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).

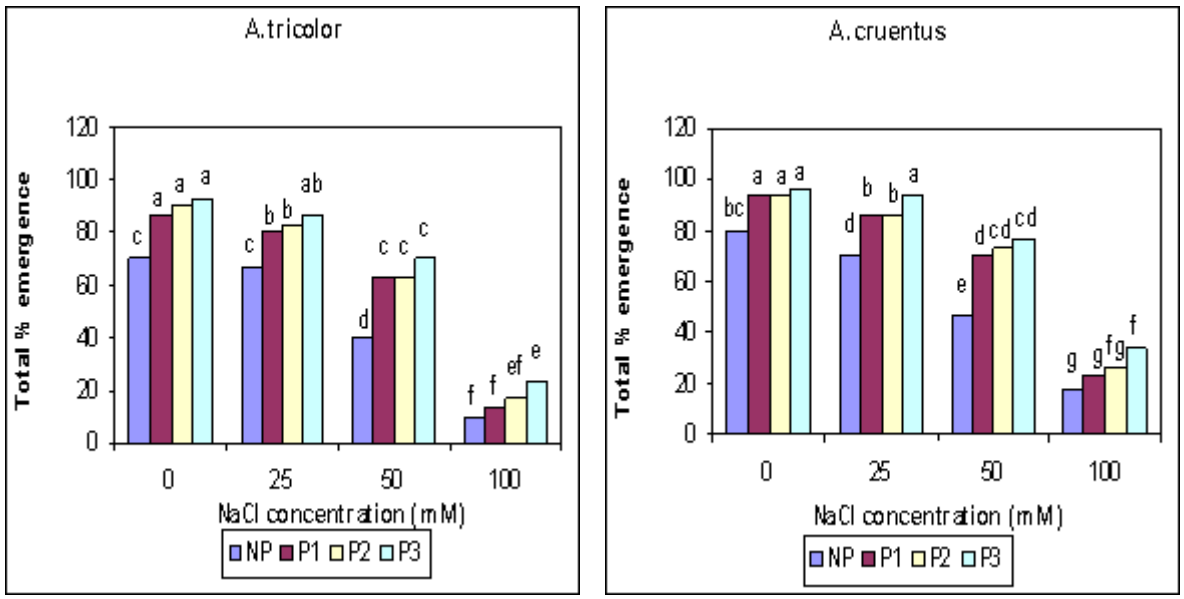

Figure 7.1 Effects of NaCl concentrations on seedling emergence of amaranth seedlings derived from non-primed seeds (NP) and seeds primed with NaCl (P1), $CaSO_4$ (P2) or NaCl + $CaSO_4$ (P3). Mean separation by Tukey's t- test. For each genotype bars followed by the same letter are not significantly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

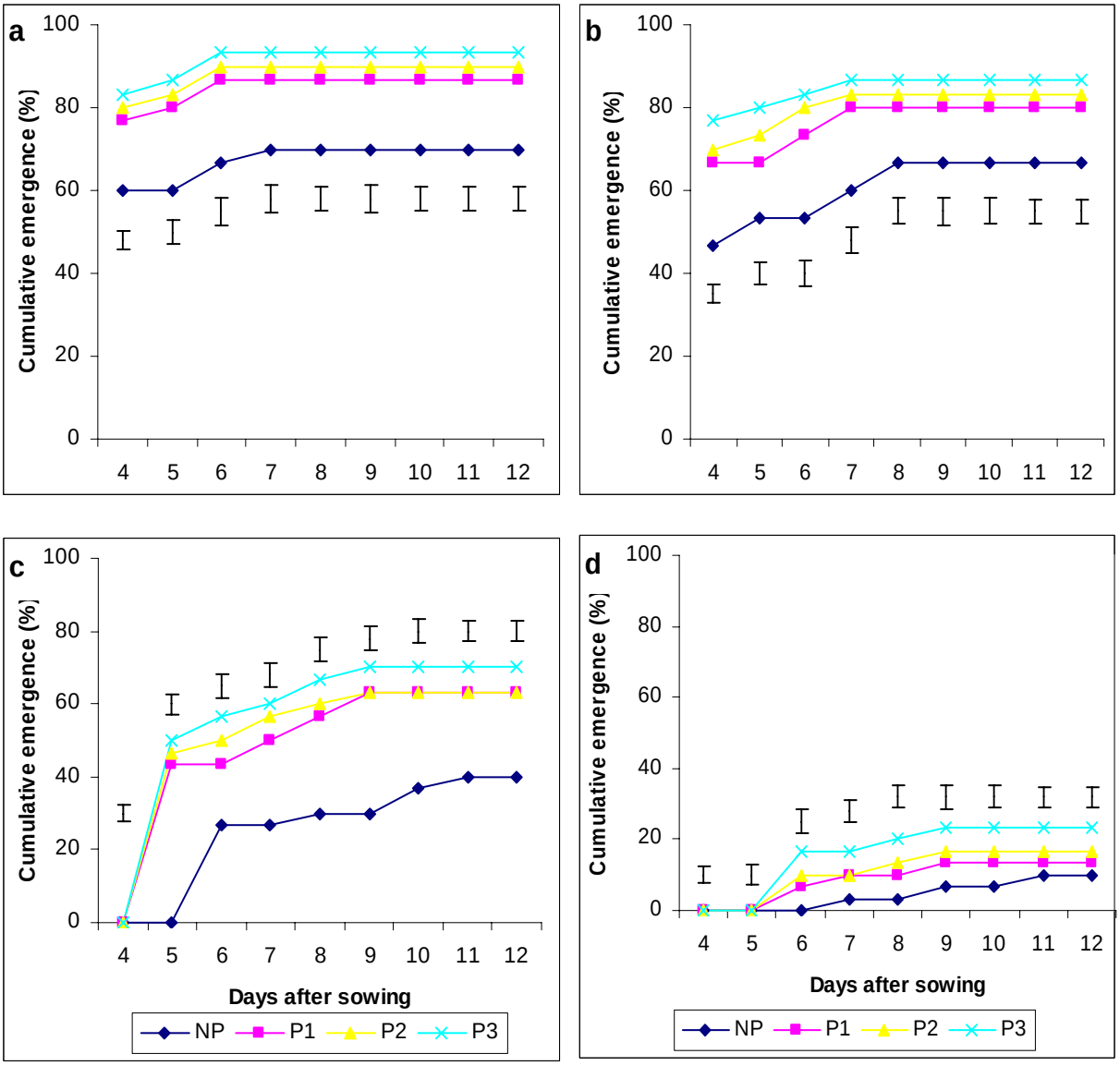

Figure 7.2 **Effect of (a) 0, (b) 25, (c) 50 and (d) 100 mM NaCl concentration on the time course of seedling emergence of *A. tricolor* derived from non-primed seeds (NP) and seeds primed with NaCl (P1), $CaSO_4$ (P2) or NaCl + $CaSO_4$ (P3). Vertical bars indicate least significant differences at P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

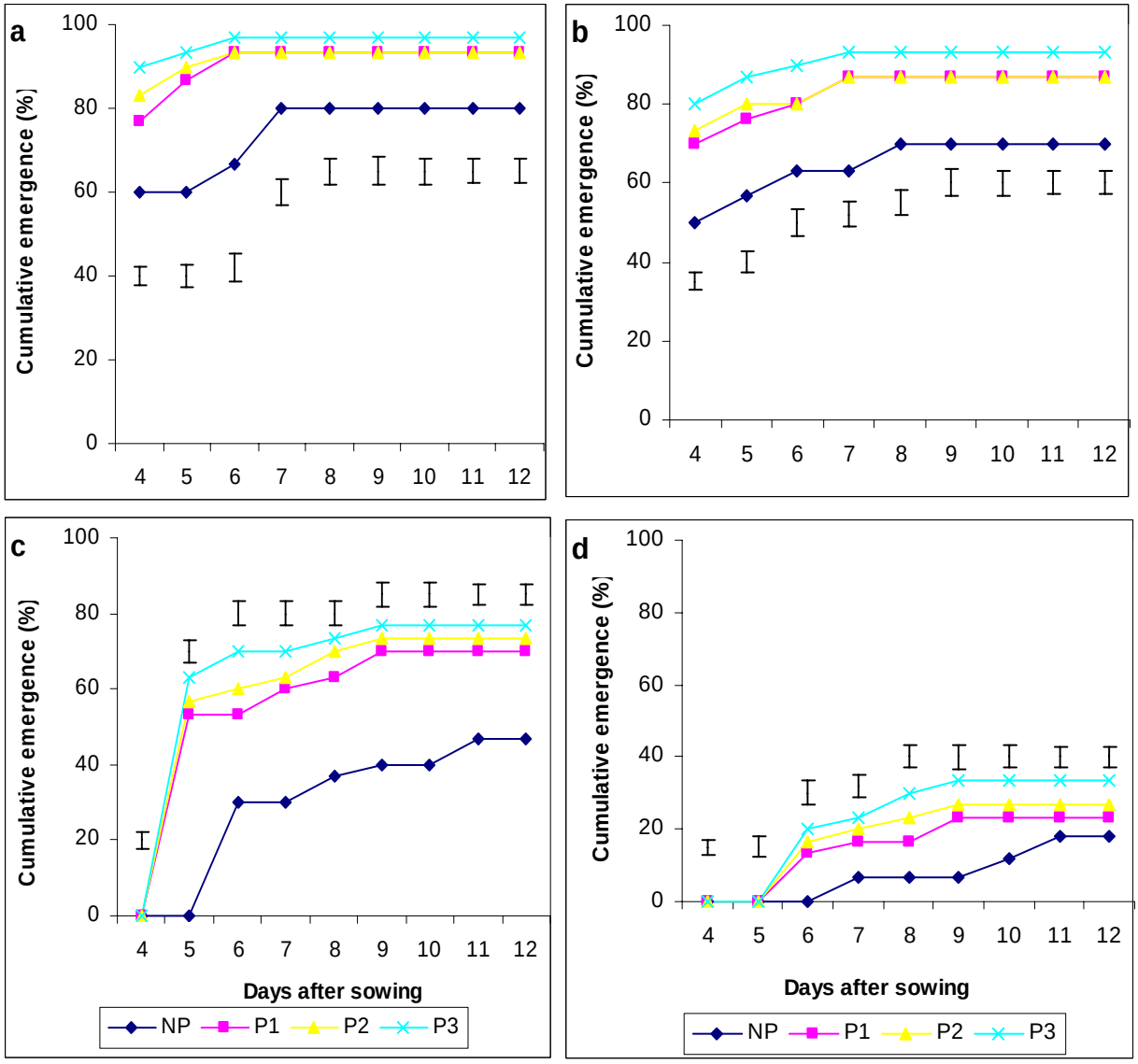

Figure 7.3 Effect of (a) 0, (b) 25, (c) 50 and (d) 100 mM NaCl concentration on the time course of seedling emergence of *A. cruentus* derived from non-primed seeds (NP) and seeds primed with NaCl (P1), $CaSO_4$ (P2) or NaCl + $CaSO_4$ (P3). Vertical bars indicate least significant differences at P =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