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

3.1 ABSTRACT

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

Keywords: Amaranth genotypes; salinity tolerance; timing of salinization

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Contributions based on study:

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3.2 INTRODUCTION

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

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

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

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

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

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

3.3 MATERIALS AND METHODS

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

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

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

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

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

Data analysis

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

3.4 RESULTS

3.4.1 Experiment 1

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

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

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

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

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

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

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

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

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

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

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

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

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

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

SEM: Standard error of the mean

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

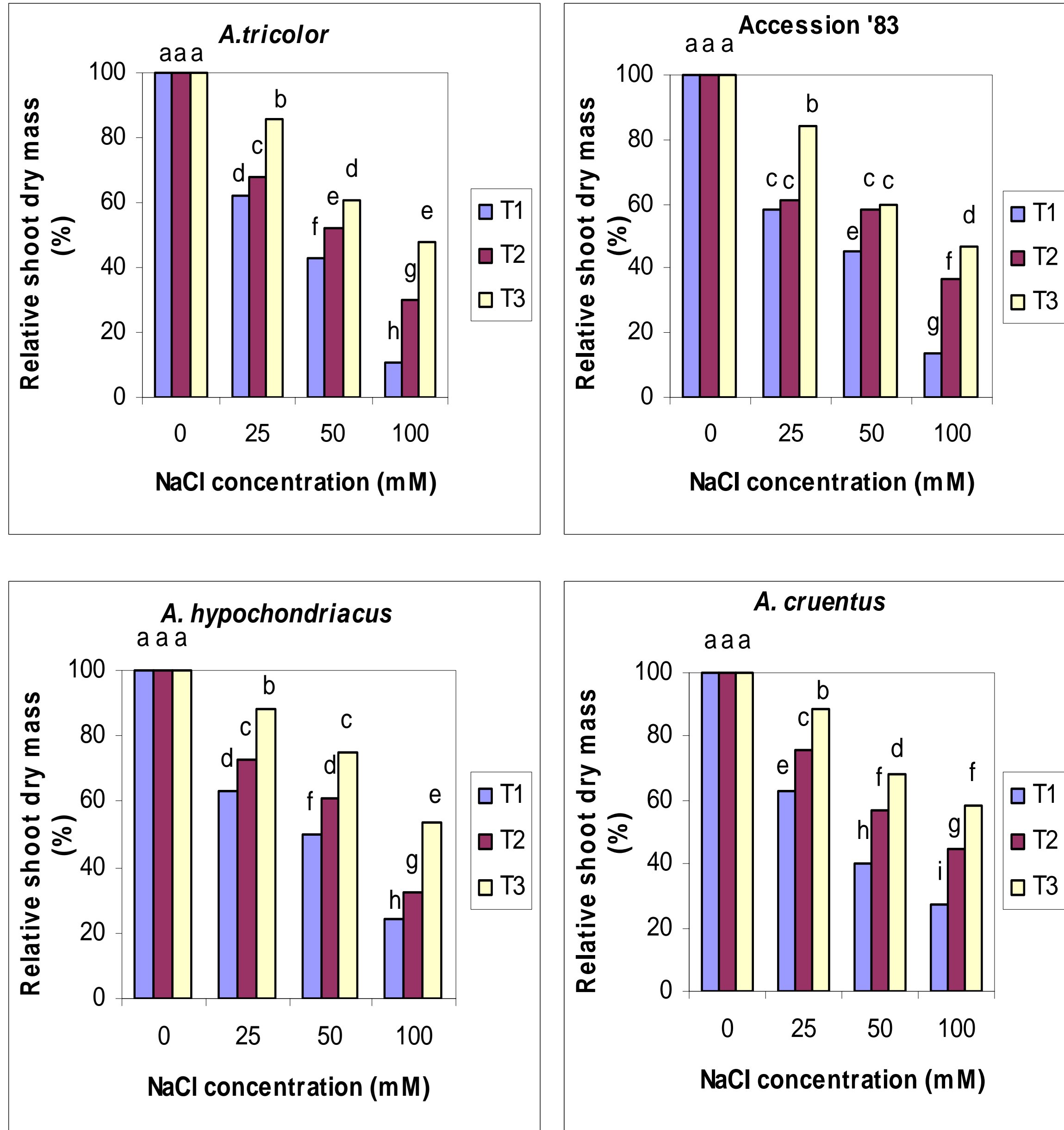

Figure 3.1 Effect of NaCl concentrations and stage of NaCl treatment application (T1- salinization at cotyledon stage, T2- at 2-leaf stage, and T3- at 4-leaf stage) on the relative shoot dry mass of different amaranth genotypes.
Mean separation by Turkey's t-test. For each genotype bars followed by the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

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

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

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

3.4.2 Experiment 2

3.4.2.1 Effect of stage of salinity treatment application on plant growth

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

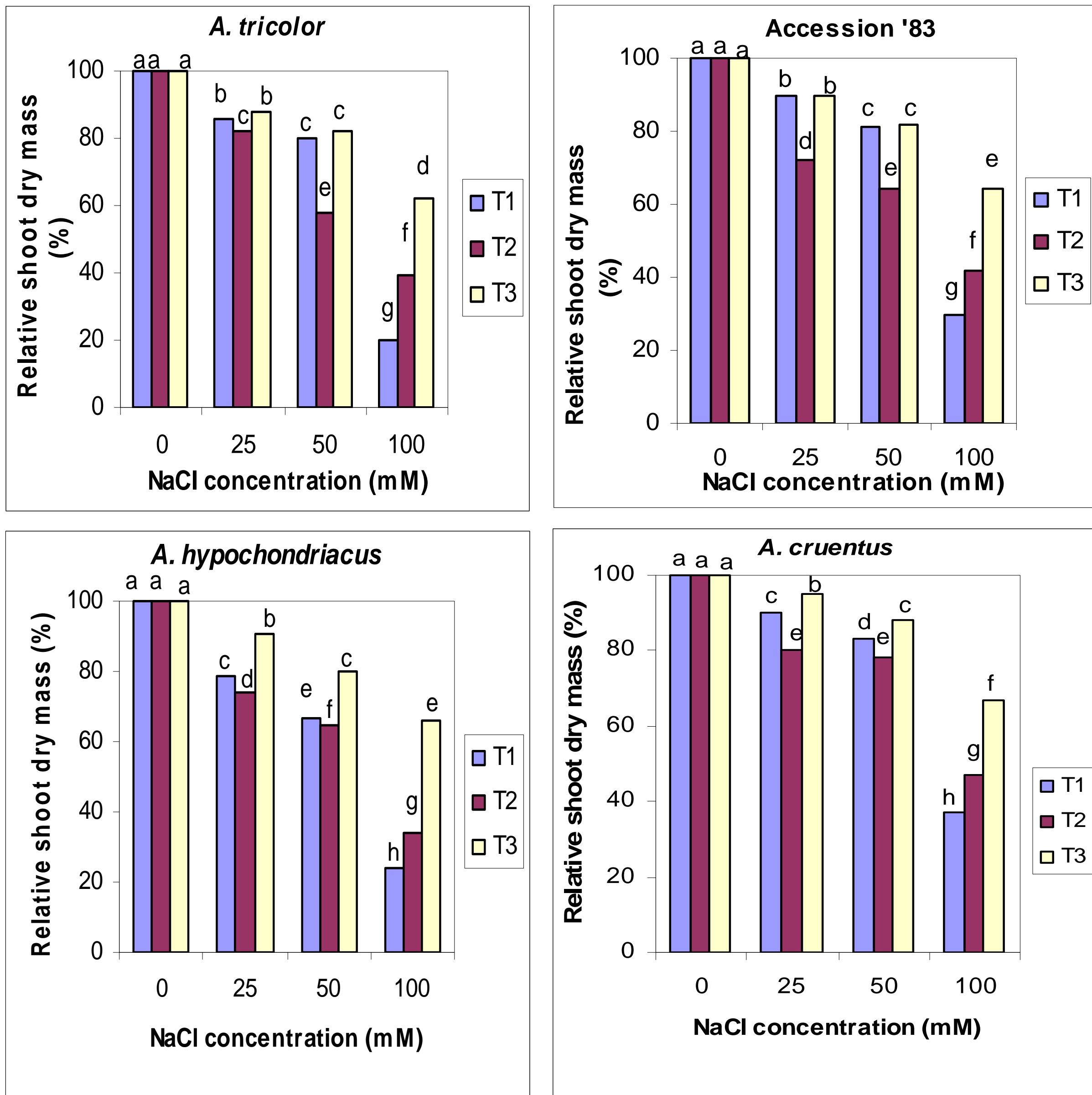

Figure 3.2 Effect of NaCl concentration applied for 14 days at different stages of growth: T1, salinization at cotyledon stage, T2, at 2-leaf stage, and T3, at 4-leaf stage, on shoot dry mass in different amaranth genotypes.
Mean separation by Turkey's t-test. For each genotype bars followed by the same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

3.4.2.2 Effect of stage of salinity treatment application on gas exchange

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

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

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

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

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

SEM: Standard error of the mean

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

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

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

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

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

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

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

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

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

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

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

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

3.5 DISCUSSION

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

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

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

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

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

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

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

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

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

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

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

3.6 CONCLUSION

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