

Salinity-induced structural and functional changes in 3 cultivars of *Alternanthera bettzickiana* (Regel) G.Nicholson

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Abstract: Three cultivars of *Alternanthera bettzickiana* (Regel) G.Nicholson have been evaluated for their tolerance and adaptability potential to salt stress. During the experiment, 5 salt regimes were maintained: 18 (control), 50, 100, 150, and 200 mM NaCl. Salinity adversely affected all growth shoot length and leaves per plant. Root fresh and dry weight decreased with an increase in salinity levels in all 3 cultivars. *A. bettzickiana* 'Green' was the most tolerant among the cultivars under study, with relatively lower ion leakage through roots, larger vascular region area, and wide metaxylem vessel in roots and stems recorded. Also observed were greater phloem and pith cell area in stems that increased midrib thickness, cortical cell area, vascular bundle area, and metaxylem area in leaves with increase in salinity level. Moreover, the vascular region area in roots, cortical cell area, vascular region thickness, metaxylem area, phloem cell area and pith cell area in stems, leaf thickness, epidermal thickness, cortical cell area, vascular bundle area, and metaxylem area in leaves were recorded in this cultivar with an increase in salinity levels. All cultivars showed increased Na⁺ and Cl⁻ content and decreased K⁺ and Ca²⁺ with an increase in the salt level of the medium.

Key words: Anatomy, ion content, salinity tolerance, vascular region, leaf thickness

1. Introduction

Salinity is a general term used to describe the presence of elevated levels of different salts, such as chlorides, sulfates and bicarbonates of sodium, magnesium, and calcium in soil and water, and it usually results from water tables rising to, or close to, the ground surface (Munns et al., 2002). Soil salinity is a major abiotic stress in agriculture worldwide. Physical indicators of dry land salinity include groundwater discharge, i.e. dampness or free surface water, seepage on the slopes, salt on the soils, and soil blackening (Duncan et al., 2009; Nadeem et al., 2012); however, these signs are not always indicative of salinity.

Soil salinity occurs in many semiarid to arid regions of the world, inhibiting the growth and yield of the plants (Touchette et al., 2009; Amirjani, 2011) and thus creating a need for salt-tolerant plants (Munns and Tester, 2008). Unfortunately, the introduction of more salinity-resistant plants has been a relatively slow process, and this fact has generated debate among physiologists, breeders, and genetic engineers about the physiological mechanism involved in salinity resistance (Flower and Yeo, 1995; Hameed et al., 2011). To achieve salt tolerance, 3 important,

interconnected aspects of plant activities need to occur: damage must be prevented or alleviated, homeostatic conditions must be reestablished in the new stressful environment, and growth must resume, albeit at a reduced rate (Flowers and Colmer, 2008).

In plants grown under salinity, epidermal cells became thickened, reduction occurred in the number of vascular bundles, and metaxylem and protoxylem elements became disorganized and deformed in shape (Rashid et al., 1999; Dolatabadian et al., 2011). The structural changes under salinity are generally related to the growth status of the plants. The plants with greater leaf area and thickness, higher ratios of palisade to leaf thickness, and higher stele to root cross-section-area proportions can tolerate salinity better (Huang and Chen, 1995; Ali et al., 2009). Secondary wall thickening, either due to lignification or suberization, can also play a critical role in salinity tolerance (Hameed et al., 2008). Some interesting and intriguing features such as successive cambia phenomenon, succulence, salt hairs, tracheids, fleshy tissues, and peculiar foliar anatomy structures are the features of plants growing under high salinity levels (Grigore and Toma, 2007).

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Landscape and vegetation management in urban and roadside settings using alternative groundcovers has gained increased attention in recent years (Anderson et al., 2010), both in terms of aesthetic appeal and environmental preservation. Ground covers are getting more common as these are low-growing plants that spread over an area (Tariq et al., 2012). They are often used to solve a problem with erosion or for maintenance of steep slopes and are suggested where shade is too dense for growing turf grass (Younis et al., 2010). Ground covers are recommended around trees when the roots are at the surface and cause mowing problems. Among ground covers, *Alternanthera bettzickiana* is a species of dwarf, tender plants native to tropical America. *Alternanthera* is hardy and is used as a perennial. It grows well in almost any type of soil, but it has an additional advantage of tolerating saline conditions (Tucker et al., 1994). *Alternanthera* has a wide range of colored foliage, ranging from red to variegated types, and for this reason it has become popular as a ground cover and for border plantation. As cultivated plants are relatively sensitive to salt stress, the present study was focused on the adaptability potential of different cultivars of *Alternanthera bettzickiana* to high salinities, and therefore, identification of a suitable ornamental for salt-affected soils.

2. Materials and methods

The effect of salinity was observed on 3 cultivars of *Alternanthera bettzickiana* (Regel) G.Nicholson 'Green', 'Red', and 'Aurea'). The plants of each cultivar were raised at nursery of the Institute of Horticultural Sciences, University of Agriculture, Faisalabad, and were thereafter transplanted in a hydroponic cultural solution at the greenhouse of the Saline Agriculture Research Center of the Institute of Soil and Environmental Sciences during 2010 and 2011.

The plants were grown in a Hoagland's nutrient solution (Hoagland and Arnon, 1950), which was provided aeration for 12 h daily. Five salinity treatments at 18 (control), 50, 100, 150, and 200 mM of NaCl were maintained during the experiment. All plants were pruned to approximately 2.5 cm to restart growth after application of salinity treatments. The pH of solution in the range of 6.0–6.5 was maintained throughout the experiment with the help of a pH meter (Model Genway 3510). Nutrient solution was changed at 15-day interval when required.

Shoot length, root length, number of leaves/plant, and fresh and dry weights of root and shoots were recorded for agromorphic characteristics. For physiological parameters, membrane permeability was studied, i.e. ion leakage through roots, by unplugging the experimental plants and placing them in deionized water for several hours. Electrical conductivity of water was measured 3 times and the average was computed (Huang et al.,

2005). The analyses for Na^+ , K^+ , Ca^{2+} , and Cl^- ions were conducted after drying the planting material in an oven. Dried planting material was converted into powder after grinding. The material was digested for the estimation of Na^+ , K^+ , and Ca^{2+} (Yoshida et al., 1976). The Cl^- ions were estimated through chloride analyzer (Sherwood Chloride Analyzer Model-926) following the method of Gomez-Cadenas et al. (1998). Total soluble sugars were determined according to the method of Yemm and Willis (1954).

Permanent double-strained (safranin and fast green) slides of root, shoot, and leaf were prepared for anatomical studies following the method of Ruzin (1999). For root anatomy, a 2-cm piece from the thickest root, for shoot anatomy, a 2-cm piece from the top internode, and for leaf anatomy, a 2-cm piece from the leaf center including the midrib was selected. Camera photographs were taken with a Carl Zeiss camera microscope. Thickness and area of dermal, mechanical, parenchymatous, and vascular tissues were recorded during the experiment.

The experiment was laid out in a factorial, completely randomized design. Data were analyzed statistically using ANOVA techniques (Steel et al., 1997) and means were compared using Tukey's test.

3. Results

Alternanthera bettzickiana demonstrated a typically glycophytic response for shoot length with decreasing growth as salinity increases (Table 1). Cultivar Green had the maximum shoot length in the control (18 mM NaCl) compared to the other 2 cultivars, Red and Aurea. Root length gradually increased in all cultivars up to the 100 mM salt level, but thereafter it significantly decreased with further increase in salt level. Cultivar Green produced the smallest roots at all salt levels. The number of leaves in all 3 cultivars decreased with increase in salinity level of the culture medium. The maximum number of leaves was recorded in cultivar Green at lower salinity levels (18 and 50 mM). Shoot and root fresh and weights consistently and significantly decreased in all cultivars with increasing salinity level, but cultivar Green was the least affected compared to the other 2 cultivars. Cultivar Red was the worst performer in relation to these characteristics under salt stress.

Ion leakage through roots means the leakage of excess absorbed ions that have been exuded through the injured parts of roots. It is also a parameter to measure root injury at very high salinity levels. Ion leakage through roots significantly and consistently increased with increase in salt level of the medium, but cultivar Green showed a little more stability than the other cultivars. The maximum ion leakage was recorded in cultivar Red (Table 2), thus showing the maximum root injury.

Table 1. Morphological characteristics of 3 cultivars of *Alternanthera bettzieckiana* (Regel) G.Nicholson subjected to salt stress (means $\pm$ SE, n = 5).

	NaCl level	Shoot length (cm)	Root length (cm)	Number of leaves per plant	Shoot fresh weight (g plant ⁻¹)	Root fresh weight (g plant ⁻¹)	Shoot dry weight (g plant ⁻¹)	Root dry weight (g plant ⁻¹)
<i>Alternanthera bettzieckiana</i> 'Green'	18 mM	45.81 a $\pm$ 4.71	4.70 b $\pm$ 0.31	39.65 a $\pm$ 3.62	24.66 a $\pm$ 0.62	3.75 a $\pm$ 0.13	6.43 a $\pm$ 0.02	0.93 a $\pm$ 0.05
	50 mM	39.63 b $\pm$ 4.33	4.84 b $\pm$ 0.29	37.44 b $\pm$ 6.64	24.32 a $\pm$ 0.32	3.68 a $\pm$ 0.09	6.29 a $\pm$ 0.02	0.92 a $\pm$ 0.02
	100 mM	24.60 c $\pm$ 2.16	9.99 a $\pm$ 1.16	4.90 c $\pm$ 0.97	19.76 b $\pm$ 0.98	2.59 b $\pm$ 0.04	5.17 b $\pm$ 0.05	0.87 a $\pm$ 0.01
	150 mM	24.87 c $\pm$ 2.60	4.83 b $\pm$ 0.38	3.64 cd $\pm$ 0.43	18.81 bc $\pm$ 0.72	1.63 c $\pm$ 0.07	4.90 c $\pm$ 0.06	0.78 b $\pm$ 0.01
	200 mM	21.33 d $\pm$ 1.85	4.98 b $\pm$ 0.45	2.15 d $\pm$ 0.18	16.92 c $\pm$ 0.53	1.53 c $\pm$ 0.09	4.50 c $\pm$ 0.04	0.58 c $\pm$ 0.01
<i>Alternanthera bettzieckiana</i> 'Red'	18 mM	39.91 a $\pm$ 2.53	5.05 c $\pm$ 0.52	23.82 a $\pm$ 1.19	26.26 a $\pm$ 0.87	3.81 a $\pm$ 0.35	7.67 a $\pm$ 0.06	1.21 a $\pm$ 0.07
	50 mM	34.17 b $\pm$ 3.41	7.83 b $\pm$ 0.21	17.23 b $\pm$ 3.28	17.81 b $\pm$ 0.93	3.88 a $\pm$ 0.21	5.26 b $\pm$ 0.05	1.12 b $\pm$ 0.01
	100 mM	21.60 c $\pm$ 3.28	9.69 a $\pm$ 0.76	6.31 c $\pm$ 0.73	9.42 c $\pm$ 0.55	2.50 b $\pm$ 0.08	4.90 b $\pm$ 0.02	0.86 c $\pm$ 0.01
	150 mM	13.89 d $\pm$ 2.11	7.77 b $\pm$ 0.47	2.15 d $\pm$ 0.17	7.65 d $\pm$ 0.39	1.23 c $\pm$ 0.01	3.11 c $\pm$ 0.01	0.63 d $\pm$ 0.01
	200 mM	13.22 d $\pm$ 1.57	7.25 b $\pm$ 0.92	1.39 d $\pm$ 0.11	6.91 d $\pm$ 0.35	1.22 c $\pm$ 0.24	2.10 d $\pm$ 0.01	0.57 d $\pm$ 0.01
<i>Alternanthera bettzieckiana</i> 'Aurea'	18 mM	40.62 a $\pm$ 2.28	6.03 a $\pm$ 0.44	13.63 b $\pm$ 1.17	26.03 a $\pm$ 0.79	4.75 a $\pm$ 0.02	7.18 a $\pm$ 0.02	1.15 a $\pm$ 0.01
	50 mM	25.70 b $\pm$ 3.55	6.80 a $\pm$ 0.39	21.40 a $\pm$ 1.75	25.94 a $\pm$ 0.84	3.14 b $\pm$ 0.04	6.12 b $\pm$ 0.02	1.10 a $\pm$ 0.01
	100 mM	20.81 c $\pm$ 3.65	6.83 a $\pm$ 0.59	9.31 c $\pm$ 1.10	16.25 b $\pm$ 0.43	2.13 c $\pm$ 0.10	5.23 b $\pm$ 0.03	0.83 b $\pm$ 0.01
	150 mM	13.91 d $\pm$ 2.49	6.31 a $\pm$ 0.54	5.10 d $\pm$ 0.66	14.92 c $\pm$ 0.74	1.42 d $\pm$ 0.02	4.13 c $\pm$ 0.05	0.88 b $\pm$ 0.01
	200 mM	12.50 d $\pm$ 2.30	5.80 a $\pm$ 0.48	1.15 e $\pm$ 0.28	8.03 d $\pm$ 0.33	1.33 d $\pm$ 0.05	2.19 d $\pm$ 0.03	0.67 c $\pm$ 0.01

Means with the same letters in each column and each cultivar area are statistically nonsignificantly different.

Table 2. Shoot ionic content of 3 cultivars of *Alternanthera bettzickiana* (Regel) G.Nicholson subjected to salt stress (means $\pm$ SE, n = 5).

	NaCl level	Ion leakage through roots(dS m ⁻³)	Shoot Na ⁺ (mg g ⁻¹ DW)	Shoot K ⁺ (mg g ⁻¹ DW)	Shoot Cl ⁻ (mg g ⁻¹ DW)	Shoot Ca ²⁺ (mg g ⁻¹ DW)
<i>Alternanthera bettzickiana</i> 'Green'	18 mM	324.66 c $\pm$ 24.77	8.54 d $\pm$ 1.21	22.37 a $\pm$ 6.51	14.01 c $\pm$ 0.34	64.26 a $\pm$ 5.82
	50 mM	318.91 c $\pm$ 43.21	11.53 c $\pm$ 0.83	20.61 b $\pm$ 4.44	14.32 c $\pm$ 0.63	54.28 b $\pm$ 9.45
	100 mM	713.52 b $\pm$ 67.36	20.23 b $\pm$ 3.91	19.92 c $\pm$ 2.63	14.01 c $\pm$ 7.34	41.73 c $\pm$ 4.58
	150 mM	708.73 b $\pm$ 53.15	29.50 a $\pm$ 2.72	19.96 c $\pm$ 1.82	37.81 b $\pm$ 4.32	42.49 c $\pm$ 7.51
	200 mM	1094.49 a $\pm$ 122.12	30.10 a $\pm$ 2.82	19.41 c $\pm$ 3.71	67.32 a $\pm$ 5.12	41.17 c $\pm$ 7.64
<i>Alternanthera bettzickiana</i> 'Red'	18 mM	241.22 e $\pm$ 43.76	7.36 e $\pm$ 6.31	20.41 b $\pm$ 5.74	6.15 e $\pm$ 0.22	62.65 a $\pm$ 7.52
	50 mM	362.78 d $\pm$ 27.34	9.14 d $\pm$ 7.32	21.53 a $\pm$ 6.91	11.04 d $\pm$ 0.21	41.26 b $\pm$ 6.21
	100 mM	871.06 c $\pm$ 18.79	20.78 c $\pm$ 4.41	20.48 b $\pm$ 2.72	24.93 c $\pm$ 0.34	41.27 b $\pm$ 3.42
	150 mM	1493.17 b $\pm$ 65.28	29.02 b $\pm$ 4.86	19.50 c $\pm$ 1.42	46.39 b $\pm$ 1.72	34.17 c $\pm$ 2.46
	200 mM	1805.14 a $\pm$ 287.38	39.43 a $\pm$ 2.82	19.53 c $\pm$ 1.40	76.18 a $\pm$ 1.83	34.22 c $\pm$ 2.91
<i>Alternanthera bettzickiana</i> 'Aurea'	18 mM	63.36 e $\pm$ 8.25	80.91 a $\pm$ 9.52	22.46 a $\pm$ 1.83	14.18 c $\pm$ 0.62	57.24 a $\pm$ 4.32
	50 mM	365.99 d $\pm$ 18.77	41.73 b $\pm$ 8.67	20.53 b $\pm$ 2.86	14.37 c $\pm$ 0.80	47.27 b $\pm$ 3.72
	100 mM	681.62 c $\pm$ 76.23	30.42 c $\pm$ 3.83	20.54 b $\pm$ 3.62	14.37 c $\pm$ 0.80	41.23 c $\pm$ 3.63
	150 mM	864.93 b $\pm$ 33.65	30.51 c $\pm$ 2.83	20.03 b $\pm$ 1.28	20.80 b $\pm$ 3.93	42.17 c $\pm$ 3.71
	200 mM	1108.65 a $\pm$ 135.23	30.03 c $\pm$ 3.14	19.47 b $\pm$ 4.74	66.13 a $\pm$ 4.63	41.24 c $\pm$ 3.52

Means with the same letters in each column and each cultivar area are statistically nonsignificantly different.

Anatomical studies of plants are important because they show adaptive features of plants under any stress environment. Anatomical studies of different parts of *Alternanthera* were conducted to explain the behavior of those plants at the cellular level that had regrowth of roots and shoots at higher salinity levels of 150 and 200 mM. Root anatomy (Figure 1) in all 3 cultivars of *A. bettzickiana* showed a diverse response to salinity stress. Epidermal thickness (Figure 2) in cultivar Green decreased gradually and significantly with an increase in salinity level (Table 3). Cultivar Red showed significant increase in this character at the 50 mM NaCl level, but thereafter decreased consistently with a further increase in salinity level. In Aurea, epidermal thickness increased gradually and significantly up to the 150 mM salt level, but significantly decreased at the highest level (200 mM NaCl).

Vascular region thickness (Figure 3) increased significantly with an increase in salt levels in cultivars Green and Aurea. In Red, this parameter decreased up to the 100 mM NaCl level, but it totally disintegrated at higher levels (Table 3). A similar trend was recorded in the case of the metaxylem vessel area as in vascular region thickness. Induction of salt in the growth medium resulted in a significant increase in the phloem area of cultivar Green, but as the salinity level increased, this parameter decreased consistently. A gradual and significant decrease in phloem area was recorded in Aurea. Salt stress did not impose significant change in Red up to the 100 mM level, but phloem completely disintegrated at higher levels.

Stem epidermal thickness disintegrated at the highest salt level (200 mM NaCl) in all 3 cultivars. This layer generally decreased with an increase in salinity level,

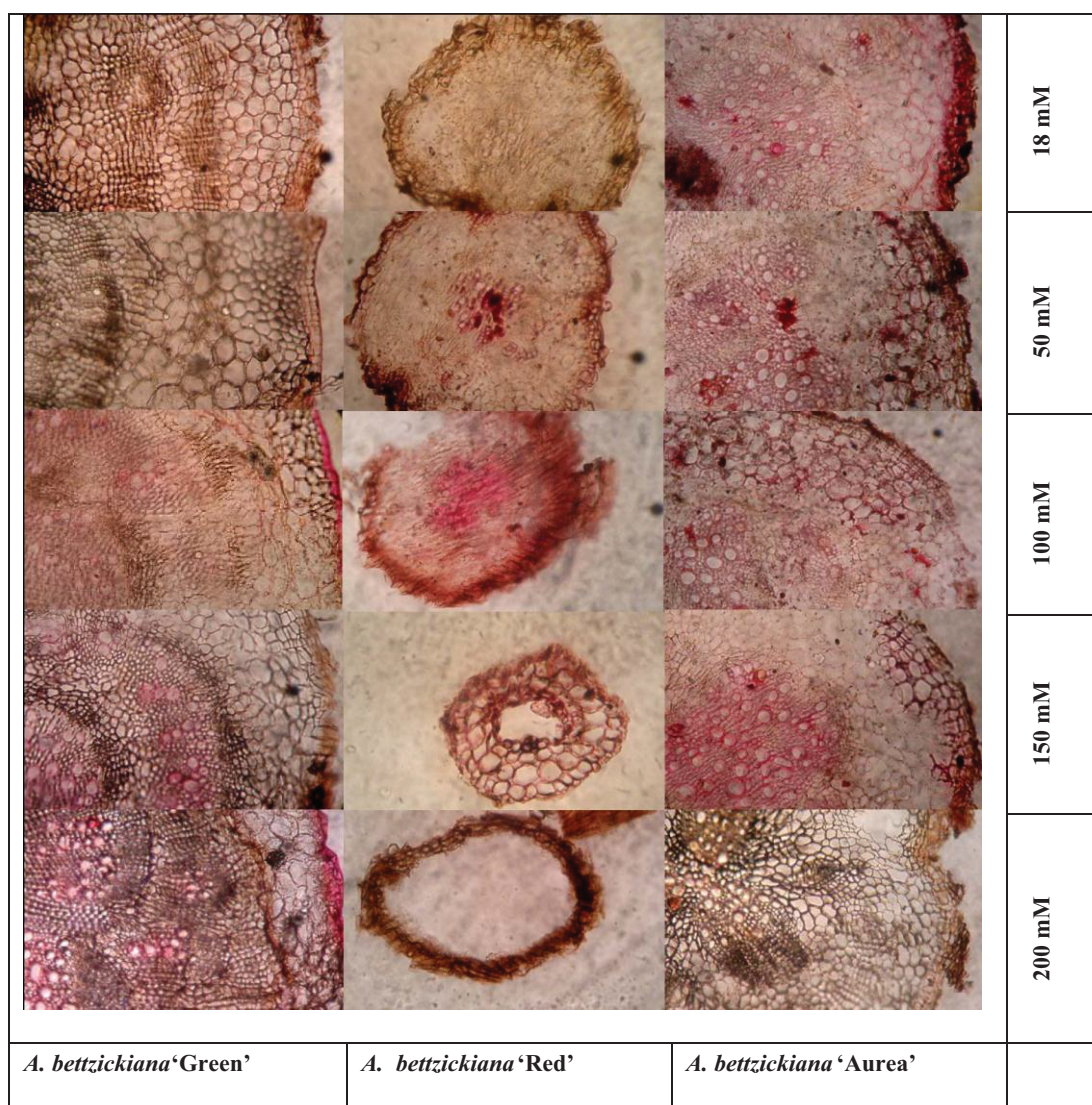


Figure 1. Root of 3 cultivars of *Alternanthera bettzickiana* (Regel) G.Nicholson subjected to salt stress.

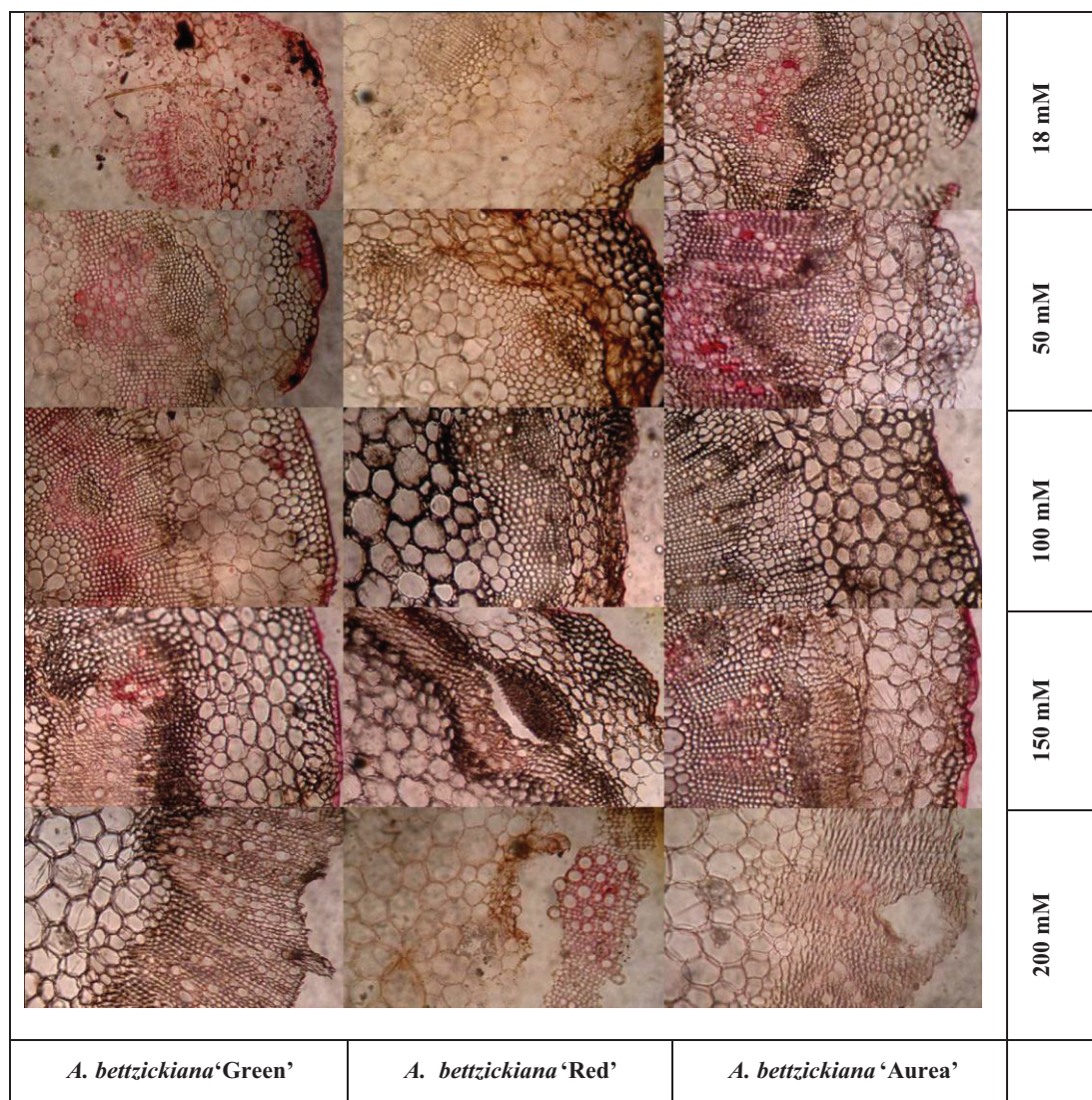


Figure 2. Stem of 3 cultivars of *Alternanthera bettzickiana* (Regel) G.Nicholson subjected to salt stress.

but cultivars Green and Aurea showed the maximum thickness at the 50 mM level (Table 4). Cortical region and cortical cell area also degenerated at the 200 mM level, like the epidermal layer. Cortical region thickness significantly and consistently decreased with increasing salinity level. The worst affected was cultivar Green. Cortical cell area, however, increased consistently with increasing salt level in Red and Aurea. Cultivar Green showed a significant increase in this parameter up to the 100 mM level, but a significant decrease at 150 mM was noted.

Thickness of the vascular region significantly increased with an increase in salt levels of the growth medium in all 3 cultivars, and the most prominent increase was recorded in Aurea. Metaxylem area, in contrast, consistently and significantly increased with the salinity level of the

medium in Red, but in Green, the vessel area increased up to the 100 mM salt level and then decreased with further increase in salt level (Table 4). In Aurea this parameter significantly increased at higher salt levels (150 and 200 mM NaCl). Phloem area was found to be significantly increased with increase in salt levels in Aurea, but in Green it increased up to the 150 mM level, whereas in Red, up to the 100 mM salt level an increase in phloem area was observed. It was found that higher salt levels resulted in significant decrease in phloem area in the cultivars. Pith cell area increased with an increase in salt levels, but in cultivar Red an increase up to the 100 mM salt level was observed.

Leaf midrib thickness increased with an increase in salt levels in 2 cultivars, Green and Aurea, but decreased

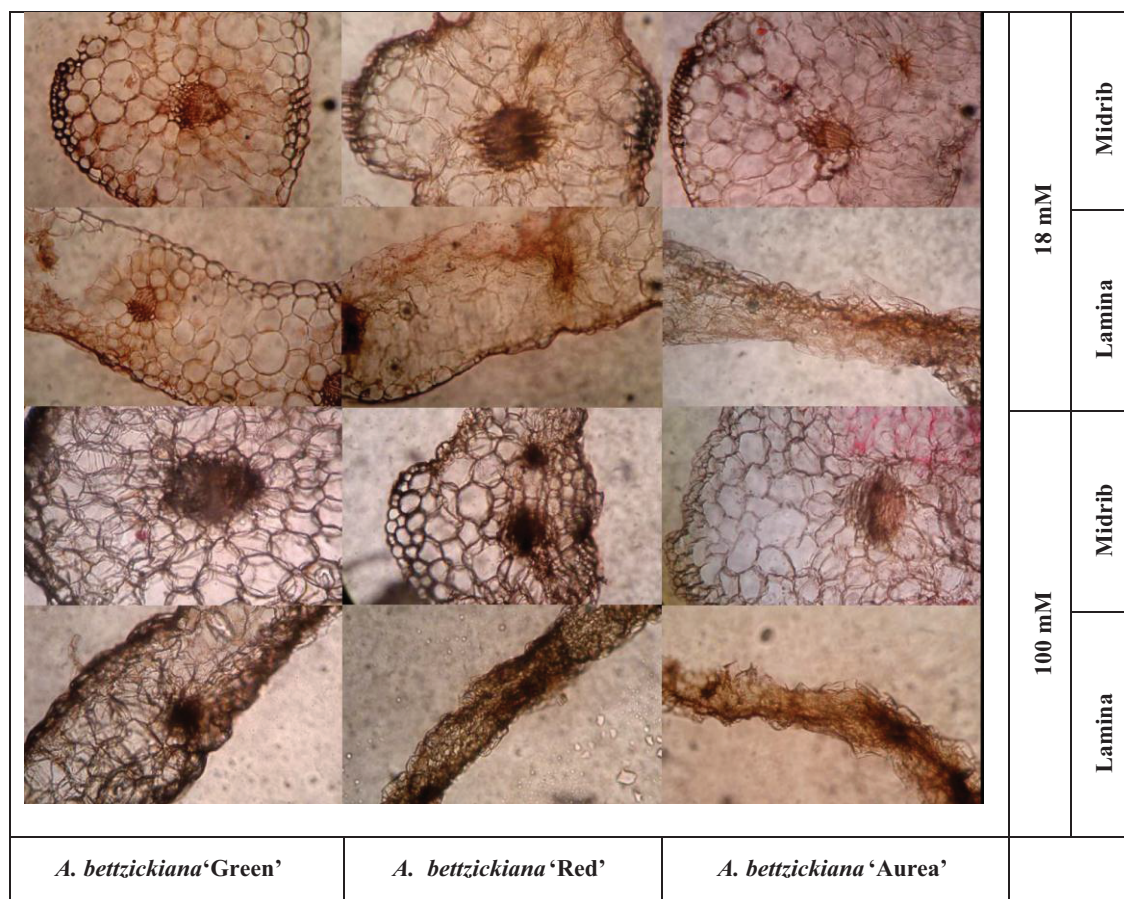


Figure 3. Leaf of 3 cultivars of *Alternanthera bettzickiana* (Regel) G.Nicholson subjected to salt stress.

in cultivar Red. In contrast, lamina thickness increased only in Green, but just at the highest level (Table 5). In the other 2 cultivars, a significant decrease in lamina thickness was recorded with an increase in salt levels. Epidermal thickness on both adaxial and abaxial leaf surfaces significantly increased with increasing salt levels. In Green, epidermal thickness increased on the adaxial side and decreased on the abaxial side, but results were not as significant for the adaxial side as the abaxial side. In Red, this character increased on the abaxial side and decreased on the adaxial side with an increase in salt levels.

Cortical cell area significantly increased in cultivars Green and Aurea, but it was more prominent in Green. In contrast, cortical cell area significantly decreased with increase in salt levels in Red.

Vascular bundle area significantly increased in cultivar Green with an increase in salt levels, but in Aurea at higher salt levels only (150 and 200 mM). This parameter significantly decreased in Red. Metaxylem area, however, significantly increased in all 3 cultivars, but larger vessels were recorded in Aurea at all salt levels.

4. Discussion

Decrease in shoot length could be attributed to the increase in NaCl concentration leading to Na⁺ and Cl⁻ toxicity because of their elevated concentration in growth medium (Pessarkali et al., 2006). Excessive accumulation of salts in the cell wall modifies the metabolic pathways and limits the cell wall elasticity. As a consequence, turgor pressure efficiency in cell enlargement declines. These processes may cause the shoot to remain smaller (Shahid et al., 2011). The reason for better performance of cultivar Red possibly may be that its root system might have developed certain mechanisms to cope with salt stress (Ogawa et al., 2011). The vigorous growth of root in cultivar Green at 100 mM NaCl proved it to be tolerant up to that level (Tucker et al., 1994; Sun et al., 2008). At higher salinity levels, the number of leaves decreased in order to preserve more water inside the plants, as if there are more leaves, the transpiration rate will be increased, which ultimately results in a high salt concentration that retards the growth (Fazeli-Nasab and Amozadeh, 2012).

Table 3. Root anatomical characteristics of 3 cultivars of *Alternanthera bettzickiana* (Regel) G.Nicholson subjected to salt stress (means $\pm$ SE, n = 5).

	NaCl level	Epidermal thickness (μm)	Cortical region thickness (μm)	Cortical cell area (μm^2)	Vascular region (μm)	Metaxylem area (μm^2)	Phloem area (μm^2)
<i>Alternanthera bettzickiana</i> 'Green'	18 mM	47.20 a $\pm$ 4.11	84.35 a $\pm$ 4.36	62.38 a $\pm$ 8.76	59.78 d $\pm$ 6.64	15.73 d $\pm$ 3.67	29.78 c $\pm$ 3.40
	50 mM	41.78 b $\pm$ 1.23	78.71 b $\pm$ 5.56	52.34 b $\pm$ 8.19	58.13 d $\pm$ 5.17	22.34 c $\pm$ 5.68	52.44 a $\pm$ 6.57
	100 mM	31.46 c $\pm$ 8.73	58.55 c $\pm$ 4.67	45.73 v $\pm$ 2.36	62.58 c $\pm$ 10.69	23.11 c $\pm$ 0.66	42.44 b $\pm$ 3.61
	150 mM	30.25 c $\pm$ 2.73	34.51 d $\pm$ 2.22	42.63 d $\pm$ 5.44	65.40 b $\pm$ 3.76	32.34 b $\pm$ 5.68	29.78 c $\pm$ 3.55
	200 mM	10.89 d $\pm$ 0.11	28.73 e $\pm$ 4.28	25.17 e $\pm$ 2.72	79.71 a $\pm$ 7.26	47.20 a $\pm$ 4.01	22.44 d $\pm$ 5.61
<i>Alternanthera bettzickiana</i> 'Red'	18 mM	15.73 d $\pm$ 2.36	37.91 a $\pm$ 5.77	27.82 b $\pm$ 4.66	20.90 a $\pm$ 3.54	28.67 b $\pm$ 4.55	27.33 c $\pm$ 4.82
	50 mM	29.46 a $\pm$ 2.73	25.72 b $\pm$ 3.33	34.67 a $\pm$ 3.66	21.46 a $\pm$ 2.34	31.47 a $\pm$ 2.28	52.44 a $\pm$ 7.61
	100 mM	28.67 a $\pm$ 4.34	24.66 b $\pm$ 1.56	21.78 c $\pm$ 1.22	12.48 b $\pm$ 1.91	29.78 b $\pm$ 3.66	48.44 b $\pm$ 5.36
	150 mM	20.78 b $\pm$ 1.23	12.06 c $\pm$ 0.67	22.02 c $\pm$ 1.71	Not recorded	Not recorded	Not recorded
	200 mM	14.89 d $\pm$ 1.11	5.67 d $\pm$ 0.92	12.19 d $\pm$ 0.16	Not recorded	Not recorded	Not recorded
<i>Alternanthera bettzickiana</i> 'Aurea'	18 mM	10.89 d $\pm$ 0.11	72.68 a $\pm$ 9.88	35.17 a $\pm$ 2.87	19.43 e $\pm$ 1.81	14.81 e $\pm$ 1.56	53.44 a $\pm$ 7.55
	50 mM	22.78 b $\pm$ 1.23	68.55 b $\pm$ 4.23	36.48 a $\pm$ 3.11	23.07 d $\pm$ 2.05	34.67 d $\pm$ 2.34	48.56 b $\pm$ 4.54
	100 mM	21.58 b $\pm$ 0.69	54.51 c $\pm$ 2.33	28.17 b $\pm$ 24.38	31.01 c $\pm$ 7.56	42.34 c $\pm$ 5.68	39.78 c $\pm$ 4.55
	150 mM	30.34 a $\pm$ 2.56	40.25 d $\pm$ 6.77	26.71 c $\pm$ 3.67	33.49 b $\pm$ 8.47	58.88 b $\pm$ 6.33	33.78 d $\pm$ 2.66
	200 mM	17.49 c $\pm$ 1.11	36.82 e $\pm$ 3.53	18.66 d $\pm$ 3.50	49.82 a $\pm$ 4.29	62.34 a $\pm$ 8.68	28.78 e $\pm$ 4.23

Means with the same letters in each column and each cultivar area are statistically nonsignificantly different.

Table 4. Stem anatomical characteristics of 3 cultivars of *Alternanthera bettzickiana* (Regel) G.Nicholson subjected to salt stress (means $\pm$ SE, n = 5).

	NaCl level	Epidermal thickness (μm)	Cortical region thickness (μm)	Cortical cell area (μm^2)	Vascular region thickness (μm)	Metaxylem area (μm^2)	Phloem area (μm^2)	Pith cell area (μm^2)
<i>Alternanthera bettzickiana</i> 'Green'	18 mM	29.78 b $\pm$ 3.55	120.42 a $\pm$ 14.47	25.17 c $\pm$ 2.87	29.78 e $\pm$ 3.55	20.78 d $\pm$ 1.23	22.44 e $\pm$ 4.35	10.48 d $\pm$ 1.11
	50 mM	34.67 a $\pm$ 7.34	101.24 b $\pm$ 18.57	33.63 b $\pm$ 2.51	33.49 d $\pm$ 4.18	28.38 c $\pm$ 2.57	25.67 d $\pm$ 2.71	15.23 c $\pm$ 2.41
	100 mM	24.67 c $\pm$ 0.74	25.31 d $\pm$ 3.45	36.66 a $\pm$ 3.15	42.58 c $\pm$ 05.94	35.67 a $\pm$ 2.71	28.67 c $\pm$ 2.66	15.80 c $\pm$ 2.84
	150 mM	19.78 d $\pm$ 1.77	36.76 c $\pm$ 3.78	21.54 d $\pm$ 3.41	48.80 b $\pm$ 4.67	34.14 a $\pm$ 5.23	64.19 a $\pm$ 5.29	22.02 b $\pm$ 1.71
	200 mM	Not recorded	Not recorded	Not recorded	65.06 a $\pm$ 7.84	31.67 b $\pm$ 2.34	52.44 b $\pm$ 4.61	67.71 a $\pm$ 5.73
<i>Alternanthera bettzickiana</i> 'Red'	18 mM	62.34 a $\pm$ 8.88	134.26 a $\pm$ 31.44	19.78 d $\pm$ 1.46	30.15 d $\pm$ 6.21	20.82 d $\pm$ 1.45	18.44 e $\pm$ 3.56	15.73 e $\pm$ 2.36
	50 mM	47.20 b $\pm$ 4.10	111.71 b $\pm$ 9.28	27.56 c $\pm$ 1.88	33.16 c $\pm$ 4.84	25.67 c $\pm$ 2.54	25.67 d $\pm$ 2.71	42.32 c $\pm$ 3.36
	100 mM	37.78 c $\pm$ 4.23	60.72 c $\pm$ 7.39	32.19 b $\pm$ 2.65	33.76 c $\pm$ 1.44	57.77 b $\pm$ 5.35	64.19 a $\pm$ 5.40	59.51 a $\pm$ 6.72
	150 mM	47.01 b $\pm$ 4.01	46.60 d $\pm$ 3.77	37.17 a $\pm$ 2.79	43.42 b $\pm$ 3.79	58.70 b $\pm$ 6.36	52.44 b $\pm$ 6.56	49.08 b $\pm$ 6.68
	200 mM	Not recorded	Not recorded	Not recorded	62.73 a $\pm$ 5.46	67.20 a $\pm$ 4.10	42.44 c $\pm$ 3.76	25.73 d $\pm$ 1.36
<i>Alternanthera bettzickiana</i> 'Aurea'	18 mM	55.33 b $\pm$ 13.67	137.26 a $\pm$ 35.43	26.21 d $\pm$ 1.81	39.33 e $\pm$ 4.17	12.83 c $\pm$ 0.85	19.67 d $\pm$ 1.71	23.89 d $\pm$ 1.56
	50 mM	62.34 a $\pm$ 8.66	104.62 b $\pm$ 15.13	37.87 c $\pm$ 2.37	45.88 d $\pm$ 3.87	21.35 b $\pm$ 4.67	25.67 c $\pm$ 2.71	25.80 c $\pm$ 3.64
	100 mM	29.78 d $\pm$ 3.77	91.23 c $\pm$ 8.67	53.19 b $\pm$ 3.11	62.93 c $\pm$ 58.46	21.35 b $\pm$ 3.43	19.67 d $\pm$ 1.71	26.45 c $\pm$ 2.21
	150 mM	34.67 c $\pm$ 2.55	76.60 d $\pm$ 4.77	65.22 a $\pm$ 5.67	77.61 b $\pm$ 7.44	32.34 a $\pm$ 5.66	48.44 b $\pm$ 6.55	62.93 b $\pm$ 8.46
	200 mM	Not recorded	Not recorded	Not recorded	94.40 a $\pm$ 9.20	32.44 a $\pm$ 4.64	64.19 a $\pm$ 6.67	65.17 a $\pm$ 2.38

Means with the same letters in each column and each cultivar area are statistically nonsignificantly different.

Table 5. Leaf anatomical characteristics of 3 cultivars of *Alternanthera bettzickiana* (Regel) G.Nicholson subjected to salt stress (means $\pm$ SE, n = 5).

	NaCl level	Midrib thickness (μm)	Lamina thickness (μm)	Adaxial epidermal thickness (μm)	Abaxial epidermal thickness (μm)	Cortical cell area (μm^2)	Vascular bundle area (μm^2)	Metaxylem area (μm^2)
<i>Alternanthera bettzickiana</i> 'Green'	18 mM	65.36 d $\pm$ 6.42	35.31 b $\pm$ 3.45	29.78 b $\pm$ 3.63	62.34 a $\pm$ 5.56	64.40 e $\pm$ 5.28	73.37 e $\pm$ 11.62	14.89 c $\pm$ 3.11
	50 mM	68.21 c $\pm$ 4.79	35.12 b $\pm$ 3.11	30.44 b $\pm$ 4.71	60.96 a $\pm$ 6.67	67.15 d $\pm$ 4.67	77.56 d $\pm$ 5.73	15.53 c $\pm$ 2.28
	100 mM	79.17 b $\pm$ 5.82	36.73 b $\pm$ 4.85	31.26 b $\pm$ 3.26	44.35 b $\pm$ 7.21	69.39 c $\pm$ 5.59	83.37 c $\pm$ 9.68	15.21 c $\pm$ 2.39
	150 mM	80.25 b $\pm$ 11.15	36.14 b $\pm$ 4.79	31.18 b $\pm$ 2.79	40.11 c $\pm$ 3.53	82.81 b $\pm$ 5.81	105.48 b $\pm$ 9.15	17.84 b $\pm$ 3.41
	200 mM	110.21 a $\pm$ 07.25	39.16 a $\pm$ 5.87	34.67 a $\pm$ 2.34	20.78 d $\pm$ 1.23	110.22 a $\pm$ 8.73	144.04 a $\pm$ 40.27	20.78 a $\pm$ 1.23
<i>Alternanthera bettzickiana</i> 'Red'	18 mM	77.15 a $\pm$ 7.44	32.68 a $\pm$ 2.66	62.34 a $\pm$ 8.68	14.11 d $\pm$ 1.56	80.22 a $\pm$ 6.89	60.04 b $\pm$ 3.78	11.51 b $\pm$ 1.37
	50 mM	63.79 b $\pm$ 6.67	25.48 b $\pm$ 1.85	45.44 b $\pm$ 5.13	21.63 c $\pm$ 3.96	58.13 b $\pm$ 3.86	77.15 a $\pm$ 8.55	10.73 b $\pm$ 1.47
	100 mM	57.81 c $\pm$ 5.81	15.76 c $\pm$ 1.17	36.72 c $\pm$ 4.62	28.13 b $\pm$ 4.23	46.73 c $\pm$ 4.93	53.63 c $\pm$ 7.29	10.36 b $\pm$ 1.39
	150 mM	54.96 d $\pm$ 4.72	11.43 d $\pm$ 0.93	34.39 c $\pm$ 3.77	30.77 a $\pm$ 4.47	41.54 d $\pm$ 5.57	48.15 d $\pm$ 5.77	11.29 b $\pm$ 1.63
	200 mM	53.15 d $\pm$ 9.54	10.21 d $\pm$ 0.56	31.67 d $\pm$ 2.27	31.67 a $\pm$ 2.34	34.40 e $\pm$ 6.20	48.66 d $\pm$ 3.13	15.32 a $\pm$ 1.15
<i>Alternanthera bettzickiana</i> 'Aurea'	18 mM	69.18 d $\pm$ 8.44	24.51 a $\pm$ 2.94	34.40 d $\pm$ 9.20	18.35 e $\pm$ 2.59	41.32 d $\pm$ 8.24	28.09 c $\pm$ 3.78	23.78 c $\pm$ 1.23
	50 mM	69.91 d $\pm$ 6.63	20.18 b $\pm$ 1.99	35.32 cd $\pm$ 4.45	21.72 d $\pm$ 3.27	44.39 c $\pm$ 6.41	26.18 d $\pm$ 5.15	24.58 c $\pm$ 1.17
	100 mM	75.42 c $\pm$ 5.28	20.73 b $\pm$ 3.17	37.15 c $\pm$ 4.18	28.68 c $\pm$ 3.95	43.34 c $\pm$ 5.27	29.93 c $\pm$ 6.21	24.11 c $\pm$ 2.63
	150 mM	77.65 b $\pm$ 8.11	18.47 c $\pm$ 5.23	40.47 b $\pm$ 5.22	35.47 b $\pm$ 4.65	47.83 b $\pm$ 5.85	35.48 b $\pm$ 3.48	26.83 b $\pm$ 3.52
	200 mM	79.24 a $\pm$ 75.67	12.25 d $\pm$ 1.62	48.46 a $\pm$ 5.73	62.34 a $\pm$ 8.68	54.16 a $\pm$ 6.32	48.66 a $\pm$ 8.35	29.78 a $\pm$ 5.23

Means with the same letters in each column and each cultivar area are statistically nonsignificantly different.

The fresh weight of plants represents the total biomass production by the plant. Reduction in shoot and root fresh weight may be due to decrease in water potential of rooting medium due to high ion concentrations, as initial growth inhibition in saline conditions is related to osmotic effect. Under salinity, plant cell turgor pressure decreases and stomatal closure takes place, resulting in decreased photosynthesis (Gale and Zeroni, 1984; Ibrahim, 2003). Na^+ and Cl^- in excess concentration in the rooting medium could cause hindrance in water and nutrient uptake by roots (Gandonou et al., 2005). Dry weight of shoot and root represents the mineral composition of the plant. Dry weights of root and shoot weight decreasing could be attributed to increased uptake of Na^+ ions and toxicity to roots (Munns et al., 1995; Aini et al., 2012). Salinity affects the growth of plants and therefore results in a decrease in dry matter production (Eker et al., 2006; Mahmood et al., 2008).

Roots actively transport nutrients across membranes against concentration gradients and into the roots by various means, which require metabolism of energy-containing compounds (Sondergaard et al., 2004). The flow of salt into the roots can be independent of water flow, especially if there is no leakage (Krishnamurthy et al., 2011). Ion leakage through roots could be attributed to ion toxicity to membranes due to high sodium and chloride influx (Cha-um et al., 2010). High amounts of NaCl may affect the availability of other ions, e.g. K^+ and Ca^{2+} , which are extremely important, and their deficiency may result in reduced plant growth and even death (Rogers, 2008). Increasing salinity resulted in the accumulation of Na^+ in all 3 cultivars. Cultivar Red showed more accumulation of Na^+ , particularly at higher salt levels (150 and 200 mM NaCl), than that recorded in the other cultivars. Na^+ accumulation caused ion toxicity, especially in glycophytes, and hence a drastic reduction of growth is a common feature (Shekhawat and Kumar, 2006; Hyun et al., 2007). The possible cause for more accumulation of Na^+ could be that cation transporters in cell membranes are somewhat permeable to Na^+ and there is constant influx of Na^+ down this electrochemical gradient that cannot be completely prevented (Pardo and Quintero, 2002).

K^+ is the preferred inorganic cation of living cells, and plants are no exception to this rule, yet almost invariably the concentration of K^+ in the soil solution is lower than the cytosolic K^+ concentration (100–200 mM), meaning that plants must actively take up and concentrate K^+ using various types of ion transporters (Munns and Tester, 2008). All salinity treatments significantly affected K^+ concentration, and this might accompany increased Na^+ uptake (Flowers and Colmer, 2008). The possible reason for decrease in K^+ concentration with increase in salinity could be that excess external Na^+ can not only impair K^+

acquisition but can also lead to accumulation of Na^+ in plant cells (Hyun et al., 2007). More Na^+ influx inhibits K^+ permeability to the cells, thus decreasing its concentration (Flowers and Colmer, 2008).

Calcium is an essential element in all plants (Marschner, 1995). The ability of Ca^{2+} to form intermolecular linkages gives it an important role in maintaining the integrity and structure of membranes and cell walls (Hanson, 1984). Ca^{2+} is also used as a second messenger in many signal transduction pathways within the cell (Hepler, 2005). Ca^{2+} accumulation in plants significantly decreased with an increase in salt level in all cultivars, and this decrease was more prominent in cultivar Red. The reason for a significant decrease in concentration of Ca^{2+} at all salinity treatments might be due to high Na^+ influx, which hampered Ca^{2+} influx (Hameed and Ashraf, 2008).

Chloride in low concentrations is an essential cofactor for photosynthesis. Considerable support for the postulated Cl^- requirement in photosynthesis came from the observation that Cl^- is essential for growth. It raises the cell osmotic pressure, affects regulation of stomata, and increases the hydration of plant tissue. Excess causes burning of plants and bronzing of leaves (Tavakoli et al., 2010). Salinity treatments significantly affected all 3 cultivars. Concentration of Cl^- significantly increased with an increase in salinity of the culture medium, and the worst affected was cultivar Red. Cl^- uptake causes ion toxicity, and thus a decline in plant growth (Lenz et al., 2006).

Growth and development of plants can be related to plasticity in anatomical characteristics for successful survival in environmental hazards (de Pereira-Netto et al., 1999). Root epidermis is directly exposed to surrounding environments and therefore important in controlling water movements through roots (Bahaji et al., 2002; Saleem et al., 2011). Increased thickness under salt stress may play a key role in drought and physiological drought conditions. Cortical region thickness and cortical cell area decreased consistently and significantly with increasing salt levels in all 3 cultivars, the worst affected due to salt stress being cultivar Red. Parenchyma (cortical region) is the important component of roots, which is a storage region for water, nutrients, and toxic ions (Lux et al., 2004; Hameed et al., 2009). Large cortical cells helped Aurea tolerate salt stress to a higher extent (Zwieniecki and Newton, 1995). Larger vessel diameter is identified as having lower resistance to water conduction (Nicotra et al., 2002). On this basis, it can be concluded that cultivars Green and Aurea are more adaptable to salt stress. Na^+ has been reported to be a major ion causing injury to cells, particularly in glycophytes (Boutilier, 2001; Rashid et al., 2001).

Leaf architecture can play an important role under salt stress (Balsamo et al., 2006). Structural modifications in leaves under limited moisture availability

contribute significantly in increasing degree of tolerance (Rhizopoulou and Psaras, 2003), and therefore they are an indicator for adaptation to salt stress (Fahn and Cutler, 1992). Leaf thickness, along with epidermal thickness, is directly related to salt tolerance in different plant species (Balsamo et al., 2006). There are a few reports of increased leaf thickness due to salt stress (Abdel and Al-Rawi, 2011). Parenchyma is a water storage tissue, and hence it is essential for survival under physiological drought (Zwieniecki and Newton, 1995; Baloch et al., 1998). Enlarged metaxylem

vessels in the stem again play a critical role for better transport of water and minerals (Steudle, 2000; Ali et al., 2009). Better development of vascular tissue, and in particular size of metaxylem vessels, may be important for efficient transport of solutes and photosynthates under salt stress (Weerathaworn et al., 1992).

It is concluded that cultivars Green and Aurea have developed specific structural and functional strategies for salinity tolerance and therefore had better growth and survival under salt stress.

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