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PHYSIOLOGICAL AND MORPHOLOGICAL RESPONSE OF POTENTIAL SALT TOLERANT TURFGRASS SPECIES TO SALINITY STRESS

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Abstract

In the present research, the growth responses and quality of turfgrass species were studied under salinity stress. Chlorophyll content, relative water content, proline accumulation, and mineral content analysis used in this study were highly related with one another, indicating their mutual effectiveness in predicting relative salinity tolerance. Relative water and chlorophyll content were found high in *Paspalum vaginatum* Sw., *Zoysia matrella* L., and *Cynodon dactylon* (L.) Pers. 'satiri', whereas, proline content was low. These three species were less affected by selectivity of saline ion (Na) uptake. Physiological parameters, indicating that *P. vaginatum, Z. matrella* and *C. dactylon* 'satiri' are more salt tolerant than *C. dactylon* 'tifdwarf'. The SEM micrograph showed salt gland excretion presence on *Z. matrella, C. dactylon* 'satiri' and *C. dactylon* 'tifdwarf' leaves. Roots cortex cell collapsed on *C. dactylon* 'tifdwarf' was greater compared to other three species

Introduction

Use of brackish ground water or saline sewage effluent for landscape irrigation in many areas of the world including Malaysia has resulted in increasing demand for salt tolerant turfgrasses (Uddin *et al.* 2011). The detrimental effects of salinity on turfgrass growth include osmotic stress, ion toxicity and nutritional disturbances (Cheeseman 1988).

Salt tolerant plants have the ability to minimize these detrimental effects by producing a series of morphological, biochemical process and physiological adaptations (Poljakoff-Mayber 1988). Under the variation of saline environments, different species have developed different adaptative mechanisms (Borsani *et al.* 2003, Sairam *et al.* 2006). Hester *et al.* (2001) observed intraspecific variation in morphological and physiological traits under salinity stress. Many attempts have been made to detect the salinity tolerance ability of crop species or cultivars by measuring photosynthetic parameters such as changes in chlorophyll content, chlorophyll fluorescens (Lee *et al*. 2008, Uddin *et al.* 2009); synthesis and accumulation of low-molecular weight organic compounds in the cytosol and organelles such as proline and glycine betaine (Ashraf and Harris 2004, Bartels and Sunkar 2005, Sairam *et al.* 2006) and water relation response of plants (Alshammary 2004). Therefore, the goal of this study was to examine the growth responses and quality of three potential salt tolerant turfgrass species under different salinity levels.

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Materials and Methods

The experiment was conducted in the glasshouse and laboratory at the Faculty of Agriculture in Universiti Putra Malaysia UPM). Three most salt-tolerant species based on genera and one medium salt-tolerant turfgrass species were selected for use in this study as listed in Table 1.

Table 1. Scientific and common names, locations and salt tolerant grade of turfgrass species used in this study.

Scientific name	Common name	Locations	Tolerant grade
Paspalum vaginatum Sw.	Seashore pasplaum	Universiti Putra Malaysia (UPM)	Salt-tolerant
Zoysia matrella L.	Manilagrass	PantaiBisikanBayu	Salt-tolerant
Cynodon dactylon (L.) Pers.	Bermudagrass (satiri)	UPM	Salt-tolerant
Cynodon dactylon (L.) Pers.	Bermudagrass (tifdwarf)	UPM	Medium salt-tolerant

The soil medium was prepared by thoroughly mixing washed river sand and Peat-Grow (KOSAS®) in the ratio of 9 : 1 (v/v). Basal fertilizer, Christmas Island Rock Phosphate (CIRP) at the rate of 0.5 kg P/100 m² and liming at the rate of 0.6 kg/100 m² were mixed into the soil mixtures before planting. The prepared soil medium was pulverized and visible insect pests and plant propagules were removed. The media was filled into plastic pots of size 14 cm diameter × 12 cm depth (1848 cm^3 volume). The adhering native soil was washed off from the turfgrasas sods (5 cm × 5 cm) and transplanted into each of the plastic pots containing the soil media. Plants were grown for 8 weeks with non-saline irrigation water in order to achieve full establishment prior to treatment. All pots were fertilized every two weeks with NPK Green (15:15:15) @ 50 kg N/ha.

The required quantity of sea water was collected from Port Dickson, Negeri Sembilan, Malaysia. The EC of the collected sea water was 48 dS/m. Five concentrations *viz.* 0, 12, 24, 36 and 48dS/m prepared from sea water were evaluated in this study. The salinity level was measured by an EC meter (HANNA® Model HI 8733). Untreated checks were irrigated with distilled water. Seawater was diluted with distilled water to obtain 12, 24, 36 and 48 dS/m salinity levels. To avoid osmotic shock, salinity levels were gradually increased by daily increments of 6d S/m salinity in all treatments until the final salinity levels were achieved. After two weeks, when the targeted salinity levels were achieved, 250 ml of the respective treatment solutions were applied to each pot on a daily basis for a period of four weeks at morning (9 a.m.) and evening time (6 p.m.).

At the end of the experiment, shoots and roots were harvested and washed with tap water and finally with distilled water. The samples were carefully washed to remove all soil particles. Samples were then dried in oven at 70^0C for 3 days until constant weight was achieved and dry weight (g/pot) was recorded.

Relative water content (RWC) was determined as described by Gonzales and Gonzales (2003) on leaf tissues excised in the morning (around 9.00 a.m.). Leaf dry weights were recorded after being oven dried for one week at 60^0C. The leaf relative water content was determined using the following formula:

$$\text{Relative water content } (\%) = \frac{(\text{Fresh} - \text{dry weight})}{(\text{Fully turgid weight} - \text{dry weight})} \times 100$$

Chlorophyll content was estimated using the method of Witham *et al.* (1986). Fresh leaf from each pot was cut into pieces by a scissors and 200 mg of cut leaves were transferred into a plastic

vial containing 20 ml of 80% acetone. The vial was quickly corked airtight and kept in the dark for 72 hrs. Absorbency of the solution was recorded at 645 and 663 nm using a scanning spectrophotometer (Model UV-3101PC, UV-VIS NIR). Chlorophyll content was estimated and expressed as mg/g of sample using the following formula (Arnon 1949):

$$\text{Total chlorophyll content (mg/g fresh leaf)} = \frac{20.2\,(A_{645}) + 8.02\,(A_{663})}{1000} \times \frac{V}{W}$$

where, A_{645} = Absorbance of the solution at 645 nm, A_{663} = Absorbance of the solution at 663 nm, V = Volume of the solution in ml and W = Weight of fresh leaf sample in gram. The respective absorption co-efficient of the following above are 12.7, 2.69, 22.9, 4.86, 20.2, and 8.02.

Proline was estimated according to the method of Bates *et al.* (1973). The proline concentration was determined from a standard curve, and calculated on a fresh weight basis as follows:

$$\mu\text{mol proline/g fresh weight} = \frac{(\mu\mu\text{ proline m/l} \times \text{ml of toluene}/115.5)}{\text{g of sample}}$$

Shoot and root morphology were observed using a Scanning Electron Microscope (SEM) (JEOL JSM-5610LV, Japan) at high vacuum and acceleration voltage of 15 kV with a working distance of 23 mm.

Plant samples were put in the ink-free envelopes and then dried in the oven at 70 ^{0}C for 72 hours. Oven-dried shoot and root samples of turfgrasses were ground and stored in plastic vials. Potassium (K), calcium (Ca), magnesium (Mg) and sodium (Na) were measured using the digestion method (Ma and Zua 1984). The digested samples were analyzed for K, Ca, Mg and Na using the Atomic Absorption Spectrophotometer (AAS) (Perkin Elmer, 5100, USA).

The data were analyzed statistically using ANOVA following RCBD design from SAS (2004). The treatment means were compared by LSD at 5% level. Regression analysis was performed as necessary by using the replicated data. Pearson's correlation analysis was performed to determine the relationship between response variables.

Results and Discussion

Four different levels of salinity stress resulted significant decreases in relative water content (RWC) of all species (Fig. 1). *Paspalum vaginatum* had the highest RWC (93%), while *C. dactylon* 'satiri' had the lowest RWC (88.15%) under control treatment. Meanwhile, *P. vaginatum* and *Z. matrella* had not experienced any changes of RWC at 12 dS/m. At the 24 dS/m salinity level and afterwards, all species gradually decreased in RWC. On the other hand *P. vaginatum* had higher RWC compared to other species throughout all the salinity levels. In a survey of 25 species of salt tolerant grasses, Glenn (1987) found that tolerance to Na was associated with leaf relative water contents. More salt tolerant plants can still function in maximizing water uptake and turgor pressure, meaning that water relations to salinity stress are key mechanisms in the saline environment (Bohnert and Jehen 1996).

Total chlorophyll content of all turf species was significantly decreased at salt stress, showing different magnitude of declining trend (Fig. 2). *Paspalum vaginatum* showed slightly decreasing in total chlorophyll content when salinity was increasing to 24 dS/m, but no significant decrease was observed with increasing salinity thereafter. Similar trend was found in *C. dactylon* 'satiri', where total chlorophyll decreased at 12 dS/m but showed insignificant change afterwards. According to the percentage reduction of total chlorophyll at 48 dS/m salinity level, species were arranged as *C. dactylon* 'tifdwarf' (75%) > *C. dactylon* 'satiri' (57%) > *Z. matrella* (54%) > *P. vaginatum* (29%). The effect of salinity on chlorophyll concentration has previously been shown by William (2007)

where chlorophyll contents were gradually decreased in respond to increasing salinity. Lee *et al.* (2004) reported that significant differences found in total chlorophyll concentration in seashore paspalum ecotypes at 50 dS/m could mainly be ascribed to changes in chlorophyll a due to non-significant changes in chlorophyll b across salinity levels and among the grass entries.

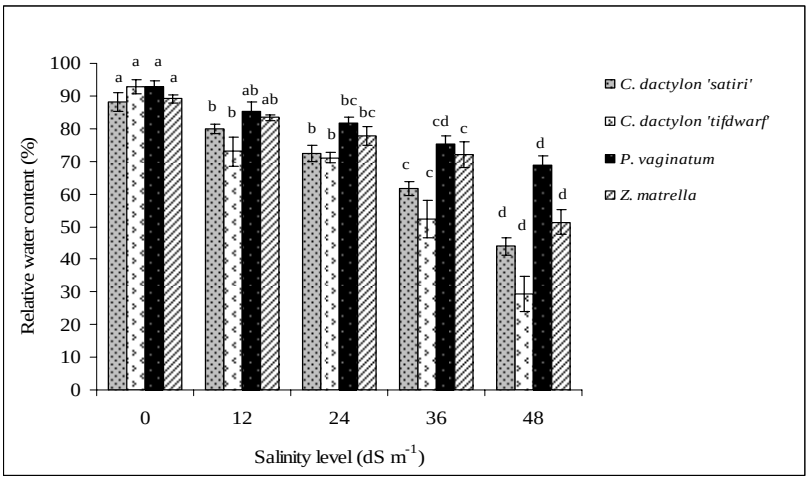

Fig. 1. Relative water content of four turfgrass species at different salinity levels. Means followed by the same letter are not significantly different at p = 0.05 (LSD test) among the salinity level of each species.

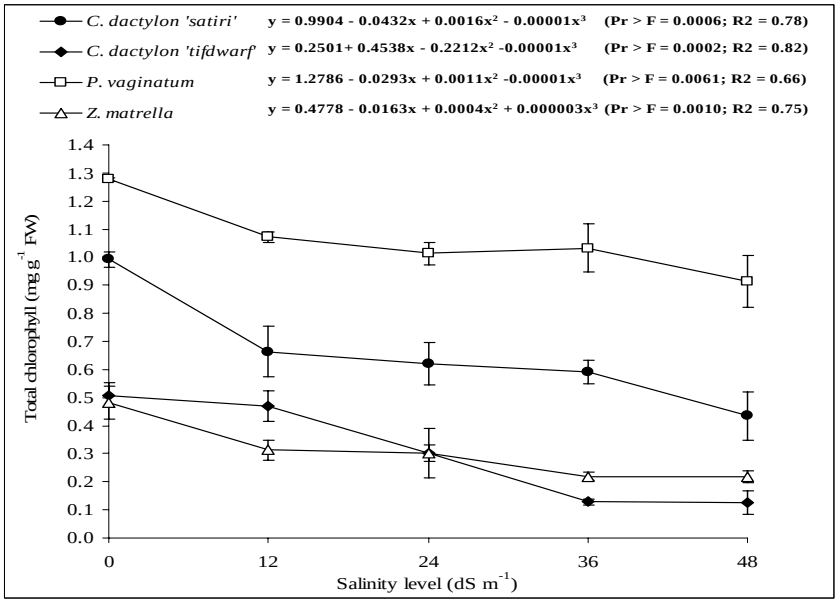

Fig. 2. Total chlorophyll content of four turfgrass species at different salinity levels. Mean ± standard error, y = Relative turf quality, and x = Salinity.

Significant differences of leaf proline content were found in all turfgrass species regarding their sensitivity to different levels of salt stress (Table 2). Proline accumulated in the turfgrass leaves increased with increasing salinity. The highest proline content under control treatment was

found in *C. dactylon* 'tifdwarf' (4.6) while the lowest was *Z. matrella* (3.73). Proline accumulation of *P. vaginatum* and *Z. matrella* seems to be insensitive as salinity increased to 12 dS/m. In contrast, sensitivity to salt brings *C. dactylon* 'tifdwarf' to have greater increasing in proline content at 12, 24 and 36 dS/m compared to others species. Qian *et al.* (2001) also found the similar result between two cultivars of Kentucky bluegrass, proline accumulation in salt tolerant 'Limousine' was less than salt sensitive 'Kenblue'. Lee *et al.* (2008) stated that the organic osmolytes like proline, Gly-betaine, and trigonelline are the most important indicators within the salt tolerant seashore paspalum genotypes.

Table 2. Effect of salinity on proline accumulation of four turfgrass species.

Salinity level (dS/ m)	Proline (µmol/g, fresh weight)				
	C. dactylon 'satiri'	*C. dactylon* 'tifdwarf'	*P. vaginatum*	*Z. matrella*	$LSD_{0.05}$
0	4.50e	4.60e	4.04d	3.73c	0.73
12	12.41d	19.79d	6.89d	4.86c	2.95
24	23.04c	31.74c	11.02c	10.66b	4.08
36	34.85b	37.26b	23.30b	12.43b	4.14
48	60.53a	56.46a	38.91a	52.37a	8.31
$LSD_{0.05}$	3.91	5.41	3.26	4.02	

Means within columns followed by the same letter(s) are not significantly different at p = 0.05%.

Table 3. Effect of salinity on leaf tissue Na concentrations of four turfgrass species.

Salinity level (dS/m)	Na (mg/g, dry weight)				
	C. dactylon 'satiri'	*C. dactylon* 'tifdwarf'	*P. vaginatum*	*Z. matrella*	$LSD_{0.05}$
0	0.97d B	0.90d B	0.96d A	0.98d C	0.33
12	18.30c B	20.46c C	18.10c A	18.59c D	2.41
24	26.35b B	27.30b C	24.16b A	25.91b D	2.16
36	27.91b B	29.39b C	26.01ab A	27.82ab C	3.36
48	31.81a B	34.57a B	27.60a A	30.20a C	3.98
$LSD_{0.05}$	1.66	2.41	2.59	3.28	

Lowercase letter(s) indicate significant differences at p = 0.05% among salinity level for each species. Uppercase letter(s) indicate significant differences at p = 0.05% among species at respective salinity.

Significant variability in shoot Na concentration was found among the tested turfgrass species. Sodium concentration in leaf tissues increased polynomially (R^2 = 0.98) with the increasing salinity levels (Table 3). Sodium content in four turfgrass species ranged between 0.9 to 0.97 mg/g in the control treatment. All of the species were observed have the same trend in increasing Na concentration; Na accumulated rapidly, as salinity increased at 12 dS/m, and gradually increase afterwards. At 48 dS/m salinity, *C. dactylon* 'tifdwarf' had the highest Na in the leaves, while the lowest was observed in *P. vaginatum*. Generally, *P. vaginatum* was the less Na accumulating species at all salinity levels followed by *Z. matrella* and *C. dactylon* 'satiri', while *C. dactylon* 'tifdwarf' was the highest Na accumulating species.

Potassium (K) content in different turfgrass species differed significantly in respond to the different levels of salinity and decreased linearly with increasing salinity (Table 4). Potassium content ranged from 37.35 to 57.93 mg/g for controls and from 32.81 to 52.1 mg/g DW at the

highest salinity treatment (48 dS/m). On an average, K uptake decreased to 91, 91, 88, and 84% at 12, 24, 36 and 48/dS salinity levels, respectively. However, there was no significant change (decreased) in K content of *P. vaginatum* up to 36 dS/m salinity level. The decrease in K content of this species was only 10% at 48 dS/m salinity level compared to the control. Over all, *P. vaginatum* was among the least K reducing species at all salinity levels while the highest K reducing species was *C. dactylon* 'tifdwarf'.

Table 4. Effect of salinity on leaf tissue K concentrations of four turfgrass species.

Salinity level (dS/m)	K (mg/g, dry weight)				
	C. dactylon 'satiri'	*C. dactylon* 'tifdwarf'	*P. vaginatum*	*Z. matrella*	$LSD_{0.05}$
0	42.72 aA	39.34 aA	57.93a A	37.35 aA	3.35
12	40.64 ab A	37.38 aA	56.85a B	35.26 b A	1.22
24	38.16 bc A	34.04 b A	55.70a B	33.62 c AB	0.81
36	35.84 cd AB	31.92 c A	55.14a B	33.11 c AB	2.44
48	34.64 d AB	31.63 c A	52.10b C	32.81 c BC	3.68
$LSD_{0.05}$	3.44	1.20	2.92	1.09	

*Lowercase letter(s) indicate significant differences at p = 0.05% among salinity level for each species. Uppercase letter(s) indicate significant differences at p = 0.05% among species at respective salinity.

Significant differences were found in Ca content among the species studied at all salinity levels (Table 5). The turfgrass species demonstrated a polynomially decreased as increasing the salinity. On an average in over all species, Ca content decreases over the control in 12, 24, 36 and 48 dS/m salinity treatments were 95, 86, 73 and 64%, respectively. Ca content in the control treatment ranged from 9.78 to 11.41 mg/g. Ca content significantly decreased (9%) in *C. dactylon* 'tifdwarf' as salinity increased to 12 dS/m salinity, while other species showed no significant difference. Calcium (Ca) content did not decrease markedly as salinity level increased from 12 to 24 dS/m in *P. vaginatum* and *C. dactylon* 'satiri'. Maximum reductions in Ca content at the highest salinity level (48 dS/m) were found in *C. dactylon* 'tifdwarf' (51%) followed by *Z. matrella* (36%) and *C. dactylon* 'satiri' (35%). The minimum reduction was recorded in *P. vaginatum* (12%).

Table 5. Effect of salinity on leaf tissue Ca concentrations of four turfgrass species.

Salinity level (dS/m)	Ca (mg/g, dry weight)				
	C. dactylon 'satiri'	*C. dactylon* 'tifdwarf'	*P. vaginatum*	*Z. matrella*	$LSD_{0.05}$
0	11.41a A	10.46a A	12.71a A	9.78a A	5.63
12	10.95a A	9.50b A	12.55a A	9.24a A	5.62
24	10.21a A	8.43c A	11.64ab A	7.89b A	5.39
36	8.54b AB	6.21d B	10.56bc A	6.97bc AB	3.98
48	7.44b AB	5.17e B	9.65c A	6.22c AB	4.34
$LSD_{0.05}$	1.62	0.74	1.40	1.05	

Lowercase letter(s) indicate significant differences at p = 0.05% among salinity level for each species. Uppercase letter(s) indicate significant differences at p = 0.05% among species at respective salinity.

Magnesium (Mg) concentration ranged in the leaf tissue was significant among the four turfgrass species at different salinity level (Table 6). Mg concentration decreased in quadratic as salinity increased up to 48 dS/m. Based on average over species result, Mg decreases over controls were 94, 88, 85 and 74% at 12, 24, 36 and 48 dS/m salinity, respectively. The results indicate that the highest Mg content was found in *P. vaginatum* (8.35 mg/g), while *C. dactylon* 'tifdwarf'

showed the lowest Mg content (6.51 mg/g) in the control treatment. Even though the Mg content of *P. vaginatum* was decreasing in numerically, but the result of ANOVA showed no significant different in up to 36 dS/m salinity level compared to the control. In contrast, *C. dactylon* 'tifdwarf' exhibited a sharp decreased in Mg content as salinity increased from 12 to 48 dS/m level. Compared to the respective controls, the percentage in Ca content in all salinity level can be ranked as: *P vaginatum* > *Z. matrella* > *C. dactylon* 'satiri' > *C. dactylon* 'tifdwarf'. High levels of sodium (Na) and other ions in salt-affected soils can induce nutrient imbalances of calcium, potassium, nitrate, magnesium, manganese, and phosphorus (Ca, K, NO_3, Mg, Mn, and P), causing deficiencies (Carrow and Duncan 1998).

Table 6. Effect of salinity on leaf tissue Mg concentrations of four turfgrass species.

Salinity level (dS/m)	Mg (mg/g, dry weight)				
	C. dactylon 'satiri'	*C. dactylon* 'tifdwarf'	*P. vaginatum*	*Z. matrella*	$LSD_{0.05}$
0	7.02a AB	6.51a B	8.35a A	6.64a B	1.37
12	6.59ab B	6.05b B	8.00ab A	6.17b B	1.07
24	6.22b B	5.45c B	7.75ab A	5.79bc B	1.02
36	6.09b B	5.04d B	7.49ab A	5.54c B	1.14
48	4.05c B	3.80e C	7.32b A	4.78d B	0.42
$LSD_{0.05}$	0.69	0.31	0.87	0.42	

Lowercase letter(s) indicate significant differences at p = 0.05% among salinity level for each species. Uppercase letter(s) indicate significant differences at p = 0.05% among species at respective salinity.

The effect of salinity on morphological of root cell was observed by scanning electron microscope (Figs 3, 4, 5 and 6). Cell damage in the root cortex of different turfgrass species under salinity treatment compared to control was evident of salt stress. Radial lines of intact living cells in root cortex were alternate with gas-filled space created by the cell death. However, no cells collapsed were observed in root cortex of *P. vaginatum* at 0, 24 and 48 dS/m salinity treatments. Meanwhile, cortical cell of *Z. matrella* and *C. dactylon* 'satiri' did not show cortical cell damage at 0 and 24 dS/m. At 48 dS/m salinity treatments, cell cortex of both species showed some collapse.

Fig. 3. Scanning electron micrographs showing root cortical tissue of *Paspalum vaginatum* under 0 dS/m (A), 24 dS/m (B), and 48 dS/m (C).

However, *Z. matrella* showed less cell collapse compared to *C. dactylon* 'satiri'. Damaged root structure of *C. dactylon* 'tifdwarf' under 24 dS/m salinity treatments is evident, as a result of least tolerant of this species to salinity. This condition became severe at the highest salinity level (48 dS/m) (Fig. 3).

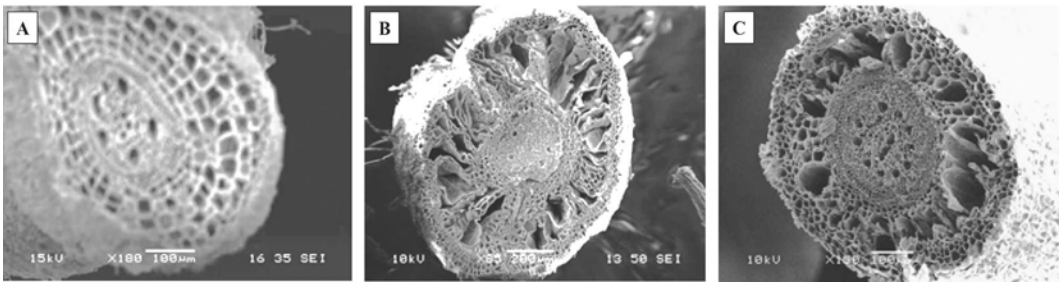

Fig. 4. Scanning electron micrographs showing root cortical tissues of *Zoysia matrella* under 0 dS/m (A), 24 dS/m (B) and 48 dS/m (C).

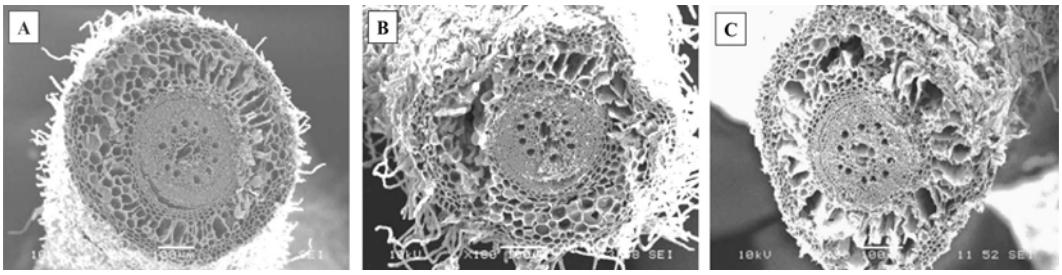

Fig. 5. Scanning electron micrographs showing root cortical tissues of *C. dactylon*'satiri' under 0 dS/m (A), 24 dS/m (B) and 48 dS/m (C).

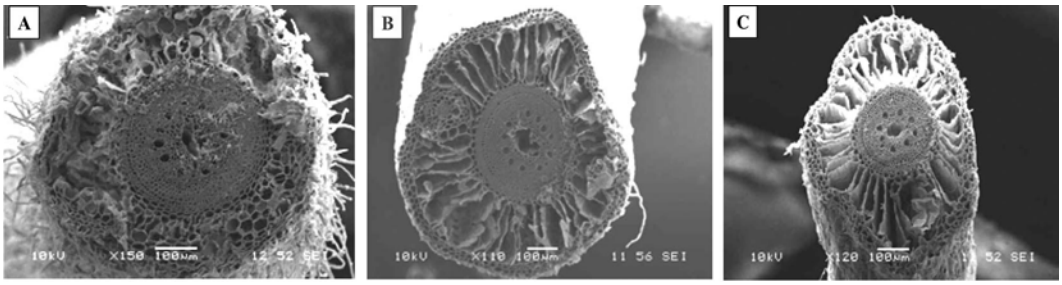

Fig. 6. Scanning electron microscopy photographs showing root cortical tissue of C. *dactylon* 'tifdwarf' under 0 dS/m (A), 24 dS/m (B), and 48 dS/m (C).

Scanning electron microscopy revealed salt glands on turfgrass leaf. Salt glands were only present on leaf surface of *Z. matrella, C. dactylon* 'satiri', and *C.dactylon* 'tifdwarf'. Scanning electron micrographs of adaxial leaf salt glands are shown in Fig. 7. Salt glands were observed in longitudinally arranged in parallel rows, adjacent to rows of stomata (Fig. 7 A, C and E). The salt glands are characterized by cutinized cell walls and surrounded by papillae (Fig. 7 B, D and F). The glands consist of two cells; a basal cell and cap cell. This cell was sticking out and lying to the leaf surfaces.

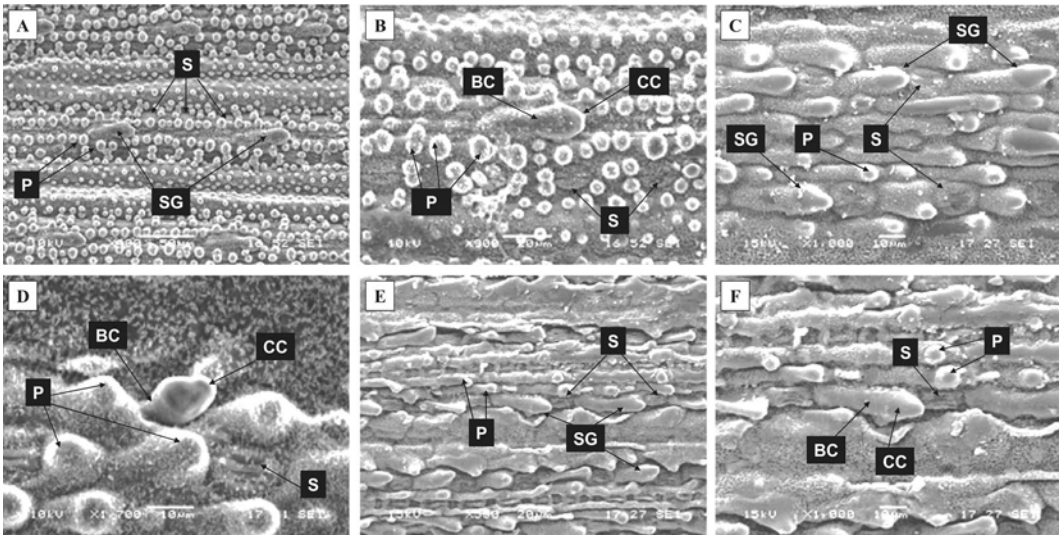

Fig. 7. Scanning electron micrographs of adaxial leaf surface. A: *Z. matrella* × 400; B: *Z. matrella* × 900 C: *C. dactylon* 'satiri' × 1000, D: *C.dactylon* 'satiri' × 1700, E: *C.dactylon* 'tifdwarf' × 550, *C. dactylon* 'tifdwarf' × 1000. Abbreviations: BC = Basal cell, CC = Cap cell, P = Papillae, S = Stomata, SG = Salt gland.

In terms of growth responsesand quality, *P. vaginatum* showed the highest performance at 48 dS/m salinity level. *C. dactylon* 'tifdwarf' was marked as moderate salt tolerant turfgrass species which tolerate at up to 12 dS/m salinity. Relative water and chlorophyll content was found high in *P. vaginatum*, *Z. matrella* and *C. dactylon* 'satiri', whereas, proline content was low. These three species were less affected by selectivity of saline ion (Na) uptake. Growth responses were in agreement with the physiological parameters, indicating that *P. vaginatum*, Z. *matrella* and *C. dactylon* 'satiri' are more salt tolerant than *C. dactylon* 'tifdwarf'.

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