Response of Amaranth to salinity stress

by

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DECLARATION

I, Elizabeth Nabwile Omami, hereby declare that this thesis for the degree Ph.D. in Horticulture at the University of Pretoria, South Africa, is my own work and has never been submitted at any other university.

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Elizabeth Nabwile Omami

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Responses of Amaranth to salinity stress

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ABSTRACT

Salinity continues to be one of the world's most serious environmental problems in agriculture. The increasing world population and urbanization are forcing farmers to utilize marginal lands as well as poor quality water. One of the strategies in dealing with salinity is growing salt tolerant plants and there has been increased need to understand the effects of salinity on crops. Owing to its high nutritive value and wide adaptability to diverse environments, amaranth is considered a promising crop for marginal lands and semiarid regions. The objective of the study was to investigate the response of amaranth to salinity stress and evaluate stress amelioration by calcium and seed priming.

Salinity tolerance during germination and early seedling growth was examined for six genotypes of amaranth (*Amaranthus* species) at different salt concentrations ranging from 0 to 200 mM NaCl or Na_2SO_4. Enhancement of germination was observed at 25 mM, while increasing salt concentrations reduced the germination percentage as well as germination rate. *A.tricolor* and Accession '83 were able to germinate in 200 mM NaCl while there was no germination at 200 mM Na_2SO_4 in all the genotypes. Overall, Accession '83 was the most resistant and *A. hybridus* the most sensitive genotype, particularly at high salt concentrations. Inhibition of germination was greater in Na_2SO_4

than in NaCl salinity treatments. Amaranth was more salt tolerant at germination than at seedling growth. Seedling emergence, survival and growth were reduced by salinity and at much lower concentrations than at seed germination. Differences in salt tolerance were noted among the genotypes.

Salinity stress was initiated at different growth stages (cotyledon stage, 2-leaf stage and 4-leaf stage) in order to determine whether tolerance of amaranth differs with the stage of development. The treatment either continued until termination of the experiment or for 14 days at each stage. Amaranth plants were less sensitive to salinity when the stress was initiated at the 4-leaf stage. Lower salt concentrations had less detrimental effects than higher concentrations when applied at the cotyledon stage. Application of low salt concentration at cotyledon stage for 14 days did not have any effect on plant growth. The results indicate that it is feasible to use saline water for growing amaranth with minimum yield losses if salt concentration, duration of exposure and time of salinization are carefully managed.

Differences in salinity tolerance among amaranth genotypes were analyzed in terms of plant survival, growth, gas exchange, water use and leaf anatomical changes. *A. hypochondriacus* and *A. cruentus* showed greater tolerance to salinity since they survived in 200 mM NaCl treatment and the reduction in growth at 50 and 100 mM was lower than that of *A. tricolor* and Accession ’83. *A. hypochondriacus* and *A. cruentus* were more efficient water users and partitioned photosynthates towards shoot growth as opposed to the other two genotypes. Photosynthetic rate, stomatal conductance, stomatal density and apertures were reduced by salinity but were higher in *A. tricolor* than in *A. cruentus*. Salinity resulted in *A. cruentus* developing thicker leaves compared to *A. tricolor*. Productivity on saline soils can be increased by growing genotypes more tolerant to salinity.

The interactive effect of salinity and water stress on amaranth plant growth was evaluated. It was found that the reduction in shoot growth was greater in plants submitted

to water stress than in those submitted to salt or salt + water stress. Water use efficiency was increased while leaf water and osmotic potentials were reduced by the salinity stress treatments. In drying soil plants previously salinized had a greater degree of osmotic adjustment, so that plants were able to continue growth for a longer period compared to water stressed plants.

The effect of calcium in ameliorating salt stress was investigated. Supplementary calcium, either as $CaSO_4$ or $CaCl_2$ ameliorated the negative effects of salinity on growth, gas exchange, membrane permeability and mineral uptake. In a separate experiment it was shown that it is feasible to mitigate the adverse effects of salinity on amaranth seed germination, seedling survival and growth by seed priming and that the positive effect of priming persisted to vegetative growth stage. Priming with $CaSO_4$ + NaCl showed a greater positive response than priming with the individual salts.

Key words: *Amaranthus*, calcium, gas exchange, germination, growth, membrane permeability, photosynthesis, salinity stress, salt tolerance, seed priming, water relations, water stress, water use efficiency (WUE).

INTRODUCTION

Salinity is one of the world's most serious environmental problems in agriculture. It is estimated that about one-third of the world's cultivated land is affected by salinity (Perez-Alfocea *et al*., 1996). The National Academy of Sciences of the USA includes salinization of soils and waters as one of the leading processes contributing to a possible worldwide catastrophe (Francois and Maas, 1994). The increasing world population, especially in arid and semi-arid regions, food shortages, and land scarcity are compelling the use of lands not utilized because of salinity and other soil stresses. Salinity and sodicity problems are characterized by an excess of inorganic salts and are common in the arid and semi-arid lands (ASAL) where they have been naturally formed under the prevailing climatic conditions and due to the high rates of evapotranspiration and lack of leaching water (Mengel and Kirkby, 1982; Shannon *et al*., 1994). In the arid and semi-arid parts of Africa, for instance, salinity and alkalinity are major problems affecting about 24% of the continent (Reich *et al*., 2004). According to Eswaran *et al*. (1997) about 30% of the population of Africa or about 250 million people are living on or are dependent on this type of land.

Although more frequent in arid lands, salt-affected soils are also present in areas where salinity is caused by poor quality of irrigation water and increases markedly during the dry season (De Pascale *et al*., 1997; Sifola and Postiglione, 2002). As competition for fresh water increases due to increasing population, water of better quality is used primarily for domestic purposes, whereas water of lower quality e.g. saline or polluted water (drainage water generated by irrigation agriculture, marginal-quality waters generated by municipalities) often is used for irrigation (Khroda, 1996; Oster, 2000; Bouwer, 2002). Wanjogu *et al*. (2001) reported that land degradation by salinization is on the increase where the use of poor quality irrigation water is a common practice in arid and semi-arid lands. However, there seems to be a general lack of information on the prevalence and composition of saline aquifers in sub-Saharan Africa (Karlberg and Penning de Vries, 2003). Although some countries, such as South Africa, Botswana and

Zimbabwe have documented the presence of saline aquifers, Karlberg and Penning de Vries (2003) reiterated that information as to what extent saline water is being used for irrigation in sub-Saharan Africa is lacking.

From the perspective of plant productivity, salinity problems accentuate year after year as a result of repeated irrigation with poor quality water. The concentration of salt in the soil rises due to evaporation. Similarly, the salt concentration in plant tissues increases as water is lost through the process of transpiration. Salt problems unrelated to irrigation are also known. Excessive fertilizer application, susceptible soil types, and drought can combine to play a major role in accentuating the toxic effect of salts on crop yield (Parker *et al*., 1983).

Agricultural production in arid and semiarid regions of the world, which depends on irrigation, faces a serious challenge because it must increase or at least maintain crop productivity while coping with ever more saline irrigation water. Irrigation with saline water is successfully practiced in many countries such as Israel, Italy and the US (Rhoades *et al*., 1992). The success of using such water is dependent on advances in the knowledge of the many factors involved in plant salt tolerance.

Salt accumulation in soils induces physiological and metabolic disturbances in crops affecting development, growth, yield and quality of crops (Pardossi *et al.*, 1999; Mavrogianopoulos *et al*., 1999; del Amor *et al*., 2000; Mer *et al*., 2000; Silveira *et al*., 2001). Reduction in growth results from salinity effects on dry matter allocation, ion relations, water status, biochemical reactions or a combination of many physiological factors. However, the severity of salt damage has been found to be dependent on the meteorological conditions, soil type (Shannon *et al*., 1994), species and cultivar (Rhoades *et al.*, 1992; Vicente *et al*., 2004), growth stages of the plant (Yeo *et al.*, 1991; Botia *et al*., 1998; Carvajal *et al*., 1998), time interval between irrigations, amount of water distributed and time of exposure to saline water (Oster, 1994). Such variability suggests that environment and species-specific assessments of plant salt tolerance are both required in order to obtain conclusive information regarding the cultivation of a certain

species using saline water of specific concentration. This is due to the fact that general guidelines for agricultural management may not respond to the species and/or environment-specific crop requirements for optimum production (Rhoades *et al*., 1992; Dalton *et al*., 2000).

The degree of salt stress on a plant depends on three responses:

- Shoot dehydration through the low water potential caused by osmotic stress (Dubey, 1997; Carvajal *et al*., 1999);
- Nutritional imbalances caused by the interference of saline ions with essential nutrients in both uptake and translocation processes or partitioning within the plant (Liu and Zhu, 1998; Grattan and Grieve, 1999);
- Specific ion toxicity due to the accumulation of ions, particularly Na^+ and Cl^- in the cytoplasm (Greenway and Munns, 1980; Yeo, 1998; Wahome *et al*., 2001).

These three effects often coexist in soils exposed to salinization, and can persist throughout the growth season (De Pascale *et al*., 2003a, b).

Sustained and profitable production of crops on salt-affected soil is possible if appropriate on-farm management practices are carried out. To be successful, growers require an understanding of how plants respond to salinity, the relative tolerances of different crops and their sensitivity at different stages of growth, and how different environmental conditions affect salt-stressed plants. A widespread practice to reduce the salt content in soils is leaching. However, with the rising cost of water, this may not continue to be a feasible method for the future. Increasing salinity has increased the need to understand the effects of salinity on crops, and genetic improvement of salt tolerance has become an urgent need for the future of agriculture in arid and semi-arid regions (Shannon, 1984; Owens, 2001). Breeding of salt tolerant crop varieties will require a clear understanding of plant response to salinity and the complex mechanisms of salt stress tolerance (Zhu, 2001; Apse and Blumwald, 2002).

As the problems of salinity become more severe Aronson (1985) and NRC (1990) have proposed the growing of alternative plants and crops suited to moderately saline conditions with the option of introducing under-exploited, salt-tolerant minor crops. Salt tolerant plants may provide a logical alternative for many developing countries.

Amaranth is used for its grain and is also consumed as a cooked vegetable in many parts of the world. Owing to its high nutritive value and a wide adaptability to diverse environments, amaranth has been considered a promising crop for marginal lands and semiarid regions (Cunningham *et al*., 1992; Allemann *et al*., 1996). The prospects for future cultivation of salt-tolerant, high-yielding genotypes of amaranth are very encouraging. However, despite a substantial amount of literature on responses of plants to salinity stress, little information is available on amaranth. The general objectives of this study were to better understand the response of amaranth to salinity stress by investigating:

- Differences in salinity tolerance in different amaranth genotypes at seed germination and vegetative growth stages.
- Salt tolerance of amaranth as affected by salt concentration and timing of salinity imposition after seedling emergence.
- Morphological and physiological traits that contribute to salinity tolerance in amaranth.
- Interactive effects of salinity and drought stress on amaranth growth and development.
- The ameliorative effects of calcium nutrition and seed priming on growth, mineral uptake and photosynthesis of salt-stressed amaranth.

CHAPTER 1
LITERATURE REVIEW

1.1 Effects of salinity in agriculture – An overview

Salinity of arable land is an increasing problem of many irrigated, arid and semi-arid areas of the world where rainfall is insufficient to leach salts from the root zone, and it is a significant factor in reducing crop productivity (Francois and Maas, 1994). Saline soils are defined by Ponnamperuma (1984) as those that contain sufficient salt in the root zone to impair the growth of crop plants. However, since salt injury depends on species, variety, growth stage, environmental factors, and nature of the salts, it is difficult to define saline soils precisely. The most widely accepted definition of a saline soil has been adopted from FAO (1997) as one that has an electrical conductivity of the saturation extract (EC_e) of 4 dS m^{-1} or more, and soils with EC_e's exceeding 15 dS m^{-1} are considered strongly saline. The common cations associated with salinity are Na^+, Ca^{2+} and Mg^{2+}, while the common anions are Cl^-, SO_4^{2-} and HCO_3^-. However, Na^+ and Cl^- ions are considered the most important, since Na^+ in particular causes deterioration of the physical structure of the soil and both Na^+ and Cl^- are toxic to plants (Dudley, 1994; Hasegawa *et al*., 2000). Soils were historically classified as saline, sodic, or saline-sodic based on the total concentration of salt and the ratio of Na^+ to Ca + Mg in the saturated extract of the soil (Dudley, 1994). However, this classification has been abandoned in favor of a management-oriented approach, and a soil that contains excessive salt is presently referred to as saline or salt-affected regardless of the specific nature of the problem.

Due to an increase in population, there is competition for fresh water among the municipal, industrial and agricultural sectors in several regions. The consequence has been a decreased allocation of freshwater to agriculture (Tilman *et al*., 2002). This phenomenon is expected to continue and to intensify in less developed, arid region countries that already have high population growth rates and suffer from serious environmental problems. For this reason there is increasing pressure to irrigate with water

of certain salt content, like ground water, drainage water and treated wastewater (Table 1.1).

Table 1.1 Classification of water (Rhoades *et al*., 1992)

Type of water	EC (dS/m)	TDS[1] (g/l)	Water class
Drinking & Irrigation water	<0.7	<0.5	Non-saline
Irrigation water	0.7-2.0	0.5-1.5	Slightly saline
Primary drainage water & groundwater	2.0-10.0	1.5-7.0	Moderately saline
Secondary drainage water & groundwater	10.0-20.5	7.0-15.0	Highly saline
Very saline groundwater	20.0-45.0	15.0-35.0	Very highly saline
Seawater	>45.0	>35.0	Brine

[1] Total dissolved solutes

According to Carvajal *et al*. (1999); Yeo (1998); Grattan and Grieve (1999) the direct effects of salts on plant growth may be divided into three broad categories: (i) a reduction in the osmotic potential of the soil solution that reduces plant available water, (ii) a deterioration in the physical structure of the soil such that water permeability and soil aeration are diminished, and (iii) increase in the concentration of certain ions that have an inhibitory effect on plant metabolism (specific ion toxicity and mineral nutrient deficiencies). The relative contribution of osmotic effects and specific ion toxicities on yield are difficult to quantify. However, with most crops, Dasberg *et al*. (1991) reported that yield losses from osmotic stress could be significant before foliar injury is apparent.

1.2 Causes of salinity

1.2.1 Primary cause

Most of the saline-sodic soils are developed due to natural geological, hydrological and pedological processes. Some of the parent materials of these soils include intermediate igneous rocks such as phenolytes, basic igneous rocks such as basalts, undifferentiated volcanic rocks, sandstones, alluviam and lagoonal deposits (Wanjogu *et al*., 2001). Climatic factors and water management may accelerate salinization. In arid and semi-arid

lands (ASAL) evapotranspiration plays a very important role in the pedogenesis of saline and sodic soils. Wanjogu *et al*. (2001) reported that most of the ASAL receive less than 500 mm of rainfall annually and this, coupled with an annual potential evapotranspiration of about 2000 mm leads to salinization. Another type of salinity occurs in coastal areas subject to tides and the main cause is intrusion of saline water into rivers (Cyrus *et al*., 1997) or acquifers (Howard and Mullings, 1996). Coastal rice crops in Asia, for instance, are frequently affected by exposure to seawater brought in by cyclones around the Indian Ocean (Sultana *et al*., 2001).

1.2.3 Secondary salinization

Secondary salt-affected soils are those that have been salinized by human-caused factors, mainly as a consequence of improper methods of irrigation. Poor quality water is often used for irrigation, so that eventually salt builds up in the soil unless the management of the irrigation system is such that salts are leached from the soil profile. Szabolcs (1992) estimated that 50% of all irrigated schemes are salt affected. Too few attempts have been made recently to assess the degree of human-induced secondary salinization and, according to Flowers and Yeo (1995) this makes it difficult to evaluate the importance of salinity to future agricultural productivity. Nevertheless, Ohara (1997) has reported increasing salinization with increasing irrigation since the 1950's, and in the Shanxi Province in China, more than one-third of the total area of irrigated land is salinized (Qiao, 1995). The land area under irrigation in Kenya is estimated to be about 84,000 ha (Ngigi, 2002) and according to Mugwanja *et al*., (1995), about 26,000 ha is considered salt degraded mainly due to poor irrigation management and poor drainage, especially in areas with a high ground water table. Anthropic salinization occurs in arid and semi-arid areas due to waterlogging brought about by improper irrigation (Ponnamperuma, 1984). Secondary salt-affected soils can also be caused by human activities other than irrigation and include, but are not limited to, the following:

(a) Deforestation is recognized as a major cause of salinization and alkalization of soils as a result of the effects of salt migration in both the upper and lower layers.

In Southeast India, for example, vast areas of former forestland became increasingly saline and alkaline a few years after the felling of the woods (Szabolcs, 1994). In Australia, a country where one-third of the soils are sodic and 5% saline (Fitzpatrick *et al*., 1994), there is serious risk of salinization if land with shallow unconfined acquifers containing water with more than 0.25% total soluble salt is cleared of trees (Bui *et al*., 1996).

(b) Accumulation of air-borne or water-borne salts in soils

Szabolcs (1994) has reported that chemical accumulation from industrial emissions may accumulate in the soil, and if the concentration is high enough, can result in salt accumulation in the upper layer of soil. Similarly, water with considerable salt concentration such as waste water from municipalities and sludge may contaminate the upper soil later causing salinization and/or alkanization (Bond, 1998; Bouwer, 2002).

(c) Salinization caused by contamination with chemicals

This kind of salinization more often occurs in modern intensive agricultural systems, particularly in greenhouses and intensive farming systems. In closed or semi closed systems (e.g. greenhouses) salts tend to accumulate if chemicals are not removed regularly, resulting in salinity or alkalinity. In countries with intensive agriculture such as Japan and the Netherlands, this type of salinization appears more frequently (Pessarakli, 1991).

(d) Overgrazing

Szabolcs (1994) reported that this process occurs mainly in arid and semi arid regions, where the natural soil cover is poor and scarcely satisfies the fodder requirement of extensive animal husbandry. Because of overgrazing, the natural vegetation becomes sparse and progressive salinization develops, and sometimes the process ends up in desertification as the poor pasture diminishes.

1.3 Salinity effects on plants

According to Dubey (1997) and Yeo (1998) salt causes both ionic and osmotic effects on plants and most of the known responses of plants to salinity are linked to these effects.

The general response of plants to salinity is reduction in growth (Romero-Aranda *et al*., 2001; Ghoulam *et al*., 2002). The initial and primary effect of salinity, especially at low to moderate concentrations, is due to its osmotic effects (Munns and Termaat, 1986; Jacoby, 1994). Osmotic effects of salts on plants are a result of lowering of the soil water potential due to increasing solute concentration in the root zone. At very low soil water potentials, this condition interferes with the plant's ability to extract water from the soil and maintain turgor. Thus, in some species salt stress may resemble drought stress. However, at low or moderate salt concentrations (high soil water potentials), plants adjust osmotically (accumulate internal solutes) and maintain a potential for the influx of water (Guerrier, 1996; Ghoulam *et al*., 2002). Plant growth may be moderated under such conditions, but unlike drought stress, the plant is not water deficient (Shannon, 1984).

At high salinity, some specific symptoms of plant damage may be recognized, such as necrosis and leaf tip burn due to Na^+ or Cl^- ions (Wahome *et al*., 2001). High ionic concentrations may disturb membrane integrity and function, interfere with internal solute balance and nutrient uptake, causing nutritional deficiency symptoms similar to those that occur in the absence of salinity (Grattan and Grieve, 1999).

Sodium and chloride, usually the most prevalent ions in saline soils or water, account for most of the deleterious effects that can be related to specific ion toxicities (Levitt, 1980). The degree to which growth is reduced by salinity differs greatly with species and to a lesser extent with varieties (Bolarin *et al.*, 1991; Ghoulam *et al.*, 2002). The severity of salinity response is also mediated by environmental interactions such as relative humidity, temperature, radiation and air pollution (Shannon *et al*., 1994). Salt accumulation in leaves causes premature senescence, reducing the supply of assimilates to the growing regions and thus decreasing plant growth (Munns *et al.*, 1995). In more sensitive varieties salt accumulates faster, and because cells are unable to compartmentalize the salt in the vacuoles to the same high degree as tolerant varieties, leaves are expected to die sooner (Munns, 1993). Neumann (1997) considered that inhibition of leaf growth by salt decreases the volume of new leaf tissues into which excess salt can be accumulated and, combined with continuous salt accumulation it could

lead to earlier build up of excess salt levels. Salt stress affects all the major processes such as growth, water relations, photosynthesis and mineral uptake.

1.3.1 Effects of salinity on plant growth

Several investigators have reported plant growth reduction as a result of salinity stress, e.g. in tomato (Romero-Aranda *et al*., 2001), cotton (Meloni *et al*., 2001) and sugar beet (Ghoulam *et al*., 2002). However, there are differences in tolerance to salinity among species and cultivars as well as among the different plant growth parameters recorded. For instance, Aziz and Khan (2001) found that the optimum growth of *Rhizophora mucronata* plants was obtained at 50% seawater and declined with further increases in salinity while in *Alhagi pseudoalhagi* (a leguminous plant), total plant weight increased at low salinity (50 mM NaCl) but decreased at high salinity (100 and 200 mM NaCl) (Kurban *et al*., 1999). In sugar beet leaf area, fresh and dry mass of leaves and roots were dramatically reduced at 200 mM NaCl, but leaf number was less affected (Ghoulam *et al*., 2002). Fisarakis *et al*. (2001), working with sultana vines recorded a larger decrease in accumulation of dry matter in shoots than in roots, particularly at high NaCl concentration, indicating partitioning of photoassimilates in favor of roots. They proposed that the results may be due to a greater ability for osmotic adjustment under stress by the roots.

1.3.2 Effects of salinity on water relations

The main cause of reduction in plant growth may result from salinity effects on water status. According to Sohan *et al*. (1999) and Romero-Aranda *et al*. (2001) increase of salt in the root medium can lead to a decrease in leaf water potential and, hence, may affect many plant processes. Osmotic effects of salt on plants are as a result of a lowering of the soil water potential due to increasing solute concentration in the root zone. At very low soil water potentials, this condition interferes with plants' ability to extract water from the soil and maintain turgor (Sohan *et al*., 1999). Thus, in some aspects salt stress may resemble drought stress. However, at low or moderate salt concentrations (higher soil

water potential), plants adjust osmotically (accumulate solutes) and maintain a potential gradient for the influx of water. Under such conditions Shannon (1984) reported that growth may be moderated, but unlike drought stress, the plant is not water deficient.

Several authors found that water potential and osmotic potential of plants became more negative with an increase in salinity, whereas turgor pressure increased (Meloni *et al*., 2001; Romero-Aranda *et al*., 2001; Gulzar *et al*., 2003). In the halophyte *Suaeda salsa*, Lu *et al*. (2002) found that leaf water potential and evaporation rate decreased significantly with increasing salt concentration. Ashraf (2001) reported similar decreases in leaf water potential with increasing salt concentration in all the six *Brassica* species studied. At 200 mM NaCl *B. campestris* and *B. carinata* maintained significantly higher leaf water potentials than the other species, and were, therefore, considered more tolerant to salt stress. With increasing salt concentration, water potential became more negative in sunflower (Sohan *et al*., 1999). According to these investigators, the results seem to stem from two factors: (1) under high salt concentration, plants sequester more NaCl in the leaf tissue than normally occurs. Increases in NaCl within the leaf tissue then result in lower osmotic potentials and more negative water potentials, and (2) the reduction in root hydraulic conductance reduces the amount of water flow from the roots to the upper portion of the canopy, causing water stress in the leaf tissue.

Salt treatment caused a significant decrease in relative water content (RWC) in sugar beet varieties (Ghoulam *et al*., 2002). According to Katerji *et al.* (1997), a decrease in RWC indicates a loss of turgor that result in limited water availability for cell extension processes.

1.3.3 Effects of salinity on leaf anatomy

Salinity has been reported to cause leaf anatomical changes in a number of plants. For instance, leaves of bean, cotton and *Atriplex* are reported to increase in epidermal thickness, mesophyll thickness, palisade cell length, palisade diameter, and spongy cell diameter with increasing salinity (Longstreth and Noble, 1979). In contrast both

epidermal and mesophyll thickness and intercellular spaces decreased significantly in NaCl-treated leaves of the mangrove *Brugueira parviflora* (Parida *et al*., 2004). In leaves of spinach salinity was found to reduce intercellular spaces (Delfine *et al*., 1998) while in tomato plants, a reduction of stomatal density occurred (Romero-Aranda *et al*., 2001).

1.3.4 Effects of salinity on photosynthesis

Growth of plants is dependent on photosynthesis and, therefore, environmental stresses affecting growth also affect photosynthesis (Salisbury and Ross, 1992; Dubey, 1997; Taiz and Zeiger, 1998). Studies conducted by a number of authors with different plant species showed that photosynthetic capacity was suppressed by salinity (Dubey, 1997; Kao *et al*., 2001; Ashraf, 2001; Romero-Aranda *et al*., 2001). A positive association between photosynthetic rate and yield under saline conditions has been found in different crops such as *Gossypium hirsutum* (Pettigrew and Meredith, 1994) and *Asparagus officinalis* (Faville *et al.*, 1999). Fisarakis *et al*. (2001) found that inhibition of vegetative growth in plants submitted to salinity was associated with a marked inhibition of photosynthesis. In contrast, there are many studies in which no or little association between growth and photosynthetic capacity is evident, as in *Triticum repens* (Rogers and Noble, 1992) and *Triticum aestivum* (Hawkins and Lewis, 1993).

The effect of salinity on photosynthetic rate depends on salt concentration and plant species. There is evidence that at low salt concentration salinity may stimulate photosynthesis. For instance, in *B. parviflora*, Parida *et al*. (2004) reported that photosynthetic rate increased at low salinity and decreased at high salinity, whereas stomatal conductance was unchanged at low salinity and decreased at high salinity.

Iyengar and Reddy (1996) attributed decreases in photosynthetic rate as a result of salinity to a number of factors:

(1) Dehydration of cell membranes which reduce their permeability to CO_2. High salt concentration in soil and water create high osmotic potential which reduces the

availability of water to plants. Decrease in water potential causes osmotic stress, which reversibly inactivates photosynthetic electron transport via shrinkage of intercellular space.

(2) Salt toxicity caused particularly by Na^+ and Cl^- ions. According to Banuls *et al.* (1990), Cl^- inhibits photosynthetic rate through its inhibition of NO_3-N uptake by the roots. Fisarakis *et al.* (2001) found that NO_3-N was significantly reduced in salt-stressed sultana vines and this reduction was correlated with photosynthetic reduction. The reduced NO_3-N uptake combined with osmotic stress may explain the inhibitory effect of salinity on photosynthesis.

(3) Reduction of CO_2 supply because of closure of stomata. The reduction in stomatal conductance results in restricted availability of CO_2 for carboxylation reactions (Brugnoli and Bjorkman, 1992). Iyengar and Reddy (1996) reported that stomatal closure minimizes loss of water by transpiration and this affects chloroplast light-harvesting and energy-conversion systems thus leading to alteration in chloroplast activity. Higher stomatal conductance in plants is known to increase CO_2 diffusion into the leaves and thereby favor higher photosynthetic rates. Higher net assimilation rates could in turn favor higher crop yields as was found by Radin *et al*. (1994) in Pima cotton (*Gossypium barbadense*). However, the results for photosynthetic rate and stomatal conductance presented by Ashraf (2001) for six *Brassica* species did not show any significant relationship. There are also reports of nonstomatal inhibition of photosynthesis under salt stress. Iyengar and Reddy (1996) reported that this nonstomatal inhibition is due to increased resistance to CO_2 diffusion in the liquid phase from the mesophyll wall to the site of CO_2 reduction in the chloroplast, and reduced efficiency of RUBPC-ase.

Other causes of reduced photosynthetic rates due to salinity have been identified by Iyengar and Reddy (1996) as: (4) enhanced senescence induced by salinity, (5) changes of enzyme activity induced by changes in cytoplasmic structure, and (6) negative feedback by reduced sink activity.

Although the rate of photosynthesis is reduced under salt stress, this is not the cause of reduction in the rate of cell expansion as suggested by several lines of evidence. According to Yeo *et al*. (1991) and Alarcõn *et al*. (1994) growth is reduced more rapidly

and at lower concentrations of sodium in the leaf than is photosynthesis. This means that plants can withstand a certain loss in photosynthetic rate without any effect on growth. The relationship between photosynthesis and growth of plants under saline conditions is not well understood. Many changes take place in plants in order to enable them tolerate saline conditions and maintain photosynthetic activity. An understanding of the mechanisms by which salinity affects photosynthesis would aid the improvement of growth conditions and crop yield and would provide useful tools for future genetic engineering.

1.3.5 Effects of salinity on ion levels and nutrient content

High salt (NaCl) uptake competes with the uptake of other nutrient ions, such as K^+, Ca^{2+}, N, P resulting in nutritional disorders and eventually, reduced yield and quality (Grattan and Grieve, 1999). Increased NaCl concentration has been reported to induce increases in Na^+ and Cl^- and decreases in Ca^{2+}, K^+ and Mg^{2+} level in a number of plants (Perez-Afocea *et al*., 1996; Khan *et al*., 2000; Bayuelo-Jiménez *et al*., 2003). Ghoulam *et al*. (2002) observed an increase in Na^+ and Cl^- content in the leaves and roots of sugar beet with increasing NaCl concentration in the rooting medium. The K^+ content of the leaves decreased in response to NaCl, but that of roots was not affected by the salt treatment. A significant increase in Na^+ and Cl^- content in leaves, stem, and root of the mangrove (*B. parviflora*) has been reported without any significant alteration of the endogenous level of K^+ and Fe^{2+} in leaves (Parida *et al*., 2004). Decreases of Ca^{2+} and Mg^{2+} content of leaves have also been reported upon salt accumulation in this species.

Under salt stress conditions, the uptake of N by plants is generally affected. A number of studies have shown that salinity can reduce N accumulation in plants (Feigin *et al*., 1991; Pardossi *et al*., 1999; Silveira *et al*., 2001). An increase in Cl^- uptake and accumulation has been observed to be accompanied by a decrease in shoot NO_3^- concentration as in eggplant (Savvas and Lenz, 1996) and sultana vines (Fisarakis *et al*., 2001). Various authors have attributed this reduction to Cl^- antagonism of NO_3^- (Bar *et al*., 1997) while others attributed the response to salinity's effect on reduced water uptake (Lea-Cox and

Syvertsen, 1993). The nitrate influx rate or the interaction between NO_3^- and Cl^- has been reported to be related to the salt tolerance of the species under investigation. Kafkafi *et al*. (1992) found that the more salt-tolerant tomato and melon cultivars had higher NO_3^- flux rates than the more sensitive cultivars.

The effect of salinity on P concentration has been reported by Grattan and Grieve (1994) to be highly dependent on plant species, plant developmental stage, composition and level of salinity, and the concentration of P in the substrate. In most cases, salinity decreased the concentration of P in plant tissue (Sonneveld and de Kreij, 1999; Kaya *et al*., 2001), but the results of some studies indicate salinity either increased or had no effect on P uptake (Ansari, 1990). The reduction in P availability in saline soils was suggested by Sharpley *et al*. (1992) to be a result of ionic strength effects that reduce the activity of phosphate, the tight control of P concentrations by sorption processes and by the low solubility of Ca-P minerals.

Salinity stress has stimulatory as well as inhibitory effects on the uptake of some micronutrients by plants. For a detailed review on this subject refer to (Vîllora *et al*., 1997; Grattan and Grieve, 1999). According to these authors nutrient imbalances may result from the effect of salinity on nutrient availability, competitive uptake, transport or partitioning within the plant, or may be caused by physiological inactivation of a given nutrient resulting in an increase in the plant's internal requirement for that essential element.

1.4 Salt tolerance

Plant salt stress resistance has been defined by Shannon and Grieve (1999) as the inherent ability of plants to withstand the effects of high salt concentrations in the root zone or on the leaves without a significant adverse effect. Sacher and Staples (1984) have defined salinity tolerance as the ability of a plant to grow and complete its life cycle on a substrate that contains high concentrations of soluble salt. In this habitat a plant has to meet two requirements: osmotic adaptation and the acquisition of the mineral elements needed for growth and functional metabolism. Levitt (1980) and Shannon *et al*. (1994)

have classified plants into halophytes and glycophytes depending on their sensitivity to salinity. Halophytes are plants that can grow in the presence of high concentrations of salt, even higher than that of seawater (ca. 500mM) and have a competitive advantage over non-halophytes in this environment. Glycophytes on the other hand are plants that are sensitive to relatively low salt concentrations. Almost all major crop species as well as most wild species are glycophytes. Although individual responses to high salinity may differ, several lines of evidence suggest that all plants use the same general salt tolerance regulatory mechanisms, and that the differences between halophytic and glycophytic species are of a quantitative rather than qualitative nature (Greenway and Munns, 1980; Zhu, 2001). Plant sensitivity to salt levels in the soil is also highly depended on environmental factors (Shannon *et al*, 1994), plant species, cultivars within a species (Greenway and Munns, 1980; Ashraf, 2002), as well as the stage of plant development (Vicente *et al*., 2004).

1.4.1 Interactions between salinity and environmental factors

The ability of plants to tolerate salinity depends on the interaction between salinity and environmental factors such as soil, water, and climatic conditions (Shannon *et al.*, 1994). For instance, many crops are less tolerant to salinity when grown under hot and dry conditions than under cool and humid conditions (Maas and Hoffman, 1977). Under hot and dry conditions yield will decrease more rapidly with increasing salinity compared to yield reduction under cool and humid conditions. This is mainly due to decreased ion accumulation and/or improved plant water relations in the latter conditions (Salim, 1989). Hence, a basic understanding of these interactions is necessary for an accurate assessment of salt tolerance.

1.4.2 Differences in salinity resistance among plant genotypes

Crop yield decreases markedly with increase in salt concentration, but the threshold concentration and rate of yield decrease vary with the species. There are marked interspecific differences in crop tolerance for salinity and within a species ecotypes exist

that can tolerate much higher salt concentrations than normal populations (Hester *et al*., 2001). The genus *Phaseolus*, for instance, includes important cultivated as well as wild species with diverse ecological adaptations. Cluster analysis by Bayuelo-Jiménez *et al*. (2002) revealed substantial intraspecific and interspecific variation in salinity tolerance. Wild species were generally found to be more salt tolerant than the cultivated species, and many tolerant accessions originated in arid, coastal, or saline areas. Such inter-and intra-crop diversity suggests that field trials should be conducted to identify local crops that are adapted to saline conditions (Shannon, 1997).

1.4.3 Influence of growth stage on salinity resistance

The response of plants to salinity varies with growth stage at which salinization is initiated. However, information about the salt tolerance of crops at different stages of growth is limited. It has been demonstrated that the reaction to salt stress varies with the stage of plant development and that a given cultivar may be tolerant at one stage and sensitive at another (Vicente *et al.*, 2004). The available data generally agree that the early seedling stage of growth is the most salt sensitive for most crops (Maas and Poss, 1989; Vicente *et al*., 2004). It is during this stage of growth of cereal crops that leaf and spikelet primodia are initiated and tiller buds are formed (Maas and Grieve, 1990). Consequently, high soil salinity during this stage can severely affect final seed yield. Significant and non-significant associations between tolerance at the germination stage and adult plant growth and development have been indicated (Lovato *et al*., 1994; Bayuelo- Jiménez *et al*., 2002). Although salt stress delays germination and emergence, most crops are capable of germinating at higher salinity levels than they would normally tolerate at the vegetative or reproductive stages of growth (Maas and Grieve, 1990). However, this high tolerance at germination stage is of little benefit when plants are less tolerant during later growth stages.

It has been argued that selection for salinity tolerance at germination, seedling stage or early vegetative growth may not produce tolerant adult plants (Kingsbury and Epstein, 1984). In contrast, the performance of seedlings under saline conditions has been

considered highly predictive of the response of adult plants to salinity (Azhar and McNeilly, 1987). Ashraf *et al*. (1986) evaluated seedlings of barley, wheat, and seven forage grass species, and demonstrated considerable tolerance of salinity at the adult stage. Similarly, in studies conducted by Bayuelo- Jiménez *et al*. (2002), five accessions of *Phaseolus filiformis* previously identified as the most tolerant at germination and early seedling growth, were also tolerant during the vegetative growth stage when exposed to 180 mM NaCl. The tolerance observed in this species, however, may or may not be expressed during reproduction. Nevertheless, tolerance observed at germination, early seedling, and vegetative growth stages is of great importance because salinity tolerance at every stage of growth is of value in determining the ultimate tolerance and performance of the species (Shannon, 1984).

1.5 Mechanisms of salt stress resistance

A variety of mechanisms contribute to salt tolerance (Gorham, 1995). Resistance is the ability of plants to adapt to salinity. It can be achieved by the ability of growing cells of a plant to avoid high ion concentrations or the ability of cells to cope with high ion concentrations (Greenway and Munns, 1980). Levitt (1980) characterized these mechanisms as avoidance and tolerance, and has used the term salt resistance to refer to a combination of tolerance and avoidance strategies. Examples of salt avoidance mechanisms include delayed germination or maturity until favorable conditions prevail; the exclusion of salt at the root zone or preferential root growth into nonsaline areas; compartmentalization of salt into and secretion from specialized organelles such as salt glands and salt hairs; or storage in older leaves (Hasegawa *et al*., 1986). These tolerance mechanisms are discussed under separate headings.

1.5.1 Selective accumulation or exclusion of ions

Both glycophytes and halophytes cannot tolerate large amounts of salt in the cytoplasm and therefore under saline conditions they either restrict the excess salts in the vacuole or

compartmentalize the ions in different tissues to facilitate their metabolic functions (Iyengar and Reddy, 1996; Zhu, 2003).

In general, exclusion mechanisms are effective at low to moderate levels of salinity, whereas ion accumulation is the primary mechanism used by halophytes at high salt levels, presumably in conjunction with the capacity to compartmentalize ions in the vacuole (Jeschke, 1984). Glycophytes limit sodium uptake, or partition sodium in older tissues, such as leaves, that serve as storage compartments which are eventually abscised (Cheeseman, 1988). Apse *et al*. (1999) reported that removal of sodium from the cytoplasm or compartmentalization in the vacuoles is done by a salt-inducible enzyme Na^+/H^+ antiporter.

Inclusion of ions in the cytoplasm can lead to osmotic adjustment that is generally accepted as an important adaptation to salinity (Guerrier, 1996). The decrease of leaf osmotic potential would compensate the salt-induced lowering of water potential, helping to maintain turgor pressure and cell functions under adverse water conditions. Under salt stress, sugar beet accumulated more inorganic ions in the leaves (Ghoulam *et al*., 2002). Such varieties are qualified as “includers” (Yeo, 1983). Similar results were reported in rice (Lutts *et al*., 1996a) and in sorghum (Colmer *et al.*, 1996). The tomato cultivar ‘Daniela’ responded to salinity by decreasing leaf osmotic potential more than ‘Moneymaker’ did and, in this sense, it was considered more adaptable to salty conditions than ‘Moneymaker’ (Romero-Aranda *et al.*, 2001). This accumulation of salt ions could play an important role in osmotic adjustment in stressed plants if they were efficiently compartmentalized. The ability to regulate salt concentration through compartmentalization is an important aspect to salt tolerance.

1.5.2 Synthesis of compatible solutes

The presence of salt in the growth media often results in accumulation of low-molecular-mass compounds, termed compatible solutes, which do not interfere with the normal biochemical reactions (Hasegawa *et al.*, 2000; Zhifang and Loescher, 2003). These

compatible solutes include mainly proline and glycine betaine (Ghoulam *et al*., 2002, Girija *et al*., 2002; Khan *et al*, 2000; Wang and Nii, 2000).). It has been reported that proline levels increase significantly in leaves of rice (Lutts *et al*., 1996b) and in sugar beet (Ghoulam *et al*., 2002). The increase in proline content in sugar beet was positively correlated to the level of salt tolerance. The proposed functions of proline under stress conditions include osmotic adjustment, protection of enzymes and membranes, as well as acting as a reservoir of energy and nitrogen for utilization during exposure to salinity (Bandurska, 1993; Perez-Alfocea *et al*., 1993a).

Exposure to saline stress results in accumulation of nitrogen-containing compounds (NCC) such as amino acids, amides, proteins, polyamines and their accumulation is frequently correlated with plant salt tolerance (Mansour, 2000). For instance, glycine betaine content has been observed to increase in green gram (Sudhakar *et al*., 1993); in amaranth (Wang and Nii, 2000) and in peanut (Girija *et al*., 2002). According to Sakamoto *et al.* (1998), subcellular compartmentation of glycine betaine biosynthesis in rice is important for increased salt tolerance. These compounds have been reported to function in osmotic adjustment, protection of cellular macromolecules, storage of nitrogen, maintenance of cellular pH, detoxification of the cells and scavenging of free radicals.

Other compatible solutes that accumulate in plants under salt stress include: (a) carbohydrates such as sugars (glucose, fructose, sucrose, fructans) and starch (Parida *et al*., 2002; Kerepesi and Galiba, 2000), and their major functions have been reported to be osmotic adjustment, carbon storage, and radicle scavenging, (b) Polyols are reported to make up a considerable percentage of compatible solutes and serve as scavengers of stress-induced oxygen radicals and are also involved in osmotic adjustment and osmoprotection (Bohnert *et al*., 1995).

According to Greenway and Munns (1980), salt sensitivity in non-halophytes may result from either (i) inability of osmoregulation, which may result from either an insufficient uptake of salt ions or a lack of synthesis of organic solutes being used as osmotica, or (ii)

injury caused by inorganic ions which are absorbed by the cell and are not compartmentalized.

1.5.3 Control of ion uptake by roots and transport into leaves

Plants regulate ionic balance to maintain normal metabolism. For example, uptake and translocation of toxic ions such as Na^+ and Cl^- are restricted, and uptake of metabolically required ions such as K^+ is maintained or increased. They do this by regulating the expression and activity of K^+ and Na^+ transporters and of H^+ pumps that generate the driving force for transport (Zhu *et al*., 1993). It is well documented that a greater degree of salt tolerance in plants is associated with a more efficient system for the selective uptake of K^+ over Na^+ (Noble and Rogers, 1992; Ashraf and O'Leary, 1996). It has been reported that a salt tolerant barley variety maintained a cytosolic Na 10 times lower than a more sensitive variety (Carden *et al*., 2003). The tomato cultivar 'Radja' seems to possess a high ability to select and translocate the major nutrients (K^+, Ca^{2+}, Mg^{2+} and NO_3^-) to young leaves under moderate salinity (Perez-Alfocea *et al*., 1996). At high salinity, however, this did not occur for NO_3^-. Thus, decreases in shoot growth observed in this genotype at high salinity could be explained not only by the great amount of toxic ions accumulated in the leaves but also by decrease of NO_3^- in young leaves. Nitrate selectivity over Cl^- in shoot has been correlated with salt tolerance in tomato cultivars (Perez-Alfocea *et al*., 1993a).

The use of plant ionic status to identify salt tolerance has been shown to be applicable (Ashraf and Khanum, 1997), and its relationship with salt tolerance is considered strong enough to be exploited as a selection tool in the breeding of salt tolerant cultivars (Omielon *et al*., 1991).

1.5.4 Changes in photosynthetic pathway under salinity

The reduction in photosynthetic rates in plants under salt stress is mainly due to the reduction in water potential. The main aim of salt tolerance is, therefore, to increase

water use efficiency under salinity. To this effect, some plants such as the facultative halophyte (*Mesembryanthemum crystallinum*) shift their C3 mode of photosynthesis to CAM (Cushman *et al*., 1989). This change allows the plant to reduce water loss by opening stomata at night, thus decreasing transpiratory water loss. In salt-tolerant plant species such as *Atriplex lentiformis,* there was a shift from the C3 to the C4 pathway in response to salinity (Zhu and Meinzer, 1999).

1.5.5 Induction of antioxidative enzymes by salinity

All environmental or man-made stresses have been reported to lead to the production of reactive oxygen species (ROS) that cause oxidative damage (Smirnoff, 1993; Schwanz *et al*., 1996). Plants possess efficient systems for scavenging active oxygen species that protect them from destructive oxidative reactions (Foyer *et al*., 1994). As part of this system, antioxidative enzymes are key elements in the defense mechanisms. Garratt *et al*. (2002) has listed some of these enzymes as catalase (CAT), glutathione reductase (GR), superoxide dismutase (SOD) and glutatione-S-transferase (GST). Superoxide dismutase, for example, metabolizes oxygen (O_2) radicals to hydrogen peroxide (H_2O_2), thus protecting cells from damage. Catalase, ascorbate peroxidase, and a variety of peroxidases catalyze the subsequent breakdown of H_2O_2 to water and oxygen (Chang *et al*., 1984; Garratt *et al*., 2002). Plants with high levels of antioxidants have been reported to have greater resistance to this oxidative damage (Spychalla and Desborough, 1990).

Garratt *et al*. (2002) and Mittova *et al*. (2002; 2003) reported increased activities of the antioxidative enzymes in plants under salt stress. They found a correlation between these enzyme levels and salt tolerance. Many changes have been detected in the activities of antioxidant enzymes in plants exposed to salinity. The activity of antioxidant enzymes was reported to increase under saline conditions in shoot cultures of rice (Fadzilla *et al*., 1997), wheat (Meneguzzo and Navarilzzo, 1999) and pea (Hernandez *et al*., 1999), but decreased in wheat roots (Meneguzzo and Navarilzzo, 1999) or was unaffected as in the case of SOD in cucumber (Lechno *et al*., 1997). The differences in these results may be due to the fact that salinity effects depend on a number of factors, for example, salt type,

concentration, plant genotype, growth stage and environmental conditions (Shannon *et al*., 1994). The mechanism by which salinity affects the antioxidant responses is not yet clear. Meneguzzo and Navarilzzo (1999), however, proposed that it might be either via (i) the effect of Cl^- toxicity on photosystem II or (ii) the change in membrane integrity caused by a high Na^+ to Ca^{2+} ratio.

The results from these studies give the possibility with which to investigate the biochemical mechanisms, in particular the role of antioxidants, underlying salt tolerance. An understanding of such mechanisms in certain plant species is essential in evaluating the potential gene flow as a means for amending and introducing salt tolerance into crop species.

1.5.6 Induction of plant hormones by salinity

The levels of plant hormones such as ABA and cytokinins increase with high salt concentration (Aldesuquy, 1998; Vaidyanathan *et al*., 1999). Abscisic acid is responsible for the alteration of salt-stress-induced genes, and these genes are predicted to play an important role in the mechanism of salt tolerance in rice (Gupta *et al.*, 1998). The inhibitory effect of NaCl on photosynthesis, growth and translocation of assimilates has been found to be alleviated by ABA (Popova *et al*., 1995). Although the nature of ABA receptor(s) remains unknown Leung and Giraudat (1998) pointed out that there is substantial evidence of the involvement of ABA in reversible protein phosphorylation and modification of cytosolic calcium levels and pH. Chen *et al*. (2001) reported that the increase of Ca^{2+} uptake is associated with the rise of ABA under salt stress and thus contributes to membrane integrity maintenance, which enables plants to regulate uptake and transport under high levels of external salinity in the longer term. ABA has been reported to reduce ethylene release and leaf abscission under salt stress in citrus probably by decreasing the accumulation of toxic Cl^- ions in leaves (Gomezcadenas *et al.*, 2002).

Other plant hormones found to accumulate in the presence of salt include jasmonates. Higher levels of jasmonates were found to accumulate in salt-tolerant tomato cultivars

compared to the salt-sensitive ones (Hilda *et al*., 2003). Jasmonates have been reported to have important roles in salt tolerance. They are generally considered to mediate signaling, such as defense responses, flowering, and senescence (Hilda *et al*., 2003). However, factors involved in the jasmonate signal-transduction pathway remain unclear.

1.6 Managing salinity in agricultural production

Saline lands can be converted to more productive croplands by preventing the influx of salt water through proper farm management practices, correcting soil toxicities and nutrient deficiencies, and leaching the salts out of the root zone. The reclamation costs can be reduced by growing salt-tolerant cultivars. These practices are discussed below.

1.6.1 Farm management practices

Salinity can be restricted by changed farm management practices. Munns *et al*. (2002) proposes that irrigated agriculture could be sustained by better irrigation practices such as adoption of partial root zone drying methodology, and drip or micro-jet irrigation to optimize use of water. They suggested that salinity could also be contained by reducing the amount of water passing beyond the roots by re-introducing deep rooted perennial plants that continue to grow and use water during the seasons that do not support annual crop plants. This may restore the balance between rainfall and water use, thus preventing rising water tables and the movement of salt to the soil surface. Deep-rooted perennial lucerne (*Medicago sativa*) has been found to lower the water table sufficiently to allow subsequent cropping (Ridley *et al.,* 2001). Such practices will rely on plants that have a high degree of salt tolerance. Salt tolerance in crops will also allow the more effective use of poor quality irrigation water. Niknam and McComb (2000) suggested that trees could be planted to take up some of the excess salt since they have high water use and can lower water tables to reduce salt discharge into streams and prevent secondary salinization of the surrounding areas. However, it has not been proven to what extend the tree planting would assist in preventing salt stress in neighboring fields.

1.6.2 Amelioration through fertilization

Salinity causes nutrient imbalances, mainly resulting in lower concentrations of the macro-elements (N, P, K and Ca) in plant tissues. Hence, the most direct way to recover the normal nutrient concentrations within the plant would be by raising their concentrations in the root zone by higher fertilizer dosages. Many studies have shown that salt stress can be alleviated by an increased supply of calcium to the growth medium (Rausch *et al*., 1996; Ebert *et al*., 2002; Kaya *et al*., 2002). Depending on the concentration ratio, sodium and calcium can replace each other from the plasma membrane, and calcium might reduce salt toxicity (Rausch *et al*., 1996). Song and Fujiyama (1996) found that tomato plants grown in saline medium with supplemental Ca^{2+} accumulated 40% less Na^{+} and 60% more K^{+} than salinized plants without such supplement.

Increased Na^{+} in the growth medium generally decreases the K^{+} content, suggesting an antagonism between Na^{+} and K^{+} (Adams and Ho, 1995). Addition of K^{+} to the nutrient solution has been found to raise K^{+} concentrations in the leaves and ameliorate salinity stress effects (Lopez and Satti, 1996; Kaya *et al*., 2001). The effect of salinity on P in plants depends on P concentration in the nutrient solution. At high P concentrations, leaf injury has been interpreted as P toxicity induced by salinity (Awad *et al*., 1990). However, at low P concentrations in the root medium, salinity was reported to inhibit P uptake by roots and translocation to the shoot (Martínez *et al*., 1996). At low P concentration in the root medium, supplementary P applied to the saline growth medium enhanced the capacity of tomato plant to regulate Na^{+}, Cl^{-} and K^{+} distribution, and improved plant growth (Awad, *et al*., 1990; Kaya *et al*., 2001). Under salt stress conditions, the uptake of N by plants is generally affected, and application of supplementary N has been found to ameliorate the deleterious effects of salinity (Gómez *et al*., 1996).

The approach of raising fertilizer dosages may work for irrigation with water at low salt concentrations. When water of high salinity is applied, however, the concentration of

antagonistic ions required is so high that it causes a marked increase in the osmotic pressure of the soil solution, compounding the stress imposed by the salinity ions (Feigin, 1985). Furthermore, Grattan and Maas (1988) reported that in some species a very high concentration of nutrients, e.g. P, could interact negatively with salinity ions, resulting in severe toxic effects.

1.6.3 Leaching

Leaching soils to remove soluble salts is the most effective method known to reclaim saline soils. This requires good permeability of the soil and good quality irrigation water. Removal of salts by leaching reduces salt hazard for plants but might cause permeability to decrease and pH to increase resulting in decomposition of roots as soil is changed from saline-sodic to sodic (Dregne, 1976). Although the best long-term solution to salinization is to provide adequate drainage, this process is expensive. Hence, many irrigation schemes, particularly in developing countries lack, adequate drainage (Toenniessen, 1984).

1.6.4 Uses of salt stress tolerant plants

Some areas have naturally occurring salinity and salt-tolerant crop plants may provide a better or perhaps the only means of utilizing these resources for food production. Salinity can possibly also be managed through biologically manipulating the plants (Shannon, 1984). Identification of plant genotypes with tolerance to salt, and incorporation of desirable traits into economically useful crop plants, may reduce the effects of salinity on productivity. Developing crop plants tolerant to salinity has the potential of making an important contribution to food production in many countries. This will permit the use of low quality water and thereby reduce some of the demand for higher quality water. Great effort is, therefore, being directed toward the development of salt-tolerant crop genotypes through the use of plant-breeding strategies involving the introgression of the genetic background from salt-tolerant wild species into cultivated plants (Shannon, 1984; Pitman and Laüchli, 2002). However, it should be borne in mind that there is also the risk that the

availability of salt tolerant genotypes will result in less effort to reclaim saline areas or to prevent salinization. In the longer term this will be counter productive.

1.7 The Amaranth

Amaranth is native to South and Central America where its cultivation by the Aztecs dates back 5000 to 7000 years ago (Kauffman and Weber, 1990; Stallknecht and Schulz-Schaeffer, 1993). Amaranth was both an important food crop for the Aztecs, and an important item in their religious ceremonies (Myers, 1996). Currently, amaranths are widely grown as a green leafy vegetable or as grain crop in many parts of sub-tropical and tropical Asia, Africa and Central America. According to Feine *et al.* (1979) this is probably due to the ability of these plants to adapt readily to new environments and extremely broad climates, as well as their competitive ability that permits culture with minimum crop management.

Amaranth comes in different forms. Some species have colored leaves, stems and flowers of purple, orange, red and gold (National Research Council [NRC], 1984). Several ornamental forms of this species are widely grown all over the world. Growth habits vary from prostrate to erect and branched to unbranched while leaf and stem colors range from red to green, with a multitude of intermediates; and seed colors range from black to white (NRC, 1984; Kochhar, 1986). This group of plants belongs to the family *Amaranthaceae*, contains about 800 species and is divided into grain and vegetable types (Allemann *et al*., 1996).

1.7.1 Grain Amaranth

Amaranth is one of the few non-grasses with the potential of becoming a cereal-like grain crop. The seedheads resemble those of sorghum (Figure 1.1) and the individual seed is extremely tiny and barely bigger than a mustard seed (0.9 to 1.7 mm in diameter). Seeds occur in massive numbers, sometimes more than 50,000 to a plant, and are cream colored, golden or pink (NRC, 1984). The three principal species considered for grain

production are: *Amaranthus hypochondriacus*, *A. cruentus* and *A. caudatus*, which are distinguished by morphological characteristics of the inflorescence and florets (NRC, 1984). *Amaranthus cruentus* is thought to be the most adaptable of all amaranth species and flowers under a wide range of day lengths than the others. It is also often grown as a vegetable and an ornamental (Figure 1.1), while *A. hypochondriacus* has excellent seed quality and shows the greatest potential for use as a food ingredient (NRC, 1984).

Figure 1.1 *Amaranthus cruentus* in flower

1.7.2 Vegetable Amaranth

There is no distinct separation between grain and vegetable amaranth types. In general, grain forms have light seeds and vegetable forms have dark seeds. However, NRC (1984) and Kochhar (1986) have listed amaranth grown for vegetables as: (a) *Amaranthus tricolor* L. (Syn. *A. oleraceus* L., *A. gangeticus* L.) which is grown mainly in East Asia and is probably the best developed of the vegetable amaranth species. The plants are

succulent, low growing, and compact, with growth habits much like spinach. Some ornamentals with very beautiful foliage also belong to this species, (b) *A. dubius* is a weedy species used as a green vegetable in West Africa and the Caribean. Its seeds are extremely small, and it has distinctive dark-green, broad ridged leaves, (c) *A. hybridus* is a weedy species commonly used as a leafy vegetable throughout tropical areas. Its size and color vary greatly. Red-stemmed varieties are usually planted as ornamentals while green varieties are commonly used as vegetables, and (d) *A. lividus* is well adapted to temperate climates. It has a number of weedy forms with either red or green leaves.

1.7.3 Weedy species

Only a limited number of all the amaranth species are used as crops. The majority of the others are considered weedy species. The main weedy types are *A. viridis, A. spinosus. A. retroflexus*, and *A. hybridus* (NRC, 1984). *Amaranthus retroflexus* (pigweed) is considered one of the world's worst weeds. The difference between the weedy species and the cultivated types is the fact that the former tend to be indeterminate and robust in growth habit.

1.7.4 Utilization and nutritional importance

1.7.4.1 Vegetable amaranth

The leaves, petioles and young tips of all *Amaranthus* species (including the weedy types) are edible and several species are already widely used as salads and as potherbs (boiled greens) (Grubben, 1976; NRC, 1984; Larkcom, 1991). As a vegetable, Amaranth is nutritionally more valuable than most spring and summer vegetables. According to Allemann *et al*. (1996), 100g portion of amaranth provides the same amount of vitamins as 600g of swiss chard or 280g of cabbage. It also provides more energy, protein, minerals and vitamins (especially vitamin A and C) than other leafy vegetables (Larkcom, 1991; Food Gardens Foundations, 1994; Allemann *et al.*, 1996). In terms of mineral content, notably iron and calcium, amaranth greens rank particularly well when

measured against other potherbs (Makus, 1984; Makus, 1990; Table 1.2). High levels of the nutritionally valuable amino acids, lysine and methionine, have been found in 13 amaranth species (Saunders and Becker, 1983). Vegetable amaranths are also an important source of vitamins especially vitamin A, the lack of which results in a serious nutritional deficiency in the tropics, and leads to blindness and even death in thousands of children each year (NRC, 1984; Okigbo, 1990).

Table 1.2 Nutrient content of selected raw vegetable leaves (per 100g of edible portion) (Saunders and Becker, 1983)

Component	Amaranth	Spinach	Basella	Chard
Dry matter (g)	13.1	9.3	6.9	8.9
Food energy (cal)	36	26	19	25
Protein (g)	3.5	3.2	1.8	2.4
Fat (g)	0.5	0.3	0.3	0.3
Carbohydrates				
Total (g)	6.5	4.3	3.4	4.6
Fiber (g)	1.3	0.6	0.7	0.8
Ash (g)	2.6	1.5	1.4	1.6
Calcium (mg)	267	93	109	88
Phosphorus (mg)	67	51	52	39
Iron (mg)	3.9	3.1	1.2	3.2
Sodium (mg)	----	71	----	14.7
Potassium (mg)	411	470	----	550
Vitamin A (IU)	6,100	8,100	8,000	6,500
Vitamin C (mg)	80	51	102	32
Riboflavin (mg)	0.16	0.2	----	0.17
Niacin (mg)	1.4	0.6	0.5	0.5
Thiamin (mg)	0.08	0.1	0.05	0.06

1.7.4.2 Grain Amaranth

According to the NRC (1984) amaranth seed can be used in several ways, for example, as cereals or as an ingredient in confection. When heated, the tiny amaranth grains pop and taste like a nutty-flavored popcorn. The seeds can be milled to produce a light-colored flour suitable for biscuits, bread, cakes and other baked goods. However, since amaranth grain is known to contain little functional gluten, it must be blended with wheat flour to make yeast leavened baked goods "rise" (NRC, 1984).

Amaranth produce a high protein seed compared to other non-legume grain crops. Its valuable source of protein exceeds that of wheat or any other cereal grain, including that of lysine, which is normally low in grains (NRC, 1984; Pedersen *et al*., 1987). Saunders and Becker (1983) reported that amaranth protein itself is low in leucine. However, this amino acid is found in excess in conventional plant protein sources, hence, it is a nutritional complement to conventional cereals. As a result, amaranth grain has been incorporated into a range of human food products, which are primarily targeted at health conscious consumers (Pedersen *et al.,* 1987; Breene, 1991).

Amaranth grain consists of approximately 5 to 9% oil, which is generally higher than in other cereals. The lipid fraction of amaranth grain is similar to that of other cereals. Detailed studies and a review on amaranth grain oil have been published (Lyon and Becker, 1987; Becker, 1989). NRC (1984) reported that unprocessed amaranth grain can be used as an animal feed, particularly for poultry.

1.7.5 Physiology of the amaranth

Evidence indicates that amaranths are adapted to many environments and tolerate adversity because they use an especially efficient type of photosynthesis (Wang *et al*., 1992; 1993). Amaranth is one of the few C4 species that are not grasses. The C4 pathway is a modification of the normal (C3) photosynthetic process that makes efficient use of the CO_2 available in the air by concentrating it in the chloroplasts of specialized

cells surrounding the leaf vascular bundles (Kanai and Edwards, 1999). In C3 species the primary carbon fixation enzyme is ribulose-1,5-biphosphate carboxilase/oxygenase (RuBPC-ase) and the first product of carboxylation is a three carbon molecule, 3-phosphoglyceric acid (3-PGA), hence the name C3. In C4 plants, the initial fixation of atmospheric carbon dioxide produces three C4 acids (malate, aspartate, oxaloacetate), hence the name C4 (Leegood, 1999). This is accomplished by the enzyme phosphoenolpyruvate carboxylase (PEPC-ase). There are a number of advantages associated with the C4 photosynthetic pathway.

The photorespiratory loss of CO_2, the basic unit for carbohydrate production, is suppressed in C4 plants. According to Gardner *et al*. (1994) and Leegood (1999), the C4 species are believed to have little or no photorespiration because movement of the four-carbon acids into the vascular sheath cells concentrates CO_2 in these cells, which would favor the RuBP carboxylase reaction over RuBP oxygenase. Consequently, plants that use the C4 pathway can convert a higher ratio of atmospheric carbon to plant sugars than those that possess the classical C3 pathway. In C4 species photosynthesis can operate at low intercellular concentrations of CO_2, and hence, lower stomatal conductance. This means that the C4 plants can restrict water loss to a minimum, and yet photosynthesize at rates equivalent to those of C3 plants (Leegood, 1999). Thus, water use efficiency of C4 plants is roughly double that of C3 plants.

One of the physiological features noted of C4 plants is their high rate of photosynthesis at full sunlight under tropical conditions (Hatch, 1992). Photosynthesis in C3 species at light saturation is often limited by the quantity of RuBPC-ase and by the capacity for regeneration of the acceptor RuBP molecule (Evans and Farquhar, 1991). The maximum rate of CO_2 assimilation that both of these limitations can support is lowered by oxygenation of RuBP and the resulting photorespiratory evolution of CO_2 (Leegood and Edwards, 1996). C4 species avoid most of this loss by concentrating CO_2 at the site of Rubisco in the bundle sheath (Leegood, 1999). This allows C4 species to attain potentially higher photosynthesis in full sunlight. C3 species reach light saturation at much lower light intensities than that of full sunlight.

As a result of CO_2 concentration at the site of Rubisco in C4 species, the requirement for nitrogen in photosynthesis is lower than in C3 species (Hocking and Meyer, 1991). The C4 pathway is particularly efficient at high temperature, in bright sunlight, and under dry conditions. Plants that use it tend to require less water than C3 carbon-fixation plants. For these reasons amaranth may be a promising crop for hot and dry areas which are also more often saline.

1.7.6 Salinity studies in amaranth

Relatively few salinity stress studies have been conducted with amaranth. Murata *et al*. (1992) and Brownell and Bielig (1996) reported that the plant family *Amaranthaceae*, to which amaranth belongs, is one of the three families of higher plants which are natrophilic, i.e., having a sodium requirement for growth. Sodium ions have been found to stimulate the regeneration of phosphoenolpyruvate in mesophyll chloroplasts of *A. tricolor* (Murata *et al*., 1992). However, excess amounts of sodium, usually in conjunction with chloride, can reduce plant growth through many physiological and biochemical processes (Match *et al*., 1986; Volkmar *et al*., 1998; Hasegawa *et al*., 2000; Kashem *et al*., 2000a, b). *Amaranthus tricolor* has been reported to germinate at concentrations of 250 mM NaCl (EC ~ 25 dS m^{-1}) (Macler *et al*., 1990), and in sand culture greenhouse experiments, *A. tricolor* has been judged 'relatively salt-tolerant' when grown with nutrient solutions containing 0 to 60 meq./L NaCl (EC ~ 0 to 6 dS m^{-1}) (Shimose *et al*., 1991). Salt tolerance has also been reported for *A. tricolor* by Wang *et al*. (1999) and Wang and Nii (2000) who suggested that amaranth may be of value as a crop for salt affected regions.

In field-grown *Amaranthus* spp., Gaikwad and Chavan (1995; 1999) found that increasing the salinity of the irrigation water from 4 to 6 dS m^{-1} reduced plant carbohydrates, soluble and total oxalates, and nitrates at both vegetative and flowering stages of growth. Increasing the salinity of irrigation water to 16 dS m^{-1} resulted in decreased leaf transpiration, diffusive resistance to CO_2, and more negative osmotic potential in field-grown *A. caudatus*, *A. hypochondriacus*, and *A. paniculatus* (Gaikwad

and Chavan, 1998). The effect of salinity on accumulation of glycine betaine (GB) in *A. tricolor* was investigated by Wang and Nii (2000). These authors found that GB increased several days after salt stress, supporting the notion that the compound is active in the process of osmotic adaptation to salinity in *Amaranthus*. The results were interpreted as evidence that the level of GB in *Amaranthus* leaves is roughly regulated by the salt concentration in the root zone (Wang and Nii (2000).

In a more recent study Makus (2003) investigated the effect of salinity and nitrogen level on agronomic performance of *A. tricolor* and found that supplemental N improved yield and leaf greenness in response to higher soil salinity. It is evident that differences in the tolerance of amaranth genotypes to salinity, particularly at different growth stages, have not been sufficiently researched. Although salinity is prevalent in arid areas the response of amaranth to the interactive effect of salinity and water stress has not yet been documented. With increasing salinity an integrated approach in dealing with the problem may be required. One approach will be to come up with ways to ameliorate salinity stress effects. However, little has been reported concerning amelioration of salinity stress in amaranth.

CHAPTER 2

EFFECT OF SALINITY STRESS ON AMARANTH SEED GERMINATION AND SEEDLING GROWTH

2.1 ABSTRACT

Good plant stands are difficult to obtain in saline environments due to poor germination and seedling emergence. The response of germination to salinity stress varies with species and variety, salt type, salt concentration and environmental conditions. Salinity tolerance during germination and early seedling growth was examined for six genotypes of amaranth namely *Amaranthus tricolor*, Accession '83, *A. dubius*, *A. hypochondriacus*, *A. cruentus* and *A. hybridus* Ten salt treatments, 0, 25, 50, 100, and 200 mM NaCl or Na_2SO_4 were applied and germination was carried out in petri dishes at 27^{o}C for 10 days. Enhancement of germination was observed at 25 mM NaCl in *A. tricolor*, *A. hypochondriacus, A. cruentus*, and at 25 mM Na_2SO_4 in *A. hybridus* and *A. dubius*. The strongest inhibition of germination occurred at the highest salt concentration (200 mM), where only 17% of *A. tricolor* and 24% of Accession '83 seeds were able to germinate in NaCl. No genotype germinated at 200 mM Na_2SO_4. Accession '83 had the highest final germination while *A. hybridus* showed the least. A seedling emergence and growth experiment was conducted in a greenhouse, in plastic pots containing sand. Four genotypes (*A tricolor*, Accession '83, *A. cruentus* and *A. hypochondriacus*) were exposed to NaCl and Na_2SO_4 at concentrations of 0, 25, 50, 100 mM. Emergence and seedling survival were reduced by increasing salt concentrations. There was no emergence at 100 mM Na_2SO_4. Stem and root lengths as well as shoot fresh mass were reduced by increasing salt stress. *A. tricolor* was the most sensitive genotype, with the seedlings surviving only in the control and 25 mM Na_2SO_4 treatments, while *A. hypochondriacus* was the most tolerant with 100% and 95% survival at 25 and 50 mM Na_2SO_4 respectively.

2.2 INTRODUCTION

An essential step in growing a successful crop is obtaining an adequate plant population, as yield is reduced by sub-optimal plant densities and uneven stands. Salinity of soil and irrigation water is a continuing threat to economic crop production especially in arid and semiarid regions of the world (Kayani *et al*., 1990). The ability of seed to germinate in saline environments, the cotyledons to break through a soil crust while emerging, and seedlings to survive in saline conditions are crucial for crop production in saline soils (Maranon *et al*., 1989).

Several investigations of seed germination under salinity stress have indicated that seeds of most species attain their maximum germination in distilled water and are very sensitive to elevated salinity at the germination and seedling phases of development (Khan and Ungar, 1996a, b; 1997; Keiffer and Ungar, 1997; Ghoulam and Fares, 2001). Plant responses to salinity also depend on the anion associated with sodium. For example, crop species such as *Hordeum vulgare* (Huang and Redmann, 1995a) and *Triticum aestivum* (Hampson and Simpson, 1990) were found to be inhibited more by sodium sulfate than by sodium chloride. For other species such as *Brassica napus*, the reverse was found (Huang and Redmann, 1995a). The detrimental effect of salinity occurs because of osmotic stress and specific ion toxicity (Ungar, 1995). The interaction of specific ion and osmotic effects induce a reduction in the number of seeds germinated and a retardation in the rate of germination.

Germination and seedling development is very important for early establishment of plants under stress conditions. Selecting cultivars for rapid and uniform germination under saline conditions can contribute towards early seedling establishment. Owing to its high nutritive value and a wide adaptability to diverse environments, amaranth has been considered a promising crop for marginal lands and semi arid regions (Cunningham *et al*., 1992; Allemann *et al*., 1996). Salinity is one of the major limiting factors in crop production in such areas. It is necessary to understand the response of amaranth to salinity stress if cultivation in saline areas is considered. Little information on the effect of salinity stress on amaranth seed germination and seedling establishment is available.

The research objectives were to:

- assess the response of amaranth seed germination and seedling growth to different salts, and levels of salinity stress, and
- evaluate genetic differences in germination and seedling development.

2.3 MATERIALS AND METHODS

2.3.1 Seed germination

Seeds of six amaranth genotypes, namely: *Amaranthus tricolor, A. hybridus, A. dubius,* Accession *'83, A. hypochondriacus,* and *A. cruentus* were supplied by Agricultural Research Council - Roodeplaat Vegetable and Ornamental Plant Institute, South Africa in May 2002, and stored at 4^{o}C until use. Germination experiments were carried out during July and August 2002.

The trials were conducted at the Experimental Farm of the University of Pretoria. Seeds were germinated in covered, sterilized, disposable petri dishes containing Whatman No. 3 filter paper moistened with either distilled water (control), or 25, 50, 100 or 200 mM of either NaCl or Na_2SO_4 solutions. The high rates of NaCl and Na_2SO_4 were included to ensure a range of germination reactions. Petri dishes were sealed with parafilm to prevent evaporation of water, thus minimizing changes in concentration of solutions. Three replicates of 50 seeds each were used for all treatments. Seeds were incubated in a growth chamber at 27^{o}C and were considered germinated with the emergence of the radicle. Germinated seeds were determined every day until the end of germination period of 10 days. Every three days, the germinated seeds were removed from the petri dishes. The first three seeds to germinate in each replicate were retained for measurements of radicle and hypocotyl lengths at the end of the experiment. In order to maintain adequate moisture 5 ml of the original salt solutions were added to each petri dish every three days.

The rate of germination was estimated by using a modified Timson index of germination velocity = ∑G/t, where G is percentage of seed germination at 2 day intervals and t is total germination period (Khan and Ungar, 1984). On the 10th day radicle and hypocotyl lengths were determined.

2.3.2 Seedling emergence and growth

This experiment was carried out in August 2002 to evaluate the effects of irrigation with various saline solutions on emergence and seedling growth. Four amaranth genotypes, namely *A. tricolor*, Accession '83, *A. cruentus*, and *A. hypochondriacus* were compared. Ten seeds for each treatment were planted at a uniform depth of 5 mm in one liter plastic pots filled with acid washed sand. The pots were placed on benches in a heated greenhouse at a temperature range of 16 to 24^{o}C (mean minimum and maximum) for 21 days and irrigated every other day with NaCl or Na_2SO_4 solutions at concentrations of 0, 25, 50, and 100 mM. High humidity was maintained by covering the pots with transparent plastic bags. The bags were removed as soon as seedlings started to emerge. The number of emerged seedlings was noted every day. After 21 days the seedlings were assessed for survival, and harvested. Shoot and root lengths, number of lateral roots and shoot fresh mass were determined. All treatments were replicated three times in a completely randomized design.

2.3.3 Statistical analysis

Data were submitted to Bartlett's test for the homogeneity of variance. Square root transformations of percent germination and emergence data were necessary to achieve homogeneity of variance and compare data from the early and late germinations. Data were analyzed by the SAS (Statistical Analysis System) (SAS Institute Inc. Cary, NC, USA 1996 Copyright) method and means were compared using Tukey's t-test at $P \leq 0.05$. Percentages without transformation are reported.

2.4 RESULTS AND DISCUSSION

2.4.1 Seed Germination

The main effects of genotype, salt type, and concentration were significant, but due to significant interactions between them, only interactive effects between the treatment combinations are presented. Seed germination in control treatments varied with genotype, from 80% in *A. hybridus* to 97% in Accession '83. With NaCl treatments the germination of *A. hypochondriacus* and *A. cruentus* was enhanced by 25 mM NaCl (Figure 2.1a). In *A. tricolor*, Accession '83 and *A. hybridus* there was no significant difference in percent germination between control and seeds submitted to 25 mM NaCl. Germination was progressively inhibited with increased NaCl concentrations. The greatest inhibition occurred with the highest salt concentrations of 100 and 200 mM. For example, at 100 mM NaCl the reduction in germination ranged from 8% in Accession '83 to 24% in *A. hypochondriacus*. Only 17% of *A. tricolor* and 24% of Accession '83 were able to germinate at 200 mM NaCl, with no germination in the other genotypes.

With Na_2SO_4 treatments enhancement of germination was observed in *A. dubius* and *A. hybridus* at 25 mM, while there was no difference in germination between the control and 25 mM treated seeds in the rest of the genotypes. A progressive decrease in germination at higher concentrations was observed. Exposure to 100 mM Na_2SO_4 depressed germination more than NaCl with germination reductions ranging from 18% in Accession '83 to 45% in *A. hybridus*. No seed germinated at 200 mM Na_2SO_4 (Figure 2.1b).

Across the treatment combinations Accession '83 had the highest germination percentage and *A. hybridus* the lowest. Significantly higher germination percentages were observed in NaCl than in Na_2SO_4 treatments, particularly at 50 and 100 mM (Figure 2.1a; 2.1b).

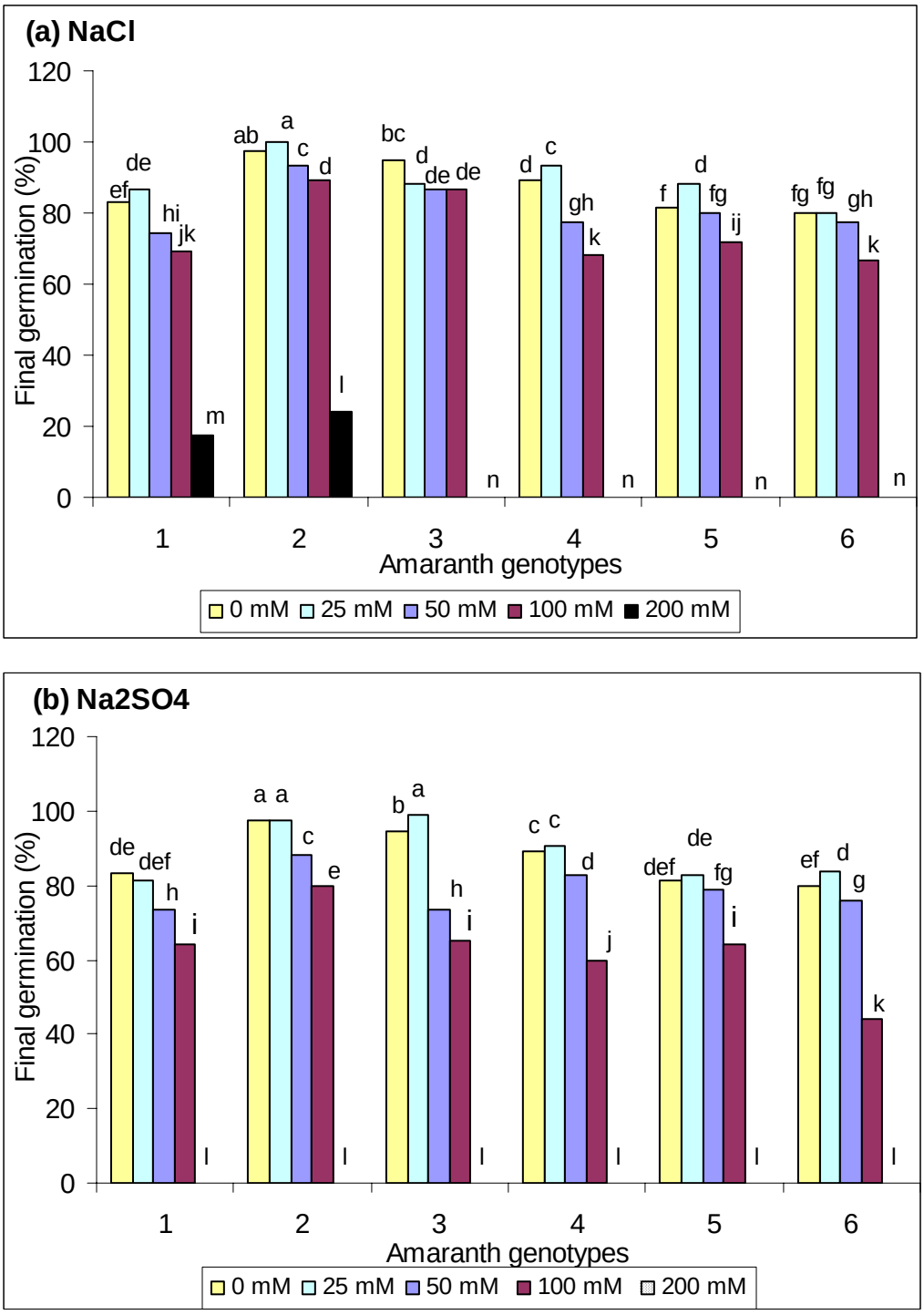

Figure 2.1 Effect of (a) NaCl and (b) Na_2SO_4 on germination of amaranth genotypes (1) *A. tricolor*, (2) Accession '83, (3) *A. dubius*, (4) *A. hypochondriacus*, (5) *A. cruentus* and (6) *A. hybridus*. Mean separation by Turkey T-test. Bars followed by the same letter are not significantly different at P = 0.05.

Figure 2.2a and 2.2b illustrate the differences in the trend of amaranth seed germination during the period of incubation in either NaCl or Na_2SO_4. In both the NaCl and Na_2SO_4 treatments germination of all the genotypes in the control and 25 mM NaCl treatments commenced after one day of incubation and was mostly completed after 4 days. Germination was delayed at the higher salt concentrations, especially at 100 mM NaCl where the delay in germination was very obvious for *A. hypochondriacus*, but also occurred in the case of the other genotypes. With the exception of *A. tricolor* and Accession '83 none of the genotypes were able to germinate in 200 mM NaCl. A delay in germination of *A. tricolor* and Accession '83 seeds at 200 mM NaCl was observed, with the first seeds only germinating on day 4. In most of the genotypes completion of germination at higher salt concentrations was also delayed to 6 to 8 days from the start of incubation.

Most of the genotypes attained more than 50% germination on day one under control and 25 mM NaCl or Na_2SO_4 treatments. At 50 mM all genotypes had less than 50% germination on day one with the exception of *A. hypochondriacus* that attained 74 and 57% germination in NaCl and Na_2SO_4 respectively. Although only 42 and 26 % of seeds of Accession '83 had germinated on day one in 50 mM NaCl or Na_2SO_4 over 80% had germinated by day 2. Germination was delayed to day 2 at 100 mM with most genotypes attaining more than 50% in NaCl. On the other hand, exposure to Na_2SO_4 resulted in 50% germination reached on day four in *A. cruentus* and *A. hypochondriacus*. A 50% germination was not attained by *A. hybridus* in 100 mM Na_2SO_4 while in NaCl it was attained by day 3 (Figure 2.2a; 2.2b).

NaCl **Na_2SO_4**

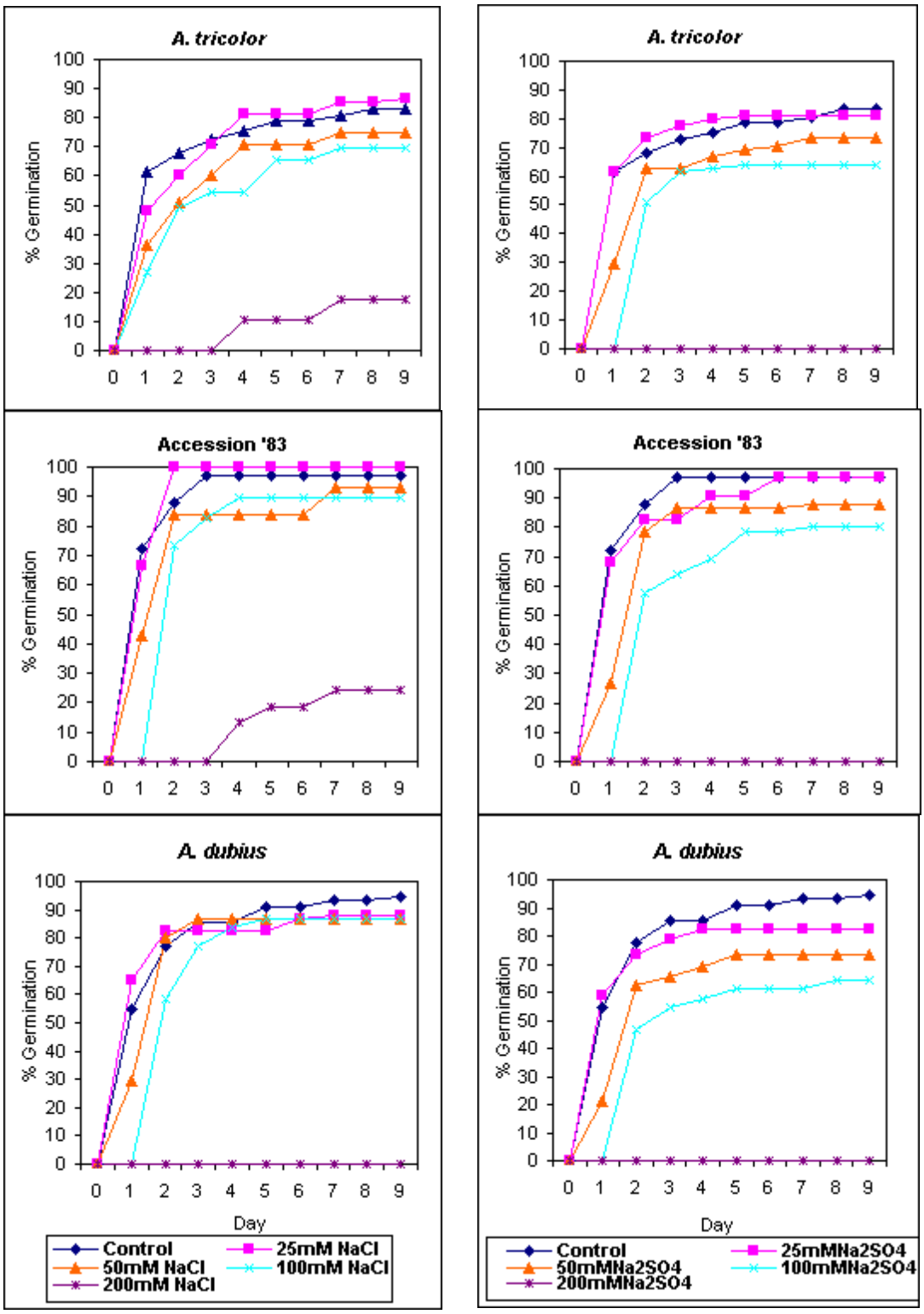

Figure 2.2a Effect of NaCl and Na_2SO_4 concentrations on the time course of germination of different amaranth genotypes.

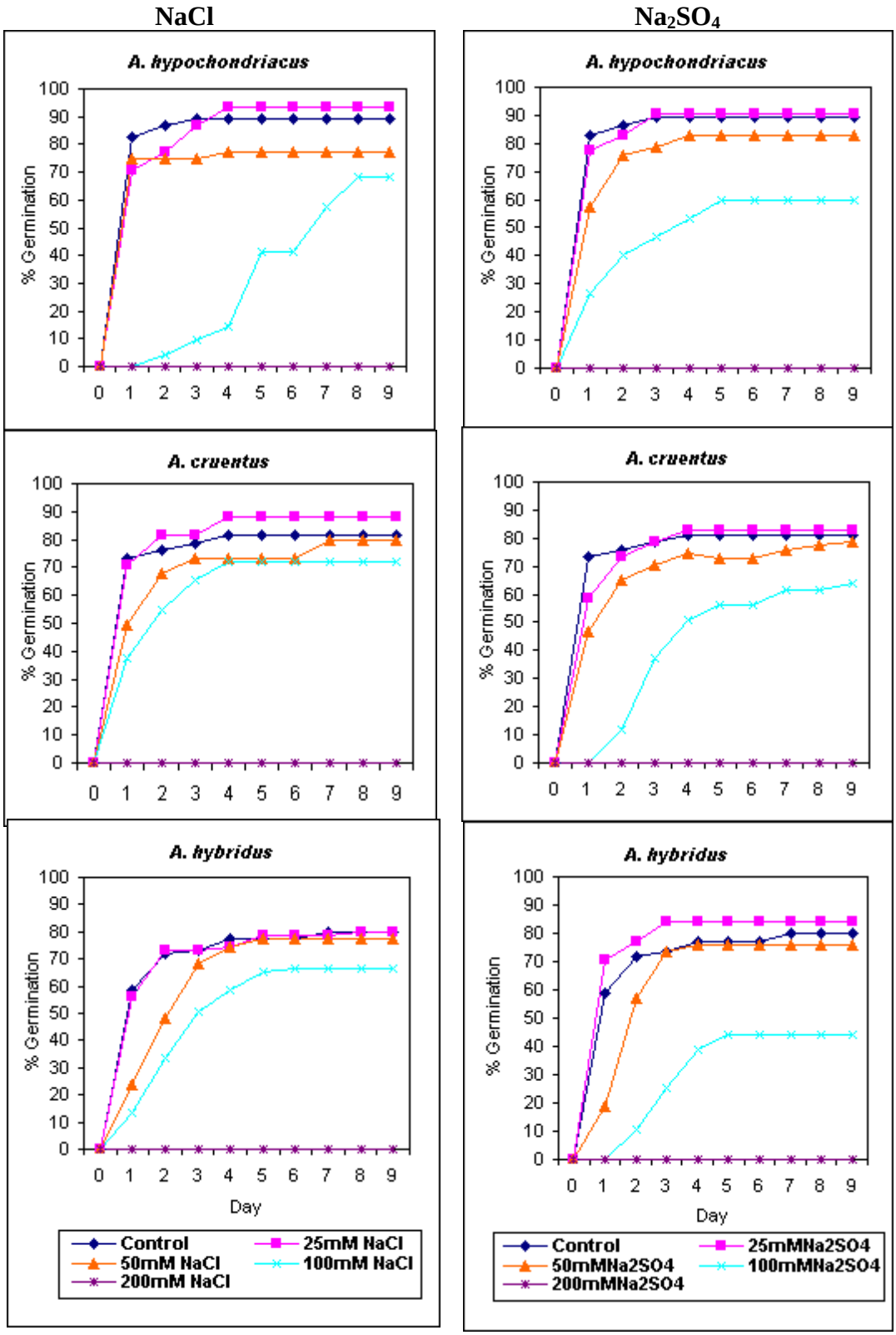

Figure 2.2b Effect of NaCl and Na_2SO_4 concentrations on the time course of germination of different amaranth genotypes.

An increase in concentration of both NaCl and Na_2SO_4 significantly affected seed germination of the six amaranth genotypes under investigation. The negative effects increased as the NaCl and Na_2SO_4 concentrations were increased. Similar reductions in germination with increasing salt concentrations were reported in *Atriplex griffithii* (Khan and Rizvi, 1994), *Haloxylon recurrum* (Khan and Ungar, 1996a) and in table beet (Uno *et al*., 1996). *Amaranthus hypochondriacus* and *A. cruentus* exhibited better germination in treatments containing 25 mM of NaCl than in the control, while germination of *A. hybridus* and *A. dubius* was enhanced by 25 mM Na_2SO_4. Germination stimulation by application of low concentrations of NaCl and Na_2SO_4 has been reported in *Cicer arietinum* and *Lens culinaris* (Mamo *et al.*, 1996) and *Pinus banksiana* (Croser *et al*., 2001). However, most species display maximum rates of germination in distilled water (Myers and Morgan, 1989; Chartzoulakis and Loupassaki, 1997). According to Dell'aquila and Spada (1993) salinity may activate or stimulate the genesis of some proteins during germination and these salt stress proteins have been associated with a protective function in wheat embryos. This may have been the reason for enhanced germination at low salt concentrations.

The delay in germination of amaranth observed in the higher salinity treatments had been reported in onion (Miyamoto, 1989), jojoba (Kayani *et al*., 1990) and sugar beet (Ghoulam and Fares, 2001). Asch and Wopereis (2001) reported that salinity levels below 4 mS cm^{-1} resulted in a delay of 1-2 days in rice, whereas higher salinity levels delayed germination by more than a week in some cases, or reduced the germination rate. It has been reported that salinity delays germination but does not appreciably reduce the final percentage germination (Ayers and Westcot, 1985). Although external salinity of 50 mM NaCl or Na_2SO_4 delayed amaranth seed germination, the final percent germination after 9 days was not affected in some genotypes, such as A. *cruentus* and *A. hybribus*. However, at 100 mM there was both a delay in germination and in the final germination percentage (Figure 2.2a; 2.2b).

It has been reported by several authors that salinity stress affects seed germination either by decreasing the rate of water uptake (osmotic effect) and/or facilitating the intake of

ions, which may change certain enzymatic or hormonal activities inside the seed (ion toxicity) (Dubey and Rani, 1990; Welbaum *et al*., 1990; Garg *et al*., 1993; Huang and Redmann, 1995b). These physico-chemical effects upon the seed results in slower and or lower rates of germination. Physiological studies to distinguish between the two effects are limited but evidence suggests that low water potential of the germination medium is a major limiting factor (Bradford, 1995). The effect of osmotic constraints on germination has been reported for wheat where salinity inhibited germination by limiting water uptake, rather than by direct Na and Cl toxity (Dell'Aquila and Spada, 1993).

In the context of this discussion, the term salt tolerance during seed germination was used only to refer to situations where the seed germinated rapidly under salt stress conditions. No distinction was made between osmotic and ionic effects of the salinity stress. The results demonstrated genotypic variation in seed germination responses of amaranth to salinity stress.

Accession '83 attained the highest final germination percentages at all the salinity levels, and the reduction in germination due to increases in salt level was much lower than in the other genotypes. This accession as well as *A. tricolor,* showed some germination at the highest NaCl concentration (200 mM). *A. hybridus* was the most sensitive genotype (Figure 2.2a; 2.2b). All the amaranth genotypes germinated rapidly under control and low salt conditions but germinated poorly at the highest salt stress levels, thus exhibiting high sensitivity. Consequently, in these genotypes, the physiological processes required for germination were sensitive to high salinity stress. According to Fooland and Lin (1997) such genotypes might be deficient in genetic elements required for coping with high salinity stress.

The rate of germination decreased progressively with an increase in NaCl and Na_2SO_4 concentrations (Figure 2.3a; 2.3b). A severe decrease in the rate of germination was observed in the two highest salt concentrations 100 and 200 mM NaCl and 100 mM Na_2SO_4. Comparison between genotypes indicated genotypic differences depending on the type of salt and concentration. There was no significant difference in the rate of

germination between control and 25 mM NaCl or Na_2SO_4 treatments in all the genotypes (Figure 2.3a; 2.3b). At low concentrations (25 and 50 mM NaCl or Na_2SO_4), the highest rates of germination were determined in Accession '83 and *A. hypochondriacus*, while *A. tricolor* and *A. hybridus* had lower rates (Figure 2.3a; 2.3b). At 100 mM the rate of germination was significantly reduced by NaCl in *A. hypochondriacus* and by Na_2SO_4 in *A. hybridus*. Accession '83 maintained a high rate of germination at 100 mM NaCl or Na_2SO_4. Differences in varietal behaviour may affect adaptability to saline environments. Genetic differences could, possibly, be exploited in breeding programs. Varietal differences in salt tolerance have been reported for other species, for example in onion (Maranon *et al.,* 1989), sugar beet (Ghoulam and Fares, 2001) and beans (Bayuelo-Jiménez *et al*., 2002).

Exposure of amaranth to high saline concentrations did not only inhibit germination but also decreased germination rate. Similar results were obtained in six plant species occurring in semi-arid climate in W. Australia (Osborne *et al*., 1993); in *Silicornia ramosissima* and *Arthrocnemum macrostachyum (*Rubio-Casal *et al*., 2003) and in *Argaria spinosa (*Bani-Aameur and Sipple-Michmerhuizen, 2001). Ungar (1996) observed that germination rate was a more sensitive parameter than germination percentage in *Atriplex patula*.

The percentage germination and rate of germination of crop seeds are of considerable agronomic importance. Reduction in the rate of germination and lengthening of the time required to reach final germination due to salinity stress would be particularly critical in semi-arid areas where favorable conditions in the seed zone may be brief. Thus, one of the more important agronomic aspects of crop establishment is the rate at which a sufficient number of seeds germinate and establish a stand during the limited period when environmental conditions are suitable.

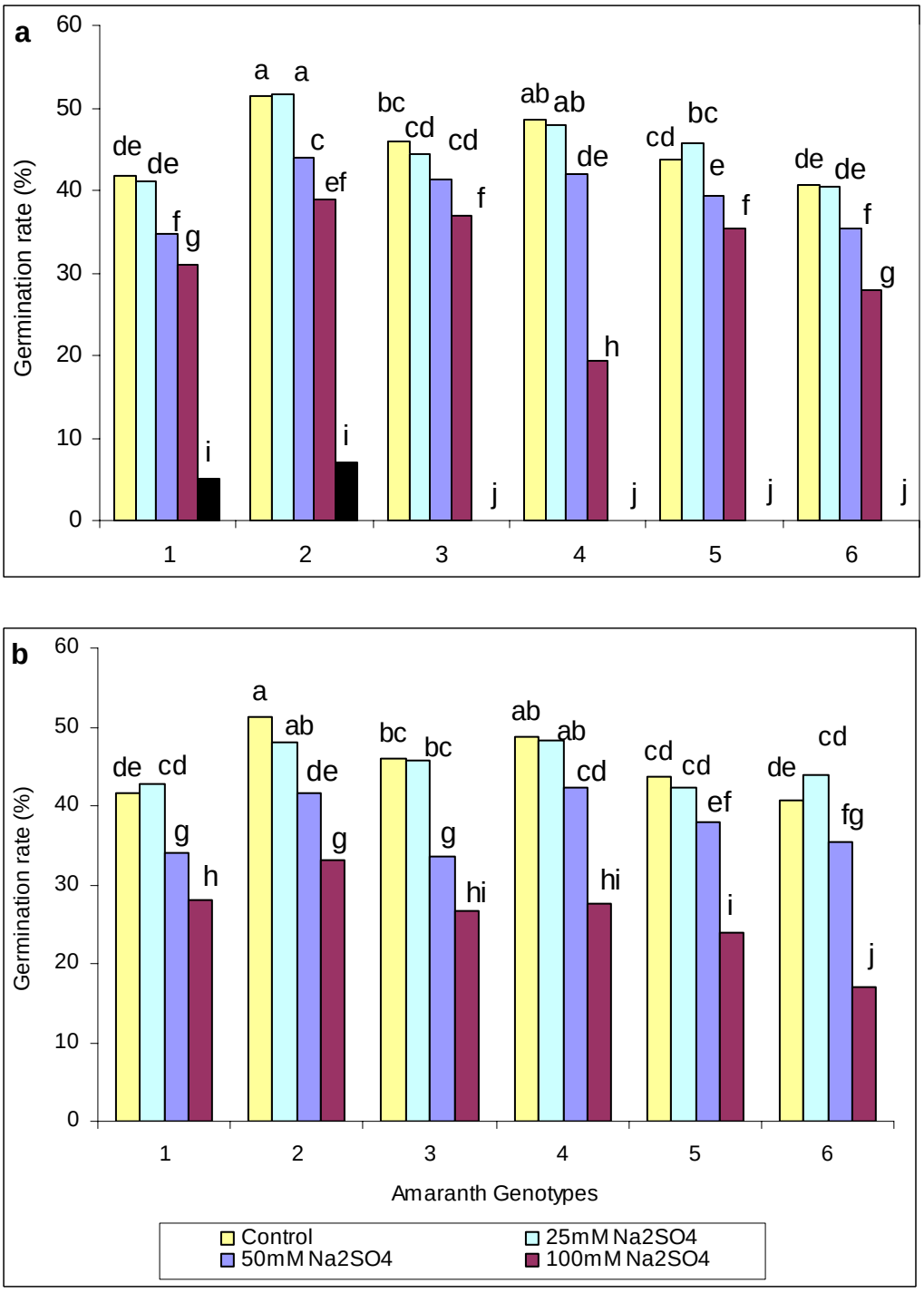

Figure 2.3 Effects of (a) NaCl and (b) Na_2SO_4 concentrations on the germination rate of (1) *A. tricolor* (2) Accession '83 (3) *A. dubius* (4) *A. hypochondriacus* (5) *A. cruentus* and (6) *A. hybridus*. Mean separation by Turkey T-test. Bars followed by the same letter are not significantly different at P = 0.05.

2.4.2 Radicle elongation

Significant differences were found in radicle lengths depending on genotype, salt type and salinity level. Increasing salt concentrations severely affected radicle elongation (Table 2.1). The 50 and 100 mM NaCl treatments resulted in *A. tricolor* radicle length reduction from 46.7 to 30.7 cm (34%) and 46.7 to 11.7 cm (75%) respectively. With Accession '83 reductions were from 43.3 to 36.7 cm (15%) at 50 mM NaCl and from 43.3 to 11 cm (74%) at 100 mM NaCl (Table 2.1). At 25 mM NaCl radicle length was enhanced by 42% in *A. hypochondriacus*, 54% in *A. cruentus* and 47% in *A. hybribus*. Enhancement by 2% in *A. cruentus* and 27% in *A. hybridus* was also determined. At 100 mM NaCl the reductions in radicle length ranged from 63% in *A. hybridus* to 90% in *A. hypochondriacus*.

Progressive decrease in radicle length with increasing Na_2SO_4 levels was also observed (Table 2.1). At 25 mM Na_2SO_4, *A. hypochondriacus* and *A. cruentus* were least affected by salinity with radicle lengths reduced by 4 and 5%. The reductions in the other genotypes ranged from 27% in *A. hybridus* to 46% in Accession '83. Reductions in the Na_2SO_4 treatments were significantly larger than in NaCl. For example, in *A. hybridus* radicle length was reduced by 88% at 100 mM Na_2SO_4 compared to 63% reduction in NaCl at the same concentration (Table 2.1). This trend was similar for all the genotypes. Bewley and Black (1994) suggested that the inhibition of the radicle under water stress is due to a reduction in the turgor of the radicle cells.

Table 2.1 Effect of NaCl and Na_2SO_4 concentrations on radicle lengths (mm) of different amaranth genotypes

Salinity (mM)	**Genotype**					
NaCl	*A. tricolor*	Accession '83	*A. dubius*	*A. hypochondriacus*	*A. cruentus*	*A. hybridus*
0	46.7a	43.3a	28.3a	43.3b	33.3c	31.7c
25	41.7a	40.3ab	20.7b	61.7a	51.3a	46.7a
50	30.7b	36.7b	20.3b	40.3b	40.0b	40.3b
100	11.7c	11.0d	6.3c	4.3d	9.3d	11.7e
200	3.7d	4.3e	0.0d	0.0e	0.0e	0.0f
Na_2SO_4						
0	46.7a	43.3a	28.3a	43.3b	33.3c	31.7c
25	31.7b	23.3c	20.0b	41.7b	31.7c	23.3d
50	10.0c	11.7d	7.3c	11.7c	7.3de	9.3e
100	5.0d	5.3e	4.0c	5.7d	3.7e	3.7f
200	0.0e	0.0f	0.0d	0.0e	0.0e	0.0f
SEM	**0.93**					

SEM: Standard error of the mean
Mean separation by Turkey T-test. Means followed by the same letter along the column are not significantly different at P = 0.05.

2.4.3 Hypocotyl length

Hypocotyl lengths were significantly reduced with increasing concentrations of both NaCl and Na_2SO_4 and clear genotypic differences were observed. Reductions ranged from 53.3 to 24.5 mm (55%) in *A. hypochondriacus* to 38.3 to 5 mm (87%) in *A. dubius* at 100 mM NaCl (Table 2.2). Greater reductions with Na_2SO_4 treatments were observed, and at 100 mM Na_2SO_4 there was no hypocotyl development in any of the genotypes except in Accession '83 where an 87% reduction was observed (Table 2.2). At 50 mM Na_2SO_4 reductions were from 53.3 to 18.3 mm (66%) in *A. hypochondriacus* to 46.7 to 5 mm (89%) in *A. hybridus* (Table 2.2). Hence, hypocotyl elongation was more sensitive to salt treatments than radicle elongation, particularly at higher salt concentrations.

Table 2.2 Effect of NaCl and Na_2SO_4 concentrations on hypocotyl lengths (mm) of different amaranth genotypes

Salinity (mM)	Genotype					
NaCl	*A. tricolor*	Accession '83	*A. dubius*	*A. hypochondriacus*	*A. cruentus*	*A. hybridus*
0	43.3a	41.7a	38.3a	53.3a	41.7a	46.7a
25	38.3b	29.3b	25.0b	45.7b	34.0b	33.3b
50	22.7d	26.7b	13.7c	40.1c	30.3b	22.0c
100	16.0d	16.3c	5.0d	24.0e	17.5d	15.7d
200	0.0e	0.0f	0.0e	0.0g	0.0f	0.0g
Na_2SO_4						
0	43.3a	41.7a	38.3a	53.3a	41.7a	46.7a
25	30.0c	26.7b	25b	28.3d	23.3c	18.7cd
50	13.0d	11.7d	5.0d	18.3f	13.3e	5.0f
100	0.0e	5.3e	0.0e	0.0g	0.0f	0.0g
200	0.0e	0.0f	0.0e	0.0g	0.0f	0.0g
SEM	**0.76**					

SEM: Standard error of the mean
Mean separation by Turkey T-test. Means followed by the same letter along the column are not significantly different at P = 0.05.

This implies that although a certain percentage of germination can be achieved under salinity stress, successful emergence and establishment may not be achieved due to weak hypocotyls elongation. Forcella *et al.* (2000) observed that the elongation rate of the coleoptile is governed by soil water potential. Genotypes *A. hypochondriacus* and *A. cruentus,* with longer hypocotyls under salinity stress, may have better potential to emerge from the soil. Cultivar selection for better salt tolerance during the germination stage should include hypocotyl elongation as a parameter in addition to the rate and percentage of germination.

Salt stress resulted in growth reduction of both hypocotyls and radicles, but hypocotyls were more sensitive since no growth was observed at high concentrations of Na_2SO_4. Similar observations have been reported in barley (Huang and Redmann, 1995a), tomato

(Fooland, 1996), pigeon pea (*Cajanus cajan*) (Subbarao *et al*., 1991) and tepary bean (*Phaseolus acutifolius* A. Gray) (Goertz and Coons, 1991).

2.4.4 Effect of salinity on emergence of amaranth seedlings

Seedling emergence began five days after seeding depending on the genotype. After 10 days the total emergence of seedlings in the control treatment was approximately 40% for *A. tricolor*, 73% for Accession '83, 70% for *A. cruentus* and 73% for *A. hypochondriacus* (Figure 2.4). Salt treatments affected the emergence of *A. tricolor* seedlings more adversely than the other genotypes. Emergence was slightly enhanced to 77% in *A. hypochondriacus* by 25 mM Na_2SO_4. A significant reduction in emergence with salinity of 25 mM and above NaCl or Na_2SO_4 was observed with genotype differences. At 25 mM NaCl, the reduction in seedling emergence ranged from 23% in *A. hypochondriacus* to 67% in *A. cruentus,* while at 50 mM emergence was reduced by 41% in *A. hypochondriacus* and 75% in *A. tricolor*. The reduction in emergence was less in Na_2SO_4 and *A. hypochondriacus* was the least sensitive genotype. At 25 mM Na_2SO_4, emergence was slightly enhanced in *A. hypochondriacus,* while the reduction in the other genotypes ranged from 5% in Accession '83 to 38% in *A. cruentus*. Emergence was reduced by 14% in *A. hypochondriacus* and by 75% in *A. tricolor* at 50 mM NaCl.

Differences in the trend of seedling emergence were also observed. Emergence started with *A. hypochondriacus* on day 5 with 70% emergence in the control treatment. With salt treatments seedlings also emerged on day 5. Emergence began on day 6 in the case of the other genotypes, and in *A. tricolor* emergence was delayed to day 8 at 25 mM and to day 10 at 50 and 100 mM NaCl, and at 50 mM Na_2SO_4. Emergence of Accession '83 was only delayed by NaCl but not by Na_2SO_4. On the other hand, emergence *of A. cruentus* was delayed by 50 mM Na_2SO_4. Seedlings that had emerged after approximately 8 days were chlorotic. At 100 mM Na_2SO_4, no seedling in any of the genotypes emerged. Emergence of all genotypes was essentially completed by day 10.

Amaranth seedlings were more sensitive to external salinity than seed germination. Upon germination, the cotyledon must push through saline soil to emerge. The data obtained here indicated that emergence of amaranth decreased when salinity was 25 mM NaCl or Na_2SO_4 and above. Seedling emergence was reduced significantly even at 25 mM NaCl, a level that enhanced seed germination. These results demonstrate that tolerance to salinity in *Amaranthus* species varies with developmental stage. Mano *et al*. (1996) reported that salt tolerance at germination was independent of salt tolerance at the seedling stages in 6646 barley genotypes. Similarly, Bayuelo-Jiménez *et al*. (2002) found that even though some *Phaseolus* species germinated rapidly in high NaCl concentrations, vigorous seedlings did not develop. Even at the lowest concentration of salt (60 mM NaCl), all the four *Phaseolus* accessions had severely reduced seedling growth relative to controls. This phenomenon has also been reported for wheat (Maas and Poss, 1989) and tomato (Fooland and Lin, 1992). According to Fooland and Jones (1993), salt tolerance at germination and seedling stages appears to be controlled by different genes and is influenced by salt concentration.

A limited number of seeds of amaranth were able to germinate and emerge in 25 and 50 mM NaCl or Na_2SO_4 salt treatments. After 2 days, however, the seedlings died. This suggests that although initial water uptake constraints were overcome, physiological processes occurring soon after germination may have been affected by ionic components of the medium. According to Al-Niemi *et al*. (1992), the effect of external salinity on emergence may be partially osmotic and that a reduction and delay in seedling emergence may be due to the inability of the seed to overcome the external osmotic potential and take up water for embryo expansion. Later stages of seedling growth may then be more susceptible to ion toxicity, which can alter physiological processes such as enzyme activation, cell division and cell differentiation (Al-Niemi *et al*., 1992; Begum *et al*., 1992). In pigeon pea, substantial genotypic variation for salinity tolerance at the germination stage has been reported (Subbarao *et al*., 1991). However, germination in pigeon pea is less sensitive to salinity than the later stages of growth.

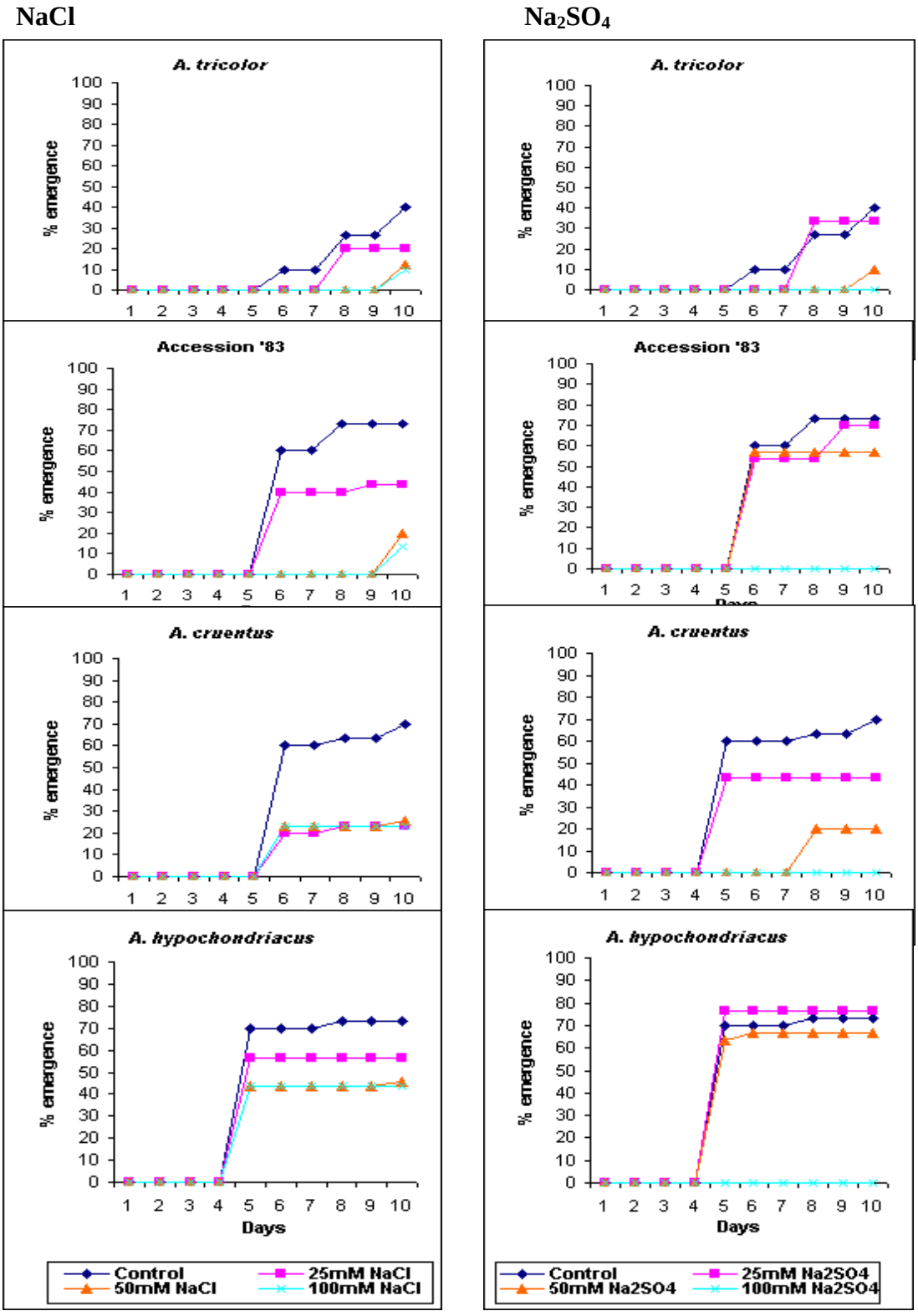

Figure 2.4 Effect of NaCl and Na_2SO_4 concentrations on the seedling emergence of different amaranth genotypes.

All the genotypes tested were able to germinate at salinity levels that are toxic at later stages of growth (Subbarao *et al.,* 1991). This is consistent with the results of the amaranth trials.

2.4.5 Survival and growth of amaranth seedlings under salinity

Salt tolerance during early seedling growth was assessed on the absolute growth at a given salt concentration relative to control under non-stress conditions. On the basis of this criterion, the results demonstrated genotypic variation in seedling growth responses of amaranth to salinity stress. Seedling survival and growth of all the genotypes were significantly affected by NaCl and Na_2SO_4 treatments and cotyledon injury was observed at the point of soil contact. *Amaranthus tricolor* appeared to be the most sensitive genotype in terms of seedling emergence and survival since no seedlings survived in NaCl treatments and in 50 and 100 mM Na_2SO_4. However, all the emerged seedlings survived at 25 mM Na_2SO_4 (Table 2.3). *Amaranthus tricolor* was, therefore, omitted in seedling growth comparisons since there was not enough data for growth parameter assessments. Of the seedlings that emerged in the 25 mM NaCl treatment, 54% of Accession '83, 43% of *A. cruentus* and 41% of *A. hypochondriacus* were able to survive, while there was no survival at higher concentrations (Table 2.3).

Seedling survival was higher in the Na_2SO_4 treatments. For example, no seedling mortality occurred in 25 mM Na_2SO_4 treatment in *A. tricolor*, *A. hypochondriacus* and Accession '83 while 46% of the seedlings survived in *A. cruentus*. At 50 mM Na_2SO_4 seedling survival ranged from 0% in *A. tricolor* to 95% in *A. hypochondriacus,* and there was no survival at 100 mM (Table 2.3).

Table 2.3 Effect of NaCl and Na_2SO_4 concentrations on seedling survival after 21 days in different amaranth genotypes

Salt treatment (mM)	Seedling survival (%)			
NaCl	*A. tricolor*	Accession '83	*A. cruentus*	*A. hypochondriacus*
0	100a	100a	100a	100a
25	0b	54b	43b	41
50	0b	0c	0c	0
100	0b	0c	0c	0
Na_2SO_4				
0	100a	100a	100a	100a
25	100a	100a	46b	100a
50	0b	59b	50b	95a
100	0b	0c	0c	0
SEM	**1.46**			

SEM: Standard error of the mean
Mean separation by Turkey T-test. Means followed by the same letter along the column are not significantly different at P = 0.05.

Since seedlings exposed to 50 and 100 mM NaCl did not survived, only results from Na_2SO_4 treatments are reported for seedling growth analysis. The interaction between genotype and Na_2SO_4 concentration was significant for root and shoot length. In the control treatment, the root and shoot lengths of *A. cruentus* and *A. hypochondriacus* were similar. Differences between genotypes were observed at higher salt concentrations, with Accession '83 showing greater sensitivity compared to the other genotypes (Figure 2.5). With increasing salt concentrations there was a decrease in both shoot and root lengths of all the genotypes and the roots of all the genotypes appeared to be hypertrophic. Similar effects of salinity in reducing plant height has been reported in *Oryza sativa* cultivars (Khan *et al*., 1997).

Root length was more affected by salinity than shoot length, particularly at high salt concentrations. For instance, at 50 mM Na_2SO_4, shoot length in *A. cruentus* was reduced by 19% compared to 31% reduction in root length (Figure 2.5). Under salinity stress,

shoot growth is frequently inhibited more than root growth (Poljakoff-Mayber and Lerner, 1994). This is in contrast with the amaranth results and could be due to differences in stage of plant development. Roots may be more sensitive than shoots to salinity when they are younger than at later stages. A greater root than shoot sensitivity to salinity was also indicated for *Cicer arietinum* and *Lens culinaris* (Mamo *et al*., 1996), and for *Picea mariana, P. glauca*, and *Pinus banksiana* (Croser *et al*., 2001).

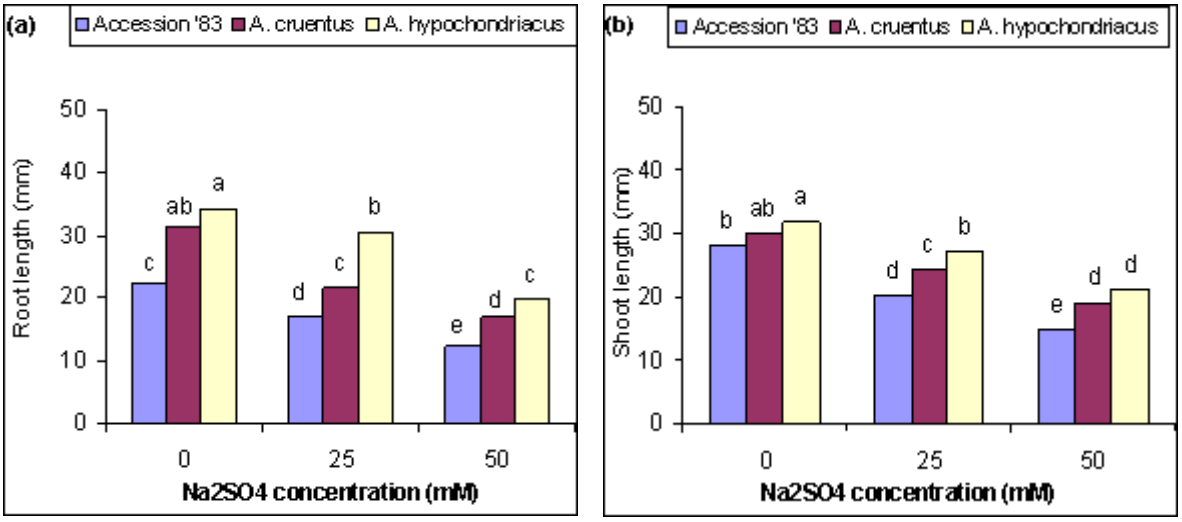

Figure 2.5 Effect of Na_2SO_4 concentrations on the (a) root length and (b) shoot length of three amaranth genotypes. Mean separation by Turkey T-test. Bars followed by the same letter are not significantly different at P = 0.05.

The main effects of genotype and Na_2SO_4 concentrations for shoot fresh mass and number of lateral roots are illustrated in Table 2.4 since the genotype x concentration interaction was not significant. Accession '83 had the least shoot fresh mass and number of lateral roots, while there was no difference in shoot fresh mass between *A. cruentus* and *A. hypochondriacus*. The highest number of lateral roots was determined in *A. hypochondriacus*.

Increasing salinity progressively reduced shoot fresh mass as well as the number of lateral roots. The reduction in the number of lateral roots was greater than shoot fresh mass (Table 2.4).

Salinity significantly reduced amaranth seedling growth. Growth reduction induced by salinity was also reported for alfalfa (McKimmie and Dobrenz, 1987, *Atriplex patula* (Ungar, 1996) and sugar beet (Ghoulam and Fares, 2001). Kayani *et al*. (1990) reported similar results in seedlings of jojoba, and attributed the growth inhibition to a reduction in the availability of soluble sugars for growth.

Table 2.4 Effect of genotype and Na_2SO_4 concentrations on seedling shoot fresh mass and number of lateral roots

Main effects	Shoot growth	
	Shoot fresh mass (g/plant)	Number of lateral roots
Genotype		
Accession '83	0.0244b	4.22c
A. cruentus	0.0344a	5.78b
A. hypochondriacus	0.0400a	6.89a
SEM	**0.0016**	**0.17**
Na_2SO_4 concentration (mM)		
0	0.047a	9.55a
25	0.037b	6.33b
50	0.015c	1.00c
SEM	**0.0016**	**0.17**

SEM: Standard error of the mean
Mean separation by Turkey T-test. Means followed by the same letter along the column are not significantly different at P = 0.05.

The results on emergence and growth revealed the sensitivity of Accession '83 to salinity stress at the seedling stage, yet this genotype was salt tolerant during germination. The reduced capacity of seedlings to survive in the same concentration of salts that they had germinated in has also been found in conifers. Croser *et al.* (2001) found that there was little effect of salinity on the emergence of *Picea glauca, P. mariana* and *Pinus banksiana*, however, later seedling growth was reduced. Lovato *et al*. (1994), in an investigation with *Stylosanthes humilis,* found that one of the most salt tolerant populations during germination was one of the most affected by salt during the initial growth phase. It has been consistently demonstrated that salt resistance is controlled by a number of genes and involves a number of component traits which are likely to be quantitative in nature (Flowers and Yeo, 1995). According to Shannon (1985), the plant's ability to respond to salt stress depends on the genes that are functioning at the stage of development during which the stress occurs.

An early tolerance to salinity stress during seed germination does not indicate that at later stages of development the plants will also be tolerant. An alternative explanation for the different responses of germination, emergence and seedling growth to the same salt concentrations is that growing seedlings are dependent on photosynthesis rather than stored food for their source of energy. Since they transpire at high rates, more salts could enter the plant in the transpiration stream, leading to salt accumulation and mortality. More research, however, is necessary to verify this hypothesis.

2.5 CONCLUSIONS

The response of amaranth seed germination and seedling growth to salinity stress is dependent on the genotype, salt type and concentration, and the parameter measured. The presence of genotypic differences in salinity tolerance during germination was demonstrated. Germination stimulation by 25 mM NaCl was observed in *A. hypochondriacus* and *A. cruentus*, while 25 mM Na_2SO_4 stimulated germination in *A. hybridus* and *A. dubius*. Increasing salt concentrations resulted in a delay and reduction in germination. The results demonstrated that *A. tricolor* and Accession '83 were the

most salt tolerant at the highest NaCl concentration (200 mM). Generally, Accession '83 was the most salt tolerant genotype. It had the highest final germination percentage at all salt levels, and the reduction in germination due to increased salinity was much less than in the other genotypes. However, *A. hybridus* was the most sensitive genotype. High percentage and rate of germination are attributes that identify tolerant cultivars at the germination stage.

Increasing salinity stress reduced seedling emergence and early growth of seedlings as indicated by hypocotyl and radicle elongation. It was observed that hypocotyl elongation was more sensitive to salinity stress than radicle elongation. Thus, it may be concluded that the rate of germination and emergence, percentage of germination, and hypocotyl and radicle lengths may be used as potential selection criteria for salinity stress tolerance at the establishment stage. These results are useful to breeders for future development of salinity tolerant cultivars and to agronomists to predict sowing rates depending upon expected saline conditions.

The amaranth genotypes were more tolerant to salinity during germination than during seedling growth. Seedling emergence as well as shoot and root lengths and fresh mass were reduced by increasing salt concentrations. Only a limited number of seedlings were able to emerge at the lowest NaCl and Na_2SO_4 concentrations, and the seedlings did not survive more than two days after emergence. *A. tricolor* and Accession '83 were tolerant during germination, but were the most sensitive at emergence and during the seedling growth stage, while *A. hypochondriacus* was less affected by salinity stress during seedling growth. Since there were genotypic differences, further work is necessary in order to determine the response of other amaranth genotypes to salinity during germination and seedling development.

The response of amaranth to salinity stress initiated after seedling emergence was investigated in order to evaluate salt tolerance of this species at different developmental stages, and the results are reported in Chapter 3.

CHAPTER 3
SALT TOLERANCE OF AMARANTH AS AFFECTED BY TIMING OF SALINITY STRESS INITIATION

3.1 ABSTRACT

Crop salt tolerance is influenced by several factors such as growth stage at which salinity is initiated and the final level of salinity achieved. This study was conducted by initiating salinity stress at different growth stages in order to determine whether tolerance of amaranth changes with the stage of development. Four amaranth genotypes, namely: *Amaranthus tricolor*, Accession '83, *A. hypochondriacus* and *A. cruentus* were used. Seeds were sown in plastic pots containing sand/vermiculite mixture in a greenhouse. The seeds were initially watered with tap water until seedling emergence and thereafter, plants were supplied with nutrient solution containing 0, 25, 50 or 100 mM NaCl. The three salinity treatments were initiated at three different growth stages, namely: 10 days after emergence (cotyledon stage), 2-leaf stage and at 4-leaf stage and monitored for the rest of growing period. Control treatments received nutrient solution only. In a separate experiment plants were salinized at cotyledon, 2-leaf and 4-leaf stage and the period of salinization was 14 days at each stage. All growth and gas exchange parameters were reduced significantly with increasing salt concentration at all timing treatments. Amaranth plants were less sensitive to salinity stress when salinity was initiated at the 4-leaf stage. The results indicate that it is feasible to use saline water for growing amaranth with minimum yield losses if salt concentration, duration of exposure and time of salinization can be carefully managed.

Keywords: Amaranth genotypes; salinity tolerance; timing of salinization

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Contributions based on study:

E.N. OMAMI & P. S. HAMMES, 2004. Salt tolerance of amaranth as affected by timing of salinity stress initiation. Submitted for publication to: *Agricultural Water Management.*,September, 2004.

3.2 INTRODUCTION

Salinity is an agricultural problem that decreases or restricts crop production in many areas. As concern about limited water resources continue to increase due to rapid expanding populations, there will be a greater need to use poor quality water in crop production. The increase in the use of saline water for irrigation poses a potential hazard to the quality of agricultural soils. Appropriate management options are required to prevent and/or relieve salinity problems in crop production. Timing of salinity stress, i.e., initiation and termination of a salinization period at different growth stages, is one such option. This option considers crop sensitivity at different growth stages, which is one of the major issues in the utilization of saline water for crop production (Shalhevet, 1994). With the identification of salt-sensitive or salt-tolerant growth stages, management options may be developed to ameliorate yield reduction under saline conditions while reducing the consumption of good quality water. Maas and Poss (1989) pointed out that such data would be useful to growers in managing their soil and water resources.

Rhoades (1987) first demonstrated the sustainability of the cyclic strategy, which involves the use of saline drainage water and nonsaline irrigation, in the Westside San Joaquin valley of California. Good quality water (EC = 0.5 dS m^{-1}) was used to irrigate cotton (*Gossypium hirsutum* L.) during germination and seedling establishment, and a saline-sodic water (EC=7.9 dS m^{-1}) was used thereafter. Other studies conducted in California involving the cyclic use of drainage waters (Rhoades, 1989; Oster, 1994; Shennan *et al*., 1995) have revealed that this strategy is sustainable for cotton, wheat, safflower (*Carthamus tinctorius* L.), sugar beet, tomato (*Lycopersicon esculentum* Mill), provided the problems of crusting, poor aeration are correctly managed. According to Qadir and Oster (2003) the cyclic strategy requires knowledge of the different salt sensitivities among the crops grown as well as the changes in salt sensitivities of crops at different stages of growth.

Many studies have been conducted to evaluate the effect of salinity applied at different growth stages. Lutts *et al.* (1995) and Zeng *et al*. (2001), for example, reported that seedling and reproductive stages in rice were more sensitive to salinity than the tillering

stage. In a study reported by Heenan *et al*. (1988), salinity was initiated at the stage of panicle initiation (PI) and relieved before booting, and yield components were significantly reduced by salinity stress between these two stages. In other crops, the responses of sorghum, wheat and tomatoes to root-zone salinity initiated at different growth stages were studied (Maas *et al*., 1986; Maas and Poss, 1989; del Amor *et al*., 2001). All these studies showed that tolerance to salinity increased as growth stages proceeded.

In studies where salt stress treatments were of equal duration during different stages of growth, Al-Tahir and Al-Abdulsalam (1997) with faba bean, and Zeng *et al*. (2001) with rice, found that plants were more sensitive to soil salinity during the vegetative and early reproductive stages of development than during later stages. In wheat, the effects of salinity on yield components were different depending on when plants were stressed (Maas and Grieve, 1990). It has been shown that salinity effects on wheat yield were most severe when yield components were developing (Francois *et al*., 1994).

In Chapter 2 it was shown that amaranth genotypes were more sensitive to salinity stress at emergence and seedling growth than at germination. The response of amaranth to salinization at different growth stages has not yet been reported. This Chapter explores the response of amaranth to saline water applied at different developmental stages after seedling emergence. By establishing the maximum salt concentration in the irrigation water to maintain crop productivity water management strategies can be developed. The objectives were to determine the effect of different degrees of salinity applied at different growth stages on amaranth growth.

3.3 MATERIALS AND METHODS

Amaranth seeds were from the same source as those used in the seed germination study (Chapter 2). Seeds of four amaranth genotypes, namely: *Amaranthus tricolor*, Accession '83, *A. hypochondriacus* and *A. cruentus* were sown in 5-liter plastic pots containing

sand/vermiculite (3:1, v/v) in a greenhouse at the University of Pretoria Experimental Farm. The experiment was conducted in October 2003. During the trial the temperature in the greenhouse ranged from 18^{o}C to 27^{o}C (mean minimum and maximum). Relative humidity ranged from 40 to 80%. The experimental design was a randomized complete block in a split-plot lay out, with factorial arrangement of all treatment combinations, and three replicates. Salt level and timing of salinity stress were main plot factors and genotype was the sub-plot factor. After emergence the seedlings were thinned to three per pot.

All pots were watered daily with tap water until seedling emergence. After emergence the seedlings were thinned to three per pot. Sodium chloride salinity treatments consisted of a control, plus three salinity levels obtained by adding 25, 50 and 100 mM NaCl to the basic nutrient solution which had the following composition (in mM): 6 KNO_3, 4 $Ca(NO_3)_2$, 1 $MgSO_4$, 1 $NH_4H_2PO_4$, 0.05 Fe-EDTA, 0.05 KCl, 0.025 H_3BO_3, 0.002 $MnSO_4$, 0.002 $ZnSO_4$, 0.005 $CuSO_4$, 0.0005 $(NH_4)_6Mo_7O_2.4H_2O$. The final nutrient solutions had electrical conductivities equivalent to 1.2, 4.1, 7.0 and 12.8 $dS.m^{-1}$ respectively. The pH of the solution was adjusted to 6.0 with sulfuric acid.

Two experiments were conducted. In experiment 1, the three salinity levels were initiated at three different times, (i) at emergence (cotyledon stage T1), (ii) at the two-leaf stage (T2) and (iii) at the four-leaf stage (T3). After salinity stress initiation the treatments continued until the end of the experiment. In the second experiment salinity treatments were the same as in Experiment 1, but the duration of salinization was 14 days at each stage. On the tenth day from sowing, salinization of treatment T1 began and continued until day 24. Salinization at the 2-leaf stage began on day 24 and continued until day 38. The 4-leaf stage was salinized from day 38 to day 52. For each treatment the salts were leached out after 14 days of salinization by changing to fresh nutrient solutions without salt and flushing the containers. In the control treatment, plants were irrigated with nutrient solution throughout the experiment. Each treatment was replicated three times with three plants per replicate.

In Experiment 2 photosynthetic rate as well as stomatal conductance were recorded at the end of every stress period and again three days before harvest, using the second and third youngest fully expanded leaves. The measurements were made with a LI-COR, 6400 portable photosynthetic system (LI-COR, Lincoln, NE). Net photosynthesis was measured at 34 MPa external CO_2 partial pressure (340 µmol CO_2 mol air $^{-1}$) and a vapor pressure deficit (VPD) of 1.8 KPa. The photosynthetic photon flux density (PPFD) was 1100 µmol m^{-2} s^{-1}. All measurements were conducted between 9:00h and 14:00h, only on bright days, when photosynthetically active radiation (PAR) intensity at the leaf surface was 1100-1200 µmol m^{-2} s^{-1}. At the end of each experiment (12 weeks after sowing), plant height, leaf number, leaf area, fresh and dry weight of leaves, stems and roots were recorded. Leaf area was recorded with a LI-3100 leaf area meter (LI-COR. Inc., Lincoln, NE, USA). Dry mass was recorded after oven drying samples at 75^{o}C to a constant weight.

Data analysis

Before analysis of variance, data of mean values for each genotype and variable were subjected to tests for heterogeneous error variables by Bartlett's Test (Gomez and Gomez, 1984). Error variances were homogeneous, thus data were not transformed. The GLM procedure of the Statistical Analysis System (SAS Institute, 1996) was used for analysis of variance of all data. F-tests for significance followed the procedures described by Ott (1988). The treatment means were compared using Tukey's t-tests at $P \leq 0.05$ significance level.

3.4 RESULTS

3.4.1 Experiment 1

The main effects of genotype, salinity and time of salinization were highly significant for all variables. The interactive effect between these factors were also significant with the exception of the interaction between salinity and timing of salinity treatments which was not significant for plant height, number of leaves and stem dry weight. Since the

interactive effects were significant main effect results are not discussed. The focus is on the first order interactions (genotype x salinity level and genotype x timing). For shoot dry mass the second order interaction (genotype x salinity level x timing) was discussed.

Table 3.1 documents the effect of salinity level on plant growth parameters of each genotype averaged across all timing treatments. Despite highly significant genotype and salinity level interactions, the general trend was a decrease in all the recorded parameters with increased salinity stress from 25 to 100 mM NaCl observed in all the genotypes. However, there were different reactions depending on genotype and the recorded parameter. For example, no significant differences were observed for root and stem dry mass with *A. tricolor,* or for plant height, root and stem dry weight with Accession '83 when plants were salinized with 25 or 50 mM NaCl (Table 3.1). All growth parameters decreased with increasing NaCl concentration in *A. hypochondriacus* and *A. cruentus*.

Most of the growth parameters such as plant height, leaf number and leaf area were less affected when 25 mM NaCl treatment was applied, and less than 20% reduction was recorded in all the genotypes. With increasing salt level, *A. tricolor* and Accession '83 were more sensitive than *A. hypochondriacus* and *A. cruentus*. For instance, a 20% reduction in leaf number and 35% in leaf area was noted in *A. hypochondriacus* when 100 mM NaCl was applied compared with 39% and 54% reductions respectively in *A. tricolor*. In terms of leaf growth *A. hypochondriacus* and *A. cruentus* were significantly affected even at the lowest NaCl concentration (25 mM). However, with an increase in the level of salinity, all the four genotypes were similarly sensitive. Root growth was significantly reduced in *A. hypochondriacus* and *A. cruentus* at all salt levels. At 25 mM NaCl root dry mass was reduced by 43% in *A. hypochondriacus* compared to a non-significant 17% reduction in *A. tricolor*.

Table 3.1 Effect of NaCl concentrations averaged across stage of salinity treatment application on vegetative growth in different amaranth genotypes

Salt level (mM)	Height (cm)	Leaf No.	Leaf area (cm^2)	Leaf mass (g/plant)	Root mass (g/plant)	Stem mass (g/plant)
A.tricolor						
Control	25.2f	69.3a	1834.7a	5.3f	3.5g	2.6g
25	22.6g	55.6c	1477.4b	4.4g	2.9ghi	1.8h
50	20.2h	52.7d	1289.1f	3.5i	2.7hi	1.5h
100	16.0j	42.0f	838.7l	2.5j	1.5k	1.0i
Accession '83						
Control	25.7f	65.7b	1844.6a	5.2f	3.3gh	2.3g
25	23.5g	55.1c	1408.2d	3.8h	2.7hi	1.8h
50	22.9g	52.0d	1318.1e	3.3i	2.4ij	1.6h
100	17.3i	45.1e	916.1k	2.6j	2.0jk	1.1i
A. hypochondriacus						
Control	45.3a	31.0h	1440.2c	8.3a	16.7a	8.4a
25	42.6b	28.3i	1234.4g	6.4c	9.3d	6.9c
50	40.0c	26.2j	1094.4h	5.8e	8.5e	6.5d
100	31.6e	24.7k	934.5j	4.3g	6.9f	4.0f
A. cruentus						
Control	45.7a	35.7g	1441.4c	8.0b	15.3b	8.4a
25	42.7b	32.6h	1236.6g	6.1d	10.5c	7.3b
50	38.9c	29.9i	1065.3i	5.3f	8.4e	5.5e
100	34.8d	27.9j	935.2j	4.2g	6.3f	4.2f
SEM	**0.27**	**0.39**	**2.71**	**0.06**	**0.15**	**0.08**

SEM: Standard error of the mean
Mean separation by Turkey's t-test. Means in each column followed by the same letter are not significantly different at P = 0.05.

The 100 mM NaCl concentration reduced the different parameters by 20% to 62%. The most sensitive parameters were stem dry mass in *A. tricolor* with a 62% reduction as well as root dry mass in *A. hypochondriacus* and *A. cruentus* (60%). The least sensitive parameter was leaf number with only 20% reduction in *A. hypochondriacus* (Table 3.1).

Table 3.2 shows the effect of timing of salinity treatments across NaCl concentrations on different plant growth parameters of different amaranth genotypes. In all genotypes investigated, the reduction in plant growth was most significant when salinity commenced at cotyledon stage (T1), and least when it commenced at the 4-leaf stage (T3) (Table 3.2). Timing of salinity stress treatment did not have any effect on stem dry mass in *A. tricolor* and Accession '83. No significant difference was recorded for plant height and leaf number in *A. tricolor* and for leaf number in *A. hypochondriacus* and *A. cruentus* between treatments T2 and T3. Although plants were most sensitive when salinity commenced at T1, only about 20% reductions were noted for plant height and leaf area in *A. hypochondriacus* and *A. cruentus* while the same parameters were reduced by 27 to 35% in *A. tricolor* and Accession '83.

The reductions in plant height, leaf number, and stem dry mass were significantly higher in *A. tricolor* and Accession '83 than in *A. hypochondriacus* and *A. cruentus* at all stages of treatment application. The reverse was true for root growth. This parameter was reduced by 42% in *A. hypochondriacus* compared to 15% reduction in Accession '83 when salinity commenced at T3. Leaf dry mass was similarly reduced in all genotypes at all stages of treatment application. The most sensitive parameters were leaf and stem dry mass in *A. tricolor* and Accession '83 and root dry mass in *A. hypochondriacus* and *A. cruentus*, while plant height and leaf number were least affected by timing of salinity treatment.

The interactions of salinity stress and stage of salinity treatment application on shoot dry weight, expressed as a percentage of the control treatment of each genotype are illustrated in Figure 3.1. Despite statistically significant interactions, the differences in genotype reactions were small, indicating similar sensitivity. Relative reductions in shoot dry mass were most pronounced when salinity was initiated at cotyledon stage (T1) while least when initiated at 4-leaf stage (T3) (Figure 3.1). This trend was similar in all the genotypes and at all NaCl concentrations with the exception of Accession '83 where no significant difference was observed between stages of NaCl application T1 and T2 when

salinized with 25 mM NaCl as well as between T2 and T3 when salinized with 50 mM NaCl.

Table 3.2 Effect of stage of salinity treatment application averaged across NaCl concentrations on vegetative growth of different amaranth genotypes: Experiment 1

Timing treatments	Height (cm)	Leaf No.	Leaf area (cm^2)	Leaf mass (g/plant)	Root mass (g/plant)	Stem mass (g/plant)
A. tricolor						
Control	25.2f	69.3a	1834.7a	5.3f	3.5g	2.6g
T1	17.9h	45.2 c	1076.6 g	3.0 ij	2.1 f	1.3 d
T2	20.2 g	51.7 b	1226.4 c	3.5 h	2.3 ef	1.4 d
T3	20.7 fg	53.4 ab	1302.2 b	3.9 g	2.7 e	1.6 d
Accession '83						
Control	25.7f	65.7b	1844.6a	5.2f	3.3gh	2.3g
T1	18.8 c	46.1 c	1034.4 h	2.9 j	2.0 f	1.4 d
T2	21.5 f	51.9 b	1219.7 c	3.2i	2.3 ef	1.5 d
T3	23.4 e	54.2 a	1388.3 a	3.6 h	2.8 e	1.6 d
A. hypochondriacus						
Control	45.3a	31.0h	1440.2c	8.3a	16.7a	8.4a
T1	35.6 d	25.0 f	961.7 i	4.8 e	7.1 d	4.9 c
T2	38.5 c	26.9 e	1119.2 e	5.5c	7.9 bc	5.8 b
T3	40.1 b	27.3 e	1182.5 d	6.2 a	9.7 a	6.7 a
A. cruentus						
Control	45.7a	35.7g	1441.4c	8.0b	15.3b	8.4a
T1	36.0 d	28.3 e	948.9 i	4.5 f	7.4c	4.8 c
T2	38.7c	30.3 d	1104.8 f	5.2 d	8.3 b	5.8 b
T3	41.7 a	31.8 d	1183.4 d	5.9 b	9.5a	6.4 a
SEM	**0.27**	**0.39**	**2.71**	**0.06**	**0.15**	**0.08**

SEM: Standard error of the mean

For timing treatment T1, T2 and T3, salinization was initiated at cotyledon stage, 2-leaf, 4-leaf stages respectively. Mean separation by Turkey's t-test. Means in each column followed by the same letter are not significantly different at P = 0.05.

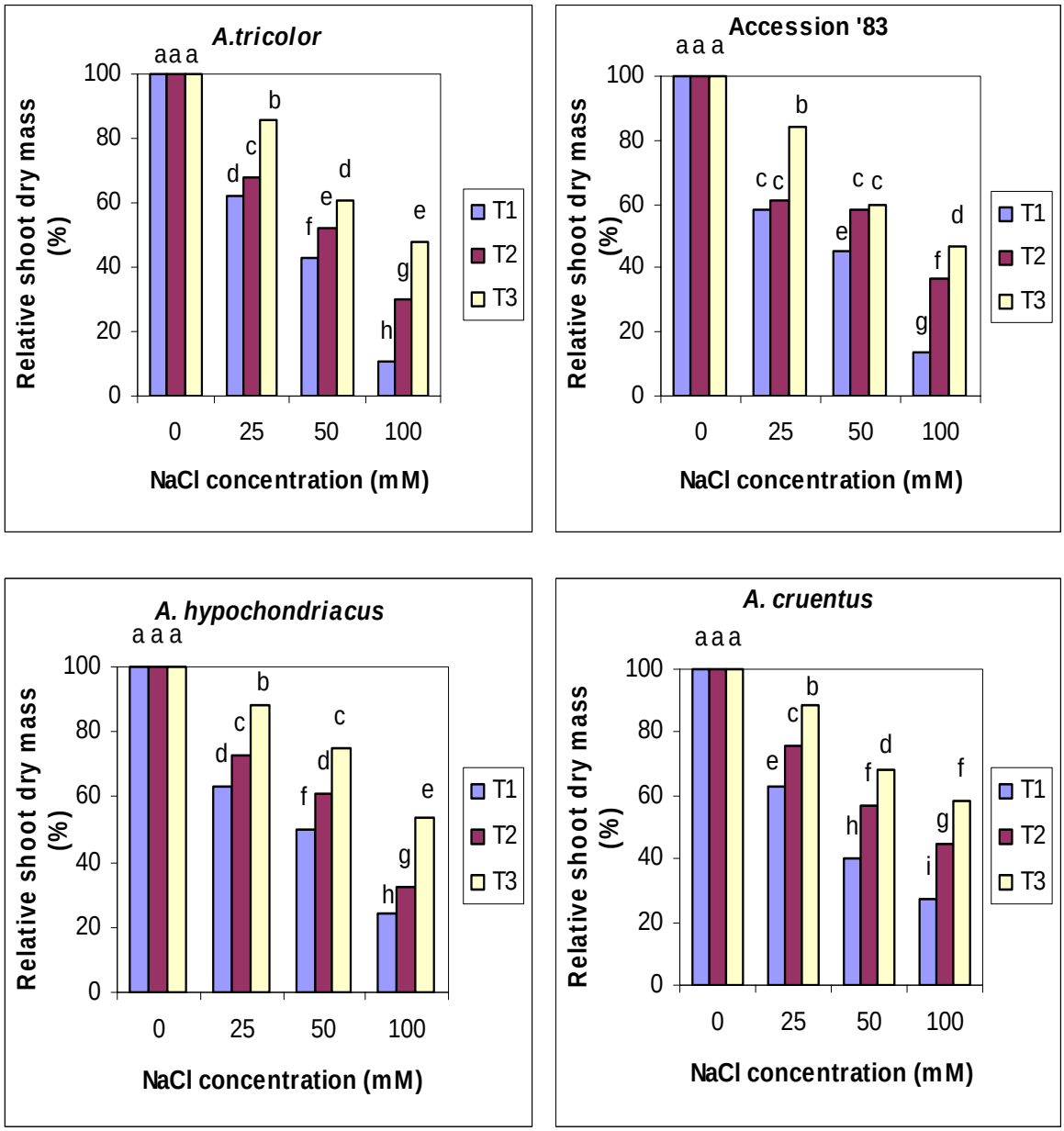

Figure 3.1 Effect of NaCl concentrations and stage of NaCl treatment application (T1- salinization at cotyledon stage, T2- at 2-leaf stage, and T3- at 4-leaf stage) on the relative shoot dry mass of different amaranth genotypes.
Mean separation by Turkey's t-test. For each genotype bars followed by the same letter are not significantly different at P = 0.05.

When plants were salinized with 25 mM NaCl, no significant difference in relative shoot dry weight was observed among genotypes when treatment was initiated at T3. At this salinity level and timing treatment, the reductions in shoot dry mass as compared to control were approximately 14% in *A. tricolor*, 16% in Accession '83, 12% in *A. hypochondriacus* and 11% in *A. cruentus.* Differences among genotypes as influenced by stage of NaCl application became more obvious with increasing NaCl concentration. When plants were salinized with 50 mM NaCl at T3, there was a 40% reduction in shoot dry mass in *A. tricolor* and Accession '83 while *A. hypochondriacus* and *A. cruentus* only showed 25 and 32% reductions respectively. Salinization at T2 with 50 mM NaCl resulted in the highest shoot mass reduction (48%) in *A. tricolor* and the least (39%) in *A. hypochondriacus* (Figure 3.1).

At the highest salt concentration (100 mM) all the genotypes were greatly affected when salinity was initiated at T1. However, no significant differences were noted in shoot mass reduction between *A. tricolor* and Accession '83 and between *A. hypochondriacus* and *A. cruentus*. The reductions in shoot dry mass ranged from 73% in *A. cruentus* to 89% in *A. tricolor*. *A. hypochondriacus* and *A. cruentus* showed a 42% reduction in shoot dry mass when the 100 mM NaCl treatment was initiated at T3, compared to 50% reduction in *A. tricolor* and Accession '83 (Figure 3.1).

Foliar injury, manifesting as marginal necrosis, became evident three weeks after the start of the treatments. It was more pronounced when 100 mM NaCl was applied and when salinity was initiated at T1 than at T2 or T3. Exposure to salinity was longer at T1 than for any of the other treatments, so injury would be more pronounced.

3.4.2 Experiment 2

3.4.2.1 Effect of stage of salinity treatment application on plant growth

In experiment 2, plants were salinized at the same stage of growth as in experiment 1; namely at cotyledon stage (T1), 2-leaf stage (T2), and 4-leaf stage (T3), but the salinity stress was applied for14 days in each case. Similar to the first experiment, data analysis

showed significant main effects as well as significant interactions. Table 3.3 documents the effect of different concentrations of NaCl treatments across different stages of salinity treatment application on various growth parameters for each of the genotypes. All the growth parameters in all genotypes were reduced with increasing NaCl concentrations. Depending on the genotype and the recorded parameter, different reactions were observed when plants were salinized with 25 and 50 mM NaCl. For example, no significant differences were observed in plant height in all the genotypes and in stem and root dry mass in *A. tricolor* and Accession '83 between the 25 and 50 mM NaCl treatments.

Exposure to 25 mM and 50 mM NaCl had very little effect on plant height and leaf area in all the genotypes with only 1 to 6% reductions observed. In *A. hypochondriacus* and *A. cruentus* leaf mass was significantly affected by salinity stress even at the lowest NaCl concentration (25 mM). However, at a high level of salinity (100 mM NaCl), these genotypes were somewhat less sensitive than *A. tricolor* and Accession '83 in terms of leaf mass. A higher root mass reduction was recorded in *A. hypochondriacus* and *A. cruentus* at all salt levels compared with that in *A. tricolor* and Accession '83. At 25 mM NaCl root dry mass was reduced by 21% in *A. hypochondriacus* compared to 8% reduction in Accession '83.

The 100 mM NaCl concentration reduced the different parameters by 15% to 53%. Salinization with 100 mM NaCl resulted in reductions ranging from 15 to 17% in plant height and 27 to 29% in leaf area. The most sensitive parameters were leaf and stem dry mass in *A. tricolor* and Accession '83 as well as root dry mass in *A. hypochondriacus* and *A. cruentus*. Plant height was the least sensitive parameter when plants were salinized with 100 mM NaCl, and was only reduced by 15 to 17% in all the genotypes (Table 3.3).

Table 3.3 Effect of exposure to salinity for 14 days averaged across stage of salinity treatment application on vegetative growth of different amaranth genotypes: Experiment 2

Salt level (mM) / Genotype	Plant height (cm)	Leaf area (cm^2/plant)	Leaf mass. (g/plant)	Stem mass (g/plant)	Root mass (g/plant)
A.tricolor					
Control	26.17f	1836c	5.81g	3.02hi	3.89hi
25	25.42fg	1834d	5.36hi	2.66ij	3.54ij
50	24.75g	1821f	4.84jk	2.32j	3.23j
100	21.67h	1314m	2.84m	1.54k	2.37k
Accession '83					
Control	26.00f	1843a	6.69de	3.84g	4.81ef
25	25.25fg	1840b	6.21f	3.36h	4.41fg
50	24.71g	1831e	5.68gh	2.99hi	4.15gh
100	22.02h	1318l	3.33l	1.89k	3.17j
A. hypochondriacus					
Control	46.33ab	1649i	8.12b	8.58b	7.69b
25	44.54cd	1620j	6.66de	7.92c	6.07d
50	43.92d	1616k	6.36ef	6.93d	5.23e
100	39.33e	1185o	4.63k	4.69f	3.61ij
A. cruentus					
Control	47.00a	1788f	8.74a	9.64a	8.56a
25	45.92ab	1768g	7.43c	8.77b	6.85c
50	45.62bc	1764h	6.90d	7.98c	5.99d
100	38.92e	1305n	5.10ij	5.49e	4.02ghi
SEM	**0.23**	**0.56**	**0.07**	**0.09**	**0.10**

SEM: Standard error of the mean

Mean separation by Turkey's t-test. Means in each column followed by the same letter are not significantly different at P = 0.05.

Table 3.4 shows the results of the effect of stage of salinity treatment application averaged across all salt concentrations for each of the genotypes. Differences among the stage of salinity application were minor, although initiation of salinity at the two-leaf stage (T2) affected growth and development more adversely than earlier or later salinization in the case of all the genotypes. Timing of the salinity treatment did not have

any significant effect on plant height and stem dry mass in *A. tricolor* as well as on root dry mass in *A. cruentus*. No significant differences were obtained between salinization at T1 and T3 for a number of parameters including root dry mass in *A. tricolor*, plant height and stem dry mass in *A. hypochondriacus*, leaf area and stem dry mass in *A. cruentus*, and for the dry mass parameters of Accession '83.

Table 3.4 Effect of exposure to salinity for 14 days at different stages of growth averaged across NaCl concentrations on vegetative growth of different amaranth genotypes: Experiment 2

Timing treatments	Height (cm)	Leaf area (cm^2)	Leaf mass (g/plant)	Root mass (g/plant)	Stem mass (g/plant)
A. tricolor					
Control	26.17e	1836b	5.81e	3.89hi	3.02i
T1	24.25gh	1658g	4.28i	2.99jk	2.11k
T2	23.29hi	1630j	4.13i	2.73k	2.00k
T3	24.29gh	1680e	4.62h	3.42ij	2.41jk
Accession '83					
Control	26.00ef	1843a	6.69c	4.81ef	3.84h
T1	23.97ghi	1662f	5.37fg	3.96gh	2.78ij
T2	23.04i	1637i	4.21i	3.36j	2.39jk
T3	24.96fg	1689d	5.64ef	4.41fg	3.07i
A. hypochondriacus					
Control	46.33ab	1649h	8.12b	7.69b	8.58b
T1	42.37cd	1507n	5.98d	5.10de	6.43fg
T2	41.96d	1401o	5.09g	4.25gh	6.34g
T3	43.46c	1514m	6.58c	5.56cd	6.77ef
A. cruentus					
Control	47.00a	1788c	8.74a	8.56a	9.64a
T1	43.33c	1618k	6.58c	5.71c	7.45cd
T2	41.87d	1600l	6.22d	5.44cd	7.04de
T3	45.25b	1619k	6.63c	5.71c	7.75c
SEM	**0.23**	**0.56**	**0.07**	**0.09**	**0.10**

SEM: Standard error of the mean
For timing treatment T1, T2 and T3, salinization was initialed at cotyledon stage, 2-leaf, 4-leaf stages respectively. Mean separation by Turkey's t-test. Means in each column followed by the same letter are not significantly different at P = 0.05.

Plant height and leaf area were less affected by stage of salinity treatment application. Initiation of salinity stress at any growth stage resulted in less than 20% reductions in plant height and leaf area in all the genotypes. These parameters were similarly reduced in all the genotypes. At all stages of salinity applications, root mass reduction in *A. hypochondriacus* and *A. cruentus* was significantly greater than in *A. tricolor* and Accession '83. When salinity commenced at T3, for instance, root dry mass was reduced by 33% in *A. cruentus* compared to 8% reduction in Accession '83. Stem dry mass was reduced to a greater extent in *A. tricolor* and Accession '83 when salinity was initiated at T1 and T2. When initiated at T3, all the genotypes showed similar reductions in stem dry mass. The most sensitive parameters were leaf and stem dry mass in *A. tricolor* and Accession '83 as well as root dry mass in *A. hypochondriacus* and *A. cruentus*.

In Figure 3.2 the interactive effects of salinity stress levels and timing of salinity treatment on shoot dry mass expressed as a percentage of the control treatment of each genotype is presented. The shoot dry mass was observed to decrease with increasing NaCl concentrations applied at different stages of growth. Despite highly significant interactions, differences between genotypes were small, indicating similar sensitivity. Relative reductions in shoot dry mass were most pronounced when salinity was initiated at the 2-leaf stage (T2), and least when salinity was initiated at the 4-leaf stage (T3) (Figure 3.2). This trend was similar in all the genotypes when 25 and 50 mM NaCl was applied. However, in *A. tricolor* and Accession '83, no significant differences were observed between T1 and T3 when salinized with 25 or 50 mM NaCl.

Application of 25 mM and 50 mM NaCl had little effect on shoot dry mass when initiated at T1 or T3. The reduction in shoot dry mass ranged from 5% in *A. cruentus* to 14% in *A. tricolor* when 25 mM NaCl was applied and 12% to 20% when 50 mM NaCl was applied. Shoot dry mass reduction was the greatest in *A. tricolor* (42%) and the least in *A. cruentus* (22%) when salinity was initiated at T2 with 50 mM NaCl. The adverse effects of 100 mM NaCl were much greater than those of the other two concentrations, and plants salinized at T1 had the greatest reduction in shoot dry mass ranging from 63% in *A. cruentus* to 80% in *A. tricolor*.

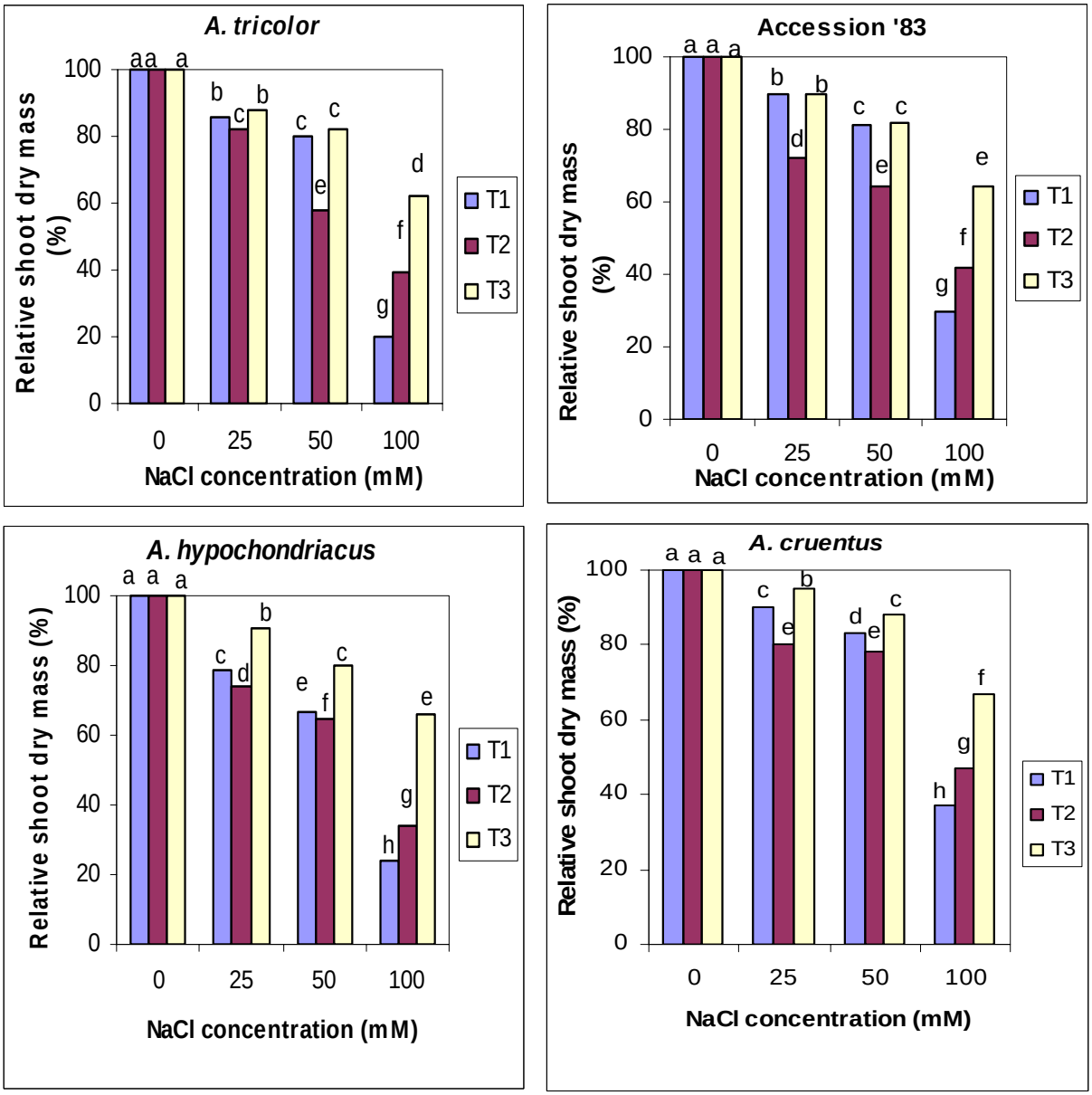

Figure 3.2 Effect of NaCl concentration applied for 14 days at different stages of growth: T1, salinization at cotyledon stage, T2, at 2-leaf stage, and T3, at 4-leaf stage, on shoot dry mass in different amaranth genotypes.
Mean separation by Turkey's t-test. For each genotype bars followed by the same letter are not significantly different at P = 0.05.

3.4.2.2 Effect of stage of salinity treatment application on gas exchange

The main effects of genotype, salinity and time of salinization treatments as well as their interaction were significant for photosynthetic rate (P_n) and stomatal conductance (g_s) (Table 3.5; 3.6). Photosynthetic rate and stomatal conductance of *A. tricolor* and Accession '83 were higher than for *A. hypochondriacus* and *A. cruentus* at all salinity treatments and stage of salinity application.

In all the genotypes both the photosynthetic rate as well as stomatal conductance decreased with increasing NaCl concentration at all times of salinization. However, the reductions were significantly higher in *A. hypochondriacus* and *A. cruentus* than in *A. tricolor* and Accession '83 especially at high NaCl concentrations. For instance, exposure of plants to 100 mM NaCl at T1 resulted in reductions in photosynthetic rate by 46 and 49% in *A. tricolor* and Accession '83 compared to 58 and 57% in *A. hypochondriacus* and *A. cruentus* (Table 3.5). The response of stomatal conductance was similar to that of photosynthetic rate. However, stomatal conductance was reduced to a lesser extent when compared to photosynthesis.

At each concentration of NaCl in the nutrient solution and for each genotype, both P_n and g_s increased at later salinization periods (T2 and T3) compared to T1 but these increases were inconsistent (Tables 3.5; 3.6).

Table 3.5 Effect of NaCl concentrations applied for 14 days at different stages of growth on photosynthetic rate (P_n) of amaranth determined at the end of each salinity stress period

	P_n (µmol $m^{-2}s^{-1}$)		
Salt level (mM)/ Genotype	**T1**	**T2**	**T3**
A. tricolor			
Control	9.9a(c)	13.1b(b)	18.6a(a)
25	8.3bc(c)	11.8c(b)	15.5c(a)
50	7.4c(c)	9.2e(b)	13.9e(a)
100	5.1de(c)	7.6fg(b)	11.5g(a)
Accession '83			
Control	10.0a(c)	15.4a(b)	18.9a(a)
25	8.6b(c)	13.7b(b)	15.7bc(a)
50	7.6c(c)	10.7d(b)	14.9d(a)
100	5.4de(c)	8.3f(b)	12.6f(a)
A. hypochondriacus			
Control	7.8b(c)	11.5c(b)	16.8b(a)
25	6.3d(c)	10.3de(b)	13.8e(a)
50	5.7de(c)	7.9fg(b)	10.6h(a)
100	3.3f(c)	5.6h(b)	8.3i(a)
A. cruentus			
Control	7.7c(c)	10.8d(b)	15.8c(a)
25	6.0d(c)	9.8e(b)	13.3e(a)
50	5.2e(c)	7.4g(b)	10.1h(a)
100	3.3f(c)	5.1h(b)	7.9i(a)
SEM	**0.16**	**0.16**	**0.16**

SEM: Standard error of the mean

For timing treatments T1, T2 and T3, salinization was initiated at cotyledon stage, 2-leaf and 4-leaf stages respectively.

Mean separation by Turkey's t-test. Means in each column followed by the same letter and in each row followed by the same letter in parenthesis are not significantly different at P = 0.05.

Table 3.6 Effect of NaCl concentrations applied for 14 days at different stages of growth on stomatal conductance (g_s) of amaranth determined at the end of each salinity stress period

Salt level (mM)/ Genotype	Gs (mmol m^{-2} s^{-1})		
	T1	**T2**	**T3**
A. tricolor			
Control	178.3a(c)	201.7c(b)	229.1f(a)
25	146.2b(c)	190.7d(b)	204.0gh(a)
50	137.3c(c)	163.4e(b)	185.6 i(a)
100	119.7de(c)	139.0f(b)	152.4j(a)
Accession '83			
Control	183.7a(c)	235.3a(b)	367.4a(a)
25	148.8b(c)	216.5b(b)	319.6b(a)
50	143.3b(c)	188.3d(b)	293.9c(a)
100	123.3d(c)	164.7e(b)	238.8e(a)
A. hypochondriacus			
Control	143.3b(c)	188.0d(b)	251.0d(a)
25	127.0b(c)	163.6e(b)	198.3h(a)
50	123.7d(c)	142.7f(b)	185.0i(a)
100	87.4g(c)	123.7g(b)	160.3d(a)
A. cruentus			
Control	136.3c(c)	184.7d(b)	242.0e(a)
25	114.2e(c)	157.0e(b)	183.3i(a)
50	108.6f(c)	144.2f(b)	177.7i(a)
100	86.4g(c)	122.3g(b)	152.7j(a)
SEM	**1.53**	**1.53**	**1.53**

SEM: Standard error of the mean
For timing treatments T1, T2 and T3, salinization was initiated at cotyledon stage, 2-leaf and 4-leaf stages respectively.
Mean separation by Turkey's t-test. Means in each column followed by the same letter and in each row followed by the same letter in parenthesis are not significantly different at P = 0.05.

Photosynthetic rate and stomatal conductance determined three days before the final harvest reflect the extent of recovery after exposure to salinity. Depending on the genotype, NaCl concentration and stage of growth, differences were noted in photosynthetic rate during the recovery phase. In *A. tricolor* and Accession '83 no differences were observed in P_n when the treatment started at T1 and T3 in plants exposed to either 25 or 50 mM NaCl. At the same concentrations, the highest P_n was attained at T3 followed by that at T1 and the lowest at T2 in *A. hypochondriacus* and *A. cruentus*. Treatment with 100 mM NaCl resulted in the highest photosynthetic rate and stomatal conductance noted at T3 followed by that at T2 and the lowest at T1 in all genotypes (Table 3.7; 3.8). Photosynthesis of 25 mM NaCl treated plants recovered fully and was similar to controls at the end of the experiment particularly when salinization was initiated at T1 and T3. The recovery of photosynthesis during recovery period was accompanied by a respective increase in stomatal conductance (Table 3.8).

Table 3.7 Effect of NaCl concentrations applied for 14 days at different stages of growth on photosynthetic rate (P_n) of amaranth after recovery from stress

Salt level (mM)/ Genotype	P_n (µmol $m^{-2}s^{-1}$)		
	T1	**T2**	**T3**
A. tricolor			
Control	18.8ab(a)	17.6a(a)	18.8ab(a)
25	18.5abc(a)	14.8bc(b)	18.7ab(a)
50	15.8de(a)	11.9d(b)	16.0c(a)
100	7.7g(c)	9.2e(b)	13.1de(a)
Accession '83			
Control	19.6a(a)	18.7a(a)	19.2a(a)
25	18.8ab(a)	16.1ab(b)	18.8ab(a)
50	16.1cd(a)	13.3c(b)	16.1bc(a)
100	7.9g(c)	10.6de(b)	13.3de(a)
A. hypochondriacus			
Control	16.8bc(a)	16.8ab(a)	16.9abc(a)
25	13.7de(b)	11.0de(c)	16.4bc(a)
50	10.9f(b)	8.2fg(c)	13.7d(a)
100	5.4gh(c)	8.1fg(b)	10.9e(a)
A. cruentus			
Control	16.4bcd(a)	15.1bc(a)	16.4bc(a)
25	13.2ef(b)	10.5def(c)	16.0c(a)
50	10.6f(b)	7.8g(c)	13.3de(a)
100	4.8h(c)	7.5g(b)	10.5f(a)
SEM	**0.47**	**0.47**	**0.47**

SEM: Standard error of the mean
For timing treatments T1, T2 and T3, salinization was initiated at cotyledon stage, 2-leaf and 4-leaf stages respectively.
Mean separation by Turkey's t-test. Means in each column followed by the same letter and in each row followed by the same letter in parenthesis are not significantly different at P = 0.05.

Table 3.8 Effect of NaCl concentrations applied for 14 days at different stages of growth on stomatal conductance (g_s) of amaranth after recovery from stress

	Gs (mmol m^{-2} s^{-1})		
Salt level (mM)/ Genotype	**T1**	**T2**	**T3**
A. tricolor			
Control	353.7b(a)	352.3b(a)	354.7b(a)
25	328.4b(a)	290.7c(b)	335.3b(a)
50	277.4cd(a)	241.2d(b)	284.3b(a)
100	149.1c(c)	194.3e(b)	239.2d(a)
Accession '83			
Control	497.2a(a)	495.3a(a)	499.0a(a)
25	457.4a(a)	347.3b(b)	464.1a(a)
50	348.2b(a)	303.1c(b)	354.1b(a)
100	211.6ef(c)	257.5d (b)	308.3c(a)
A. hypochondriacus			
Control	288.7c(a)	292.0c(a)	292.3c(a)
25	236.9de(b)	192.6e(c)	281.7c(a)
50	193.5f(b)	149.4f(c)	236.7d(a)
100	104.8d(c)	148.1f(b)	191.3e(a)
A. cruentus			
Control	285.3c(a)	286.0c(a)	285.8c(a)
25	237.5de(b)	193.9e(c)	282.3c(a)
50	192.7f(b)	149.5f(c)	236.5d(a)
100	102.9g(c)	146.2f(b)	189.3e(a)
SEM	**7.47**	**7.47**	**7.47**

SEM: Standard error of the mean

For timing treatments T1, T2 and T3, salinization was initiated at cotyledon stage, 2-leaf and 4-leaf stages respectively.

Mean separation by Turkey's t-test. Means in each column followed by the same letter and in each row followed by the same letter in parenthesis are not significantly different at P = 0.05.

3.5 DISCUSSION

Amaranth growth decreased with increasing NaCl concentration. All the genotypes were less sensitive to salinity when the treatments were applied at later developmental stages (Tables 3.2; 3.4). Similar observations were made by Al-Tahir and Al-Abdulsalam (1997) who reported significant reductions in faba bean yield when salinity was applied during early vegetative growth rather than at later stages. They attributed this to the fact that a major proportion of vegetative growth had occurred before salinization of the later period, thus decreasing the effect of salinity.

Contrary to the first experiment where plants salinized at T1 were the most sensitive, experiment 2 recorded less detrimental effects when plants were salinized at this stage than at T2 with the low salt concentration. The results show that plants stressed at the cotyledon stage for 14 days with a 25 mM NaCl solution were able to recover from salinity stress effects. It is not clear why plants salinized at T2 were more sensitive to stress than those salinized at T1 although plants stressed at T2 had more vegetative growth at the time of stress initiation. It may be due to the fact that these plants had less time for recovery after termination of stress.

When plants were exposed to salinity from the cotyledon stage (T1) to the end of the experiment, treatment with 25 mM NaCl reduced shoot dry weight by 37% to 42%. Less detrimental effects were noted when salinization was applied for 14 days only when shoot dry mass declined by 10% to 21%. All amaranth genotypes were sensitive to irrigation water with 100 mM NaCl (12.8 $dS.m^{-1}$) since shoot dry mass decreased by 42-53% when salinity treatment continued from the 4-leaf stage till termination of the experiment (Figure 3.1), and 33% to 38% when salinization was applied for only 14 days (Figure 3.2). These results are in agreement with those of del Amor *et al*. (2001) who observed yield reductions of up to 30% in tomato when irrigated with water of 6 $dS.\ m^{-1}$ when salinity was applied 66 days after transplanting. Amaranth shoot dry mass was significantly reduced by a 14-day period of salinization with 100 mM NaCl solution at the cotyledon and 2-leaf stages. An important observation from this study is that

salinization with 100 mM NaCl for only 14 days at the cotyledon and 2-leaf stages significantly reduced shoot dry mass, whereas 14-day period of salinization had less detrimental effects at the 4- leaf stage.

Photosynthetic rates (P_n) as well as stomatal conductances (g_s) of amaranth decreased with increasing NaCl concentration. It has been reported by Walker *et al* (1981) that inhibition of vegetative growth due to salinity effects was associated with a marked inhibition of photosynthesis. Stomatal closure appeared to limit CO_2 assimilation. Similar observations were made by Fisarakis *et al*. (2001) who reported a linear relationship between P_n and g_s in sultana vines. Non-stomatal factors have also been reported to be involved in P_n decreases. Walker *et al*. (1981) and Fisarakis *et al.* (2001) reported that the decrease of photosynthesis was primarily caused by stomatal closure, followed by non-stomatal inhibition due to high Cl^- accumulation. The contribution of non-stomatal factors in decreasing photosynthesis increases with increasing external salinity.

Photosynthetic rates and stomatal conductance data recorded at the end of every stress cycle indicated that the values increased as time of salinity stress initiation advanced (Tables 3.5; 3.6). As plants matured, P_n and g_s increased and, irrespective of the time of stress initiation these parameters were the highest after T3. Timing of salinity stress did not have any effect on P_n and g_s when plants were exposed to 25 mM NaCl. However, exposure to 50 mM resulted in the lowest values of these parameters noted when salinization commenced at T2 followed by those salinized at T1 and the highest when salinization commenced at T3 (Tables 3.7; 3.8). Apparently, plants salinized at T2 did not have enough time to recover from stress compared to those salinized at T1, while those salinized at T3 were least affected by the stress since they had greater vegetative growth at the time of salinization.

When salinity was relieved after 14 days of stress, photosynthetic rates and stomatal conductance recovered to values similar to those of control plants, particularly when plants were salinized with 25 mM NaCl (Tables 3.7; 3.8). Recovery of photosynthetic

rates within 2 days after removing vines from 90 mM NaCl has been reported by Walker *et al*. (1981). They found that the carboxylating efficiency of Rubisco was insensitive to salt stress, and this was the key factor for the recovery of P_n during relief. Similar results during the relief period have also been reported for salt-stressed olives (Tattini *et al*., 1995) and for sultana vines (Fisarakis *et al*., 2001).

Full recovery of the photosynthetic rate of the amaranth plants treated with 100 mM NaCl was not attained. Hence, although this study could not ascertain whether P_n decreases were due to stomatal or non-stomatal factors, or both, these results indicate that the high salt level probably caused some injury to the photosynthetic apparatus impeding the recovery of the plants from saline stress. De Herralde *et al*. (1998) made similar observations with *Argyranthemum coronopifolium* plants. They observed that photosynthetic rate and stomatal conductance of plants salinized with 140 mM NaCl did not recover when the stress was removed, and suggested that there was a toxic effect of salt concentration.

Duration of salinization, level of salinity and sensitivity at different growth stages are important factors in the determination of salinity effects during the utilization of saline water for crop irrigation. The identification of differential sensitivity to salinity at different growth stages will help saline water management. For example, during early growth stages, good quality water is critical for a normal amaranth production without significant yield reduction. Poor quality water with low to moderate levels of salinity can probably be used after the 4-leaf stage. Appropriate management options should be developed by incorporating the effects of timing of salinity treatments with factors such as the thresholds of salinity effects on yield, irrigation practices and water resources.

The response of amaranth to time of salinity stress initiation was similar in all the genotypes at different growth stages. This indicates the close genetic backgrounds in these genotypes. For the management of saline water in irrigation, this indicates that uniform management options can be developed in controlling salinity problems for the cultivars with similar genetic backgrounds.

3.6 CONCLUSION

Increasing NaCl concentrations in the growth medium resulted in decreases in amaranth growth. Plants were less sensitive to salinity when the stress was initiated at the 4-leaf stage. However, when salinity was initiated at the cotyledon stage for 14 days with 25 mM NaCl, plants were able to recover from stress. Saline water can be used with less deleterious effects on yield when salinity is initiated at the later stages of growth, or during early plant growth when saline water of low EC can be used for a short period of time. Identifying more tolerant plant growth stage is important for irrigated agriculture under saline conditions.

CHAPTER 4

DIFFERENCES IN SALINITY STRESS TOLERANCE IN TERMS OF GROWTH AND WATER USE EFFICIENCY AMONG FOUR AMARANTH GENOTYPES

4.1 ABSTRACT

Amaranth is a promising C_4 crop for semi-arid regions due to its high nutritive value and the ability to adapt to diverse environments. Such areas are also prone to soil salinity. Crop production can be limited by saline irrigation water. Data on differential tolerance of amaranth genotypes to salinity stress is lacking. In this study the response of four amaranth genotypes, viz. *A. tricolor,* Accession '83, *A. hypochondriacus* and *A. cruentus* to saline water with different NaCl concentrations were analyzed in terms of growth, gas exchange, water use and leaf anatomical changes. The study was conducted in a greenhouse. The treatments consisted of saline water at 0, 25, 50, 100 and 200 mM NaCl, equivalent to electrical conductivities of 1.2, 4.1, 7.0, 12.8 and 24 dS. m^{-1} respectively. Plant growth, photosynthetic rate and stomatal conductance were significantly reduced at all salinity levels. *A. tricolor* and Accession '83 did not survive in the 200 mM NaCl treatment. At 50 and 100 mM NaCl the reduction in shoot growth was greater in *A. tricolor* and Accession '83 than that in *A. hypochondriacus* and *A. cruentus.* Water use efficiency increased with increasing salinity and ranged from 3.9 in *A. tricolor* to 6.7 g DM kg^{-1} H_2O in *A. cruentus* when plants were salinized with 100 mM NaCl. Specific leaf area (SLA) was decreased by salinity and differed between genotypes. A negative relationship between SLA and WUE was observed in the four amaranth genotypes. *A. tricolor* and Accession '83 had thinner leaves, more stomates per leaf area and larger stomatal apertures than *A. hypochondriacus* and *A. cruentus.*

Keywords: *Amaranthus*; gas exchange; growth; salinity tolerance; water use efficiency

Publication and conference contributions based on study:

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E.N. OMAMI, P. S. HAMMES & P. J. ROBBERTSE, 2004. Differences in salinity stress tolerance in terms of growth and water use efficiency in amaranth genotypes. Submitted for publication to: *New Zealand Journal of Crop & Horticultural Science,* September, 2004.

4.2 INTRODUCTION

Amaranthus spp. is used for its grain and as a vegetable. The leaves are high in protein, vitamins and minerals (Allemann *et al*., 1996). Its ability to adapt to diverse growing conditions such as low nutrient soils and a wide range of temperature and irradiation, as well as its tolerance to drought stress, emphasize the possible use of this species as a nutritious green crop in semi-arid regions (Myers, 1996). However, soils in such areas are often characterized by an excess of inorganic salts due to high evaporative water losses that exceed precipitation (Mengel and Kirkby, 1982). Hence, to enable amaranth cultivation in salt-prone regions, a better understanding of the strategies employed by amaranth genotypes in adaptation to salinity stress is required.

Salinity can affect growth, dry matter accumulation and yield (Sultana *et al*., 1999; Asch *et al*., 2000). It is well known that dry mass of plants is reduced in proportion to the increase in salinity (Pardossi *et al*., 1999; Romero-Aranda *et al*., 2001). The reduction in growth of salinized plants may be related to salt-induced disturbance of the plant water balance, and in the extreme to a loss of leaf turgor which can reduce leaf expansion and therefore, photosynthetic leaf area (Erdei and Taleisnik, 1993; Huang and Redmann, 1995b). Other causes of growth reduction under salinity stress include ionic imbalances, changes in nutrient and phytohormonal status, physiological processes, biochemical reactions, or a combination of such factors (Volkmar *et al*., 1998; Hasegawa *et al*., 2000;

Kashem *et al*., 2000a, b), accompanied by a reduction in photosynthesis (Sultana *et al*., 1999).

Although the factors that limit photosynthesis in salt stressed plants have been investigated for a number of species, the mechanistic nature of inhibition is unclear (Steduto *et al*., 2000). Salt may affect growth indirectly by decreasing the rate of photosynthesis. Photosynthesis may decrease due to stomatal closure or by a direct effect of salt on the photosynthetic apparatus. However, conflicting results on stomatal and non-stomatal limitation of photosynthesis are reported. For instance, in bean (a salt sensitive species) and cotton (a salt-tolerant species) the reduction in assimilation was found to be mostly due to stomatal limitation (Brugnoli and Lauteri, 1991), whereas other authors ascribed the reduction in photosynthesis to non-stomatal limitation (Dunn and Neales, 1993).

Among several strategies devised to overcome the problem of salinity stress, the selection of crop species or cultivars with salinity tolerance traits has been considered an economical and efficient strategy. Hence, the challenges for using salty water profitably will depend on greater knowledge of salt tolerance (Shannon and Grieve, 1999). Various workers have tried to identify physiological and biochemical differences between salt tolerant and sensitive plants in an effort to develop rapid screening methods for salt tolerance (Alian *et al*., 2000). It is well established that salt tolerance ability depends on genetic and biochemical characteristics of the species and sufficient genetic variability in relation to salinity exist in many agricultural crops (Alian *et al*., 2000; Bayuelo-Jiménez *et al*., 2003; Misra and Dwivedi, 2004).

Water use efficiency (WUE) may be one trait that can contribute to productivity when water resources are limited (Wright *et al*., 1994). Specific leaf area (SLA), an indicator of leaf thickness, is reduced under saline conditions (Bayuelo-Jiménez *et al*., 2003). Reduction of SLA is assumed to be a way to improve WUE (Wright *et al*., 1994; Craufurd *et al*., 1999). According to Liu and Stützel (2004) this is because thicker leaves usually have a higher density of chlorophyll and proteins per unit leaf area, hence, have a

greater photosynthetic capacity than thinner leaves. Nageswara Rao *et al*. (1995) recommended leaf thickness as a selection criterion for enhancing WUE in groundnut.

Several studies have shown that water uptake, and hence water use and transpiration, declined as the salt concentration in the irrigation water increased (Soria and Cuartero, 1997; Bayuelo-Jiménez *et al*., 2003). Reduction of water uptake with salinity could be related to reductions in morphological and/or physiological parameters like leaf area, stomatal density, and stomatal closure (stomatal conductance and transpiration). Since response to saline water varies greatly with species or cultivar (Bayuelo-Jiménez *et al*., 2003; Misra and Dwivedi, 2004), there is need for assessment of salinity tolerance among different species. Information on differences in salinity tolerance, and especially the relationship between WUE and stomatal conductance, photosynthesis and growth of amaranth genotypes is lacking. The objectives of this study were to (i) investigate differences in salinity stress tolerance in terms of WUE and growth among amaranth genotypes (ii) identify characteristics contributing to differences in water use and (iii) evaluate the significance of changes in these features for plant performance in a saline environment.

4.3 MATERIALS AND METHODS

4.3.1 Plant culture

This research was conducted in a greenhouse at the Experimental Farm, University of Pretoria between April and June 2002. The temperature ranged from 20^{o}C to 30^{o}C and relative humidity mainly between 60 to 70%. Seeds of four amaranth genotypes (*A. tricolor*, Accession '83, *A. hypochondriacus* & *A. cruentus*) were sown in separate seed trays. One month after sowing, one seedling per pot was transplanted into 5-liter plastic pots containing a sand/vermiculite mixture (3:1, v/v). The seedlings were irrigated daily with nutrient solution for 10 days after transplanting before commencement of the treatments. The nutrient solution used was the same as that specified in Chapter 3.

4.3.2 Salinity treatments

The nutrient solution for plants exposed to salt stress was identical to that of the control except for the addition of NaCl. The treatments consisted of a control, plus four salinity levels that were obtained by adding 25, 50, 100 and 200 mM NaCl to the basic nutrient solution. The different solutions had electrical conductivities (EC) equivalent to 1.2, 4.1, 7.0, 12.8 and 24 dS. m^{-1} respectively. In order to avoid osmotic shock, NaCl salinization initiated 10 days after transplanting of the seedlings was stepped up in daily increments of 25 mM until the final concentration was reached. The seedlings were watered daily until the solution drained freely.

A randomized complete block design with a split plot arrangement of treatments was used with the five NaCl levels as the main plots. Genotypes were allocated to the subplots and were randomized within each main plot. There were three replications.

4.3.3 Gas exchange measurements

Photosynthetic rate (P_n), stomatal conductance (g_s) and transpiration (E) were measured 28 days after initiation of the salt treatments on the second and third youngest fully expanded leaves. Measurements were made with a LI-COR, 6400 portable photosynthetic system (LI-COR, Lincoln, NE) following the same procedure as in Chapter 3.

4.3.4 Water use

Plant water use was measured gravimetrically. Everyday between 7:00h and 9:00h the plants were irrigated and pots left to drain to a constant weight before they were weighed. The pots were again weighed on the following day before irrigation in order to determine water loss per day. This procedure was carried out until termination of the experiment. Aluminium foil was placed on the surface of each pot to limit evaporation. Blank pots were prepared, one at each salinity level, to correct for water loss from plots in the absence of plants. Differences in water loss among the blank pots were small and their mean value was used as the estimate of evaporation.

Water loss through evapotranspiration was estimated during the experiment by measuring the daily mass loss of each pot. The daily increment of plant mass was small in comparison to water loss and was not taken into account in the estimate. Transpiration was estimated by subtracting the water loss from the blank pots from water loss from planted pots. Water use efficiency was calculated by dividing dry mass of shoots by the amount of water transpired (Glen and Brown, 1998).

4.3.5 Plant growth measurements

At the end of the experiment (eight weeks from the start of treatments), plant height was measured. The plants were then separated into leaves, stems and roots, and the number of leaves recorded. The roots were separated from the growth medium by washing in running water. Total leaf area per plant was measured with a LI-3100 leaf area meter (LI-COR. Inc., Lincoln, NE, USA). Fresh mass of shoots and roots were determined, and dry mass was obtained after oven drying the samples at 75^{o}C until constant weight. Relative shoot and root growth (percentage of growth of salinized vs unsalinized treatments) were determined. Root, stem and leaf mass ratio was determined as root, stem or leaf dry mass divided by plant total dry mass. Specific leaf area (SLA), the ratio of leaf area to leaf dry mass, was calculated.

4.3.6 Leaf anatomy

At the end of the experiment small leaf pieces of *A. tricolor* and *A. cruentus* from the control and 100 mM NaCl treated plants were fixed for two hours in 5% glutaraldehyde buffered with 0.075 M sodium phosphate (pH 7.4). Post-fixation followed for 2 hours in 2½% osmium tetroxide similarly buffered. The leaf segments were then dehydrated in alcohol series and specimens subsequently dried in liquid carbon dioxide (CO_2). Specimens for scanning electron microscopy (SEM) observation were mounted on aluminium stubs, coated with gold and viewed with a JEOL JSM-840 scanning electron microscope (JEOL, Tokyo). Stomatal density and dimensions were determined on three fields taken at random from each sample. Specimens for light microscopy (LM)

observation were embedded in Quetol 651 resin. Thin cross-sections of leaves (1 µm thick) were obtained with a Reichert Om U_2 ultramicrotome, stained with 1% toluidine blue O in borax, and examined with a Nikon light microscope. Total leaf thickness and mesophyll thickness were measured from three specimens taken at random.

4.3.7 Statistical analysis

All data were subjected to standard analyses of variance using the General Linear Model (GLM) procedure of the Statistical Analysis System (SAS, 1996) to determine the effect of main factors and the interaction between them. Differences at the $P \leq 0.05$ level were used as a test of significance and means were separated using Tukey's t-test.

4.4 RESULTS

4.4.1 Plant growth

Salinity stress had significant effects on all the growth parameters, and differences among genotypes for all characteristics were highly significant (Table 4.1). The interactions between genotype and salinity stress levels were also significant. All growth parameters decreased with increasing NaCl concentrations. However, their sensitivity to salinity stress varied with the level of stress and genotype. *A. tricolor* and Accession '83 did not survive in the 200 mM NaCl solution. Plants died about three weeks after the start of the treatments hence there was no data available for plant growth measurements at the end of the experiment. Although *A. hypochondriacus* and *A. cruentus* survived in the 200 mM NaCl treatment, plant growth was significantly reduced. Figure 4.1a and 4.1b illustrate the general reaction of *A. tricolor* and *A. cruentus* plants exposed to different concentrations of NaCl. Similar responses were observed with Accession '83 and *A. hypochondriacus* respectively.

a

Figure 4.1a ***Amaranthus tricolor*** **plants salinized with (A) 0 mM, (B) 25 mM, (C) 50 mM and (D) 100 mM NaCl solution.**

b

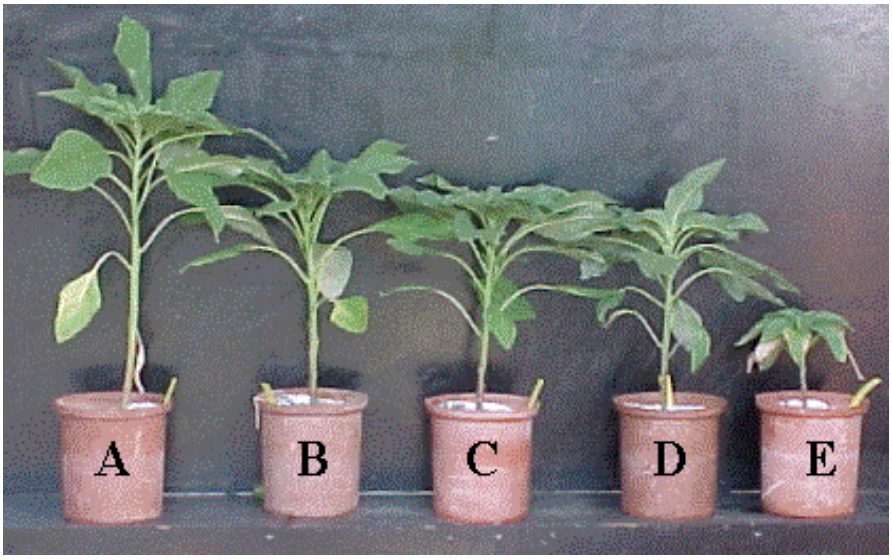

Figure 4.1b ***Amaranthus cruentus*** **plants salinized with (A) 0 mM, (B) 25 mM, (C) 50 mM, (D) 100 mM and (E) 200 mM NaCl solution.**

The most sensitive parameter was leaf area where 75% and 69% reduction was noted in *A. hypochondriacus* and *A. cruentus* respectively (Table 4.1). The least sensitive parameter at this concentration was leaf number with 54% reduction in *A. hypochondriacus*, and 49% in *A. cruentus*. Necrosis was observed at the leaf margins at 100 mM and 200 mM NaCl in *A. tricolor* and *A. cruentus* respectively (Figure 4.1).

Salinization with 25 mM NaCl did not have any significant effect on plant height in *A. tricolor*, Accession '83 and *A. cruentus*. In *A. hypochondriacus* plant height was reduced by 10%. Differences among genotypes were noted with application of higher NaCl concentrations. The reduction in plant height was more pronounced at 100 mM NaCl and especially in *A. tricolor* and Accession '83 than in *A. hypochondriacus* and *A. cruentus*. The reduction in *A. tricolor,* for instance, was 37% compared to 25% in *A. cruentus* (Table 4.1).

The effect of salt stress on the number of leaves was similar to that on plant height. Salinization with 25 mM NaCl did not have any effect on leaf number in *A. hypochondriacus* and *A. cruentus*. The number of leaves decreased with increasing NaCl concentration. *A. tricolor* and Accession '83 were more sensitive to salinity. They showed reductions in the number of leaves compared to *A. hypochondriacus* and *A. cruentus* at all concentrations of NaCl. The reduction in leaf area was similar in all the genotypes when salinized with 25 or 50 mM NaCl. At 100 mM NaCl, the reduction in leaf area in *A. tricolor* and Accession '83 was significantly higher than that in *A. hypochondriacus* and *A. cruentus*. For example, leaf area was reduced by 58% in *A. tricolor* and 49% in *A. cruentus* (Table 4.1).

Specific leaf area (SLA), an indicator of leaf thickness decreased with increasing salinity stress and varied among genotypes and salinity stress levels (Table 4.1). *A. tricolor* and Accession '83 had higher SLA values than *A. hypochondriacus* and *A. cruentus* for unstressed plants and at all concentrations of NaCl. Specific leaf area decreased with increasing NaCl, however, the reductions were higher in *A. hypochondriacus* and *A.*

cruentus than in *A. tricolor* and Accession '83. At 100 mM NaCl, for instance, the reduction in SLA was 14% in *A. hypochondriacus* compared to 10% in Accession '83.

Table 4.1 Effect of NaCl concentrations in the nutrient solution on plant height, leaf number, leaf area and specific leaf area of four amaranth genotypes

Genotype/NaCl concentration (mM)	Plant height (cm)	Leaf number	Leaf area (cm^2/plant)	Specific leaf area (cm^2 g^{-1})
A. tricolor				
Control	26gh	70a	2176b	374a
25	24gh	58b	1828f	369b
50	21hij	53c	1389j	358c
100	17j	42d	912o	333e
200	0k	0l	0r	0n
Accession '83				
Control	27g	67a	2199a	365b
25	25gh	56b	1869e	354c
50	22hi	53c	1408i	349d
100	19ij	41d	998m	327f
200	0k	0l	0r	0n
A. hypochondriacus				
Control	62a	33fg	1998d	254g
25	56bc	30gh	1690h	248h
50	50de	28hi	1314k	230j
100	44f	25i	935n	219k
200	23gh	15k	501q	196l
A. cruentus				
Control	62a	37e	2014c	246h
25	58ab	34ef	1752g	239i
50	53cd	31fgh	1324k	228j
100	46ef	29h	1030l	215k
200	25gh	19j	633p	187m
SEM	**0.78**	**0.62**	**2.8**	**0.9**

SEM: Standard error of the mean

Mean separation by Tukey's t- test. Means within each column followed by the same letter are not significantly different at P = 0.05.

Shoot and root dry masses expressed as a percentage of the control for each genotype was significantly reduced by salinity stress, and genotypic differences were observed. When plants were salinized with 25 mM NaCl the reductions in shoot dry mass ranged between 15 to 16% and no significant differences were observed among genotypes. With increasing salinity, shoot dry mass in *A. hypochondriacus* and *A. cruentus* was reduced to a lesser extend than in *A. tricolor* and Accession '83 (Figure 4.2). Root growth of *A. tricolor* and Accession '83 was less sensitive to salinization with low NaCl concentration since dry mass was reduced by only 17% when plants were salinized with 25 mM. At high NaCl concentration (100 mM) root dry mass was reduced by 57% in both genotypes. *A. hypochondriacus* and *A. cruentus* were highly sensitive to salinity stress since reductions in root dry masses of 43 and 32% were recorded even at the lowest NaCl level. Root dry mass was reduced by 71 and 68% respectively when plants were salinized with 100 mM NaCl (Figure 4.2).

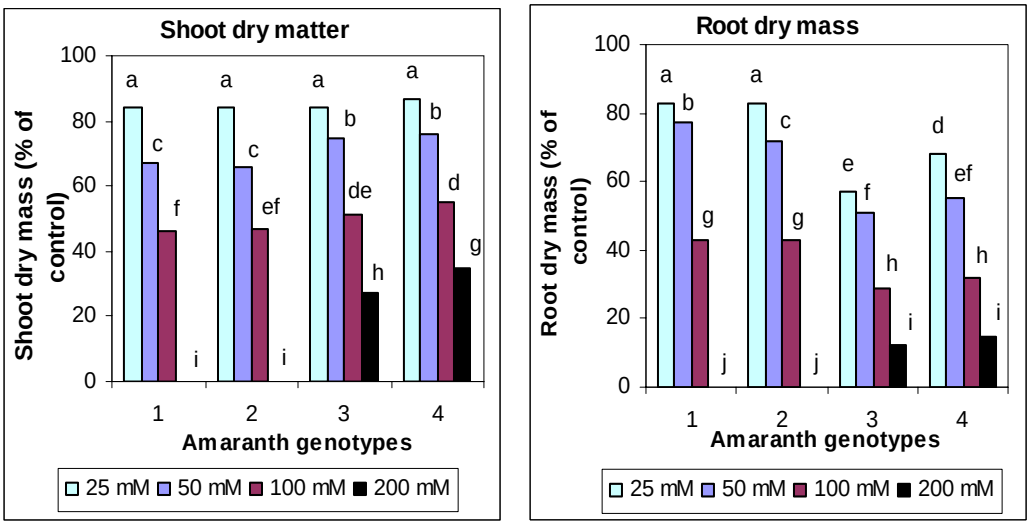

Figure 4.2 Effect of NaCl concentrations in the nutrient solution on shoot and root dry mass of amaranth genotypes: (1) *A. tricolor*, (2) Accession '83, (3) *A. hypochondriacus* and (4) *A. cruentus*. Mean separation by Tukey's t- test. For each parameter bars followed by the same letter are not significantly different at P = 0.05.

Dry matter partitioning into roots, stems and leaves expressed as a ratio of plant total dry mass for each genotype is presented in Figure 4.3. Under control conditions, all four amaranth genotypes had a similar root dry mass ratio (Figure 4.3). Salinity stress did not affect root dry mass ratio in *A. tricolor*, but it had significant effect on that of the other three genotypes. In Accession '83 salinization with 25 mM NaCl increased root dry mass ratio from 0.3 to 0.4. No further increase was noted when plants were salinized with 50 mM NaCl. At 100 mM NaCl root dry mass ratio was again reduced and was similar to that of the control. In *A. hypochondriacus* and *A. cruentus*, salinity stress decreased root dry mass ratio with the greatest reduction (from 0.3 to 0.1) obtained when plants were salinized with 200 mM NaCl.

Stem dry mass ratio was not affected by salinity stress in any of the genotypes (Figure 4.3). *A. tricolor* and Accession '83 had a higher leaf dry mass ratio than *A. hypochondriacus* and *A. cruentus* under control conditions. Salinity stress did not affect leaf dry mass ratio in *A. tricolor*, but significantly increased it in *A. hypochondriacus* and *A. cruentus*. In Accession '83 leaf dry mass ratio decreased with salinity stress at 25 and 50 mM NaCl and increased to the control value at 100 mM NaCl.

It is worthwhile to note that in the vegetable type amaranth (*A. tricolor* and Accession '83) the ratio of stem dry mass was generally lower than that of the leaf dry mass at all NaCl concentrations, while the opposite was observed for the grain types (*A. hypochondriacus* and *A. cruentus*). Figure 4.3 shows that salinity stress affected leaves, stems and roots of *A. tricolor* to the same extent, but for *A. hypochondriacus* and *A. cruentus* root growth was more affected by increasing salinity with the results that an increasing fraction of the limited assimilates were utilized for leaf growth.

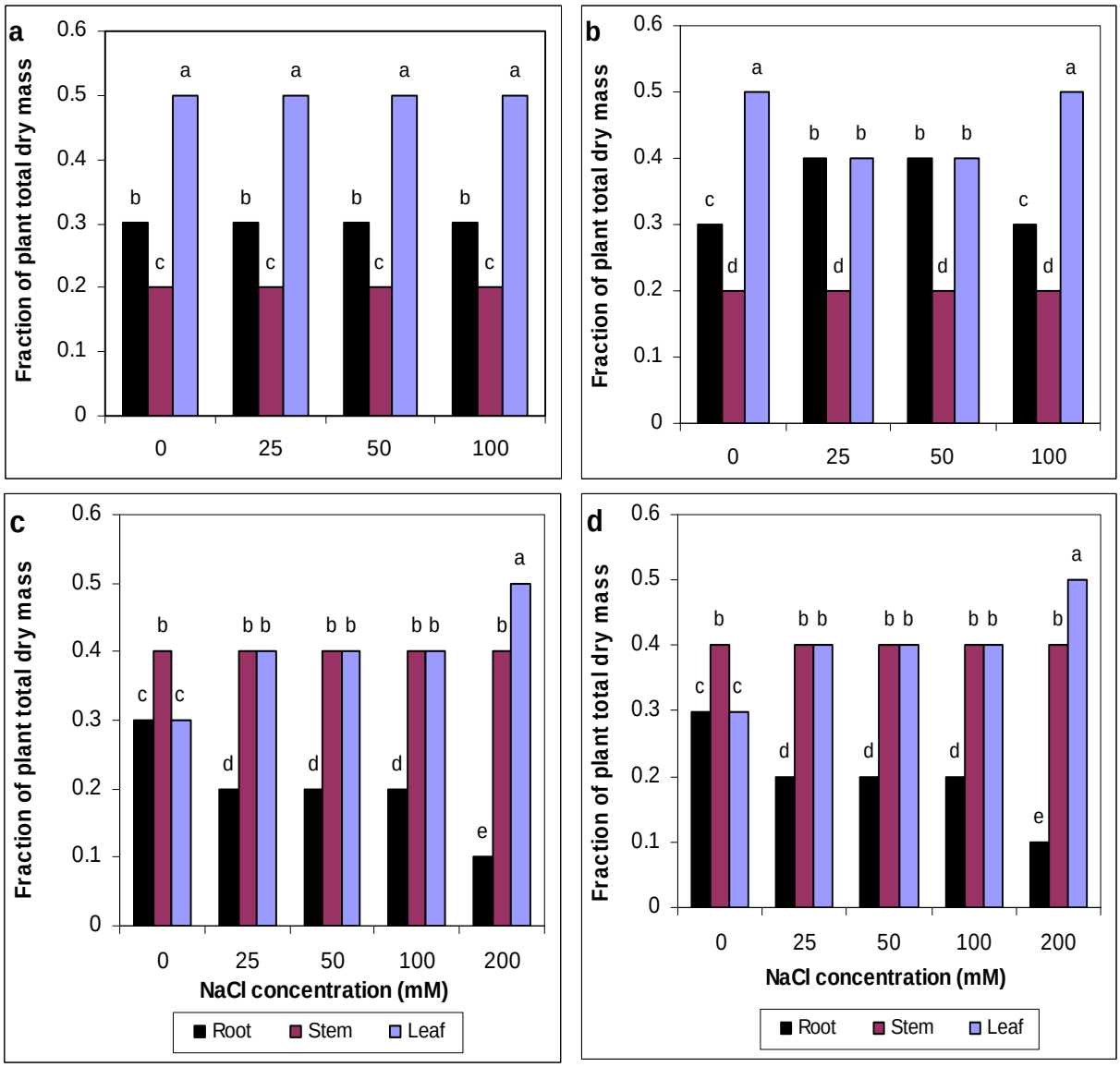

Figure 4.3 Effect of NaCl concentrations in nutrient solution on dry matter partitioning in root, stem and leaves of (a) *A. tricolor*, (b) Accession '83, (c) *A. hypochondriacus* and (d) *A. cruentus* grown under different NaCl concentrations. Mean separation by Tukey's t- test. For each genotype bars followed by the same letter are not significantly different at P = 0.05.

4.4.2 Gas exchange

Salinity significantly affected stomatal conductance (g_s) and photosynthetic rate (P_n). The interaction between salinity and genotype was also highly significant, indicating that genotypes differed in response to salinity stress. Stomatal conductances of *A. tricolor* and Accession '83 were higher than for *A. hypochondriacus* and *A. cruentus* at all salinity treatments (Figure 4.4a). Salinity stress significantly reduced stomatal conductance. The average reduction in all genotypes was 28% when 25 mM NaCl was applied. However, at 100 mM NaCl, the reduction in g_s was greater in *A. hypochondriacus* and *A. cruentus* (53%) in comparison to that of *A. tricolor* and Accession '83 (47%) (Figure 4.4a). The relative reduction of stomatal conductance exceeded that of CO_2 assimilation. A significant correlation between CO_2 assimilation rate and stomatal conductance ($r = 0.94$) indicated that the response of CO_2 assimilation to salinity was strongly associated with stomatal conductance.

The response of P_n to salinity stress was similar to that of g_s. Increasing salinity progressively reduced CO_2 assimilation rate. At the lowest NaCl concentration (25 mM) the reduction in photosynthetic rate ranged from 14% in Accession '83 to 21% in *A. cruentus* (Figure 4.4b). With increasing NaCl concentrations, the reduction in P_n was much greater in *A. hypochondriacus* and *A. cruentus* than in *A. tricolor* and Accession '83. For instance, at 50 mM NaCl, P_n was reduced by 19% in *A. tricolor* compared to 28% in *A. cruentus*.

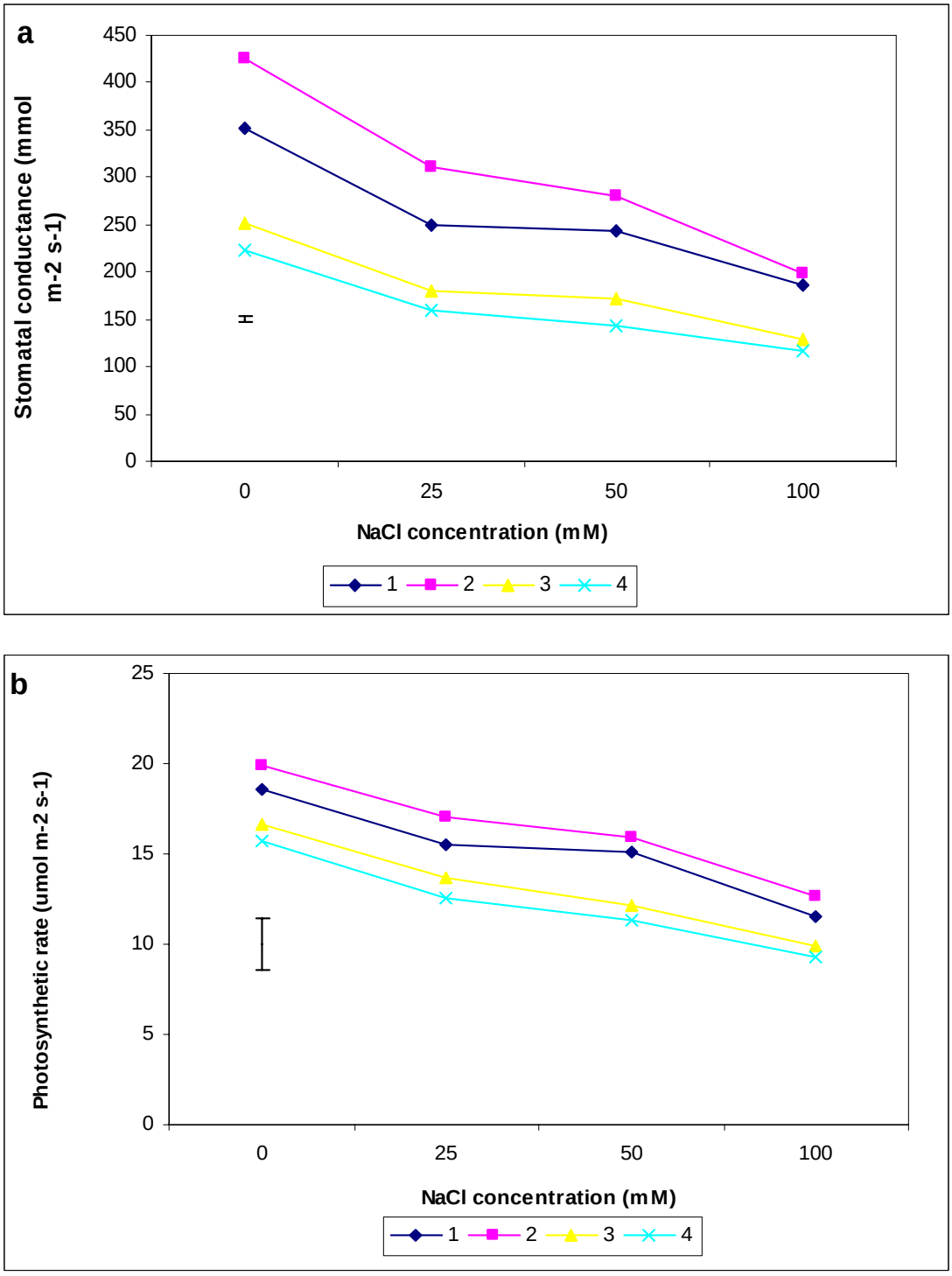

Figure 4.4 Effect of NaCl concentrations in the growth medium on (a) stomatal conductance and (b) photosynthetic rate of four amaranth genotypes (1) *A. tricolor*, (2) Accession ‘’83, (3) *A. hypochondriacus* and (4) *A. cruentus*. Mean separation by Tukey’s t- test. Verttical bars indicate least significant differences at P = 0.05.

4.4.3 Transpiration and water use

Total water loss by transpiration as well as transpiration rates (E) were significantly reduced by increasing NaCl concentrations (Table 4.2). The reduction in total water loss by transpiration was similar in all the genotypes when 25 mM or 100 mM NaCl was applied. However, when 50 mM was applied, the reduction was lower in *A. hypochondriacus* and *A. cruentus* (38%) compared to that in *A. tricolor* and Accession '83 (44%). In *A. hypochondriacus* and *A. cruentus* the rate of transpiration was reduced to a greater extent than in *A. tricolor* and Accession '83. Salinization with 50 mM NaCl, for instance resulted in E being reduced by 35% in Accession '83 and 47% in *A. cruentus*.

Water use efficiency (WUE) increased with increasing salinity, and differences among genotypes occurred. Photosynthetic water use efficiency (WUE^a) derived from instantaneous gas exchange parameters (P_n/E) increased with increasing salinity since salinity reduced leaf transpiration rate more than that of CO_2 assimilation (Table 4.2). Water use efficiency (WUE^b) determined as the amount of dry matter produced per unit of water transpired also increased with salinity. Among genotypes, *A. cruentus* followed by *A. hypochondriacus* were the most efficient in water use, while *A. tricolor* was the least efficient genotype. The increases in WUE^b between 0 and 100 mM NaCl ranged from 21% in *A. tricolor* to 34% in *A. cruentus* (Table 4.2).

Table 4.2 Effect of NaCl concentrations in the nutrient solution on total water loss by transpiration, transpiration rate (E), and water use efficiency (WUE) of different amaranth genotypes

Genotype/NaCl concentration (mM)	Total transpiration (kg/plant)	Transpiration rate (E) (mmol m^{-2} s^{-1})	WUE[a]	WUE[b] g/kg
A. tricolor				
Control	2.5c	5.5ab	3.4de	3.3l
25	1.9f	3.9d	3.9c	3.6k
50	1.4g	3.6de	4.2bc	3.8j
100	0.9h	2.6fg	4.4ab	3.9j
200	0.0j	0.0i	0.0f	0.0m
Accession '83				
Control	2.6c	5.8a	3.4de	3.5k
25	2.0ef	4.5c	3.8cd	3.9j
50	1.5g	3.8d	4.2bc	4.1i
100	1.0h	2.8f	4.5ab	4.3h
200	0.0j	0.0i	0.0f	0.0m
A. hypochondriacus				
Control	3.5a	5.3b	3.1e	4.9g
25	2.6c	3.7de	3.7cd	5.5f
50	2.1de	2.9f	4.1bc	5.9e
100	1.4g	2.2g	4.5ab	6.3c
200	0.7i	1.6h	4.7a	6.9b
A. cruentus				
Control	3.5a	5.1b	3.1e	5.0g
25	2.8b	3.3e	3.8cd	5.6f
50	2.2d	2.7f	4.2bc	6.1d
100	1.5g	2.0g	4.6ab	6.7b
200	0.9h	1.5h	4.7a	7.3a
SEM	**0.51**	**0.92**	**0.10**	**0.04**

[a] - Expressed as the ratio P_n/E
[b] - Expressed as dry matter per kg of water used
SEM: Standard error of the mean
Mean separation by Tukey's t- test. Means within each column followed by the same letter are not significantly different at P = 0.05.

4.4.4 Effect of salinity stress on leaf cell ultrastructure

4.4.4.1 *Stomatal density and pore size*

In both genotypes, increasing NaCl concentration resulted in a decrease in the number of stomata (Table 4.3). *A. tricolor* had a higher number of stomata than *A. cruentus.* Salinity stress reduced the number of stomata on the upper and lower leaf surface by 47 and 45% in *A. cruentus* compared to 20 and 19% in *A. tricolor*. The length of stomatal aperture on the upper leaf surface was not affected by salinity in *A. cruentus,* while that of *A. tricolor* was reduced by 45%. On the lower leaf surface, the length of stomatal aperture was reduced by 38% in *A. cruentus* and 42% in *A. tricolor* (Table 4.3). Differences between the effect of salinity stress on stomatal density and closure are shown in the micrographs (Figure 4.5). The stomata of plants under salt treatment were either completely or partially closed when compared to plants in control (Figure 4.5). The effect of salinity stress on epidermal cell size is also clear in the micrographs. Epidermal cells were reduced in size with increasing NaCl concentration in both genotypes (Figure 4.5).

Table 4.3 Effect of NaCl concentrations on the number of stomata and stomatal aperture on the upper and lower leaf surfaces of two amaranth genotypes

Genotype/NaCl concentration	Upper leaf surface		Lower leaf surface	
	Stomatal number per mm^2	Length of stomatal aperture (µm)	Stomatal number per mm^2	Length of stomatal aperture (µm)
A. tricolor				
0 (control)	267a	9a	272a	12a
100 mM	213b	5b	221b	7bc
A. cruentus				
0 (control)	152c	6b	163c	8b
100 mM	80d	4b	89d	5c
SEM	**0.57**	**0.54**	**0.61**	**0.56**

SEM: Standard error of the mean
Mean separation by Tukey's t- test. Means within each column followed by the same letter are not significantly different at P = 0.05.

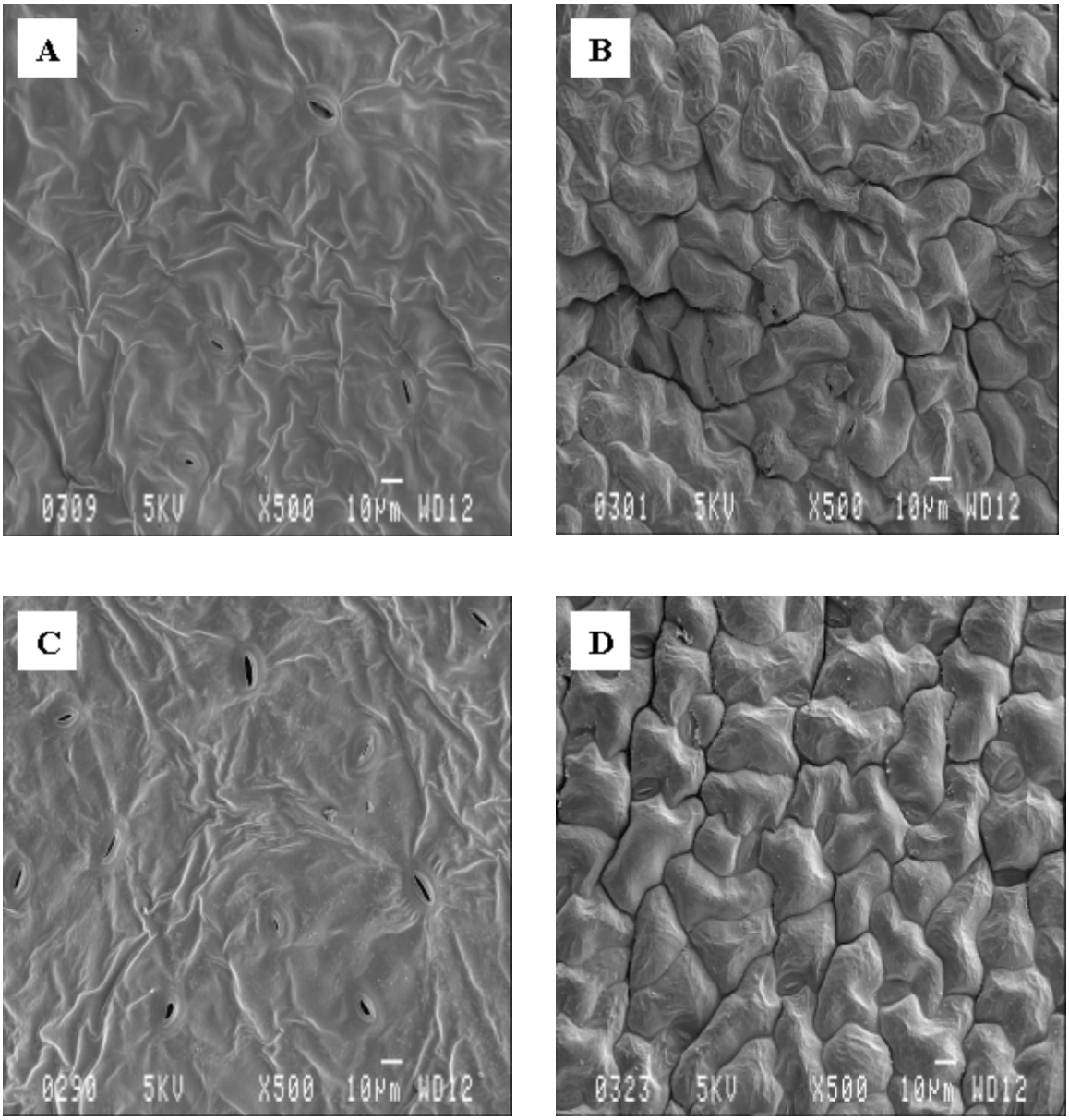

Figure 4.5 Scanning electron micrographs showing stomates and cell size on the upper leaf surface of two amaranth genotypes. A, B (*A. cruentus* at control and salinized with 100 mM NaCl); C, D (*A. tricolor* at control and salinized with 100 mM NaCl). Bars = 10 µm.

4.4.4.2 Mesophyll thickness

Salinity stress induced changes in leaf anatomical characteristics. In particular, it resulted in an increase in the size of almost all the cells of the mesophyll, as well as the entire lamina thickness (Table 4.4; Figure 4.6). The main treatment effects (genotype and salt level) were significant but the interaction between these factors was not significant indicating similar reactions in the two genotypes. The palisade layer and total leaf thickness of *A. cruentus* were greater than in *A. tricolor*. However, when other leaf histological components were considered no genotypic differences were found. Salinity stress induced an increase in the thickness of all the leaf components compared to the control (Table 4.4).

Table 4.4 Main effects of genotype and salinity stress on leaf tissue thickness of amaranth

Main effect	Tissue thickness (µm)				
	Upper epidermis	Palisade	Spongy	Lower epidermis	Total thickness
Genotype					
A. tricolor	17a	29.0b	30.5a	13.0a	90.0b
A. cruentus	18a	43.5a	30.0a	12.5a	104.0a
SEM	**0.73**	**0.64**	**0.64**	**0.64**	**0.54**
NaCl level (mM)					
0	15.5b	31.0b	29.0b	11.0b	86.5b
100	20.0a	41.5a	31.5a	14.5a	107.5a
SEM	**0.73**	**0.64**	**0.64**	**0.64**	**0.54**

SEM: Standard error of the mean
Mean separation by Tukey's t- test. Means within each column followed by the same letter are not significantly different at P = 0.05.

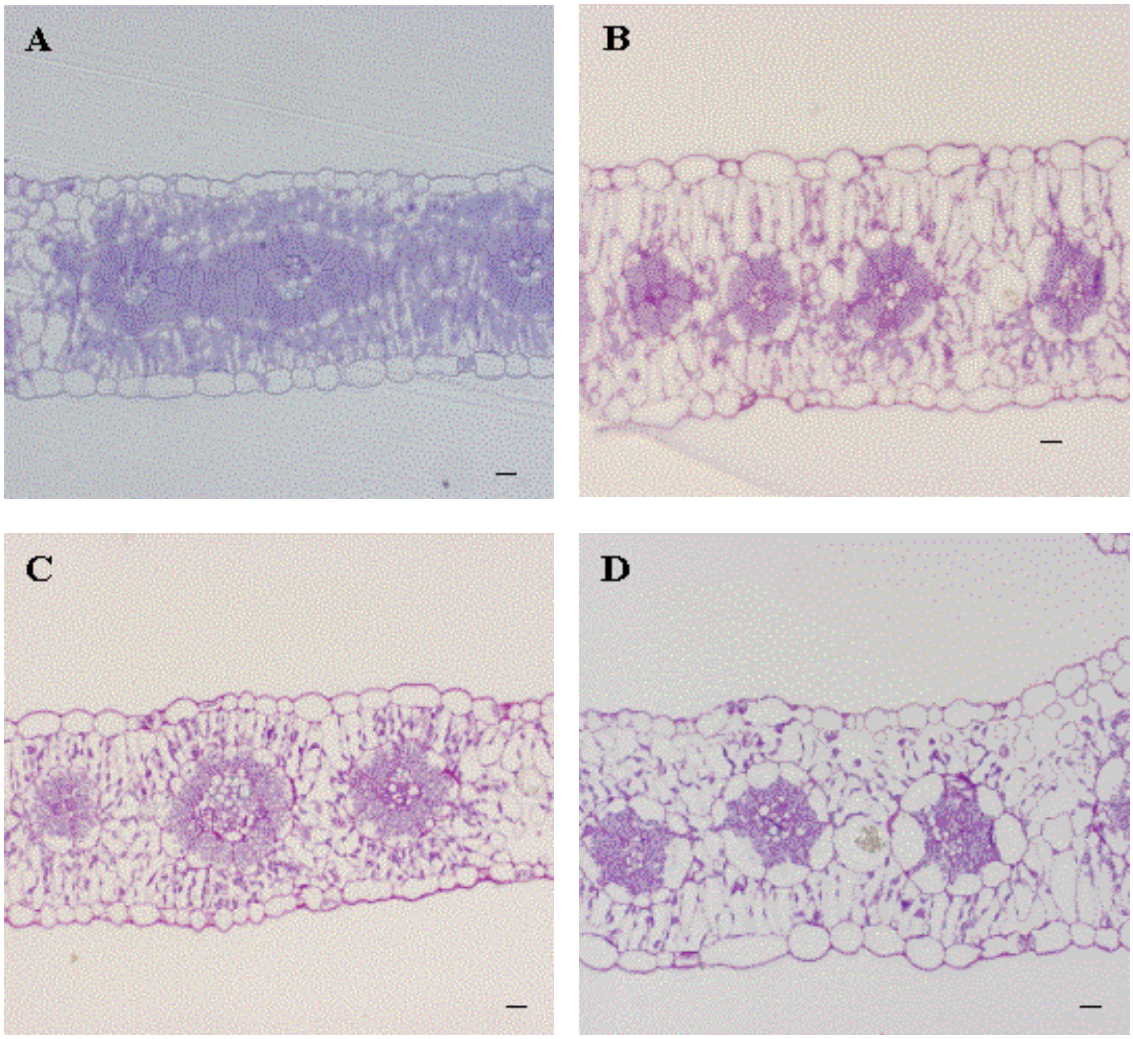

Figure 4.6 Leaf cross sections of *A. cruentus* (A – control, B – salt stressed) and *A. tricolor* (C – control, D – salt stressed) showing different tissue thickness. Bars = 10 µm

4.5 DISCUSSION

4.5.1 Plant growth

Salinity stress caused reductions in plant height, leaf area shoot and root dry mass in all amaranth genotypes, although the relative effects varied and the classification of the genotype for its salt tolerance would vary according to the parameter used. For example, on the basis of leaf area, plant height and shoot dry mass *A. hypochondriacus* and *A. cruentus* were more tolerant than *A. tricolor* and Accession '83 at 100 mM NaCl, while the reverse was true for root dry mass. Differences in sensitivity among genotypes could not be distinguished when 25 mM NaCl was applied. At the highest NaCl concentration (200 mM), *A. hypochondriacus* and *A. cruentus* were more tolerant. The effect of salinity on leaf area was greater than on shoot length (Table 4.1). It is well known that salinity reduces plant growth and that there are differences in tolerance to salinity among species and among cultivars (Cruz *et al*., 1990; Bolarin *et al.,* 1991; Romero-Aranda *et al*., 2001). Ashraf (2001) recorded a significant reduction in mean fresh and dry mass of shoots of *Brassica* species with increase in salt concentration and observed that the response to salinity stress differed with species and the measured growth variable.

Leaf area was significantly correlated to leaf number (r = 0.88), suggesting a lower leaf production as one of the reasons for reduced leaf area development under salinity stress. The size of individual leaves also decreased as salinity increased (Figure 4.1). The reduction in leaf area implies that the area available for transpiration and assimilate production would be reduced, thereby reducing the growth of plants. Thus, assessment of the ability of a genotype to maintain a large leaf area, during salinity stress would help in characterizing the genotypes as salinity stress tolerant or susceptible. At 100 mM NaCl, the reduction in leaf area in *A. tricolor* and Accession '83 was greater than that in *A. hypochondriacus* and *A. cruentus*. Hence, the latter two amaranth genotypes may be considered more salt tolerant than the former genotypes (Table 4.1).

The response of root growth to salinity stress in relation to shoot growth differed with the level of NaCl used and the genotype. Genotypic differences in dry matter production and partitioning under stress can be used as indicators of tolerance to salinity stress. Similar sensitivity in terms of root and shoot growth was noted in *A. tricolor* and Accession '83 when plants were salinized with either 25 or 100 mM NaCl. However, at 50 mM NaCl root biomass was higher than shoot biomass (Figure 4.2). Sobrado and Turner (1986) found similar root to shoot ratios in well-watered and water-stressed plants of *Helianthus petiolaris* Nutt. and *Helianthus annuus* L. and suggested that it might have been due to a similar degree in osmotic adjustment in root and leaf cells. A high root dry mass could indicate an increased capacity of water uptake, thereby maintaining the shoot in a well-hydrated condition (Blum, 1996). In *A. hypochondriacus* and *A. cruentus* shoot biomass was higher than root biomass at all levels of NaCl. Sustaining shoot growth during salinity stress is particularly important for genotypes cultivated as leafy vegetable crops. A reduction in root growth with increasing root zone salinity was also observed by Bassil and Kaffka (2002) with safflower. According to Bassil and Kaffka (2001) earlier physiological maturity could have accounted for some of the observed reduction in root growth of safflower grown in saline plots.

The results from this study contrast with observations from most investigators who found that roots were less affected by salinity than shoots (Brugnoli and Bjorkman, 1992; Chartzoulakis *et al*., 1995; Perez-Alfocea *et al*., 1996). Fisarakis *et al*. (2001) reported that at 50 mM and especially at 100 mM NaCl, root growth of sultana vines was less affected than that of shoots resulting in high root/shoot ratios. Dalton *et al*. (1997; 2001) have discussed in great detail functional drawbacks of such a response in saline versus drought environments. More recently, Munns (2002) pointed out that under certain conditions high root/shoot ratios may actually enhance the accumulation of toxic ions into the shoot. De Pascale *et al*. (2003a) proposed that the smaller root/shoot ratio observed in salinized vs. drought affected plants may be functionally associated with the need of salt stressed plants to restrict the uptake of toxic ions to the shoot while still maintaining high turgor and a positive growth rate. According to Gunes *et al*. (1996); Hayashi *et al*. (1997); Shen *et al*. (1997) and Maggio *et al*. (2001) this may be

accomplished by simultaneously reducing root vs. shoot development and activating specific metabolic pathways (i.e., osmolyte biosynthesis), both of which occur in saline environments.

At the highest NaCl concentration (200 mM), the leaf dry mass was higher than stem dry mass in *A. hypochondriacus* and *A. cruentus*. Similar results were obtained by Sifola and Postiglione (2002) who found that increasing salt concentration in the irrigation water increased assimilate partitioning towards the leaf and decreased that towards the stem.

4.5.2 Transpiration and water use

In the four amaranth genotypes, total transpiration as well as transpiration rate (E) decreased with increasing NaCl concentration. Similar decreases in transpiration rate with increasing salinity were recorded by Ashraf (2001) with *Brassica* species. Bassil and Kaffka (2002) observed that consumptive water use and biomass declined at high EC and that safflower's evaporative demand was correlated with reduced height and leaf area in saline plots. According to Pang and Letey (1998) increasing soil or water salinity reduces transpiration and increases drainage for a given irrigation volume.

The reduction in transpiration with salinity should be related to the reduced g_s and the lower stomatal density of leaves developed under saline conditions as indicated by the close correlation found between these parameters. Amaranth growth reduction resulting into reduced leaf number and leaf area could probably be the main origin of the observed reduction in water uptake and transpiration. Reduction in water uptake has also been related to reduction in hydraulic conductance of the root system (Rodriguez *et al*., 1997). This may explain the reduction in water absorption rate and may contribute to a similar reduction in nutrient uptake, resulting in retarded plant growth and decreased dry-matter yield under salt stress conditions.

Water use efficiency in the four amaranth genotypes increased with increasing NaCl concentration. This increase may be due to the large decrease in transpiration rate

compared to photosynthetic rate. Ashraf (2001) recorded increasing water use efficiency of the salt tolerant *Brassica* species with increasing external salt concentration and attributed this increase to relatively higher assimilation rates and lower stomatal conductance in these species. Studies have shown that WUE of a crop is related to the morphological characteristics of leaves. Wright *et al*. (1994) proposed that under field conditions at moderate temperatures, there is a close negative relationship between WUE and SLA. Salinity stress reduced SLA and increased WUE in amaranth. This is probably part of an adaptive mechanism to reduce leaf area and transpiration (Craufurd *et al*., 1999). According to Thumma *et al*. (2001) the relationship between WUE and SLA may be due to the fact that plants with low SLA (thicker leaves) have more mesophyll cells per unit area, leading to higher rates of CO_2 assimilation, and consequently, higher biomass production. However, CO_2 assimilation in amaranth was also reduced with increasing salinity. Bayuelo-Jiménez *et al*. (2003) argued that the lower SLA of salt stressed plants probably reflects an overloading of the leaves with inorganic and organic solutes, which allows osmotic adjustment but reduces the efficiency for gaining carbon.

4.5.3 Gas exchange

Salinity significantly reduced P_n and g_s of all the genotypes and the reduction was proportional to the increase in NaCl level. Similar results were reported by Ashraf (2001) with *Brassica* species where both photosynthetic rate and stomatal conductance showed significant decreasing trends with increase in salt concentration in the rooting medium. Gas exchange response of tobacco to saline treatments was markedly decreased even at the lowest level of salinity (2.5 dS m^{-1}) (Sifola and Postiglione, 2002). Similarly, Bayuelo-Jiménez *et al*. (2003) reported a reduction in photosynthetic carbon assimilation in *Phaseolus* species and attributed this decrease to reduced stomatal conductance.

The reduction in net carbon dioxide assimilation by increased salinity could be due to a limitation of CO_2 supply as a result of stomatal closure (Perera *et al*., 1994; Steduto *et al*., 2000); to non-stomatal factors related to the toxic effect of salts in the activity of the photosynthetic mesophyll thus depressing specific metabolic processes in carbon uptake

(Seemann and Critchly, 1985; Sultana *et al*., 1999; Chen *et al*., 1999); inhibition in photochemical capacity or a combination of these factors (Everard *et al.*, 1994; Dubey, 1997). Although the role of stomatal vs non-stomatal responses to NaCl salinity was not distinguished in amaranth, the results showed a close relationship between P_n, g_s and stomatal density making it clear that the reduction in net CO_2 assimilation with salinity could be explained by the reduction in g_s and stomatal density. Xu *et al* (1994) and Romero-Aranda *et al.* (2001) demonstrated for tomato exposed to high electrical conductivity in the root medium, that net CO_2 assimilation is more affected by the limitation of CO_2 supply than by biochemical processes in the leaf mesophyll. Meloni *et al*. (2003) also reported that the stomatal closure limited leaf photosynthetic capacity in the NaCl-treated cotton plants.

Higher stomatal conductance in plants is known to increase CO_2 diffusion into leaves thereby favoring higher photosynthetic rates. Higher net assimilation rates could in turn result in a higher biomass and higher crop yields (Taiz and Zeiger, 1998). However, this may not always be the case as was observed by Ashraf (2001). In his study with six *Brassica* species he found no significant relationship between photosynthetic rate and stomatal conductance although these two variables declined consistently with increase in salt concentration of the growth media. It was similarly shown by Melesse and Caesar (1992) with *Vicia faba* that stomatal conductance bore little relationship with photosynthetic rate.

It is noteworthy that higher CO_2 assimilation rates and stomatal conductance occurred in *A. tricolor* and Accession '83 than in *A. hypochondriacus* and *A. cruentus*. The characteristic of higher stomatal conductance and CO_2 assimilation can be regarded as an adaptive mechanism to salinity. According to Plaut *et al*. (1990) a higher stomatal conductance could be associated with a higher stomatal density. A higher stomatal density was observed in *A. tricolor* compared to *A. cruentus* (Table 4.3). This trait may allow maintenance of CO_2 exchange (Lynch *et al*., 1992). Stomatal density is a character of special interest in terms of its potential utility in genetic improvement since it may be related to leaf water use and hence, water use efficiency. Further studies to determine the

extent of variability in physiological traits related to leaf photosynthesis for salinity tolerance are greatly needed.

4.5.4 Leaf anatomy

In amaranth, salt-stressed leaves were thicker than in control plants (Table 4.4, Figure 4.6). The dense arrangement of palisade and spongy cells may result in reduction of the diffusion conductance in salt-stressed amaranth leaves. According to Evans *et al*. (1994) and Syvertsen *et al*. (1995) changes in leaf anatomy are likely to affect the conductance to CO_2 diffusion. Reduction of mesophyll conductance was related to mesophyll thickening in olive leaves (Bongi and Loreto, 1989). Salt-induced increase of mesophyll thickness may have contributed to reduced mesophyll conductance and photosynthesis in cotton (Brugnoli and Bjorkman, 1992). However, it is possible that the observed reduction in mesophyll conductance in salt-stressed leaves is caused in part by ultrastructural features related to the stress such as reduced chloroplast adherence to the cell wall (Sharkey *et al.*, 1991). Contrary to the results obtained with amaranth, low salt accumulation slightly decreased the thickness of spinach leaves (Delfine *et al*., 1998). However, the reduction in P_n in the salt-stressed spinach leaves was suggested to be associated with a reduction of the intercellular spaces in the mesophyll with respect to controls and may have caused a restriction of carbon flow towards the chloroplasts. These results support the idea that a direct relationship between leaf porosity and mesophyll conductance exists (Loreto *et al*., 1992; Evans *et al*., 1994; Syvertsen *et al.*, 1995).

A reduction in the number of stomata as well as size of stomatal apertures with salinity stress was noted in amaranth. A mechanism of water economy in salt-stressed plants is the reduction of transpiration by closure of stomata. On the other hand, the rate of photosynthesis is also reduced since CO_2 is prevented from entering the mesophyll. This conforms to data obtained from this study. *Amaranthus tricolor* which had a higher number of stomata and larger stomatal apertures also had a higher photosynthetic rate and transpiration rate compared to *A. cruentus* (Table 4.2, 4.3, Figure 4.4). Salinity stress also resulted in a decrease of the cell size (Figure 5). According to Oertli *et al*. (1990), the

small size contributes to a resistance against cell collapsing due to arid conditions. Small epidermal cells have been found to be at least 20 times more resistant to collapse than large ones. Cutler *et al*. (1977) and Steudle *et al*. (1977) have considered the reduction in cell size under water stress as a drought adaptation mechanism. According to Cutler *et al*. (1977) the reduction in cell size appears to be a major response of cells to water deficiency that may be caused either by drought or salinity stress.

The salinity level at which amaranth shoot yield was reduced by 50% was approximately 75 mM NaCl (10 dS m^{-1}) for *A. tricolor* and Accession'83, 100 mM (12 dS m^{-1}) for *A. hypochondriacus* and 125 mM (14.6 dS m^{-1}) for *A. cruentus.* According to the classification of salt tolerance of herbaceous crops (Maas, 1986) and vegetable crops (Shannon and Grieve, 1999), amaranth can be classified as moderately tolerant and compares well with other vegetables crops such as cowpea and Brassica.

4.6 CONCLUSIONS

In all four amaranth genotypes, salinity stress decreased plant growth. However, the sensitivity to stress differed with the level of salinity, genotype and the measured parameter. The reduction in growth due to salinity in most of the parameters was more severe for *A. tricolor* and Accession '83 than for *A. hypochondriacus* and *A. cruentus*. Salinity stress resulted in genotypic differences in dry matter partitioning. High salt tolerance of *A. hypochondriacus* and *A. cruentus* was found to be associated with their high water use efficiency, but there was little association of the tolerance of these genotypes with respect to stomatal conductance and photosynthetic rate.

The effect of salinity stress on photosynthetic rate and water use efficiency was closely related to leaf anatomical features. Salt stress induced a reduction of stomatal conductance in amaranth leaves and this reduction may have contributed to the inhibition of photosynthesis. The reduction of mesophyll conductance was associated with leaf thickness and less intercellular spaces in the mesophyll of salt-stressed leaves, which may have made the path towards the sites of CO_2 fixation more difficult.

According to the classification of salt tolerance of herbaceous crops, amaranth can be rated as moderately salt tolerant similar to other leafy vegetable crops.

CHAPTER 5
INTERACTIVE EFFECTS OF SALINITY AND WATER STRESS ON GROWTH, WATER RELATION AND GAS EXCHANGE IN AMARANTH

5.1 ABSTRACT

Amaranth is a promising vegetable crop species often grown under semi-arid conditions prone to both drought and salinity. However, the response of amaranth to combined water and salt stress has not been investigated. This study was initiated to determine the effects of water and salinity stress, both individually and in combination, on leaf water relations, gas exchange and growth of two amaranth genotypes, viz. *Amaranthus tricolor* and *A. cruentus*. The plants were grown in a temperature-regulated greenhouse in plastic pots filled with a sand/vermiculite mixture. Plants were exposed to 8 days of drought and/or salinity stress, a recovery period of 8 days, followed by a final two weeks of stress. The treatments consisted of (1) unstressed control, (2) 100 mM NaCl, (3) PEG (polyethylene glycol M_W 6000) iso-osmotic to 100 mM NaCl, (4) 50 mM NaCl + PEG iso-osmotic to 50 mM NaCl. Plant growth, photosynthetic rate, stomatal conductance and water loss were reduced by all stress treatments. The reduction in shoot growth was greater in plants submitted to PEG-induced water stress (41% in *A. tricolor* and 44% in *A. cruentus*) than in salinized plants (37% in *A. tricolor* and 27% in *A. cruentus*). Water use efficiency was increased since water loss was reduced more than photosynthetic rate. Leaf water and osmotic potentials were reduced by the stress treatments. Salinized plants had a greater degree of osmotic adjustment, so that plants were able to continue growth for a longer period compared to water stressed plants. Most parameters recovered when the stress treatments were discontinued. However, photosynthesis in salt stressed plants did not recover indicating a toxic effect of salt on the photosynthetic apparatus.

Key words: *Amaranthus tricolor*, *A. cruentus*, gas exchange, growth, photosynthesis, salinity, water relations, water stress

Contributions based on study:

E.N. OMAMI & P. S. HAMMES , 2005. Amaranth response to interactive effect of salinity and water stress. Submitted for publication to: *New Zealand Journal of Crop & Horticultural Science*. 1st February, 2005.

5.2 INTRODUCTION

Excessive soil salinity occurs in many semi-arid regions of the world inhibiting plant growth due to water deficiency and salinity problems (Neumann, 1997). A plant in drying soil is exposed to increasing levels of both water stress and osmotic stress, because the matrix potential and the osmotic potential decrease simultaneously with decreasing soil moisture (Shalhevet, 1993; Glen and Brown, 1998). This is common in arid soils, in which salts often concentrate near the surface as the soil dries between rains (McNaughton, 1991) and in irrigated soils, which can accumulate damaging levels of salts between irrigations (McCree and Richardson, 1987; Shalhevet, 1993). Futhermore, irrigation with poor quality water often results in both salt stress and water stress in the dryer parts of the irrigation cycle.

Both low soil osmotic potentials (due to dissolved salts) and low soil matric potentials (associated with reduced water content) cause low water potentials in plants resulting in reduced leaf expansion rates, lower photosynthetic rates per unit leaf area and reduced growth (Rawson and Munns, 1984). The soil matrix and osmotic potentials are additive in lowering the free energy of water in soil (Shalhevet, 1993), and the primary physiological response of plants to both conditions is to lower the cell water potential through the accumulation of organic and inorganic solutes so that the roots can continue to extract water from the soil solution (Flowers and Yeo, 1986; Pitman, 1988). Hence, it is logical to think that the two stress factors could be additive in affecting plant performance (Shalhevet, 1993). However, studies in which plants are grown in drying soils at different salinities show a more complicated response, in which soil salts actually mitigate some of the negative effects of water stress. For example, plants in drying soils

usually survive longer in saline than in non-saline soils, because salt-stressed plants grow less and, therefore, deplete soil moisture more slowly than non-stressed plants (McCree and Richardson, 1987; Richards, 1992; Shalhevet, 1993). Studies of the combined effects of salt and water stresses on growth of maize (Stark and Jarrell, 1980) and sorghum (Richardson and McCree, 1985) showed that although salinity reduced the rates of leaf expansion under well-irrigated conditions, it also allowed leaf expansion to continue down to lower leaf water potentials under water stress. Furthermore, salt stress can increase leaf instantaneous water use efficiency by reducing stomatal conductance to a greater extent than photosynthesis (Guy and Reid, 1986; Ayala and O'Leary, 1995), thereby allowing plants under salt stress to produce more dry matter than plants in nonsaline soil on the same quantity of water (Richards, 1992).

Finally, salt stress can precondition plants to low soil water potential by allowing them to osmotically adjust, enhancing their ability to survive as the soil dries (Shalhevet, 1993). Thus the combined effects of salinity and water stress may be less detrimental to plant growth than the sum of the separate effects. These generalizations have practical implications with regard to irrigation strategies for crop plants in salt affected soils (Richards, 1992; Shalhevet, 1993), and they may also be relevant to the growth strategies of adapted native plants in saline arid soils (McNaughton, 1991).

In several plants, salt tolerance and drought tolerance are linked through a common mechanism of salt uptake for osmotic adjustment (Flowers and Yeo, 1995; Glen and Brown, 1998). Physiological studies have often dealt separately with salt and water stresses, but in the field, salt stress is usually accompanied by water stress. Despite their importance, relatively few studies have considered the combined effects of water and salt stress on plants (McNaughton, 1991; Richards, 1992; Shalhevet, 1993).

Owing to its high nutritive value and a wide adaptation to diverse environments, amaranth has been considered a promising crop for marginal lands and semiarid regions. Whitehead and Singh (1992) described this species as drought tolerant with the ability to adapt to low moisture. Experiments involving the comparative analysis of eight different

crops with respect to drought tolerance and the physiological response to soil water deficits showed that amaranth plants have a stunning capacity to recover after a spell of severe drought stress (Myers, 1996). This observation indicated that amaranth might owe part of its reputed drought tolerance to the ability to shut down transpiration through wilting, then recovering easily when moisture is available. More studies on the effect of water deficit on amaranth growth have also been conducted by Liu and Stützel (2002a; 2004). Considering that most of the world's arable land is classified as semiarid and that drought is the major limiting factor in crop production, the prospects for future cultivation of drought-resistant amaranth are very encouraging. However, semiarid areas are also prone to salinity problems, yet there is little information on the response of amaranth to the combined effects of water and salinity stress.

This study was conducted to evaluate the effects of salinity stress both alone and in combination with water stress on water relations, gas exchange and growth of amaranth and to test the hypothesis that the combined effects of salinity and water stress may be less detrimental to amaranth plant growth than the sum of the separate effects of salinity and water stress.

5.3 MATERIALS AND METHODS

5.3.1 Plant material and growth conditions

Seeds of two amaranth genotypes (*A. tricolor* and *A. cruentus*) were sown in germination trays in a greenhouse at the Experimental Farm, University of Pretoria in September 2003. Temperatures ranged from 25^{o} to 35^{o}C (day) and 16 to19^{o}C (night). After about three weeks, seedlings selected for uniform size were transplanted into 5-liter capacity plastic pots containing a sand and vermiculite mixture (3:1, v/v). The pots had bottom drain holes to allow for draining of excess solution. Three seedlings were planted per pot. Seedlings were irrigated daily with nutrient solution for 10 days before commencement of the treatments.

Two different osmotic compounds: NaCl and polyethylene glycol (PEG) at an iso-osmotic potential were used. The osmotic potential of the solutions was verified with a Wescor-5500 vapor pressure osmometer (Wescor, Logan, UT, USA). Each genotype was divided into four groups for the four treatments:

(a) Nutrient solution (control)
(b) 100 mM NaCl (salt stress)
(c) PEG (M_W 6000) iso-osmotic to 100 mM NaCl (water stress)
(d) 50 mM NaCl + PEG iso-osmotic to 50 mM NaCl (both salt and water stress).

The osmotic potential of the various stress solutions was equivalent to –0.4 MPa.
Plants were submitted to the various stresses in two cycles. The first treatment cycle commenced 10 days after transplanting (approximately 30 days after emergence) and lasted for 8 days. This was followed by an 8 day recovery period before the second stress cycle was initiated. During the recovery period plants were irrigated with nutrient solution without stress compounds. The second stress cycle lasted for 16 days before the experiment was terminated.

Leaf water relations and gas exchange measurements were taken midway during the first stress cycle (day 4) and recovery period (day 12), and at the end of each period (days 8 and 16). During the second stress cycle, measurements were taken once in a week (days 24 and 32). Plant growth measurements expressed as dry mass of leaves, stems and roots were taken at the end of the experiment after oven drying the samples at 75^{o}C to constant weight. Leaf area was determined with a LI-3100 leaf area meter (LI-COR. Inc., Lincoln, NE, USA).

5.3.2 Water relations

Xylem water potential was measured at midday with a pressure chamber (Model 3000, Soil Moisture Equipment Corp., Santa Barbara, CA 93105, USA) to show leaf water potential (Turner, 1988). The youngest expanded leaf was cut, immediately put into

polyvinyl bag and then placed in the pressure chamber. Air humidity in the pressure chamber was maintained at saturation, to prevent transpirational water loss. The speed of pressure application was fast at the beginning of the measurement and then slowed to 0.01 MPa s^{-1}, when the pressure was close to the level of leaf water potential. When water exuded from the xylem and saturated the cut end of the petiole, pressure application was stopped. The balance pressure was then assumed to be the leaf water potential (Ψ_w). Leaf osmotic potential (Ψ_π) measurements were made in the remainder of the leaf that had been used for leaf water potential. Leaf samples which were initially frozen and thawed were centrifuged for 5 min at 2000 x g to extract cell sap, and the osmotic potential (Ψ_π) of the cell sap was measured with a Wescor-5500 vapor pressure osmometer (Wescor, Logan, UT, USA). The osmometer was calibrated after every pair of readings using commercial standards. Readings were converted to pressure units by using the van't Hoff equation ($\pi = -cRT$), where c is the osmolality (mosmol kg^{-1}), R the gas constant and T the temperature (K) (Nobel, 1991). Turgor pressure was estimated as the difference between water potential and osmotic potential: $\Psi_p = \Psi_w - \Psi_\pi$.

For the determination of relative water content (RWC-the water content of leaf tissue expressed as a percentage of the water content of the fully turgid tissue), fully expanded leaves of two plants per replicate were used. Three leaf discs (10 mm in diameter) were punched from the interveinal area of each plant using a cork borer and the fresh mass (FM) of pooled discs per replicate was determined immediately. Weighed leaf discs were then placed in distilled water for 4 hours at 20°C under dim illumination to avoid respiratory losses. Four hours of floating in water was found to be sufficient for complete hydration of leaf discs (Jensen *et al.,* 1996; Liu and Stützel, 2002a; Ghoulam *et al*., 2002). The leaf discs were then carefully blotted to remove surface water and turgid mass (TM) was taken to calculate water uptake. Dry mass (DM) of the leaf discs was determined by drying the tissues at 75°C to constant mass. Fresh mass, turgid mass and dry mass data of leaf discs were recorded for the determination of RWC. Relative water content (%) was determined as:

$$RWC = [(FM-DM)/(TM-DM)] \times 100$$

5.3.3 Gas exchange

Photosynthetic rate (P_n), stomatal conductance (g_s) and transpiration (E) were measured instantaneously with a LI-COR, 6400 portable photosynthetic system (LI-COR, Lincoln, NE). Net photosynthesis was measured as described in Chapter 3.

5.3.4 Water loss and water use efficiency

Transpirational water loss (E) and photosynthetic rates (P_n) were taken from gas exchange measurements on the last day of assay. These values were used to determine photosynthetic water use efficiency as (P_n/E).

5.3.5 Statistical methods

The results were analyzed as a completely randomized design with three replications using the General Linear Models (GLM) procedure of Statistical Analysis System (SAS Institute Inc. Cary, NC, USA 1996 Copyright). Differences among treatments were determined with Tukey's t-test at $P \leq 0.05$.

5.4 RESULTS

5.4.1 Plant growth

Water stress, salt stress or a combination of water and salt stress affected the dry mass of leaves, stems, and roots as well as leaf area and root/shoot ratio. Differences between genotypes for all characters were highly significant. Interactions between genotype and stress were also significant for stem dry mass, root dry mass and leaf area.

Plants submitted to water stress were more necrotic compared to those under saline stress or combined water and salt stress (Figure 5.1). All the stress treatments reduced leaf mass compared to the control (Table 5.1). However, plants under water stress had the lowest biomass compared to those under the other two stress treatments. *A. cruentus* had a

higher leaf mass than *A. tricolor* and the reduction due to the different stresses was lower than in *A. tricolor*. For instance, the reduction in leaf dry mass of plants submitted to salt stress was 33% in *A. tricolor* compared to 23% in *A. cruentus*.

Stem mass was reduced by the stress treatments in both genotypes. Stem mass of *A. tricolor* was significantly lower than that of *A. cruentus* and the different stress treatments reduced it more in *A. tricolor* than in *A. cruentus*. In *A. tricolor* no differences were noted among the stress treatments, while in *A. cruentus* the water stress reduced stem dry mass by 45%, salinity stress reduced stem dry mass by 12%, and a combination of water and salt stress reduced it by 29% (Table 5.1).

The reduction in root dry mass was the least in plants exposed to water stress. A significant reduction in root dry mass occurred in plants submitted to salinity stress and was greater in *A. cruentus* (61%) compared to that in *A. tricolor* (53%). Although root/shoot ratios were not statistically different a clear trend could be observed. The root/shoot ratio of water stressed plants was increased by 25% in both amaranth genotypes. In plants submitted to salinity stress root/shoot ratio was reduced by 25% in *A. tricolor* and 50% in *A. cruentus*. A combined salt and water stress treatment had no effect on root/shoot ratio in *A. tricolor* but reduced that in *A. cruentus* by 25% (Table 5.1).

Leaf area was reduced by the stress treatments with respect to control plants and at any particular treatment the reduction was greater in *A. tricolor* compared to that in *A. cruentus*. In plants exposed to water stress, leaf area was reduced by 64% in *A. tricolor* and 57% in *A. cruentus*. The reduction in leaf area was 37% in *A. tricolor* and 28% in *A. cruentus* in plants submitted to salinity stress, and 44% in *A. tricolor* and 34% in *A. cruentus* in plants under combined water and salt stress (Table 5.1).

Table 5.1 Effect of water stress, salinity stress and salinity + water stress on leaf, stem and root dry mass (g/plant) and leaf area (cm^2/plant) of two amaranth genotypes

Experiment/ Genotype	Leaves dry mass (g/plant	Stem dry mass (g/plant)	Root dry mass (g/plant)	Root/shoot ratio	Leaf area (cm^2/plant)
A. tricolor					
Control	4.8b	2.7e	3.4c	0.4a	1434a
Water Stress	2.6f	1.8f	2.2e	0.5a	516h
%	54.2	66.7	64.7	125	36
Salinity Stress	3.2cd	1.5f	1.6f	0.3a	902d
%	66.7	55.5	47.0	75	63
Water + salt stress	2.9bdf	1.8f	1.9ef	0.4a	798f
%	60.4	66.7	55.9	100	56
A. cruentus					
Control	6.2a	7.6a	5.9a	0.4a	1281b
Water Stress	3.6c	4.2d	3.9b	0.5a	549g
%	58.1	55.3	66.1	125	43
Salinity stress	4.8b	6.7b	2.3e	0.2a	922c
%	77.4	88.1	38.9	50	72
Water + salt stress	4.2b	5.4c	2.9d	0.3a	851e
%	67.7	71.0	49.1	75	66
SEM	**0.12**	**0.11**	**0.10**	**0.07**	**3.32**

SEM: Standard error of the mean
Percentages (%) are values of treated plants in respect to controls.
Mean separation by Tukey's t- test. Means within each column followed by the same letter are not significantly different at P = 0.05.

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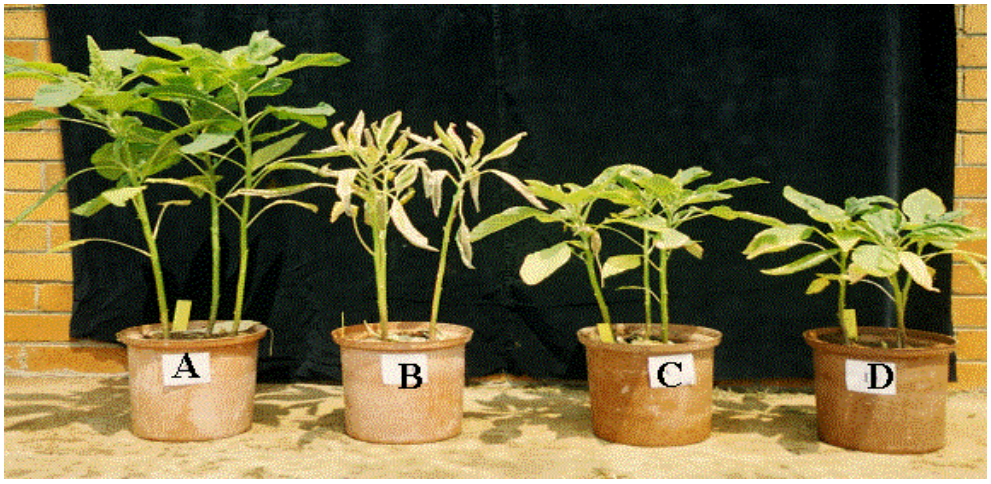

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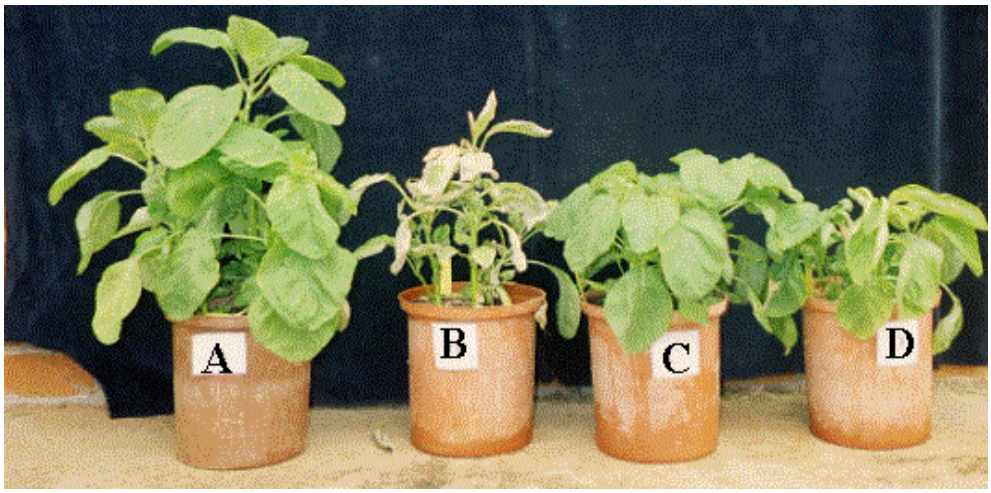

Figure 5.1 Growth of *A. cruentus* (1) and *A. tricolor* (2) as affected by iso-osmotic water and salt stresses: (A) control, (B) water stress, (C) salt stress, (D) water + salt stress.

5.4.2 Water relations

Salinity, water stress and a combination of salt and water stress treatments significantly affected leaf water potential, osmotic potential and turgor potential of both amaranth genotypes (Figure 5.2). Imposition of different stress treatments induced a progressive decrease in Ψ_w. At the end of the first stress cycle of 8 days, the Ψ_w decreased by –0.5 to ─ 0.8 MPa in *A. tricolor* and by –0.4 to -0.7 MPa in *A. cruentus* depending on the type of stress. At this stage plants submitted to salt stress had higher Ψ_w values. During the recovery period (day 8-16) water stressed plants achieved values similar to those registered in the control plants. Salt-treated plants and those submitted to combined water and salt stress did not fully recover and Ψ_w values were significantly lower than those of control plants (Figure 5.2). Leaf water potential decreased sharply during the second stress cycle and on the last day of the experiment salt treated plants had the highest Ψ_w values (–1.5 MPa in both genotypes) while plants submitted to water stress had the lowest values (-1.9 MPa in *A. cruentus* and –2.2 MPa in *A. tricolor*).

The behavior of leaf osmotic potential (Ψ_π) was similar to that of Ψ_w, since it was also reduced by stress and differences between genotypes were significant. The interaction between genotype and stress was significant at the end of every stress period (day 8 and day 32). At the end of the first stress cycle osmotic potential was reduced from –0.7 to -1.7 MPa in *A. tricolor* and from –0.6 to –2.0 MPa in *A. cruentus* (Figure 5.2). In both genotypes, plants submitted to salt stress had the lowest Ψ_π values, and those submitted to water stress had the highest. Osmotic potential increased during the recovery period and water stressed plants achieved values similar to those registered in the control plants (Figure 5.2). A significant decrease in osmotic potential followed during the second stress cycle with reductions ranging from –0.7 to –3.1 MPa in *A. tricolor* and –0.6 to –3.5 MPa in *A. cruentus*.

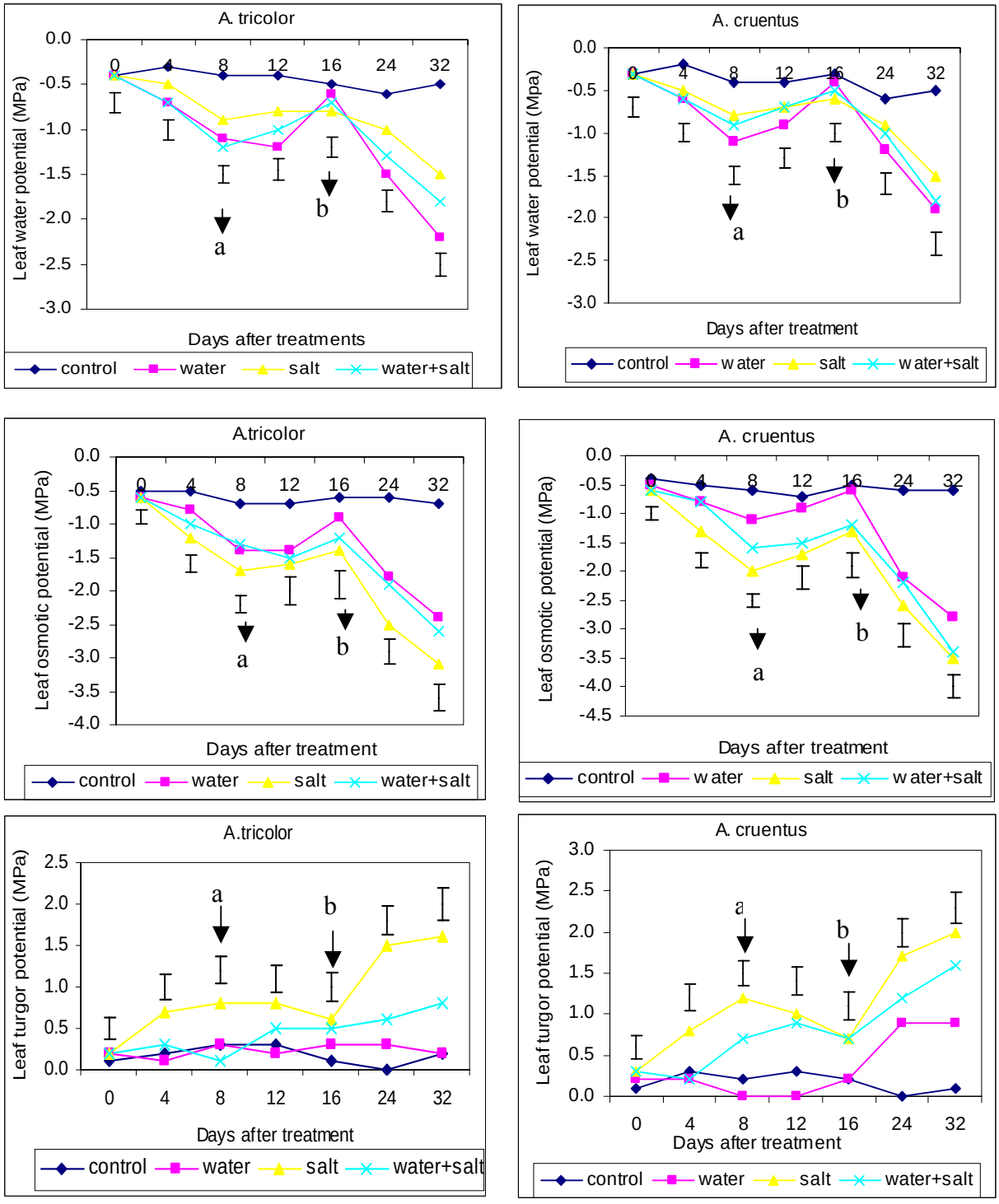

Figure 5.2 Leaf water potential, osmotic potential and turgor potential of *A. tricolor* and *A. cruentus* exposed to iso-osmotic stress treatments. Arrows indicate the beginning of the recovery period (a) and the beginning of the second stress period (b). Vertical bars indicate least significant differences at P = 0.05.

The different stress treatments decreased leaf osmotic potential to a greater extent than water potential. This difference was reflected in turgor potential increase as stress proceeded. These reductions in water and osmotic potentials are related to the maintenance of leaf turgor under stress conditions. The leaf turgor potential (Ψ_p) was maintained almost constant in control plants throughout the experimental period. In *A. tricolor* there was no significant difference in Ψ_p between control and plants submitted to water stress or water plus salt stress. However, during the second stress period Ψ_p of plants under water plus salt stress was higher than that of control. Turgor potential of plants submitted to salt stress increased gradually during the first stress cycle to 0.8 MPa, decreased slightly during the recovery period and increased sharply during the second stress period. A similar trend was observed in *A. cruentus*. Turgor potential of water stressed plants did not differ from that of control plants during the first stress cycle but was higher during the second. In salt treated plants turgor potential increased to 1.2 MPa and to 0.7 MPa in plants submitted to combined water and salt stress at the end of the first stress cycle. This was followed by a decrease during the recovery and a sharp increase during the second stress period. The highest Ψ_p on the last day of treatment was obtained in salt treated plants (1.6 MPa in *A. tricolor* and 2.0 MPa in *A. cruentus)* followed by that in plants submitted to a combined water and salt stress (Figure 5.2).

Relative water content (RWC) of amaranth was also affected by salinity, water stress or a combination of salt and water stress. In the control plants, RWC was maintained at 83-92% in *A. tricolor* and 86-94% in *A. cruentus* (Figure 5.3). The different stress treatments caused a significant decrease in RWC. At the end of the first stress cycle, for instance, the RWC in *A. tricolor* was reduced from 86% in control plants to 58%, 76% and 65% in plants under water stress, salt stress or combined water and salt stress, respectively. In *A. cruentus*, the reductions were from 87% in control plants to 60%, 79% and 71%. The relative water content of stressed plants in both genotypes recovered to the levels of the control plants when the stress was removed and decreased again during the second stress cycle.

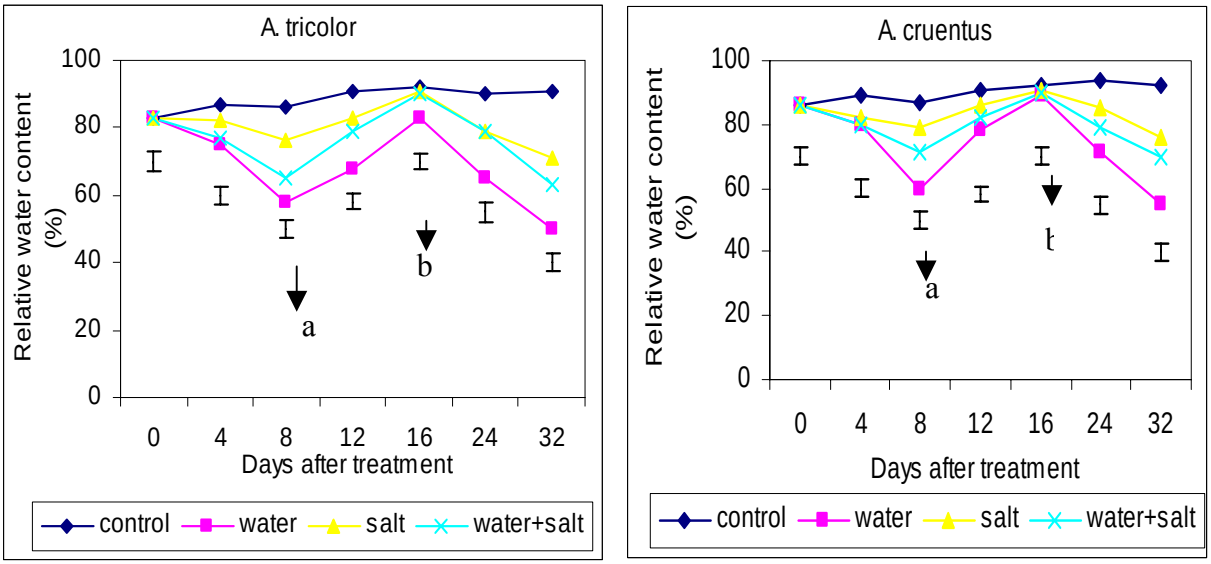

Figure 5.3 Effect of water stress, salt stress and combined water and salt stress on relative water content of *A. tricolor* and *A. cruentus*. Arrows indicate the beginning of the recovery period (a) and the beginning of the second stress period (b). Vertical bars indicate least significant differences at P = 0.05.

5.4.3 Water loss and water use efficiency

Salinity, water stress or a combination of salt and water stress significantly affected water loss and water use efficiency. The different stress treatments reduced transpiration rate (E) in both genotypes compared to the control (Table 5.2). However, the reduction in plants submitted to salt stress was greater (71% in *A. tricolor* and 75% in *A. cruentus*) than in the other two stress treatments. The water use efficiency of stressed plants was higher than that of control plants. In both amaranth genotypes the increase in WUE was greater in plants submitted to salinity or a combination of salt and water stress (58% and 50% in *A. tricolor*, and 64% and 46% in *A. cruentus* respectively) than in those submitted to water stress (23% in *A. tricolor* and 25% in *A. cruentus*).

Table 5.2 Effect of water stress, salinity stress and salinity + water stress on transpiration and water use efficiency of *A. tricolor* and *A. cruentus* determined at the end of experimental period (32 days after start of the treatments)

Genotype/stress	Transpiration rate (E) mmol m^{-2} s^{-1}	Water use efficiency (WUE) (P_n/E)
A. tricolor		
Control	6.5a	2.6d
Water Stress	2.4c	3.2c
% of control	37	123
Salinity Stress	1.9d	4.1ab
% of control	29	158
Water + salt stress	2.2cd	3.9b
% of control	34	150
A. cruentus		
Control	5.2b	2.8d
Water Stress	1.8d	3.5c
% of control	35	125
Salinity stress	1.3e	4.6a
% of control	25	164
Water + salt stress	1.5de	4.1ab
% of control	29	146
SEM	**0.11**	**0.12**

SEM: Standard error of the mean

Percentages (%) are values of treated plants in respect to controls. Mean separation by Turkey's t-test. Means within each column followed by the same letter are not significantly different at P = 0.05.

5.4.4 Gas exchange

The effect of salinity, water stress or a combination of salt and water stress on stomatal conductance (g_s) and photosynthetic rate (P_n) is shown in Figure 5.4. A reduction in g_s values occurred in stressed plants in both genotypes during stress periods. The g_s in *A. tricolor* was higher and was reduced to a lesser extent than that in *A. cruentus*. At the end of the second stress cycle, for instance, stomatal conductance in *A. tricolor* was reduced by 18, 35 and 27% in plants submitted to water stress, salt stress and a combination of salt and water stress, respectively, while that in *A. cruentus* was reduced by 22, 51 and 40%. During recovery period, the g_s of plants under all stress treatments increased, achieving practically the control plant values (Figure 5.4).

Photosynthetic rate was also reduced by the different stress treatments in both amaranth genotypes. However, at any stress, P_n was significantly higher in *A. tricolor* than in *A. cruentus*. On day four no significant difference in P_n were recorded among the different stress treatments. However, on the last day of the first and the second stress cycles plants under salt stress and those under combined salt and water stress had higher P_n values than those under water stress. Compared to the control treatment, P_n on day 8 in *A. tricolor* was reduced by 25 in water stressed plants, 8% in salt stressed and 8% in plants exposed to both water and salt stress. In *A. cruentus,* P_n was reduced by 33 in water stressed plants, 11% in salt stressed and 22% in plants exposed to both water and salt stress (Figure 5.4). After re-watering, the P_n of water stressed plants increased reaching values similar to that of control plants (Figure 5.4). However, P_n values did not show any recovery in the salt stressed plants and those under combined water and salt stress, resulting in a reduction of net photosynthesis and the photosynthetic rate declined further during the second stress cycle (Figure 5.4).

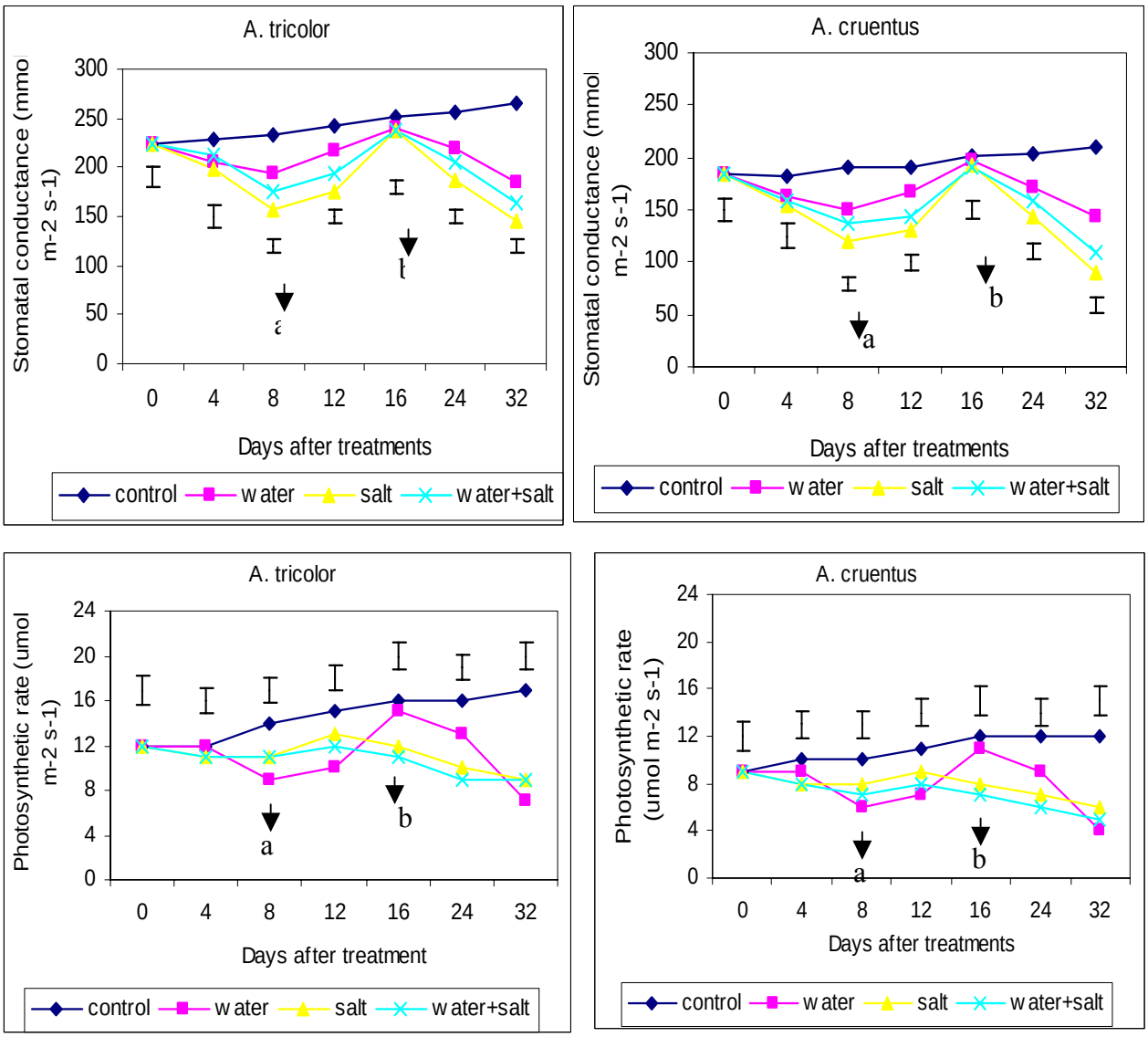

Figure 5.4 Effect of water stress, salt stress and combined water and salt stress on stomatal conductance and photosynthetic rate of *A. tricolor* and *A. cruentus*. Arrows indicate the beginning of the recovery period (a) and the beginning of the second stress period (b). Vertical bars indicate least significant differences at P = 0.05.

5.5 DISCUSSION

5.5.1 Effect of salinity, water stress and salinity and water stress on plant growth and development

Amaranth plants exposed to the three types of stress had reduced growth. The lower leaf biomass was due to senescence and death of leaves as well as formation of smaller leaves, typical of many species under osmotic stress (Chaves and Pereira, 1992). The reduction in leaf canopy surface due to water and salt stress has been considered an avoidance mechanism that permits minimizing water loss through transpiration (Blum, 1997). However, plants under salt stress and those in combined water and salt stress had higher biomass than those under water stress. Shalhevet and Hsiao (1986) found a similar response to salinity and drought on cotton and pepper. They observed that at the same water potential plants under saline conditions produced more biomass than plants exposed to drought. Pérez-Alfocea *et al*. (1993b) also observed that for some tomato genotypes a PEG-induced stress reduced growth more than a comparable NaCl-induced stress.

The root/shoot ratio was higher in water stressed plants compared to control and salinized plants. Similar results were obtained in pepper (De Pascale *et al*., 2003a). These results suggest that stress tolerance may involve differences in the partitioning of photosynthates in salt and water stressed plants. The water stressed amaranth had larger root systems that most likely optimized water uptake by exploring a larger volume of soil. In contrast, the lower root/shoot ratio observed in salinized plants may have been functionally associated with the need of salt stressed plants to restrict the uptake of toxic ions to the shoot while still maintaining high turgor and a positive growth rate (Dalton *et al.*, 1997; Maggio *et al*., 2001). This may be accomplished by simultaneously reducing root development and activating specific metabolic pathways (i.e., osmolyte biosynthesis), both of which occur in saline environments (Gunes *et al.*, 1996; Hayashi *et al*., 1997; Shen *et al*., 1997: Maggio *et al.*, 2001).

5.5.2 Effect of salinity, water stress and salinity and water stress on water relations

Water and saline stress promoted significant differences in stressed plants versus control plants in terms of water relations. Leaf water potential, osmotic potential and relative water content decreased with water stress, salt stress and water plus salt stress (Figure 5.2; 5.3). The decrease in leaf osmotic potential always exceeded that of leaf water potential, resulting in positive turgor potential. The reduction was higher in salt treated plants than in those exposed to water stress. As turgor potential was maintained or enhanced by salinity or water stress, osmotic adjustment was maintained. There is substantial evidence that plants adjust to high salt concentrations or water stress by lowering tissue osmotic potential by accumulation of inorganic ions and /or organic substances to permit the maintenance of turgor (Morgan, 1984; 1992; Pérez-Alfocea *et al*., 1993b; Cachorro *et al.,* 1995; Premachandra *et al*., 1995). The former occurs in plants under salt stress, whereas under drought stress the latter is more significant (Erdei *et al*., 1990; Alarcón *et al*., 1993; Torrecillas *et al*., 1995). Wyn Jones (1981) and Raven (1985) suggested that the osmotic adjustment by salt accumulation is less energy and carbon demanding than the adjustment by organic solutes. This may explain why the reduction in osmotic potential was more in salt treated plants than in water stressed plants. Similar observations were reported in tomato cultivar 'Fireball', that fully adjusted osmotically to salt stress but exhibited no adjustment to water stress (Alian *et al*., 2000). Leigh and Storey (1993), on the other hand, proposed that this capacity to accumulate salts is a beneficial trait only when the absorption of salts is accompanied by a plant's ability to regulate internal Na^+ and Cl^- concentrations.

Increased salt concentration in the root medium or water stress led to an osmotic adjustment (lowering of leaf Ψ_π) that is generally accepted as an adaptation to salinity or water stress (Guerrier, 1996; Ghoulam *et al*., 2002; Iannucci *et al*., 2002). The decrease of leaf Ψ_π is thought to compensate for the salt or water stress-induced lowering of Ψ_w and helps to maintain turgor pressure and cell functions under adverse water conditions. *A. cruentus* responded to salinity and water stress by decreasing Ψ_π more than *A. tricolor*

did and, in this sense, it may be more adaptable to salinity and water stress conditions than *A. tricolor*.

Several studies have suggested that osmotic adjustment following water deficit stress improves cell turgor and subsequently aids in growth recovery (Morgan, 1984; Turner, 1986). Ludlow and Muchow (1988) have cautioned, however, that although plants exhibiting osmotic adjustment may have an advantage with respect to enhanced soil water extraction, continued water extraction by these plants could exhaust the supply of soil water and contribute to premature soil dehydration. Due to osmotic adjustment both amaranth genotypes showed an increase of leaf turgor with stress, nevertheless, leaf expansion was reduced. These results confirm the idea of Munns (1988; 1993) that although turgor is the potential energy which powers cell extension, it is not the parameter that controls the growth process. He emphasized that the solutes that account for osmotic adjustment must be diverted from growth processes such as protein and cell wall synthesis, and therefore, osmotic adjustment should not necessarily be expected to promote growth. In contrast, Richardson and McCree (1985) demonstrated in sorghum that the metabolic cost of storing and using photosynthate for osmotic adjustment was less than the cost of converting it into new biomass.

5.5.3 Effect of salinity, water stress and salinity and water stress on water loss and water use efficiency

Water loss of plants submitted to salinity, water stress or a combination of salt and water stress was lower than that of control plants in *A. tricolor* and *A. cruentus* (Table 5.2). The reduction in water loss through transpiration was due mainly to a reduction of stomatal conductance. Plants submitted to salinity stress had lower stomatal conductance and hence, lower rate of transpiration than plants under water stress. Similar decreases in transpiration rate with increasing salinity or water stress have been reported in tomato (Xu *et al.*, 1994 and Romero-Aranda *et al.*, 2001); *Brassica* species (Ashraf, 2001); safflower (Bassil and Kaffka, 2002) and amaranth (Liu and Stützel, 2002b). They all attributed the reduction in transpiration to lower stomatal conductance.

Photosynthetic water use efficiency of stressed plants was significantly higher than that of control plants (Table 5.2). Plants under salt and water deficit conditions usually minimize transpirational water loss and maximize photosynthesis and show a higher water use efficiency as a consequence (Xu *et al*., 1994). This is one kind of adaptation mechanism that allows plants to survive water deficit conditions. Plants exposed to salt stress had higher WUE than those under water stress since transpiration rate in these plants was the least and P_n much higher than in water stressed plants.

One way in which salts may enhance plant performance in drying soil is by increasing their water use efficiency. Both halophytes (Guy and Reid, 1986; Ayala and O'Leary, 1995) and nonhalophytes (Brugnoli and Lauteri, 1991) were found to have higher photosynthetic WUE when grown in the presence of salt, and this led to higher biomass production under water-limiting conditions (Richards, 1992). *Atriplex canescens* seedlings had 20% greater WUE and greater organic matter production at an optimal compared to the suboptimal salt level (Glen and Brown, 1998). Tomato plants submitted to either salt or water stress treatments were found to have higher WUE compared to control plants (Xu *et al*., 1994). Similarly, Ayala and O'Leary (1995) observed that instantaneous and long-term WUE of *Salicornia bigelovii* Torr., measured by gas exchange and ^{13}C ratios, respectively, both increased with salinity.

The enhanced WUE of nonhalophytes under salt stress is generally regarded as a sodium avoidance mechanism (Greenway and Munns, 1980; Brugnoli and Bjorkman, 1992). Sodium enters plants in proportion to the transpiration rate (Pitman, 1988), and by lowering stomatal conductance plants can reduce the rate of Na entry into leaves. Photosynthesis and growth are also lowered at lower stomatal conductance but not in direct proportion, so WUE increases, though net primary production decreases (Brugnoli and Lauteri, 1991; Brugnoli and Bjorkman, 1992). Ashraf (2001) recorded increasing water use efficiency of the salt tolerant *Brassica* species with increasing external salt concentration and attributed this increase to relatively higher assimilation rates and lower stomatal conductance in these species.

5.5.4 Effect of salinity, water stress and salinity and water stress on gas exchange

Stomatal conductances (g_s) as well as photosynthetic rates (P_n) were reduced in plants submitted to salinity, water stress or a combination of salt and water stress compared to control plants (Figure 5.4). The reduction in P_n was attributed mainly to decreases in g_s. Similar decreases in photosynthetic capacity in NaCl-treated plants were observed in cotton Meloni *et al*. (2003) and *Phaseolus* species (Bayuelo-Jiménez *et al*., 2003). In both studies, the depressions in P_n were attributed to reductions in stomatal conductance. Under water stress, a continual decline in stomatal conductance was noted in tomato (Xu *et al*., 1994) and in *A. cruentus* and *Zea mays* (Lal and Edwards, 1996) suggesting that it was a major limitation to photosynthesis.

Among the three types of stresses, P_n was the highest in salt treated plants especially during the first stress cycle (Figure 5.4). This may be related to the high leaf water potential and turgor maintenance in salt treated plants compared to water stressed plants. According to Xu *et al*. (1994), photosynthetic performance under water or salt stress is related to leaf water potential and also to turgor maintenance, and decreases in leaf water potential accounted, in part, for the P_n depressions. There was a marked difference in the ability of the amaranth plants to recover from these stresses. Water stressed plants showed a remarkable recovery of P_n and g_s to control levels after re-watering. This suggests that during the course of the stress treatment there was no irreversible damage to the photosynthetic capacity. According to Bjorkman and Demmig (1987) the reduction in photosynthetic rates under water stress can be mainly attributed to stomatal conductance reduction, and not to injuries to the photosynthetic apparatus. In salt treated amaranth plants, g_s recovered when the salt was removed from the medium. However, failure of P_n recovery, suggested that there was a toxic effect of salt concentration on the photosynthetic apparatus, as was also reported by De Herralde *et al*. (1998).

The performance of amaranth plants was enhanced by salt in water stressed soil, contrary to the initial expectation that it would be an additive stress factor. Richards (1992) also reported a beneficial effect of salinity on plants grown to the wilting point in drying soils.

His experiments included crop plants such as wheat, barley, and sunflower as well as halophytes and nonhalophytes. All the plants reached a higher final mass in saline than nonsaline soil, although they grew more slowly. Wheat and barley were able to produce viable seed in the saline but not in the nonsaline treatments.

One way in which soil salinity can enhance plant performance is by lowering the leaf area and growth rate of plants, thereby decreasing the rate at which soil water is depleted and thus enhancing the longevity of plants (Richards, 1992; Shalhevet, 1993). Eshel and Waisel (1984) found that similar-sized *Salsola kali* plants took twice as long to reach the wilting point on saline compared to nonsaline medium. *Atriplex canescens* seedlings grew much more slowly at 520 mol/m^3 NaCl than at lower salinities but lasted much longer before wilting (Glen and Brown, 1998). Hence, the increased longevity could aid the survival of plants in saline soils between scattered rain.

5.6 CONCLUSION

Salinity, water stress and a combination of salt and water stress decreased amaranth plant growth, transpiration and photosynthetic rates, leaf water and osmotic potentials. Water use efficiency was increased since transpiration was more depressed than photosynthesis. Decreases in stomatal conductance and leaf water potential accounted for the Pn depressions. Plants submitted to salinity stress alone or in combination with water stress had a higher biomass, and the rate of leaf senescence was less, compared to water stressed plants.

The results from this study indicate that amaranth developed tolerance and avoidance mechanisms in response to water and saline stress. The avoidance mechanisms consisted of the reduction of water loss via transpiration due mainly to the senescence of leaves and reduction of leaf stomatal conductance. The resistance mechanisms included the development of osmotic adjustment which resulted in leaf turgor maintenance. The agronomic implication of these responses is that at moderate salinity levels, amaranth plants are able to acclimatize and survive.

The ability of salinized amaranth plants to continue leaf expansion and carbon gain under water stress can be attributed primarily to a greater ability of osmotic adjustment, and a lower water loss rate per plant, which in turn was due to decreased leaf area and reduced water loss per unit leaf area. These physiological adjustments could mitigate the effect of poor quality irrigation water in the field to some extent.

CHAPTER 6
AMELIORATIVE EFFECTS OF CALCIUM ON MINERAL UPTAKE AND GROWTH OF SALT-STRESSED AMARANTH

6.1 ABSTRACT

The detrimental effects of salinity stress in plants may be ameliorated by calcium (Ca). However, little is known concerning the efficacy of different calcium sources. Two amaranth genotypes (*Amaranthus tricolor* and *A. cruentus*) were grown in a greenhouse to investigate the effectiveness of supplementary calcium (Ca) applied into the nutrient solution in ameliorating salinity stress effects. Treatments were (1) nutrient solution alone (C); (2) nutrient solution plus 100 mM NaCl (C+S); (3) nutrient solution + 100 mM NaCl + supplemental 10 mM Ca as $CaSO_4$ (C+S+$CaSO_4$) and (4) nutrient solution + 100 mM NaCl + supplemental 10 mM Ca as $CaCl_2$ (C+S+$CaCl_2$) supplied in the nutrient solution. The effect of supplementary Ca^{2+} on growth, gas exchange, membrane permeability and mineral uptake of NaCl-stressed amaranth plants was investigated. Dry matter production, relative water content, stomatal conductance and photosynthetic rate of salt-stressed plants were less than those of control. Supplementary Ca^{2+} ameliorated the negative effects of salinity on these parameters. Membrane leakage, as well as sodium (Na^+) and chlorine (Cl^-) concentrations in plant tissues increased in both genotypes in NaCl-treated plants but to a greater extent in *A. tricolor* than in *A. cruentus*. The concentrations of K^+, Ca^{2+} and N were reduced in shoots whereas they increased in roots of NaCl-treated plants. In general, supplemental Ca^{2+} partly ameliorated the negative salt stress effects in amaranth regardless of the source of calcium.

Keywords: Amaranth; Calcium; Gas exchange; Growth; Membrane permeability; Salinity

6.2 INTRODUCTION

Salinity is one of the world's most serious environmental problems in agriculture. Salinity stress affects many metabolic aspects of plants and induces anatomical and morphological changes resulting in reduced growth (Seeman and Critchley, 1985). This reduction in growth may result from salinity effects on dry matter allocation, ion relations, water status, biochemical reactions or a combination of many physiological factors (Volkmar *et al*., 1998; Asch *et al*., 2000; Hasegawa *et al*., 2000; Kashem *et al*., 2000a, b; Romero-Aranda *et al*., 2001). Some researchers have linked NaCl stress with macro-nutrient deficiencies, e.g., high NaCl concentration has been shown to induce calcium deficiency in tomato (Navarro, *et al*., 2000), wheat and barley (Ehret *et al*., 1990) and maize (Evlagon *et al*., 1990). Adverse salinity effects on plants can be reduced by adequate mineral nutrition (Cerdă and Martinez, 1988; Grattan and Grieve, 1992; 1999). In a more recent study, Makus (2003) investigated the effect of salinity and nitrogen level on agronomic performance of *A. tricolor* and found that supplemental N improved yield and leaf greenness in response to higher soil salinity.

Enhanced supply of Ca^{2+} as well as NO_3^- is known to restrict the uptake of Na^+ and Cl^- in plants and ameliorate growth under saline conditions (Bar *et al*., 1997; Bãnuls *et al*., 1991; El-Siddig and Lüdders, 1993). The interaction of Na^+ and Ca^{2+} on plant growth and ion relations is well established (Rengel, 1992). Many studies have indicated that the primary effect of salt stress is a disruption of membrane integrity caused by the displacement of Ca^{2+} from the cell surface by Na^+ (Cramer *et al*., 1985; Lynch and Lauchli, 1985; Lynch *et al*., 1987). Cramer *et al*. (1987) demonstrated evidence for the displacement of membrane-associated Ca^{2+} by Na^+ in root hairs of salinized cotton (*Gossypium hirsutum* L.) seedlings.

A widespread practice to reduce the salt content in the soil is leaching. However, excess irrigation in order to leach salts is already becoming a less viable option due to the cost and unavailability of water. One possible approach to reduce the effect of salinity on plant productivity is through the addition of calcium supplements to irrigation water. Calcium is well known to have regulatory roles in plant metabolism (Cramer *et al*.,

1986), and Na^+ ions may compete with calcium ions for membrane binding sites. It has, therefore, been hypothesized that high calcium levels can protect the cell membrane from the adverse effects of salinity (Busch, 1995). High calcium levels were found to protect the cells of the maritime halophyte *Aster tripolium* L. from adverse effects of salinity (Perera *et al*., 1995) and Cramer *et al*. (1988) observed that 10 mM supplemental calcium ameliorated the effects of salinity on root growth in cotton exposed to 75 mM NaCl. External supplied Ca^{2+} has been shown to ameliorate the adverse effects in plants presumably by facilitating higher K^+/Na^+ selectivity (Hasegawa *et al*., 2000). However, calcium supplements were unable to ameliorate NaCl damage in blueberry (*Vaccinium ashei* L.) (Wright *et al*., 1993) and sunflower (*Helianthus annuus* L) (Sohan *et al*., 1999).

An alternative strategy for coping with salinity could be to supplement calcium where the growth medium is saline. Although a wide range of investigations have been carried out on the effect of salinity in a number of crops, this problem and how to overcome it, has not been sufficiently investigated in amaranth. Reports on mineral nutrition schemes to combat salinity effects in amaranth are lacking and no information as to whether different forms of Ca^{2+} vary in their beneficial effects on salt-stressed plants is available. Hence, the objective of this study was to test the hypothesis that supplemental calcium can at least partially reverse the adverse effects of salinity on plant growth, gas exchange, membrane permeability and mineral uptake in amaranth.

6.3 MATERIALS AND METHODS

6.3.1 Plant material and culture

The experiment was carried out in a greenhouse at the University of Pretoria Experimental Farm in September 2002. The mean air temperature was 30/20 ±2°C (day/night) and relative air humidity of 60±10%. Seeds of *A. tricolor* and *A. cruentus* were sown in seed trays and one month after germination three seedlings were selected for uniformity and transplanted into 5-liter plastic pots containing sand/vermiculite mixture (3:1, v/v). The pots were irrigated with nutrient solution for seven days before

commencement of the treatments. The nutrient solution was similar to that used in Chapter 3.

The treatments consisted of plants receiving: (i) nutrient solution alone (Control); (ii) nutrient solution containing 100 mM NaCl (C+S); (iii) nutrient solution containing 100 mM NaCl plus 10 mM $CaSO_4$ (C+S+$CaSO_4$) and (iv) nutrient solution containing 100 mM NaCl plus 10 mM $CaCl_2$ (C+S+$CaCl_2$). The pots were irrigated in excess of their capacity every two days, and the EC of leachates determined to ensure efficient displacement of the old solution and to avoid accumulation of salts. Each treatment was replicated three times and each replicate included three plants.

6.3.2 Plant growth measurements

The experiment was terminated four weeks after treatments commenced. Plant height was recorded and leaf area determined with a LI-3100 leaf area meter (LI-COR. Inc., Lincoln, NE, USA). Dry mass of leaves, stems and roots was determined after oven drying the samples at 75^{o}C to a constant mass.

6.3.3 Gas exchange

Photosynthetic rate (P_n), stomatal conductance (g_s) and transpiration (E) were measured at the end of the experiment on the second and third youngest fully expanded leaves. Measurements were made with a LI-COR, 6400 portable photosynthetic system (LI-COR, Lincoln, NE). Net photosynthesis was measured at 34 MPa external CO_2 partial pressure (340 µmol CO_2 mol air $^{-1}$) and a VPD of 1.8 KPa. All measurements were conducted between 9:00h and 14:00h on bright days when the photosynthetically active radiation (PAR) intensity at the leaf surface was 1100-1200 µmol m^{-2} s^{-1}.

6.3.4 Relative water content

For the determination of relative water content the procedure used in Chapter 5 was followed.

6.3.5 Electrolyte leakage

Electrolyte leakage was used to assess membrane permeability. It was determined as described by Lutts *et al*. (1995). Two leaf samples per plant were taken and cut into 1 cm segments. Leaf samples were washed with distilled water to remove surface contamination then placed in individual stoppered vials containing 10ml of distilled water. The samples were incubated at room temperature (25^{o}C) on a shaker (100rpm) for 24 hours and the EC of the bathing solution (EC1) recorded. The same leaf samples were then placed in an autoclave at 120^{o}C for 20 minutes and the second reading (EC2) was taken after cooling the solution to room temperature. Electrolyte leakage was calculated as EC1/EC2 and expressed as a percentage.

6.3.6 Chemical analysis

At the end of the experiment leaves, stems and roots were sampled and analyzed for N, P, K^+, Ca^{2+}, Na^+ and Cl^-. After rinsing with distilled water the samples were dried at 75^{o}C for 48 h to constant weight. Ground samples were ashed at 550^{o}C in a porcelain crucible for 6 h. K^+, Ca^{2+} and Na^+ were determined after extraction in HCl, using an atomic absorption spectrophotometer. Cl^- was assessed with a chloride meter by silver ion titration. Phosphorus was analyzed by a vanadate-molybdate method using a spectrophotometer. Reduced N was determined by the Kjeldahl method.

6.3.7 Data statistical analysis

The experiment was arranged in a completely randomized design with each treatment replicated three times. Pots were placed in an equidistant pattern and their position was randomly changed daily to expose all plants at comparable growth conditions. Data were analyzed for significance using the general linear model (GLM) procedure in the SAS statistical program (Statistical Analysis Systems Institute, Inc., 1996) and means separated with Tukey's t- test at a significance level of 0.05.

6.4 RESULTS

6.4.1 Plant growth

The main effects (genotype and treatments) were significant for plant height, shoot dry mass and root dry mass. However, the interactions between these effects were not significant. Across all the treatments, plant height, shoot dry mass and root dry mass were higher in *A. cruentus* than in *A. tricolor* (Table 6.1). These parameters were reduced by 33, 49 and 64%, respectively in the 100 mM NaCl (C+S) treatment compared to unstressed control (C) plants (Table 6.1). Supplementary Ca applied either as $CaSO_4$ or $CaCl_2$ resulted in significant increases in dry matter production of plants exposed to NaCl. Root dry mass from these treatments was not different from that for the control (C) treatment (Table 6.1).

Table 6.1 Effect of calcium supplementation on amaranth plant growth

Main effects	Plant height (cm)	Shoot dry mass (g/plant)	Root dry mass (g/plant)
Genotype			
A. tricolor	30.0b	6.6b	2.9b
A. cruentus	56.5a	9.5a	3.5a
SEM	**0.7**	**0.3**	**0.1**
Treatments			
C	51.9a	10.0a	4.2a
C + S	34.7c	5.1c	1.5b
C+S+$CaSO_4$	45.0b	9.0ab	3.7a
C+S+$CaCl_2$	41.3b	7.9b	3.4a
SEM	**1.0**	**0.5**	**0.2**

SEM: Standard error of the mean

Mean separation by Tukey's t- test. Means within each column followed by the same letter are not significantly different at P = 0.05.

C=Plants receiving nutrient solution; **C + S**=Plants receiving nutrient solution plus 100 mM NaCl; **C+S+$CaSO_4$** =Plants receiving nutrient solution plus 100 mM NaCl plus 10 mM $CaSO_4$; **C+S+$CaCl_2$**=Plants receiving nutrient solution plus 100 mM NaCl plus 10 mM $CaCl_2$.

.The interaction between genotype and treatment was significant for leaf area. In general, *A. tricolor* had a higher leaf area than *A. cruentus*. Treatment with 100 mM NaCl reduced the leaf area in both genotypes. However, the reduction was greater in *A. tricolor* (54%) compared to that in *A. cruentus* (49%) (Table 6.2). Supplementary calcium increased leaf area. In general, $CaSO_4$ ameliorated the Na-induced salinity effects more effectively than did $CaCl_2$. In *A. tricolor*, leaf area was increased to 85% and 83% of control when supplemented with either 10 mM $CaSO_4$ or $CaCl_2$ respectively, while that of *A. cruentus* was increased to 89% and 84%.

Table 6.2 Effect of calcium supplementation on leaf area of two amaranth genotypes

Genotype/Treatment	Leaf Area (cm^2/plant)
A. tricolor	
C	1550.6a
C+S	717.8g
C+S+$CaSO_4$	1317.6c
C+S+$CaCl_2$	1280.9d
A. cruentus	
C	1426.8b
C+S	727.4g
C+S+$CaSO_4$	1265.5e
C+S+$CaCl_2$	1198.7f
SEM	**2.17**

SEM: Standard error of the mean

Mean separation by Tukey's t- test. Means within each column followed by the same letter are not significantly different at P = 0.05.

C=Plants receiving nutrient solution; **C + S**=Plants receiving nutrient solution plus 100 mM NaCl; **C+S+$CaSO_4$** =Plants receiving nutrient solution plus 100 mM NaCl plus 10 mM $CaSO_4$; **C+S+$CaCl_2$**=Plants receiving nutrient solution plus 100 mM NaCl plus 10 mM $CaCl_2$.

6.4.2 Gas exchange

The genotype x treatment interaction was significant for stomatal conductance. Although *A. tricolor* had higher stomatal conductance than *A. cruentus* at all treatments, salinity stress reduced this parameter in both genotypes compared to control plants. However, the effect was more pronounced in *A. cruentus* with a 50% reduction compared to a 42% reduction in *A. tricolor* (Table 6.3). The response to supplemental Ca was similar in both genotypes. Calcium sulfate ameliorated NaCl-induced salinity effects on g_s more effectively than did $CaCl_2$. Treatment with $CaSO_4$ or $CaCl_2$ resulted in increased stomatal conductance to 91% and 81% of control, respectively in *A. tricolor* and 89% and 78% in *A. cruentus*.

Table 6.3 Effect of calcium supplementation on stomatal conductance of two amaranth genotypes

Genotype/Treatment	Stomatal conductance (mmol m^{-2} s^{-1})
A. tricolor	
C	325.6a
C+S	188.8e
C + S + $CaSO_4$	297.4b
C + S + $CaCl_2$	262.7c
A. cruentus	
C	217.4d
C + S	109.8g
C + S + $CaSO_4$	193.5e
C + S + $CaCl_2$	169.6f
SEM	**1.7**

SEM: Standard error of the mean

Mean separation by Tukey's t- test. Means within each column followed by the same letter are not significantly different at P = 0.05.

C=Plants receiving nutrient solution; **C + S**=Plants receiving nutrient solution plus 100 mM NaCl; **C+S+$CaSO_4$** =Plants receiving nutrient solution plus 100 mM NaCl plus 10 mM $CaSO_4$; **C+S+$CaCl_2$**=Plants receiving nutrient solution plus 100 mM NaCl plus 10 mM $CaCl_2$.

Figure 6.1 shows the effects of treatment with 100 mM NaCl and supplemental $CaSO_4$ on the growth of *A. tricolor* and *A. cruentus*.

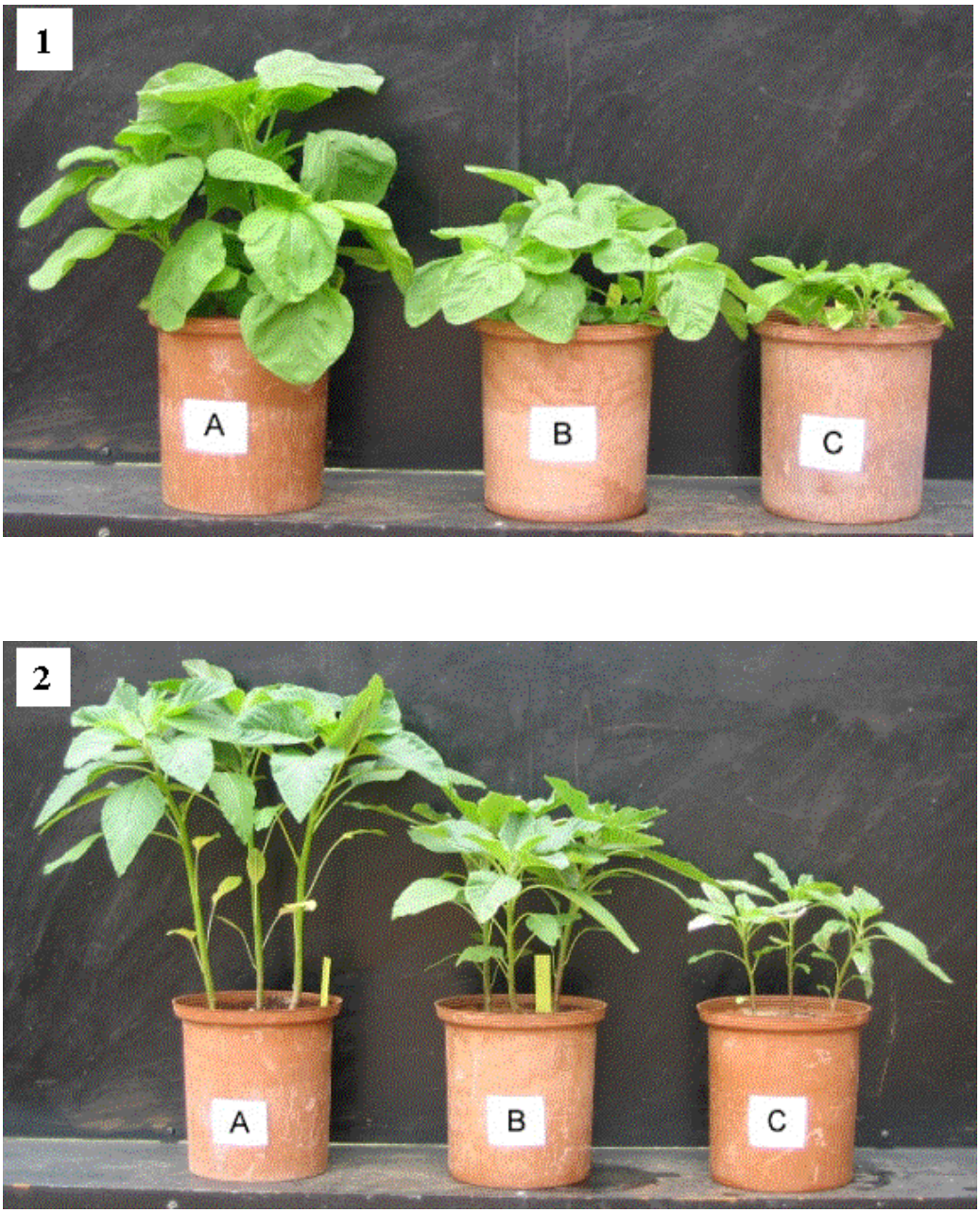

Figure 6.1 Growth of (1) *A. tricolor* and (2) *A. cruentus* supplied with (A) nutrient solution alone, (B) Nutrient solution plus 100 mM NaCl plus additional 10 mM Ca and (C) Nutrient solution plus 100 mM NaCl.

The main effects (genotype and treatment) were significant on photosynthetic rate (P_n), while the interaction between these effects was not significant. *A. tricolor* had higher P_n than *A. cruentus* across all the treatments. The presence of NaCl in the nutrient solution decreased P_n by 39% (Table 6.4). Supplemental calcium increased P_n to control values and no difference was noted between the different calcium sources.

Table 6.4 Effect of calcium supplementation on photosynthetic rate of amaranth

Main effects	Photosynthetic rate ($\mu m\ m^{-2}\ s^{-1}$)
Genotype	
A. tricolor	14.8a
A. cruentus	12.2b
SEM	**0.6**
Treatments	
C	16.3a
C + S	9.9b
C+S+$CaSO_4$	14.3a
C+S+$CaCl_2$	13.4a
SEM	**0.8**

SEM: Standard error of the mean
Mean separation by Tukey's t- test. Means within each column followed by the same letter are not significantly different at P = 0.05.
C=Plants receiving nutrient solution; **C + S**=Plants receiving nutrient solution plus 100 mM NaCl; **C+S+$CaSO_4$** =Plants receiving nutrient solution plus 100 mM NaCl plus 10 mM $CaSO_4$; **C+S+$CaCl_2$**=Plants receiving nutrient solution plus 100 mM NaCl plus 10 mM $CaCl_2$.

6.4.3 Relative water content

Salt treatment caused a high significant decrease in RWC in the two genotypes (Table 6.5). The relative change between control and NaCl treatment was more pronounced for *A. tricolor* (from 80% in the control to 58% in 100 mM NaCl treatment compared to that in *A. cruentus* with 86% for the control and 74% at 100 mM NaCl. Application of supplemental calcium as either $CaSO_4$ or $CaCl_2$ increased the relative water content and no significant difference in their effect was noted.

Table 6.5 Effect of calcium supplementation on relative water content of two amaranth genotypes

Treatment	Relative water content (%)	
	A. tricolor	*A. cruentus*
C	80ab	86a
C+S	58d	74bc
C+S+$CaSO_4$	76bc	82ab
C+S+$CaCl_2$	69c	79ab
SEM	**1.8**	**1.8**

SEM: Standard error of the mean
Mean separation by Tukey's t- test. Means within each column followed by the same letter are not significantly different at P = 0.05.
C=Plants receiving nutrient solution; **C + S**=Plants receiving nutrient solution plus 100 mM NaCl; **C+S+$CaSO_4$** =Plants receiving nutrient solution plus 100 mM NaCl plus 10 mM $CaSO_4$; **C+S+$CaCl_2$**=Plants receiving nutrient solution plus 100 mM NaCl plus 10 mM $CaCl_2$.

6.4.4 Membrane permeability

Membrane permeability was determined by measuring electrolyte leakage. Electrolyte leakage did not differ between genotypes in the absence of stress (Table 6.6). The presence of 100 mM NaCl in the nutrient solution induced significant increases in

electrolyte leakage for both genotypes and was higher in mature leaves (ML) than in developing leaves (DL). Increases in membrane permeability with 100 mM NaCl were higher in *A. tricolor* than in *A. cruentus*. Supplementary Ca^{2+} resulted in a decrease in membrane permeability restoring it to the levels not significantly different from control (C) values in all cases.

Table 6.6 Effect of calcium supplementation on electrolyte leakage (%) in leaves of two amaranth genotypes

Genotype/Treatment	Developing leaf (DL)	Mature leaf (ML)
A. tricolor		
C	8.5c	11.6d
C + S	43.6a	52.4a
C + S + $CaSO_4$	9.7c	13.7cd
C + S + $CaCl_2$	13.5c	19.8c
A. cruentus		
C	7.5c	10.4d
C + S	35.6b	43.2b
C + S + $CaSO_4$	9.6c	12.3d
C + S + $CaCl_2$	11.2c	15.4cd
SEM	**1.4**	**1.5**

SEM: Standard error of the mean

Mean separation by Tukey's t- test. Means within each column followed by the same letter are not significantly different at P = 0.05.

C=Plants receiving nutrient solution; **C + S**=Plants receiving nutrient solution plus 100 mM NaCl; **C+S+$CaSO_4$** =Plants receiving nutrient solution plus 100 mM NaCl plus 10 mM $CaSO_4$; **C+S+$CaCl_2$**=Plants receiving nutrient solution plus 100 mM NaCl plus 10 mM $CaCl_2$.

6.4.5 Ionic content

The interaction between genotype and treatment was not significant for Na^+ and Cl^- accumulation and only main effects are reported. Concentrations of Na^+ and Cl^- in amaranth tissues (shoots and roots) were higher in *A. tricolor* than in *A. cruentus* (Table 6.7). Sodium and Cl^- concentrations increased in shoots and roots in the presence of NaCl stress. Roots accumulated higher amounts of both Na^+ and Cl^- than shoots. The effect of supplementary calcium differed with the source of calcium used, plant part and the ion in question. For instance, in shoots, application of Ca^{2+} either as $CaSO_4$ or $CaCl_2$ lowered sodium (Na) concentration to the control value, whereas in roots, the Na^+ concentration was lowered but remained significantly higher than in the control (Table 6.7). Calcium sulfate similarly reduced Cl^- concentration in shoots to that of control value while in the roots Cl^- concentration was lowered but remained significantly higher than in the control. Supplementary Ca^{2+} as $CaCl_2$, on the other hand, had no significant effect on the concentration of Cl^- in either shoots or roots.

No significant differences were observed between *A. tricolor* and *A. cruentus* in the accumulation of Ca^{2+} and K^+. Concentrations of Ca^{2+} and K^+ decreased in leaves by 45% and 37% in the presence of NaCl stress, but increased in the roots (Table 6.7). Application of supplementary Ca^{2+} resulted in increased Ca^{2+} and K^+ levels in the roots.

Table 6.7 Effect of calcium supplementation in salt stressed plants on the concentration of different ions in shoot and roots of two amaranth genotypes

	Na^+ (% dry weight)		Cl^- (mg/kg)		Ca^{2+} (% d.w)		K^+ (% d.w)	
Main effects	Shoots	Roots	Shoots	Roots	Shoots	Roots	Shoots	Roots
Genotype								
A. tricolor	0.74a	2.5a	100.9a	130.7a	2.4a	1.9a	3.1a	2.5a
A. cruentus	0.33b	1.5b	87.7b	108.2b	2.3a	1.5a	3.0a	2.1a
SEM	**0.05**	**0.08**	**1.50**	**1.30**	**0.12**	**0.10**	**0.18**	**0.14**
Treatments								
C	0.17b	0.8c	85.5c	102.5c	2.7a	1.1b	3.5a	1.8b
C + S	1.37a	3.2a	102.6a	133.0a	1.5b	2.1a	2.2b	2.7a
C+S+$CaSO_4$	0.26b	1.8b	92.2bc	115.5b	2.6a	1.5b	3.4a	2.2ab
C+S+$CaCl_2$	0.32b	2.2b	97.0ab	127.0a	2.5a	2.0a	3.3a	2.5ab
SEM	**0.07**	**0.11**	**2.13**	**1.84**	**0.17**	**0.14**	**0.25**	**0.21**

SEM: Standard error of the mean

Mean separation by Tukey's t- test. Means within each column followed by the same letter are not significantly different at P = 0.05.

C=Plants receiving nutrient solution; **C + S**=Plants receiving nutrient solution plus 100 mM NaCl; **C+S+$CaSO_4$** =Plants receiving nutrient solution plus 100 mM NaCl plus 10 mM $CaSO_4$; **C+S+$CaCl_2$**=Plants receiving nutrient solution plus 100 mM NaCl plus 10 mM $CaCl_2$.

Treatment with either $CaSO_4$ or $CaCl_2$ reduced the Na^+ translocation to the shoots and retained it in roots. As a consequence the Ca^{2+}/Na^+ and K^+/Na^+ ratios in leaves increased (Table 6.8). In general, the Ca^{2+}/Na^+ and K^+/Na^+ ratios were higher in *A. cruentus* than in *A. tricolor*. Although these ratios increased to a greater extent with application of $CaSO_4$ than with $CaCl_2$, no differences were observed between the effects of the two Ca^{2+} sources. For instance, in *A. tricolor*, the Ca^{2+}/Na^+ ratio increased from 0.7 to 8.3 in plants treated with 100 mM NaCl alone and those supplemented with 10 mM $CaSO_4$, and from 0.7 to 6.5 in plants supplemented with $CaCl_2$ (Table 6.8). In *A. cruentus*, the increase was from 2.2 in NaCl treated plants to 12.3 and 9.9 in plants supplemented with $CaSO_4$ or $CaCl_2$ respectively (Table 6.8).

Table 6.8 Effect of calcium supplementation on Ca^{2+}/Na^{+} and K^{+}/Na^{+} ratios of two salt stressed amaranth genotypes

Genotype/Treatment	**Ca^{2+}/Na^{+} ratio**	**K^{+}/Na^{+} ratio**
A. tricolor		
C	13.1b	17b
C + S	0.7e	1.1e
C + S + $CaSO_4$	8.3c	10.6c
C + S + $CaCl_2$	6.5cd	8.3cd
A. cruentus		
C	20.3a	26a
C + S	2.2de	2.9de
C + S + $CaSO_4$	12.3b	16.0b
C + S + $CaCl_2$	9.9bc	13.2bc
SEM	**0.91**	**1.15**

SEM: Standard error of the mean
Mean separation by Tukey's t- test. Means within each column followed by the same letter are not significantly different at P = 0.05.
C=Plants receiving nutrient solution; **C + S**=Plants receiving nutrient solution plus 100 mM NaCl; **C+S+$CaSO_4$** =Plants receiving nutrient solution plus 100 mM NaCl plus 10 mM $CaSO_4$; **C+S+$CaCl_2$**=Plants receiving nutrient solution plus 100 mM NaCl plus 10 mM $CaCl_2$.

Differences in N and P accumulation between genotypes and treatments were significant. *A. tricolor* accumulated more N in shoots than *A. cruentus* while there was no difference between genotypes in N accumulation in the roots and in P accumulation in both shoots and roots (Table 6.9). Concentrations of N and P decreased by 47 and 40% respectively in shoots but increased in roots in the presence of NaCl stress. Nitrogen assimilation was enhanced in shoots in response to Ca^{2+} application and $CaSO_4$ was more effective than $CaCl_2$. In roots N-assimilation of salt stressed plants was enhanced but was reduced by supplementary Ca^{2+} (Table 6.9). The effect of supplementary Ca^{2+} was not significant on the accumulation of P in the shoots as well as in roots.

Table 6.9 Effect of calcium supplementation on nitrogen and phosphorus content of two salt stressed amaranth genotypes

	N (% d.w)		P (% d.w)	
Main effects	Shoots	Roots	Shoots	Roots
Genotype				
A. tricolor	3.7a	1.8a	0.4a	0.4a
A. cruentus	3.4b	1.9a	0.4a	0.3a
SEM	**0.04**	**0.06**	**0.04**	**0.04**
Treatments				
C	4.3a	1.8c	0.6a	0.2b
C + S	2.3d	3.0a	0.3b	0.5a
C+S+$CaSO_4$	4.0a	2.2b	0.4ab	0.3ab
C+S+$CaCl_2$	3.5b	2.5b	0.3b	0.4ab
SEM	**0.06**	**0.08**	**0.06**	**0.06**

SEM: Standard error of the mean
Mean separation by Tukey's t- test. Means within each column followed by the same letter are not significantly different at P = 0.05.
C=Plants receiving nutrient solution; **C + S**=Plants receiving nutrient solution plus 100 mM NaCl; **C+S+$CaSO_4$** =Plants receiving nutrient solution plus 100 mM NaCl plus 10 mM $CaSO_4$; **C+S+$CaCl_2$**=Plants receiving nutrient solution plus 100 mM NaCl plus 10 mM $CaCl_2$.

6.5 DISCUSSION

6.5.1 Effect of calcium supplementation on vegetative growth of salt stressed amaranth

Salt stress had a significant inhibitory effect on dry mass of shoots and roots (Table 6.1), and genotypes differed significantly in their response with less reductions observed in *A. cruentus* than in *A. tricolor*. Similar reductions in dry matter production have been shown in for bell pepper (Chartzoulakis and Klapaki, 2000); tomato (Navarro *et al*., 2000) and *Phaseolus* spp (Bayuelo-Jiménez *et al.*, 2003).

In this investigation it was shown that Ca^{2+} application, either as $CaSO_4$ or $CaCl_2$ to saline stressed plants enhanced plant height and dry mass of shoots and roots. However, the effect of $CaCl_2$ in ameliorating leaf area expansion was less than that of $CaSO_4$. The weaker ameliorative effect of $CaCl_2$ than $CaSO_4$ may have been due to the toxic effect of additional Cl^- ions from $CaCl_2$. Alternatively, the SO_4^- ions may have enhanced growth as was observed in snap bean (Awada *et al*., 1995). Similarly supplementary Ca^{2+} was found to enhance dry matter production in tomato (Navarro *et al*. 2000), strawberry (Kaya *et al*., 2002) and guava (Ebert *et al*., 2002) exposed to salinity stress.

6.5.2 Effect of calcium supplementation on gas exchange

Salinity reduced stomatal conductance (g_s) and photosynthetic rate (P_n) (Table 6.3 and 6.4). The detrimental effect of salinity on CO_2 assimilation has been shown in numerous studies (Ebert *et al*., 2002, Bayuelo-Jiménez *et al*. (2003). Salinity first reduces stomatal conductance and then impairs net photosynthetic rate. It has been proposed that the reduction of leaf gas exchange in response to salinity is due to increase in leaf Na^+ concentration (García-Legaz *et al*., 1993; Walker *et al*., 1993). Toxic accumulation of Na^+ and Cl^- in the leaves has been correlated with stomatal closure and with non-stomatal factors such as reduction in total chlorophyll content, both of which limit the amount of photo-assimilate production (Seemann and Critchley, 1985; Romero-Aranda and Syvertsen, 1996). In the present study, P_n and g_s were negatively correlated ($P<0.001$) with leaf Na^+ and Cl^- in both genotypes. This correlation between gas exchange in leaves and foliar concentrations of Na^+ and Cl^- suggests that the toxic effect of the accumulated ions could be involved in the reduction of photosynthesis and stomatal conductance (Lloyd *et al*., 1990).

Supplementary Ca^{2+} partly alleviated the detrimental effects induced by salinity on g_s and P_n. Supplementation of Ca^{2+} increased the rate of photosynthesis by increasing the concentration of Ca^{2+} and K^+, as was also found in sorghum (Colmer *et al*., 1996). The greater CO_2 assimilation rates after application of supplementary Ca^{2+} at high salinity could be attributable to high N concentrations in leaves, which might have induced

chlorophyll synthesis (Shadad *et al*., 1988; Ebert *et al*., 2002). In addition, the ability of leaves to maintain low Na^+ concentrations and the preference to accumulate K^+ may contribute to a better regulation of stomatal opening to achieve their normal regulation of turgor under salt stress. A reduction in the K^+ content is typical of plants grown under salt stress (Long and Baker, 1986) and may cause damage to the photosynthetic apparatus (Chow *et al*., 1990). The role of K^+ is vital for osmoregulation, maintaining cell turgor and stimulating photosynthesis (Peoples and Koch, 1979). Calcium has also been shown to regulate guard-cell turgor and stomatal aperture (Webb *et al.,* 1996). The amaranth results are in agreement with the initial hypothesis that extra calcium may ameliorate the effect of salinity on g_s and P_n as has been found in rice (Sultana *et al*., 2001), quava (Ebert *et al*., 2002) and cucumber (Kaya and Higgs, 2002).

6.5.3 Effect of calcium supplementation on relative water content

A reduction in relative water content (RWC) was observed in salt stressed amaranth plants (Table 6.5). This reduction was more pronounced in the less tolerant genotype, *A. tricolor*, than in the more tolerant *A. cruentus*. The decrease in RWC indicated a loss of turgor that resulted in limited water availability for cell extension processes (Katerji *et al*., 1997). Thus the growth inhibition in *A. tricolor* could be related to the decrease of RWC induced by salt treatment. Several studies have shown that water uptake, and hence water content in the leaves declined as the salt concentration in the irrigation water increased (Soria and Cuartero, 1997; Bayuelo-Jiménez *et al*., 2003; Cabañero *et al*., 2004).

Application of supplementary Ca^{2+}, either as $CaSO_4$ of $CaCl_2$, resulted in increased RWC in both amaranth genotypes. These results are in agreement with the initial hypothesis that extra supply of calcium would ameliorate the effect of salinity in water relations in amaranth plants, as has been found in cucumber (Kaya and Higgs, 2002) and pepper (Cabañero *et al*., 2004). The role of Ca^{2+} in plant-water relations has been demonstrated in melon (Carvajal *et al*., 2000) and pepper (Cabañero *et al*., 2004). They reported that NaCl decreased the passage of water through the membrane and roots by reducing the

activity of aquaporins, and that Ca^{2+} ameliorated the negative effect of NaCl stress. Aquaporins allow water to pass freely across cellular membranes, following osmotic or hydrostatic pressure gradients (Chispeels and Maurel, 1994).

6.5.4 Effect of calcium supplementation on membrane permeability

The presence of 100 mM NaCl in the rooting medium caused a disturbance in membrane permeability expressed by an increase in solute leakage in both amaranth genotypes (Table 6.6). The leakage was higher in *A. tricolor* than in *A. cruentus*, indicating severe membrane damage for the former genotype under salt stress where reductions in shoot dry weight was also higher. This may indicate a link between dry matter production and membrane permeability and may also suggest that a great part of leaf ion content in *A. tricolor* did not contribute to the osmotic adjustment of the cells. The opposite may have been true for the more tolerant genotype, *A. cruentus*. Supplementary Ca^{2+} decreased membrane permeability and restored it to the levels not significantly different from control values in all cases. These results are in agreement with the findings obtained in rice (Lutts *et al*., 1996a), sugar beet (Ghoulam *et al.,* 2002) and strawberry (Kaya *et al*., 2002). These authors also reported that high salt concentration increased the membrane permeability, with solute leakage high for salt sensitive and low for salt tolerant cultivars, and that supplementary Ca^{2+} alleviated the negative effect of salt stress.

6.5.5 Effect of calcium supplementation on ionic regulation

The presence of NaCl in the nutrient solution resulted in accumulation of Na^+ and Cl^- in amaranth and this accumulation was greater in the roots than in shoots (Table 6.7). The regulation of transport and distribution of ions in the different organs of the plant and within the cell is an essential factor of the mechanism of salt tolerance (Greenway and Munns, 1980). This is because the accumulation of Cl^- and/or Na^+ in plant tissues is toxic and may be one of the main causes for growth inhibition under high salinity (Greenway and Munns, 1980; Yeo and Flowers, 1986). Salt tolerance in glycophytes is associated with the ability to limit uptake and/or transport of saline ions (mainly Na^+ and

Cl^- from the root zone to the aerial parts (Greenway and Munns, 1980). Data recorded in this study suggests that this occurs in amaranth. The accumulation of Na^+ and Cl^- in roots provides a mechanism for amaranth to cope with salinity in the rooting medium. At high external salinity (100 mM), the accumulation of Na^+ and Cl^- in the roots may indicate the existence of an inhibition of transport of these ions to the leaf laminae.

It has been indicated that the capacity of Na^+ and/or Cl^- exclusion from the shoots is well correlated to the salt tolerance degree (Gorham *et al*., 1993). *A. cruentus* accumulated less Na^+ and Cl^- and may be considered more tolerant than *A. tricolor*. Similar results were obtained in tomato hybrid 'Radja' (Perez-Alfocea *et al*., 1996). It was noted that this hybrid avoided Na^+ accumulation in leaves at moderate salinity, hence, its salt tolerance seems to be related to the capacity of Na^+ exclusion from the shoot. This is in accordance with the negative relationship found between the accumulation of toxic ions (Na^+ and Cl^-) in leaves and the shoot growth of tomato plants growing under salinity (Perez-Alfocea *et al*., 1993c). Similarly, sodium ions accumulated in roots and pith cells in the lower part of the stem of sweet pepper plants grown under salt stress rather than in the leaves (Zandstra-Plom *et al*., 1998). The noticed difference between genotypes fits with the general findings that differences in the capacity of sodium retention in roots reflect differences in salt tolerance (Reimann, 1992). Tattini *et al*. (1995) studied the ionic relations of two olive cultivars during salt stress and reported that the resistance mechanism of the salt-tolerant cultivar was related to Na^+ exclusion by roots and the ability to maintain an appropriate K^+/Na^+ ratio in actively growing tissue. In this study, it was shown that there are significant genotypic differences in salt tolerance among amaranth genotypes, which seems to be related to the salt exclusion mechanisms at root level, which prevent Na^+ and Cl^- translocation to the aboveground parts. In general, salt tolerance has been positively correlated with ion exclusion in some crop species, e.g., wheat (Ashraf and O'Leary, 1996), sunflower (Ashraf and Tufail, 1995) and *Brassica carinata* (Ashraf and Sharif, 1997). In contrast, a negative correlation between ion concentration and salt tolerance has also been reported in other crops e.g., *Vigna* spp. (Gulati and Jaiwal, 1993), and lentil (Ashraf and Waheed, 1993). Leidi and Saiz (1997) observed the association of high shoot Na^+ with salt tolerance of cotton. The

accumulation of salt ions could play an important role in osmotic adjustment if they were efficiently compartmentalized at the cell level.

Supplementary calcium resulted in reduced Na^+ and Cl^- accumulation in the leaves. However, the Cl^- concentration of $CaCl_2$ supplied plants was still higher than the control although there was no effect on dry matter accumulation. This clearly shows that Ca^{2+} facilitates retention of both Na^+ and Cl^- in the root system or the stem of amaranth, as was reported in quava (Ebert *et al*., 2002). The decrease in leaf Na^+ and Cl^- with application of Ca^{2+} may partially be explained by a "dilution effect", i.e. increase in dry matter accumulation. These results are in agreement with the findings obtained in tomato (Satti and Al-Yahyai, 1995) and strawberry (Kaya *et al*., 2002). Calcium is thought to improve the K^+/Na^+ selectivity of membranes (Marschner, 1995) and prevent the cell from invasion of toxic ions (Cramer *et al*., 1987).

Salt treatment was found to alter mineral nutrient distribution and decrease absorption of all the nutrients studied (Table 6.7 and 6.9). In particular, salinity decreased Ca^{2+}, K^+ and N uptake in both amaranth genotypes. Salt-induced nutrient deficiency has been reported by many researchers (Sultana *et al.,* 2001; Kaya *et al*., 2001, 2002). The possible cause of reduced nutrient uptake under salinity is that ions in present high concentrations in the external solution (i.e. Na^+ or Cl^-) are taken up at a high rate, which may lead to excessive accumulation in the tissue. These ions may inhibit the uptake of other ions into the roots (i.e. K^+ or Ca^{2+}) and their transport into the shoot, eventually leading to deficiency in the tissue (Mengel and Kirkby, 1987). The reduction in K^+ uptake caused by Na^+ is a well-known competitive process in plant roots (Cerdá *et al*., 1995). It has also been reported that Na^+ competes with K^+ for intracellular influx because these cations are transported by common protein (Hasegawa *et al*., 2000). However, salt-stressed amaranth plants were able to maintain a relatively high K^+ content independent of Na^+ accumulation. These results are consistent with data obtained in cotton (Brugnoli and Bjorkman, 1992) and spinach (Delfine *et al*., 1998) suggesting that K^+ maintenance may be a common mechanism of protection against salt damage in glycophytes. It also suggests that the high K^+ levels in leaves may act as the major monovalent cationic osmoticum in the presence

of external salt and could be a regulatory mechanism to maintain osmotic balance under salinity stress (Grieve and Walker, 1983). Salinity has also been reported to lower Ca^{2+} uptake in tomato (Cuartero and Fernández-Muñoz, 1999; Navarro *et al*., 2000) and in strawberry (Kaya *et al*., 2002). Reduced assimilation of N and Ca^{2+} in cowpea plants due to salt stress has been reported by Silveira *et al*. (2001). The decreased amount of macronutrients in salt-treated plants may also be explained by the lower accumulation of dry matter production.

With regard to nutrient uptake, accumulation and translocation, Ca-supplied plants were found to accumulate more K^+, Ca^{2+} and N (Table 6.7 and 6.9). This finding is in agreement with several other investigators who showed that application of additional Ca^{2+} corrected nutrient deficiencies in tomato (Navarro *et al*., 2000), strawberry (Kaya *et al*., 2002) and guava (Ebert *et al*., 2002). Calcium is a non-toxic inorganic nutrient that is very effective in detoxifying high concentrations of other elements in plants under saline conditions (Greenway and Munns, 1980). The results for Ca^{2+}/Na^+ ratios suggest that Ca^{2+} may have played an important role in maintaining the proper functioning of biological membranes and their permeability (Kent and Läuchli, 1985), thereby resulting in relatively normal growth. The ability of Na^+ and Cl^- exclusion from the leaf lamina combined with the ability to maintain relatively high Ca^{2+} and K^+ concentrations in leaves may provide amaranth with a tolerance mechanism for low and moderate salinity levels. Selectivity of K^+ over Na^+ has been found to be an important physiological trait for tomato (Perez-Alfocea *et al*., 1993c).

6.6 CONCLUSION

High NaCl concentration in the nutrient solution reduced plant growth, stomatal conductance, photosynthetic rate, relative water content and induced deficiencies of Ca^{2+}, K^+, N. Membrane leakage and accumulation of Na^+ and Cl^- were increased with salinity. *A. cruentus* showed more tolerance to salinity stress than *A. tricolor* and accumulated less of the toxic ions (Na^+ and Cl^-), exhibited less membrane leakage, and growth was less

affected. In both genotypes Na^+ and Cl^- ions accumulated to a greater extent in roots than in shoots.

Supplementary Ca^{2+} ameliorated the parameters affected by salinity and no significant differences were observed in the ameliorating effects of $CaSO_4$ and $CaCl_2$. The two amaranth genotypes responded in a similar manner to amelioration of salinity stress by Ca^{2+}. The potential of Ca^{2+} to alleviate NaCl-induced growth reductions in amaranth was confirmed.

CHAPTER 7

SALT TOLERANCE OF AMARANTH AS AFFECTED BY SEED PRIMING

7.1 ABSTRACT

Due to increased salinity problems, efforts are being made to develop strategies to ameliorate salt stress. This study was conducted to evaluate the effectiveness of seed priming in ameliorating salinity stress effects in amaranth during seedling development and the early vegetative stage. Two experiments were conducted with seeds of two amaranth genotypes namely *A. tricolor* and *A. cruentus*. Seeds were primed for 3 hours with solutions of NaCl, $CaSO_4$, or a combination of the two salts, with similar osmotic potentials (-1.3 MPa). In experiment 1, non-primed and primed seeds were sown in 1-liter plastic pots filled with sand. The pots were placed in a greenhouse and exposed to 0, 25, 50 and 100 mM NaCl for a period of 21 days. Experiment two was conducted in a similar manner as experiment 1 but without the 25 mM NaCl treatment. At 21 days after emergence, three seedlings from each treatment were transplanted into each 5-litre plastic pot containing sand/vermiculite and watered with 0, 50 and 100 mM NaCl solutions for 28 days. Seedlings from primed seed emerged earlier and attained a higher total emergence than non-primed seed. Seed priming enhanced photosynthesis, water relations, and general plant growth, and prevented toxic and nutrient deficiency effects of salinity because less Na but more Ca and K accumulated in the amaranth plants. Plants from primed seeds had significantly higher Ca:Na balances than those from non-primed seeds. Priming with $CaSO_4$ + NaCl was more effective than priming with the individual salts. The results suggest that seed priming increased salt tolerance of amaranth at the seedling and early vegetative growth stage by promoting K and Ca accumulation, besides inducing osmoregulation.

Keywords: Amaranth, priming, salt tolerance

7.2 INTRODUCTION

Due to increased salinity problems, the need to develop crops with higher salt tolerance has increased strongly within the last decade. Generally, plants do not develop salt tolerance unless they are exposed to saline conditions. Salt tolerance of plants can be increased by treatment of seeds with NaCl solution prior to sowing (Levitt, 1980; Sivritepe *et al*., 2003).

Seed priming or osmoconditioning is one of the physiological methods which improves seed performance and provides faster and synchronized germination (Sivritepe and Dourado, 1995). It entails the partial germination of seed by soaking in either water or in a solution of salts for a specified period of time, and then re-drying them just before the radicle emerges (Copeland and McDonald, 1995; Desai *et al*., 1997). Seed priming stimulates many of the metabolic processes involved with the early phases of germination, and it has been noted that seedlings from primed seeds emerge faster, grow more vigorously, and perform better in adverse conditions (Desai *et al*., 1997).

Some of the factors that affect seed priming response are solution composition and osmotic potential (Bradford, 1986; Smith and Cobb, 1991). However, osmotic potential is not mentioned in most of the seed priming studies (Bradford *et al*., 1988; Yeoung *et al*., 1996; Sivritepe *et al*., 2003). It has been shown that NaCl seed priming could be used as an adaptation method to improve salt tolerance of seeds. In studies conducted by Cano *et al*. (1991) and Cayuela *et al*. (1996) with tomatoes, Pill *et al*. (1991) with asparagus and tomatoes, and Passam and Kakouriotis (1994) with cucumber, it was concluded that seed priming improves seed germination, seedling emergence and growth under saline conditions. However, the possible beneficial effects of NaCl priming for mature plants remain unclear. Passam and Kakouriotis (1994) reported that benefits of NaCl seed priming did not persist beyond the seedling stage in cucumber, while Cano *et al*. (1991) found that NaCl seed priming had positive effects on mature plants and on yield of tomato.

Since NaCl seed priming has become an important technique to increase salt tolerance of plants, it is important to understand the physiological effects which mediate the responses to salinity. However, studies on physiological changes induced by NaCl seed priming have seldom been conducted. According to Cano *et al*. (1991), the higher salt tolerance of plants from primed seeds seems to be the result of a higher capacity for osmotic adjustment since plants from primed seeds have more Na^+ and Cl^- ions in their roots and more sugars and organic acids in leaves than plants from non-primed seeds.

External Ca^{2+} has been shown to ameliorate the adverse effects of salinity in plants (Sultana *et al*., 2001; Kaya *et al*., 2002; Ebert *et al*., 2002). According to Hasegawa *et al*. (2000), this amelioration is presumably by facilitating higher K^+/Na^+ selectivity. Calcium has often been used as a pelleting (seed coating) material. Baker and Hatton (1987), for instance, documented that coating rice seed with calcium peroxide increased germination and plant establishment. In their various forms, seed coatings have become an important part of modern agriculture, and some have been shown to improve emergence and seedling growth in agronomic crops (Mikkelsen, 1981; Spilde, 1997). However, little is known concerning the use of calcium in seed priming and whether this treatment can ameliorate the adverse effects of salinity on plants.

Although priming seed has been successively practiced on some agronomic crops (Cano *et al.,* 1991; Passam and Kakouriotis, 1994; Cayuela *et al.,* 1996; Sivritepe *et al*., 2003), information on the effects of this technique on amaranth is limited. Two greenhouse experiments were conducted to examine the effects of seed priming with NaCl, alone and in combination with Ca^{2+}, on salt tolerance of amaranth at the seedling and early vegetative growth stages.

7.3 MATERIALS AND METHODS

The effect of seed priming on seedling emergence, survival and plant growth of amaranth in a saline environment was studied in two experiments conducted in a greenhouse at the

University of Pretoria in February 2004. In the first experiment the effect of seed priming on seedling emergence and survival was determined. The second experiment was conducted to evaluate whether the ameliorative effects of priming persists up to the vegetative growth stage of amaranth.

7.3.1 Seed priming

Three different salt solutions with the same osmotic potential (–1.3 MPa) were used for seed priming. These solutions were prepared by dissolving the appropriate quantity of NaCl, $CaSO_4$ or NaCl + $CaSO_4$ in distilled water. To ensure that the salts were thoroughly dissolved, the solutions were placed on a shaker until completely dissolved. The osmotic potential of the solutions was verified with a Wescor-5500 vapor pressure osmometer (Wescor, Logan, UT, USA). This concentration was chosen on the basis of preliminary experiments and showed no inhibition of germination. Seeds of two amaranth genotypes (*A. tricolor* and *A. cruentus*) were imbibed for 3 hours at room temperature in the different priming solutions. Non-primed seeds (NP-seeds) were pre-hydrated in distilled water under the same conditions as primed (P-seeds) in order to avoid the effect of seed priming on plant growth by differences in seed development (Taylor *et al*., 1992). After priming, seeds were washed with distilled water and spread out on a paper towel to dry in the shade for 48 hrs.

7.3.2 Experiment 1

Two days after priming, seeds were sown in 1-liter plastic pots containing washed silica sand. The pots were placed in a greenhouse where the temperature ranged between 18 and 31^{o}C and relative humidity between 70 and 85%. The pots, containing 20 seeds each, were irrigated daily with nutrient solution in which 0, 25, 50 and 100 mM NaCl was supplied. The 50 and 100 mM NaCl solutions were applied in daily increases of 25 mM NaCl until the desired concentration was reached in order to avoid shock. Electrical conductivities (EC) of these solutions were 1.2, 4.1, 7.0 and 12.8 dS. m^{-1} respectively. Surplus water drained from the bottom of the pots to avoid build-up of salt in the growth

media. There were three replications (pots) of each treatment combination and the pots were arranged randomly.

The pots were inspected daily and emergence recorded as the appearance of the cotyledons. The total number of emerged seedlings in each replicate was determined and expressed as a percentage. The seedlings were allowed to grow for 21 days during which seedling survival was assessed at 7-day intervals. At 21 days after emergence the surviving seedlings were harvested and root and shoot lengths were recorded. For determination of dry mass the shoots and roots were oven dried at 75^{o}C to a constant mass.

7.3.3 Experiment 2

This experiment was conducted in the same manner as Experiment 1. However, the 25 mM NaCl treatment was omitted. At 21 days after sowing, three seedlings from each treatment were selected for uniformity and transplanted into 5-liter plastic pots containing sand-vermiculite mixture. In order to collect satisfactory amounts of plant material for chemical analyses in non-primed seeds exposed to 50 and 100 mM NaCl salinity levels, extra seedlings were grown along with the main experiment. The experiment was carried out for 28 days after transplanting.

7.3.3.1 Determination of photosynthetic rate

Photosynthetic rate (P_n) was measured 14 and 28 days after transplanting on the second and third youngest fully expanded leaves with a LI-COR, 6400 portable photosynthetic system (LI-COR, Lincoln, NE). Photosynthetic measurements followed the same procedure as described in Chapter 3.

7.3.3.2 Determination of relative water content

The relative water content (RWC) was recorded 14 and 28 days after transplanting as described in Chapter 5.

7.3.3.3 Determination of vegetative growth parameters

At the end of the experiment (4 weeks from transplanting) plant height was recorded. Plants were then harvested and separated into shoots and roots. Leaf area was determined with a LI-3100 leaf area meter (LI-COR. Inc., Lincoln, NE, USA). Dry mass (after oven drying the samples at 75^{o}C to constant mass) was recorded.

7.3.3.4 Determination of nutrient content in plant materials

Chemical analysis was carried out on the oven-dry plant material. Ground samples were ashed at 550^{o}C in a porcelain crucible for 6h. Potassium, calcium and sodium were determined after extraction in HCl, using an atomic absorption spectrophotometer.

7.3.3.5 Data analysis

Data were submitted to Bartlett's test for the homogeneity of variance. Log transformations of percent emergence data were necessary to achieve homogeneity of variance and to compare data from the early and late emergence. All data were subjected to standard analyses of variance using the General Linear Model (GLM) procedure of the Statistical Analysis System (SAS, 1996) to determine the effect of main factors and the interaction between them. Differences at the $P \leq 0.05$ level were used as a test of significance and means were separated using Tukey's t-test.

7.4 RESULTS

7.4.1 Experiment 1

7.4.1.1 Effect of seed priming on seedling emergence under salinity

The response of amaranth genotypes to seed priming differed with the priming treatment and NaCl concentration in the irrigation water. In general, increased NaCl salinity decreased total emergence of seedlings derived from either primed or non-primed seeds in both genotypes (Figure 7.1). However, total emergence percentages of the primed seed were higher than for non-primed ones. For instance, at 0 mM NaCl total emergence of

non-primed seed was 70% in *A. tricolor* and 80% in *A. cruentus*. Seed priming resulted in increased total emergence of 87 to 93% in *A. tricolor* and 93 to 97% in *A. cruentus* depending on the type of salt used for priming. Seedling emergence of seeds derived from non-primed seed was reduced to less than 50% when plants were treated with 50 mM NaCl, while emergence of seedlings from primed seed ranged from 63 to 70% in *A. tricolor* and 70 to 77% in *A. cruentus.* A significant decrease in total emergence occurred at 100 mM where total emergence was less than 40% in all treatments (Figure 7.1). Although seedling emergence from seeds primed with $CaSO_4$ + NaCl (P3) was higher than seeds primed with NaCl (P1) or $CaSO_4$ (P2), no significant differences were noted among these treatments when plants were supplied with 0, 25 or 50 mM NaCl. With 100 mM NaCl emergence of seeds primed with $CaSO_4$ + NaCl was significantly higher than that of control and seeds primed with individual salts (Figure 7.1).

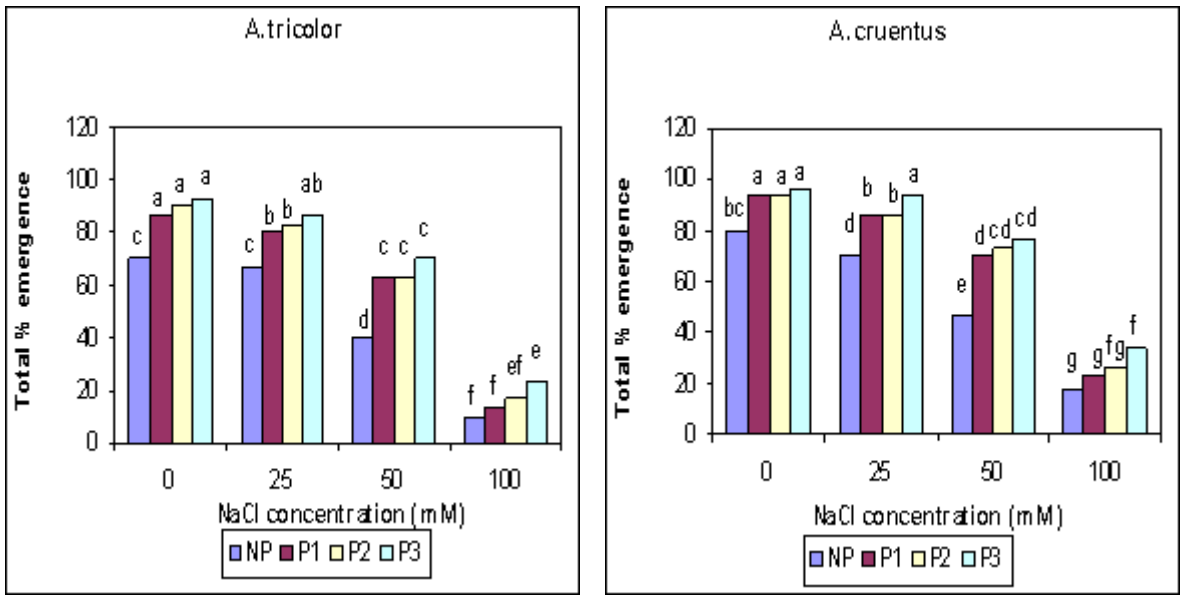

Figure 7.1 Effects of NaCl concentrations on seedling emergence of amaranth seedlings derived from non-primed seeds (NP) and seeds primed with NaCl (P1), $CaSO_4$ (P2) or NaCl + $CaSO_4$ (P3). Mean separation by Tukey's t- test. For each genotype bars followed by the same letter are not significantly different at P = 0.05.

The kinetics of emergence of *A. tricolor* (Figure 7.2) and *A. cruentus* (Figure 7.3) varied and was affected by NaCl concentration and priming treatments. When 0 or 25 mM NaCl was applied, seedlings first emerged on day four in both genotypes for non-primed and primed seeds (Figure 7.2). However, with increasing NaCl concentration seedling emergence was delayed in NP than in P seeds. For instance, when 50 mM NaCl was applied, seedling emergence was delayed to day five in primed and day six in non-primed seeds (Figure7.2c; 7.3c), while exposure to 100 mM NaCl resulted in emergence delayed to day six in primed and day seven in non-primed seed (Figure 7.2d; 7.3d).

Time to completion of emergence varied with priming treatment and the level of NaCl applied. Emergence was complete within two days from the start of emergence in primed seeds and 4 to 5 days in NP seeds when 0 or 25 mM NaCl was applied (Figure. 7.2a; b and 7.3a; b). Seedling emergence spread over a longer period in NP seeds and at higher NaCl concentration. At 50 and 100 mM NaCl, emergence in P and NP seeds was completed in 5 and 7 days respectively from the beginning of emergence (Figure 7.2c; d and 7.3c; d).

7.4.1.2 Effect of seed priming on seedling survival under salinity

The genotype x salt and genotype x priming interactions were not significant, indicating that the two genotypes reacted similarly. In Table 7.1 data on the salinity x priming interaction on seedling survival is presented. Regardless of the priming treatments, survival of seedlings was reduced as NaCl concentration and days after emergence increased. The effect of seed priming depended on the concentration of NaCl applied and the time the data was recorded. At 7 days after emergence, seedling survival at 0 mM NaCl was not affected by priming. At 14 and 21 days, seedlings from primed seeds tended to have a higher survival percentage compared to those from non-primed seeds or the control treatment (Table 7.1). When 25 mM NaCl was applied survival of seedlings from seeds primed with $CaSO_4$ or $CaSO_4$+ NaCl was higher than that of non-primed seeds and seeds primed with NaCl. There was no significant difference in seedling survival between plants exposed to 0 mM and those exposed to 25 mM NaCl.

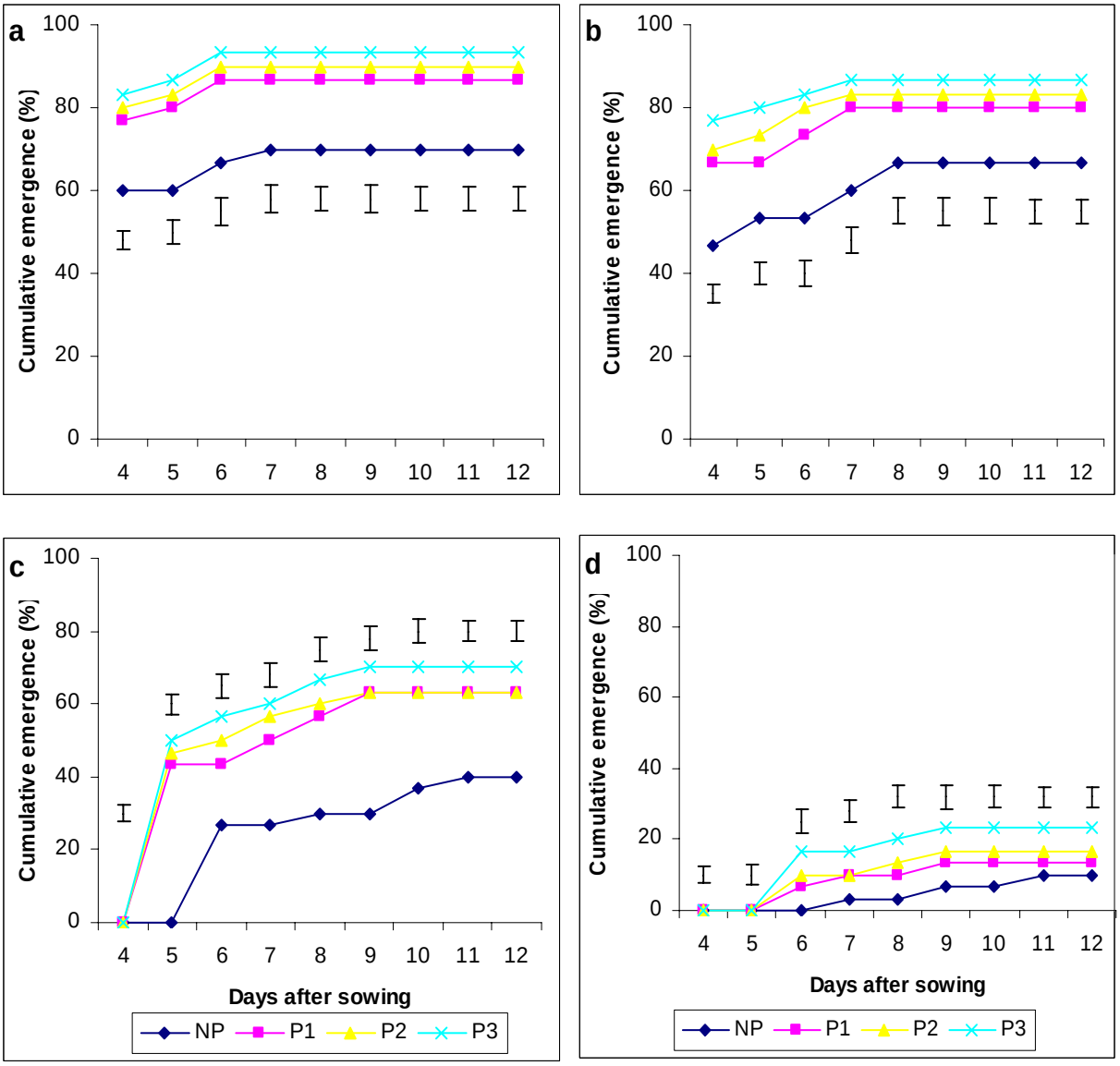

Figure 7.2 **Effect of (a) 0, (b) 25, (c) 50 and (d) 100 mM NaCl concentration on the time course of seedling emergence of *A. tricolor* derived from non-primed seeds (NP) and seeds primed with NaCl (P1), $CaSO_4$ (P2) or NaCl + $CaSO_4$ (P3). Vertical bars indicate least significant differences at P = 0.05.**

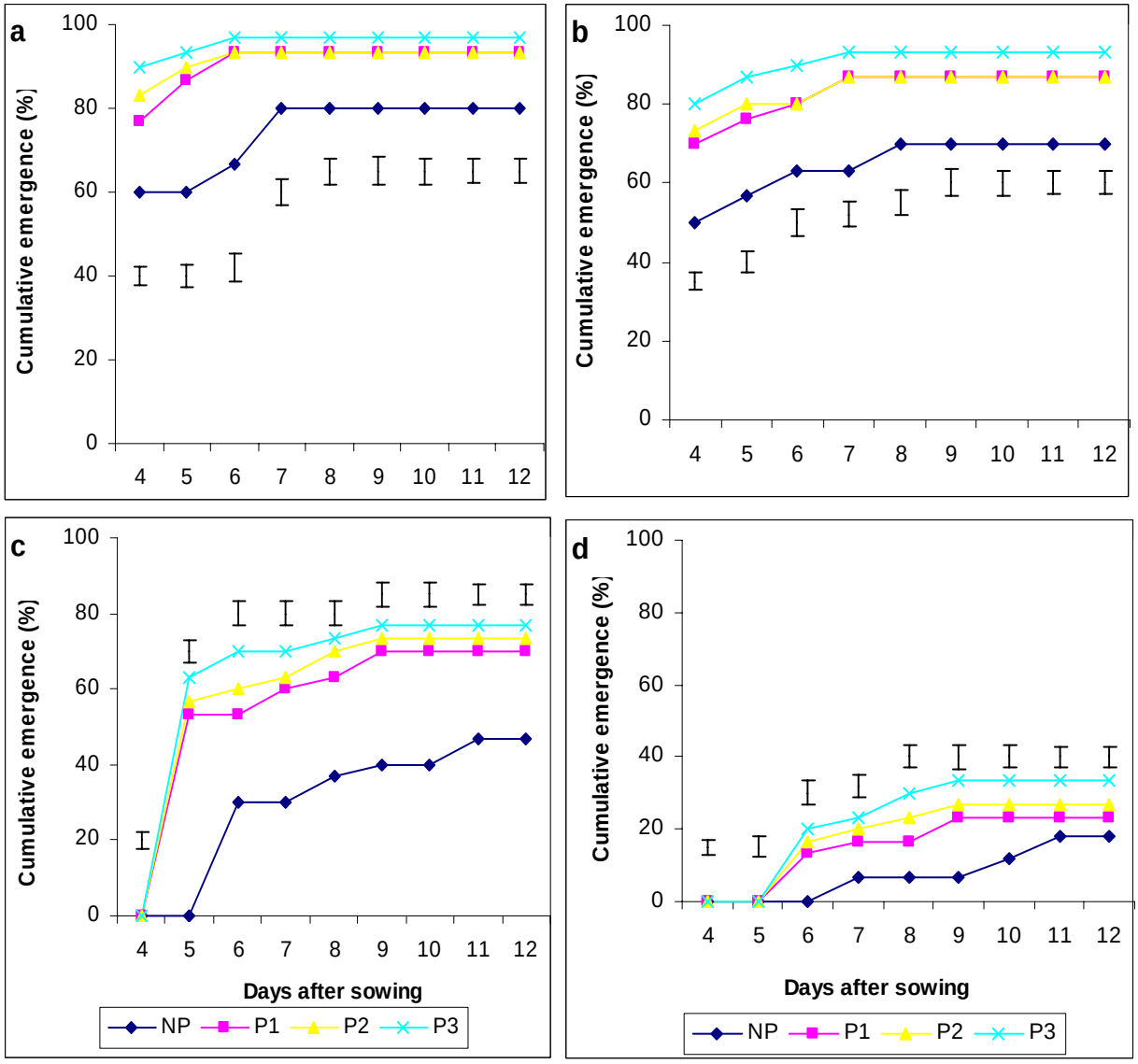

Figure 7.3 Effect of (a) 0, (b) 25, (c) 50 and (d) 100 mM NaCl concentration on the time course of seedling emergence of *A. cruentus* derived from non-primed seeds (NP) and seeds primed with NaCl (P1), $CaSO_4$ (P2) or NaCl + $CaSO_4$ (P3). Vertical bars indicate least significant differences at P = 0.05.

A significant reduction in seedling survival was observed when plants were exposed to 50 mM NaCl and the decline in seedling survival was lower for primed seeds. When the seed was not primed, seedling survival was 81% at 7 days after emergence, and declined to 65% at 21 days after emergence. In the primed seeds survival ranged between 85 and 90% at 7 days after emergence and declined to 73 to 80% at 21 days after emergence. At 100 mM NaCl the seedling survival trend was similar to that observed at 50 mM NaCl (Table 7.1). It is interesting to note that although application of 100 mM NaCl resulted in the least survival percentages on day 7, 100% of seedlings in the NP treatment survived since this was the day they first emerged. However, survival was reduced to 35% at 21 days after emergence. Survival in primed seeds was reduced from more than 75% at day 7 to less than 62% at 21 days after emergence, and seeds primed with $CaSO_4$+ NaCl had better seedling survival rates than those primed with either NaCl alone or $CaSO_4$ (Table 7.1). The adverse effect of high NaCl concentration on seedling survival was ameliorated when seed was primed.

7.4.1.3 Effect of seed priming on seedling growth under salinity

The main effects of genotype, NaCl salinity and seed priming were significant on shoot and root length, as well as on shoot and root dry mass of seedlings 21 days after emergence while the interactions between these factors were not significant. All the parameters under observation were significantly higher in *A. cruentus* compared to *A. tricolor* (Table 7.2). Across amaranth genotypes and priming treatments, increasing NaCl concentration resulted in significant reductions in shoot and root length. The average length of the shoot was reduced by 27% at 25 mM, 45% at 50 mM and by 61% at 100 mM NaCl. The reduction in root length was less than that of shoot length. Root length was reduced by 17% at 25 mM, 36% at 50 mM and by 51% at 100 mM NaCl (Table 7.2). Shoot and root dry mass was similarly reduced with increasing NaCl concentration and root dry mass was reduced to a greater extent than shoot dry mass.

Table 7.1 Effects of seed priming on the survival rates of seedlings of amaranth under salinity 7, 14 and 21 days after emergence

NaCl salinity (mM)	Priming	Seedling survival (%)		
		7days	14 days	21 days
0	NP	93 bc	87b	86.5c
	P1	97 ab	95a	93ab
	P2	98.5ab	97 a	93 ab
	P3	100a	97 a	97a
25	NP	93 bc	87 b	83cd
	P1	93 bc	88.5b	88.5bc
	P2	97a	97a	93 ab
	P3	98.5a	95a	95a
50	NP	81.5 e	70e	65 g
	P1	85de	75de	73f
	P2	88.5cd	77cd	75ef
	P3	90cd	81.5bc	80de
100	NP	100a	45g	35j
	P1	77f	58.5f	41.5i
	P2	87cd	75de	58h
	P3	90cd	77cd	62g
SEM		**1.26**	**1.22**	**1.12**

SEM: Standard error of the mean
Seeds were either not primed (NP) or primed with NaCl (P1), $CaSO_4$ (P2) or NaCl+$CaSO_4$ (P3). Mean separation by Turkey's t-test. Means followed by the same letter along the column are not significantly different at P = 0.05.

Seed priming increased the length of both shoots and taproots compared to the non-primed control (Table 7.2). The increases were greater in the NaCl + $CaSO_4$ primed treatment than for the other priming treatments. Root length of plants derived from seeds primed with NaCl + $CaSO_4$ averaged 40.9 mm. In comparison, plants primed with NaCl or $CaSO_4$ alone achieved total root lengths of 32.1 and 36.2 mm respectively. Significant increases in shoot dry mass were observed for primed seeds. Shoot dry mass ranged from

0.13 g/plant in the control treatment to 0.24 g/plant in the NaCl + $CaSO_4$ primed treatment. Priming the seed resulted in significant increases in root dry mass of up to 39% in seeds primed with NaCl to 132% in those primed with NaCl + $CaSO_4$ compared to the control treatment (Table 7.2).

Table 7.2 Main effects of genotype, NaCl salinity and seed priming on shoot length, root length, shoot dry mass and root dry mass of amaranth 21 days after emergence

Main effects	Shoot length (mm)	Root length (mm)	Shoot dry mass (g/plant)	Root dry mass (g/plant)
Genotype				
A. tricolor	27.12b	31.37b	0.16b	0.09b
A. cruentus	31.81a	37.19a	0.21a	0.13a
SEM	**0.41**	**0.41**	**0.0043**	**0.0031**
NaCl level (mM)				
0	44.12a	46.25a	0.27a	0.18a
25	32.25b	38.49b	0.20b	0.12b
50	24.12c	29.87c	0.16c	0.09c
100	17.37d	22.50d	0.12d	0.06d
SEM	**0.58**	**0.58**	**0.006**	**0.004**
Priming				
NP	24.37d	27.87d	0.13d	0.07d
P1	27.62c	32.12c	0.16c	0.10c
P2	31.12b	36.25b	0.21b	0.13b
P3	34.75a	40.87a	0.24a	0.16a
SEM	**0.58**	**0.58**	**0.006**	**0.004**

SEM: Standard error of the mean
Seeds were either not primed (NP) or primed with NaCl (P1), $CaSO_4$ (P2) or NaCl+$CaSO_4$ (P3). Mean separation by Turkey's t-test. Means followed by the same letter along the column are not significantly different at P = 0.05.

7.4.2 Experiment 2

7.4.2.1 Effect of seed priming on photosynthetic rate of amaranth under salinity

The genotype, salinity and priming main effects were significant for photosynthetic rate (P_n) while the interactions between these factors were not significant. Photosynthetic rates recorded 28 days after transplanting were higher than those recorded at 14 days (Table 7.3). Across NaCl concentrations and priming treatments, P_n was greater in *A. tricolor* than in *A. cruentus.* Increasing NaCl concentration resulted in a decrease in P_n. For instance, P_n was reduced by 28% at 50 mM and by 35% at 100 mM NaCl 14 days after transplanting. At 28 days, the reduction in P_n was 15% and 32% when plants were exposed to 50 and 100 mM NaCl respectively.

Seed priming treatments resulted in increased photosynthetic rates. However, the effect depended on the type of salt used for priming and the time after transplanting when data was recorded. The effect of priming was observed to be greater 14 days after transplanting than 28 days after transplanting. At 14 days P_n was increased by 21% when NaCl was used for priming, 31% when $CaSO_4$ was used and by 42% when NaCl + $CaSO_4$ was used (Table 7.3). These increases were significantly lower 28 days after transplanting (19, 24 and 28% respectively).

7.4.2.2 Effect of seed priming on relative water content of amaranth under salinity

The response of relative water content (RWC) was similar to that of P_n with main effects being significant but not their interactions. The RWC increased as the number of days after transplanting increased and was significantly higher (8%) in *A. cruentus* compared to *A. tricolo*r at 14 and 28 days after transplanting (Table 7.4). Increasing NaCl concentration resulted in reductions in RWC. For instance, at 14 days after transplanting RWC was reduced by 13% when plants were supplied with 50 mM NaCl and by 20% when supplied with100 mM NaCl.

Table 7.3 Main effects of genotype, NaCl salinity and seed priming on photosynthetic rate of amaranth 14 and 28 days after transplanting

Main effects	Photosynthetic rate (µmol m^{-2} s^{-1})	
	14 days	28 days
Genotype		
A. tricolor	14.05a	15.82a
A. cruentus	10.50b	11.90b
SEM	**0.32**	**0.42**
NaCl level (mM)		
0	14.85a	16.47a
50	12.26b	13.96b
100	9.71c	11.14c
SEM	**0.39**	**0.51**
Priming		
NP	10.15c	11.97b
P1	12.28b	14.22a
P2	13.27ab	14.85a
P3	14.45a	15.33a
SEM	**0.45**	**0.59**

SEM: Standard error of the mean
Seeds were either not primed (NP) or primed with NaCl (P1), $CaSO_4$ (P2) or NaCl+$CaSO_4$ (P3). Mean separation by Turkey's t-test. Means followed by the same letter along the column are not significantly different at P = 0.05.

Seed priming increased RWC at both 14 and 28 days after transplanting. The effect of priming was more pronounced when NaCl + $CaSO_4$ was used for priming than the other priming treatments. For instance, at 14 days after emergence the RWC was increased by 8, 15 and 22% when NaCl, $CaSO_4$ or NaCl + $CaSO_4$ respectively, were used for priming (Table 7.4).

Table 7.4 Main effects of genotype, NaCl salinity and seed priming on relative water content of amaranth 14 and 28 days after transplanting

Main effects	Relative water content (%)	
	14 days	28 days
Genotype		
A. tricolor	71.25b	76.42b
A. cruentus	76.83a	82.33a
SEM	**0.73**	**1.14**
NaCl level (mM)		
0	82.75a	90.5a
50	72.37b	77.62b
100	67.00c	70.00c
SEM	**1.14**	**0.90**
Priming		
NP	67.12c	74.67c
P1	72.33b	78.77b
P2	77.41ab	82.34ab
P3	81.83a	84.83a
SEM	**1.31**	**1.04**

SEM: standard error of the mean
Seeds were either not primed (NP) or primed with NaCl (P1), $CaSO_4$ (P2) or NaCl+$CaSO_4$ (P3). Mean separation by Turkey's t-test. Means followed by the same letter along the column are not significantly different at P = 0.05.

7.4.2.3 Effect of seed priming on vegetative growth of amaranth under salinity

The genotype x seed priming and salinity x seed priming interactions on vegetative growth were not significant, indicating that the main effect of seed priming is representative of both genotypes, and was similar at the different salinity levels. The vegetative growth parameters increased significantly when the seed was primed (Table 7.5). Plants in the control treatments were the shortest (25 cm), produced the least number of leaves (36) and had the smallest total leaf area per plant (1267.4 cm^2). All the

priming treatments resulted in increases in the recorded plant growth parameters. However, the highest increases were observed in plants derived from seeds primed with NaCl + $CaSO_4$. Priming increased plant height by 18% in NaCl primed seeds to 48% in the NaCl + $CaSO_4$ treatment. The number of leaves was increased by 15 to 35%. The least effect of priming was noted in leaf area, with increases of between 2 to 4% (Table 7.5).

Table 7.5 Effects of seed priming on plant height, leaf number and total leaf area of amaranth under salinity taken 28 days after transplanting

Main effect	Plant height (cm)	Leaf number	Leaf area (cm^2/plant)
Priming			
NP	25.0d	36.5d	1267.4d
P1	29.5c	41.9c	1291.5c
P2	33.6b	46.3b	1308.8b
P3	37.0a	49.3a	1322.3a
SEM	**0.58**	**0.52**	**3.19**

SEM: Standard error of the mean
Seeds were either not primed (NP) or primed with NaCl (P1), $CaSO_4$ (P2) or NaCl+$CaSO_4$ (P3). Mean separation by Turkey's t-test. Means followed by the same letter along the column are not significantly different at P = 0.05.

The interactive effect of genotype and salinity was significant for plant growth parameters. In general, increasing the NaCl concentration resulted in reductions in plant height, leaf number and leaf area. Plant height and leaf number were reduced to a greater extent in *A. tricolor* than in *A. cruentus*. For instance, in *A. tricolor* plant height was reduced by 29% when plants were supplied with 50 mM NaCl and by 40% when supplied with 100 mM. On the other hand, plant height in *A. cruentus* was reduced by 13

and 28% respectively (Table 7.6). No difference in plant height was noted between plants supplied with 50 mM and those supplied with 100 mM NaCl in *A. tricolor*.

Leaf area was reduced to the same extent in both genotypes when 50 mM NaCl was supplied. However, when 100 mM was supplied, the reduction in leaf area was higher in *A. tricolor* (58%) than in *A. cruentus* (49%) (Table 7.6).

Table 7.6 Interactive effects of NaCl salinity and genotype on amaranth plant height, leaf number and leaf area

Genotype	NaCl salinity (mM)	Plant height (cm)	Leaf number	Leaf area (cm^2/plant)
A. tricolor	0	25.1d	66.2a	1904.8a
	50	17.9e	52.0b	1238.5c
	100	15.1e	39.0c	8.6.3f
A. cruentus				
	0	50.0a	40.0c	1760.2b
	50	43.5b	35.2d	1168.1d
	100	36.0c	28.6e	906.9e
SEM		**0.71**	**0.64**	**3.91**

SEM: Standard error of the mean
Mean separation by Turkey's t-test. Means followed by the same letter along the column are not significantly different at P = 0.05.

Genotype and NaCl salinity had a significant effect on shoot and root dry mass, as well as on shoot:root ratio while seed priming did not have any significant effect on shoot:root ratio (Table 7.7). *A. cruentus* had higher shoot dry mass, root dry mass and shoot:root ratio when compared to *A. tricolor*. Shoot dry mass, root dry mass and shoot:root ratio were reduced by increasing concentration of NaCl. Root dry mass was the most sensitive parameter with reductions of 45% at 50 mM and 62% at 100 mM NaCl. In comparison, shoot dry mass was reduced by 24 and 31% at 50 and 100 mM NaCl, respectively.

Shoot:root ratio was reduced by 10% and 35% in plants supplied with 50 and 100 mM NaCl.

All the priming treatments resulted in increases in shoot and root dry mass compared to the controls. However, priming with NaCl + $CaSO_4$ had the highest effect. The increases in shoot dry mass ranged from 42% in plants derived from seeds primed with NaCl to 122% in plants derived from seeds primed with NaCl + $CaSO_4$. Similarly, root dry mass was increased by 48 to 122%. Shoot: root ratio was not affected by priming (Table 7.7).

7.4.2.4 Effect of seed priming on ion content of amaranth under salinity

The effects of genotype, NaCl salinity and priming were significant on shoot Ca^{2+} and K^+ content while the interactive effect of salinity and priming was significant on Na^+ content, Ca:Na ratio and K:Na ratio. *A. tricolor* contained higher levels of shoot Ca and K^+ than *A. cruentus* (Table 7.8). Increasing the NaCl concentration reduced Ca^{2+} content by 17% and K^+ content by 11% when plants were supplied with 50 mM NaCl. At 100 mM NaCl the reductions were 42 % and 28% (Table 7.8).

The effect of priming on Ca^{2+} and K^+ content varied with the priming treatment. Priming with NaCl resulted in a 25% increase in Ca^{2+} content and 29% in K^+ content. Greater increases in Ca and K^+ were observed when $CaSO_4$ or NaCl + $CaSO_4$ were used for priming. Priming with $CaSO_4$ or NaCl + $CaSO_4$ increased shoot Ca^{2+} content by 60 and 43% and K content by 52 and 37% (Table 7.8).

Generally, priming tended to reduce the accumulation of Na^+ in amaranth leaves (Table 7.9). However, its effect varied with NaCl concentration in the irrigation water. At 0 mM NaCl priming did not have any effect on Na^+ content which ranged between 0.1% in plants primed with $CaSO_4$ to 0.19% in NP seeds. Sodium content was significantly reduced in plants derived from seeds primed with $CaSO_4$ or NaCl + $CaSO_4$ compared to NP seeds or those primed with NaCl when 50 or 100 mM NaCl was supplied. For instance, at 50 mM NaCl, the Na^+ content was reduced by 48% following priming with

$CaSO_4$, and by 41% in NaCl + $CaSO_4$ priming. There was no significant difference in Na^+ content in plants derived from NP seeds and those primed with NaCl.

Table 7.7 Main effects of genotype, NaCl salinity and seed priming on shoot dry mass, root dry mass and shoot: root ratio of amaranth 28 days after transplanting

Main effects	Shoot dry mass (g/plant)	Root dry mass (g/plant)	Shoot: root ratio
Genotype			
A. tricolor	6.8b	3.14b	2.30b
A. cruentus	10.6a	4.35a	2.63a
SEM	**0.25**	**0.17**	**0.08**
NaCl level (mM)			
0	11.0a	5.8a	2.9a
50	8.4b	3.2b	2.6a
100	6.7c	2.2c	1.9b
SEM	**0.30**	**0.21**	**0.10**
Priming			
NP	5.4d	2.3c	2.4a
P1	7.7c	3.4b	2.4a
P2	9.8b	4.2b	2.5a
P3	12.0a	5.1a	2.6a
SEM	**0.35**	**0.24**	**0.12**

SEM: Standard error of the mean
Seeds were either not primed (NP) or primed with NaCl (P1), $CaSO_4$ (P2) or NaCl+$CaSO_4$ (P3). Mean separation by Turkey's t-test. Means followed by the same letter along the column are not significantly different at P = 0.05.

The ratios of Ca:Na and K:Na decreased with increasing NaCl concentration. However, they were higher in primed than in NP seeds. At 0 mM NaCl plants derived from seeds primed with $CaSO_4$ had the highest Ca:Na and K:Na ratios (27.7 and 34.1) followed by

those from seeds primed with NaCl + $CaSO_4$ (18.2 and 22.1). The lowest ratios were observed in plants derived from NaCl primed seeds (13.9 and 18.1) (Table 7.9). When plants were supplied with 50 mM NaCl there was no significant difference in Ca:Na and K:Na ratios between the different priming treatments. At 100 mM NaCl, seed priming did not have any effect on Ca:Na and K:Na ratios (Table 7.9).

Table 7.8 Main effects of genotype, NaCl salinity and seed priming on ion content in leaves of amaranth 28 days after transplanting

Main effects	Ion content (% of dry weight)	
	Ca	K
Genotype		
A. tricolor	2.07a	2.72a
A. cruentus	1.84b	2.48b
SEM	**0.05**	**0.05**
NaCl level (mM)		
0	2.4a	2.99a
50	1.99b	2.65b
100	1.40c	2.15c
SEM	**0.06**	**0.07**
Priming		
NP	1.45c	2.00d
P1	1.81b	2.38c
P2	2.32a	3.05a
P3	2.08a	2.75b
SEM	**0.07**	**0.08**

SEM: Standard error of the mean
Seeds were either not primed (NP) or primed with NaCl (P1), $CaSO_4$ (P2) or NaCl+$CaSO_4$ (P3). Mean separation by Turkey's t-test. Means followed by the same letter along the column are not significantly different at P = 0.05.

Table 7.9 Interactive effects of NaCl salinity and seed priming on Na content Ca:Na and K:Na ratios in leaves of amaranth determined 28 days after transplanting

NaCl salinity (mM)	Priming	Ion content		
		Na (% d.w)	Ca:Na ratio	K:Na ratio
0	NP	0.19d	10.00d	12.35d
	P1	0.15d	13.95c	18.15c
	P2	0.10d	27.75a	34.10a
	P3	0.14d	18.25b	22.10b
50	NP	0.86b	1.75f	2.35f
	P1	0.83b	4.65e	5.90e
	P2	0.45c	5.96e	8.42e
	P3	0.51c	5.62e	7.35e
100	NP	1.34a	0.70f	1.10f
	P1	1.25a	1.15f	1.90f
	P2	0.86b	2.05f	3.15f
	P3	0.94b	1.70f	2.55f
SEM		**0.06**	**0.56**	**0.70**

SEM: Standard error of the mean
Seeds were either not primed (NP) or primed with NaCl (P1), $CaSO_4$ (P2) or NaCl+$CaSO_4$ (P3). Mean separation by Turkey's t-test. Means followed by the same letter along the column are not significantly different at P = 0.05.

7.5 DISCUSSION

7.5.1 Experiment 1

Seedling emergence, survival and growth

Sodium chloride salinity caused decreases in total emergence and seedling survival, and inhibited growth in amaranth seedlings. These negative effects of salinity on amaranth growth are similar to those reported in tomato (Cayuela *et al*., 1996) and melon (Botia *et*

al., 1998; Carvajal *et al*., 1998). Seed priming counteracted the inhibition effect of salinity on seedling emergence and growth in amaranth, as has been shown in priming treatments in cucumber (Passam and Kakouriotis, 1994) tomato (Cayuela *et al*., 1996) and melon (Svritepe *et al*., 2003). The total emergence and dry mass were higher in amaranth seedlings derived from primed seeds which emerged earlier than those from non-primed seeds. The results suggest that in both amaranth genotypes, seedlings derived from primed seed adapt better to salinity. Levitt (1980) states that salt resistant plants possess adaptation mechanisms originating from osmoregulation, which is the basis of their tolerance to salt-induced osmotic stress. Osmoregulation can occur in plants by active uptake of inorganic ions (such as Na^+, K^+ and Cl^-) or synthesis of organic solutes (such as sugars, organic acids, free amino acids and proline) depending on the species (Levitt, 1980; Hasegawa *et al*, 1986). According to Cayuela *et al*. (1996) working with tomatoes and Sivritepe *et al*. (2003) working with melon, the higher adaptation capacity of seedlings from primed seed to salinity could be due to osmoregulation induced by organic solutes.

The positive effect of seed priming on plant growth in short-term experiments may be due to the earlier emergence of the seedlings from primed seeds than from non-primed seeds (Figure 7.2; 7.3). Similar observations were reported in cucumber (Passam and Kakouritis, 1994) and muskmelon (Nascimento and West, 1999). These authors observed that the major effects of seed priming on seedling growth were due to earlier germination. In addition, seed priming was found to minimize seed coat adherence during emergence of muskmelon seeds (Nascimento and West, 1998).

Seedling survival decreased with increasing NaCl concentration. However, seed priming alleviated the detrimental effects of salinity on survival (Table 7.1). A higher effect of priming on survival was observed in seedlings derived from seeds primed with $CaSO_4$ + NaCl and $CaSO_4$ compared to those primed with NaCl. Buerkert and Marschner (1992) postulated that the main effect of Ca^{2+} supply on survival of bean seedlings was to decrease exudation of amino acids and carbohydrates from seeds and seedlings. Exudates

attract and activate zoospores, thereby resulting in increased fungal infection (Kuan and Erwin, 1980) and, hence, reduced seedling survival.

The results showed a tendency for the shoot and root of primed seeds to elongate at a faster rate than those of non-primed seeds (Table 7.2). This outcome would be expected, since many of the metabolic processes involved with the early phases of germination had already been initiated during priming. With a faster rate of hypocotyl elongation, the primed seeds emerged earlier and attained a greater shoot length than the non-primed seeds. This implies that primed seed will be less vulnerable to soil fungal and bacterial pathogens since it emerges faster, and can also lead to a more uniform plant stand. Furthermore, rapid seedling establishment minimize crop risk due to environmental conditions in the field. A uniform stand of healthy, vigorous plants is important for profitable amaranth production under saline conditions. A greater increase in root length in seedlings derived from seeds primed with $CaSO_4$ + NaCl and $CaSO_4$ compared to those primed with NaCl may be due to the availability of Ca which improved the conditions for root growth in the microenvironment around the seed. Kirkby and Pilbeam (1984) stated that calcium is involved in cell division and elongation.

7.5.2 Experiment 2

7.5.2.1 Effect of seed priming on photosynthetic rate and relative water content of amaranth under salinity

Photosynthetic rate (P_n) and relative water content (RWC) were determined at two-week intervals in order to determine whether adaptation to salinity in plants derived from primed seeds persist to later stages of growth. These parameters were higher in plants derived from primed seed than those from non-primed seed 14 days after transplanting. At 28 days, P_n and RWC were still significantly higher in the primed than in the non-primed treatments (Table 7.3 and 7.4). This suggests that plants from primed seeds were more tolerant to salinity and maintained tolerance by attaining higher photosynthetic rates and relative water content at least until 28 days after transplanting.

Physiological changes induced by seed priming have seldom been studied in seeds (Nonogaki *et al*., 1992; Lanteri *et al*., 1993), or in plants from seeds primed with NaCl or $CaSO_4$. The hypothesis that seed priming induces physiological changes in plants, and these changes are more clearly shown at advanced stages of development was verified. This is in accordance with Amzallag and Lerner (1995) who defined adaptation as a long-term response during which the plant adjusts its physiology to the environmental conditions.

7.5.2.2 Effect of seed priming on vegetative growth of amaranth under salinity

Plant height, leaf number, leaf area, and shoot and root dry mass were significantly higher in the primed compared to non-primed treatments 28 days after transplanting (Table 7.7). In order to study the effect of seed priming on plant growth it is necessary to determine plant growth over a longer time period, as short-term results may only reflect the earlier emergence. The results from this study show that tolerance to salinity was maintained during the growth period, hence, plant growth parameters remained significantly higher in primed than in non-primed treatments at 28 days after transplanting. Similarly, Cano *et al*. (1991) and Cayuela *et al*. (1996) showed that in some tomato cultivars grown under saline conditions, fruit yield was higher in plants from primed seeds than from non-primed seeds. According to Cayuela *et al*. (1996) the better growth of plants from primed seeds seems to result from a higher capacity for osmotic adjustment. Moreover, a better adaptation capacity was found at moderate levels of salinity than at high levels. This could be due to the negative effect of high salt level during the growing period predominating over the positive effect of salt priming of seeds, as indicated by Cano *et al*. (1991).

Differences in adaptation induced by seed priming were noted between amaranth genotypes with *A. cruentus* showing a better adaptation to saline conditions than *A. tricolor*. Amzallag *et al*. (1993) indicated that different sorghum genotypes exposed to similar adaptation-inducing conditions showed different degrees of adaptation, suggesting a genetic component in the capacity for adaptation. Thus, it would be interesting to repeat this study using other amaranth genotypes showing different

physiological responses to salinity in order to determine whether the capacity for adaptation varies between other genotypes within the species.

7.5.2.3 Effect of seed priming on leaf ionic content of amaranth under salinity

Salt induced injuries can occur not only due to osmotic and oxidative effects, but also due to toxic and nutrient deficiency effects of salinity. Exposure of amaranth to NaCl caused an increase in Na^+ and a decrease in K^+ and Ca^{2+} concentrations in leaves in all the treatments (Tables 7.8 and 7.9). Similar effects of salinity on ion content was reported in Chapter 6, and was also found in celery (Pardossi *et al*., 1999), eggplant (Chartzoulakis and Klapaki, 2000) and tomato (Alian *et al*., 2000; Romero-Aranda *et al*., 2001). In addition, accumulation of Na^+ changes ion balances such as Ca: Na and K:Na in plant cells under saline conditions. Reductions in these ratios were noted with increasing NaCl concentration (Table 7.9). Similarly, in melon seedlings from non-primed seeds, Na:Ca ratio increased while K:Na ratio decreased depending on salinity level (Sivritepe *et al*., 2003). According to Levitt (1980), increase in the Ca:Na balance results in increased cell permeability, while an increased K:Na ratio causes decreased use of metabolic energy.

Seed priming resulted in reduced Na^+ accumulation in amaranth leaves and increased the Ca^{2+} and K^+ content and the Ca:Na and K:Na ratios (Tables 7.8 and 7.9). The results showed that seed priming decreased the detrimental effects of salinity on ion metabolism by decreasing Na^+ and increasing K^+ and Ca^{2+} accumulation. Sivritepe *et al*. (2003) made similar observations with melon seedlings derived from seeds primed with NaCl. Numerous studies indicated that an increase in the concentration of Ca^{2+} in plants challenged with salinity stress could ameliorate the inhibitory effects on growth (Navarro *et al*., 2000; Kaya *et al*., 2002). Furthermore, higher Ca^{2+} accumulation capacity under saline conditions can sustain the Na:Ca balance, which is responsible for the semi-permeability of cell membranes (Greenway and Munns, 1980). The results suggest that priming of amaranth seeds increased salt tolerance by promoting K^+ and Ca^{2+} accumulation.

7.6 CONCLUSIONS

Sodium chloride salinity had detrimental effects on amaranth seedling emergence and survival, as well as on relative water content, photosynthetic rate, ion accumulation and plant growth. Sodium concentration increased in shoots while K^+ and Ca^{2+} concentrations decreased with salinity. Seed priming increased amaranth salt tolerance at moderate salinity by partly alleviating the detrimental effects of salinity on the studied parameters. The most effective priming treatment was with NaCl + $CaSO_4$. Apparently, priming seeds with small amounts of Ca^{2+} appeared to provide sufficient Ca^{2+} to enable amaranth to establish well in saline soils. This study showed that seed priming can be used to increase salt tolerance in amaranth. Hence, seed priming to optimize seedling establishment and plant growth in saline soils deserves more attention.

CHAPTER 8

GENERAL DISCUSSION

Due to the problem of salinity worldwide efforts are being made to combat it. One strategy in dealing with salinity has been suggested to be the growing of salt tolerant plants and this has increased the need to understand salt tolerance in plants. Amaranth adapts well to diverse environments, is relatively free from serious pests and diseases, is easy to cultivate and has high nutritive value. It is, therefore, a promising crop for arid and semi-arid areas. However, such areas suffer from salinity problems. Compared to many other crops, amaranth has received relatively little research attention regarding salinity effects. The approach of this study was to focus on some of the responses of amaranth genotypes to salinity in order to assess salt tolerance in this species.

8.1 Salinity tolerance at different stages of plant development

In a study evaluating the effect of salinity stress on amaranth seed germination and seedling growth (Chapter 2), it was found that salinity reduced seed germination and seedling growth. However, amaranth was more tolerant during germination than during emergence and seedling growth. These results are in agreement with reports on Lentil (*Lens culinaris* Medic.) (Ashraf and Waheed, 1990), *Hordeum* spp (Mano and Takeda, 1998) and *Phaseolus* species (Bayuelo-Jiménez *et al*., 2002). These authors also demonstrated that salinity tolerance varies with plant ontogeny. Furthermore, initiation of salinity at different growth stages after seedling emergence (Chapter 3) showed that salinity tolerance in amaranth differs with the growth stage at which salinity is initiated. Amaranth was more tolerant to salinity when it was initiated at the 4-leaf stage than at cotyledon stage. Epstein and Rains (1987) has reported that during plant growth, the form and function of various organs change and that the plant's ability to respond to the salt stress depends upon the genes that are functioning at the stage of development during which the stress occurs. The relative sensitivity could change from one developmental stage to another. One of the reasons for decreasing sensitivity with age could be a gradual acclimation of the crop to salinity.

Evidence collected from various species suggests that salt tolerance is a developmentally regulated stage-specific phenomenon, so that tolerance at one stage of development may not be correlated with tolerance at other developmental stages (Shannon, 1986). Therefore, specific stages throughout the ontogeny of the plant, such as germination and emergence, seedling survival and growth should be evaluated separately during the assessment of germplasm for salt tolerance. Such assessments may facilitate development of cultivars with salt-tolerance characteristics throughout the ontogeny of the plant.

Information on the growth stage response to salinity within a crop is important in adopting suitable genetic and management strategies for saline soils. For example, if a crop is more sensitive during one stage than another, there is an opportunity to regulate the salinity of irrigation water during the season to minimize salt injury at the sensitive stage. One way is to substitute fresh water for saline for the duration of the sensitive stage. This procedure has been reported to markedly increase the apparent salt tolerance of some crops such as maize (Pasternak *et al.*, 1985).

8.2 Genotypic variability

Various strategies have been adopted by plant scientists in overcoming salinity and one important component is the evaluation of genetic variability of the cultivated species or its wild relatives to identify a tolerant genotype that may sustain a reasonable yield on salt affected soils (Kingsbury and Epstein, 1984). Genotypic variation in salinity tolerance at the germination stage and later amaranth growth stage was reported in Chapter 2 and 3. It was demonstrated that *A. tricolor* and Accession '83 were among the most salt tolerant genotypes during germination at 100 and 200 mM NaCl, but they were sensitive during emergence and early seedling growth. In Chapter 4, it was found that when amaranth plants were exposed to salinity one month after seeding *A. hypochondriacus* and *A. cruentus* were more tolerant than *A. tricolor* and Accession '83. The latter two genotypes did not survive in 200 mM NaCl treatment and the reduction in dry mass due to lower levels of salinity was much higher than in the former two genotypes. Thus, selection of plants for salinity tolerance should not only be based on

tolerance at seed germination since ultimately the final yield is the most important determinant factor for salinity tolerance.

Some of the strategies used by amaranth for salinity tolerance included efficient use of water and assimilate partitioning. *A. hypochodriacus* and *A. cruentus* utilized water more efficiently than *A. tricolor* and Accession '83, and this was related to lower stomatal conductance and reduced transpiration in the former genotypes. Although *A. tricolor* and Accession '83 had higher photosynthetic rates, dry mass was reduced to a greater extent than in *A. hypochondriacus* and *A. cruentus*. This shows that factors other than photosynthesis such as osmotic adjustment and protein synthesis may have contributed to plant growth (Wang and Nii, 2000). In plant production the marketable component of the plant is the most important. Under salinity stress *A. hypochondriacus* and *A. cruentus* partitioned most of the assimilates towards shoot growth and were able to maintain higher dry mass production than *A. tricolor* and Accession '83.

8.3 Salinity and water stress interactions

Salinity is normally associated with aridity, yet there is little or no information concerning the interactive effect of salinity and water stress in amaranth. This initiated the trials presented in Chapter 5. When amaranth plants were submitted to water stress, salt stress and a combination of water and salt stress, plants under salt stress and those in combined water and salt stress had higher biomass than those under water stress. This may have been due to a number of reasons:

a) Salt and salt + water stressed plants maintained higher water use efficiencies as a result of reduced stomatal conductance and transpiration rates compared to water stressed plants.

b) Increased salt concentration in the root medium or water stress led to an osmotic adjustment (lowering of leaf Ψ_π) that is generally accepted as an adaptation to salinity or water stress (Ghoulam *et al*., 2002; Iannucci *et al*., 2002). However, there was a greater adjustment in salt and salt + water stressed plants than in water stressed plants. According to Wyn Jones (1981) and Raven (1985), osmotic adjustment by salt accumulation, which

may have occurred in salt and salt + water stressed plants, is less energy and carbon demanding than the adjustment by organic solutes that may have taken place in water stressed plants.

Furthermore, salt-treated plants and those submitted to salt + water stress were able to survive for a longer period before wilting because of the reduced growth rate of plants, thereby decreasing the rate at which soil water is depleted (Glen and Brown, 1998). This implies that in areas with erratic rainfall, plants irrigated with saline water of low EC may be able to survive the dry period before the next rain compared to those irrigated with high quality water.

8.4 Amelioration of salinity stress effects by Ca nutrition and seed priming

One possible approach to reduce the effect of salinity on plant productivity is through the addition of calcium supplements to irrigation water. Several reports have indicated that supplemental Ca^{2+} (usually up to at least 5 mM) may alleviate the reduced growth caused by NaCl salinity. The effect of Ca^{2+} in ameliorating salinity stress in amaranth was investigated in Chapter 6. Both $CaSO_4$ and $CaCl_2$ were effective in partly ameliorating salinity stress effects on photosynthesis, growth and mineral uptake. The positive effect of Ca^{2+} may be due to its role in the protection of cell membranes from the adverse effects of salinity (Busch, 1995). This topic deserves further research attention. The application of different Ca^{2+} concentrations in order to determine the optimum is required. Furthermore, it would be interesting to establish at what developmental stage application of supplemental Ca^{2+} is more effective, as well as the duration of application.

The effect of seed priming in salt tolerance in amaranth was investigated in Chapter 7. Seed priming improved seedling emergence, survival and growth. However, the greatest positive effect was from seeds primed with $CaSO_4$ + NaCl compared to those primed with the individual salts. This may have been partly due to improved salt adaptation from NaCl (Cayuela *et al*., 1996) combined with the protective effect from Ca^{2+} (Busch, 1995). In the trial reported in Chapter 7, seeds were imbibed for three hours and left to dry for two days before planting. Although there were positive effects of priming, it would be

interesting to determine differences in the response with different periods of imbibition, as well as the time from priming to planting. On-farm field trials on the use of Ca^{2+} and seed priming for salinity stress amelioration will be necessary before final recommendations can be made.

8.5 Concluding remarks

This investigation evaluated some of the responses of amaranth to salinity stress and definite contributions were made:

1. Differences in salinity stress tolerance among amaranth genotypes were demonstrated and these differences varied with the stage of plant growth. Tolerance at one stage of development did not imply tolerance at another stage. Generally, amaranth was more salt tolerant at germination than at emergence and seedling growth, and more tolerant during the vegetative growth stage than at the cotyledon stage.
2. One of the responses to salinity stress involved changes in leaf anatomical features. This contributed to a better understanding of reasons for reduced photosynthetic rates with increasing salinity. In comparing two genotypes (*A. tricolor* and *A. cruentus*), it was found that although *A. tricolor* maintained relatively higher photosynthetic rates under saline conditions than *A. cruentus*, the reduction in growth was higher in the former genotype, showing that factors other than photosynthesis are involved in growth processes. There were negative correlations between leaf anatomical features (stomatal density, stomatal apertures and specific leaf area) and water use efficiency. From the results of this study, it may be concluded that *A. cruentus* may have attributed its salt tolerance to higher water use efficiency compared to *A. tricolor*.
3. It was demonstrated that salinity stress caused changes in the pattern of dry matter accumulation and partitioning to different plant parts of amaranth and genotype differences were observed. This may be one of the salt tolerance strategies.
4. The interactive effect of salinity and water stress was found to be less detrimental than the sum effects of the individual stresses. Hence, in drying soils, amaranth plants initially exposed to salinity stress may be able to survive longer than those supplied with high quality water under the same condition. Failure of photosynthesis to recover after the

removal of salt stress compared to water stress suggested that there was a toxic effect of salt concentration on the photosynthetic apparatus.

5. The capacity for supplementary calcium to ameliorate salinity stress effects on photosynthesis, growth and mineral uptake was demonstrated. One of the effects of calcium was found to be the reduction of Na^+ and Cl^- uptake.

6. The Priming experiment revealed that priming amaranth seed with NaCl, $CaSO_4$ or NaCl+$CaSO_4$ improved seedling emergence, survival and growth, and the positive effect of priming persisted to later plant growth. The priming effect of NaCl+$CaSO_4$ was higher than that of the other priming treatments.

8.6 Recommendations

- Further studies to determine the extent of variability for salt tolerance among other amaranth genotypes are greatly needed.
- A number of field studies are recommended since many physiological responses underlying adaptation may be overlooked when operating outside the field context.
- More information on the effect of salinization on productivity of land and the economic implications of the amelioration measures is necessary.

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