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Tolerance and avoidance responses to salinity and water stresses in *Calotropis procera* and *Suaeda aegyptiaca*

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Abstract: This study was designed to evaluate the response of 2 wild shrubs, *Calotropis procera* and *Suaeda aegyptiaca*, to salinity (100% seawater), drought, and waterlogging stresses. The 90-day-old plants were subjected to the stress treatments for 3 weeks, and growth and some physiological parameters were evaluated. The total plant dry mass of *C. procera* plants was reduced by 40%, whereas *S. aegyptiaca* was not significantly affected by salinity stress. Water deficit and waterlogging stresses significantly reduced the total dry mass of both species. Under all conditions, the root/shoot ratio in *C. procera* was 3-fold higher than in *S. aegyptiaca*. All applied stresses markedly increased leaf shedding in *C. procera* plants only. These plants appeared to have a higher salinity and waterlogging stress intensity index as manifested by chlorophyll levels lower than those in *S. aegyptiaca*. Under all conditions, Na⁺ levels of *S. aegyptiaca* were twice those of *C. procera* plants. All stresses reduced the K⁺/Na⁺ ratio in *C. procera* leaves. On the other hand, *S. aegyptiaca* plants were able to maintain this ratio near control levels under salinity and drought stresses. Consequently, *S. aegyptiaca* leaves had higher partial osmotic pressure than *C. procera*. The proline and total free amino acids levels in *C. procera* were between 1.3- and 2-fold higher than in *S. aegyptiaca*. Among all amino acids, the common change in both species under all stresses was an accumulation of free proline and a decrease in methionine levels. The results revealed that the response of both species to salinity and water stresses included avoidance and tolerance mechanisms with some differences between them.

Key words: Amino acids, *Calotropis*, drought, leaf shedding, osmotic pressure, proline, salinity, *Suaeda*, waterlogging

1. Introduction

The responses of plants to environmental stresses are complex and involve many kinds of physiological and biochemical changes. Maintaining both the balance of ecosystems and the efficient economic use of them is based on understanding the physiological/biochemical mechanisms of plant stress response and plant resistance to stress (Mandre 2002). This resistance is active (tolerance) or passive (avoidance), with most plants using a combination of the 2 (Levitt 1980; Mandre 2002).

Most stresses appear with similar symptoms, including leaf area index reduction, chlorophyll degradation, and canopy temperature increase (Baret et al. 2007). During stress, active solute accumulation of compatible solutes as amino acids and carbohydrates may be an effective stress tolerance mechanism (Kerepesi and Galiba 2000). Accumulation of the amino acid proline is one of the most frequently reported modifications induced by water deficit, salt, and waterlogging stresses in plants and is thought to be involved in stress tolerance mechanisms,

although its precise role remains controversial (Gadallah 1995; Lutts et al. 1999; Yiu et al. 2009). Additionally, homeostasis of K⁺ and Na⁺ ions and the K⁺/Na⁺ ratio are vital for plant tolerance to many stresses. The need for K⁺/Na⁺ homeostasis under salt or water stress is indicated by the fact that high concentrations of Na⁺ in the cytoplasm are toxic for metabolically active cells, and Na⁺ toxicity is closely related to disturbances in essential K⁺ function (Stevens and Prior 1994; Rejili et al. 2007).

Avoidance responses to water deficit stress include increased biomass allocation to roots, leaf shedding, leaf rolling, and low stomatal conductance; responses to salinity stress include exclusion, secretion, shedding, and succulence mechanisms (Levitt 1980; Touchette et al. 2009). Under waterlogging stress, avoidance of oxygen deficiency through effective internal aeration and formation of adventitious roots is a key adaptation criterion (Jackson and Armstrong 1999).

Calotropis procera is a woody evergreen shrub that is widely distributed in arid and semiarid regions and has

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sustained commercial uses as a fiber source, as a ruminant feedstuff, and in medicinal drug preparation (Erdman and Erdman 1981). This species grows profusely and survives well under drought conditions (Boutraa 2010). The growth and chlorophyll levels of these plants were adversely affected by 80 mM NaCl stress (Al-Sobhi et al. 2006). To date, no study has examined the impact of waterlogging stress on this species. Investigating the effects of waterlogging on *C. procera* would extend our information about the mechanisms of plant resistance to this stress. Furthermore, such a study may identify common morphological or physiological modifications in this species in response to salinity, drought, and waterlogging stresses.

Suaeda aegyptiaca is a salt-tolerant plant that grows naturally as a perennial and leaf succulent shrub in the west of Saudi Arabia. In addition to its ecological importance, some medicinal uses were also reported for this species (Al-Ani et al. 2011). The effect of salinity on most *Suaeda* species has been studied (Khan et al. 2000; Guan et al. 2010; Al-Ani et al. 2011). However, few studies have examined the impact of salinity stress on *S. aegyptiaca* species. Eshel (1985) reported that Na⁺ salts in the medium lowered K⁺ and the transpiration rate in this species and simultaneously increased growth by as much as 900%. This enhancement is probably due to the fact that *S. aegyptiaca* is a true halophyte that uses Na⁺ as an essential nutrient (Askari et al. 2006). To date, no information is available on the physiological response of this species to water deficit or waterlogging stress.

The present short-term study was conducted to investigate some tolerance and avoidance responses to salinity, water deficit, and waterlogging stresses in a xerophytic plant, *C. procera*, and a halophytic plant, *S. aegyptiaca*. This has been achieved by studying the effect of these stresses on growth, chlorophyll, the K⁺/Na⁺ ratio, osmotic pressure, and free amino acid content in both species. Seawater salinity was used in this study because seawater is relevant to the field conditions of *S. aegyptiaca*.

2. Materials and methods

2.1. Seed collection

Seeds of *Calotropis procera* (Aiton) and *Suaeda aegyptiaca* (Hasselq.) Zohary were collected from the Al-Qunfida area of Saudi Arabia in October, when the seeds of these plants were available. A germination test was carried out to check the viability of the seeds; viability was about 95% for both species.

2.2. Growth conditions

The seeds were surface-sterilized in 0.005 M HgCl₂ solution for 3 min and subsequently rinsed with sterilized distilled water. The seeds were soaked in distilled water for 8 h. From each species, 4 homogeneous seeds were sown in plastic pots (20 cm × 25 cm × 30 cm) containing

5 kg of loamy sand soil (70% sand, 20% silt, 10% clay; pH 7.1). This soil was taken from an agricultural field in Al-Qunfida Governorate. All pots were irrigated with tap water (electrical conductivity [EC] = 0.2 dS m⁻¹) for 1 month, and then the plants were thinned to 1 plant pot⁻¹ and each received 1 g of Ca(NO₃)₂ and 1 g of K₂HPO₄ as inorganic fertilizers. The plants were irrigated with tap water for another 2 months. Each species was then allocated into 4 treatments for 3 weeks: control (tap water), salinity stress (100% seawater), drought stress, and waterlogging stress. Salinity was applied by irrigation with seawater. The initial concentration of seawater was 10% and it increased gradually to 20%, 40%, and 80% seawater for 1 week, to continue with 100% seawater (EC = 60.5 dS m⁻¹) until harvest. Water deficit stress was obtained by withholding water. Waterlogging stress was applied by immersing the pots in plastic dishes filled with tap water so that the water was 3 cm above the soil surface of each pot. These 4 treatments were replicated 12 times for a total of 48 pots for each species. The plants were grown in a greenhouse at Al-Qunfida College and subjected to natural day/night conditions. Minimum/maximum temperatures and average photoperiods (day/night) were 20/35 °C and 11.5/12.5 h, respectively, during the study. Irrigation to field capacity for control and salinity treatments was carried out when soil moisture content had fallen to 60% of its initial value. At the end of the stress period, samples were taken for soil moisture content (SMC), growth, and biochemical analyses. Only the leaves that were budding at the same time among the 4 treatments were used for the physiological analyses.

2.3. Monitoring SMC

SMC was determined by obtaining the wet weight of a soil sample from each pot (W1), oven-drying the soil sample for 2 days at 105 °C, and weighing the dry soil sample (W2). The SMC is expressed as mass (g) of soil water / 100 g of dry soil. $SMC (\%) = (W1 - W2) / W2 \times 100$.

2.4. Determination of chlorophyll content

Chlorophylls *a* and *b* from leaf samples were extracted rapidly in ammoniacal acetone, and their concentrations were determined spectrophotometrically according to the method of Lichtenthaler (1987).

2.5. Estimation of stress intensity index

Stress intensity index (SII) was calculated using a formula modified from Fischer and Murrer (1978). The modification involved the use of chlorophyll content instead of plant yield.

$$SII = 1 - (\text{total chlorophyll under stress conditions} / \text{total chlorophyll under control conditions})$$

2.6. Determination of K⁺ and Na⁺ concentrations

A known dry weight of leaves was digested in boiling concentrated HNO₃ and brought to a known volume with deionized water. K⁺ and Na⁺ concentrations were

measured by flame photometer (PerkinElmer, Model 2100, Germany).

2.7. Analysis of amino acids

Free amino acids in plant leaves were extracted with ethanol, and amino acid contents were determined as described by Spackman et al. (1958) and Moore et al. (1958). The analysis was performed in the Central Service Unit of the National Research Center, Egypt, using an amino acid analyzer (LC3000, Eppendorf-Biotronik, Germany). The technique was based on the separation of amino acids using strong cation exchange chromatography followed by ninhydrin color reaction and photometric detection at 570 nm.

2.8. Determination of partial osmotic pressure

One gram of powdered leaf dry weight was heated at 90 °C in deionized water for 1 h. After centrifugation at 7000 rpm for 15 min, the total volume of the supernatant was adjusted to 25 cm³ and the EC was measured using a conductivity meter (Mettler-Toledo). The partial osmotic pressure (POP) was estimated with the following formula: $POP \text{ (bar)} = 0.36 \times EC \text{ (mmho cm}^{-1}\text{)}$ (Bresler et al. 1982).

2.9. Statistical analysis

The experiment had a completely random and factorial design. Results were based on 6 replicates for growth analysis and 3 replicates for physiological parameters. The results were subjected to an analysis of variance using the general linear model and one-way ANOVA. The significant differences between means (least significant difference a posteriori test) at $P < 0.05$ were calculated with SPSS 15 (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Changes in SMC

C. procera plants caused more depletion in SMC than *S. aegyptiaca* plants in control and water deficit conditions (Figure 1). In plants grown under water deficit stress, *C. procera* and *S. aegyptiaca* lowered SMC to 0.98% and 1.93%, respectively. SMC under waterlogging stress was about twice that of the control. The SMC of *C. procera* increased significantly, whereas *S. aegyptiaca* was not affected by salinity stress at the end of the experiment compared to control plants (Figure 1).

3.2. Changes in growth parameters

Salinity stress reduced *Calotropis* dry mass by about 40% and nonsignificantly affected dry mass in *Suaeda* plants compared to control plants (Figure 2a). Drought and waterlogging stresses significantly reduced the total dry mass of both species (Figure 2a). Salinity stress appeared to be the most effective stress for reducing *Calotropis* growth, while in *Suaeda* growth was more adversely affected by both drought and waterlogging stresses (15% reduction by both).

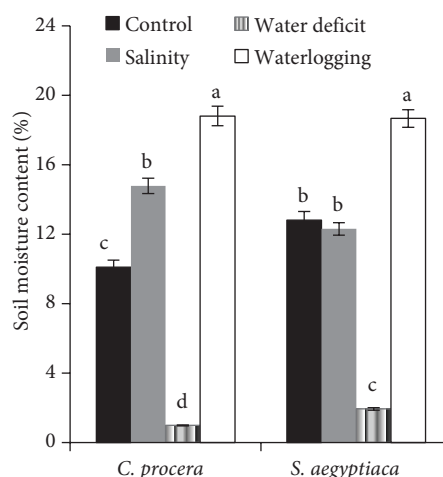


Figure 1. Changes in soil moisture content (% oven-dried soil dry weight) under different stress conditions. Values are means \pm SE. Values in a group with the with the same letter(s) are not significantly different at $P < 0.05$.

The application of drought and salinity stresses significantly increased the root/shoot ratio of the 2 species compared to control plants (Figure 2b). However, waterlogging stress nonsignificantly affected this ratio in both species (Figure 2b). Under all conditions, *C. procera* plants had a higher (about 3-fold increase) root/shoot ratio than *S. aegyptiaca* plants. Visual examination indicates that *C. procera* root was very blackish with some rotting under waterlogging conditions. On the other hand, the root system of *S. aegyptiaca* was without rotting and had many long adventitious roots. Leaf shedding in *C. procera* increased markedly in response to all applied stresses (Figure 2c). Salinity, drought, and waterlogging increased leaf loss in this species to 200%, 250%, and 150% of the control, respectively. Conversely, all applied stresses did not significantly affect leaf shedding in *S. aegyptiaca* (Figure 2c).

3.3. Changes in chlorophyll content

C. procera plants had higher total chlorophyll content than *S. aegyptiaca* plants. The chlorophyll content in *Calotropis* leaves was twice as high as in *Suaeda* leaves (Figure 3a). Water deficit and waterlogging stresses significantly reduced these photosynthetic pigments in both species compared to control plants. The administration of salinity reduced chlorophyll content in *C. procera* and nonsignificantly affected *S. aegyptiaca* (Figure 3a).

3.4. Changes in SII

The SII of *C. procera* on a total chlorophyll basis was much higher in response to salinity and waterlogging than drought stress (Figure 3b). Conversely, the SII of *S. aegyptiaca* due to salinity was much lower than with other stresses. The SII of *Calotropis* was 20-fold and 1.5-

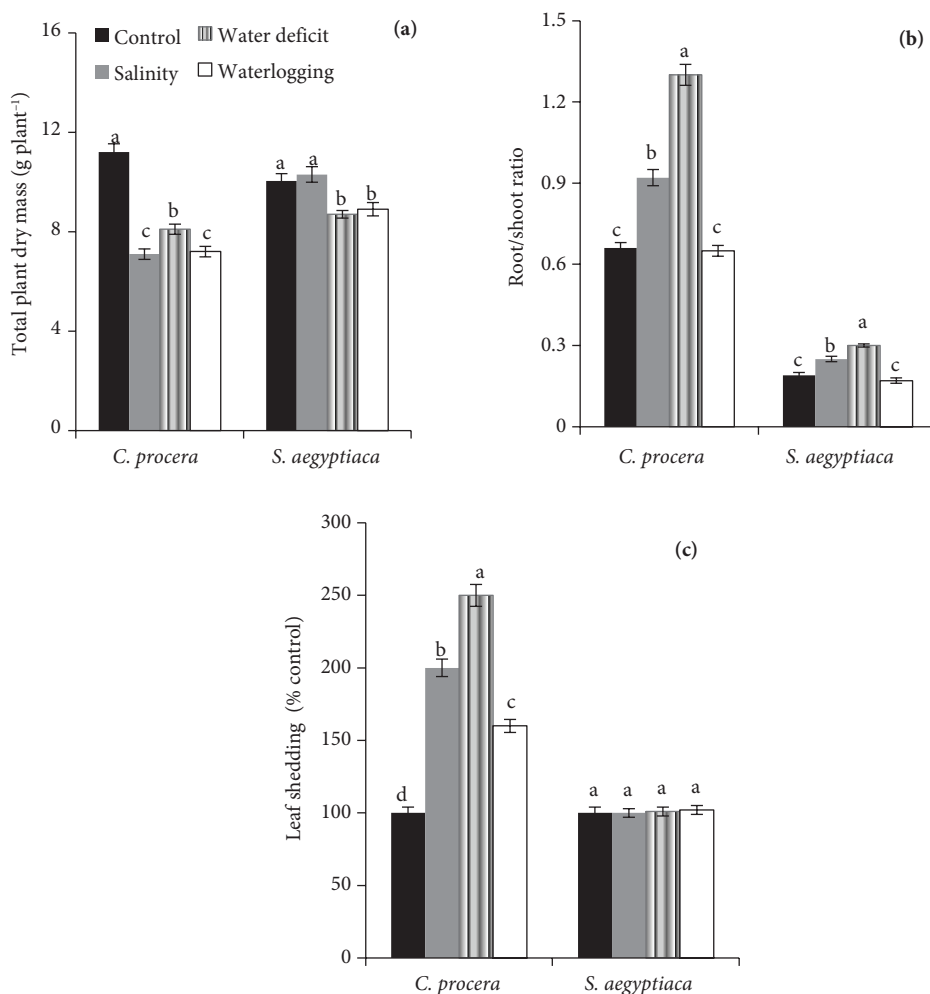


Figure 2. Effects of different stress conditions on growth parameters of *C. procera* and *S. aegyptiaca* plants. Values are means \pm SE. Values in a group with the same letter(s) are not significantly different at $P < 0.05$.

fold greater than that of *Suaeda* in response to salinity and waterlogging stresses, respectively. On the other hand, in response to drought stress the SII in *Suaeda* was 3-fold greater than in *Calotropis* (Figure 3b).

3.5. Changes in K⁺ and Na⁺

Salinity and drought stresses significantly increased leaf K⁺ concentration in both species compared to control plants (Figure 4a). On the other hand, waterlogging significantly increased leaf K⁺ content in *C. procera* and significantly lowered it in *S. aegyptiaca*.

The results show that *S. aegyptiaca* leaves under all conditions had higher (by approximately 2-fold) leaf Na⁺ content than *C. procera* (Figure 4b). Salinity stress significantly increased Na⁺ content in both species compared to control plants. Na⁺ concentration in *S. aegyptiaca* was not significantly affected, whereas the concentration in *C. procera* increased significantly in

response to water stress (Figure 4b). Waterlogging stress raised Na⁺ levels in *C. procera* by about 90%.

C. procera appeared to have a higher K⁺/Na⁺ ratio than *S. aegyptiaca* (Figure 4c). All types of stress reduced this ratio in *Calotropis* plants, and the effect was more pronounced under waterlogging stress. *S. aegyptiaca* plants were able to maintain this ratio near control levels under salinity and drought stresses. Additionally, the K⁺/Na⁺ ratio was reduced by 40% and 15% in *Calotropis* and *Suaeda*, respectively, in response to waterlogging stress.

3.6. Changes in POP

The results for POP are shown in Figure 5. In general, *S. aegyptiaca* leaves had higher osmotic pressure than *C. procera* leaves. The POP of *Suaeda* was 2-fold higher than in *Calotropis*. Salinity and drought stresses significantly increased the POP in both species compared to control plants. The POP of *Calotropis* increased significantly,

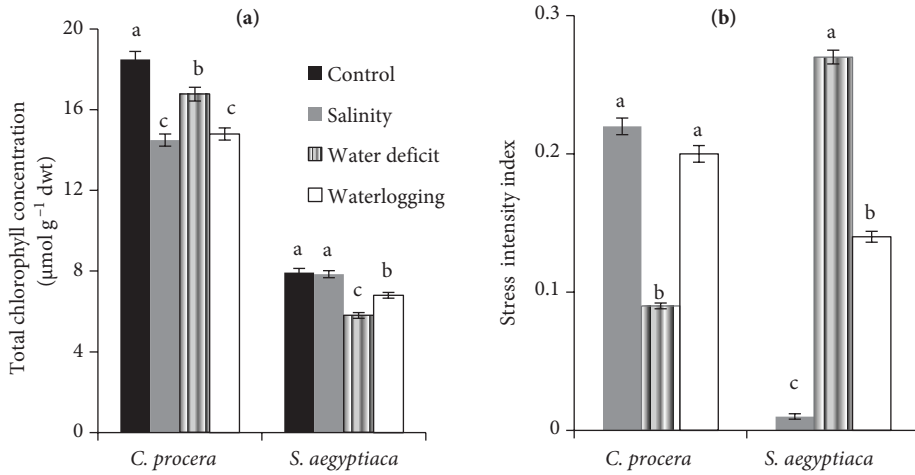


Figure 3. Effects of different stress conditions on total chlorophyll content and stress intensity index of *C. procera* and *S. aegyptiaca* plants. Values are means \pm SE. Values in a group with the same letter(s) are not significantly different at $P < 0.05$.

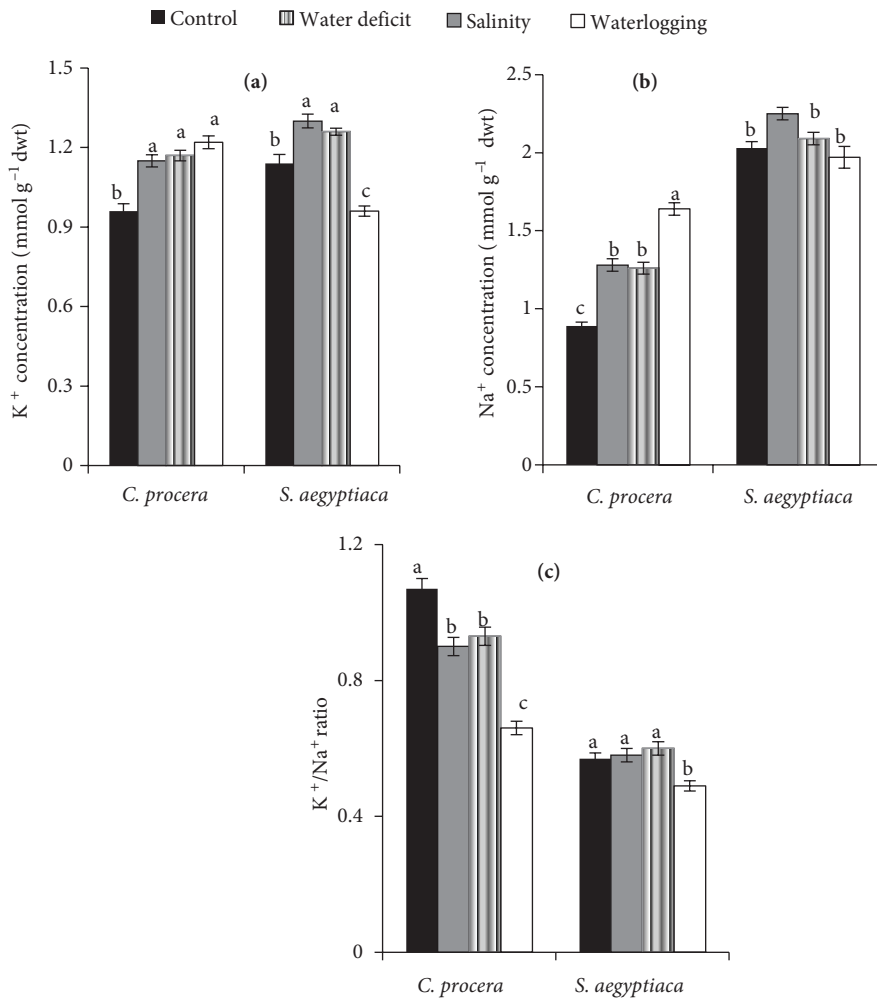


Figure 4. Effects of different stress conditions on K and Na ion content of *C. procera* and *S. aegyptiaca* plants. Values are means \pm SE. Values in a group with the same letter(s) are not significantly different at $P < 0.05$.

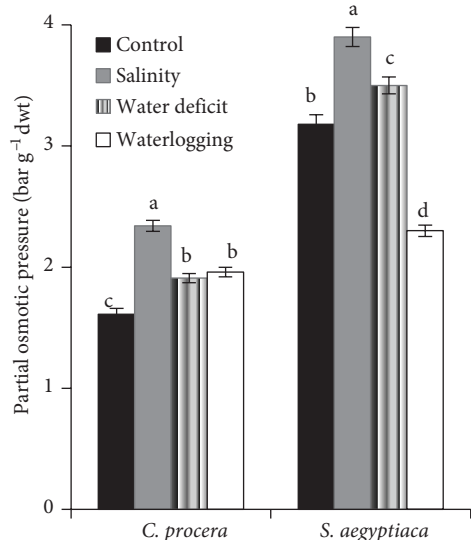


Figure 5. Effects of different stress conditions on partial osmotic pressure of *C. procera* and *S. aegyptiaca* plants. Values are means \pm SE. Values in a group with the same letter(s) are not significantly different at $P < 0.05$.

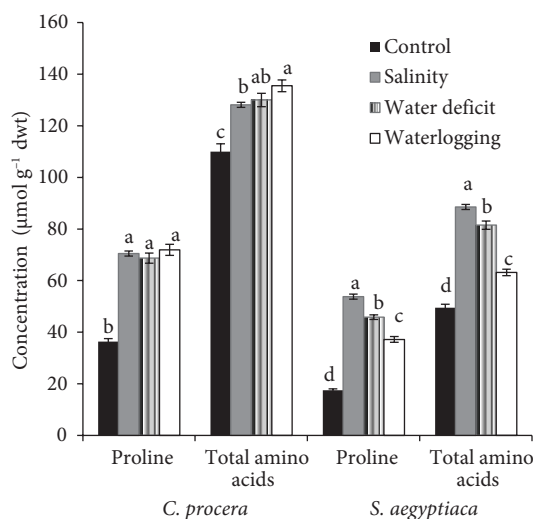


Figure 6. Effects of different stress conditions on proline and total amino acids content of *C. procera* and *S. aegyptiaca* plants. Values are means \pm SE. Values in a group with the same letter(s) are not significantly different at $P < 0.05$.

whereas that of *Suaeda* was greatly reduced in response to waterlogging stress (Figure 5).

3.7. Changes in free amino acids

Results show that proline and total free amino acids levels in *C. procera* were between 1.3- and 2-fold higher than in *S. aegyptiaca* (Figure 6). However, the rates of increase in *Suaeda* were much higher than in *Calotropis* in response to all applied stresses. The rates of proline increase were as much as 194%, 189%, and 198% in *C. procera* and 307%, 262%, and 213% in *S. aegyptiaca* in response to salinity, drought, and waterlogging, respectively.

The pattern of changes in amino acids appeared to depend on the species and type of applied stress (Table). In general, the effect of salinity and drought on amino acids was similar. Imposition of salinity and drought stress significantly increased glutamic acid, alanine, and proline levels in both species. On the other hand, methionine, phenylalanine, and histidine decreased significantly in both species in response to these stresses. The pattern of change in other measured amino acids was not similar in the tested species. For example, the amino acid cysteine decreased significantly in *C. procera* and increased significantly in *S. aegyptiaca* (Table).

Concerning the effect of waterlogging stress, only proline increased significantly in relation to control in both species (Figure 6). Conversely, glutamic acid, glycine, leucine, histidine, and methionine decreased significantly, whereas alanine was not significantly affected in response to this stress in either species (Table). The pattern of change in other measured amino acids was not similar across species.

For example, lysine decreased significantly in *C. procera* and increased nonsignificantly in *S. aegyptiaca* (Table).

4. Discussion

The observed negative effect of salinity stress on the total plant biomass of *C. procera* is compatible with the results of Al-Zahrani (2002). The depressive effect of salinity is apparently due to increased Na^+ with a correspondingly lower K^+/Na^+ ratio in plant leaves, as the cellular ionic imbalance results in osmotic damage and disorders in enzyme activation and protein synthesis (Tester and Davenport 2003; Munns and Tester 2008). Concerning *S. aegyptiaca*, it was able to grow in 100% seawater without significant changes to growth, indicating that this species is an obligate halophyte and can establish itself in most saline environments. Eshel (1985) reported the enhancement effect of NaCl salinity stress on the growth of this species. The tolerance of *Suaeda* and other halophytes to salinity relies on controlled uptake and compartmentalization of Na^+ , K^+ , and Cl^- as well as the synthesis of organic compatible solutes, even where salt glands are operative (Flowers and Colmer 2008).

In this study, drought stress had a negative effect on total biomass in both species. This could be attributed to loss of turgor, which affects the rate of cell expansion and ultimate cell size, consequently decreasing growth rate, stem elongation, leaf expansion, and stomatal aperture (Hale and Orcutt 1987). The adverse effect of waterlogging stress on growth vigor in both species appeared to be due to a decrease in the K^+/Na^+ ratio, resulting from O_2 deprivation

Table. Effects of different stress conditions on amino acid concentrations in *Calotropis* and *Suaeda* plants. Values are means \pm SE. For each species, values in each row with the same letter(s) are not significantly different at $P > 0.05$.

	Treatments							
	<i>Calotropis procera</i>				<i>Suaeda aegyptiaca</i>			
	Control	Salinity	Water deficit	Waterlogging	Control	Salinity	Water deficit	Waterlogging
Amino acids concentration ($\mu\text{mol g}^{-1}$ dwt)								
Aspartic acid	7.3b \pm 0.15	7.4b \pm 0.16	7.6b \pm 0.14	8.8a \pm 0.18	5.3a \pm 0.09	4.2b \pm 0.08	4.2b \pm 0.06	3.8c \pm 0.07
Glutamic acid	8.0b \pm 0.20	11.2a \pm 0.22	10.1a \pm 0.20	6.4c \pm 0.13	6.4c \pm 0.13	7.5b \pm 0.15	9.7a \pm 0.20	4.9d \pm 0.10
Glycine	5.7a \pm 0.09	4.0c \pm 0.08	4.6bc \pm 0.09	5.0b \pm 0.10	2.6b \pm 0.05	3.4a \pm 0.07	3.2a \pm 0.06	2.0c \pm 0.04
Alanine	5.0c \pm 0.08	6.4a \pm 0.09	5.7b \pm 0.10	5.2c \pm 0.08	3.1b \pm 0.06	3.8a \pm 0.08	3.5a \pm 0.07	3.0b \pm 0.05
Cystine	15.2b \pm 0.27	11.5c \pm 0.23	11.4c \pm 0.22	16.5a \pm 0.28	4.3b \pm 0.09	5.8a \pm 0.12	5.5a \pm 0.11	4.5b \pm 0.10
Methionine	1.30a \pm 0.04	0.53b \pm 0.01	0.56b \pm 0.02	0.55b \pm 0.01	0.58a \pm 0.02	0.42b \pm 0.03b	0.49b \pm 0.01	0.48b \pm 0.01
Leucine	10.8a \pm 0.21	5.0c \pm 0.08	5.9b \pm 0.12	6.2b \pm 0.12	2.9b \pm 0.06	3.8a \pm 0.08	3.1b \pm 0.06	2.5c \pm 0.05
Phenylalanine	6.9a \pm 0.14	4.4b \pm 0.09	3.4c \pm 0.07	4.6b \pm 0.10	0.78b \pm 0.02	1.4a \pm 0.03	1.5a \pm 0.04	1.4a \pm 0.03
Histidine	3.8a \pm 0.09	1.9c \pm 0.04	2.2c \pm 0.06	2.9b \pm 0.07	2.4a \pm 0.05	1.2b \pm 0.02	1.3b \pm 0.03	0.9b \pm 0.02
Lysine	5.7a \pm 0.12	2.4d \pm 0.05	3.4b \pm 0.07	3.0c \pm 0.06	1.4c \pm 0.06	1.9a \pm 0.06	1.7ab \pm 0.04	1.6bc \pm 0.05
Arginine	3.9c \pm 0.08	2.9d \pm 0.06	6.6a \pm 0.10	4.5b \pm 0.09	2.2a \pm 0.08	1.3c \pm 0.05	1.6b \pm 0.06	0.8d \pm 0.03

in flooded soil and the resulting energy deficits (Colmer and Flowers 2008). Salinity and drought stress appeared to improve the root/shoot ratio of both species, and the effect was more pronounced in *Calotropis* plants. This supports the findings of Kalapos et al. (1996), who reported that decreasing water availability substantially reduced the relative growth rate, while biomass partitioning changed to favor root growth so that the plant could exploit the limited water resources more efficiently.

Leaf shedding was clear in *C. procera* plants under all stress conditions. This shedding can be viewed as a beneficial phenomenon since it removes organs that are no longer of benefit to the plant and increases photosynthetic efficiency per unit area of land (McLaurin and Kays 1993). Furthermore, shedding minimizes water loss and transpiration during the dry season (Hasselquist et al. 2010). Thus, leaf shedding is a mechanism to prevent plant water deficit and contributes to drought resistance in *C. procera*. The increase in leaf shedding in xerophytic plants under salinity stress may occur because the leaf sap salt concentration reaches toxic levels, and this accelerates leaf senescence and reduces plant growth (Munns and Tester 2008). Under waterlogging stress, however, soil O_2 deprivation may induce leaf shedding in *C. procera* as a consequence of hormonal disorder and limited energy.

Chlorophyll content may be one of the parameters determining salt tolerance in plants (Srivastava et al. 1998), and its levels may determine the relative salt tolerance of the plants. The reduction of chlorophyll content in both

species in response to water deficit and waterlogging stresses is consistent with other studies (Naumann et al. 2008; Boutraa 2010). Furthermore, the deleterious effect of salt stress on *Calotropis* chlorophyll is in accord with the results of Al-Sobhi et al. (2006). Changes in the SII revealed the degree of reduction in chlorophyll content, indicating the difference in stress resistance between the species. Consequently, *S. aegyptiaca* was more resistant to salinity and waterlogging than *C. procera*, which had high drought resistance.

In spite of their physiochemical similarities, it is K^+ rather than Na^+ that is essential to plant life (Maathuis and Amtmann 1999). In this study, the observed increases in K^+ and Na^+ levels in *C. procera* and *S. aegyptiaca* in response to salinity stress are consistent with the results of Ibrahim (2004) and Guan et al. (2010). This increase in K^+ and Na^+ led to the observed increase in leaf POP, which contributes in osmotic adjustment to maintaining turgor and hence sustains metabolic activity during stress (Flower and Ludlow 1986; Flowers and Colmer 2008). The increase in Na^+ in *C. procera* leaves under drought stress could be an effective strategy for adapting to arid environments, whereas xerophytes use Na^+ as an osmoticum to increase plant water content (Wang et al. 2004). High Na^+ and osmotic pressure in *S. aegyptiaca* (on a dry weight basis) shows that this species is a salt-includer halophyte, and the leaf succulence of these plants tends to lower intracellular ion concentrations and prevent excessive accumulation of ions in leaf sap (Flowers and Colmer 2008).

The reduction of K^+/Na^+ in *C. procera* under salinity and drought stresses indicates that under these conditions, this species is less selective regarding K^+ than Na^+ . On the other hand, the halophyte *S. aegyptiaca* maintained this ratio under salinity and drought stresses, and this is a stress tolerance criterion (Gorham et al. 1990; Rejili et al. 2007). Others found that salinity stress decreased the K^+/Na^+ ratio in another *Suaeda* species (Khan et al. 2000; Guan et al. 2010). Recently it has been demonstrated that some Na^+ excretion at the leaf surface helps to confer high salinity tolerance in *S. fruticosa* (Labidi et al. 2010). The ability of *S. aegyptiaca* to maintain its K^+/Na^+ ratio may be attributed, in part, to this mechanism. The decrease in the K^+/Na^+ ratio in both species in response to waterlogging stress agrees with other reports in glycophytes (Stevens and Prior 1994; Zhang et al. 2011) and halophytes (Colmer and Flowers 2008) and could be attributed to O_2 deficiency, which restricts respiration, inhibiting the uptake of K^+ and the exclusion of Na^+ (Barrett-Lennard 2003). This low K^+/Na^+ ratio can disturb various enzymatic processes in the cytoplasm as well as protein synthesis (Tester and Davenport 2003).

The increase in free amino acids may aid plant tolerance of biotic stress by contributing to the osmotic balance when electrolytes are lower in the cytoplasm than in the vacuole, or as a reserve of N, principally for synthesis of specific enzymes (Greenway and Munns 1980). The observed increase in some free amino acids (mainly proline, glutamic acid, and alanine) in leaves of *Calotropis* and *Suaeda* under salinity and water deficit stress conditions is in agreement with other data in the literature (Zagdanska 1984; Fayek et al. 2010). The increase in glutamic acid content is likely derived from the fact that this amino acid is the precursor of proline (Lea and Ireland 1999). In addition to proline increase, another common finding

among the effects of salinity, drought, and waterlogging was a decrease in methionine levels in both species. This decrease in methionine levels may be attributed to the increase in ethylene and polyamine biosynthesis under stress conditions (Quinet et al. 2010). The concentrations of proline and total free amino acids in *Calotropis* were higher than in *Suaeda*. Why the xerophytes have high levels of proline is unknown. Perhaps it is because they are intolerant to high Na^+ concentrations, and proline serves as an alternative compatible solute for osmotic adjustment (Wang et al. 2004).

In conclusion, *Calotropis procera* was more resistant to water deficit stress than *Suaeda aegyptiaca*, and the converse was true for salinity and waterlogging stresses. The resistance of both species to these stresses included avoidance and tolerance mechanisms. The avoidance responses to water deficit stress were the increases in root/shoot ratio in both species and leaf shedding in *C. procera* only. *S. aegyptiaca* was tolerant to Na^+ toxicity by K^+ and K^+/Na^+ ratio homeostasis, while *C. procera* was intolerant. Avoidance responses to waterlogging stress included the formation of adventitious roots by *S. aegyptiaca*, a mechanism that did not appear in *C. procera*. A common tolerance mechanism in both species was an increase in proline and total free amino acids under all applied stresses. Although stress processes have been well characterized in many plants, this is one of the first studies to compare morphological and physiological responses in xerophytes and halophytes receiving similar salinity, water deficit, and waterlogging treatments.

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