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

5.1 ABSTRACT

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

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

Contributions based on study:

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5.2 INTRODUCTION

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

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

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

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

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

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

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

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

5.3 MATERIALS AND METHODS

5.3.1 Plant material and growth conditions

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

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

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

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

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

5.3.2 Water relations

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

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

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

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

5.3.3 Gas exchange

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

5.3.4 Water loss and water use efficiency

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

5.3.5 Statistical methods

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

5.4 RESULTS

5.4.1 Plant growth

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

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

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

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

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

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

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

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

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

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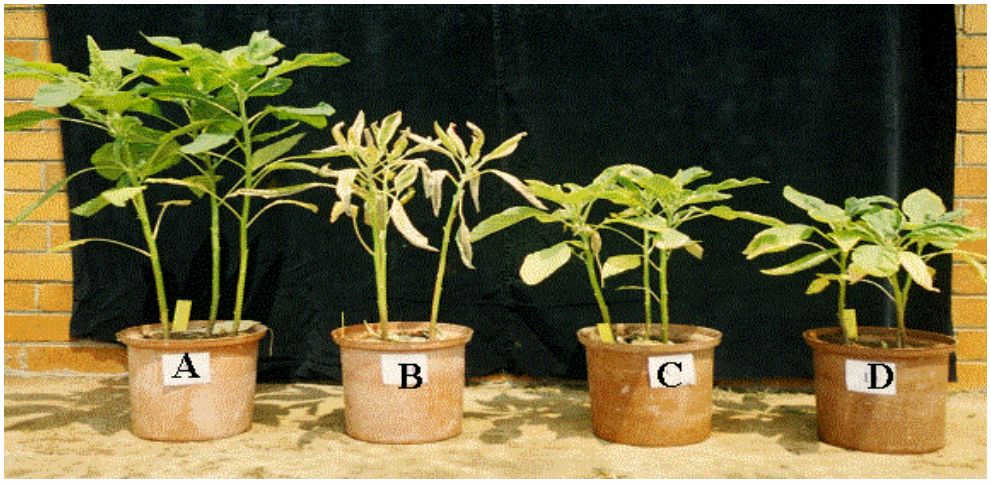

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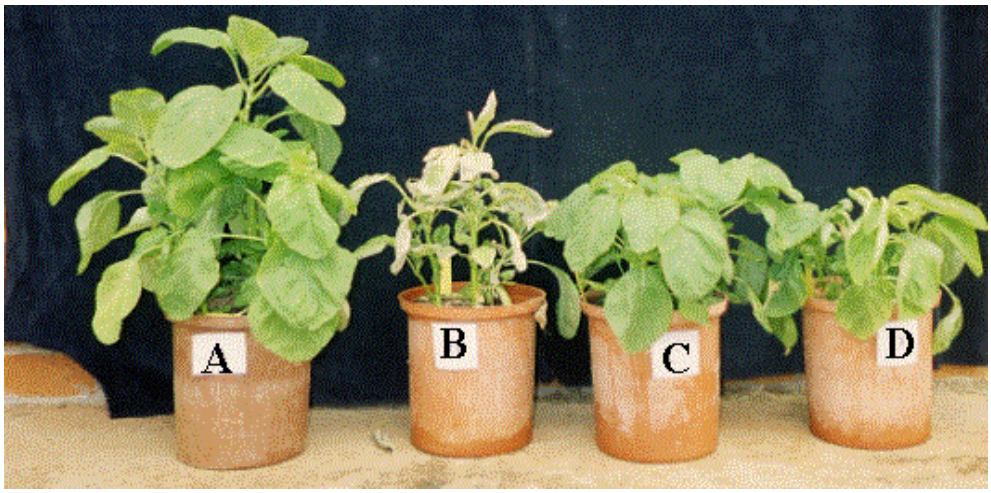

Figure 5.1 Growth of *A. cruentus* (1) and *A. tricolor* (2) as affected by iso-osmotic water and salt stresses: (A) control, (B) water stress, (C) salt stress, (D) water + salt stress.

5.4.2 Water relations

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

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

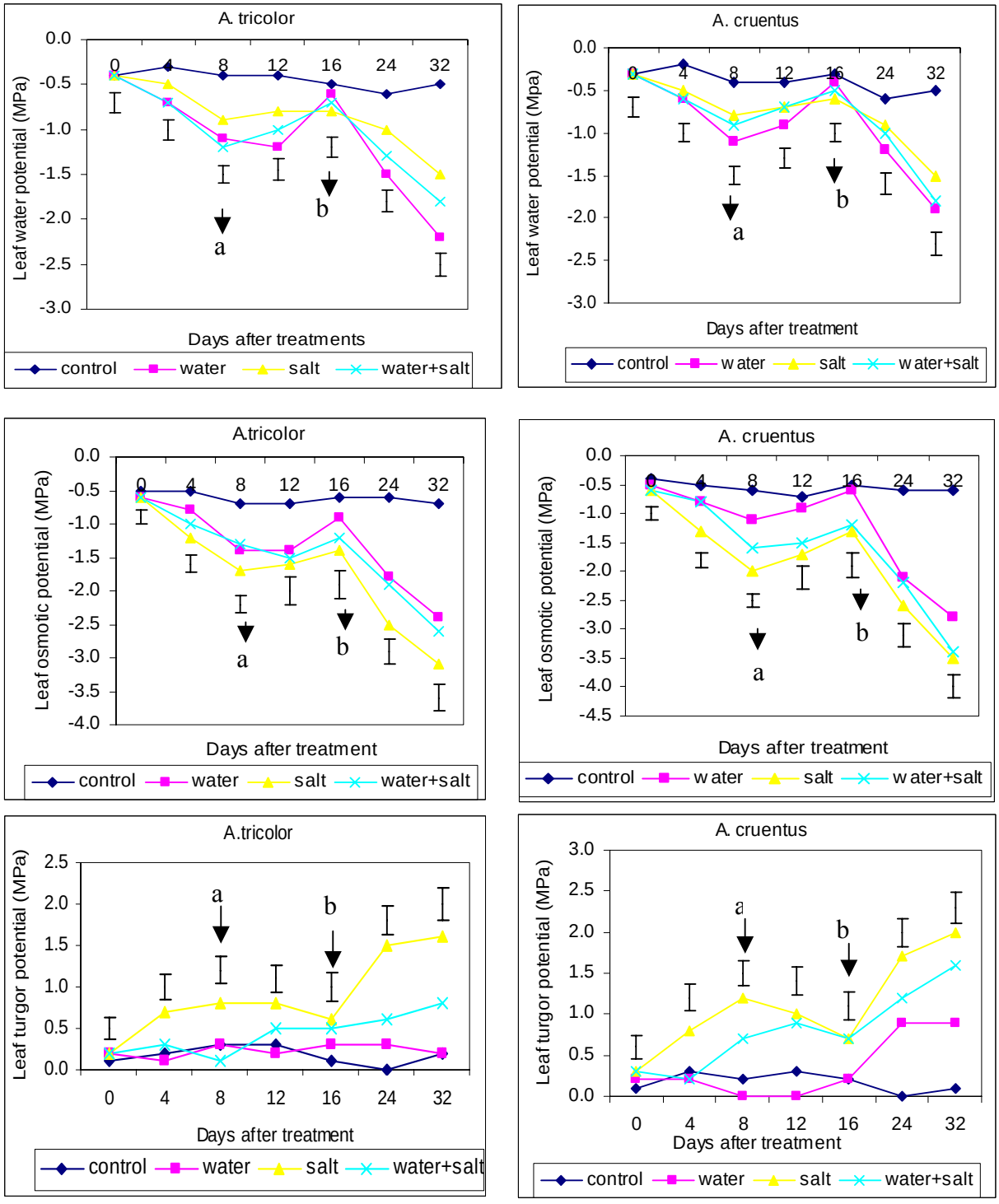

Figure 5.2 Leaf water potential, osmotic potential and turgor potential of *A. tricolor* and *A. cruentus* exposed to iso-osmotic stress treatments. Arrows indicate the beginning of the recovery period (a) and the beginning of the second stress period (b). Vertical bars indicate least significant differences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

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

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

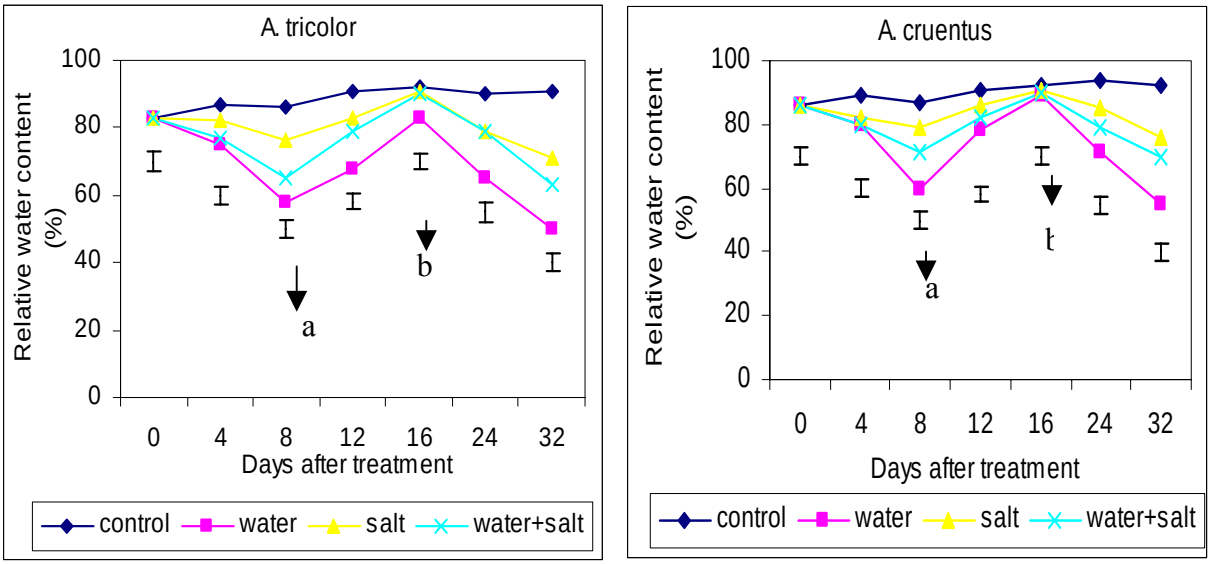

Figure 5.3 Effect of water stress, salt stress and combined water and salt stress on relative water content of *A. tricolor* and *A. cruentus*. Arrows indicate the beginning of the recovery period (a) and the beginning of the second stress period (b). Vertical bars indicate least significant differences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

5.4.3 Water loss and water use efficiency

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

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

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

SEM: Standard error of the mean

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

5.4.4 Gas exchange

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

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

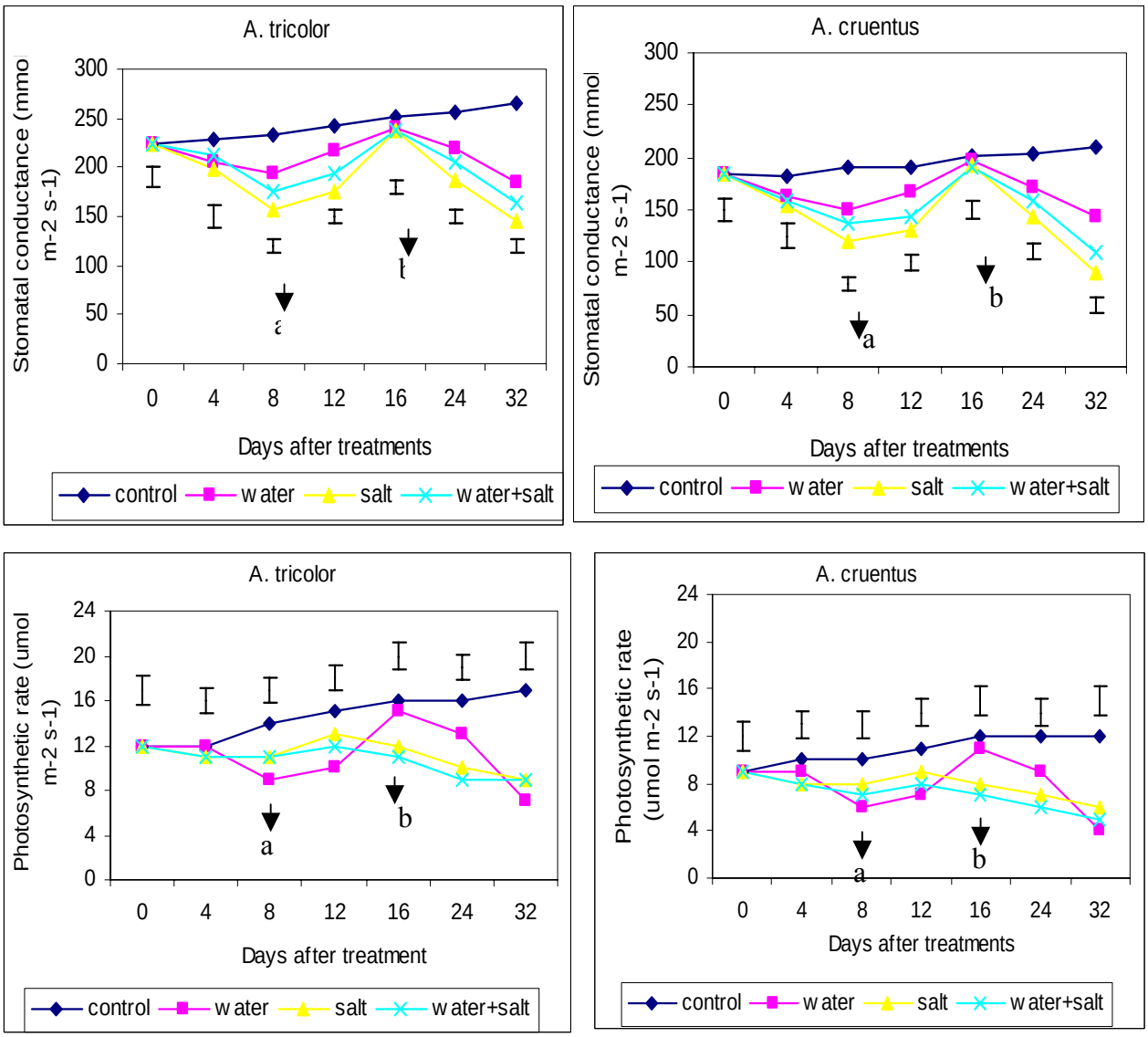

Figure 5.4 Effect of water stress, salt stress and combined water and salt stress on stomatal conductance and photosynthetic rate of *A. tricolor* and *A. cruentus*. Arrows indicate the beginning of the recovery period (a) and the beginning of the second stress period (b). Vertical bars indicate least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

5.5 DISCUSSION

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

5.6 CONCLUSION

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

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

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