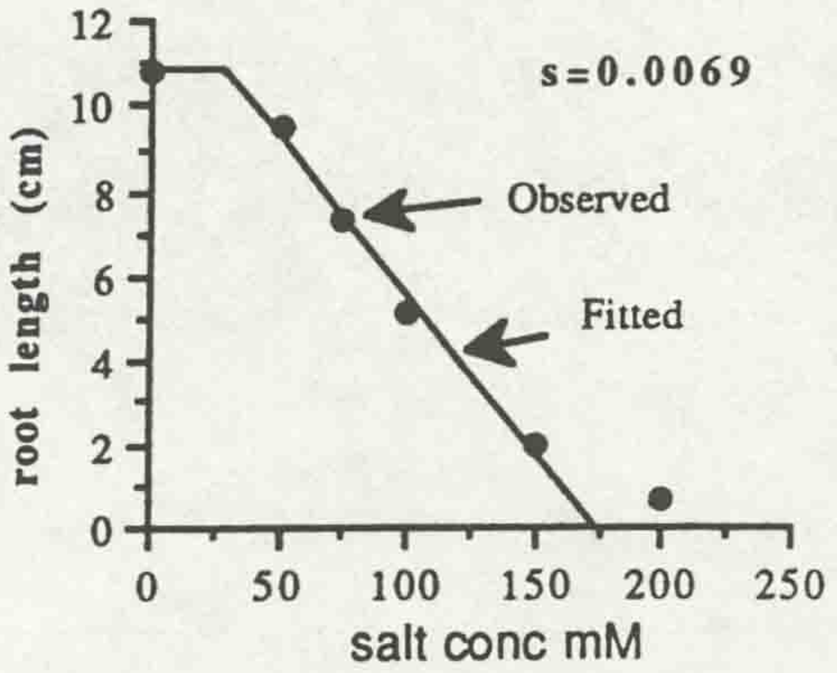
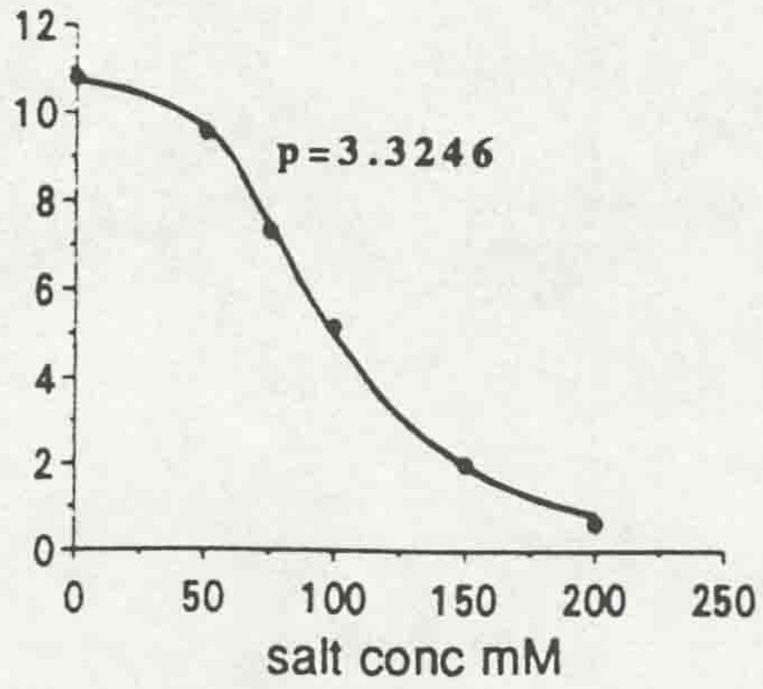
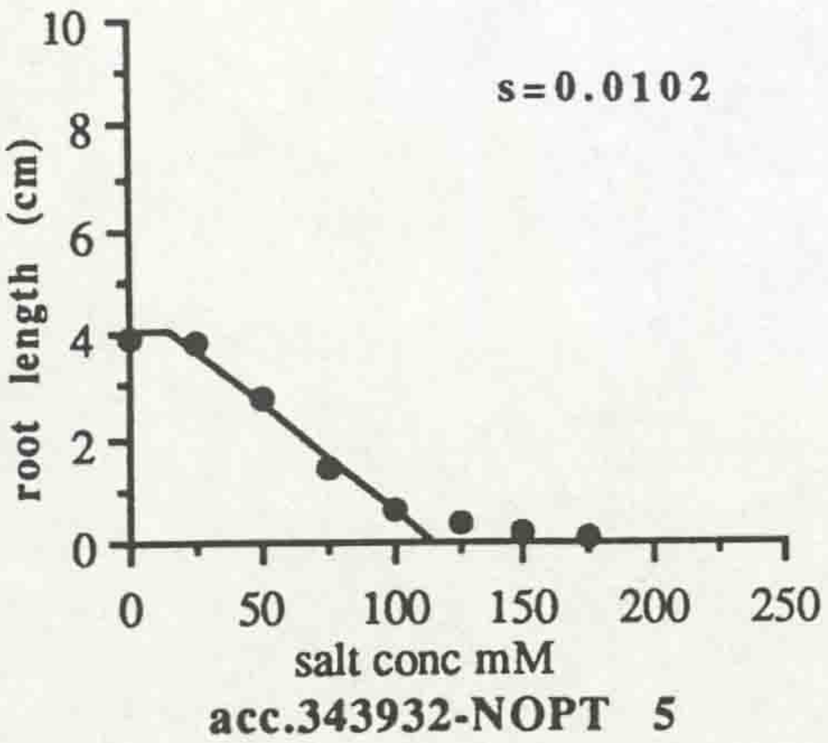


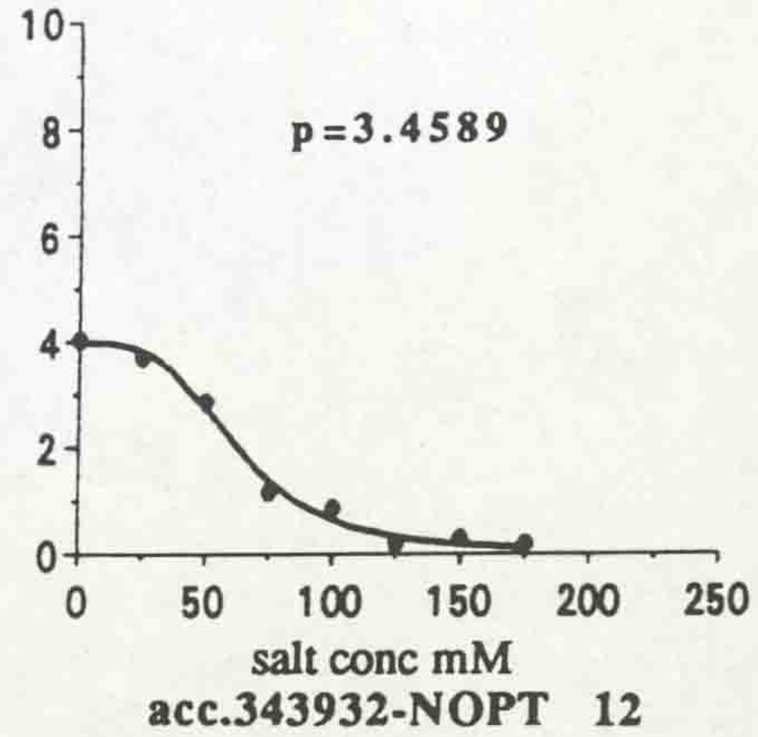
acc.221726-NOPT 5

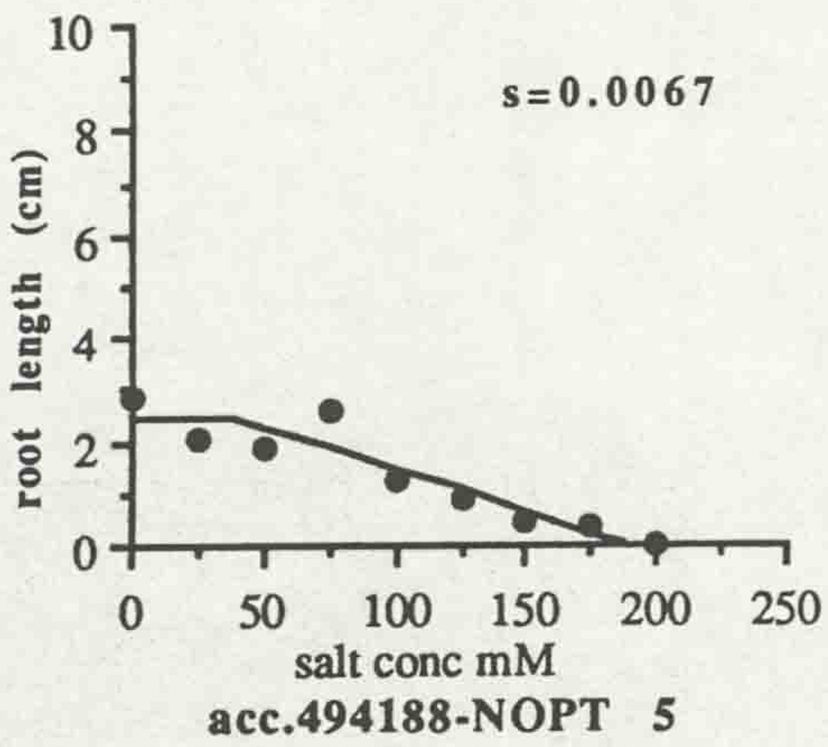


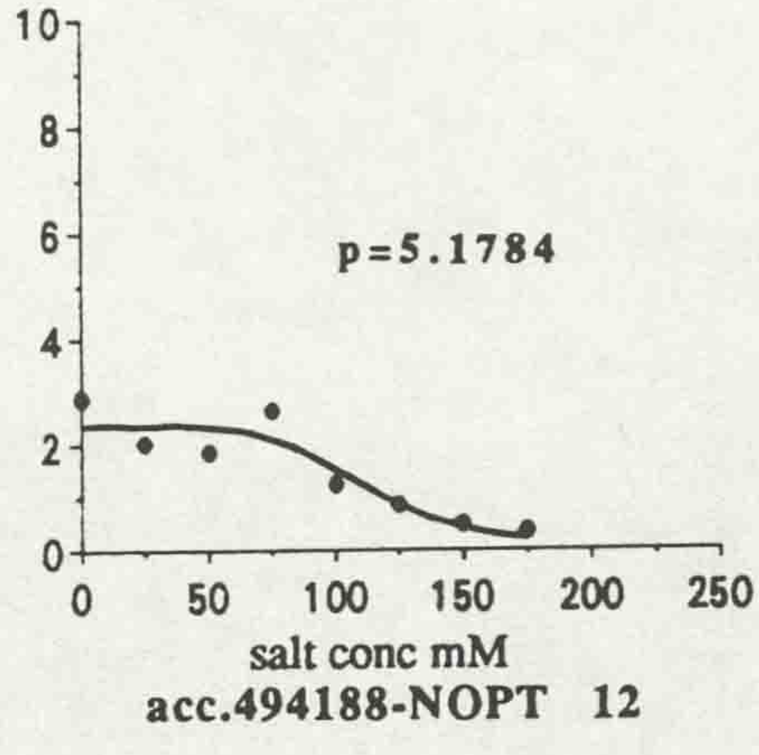
acc.221726-NOPT 12

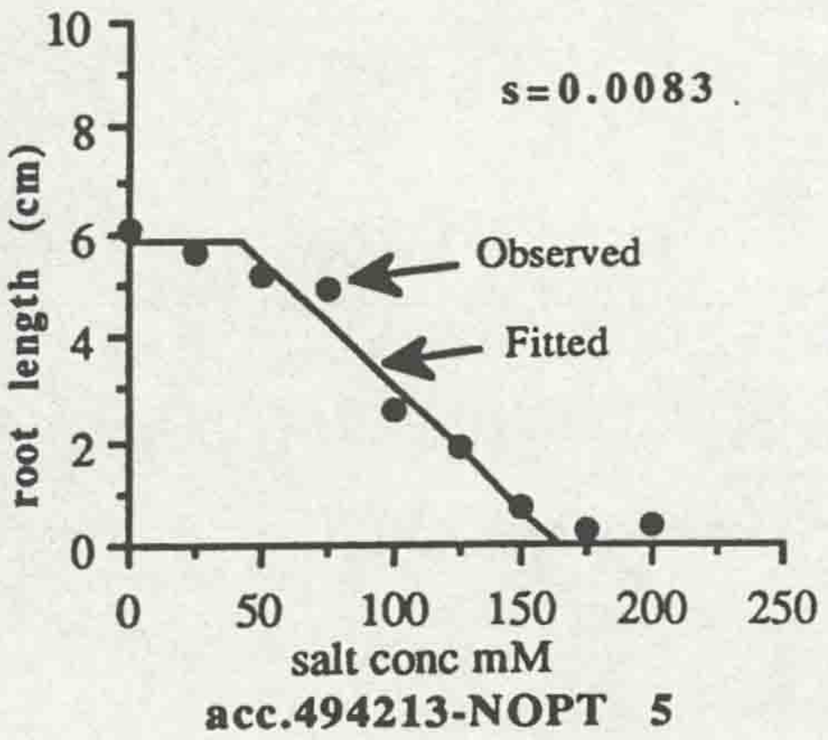
Figure 2.3. Response functions between salt solution (mM) and root length (cm) of 14-day-old seedlings of *E. tef* with slope, s (see NOPT 5 in materials and methods) and empirical constant, p (see NOPT 12 in materials and methods)

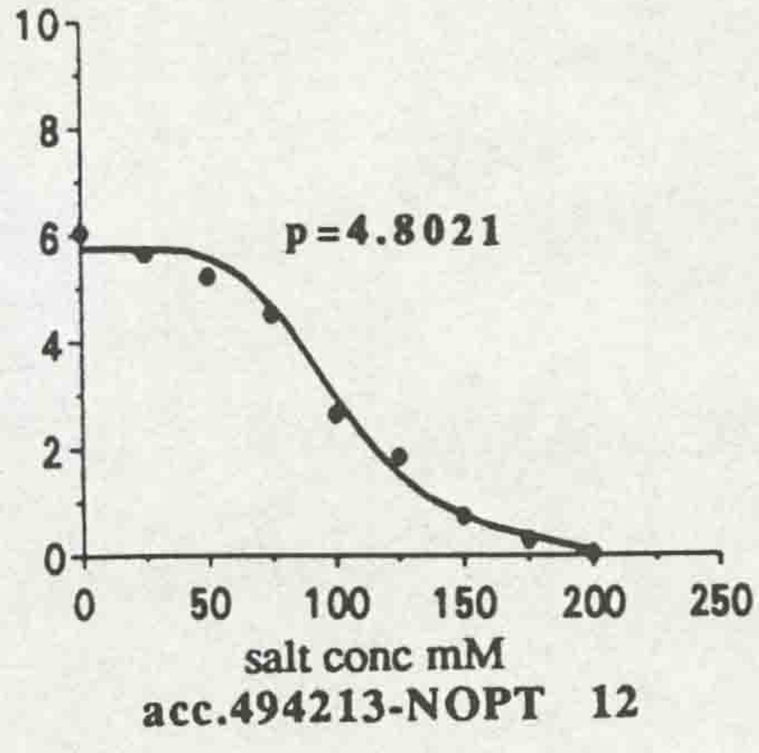












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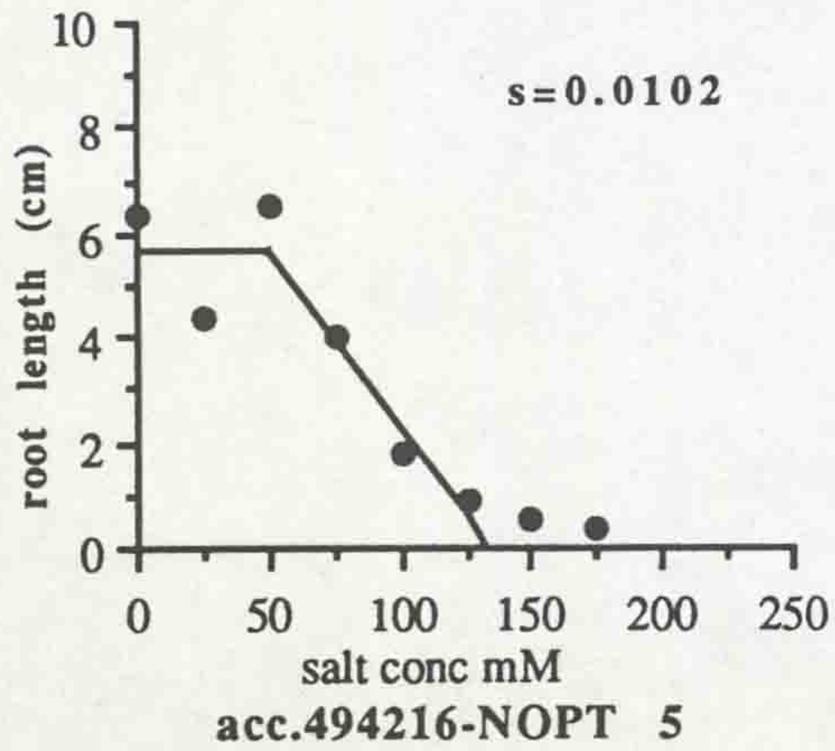

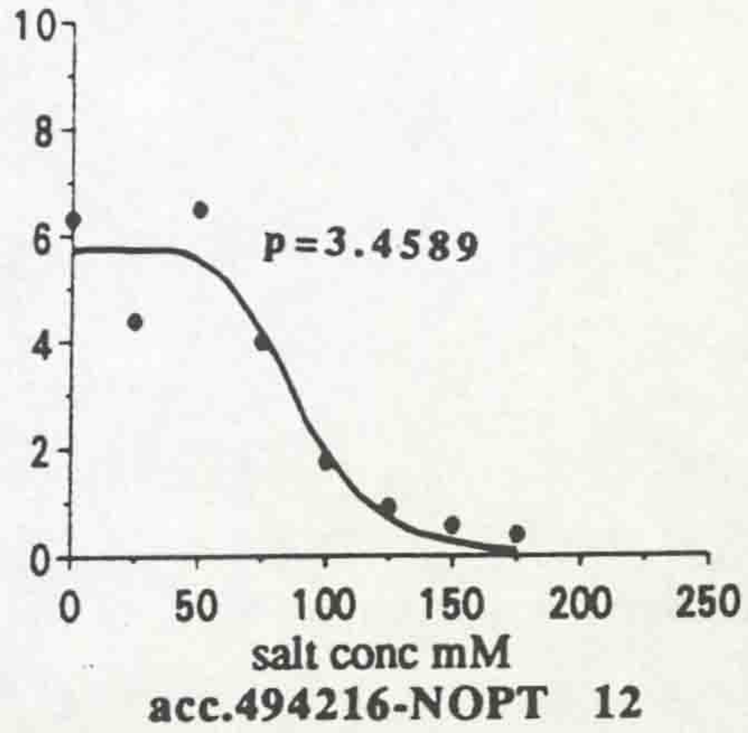

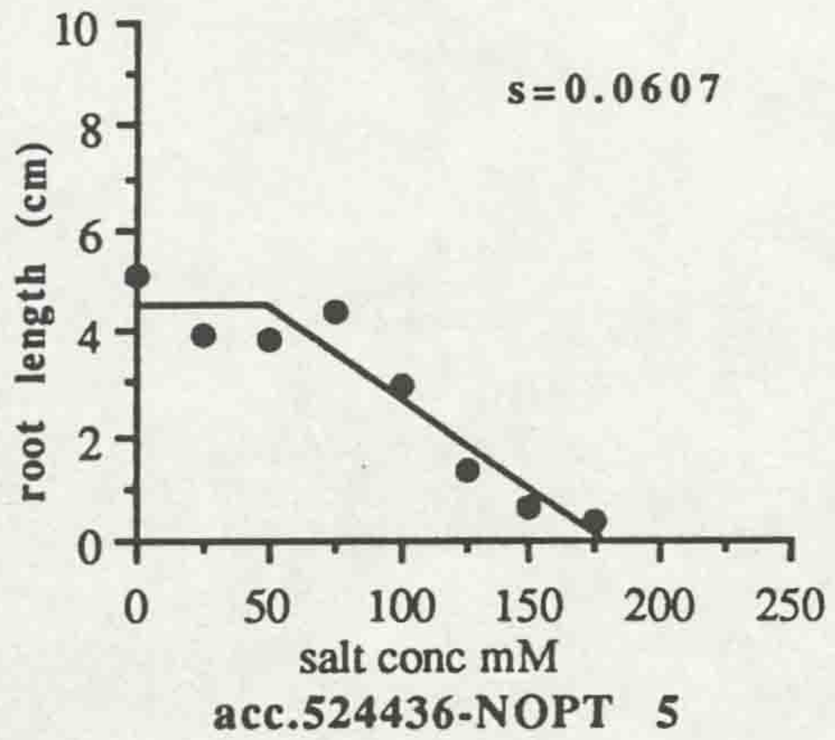

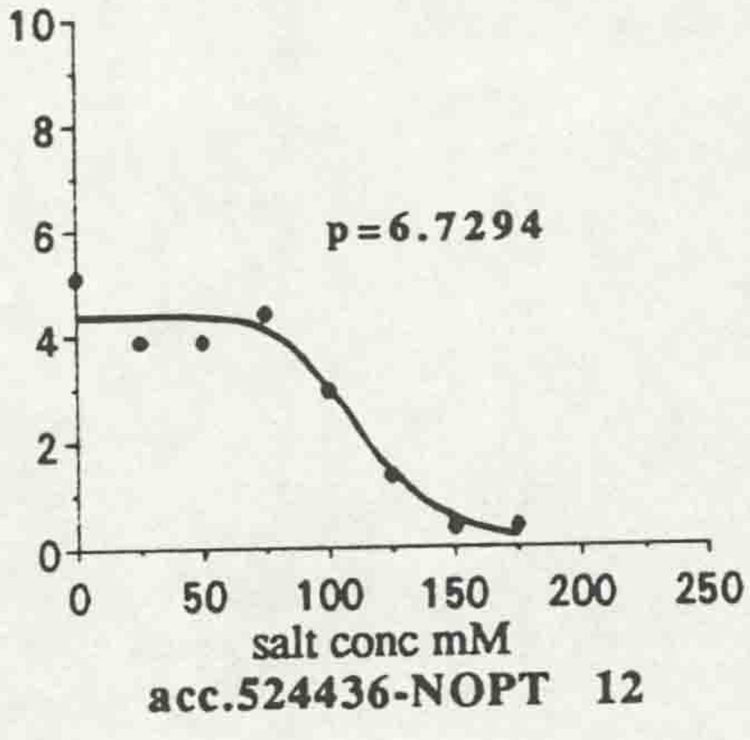

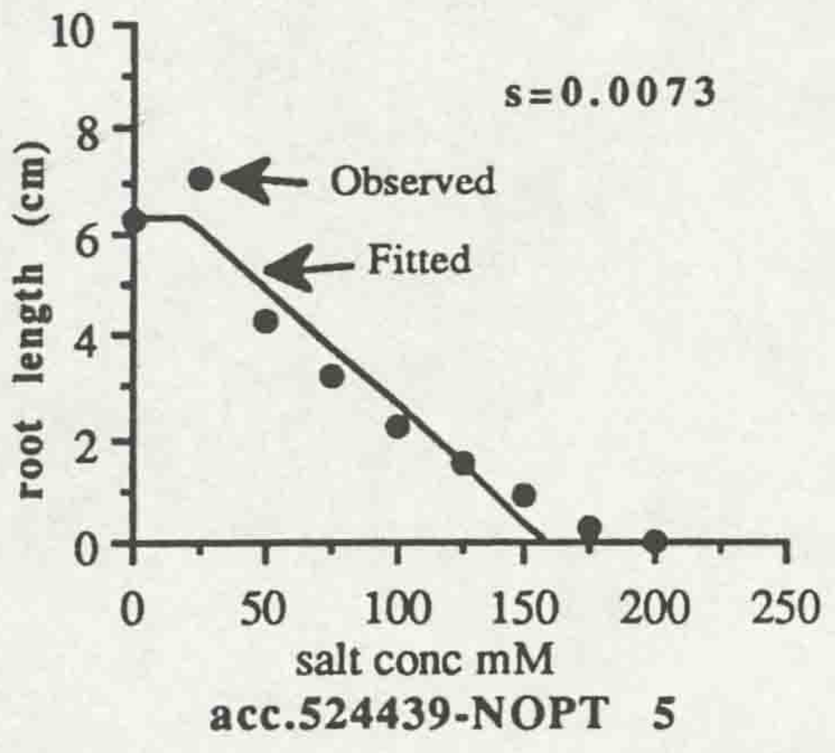

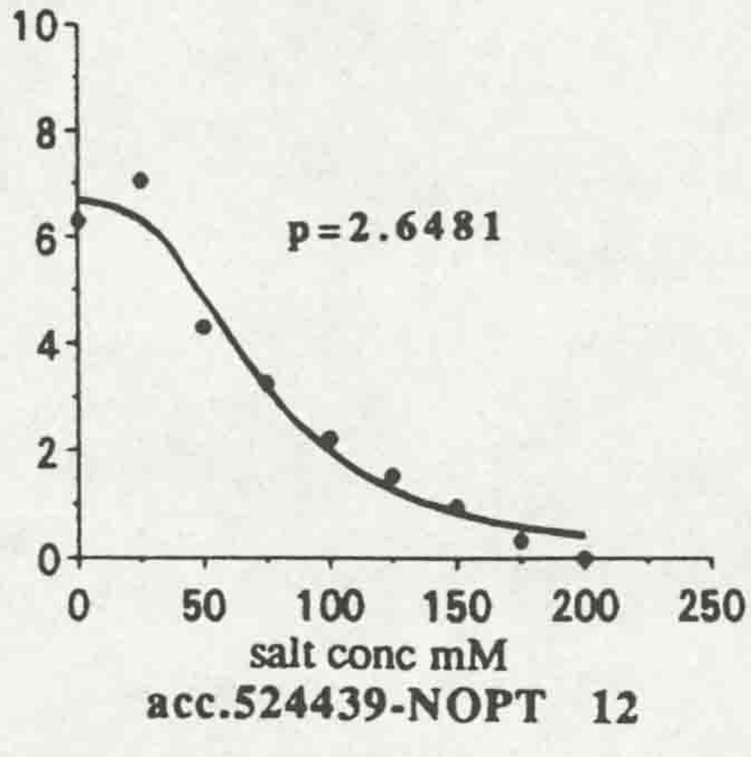

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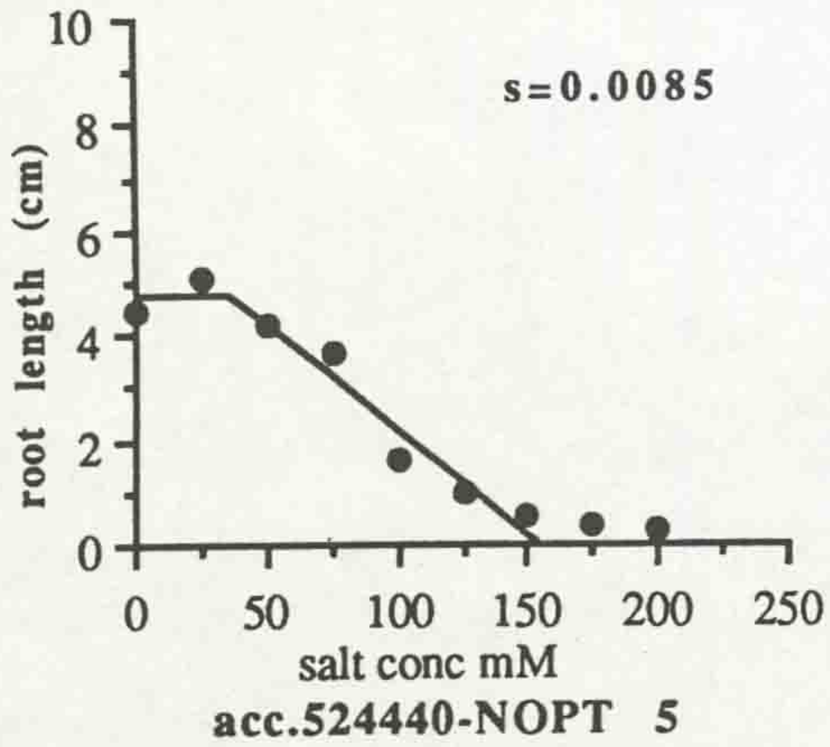
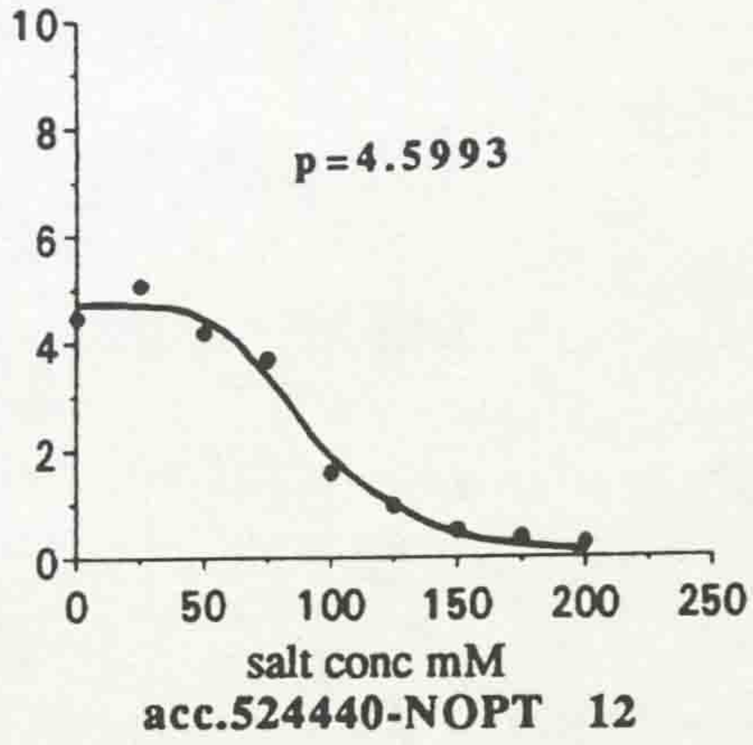


acc.524440-NOPT 5



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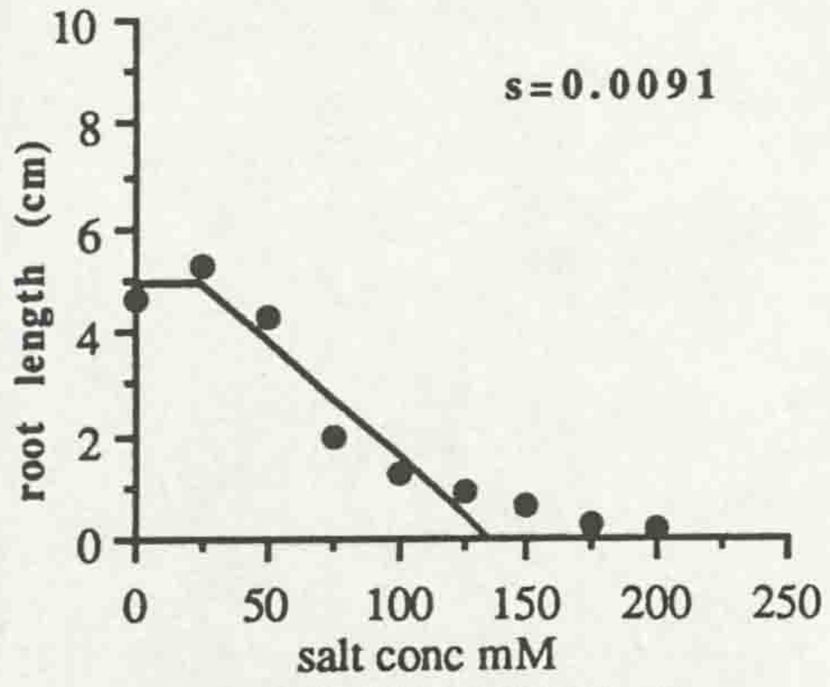

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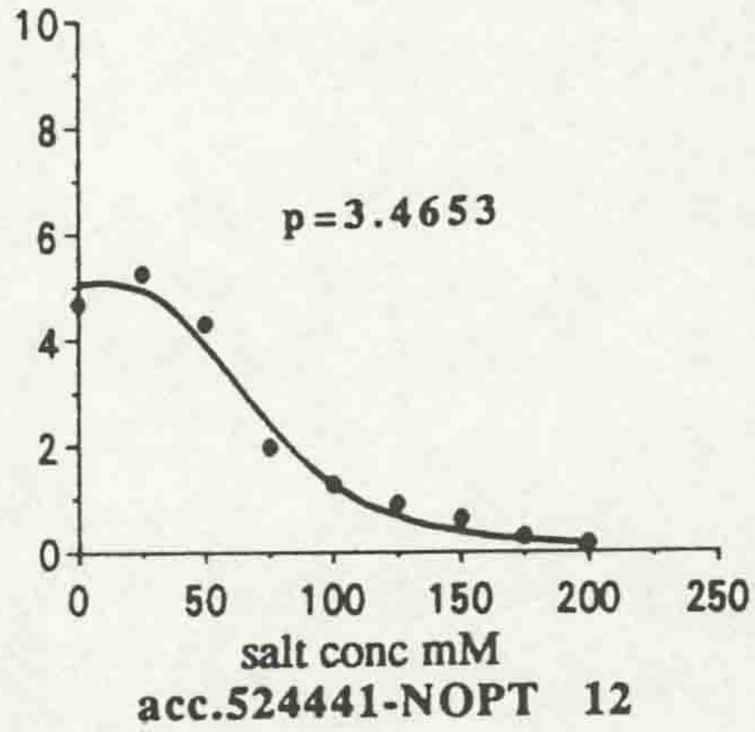

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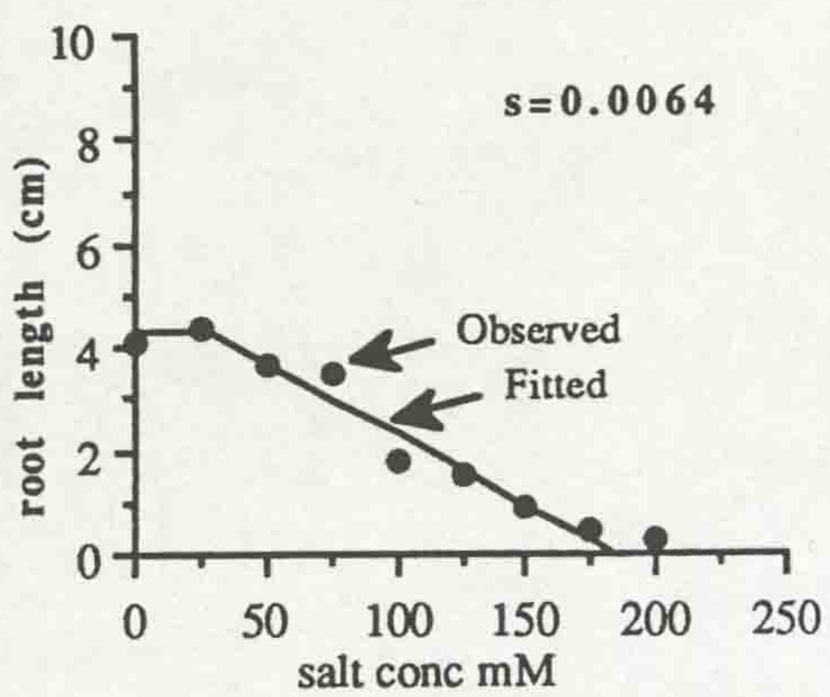

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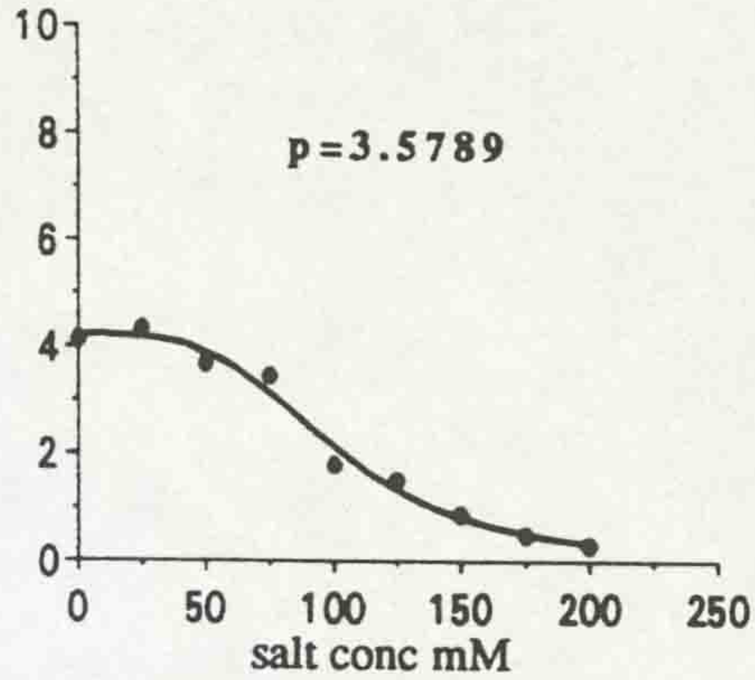

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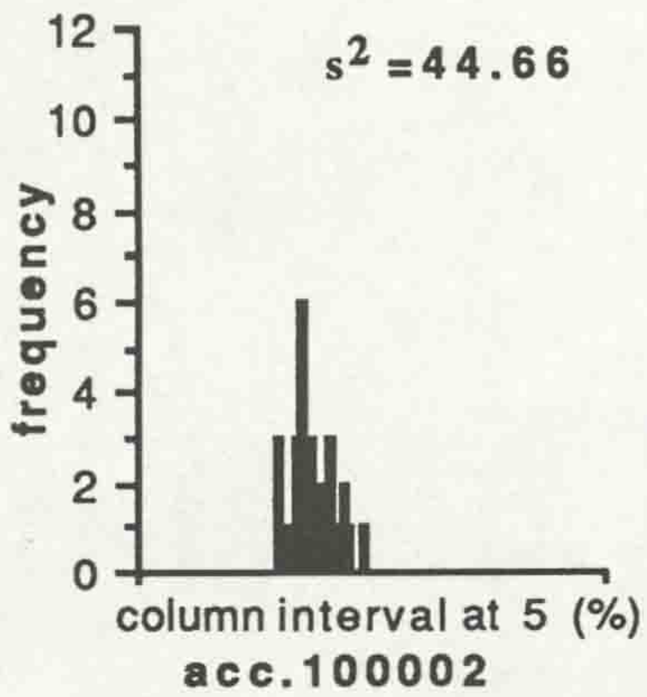
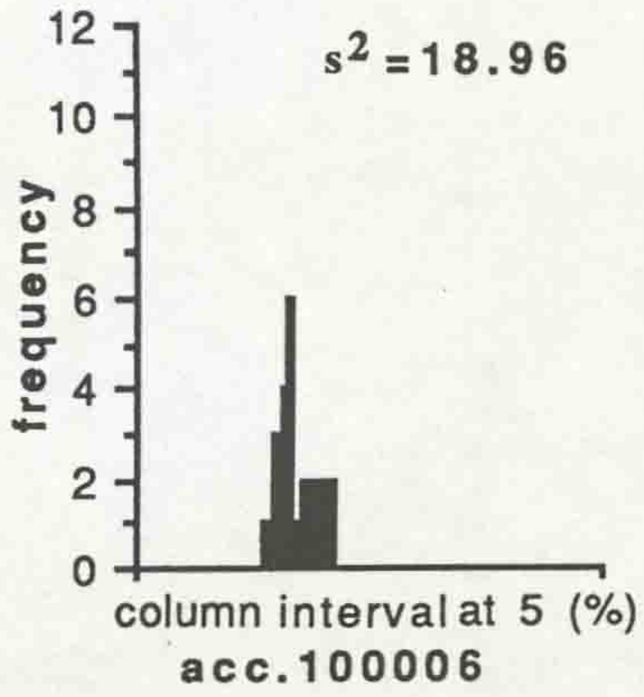
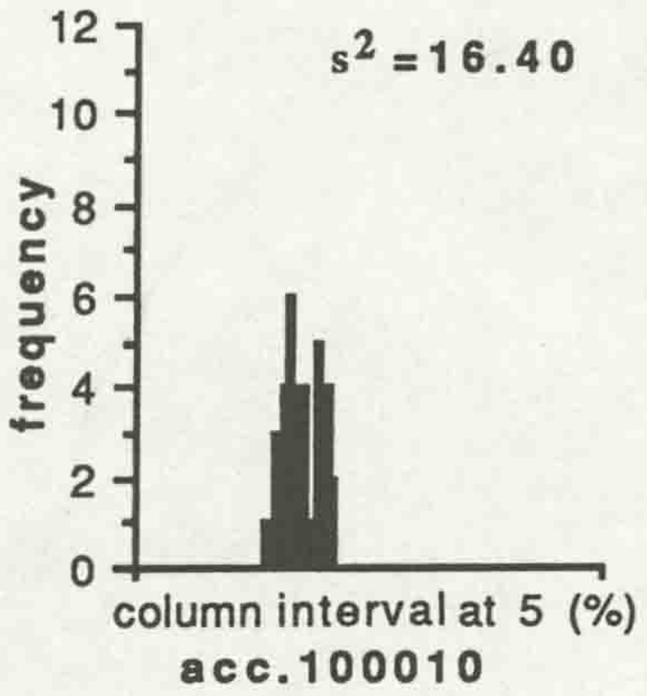
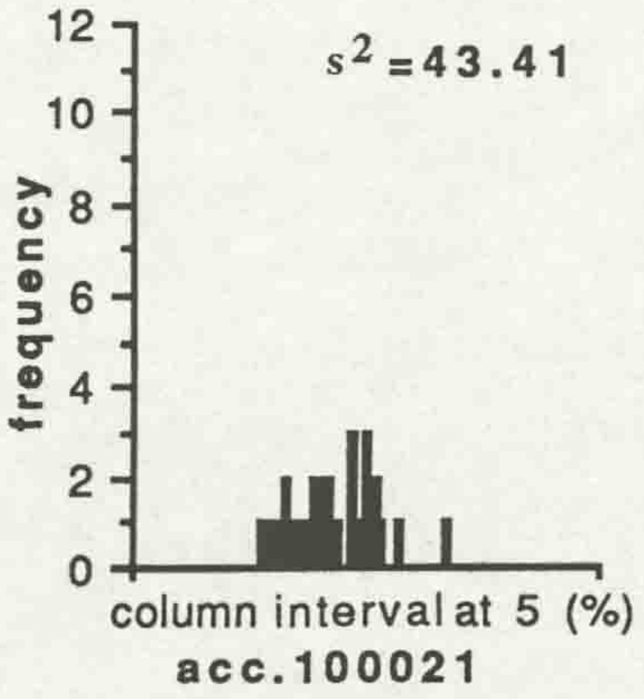
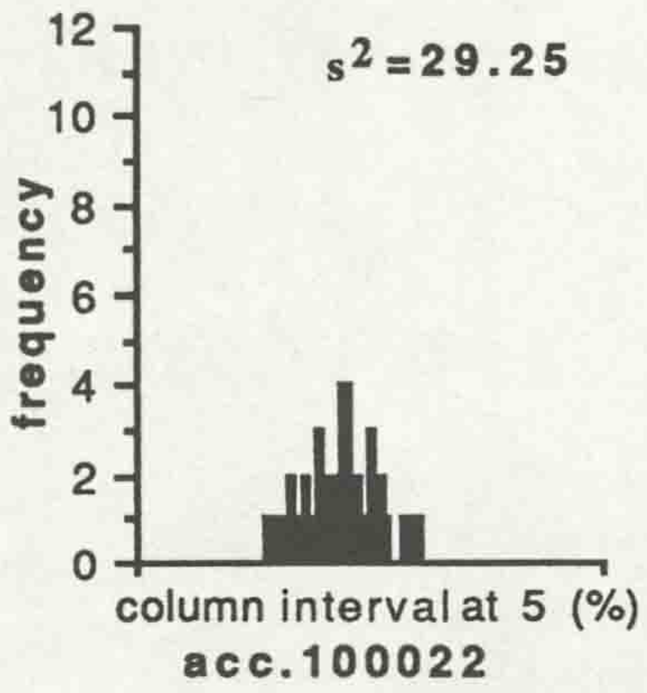
Figure 2.4. Frequency distribution of relative tolerance of root length of 30 seedlings of *E. coracana* grown at 100 mM NaCl [with variance (s^2)]

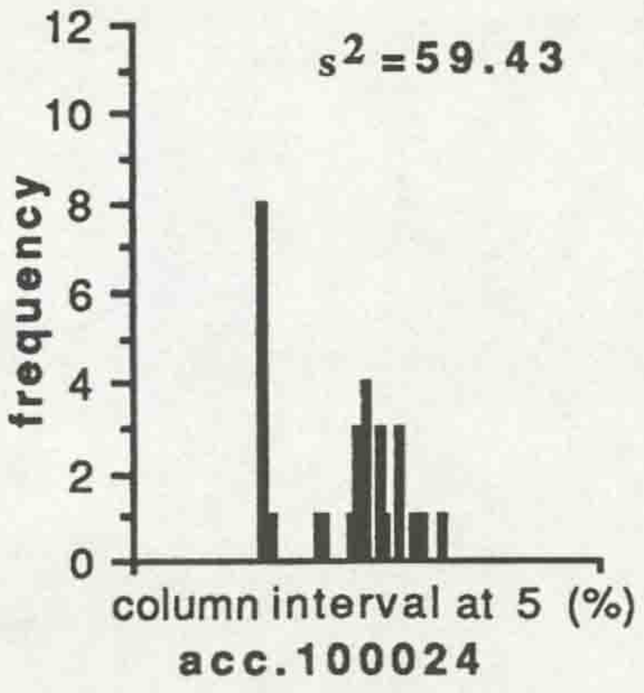












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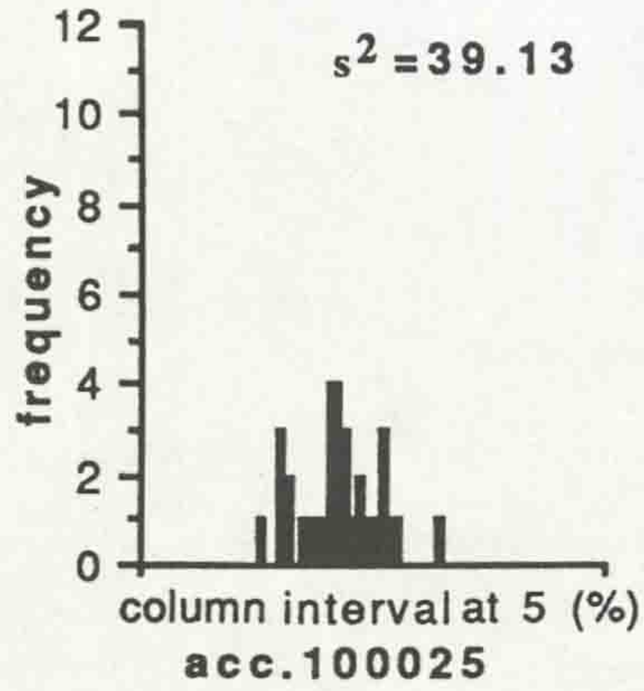
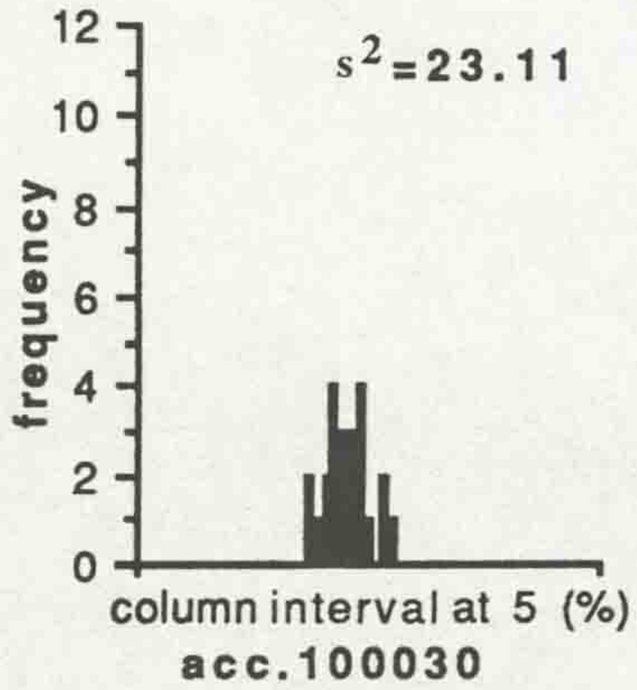




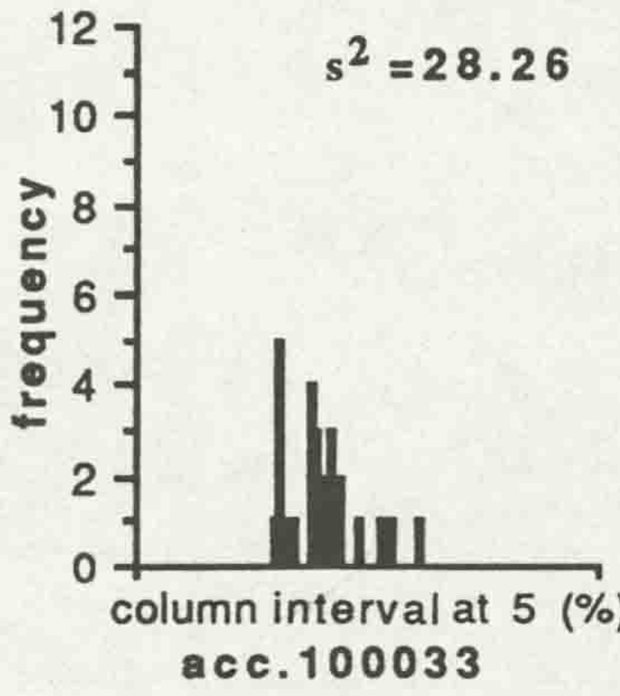
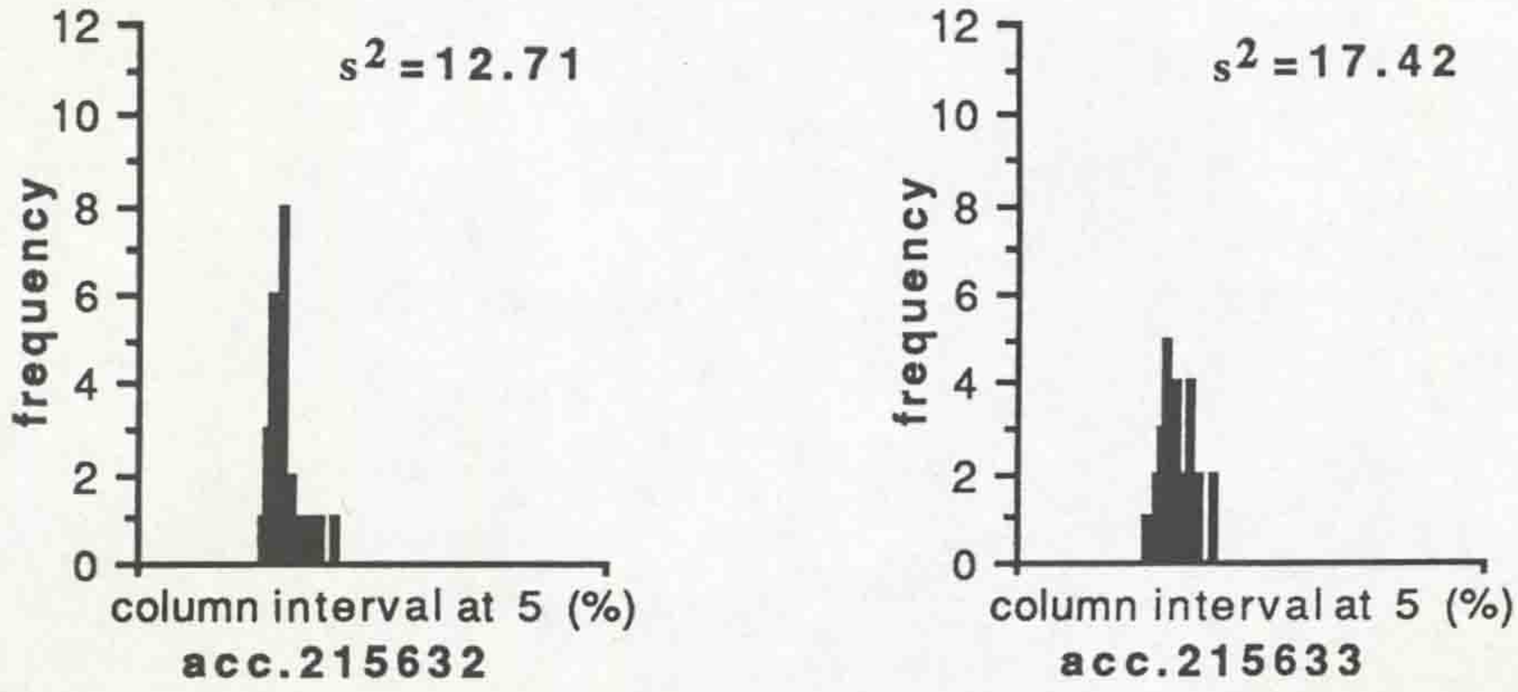
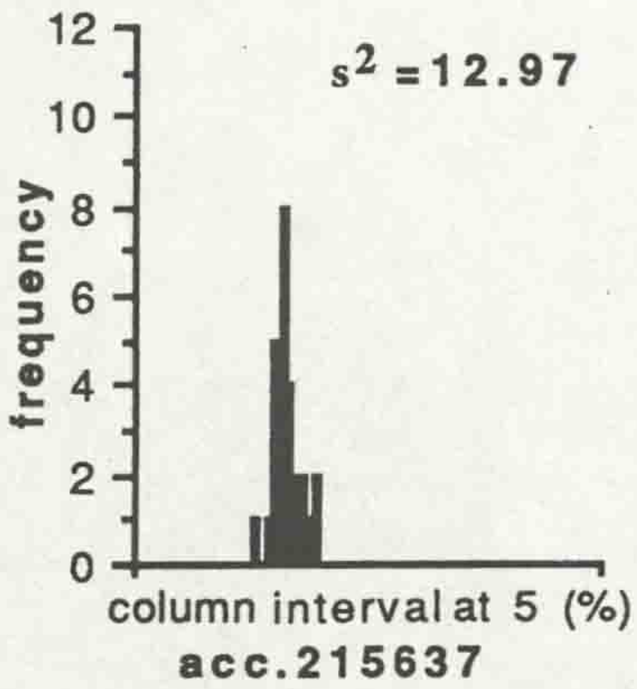
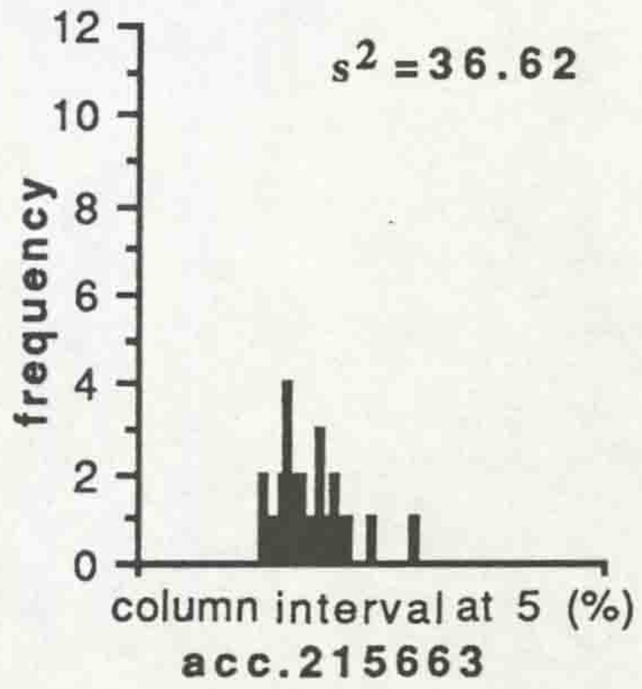
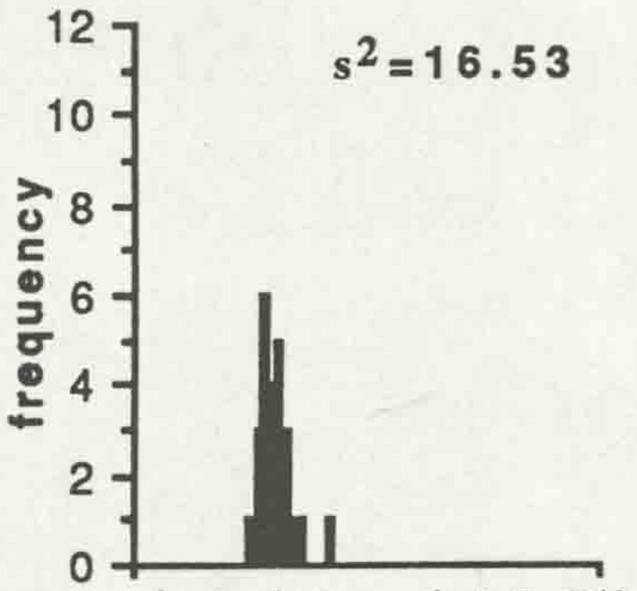


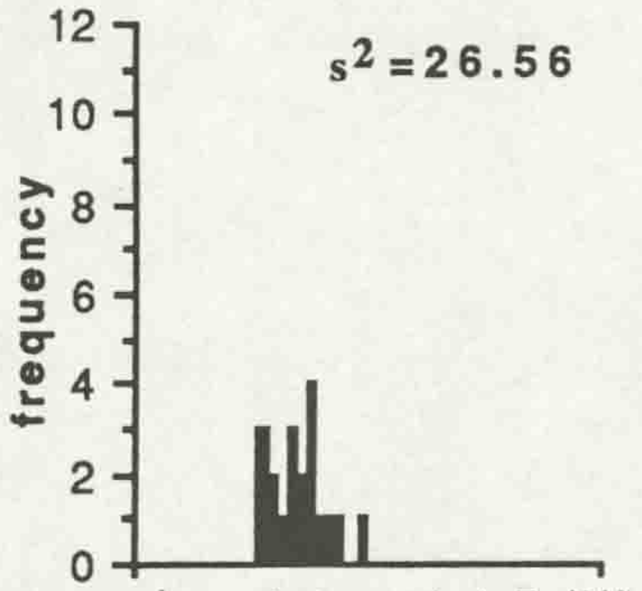
Figure 2.5. Frequency distribution of relative tolerance of root length of 30 seedlings of *P. americanum* grown at 100 mM NaCl [with variance (s^2)]



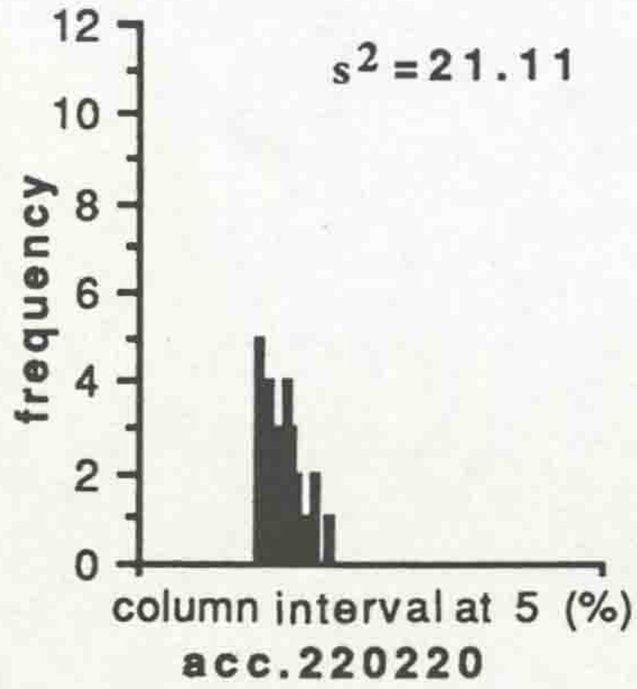


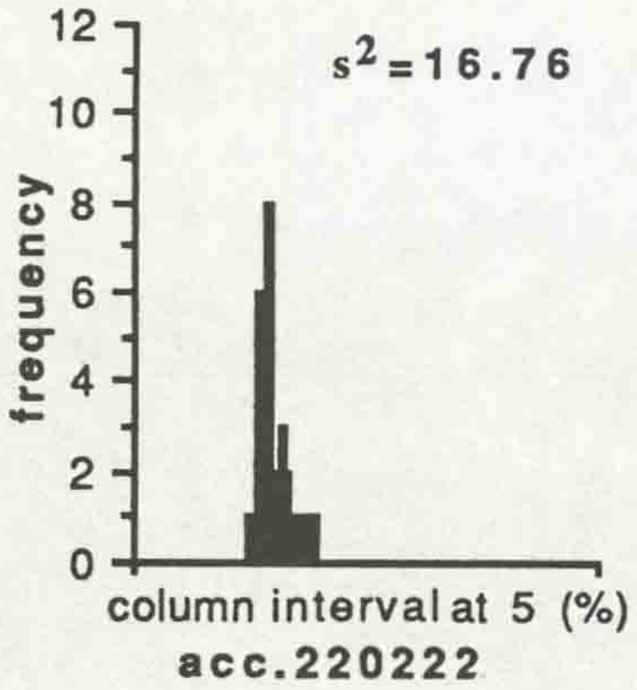






(Figure 2.5 continued)





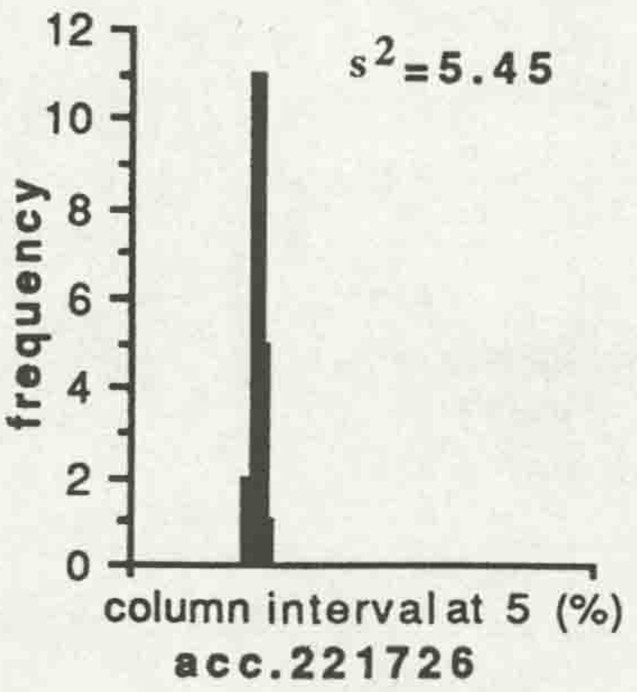
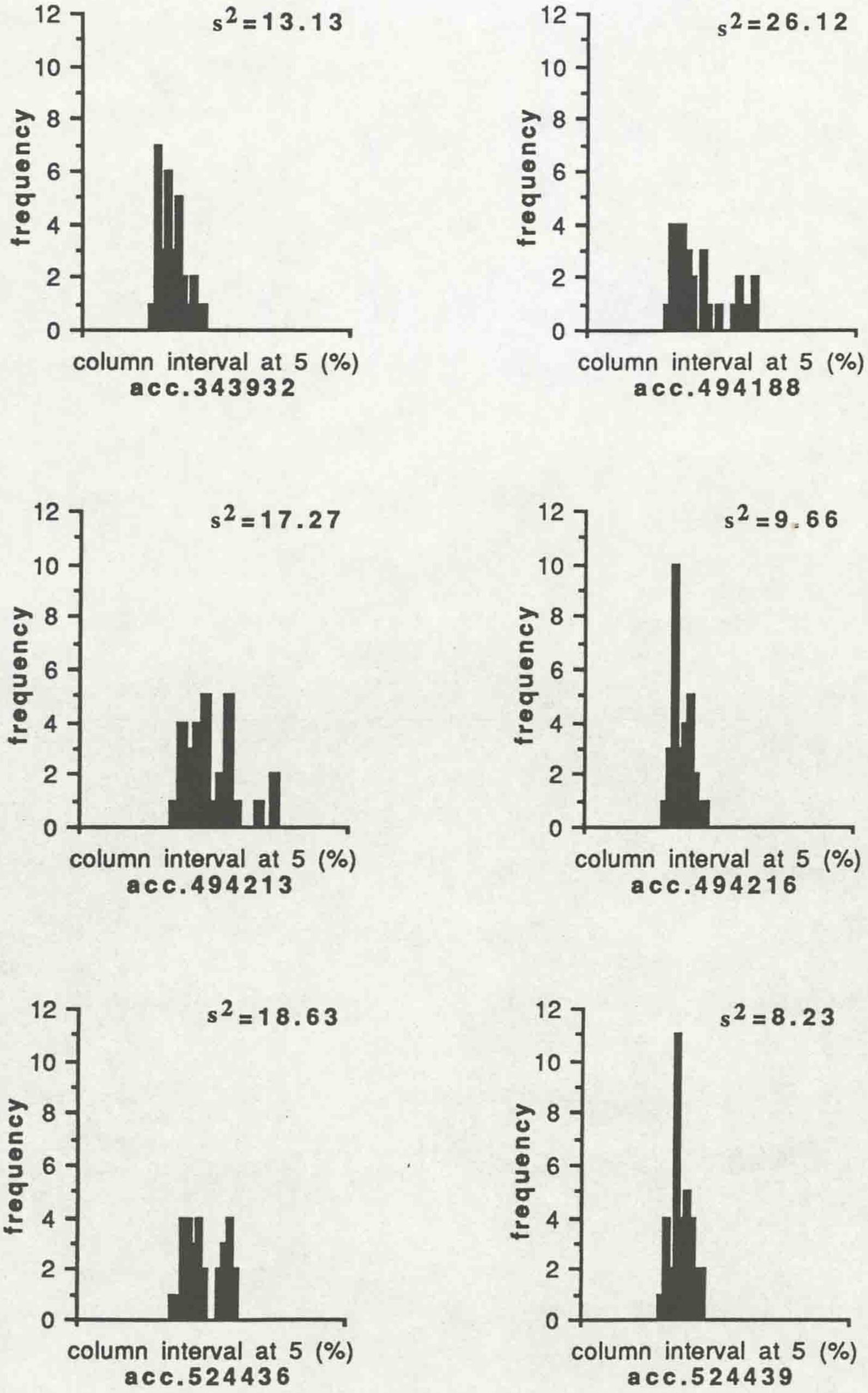
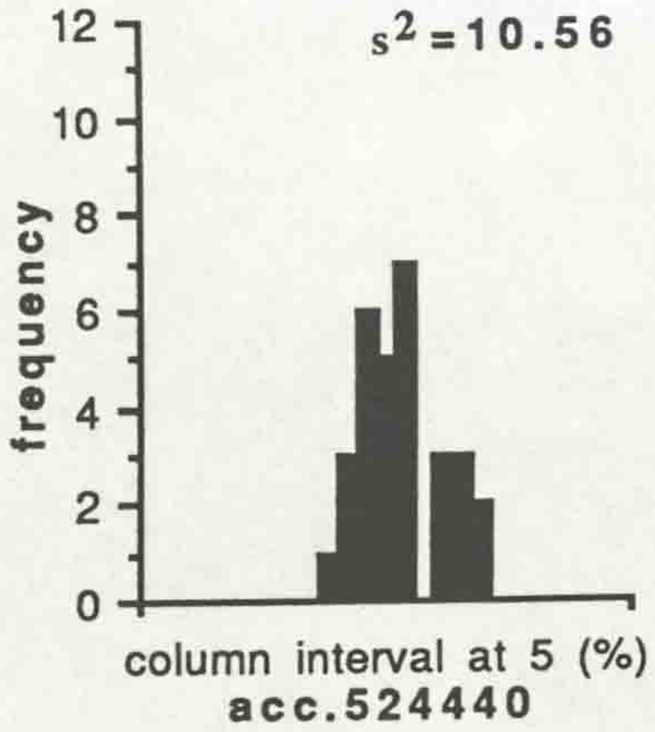
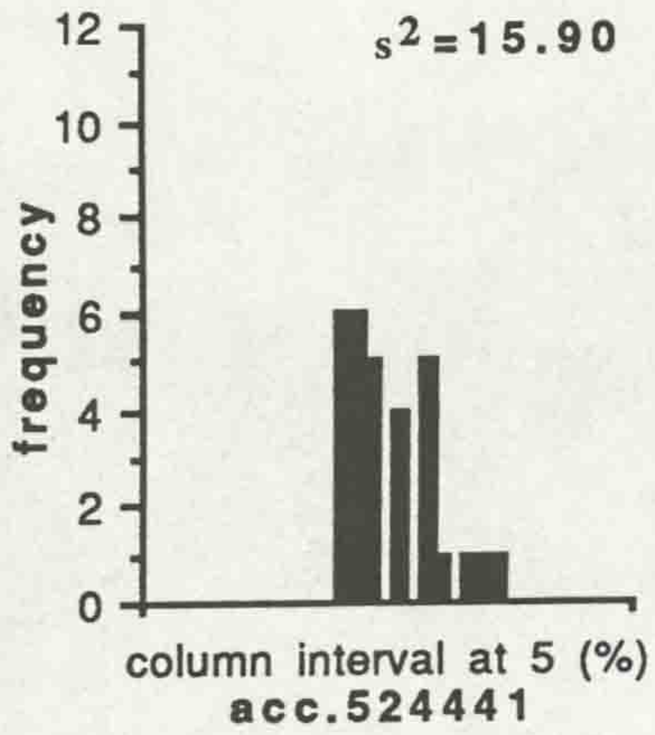


Figure 2.6. Frequency distribution of relative tolerance of root length of *E. tef* grown at 100 mM NaCl [with variance (s^2)]



(Figure 2.6 continued)





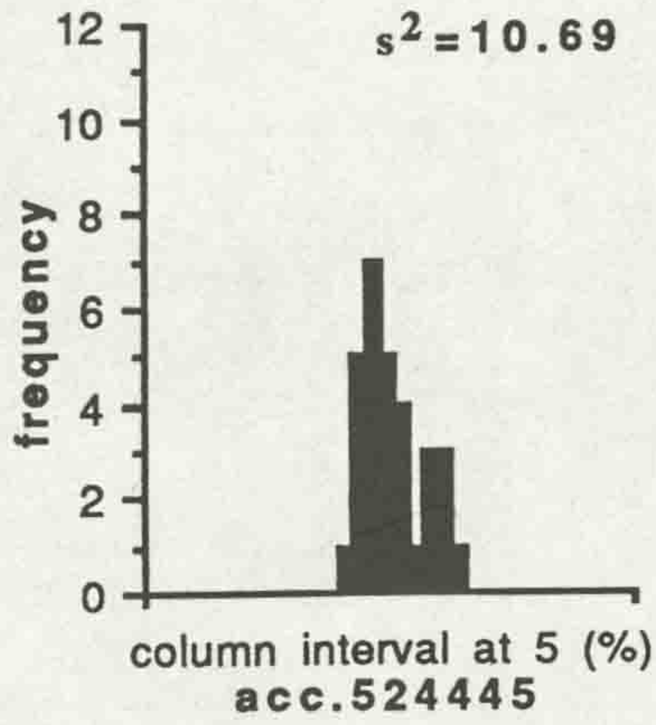


Table 2.7. Relative variance for within accessions variability in root length in 100 mM NaCl solution culture of two weeks old seedlings of finger millet (following Lewontin, 1966)

Accessions	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
1-100001	–																								
2-100002	NS	–																							
3-100004	NS	NS	–																						
4-100005	NS	NS	NS	–																					
5-100006	NS	NS	**	NS	–																				
6-100007	NS	NS	NS	NS	NS	–																			
7-100008	NS	NS	NS	NS	NS	NS	–																		
8-100009	NS	NS	*	NS	NS	NS	NS	–																	
9-100010	*	**	**	*	NS	*	*	*	–																
10-100012	NS	*	**	*	NS	NS	NS	NS	NS	–															
11-100014	NS	NS	**	NS	NS	NS	NS	NS	NS	NS	–														
12-100015	NS	NS	NS	NS	NS	NS	NS	NS	*	*	NS	–													
13-100016	NS	NS	*	NS	NS	NS	NS	NS	*	NS	NS	NS	–												
14-100017	NS	NS	NS	NS	NS	NS	NS	NS	*	NS	NS	NS	NS	–											
15-100018	NS	NS	*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	–										
16-100019	NS	NS	NS	NS	NS	NS	NS	NS	*	*	NS	NS	NS	NS	NS	–									
17-100021	NS	NS	*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	–								
18-100022	*	*	**	NS	NS	NS	NS	NS	NS	NS	NS	*	NS	NS	NS	*	NS	–							
19-100024	***	*	NS	*	**	*	*	*	**	**	**	**	*	*	*	**	*	**	–							
20-100025	***	NS	NS	NS	*	NS	NS	NS	**	*	*	NS	NS	NS	*	NS	*	*	NS	–						
21-100030	*	**	**	*	NS	*	*	*	NS	NS	NS	*	*	*	NS	*	NS	NS	***	**	–					
22-100031	*	*	**	*	NS	*	*	*	NS	NS	NS	*	*	*	NS	*	NS	NS	**	*	NS	–				
23-100032	NS	NS	NS	NS	NS	NS	NS	NS	NS	*	*	NS	NS	NS	NS	NS	NS	*	*	NS	*	*	–		
24-100033	NS	NS	NS	NS	NS	NS	NS	NS	*	NS	NS	NS	NS	NS	NS	NS	NS	NS	*	NS	*	NS	NS	–	
25-100034	NS	*	**	*	NS	NS	NS	NS	NS	NS	NS	*	NS	NS	NS	*	NS	NS	**	*	NS	NS	*	NS	–

Table 2.8. Relative variance for within accessions variability in root length in 100 mM NaCl solution culture of two weeks old seedlings of pearl millet (following Lewontin, 1966)

Accessions	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
1-203654	–																								
2-203655	NS	–																							
3-203657	NS	NS	–																						
4-203658	NS	NS	NS	–																					
5-203659	NS	NS	NS	NS	–																				
6-203661	NS	NS	NS	NS	NS	–																			
7-203662	NS	NS	NS	NS	NS	NS	–																		
8-215631	NS	NS	*	NS	*	*	*	–																	
9-215632	NS	NS	*	*	*	*	*	*	–																
10-215633	NS	NS	*	*	*	*	*	NS	NS	–															
11-215634	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	–														
12-215637	NS	NS	NS	NS	NS	NS	NS	*	*	*	NS	–													
13-215663	NS	NS	NS	NS	NS	NS	NS	NS	*	NS	NS	NS	–												
14-219936	NS	NS	NS	NS	NS	NS	NS	NS	*	*	NS	NS	NS	–											
15-219569	NS	NS	NS	NS	NS	NS	NS	*	*	*	NS	NS	NS	NS	–										
16-219975	NS	NS	*	*	*	*	*	NS	NS	NS	NS	*	*	NS	*	–									
17-219979	*	*	*	**	**	**	**	NS	NS	NS	*	**	**	**	**	NS	–								
18-219984	NS	NS	*	*	*	*	*	NS	NS	NS	NS	*	NS	NS	*	NS	NS	–							
19-219985	NS	NS	**	*	*	*	*	NS	NS	NS	NS	*	*	*	*	NS	NS	NS	–						
20-220134	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	**	NS	NS	NS	NS	NS	*	NS	NS	–					
21-220139	NS	NS	NS	NS	NS	NS	NS	NS	*	*	NS	NS	NS	NS	NS	*	**	*	NS	NS	–				
22-220164	*	*	NS	NS	NS	NS	NS	**	**	**	*	NS	NS	NS	NS	**	**	**	*	*	NS	–			
23-220220	NS	NS	NS	NS	NS	NS	NS	NS	*	*	NS	NS	NS	NS	NS	*	**	*	**	NS	NS	NS	–		
24-220222	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	**	NS	*	NS	NS	*	NS	–	
25-221726	NS	NS	**	*	*	*	*	NS	NS	NS	NS	*	*	*	*	NS	NS	NS	*	NS	*	**	*	NS	–

Table 2.9. Relative variance for within accession variability in root length in 100 mM NaCl solution culture of two weeks old seedlings of tef (following Lewontin, 1966)

Accessions	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1-343932	_														
2-494188	**	_													
3-494197	***	***	_												
4-494205	**	***	***	_											
5-494213	**	NS	***	***	_										
6-494215	***	***	***	***	***	_									
7-494216	*	***	***	NS	***	***	_								
8-524433	***	***	NS	***	***	***	***	_							
9-524436	***	***	**	***	***	NS	***	*	_						
10-524437	***	***	*	***	***	***	***	*	***	_					
11-524438	***	NS	***	***	NS	**	***	***	**	***	_				
12-524439	***	***	NS	***	***	**	***	NS	**	*	***	_			
13-524440	***	***	NS	***	***	**	***	NS	*	**	**	NS	_		
14-524441	***	***	NS	***	***	**	***	NS	*	**	***	NS	NS	_	
15-524445	***	***	***	***	***	***	***	***	***	***	***	***	***	***	_

Table 2.10. Mean squares (MS) from analysis of variance of absolute root length of individual seedling of finger millet at each NaCl concentration

Item	Df	Control	50 mM	75 mM	100 mM	150 mM	200 mM	Expected MS
Between accessions	24	305.72***	249.49***	189.29***	51.06***	24.78***	39.43***	$V_W + 30V_b$
Within accession	715	6.19	7.39	6.80	5.91	1.90	0.86	V_W

Table 2.11. Components of variance and broad sense heritabilities (h^2_B) of NaCl tolerance in finger millet seedlings at each NaCl concentration

Components	Control	50 mM	75 mM	100 mM	150 mM	200 mM
$V_b = V_G$	9.98	8.07	6.08	1.51	0.76	1.29
$V_b + V_W = V_P$	16.17	15.46	12.88	7.42	2.66	2.15
$h^2_B = V_G/V_P$	0.62	0.52	0.47	0.20	0.29	0.60

Table 2.12. Mean squares (MS) from analysis of variance of absolute root length of individual seedling of tef at each NaCl concentration

Item	Df	Control	25 mM	50 mM	75 mM	100 mM	125 mM	150 mM	175 mM	200 mM	Expected MS
Between accessions	14	79.70***	61.31***	49.15***	38.93***	14.20***	5.46***	1.48***	0.48***	0.49***	$V_W + 30V_b$
Within accession	435	1.51	1.25	0.61	0.88	0.51	0.10	0.04	0.04	0.01	V_W

Table 2.13. Components of variance and broad sense heritabilities (h^2_B) of NaCl tolerance in tef seedlings at each NaCl concentration

Components	Control	25 mM	50 mM	75 mM	100 mM	125 mM	150 mM	175 mM	200 mM
$V_b = V_G$	1.27	2.00	1.62	1.26	0.46	0.18	0.05	0.02	0.2
$V_b + V_W = V_P$	2.78	3.15	2.23	2.14	0.97	0.28	0.09	0.06	0.03
$h^2_B = V_G/V_P$	0.46	0.63	0.73	0.59	0.47	0.64	0.56	0.33	0.67

2.4. Discussion

Measurements of seedling root lengths in saline solution cultures have been successfully used to distinguish salt-tolerant and salt sensitive populations of several grass species (Hannon and Bradshaw 1968; Ashraf *et al*., 1986a). The technique was subsequently adapted to assess salt tolerance in seedlings of several crop species with the aim of using it to select within them for improved salt tolerance (e.g. sorghum, Azhar and McNeilly, 1987; maize, Ashraf and McNeilly, 1989; lentil, Ashraf and Waheed, 1990; pearl millet, Ashraf and McNeilly, 1992; lucerne, Al-Khatib *et al*., 1993). Previous experience of selecting for improved salinity tolerance in these species has shown that selection at seedling stage on the basis of 14-day-old seedling root and shoot length differences is effective in producing individuals which are more tolerant at all subsequent growth stages than unselected control individuals (Ashraf and McNeilly, 1992; Al-Khatib *et al*.,1993).

Roots are more sensitive to salinity than other plant components (Levitt, 1980; Abdul-Halim *et al*., 1988), and thus the inhibition of root growth adversely affects the survival and productivity of plants. Root growth as an indicator for the whole complex of characteristics determining salt resistance is especially useful in the first steps of screening programmes for salt resistance (Kik, 1989). The method provides a quick and accurate method of screening for enhanced salt tolerance, one of the necessities for the development of a protocol leading to the production of salt-tolerant crop varieties. The evaluation of that tolerance has been considerably facilitated in the present and other cases by the use of the non-linear least square inversion model developed by van Genuchten and Hoffman (1984). Combining these two techniques has provided useful preliminary information for the development of salinity tolerant lines from within the three species examined here. Thus the absolute root lengths of a subsample of 9 accessions from each species plotted as functions of salt concentration in the nutrient solution, use of this analysis revealed significant differences in growth response curves for the two minor millets (Figures 2.1 - 2), and tef (Figure 2.3). Differences between mean values for C_t, C_0, and C_{50}, in the three species are significant, showing that finger millet (*E. coracana*) has a greater innate tolerance than tef (*E. tef*), which is itself innately more tolerant than pearl millet (*P. americanum*). It is also clear that there are differences between accessions within the three species, allowing the identification of

those, e.g. 215663, and 221726 in pearl millet, and 100021, 100022, 100024, and 100030 in finger millet, and 494188, 494213 and 524436 in tef, from which selection for enhancement of salt tolerance would seem to be worthwhile. In a similar study examining 24 barley cultivars at the germination stage, Martinez-Cob *et al.* (1987) assessed tolerance using threshold salinity (C_t) as a reference parameter, and identified some cultivars such as Mari, Viva, and Kim, which proved to be highly tolerant to salinity. They suggested that C_t is the most appropriate parameter for determining salinity tolerance.

Variability has been found to be higher in non-saline conditions than under saline stress due to the fact that decreased stress permits a greater range of phenotypic expression and increases variance between and within lines (Shannon *et al.*, 1983). The effectiveness of selection clearly depends on the amount of intra-population variability, in particular the presence of variability within those accessions at a particular - preferably high - salt concentration. Differing degrees of variability can be seen in the data for individual seedling root growths at 100 mM NaCl presented in Figures 2.4 - 6.

Statistical comparison of variance data can be undertaken using the method of Lewontin (1966) for estimation of what he termed 'relative variation' or 'intrinsic variation' for comparison of variation. This method allows comparison of the patterns of variation between accessions or populations regardless of large differences between means. From this analysis it has been shown that in tef, whilst some cultivars have similar intrinsic variation to others, other accessions have significantly greater or lesser amounts of variation within them. This differences may be related to differences in history of the accessions and in particular it may reflect the presence of ranges of lines within these land race accessions and/or differences in root growth rates reflecting differences in soil fertility in their sites of origin. By contrast however, in finger and pearl millet most of the accessions have similar intrinsic variation, although some still differ significantly in their inherent variability.

For selection to be successful it is of course necessary that the variability observed in root lengths has a genetic basis. In *P. americanum* this has been shown to be the case (Ashraf and McNeilly 1992), based upon estimates of realised heritabilities, and current evidence from a diallel crossing programme (see Chapter 3), has shown that the character is under polygenic control, with a significant dominance component,

dominance being towards tolerance. Comparable data for finger millet or tef are not yet available, but it would seem not unreasonable to assume that there is at least some degree of genetic basis to the observed variability.

Previous broad sense heritability estimates for salinity tolerance in other crop species show, as might be expected, considerable differences. Thus in alfalfa estimated h^2_B was 0.5 (Allen *et al.*, 1985), while in seven grass and four forage species estimates ranged from 0.23 to 0.77, and 0.31 to 0.62 respectively (Ashraf *et al.*, 1986b, 1987). Based upon these estimates of these heritablities, Ashraf *et al*. (1986b, 1987) suggested that a significance advance in salinity tolerance in these species may be possible using high artificial selection pressures. Similarly, in the present study the broad sense heritability estimates for finger millet at six NaCl concentrations (h^2_B = 0.20 to 0.62) and tef at nine NaCl concentrations (h^2_B = 0.33 to 0.67) suggest that prospects of improving the character through selection and breeding are considerable, provided the genetic system controlling the variation is predominantly affected by genes with additive effects. Unfortunately no evidence is available for this at present.

The data are encouraging from a plant breeding point of view since it appears that sufficient variation exists both between and within cultivars of the three species to make selections for improved salt tolerance feasible, provided a link can be established between tolerance of 14-day-old seedling as estimated in this Chapter, and tolerance of those seedlings at adult plant stage. In *P. americanum*, this aspect is being examined in Chapter 4.

THE GENETIC BASIS OF VARIATION IN SALT TOLERANCE

CHAPTER THREE

CHAPTER 3

THE GENETIC BASIS OF VARIATION IN SALT TOLERANCE

3.1. Introduction

Because salinity tolerance is complex, its nature is not well understood, and its expression changes with plant age and possibly with preconditioning, it is not surprising that good data about the inheritance of tolerance are difficult to obtain (Blum, 1988). A proper genetic evaluation to produce data relevant to a practical breeding solution, should perhaps follow the general approach of evaluating different agricultural crops for adaptation to salinity, i.e., the evaluation of genetically different materials under a wide range of salinities. An understanding of the genetic basis of desirable attributes and the identification of parent lines superior in those attributes can reveal both the prospect of possibilities for improving these attributes and particular parent lines for their use in achieving such improvements.

Salt effects are seen in many aspects of plant life and in different tissues of the plant and a measurable genetic variation has been reported for different salt tolerant attributes in different plant species. For example the salinity resistance of rice in terms of root growth at 80 mM NaCl solution, was studied in F_1, F_2, and backcrosses of two crosses between tolerant and non tolerant parents (Jones and Stenhouse, 1984). Additive genetic variance had an important effect whilst dominance variance was more important in one cross than in the other. Broad sense heritability ranged from 49% to 83%. The slight bimodal distribution of F_2 indicated that only a small number of genes controlled salinity resistance.

Crosses between soybean varieties differing in Cl^- exclusion (Cl^- exclusion being a feature of tolerant lines) were studied for salt resistance in terms of leaf necrosis and leaf Cl^- concentrations (Abel and Mackenzie, 1964). Their results from segregating generations confirmed a single dominant gene model for the control of Cl^- exclusion. These results are probably the only solid case for a single-gene control of salinity resistance in plants (Blum, 1988).

Leaf necrosis symptoms were used to evaluate salinity resistance in cucumber (Jones, 1984). In this case, segregation data from a resistant x susceptible cross

suggested that resistance was controlled by a single dominant major-gene locus, with additional effects from many minor genes. Based upon these data, narrow sense heritability for salinity resistance ranged from 41 to 86%.

Data in Chapter 2 provide evidence for considerable amounts of variation in salinity tolerance in pearl millet. Moreover the potential for exploiting variation in salinity tolerance in *Pennisetum americanum* (L.) Leeke has been illustrated by Ashraf and McNeilly (1992). In order to select genotypes which are likely to lead to acceptable advancement in tolerance, the use of certain biometrical techniques is important for detecting those genotypes possessing combination of these desirable traits.

Information about the genetic basis of salinity tolerance and its components is an obvious imperative for the synthesis of genetically superior pearl millet cultivars. This is necessary since the type of breeding programme for a particular crop will be determined by the nature and relative magnitudes of genetic/non genetic variation associated with the plant population and the nature of gene action governing those characters of importance in selection and breeding. The diallel crossing procedure and analysis developed by Hayman (1954a, b) and Jinks (1954, 1955, 1956) as applied by Whitehouse *et al*. (1958) and Mather and Jinks (1977) provides such a possibility, and was the system used in the investigations to be described in this Chapter to assess the mode of inheritance of tolerance to salinity in F_1 generation material of *Pennisetum americanum* (L.) Leeke.

3.1.1. Statistical analysis

The data in each progeny were subjected to a standard analysis of variance to determine significance of difference of mean tolerance, since only where there are significant genotypic differences between the parents and between their F_1 hybrids can the data be used for further analysis.

3.1.1.1. Genetic analysis

The general requirements of any analysis of variance of a diallel table are that it provides appropriate tests of significance of the principal genetic components, namely additive, dominance, maternal, and reciprocal effects. The most satisfactory analysis for a complete diallel set of crosses from this point of view is the diallel analysis of

Hayman (1954a, b) and Jinks (1954, 1955, 1956). This analysis further subdivides the dominance effect ('b') into directional dominance (b_1) which tests the mean deviation of the F_1's from the mid-parental value; effects due to parents contributing varying numbers of dominant genes (b_2) which tests if some parents contain considerably more dominant alleles than others; and specific gene interaction (b_3) which indicates whether part of the dominance deviation is unique to each particular F_1.

The ability of an inbred line to transmit desirable performance to the hybrid progeny is referred to as its combining ability (Poehlman, 1987). Crossing a particular line to several other lines provides an additional genetic assessment of that line, i.e. the mean performance of the line in all its crosses. This mean performance, when expressed as a deviation from the mean value of all crosses, is called the general combining ability of the line (Falconer, 1989). It is the average value of all F_1's having the line as one parent, the value being expressed as a deviation from the overall mean of crosses. General combining ability effects represent fixable (additive gene action) genetic component. Any particular cross, then, has an expected value which is the sum of the general combining abilities of its two parental lines. The cross may, however, deviate from the expected value to a greater or lesser extent. This deviation is called the specific combining ability of the two lines in combination (Falconer, 1989). Specific combining ability effects represent pre-dominance gene action. Falconer also summarised that in statistical terms, the general combining abilities are main effects whilst the specific combining ability is an interaction. Estimation of general combining ability effects and specific combining ability constants were performed for the data in the experiment described here using Griffings (1956) Method I Model I.

Further statistics may be obtained for the variance components of each array (V_r), the covariance of the family means within the array with the phenotypes of their respective non-recurrent parents (W_r), variance of parental means ($V_{oLo} = V_p$), estimation of means of array variances (V_{1L1}), variance of means of arrays (V_{oL1}), and means of array covariances (W_{oLo1}). These statistics have further use in the estimation of the relative size of D, an estimate of the additive effects; H_1, variation due to dominance effects of genes; H_2, variation due to dominance effects of genes corrected for gene distribution; F, which provides an estimate of the relative frequency of dominant to recessive alleles in the parental lines and the variation in dominance over

loci. Hence F will be positive whenever the dominant alleles are more frequent than the recessive alleles, irrespective of whether or not the dominant alleles have increasing or decreasing effects.

In addition, the diallel provides estimates of essential parameters to provide estimates of both narrow sense (h^2_N) and broad sense (h^2_B) heritabilities (Kearsey, 1965; Mather and Jinks, 1982; Lawrence, 1984).

Genetic correlation among NaCl treatments was estimated from the additive, phenotypic, and environmental components of variance in the respective NaCl concentrations, following Lothrop *et al.* (1985).

3.1.2. Definitions, assumptions and adequacy of the additive-dominance model

3.1.2.1. Diallel definitions

A diallel cross is the set of all possible matings between several genotypes which may be individuals, clones, homozygous lines, etc., such that if there are **n** lines there are $\mathbf{n^2}$ mating combinations, counting reciprocals separately. A diallel table is therefore an arrangement of $\mathbf{n^2}$ observations from a set of diallel crosses between **n** parental lines. Each row and column of the square corresponds to measurements of offspring with a common parental genotype so that the **n** parents form the leading diagonal of the table and each male array (row) has a common male parent, just as each female array (column) which has a common female parent. Thus the $\mathbf{n}^2$ combinations can be divided into three groups:

1. the **n** parental lines themselves,
2. one set of 1/2[**n**(**n**-1)] F_1's, and
3. the set of 1/2[**n**(**n**-1)] reciprocal F_1's.

Diallel crossing techniques may vary depending upon whether or not the parental inbreeds or the reciprocal F_1's are included or both. With this in mind Griffing (1956) classified four possible experimental methods:

1. parents, one set of F_1's and reciprocal F_1's are included (all $\mathbf{n}^2$ combinations),

2. parents and one set of F_1's are included but reciprocal F_1's are not (1/2[**n**(**n**+1)] combinations),
3. one set of F_1's and reciprocals are included but not the parents (**n**(**n**-1) combinations), and
4. one set of F_1's but neither parents nor reciprocal F_1's is included (1/2[**n**(**n**-1)] combinations).

Each method necessitates a different form of analysis.

3.1.2.2. Assumptions

The following assumptions are involved in deriving genetical interpretations from diallel designs (Hayman, 1954b; Crumpacker and Allard, 1962; Kearsey, 1965).

1. Homozygous parents
2. Regular diploid behaviour at meiosis
3. No differences between reciprocal crosses
4. No non-allelic interaction
5. No multiple allelism
6. Uncorrelated gene distributions

Failure to meet some of these conditions will cause characteristic disturbances of the array variance and array covariance regression (Jinks, 1954; Dickinson and Jinks, 1956) and helps in identifying those assumptions which are not fulfilled. Nevertheless, regarding the homozygosity of parents, Griffing (1956) and Oakes (1967) have shown that the technique may be applied to crosses between heterozygous individuals as in the case of diallel crosses in potato using heterozygous clones (Tai, 1976; Kaminski, 1977; Killick, 1977).

3.1.2.3. Adequacy

The adequacy of the additive-dominance model and hence the fulfilment of assumptions for the model can be determined with the use of two tests. The consequence of the failure of those assumptions makes the model inadequate as follows.

Firstly a general test of assumptions is gained from joint regression analysis of

W_r on V_r. The regression coefficient of W_r on V_r should be significantly different from zero but not from unity if all the assumptions are met (Mather and Jinks, 1982). Failure of this test means that either genes show non-allelic interaction i.e. are not independent in their action, or that they show non-random association among the parents, i.e. are non-independent in their distribution.

The second test of adequacy of the diallel analysis is the analysis of variance of W_r+V_r and W_r-V_r. The presence of dominance or certain types of non-allelic interaction leads to the arrays having different W_r+V_r values. In the presence of non-allelic interaction, the difference in the magnitude of W_r-V_r over arrays is significant, whereas in the presence of dominance, W_r-V_r will not vary more than would be expected from error variation (Mather and Jinks, 1977).

Finally from the graphic representation of the regression W_r on V_r provides an indication of the type of dominance. Full dominance at all loci is indicated when the regression line passes through the origin ($D = H_1$); partial dominance is revealed when the line intercepts the W_r axis above the origin ($D > H_1$); overdominance is indicated when regression line intercepts the W_r axis below the origin ($D < H_1$). Hayman (1954a), Jinks (1955, 1956) and Whitehouse *et al.* (1958) demonstrated that an easier way of extracting information from diallel cross is to plot the covariance (W_r) of each array against its variance (V_r). The slope and positions of the limiting parabola indicate the degree of dominance and the presence or absence of gene interaction. The positions of the array points on the regression line give a measure of the relative frequency of recessive alleles in any array. Arrays nearest to the point of origin possess most of the dominant genes, while the arrays furthest from the origin possess most recessive genes and arrays in an intermediate position possess both dominant and recessive genes.

3.2. Materials and methods

The F_1 material used in the present study was obtained from crossing twelve accessions/lines following a diallel crossing programme. Eight of the parents were chosen from among the accessions used for assessment of variability in salinity tolerance (Chapter 2) and the other four were from material examined by previous workers. The parental accessions/lines were Kitui Local, Selection 2, 93611, 93614, 203656, 203658, 203659, 203662, 215634, 219885, 219975 and 221726 (see

Appendix 1.1 for origins of accessions).

Each of the parental lines was grown in Jonn Innes No 2 compost in 18 cm diameter plastic pots during summer 1991 under glasshouse conditions, selfed, and seeds harvested separately for each parent. Selfing was accomplished by removing the top one or two leaf blades of the culm and enclosing the head and culm in a glassine bag before style exsertion. The bag was stapled closed and left on the head until it had been harvested, dried, and prepared for threshing. The harvested seeds with the diallel experimental design were sent to Ethiopia for subsequent planting and crossing. In January 1992, the parental materials were planted in a glasshouse in 18 cm pots using a natural sandy loam soil in the Plant Genetic Resources Centre (PGRC), Addis Ababa. Grid planting at an interval of two weeks was exercised to ensure synchrony of flowering, plants were watered daily, and the temperature of the glasshouse was maintained between 28° and 32°C. The plants flowered from mid April to early June and parental lines were control-crossed according to a diallel design.

Protogyny (carpels emerge and mature before stamens) is particularly conspicuous in pearl millet, and provides an excellent means to ensure almost 100% cross-pollination without emasculation. The crossing was effected as follows. Heads were enclosed in glassine bags on emergence from the flag leaf sheath, and were examined daily through the glassine bag for the presence of exserted styles. When styles were exserted the head was ready to be pollinated (Burton, 1980). In some genotypes anther exsertion began before the florets at the top and the bottom of the head were exserted, any florets without stigmas were removed at the time of pollination, a procedure which does not adversely affect the pollination and seed set of those florets remaining on the head.

Pollen from the respective male parent was collected by gently shaking the panicle at anther dehiscence and the pollen was collected in glassine bags. Pollination was effected by enclosing the female head in the pollen-collecting glassine bag and shaking it.

At maturity, seed was harvested separately for each parent. A count of seed obtained in each cross was made in order to asses the extent of possible testing under different salinity levels. In some of the crosses involving parents Selection 2, 93614, 203656, 203658, 215634, 219885 and 219975 seed number set was insufficient to

include in the testing programme. Therefore, F_1 families and their parents comprising a 5 x 5 diallel were assessed for their response to varying concentrations of NaCl.

3.2.1. Assessment of F_1 progenies in NaCl solutions

25 F_1 families and the selfed parents were assessed in four treatment solutions containing 0 (control), 75, 125 and 175 mM NaCl prepared in 1/10 strength Rorison nutrient solution as used in Chapter 2 (see Appendix 2.1 for composition of Rorison solution). Each family in each treatment was replicated twice in a completely randomised design. Seeds were surface sterilised by soaking in a 2% solution of sodium hypochlorite (v/v) for ten minutes prior to planting. The conditions in the growth room were similar to those described in Chapter 2.

Root lengths of ten randomly chosen seedlings from each replicate in each treatment were measured after 14 days. F_1 family means in each treatment were thus based upon 20 progeny values. Root lengths were expressed as relative root growth (Dewy, 1960; Maas, 1985) where root length in each NaCl solution is expressed as a percentage of control root length. The data on relative root length under three salinity levels were used to investigate the genetic basis of salt tolerance.

3.2.2. Validity of assumptions

Natural out crossing in *Pennisetum americanum* (L.) Leeke is not complete because plants have several culms that flower in succession. This allows the head that reaches anthesis first to pollinate other heads on the same plant that are just exserting anthers. As a consequence, self-pollination as high as 31% has been observed (Burton, 1974). However, a further generation of controlled selfing was used to achieve a higher degree of homozygosity in the parental generation, than would be available in the seed obtained from the gene bank sources involved.

Its diploid chromosome number of *P. americanum* is 2n = 2x with x = 7 and chromosomal segregation is of normal diploid type (Purseglove, 1976).

Hence a prerequisite check of the validity of a set of assumptions for diallel analysis was fulfilled. Reciprocal differences could be detected, if any, after Hayman's (1954b) analysis of variance of diallel table. The remaining three assumptions of non-allelic interaction, multiple allelism, and uncorrelated gene distribution were satisfied

through the analysis of variance of W_r+V_r and W_r-V_r entities for the arrays of each replicated diallel table.

3.3. Results

Analysis of variance indicated significant ($p<0.001$, Table 3.1) differences in relative NaCl tolerance data for parents and crosses permitting further data analyses.

The magnitude of the components of genetic variation for each of the three NaCl concentrations summarised in the form of mean squares, are given in Table 3.2. Differences between duplicate observations on each of the 25 entries were used as the appropriate error term for each NaCl concentration (Mather and Jinks, 1977).

From the analysis of variance (Table 3.2) there is evidence of significant additive genetic effects (a) and general dominance effects (b) at the three NaCl concentrations (both at $p<0.001$).

Among the three non-additive components indicated in Table 3.2, the b_1 item was significant for the 75 and 175 mM NaCl treatments ($p<0.01$) indicating unidirectional dominance effects. The b_1 item was not significant however at 125 mM salinity level. The b_2 item, test of gene asymmetry, was highly significant ($p<0.001$) at 75 and 125 mM NaCl levels and at 175 mM NaCl ($p<0.05$). Thus the variation in NaCl tolerance was due to the parents containing differing numbers of dominant genes. The b_3 item was significant at 75 and 175 mM ($p<0.01$) and 125 mM ($p<0.001$) NaCl suggesting that only certain crosses showed significant deviation from the mid parent (i.e. dominance was specific to certain crosses). Maternal effects (c), the 'd' item and reciprocal differences in the crosses were shown to be non significant at all salinity levels.

The adequacy of the additive-dominance model, and validity of three of the assumptions (no non-allelic interaction, no multiple allelism, and uncorrelated gene distribution) were assessed using joint regression analysis of variance of W_r+V_r and W_r-V_r. The results of the two tests for each of the three salt concentrations are presented in Table 3.3.

Table 3.1. Mean squares from the analysis of variance of 16 F_1 hybrids and their parents used in a 5 x 5 diallel cross

Item	Df	Mean squares
Replicates	1	86.12^{NS}
Genotypes (G)	24	494.56***
NaCl solutions (S)	2	12321.25***
G x S	48	99.77***
Residual	74	24.52

Table 3.2. Mean squares of components of variation in 5-parent diallel cross assessed in three NaCl concentrations

	Components of variation	Df	NaCl concentrations (mM)		
			75	125	175
a.	Additive effects	4	1200.3***	377.9***	279.9***
b.	General dominance effects	10	366.4***	202.4***	71.8***
b_1.	Directional dominance effects	1	352.5***	66.7^{NS}	113.8**
b_2.	Effects due to unequal distribution of dominance	4	599.6***	317.6***	50.8*
b_3.	Effects due to dominance deviation unique to F_1's	5	182.6**	137.4***	80.2**
c.	Maternal effects	4	33.2^{NS}	39.3^{NS}	11.0^{NS}
d.	Non-maternal reciprocal differences	6	45.1^{NS}	69.2^{NS}	9.5^{NS}
	Error	24	39.71	21.56	13.50

At 75 mM the slope of the regression line did not deviate significantly either from zero or unity (b = 0.545 $\pm$ 0.395) suggesting intra-allelic interaction. The details of the two analyses of variance for W_r+V_r and W_r-V_r respectively so obtained are given in Appendix 3.2. The mean squares between arrays for W_r-V_r was not significant when tested against that within arrays and indeed was smaller than it. There is thus no evidence of any non-allelic interaction (epistasis). This also confirms the adequacy of the additive-dominance model. Turning to the analysis of variance of W_r+V_r, it can be seen that the mean square between arrays was greater than that within array, but not significantly so. On this evidence alone, therefore, it cannot be assumed that dominance is present. However, there is evidence for non-additive effects from the initial analysis of variance (Table 3.2) and this must be accounted for in some way. Since there is no evidence of interaction between non-allelic genes, it may be concluded that although not formally significant by itself, the higher value for the mean square between arrays for W_r+V_r (Appendix 3.2), does in fact reflect dominance (Mather and Jinks, 1977). Thus the model was adequate for analysis of the data obtained at 75 mM NaCl.

At 125 mM NaCl, the slope of the regression line did not deviate significantly (b = 0.569 $\pm$ 0.767) both from zero and from unity (Table 3.3). The W_r+V_r item however was non significant ($p>0.05$) indicating the absence of dominance (Table 3.3). However, as in the case of the data for 75 mM NaCl considered above, the mean square between arrays for W_r+V_r was greater than that within arrays (Appendix 3.2) suggesting the presence of either dominance or non-allelic interaction. However, the W_r-V_r item was significant ($p<0.01$) suggesting the presence of non-allelic interaction (Table 3.3). As a consequence, analysis of the data using the Hayman-Jinks model was rejected.

At 175 mM NaCl the slope of the regression line (b = 0.815 $\pm$ 0.121) deviated significantly from zero but not from unity (Table 3.3). This confirmed two things: firstly, the absence of non-allelic interaction and secondly, independent distribution of genes among parents. Separate test of dominance carried out on these data indicated significant differences ($p<0.001$) for W_r+V_r and non-significant differences ($p>0.05$) for W_r-V_r (Appendix 3.2, Table 3.3), the former suggesting the presence of dominance

Table 3.3. Scaling test for adequacy of additive-dominance for 5-parent diallel data in three NaCl concentrations

NaCl	Regression analysis	Analysis of variance		Conclusions
		$W_r + V_r$	$W_r - V_r$	
75 mM	b = 0.545 ± 0.395	1.8535^{NS}	3.6578^{NS}	Model adequate for data analysis
	The slope of the regression line did not deviate significantly either from zero or from unity			
125 mM	b = 0.569 ± 0.767	1.7014^{NS}	11.756**	Data unfit for analysis using the model
	The slope of the regression line did not deviate significantly either from zero or from unity			
175 mM	b = 0.815 ± 0.121	44.6485***	1.2144^{NS}	Both tests suggested adequacy of the model for data analysis
	Regression line deviated from zero but not from unity			

and the latter the absence of non-allelic interaction. Therefore, this set of data was adequate for further analysis using the Hayman-Jinks model.

Variance of the components of each array (V_r) and covariance of all the offspring included in each parental array with non recurrent parent (W_r), and their means which are involved in the analyses are given in Appendix 3.1. Other statistics, variance of parental means ($V_{oLo} = V_p$) and variance of means of arrays (V_{oL1}) are given in the Figures.

The two analyses carried out above provided no reason to doubt the adequacy of the model for the data analysis at 75 and 175 mM NaCl.

For genetic interpretation of variation in salt tolerance in the accessions examined, the diallel provides statistics from which estimates of the components of variation can be obtained. In the present analysis, the genetic components, D, H_1, H_2 and F and the environmental component, E were estimated for the data from both NaCl concentrations, and are presented in Table 3.4.

3.3.1. Estimation of genetic components

3.3.1.1. 75 mM NaCl

Data for components of variation and statistical ratios are given in Table 3.4. The additive variance component (D) was significant. However, since $H_1 > D$, the effect of genes with dominance properties (H_1 or H_2) appeared to be more pronounced. The average degree of dominance indicated by the $(H_1/D)^{0.5}$ was more than unity, suggesting a degree of over-dominance. However one cannot be confident about the presence of overdominance since the regression line (Figure 3.1) intersects the covariance axis above the origin. Testing mean squares between arrays against that within arrays for W_r+V_r was non significant (Table 3.3 and Appendix 3.2) indicating additive type of variation. Thus, additive effects with partial dominance was shown for NaCl tolerance at this NaCl concentration.

Table 3.4. Estimates of genetic parameters controlling root length measurements in 75 and 175 mM NaCl concentrations

Components	75 mM	175 mM
E	39.71 ± 1.41	13.50 ± 0.48
D	320.71 ± 12.75	51.28 ± 2.77
H_1	483.34 ± 11.63	70.79 ± 2.54
H_2	482.31 ± 11.64	111.47 ± 2.53
F	242.16 ± 12.79	11.81 ± 3.25
h	6.64 ± 1.71	3.77 ± 0.56
Narrow sense heritability	0.20	0.02
Broad sense heritability	0.80	0.67
$(H_1/D)^{0.5}$	1.23	1.18
$H_2/4H1$ (uv)	0.25	0.39
$1/2F/[D(H_1-H_2)]$	0.37	0.003
$[(4DH_1)+F]/[(4DH_1)-F]$	1.00	1.00

E = environmental component of variation
D = additive effects of genes
H_1 = dominance effects of genes
H_2 = dominance effect of genes corrected for gene distribution
F = frequency of dominance alleles
h = overall dominance effects of heterozygous loci

Figure 3.1. W_r/V_r regression for relative root length of *P. ammericanum* (L.) Leeke seedlings in 75 mM NaCl from an 5 x 5 diallel

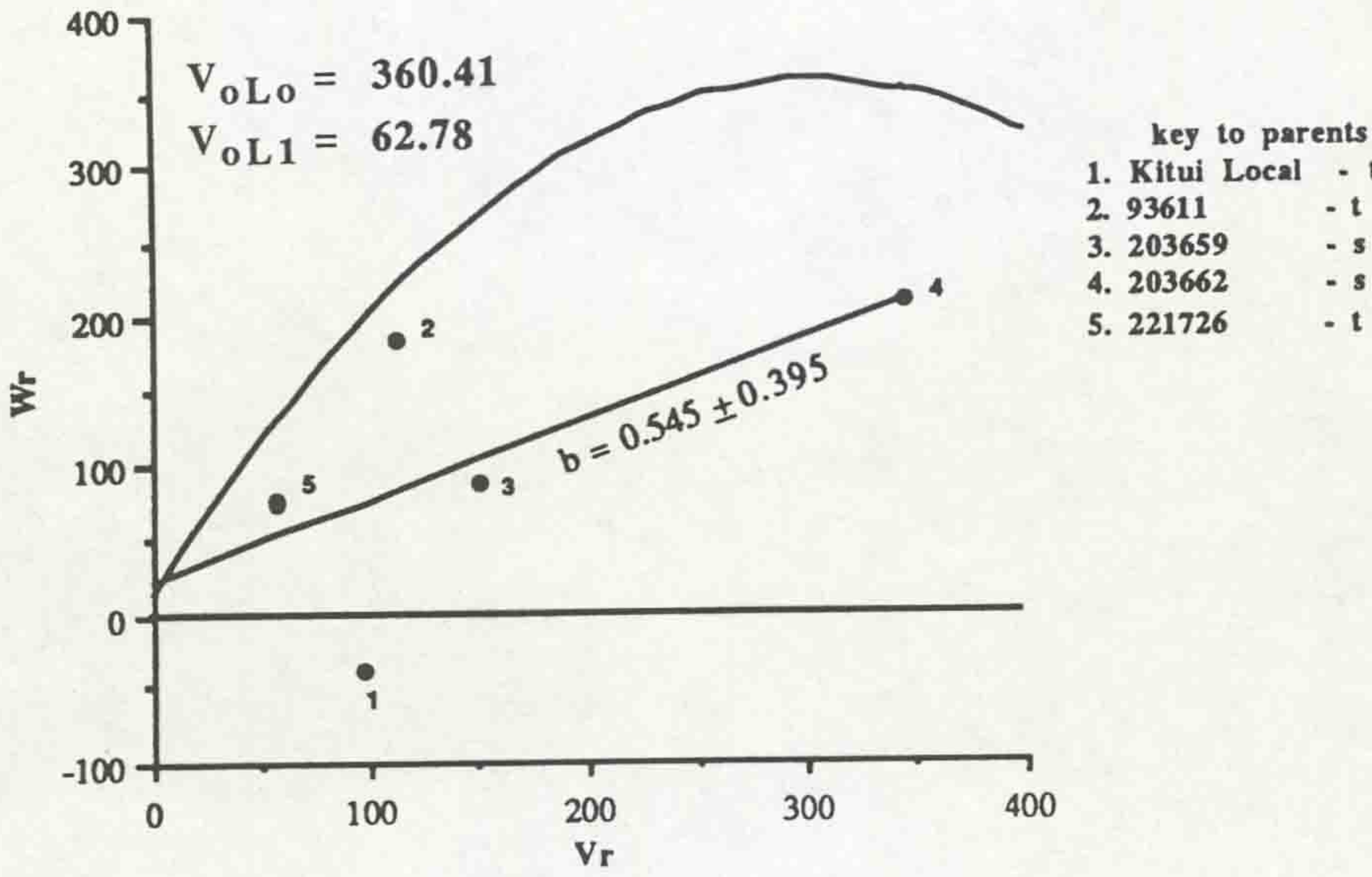
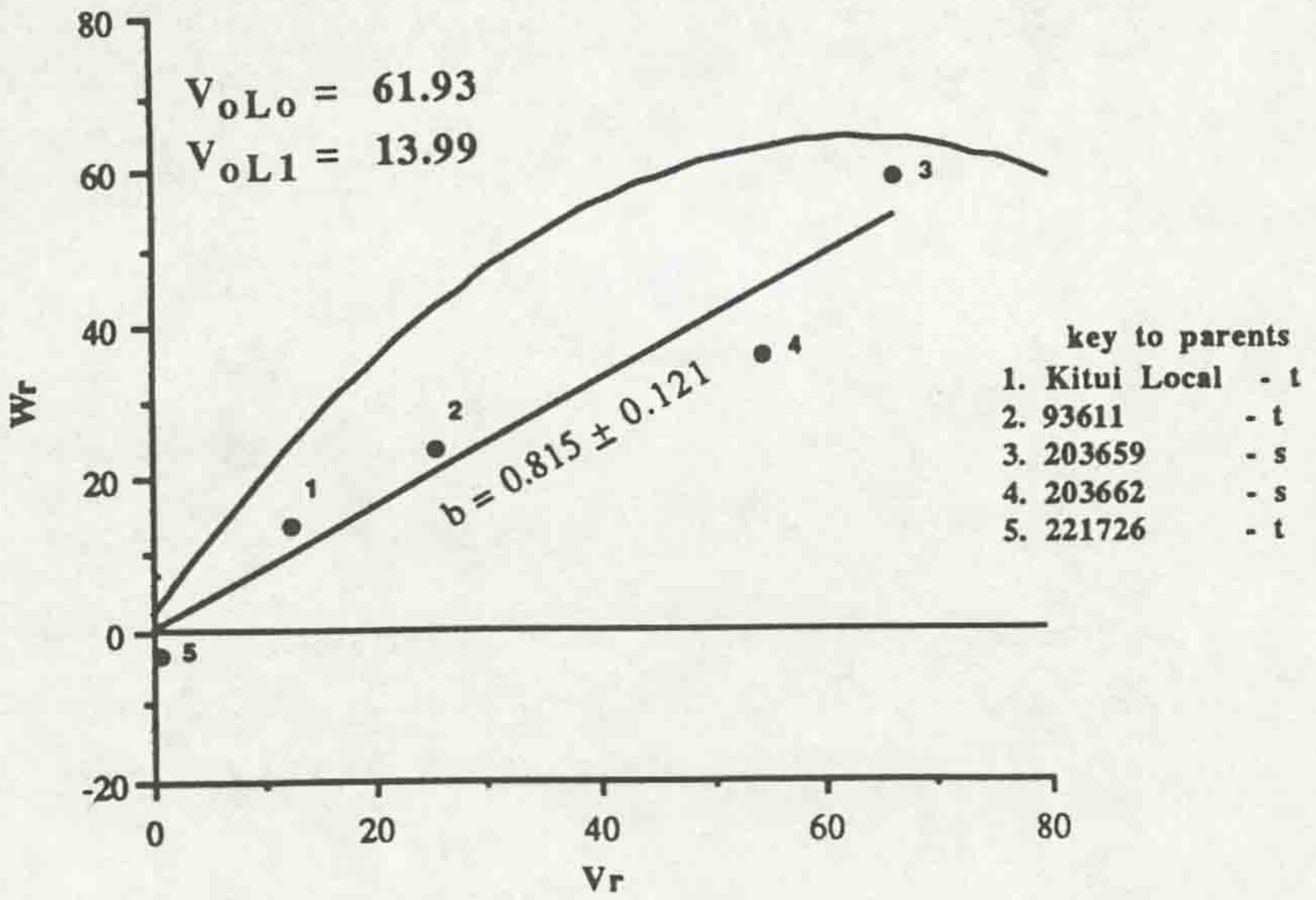


Figure 3.2. W_r/V_r regression for relative root length of *P. ammericanum* (L.) Leeke seedlings in 175 mM NaCl from an 5 x 5 diallel



H_1 and H_2 did not differ indicating equal gene frequencies at all loci (Table 3.4). Proportion of genes, $H_2/4H_1 = 0.25$ which arises when $u = v = 0.5$ also showed symmetry of positive and negative effects at the loci. The estimate of $[(4DH_1)+F]/[(4DH_1)-F] = 1$, implied equal distribution of dominant and recessive alleles within the parents.

The positive sign of h (F_1 mean minus parental means) indicated the trend of dominance being towards tolerance to NaCl. The relatively low value 0.37 for $1/2F/[D(H_1-H_2)]$ provided little evidence that the dominance deviation at one locus was particularly consistent in sign or magnitude. Narrow sense heritability, a reflection of the additive variation, was estimated 0.20, whilst for broad sense heritability the value was 0.80.

When plotting the regression of W_r on V_r, the presence of only dominance and additive effects is shown when the points are dispersed along a line of unit slope, the parents having the dominant character located towards the origin and the parents with recessive character located distant from the origin. Absence of dominance is shown when the arrays are clustered at random around the mid point of the regression line. Thus from the relative position of the array points along the regression line (Figure 3.1), accessions Kitui Local and 221726 possess the most dominant genes, whereas accession 203662 contained most recessive genes because of its distal position from the origin. Accessions 93611 and 203659 contained both dominant and recessive genes.

3.3.1.2. 175 mM NaCl

Examination of Table 3.4, shows that both additive and dominance gene effects appear to be involved in controlling tolerance at 175 mM NaCl. $H_1 > D$, indicating that the effects are in the main of dominance type. The ratio 1.18 of H_1/D showed dominance slightly tending towards overdominance. The regression line however intersects the covariance axis at the origin suggesting complete dominance type of gene action.

Unequal gene frequencies among the parents was confirmed by both (H_1-H_2) and the ratio 0.39 of $H_2/4H_1$ (Table 3.4). Again the positive value of h suggests dominance towards high salt tolerance. The smaller estimate of 0.003 for $1/2F/[D(H_1-H_2)]$ suggested that the level of dominance across the loci was almost constant. The

estimate of narrow sense heritability was very low, the value being 0.02, whilst the broad sense heritability was considerably higher at 0.67.

A comparison of the array distribution in Figure 3.2 showed that accessions 221726 and Kitui Local formed one group which contained most dominant genes for salt tolerance, whilst accessions 203659 and 203662 had the maximum number of recessive alleles, accession 93611 being intermediate.

3.3.2. Combining ability

Combining ability analysis provides estimates of combining ability effects and assists the choice of suitable parents and crosses for further exploitation of genes in a population. Combining ability was therefore examined for the parental materials considered here, and the crosses made between them. Data for general combining ability (gca) effects, and specific combining ability (sca) constants are presented in Tables 3.5 and 3.6 respectively.

At 75 mM NaCl, Kitui Local and 93611 had significantly greater gca effects than the rest of the parents, whilst 203659 and 203662 had the lowest gca effects. At 175 mM NaCl, 221726 had the greatest gca effects followed by parents Kitui Local and 93611, whilst 203659 and 203662 showed lower gca effects.

It was shown in Table 3.6 that at 75 mM NaCl all crosses involving Kitui Local and the other parents had lower sca estimates. By contrast the crosses, 203662 x 203659, 203662 x 221726 and 203659 x 221726 had relatively high sca constants.

At 175 mM NaCl, the cross Kitui Local x 221726 had a low sca constant whereas the crosses Kitui Local x 203659 and Kitui Local x 203662 had relatively higher sca constants. Genotype 203659 x 203662 had also relatively higher sca constant.

3.3.3. Genetic correlation

To estimate genetic correlation between the F_1 families grown at 75 mM and 175 mM NaCl, components of variances and the covariances were calculated from them and are presented in Table 3.7. The estimate of 'r', correlation coefficient was 0.54.

Table 3.5. Estimates of general combining ability effects

Parent	General combining ability effects	
	75 mM NaCl	175 mM NaCl
Kitui Local	10.13	1.25
93611	5.74	0.93
203659	-5.61	-3.03
203662	-8.34	-4.28
221726	-1.93	5.08
S.E. $(\hat{g}_i - \hat{g}_j)$	1.55	1.03

Table 3.6. Estimates of specific combining ability constants at 75 mM and 175 mM NaCl

Parent	NaCl	Parent			
		93611	203659	203662	221726
Kitui Local	75 mM	-9.09	-3.23	-8.57	-4.26
	175 mM	-0.65	3.50	2.49	-3.36
93611	75 mM		0.14	2.80	-0.89
	175 mM		1.34	1.77	-2.96
203659	75 mM			8.66	4.06
	175 mM			3.83	-1.25
203662	75 mM				4.65
	175 mM				-0.80

Standard error	75 mM	175 mM
S.E. $(\hat{S}_{ij} - \hat{S}_{ik})$	4.84	2.30
S.E. $(\hat{S}_{ij} - \hat{S}_{kl})$	3.63	1.73

Table 3.7. Genetic (G), phenotypic (P) and environmental (E) variances and their respective covariances of families tested in 75 and 175 mM NaCl concentrations, and their genetic correlation

Families	Components of variances			Components of covariances			'r'
	G	E	P	G_{xy}	E_{xy}	P_{xy}	r_G
75 mM	369.88	37.87	407.75	92.83	20.02	112.85	0.54**
175 mM	79.90	13.81	93.71				

3.4. Discussion

Diallel analysis provides means for the detection of direction of dominance, estimation of the relative frequency of dominant increasing and decreasing genes, the grouping of parents in terms of the number of dominant genes they carry (W_r/V_r graph), and a test of the adequacy of the additive-dominance model for data interpretation. Dickinson and Jinks (1956) have discussed the tests for linkage, correlated gene distribution, and non-allelic interaction for the heterozygous diallel. No other design includes a test for the presence of these effects nor do they detect the presence of multiple alleles (Kearsey, 1965). The diallel analysis carried out here provides such information of potential value for breeding programmes in salinity tolerance in pearl millet.

The additive-dominance model of Hayman (1954a, b), and Jinks (1954) was shown to be adequate for analysis of the data set at 75 mM and 175 mM NaCl. At 125 mM NaCl level, however, convergence of variance was observed (Table 3.2). As a result the data were not appropriate for analysis using the diallel models of Hayman and Jinks.

In the present study, the data set at 75 mM and 175 mM NaCl treatment were further analysed and components of variation were estimated (Table 3.4). Parameter estimates indicated both additive and non-additive gene effects in controlling the expression of salt tolerance. The magnitudes of these genetic components differed at the two salinity levels (Table 3.4), estimates being higher at lower salinity (75 mM) whilst lower at higher salinity (175 mM). The data obtained suggest some degree of additive component controlling tolerance. In the main however, genes showing dominance effects appeared to be more important at both salinity levels. In a similar study in sorghum (Azhar and McNeilly, 1988), both additive and dominance gene effects were involved in controlling the expression of salt tolerance, while genes with dominance properties appeared to be more important at both 100 mM and 150 mM NaCl levels. In rice (Gregoria and Senadhira, 1993), the low level of Na^+/K^+ ratio found in the shoots of seedlings grown in $NaCl+CaCl_2$ (16:1 by weight) at EC 12 dS m^{-1} was governed by both additive and dominance gene effects.

An examination of the dominance relations of the different accessions based

upon the W_r/V_r regression would be useful. At 75 mM NaCl, one of the salt sensitive accessions (203662) contained most of the recessive genes whereas the other salt sensitive accession (203659) possessed both dominant tolerant and recessive susceptible genes, whilst the salt-tolerant accessions (221726 and Kitui Local) contained most of the dominant genes (Figure 3.1). However, at 175 mM the pattern of dominance was straight forward. The two salt-tolerant accessions 221726 and Kitui Local had the maximum number of dominant genes (Figure 3.2) dominance being predominantly towards higher tolerance. Likewise the two salt sensitive accessions (203659 and 203662) contained the maximum number of recessive genes (Figure 3.2).

An indication of the overall direction of the deviation of the F_1 means from their corresponding mid-parents can be obtained by comparing the mean of all parental lines with that of all F_1's. Indeed positive heterosis was noted (Appendix 3.3). This may be due to combinations of different dominant gene effects within a single genotype, and there is potential to stabilise these effects in inbred lines with high salinity tolerance.

In general F_1 progenies of individual crosses performed better than predicted from their mid parental values (Appendix 3.3). At both salinity levels the F_1, between Kitui Local x 221726 showed a degree of heterosis. Both parents were considered to be relatively tolerant. A similar situation was reported by Burton (1958) where in 'Gahi 1' pearl millet grown for forage, most of the increased production resulted from hybrid vigour. By contrast crosses between the relatively sensitive accessions, 203659 x 203662 showed as would be expected poor performance. The better performance of hybrid progeny was similar at both salinities, all the F_1s having greater tolerance than their parentals.

The finding that variation in salt tolerance is predominantly due to genes with dominance effects, is confirmed by the estimated narrow sense heritability of 0.20 at 75 mM NaCl and 0.02 at 175 mM NaCl. Estimates for heritability in the broad sense of 0.80 at 75 mM NaCl and 0.67 at 175 mM NaCl further confirm the role of dominance effects in the genetic control of NaCl tolerance. Though estimates of heritability have notoriously high standard errors (Falconer, 1981; Lawrence, 1984), they may be used to estimate progress through selection (Hanson, 1963; Liang *et al.*, 1972). In doing so however it must be remembered that, as stated by Falconer (1989), the heritability value of a given character refers only to a particular population under particular

conditions, and therefore ambiguity in estimation of heritability is to be expected, i.e. heritability estimates are not constant. Dominance towards tolerance is also suggested from the magnitudes of the non-additive components and confirmed by the positive value for the potence ratio (Table 3.4).

The main advantage of the diallelmay be that it permits in some circumstances the estimation of specific combining ability effects, and frequently the breeding value or general combining ability (Mayo, 1987). Genetic advances among hybrids are usually the result of making crosses between selected inbred lines that have been chosen for their individual characters as well as their combining abilities (Falconer, 1989). Differences of general combining ability are due to the additive variance and interactions in the base population; and differences of specific combining ability are attributable to the non-additive genetic variance. The objective of the present study was, however, to compare combining abilities of the parents when the parents themselves are used as testers and to identify the higher yielding combinations. From the comparison of the parents (Table 3.5), the general combining ability of parent Kitui Local was relatively higher. At the same time the relatively lower specific combining ability associated with Kitui Local (Table 3.6) indicated that Kitui Local uniformly transmitted its relatively salt tolerance ability to all of its F_1's and for this reason Kitui Local is probably superior to others for inclusion in the production of a synthetic salt-tolerant variety (Griffings, 1956).

An estimate of genetic correlation, defined as correlation of breeding values for families from the same cross tested at 75 mM and 175 mM NaCl showed positive correlation (Table 3.7). This may be due to the genes operating at high and low salinity conditions being the same (Shannon, 1985).

The diallel cross method followed here in elucidating the genetic architecture of salt tolerance in pearl millet has previously been used by breeders in an attempt to determine the inheritance mechanism of salt tolerance in rice (Moeljopawiro and Ikehashi, 1981) and salinity tolerance in sorghum (Azhar and McNeilly, 1988). The results of these studies indicated that both additive and dominance genetic effects were important for controlling NaCl tolerance determined from root length growth in the stress conditions applied. The findings of Ekanayake *et al.* (1985) showed that drought tolerance in rice is also controlled by both dominance and additive effects whilst the

same appears to be true of copper tolerance in the wild *Silene vulgaris* (Schat and Ten-Bookum, 1992), and other wild metal tolerant species Given that these stress factors impose very high selected pressures such a genetic architecture might be expected with respect to the evolution of dominance.

The analysis described here has provided two important pieces of information about the genetic basis of salinity tolerance in pearl millet.

1. The trait is governed by both additive and dominance genetic effects at both 75 mM 175 mM NaCl levels with the dominance genetic effects being predominant.
2. Selection at 75 mM NaCl would be of paramount importance towards improvement of salinity tolerance in pearl millet, whilst exploitation of non-additive variance through heterosis might be rewarding by testing at both 75 and 175 mM NaCl levels.

THE RESPONSE OF TWELVE PEARL MILLET ACCESSIONS TO NaCl DURING ONTOGENY OF THE WHOLE PLANT

CHAPTER FOUR

CHAPTER 4

THE RESPONSE OF TWELVE PEARL MILLET ACCESSIONS TO NaCl DURING ONTOGENY OF THE WHOLE PLANT

4.1. Introduction

Variability in salinity tolerance within species has been reported with increasing frequency in recent years; however, the choice of criteria by which tolerance is measured has not been consistent among investigators (Rush and Epstein, 1976; Shannon, 1978; Pasternak *et al*., 1979; Norlyn, 1980; Shannon *et al*., 1983). Plant response to salinity may change with age, and salt stress increases as the plant continues to grow and transpire under saline conditions due to increased salt load on the root as time passes (Blum, 1988). A plant's response, and consequently its effective salt tolerance, are influenced by its ontogenic stage, and salinity effects may vary depending upon the growth stage at the time of stress (Ashraf and Waheed, 1990), suggesting that the plants ability to respond to salt stress depends upon the genes that are functioning at the stage of development during which the stress occurs (Shannon, 1985).

The effects of and responses to salt stress may also be modified by changes that have occurred due to previous stress, e.g. during rapid vegetative growth the plant strives to maintain as large a photosynthetic area as possible to maintain a root system that will support the plant and provide water and nutrients (Shannon, 1985). Salinity affects this balance, typically reducing vegetative top growth more than root growth (Maas and Nieman, 1978). Such changes may affect the severity of, and response to, subsequent stresses.

The small amount of available information with respect to effect of plant age on salinity resistance (Blum, 1988) does not allow development of any generalisation. Therefore, for varietal improvement in salinity tolerance to be effective, availability of information about the effects of salinity on all phases of plant growth are essential and equally it would be worthwhile to identify the life stage most susceptible to the effect of salinity in order to maximise selection efficiency (Azhar and McNeilly, 1989).

The desired adaptive response would therefore be one in which plants become more resistant with age, either as a function of age *per se* or as a function of hardening

(Blum, 1988). For example, in barley, salinity tolerance has been found to both increase (Greenway, 1965) and decrease (Lynche *et al.*, 1982) with plant age; in sugar beet, salinity tolerance was found to be lowest during germination (Bernstein and Hayward, 1958); in wheat, reduction in total grain yield in response to salinity occurred primarily through inhibition of tillering capacity (Maas and Grieve, 1990); in tomato, the seedling stage of seven cultivars was more sensitive to salinity stress than their adult stage (Pasternak *et al.*, 1979); in sorghum, grain yield decreased most when stress was imposed during the vegetative and reproductive stage of development (Maas, *et al.*, 1986).

This Chapter describes an experimental assessment of the effects of various levels of salinity applied throughout the whole course of plant development on the growth and yield of twelve accessions of pearl millet (*P. americanum*) at different growth stages.

4.2. Materials and methods

Twelve pearl millet accessions Kitui Local (t), Selection 2 (t), 93611 (t), 93614 (t), 203656 (s), 203658 (s), 203659 (s), 203662 (s), 215631 (s), 215632 (s), 215634 (s) and 221726 (t) were used in this experiment (t = tolerant, s = sensitive).

Seedlings of the twelve accessions were raised in washed river sand, irrigated with nutrient solution half-strength (following Rorison in Hewitt, 1966) in 30 x 60 cm plastic trays. Four one-week-old of similar size seedlings were transplanted into 18 cm plastic pots containing dry river sand washed for one week, on days 1 - 5 with tap water and then for two days using nutrient solution prior to planting and transplanting. The seedlings were fed with half-strength nutrient solution every two days for five weeks. Holes in the base of the plastic pots allowed quick drainage of the solutions through cheesecloth which lined the base of the pots. To retain leachate, plastic saucers were placed under each pot.

Imposition of salinity stress upon the seedlings began five weeks after transplanting, with addition of NaCl as appropriate to the half-strength Rorison solutions. Excess solution was added to each pot to avoid NaCl accumulation. The NaCl concentration was increased in aliquots of 25 mM NaCl on alternate days until the appropriate salt treatment was reached. The salinity treatments were of 75 mM (EC = 7

dS m^{-1}), 100 mM (EC = 10 dS m^{-1}) and 150 mM (EC = 13 dS m^{-1}). Controls consisted of plants grown in the nutrient solution without salt addition (EC = 0.8 dS m^{-1}). Treatments continued with the addition of the appropriate solution on alternate days. To monitor levels of salinity, the first 50 to 75 ml of solution flowing from the bases of twenty randomly chosen pots was collected at each alternate irrigation and solution electrical conductivity determined. To minimise salt concentration fluctuations, all pots were flushed with non-saline nutrient solution every two weeks, following which the pots were immediately flushed with their respective NaCl nutrient solution until the electrical conductivity of the effluent solution was equal to that of the solution being added.

Daytime glasshouse temperatures ranged from 21° to 40°C (mean = 30°C); night temperatures, from 14° to 30°C (mean = 20°C). Relative humidity ranged from 40% to 80% with a mean of 60% during the day, and 70% during the night. Sixteen hours natural daylength was provided, natural daylight being supplemented using 400 Watt mercury vapour lamps.

The experiment was of complete randomised block design consisting of four treatments (0, 75, 100 and 150 mM NaCl) imposed throughout plant development. The physiological development of the plants from seedling emergence to maturity was rated as below following Jauhar (1981).

(i) Growth stage 1

The vegetative stage, included the periods of leaf growth and expansion, tillering, and stem elongation.

(ii) Growth stage 2

The reproductive stage, including booting, inflorescence emergence, and anthesis.

(iii) Growth stage 3

The maturation stage, milk and dough development, and ripening.

At each stage of growth, four experimental units representing 0 (control), 75, 100 and 150 mM NaCl treatments at each of the replications were measured for plant height, number of leaves, and percentage live leaves. Dry weights of roots, stems, leaf

sheathes and leaf blades were measured at stage 3.

Heights of plants were measured from base to the tip of the stem for measurements taken at stages 1 and 2, while mature plant height at stage 3 was measured to the tip of the inflorescence. Mean plant height was calculated for each stage of growth.

Total number of leaves per plant was counted, and averaged over number of plants in each replicate in each treatment. Similarly, number of live leaves (green leaves without necrosis) per plant was counted at the three different growth stages, and the percentage of live leaves calculated.

At stage 3 all four plants were harvested and bulk samples of roots, stems, leaf sheathes and leaf blades of all four plants were made oven dried at 50^{o}C for ten days, weighed, and mean shoot and root dry weight per plant calculated.

Degree of salt tolerance was estimated as relative values (treatment estimates expressed as percentage of controls) for each character in each replicate in the three NaCl treatments (75, 100 and 150 mM).

Data for both absolute and relative values for the twelve accessions for the three characters measured at the three growth stages, and for the four characters measured only at growth stage 3 were subjected to analysis of variance.

The salt sensitive growth stage for absolute plant height and percentage live leaves was assessed using a non-linear least squares method of van Genuchten and Hoffman (1984), option 12, as used in Chapter 2. Similarly absolute dry weights of root, stem, leaf sheath and leaf blade of the accessions as a function of the NaCl of the nutrient solution imposed throughout whole plant development were used to further compare the responses of the accessions.

4.3. Results

Salinity response was assessed both as absolute (Dewey, 1960) and as relative salt tolerance values (Maas and Hoffman, 1977; Maas, 1985). To simplify presentation, only data for six accessions are presented; the data of the remaining six accessions are given in Appendices 4.1 - 2.

Table 4.1. Mean squares and significances from the analysis of variance of absolute values of plant height, number of leaves per plant, and live leaves percentage per plant at three different growth stages

Item	Df	Plant height	Number of leaves per plant	Percentage live leaves per plant
Blocks	2	126.88^{NS}	7.17***	107.53^{NS}
Accessions (Acc)	11	934.94***	9.57***	299.43***
NaCl solutions (Sol)	3	16726.37***	45.94***	4456.34***
Growth stages (Gst)	2	492.18***	215.63***	27731.39***
Acc x Sol	33	359.40***	2.46***	27731.39***
Acc x Gst	22	82.33^{NS}	0.76^{NS}	166.51***
Sol x Gst	6	751.81***	7.33***	755.33***
Acc x Sol x Gst	66	77.41^{NS}	0.55^{NS}	33.46^{NS}
Residual	286	58.65	0.97	36.50

Table 4.2. Mean squares and significances from the analysis of variance of absolute values of dry weights of root, stem, leaf sheath and leaf blade recorded at growth stage 3

Item	Df	Root	Stem	Leaf sheath	Leaf blade
Blocks	2	7.83^{NS}	2.34*	0.18^{NS}	0.25^{NS}
Accessions (Acc)	11	47.64***	5.10***	0.79***	11.17***
NaCl solutions (Sol)	3	853.96***	383.92***	37.04***	111.46***
Acc x Sol	33	12.48***	2.65***	0.44***	2.49***
Residual	94	3.998	0.565	0.116	0.428

4.3.1. Absolute salt tolerance

The results obtained from the analysis of variance of absolute values for the three characters, mean plant height, mean number of leaves per plant, and mean percentage live leaves per plant, collected at three different growth stages, and for the four characters, mean dry weights per plant of roots, stems, leaf sheathes, and leaf blades taken at growth stage 3 are presented in Tables 4.1 and 4.2 respectively.

4.3.1.1. Mean Plant height

The results of the analysis (Table 4.1) show that accessions differed significantly ($p<0.001$) in plant height, increasing NaCl concentrations significantly ($p<0.001$) reduced mean plant height and there were significant ($p<0.001$) difference in plant height at different growth stages. Accessions heights differed significantly in different NaCl concentrations (interaction accessions x NaCl concentrations significant at $p<0.001$), and NaCl concentrations affected plant heights differently at different growth stages (interaction NaCl concentrations x growth stages significant at $p<0.001$).

Mean plant height of the accessions at the three growth stages was affected by NaCl treatments (Figure 4.1). At 100 and 150 mM NaCl across all growth stages, plants of accession 221726 were the tallest plants, whilst plants of accession 93611 did not show substantial reduction in height with increasing salinity at any of the three growth stages.

The NOPT 12 fitted curve for mean plant height of each of the six accessions as function of NaCl concentrations imposed during each growth stage is presented in Figure 4.2. Mean height per plant was affected most by salt imposed during maturation (growth stage 3), less during the reproductive stage (growth stage 2) and least during the vegetative stage (growth stage 1).

4.3.1.2. Mean number of leaves per plant

Data for mean number of leaves per plant of six accessions are presented in Figure 4.3. Mean number of leaves per plant of the accessions differed significantly from each other ($p<0.001$, Table 4.1), increasing salinity caused overall significant

Figure 4.1. Mean plant height (cm) at three different growth stages

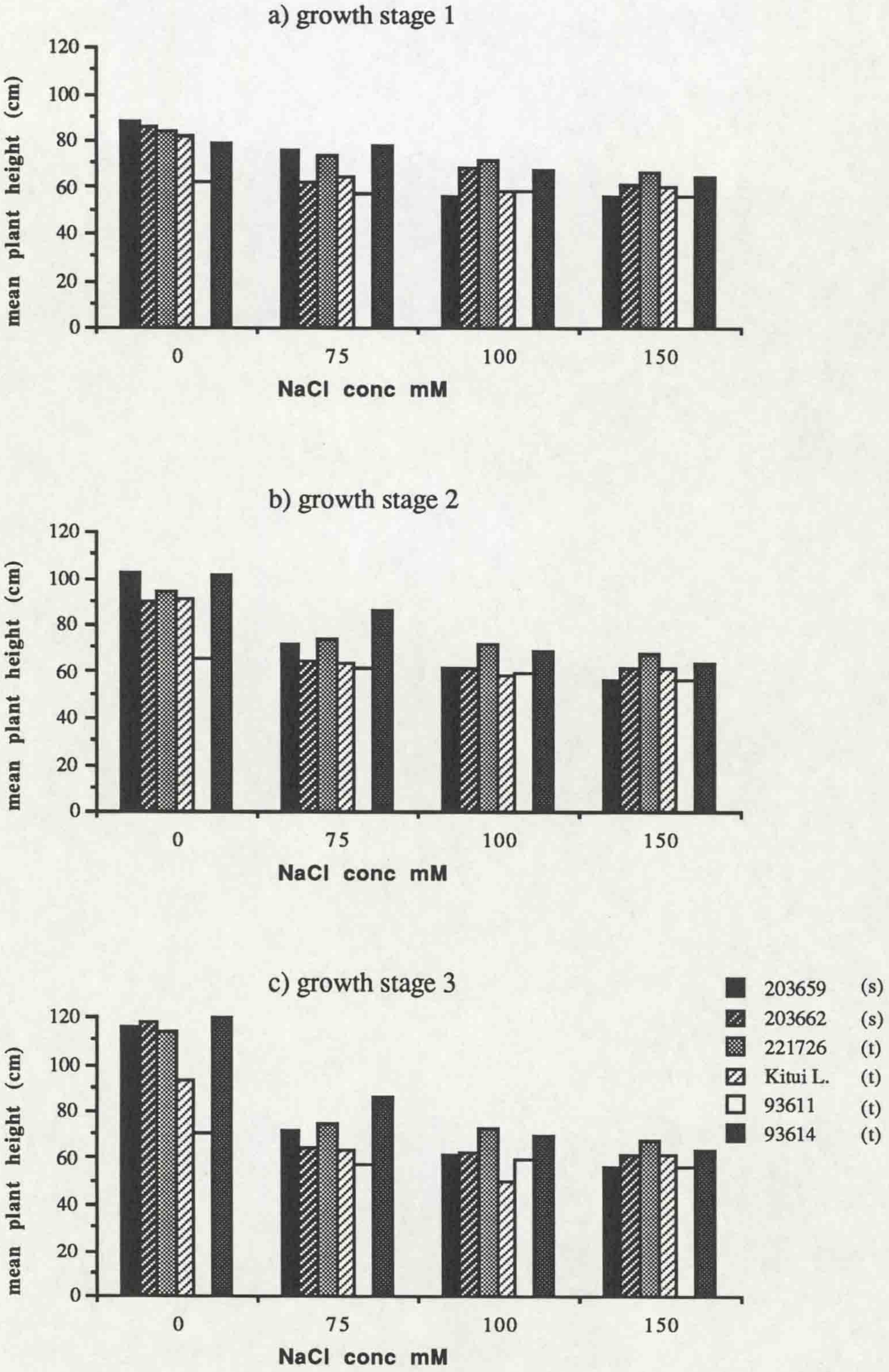
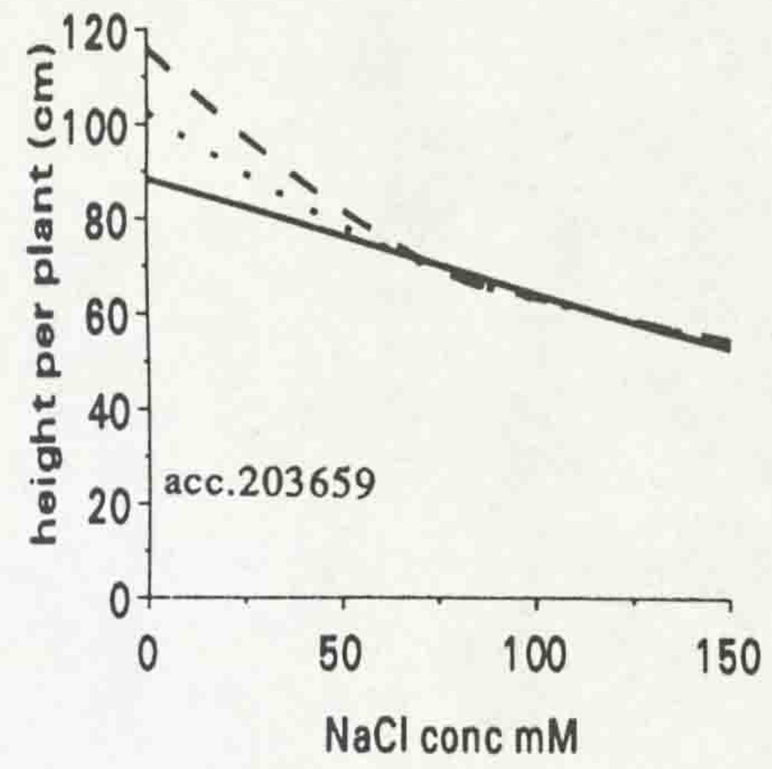
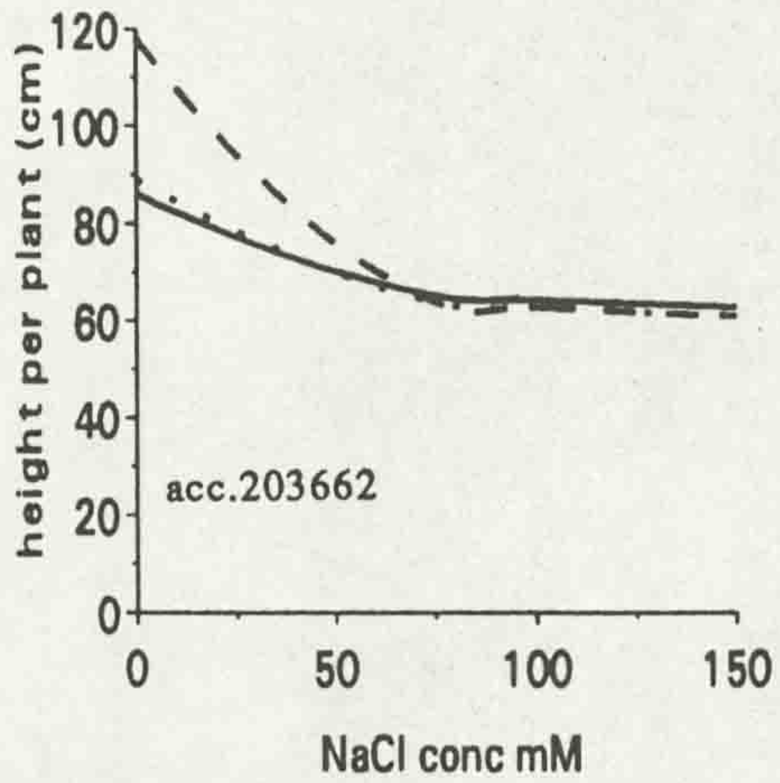
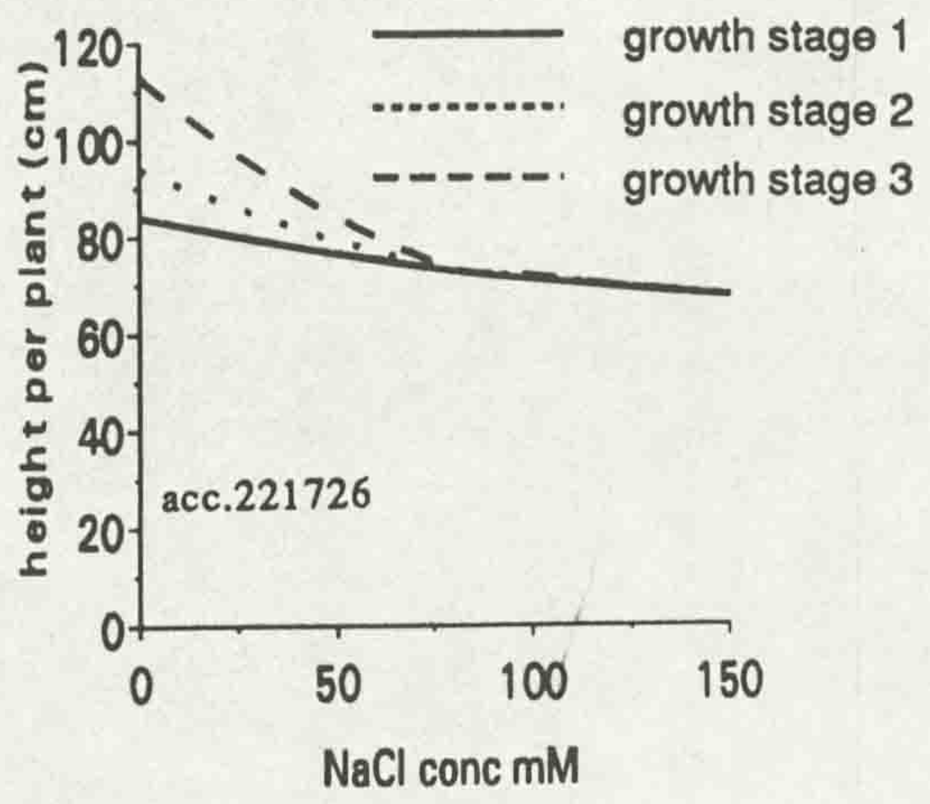
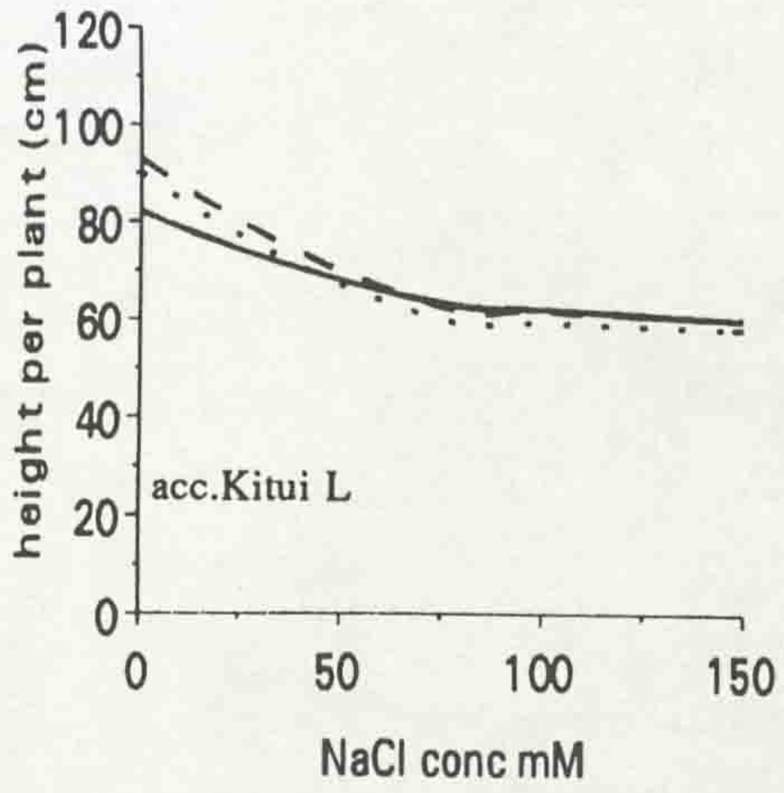


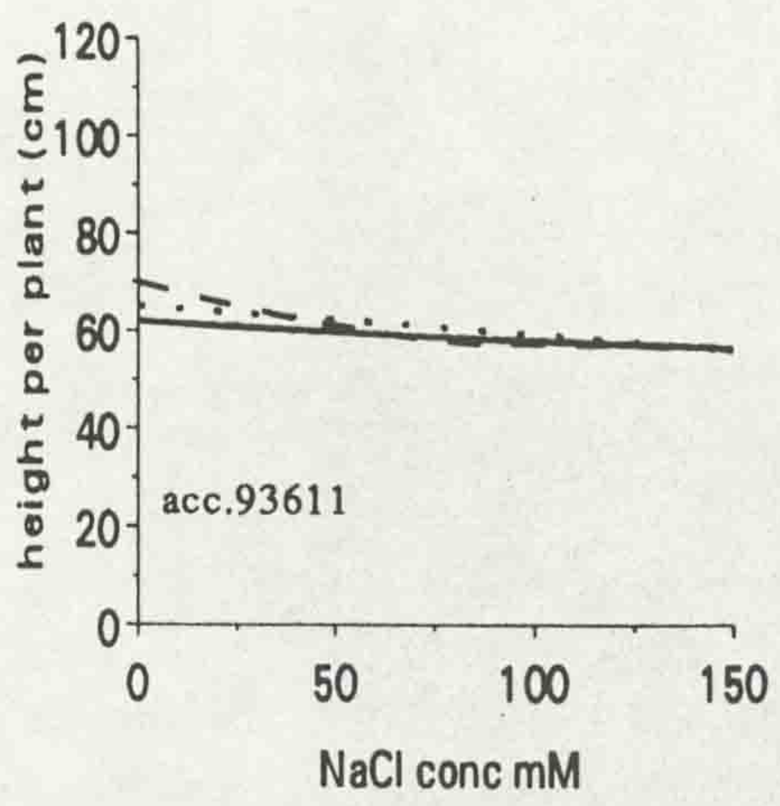
Figure 4.2. Plant height at three different growth stages as a function of NaCl concentration (fitted curve)











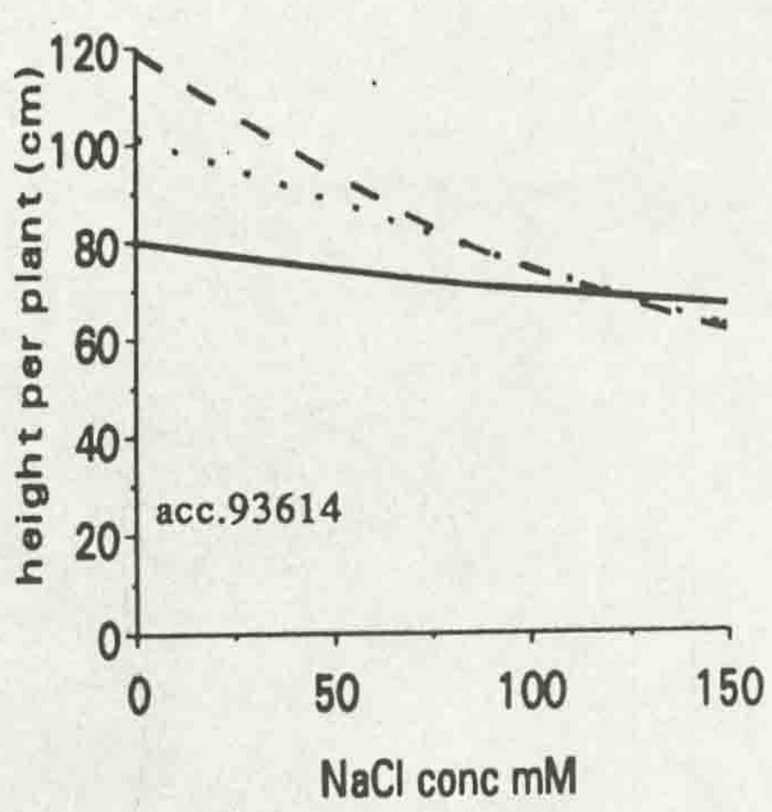
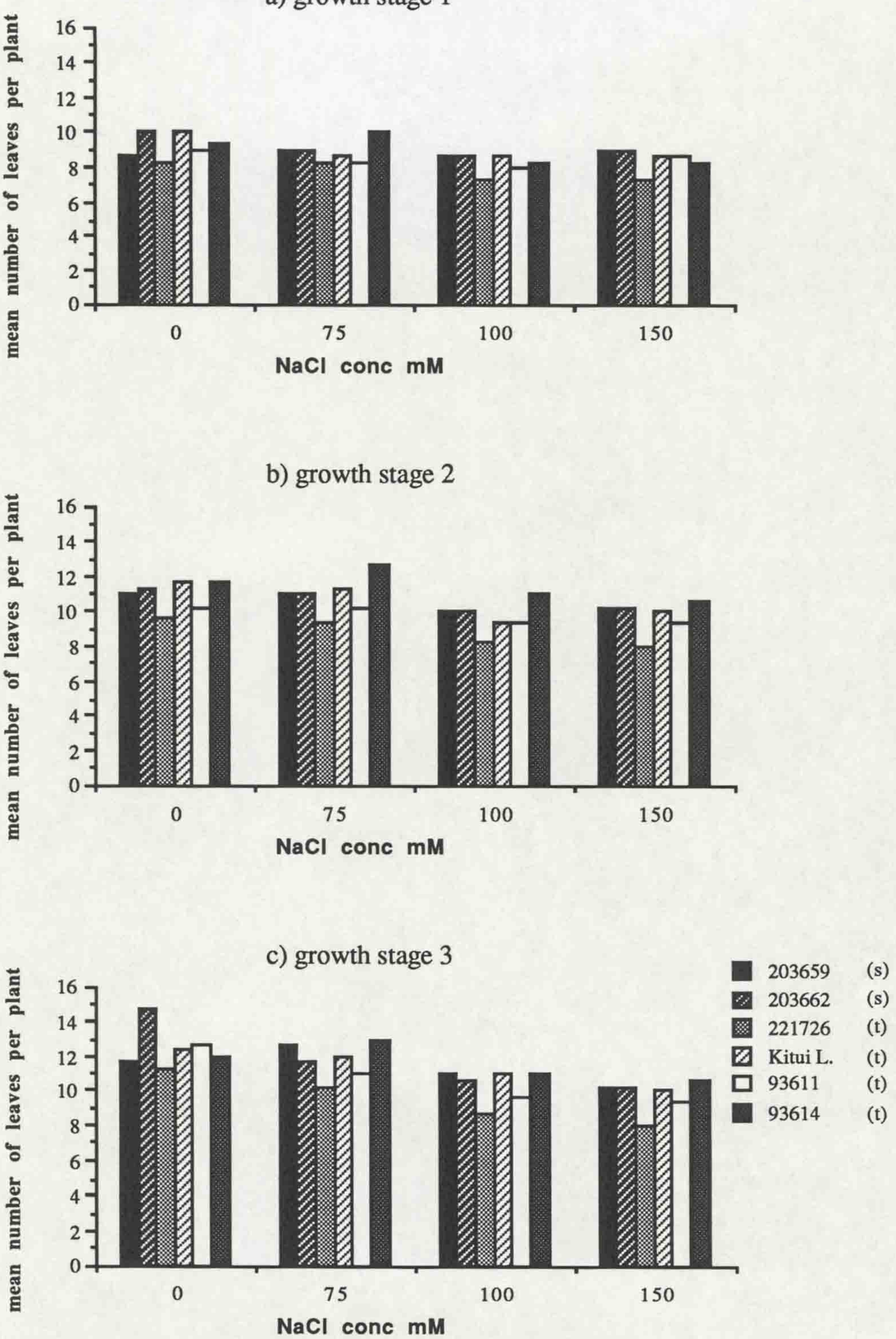


Figure 4.3. Mean number of leaves per plant at three different growth stages



reductions in number of leaves per plant ($p<0.001$), whilst number of leaves per plant was different at different growth stages ($p<0.001$). However the accessions did not differ significantly in overall mean number of leaves per plant at different growth stages. The interaction, accessions x NaCl solutions was significant ($p<0.001$) suggesting that the degree of reduction in leaf number per plant differs between accessions in response to different NaCl concentrations.

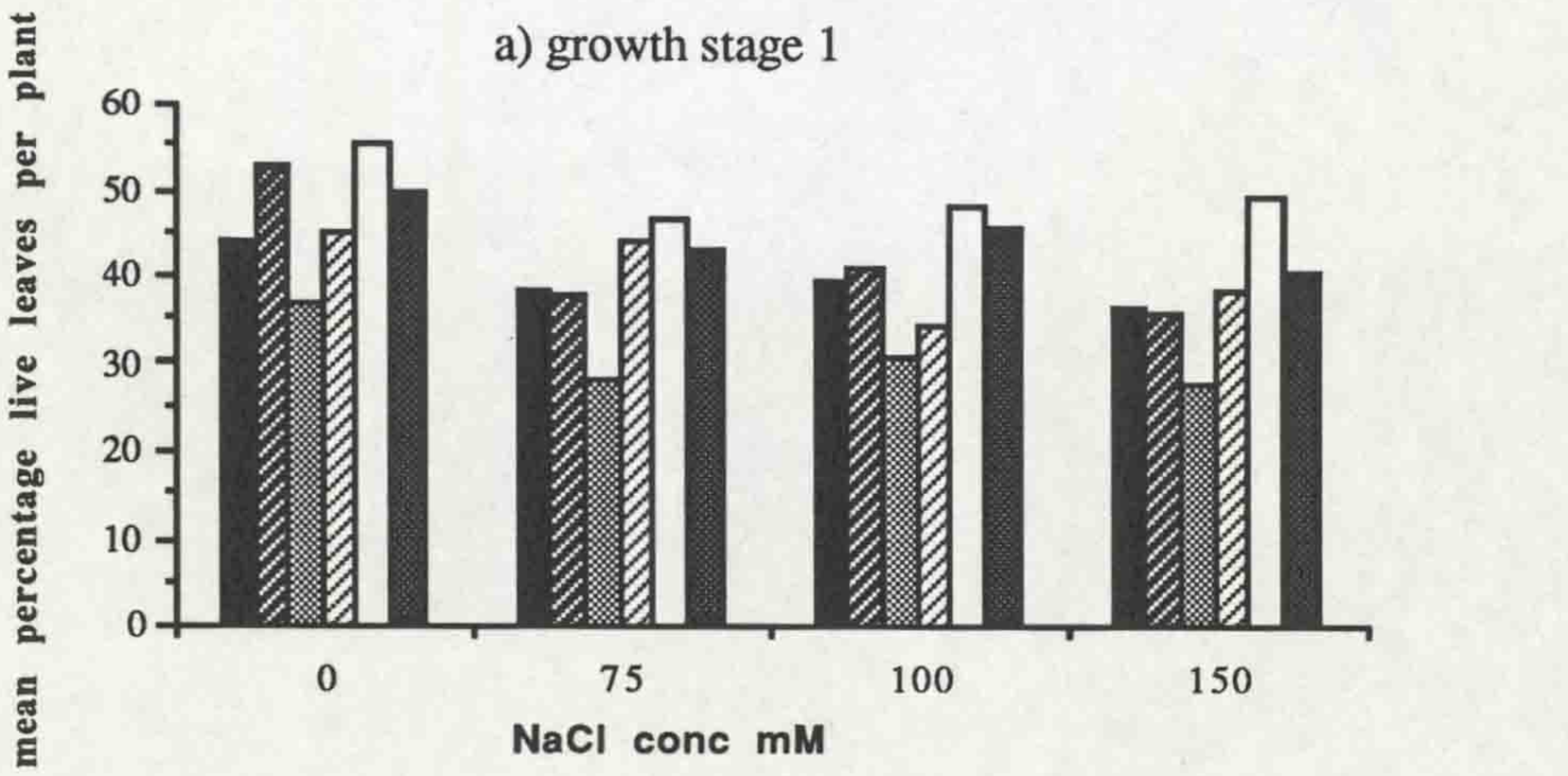
4.3.1.3. Mean percentage live leaves per plant

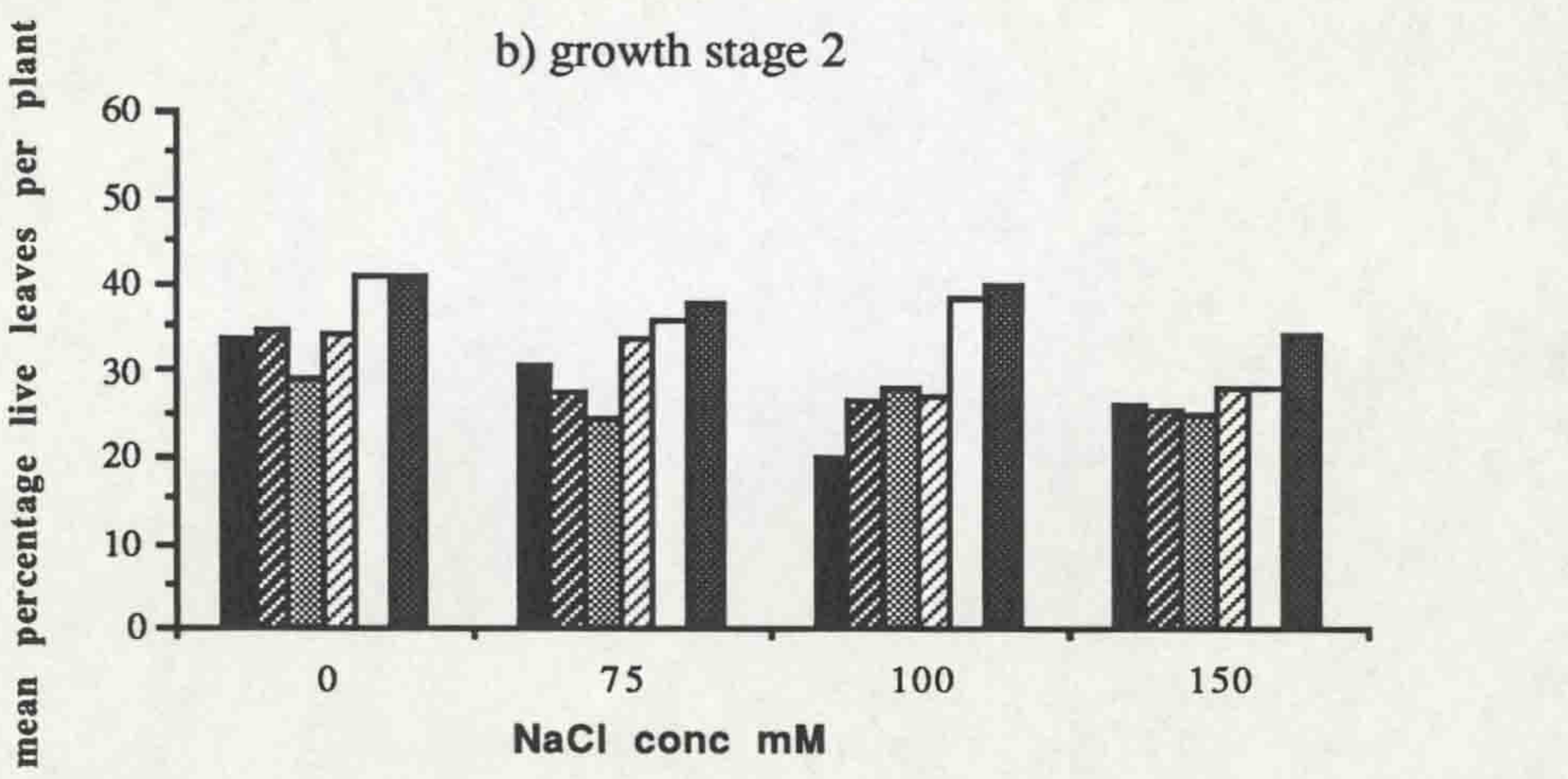
The results of the analysis of variance in Table 4.1 show that accessions differed significantly ($p<0.001$) in number of live leaves throughout the experiment, and increasing NaCl concentrations significantly ($p<0.001$) reduced the number of live leaves. Mean percentage live leaves per plant was significantly ($p<0.001$) different at different growth stages, and the interaction, and accessions differed in mean percentage live leaves per plant at different growth stages. Accession response in number of live leaves to changing NaCl concentrations was significant (interaction accessions x NaCl concentrations at $p<0.001$), and the number of live leaves on different accessions differed significantly at different growth stages (interaction accessions x growth stages at $p<0.001$). Different NaCl concentrations had significantly ($p<0.001$) different effects on live number of leaves at different growth stages.

The negative impact of NaCl on mean number of live leaves per plant was greater at growth stages 2 and 3 than at growth stage 1 (Figure 4.4). The salt sensitive accessions (203659 and 203662) were markedly affected at 150 mM NaCl solution at growth stage 3, whilst accession 221726 had the smallest number of dead leaves (Figure 4.4).

The fitted curve for mean percentage live leaves per plant of each of the six accessions as a function of the NaCl solution imposed during each growth stage (Figure 4.5) showed that growth stage 3 was the most sensitive stage for this character. These data also confirms the greater tolerance of accession 221726.

Figure 4.4. Mean percentage live leaves per plant at three different growth stages





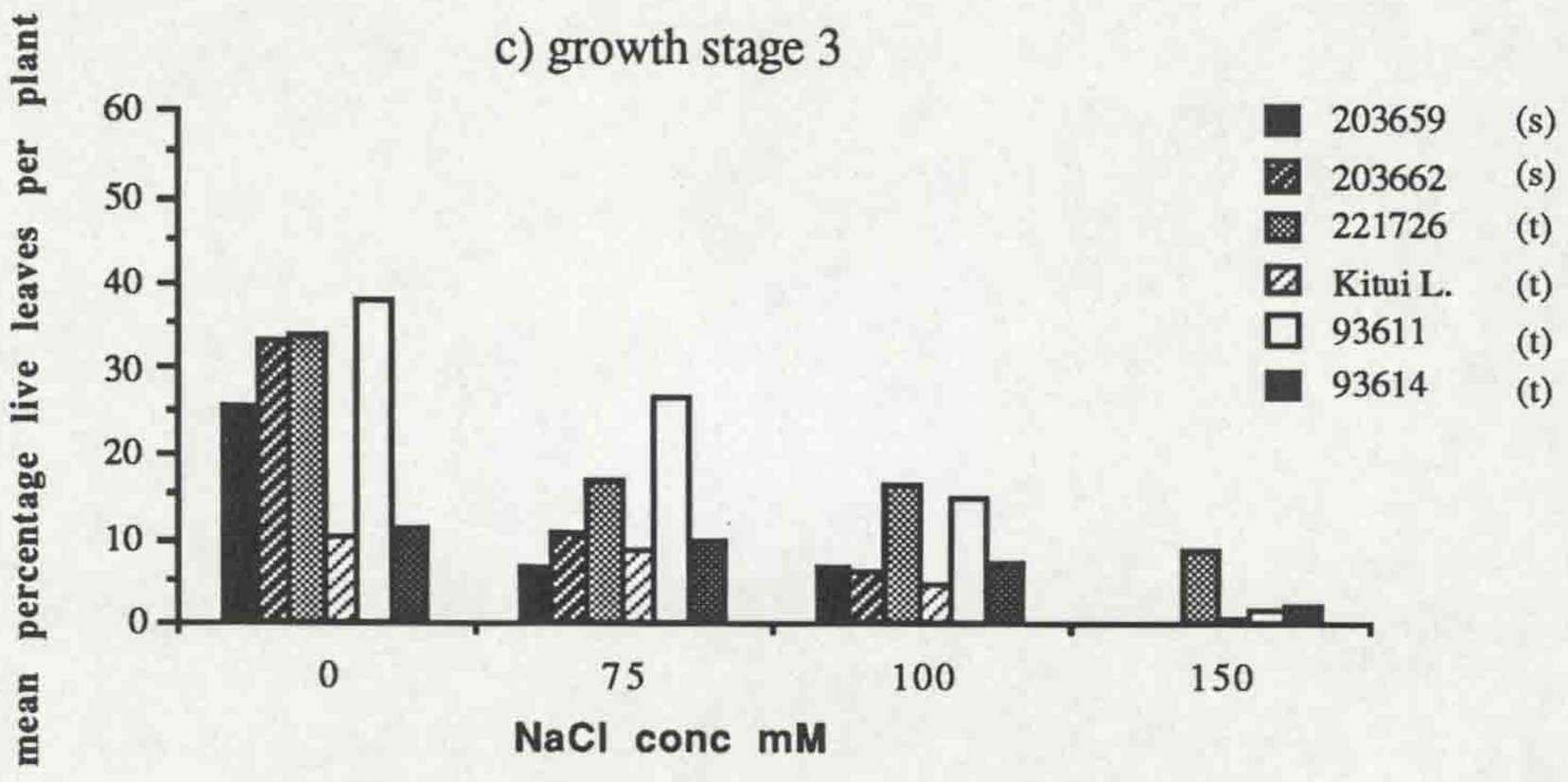
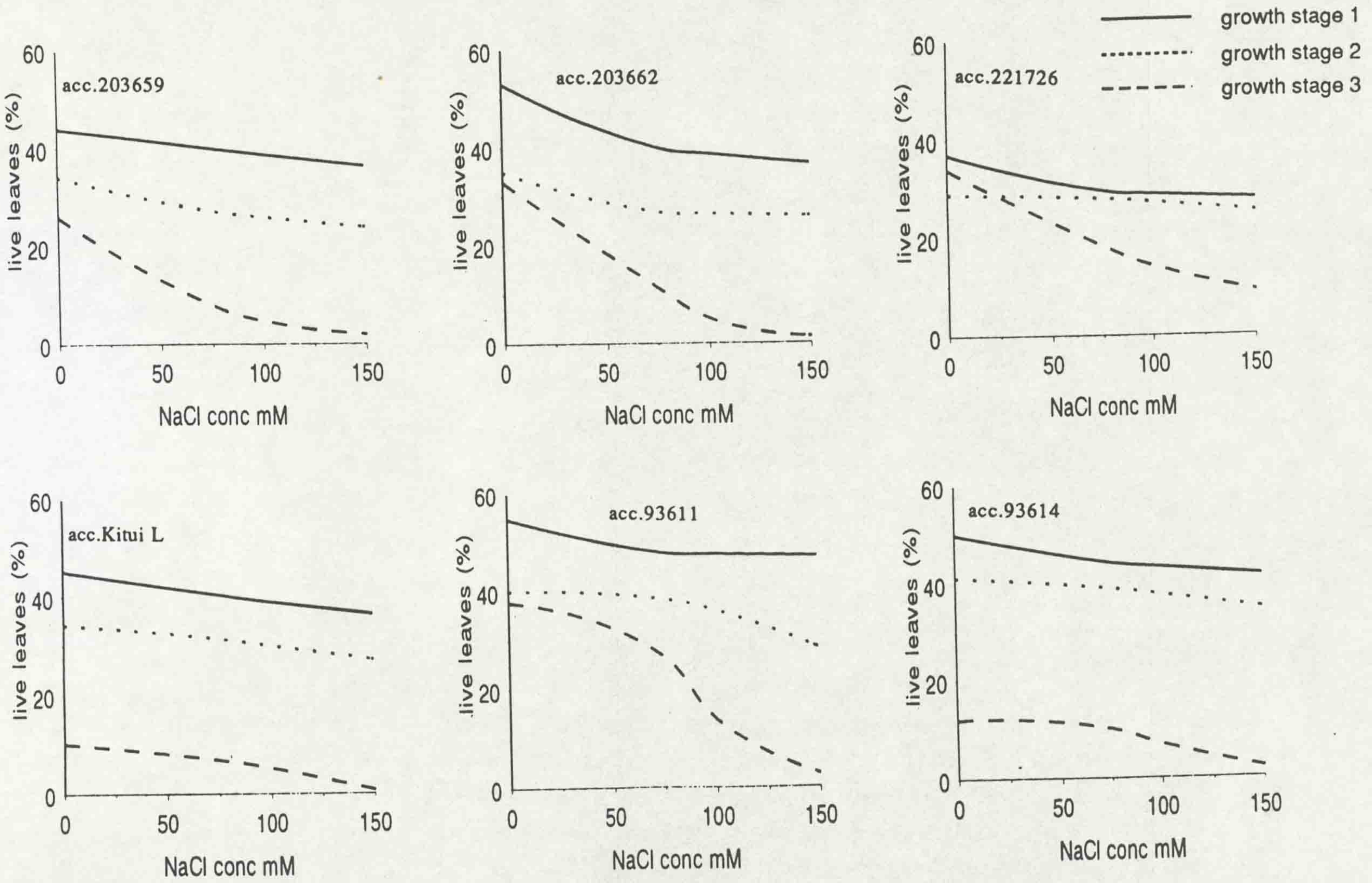


Figure 4.5. Percentage live leaves at three different growth stages as a function of NaCl concentration (fitted curve)



4.3.1.4. Mean root dry weight per plant

Mean root dry weight per plant of the accessions differed significantly ($p<0.001$, Table 4.2) from each other, and was reduced significantly by increasing NaCl concentrations ($p<0.001$, Table 4.2). Accessions differed significantly in mean root dry weight per plant in response to different NaCl concentrations, interaction term, accessions x NaCl solutions was significant ($p<0.001$, Table 4.2).

Figure 4.6a shows that the tolerant accession 221726 had greater root dry weight than the rest of the accessions across all NaCl concentrations, whilst at 150 mM NaCl, the sensitive accession 203659 had the lowest root dry weight.

The response function curve for mean root dry weights of the six accessions and NaCl concentrations is presented in Figure 4.7, and shows that two of the tolerant accessions (221726 and Kitui Local) had greater root dry weight at 150 mM NaCl, whilst accession 203659 (susceptible) had the lowest root dry weight.

4.3.1.5. Mean stem dry weight per plant

From the analysis of variance in Table 4.2, the difference between accessions in mean stem dry weight per plant was significant ($p<0.001$). Increasing NaCl concentrations significantly ($p<0.001$) reduced stem dry weight, and accession dry weights differed significantly ($p<0.001$) in different saline solutions.

Data for the six accessions are presented in Figure 4.6b. The effect of NaCl on stem dry weight was greater in accession 203659 at 100 mM NaCl and in both the sensitive accessions (203659 and 203662) at 150 mM NaCl. On the other hand, accession 221726 (tolerant) consistently maintained a greater stem dry weight across all NaCl concentrations.

Mean stem dry weight per plant as a function of NaCl in the nutrient solution imposed during each growth stage (Figure 4.8) showed that accession 221726 (tolerant) had greater stem dry weight at the highest salinity (150 mM). In contrast, accession 203659 (salt sensitive) showed a very sensitive response and had the lowest stem dry weight.

Figure 4.6. Mean dry weights (g) of a) root, b) stem, c) leaf sheath, and d) leaf blade per plant at growth stage 3 [with standard error (S.E.)]

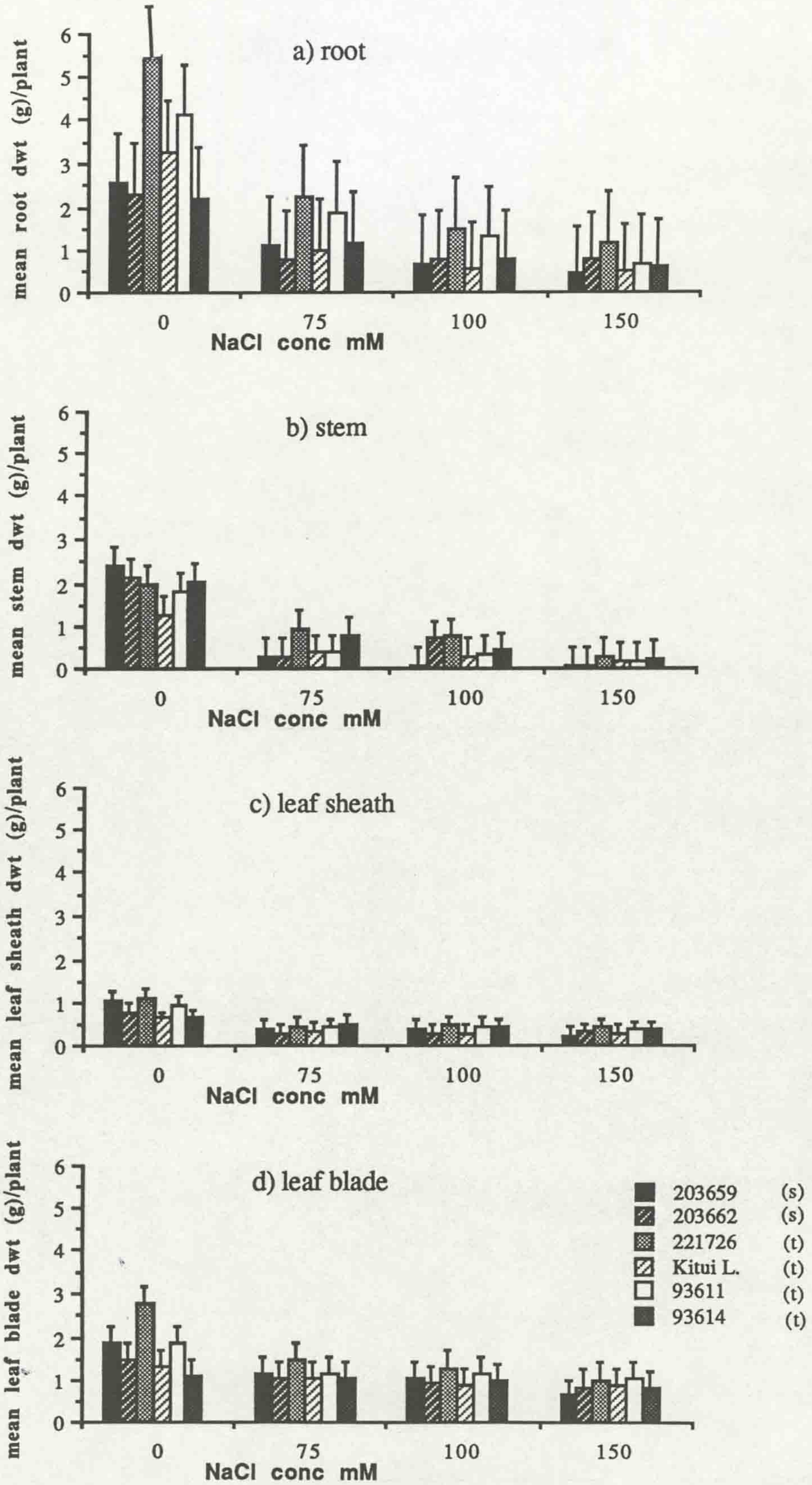


Figure 4.7. Root dry weight at growth stage 3 as a function of NaCl concentration (fitted curve)

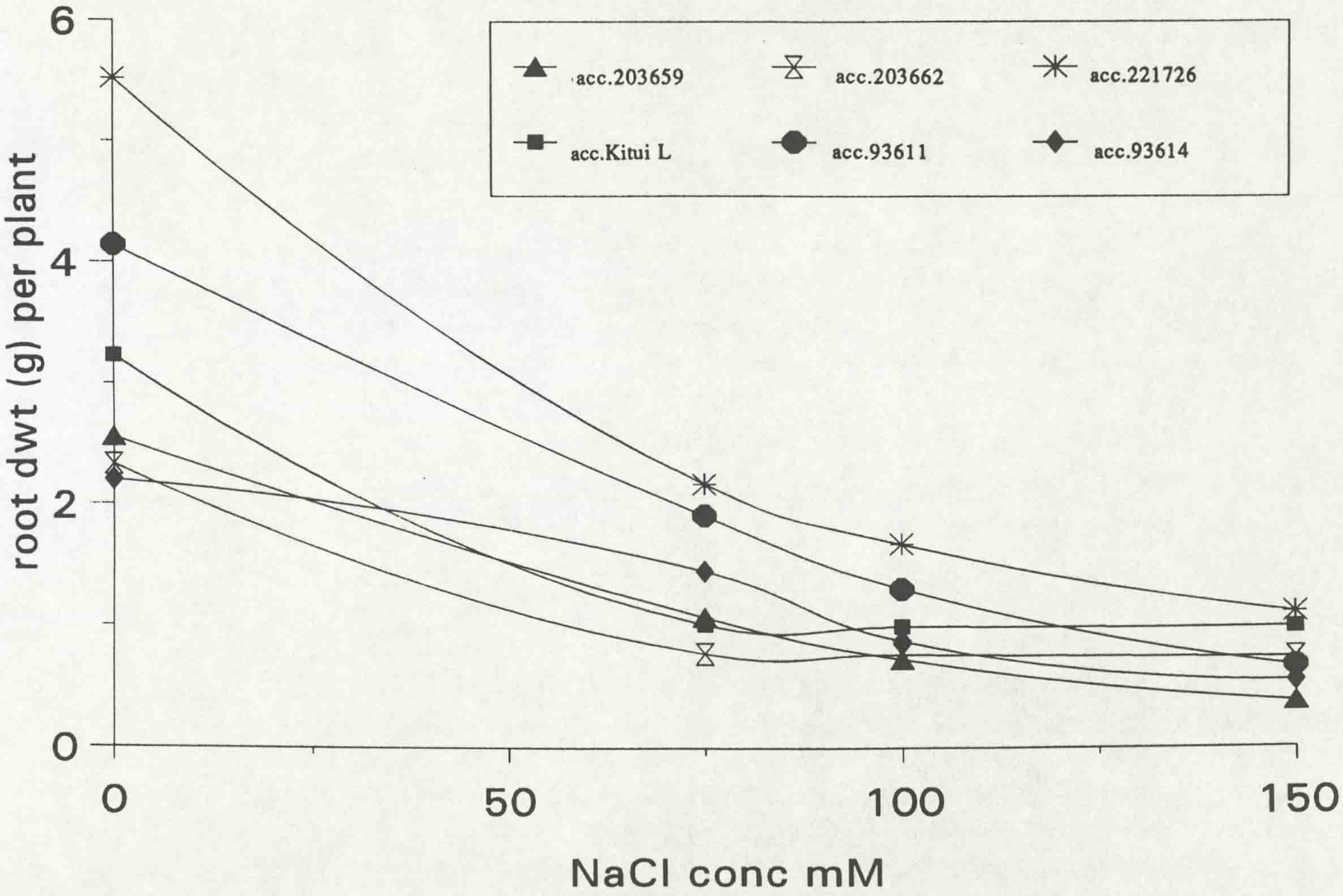


Figure 4.8. Stem dry weight at growth stage 3 as a function of NaCl concentration (fitted curve)

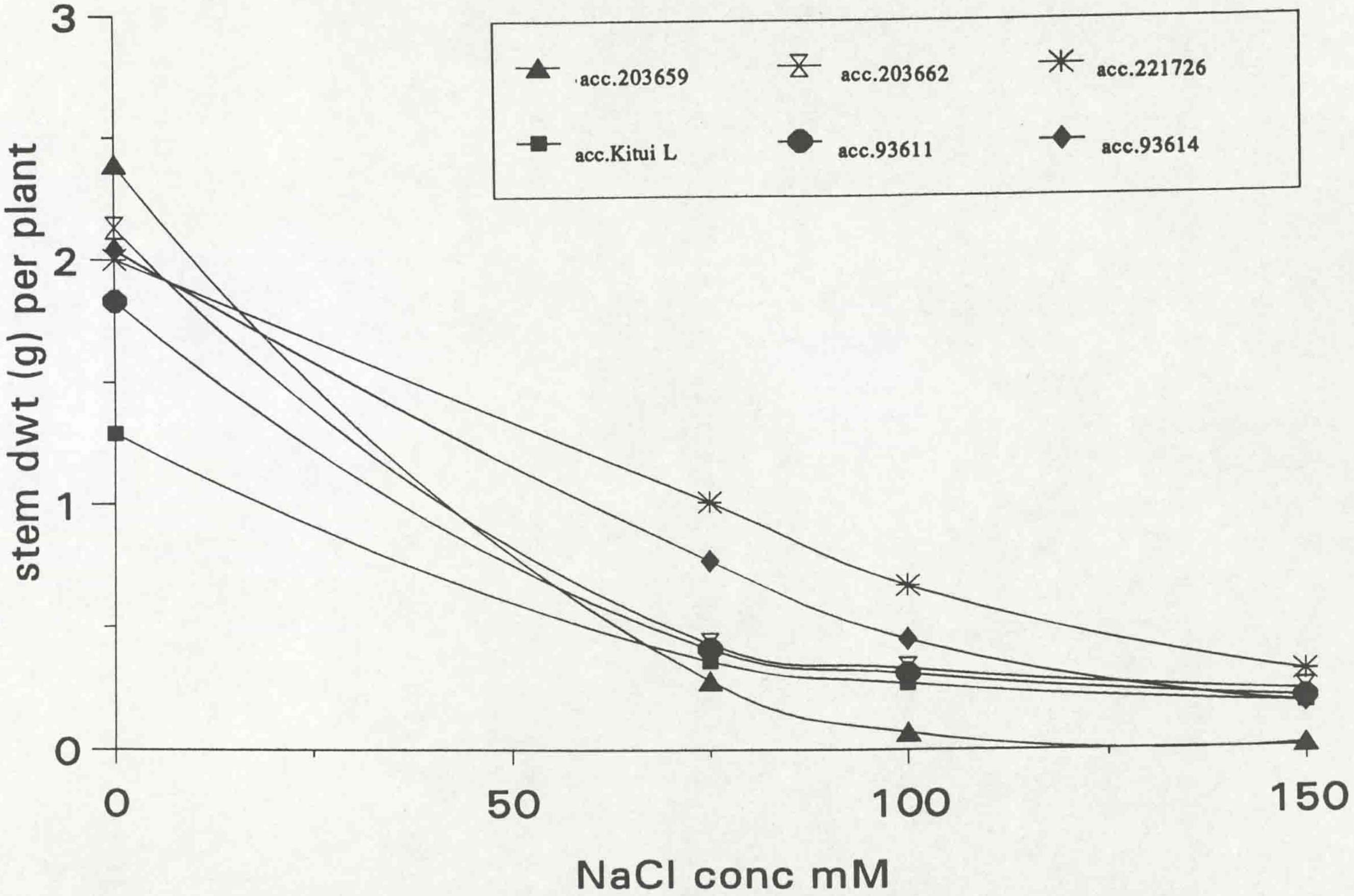


Figure 4.9. Leaf sheath dry weight at growth stage 3 as a function of NaCl concentration (fitted curve)

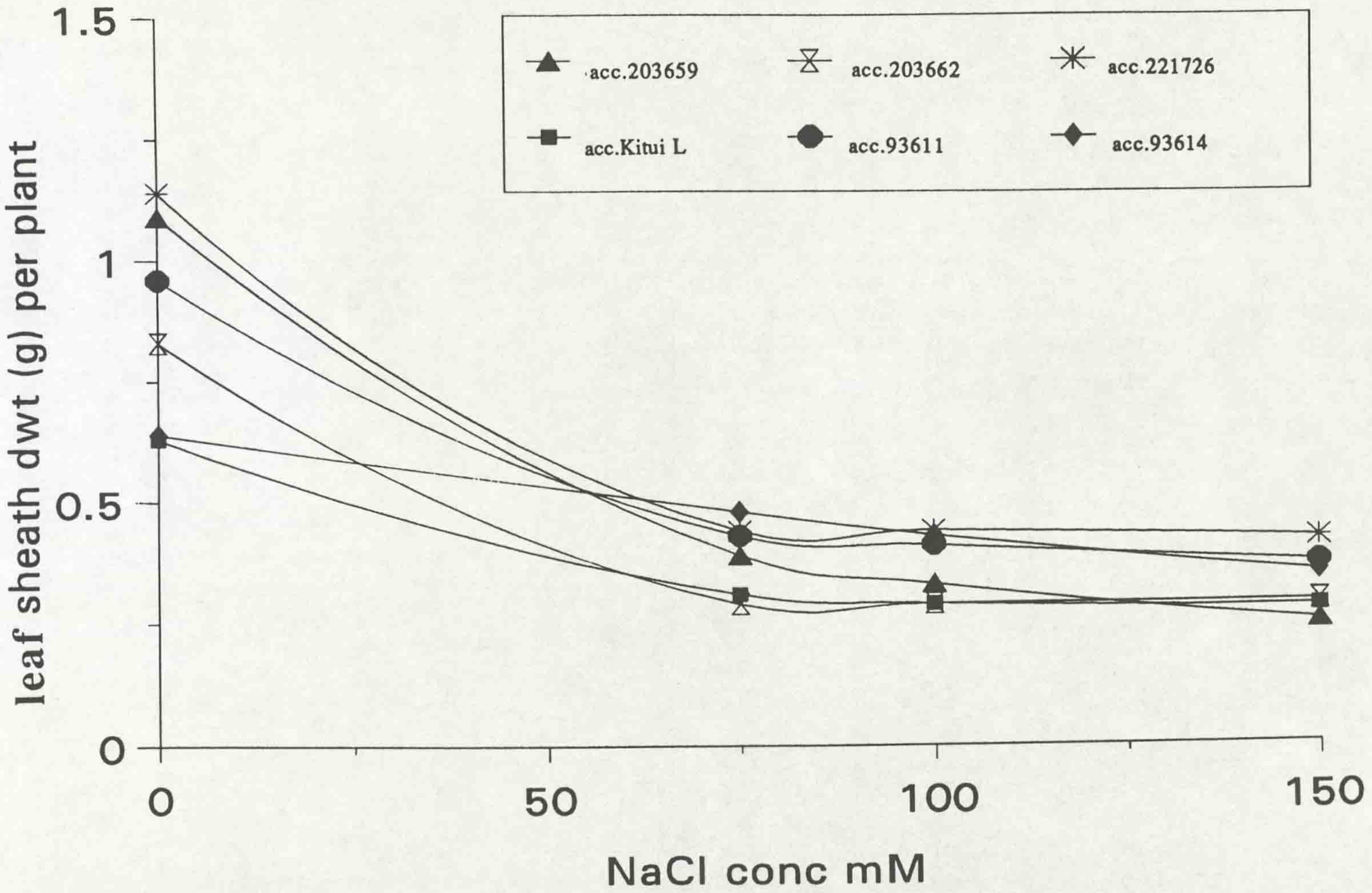
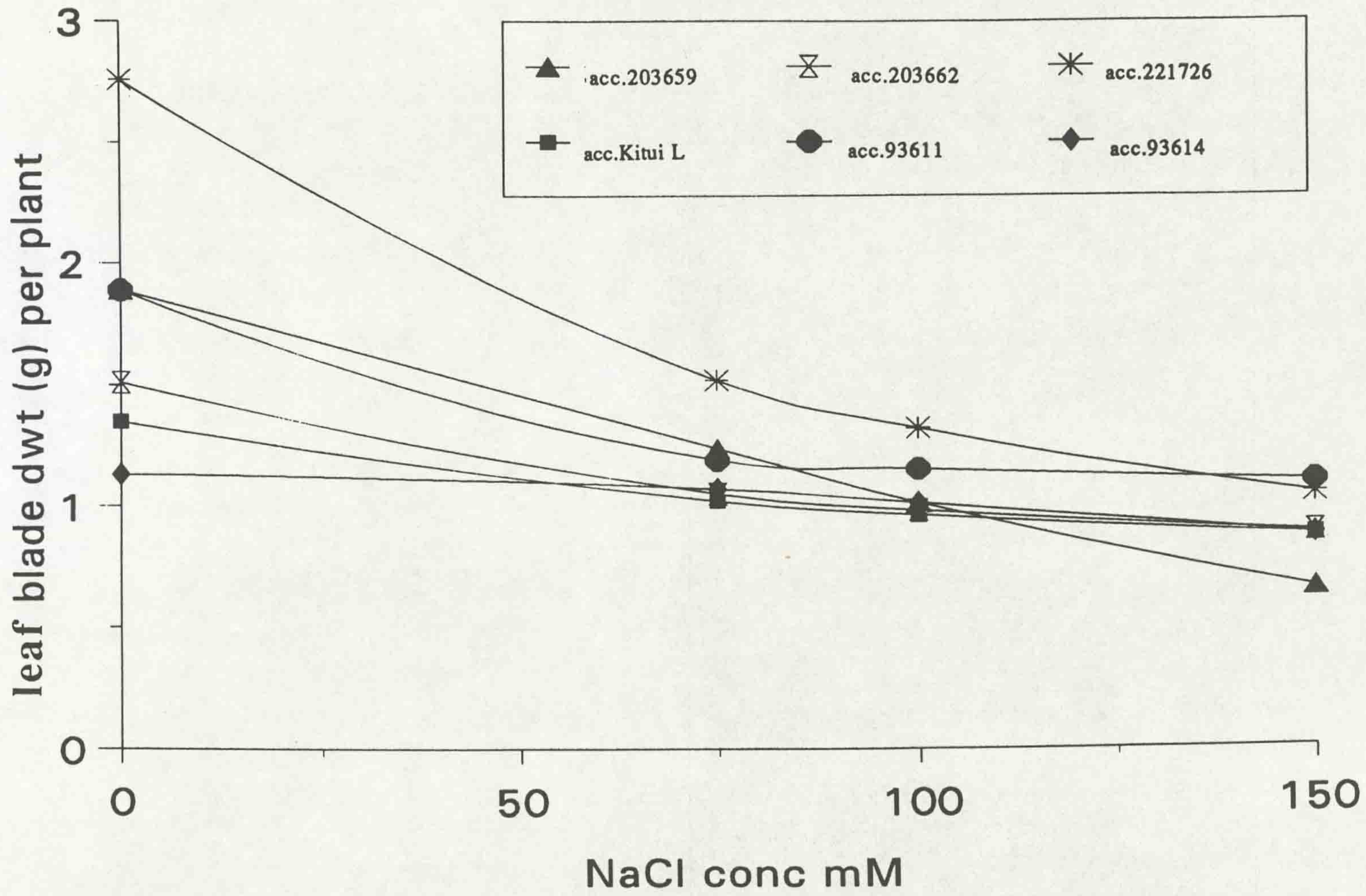


Figure 4.10. Leaf blade dry weight at growth stage 3 as a function of NaCl concentration (fitted curve)



4.3.1.6. Mean leaf sheath dry weight per plant

Table 4.2 and the data presented in Figure 4.6c show that NaCl treatments caused a considerable reduction in leaf sheath dry weight of the accessions ($p<0.001$), and differences between accessions were significant ($p<0.001$). The two-factor interaction (accessions x NaCl solutions) was also significant ($p<0.001$) showing that NaCl treatments significantly reduced leaf sheath dry weight by different degrees in different accessions.

Although accessions differed significantly ($p<0.001$, Table 4.2) in mean leaf sheath dry weight per plant, two of the three salt-tolerant accessions (93611 and 93614) showed similar response at 75, 100 and 150 mM NaCl (Figure 4.6c). However yet again accession 221726 (tolerant) had greater leaf sheath dry weight at 150 mM NaCl, whilst accession 203659 (susceptible) had relatively lower leaf sheath dry weight (Figure 4.6c).

The fitted relationship of mean leaf sheath dry weight per plant and NaCl concentration imposed during each growth stage (Figure 4.9) shows the following: a) accession 93614 was the least affected, b) accession 221726 had relatively greater leaf sheath dry weight at 150 mM NaCl, whilst accession 203659 had the smallest leaf sheath dry weight, and c) accessions 93611 and 93614 were second to accession 221726 in their leaf sheath dry weight at 150 mM NaCl.

4.3.1.7. Mean leaf blade dry weight per plant

Leaf blade dry weights of the accessions differed significantly when averaged across all salinity treatments ($p<0.001$, Table 4.2). Mean leaf blade dry weight of all accessions was significantly reduced ($p<0.001$, Table 4.2) with increasing salinity. The interaction term, accessions x NaCl solutions was significant ($p<0.001$, Table 4.2) suggesting that the degree of reduction in leaf blade dry weights in response to increasing NaCl concentration differed significantly between accessions.

Figure 4.6d plots mean leaf blade dry weight per plant in response to increasing salt concentrations, showing clearly the reduction in weight at 150 mM NaCl. Although leaf blade dry weight differed significantly between accessions ($p<0.001$, Table 4.2) the salt sensitive accessions (203659 and 203662) did not differ from others at all across all NaCl concentrations (Figure 4.6d).

The response function curve (Figure 4.10) shows that the two tolerant accessions 93611 and 221726 had greater leaf blade dry weights at 150 mM NaCl, whilst the salt sensitive accession (203659) had the smallest leaf blade dry weight at 150 mM NaCl. At 100 mM NaCl, 221726 had the highest leaf blade weight, 93611 the intermediate leaf blade weight, and the remaining accessions, tolerant and sensitive, had similar leaf blade weights.

4.3.2. Relative salt tolerance

To evaluate the salinity tolerance of the data for three different plant characters at three different growth stages, and for four other different characters at growth stage 3 accessions have been compared on the basis of yields under saline conditions expressed as a fraction of their yields obtained under non saline condition, a criterion for providing comparable measure of salt tolerance suggested by Maas and Hoffman (1977) and Maas (1985). Relative salt tolerance of the accessions have been subjected to analysis of variance, and the results are presented in Tables 4.3 - 4.

4.3.2.1. Relative plant height

The results obtained from the analysis of variance of relative salt tolerance data (Table 4.3) showed that accessions differed significantly in relative plant height. Increasing NaCl concentrations significantly reduced accessions' relative plant height values ($p<0.001$). The interaction, accessions x growth stages was significant ($p<0.01$) suggesting that accessions relative plant heights differed significantly at different growth stages.

Mean relative plant height data of six accessions is presented in Figure 4.11. The effect of NaCl on relative plant height was moderate at growth stage 1. As plants grew, plant height as percentage of control decreased, with greater effect at higher NaCl concentration. Accession 203659 had the lowest relative plant height values. One of the tolerant accessions (93611) was less affected than the others, and its relative plant height was greater than 80% of controls in each NaCl concentration at each growth stage.

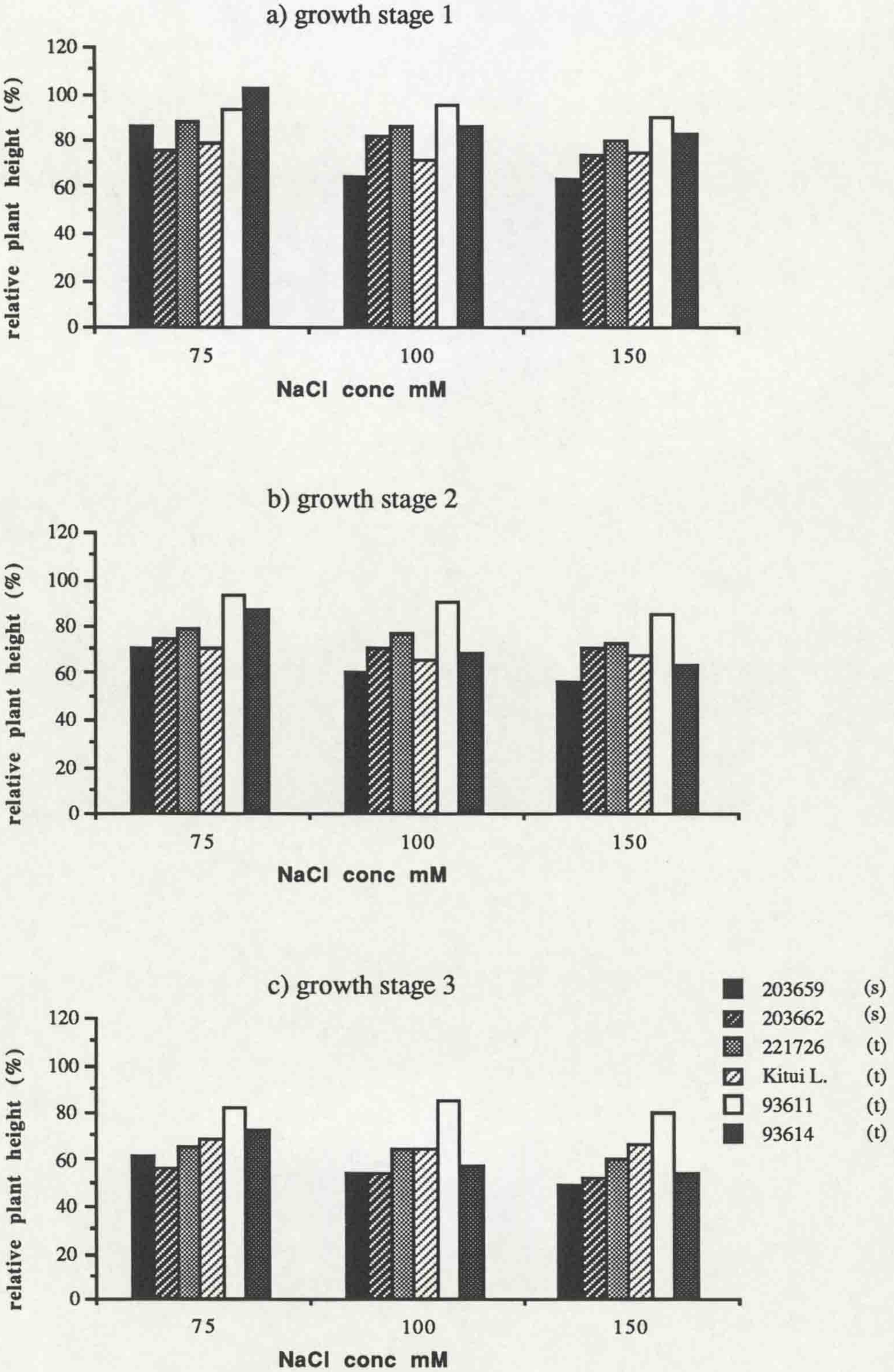
Table 4.3. Mean squares and significances from the analysis of variance of relative values of plant height, number of leaves per plant, and live leaves per plant at three different growth stages

Item	Df	Relative Plant height	Relative number of leaves per plant	Relative percentage live leaves per plant
Blocks	2	563.79***	515.75*	226.14^{NS}
Accessions (Acc)	11	2147.07***	1057.27***	1193.08**
NaCl solutions (Sol)	2	2012.80***	4843.33***	11287.47***
Growth stages (Gst)	2	5935.01***	1797.01***	118530.20***
Acc x Sol	22	136.24^{NS}	141.40^{NS}	866.03*
Acc x Gst	22	166.51**	207.46^{NS}	493.92^{NS}
Sol x Gst	4	16.87^{NS}	310.00^{NS}	4085.07***
Acc x Sol x Gst	44	17.96^{NS}	56.60^{NS}	207.82^{NS}
Residual	214	86.15	138.21	469.24

Table 4.4. Mean squares and significances from the analysis of variance of relative values of dry weights of root, stem, leaf sheath and leaf blade recorded at growth stage 3

Item	Df	Root	Stem	Leaf sheath	Leaf blade
Blocks	2	158.91^{NS}	92.18*	130.20^{NS}	185.31^{NS}
Accessions (Acc)	11	485.74***	752.56***	1162.47***	1705.27***
NaCl solutions (Sol)	2	3026.05***	2490.66***	938.36***	1923.50***
Acc x Sol	22	104.42^{NS}	79.83***	76.81^{NS}	75.97^{NS}
Residual	70	167.93	23.73	87.18	168.73

Figure 4.11. Relative plant height (%) at three different growth stages



4.3.2.2. Mean relative number of leaves per plant

Accessions differed significantly in relative number of leaves ($p<0.001$, Table 4.3). Increasing NaCl concentrations significantly ($p<0.001$, Table 4.3) reduced mean relative number of leaves per plant. Overall mean relative number of leaves was significantly lower at growth stage three ($p<0.001$, Table 4.3). None of the interactions were significant ($p>0.05$, Table 4.3). However there was some suggestion that relative leaf number is reduced to a greater degree at growth stage 3, particularly accession 203662 was affected at 100 mM NaCl (Figure 4.12). On the other hand however, at growth stage 3 accessions 203659 and 203662 (sensitive) were having relative values of 85% and 67% respectively at 150 mM NaCl which were as good as the four tolerant accessions, whilst accession 93614 was unaffected (Figure 4.12).

4.3.2.3. Mean relative percentage live leaves per plant

Differences between accessions in relative number of live leaves was significant ($p<0.01$, Table 4.3). Different NaCl treatments significantly ($p<0.001$, Table 4.3) reduced the relative percentage of live leaves per plant. There was significant ($p<0.001$) reduction in relative percentage live leaves at different growth stages, and the effect of increasing NaCl concentrations was significantly greater ($p<0.001$) at growth stage 3 than at growth stages 1 and 2. Accessions responded differently ($p<0.05$, Table 4.3) to different NaCl solutions.

The negative impact of NaCl was greater at growth stage 3 (Figure 4.13). At growth stage 3, there was marked and significant reduction of relative percentage live leaves per plant in all the accessions in all NaCl concentrations. This was particularly the case at the highest concentration of 150 mM NaCl. The two salt sensitive accessions (203659 and 203662) were the most affected having no live leaves at growth stage 3 at 150 mM NaCl. Accession 221726 (tolerant) was least affected followed by accession 93614 (also tolerant).

4.3.2.4. Mean relative root dry weight per plant

The results of the analysis of variance in Table 4.4 show that accessions

Figure 4.12. Mean relative number of leaves per plant (%) at three different growth stages

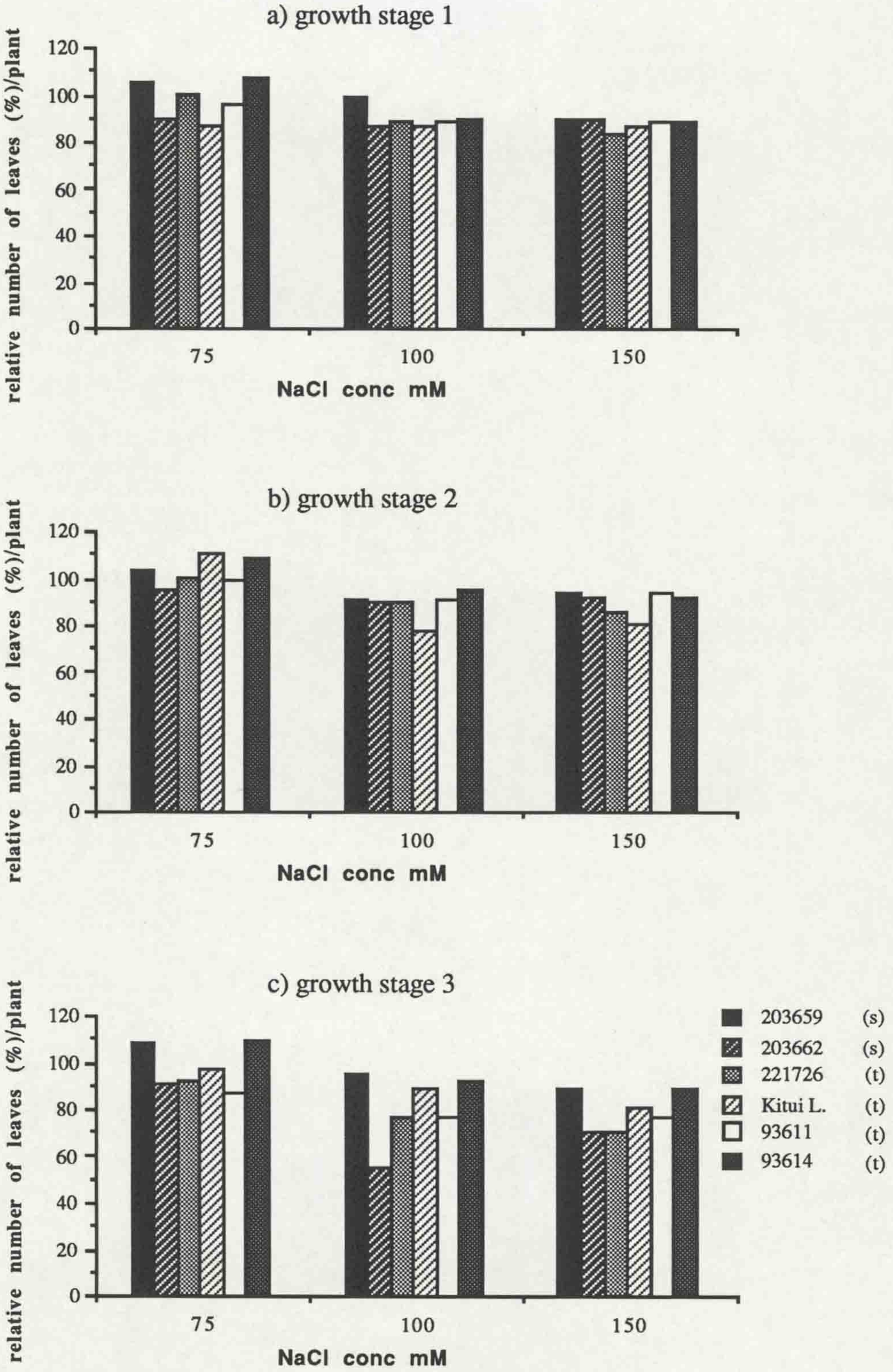
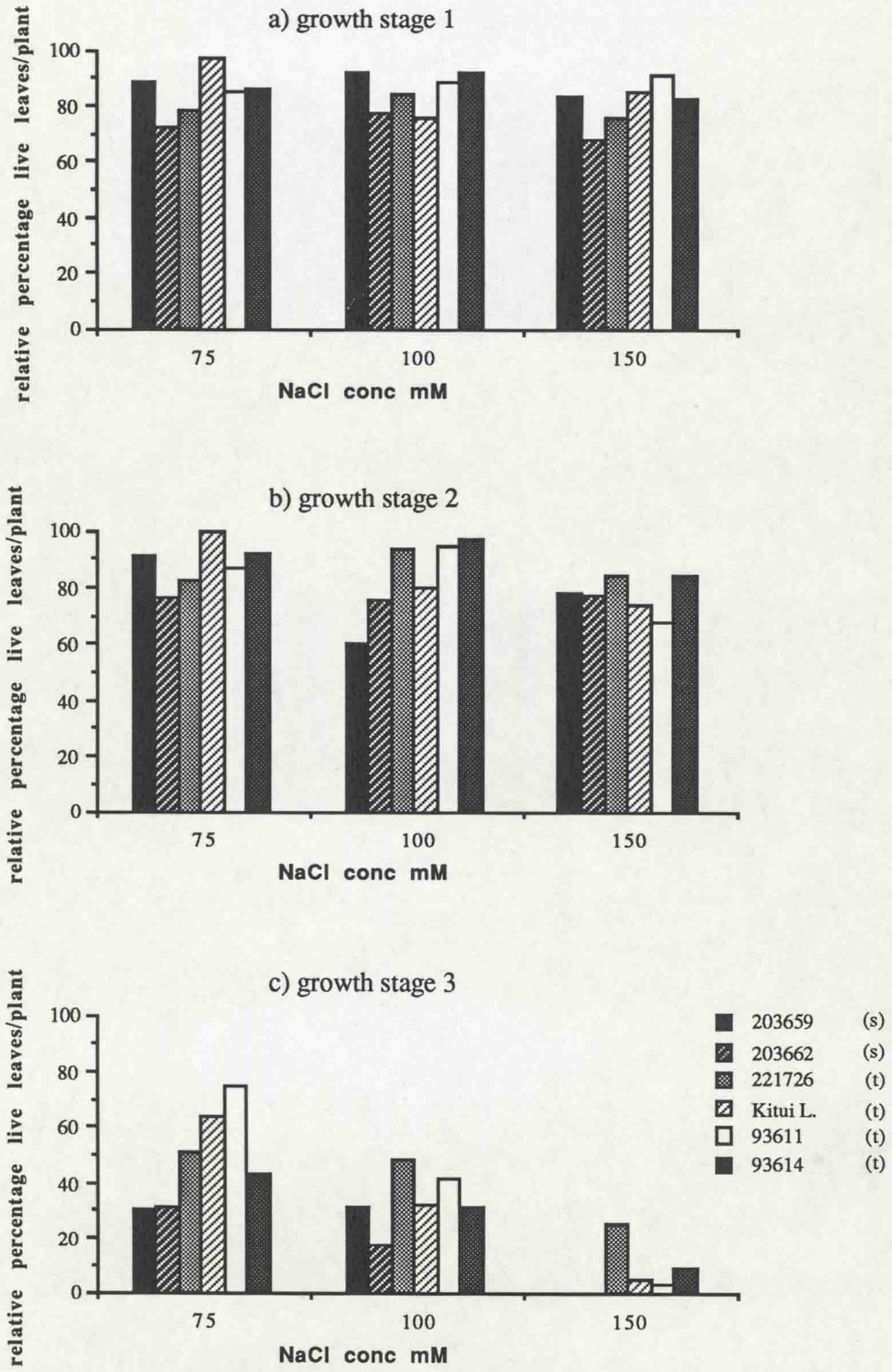


Figure 4.13. Mean relative percentage live leaves (%) per plant at three different growth stages



differed significantly ($p<0.001$) in relative root dry weight. Increasing NaCl concentrations significantly ($p<0.001$) reduced mean relative root dry weight per plant.

Data for mean relative root dry weight per plant for six accessions are presented in Figure 4.14a. Although the interaction term accessions x NaCl concentrations was not significant there appear to be some indication of accession differences in response. Kitui Local (tolerant) had the lowest value across all NaCl concentrations, the susceptible accession 203662 had the highest relative root weight at 150 mM NaCl concentration. Accession 93614 (also tolerant) had relatively greater mean relative root dry weight than the rest of the accessions at 75 and 100 mM NaCl.

4.3.2.5. Mean relative stem dry weight per plant

Table 4.4 shows that accessions differed significantly ($p<0.001$) in relative stem dry weight, and NaCl concentrations significantly ($p<0.001$) reduced relative stem dry weight. The significant ($p<0.001$) interaction term, accessions x NaCl concentrations showed that the twelve accessions responded differently in relative stem dry weight in different NaCl solutions.

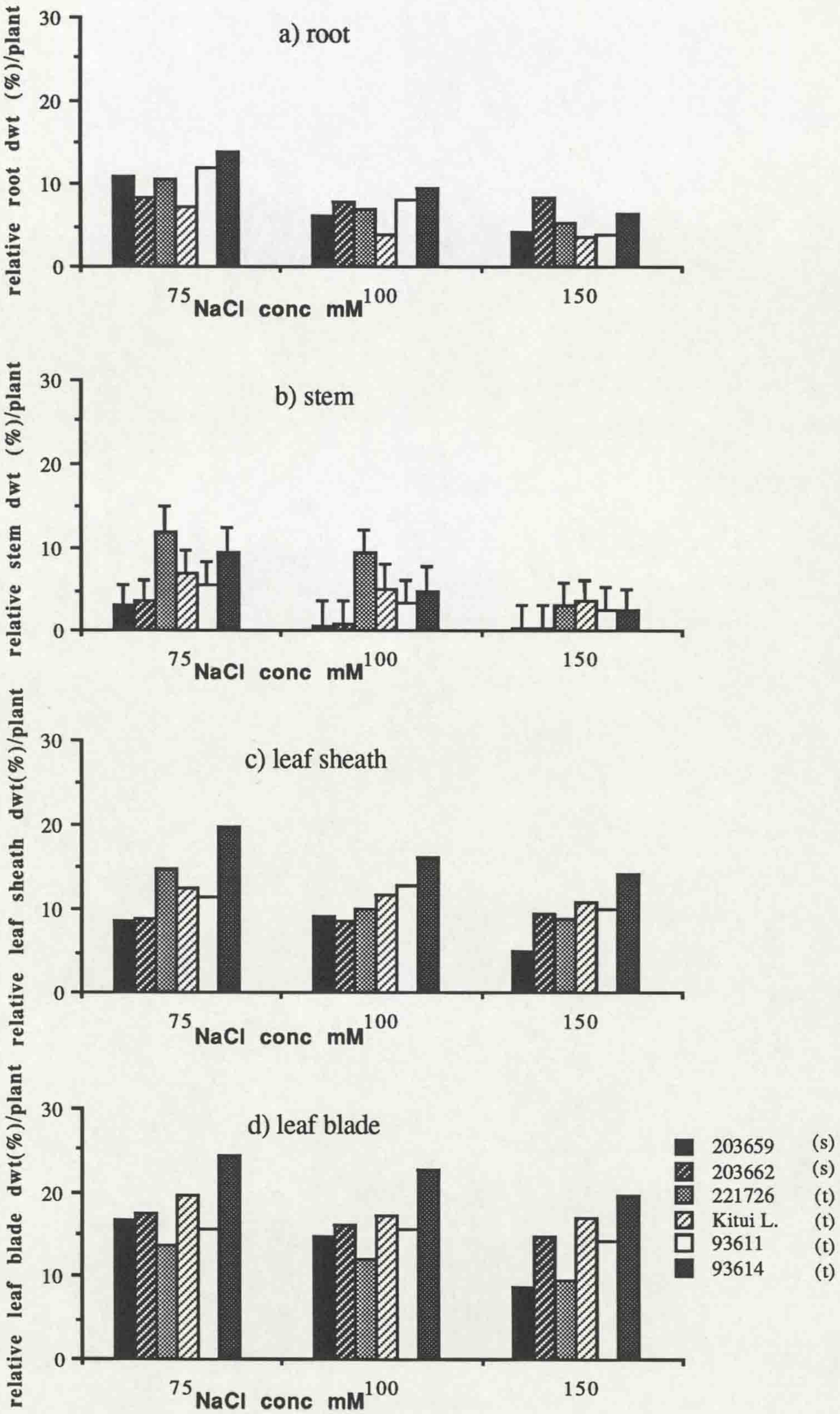
The salt sensitive accessions were clearly separated from the salt-tolerant accessions by this parameter (Figure 4.14b) at all three NaCl concentrations, the two salt sensitive accessions (203659 and 203662) having markedly lower relative stem dry weight than the four salt-tolerant accessions.

4.3.2.6. Mean relative leaf sheath dry weight per plant

Significant difference ($p<0.001$) in relative leaf sheath dry weight was observed between accessions (Table 4.4), and NaCl concentrations significantly reduced mean relative leaf sheath dry weight per plant ($p<0.001$).

Data for mean relative leaf sheath dry weight per plant of six accessions is presented in Figure 4.14c. The ranking of the six accessions did not change in response to increasing salinity, the accession x NaCl solutions interaction being non significant. It is interesting to note however that the tolerant accession 93614 had greater mean relative leaf sheath dry weight across all NaCl concentrations, whereas the sensitive accession 203659 had the lowest leaf sheath dry weight at 150 mM NaCl.

Figure 4.14. Mean relative dry weights (%) of a) root, b) stem (with S.E.), c) leaf sheath, and d) leaf blade per plant at growth stage 3



4.3.2.7. Mean relative leaf blade dry weight per plant

Accessions were significantly different from each other ($p<0.001$, Table 4.4) in relative leaf blade dry weight, and the effect of increasing salinity in reducing relative leaf blade dry weight was also significant ($p<0.001$, Table 4.4). The six accessions did not respond differently to different NaCl concentrations (Figure 4.14d), interaction, accessions x NaCl concentrations was non significant. Once again however the data show that the sensitive accession 203659 had the greatest reduction in relative leaf blade dry weight in response to NaCl increase, whilst the tolerant accessions 93611 and Kitui Local had the least reduction in relative leaf blade dry weight.

4.3. Discussion

The complexities of salinity effects are formidable constraints on the articulation of any specific salt tolerance mechanism. Information is needed about the impact of different levels of salinity at various stages of plant development for different crops. Identifying those growth stages most susceptible and/or not susceptible to salinity will assist the breeder in determining target characters for improvement through selection and breeding. When a specific and readily quantifiable physiological mechanism conferring salt tolerance is not available, assessment of plant material according to the amount of salt injury reflected in partial or complete necrosis, or in measurement of other plant characters of importance, yield of green matter and grain yield, appear to be practical alternative methods (Noble *et al.*, 1984).

Salinity is known to affect plant growth during all developmental stages and crop responses to salinity vary during ontogeny (Maas and Hoffman, 1977; Shannon, 1985; Maas *et al.*, 1986; Maas and Poss., 1989; Azhar and McNeilly 1989). Because of these differences in salinity tolerance during the ontogeny, some studies have been concerned with selecting for tolerance through the complete life cycle of the plant, as with tomato and barley (Epstein *et al.*, 1980). In the present study the data presented describe the salinity tolerance of twelve pearl millet accessions irrigated with nutrient solution containing 75, 100, and 150 mM NaCl applied from the seedling stage to maturity, with measurements taken at growth stage 1, growth stage 2, and growth stage 3 for plant height, number of leaves, and percentage live leaves, and with measurements taken only at growth stage 3 for dry weights of root, stem, leaf sheath,

and leaf blade. Such a procedure would seem to provide a good evaluation of potential salinity tolerance (Poljakoff-Mayber, 1982).

Salt tolerance data have been expressed by some workers using yield as a function of the average salt concentration in the root zone, providing data which generally apply only if salinity is fairly uniform from the seedling stage to maturity (Maas *et al.*, 1986). In the experiment described in this Chapter, salinity was maintained uniform and consistent from the seedling stage to maturity. Plant height seemed to be affected little during the growth stage 1 (vegetative stage). However salt stress during stage 2 reduced stem elongation, but had less effect on plant height than during stage 3. At the third growth stage, salinisation caused a markedly stunted growth, a common feature of salt stressed plants (Gale, 1975), significantly reduced panicle elongation and even panicle differentiation after booting. Thus accession plant height was most sensitive during growth stage 3 and least sensitive during growth stage 1 (Figure 4.2). However it should be noted that sensitivity at different stages was not independent of the previous treatment.

Assessment of relative tolerance of the accessions in relation to control plants yielded non significant effects of various interactions (Tables 4.3 - 4). Thus the relative yield/salinity relationship does not appear to provide a useful measure of plant tolerance. Absolute values permit direct estimations of economic returns under specific salinity conditions independent of control values (Maas, 1985). For this reason absolute values are often preferred in discussion as the criterion for selection (Ashraf and McNeilly, 1992). Use of relative data for mean total number of leaves (live and dead) per plant again has not provided a good comparison of accessions and growth stages. However data for percentage live leaves whether absolute or relative gave good separation of accessions and growth stages, the separations corresponding with the tolerant and non tolerant classification of the accessions at the seedling stage. Salinity caused leaf necrosis throughout the whole course of plant development (Figures 4.4 and 4.13), but the greatest leaf senescence and necrosis was recorded at growth stage 3 (Figures 4.13). Salinisation through the whole sequence of plant development had a much greater impact on the number of live leaves than on the total number of leaves per plant. This impact on percentage live leaves increased with plant age, all twelve pearl millet accessions examined being most sensitive to NaCl at growth stage 3 whereas at

growth stages 1 and 2 only small reductions in live leaf numbers were recorded with increasing NaCl concentrations (Figure 4.13). This is in agreement with the findings of Maas *et al*. (1986) on sorghum. In contrast, however, studies with maize (Maas *et al*., 1983), wheat (Maas and Poss, 1989) and sorghum (Azhar and McNeilly, 1989) showed that growth stage 1 was the most sensitive to salinity, whilst Yoshida (1967) and Akbar *et al*. (1972) showed that growth stage 2 was the most critical in rice. The present results do not fit in either of the two groups. However, it is partially in agreement with the findings of Azhar and McNeilly (1989) where two accessions, Double TX and Giza 114, which were comparatively tolerant at growth stage 1 were more sensitive at growth stage 3 suggesting variability in tolerance mechanism within this species. Paralleling these from the absolute percentage live leaves data presented in Appendix 4.1c, accession 203656, which showed moderately tolerant response with respect to this character at growth stages 1 and 2, became more sensitive at growth stage 3.

As salt concentrations increase above a threshold level, which will vary with the species, there is a progressive decrease in growth rate and final plant size (Maas and Hoffman, 1977). The reduction in dry weights of root, stem, leaf sheath, and leaf blade was almost linear in the data presented here, and was a consequence of reduced total plant biomass, comparing plant height, number of leaves, and percentage live leaves. Loss of leaf area for photosynthesis, and eventual leaf death in response to salinity is clearly disadvantageous for assimilate production as the photosynthetic area is reduced to such an extent that carbohydrate supply becomes a limiting factor in plant growth (Munns and Termaat, 1986).

The reduction in plant tissues and dry matter weights due to salinity in the accessions studied here may be attributable to three phenomena (Poljakoff-Mayber, 1982). Firstly, osmotic effects resulting from soil salinity may cause disturbances in the water balance leading to growth inhibition either directly, or through other processes, such as stomatal closure and reduction in photosynthesis. Secondly, toxic effects caused by specific ions which affect metabolism. Thirdly, solute accumulation which may induce an internal imbalance in plant nutrition. Shannon (1984) stated that salinity causes a combination of osmotic and ionic effects in plants. The osmotic effects interfere with the plant's ability to extract water from the soil and maintain an internal

water balance, whereas the ionic effects may interfere with solute balance in the cytoplasm, or in some cases, disturb membrane function and cause specific ion toxicities.

The results of the experiment described here showed that the accessions were able at least to some degree to respond to external concentration of NaCl in order to limit its damaging effect. Normally salt accumulation in shoots occurs through the transpiration process, and therefore tends to be highest in mature leaves as they have larger leaf areas (Greenway and Munns, 1980). Total death of leaves was observed in the two salt sensitive accessions (203659 and 203662) in 150 mM NaCl at growth stage 3. When salinity was imposed throughout the whole plant development, Munns and Termaat (1986) showed that prolonged transpiration brings large amounts of Na^+ and Cl^- ions into the shoot, especially into the old leaves, thus killing them and they suggested that this process must eventually limit the supply of assimilates to the growing regions and may be the main factor determining yield .

Variation in whole plant reaction to salinity might provide best means of selection for salinity tolerance. While based on somewhat limited experimental data, no single accession was however found to be consistently superior across growth stages. This suggests that salt tolerance may be under separate genetic control at each of the developmental stages, as suggested by Jones and Qualset (1984). Most importantly the responses of the accessions examined in this Chapter appeared to be in great part consistent with those observed when performance was assessed after two weeks growth in solution culture (Chapter 2). It was clear in Chapter 2 that based upon mean overall values of C_t, C_0, and C_{50} pearl millet accession 221726 was relatively tolerant and therefore a good potential target from which selection for enhancement of salt tolerance would seem to be worthwhile. In this sand culture experiment accession 221726 was again the most tolerant accession based on six whole plant measurements namely plant height, percentage live leaves, and root, stem, leaf sheath and leaf blade dry weights. Again, based on two-week-old seedlings growth data, accessions 203659 and 203662 were shown to be among the most salt sensitive accessions ranking III in C_t, C_0, and C_{50} (Table 2.4 in Chapter 2). On the basis of plant height, percentage live leaves, and dry weights of root, stem, leaf sheath, and leaf blade data these two

accessions were shown to be salt sensitive. This information would seem to be of value for future work in improving salinity tolerance in pearl millet through selection, in that it suggests strongly that selection based upon the growth of two-week-old seedlings in solution culture is likely to provide a good correlation with performance of the adult plant under saline conditions. Even used as an initial screening procedure much time and effort in effecting that screening would be saved.

THE PHYSIOLOGY OF SALT TOLERANCE IN PENNISETUM AMERICANUM (L.) LEEKE

CHAPTER FIVE

CHAPTER 5

THE PHYSIOLOGY OF SALT TOLERANCE IN PENNISETUM AMERICANUM (L.) LEEKE

5.1. Introduction

Two major reasons have been suggested for salt sensitivity in nonhalophytes (Greenway and Munns, 1980). Firstly, the inability of cells to osmoregulate which may result from either an insufficient uptake of ions, and secondly, the ability of toxic ions to interfere with physiological and biochemical processes of the organism.

Amino acids and amine accumulation may occur not only as a result of salinity *per se*, but also under conditions of water stress in higher plants and the phenomenon is also well known in animal cells. Strogonov (1970) took the view that changes in nitrogen metabolism under saline conditions were indicative of salt injury, and suggested that necrosis caused by salt poisoning is normally accompanied by an increased amino acid content. According to Stewart and Lee (1974) proline functions as a compatible solute in the important role of balancing cytoplasmic and vacuolar water potentials. Support for this view came from the work of Treichel (1975) who showed that the amount of proline present in plant tissues was dependent on the degree of osmotic stress, and was positively correlated with the amount of Na^+ and Cl^- in the plant sap. Wyn Jones *et al.* (1976) suggested that in some species where proline accumulation is not correlated with the external salt concentration, betaine, choline, or other quaternary ammonium compounds are accumulated. In wheat grass (*Agropyrum desertorum*) proline increased in response to salinity increase, but no differences were observed in proline amounts between salt-tolerant and salt sensitive lines (Shannon, 1978). In another study Tal *et al.* (1979) found that salt and drought tolerant species of *Lycopersicon peruvianum* accumulated less proline than the cultivated tomato (*L. esculentum*) under both salt and water stress. They suggested, therefore, that the accumulation of proline does not play an important role in the stress tolerance of the wild tomato.

Previous studies in halophytes have demonstrated that sucrose is accumulated rather than glucose in response to salinity (Briens and Larher, 1982). It was suggested that this organic solute may stabilise enzymes against high temperature and salts (Paleg

et al., 1981) and function as a protein stabiliser (Wyn Jones, 1984). Shannon (1978) found that sugar content of wheat grass did in fact increase at higher salinity but sensitive and tolerant lines did not differ. By contrast, Rathert (1984) in comparing sucrose and starch found that under salt treatment, sucrose content increased considerably in bush beans, slightly in rice, but decreased in soybeans and cotton. The starch content of leaves increased in sensitive bush bean, increased less in moderately sensitive rice, increased little in soybean and actually decreased in leaves of the relatively tolerant cotton.

Polyols and their close derivatives are also considered as important organic cytosolutes (Wyn Jones, 1984). Nonetheless, whilst the roles of soluble carbohydrates and amino acids are well established as solutes for osmoregulation in halophytic higher plants (Briens and Larher, 1982), it is still not clear whether the same solutes are involved in osmoregulation in glycophytes.

The mechanism of tolerance to salinity in nonhalophytes has not been extensively reviewed. In nonhalophytes tolerance to salinity is commonly correlated with ability to restrict the entry of ions to shoots or to an ability to exclude entry of excess amounts of specific ions (Greenway and Munns, 1980). This has been observed by Abel and Mackenzie (1964) in a salt sensitive cultivar of soybean which accumulated more Cl^- in its shoot than a tolerant cultivar. Later, Abel (1969) showed that the capacity for inclusion or exclusion of Cl^- in the leaves of these soybean varieties was inherited and controlled by a single gene pair. Läuchli and Wieneke (1979) in an investigation with two soybean cultivars confirmed these findings of Abel and Mackenzie (1964), but also showed that the salt sensitive cultivars accumulated both Cl^- and Na^+ in the leaf to such a degree that it caused leaf injury. Similarly, Winter and Läuchli (1982) reported that in the relatively salt-tolerant *Trifolium alexandrinum* (L.) there was less Na^+ and Cl^- in the leaves than in the more sensitive *T. pratense* (L.). This general principle of avoidance appears to be essential to glycophytes which as a group are unable to accumulate concentrations of inorganic ions sufficient for osmotic adjustment, although there are considerable differences within them as a group. Lessani and Marschner (1978) found no correlation between the extent of Cl^- translocation and

growth depression caused by salinity for several different species which differ in salt tolerance. A similar finding was reported for wheat by Kingsbury *et al.* (1984) who found no differences between sensitive and tolerant lines in Cl^- accumulation.

Two requirements for a plant to survive in a saline habitat are osmotic adaptation and functional metabolism. In the case of NaCl salinity, this pertains particularly to uptake of K^+ (Jeschke, 1984). Since active Na^+ efflux is considered a universal property of plant roots, K^+-dependent Na^+ extrusion should be found in salt-tolerant species (Jeschke, 1984). *Hordeum distichon*, which is considered as one of the most salt-tolerant crop species, and *Triticum aestivum*, which is considered as a moderately salt-tolerant crop species showed a remarkably efficient K^+–Na^+ exchange system when grown under NaCl stress conditions (Maas and Hoffmann, 1977). The *Triticale* cultivar, GLT 176, on the other hand, was an efficient salt excluder (Wyn Jones, 1984). Salt excluders possess mechanisms that ensure that salt reaches the shoot only in very small amounts, and this might be due to a very efficient selectivity towards K^+ during absorption (Albert and Popp, 1977), and/or another possibility is that Na^+ is absorbed in significant amounts but is reabsorbed from the xylem sap in proximal parts of the root (Jacoby and Ratner, 1973) and is then either stored or retranslocated (Winter, 1982b). However, the salt-tolerant *Atriplex hortensis* showed comparatively low K^+-Na^+ exchange and was similar in this respect to the salt-sensitive species onion (Maas and Hoffmann, 1977).

The objective of this section of this study is to examine the effect of salinity on the content of organic and inorganic metabolites in roots and shoots of 14-day-old seedlings of pearl millet accessions of different salt sensitivities, and thus to provide preliminary information about a possible mechanism of tolerance in that species. The approach was to examine the physiological responses to salinity in salt-tolerant and salt sensitive accessions of the same species identified during an earlier study, 93611 and 93614, both tolerant (Ashraf and McNeilly, 1992) and 203662 identified as susceptible in Chapter 2 of this thesis, in the supposition that the observed differences in the physiological response to salt are more likely to be related to the salt tolerance

phenomenon than are differences between different species.

5.2. Materials and methods

Three accessions of *Pennisetum americanum* (L.) Leeke were used in the experiment.

Accessions 93611 and 93614 which were considered as tolerant, and 203662 which was sensitive to NaCl, were used in this experiment. Four NaCl concentrations, 0 (control), 75, 100 and 125 mM, were used. Twenty surface sterilised seeds of each accession were sown on rafts of black alkathene beads, three layers deep, floating on 1/10 strength Rorison nutrient solution in 300 cm^3 plastic beakers as used in Chapter 2. Three replicates per accession in each NaCl treatment were placed in a completely randomised design, and the experiment was carried out under the same controlled environmental conditions as used in Chapter 2.

After 14 days, roots and shoots from the seedlings of each replicate were harvested separately.

5.2.1. Measurement of metabolites

5.2.1.1. Amino acids

Fresh root and shoot material of each replicate was weighed, cut into small pieces and placed separately in test tubes. 20 cm^3 of 80% (v/v) ethanol was added to each test tube containing the material and heated at 60°C for 30 minutes. The extract was filtered and the volume made up to 25 cm^3 with 80% (v/v) ethanol.

The colorimetric method of Rosen (1957) was used to measure the total amino acids. Ninhydrin reagent (Sigma Chem. Co. 20g dm^{-3} hydrindantin 75% (v/v) dimethylsulfoxide, mol dm^3 lithium acetate at pH 5.2) was diluted in the ratio 1 part reagent to 4 parts double distilled water. 0.5 cm^3 ninhydrin reagent was added to 1 cm^3 of aqueous extract, mixed, and incubated in a water bath at 100°C for 15 minutes. Samples were cooled, diluted with 10 cm^3 of 50% (v/v) n-propanol, mixed and left for 15 minutes for colour development. Total amino acids were measured using a Linear

Readout Grating Spectrophotometer at 570 nm.

Glutamic acid at 0, 0.5, 1.0, 1.5, 2.0 and 2.5 mM was used as standard solution for calibration.

5.2.1.2. Proline

Samples were extracted as for total amino acids. The method adopted by Troll and Lindsley (1955) was used to quantify proline content. Acid ninhydrin reagent was made up by dissolving 1.259 g ninhydrin in 30 cm^3 of glacial acetic acid and 20 cm^3 6M orthophosphoric acid. 2 cm^3 acid ninhydrin reagent was added to 2 cm^3 of the aqueous extract and incubated in a water bath at 95°C for 1 hour. Samples were cooled, and 2 cm^3 of toluene was added to each replicate and left until aqueous and toluene layers separated. The toluene layer was removed carefully and the proline concentration determined at an absorbance of 518 nm.

Proline standard solutions were made up from *l*-proline (Sigma Chemicals) at concentrations of 0, 0.01, 0.05, 0.12, 0.30 and 0.40 mM.

5.2.1.3. Polyols

Samples were again extracted as for total amino acids.The colorimetric method of Bok and Demain (1977) was used for determination of polyol content. The reagents used were (1) Nash reagent, (2) 0.015M sodium periodate and (3) 0.1% (w/v) *l*-rhamnose. Nash reagent was prepared by dissolving 7.5 g of ammonium acetate in 10 cm^3 glacial acetic acid and 10 cm^3 acetylacetone. 0.015M sodium periodate was prepared by dissolving 0.1604 g of sodium periodate in 50 cm^3 of 0.12M HCl. 0.1% (w/v) *l*-rhamnose was prepared by dissolving 0.05 g of *l*-rhamnose in 50 cm^3 of deionised distilled water.

0.2 cm^3 of 0.015 M sodium periodate was added to 0.2 cm^3 of the aqueous extract, mixed, and left to stand for 10 minutes at room temperature. 0.4 cm^3 of 0.1% (w/v) *l*-rhamnose was added and mixed; finally 0.8 cm^3 of Nash reagent was added

and the samples incubated in a water bath at 53°C for 15 minutes, cooled and absorbance read at 412 nm.

Mannitol was used as a standard solution at concentrations of 0, 0.1, 0.2, 0.4, 0.8 and 1.2 mM.

5.2.1.4. Water soluble carbohydrates

These samples were again extracted as for amino acids. Carbohydrates were quantified using the method of Plummer (1987). Anthrone reagent was prepared by dissolving 1g anthrone carefully in 500 cm^3 concentrated sulphuric acid. 0.1 cm^3 of aqueous extract was diluted with 0.9 cm^3 80% (v/v) ethanol and 4 cm^3 anthrone reagent was added drop by drop and the contents mixed. The test tubes and their contents were incubated with a marble on top in a water bath at 95° - 100°C for 10 minutes. Tubes were cooled and water soluble carbohydrates measured at an absorbance of 620 nm.

Sucrose at 0, 0.1, 0.3, 0.5, 0.7 and 1.0 mM was used as a standard solution.

5.2.1.5. Sodium (Na^+), Potassium (K^+) and Chloride (Cl^-)

Fresh roots and shoots harvested from 0, 75, 100 and 125 mM NaCl were subjected to a quick rinse in calcium nitrate solution of 10, 50, 70 and 85 mM concentration respectively in order to wash (out) other ions from the surface of the plant tissues, and subsequently oven dried at 50°C for 5 days. 20 mg sub-samples of the dried material from each replicate were placed in test tubes and 2 cm^3 of concentrated nitric acid was added. After digestion was completed on a hot plate at 70°C, samples were filtered, and the volume of each was made up to 10 cm^3 with deionised distilled water. Na^+ was measured at 589 nm, and K^+ at 766 nm by flame emission.

For Cl^- analysis, 10 cm^3 of double distilled deionised water was added to 20 mg of each dried sample. Digestion was carried on a hot plate maintained at 70° - 80°C for 1 hour. The samples were then cooled and filtered. The volume of each sample was

made up to 10 cm^3 with double distilled water. Cl^- was determined using CMT Chloride Titrator (Radiometer).

Data for tissue organic and inorganic cation contents were subjected to analysis of variance.

5.3. Results

5.3.1. Organic solutes

Analysis of variance of the data for amino acids, proline, polyols and water soluble carbohydrates in root and shoot fractions are given in Table 5.1, and the mean values for those data are presented in Figures 5.1 - 4.

5.3.1.1. Amino acids

There were no significant differences between the three accessions in the accumulation of amino acids in the shoots ($p>0.05$, Table 5.1b). However there were significant differences between accessions in amino acid content of the roots ($p<0.001$, Table 5.1a). Amino acid concentration increased significantly in both shoots and roots ($p<0.001$, Table 5.1a, b) with increasing NaCl concentration in the growth medium (Figure 5.1a, b). The interaction term, accessions x NaCl concentrations was non-significant for root data ($p>0.05$, Table 5.1a), but was significant for shoot data ($p<0.05$, Table 5.1b). This indicates concentrations of amino acids in shoots of the three accessions increased significantly with increased salinity, but was greater in the tolerant accessions 93611 and 93614 at 125 mM NaCl than the sensitive accession (Figure 5.1b).

Table 5.1. Mean squares and significances from the analysis of variance of concentrations of amino acids, proline, polyols and water soluble carbohydrates in roots and Shoots of 14-day-old seedlings grown at four NaCl levels

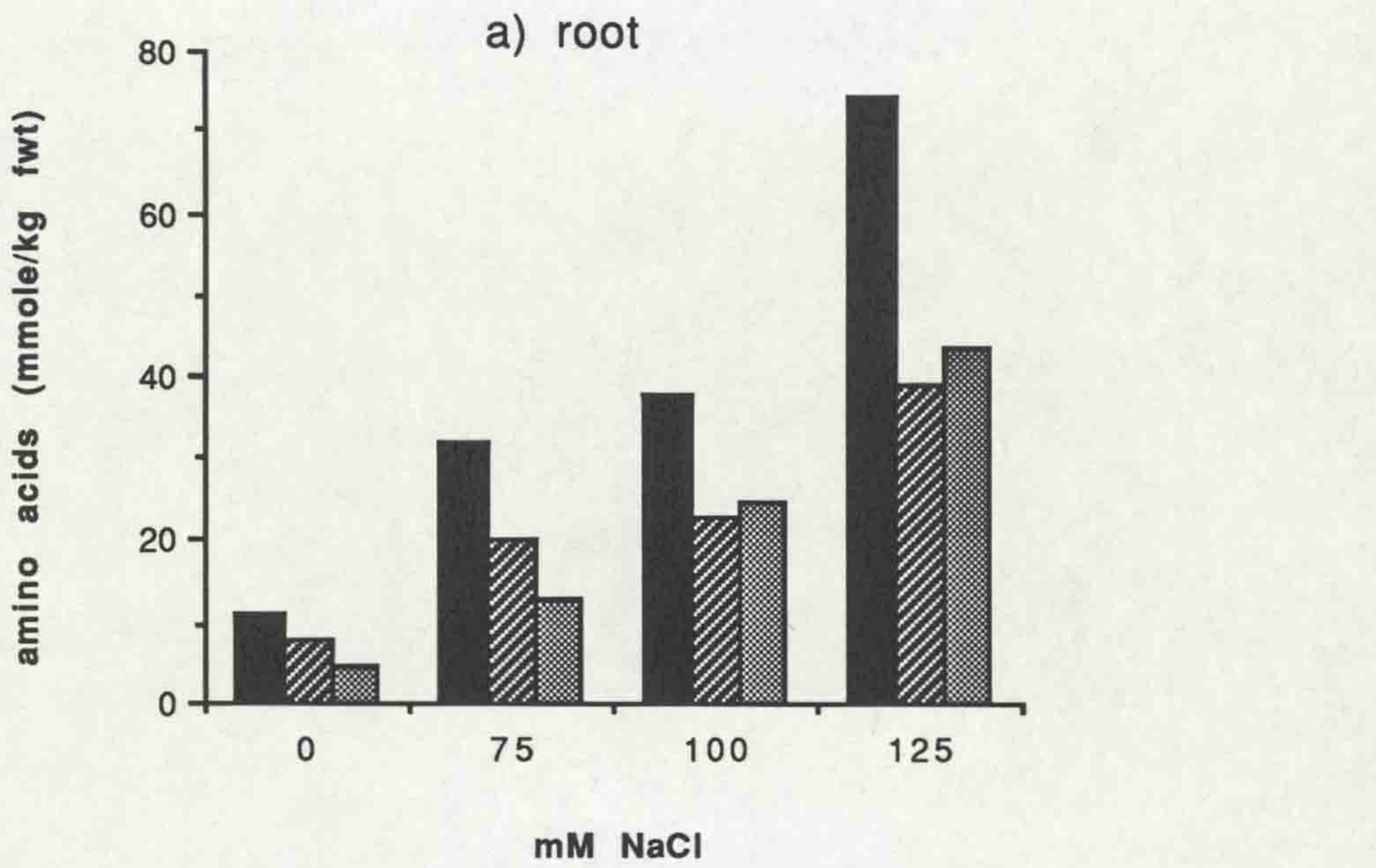
a) Root

Item	Df	Amino acids	Proline	Polyols	Carbohydrates
Blocks	2	93.5^{NS}	172.9^{NS}	71.6^{NS}	120.2^{NS}
Accessions	2	1169.2***	456.5**	169.2*	1708.8**
NaCl (Sol)	3	3117.9***	640.8**	1017.3***	5448.5***
Acc x Sol	6	162.5^{NS}	110.4^{NS}	245.2^{NS}	355.4^{NS}
Residual	22	118.08	84.31	34.38	233.26

b) Shoot

Item	Df	Amino acids	Proline	Polyols	Carbohydrates
Blocks	2	7.01^{NS}	415.6^{NS}	26.5^{NS}	1326.3^{NS}
Accessions	2	145.9^{NS}	458.1^{NS}	205.5*	890.7^{NS}
NaCl (Sol)	3	4666.1***	1567.7***	289.23**	14101.0***
Acc x Sol	6	255.51*	16.36^{NS}	10.34^{NS}	308.6^{NS}
Residual	22	73.33	168.9	41.81	495.2

Figure 5.1. Accumulation of amino acid in roots and in shoots (with LSD 5%) of 14-day-old seedlings grown at four NaCl levels



5.3.1.2. Proline

Accessions differed significantly in the amounts of proline accumulated in their roots ($p<0.01$, Table 5.2a), the sensitive accession 203662 accumulating approximately half the amount of the tolerant accessions 93611 and 93614. Differences between shoot proline contents, however, were not significant. Nonetheless proline levels increased both in the roots and shoots ($p<0.01$; Table 5.1a, b; Figure 5.2a, b) in all the accessions with increasing treatment salinity. Proline concentrations in both roots and shoots of the three accessions increased in parallel with increasing NaCl concentrations, the interaction term being non significant (Table 5a, b).

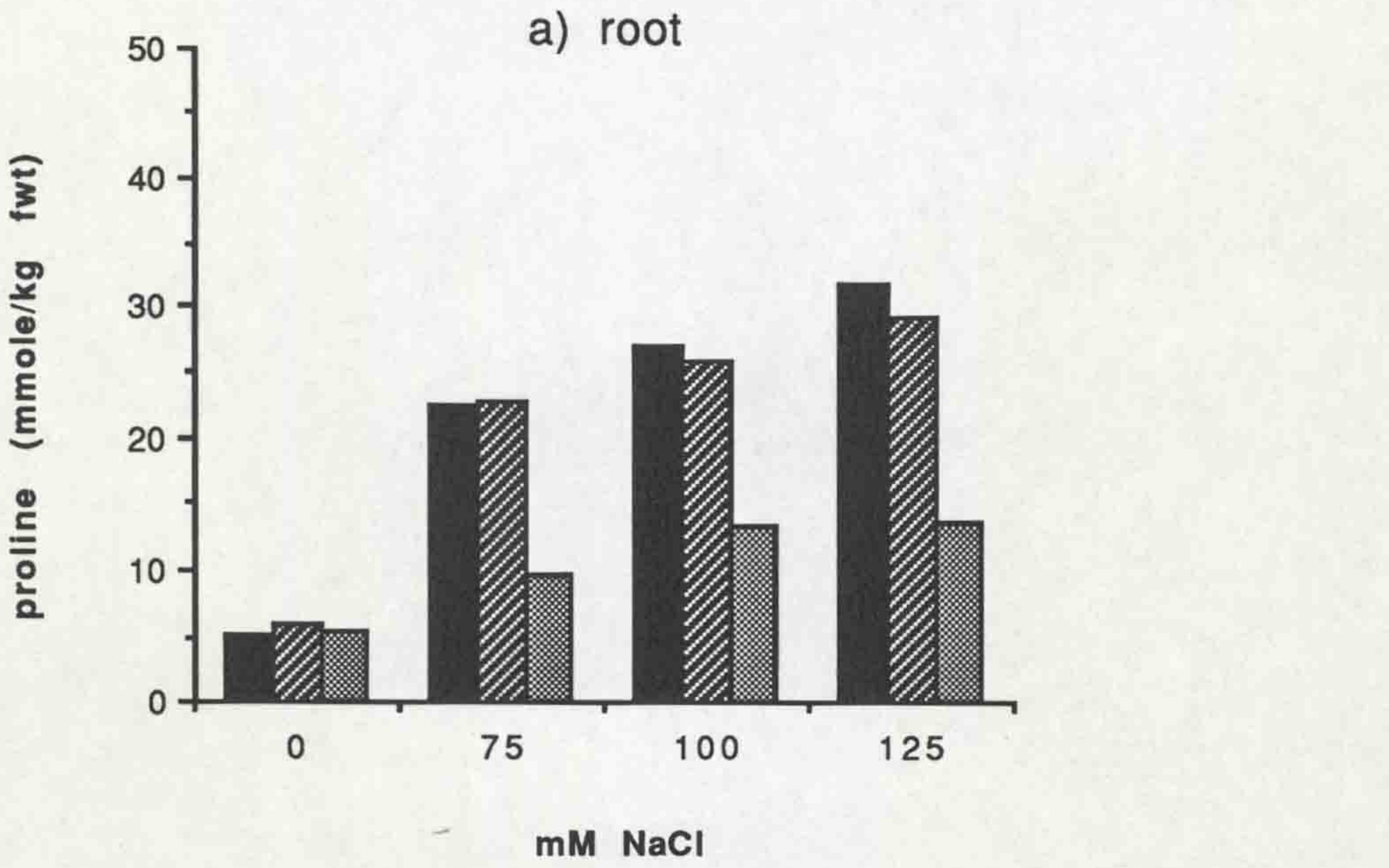
5.3.1.3. Polyols

The accessions were significantly different in accumulation of polyols in both roots and shoots ($p<0.05$, Tables 5.1a, b), the susceptible accession 203662 having lower polyol concentrations than in the tolerant accessions 93611 and 93614 (Figure 5.3a, b). The concentration of polyols in the roots of both the tolerant (93611 and 93614) and susceptible (203662) accessions increased significantly with increase in solution salinity ($p<0.001$, Table 5.1a, Figure 5.3a). This was also observed in the shoots ($p<0.01$, Table 5.1b, Figure 5.3b). None of the interaction factors was significant ($p>0.05$, Table 5.1a, b), indicating no significant difference between accessions in polyol concentrations in roots or shoots in the four salt treatments.

5.3.1.4. Water soluble carbohydrates

Accessions differed significantly in the amount of water soluble carbohydrate accumulated in their roots ($p<0.01$, Table 5.1a). However differences were not significant in the shoots ($p>0.05$, Table 1b). In general, there was a considerable increase in water soluble carbohydrates in both roots and shoots of the accessions with increased salinity concentration ($p<0.001$, Table 5.1a, b; Figure 5.4a, b). A relatively greater proportion of water soluble carbohydrate was retained in roots (Figure 5.1a). However, there were similar patterns of increase in water soluble carbohydrate in the three accessions at four NaCl level, (accessions x NaCl solutions was non significant).

Figure 5.2. Accumulation of proline in roots and shoots of 14-day-old seedlings grown at four NaCl levels



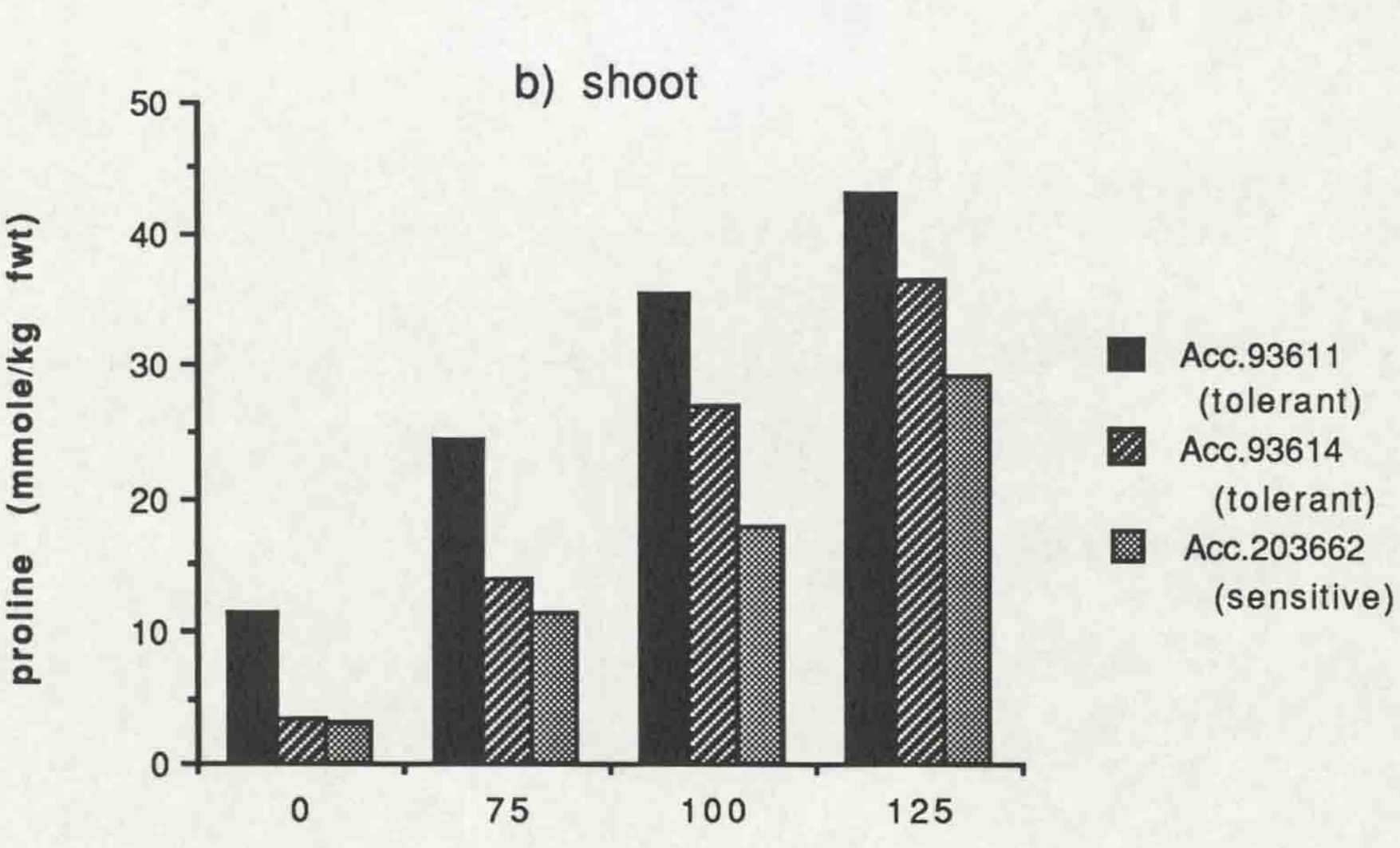
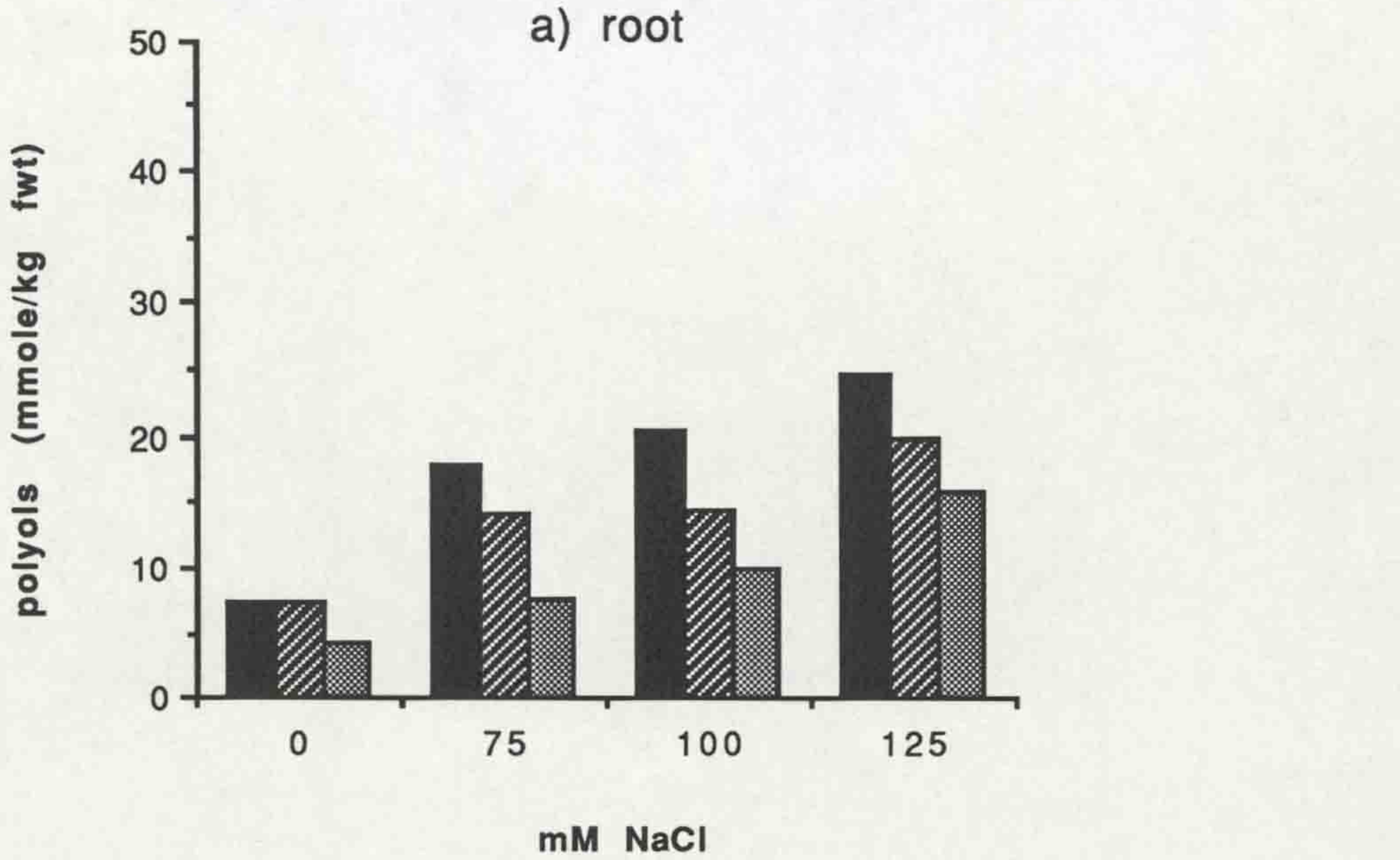


Figure 5.3. Accumulation of polyols in roots and shoots of 14-day-old seedlings grown at four NaCl levels



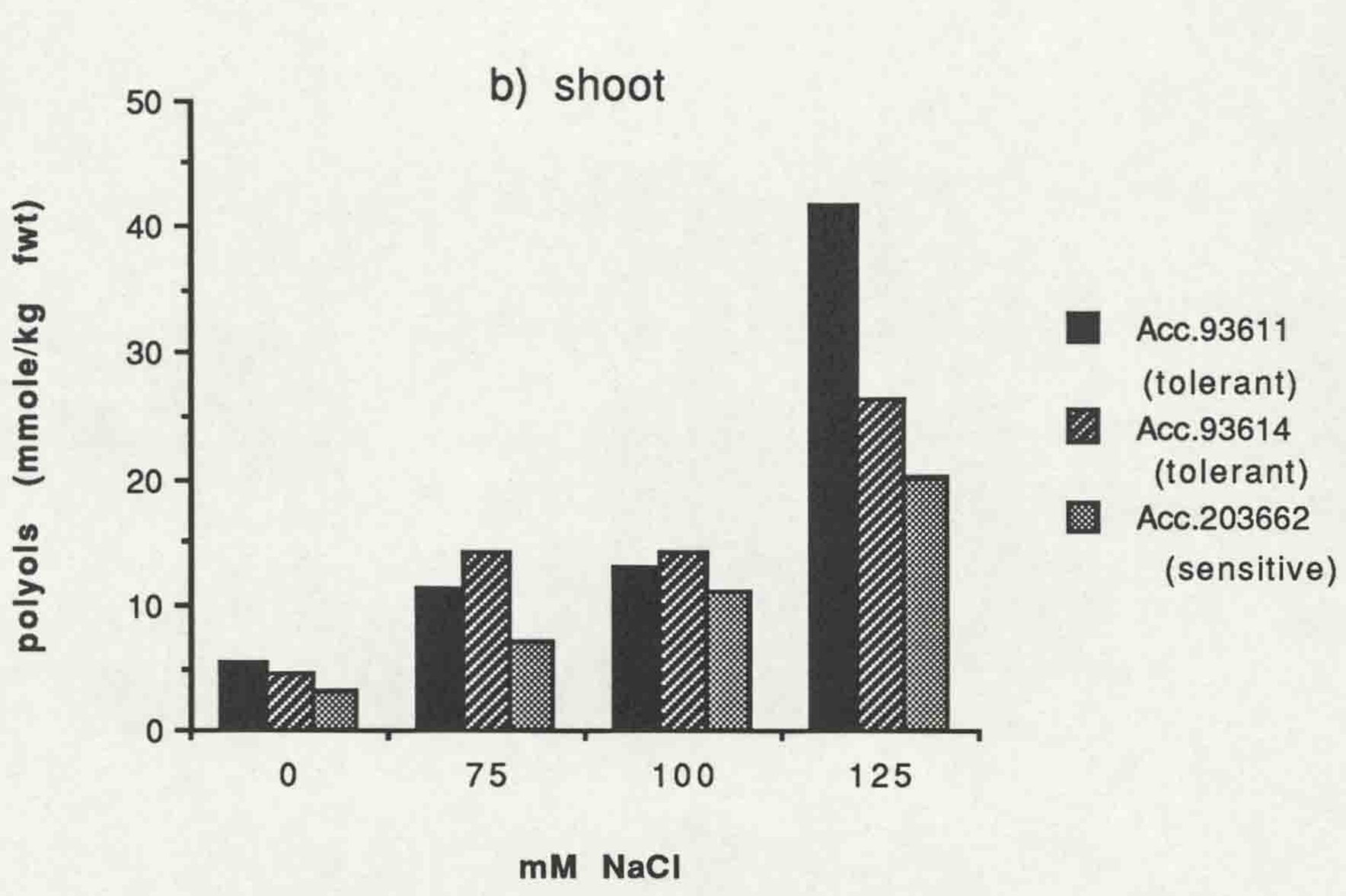
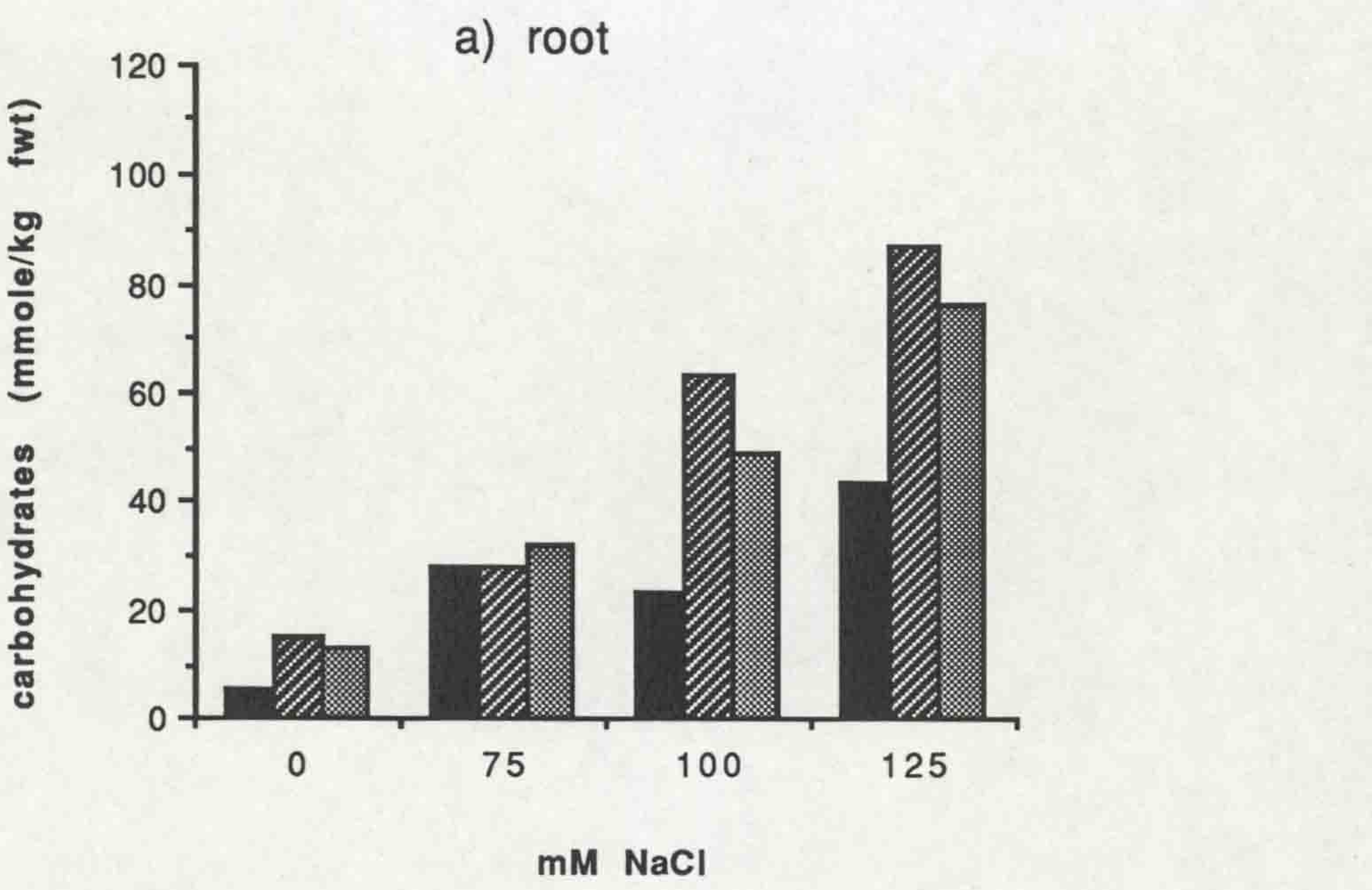


Figure 5.4. Accumulation of water soluble carbohydrates in roots and shoots of 14-day-old seedlings grown at four NaCl levels



5.3.2. Inorganic ions

5.3.2.1. Sodium (Na^+)

Root tissues of all the three accessions showed a marked increase in Na^+ levels with increasing NaCl concentrations ($p<0.001$, Table 5.2a, Figure 5.5a) although the accessions were not significantly different from each other in Na^+ accumulation ($p>0.05$, Table 5.2a). In shoot tissues a sharp increase in Na^+ levels occurred with increasing NaCl concentration for all the three accessions (Figure 5.5b). Accessions were significantly different from each other in their degree of accumulation of Na^+ ($p<0.05$, Table 5.2b), the susceptible accession (203662) accumulating relatively higher levels of Na^+ in shoots (Figure 5.5b) than the two tolerant accessions (93611 and 93614). The two tolerant accessions had significantly higher Na^+ contents at the higher NaCl concentrations in their shoots, whereas in the control, the sensitive accession 203662 had the lowest Na^+ content. Differences in root Na^+ were found only in response to increasing NaCl concentrations in the growth media.

5.3.2.2. Potassium (K^+)

Increasing NaCl concentrations caused a progressive reduction in the levels of K^+ in both roots and shoots (Figure 5.6a, b) and accessions differed significantly ($p<0.001$, Table 5.2a, b). There were marked overall differences between accessions in the accumulation of K^+ in root tissues in response to increase in treatment salinity up to 100 mM NaCl ($p<0.001$, Table 5.2a). The same was not however observed for shoots ($p>0.05$, Table 5.2a). The amounts of K^+ accumulated by the three accessions differed significantly at different NaCl treatments (Figure 5.6a, b) in both roots ($p<0.001$, Table 5.2a) and shoots ($P<0.05$, Table 5.2b). The K^+ concentration in both roots and shoots of the sensitive accession 203662 was lower than that of the tolerant

Table 5.2. Mean squares and significances from the analysis of variance of concentrations of Na^+, K^+, Cl^- and Na^+:K^+ ratio in roots and shoots of 14-day-old seedlings grown at four NaCl levels

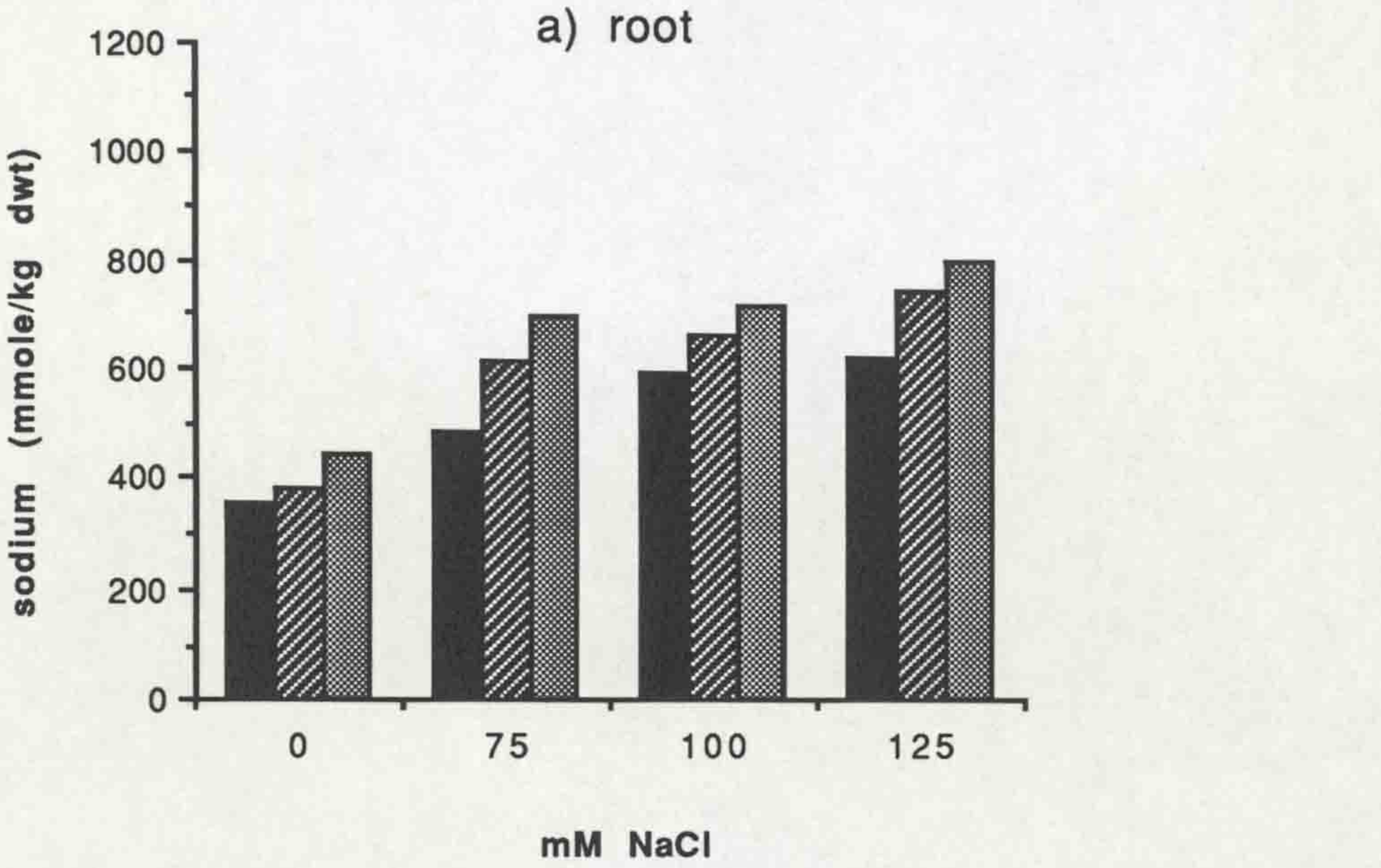
a) Root

Item	Df	Na^+	Cl^-	K^+	Na^+:K^+
Blocks	2	33546.1^{NS}	3424.6^{NS}	561.14^{NS}	0.8^{NS}
Accessions	2	64840.2^{NS}	7153.6^{NS}	82369.1***	20.0^{NS}
NaCl (Sol)	3	180439.0***	96038.8**	527321***	95.8*
Acc x Sol	6	2198.81^{NS}	689.2^{NS}	63736.1***	3.4^{NS}
Residual	22	22328.84	20838.34	4466.30	2.64

b) Shoot

Item	Df	Na^+	Cl^-	K^+	Na^+:K^+
Blocks	2	1192.2^{NS}	40320.2^{NS}	118449.3^{NS}	0.34^{NS}
Accessions	2	69133.0*	47095^{NS}	24942.3^{NS}	21.7^{NS}
NaCl (Sol)	3	379395.0***	169165.8*	1020685.0***	174.5^{NS}
Acc x Sol	6	40776.6*	6511.3^{NS}	46866.8*	16.9^{NS}
Residual	22	13442.11	38277.22	17591.68	9.58

Figure 5.5. Accumulation of sodium in roots and shoots of 14-day-old seedlings grown at four NaCl levels



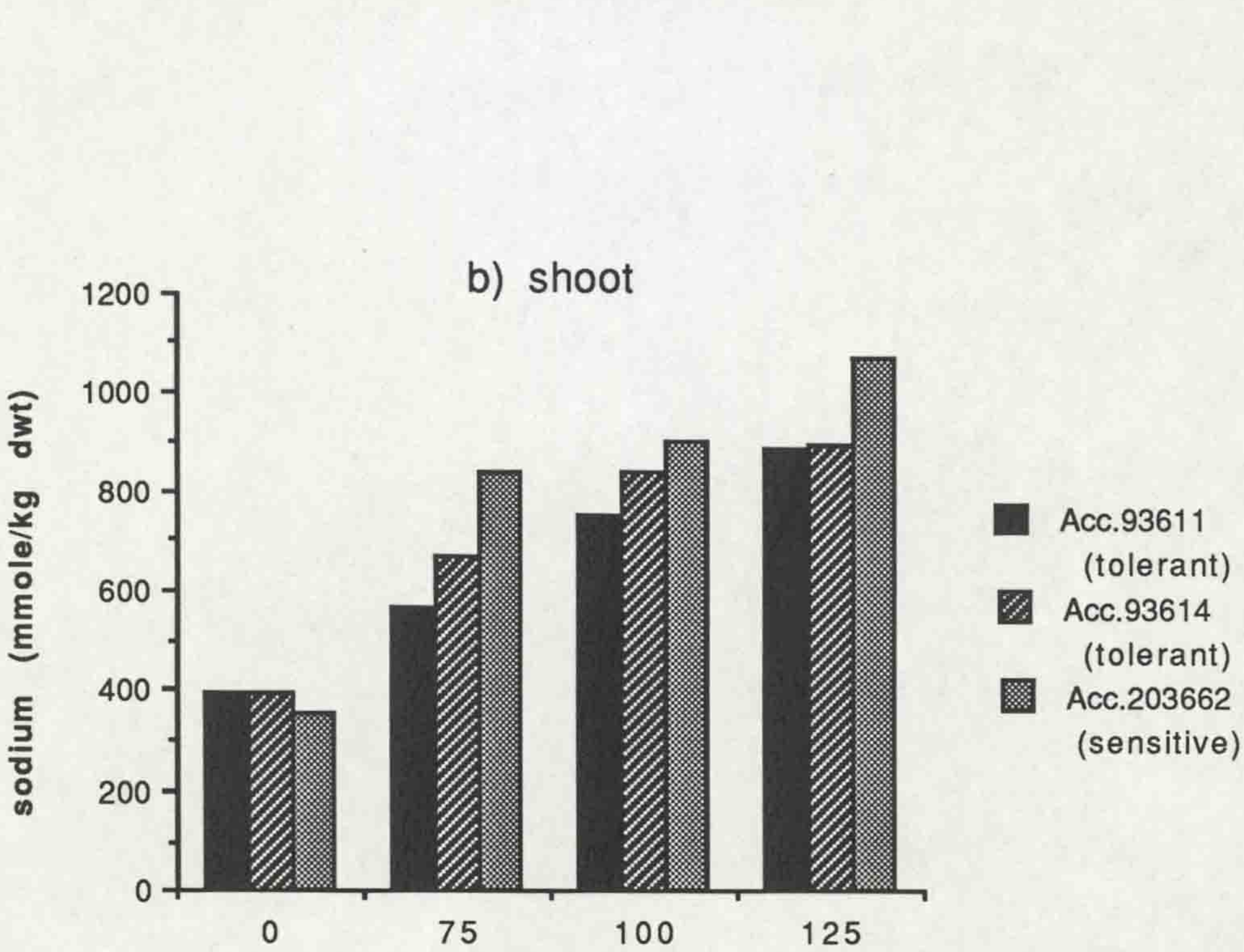
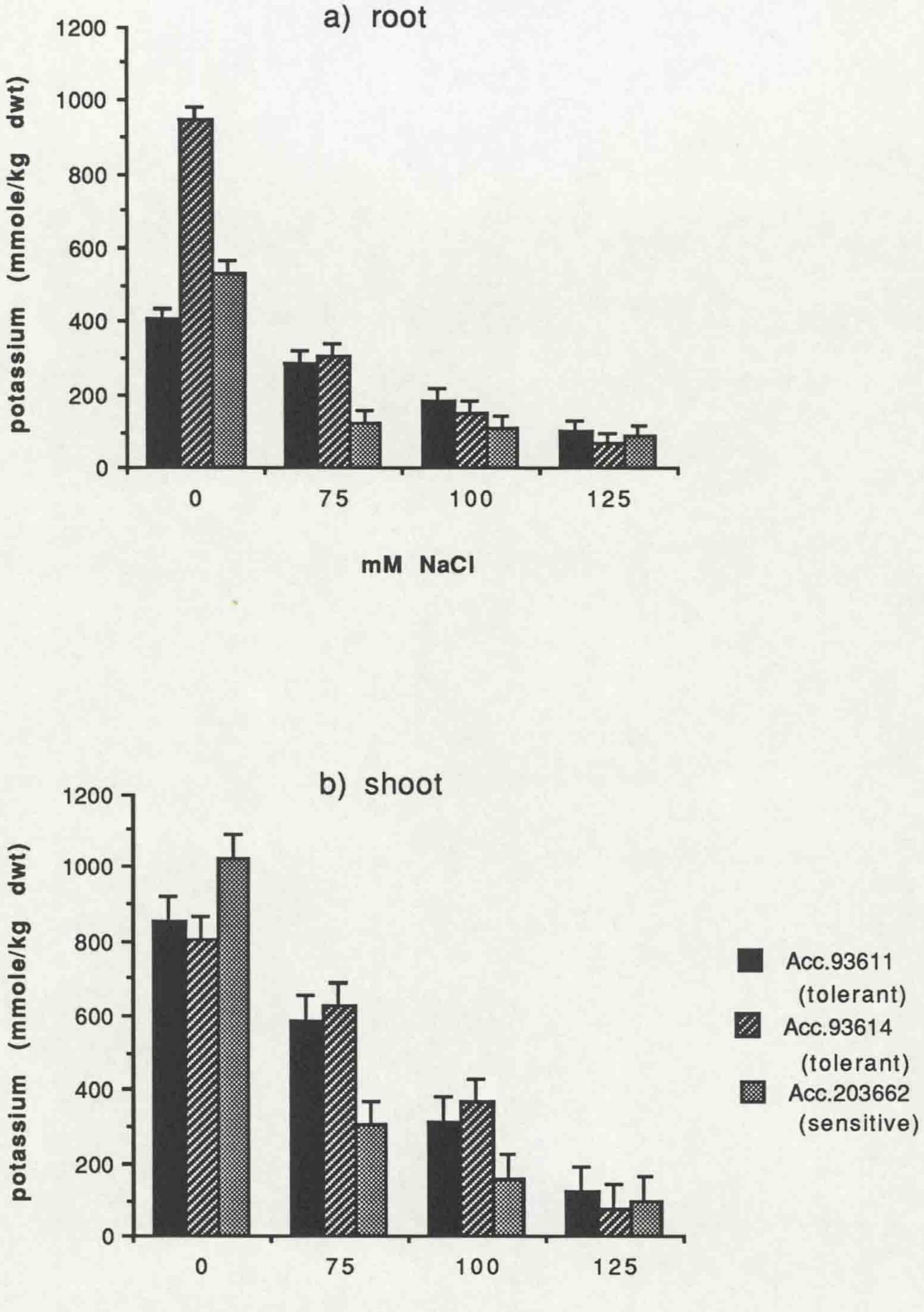


Figure 5.6. Accumulation of potassium in roots and shoots of 14-day-old seedlings grown at four NaCl levels (with LSD 5%)



accessions 93611 and 93614 at 75 mM NaCl and 93611 at 100 mM NaCl. However there was no difference between accessions at 125 mM NaCl for either root or shoot K^+ contents.

5.3.2.3. Chloride (Cl^-)

With increasing NaCl concentration, there were considerable increases in the concentration of Cl^- in both roots ($p<0.01$, Table 5.2a, Figure 5.7a) and shoots ($p<0.05$, Table 5.2b, Figure 5.7b). However the accessions were not significantly different in the accumulation of Cl^- with increasing NaCl in the growth media. There is some suggestion that more Cl^- was accumulated in shoots of the susceptible accession (203662) than in the roots and shoots of the tolerant accessions (Figure 5.7) although difference was not significant. Cl^- content of roots and shoots of the three accessions responded in similar manner to increasing NaCl in the growth medium.

5.3.2.4. Sodium : Potassium ratio (Na^+ : K^+)

There was no significant differences between accessions with respect to Na^+:K^+ ratios both in roots and shoots. Na^+:K^+ ratio increased significantly in roots ($p<0.05$, Table 5.2a) and similarly in all three accessions.

Figure 5.7. Accumulation of chloride in roots and shoots of 14-day-old seedlings grown at four NaCl levels

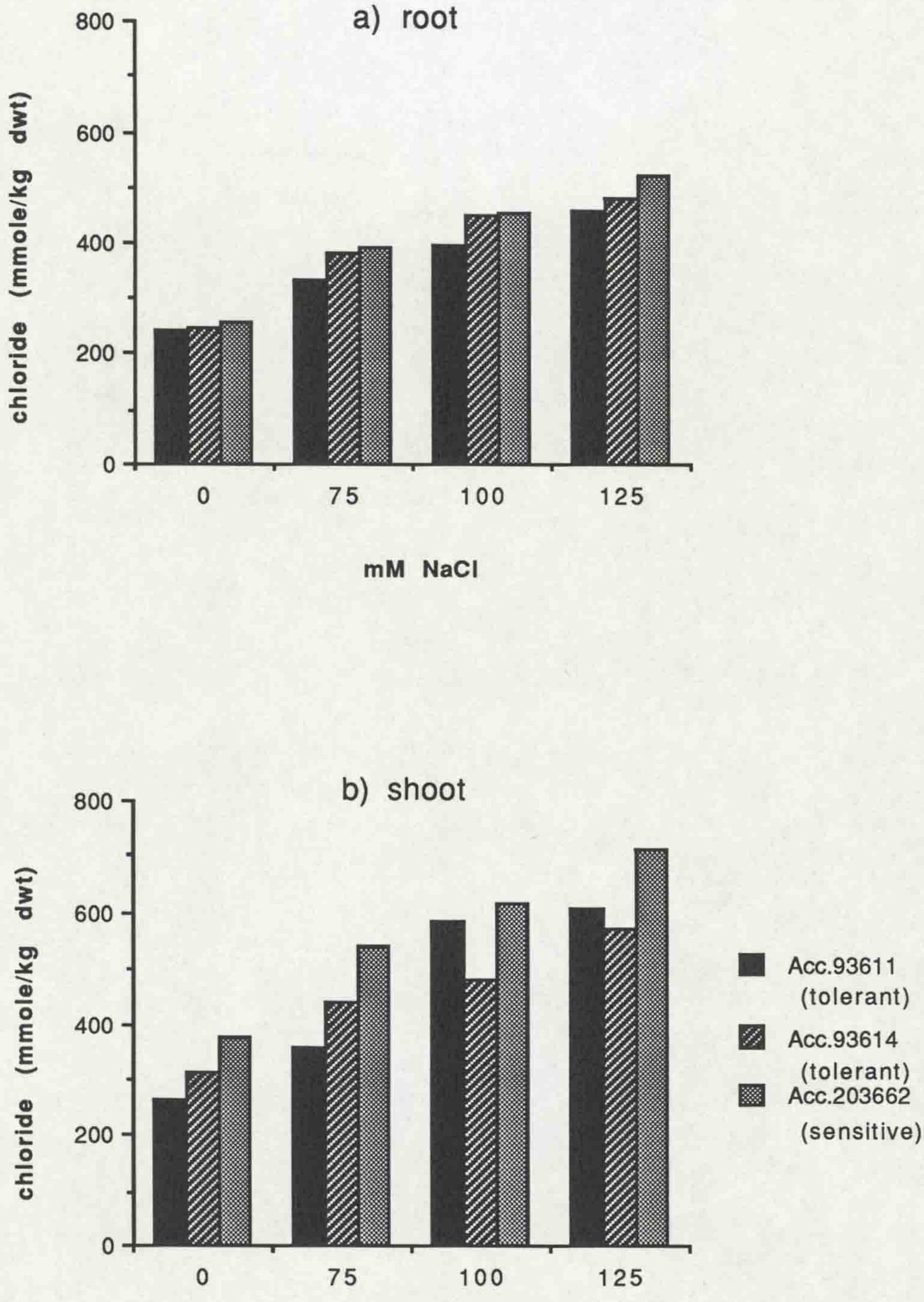
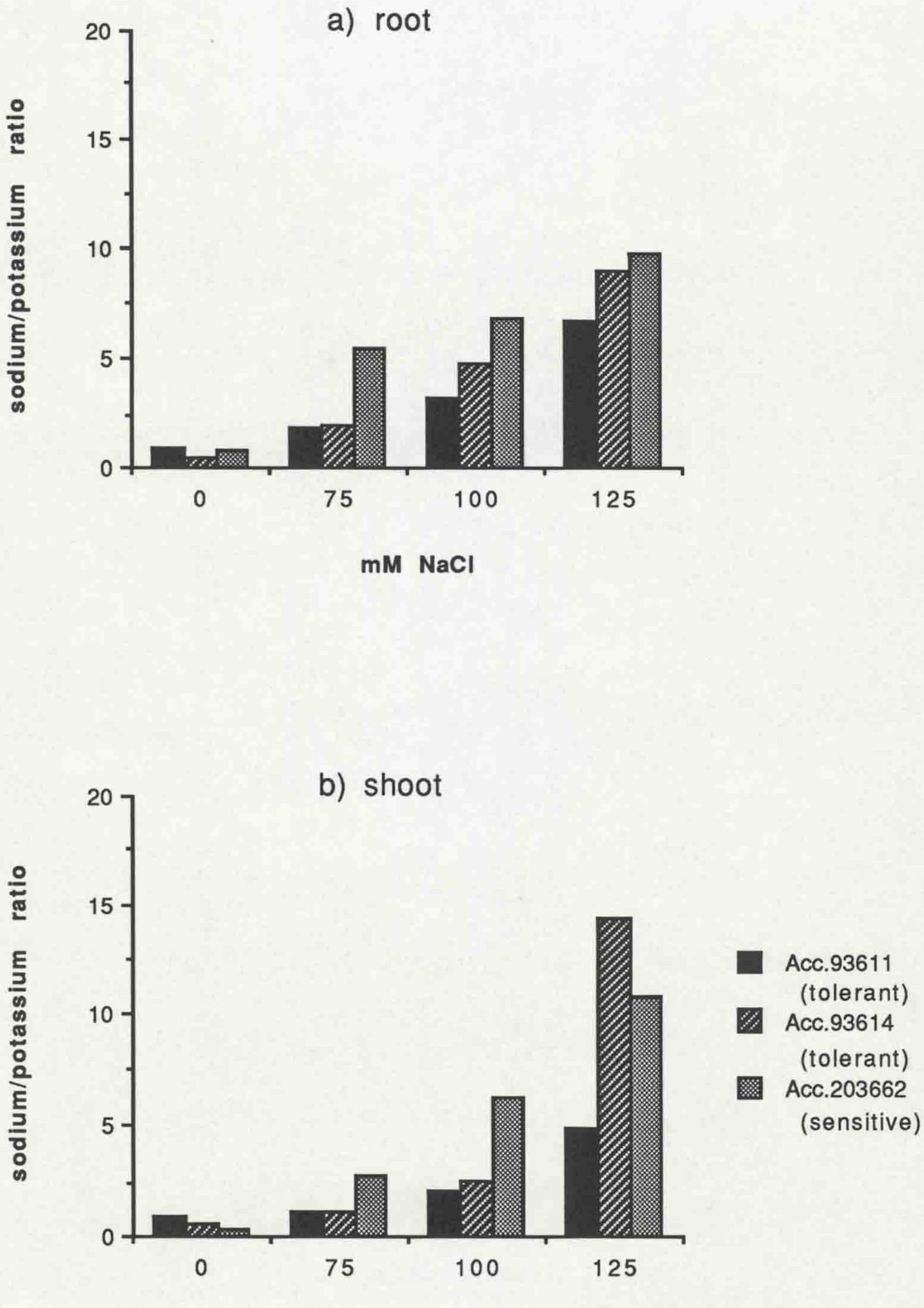


Figure 5.8. Sodium/potassium ratio in roots and shoots of 14-day-old seedlings grown at four NaCl levels



5.4. Discussion

Studies on the metabolic responses of plants to salinity stress may provide evidence about the osmoregulatory role of organic cations and physiological mechanisms involved in uptake, distribution and/or exclusion of inorganic cations.

Both the tolerant accessions 93611 and 93614, and the susceptible 203662 showed significantly increased levels of amino acid concentrations in roots and shoots with increasing salinity stress (Tables 5.1a, b; Figures 1a, b). The pattern of increase was however much higher in the tolerant accession 93611 (Figure 5.1a, b). This is in agreement with the findings of Strogonov (1973), who reported an increase in amino acid content of pea plants with increasing salinity. Similarly, Wainwright (1980) reported that accumulation of amino acids in salt-tolerant and salt sensitive ecotypes of *Agrostis stolonifera* in response to salinity stress differed in degree, and suggested that it was possible that salt-tolerant plants had utilised a normal response, but were hypersensitive to the salt stress. In this way the plants were able to accumulate potentially protective compounds before damage occurred. There is an interesting parallel here with hypersensitive reactions to pathogens often being involved in the tolerance mechanism. In the present study also, it was possible that the increase in amino acid content of the accessions observed may be due to their ability to decrease osmotic potential, or improve osmotic adjustment by accumulating organic solutes (Maas and Nieman, 1978; Aspinall and Paleg, 1981), a process in which the tolerant accession 93611 clearly had a considerable advantage, being able to accumulate greater amounts of amino acids.

There have been suggestions about a possible role of proline in conferring salt tolerance, and its potential as a physiological marker for tolerance, and it has been reported that accumulation of proline under stress may act as a protective solute by maintaining an intracellular equilibrium between cytoplasm and vacuole (Aspinall and Paleg 1981). In wheat grass, Shannon (1978) reported increased proline production under saline stress conditions and he attributed this to osmotic adjustment by the plant, however he found no differences between sensitive and tolerant lines. More recently it has been shown that its presence in plant tissues is associated with general environmental stress and not particularly to salinity, and its amount depends on plant species (Gorham *et al*., 1985). In the present work, proline accumulation increased

significantly in roots and shoots (Tables 5.1a, b, Figure 5.2a, b) in all the three tolerant and non-tolerant accessions in response to increasing NaCl concentration and accessions also differed significantly in root proline content (Table 5.1a). However in contrast to the findings of Shannon (1978) both roots and shoots of the tolerant accessions 93611 and 93614 had higher proline concentrations than the sensitive 203662 (Figure 5.2a, b). This could again be of adaptive significance for the tolerant accessions, on the assumption that a higher proline concentration may offer protection to cells from salt damage. If tolerant accessions consistently accumulate proline levels that are higher than those in susceptible accessions, this could be a suitable physiological marker for tolerance. However in *Nicotiana sylvestris* susceptible callus cultures accumulated significantly higher levels of proline than selected tolerant cell lines (Dix and Pearce, 1981) suggesting that proline presence in plant tissues depends on plant species as has been suggested previously by Gorham *et al*. (1985). It is also possible that evidence from callus tissue may not be consistent with events in whole plant material.

Polyols may also contribute to enhancement of salt tolerance by osmotically balancing the cytoplasm with the vacuole or with other sites outside the cells such as the apparent free space of the cell walls where salt is sequestered (Wyn Jones, 1984). All three accessions studied here accumulated increased amount of polyols in roots and shoots as salinity levels increased (Figure 5.3a, b). A similar pattern of polyol accumulation was observed previously in cell cultures of *Coleus blumei* (Ibrahim, 1990). The increases in polyol concentrations in roots and shoots were significant and the two tolerant accessions accumulated markedly greater polyol in their roots and shoots at each NaCl concentration than the non-tolerant accession (Tables 5.1a, b; Figures 5.3a, b) which suggests a role for polyols as a physiological indicator associated with salt tolerance.

Carbohydrate metabolism, the most important energy source for plant growth and development, is considerably inhibited during salinity stress which causes an increase in concentrations of carbohydrates in plant tissues (Rathert, 1982). The relatively salt-tolerant cotton cultivar, Giza 45, was not only characterised by restricted Na^+ and Cl^- uptake and translocation throughout the plant, but also by high sucrose content in the roots which is closely associated with carbohydrate metabolism for

osmotic adaptation. Evidently, this is an additional mechanism to prevent salt injury (Rathert, 1982). Likewise in the present study, the tolerant accession 93611 had relatively lower levels of water soluble carbohydrates in roots and shoots across all levels of NaCl treatments (Figure 5.4a, b), a possible means of resistance of cell metabolism to NaCl stress.

A significant increase in Na^+ was observed in roots and shoots of all three accessions in response to increasing NaCl concentrations in the growth medium (Table 5.2a, b, Figure 5.5a, b). Similar results were reported in alfalfa by several workers (Bernstien and Pearson, 1956; Noble *et al.*, 1984; Ashraf *et al.*, 1987; Al-Khatib, 1991). Na^+ concentration was however lower in the two tolerant accessions compared with the susceptible accession 203662 although the difference was significant only for shoot tissues (Figure 5.5a, b, Table 5.2a, b).

Pennisetum americanum (L.) Leeke is moderately tolerant to salinity and there is considerable variation for salinity tolerance within it (Ashraf and McNeilly, 1987, 1992). This is substantiated in the present work. Whilst the two tolerant accessions examined here accumulate low concentrations of Na^+ ions in their tissues in response to NaCl stress, this does not appear to be a general phenomenon in salt-tolerant species (Greenway and Rogers, 1963; Yeo and Flowers, 1982). The lower Na^+ concentrations in roots and shoots of the tolerant accessions suggested that there was some exclusion of Na^+ ions in these accessions, a situation which seems to follow the general pattern of ion exclusion described for glycophytes (Läuchli and Wieneke, 1979; Greenway and Munns, 1980; Winter and Läuchli, 1982). The mechanism of Na^+ uptake in salt-tolerant plants may well be due to differential potentials for ion uptake as shown by Lessani and Marschner (1978) in various crops. In salt sensitive plants, in particular it has been observed that an increase in Na^+ results when control of uptake fails and this failure is correlated with reduction in growth (Wainwright, 1980). Wainwright (opp. cit.) suggested that in glycophytes, unlike the situation in halophytes, Na^+ is excluded from the shoot and they do show increase in growth. The relatively higher Na^+ uptake and its increased accumulation in roots and shoots of the accessions may be the main

cause for growth depression, either because of osmotic stress due to decreased external water potential, or the effects of specific ions on metabolic processes, ranging from absorption of nutrient to enzyme activation or inhibition (Kingsbury *et al*., 1984). The mineral composition of the seedlings also indicates the extent to which Na^+ and Cl^- accumulation, and ion imbalance may have been involved in growth retardation of these accessions.

Although K^+ is not involved specifically in photosynthetic metabolism, it is required in relatively high concentrations for other biophysical and biochemical processes which affect photosynthesis (Huber, 1985). In this experiment K^+ accumulation in roots and shoots decreased with increasing NaCl concentration (Figures 5.6a, b). This agrees with the results of Umiel *et al*. (1980) who found that K^+ uptake decreased with increasing NaCl concentration in tobacco callus cultures. Figures 5.6a, b showed that the two tolerant accessions maintained higher levels of tissue or root and shoot K^+ than the susceptible accession, suggesting that they were able to preferentially absorb K^+ under conditions of increasing Na^+ levels. Binzel *et al*. (1987) reported similar differences between salt adapted and salt sensitive tobacco cell cultures.

A decrease in K^+ content with increasing salinity stress could on the other hand be due to leakage of K^+ from roots caused by NaCl as shown by Nassery (1975, 1979) and Bates (1976). Wyn Jones and Storey (1978) have suggested that barley is less adapted to salinity than *Spartina* spp. because it is unable to exploit the osmotic benefits of Na^+ and Cl^-, possibly due to Na^+ induced membrane damage and subsequent solute leakage. Nassery (1975) showed that K^+ leakage from barley and bean roots was NaCl induced rather than osmotically induced, and NaCl treatment was subsequently found to induce K^+ leakage at concentrations which cause substantial reduction of root growth (Ahmed, 1978). Wainwright (1980) suggested that LiCl, which inhibits root growth, did not induce K^+ leakage and suggested a significant role

for salt induced K^+ leakage in salt toxicity. In the present study, however, there was no evidence of K^+ leakage, but maintenance of high K^+ may be the result of either selectivity for K^+ or avoidance of Na^+. The patterns of Na^+/K^+ ratios also confirmed this view (Tables 5.2a, b; Figures 5.8a, b).

Salinity effects on three cultivars of barley of different salt sensitivities were examined by Wyn Jones and Storey (1978), California Mariout being the most resistant, Arimar being intermediate and Chevron the most sensitive. When the cultivars were grown in media containing NaCl, California Mariout and Arimar showed decreased root and shoot Cl^- levels when compared with the more sensitive Chevron. In the present study the major difference was that of significantly increased Cl^- accumulation. Such an increase in Cl^- concentrations might have caused growth depression either because of an osmotic stress or the effect of specific ions.

In toto the 14-day-old seedlings of both tolerant and susceptible accessions accumulated more organic solutes (amino acids, proline, polyols and water soluble carbohydrates) with increasing salinity levels. The same pattern was observed in the accumulation of Na^+ and Cl^-. By contrast K^+ concentrations decreased with increasing levels of treatment salinity.

Although all the components (except K^+) increased with increased salinity the most significant findings were the following.

1. The major differences between responses of the accessions to salinity stress were the varying concentrations of both organic solutes and inorganic ions, the sensitive accession 203662, the sensitivity of which was found from tolerance testing in Chapter 2, accumulating more Na^+ in shoots, but less K^+, amino acids and polyols both in roots and shoots, and proline and carbohydrates in roots, than the two salt-tolerant accessions.
2. Accession 93611, which proved to be moderately tolerant from the genetic study in Chapter 2, showed both avoidance/selectivity and better osmotic adaptation through synthesis of organic solutes and modified carbohydrate metabolism.
3. The extent of polyol accumulation in response to increasing salinity, accessions

being significantly different from each other in both root and shoot polyol contents and the two tolerant accessions consistently maintaining greater polyol levels across NaCl concentrations, could be a possible physiological marker for enhanced salinity tolerance.

RESPONSE OF PEARL MILLET ACCESSIONS TO NaCl ALONE AND WITH $CaCl_2$

CHAPTER SIX

CHAPTER 6

RESPONSES OF PEARL MILLET ACCESSIONS TO NaCl ALONE AND WITH $CaCl_2$

6.1. Introduction

Salt is a general term and salinity problems in the field may be caused primarily by one salt or by a combination of several salts. Saline soils are dominated by NaCl but may contain Na_2SO_4, $MgSO_4$, $CaSO_4$, $MgCl_2$, KCl and $CaCl_2$ (Flowers, 1972). Single salt salinity or alkalinity rarely occurs in nature, and salts, if present as a mixture, interact strongly with each other in their effect on germination (Paleg and Aspinall, 1981). By and large most selection work has involved NaCl as a common salt. NaCl solutions are frequently supplemented by $CaCl_2$, at the ratio of about 2:1 respectively (Blum, 1988), although the USDA Salinity Laboratory recommends use of a 1:1 by weight combination of salts (Francois, pers comm).

There is evidence from several species that the salt sensitivity of certain varieties is due to the absorption of relatively high amounts of Cl^- and/or Na^+, i.e. these varieties suffer from excess of these ions in their expanded leaves. Both varietal and species comparisons show that sensitivity towards high leaf Cl^- and/or Na^+ concentrations is much greater for nonhalophytes than for halophytes. This difference is almost certainly based on inadequate cellular compartmentation of ions in the leaves of nonhalophytes, or alternatively, metabolism in halophytes may be very tolerant to high levels of electrolytes (Greenway and Munns, 1980).

Recent data from salt tolerance studies in pearl millet (Ashraf and McNeilly, 1987, 1992) have shown considerably differing responses of some accessions to NaCl+$CaCl_2$, and they related the tolerance of some of these genotypes to the concentration of specific ions in plant tissues. Azhar (1988) investigated the response of some sorghum accessions to NaCl alone and with $CaCl_2$. The data showed that the accessions differed in their response to EC (Electrical Conductivity) levels due to NaCl and NaCl+$CaCl_2$, and in general root growth was more profoundly affected than shoot growth. Information on the responses of crop accessions/cultivars to salinity due to different salts which are components of saline soils is necessary for development of

material suitable for conditions resulting from the combinations of different salts in such soils.

In the study pursued here, a comparison was made of the response and ionic contents of two-week-old seedlings of twelve pearl millet accessions to NaCl alone and to a combination of NaCl+$CaCl_2$ with a view to determining reasons for differences, if any.

6.2. Materials and methods

Twelve pearl millet accessions/cultivars, Kitui Local (t), Selection 2 (t), 93611 (t), 93612 (t), 93614 (t), 203658 (s), 203659 (s), 203662 (s), 215634 (s), 219975 (s), 220220 (s) and 221726 (t) were used in this experiment (see Appendix 1.1 for origins of accessions). Each accession was grown in NaCl alone, and NaCl+$CaCl_2$ 1:1 by weight (NaCl 1g:1g $CaCl_2.6H_2O$ following the recommendation of L.E. Francois, USDA Salinity Laboratory, pers comm), in 0.1 strength nutrient solution as described in previous chapters. There were three salinity levels quantified as electrical conductivity (EC) of EC 4.0, EC 8.0, and EC 12.0 dS m^{-1}. The 0.1 strength nutrient solution, EC 0.3 dS m^{-1}, was used as a non-saline control. The equivalent level in mM of each EC in each salinity is given in Appendix 6.2.

The experimental procedures and conditions were identical to those used in the experiments described in Chapter 2 (p. 18), and the experiment had three replicates in a completely randomised design.

After 14 days, seedlings were harvested and again as in previous experiments (Chapters 2 and 3) shoot length and longest root length were obtained for a total of 30 randomly chosen seedlings (10 per replicate) from each treatment.

Of the twelve accessions assessed, shoots and roots of six, 93611, 93614, 203659, 203662, 219975 and 221726 were analysed for Na^+, K^+ and Cl^- contents after bulk drying separately of all shoot and root materials of the 10 measured seedlings of each replicate at 50°C for five days. The methods followed for preparing the samples, and the procedures to determine the concentrations of the cations and Cl^- were identical with those described in Chapter 5 (pp. 111–112).

6.3. Results

6.3.1. Root and shoot lengths data

Results of analysis of variance of data for root and shoot lengths are given in Table 6.1. Absolute root and shoot lengths of the twelve accessions in the respective salinities and concentrations are given in Appendix 6.1. The relative root and shoot length data for the accessions due to increasing solution EC from NaCl alone and NaCl+$CaCl_2$ are presented in Figures 6.1 (roots) and 6.2 (shoots).

There were overall differences in seedling root and shoot length data in NaCl and NaCl+$CaCl_2$ treatments ($p<0.01$ roots, $p<0.001$ shoots). Further, the response of seedling root lengths and shoot lengths of different accessions differed significantly between the NaCl alone or NaCl+$CaCl_2$ treatments (Acc x T significant at $p<0.05$ root, $p<0.001$ shoots).

Root lengths at EC 4 were greater in the mixed salt treatments than in NaCl alone. However, in accessions 93611, 203658, 203659, and 220220, they did not differ. At EC 8 root length of most accessions (other than 93611, 215634, and 221726) was greater in the mixed salt solution than when grown in NaCl alone, whilst at EC 12 accessions 93611, 93614, Kitui Local, and Selection 2 had similar root lengths in the two solutions, the remaining accessions again having longer roots in the mixed salt treatments. As would be expected from previous evidence accessions differed in their response to increasing conductivity whether due to NaCl alone or NaCl+$CaCl_2$ solutions.

Whilst overall shoot length for the accessions grown in NaCl alone and NaCl+$CaCl_2$ differed significantly, the responses of different accessions to increasing EC due to those two salinity sources did not differ significantly, reflecting the almost parallel responses in shoot growth reduction caused by NaCl alone and NaCl+$CaCl_2$. Some accessions, 93611, 93614, 203658, 215634, and 220220 had overall very similar relative shoot lengths in the two solutions, whilst others, 221726, Kitui Local, and Selection 2 had almost identical relative shoot lengths at certain EC levels. Other accessions, 93612 and 219975 differed markedly in the impact of NaCl and NaCl+$CaCl_2$ treatments (Figure 6.2). Accessions 93611 and 215634 were the only

Table 6.1. Mean squares (MS) and significances from the analysis of variance of the response of twelve pearl millet accessions to increasing EC levels due to NaCl alone, and NaCl+$CaCl_2$

Source of variation	Df	Root MS	Shoot MS
Blocks	2	21819.89*	8974.67**
Accessions (Acc)	11	30236.58***	7984.86***
NaCl vs NaCl+$CaCl_2$ (T)	1	88112.97**	36667.72***
Solution conductivity (Cond)	2	99664.37***	23194.89***
Acc x T	11	11045.22*	4954.33***
Acc x Cond	22	11028.36*	1582.84*
T x Cond	2	45198.99***	142.08^{NS}
Acc x T x Cond	22	1372.53^{NS}	409.58^{NS}
Residual	142	4818.81	1243.65

ones in which shoot length in NaCl was greater than in NaCl+$CaCl_2$, no statistical significance can be attached to these data.

The significant treatments (NaCl vs NaCl+$CaCl_2$) x solution conductivity interaction ($p<0.001$) for root suggested that increasing EC level due to NaCl or NaCl+$CaCl_2$ reduced root length differently. However the same interaction for shoot was not significant ($P>0.05$).

6.3.1.1. Root length in NaCl and NaCl+$CaCl_2$

a) NaCl (Table 6.2, Figure 6.1)

At EC 4, accessions 93611, 93612, 93614, Kitui Local, Selection 2, 215634, and 221726 had greater relative root length than accessions 203658, 203659, 203662, 219975, and 220220.

At EC 8, accessions Kitui Local, Selection 2, and 215634 had longer roots than the rest of the accessions. Accessions 93611, 93612, 93614, and 221726 had intermediate root lengths, whilst the remaining accessions were markedly affected at this EC level and their relative root length values ranged between 8 - 25%.

Accessions 93611, 93612, 93614, Kitui Local, Selection 2, and 221726 still had higher relative root length values at EC 12. Based on root length data at EC 12, the accessions have been ranked for relative tolerance (Table 6.2), relative root length less than 20% classified as category III, 20% - 50% as category II, and values in excess of 50% were classified category I.

b) NaCl+$CaCl_2$ (Table 6.3, Figure 6.1)

There was marked contrast in the responses of accessions at EC 4. In accessions 93612, 93614, Kitui Local, and Selection 2, it appears to have stimulated root lengths, whilst in accessions 203658, 203659, 203662, and 220220, it resulted in a marked reduction in relative root length.

At EC 8, accessions 93612, 93614, Kitui Local, and Selection 2 had greater relative root length values than the remaining accessions. Accessions 93611, 215634, and 221726 showed intermediate relative root lengths, whilst the remaining accessions had lower relative root lengths ranging from 42.5% to 46.26%.

Table 6.2. Growth parameters, their relative tolerance, and tolerance ranking of twelve pearl millet accessions after 14 days growth in control (EC 0.03 dS m^{-1}) and salinised (EC 12.0 dS m^{-1}) solution cultures of NaCl (Relative values are salinised values expressed as per cent of control)

Accession number	Mean root length(cm) control	Relative root length (%) 12.0 dS m^{-1}	Tolerance ranking	Mean shoot length (cm) control	Relative shoot length (%) 12.0 dS m^{-1}	Tolerance ranking
Kitui Local	4.68	57.69	I	7.82	57.54	I
Selection 2	7.70	62.47	I	13.26	68.48	I
93611	10.94	57.86	I	12.01	62.03	I
93612	5.59	57.25	I	9.30	53.76	I
93614	6.59	63.43	I	9.37	60.19	I
203658	11.10	8.20	III	11.34	18.87	III
203659	9.27	12.62	III	10.36	23.17	II
203662	10.34	12.89	III	11.78	21.56	II
215634	3.41	25.51	II	7.60	53.42	I
219975	6.62	8.91	III	9.31	19.87	III
220220	10.42	20.06	II	11.31	34.75	II
221726	4.43	49.21	I	7.35	70.07	I

Tolerance rank	**I**	**Relative root length**	**> 50%**
	II		**20% - 50%**
	III		**< 20%**

Table 6.3. Growth parameters, their relative tolerance, and tolerance ranking of twelve pearl millet accessions after 14 days growth in control (EC 0.03 dS m^{-1}) and salinised (EC 12.0 dS m^{-1}) solution cultures of NaCl+$CaCl_2$ (Relative values are salinised values expressed as per cent of control)

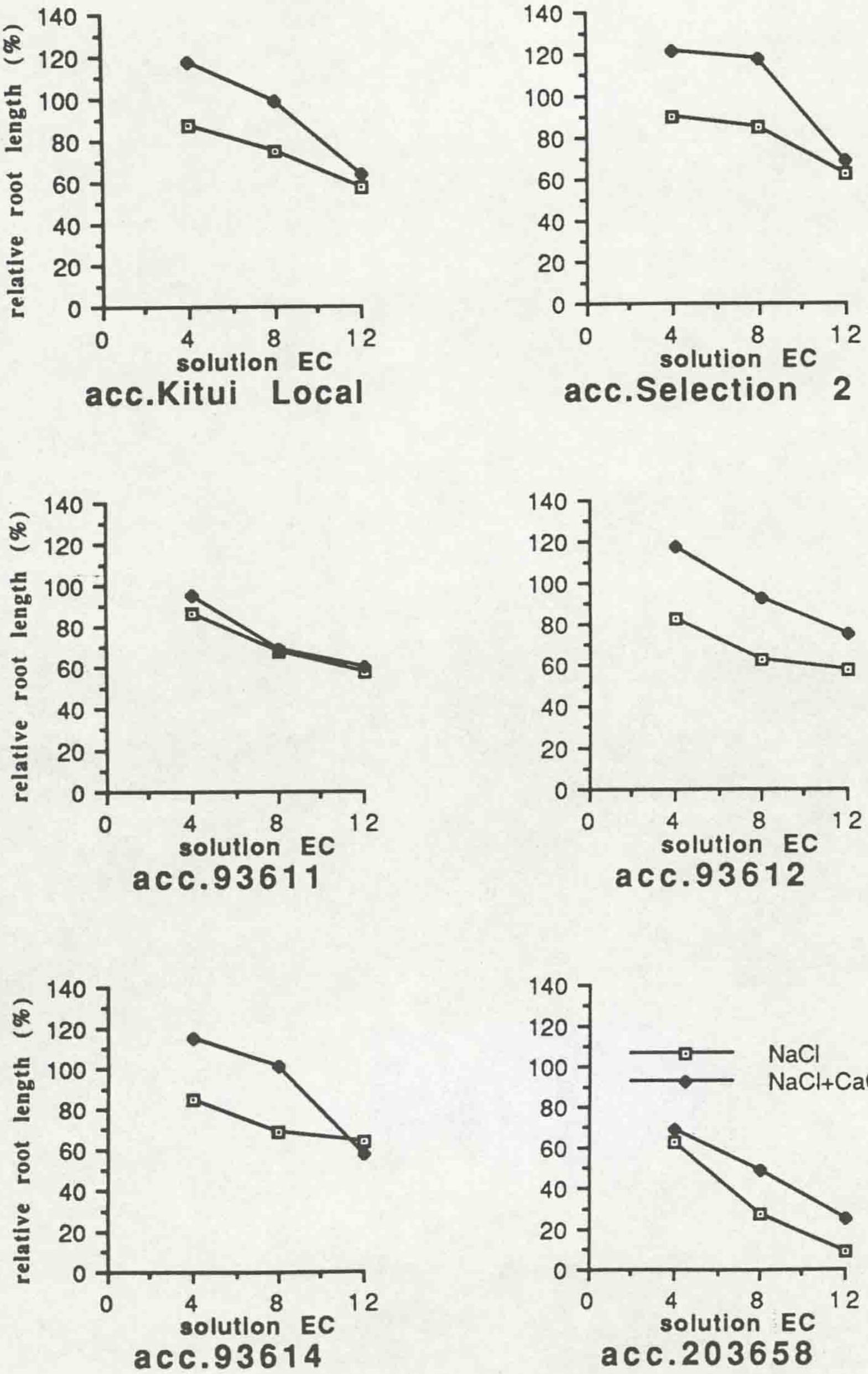
Accession number	Mean root length(cm) control	Relative root length (%) 12.0 dS m^{-1}	Tolerance ranking	Mean shoot length (cm) control	Relative shoot length (%) 12.0 dS m^{-1}	Tolerance ranking
Kitui Local	4.68	64.10	I	7.82	71.10	I
Selection 2	7.70	69.33	I	13.26	71.04	I
93611	10.94	60.01	I	12.01	60.00	I
93612	5.59	75.13	I	9.30	76.56	I
93614	6.59	56.90	II	9.37	54.00	II
203658	11.10	24.86	III	11.34	33.95	III
203659	9.27	28.88	III	10.36	43.05	II
203662	10.34	35.01	III	11.78	40.92	II
216534	3.41	44.28	II	7.60	46.97	II
219975	6.62	33.99	III	9.31	81.74	I
220220	10.42	40.69	II	11.31	51.37	II
221726	4.43	65.46	I	7.35	63.27	I

Tolerance rank		Relative root length
	I	> 60%
	II	40% - 60%
	III	< 40%

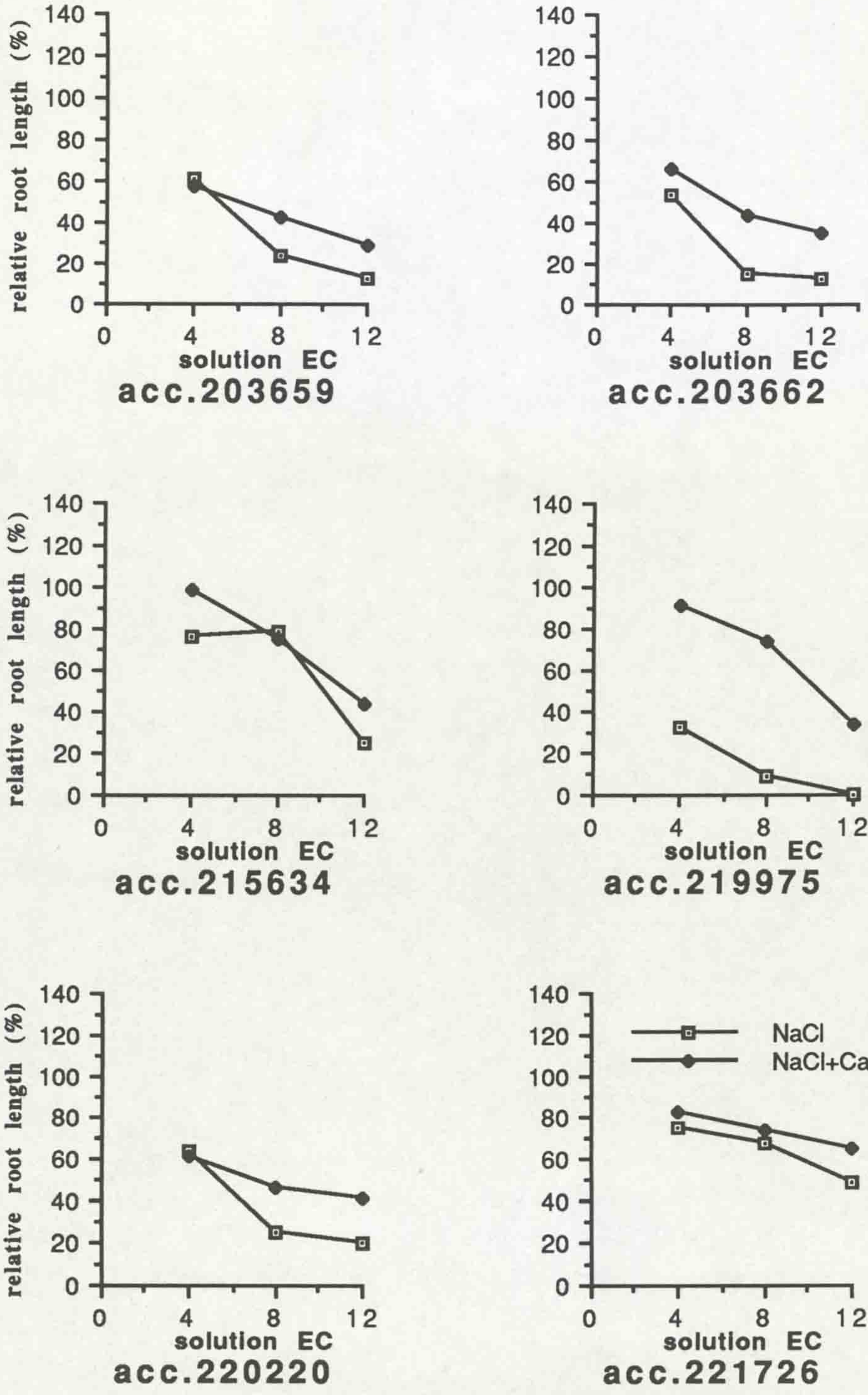
Table 6.4. Correlation coefficient (r) for relative root and shoot lengths of twelve pearl millet accessions at EC 12 dS m^{-1} due to NaCl alone, and NaCl+$CaCl_2$

Item	r (correlation coefficient)
Relative root length	0.94***
Relative shoot length	0.58**

Figure 6.1. Relative root lengths of twelve pearl millet accessions at increasing EC due to NaCl alone (—□—), and NaCl+$CaCl_2$ (—●—)



(Figure 6.1 continued)



Accessions were again ranked for tolerance at EC 12 (Table 6.2). Accessions 93611, 93612, Kitui Local, Selection 2, and 221726 had greater relative root length than the remaining accessions and their tolerance ranking was I. On the other hand accession 93614 had intermediate relative root length and its tolerance ranking was II (Table 6.3). The relative root lengths of the remaining five accessions ranged from 24.86% to 44.28% and their tolerance ranking was III (Table 6.3).

6.3.1.2. Shoot length in NaCl and NaCl+$CaCl_2$

a) NaCl (Table 6.2, Figure 6.2)

Shoot growth of accession 215634 appears to have been stimulated by increased solution EC, and apart from Kitui Local the remaining accessions had relative values less than 90% at EC 4. Most noticeable was the markedly reduced relative shoot length of accession 219975 (48.23%) at this EC level.

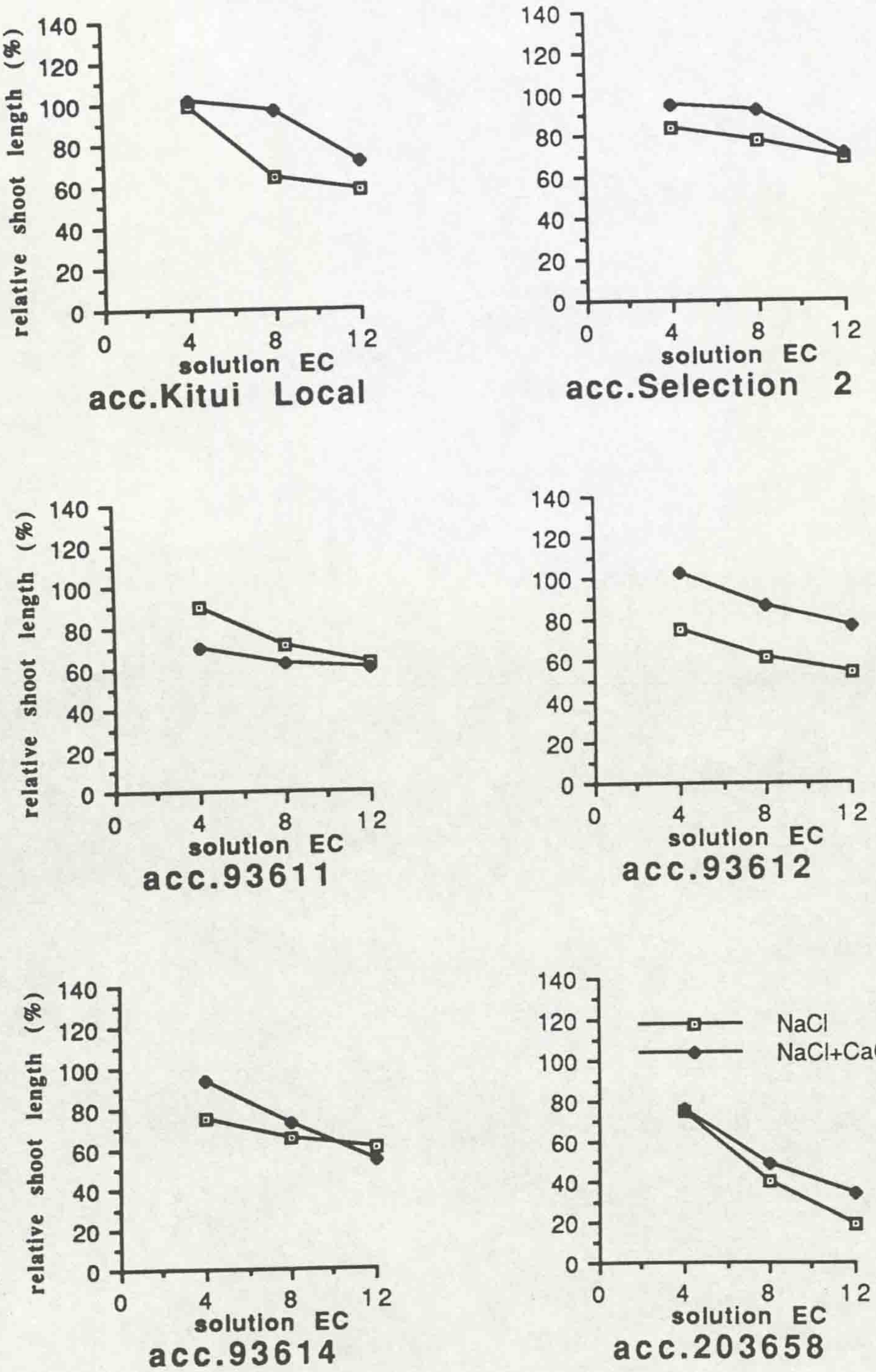
At EC 8, the relative shoot length of accessions 93611, Selection 2, 215634, and 221726 were greater than those of the other accessions. Accessions 93612, 93614, and Kitui Local had intermediate relative shoot lengths, whilst the remaining five accessions had relative shoot lengths which ranged from 21.16% to 49.98%.

At EC 12, there was a marked reduction in shoot length of the accessions 203659 and 215634 whereas in others (Kitui Local, 93611, and 219975) there was virtually no reduction. Relative shoot length of accessions 93611, 93614, Selection 2, and 221726 were greater than those of the other accessions and their tolerance ranking was I (Table 6.2). Accessions 93612, Kitui Local, and 215634 with relative shoot length of 53.76%, 57.54% and 53.42% respectively were ranked II in their tolerance, whilst the remaining accessions with relative shoot length ranging from 19.87% to 34.75% were ranked in the sensitive response category III (Table 6.2).

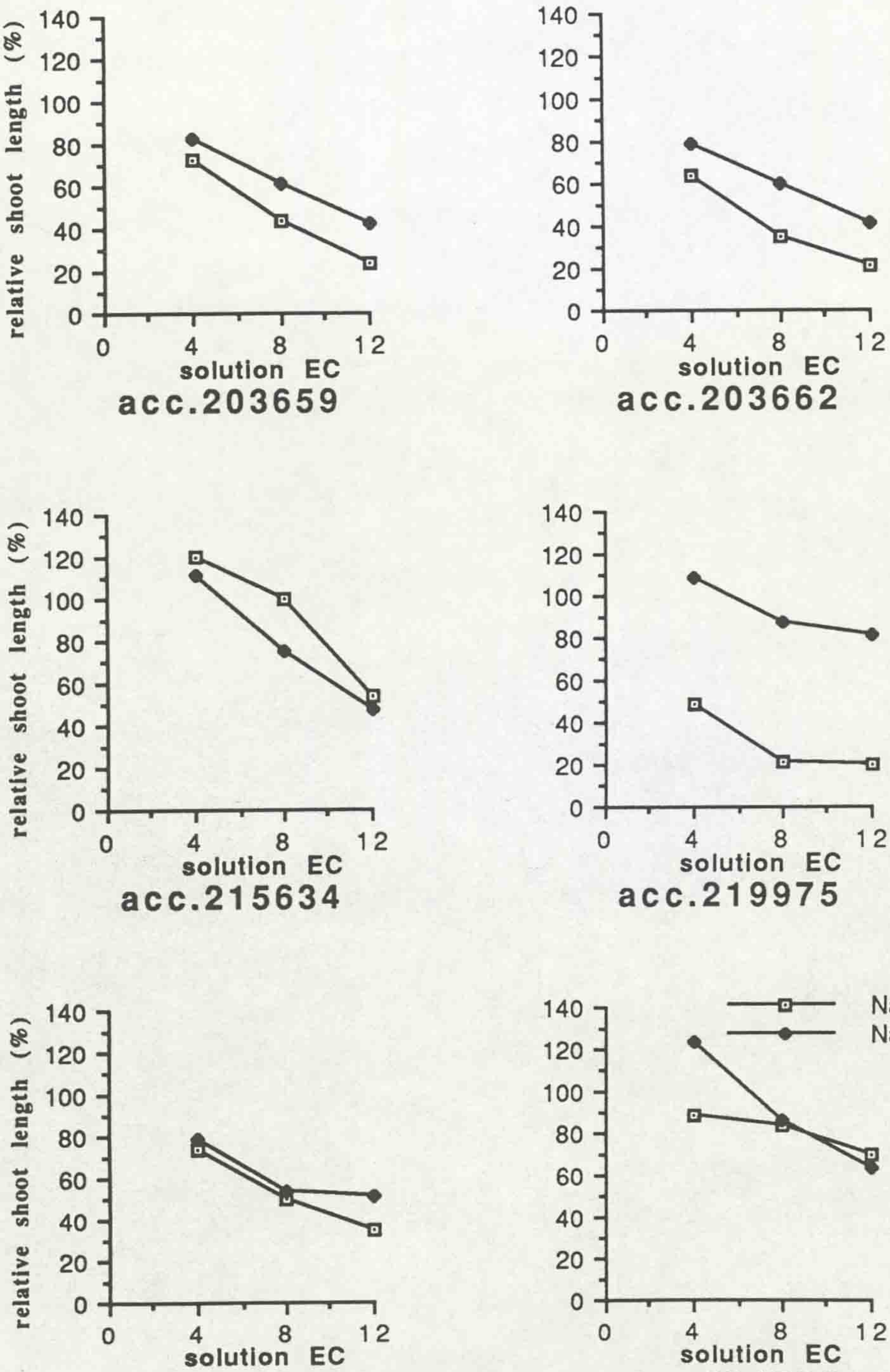
b) NaCl + $CaCl_2$ (Table 6.3, Figure 6.2)

The growth of shoots of accessions 93612, Kitui Local, 215634, 219975 and 221726 remained unaffected or was stimulated at EC 4, having relative tolerance of greater than 100%. There was not a marked reduction in relative shoot lengths of the

Figure 6.2. Relative shoot lengths of twelve pearl millet accessions at increasing EC due to NaCl alone (—□—), and NaCl+$CaCl_2$ (—●—)



(Figure 6.2 continued)



remaining accessions up to EC 8 either except for accessions 215634 and 221726. At EC 8, however, the relative shoot lengths of accessions 203658 and 220220 were markedly reduced.

At EC 12, Accessions 93611, 93612, Kitui Local, Selection 2, 219975, and 221726 had greater relative shoot lengths in excess of 60% and were ranked I in tolerance ranking (Table 6.3). The relative shoot lengths of the remaining accessions were markedly reduced and were II in their tolerance ranking, whilst accession 203658 ranked III in tolerance ranking with 33.95% relative shoot length (Table 6.3).

6.4. Na^+, K^+ and Cl^- content of accessions grown in NaCl and $NaCl+CaCl_2$ (Table 6.5, Figure 6.3-5)

The results of analysis of variance of ion contents of roots and shoots of the six pearl millet accessions, 93611, 93614, 203659, 203662, 219975, and 221726 grown at increasing EC due to NaCl alone, and $NaCl+CaCl_2$ are presented in Table 6.5. Overall NaCl uptake was greater for both roots and shoots in NaCl pure vs $NaCl+CaCl_2$, apart from controls (T significant at $p<0.001$ for shoots and roots). Shoots and roots accumulated more Na^+ as EC increased in NaCl alone than with $NaCl+CaCl_2$ (T x Cond significant at $p<0.001$ roots, $p<0.05$ shoots). Accessions differed significantly in Na^+ uptake overall in roots ($p<0.001$) and shoots ($p<0.05$).

The analysis of variance showed that the six accessions differed significantly ($p<0.001$) from each other in the accumulation of Cl^- ions in both roots and shoots. Cl^- contents were greater for both roots and shoots in NaCl alone, and $NaCl+CaCl_2$ (T significant at $p<0.001$ for roots and shoots). Roots and shoots took up more Cl^- as EC increased in $NaCl+CaCl_2$ than with NaCl alone (T x Cond significant at $p<0.001$ for roots and shoots).

Treatment (NaCl vs $NaCl+CaCl_2$) for K^+ both in shoots and roots was significant ($p<0.001$) suggesting that K^+ contents were lesser for both roots and shoots in NaCl pure vs $NaCl+CaCl_2$, apart from controls. Shoots accumulated lesser

Table 6.5. Mean squares and significances from the analysis of variance of ion concentration in roots and shoots of six pearl millet accessions grown at increasing EC due to NaCl alone, and NaCl+$CaCl_2$

Item	Df	Roots			Shoots		
		Na^+	K^+	Cl^-	Na^+	K^+	Cl^-
Blocks	2	5427.12^{NS}	334.54^{NS}	10991.43^{NS}	127945.07^{NS}	1289.73^{NS}	53611.11^{NS}
Accessions (Acc)	5	63081.23***	30569.05***	929698.57***	200817.01*	117846.06***	916650.17***
NaCl vs NaCl+$CaCl_2$ (T)	1	1432162.69***	9692.73***	537210.82***	140622.81***	41606.14***	1046358.51***
Solution conductivity (Cond)	3	765125.28***	21161.81***	2859671.48***	447626.59***	43847.58***	1856057.58***
Acc x T	5	29303.62^{NS}	7365.70***	282835.28***	51698.18^{NS}	7790.51***	265983.51***
Acc x Cond	15	14584.09^{NS}	10760.01***	52277.01**	68772.04^{NS}	5880.15***	63281.47^{NS}
T x Cond	3	311320.92***	1084.13^{NS}	149121.41***	206840.32*	4257.83**	232955.73***
Acc x T x Cond	45	18117.84^{NS}	2210.46^{NS}	47022.76**	60631.61^{NS}	1548.66*	52580.73^{NS}
Residual	94	13903.30	780.71	20002.49	13903.30	780.71	20002.49

K^+ as EC increased in NaCl alone than with NaCl+$CaCl_2$ ($p<0.01$), whilst roots took up similar amounts of K^+ in both sources of salinity ($p>0.05$). However accessions differed significantly in K^+ uptake overall in roots and shoots ($p<0.001$ for roots and shoots).

The significant three-factor interaction, accessions x treatments (NaCl vs NaCl+$CaCl_2$) x solution conductivity for Cl^- in roots ($p<0.01$), and for K^+ in shoots ($p<0.05$) suggested that concentrations of Cl^- and K^+ in roots and shoots respectively, of the six accessions grown at four increasing levels of each NaCl and NaCl+$CaCl_2$ were different.

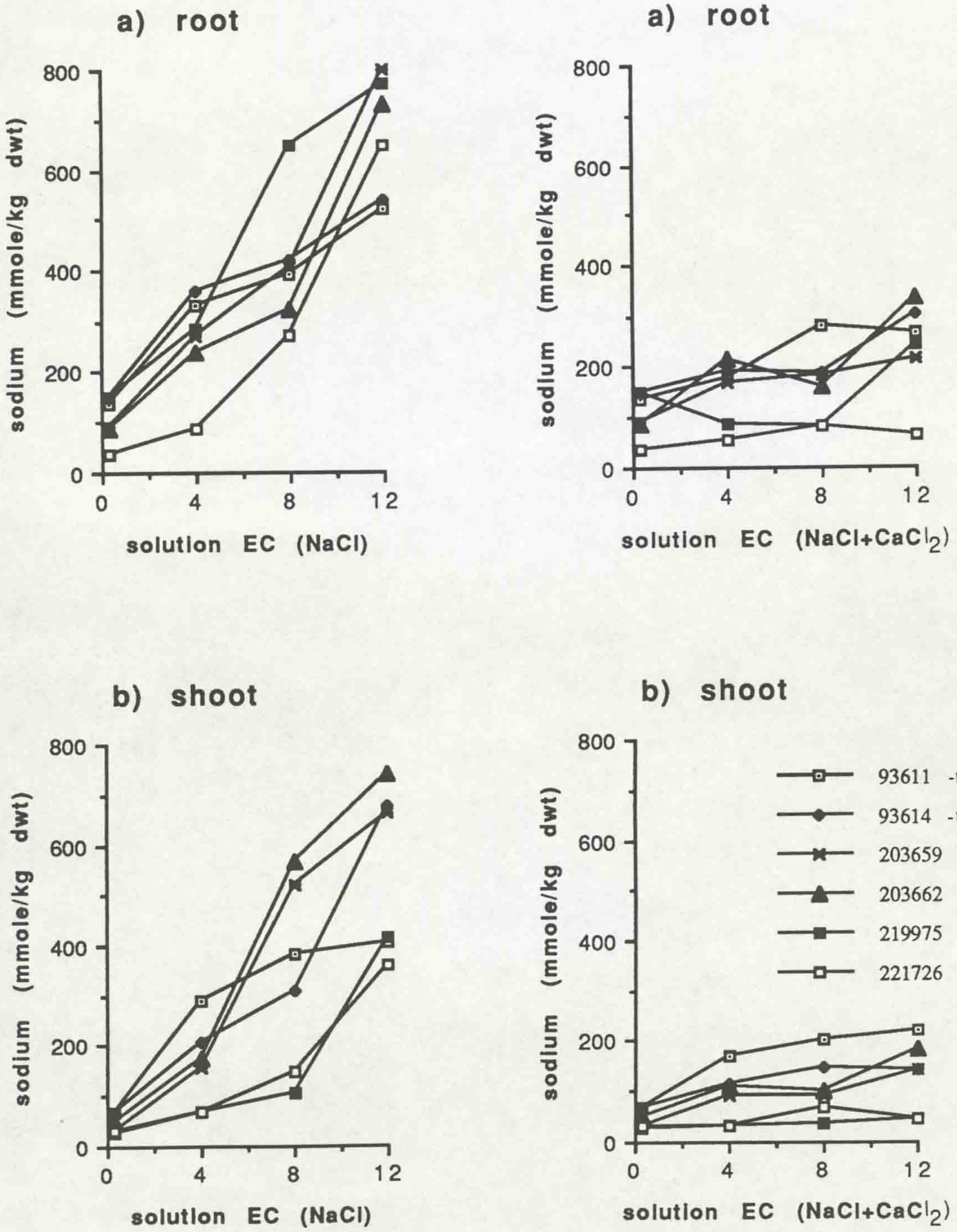
Accession-comparison for K^+ in shoots and Cl^- in roots have been made based upon the three-factor interaction in Figures 6.4b and 6.5a respectively. The concentration of Na^+ and K^+ in roots, and Na^+ and Cl^- in shoots of accessions have been compared in Figures 6.3 - 5.

6.4.1. Sodium content (Figure 6.3)

a) NaCl salinisation

In both roots and shoots accumulation of Na^+ increased significantly as the EC level increased. However there was no overall consistency in patterns of uptake. At EC 4, lowest concentration was observed in roots and shoots of the tolerant accession 221726. At EC 8, the increase in the concentration of Na^+ was greater in the roots of the sensitive 219975 and shoots of the other two sensitive accessions 203662 and 203659. In roots again the tolerant 221726 maintained the smallest Na^+ level while in shoots accessions 219975 (s) and 221726 (t) accumulated smaller level of Na^+. At EC 12 in roots, concentration of Na^+ was greater in the sensitive accessions 203659, 219975, and 203662 , and the tolerant 221726, whilst there was markedly increased Na^+ accumulation in shoots of accessions 93614 (t), 203659 (s), and 203662 (s). In

Figure 6.3. Accumulation of Na^+ in roots and shoots of six accessions grown at increasing EC levels due to NaCl, and $NaCl+CaCl_2$



roots the tolerant accessions 93611 and 93614 maintained lowest level of Na^+, whilst in shoots the two tolerant accessions 221726 and 93611, and the sensitive 219975 accumulated a lower levels of Na^+ at EC 12. Thus some degree of relationship can be observed with tolerance at EC 12, sensitives have highest Na^+.

b) $NaCl+CaCl_2$ salinisation

Figure 6.3 showed that Na^+ contents in plants grown in $NaCl+CaCl_2$ solutions was greater in roots than in shoots. Again the accessions did not show a consistent pattern in the increase of Na^+ as EC increases. At EC 12 in roots (Figure 6.3), the sensitive 203662 had greater Na^+ than the other accessions, whilst in shoots, the tolerant 93611 had greater Na^+ than the remaining accessions across all EC levels apart from the control. In contrast the other tolerant accession 221726 accumulated the smallest level of Na^+ both in roots and shoots (with exception at EC 8 in shoots), whilst the sensitive 219975 had a similar low Na^+ level in its shoots.

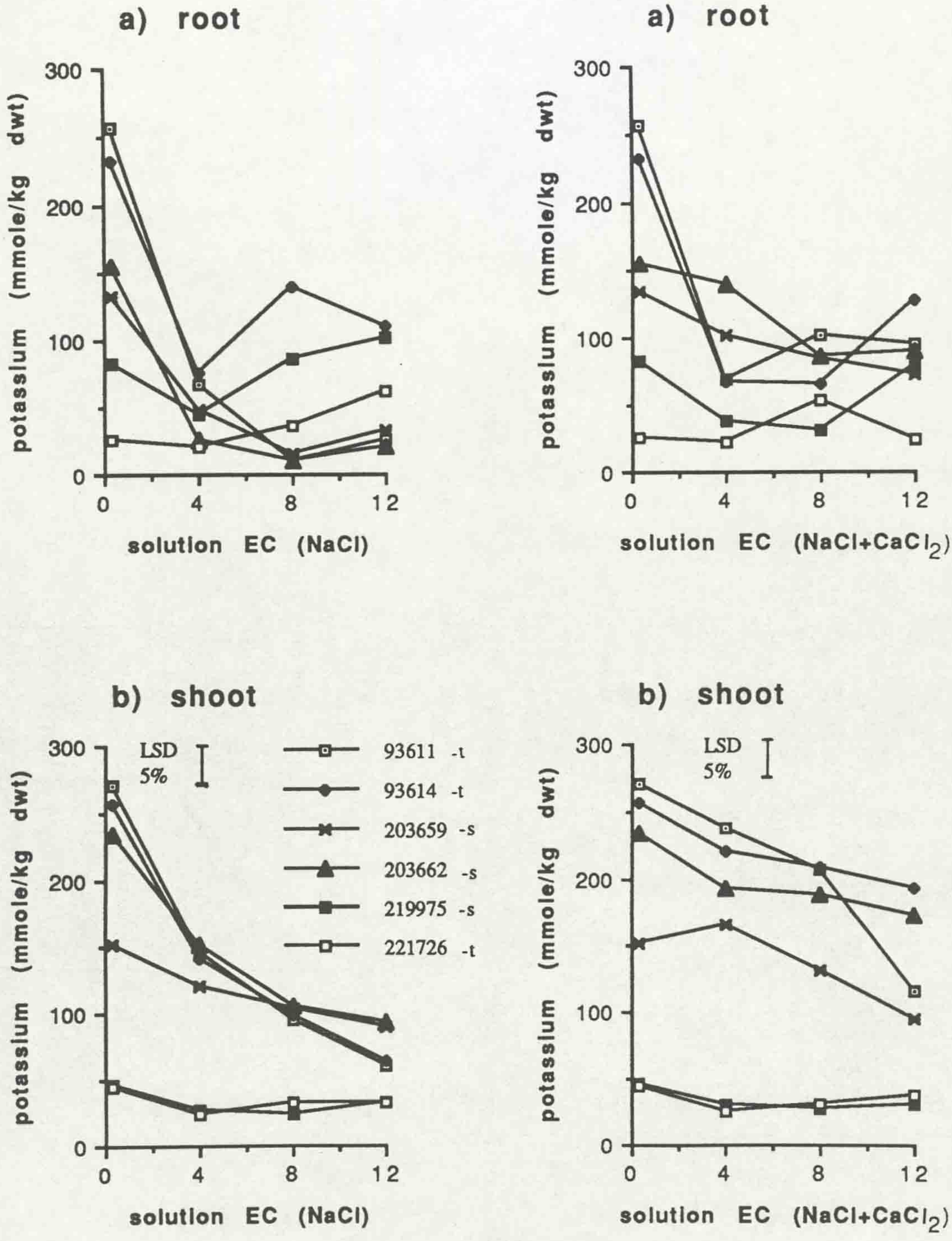
6.4.2. Potassium content (Figure 6.4)

a) NaCl salinisation

Differences were observed amongst accessions in K^+ concentration in roots and shoots and in general there was a decreasing pattern of K^+ content with increasing EC. However there was no consistent relationship with tolerance.

Both in control (EC 0.3) and EC 4, the tolerant accessions 93611 and 93614 had higher K^+ concentration in roots and shoots than the other four accessions. At EC 8, the roots of the tolerant 93614 accumulated markedly greater K^+ than the remaining accessions, whereas the equally tolerant 93611 had the lowest K^+ content in its roots as did the sensitive 203662. At EC 12, the tolerant 93614 and sensitive 219975 had greater K^+ level in their roots.

Figure 6.4. Accumulation of K^+ in roots and shoots of six accessions grown at increasing EC levels due to NaCl, and $NaCl+CaCl_2$



In shoots however accessions 219975 (s) and 221726 (t) had significantly lower K^+ concentration than the remaining accession at all EC levels). However at EC 12 in shoots, the sensitives 203659 and 203662 had significantly higher K^+ than both tolerant 221726 and sensitive 219975, whilst the other two tolerant accessions 93611 and 93614 had intermediate K^+ level at this EC level.

b) $NaCl+CaCl_2$ salinisation

Looking at Figure 6.4 again in general K^+ concentration in root and shoot of four of the six accessions decreased with increasing EC level whereas in accessions 221726 (t) and 219975 (s) there was no clear change in shoot K^+ contents. On the other hand in the roots of accessions 93614 (t) and 219975 (s) increasing EC reduced concentration up to EC 8, but K^+ content doubled at EC 12. Marked decreases of more than 100% in K^+ content of roots occurred between control EC and EC 4 in the tolerant accessions 93611 and 93614. At EC 12, the tolerant 93614 had highest K^+ level in roots, whilst the equally tolerant 221726 had by far the lowest root K^+ concentration.

In shoots, increasing EC levels did not affect the very low K^+ concentrations in accessions 219975 (s) and 221726 (t) and both differed significantly from the remaining accessions across all EC levels. The tolerant accessions 93611 and 93614 accumulated a higher K^+ concentrations in their shoots in control and EC 4, but they did not differ from the sensitive 203662 at EC 8, whilst at EC 12 the tolerant 93611 had a significant lower K^+ shoot content than either 93614 (t) and 203662 (s).

6.4.3. Chloride content (Figure 6.5)

a) NaCl salinisation

The Cl^- content of roots differed between accessions as a consequence of their being grown in NaCl alone, and NaCl+CaCl2, and the EC imposed by those solutions

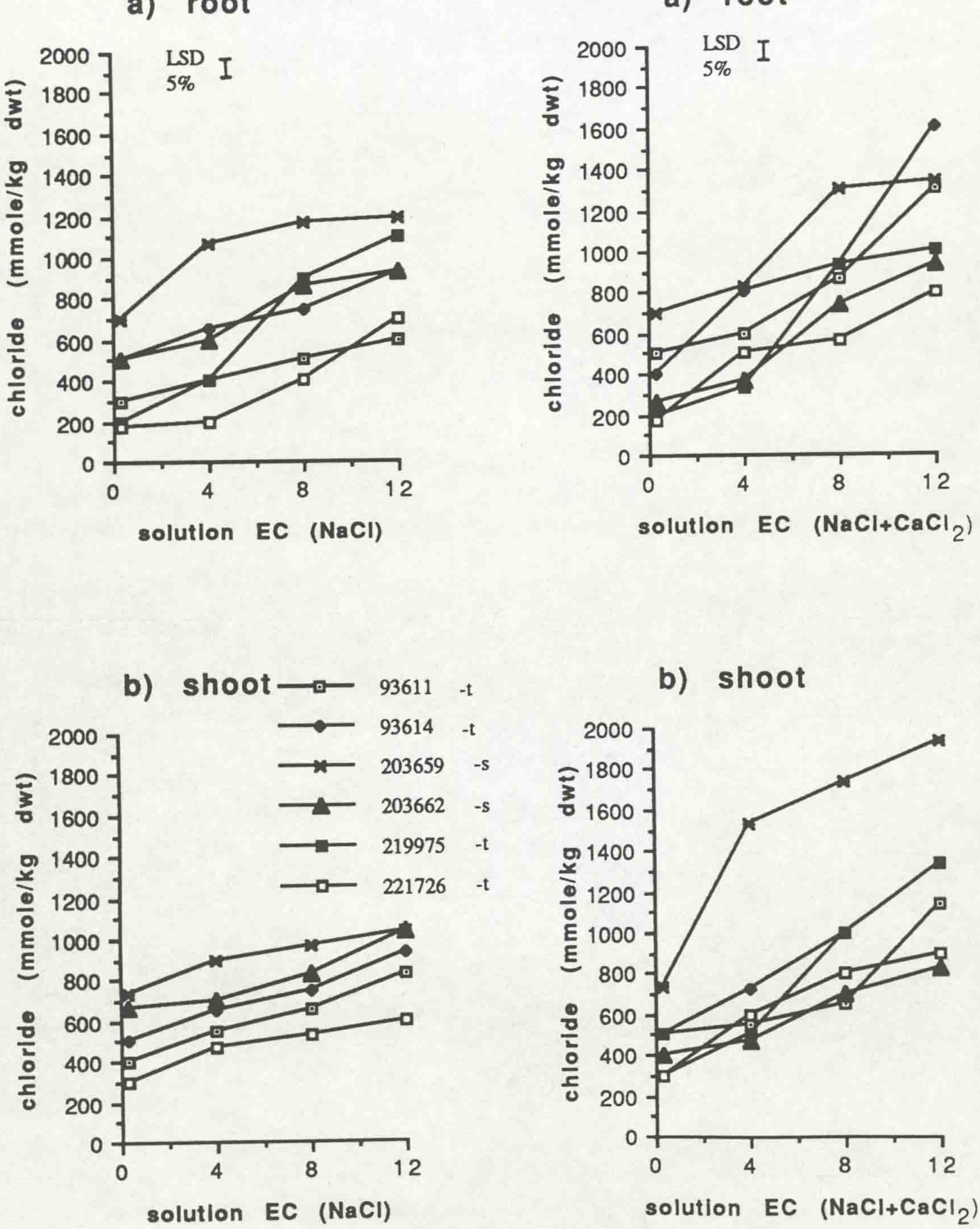
(Acc x Cond significant at $p<0.01$). Concentration of Cl^- in the tissues increased with increasing EC levels in both roots and shoots. In roots, the sensitive 203659 had significantly greater Cl^- than the remaining accessions up to EC 8, but had a similar Cl^- content as the sensitive 219975 at EC 12. A similar pattern was observed in the shoots, accession 203659 (sensitive) also accumulated the highest level of Cl^- in shoots up to EC 8, but had a similar Cl^- content as the sensitive 203662 at EC 12. By contrast the two tolerant accessions 221726 and 93611 accumulated significantly lower Cl^- in roots at EC 8 and 12 than the remaining accessions, and also had relatively smaller shoot Cl^-, as the other sensitive 219775, than the remaining accessions across all EC levels.

b) $NaCl+CaCl_2$ salinisation

The overall pattern of accumulation of Cl^- in both roots and shoots of accessions grown in $NaCl+CaCl_2$ was similar to that in NaCl alone, Cl^- content increasing with increasing EC. However the majority of accessions had a greater overall Cl^- content in roots and shoots at EC 8 and 12 than at the same EC in NaCl solutions (T significant at $p<0.001$). Accessions mean Cl^- content was greater in solutions with $NaCl+CaCl_2$ than in those with NaCl alone, and some accessions such as 203659 (sensitive) in shoots and at EC 12 accession 93614 (tolerant) in roots. In contrast the sensitive 203662 had lower Cl^- content in its roots and shoots in $NaCl+CaCl_2$ than in NaCl alone treatments.

Root Cl^- content differed significantly ($p<0.01$) in different accessions at different EC levels depending upon whether they were grown in NaCl alone or $NaCl+CaCl_2$ containing solutions.

Figure 6.5. Accumulation of Cl^- in roots and shoots of six accessions grown at increasing EC levels due to NaCl, and $NaCl+CaCl_2$



6.5. Discussion

Although saline soils are predominantly affected by Na^+ or Cl^- ions, cations of other salts, particularly of Ca^{2+}, are also of frequent occurrence (Shannon, 1984). This investigation was carried out to determine whether the responses of twelve pearl millet accessions to salinity due to NaCl alone, and NaCl+$CaCl_2$ differed, and whether ionic contents differed in these different solutions, with a view to determining reasons for differences, if any, in response.

Specific ion content and distribution within the plant has been suggested as a useful measure of salinity tolerance (Shannon, 1984). Previous studies on salt tolerance (Kawasaki and Moritusigu, 1979) showed that crop plants grown in saline substrates containing NaCl alone developed blade deformation and necrosis, characteristic of calcium deficiency, and NaCl salinity is also known to decrease the uptake of Ca^{2+} ions to the shoots in several plant species (Lynch and Läuchli, 1985). The elevated concentrations of Na^+ ions accumulated in the pearl millet accessions grown in solutions containing NaCl alone (Figure 6.3) compared with the same accessions grown in NaCl+$CaCl_2$ is in agreement with Wieneke and Läuchli (1980) who observed that increasing Ca^{2+} concentrations in the growth medium markedly decreased Na^+ uptake and translocation.

Yeo and Flowers (1986) suggested several physiological attributes such as leaf necrosis, and Na^+ and Cl^- concentrations in the tops of seedlings, that might correlate with salinity resistance in rice. They argue that individually these attributes are unlikely to be selected for in a conventional breeding programme, because each on its own will have little impact on the phenotype, but that when several such attributes are combined in a single genotype there will be a substantial effect. In the present study where accessions performance is examined across a range of characters accession 221726 had better performance in both NaCl and NaCl+$CaCl_2$ combined than the remaining accessions (Tables 6.2 - 3, Figures 6.1 - 5). Differences in salt tolerance between varieties within species are well documented, and genetically based salt tolerance clearly must be associated with mechanisms of mineral nutrient uptake (Rathert, 1982).

The results of previous investigations, for example in soybean (Wieneke and Läuchli, 1979; 1980) and in wheat (Ashraf and McNeilly, 1988) suggest that higher salt tolerance is associated with reduced shoot Na^+ and Cl^- concentrations in plants. However the latter authers found equivalent tolerance to NaCl in two wheat cultivars one of which excluded, and the other accumulated Na^+, yet both were tolerant. From the low Na^+ and Cl^- content of the plants of accession 221726, increased salinity tolerance may be related to exclusion of both these ions from roots and shoots, a characteristic of glycophytes (Shannon, 1984). In contrast accessions 203659 and 203662 are less tolerant particularly at the highest solution EC, and both had high Na^+ (Figure 6.3) and Cl^- (Figure 6.5) concentrations in their roots and shoots. Greater growth depression of seedlings of these two accessions (203659 and 203662) at higher salt concentration may have occurred due either to osmotic effects of salt stress, ion imbalance, or excessive accumulation of ions (Kingsbury *et al.*, 1984). The elevated Cl^- concentration in accession 203659 grown in NaCl+$CaCl_2$ (Figure 6.5) suggests the extent to which an inability to control ion accumulation may influence degree of salinity tolerance. This is in agreement with the findings of Bottacin *et al.* (1985), who reported that salt resistant genotypes of pearl millet contained less Na^+ and Cl^- in their leaves than susceptible genotypes when grown at 300 mM NaCl.

Variation in salt tolerance also affects the foliar concentrations of K^+, a nutrient required in relatively high concentrations for photosynthetic metabolism (Huber, 1985). In this study the concentration of K^+ in tissues declined with increase in salinity, a decline which may be due to high concentrations of Na^+ interfering with K^+ uptake by roots (Munns *et al.*, 1983).

As can be observed (Figures 6.1 - 2), on the whole, relative root and shoot lengths of the plants grown in NaCl+$CaCl_2$ were greater than when the same material was grown in NaCl alone at the same EC levels, whilst Na^+ concentration in plants grown in NaCl+$CaCl_2$ were lower than the plants grown in NaCl alone. Wieneke and

Läuchli (1985) suggested that in saline systems Ca^{2+} may become even more important, based on their observation that Na^{+} uptake in a salt sensitive soybean variety Jackson was inhibited by increasing Ca^{2+} concentrations in the medium. Matar *et al.* (1975) also ascribed symptoms of growth depression of plants under salt stress in part to insufficient Ca^{2+} supply.

Toxic effects of both Na^{+} and Cl^{-}, directly or indirectly, are well documented. Na^{+} and Cl^{-} exclusion from tolerant accessions of cotton during salinity stress is probably associated with metabolic reactions for osmotic adaptation, e.g. carbohydrate metabolism (Rathert *et al.*, 1981; Rathert, 1982). Na^{+} has been shown to inhibit enzyme activity during carbohydrate metabolism in cotton (Hawker *et al.*, 1974), whilst restricted plant growth under salinity stress may in part be due to Cl^{-} affected invertase activity (Rathert, 1982), and reduced invertase activity was correlated with inhibited leaf expansion rates in NaCl treated bushbeans and corn (Hawker and Walker, 1978).

Weimburg (1970) did not find any significant differences in the levels of 18 different enzymes from pea seedlings (cultivar Alasca) growth either in a liquid medium or in the same medium salinised with NaCl, KCl, Na_2SO_4 or K_2SO_4. On another occasion, Porath and Poljakoff-Mayber (1964, 1968) using pea root tips of the cultivar Laxton Progress investigated the effect of a range of salinities, either as NaCl or as Na_2SO_4. The changes that occurred led to an increase of the pentose-phosphate pathway under salinities caused by NaCl, whereas Na_2SO_4 had little effect in this respect. From comparisons of responses of the twelve accessions to NaCl alone, and NaCl+$CaCl_2$, it is evident that various salts may inhibit plant growth to different degrees (Figures 6.1 - 2), and also genotypes showing a better response to one salt may not necessarily do so in another (see Figures). However the response of accession 221726 (which was shown in this work to be tolerant) was consistent, being tolerant to salinity due both to NaCl alone, and NaCl+$CaCl_2$. Also the average measure of relationship between the relative values of accessions grown in NaCl alone, and NaCl+$CaCl_2$ at EC 12, the correlation coefficient (r), values 0.94 and 0.58 for relative

root length and relative shoot length respectively (Table 6.4), suggested at least group of accessiuons are equally tolerant to NaCl and the mixture of NaCl : $CaCl_2$, 1:1 by weight.

In conclusion it seems possible that Na^+ and Cl^- accumulation in roots or leaves may be used as an indicator for salt tolerance in *Pennisetum americanum* (L.) Leeke genotypes at least at the onset of salinity stress. Secondly the relative root and shoot lengths of the accessions grown in NaCl alone were markedly smaller than the relative root and shoot lengths of the accessions grown in NaCl+$CaCl_2$, and thirdly that some accessions differ in response to NaCl alone and NaCl+$CaCl_2$ whilst others do not.

GENERAL DISCUSSION

CHAPTER SEVEN

CHAPTER 7

GENERAL DISCUSSION

Excessive salinity is a major factor limiting plant life, and hence all life particularly in arid and semi-arid parts of the world and terrestrial environments subject to irrigation by sea water. Crops grown in these areas are frequently irrigated and irrigation frequently compounds difficulties with soil salinity. Irrigation water may contain from 100 to 1000 g of salt per cubic meter of water and since the annual application of water may amount to 10,000 m^3/ha, the annual addition of salt to the soil may be between 1 and 10 tons/ha (Shainberg, 1975). Of the possible strategies for coping with it, only that of ameliorating the soil and water has been extensively applied (Gates and Grismer, 1989). Expensive agricultural engineering and management techniques seem to be worldwide an impractical objective to cope with a continuously increasing demand for the burgeoning human population in arid and semi-arid areas. The alternative option is genetic manipulation of crops to adapt them to saline conditions (Epstein *et al.*, 1980), a procedure which would allow the use of brackish water for irrigation (Ramage, 1980).

As stated previously, in Ethiopia, salt land covers 200,000 km^2 and about 9% of the total population live in this area (Sissay, 1986). Since any technological approach for manipulating environmental factors to remove the problem of salinity (in this case manipulating both soil and water), is quite impossible financially for Ethiopia, the alternative option is to develop salt-tolerant plant material to suit saline conditions. An option which Epstein *et al*. (1980) term 'better crops for the soils we have than better soils for the crops we have'.

Vavilov in 1951 concluded that the Ethiopian region was an important primary or secondary centre of domestication for some 38 different crop species. Other scientists have also reported the existence within many of the cultivated crops in Ethiopia of considerable genetic diversity, with some of the variations being rare and possibly unique (Zohary, 1970; Munk, 1972; Frankel, 1973). However, whether or not this genetic diversity encompasses salt tolerance would be a subject for investigation.

The potential for increase in salt tolerance within a species is of course constrained by the extent of variability for that tolerance within the existing gene pool. Variation in salt tolerance exists between and within species, especially among species with halophilic tendencies such as sugar beet and date palm. In some species the variability in salt tolerance that is available may be inadequate, or their general lack of tolerance may be too low to result in a successful breeding programme. In such cases it might be possible to find salt tolerance wild relatives which can then be exploited as a source of germplasm. Often wild relatives of crop plants have greater levels of salt tolerance, and these are being used in crosses to increase the range of genetic variability in crop breeding programmes (Downton, 1984). Because crop plants differ quite markedly in their level of salt tolerance, the effect of salinity on yield is a function of the threshold salinity level above which yield declines and the percentage yield decrease per unit of salinity increase above the threshold (McWilliams, 1986).

The work described in this thesis was designed to gain initial understanding of the relative tolerances of the three minor millets, *Pennisetum americanum*, *Eleusine coracana* and *Eragrostis tef*, and of the extent and nature of genetic variability within them, assessing material mainly of Ethiopian origin, with a longer term aim to exploit such variation in the development of lines with considerably enhanced salt tolerance.

The water culture technique employed in the series of experiments for assessing variability ensures that the chemical features of the root environment - concentrations of individual ions, total salinity and pH-are defined and such experiments are conducted in controlled environments in a growth room. The entire root system is uniformly exposed to a saline medium and the plants can be recovered without injury to the roots or shoots, for measurement, chemical analysis, or transfer to other medium (Epstein *et al.*, 1980). Use of simple measures of root lengths or shoot lengths of two or three week old seedlings has revealed genetically based variation in response to metals as well as to salinity in a range of, for example aluminium tolerance in barley and wheat varieties (Foy *et al*., 1965), copper tolerance in *Agrostis capillaris* (McNeilly and Bradshaw, 1968), salt tolerance in *Agrostis stolonifera* and *Festuca rubra* (Hannon and Bradshaw, 1968), in seven grass species (Ashraf *et al*., 1986a, b), and in *Sorghum bicolor* (Azhar and McNeilly, 1987).

From the results of the series of experiments described in Chapters 2 and 6 it is

clear that there is considerable variability in root and shoot growth in saline solution cultures in the three species examined. In analysing the response functions of the accessions of the three species, the computer programme, 'SALT' (van Genuchten and Hoffman, 1984) allows estimates of the parameters that quantify accession response to increasing salinity. In this particular study two statistical analysis options (NOPT 5 and NOPT 12) were used to estimate the C_t (the threshold concentration defined as the maximum soil salinity at which there is no yield/growth reduction when compared with the yield/growth under non saline control conditions), and C_{50} (the salinity at which the yield/growth is reduced by 50%). Both options (Chapter 2) give similar results although option 12, a sigmoid - form curve, fits the observed data slightly better than option 5, which van Genuchten and Hoffman term a piecewise response function. The results also suggest that their ranking based on a simple linear response to salt, and C_t values obtained from option 5, and C_{50} value obtained from option 12 seem to be reliable parameters for screening minor millets and tef germplasm because they clearly reflect the salt tolerance ranking of the accessions of the three species. Furthermore the combination of the two parameters would provide a better evaluation for selection for salinity tolerance. C_0 seems to be less relevant because of lack of consistency between the values generated by the two analysis options.

The existence of variability in any character, salinity tolerance in the present case, which is the subject of a plant breeding programme aiming to improve the character is of fundamental importance and has been emphasised by all workers interested in salinity tolerance improvements. The variation found in the seedling responses of 25 accessions of each of *P. americanum* and *E. coracana*, and 15 accessions of *E. tef* to increasing NaCl concentrations (Chapter 2) is clearly an indication of their differing abilities to grow under salinity stress and hence potential inter-accession variability within these species. From the differences between accessions found within the three species, accessions 215663 and 221726 in pearl millet, 100021, 100022, 100024, and 100030 in finger millet, and 494188, 494213, and 524436 in tef are those from which selection for enhancement of salt tolerance would seem worthwhile (see Chapter 2). These data also indicate that finger millet is the most inherently tolerant species, and pearl millet the least tolerant, tef being intermediate.

For selection to be successful it is of course necessary that the variability observed in root lengths in response to salinity has a genetic basis. The diallel crossing and analysis (Chapter 3) provide preliminary information about the inheritance of salt tolerance in *P. americanum*. Genetic variation for the character appeared to be influenced predominantly by genes with dominance effects. The additive variation was much greater at 75 mM NaCl than at 175 mM NaCl. This clearly relates to the arguments of Lawrence (1984) that population under strong directional selection (in this case higher concentration of 175 mM NaCl) would always tend to show relatively low values for the additive component of variation (narrow sense heritability) for the character under selection. It is also clear that two of the salt-tolerant accessions (221726 and Kitui Local) possessed maximum number of dominant genes, whilst the two salt-sensitive accessions (203659 and 203662) maintained the maximum number of recessive genes. The results thus suggest that selected salt-tolerant phenotypes may maintain their tolerance through subsequent generations and that this phenomenon is under polygenic control, with significant dominance being towards tolerance, confirming the view of Ashraf and McNeilly (1992). Comparable data for finger millet and tef are not yet available. Nonetheless, estimated broad sense heritability (h^2_B) values for salt tolerance in *E. coracana* and *E. tef* under different salt concentrations indicated that variation in root growth in response to salinity was largely genetically controlled.

It is clear that salinity affects plant growth during all developmental stages (Maas and Hoffman, 1977; Shannon, 1985; Maas *et al*., 1986; Maas and Poss, 1989; Azhar and McNeilly, 1989). From the sand culture experiment assessing salinity tolerance of pearl millet accessions throughout the whole plant development, it is of interest that the tolerant accession 221726, the tolerance of which was suggested from the findings in Chapter 2, was also the most tolerant accession based on measurements in mature adult plants of plant height, percentage live leaves and dry matter weights (root, stem, leaf sheath and leaf blade). This is in agreement with the work on artificial selection for increased salt tolerance in seven grass species reported by Ashraf *et al*. (1986a, b) which was based upon selecting three-week-old seedlings with longest root lengths under salt stress conditions in solution culture. Individuals expressing the greatest root growth in saline solutions subsequently yielded more dry matter and

higher tiller numbers at the adult plant stage in saline irrigated sand culture than those of the unselected lines/individuals. Seedlings from a number of species selected on this basis have superior performance in saline conditions as adults, and as adults obtained from the intercrossing of lines selected originally as seedlings (McNeilly, 1990).

It is well documented that salinity induces metabolic changes in plants and these cause suppression of growth, and may ultimately lead to the death of the plant at higher salinity levels (Shannon, 1978). The cytoplasm of a tolerant plant can adapt to high salt concentrations, or it may exclude toxic salts while accumulating or producing other osmotica to prevent osmotic water loss (Flowers, *et al*., 1977; Greenway and Munns, 1980). However, to date the identification of a common mechanism for the physiological basis of salinity tolerance in crops is far from being fully understood.

It is widely known however that salt tolerance can involve both avoidance and tolerance mechanisms. Analysis of plant material for specific ions has been suggested as a useful measure of salinity tolerance since differences in the degree of ion exclusion has been cited as a major differences between salt-sensitive and salt-tolerant crop cultivars (Shannon, 1984). In the present study (Chapter 5), in general the amount of Na^+ and Cl^- accumulated in roots and shoots of 14-day-old seedlings of accessions of *P. americanum* increased with increasing salinity, and the increase was much higher in the salt-sensitive accessions than in salt-tolerant accessions which is in agreement with Shannon (1984). By contrast K^+ concentration decreased with increasing salinity, but the extent of K^+ selectivity in the tolerant accessions was also higher than in the sensitive accessions, which was also confirmed by the pattern of Na^+/K^+ ratios.

The results in chapter 6 were also quite interesting. When parameter estimates of relative root lengths, relative shoot lengths, and Na^+ and Cl^- contents of both roots and shoots are combined to assess tolerance of accessions under two different salinity regimes (NaCl and $NaCl+CaCl_2$) across a range of concentrations, the tolerant accession 221726 showed better performance in both solutions. When the same comparison was made, particularly, at the highest salinity level the sensitive accessions (203659 and 203662) had less relative root and shoot lengths, but higher Na^+ and Cl^- concentrations in both roots and shoots. This is in general agreement with a general

situation described in glycophytes that the exclusion of Na^+ and Cl^- from the tissues, and an assured supply of K^+ in the tissues, are amongst the most important features of salinity tolerant material. A similar observation was reported by Bottacin *et al.* (1985), who showed that salt-tolerant genotypes of pearl millet maintained less Na^+ and Cl^- in their leaves than sensitive genotypes when grown at 300 mM NaCl. Another feature associated with salinity tolerance which is also considered as an important component of tolerance is the accumulation of organic solutes which function in osmotic adjustment. However in the present study it was shown that both tolerant and susceptible accessions accumulated more organic solutes (amino acids, proline and polyols) with increasing salinity levels, the pattern of increase being higher in the tolerant accessions than in the sensitive accessions. The extent of polyol accumulation with respect to increasing salinity would make polyols a possible candidates as markers. In conclusion it can be assumed that Na^+ and Cl^- accumulation in combination with increased polyol concentration could be indicators for salt tolerance in *P. americanum*. However this study was carried out only on two-week-old seedlings, and any generalisation of this nature should involve information about responses at the whole plant level. Clearly further investigation throughout the whole of plant development would be required to determine the usefulness of such characteristics. Nevertheless the summary made by McNeilly (1990), based on the knowledge and experience acquired from previous workers in heavy metal tolerance (Bradshaw and McNeilly, 1981) and in salinity tolerance (Ashraf *et al*., 1986a), emphasises the vital role of simple selection and breeding experiments using root length differences as the basis of selection to achieve tolerant accessions and/or genotypes with or without existence of background physiological knowledge. This is indeed undisputable and might offer a degree of optimism for sensitive tolerance breeding.

Salinity is a perennial problem as long as man continues to practice conventional field - based agricultural production. In developing countries the problem is more acute (McWilliams, 1986). More tolerant crop varieties are required to help improve and stabilise agricultural production. Adaptation to salt stress will require integration and co-ordination of many individual responses at the whole plant level, and a similar

relationship is required between plant breeders, geneticists, plant physiologists and biochemists in combining within single lines salt tolerance with good agronomic characteristics and pest/insect resistance.

The work described in this thesis is a systematic application of basic procedure to improve salt tolerance in particular in pearl millet, starting with examining variation between and within accessions. The use of the rooting method and selection at seedling stage seem to be a valid and worthwhile means of identifying tolerant individuals and ultimately enhancing salinity tolerance in pearl millet, where accessions selected at the seedling stage were shown to be tolerant also at the adult stage, and this character is under polygenic control with significant dominance being towards tolerance.

Finally future germplasm exploration and collection expeditions in Ethiopia will concentrate in collecting germplasm of millet and other species from salt affected regions of the country.

REFERENCES

REFERENCES

Abel, G.M. 1969. Inheritance of the capacity for chloride inclusion and chloride exclusion by soybean. Crop Sci. 9, 697–698.

Abel, G.H. and McKenzie, A.J. 1964. Salt tolerance of soybean varieties (*Glycine max* L. Merril) during germination and later growth. Crop Sci. 4, 157–161.

Abdul-Halim, R.k., Salih, H.M., Ahmed, A.A. and Abdul Rahem, A.M. 1988. Growth and development of maxipak wheat as affected by soil salinity and moisture levels. Plant and Soil 112, 255–259.

Akbar, M., Yabuno, T. and Nakao, S. 1972. Breeding for saline resistant varieties of rice. I. Variability for salt tolerance among some rice varieties. Japan J. Breeding 22, 277–284.

Akbar, M. and Ponnamperuma, F.N. 1982. Saline soils of south and south east Asia as potential rice land. Rice research strategies for the future, Los Banõs, Philippines, pp. 265–281.

Albert, R. and Popp, M. 1977. Chemical composition of halophytes from the Neusiedles lake region in Australia. Oecologia (Berl.) 27, 15–70.

Al-Khatib, M.M. 1991. An assessment of the potential for improving salt tolerance in alfalfa, *Medicago sativa* (L.). PhD. Thesis, the University of Liverpool, UK, pp. 146–175

Al-Khatib, M., McNeilly, T. and Collins, J.C. 1993. The potential of selection and breeding for improved salt tolerance in lucerne (*Medicago sativa* L.). Euphytica 65, 43–51.

Allen, S.G., Dobrenz, A.K., Shonhorst, M.H. and Stoner, J.E. 1985. Heritability of NaCl tolerance in germinating alfalfa seeds. Agron. J. 77, 99–101.

Ashraf, M. and McNeilly, T. 1987. Salinity effects on five cultivars/lines of pearl millet [*Pennisetum americanum* (L.) Leeke]. Plant and Soil 103, 13–19.

Ashraf, M., McNeilly, T. and Bradshaw, A.D. 1986a. The potential for evolution of salt (NaCl) tolerance in seven grass species. New Phytol. 103, 299–309.

Ashraf, M., McNeilly, T. and Bradshaw, A.D. 1986b. The response of selected salt-tolerant and normal lines of grass species to NaCl in sand culture. New Phytol. 104, 453–461.

Ashraf, M., McNeilly, T. and Bradshaw, A.D. 1987. Selection and heritability of tolerance to sodium chloride in four forage species. Crop Sci. 27, 232–234.

Ashraf, M. and McNeilly, T. 1989. Effect of salinity on some cultivars of maize. Medica 34, 179–189.

Ashraf, M. and Waheed, A. 1990. Screening of local/exotic accessions of lentil (*Lens culinaris* Medic.) for salt tolerance at two growth stages. Plant and Soil 128, 167–176.

Ashraf, M. and McNeilly, T. 1992. The potential for exploiting variation in salinity tolerance in pearl millet [*Pennisetum americanum* (L.) Leeke]. Plant Breeding 108, 234–240.

Aspinall, D. and Paleg, L.G. 1981. Proline accumulation: Physiological aspects. In: L.G. Paleg and D. Aspinall (Eds.), Physiology and biochemistry of drought resistance in plants, Academic Press, Australia, pp. 206–207.

Azhar, F.M. 1988. The potential for breeding *Sorghum bicolor* (L.) Moench for saline conditions. PhD. Thesis, The University of Liverpool, UK, pp. 132–161.

Azhar, F.M. and McNeilly, T. 1987. Variability for salt tolerance in *Sorghum bicolor* (L.) Moench under hydroponic conditions. J. Agron. and Crop Sci. 159, 269–277.

Azhar, F.M. and McNeilly, T. 1988. The genetic basis of variation for salt tolerance in *Sorghum bicolor* (L.) Moench seedlings. Plant Breeding 101, 114–121.

Azhar, F.M. and McNeilly, T. 1989. The response of four sorghum accessions/cultivars to salinity during whole plant development. J. Agron. and Crop Sci. 163, 33–43.

Bal, A.R. and Dutt, S.K. 1986. Mechanism of salt tolerance in wild rice (*Oryza coarctata* Roxb). Plant and Soil 92, 399–404.

Binzel, M.L., Hasegawa, P.M., Rhodes, D., Handa, A.K. and Bressan, R.A. 1987. Solute accumulation in tobacco cells adapted to NaCl. Plant Physiol. 84, 1408–1415.

Bernstein, L. and Pearson, G.A. 1956. Influence of exchangeable sodium on the yield and chemical composition of plants. Green beans, garden beets, clover and alfalfa. Proceedings, Soil Sci. Soc. America 82, 247–258.

Bernstein, L. and Hayward, H.E. 1958. Physiology of salt tolerance. Ann. Rev. Plant

Physiol. 9, 25.

Black, R.F. 1956. Effect of NaCl in water culture on the ion uptake and growth of *Atriplex hastata* L. Aust. J. Biol. Sci. 9 (1), 67–80.

Blum, A. 1988. Plant breeding for stress environments. CRC Press. Inc. Boca Raton, Florida, pp.163–179.

Bok, S.H. and Demain, A.L. 1977. An improved colorimetric assay for polyols. Analytical Biochemistry 81, 18–20.

Bottacin, A., Cacco, G. and Saccomani, M. 1985. Nitrogen absorption and assimilation in NaCl-resistant and NaCl-sensitive pearl millet genotypes (*Pennisetum americanum*). Cand. J. Bot. 63, 517–520.

Bradshaw, A.D. and McNeilly, T. 1981. Evolution and Pollution. Edward Arnold, London.

Briens, M. and Larher, F. 1982. Osmoregulation in halophytic higher plants: A comparative study of soluble carbohydrates, polyols, betaines and free proline. Plant, Cell and Environment 5, 287–292.

Burton, G.W. 1952. Quantitative inheritance in grasses. Proceedings of Sixth International Grassland Congress, Pennsylvania State College, USA, pp. 277–283.

Burton, G.W. 1958. Cytoplasmic male sterility in pearl millet, *Pennisetum Americanum* (L.). R. Br. Agron. J. 40, 230.

Burton, G.W. 1974. Factors affecting pollen movement and natural crossing in pearl millet. Crop Sci. 14, 802–805.

Burton G.W. 1980. Pearl millet. In: W.R. Fehr and H.H. Hadley (Eds.), Hybridyzation of crop plants. American Society of Agronomy–Crop Science Society of America, Madison, Wisconsin, USA, pp. 457–469.

California Department of Water Resources, 1984. Office memorandum on lands under irrigation in the drainage problem. San Joaquin District.

Chapman, V.J. 1968. Vegetation under saline conditions. In: H. Boyko (Ed.), Saline irrigation for agriculture and forestry. Junk Publisher, The Hague, pp. 201–216.

Chapman, V.J. 1975. The salinity problem in general, its importance and distribution with special reference to natural halophytes. In: A. Poljakoff-Mayber and J.

Gale (Eds.), Plant in saline environments. Springer-Verlog, New York, pp. 7–21.

Clipson, N.J.W., Lachno, D.R. and Flowers, T.J. 1988. Salt tolerance in halophyte *Suaeda maritima* L. Dum–Abscisic acid concentrations in response to constant and altered salinity. J. Exp. Bot. 39 (207), 1381–1388.

Crumpacker, D.W. and Allard, R.W. 1962. A diallel cross analysis of heading data in wheat. Hilgardia 32, 275–318.

Dix, P.J. and Pearce, R.S. 1981. Proline accumulation in NaCl resistant and sensitive cell lines of *Nicotiana sylvestris*. Z. Pflanzenphysiolgie 102, 243–248.

Dewey, D.R. 1960. Salt tolerance of twenty five strains of *Agropyron*. Agron. J. 52, 631–635.

Dickinson, A.G. and Jinks, J.L. 1956. A generalised analysis of diallel cross. Genetics 41, 65–78.

Downton, W.J.S. 1984. Salt tolerance of food crops: perspectives for improvements. CRC Critical. Rev. Plant. Sci. 1, 183–201.

Dobzhansky, Th. 1970. Genetics of evolutionary process. Columbia Univ. Press, New York.

Ebba, T. 1969. Tef (*Eragrostis tef*): The cultivars usage and some of the known diseases and insect pests, Part I, Agri. Expt. Sta. Bull. No. 60, HSIU, College of Agriculture, Dire Dawa, Ethiopia, pp.1–56.

Ekanayake, I.J., O'Toole, J.C., Garrity, D.P. and Masajo, T.M. 1985. Inheritance of root characters and their relations to drought resistance in rice. Crop Sci. 25, 927–932.

Epstein, E. 1977. Adaptation of crops to salinity. In: M.J. Wright (Ed.), Proceedings of the Workshop on Plants Adaptation to Mineral Stress in Problem Soils, Agric. Exp. Stn., Special Publ., Ithaca, New York, pp. 73–82.

Epstein, E. 1980. Response of plants to saline environments. In: D.W. Rains, R.C. Valentine and A. Hollaender (Eds.), Genetic engineering of osmoregulation: Impact on plant productivity for food, chemicals and energy, Plenum Press, New York and London, 14, 7–21.

Epstein, E. and Norlyn, J.D. 1977. Sea-water based crop production: A feasibility study. Sci. 197, 249–251.

Epstein, E., Norlyn, J.D., Rush, D.W., Kingsbury, R.W., Kelley, D.W., Cunningham, G.A. and Wrona, A.F. 1980. Saline culture of crops: A genetic approach. Sci. 210, 399–404.

Ethiopian Statistical Abstract, 1980. Central Statistical Office, Addis Ababa, pp. 304.

FAO-UNESCO (Food and Agriculture Organisation-United Nations Educational, Scientific and Cultural Organization), 1974. Soil map of the world, Legend 1:500,000, Paris.

Falconer, D.S. 1981. Introduction to quantitative genetics. 2nd. Ed. Longman, London and New York.

Falconer, D.S. 1989. Introduction to quantitative genetics. 3rd Ed. Longman, Hong Kong.

Fitzpatrick, E.A. 1980. Properties of soil horizon. In: Soils-their formation, classification and distribution, Longman Inc., New York, pp. 81–119.

Flowers, T.J. 1972. Salt tolerance in *Suaeda maritima* (L.) Dum: the effect of NaCl on growth, respiration and soluble enzymes in a comparative study with *Pisum sativum* L. J. Exp. Bot. 23, 310–321.

Flowers, T.J. 1975. Halophytes. In: D.A. Bakes and J.L. Hall (Eds.), Ion transport in plant cells and tissues, Elsevier: Amsterdam, pp. 309–334.

Flowers, T.J. 1985. Physiology of halophytes. Plant and Soil 89, 41–56.

Flowers, T.J. and Yeo, A.R. 1986. Ion relations of plants under drought and salinity. Aust. J. Plant Physiol. 13, 75–91.

Flowers, T.J., Troke, P.F. and Yeo, A.R. 1977. The mechanism of salt tolerace in halophytes. Ann. Rev. Plant Physiol. 28, 89–121.

Foy, C.D., Armiger, W.H., Briggle, L.W. and Reid, D.A. 1965. Differential aluminium tolerance of wheat and barley varieties on acid soils. Agron. J. 57, 413–417.

Francois, L.E. 1985. Salinity effects on germination, growth and yield of two squash cultivars. HortScience 20 (6), 1102–1104.

Francois, L.E., Maas, E.V., Donovan, T.J. and Younger, V.L. 1986. Effect of salinity on grain yield and quality, vegetative growth and germination of semi-dwarf and durum wheat. Agron. J. 78, 1053–1058.

Frankel, O.H. 1973. Genetic resources survey as a basis for exploration. In: O.H.

Frankel and J.G. Hawkes (Eds.), Crop genetic resources for today and tomorrow, IBP2, Cambridge University Press, Cambridge, pp. 99–109.

Frankel, O.H. 1977. Natural variation and its conservation. In: A. Muhammed, R. Aksel and R.C. von Borstel (Eds.), Genetic diversity in plants, Plenum Press, New York and London, pp. 21–44.

Frankel, O.H. and Bennett, E. 1970. Genetic resources. In: O.H. Frankel and E. Bennett (Eds.), Genetic resources in plants: their exploration and conservation, Blackwell, Oxford, pp. 7–17.

Gale, J. 1975. Water balance and gas exchange of plants under saline conditions. In: A. Poljakoff-Mayber and J. Gale (Eds.), Plant in saline environments. Springer-Verlog, New York, pp. 168–185.

Gates, T.K. and Grismer, M.E. 1989. Irrigation and drainage strategies in salinity-affected regions. Journal of Irrigation and Drainage Engineering 115 (2), 255–284.

Gibbon, D. and Pain, A. 1985. Crops of the drier regions of the tropics. Longman, and New York, pp. 90–95.

Greenway, H. 1965. Plant response to saline substrates. III. Growth and ion uptake throughout plant development in two varieties of *Hordeum vulgare*. Aust. J. Biol. Sci. 18, 763–774.

Gorham, J., Wyn Jones, R.G. and McDonnell, M. 1985. Some mechanism of salt tolerance in crop plants. Plant and Soil 89, 15–40.

Greenway, H. and Munns, R.G. 1980. Mechanism of salt tolerance in nonhalophytes. Ann. Rev. Plant Physiol. 31, 149–190.

Greenway, H. and Rogers, A. 1963. Growth and ion uptake of *Agropyron elongatum* on saline substrates, as compared with a salt-tolerant *Hordeum vulgare*. Plant and Soil 18, 21–30.

Gregoria, G.B. and Senadhira, d. 1993. Genetic analysis of salinity tolerance in rice (*Oryza sativa* L.). Theor. Appl. Genet. 86, 333–338.

Griffing, B. 1956. Concept of general and specific combining ability in relation to diallel crossing systems. J. Biol. Sci. 9, 463–493.

Hannon, N. and Bradshaw, A.D. 1968. Evolution of salt tolerance in two coexisting pecies of grass. Nature 220, 1342–1343.

Hanson, W.D. 1963. Heritability. In: W.D. Hanson and H.F. Robinson (Eds.), Statistical genetics and plant breeding. Natural Acad. Sci.–Natural Research Council, Washington D.C., Publ. 982, pp. 125–139.

Hawker, J.S. and Walker, R.R. 1978. The effect of sodium and chloride expansion rates and invertase activity of leaves. Aust. J. Plant Physiol. 5, 73–80.

Hawker, J.S., Marschner, H. and Downton, W.J.S. 1974. Effect of sodium and potassium on starch synthetase in leaves. Aust. J. Plant Physiol. 1, 491–501.

Hayman, B.I. 1954a. The theory and analysis of diallel crosses. Genetics 39, 789–809.

Hayman, B.I. 1954b. The analysis of variance of diallel cross. Biometrics 10, 235–245.

Hannon, N. and Bradshaw, A.D. 1968. Evolution of salt tolerance in two coexisting species of grass. Nature 220, 1342-1343.

Hellebust, J.A. 1976. Osmoregulation. Ann. Rev. Pl. Physiol. 27, 485–505.

Hewitt, E.J. 1966. Sand and water culture methods used in the study of plant nutrition. 2nd. Ed. Comm. Agri. Bur. Tech. Comm. No. 22.

Huber, S.C. 1985. Role of potassium in photosynthesis and respiration. In: R.D. Munson (Ed.), Potassium in agriculture, American Soc. Agron., Madison, Wisconsin, pp. 369–396.

Ibrahim, K. M. 1990. Production of variation in salt tolerance in ornamental plants. Ph.D. Thesis, University of Liverpool, UK, pp. 98–120.

Jacoby, B. and Ratner, A. 1974. Mechanism of sodium exclusion in beans and crop plants–a re-evaluation. In: J. Wehman (Ed.), Plant analysis and fertiliser problems, German Society of Plant Nutrietion, Hannover, 1, 175–184.

Jafari, M. 1990. The potential for improvement of salinity and drought tolerance in some semi-arid forage grasses. Ph.D. Thesis, University of Liverpool, UK, pp. 11–14.

Jauhar, P.P. 1981. Cytogenetics and breeding of pearl millet and related species. Alan R. Liss. Inc. New York.

Jeschke, W.D. 1984. K^+ - Na^+ exchange at cellular membranes, intracellular compartmentation of cations, and salt tolerance. In: R.C. Staples and G.H. Toenniessen (Eds.), Salinity tolerance in plants: Strategies for crop

improvement, A Willey–Interscience Publication, New York, Chichester, Brisbane, Toronto, Singapore, pp. 37–66.

Jinks, J.L. 1954. The analysis of continuous variation in a diallel cross of *Nicotiana rustica*. Genetics 39, 767–788.

Jinks, J.L. 1955. A survey of genetical basis of heterosis in a variety of diallel crosses. Heredity 9, 223–238.

Jinks, J.L. 1956. The F_2 and backcross generation from a set of diallel crosses. Heredity 10, 1–30.

Jones, R.W. 1984. Studies related to salt tolerance in cucumber. Diss. Abstr. Int. B. 45, 1376.

Jones, R.A. and Qualset, C.O. 1984. Breeding crops for environmental stress tolerance. In: G.B. Collins and J.G. Petolino (Eds.), Application of genetic engineering to crop improvement, Junk Publisher, Dordrecht, Boston, Cancaster, pp. 305–340.

Jones, R.W. and Stenhouse, J.W. 1984. Inheritance of salt tolerance in mangrove rice. Int. Rice Res. Newsl. 9 (3), 9.

Johnston, W.R., Steninert, B.C. and Stroh, C.M. 1982. Benefits from drainage of heavy irrigated soils. Advances in Drainage, ASAE, St. Joseph. Mich.

Kaminiski, R. 1977. Variability and heritability of morphological and physiological characters of potato. Genet. pol. 18, 115–123.

Kawasaki, T. and Moritsugu, M. 1979. A characteristic symptom of calcium deficiency in maize and sorghum. Comm. Soil Sci. Pl. Anal. 101, 41–56.

Kearsey, M.J. 1965. Biometrical analysis of a random mating population: Comparison of five experimental designs. Heredity 20, 205–235.

Kebebew, F. and Adhanom, T.M. 1986. The status of plant genetic resources in the northern Ethiopia. PGRC/E–ILCA Germplasm Newsletter, No. 11, pp. 32.

Kernick, M.D. 1986. Forage plants for salt-affected areas in developing countries. Reclamation and Revegetation Research 5, 451–459.

Kik, C. 1989. Ecological genetics of salt resistance in the clonal perennial, *Agrostis stolonifera* L. New Phytol. 113, 453–458.

Killick, R. 1977. Genetic analysis of several traits in potato by means of diallel cross. Ann. App. Biol. 86, 279–289.

Kingsbury, R.W. and Epstein, E. 1984. Selection for salt-tolerant spring wheat. Crop Sci. 24, 310–315.

Kingsbury, R.W., Epstein, E. and Pearcy, R.W. 1984. Physiological responses to salinity in selected lines of wheat. Plant Physiol. 74, 417–423.

Läuchli, A. and Wieneke, J. 1979. Studies on growth and distribution of Na^+, K^+ and Cl^- in soybean varieties differing in salt tolerance. Z. Pflazeneraehr 142, 3–13.

Lawrence, M.J. 1984. The genetical analysis of ecological traits. In: B. Shorrocks (Ed.), Evolutionary ecology, Blackwell Sci. Publ., Oxford, London, Edinburgh, pp. 27–63.

Lessani, H. and Marschner, H. 1978. Relation between salt tolerance and long-distance transport of sodium and chloride in various crop species. Aust. J. Plant Physiol. 5, 27–37.

Liang, G.H., Reddy, C.R. and Dayton, A.D. 1972. Heterosis inbreeding depression and heritability estimation in a systematic series of grain sorghum genotypes. Crop Sci. 12, 409–411.

Lothrop, J.E., Atkins, R.E. and Smith, O.S. 1985. Variability for yield and yield components in IAPIR grain sorghum random mating populations. II. Correlation, estimated gains from selection, and correlated response to selection. Crop Sci. 25, 240–244.

Levitt, J. 1978. Crop tolerance to suboptimal land conditions–A historical overview. In: G.A. Jung (Ed.), Crop tolerance to suboptimal land conditions, ASA, CSSA, SSSA, Madison, WI, pp. 161–171.

Lewontin, H.W. 1966. On the measurement of relative variability. Systematic Zoology 15, 141–142.

Lyon, C.B. 1941. Response of two species of tomatoes and their F_1 generation to sodium sulphate in the nutrient medium. Bot. Gaz. 103, 107–122.

Lynch, J. and Läuchli, A. 1985. Salt stress disturbs the calcium nutrition of barley (*Hordeum vulgare* L.). New Phytol. 99, 345–354.

Lynch, J., Epstein, E. and Läuchli, A. 1982. Na^+ - K^+ relationships in salt stressed barley. In: A. Scaif (Ed.), Proceedings. 9th. Int. Plant Nutr. Colloq. pp. 347–352.

Maas, E.V. 1985. Salt tolerance of plants. Applied Agriculture Research. 1, 12–26.

Maas, E.V. and Hoffman, G.J. 1977. Crop salt tolerance current assessment. J. Irrig.and Drain. Divi. ASCE 103, 115–134.

Maas, E.V. and Nieman, R.H. 1978. Physiology of plant tolerance to salinity. In: G.A. Jung (Ed.), Crop tolerance to suboptimal land conditions: A Special Publ. 32, Amer. Soc. Agron. pp. 277–299.

Maas, E.V. and Poss, J.A. 1989. Salt sensitivity of wheat at various growth stages. Irrig. Sci. 10, 29–40.

Maas, E.V. and Grieve, C.M. 1990. Spike and leaf development in salt stressed wheat. Crop Sci. 30, 1309–1313.

Maas, E.V., Hoffman, G.J., Chaba, G.D., Poss, J.A. and Shannon, M.C. 1983. Salt sensitivity of corn at various growth stages. Irrig. Sci. 4, 45–57.

Maas, E.V., Poss, J.A. and Hoffman, G.J. 1986. Salinity sensitivity of sorghum at three growth stages. Irrig. Sci. 7, 1–11.

Martinez-Cob, A., Aragües, R. and Royo, A. (1987). Salt tolerance of barley (*Hordeum vulgare* L.) cultivars at germination stage: Analysis of the response functions. Plant and Soil 104: 53–56.

Matar, Y., Doering, H.W. and Marschner, H. 1975. Auswirkungen von NaCl and Na_2SO_4 auf substanzbildung, salat. Z. Pflanzenernaer Bodenk 138, 295–307.

Mather, K. and Jinks, J.L. 1977. Introduction to biometrical genetics. Chapman and Hall, London.

Mather, K. and Jinks, J.L. 1982. Biometrical genetics. 3rd. Ed. Chapman and Hall, London.

Mayo, O. 1987. The theory of plant breeding. 2nd. Ed. Clarendon Press, Oxford.

McNeilly, T. 1990. Selection and breeding for salinity tolerance in crop species-A case for optimism ?. Acta Ecologica 11 (4), 595–610.

McNeilly, T. and Bradshaw, A.D. 1968. Evolutionary process in population of copper tolerant *Agrostis tenuis* Sibth. Evolution 22, 108–118.

McWilliams, J.R. 1986. The national and international importance of drought and salinity effects on agricultural production. Aust. J. Plant Physiol. 13, 1–13.

Milijikovic, N. 1965. General review of the salt affected ('slatina') soils of Yugoslavia and their classification. Agrokemia es Talajitan, 14 Supplement, 235–242.

Ministry of Agriculture of Socialist Ethiopia. 1983. Economic analysis of land use. Technical Report, Vol. 2, No. 3, Food and Agriculture Organisation/Land Use and Regulatory Department of Ethiopia, Addis Ababa, pp. 1–94.

Moeljopawiro, S. and Ikehashi, H. 1981. Inheritance of salt resistance in rice. Euphytica 30, 291–300.

Munk, L. 1972. High lycine barley–A summary of the present development in Sweden. Barley Genetics Newsl. 2, 54–59.

Munns, R. and Termaat, A. 1986. Whole-plant responses to salinity. Aust. J. Plant Physiol. 13, 143–160.

Munns, R., Greenway, H. and Kirst, G.O. 1983. Halotolerant eukaryotes. In: O.L. Lange, P.S. Nobel, C.B. Osmond and H. Ziegler (Eds.), Physiological plant ecology III. Responses to the chemical and biological environment, Encycl. Plant Physiol. New Ser., Vol. 12c, pp. 59–135.

Nassery, H. 1975. The effcte of salt and osmotic stress on the retention of potassium by excised barley and been roots. New Phytol. 75, 63–67.

Nassery, H. 1979. Salt induced loss of potassium from plant roots. New Phytol. 83, 23–27.

Noble, C.L., Halloran, G.M. and West, D.W. 1984. Identification and selection for salt tolerance in lucerne (*Medicago sativa* L.). Aust. J. Agri. Res., 35, 239–252.

Norlyn, J.D. 1980. Breeding salt-tolerant crop plants. In: D.W. Rains, R.C. Valentine and A. Hollaender (Eds.), Genetic engineering of osmoregulation: Impact on plant productivity for food, chemicals and energy, Plenum Press, New York and London, 14, 293–309.

Norlyn, J.D. and Epstein, E. 1984. Variability in salt tolerance of four triticale lines at germination and emergence. Crop Sci. 24, 1090–1092.

Oakes, M.W. 1967. The analysis of diallel cross of heterogeneous or multiple allelic lines. Heredity 22, 83–95.

Osmond, C.B., Lüttge, U., West, K.R., Pallaghy, C.K. and Schachar-Hill, B. 1969. Ion absorption in *Atriplex* leaf tissue. II. Secretion of ions to epidermal bladders. Aust. J. Biol. Sci. 22, 797–814.

Paleg, L.G., Douglas, T.J., VanDaal, A. and Keech, D.B. 1981. Proline, betaine and

other organic solutes protect enzymes against heat inactivation. Aust. J. Plant Physiol. 8, 107–114.

Passioura, J.B. 1986. Resistance to drought and salinity: Avenue for improvement. Aust. J. Plant Physiol. 13, 191–201.

Pasternak, D., Twersky, M. and De Malch, Y. 1979. Salt resistance in agricultural crops. In: H. Mussell and R.C. Staples (Eds.), Stress physiology in crop plants, John Wiley and Sons, New York, pp. 128–142.

Pasternak, D., Demalach, Y. and Borovic, I. 1984. Irrigation with brackish water under desert conditions. I. Problems and solutions in production of onion (*Allium cepa* L.). Agri. water management 9, 225–235.

Pasternak, D., Demalach, Y. and Borovic, I. 1985. Irrigation with brackish water under desert conditions. II. Physiological and yield response of maize (*Zea mays* L.) to continuous irrigation with brackish and to alternating brackish–fresh–brackish water irrigation. Agri. water management 10, 47–60.

Pitman, M.G. 1984. Transport across the root and shoot/root interactions. In: R.C. Staples and G.H. Toenniessen (Eds.), Salinity tolerance in plants: Strategies for crop improvement, A Wiley-Interscience Publication, New York, Chichester, Brisbane, Toronto, Singapore, pp. 93–123.

Plummer, D.T. 1987. Practical biochemistry. 3rd. Ed. McGraw-Hill Book Company, 179–180.

Poehlman, J.M. 1987. Breeding field crops. AVI Publishing Company. Westport, Connecticut, USA.

Ponnamperuma, F.N. 1984. Role of cultivar tolerance in increasing rice production on saline lands. In: R.C. Staples and G.H. Toenniessen (Eds.), Salinity tolerance in plants–Strategies for crop improvement. A Willey–Interscience Publication, New York, Chichester, Brisbane, Toronto, Singapore, pp. 255–271.

Poljakoff-Mayber, A. 1982. Biochemical and physiological responses of higher plants to salinity stress. In: A. San Pietro (Ed.), Biosaline research, Plenum Press, New York, pp. 245–269.

Porath, E. and Poljakoff-Mayber, A. 1964. Effect of salinity on methabolic pathways in pea root tips. Israel J. Botany 13, 115–121.

Porath, E. and Poljakoff-Mayber, A. 1968. The effect of salinity in the growth medium

on carbohydrate metabolism in pea root tips. Plant Cell Physiology 9, 195–203.

Prasad, S.R., Prakash, R., Sharma, C.M. and Haque, M.F. 1981. Genotypic and phenotypic variability in quantitative characters in oat. Indian Journal of Agric. Sci. 51, 480–482.

Purseglove, J.W. 1976. In: N.W. Simonds (Ed.), Evolution of crop plants, Longman, UK.

Rains, D.W. 1979. Salt tolerance of plants. In: A. Hollaender, J.C. Aller, E. Epstein and A. San Pietro (Eds.), The Biosaline Concept–Approach to the utilization of underexploited resources, Plenum Press, New York, pp. 47–67.

Rathert, G. 1982. Influence of extreme K^+ : Na^+ ratios and high substrate salinity on plant metabolism of crops differing in salt tolerance. VII. Relations between carbohydrates and degradative enzymes in salt tolerant and salt sensitive cotton genotypes during initial salinity stress. J. Plant Nutr. 5 (12), 1401–1413.

Rathert, G. 1984. sucrose and starch content of plant part as a possible indicator for salt tolerance of crops. Aust. J. Plant Physiol. 11, 491–495.

Rathert, G., Doering, H.W. and Witt J. 1981. Influence of K : Na ratios and high substrate salinity in plant metabolism of crops differing in salt tolerance. III. K/Na effects on carbohydrate pattern of bushbean and sugar beet plants in response to the salt tolerance of the species. J. Plant Nutr. 4, 131–141.

Rosen, H. 1957. A modified ninhydrin colorimetric analysis for amino acids. Archives of Biochemistry and Biophysics 67, 10–15.

Rush, D.W. and Epstein, E. 1976. Genotypic response to salinity: Differences between salt-sensitive and salt-tolerant genotypes of tomato. Plant physiol. 57, 162–166.

Rush, D.W. and Epstein, E. 1981. Breeding and selection for salt tolerance by incorporation of wild germplasm into domestic tomato. J. American. Soc. Hort. Sci. 106, 699–704.

San Joaquin Valley Interagency Drain. Programme, 1979. Agricultural drainage and salt management in San Joaquin valley: Final report including recommended plan and first-stage environmental impact report. U.S. Bureau of Reclamation, California Dept. of Water Resources and California State Water Resource Control Board.

Schat, H. and Ten-Bookum, W.M. 1992. Genetic control of copper tolerance in *Silene vulgaris*. Heredity 68, 219–229.

Shannon, M.C. 1978. Testing salt tolerance variability among tall wheat grass lines. Agron. J. 70, 719-722.

Shannon, M.C. 1984. Breeding, selection, and the genetics of salt tolerance. In: R.C. Staples, and G.H. Toenniessen (Eds.), Salinity tolerance in plants: Strategies for crop improvement, A Willey–Interscience Publication, New York, Chichester, Brisbane, Toronto, Singapore, pp. 231–254.

Shannon , M.C., McCrigh, T. and Drapes, J.H. 1983. Screening test for salt tolerance in lettuce. J. American Soc. Hort. Sci. 108, 225–230.

Shannon, M.C. 1985. Principles and strategies in breeding for higher salt tolerance. Plant and Soil 89, 227–281.

Shainberg, I. 1975. Salinity of soils–Effects of salinity on physics and chemistry of soils. In: A. Poljakoff-Mayber and J. Gale (Eds.), Plant in saline environments. Springer-Verlog, New York, pp. 39–55.

Sissay, B. 1986. Salt affected wasteland in Ethiopia: Potential for production of forage and fuel. In: E.G. Barrett-Lennard, C.V. Malcolm, W.R. Stern and S.M. Wilkins (Eds.), Forage and fuel production from salt affected wasteland. Reclamation and Revegetation Research 5, 59–64.

Skelding, A.D. and Winterbotham, 1939. The structure and development of the hydathodes of *Spartina townsendii* groves. New Phytol. 38, 69–79.

Stavarek, S.J. and Rains, D.W. 1984. Cell culture techniques: Selection and physiological studies of salt tolerance. In: R.C. Staples and G.H. Toennissen (Eds.), Salinity tolerance in plants: Strategies for crop improvement, A Wiley-Interscience Publication, New York, Chichester, Brisbane, Toronto, Singapore, pp. 321–333.

Steponkus, P.L. 1980. A unified concept of stress in plants. In: D.W. Rains, R.C. Valentine and A. Hollaender (Eds.), Genetic engineering of osmoregulation: Impact on plant productivity for food, chemicals and energy, Plenum Press, New York and London, 14, 235–255.

Stewart, G.R. and Lee, J.A. 1974. The role of proline accumulation in halophytes. Planta, 120, 279–289.

Strogonov, B.P. 1970. structure and function of plant cells in saline habitats. Israel programme for scientific translation, Jerusalem.

Strogonov, B.P. 1973. Structure and function of plant cells in saline habitats. In: A. Mercado and B. Gollek (Eds.), New trends in the study of salt tolerance, Translated from Russian, John Wiley and Sons, New York.

Szabolcs, I. 1989. Salt-affected soils. CRC Press, Boca Raton, Florida.

Tai, G.C.C. 1976. Estimation of general and specific combining abilities in potato. Cand. J. Genet. Cytol. 18, 463–470.

Tal, M. 1984. Physiological genetics of salt resistance in higher plants: Studies on the level of the whole plant and isolated organs. In: R.C. Staples and G.H Toenniessen (Eds.), Salinity tolerance in plants: Strategies for crop improvement, A Wiley-Interscience Publication, New York, Chichester, Brisbane, Toronto, Singapore, pp.301–315.

Tal, M., Katz, A., Heinken, H. and Dehan, K. 1979. Salt tolerance in the wild relatives of the cultivated tomato: Proline accumulation in *Lycopersicon esculentum* mill., *L. peruvianum* mill. and *Solanum pennellii* cor. treated with NaCl and polythylene glycole, New Phytol. 82, 349–355.

Tefera, H., Ketema, S. and Tesemma, T. 1990. Variability, heritability and genetic advance in tef [Eragrostis tef (Zucc.) Trotter] cultivars. Trop. Agric. (Trinidad) 67, 317–320.

Treichel, S. 1975. Der einfuss von NaCl auf die prolinkonzentration verschiendener halophytes. Z. Pflanzen Physol. 76, 56–68.

Troll, W. and Lindsly, J. 1955. A photometric method for the determination of proline. Journal of Biological Chemistry 255, 655–660.

Umeil, N., Zahavi, E. and Chen, Y. 1980. Effects of salinity stress in tobacco. II. Short term kinetics of sodium and potassium uptake by callus cultures grown on media containing NaCl. Z. Pflanzenphysiologie 100, 363–367.

U.S. Salinity Laboratory Staff, 1954. R.A. Richard (Ed.), Diagonosis and improvement of saline and alkaline soils. USDA Handbook 60.

van Genuchten, M.T. 1983. Analysing crop salt tolerance data: Model description and users manual USDA-ARS, USSL, Riverside, California, Research Report No. 120, pp. 1-50.

van Genuchten, M.T. and Hoffman, G.J. 1984. Analysis of crop salt tolerance data. In: I. Shainberg, and J. Shalhevet (Eds.), Soil salinity under irrigation, Spriger Verlag, Berlin, pp. 258–271.

Vavilov, N.I. 1926. Studies on the origin of cultivated plants. Institute of Applied Botany and Plant Breeding, Leningrad.

Vavilov, N.I. 1951. The origin, variation, immunity and breeding of cultivated plants. Translated from the Russian by K. Starrchester, Ronald Press, New York, pp. 37–38.

Wainwright, S.J. 1980. Plants in relation to salinity. Adv. Bot. Res. 8, 221–285.

Waisel, Y. 1972. Biology of halophytes. Academic Press, New York and London.

Weimburg, R. 1970. Enzyme levels in pea seedlings grown on highly salinised media. Plant Physiol. 46, 466–470.

Whitehouse, R.N.H., Thompson, J.B. and Ribeiro, M.A.N. 1958. Studies in the breeding of self pollinated cereals. The use of diallel cross analysis in yield prediction. Euphytica 17, 147–169.

Wieneke, J. and Läuchli, A. 1979. short-term studies on the uptake and transport of Cl^- by soybean cultivars differing in salt tolerance. Z. Pflanzenernaehr. Bodenkd. 142, 799–814.

Wieneke, J. and Läuchli, A. 1980. Salt stress on distribution of Na^+ and some other cations in two soybean varieties differing in salt tolerance. Z. Pflanzenernaehr. Bodenkd. 143, 55–67.

Winter, E. 1982. Salt tolerance of *Trifolium alexandrinum* (L.). III. Effect of salt on ultrastructure of phloem and xylem transfer cell petioles and leaves. Aust. J. Plant Physiol. 9, 239–250.

Winter, E. and Läuchli, A. 1982. Salt tolerance of *Trifolium alexandrinum* (L.). I. Comparison of the salt response of *T. alexandrinum* (L.) and *T. pratense* (L.). Aust. J. Plant Physiol. 9, 221–226.

Witt, S.C. 1985. A brief book–Biotechnology and genetic diversity. California Agricultural Lands Project, San Francisco.

Wyn Jones, R.G 1984. Phytochemical aspects of osmotic adaptation. In: B.N. Timmermann, C. Steelink and F.A. Loewus (Eds.), Recent advances in

phytochemistry: Phytochemical adaptations to stress, 18, 55–78.

Wyn Jones, R.G. and Gorham, J. 1986. The potential for enhancing the salt tolerance of wheat and other important crop plants. Outlook in Agriculture 15 (1), 33–39.

Wyn Jones, R.G. and Storey, R. 1978. Salt stress and comparative physiology in the *Gramineae*. IV. Comparison of salt stress in *Spartina* x *townsendii* and three barley cultivars. Aust. J. Plant Physiol. 5, 839–850.

Wyn Jones, R.G., Storey, R. and Pollard, A. 1976. Ionic and osmotic regulation in plants particularly halophytes. Presented at Int. Workshop Transmembrane Ionic Exchanges in Plants, Rouen.

Wyn Jones, R.G., Gorham, J. and McDonnell, E. 1984. Organic and inorganic solute contents as selection criteria for salt tolerance in the *Triticeae*. In: R.C. Stapples and G.H. Toenniessen (Eds.) Salinity tolerance in plants: Strategies for crop improvement, A Willey-Interscience Publication New York, Chichester, Brisbane, Toronto, Singapore, pp. 189–205.

Yeo, A.R. and Flowers, T.J. 1986. Salinity resistance in rice (*Oryza sativa* L.) and pyramiding approach to breeding varieties for saline soils. Aust. J. Plant Physiol. 13, 161–173.

Yoshida, S. 1967. Salt tolerance of rice plant. Ann. Rept. IRRI, pp. 32–36.

Zaborsky, O. 1980. The biosaline concept an opportunity for research and development. In: D,W. Rains, R.C. Valentine and A. Hollaender (Eds.), Genetic engineering of osmoregulation: Impact on plant productivity for food, chemicals, and energy, Plenum Press, New York and London, 14, 339–344.

Ziegler, H. and Lüttge, U. 1967. Die salzdrüsen von *Limonium vulgare*. II. Mitteilung die lokalisierung chloride. Planta 74, 1–17.

Zohary, D. 1970. Centres of diversity and centres of origin. In: O.H. Frankel and E. Bennet (Eds.), Genetic resources in plants: Their exploration and conservation, Blackwell Scientific Publication, Oxford pp. 33–42.

APPENDICES

APPENDICES

Appendix 1.1. Lists of accessions, their sources, and origins of the species used in the series of the experiments

P. americanum			*E. coracana*			*E. tef*		
Acc. No.	Source	Origin	Acc. No.	Source	Origin	Acc. No.	Source	Origin
203654	PGRC/E	Ethiopia	100001	PGRC/E	Ethiopia	343932	USA	Ethiopia
203656	PGRC/E	Ethiopia	100002	PGRC/E	Ethiopia	494188	USA	Ethiopia
203657	PGRC/E	Ethiopia	100004	PGRC/E	Ethiopia	494197	USA	Ethiopia
203658	PGRC/E	Ethiopia	100005	PGRC/E	Ethiopia	494205	USA	Ethiopia
203659	PGRC/E	Ethiopia	100006	PGRC/E	Ethiopia	494213	USA	Ethiopia
203661	PGRC/E	Ethiopia	100007	PGRC/E	Ethiopia	494215	USA	Ethiopia
203662	PGRC/E	Ethiopia	100008	PGRC/E	Ethiopia	494216	USA	Ethiopia
215631	PGRC/E	Ethiopia	100009	PGRC/E	Ethiopia	524433	USA	Ethiopia
215632	PGRC/E	Ethiopia	100010	PGRC/E	Ethiopia	524436	USA	Ethiopia
215633	PGRC/E	Ethiopia	100012	PGRC/E	Ethiopia	524437	USA	Ethiopia
215634	PGRC/E	Ethiopia	100014	PGRC/E	Ethiopia	524438	USA	Ethiopia
215637	PGRC/E	Ethiopia	100015	PGRC/E	Ethiopia	524439	USA	Ethiopia
215663	PGRC/E	Ethiopia	100016	PGRC/E	Ethiopia	524440	USA	Ethiopia
219336	PGRC/E	Ethiopia	100018	PGRC/E	Ethiopia	524445	USA	Ethiopia
219569	PGRC/E	Ethiopia	100017	PGRC/E	Ethiopia	524441	USA	Ethiopia
219975	PGRC/E	Ethiopia	100019	PGRC/E	Ethiopia			
219979	PGRC/E	Ethiopia	100021	PGRC/E	Ethiopia			
219984	PGRC/E	Ethiopia	100022	PGRC/E	Ethiopia			
219985	PGRC/E	Ethiopia	100024	PGRC/E	Ethiopia			
220134	PGRC/E	Ethiopia	100025	PGRC/E	Ethiopia			
220139	PGRC/E	Ethiopia	100030	PGRC/E	Ethiopia			
220164	PGRC/E	Ethiopia	100031	PGRC/E	Ethiopia			
220220	PGRC/E	Ethiopia	100032	PGRC/E	Ethiopia			
220222	PGRC/E	Ethiopia	100033	PGRC/E	Ethiopia			
221726	PGRC/E	Ethiopia	100034	PGRC/E	Ethiopia			
93611	Bari	Libya						
93612	Bari	Libya						
93614	Bari	Libya						
Kitui L.	Bari	Kenya						
Selection 2	Bari	Pakistan						

Appendix 2.1. Composition of Rorison solution

Nutrient source	Stock solution g l^{-1}	Make-up volume (per litre)	Final concentration µg ml	
$Ca(NO_3)_2.4H_2O$	472.00	1 ml	N	56.00
K_2HPO_4	175.00	1 ml	Ca	80.16
$MgSO_4.7H_2O$	123.00	2 ml	Mg	24.31
			S	32.06
Fe Na EDTA	12.50	1 ml	Fe	3.00
Trace elements		**1 ml**		
$MnSO_4.4H_2O$	2.028		Mn	0.50
			S	0.30
H_3BO_3	2.863		B	0.50
$(NH_4)_6.MO_7O_{24}$	0.184		MO	0.11
			N	0.01
$ZnSO_4.7H_2O$	0.44		Zn	0.10
$CuSO_4.5H_2O$	0.390		Cu	0.10

K_2HPO_4 can be replaced by KCl to the required strength.

Appendix 2.2. Absolute root length (cm) of sixteen accessions of *P. americanum* at six different NaCl concentrations

Acc. No.	Control	50mM	75mM	100mM	150mM	200mM
203654	5.66	4.60	3.06	2.52	1.22	0.00
203655	6.79	4.94	2.75	1.71	0.27	0.00
203657	5.85	4.57	1.94	1.06	0.35	0.00
203658	11.7	7.68	4.85	1.86	0.22	0.00
203659	10.4	6.66	3.96	1.90	0.25	0.00
203661	6.63	5.16	2.21	0.93	0.22	0.00
203662	10.2	6.16	4.00	2.00	0.30	0.00
215631	8.77	7.39	4.21	2.47	1.07	0.27
215634	5.03	5.10	2.98	2.04	0.78	0.49
219975	8.00	6.27	5.00	3.50	1.96	0.30
219979	8.00	6.11	5.47	2.93	1.67	0.48
219984	10.3	8.23	5.70	3.92	1.84	0.00
219985	11.7	8.70	6.27	3.92	1.11	0.64
220134	9.00	7.41	5.67	3.70	2.56	0.26
220139	6.94	5.88	2.94	1.45	0.64	0.49
220164	10.2	8,57	5.96	3.65	1.51	0.18

Appendix 2.3. Absolute root length (cm) of sixteen accessions of *E. coracana* at six different NaCl concentrations

Acc. No.	Control	50mM	75mM	100mM	150mM	200mM
100001	15.2	12.2	10.9	9.30	4.09	1.10
100004	15.0	12.2	11.1	7.35	1.86	0.65
100005	15.7	12.2	11.6	7.00	1.54	0.48
100007	10.7	10.6	10.2	7.20	1.17	0.23
100008	10.4	8.40	7.50	6.23	1.61	0.41
100009	6.93	5.89	5.50	4.49	1.51	0.24
100012	9.39	7.73	6.47	6.06	2.26	0.20
100014	9.15	8.14	7.60	6.05	2.11	1.07
100015	13.0	11.1	9.50	7.97	2.10	0.59
100016	7.40	6.33	5.64	4.60	2.72	0.50
100017	9.63	8.70	7.50	5.90	2.84	0.32
100018	6.76	6.00	4.94	3.95	1.57	0.18
100019	4.51	3.52	2.90	2.50	1.30	0.29
100031	8.11	7.90	7.62	5.37	4.59	1.56
100032	9.19	8.25	6.99	4.75	1.87	0.49
100034	10.0	9.09	9.05	6.49	2.00	1.89

Appendix 2.4. Absolute root length (cm) of six accessions of *E. tef* in nine different NaCl concentrations

Acc. No.	Control	25mM	50mM	75mM	100mM	125mM	150mM	175mM	200mM
494197	6.10	5.16	4.62	3.64	1.77	0.79	0.45	0.50	0.24
494205	7.71	6.49	5.37	4.22	2.57	1.43	0.80	0.62	0.00
494215	4.61	4.17	3.76	3.21	1.90	0.80	0.63	0.35	0.25
524433	3.99	4.42	2.29	1.94	1.38	0.86	0.65	0.43	0.35
524437	6.97	6.27	5.16	4.98	3.40	1.65	0.91	0.60	0.38
524438	9.23	7.79	5.66	4.51	2.71	1.21	0.18	0.15	0.00

Appendix 2.5. Mean for parameters C_t, C_0 and C_{50} of *P. americanum*, *Eleusine coracana* and *E. tef*

Parameter	*P. americanum*	*E. coracana*	*E. tef*
C_t	22.04mM	52.44mM	23.54mM
C_0	150.07mM	190.97mM	158.74mM
C_{50}	80.29mM	113.78mM	86.66mM

Appendix 3.1. Variance of the component of each array (V_r) and covariance of all the offspring included in each parental array with non recurrent parent (W_r), and their means

Parents	75mM NaCl			125mM NaCl			175mM NaCl		
	Array means	Vr	Wr	Array means	Vr	Wr	Array means	Vr	Wr
Kitui Local	56.95	96.51	-38.71	31.22	39.58	14.27	16.17	12.25	13.69
93611	52.56	111.4	182.6	30.22	106.3	129.6	15.93	25.51	23.40
203659	41.22	149.4	86.45	22.67	65.48	12.89	11.87	66.47	58.91
203662	38.49	57.00	75.86	21.52	29.10	31.28	10.63	54.67	35.77
221726	44.90	343.7	208.8	26.23	107.0	-0.30	20.00	0.79	-3.43
Mean	46.82	151.6	103.0	26.37	69.50	37.55	14.92	31.94	25.67

Appendix 3.2. Details of analysis of variance of $W_r + V_r$ and $W_r - V_r$

Item	Df	75mM NaCl		125mM NaCl		175mM NaCl	
		MS	VR	MS	VR	MS	VR
$W_r + V_r$							
Between arrays	4	73543.6	1.85^{NS}	15198.8	1.70^{NS}	4989.3	44.65***
Within arrays	5	40650.2		8933.1		111.8	
$W_r - V_r$							
Between arrays	4	170805.6	3.66^{NS}	5806.0	11.8**	159.7	1.21^{NS}
Within arrays	5	4669.7		493.9		131.5	

Appendix 3.3. Family means of relative root lengths of 20 F_1 hybrids of *P. americanum* and their parents in a 5 x 5 diallel crosses in three NaCl concentrations

Genotypes	75mM	125mM	175mM
Kitui Local x 93611	58.1	33.8	18.0
Kitui Local x 203659	47.7	25.1	16.9
Kitui Local x 203662	49.3	29.8	20.4
Kitui Local x 221726	71.6	30.4	20.6
93611 x 203659	48.7	26.2	17.4
93611 x 203662	45.2	23.7	7.82
93611 x 221726	46.3	23.4	17.6
203659 x 203662	23.3	12.2	1.55
203659 x 221726	52.7	35.0	22.1
203662 x 221726	36.6	27.9	19.0
Parents			
Kitui Local	54.1	25.9	10.5
93611	66.5	45.9	19.4
203659	30.0	15.2	3.08
203662	37.3	37.3	5.89
221726	19.7	14.9	22.7

Appendix 4.1a. Absolute mean height (cm) per plant of six accessions of *P. americanum* at three different stages of growth in four different NaCl concentrations (mM)

Accession number	Growth stage 1				Growth stage 2				Growth stage 3			
	0.0	75	100	150	0.0	75	100	150	0.0	75	100	150
203656	84.3	60.1	60.2	49.7	93.9	60.8	64.9	49.3	110.2	62.1	64.7	49.3
203658	83.2	59.8	62.6	53.6	81.9	59.0	58.8	52.6	92.9	57.8	58.9	53.4
215631	72.6	66.8	61.7	62.2	75.8	64.5	61.6	62.3	82.3	65.5	60.9	62.2
215632	71.9	66.3	62.3	57.1	76.0	66.6	60.3	57.8	82.6	66.8	61.7	61.3
215634	66.6	64.1	61.3	57.5	71.3	64.3	61.8	57.0	74.3	64.5	61.7	57.0
Selection 2	77.7	62.7	62.8	57.9	92.2	62.8	62.9	58.1	112.3	62.5	63.4	58.8

Appendix 4.1b. Absolute mean number of leaves per plant of six accessions of *P. americanum* at three different stages of growth in four different NaCl concentrations (mM)

Accession number	Growth stage 1				Growth stage 2				Growth stage 3			
	0.0	75	100	150	0.0	75	100	150	0.0	75	100	150
203656	9.33	9.33	9.33	8.0	11.0	10.7	10.7	8.33	14.0	11.3	10.7	8.33
203658	8.67	9.33	8.33	8.0	10.3	11.0	10.0	9.67	11.7	11.0	`0.0	9.67
215631	9.0	8.67	8.67	8.67	11.0	10.0	10.3	9.33	13.0	11.7	11.3	9.33
215632	9.0	10.0	9.0	8.0	10.0	12.0	10.7	10.0	11.0	13.7	11.0	10.0
215634	9.0	9.0	8.33	8.33	10.0	11.3	10.0	10.0	12.0	11.7	10.0	10.0
Selection 2	9.0	8.33	8.0	9.33	10.3	10.7	11.0	11.0	11.7	12.7	11.3	11.0

Appendix 4.1c. Absolute mean percentage live leaves (%) per plant of six accessions of *P. americanum* at three different stages of growth in four different NaCl concentrations (mM)

Accession number	Growth stage 1				Growth stage 2				Growth stage 3			
	0.0	75	100	150	0.0	75	100	150	0.0	75	100	150
203656	44.9	34.7	35.7	32.1	32.0	24.4	22.9	21.7	36.9	5.57	0.00	0.00
203658	42.9	37.3	46.5	40.42	36.56	24.8	31.8	29.4	24.2	0.91	12.0	0.00
215631	42.8	42.3	45.6	33.8	31.1	27.7	34.7	25.3	30.8	15.1	14.5	0.00
215632	42.7	34.7	40.4	24.2	37.4	29.8	30.8	24.1	36.0	12.0	13.5	0.00
215634	46.5	35.4	43.7	33.2	33.2	28.2	32.8	25.6	30.0	13.4	10.3	0.00
Selection 2	46.8	46.7	44.6	37.9	38.6	33.3	30.5	24.2	26.1	18.1	11.5	1.30

Appendix 4.1d. Absolute mean root dry weight (g) per plant in four NaCl concentrations at growth stage 3 (maturity)

Accession No.	Control	75mM	100mM	150mM
203656	2.62	0.86	0.77	0.65
203658	2.19	0.86	0.42	0.41
215631	4.57	1.40	1.06	0.68
215632	4.73	1.13	0.95	0.83
215634	3.59	1.42	0.68	0.64
Selection 2	1.96	0.92	0.75	0.58

Appendix 4.1e. Absolute mean stem dry weight (g) per plant in four NaCl concentrations at growth stage 3 (maturity)

Accession No.	Control	75mM	100mM	150mM
203656	2.58	0.11	0.09	0.03
203658	1.43	0.11	0.06	0.02
215631	1.70	0.29	0.20	0.03
215632	1.90	0.38	0.08	0.04
215634	1.39	0.29	0.13	0.08
Selection 2	1.61	0.50	0.32	0.11

Appendix 4.1f. Absolute mean leaf sheath dry weight (g) per plant in four NaCl concentrations at growth stage 3 (maturity)

Accession No.	Control	75mM	100mM	150mM
203656	1.06	0.29	0.30	0.19
203658	0.80	0.28	0.26	0.19
215631	0.80	0.46	0.39	0.30
215632	0.79	0.43	0.37	0.31
215634	0.74	0.35	0.34	0.33
Selection 2	0.70	0.32	0.38	0.33

Appendix 4.1g. Absolute mean leaf blade dry weight (g) per plant in four NaCl concentrations at growth stage 3 (maturity)

Accession No.	Control	75mM	100mM	150mM
203656	1.76	1.03	1.03	0.61
203658	1.83	0.99	0.98	0.81
215631	2.51	1.54	1.16	1.23
215632	2.59	1.38	1.15	1.12
215634	2.46	1.41	1.41	1.06
Selection 2	1.34	0.79	1.01	0.86

Appendix 4.2a. Relative mean height (%) per plant of six accessions of *P. americanum* at three different growth stages in three different NaCl concentrations (mM)

Accession number	Growth stage 1			Growth stage 2			Growth stage 3		
	75	100	150	75	100	150	75	100	150
203656	71.4	71.7	58.2	65.2	69.3	52.6	60.3	58.6	44.7
203658	72.1	75.6	64.6	72.8	72.5	64.9	62.4	63.4	57.5
215631	92.3	85.0	85.9	85.3	81.2	82.3	79.9	74.1	75.91
215632	92.2	86.8	79.7	87.6	79.1	76.3	80.9	74,6	74.3
215634	93.6	89.6	84.0	90.3	86.8	80.3	87.2	83.4	77.4
Selection 2	80.6	81.0	74.7	68.1	68.8	63.8	55.8	56.8	53.1

Appendix 4.2b. Relative mean number of leaves (%) per plant of six accessions of *P. americanum* at three different growth stages in three different NaCl concentrations (mM)

Accession number	Growth stage 1			Growth stage 2			Growth stage 3		
	75	100	150	75	100	150	75	100	150
203656	100.4	100.4	88.9	98.1	97.8	76.5	81.9	76.6	59.9
203658	107.9	100.5	92.1	103.3	97.0	93.6	94.5	85.9	82.8
215631	96.3	96.3	96.3	100.0	93.6	87.9	91.7	87.9	75.5
215632	82.4	101.2	89.2	116.4	106.7	100.0	124.9	101.1	92.7
215634	103.7	92.6	92.6	110.0	97.0	96.7	104.1	102.3	83.9
Selection 2	93.0	88.4	103.3	103.7	107.4	106.7	108.6	97.2	94.2

Appendix 4.2c. Relative mean percentage live leaves (%) per plant of six accessions of *P. americanum* at three different growth stages in three different NaCl concentrations (mM)

Accession number	Growth stage 1			Growth stage 2			Growth stage 3		
	75	100	150	75	100	150	75	100	150
203656	77.9	79.9	71.7	76.7	71.6	66.9	13.6	0.00	0.00
203658	87.6	109.5	96.6	67.2	92.4	86.3	2.35	35.1	0.00
215631	99.3	107.0	79.9	90.4	112.1	83.1	48.9	46.6	0.00
215632	83.1	96.4	58.2	82.0	83.8	67.9	45.8	46.6	0.00
215634	81.7	101.8	80.0	85.2	98.7	78.1	45.9	34.9	0.00
Selection 2	87.3	96.0	81.1	86.7	79.3	62.9	83.1	44.1	4.99

Appendix 4.2d. Relative mean root dry weight (g) per plant in three NaCl concentrations at growth stage 3 (maturity)

Accession No.	75mM	100mM	150mM
203656	8.28	6.98	6.19
203658	10.2	5.37	4.73
215631	7.92	5.79	3.98
215632	8.41	4.99	4.58
215634	13.6	4.97	4.75
Selection 2	14.0	11.4	8.39

Appendix 4.2e. Relative mean stem dry weight (g) per plant in three NaCl concentrations at growth stage 3 (maturity)

Accession No.	75mM	100mM	150mM
203656	1.07	0.77	0.19
203658	1.93	0.94	0.35
215631	4.08	0.65	0.30
215632	4.81	1.21	0.54
215634	5.32	2.33	1.40
Selection 2	7.86	5.12	1.61

Appendix 4.2f. Relative mean leaf sheath dry weight (g) per plant in three NaCl concentrations at growth stage 3 (maturity)

Accession No.	75mM	100mM	150mM
203656	6.90	6.93	4.54
203658	8.58	7.98	5.92
215631	14.4	12.0	10.8
215632	13.7	11.6	9.76
215634	14.7	11.6	11.2
Selection 2	11.7	14.0	12.0

Appendix 4.2g. Relative mean leaf blade dry weight (g) per plant in three NaCl concentrations at growth stage 3 (maturity)

Accession No.	75mM	100mM	150mM
203656	14.7	13.9	8.69
203658	13.8	13.7	11.2
215631	15.6	11.6	12.3
215632	13.4	11.17	10.83
215634	14.4	11.8	10.5
Selection 2	20.5	20.6	18.5

Appendix 6.1a. Absolute root length of twelve pearl millet accessions in NaCl, and NaCl+$CaCl_2$ at three different concentrations and the control (EC 0.3 dS m^{-1})

		NaCl alone (dS m^{-1})			NaCl+$CaCl_2$ (dS m^{-1})		
Acc. No.	Control	EC 4	EC 8	EC 12	EC 4	EC 8	EC 12
Kitui Local	4.68	4.07	3.50	2.70	5.50	4.60	3.00
Selection 2	7.70	6.91	6.53	4.81	9.33	9.03	5.33
93611	10.94	9.44	7.42	6.33	10.4	7.50	6.57
93612	5.59	4.59	3.50	3.20	6.59	8.16	8.92
93614	6.59	5.59	4.54	4.18	7.55	6.71	3.75
203658	11.1	6.79	3.09	0.91	7.58	5.35	2.76
203659	9.27	5.63	2.15	1.17	5.29	3.94	2.67
203662	10.3	5.59	1.55	1.20	6.89	4.53	3.62
215634	3.41	2.60	2.69	0.87	3.36	2.55	1.51
219975	6.62	2.13	0.59	0.59	6.01	4.88	2.25
220220	10.4	6.60	2.59	2.09	6.34	4.82	4.24
221726	4.43	2.99	3.32	2.18	3.64	3.25	2.90
Overall mean	**7.59**	**5.24**	**3.46**	**2.52**	**6.54**	**5.44**	**3.96**
Mean tolerants	**6.66**	**5.60**	**4.80**	**3.90**	**7.17**	**6.54**	**5.08**
Mean sensitives	**8.51**	**4.89**	**2.11**	**1.14**	**5.91**	**4.35**	**2.84**

Appendix 6.1b. Absolute shoot length of twelve pearl millet accessions in NaCl, and NaCl+$CaCl_2$ at three different concentrations and the control (EC 0.3 dS m^{-1})

		NaCl alone (dS m^{-1})			NaCl+$CaCl_2$ (dS m^{-1})		
Acc. No.	Control	EC 4	EC 8	EC 12	EC 4	EC 8	EC 12
Kitui Local	7.82	7.71	5.00	4.50	7.91	7.52	5.56
Selection 2	13.2	11.2	10.2	9.08	12.6	12.3	9.42
93611	12.0	10.8	8.62	7.45	8.39	7.50	7.16
93612	9.30	7.00	5.70	5.00	9.54	8.05	7.12
93614	9.37	7.02	6.11	5.64	8.73	6.84	5.06
203658	11.3	8.44	4.53	2.14	8.59	5.56	3.85
203659	10.4	7.53	4.47	2.40	8.55	6.40	4.46
203662	11.8	7.49	4.10	2.54	9.29	7.08	4.82
215634	7.60	9.12	7.54	4.06	8.48	5.71	3.57
219975	9.31	4.79	1.97	1.85	10.1	8.09	7.61
220220	11.3	8.32	5.54	3.93	8.93	6.14	5.81
221726	7.35	6.51	6.15	5.15	9.08	6.36	4.65
Overall mean	**10.06**	**7.99**	**5.83**	**4.98**	**9.18**	**7.30**	**5.76**
Mean tolerants	**9.84**	**8.37**	**6.96**	**6.14**	**9.38**	**8.10**	**6.50**
Mean sensitives	**10.30**	**7.62**	**4.70**	**2.82**	**8.99**	**6.49**	**5.02**

Appendix 6.2. The level in mM of each EC (Electrical Conductivity) in each salinity, and Ca^{2+} concentration in $NaCl+CaCl_2$

NaCl alone		$NaCl+CaCl_2$		
EC (dS m^{-1})	mM	EC (dS m^{-1})	mM	Ca^{2+} (mM)
0.30 (Control)	0.00	0.50 (Control)	0.00	-
4.00	35.0	4.00	28.00	9.13
8.00	75.0	8.00	62.00	18.26
12.00	125.0	12.00	100.0	24.78