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COMPARATIVE RESPONSE OF THREE TROPICAL GROUNDCOVERS TO SALT STRESS

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ABSTRACT

The increasing interest in cultivating groundcover plants in warm and semiarid areas requires a better understanding of the salinity effects on landscape plants. This work aimed to study the response of three groundcovers (*Alternanthera dentate*, *Sphagneticola trilobata*, and *Alternanthera amoena*) to high sodium chloride concentrations. The trial was conducted in the natural greenhouse environment. Plants were raised in pots filling clay-loamy soil. Hewitt's nutrient solution containing 0, 25, 50, 75, and 100 mM NaCl irrigated the plants. Plant growth, antioxidative enzyme activity, and the relative water content (RWC), proline, sodium, potassium, and chloride were determined. The study indicated that increasing NaCl concentration in the nutrient solution led to: a) significant differences in the fresh weight of shoots among salinity treatments and among species; b) increased root growth with increasing salinity stress up to the mild stress level of 25 mM NaCl, however at different rates with three species; c) reduced RWC of the leaves of three species grown under salinity-induced stress; d) the increased proline content of the leaves, and more pronounced increases with *A. dentate* and *A. amoena* from 0 to 50 mM NaCl, and with *S. trilobata* from 0 to 100 mM NaCl; e) significant changes in the activities of antioxidative enzymes including superoxide dismutase, peroxidase, and catalase; f) significant decrease of the K^+/Na^+ ratio along with increase of salinity stress; g) increased ratio of leaf/ root content of Cl^- in *A. dentate* and in particular, *A. amoena*; h) a significant reduction in visual qualities of all examined plants. Therefore, because of its ability to maintain leaf characteristics, visual quality, and salt-tolerance mechanisms even under high salinity, *S. trilobata* can be considered for urban landscaping projects in semiarid and saline areas where low-quality water is used for irrigation.

Keywords: *Alternanthera amoena, Alternanthera dentate,* antioxidative enzymes, landscape, salinity, *Sphagneticola trilobata*

INTRODUCTION

Today, the availability of high-quality irrigation water is the number one issue confronting Iran's urban landscape management of water shortage areas. Therefore, saline groundwater will irrigate large landscape sites in the future. Moreover, many cities in Iran, especially Ahwaz, have extremely variable environments with a wide range of temperature, humidity, and soil moisture conditions. With the increased use of alternative irrigation water sources on the landscape, in this area of Iran, groundcovers are the prominent species that are selected to plant in many urban landscapes instead of turfgrass. *Alternanthera dentate*, *Alternanthera amoena*, and *Sphagneticola trilobata* are important groundcover plants for tropical

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urban landscapes. *A. dentate* and *A. amoena* belong to the Amaranthaceae family, which includes 80 species of evergreen, perennial herbs, native to sub-tropical and tropical areas of South America and Australia. Those plants can withstand trimming and quickly propagate by cutting or division [Kumar et al. 2011]. *Sphagneticola trilobata* belongs to the *Asteraceae* family and is a creeping plant native to South and Central America's tropical and sub-tropical regions. It has been naturalized in many humid tropical areas of the world [Fucina et al. 2016]. However, little information exists about the underlying physiological mechanism and related vital components under salt stress.

Many studies have been conducted on introducing suitable plants for landscapes. However, a broad range of salinity tolerance exists among landscape plants. Selection of the proper species for use in the landscape has been troublesome [McFarland et al. 1990]. Moreover, many factors, such as plant type, environmental factors, and edaphic interaction with root-exposed salinity levels, influence plant salinity tolerance. Plant salinity response often differs with environmental factors such as temperature, relative humidity, and light. Also, the stage of plant development can influence salinity tolerance [Rouphael et al. 2017]. Saline water causes multiple direct and indirect effects on plant growth and metabolism [Carillo et al. 2019], along with visible symptoms like stunted growth and small leaves. Recent results showed that *Sesuvium portulacastrum*, a vegetation cover plant, can express high growth potential, even under severe salinity [Slama et al., 2007]. In some other studies, groundcover plants were shown to be affected by salinity. Results demonstrated that salt stress-induced oxidative stress in *Artemisia annua* L. may have damaged the photosynthetic process and caused a decrease in chlorophyll concentration and a decline in photosynthetic rate biomass productivity [Irfan Qureshi et al. 2005].

However, there is still a dearth of knowledge regarding the ranges of these plants' complex salt reactions. Unavoidably, some salt components are anions or cations with nutritional value for plants, which only cause destructive effects in high ionic concentrations. Under greenhouse conditions, this study evaluates the tolerance potentials and morpho-physiological characteristics of three tropical species of popular groundcovers with varying salt sensitivity.

MATERIAL AND METHODS

Plant materials and experimental design

Stem cuttings of *Alternanthera dentate* (Moench), *Sphagneticola trilobata* (L.) Pruski, *Alternanthera amoena* (Lemo) Voss obtained from mother plants were cultivated in a field grown at the campus of agricultural sciences and natural resources university of Khuzestan, Ahwaz, Iran. The cuttings of each species, approximately equal in diameter and length, consisting of three node sections of the stem, were prepared and immediately planted in pots containing 5 L of clay-loamy farm soil. Pots were placed in a greenhouse under sunlight conditions and a temperature of 30 ±5 °C and were watered daily. Propagules were grown until they had a well-developed root system and a stem size of 8 to 10 nodes (approximately 12–13 weeks). Three months later, these plants were treated with Hewitt's [1966] nutrient solution containing 25, 50, 75, and 100 mM NaCl alternative days. Plants irrigated with Hewitt's nutrient solution served as the control. All species were manually irrigated 1–3 times per week, depending on the evaporative demand inside the greenhouse. Drainage of 15–20% of the applied water was assured at each supply. Experimental values were collected in the third month after stress induction. The top second to fourth pair of leaves were selected for experimentation.

Growth and water content measurement

At the end of the experiment, all plants were uprooted and washed under running tap water, divided into root and shoot, and separately weighted (FW). Relative water content (RWC) was determined in the third or fourth youngest fully expanded leaf harvested in the morning. This parameter was determined using the following equation:

$$RWC = (L_{FW} - L_{DW}) / (L_{TW} - L_{DW})$$

[Schonfeld et al. 1988]

where FW is the leaf fresh weight recorded during collection, dry weight (DW), leaf parts were kept in an oven run at 75 °C for 72 h and LTW is the leaf turgid weight. To determine TW, a disc from leaves was kept in distilled water in darkness at 4ºC to minimize respiration losses until they reached a constant weight (after 24 h).

Proline content

The amounts free in the fresh leaf were determined spectrophotometrically using the Bates et al. [1973] method. The residue was eliminated by centrifuging the sample at 13,000 g for 8 minutes at 4 °C after homogenizing it in a mortar with 5 mL of sulphosalicylic acid (3% w/v) using about 0.5 g of the sample. The above extract (2 mL) was added to the reaction solution with 2 mL each of acid ninhydrin and glacial acetic acid. Then, this mixture was heated for one hour at 100 °C in a hot water bath. The reaction mixture could cool by placing the test tube in a cold bath. Toluene (4 mL) was added to the reaction mixture and vibrated rapidly with a stirrer for 20–30 seconds. The toluene-containing chromophore was aspirated and warmed to room temperature. The optical density was measured by absorbance of red color at 520 nm against a reagent blank.

Enzyme extractions and assays

Leaf fresh material (0.5 g) was homogenized in 1 mL of ice-cold buffer (50 mM sodium phosphate, pH 7.0) containing 1% soluble polyvinylpyrrolidone using a pre-chilled mortar and pestle. The supernatant was centrifuged at 15.000 rpm for 15 min at 4 °C. The supernatant was used for superoxide dismutase (SOD), peroxidase (POX) and catalase (CAT) assay. The SOD activity was measured following Beauchamp and Fridovich [1971] method. A 3 mL reaction mixture containing 50 mM phosphate buffer (pH 7.8) with 2 mM riboflavin, 13 mM methionine, 0.1 mM ethylenediaminetetraacetic acid (EDTA), 75 µM nitroblue tetrazolium (NBT) and enzyme extract (20 mM) was prepared. The reaction mixture was exposed for 10 minutes at low fluorescent light, and a spectrophotometer (NanoDrop 2000, Germany) was used to measure the reaction mixture's decrease in absorbance at 560 nm. One unit of SOD was defined as the amount of enzyme which caused 50% inhibition of NBT reduction under the assay condition, and the results were expressed as U mg−1 protein. CAT activity was assayed according to Aebi and Lester [1984]. The reaction mixture consisted of 50 mM potassium phosphate buffer (pH 7.0), 10.5 mM H_2O_2, and 20 µL enzyme extract. The decrease in H_2O_2 level was monitored and quantified by its molar extinction coefficient (36 M^{-1} cm^{-1}), and the results were expressed as µmol H_2O_2 min^{-1} mg^{-1} protein. POX activity was evaluated by adding 0.1 mL enzyme extract to the reaction mixture containing 1% H_2O_2 and pyrogallol phosphate buffer (pH 6.8). The change in the absorbance was recorded for 2 minutes at intervals of 20 seconds, at 420 nm, on a spectrophotometer (NanoDrop 2000, Germany) [Chance and Maehly 1956].

Sodium, potassium, and chloride determination

Fresh leaves and roots (only for chloride determination) were washed with distilled water immediately after harvesting, dried for 72 h in an oven at 65 °C, and powdered into fine particles with a mortar and pestle. The powders (300 mg) were added into 10 mL extraction solution (HNO_3, 0.5%) and then incubated at 75 °C for 60 minutes. The total K^+ and Na^+ were directly assayed in the mineralized by flame emission spectrophotometry [Lachica et al. 1973]. Chloride was assayed by the same extract by colorimetry [Alvarez and Sanchez-Blanco 2014].

Visual quality

This characteristic was assessed at the end of the experiment using a ranking scale from 0 to 10, 0 = no live, 10 = ideal shoot density, and uniformity [Salehi Salmi and Salehi 2013].

Data analysis

The experimental design was a completely randomized 3 × 5 factorial with five replicates per treatment. Two-way ANOVA analyzed the data, and all the analyses were carried out using SPSS 12.0 (SPSS Inc., Chicago, IL, USA). Treatment means were separated with Duncan's Multiple Range Test. Statistical comparisons were considered significant at $P \leq 0.05$.

RESULTS

Plant growth and water content responses to salinity

Significant differences were observed among salinity treatments and species for fresh shoot weight. *A. amoena* showed the highest reduction, with a shoot relative FW of 0.61, lower than that for *A. dentate* and *S. trilobata,* 0.73 and 0.92, respectively. Under treatment 50 mM, the three species showed different changes in shoot weight to control treatment, so that relative FW of shoot *A. amoena* was decreased (to 0.84),

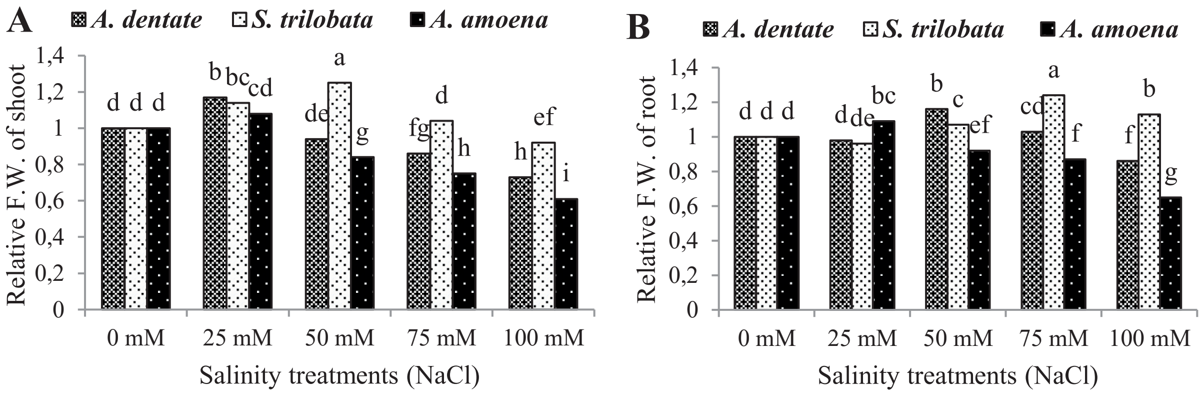

Fig. 1. Plant growth responses: A) relative fresh weight of shoot; B) relative fresh weight of root) of the three species under different saline water treatments at the end of the experiment. In each chart, columns followed by the same letter are not significantly ($p \leq 0.05$) different by Duncan's test

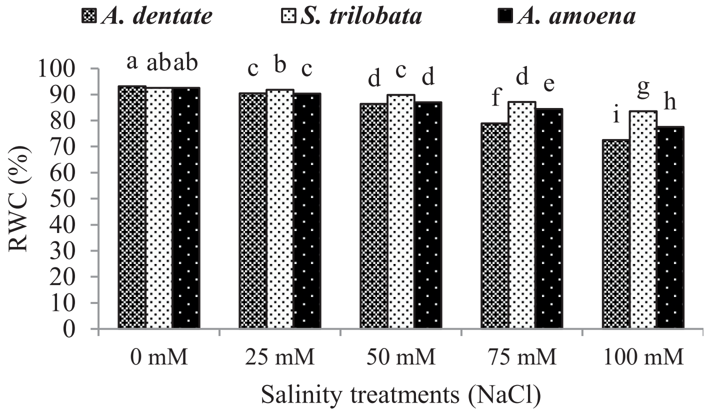

Fig. 2. Changes in RWC in leaves of three species grown under different salinity treatments. Columns followed by the same letter are not significantly ($p \leq 0.05$) different by Duncan's test

S. trilobata was increased (to 1.25), and *A. dentate* had no change (Fig. 1A). Root growth changed progressively with increasing salinity stress, from the mild stress level of 25 mM NaCl, but at different rates of changes for the three species (Fig. 1B). No significant differences were observed between the control and treatment 25 mM NaCl for *A. dentate* and *S. trilobata*. However, the sensitive species *A. amoena* showed a significant increase in its root growth. A more severe and significant decrease in root weight was observed under treatment with 75 mM NaCl for *A. dentate* and *A. amoena*. In contrast, most fresh weight of root was observed for *S. trilobata* in this treatment.

Saline water stress reduced RWC in the leaves of the three species grown at different levels. However, the effect was more significant at higher NaCl concentrations (Fig. 2). *S. trilobata* was less affected by NaCl than other species. The highest reduction in RWC was observed for *A. dentate*, with a relative value of 72.5% at 100 mM of NaCl.

Proline accumulation in leaves

Salinity increased the proline concentration in the leaves of all species, but the effect was more pronounced for *A. dentate* and *A. amoena* up to 50 mM NaCl and for *S. trilobata* up to 100 mM NaCl. At the

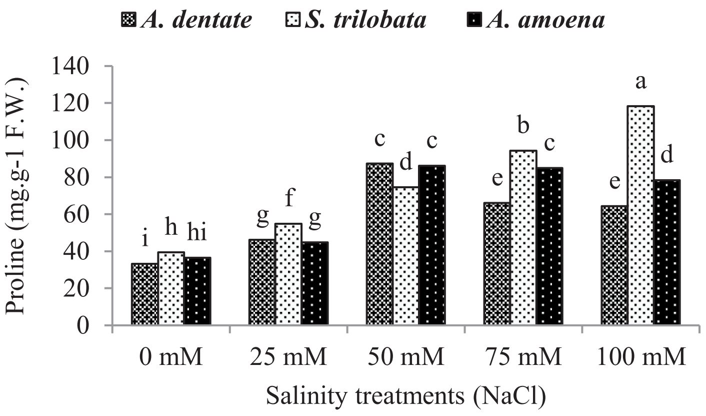

Fig. 3. Proline contents of three groundcovers at increasing NaCl concentrations (mM) in the irrigation water. Columns followed by the same letter are not significantly ($p \leq 0.05$) different by Duncan's test

high salt concentration, *S. trilobata* showed the most proline accumulation in leaves (Fig. 3).

Changes in the antioxidant enzyme

To study whether salinity influenced the antioxidant defense system in the groundcovers, the activities of major ROS-scavenging antioxidant enzymes, including SOD, POX, and CAT, were determined. In response to salinity stress, antioxidant enzyme activities were significantly changed. The SOD activity variation is illustrated in Table 1. SOD activity increased as NaCl further increased from 0 mM to 50 mM and declined as NaCl concentration increased from 75 to 100 mM. In all the species, POX activities showed a reducing trend with increasing salt concentrations irrespective of salt tolerance levels (Table 1). At any given concentration

Table 1. The means comparing the superoxide dismutase (SOD), peroxidase (POX), and catalase (CAT) of potted groundcover plants irrigated with five saline water concentrations

NaCl (mM)	SOD (U/g FW/min)			POX (µmol/min.g)			CAT (µmol/min.g)		
	A. dentate	*S. trilobata*	*A. amoena*	*A. dentate*	*S. trilobata*	*A. amoena*	*A. dentate*	*S. trilobata*	*A. amoena*
0	429	412	438	40.0	30.7	49.7	2.88	2.52	3.01
25	629	432	559	37.5	22.4	37.5	3.36	2.66	3.28
50	707	456	681	32.9	17.6	28.4	4.51	3.12	3.69
75	495	468	518	24.5	15.9	29.1	6.10	3.25	4.15
100	448	443	462	22.6	12.7	14.3	6.94	3.31	4.73
LSD Value	12.7	–	–	3.9	–	–	1.04	–	–

In each characteristic, treatment means were separated according to the LSD values.

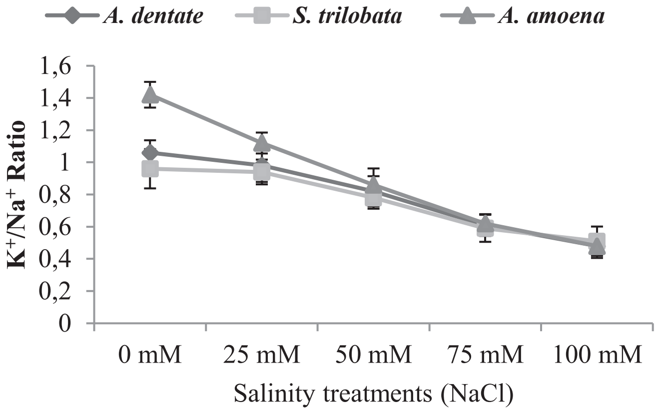

Fig. 4. The effect of different NaCl concentrations on the K^+/Na^+ ratio. Bars represent standard error values

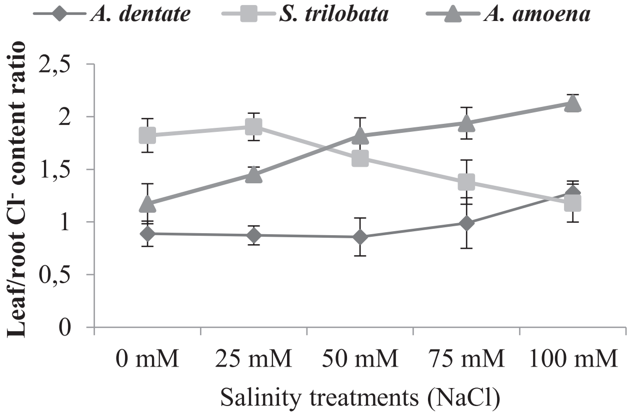

Fig. 5. Leaf/root Cl^- content ratio at increasing NaCl concentrations (mM) in the irrigation water. Bars represent standard error values

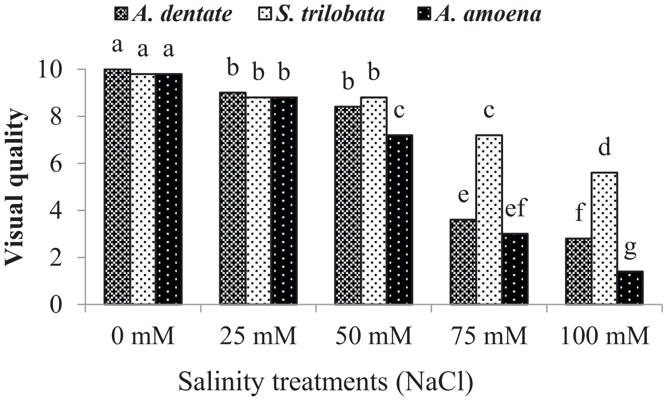

Fig. 6. Visual qualities of three groundcover plants at increasing NaCl concentrations (mM) in the irrigation water. Columns followed by the same letter are not significantly ($p \leq 0.05$) different by Duncan's test

of NaCl, the decrease or increase in enzyme activities was more pronounced in *A. dentate* than in other species. CAT activities in three groundcovers increased with increasing salinity levels. So, the CAT activity at 100 mM NaCl was increased by 240% in *A. dentate*, 131% in *S. trilobata*, and 157% in *A. amoena*, respectively, relative to the control (Table 1).

Potassium/sodium ratio and chloride changes

The ratio of potassium to sodium ions indicates plant salt tolerance, as it reflects the ability of plants to maintain a high K^+ uptake and a low Na^+ accumulation under saline conditions. The results showed a significant difference in the K^+/Na^+ ratio among the species in the control condition (without salinity), ranging from 1.4 for *A. dentata* to 1 for *S. trilobata*. However, as the salinity increased, the K^+/Na^+ ratio decreased for all species, indicating that they could not prevent Na^+ influx and/or enhance K^+ efflux under high NaCl concentrations. The plants irrigated with 100 mM NaCl had the lowest K^+/Na^+ ratio and the highest Na^+ levels compared to the plants of the other treatments (Fig. 4).

Leaf/root $Cl^–$ content ratio increased in *A. dentate* and especially in *A. amoena* (Fig. 5). These increases were due to the $Cl^–$ concentration of root in salt-stressed plants (data not shown). However, $Cl^–$ concentrations increased in the roots of stressed plants of *S. trilobata* (Fig. 5). In *A. dentate*, the $Cl^–$ concentration changes were small and primarily non-statistically significant. There were no changes in $Cl^–$ content in the roots (data not shown).

Visual qualities of three groundcover plants

The addition of salt in the nutrient solution caused a significant reduction in the visual qualities of all examined plants. These reductions were slight in *S. trilobata* and considerable in *A. amoena* (Fig. 6). The 50 mM NaCl concentration in *S. trilobata* and *A. dentate* showed only slight necrosis (data not shown). The reduction of visual quality was proportional to the increase in irrigation water salinity and reached, at the highest salt level, 2.8 in *A. dentate*, 5.6 in *S. trilobata,* and 1.4 in *A. amoena* (Fig. 6).

DISCUSSION

This study detected significant plant growth and RWC differences between the control and the four different salinity treatments during the vegetative stage. The threshold differs with species and cultivars, and most plants showed growth reductions at salinity levels above those values [Cai et al. 2014]. Three species of *Rose* were examined in response to salt toxicity in research by Niu et al. [2008]. Unlike the control, shoot growth in all species declined at high salinity and moderate levels. At the higher salinities, *R. fortuniana* exhibited less of a decrease in shoot growth than *R. multiflora* and *R. odorata*. In the current investigation, the fresh weight of the shoot varied significantly among the salinity treatments and species. *A. amoena* exhibited the highest reduction. Under moderate treatment, the three species showed different changes in shoot weight compared to the control treatment. The relative fresh weight of the shoot for *A. amoena* decreased to 0.84, *S. trilobata* increased to 1.25, and *A. dentate* had no change. These findings agree with the results reported by Paludan-Müller et al. [2002] and Hai Jing et al. [2009], showing salt-tolerant species gain more relative fresh weight than the susceptible ones under stress conditions.

RWC is one of the most significant physiological and reliable indices that can be used to assess the extent of various genotypes' resistance to stress [Hasheminasab et al. 2014]. According to research on several species of ornamental plants, including melaleuca and myrtle [Alvarez and Sánchez-Blanco 2014, Acosta-Motos et al. 2016], salinity decreases RWC. According to research by Rai et al. [2011], resistant species can retain their water potential under salt stress conditions for a more extended time than susceptible species. According to Sucre and Suárez [2011], the RWC of *Ipomoea pes-caprae* decreased as the severity of the salt stress increased. These findings agree with our results on various species of groundcovers.

Plants can modify their osmotic balance by accumulating osmolytes, such as proline, suitable for cell metabolism [Hasegawa et al. 2000]. In the present study, salinity increased the proline concentration in the leaves of all species, indicating that they activated the proline biosynthesis pathway to cope with salt stress. However, the effect of salinity on proline accumulation was more pronounced for *A. dentata* and *A. amoena* up to moderate salinity and for *S. trilobata* up to high level of NaCl, suggesting that these species had different thresholds of salt tolerance and proline response. As reported in some ornamental species

[Patel et al. 2009, Don et al. 2010], the plants may use proline to decrease the osmotic potential and maintain turgor. The mechanism in saline conditions enables plants to maintain a relative level of water content and achieve a turgor loss point at more negative water potential values [Acosta-Motos et al. 2016]. Similar findings were observed in reaction to salt stress in *Alternanthera philoxeroides* [Bolanos and Longstreth 1984] and *Melissa officinalis* [Khalid and Cai 2011].

Along with SOD and POX, CAT also plays a significant role in scavenging free radicals, especially when it comes to protecting against oxidation resulting from organ cell injury [Kumar et al. 2018]. Reactive oxygen species (ROS) cause membrane lipid peroxidation and chlorophyll degradation, reducing membrane fluidity [Charles et al. 2007]. The findings of this study demonstrated that SOD activity increased significantly as NaCl concentrations increased to 50 mM, and CAT activity increased as NaCl concentrations rose to 100 mM. Higher SOD and CAT activity under higher NaCl concentrations may contribute to greater salt tolerance of *A. dentate* when compared to *A. amoena*. As a result, *A. dentate* has developed a robust antioxidant defense mechanism to protect it from oxidative stress, so the increase in CAT and SOD activity could effectively reduce the damage. According to Wang et al. [2017], under conditions of severe salt stress, antioxidant enzyme activity was more significant in salt-tolerant cultivars than salt-sensitive cultivars. In reaction to higher salinity, POX activity was considerably reduced in all three species. Additionally, Kibria et al. [2017] demonstrated that the POX activity of rice genotypes is substantially reduced by high salinity. From the low antioxidant enzyme activity in *S. trilobata*, it can be concluded that the species has other biochemical and molecular mechanisms, such as proline compartmentalization and ions accumulation at the root, to counteract salt stress's effects [Garcia-Caparros et al. 2016].

In our study, differences in ion accumulation also varied in leaves, where acclimated *A. amoena* showed higher potassium content in leaves compared to two other species under normal conditions. However, in response to NaCl stress, potassium to sodium ratio was decreased gradually, especially in *A. amoena*. Our results are consistent with many researchers' observations that saline induces increased Na^+ concentrations in leaves [Cassaniti et al. 2009, Garcia-Caparros et al. 2016, Zhao et al. 2017]. Research on salt-tolerant plants like *Chenopodium quinoa* [Hariadi et al. 2011] showed that the buildup of these ions in roots could prevent Na^+ accumulation in shoots. This Na^+ retaining in the roots plays a significant role in the species' ability to adapt to salt conditions [Wahome 2000], even though glycophytes typically cannot prevent Na^+ accumulation in their shoots, particularly under long-term conditions. Also, plants maintain a K^+ to Na^+ ratio under salt stress in the cytosol. They do this by regulating the expression and activity of Na^+ and K^+ transporters and H^+ pumps, which create the driving force for transport [Zhao et al. 2017].

According to research by Tavakkoli et al. [2005], species followed different strategies based on the $Cl^–$ accumulation in the roots and leaves. The comparatively low $Cl^–$ concentration in the roots of *A. dentate* and *A. amoena* may indicate the involvement of $Cl^–$ exclusion, while the salt-tolerant species, *S. trilobata*, behaved as accumulators. Similar observations have been reported in *Rosa* sp. [Wahome et al. 2000]. They found that the salt-sensitive genotype, *R. chinensis* 'Major', had a higher chloride accumulation in the shoots than the salt-tolerant genotype, *R. rubiginosa*. On the other hand, R. *rubiginosa* had a higher $Cl^–$ accumulation in the roots, indicating that it could restrict the $Cl^–$ transport to the shoots. Therefore, *R. chinensis* 'Major' suffered more salt injury and ion imbalance than *R. rubiginosa*.

Our research findings indicate that introducing salt into the nutrient solution markedly reduced the visual quality of all plants under examination. The effect was relatively subdued in *S. trilobata* but notably more pronounced in *A. amoena*. This reduction in visual quality correlated directly with the increased salinity of the irrigation water. The indications of visual quality reduction are wilting and changes in leaf color [Sera 2017]. Electron microscopy in plants treated with NaCl reveals that the starch content of the chloroplasts declines, the size and number of plastoglobuli increase, and the thylakoidal structure of the chloroplasts becomes disorganized [Hernandez et al. 1999]. Necrosis typically advances from the tip leaf to its basis [Wahome 2000]. Other signs include wilting, necrosis of bud and shoot, and early leaf and needle fall-off. Similar observations have been reported in *Rosa × hybrida* L. [Cai et al. 2014] and *Ipomoea pes-caprae* [Sucre and Suarez 2011]. Irfan Qureshi et al. [2005] observed the occurrence of necrosis and chlorosis on the leaves of *Artemisia annua* L. after 45 days of treatment.

CONCLUSIONS

In this study, we evaluated the visual quality, biochemical, and physiological changes of three tropical groundcover species, *Alternanthera dentata*, *Sphagneticola trilobata*, and *Alternanthera amoena*, under different levels of sodium chloride (NaCl) stress. We used a multifactorial approach that considered the effects of increasing NaCl concentration in the irrigation water on the plant responses. This study showed that *S. trilobata* was a moderately salt-tolerant ornamental species, as it exhibited only slight reductions in the relative fresh weight of shoot and root, relative water content (RWC), and visual quality compared to the other two species. We also investigated the antioxidative enzyme activity under salinity stress, which is a vital indicator of the plant adaptation to environmental stress. We found that the increased proline content and antioxidative enzyme activity (catalase – CAT, and superoxide dismutase – SOD), as well as the maintenance of the K^+/Na^+ ratio, might be some of the physiological and biochemical mechanisms underlying the salinity tolerance of the plants. Therefore, we suggest that *S. trilobata* can be a suitable candidate for landscaping projects in saline areas and semiarid regions and in urban landscape design where the irrigation water quality is low. For future research, we recommend identifying the critical pathways involved in plant salinity tolerance and exploring the biomolecular mechanisms of salinity stress tolerance. Moreover, we propose that enhancing the antioxidative capacity and Na^+/K^+ ion compartmentalization may be linked to improving plant salt stress tolerance.

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