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*.

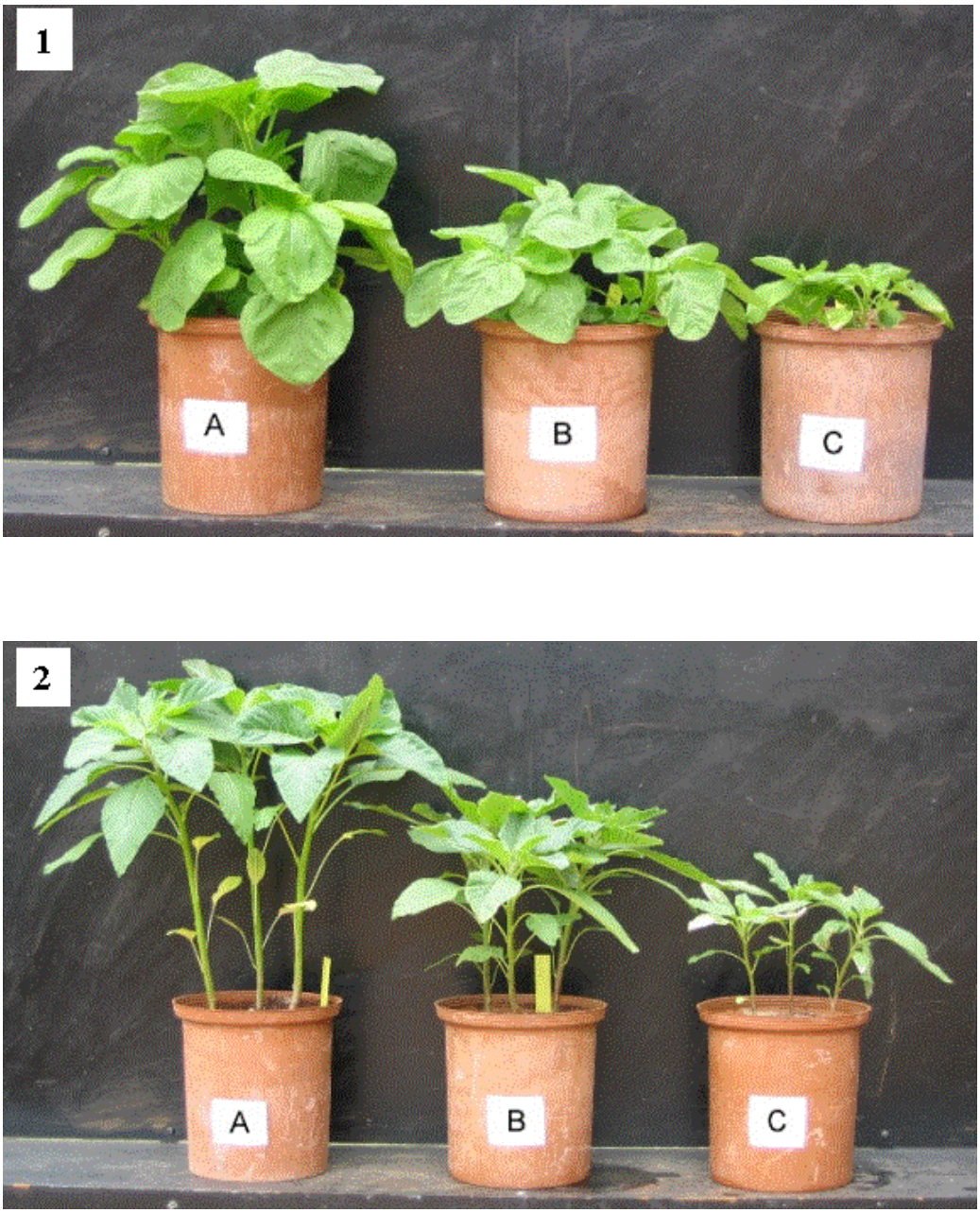

Figure 6.1 Growth of (1) *A. tricolor* and (2) *A. cruentus* supplied with (A) nutrient solution alone, (B) Nutrient solution plus 100 mM NaCl plus additional 10 mM Ca and (C) Nutrient solution plus 100 mM NaCl.

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.