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).

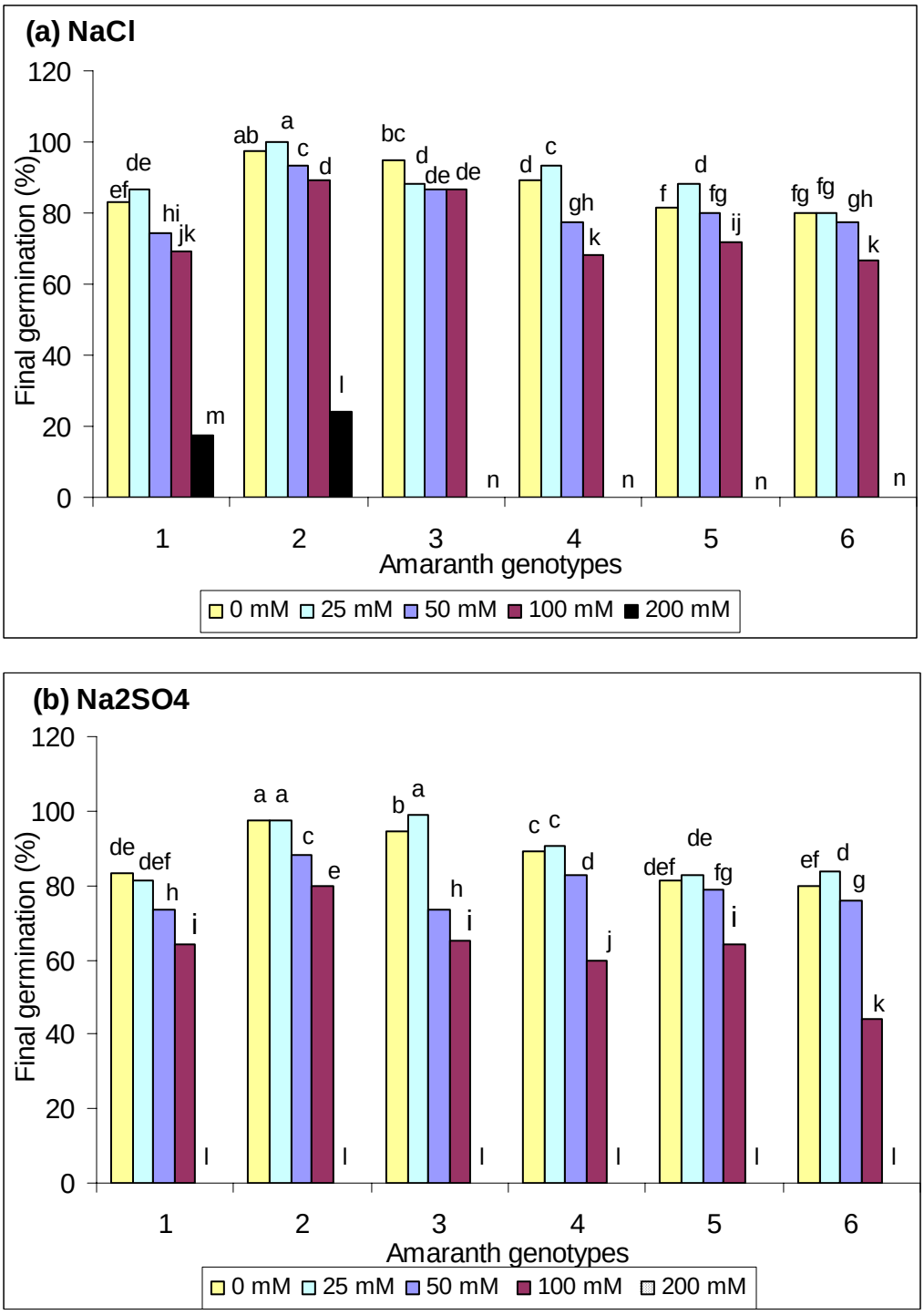

Figure 2.1 Effect of (a) NaCl and (b) Na_2SO_4 on germination of amaranth genotypes (1) *A. tricolor*, (2) Accession '83, (3) *A. dubius*, (4) *A. hypochondriacus*, (5) *A. cruentus* and (6) *A. hybridus*. Mean separation by Turkey T-test. Bars followed by the same letter are not significantly different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

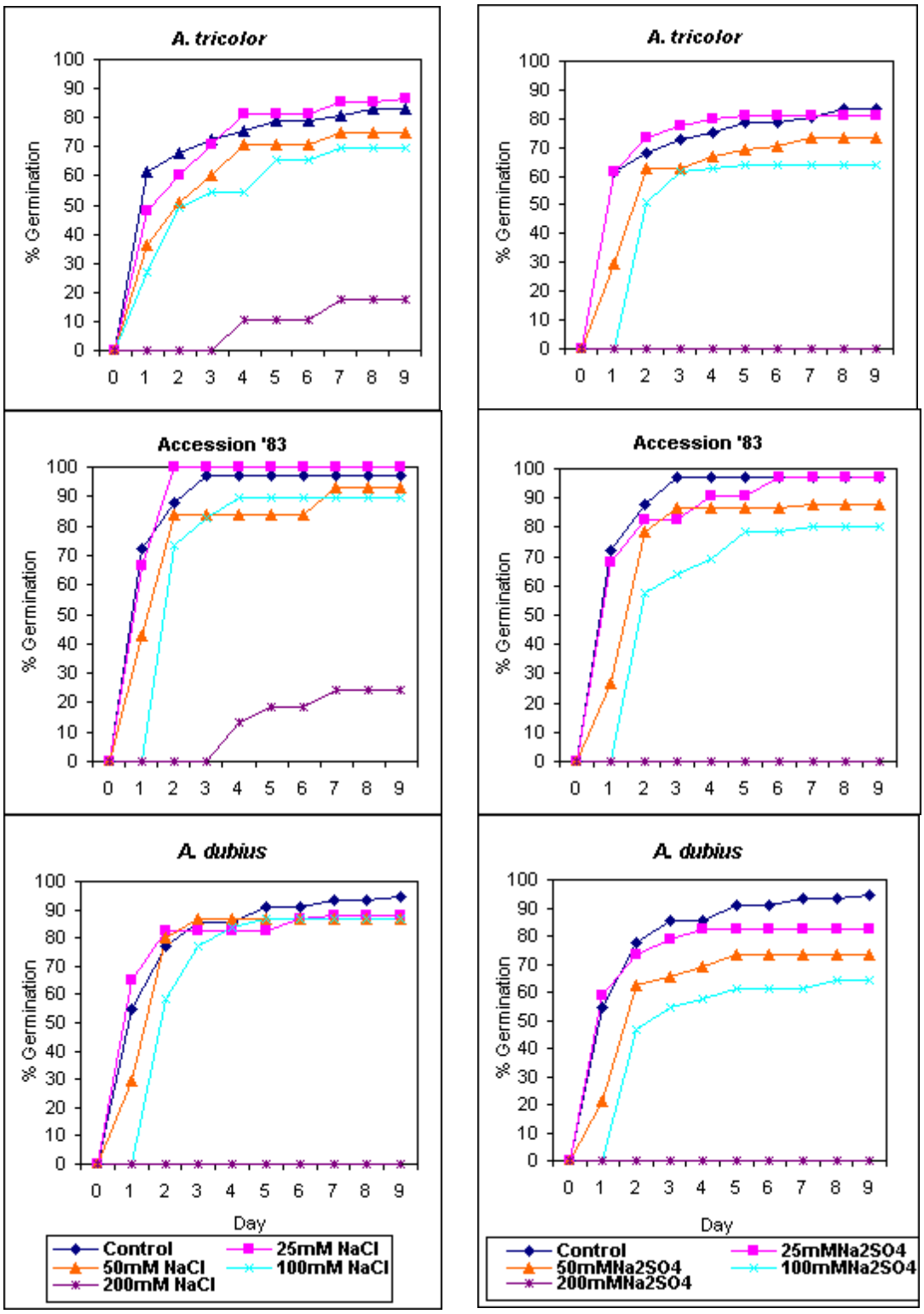

Figure 2.2a Effect of NaCl and Na_2SO_4 concentrations on the time course of germination of different amaranth genotypes.

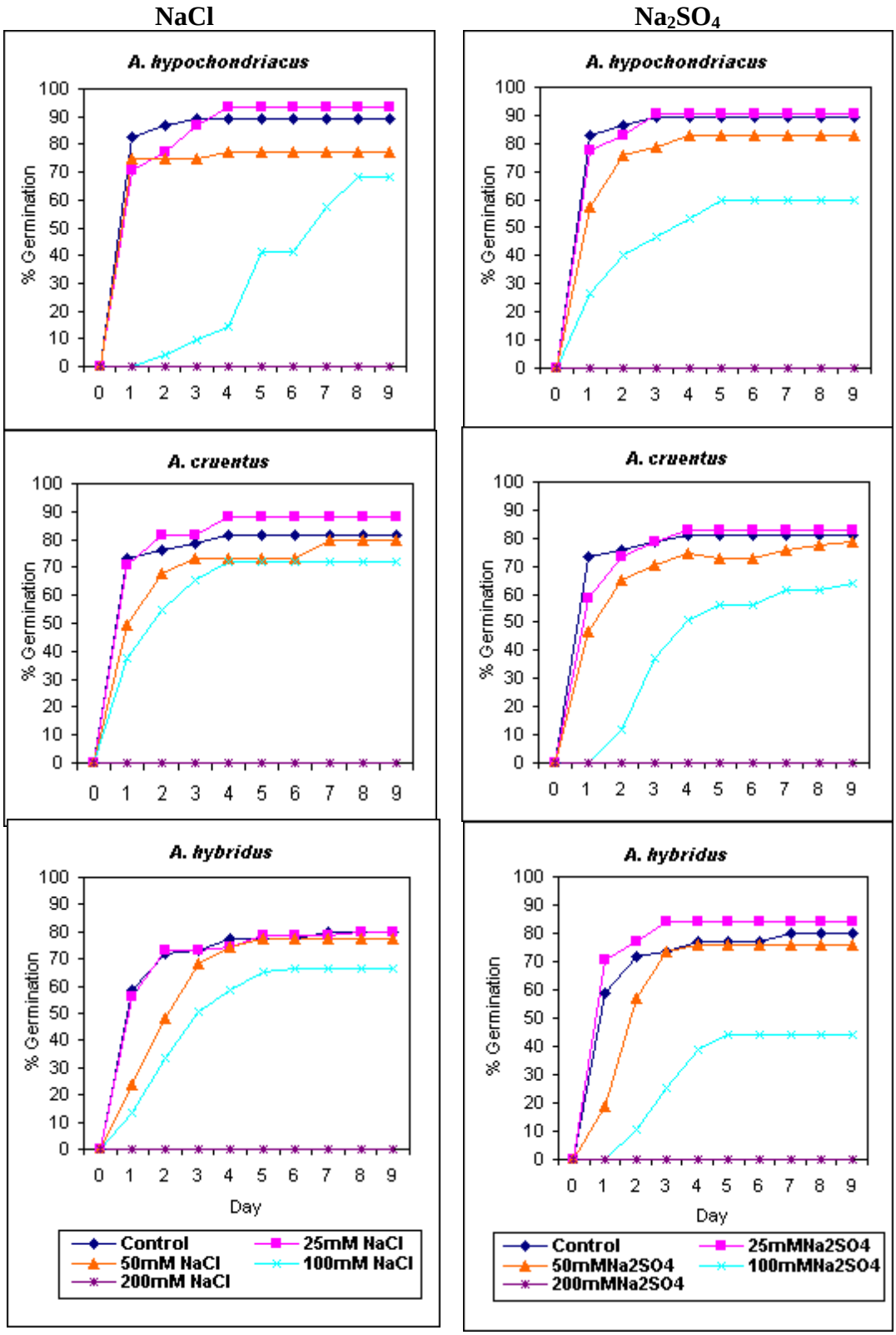

Figure 2.2b Effect of NaCl and Na_2SO_4 concentrations on the time course of germination of different amaranth genotypes.

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.

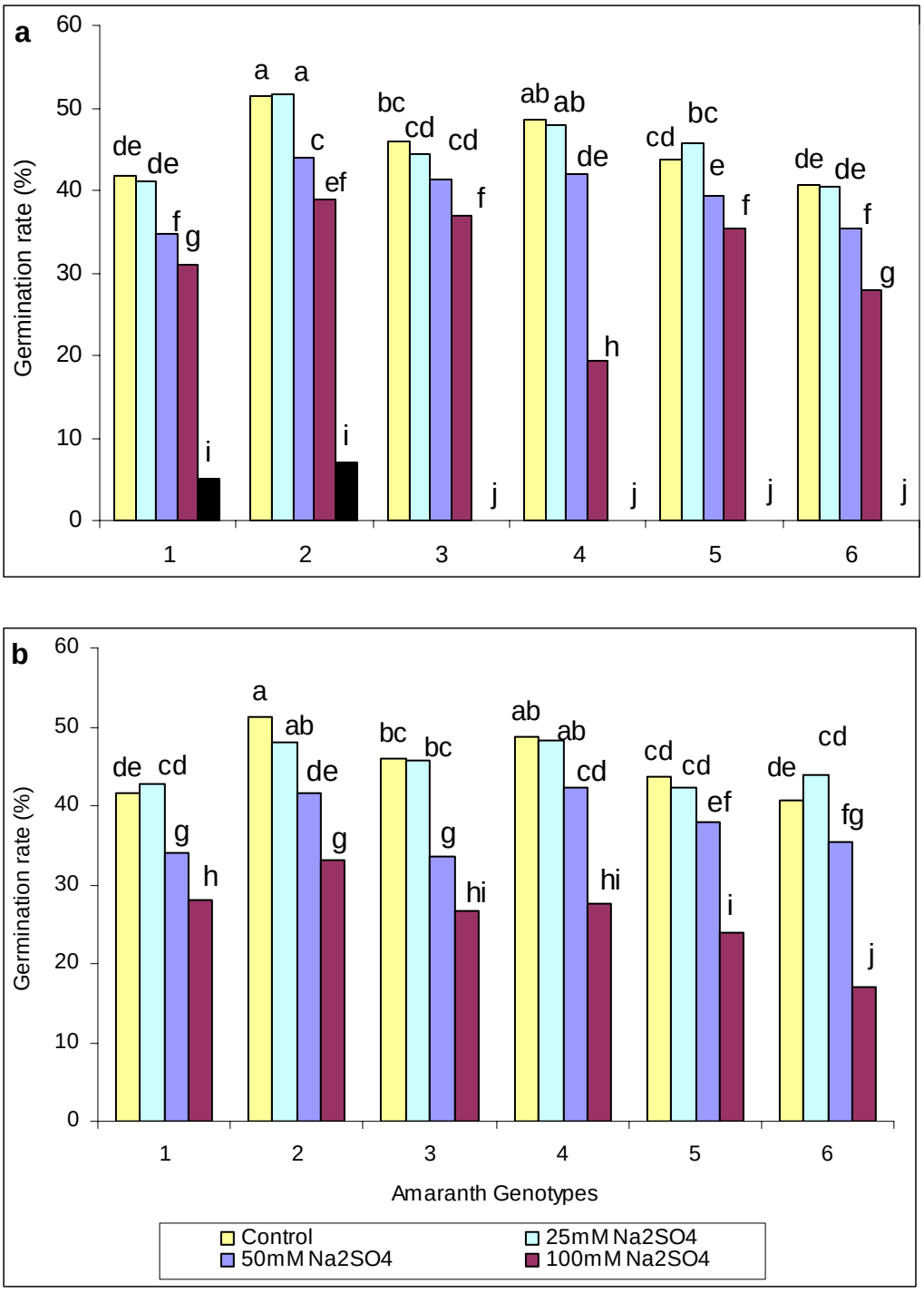

Figure 2.3 Effects of (a) NaCl and (b) Na_2SO_4 concentrations on the germination rate of (1) *A. tricolor* (2) Accession '83 (3) *A. dubius* (4) *A. hypochondriacus* (5) *A. cruentus* and (6) *A. hybridus*. Mean separation by Turkey T-test. Bars followed by the same letter are not significantly different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

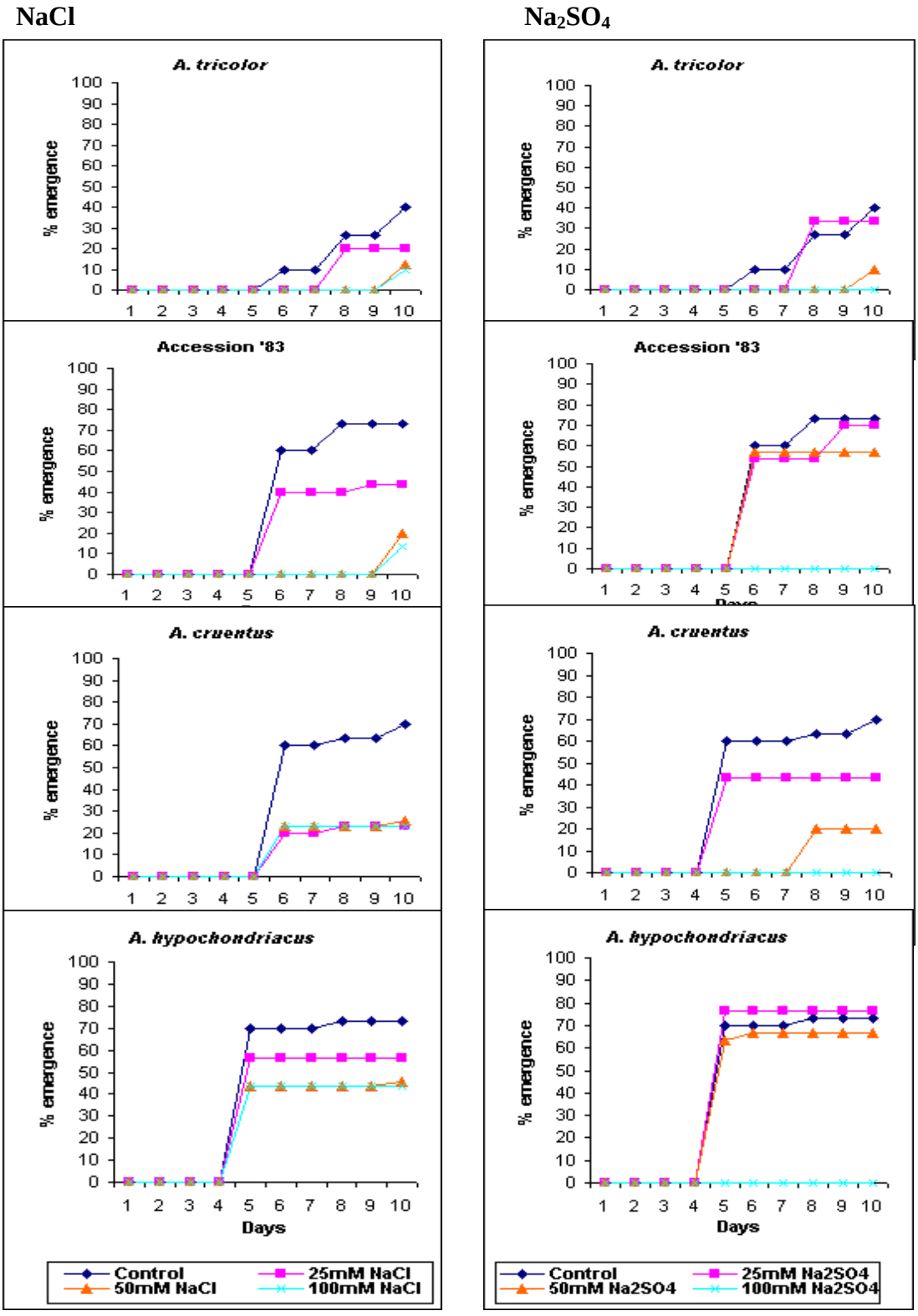

Figure 2.4 Effect of NaCl and Na_2SO_4 concentrations on the seedling emergence of different amaranth genotypes.

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).

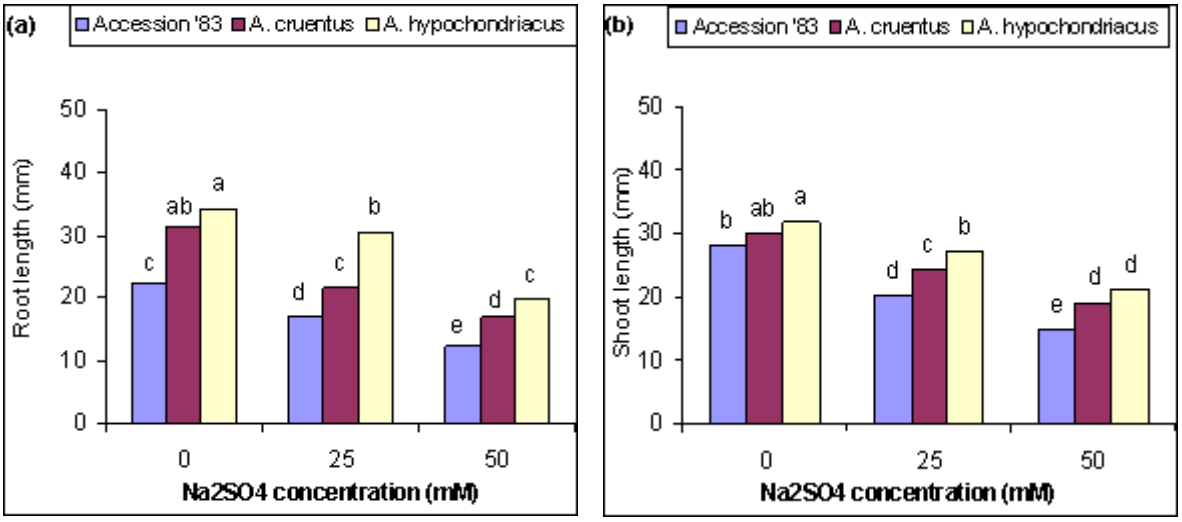

Figure 2.5 Effect of Na_2SO_4 concentrations on the (a) root length and (b) shoot length of three amaranth genotypes. Mean separation by Turkey T-test. Bars followed by the same letter are not significantly different at P = 0.05.

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.