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Full Length Research Paper

Growth indices and salinity tolerance threshold in a medicinal plant *Andrographis paniculata* Nees.

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Salt tolerance threshold is a critical parameter for establishing plant salt tolerance, which is generally known as the relative yield response to increase root area salinity. In this study, we focused on relative growth rate (RGR) and salt tolerance index (STI) in *Andrographis paniculata* (AP). For this purpose, after 30 days from culturing, the seedlings were placed in different salinity levels (0, 4, 8, 12, and 16 dSm^{-1}) on Hoagland medium and were compared at various exposure times (0, 5, 10, 15, and 20 days). The results revealed that salinity, exposure times and their interaction had significant effect on the measured traits. The injurious effects of salinity on RGR during exposure time indicated two specific outcomes: (1) at five days salt exposure during which growth began to decrease due to low osmotic potential, and (2) at 20 days salt exposure which identified reduction rate of yield, which was attributed to toxicity of specific ion. Although, salinity effects on STI were the same among accessions at 5 and 10 days salt exposure; it affected STI at 15 and 20 days, significantly. In view that all the growth indices decreased in sequence of increasing salinity levels, 12 dSm^{-1} salinity and 15 days stress could be concluded as the extreme salinity level and exposure time.

Key words: *Andrographis paniculata*, salinity, salt tolerance threshold, exposure times, salt tolerance index.

INTRODUCTION

Andrographis paniculata is an important medicinal plant commonly known as “Kalmegh or King of Bitters” in the family Acanthaceae (Gomathinayagam et al., 2009; Valdiani et al., 2012). The leaves and aerial parts of the plant have been applied in traditional medicine for the treatment of common cold, fever, sore throat, and diarrhea (Saralamp et al., 1996). The importance of the plant lies in its diterpenoids of which Andrographolide is the main compound with immunostimulant (Puri et al., 1993), antipyretic, anti-inflammatory, and anti-diarrheal properties (Gupta et al., 1993) and other related diterpenoids constituents, including 14-deoxy-11, 12-didehydroandrographolide, neoandrographolide, and andrographiside that possess various medicinal properties (Lattoo et al., 2008; Matsuda et al., 1994). The plant is an erect annual herb with a dark-green quadrangular stem; lanceolate and pinnate leaves; small and white flowers; linear-oblong capsules; and tiny yellowish brown seeds (Jiang et al., 2009). It grows abundantly in tropical climatic conditions, that is, moist and sunny situations in any soil having reasonable amount of organic material (Lattoo et al., 2008; Valdiani et al., 2012).

Soil salinity is one of the major environmental stresses

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that causes many adverse effects on plant growth, due to low osmotic potential of soil solution (osmotic stress), specific ion effects (salt stress), nutritional imbalance or a combination of these factors (Ashraf, 2004; Ashraf et al., 2005; Sahi et al., 2006). All these factors alter various biochemical and physiological responses in plants and cause adverse effects on all plant processes, including photosynthesis, growth and development by reducing hormone delivery from roots to leaves which may lead to toxicity in non-salt-tolerant plants, known as glycophytes (Ashraf and Sarwar, 2002; Iqbal et al., 2006; Munns and James, 2003; Sahi et al., 2006). Growth and development of glycophytes are negatively affected, while halophytes can tolerate high salt concentrations (Parvaiz and Satyawati, 2008). Agricultural productivity in arid and semiarid regions of the world, where 25% of the irrigated lands is affected by salts, is very low due to the accumulation of salts in soils (Munns, 2002).

Plant salt tolerance may be expressed by plotting the relative yield as a continuous function of root area salinity (Maas, 1990). This relationship is represented by two intersecting linear regions, which identify the threshold, after which the yield begins to decline, as well as the yield reduction rate (slope) at increasing salinity. The salinity tolerance threshold is a specific target for improving plant salt stress tolerance (Maggio et al., 2007).

Understanding the ways that plants can tolerate salt stress conditions is necessary for growing and developing plants on marginal agricultural lands that are otherwise non productive. The ability of different plant species to withstand salinity stress is different and depends on several interacting variables, including environmental conditions, plant growth stages, salinity levels and exposure times (Munns, 2002). Sometimes, these factors may act as stresses leading to injury and in extreme cases, death of the plant (Jaleel et al., 2007a). For many salt sensitive plants, excess sodium is the main contributing factor for growth inhibition (Jaleel et al., 2007b). High sodium disturbs potassium (K) nutrition when the sodium accumulates in cytoplasm, preventing many enzyme activities (Jaleel et al., 2007a). The effects of salinity levels on plant growth had been studied in different plants. Some medicinal and aromatic plants have been reported to be tolerant to salinity conditions (Ashraf and Orooj, 2006), which indicate the potential of producing medicinal plants in saline areas.

Due to the increasing demand of medicinal plants and traditional medical systems throughout the world as well as for the pharmaceutical industry, some medicinal plants need to be grown commercially, but soil salinity and other forms of pollution are serious threats to crop production (Qureshi et al., 2005). So, the study of plant response to salinity in medicinal plants, such as *A. paniculata* is valuable. On the other hand little research has been done on *A. paniculata* under salinity conditions. Rajpar et al. (2007) reported that by increasing the salinity levels, some morphological traits of the plant were suppressed, and the seedlings could not survive at 12 dSm^{-1}. Our understanding of the quantitative effects of salinity levels and exposure times on important salt tolerance parameters like threshold (EC dSm^{-1}) and slope (% per dSm^{-1}) values were not determined for *A. paniculata* at seedling stage. The determination of salinity effects on growth rate, salt tolerance index and the interrelationships among different morphological traits under salinity stress will help us to develop a better growing condition for this plant under salinity as well as improving our understanding on the mechanisms underlying salt tolerance in plants. Here, we present a study done on the effects of salinity levels and exposure times on the growth of *A. paniculata* seedlings with the objectives to determine the salt tolerance threshold and to identify the salt-tolerant and salt-sensitive accessions based on the salt tolerant index.

MATERIALS AND METHODS

Seeds and chemicals

Seeds of six *A. paniculata* accessions, namely, 11179,11216, 11241,11249,11264, and 11329 were obtained from the Agro Gene Bank, University Putra Malaysia, Serdang, Selangor, Malaysia. The seeds were kept at 4°C until use. The chemicals used for the treatments were of analytical grade obtained from Fisher Chemicals.

Experimental design

The experiment was conducted in a controlled growth chamber in the Department of Crop Science, University Putra Malaysia during September to December, 2011. Since there has been insufficient study on salt tolerance in *A. paniculata*, we decided to test the salt tolerance of seedlings under different concentrations of salinity at various exposure times. For this purpose, an experiment with a split plot arrangement based on the randomized complete block design (RCBD) with three factors in three replications was carried out. The three factors evaluated in the experiment were: different concentrations of saline water (control, 4, 8, 12, and 16 dSm^{-1}) in the main plots, the genetic of the plant based on six different accessions (11179, 11216, 11241, 11249, 11264, and 11329) in the sub-main plots, and five different exposure times (control, 5, 10, 15, and 20 days). Therefore, there were 30 treatment combinations for each exposure time.

The seeds were first scarified using sand paper (Talei et al., 2012), and were then placed and distributed evenly on filter papers No. 2 in sterile 15 cm diameter Petri dishes, separately. The Petri dishes were sealed with parafilm to prevent any water loss during incubation. The average temperature in the growth chamber was set between 28 and 30°C, and the relative humidity (RH) varied between 60 and 75%.

Ten days after being soaked (two leaves stage), the seedlings were transferred into the Jiffy medium. Thirty days later (six to eight leaves stage), the seedlings of each accession were placed in different concentrations of NaCl (0, 4, 8, 12, and 16 dSm^{-1}) in separate trays. Before the treatment, the shoot and root length, number of leaves, and total fresh and dry weight of the seedlings were measured. At the end of different exposure times (control, 5, 10, 15, and 20 days), all seedlings were harvested by cutting them at the jiffy level, and the data on morphological traits including; plant height (PH), number of leaves on main stem (NL), root length (RL), fresh and dry shoot weight (SFW, SDW), and fresh and dry root weight, (RFW, RDW) after drying at 68°C for 48 h were measured. Also, the growth analysis was evaluated according to Benincasa (1988), and the relative growth rate (RGR) and the salt tolerance index (STI) were calculated as follow:

$$RGR = (\ln DM_2 - \ln DM_1)\ (t_2 - t_1)^{-1}\ (g.g^{-1}.d^{-1})$$

$$STI = [(TDW \text{ at } S_x / TDW \text{ at } S_1) \times 100]$$

Where DM_1, the initial total (shoot + root) dry weight; DM_2, the final total dry weight; $(t_2 - t_1)$, the difference in time interval between two samplings; TDW, total dry weight; S_1, control; and S_x, saline treatment (Baci et al., 2003).

Statistical analysis

Initially, the raw data were tested for normality using the Statistical Package for Social Sciences (SPSS) software No.19 and the square transformation method was employed. The transformed data were analyzed by analysis of variance (ANOVA) according to the design, and the means were compared by Duncan's multiple range test ($P \leq 0.01$).

RESULTS

Evaluation of morphological traits of *A. paniculata* before applying salinity stress

Variance analysis of morphological traits comprising plant height (PH), number of leaves (NL), root length (LR) and total dry weight (TDW) before applying salinity stress showed that there were no significant differences between the accessions. The coefficient of variation (CV%) showed that within accessions, all groups were homogeneous and the range of CV within accessions in terms of all studied traits was low and between 0 to 20.13% (Table 1).

Effects of different salinity levels and exposure times on morphological traits in *A. paniculata*

Salinity levels and exposure times affected the morphological traits of *A. paniculata* seedlings. Variation due to salinity levels (SL), exposure times (ET), accessions (AC) and their interaction were highly significant ($P < 0.01$). The interaction of SL × AC was not significant in terms of relative growth rate (RGR) (Table 2). All morphological traits, including RGR and salt tolerance index (STI) varied with SL and ET.

The salinity levels and exposure times significantly affected accessions. Among the accessions, the highest STI and RGR (83.72% and 0.09 g/g/day) were observed in accession No. 11249, whereas the lowest (78.12% and 0.08 g/g/day) with the same condition belonged to accession No. 11216 (Figure 1). In all accessions plant height (PH), number of leaves on main stem (NL), root length (RL), fresh and dry shoot weight (SFW, SDW) and fresh and dry root weight, (RFW, RDW), total dry weight (TDW), salt tolerance index (STI) and relative growth rate (RGR) decreased under the effect of salt treatment (Table 3). The first day where leaves started to fall was observed on the 10th day at 16 dSm^{-1} and the 15th day at 12 dSm^{-1} salinity levels. The RGR at 20 days after applying salinity stress in high salinity level (16 dSm^{-1}) was negative (Figure 2). As shown in Figure 2, the greatest decrease in STI and RGR were observed at 16 dSm^{-1} and 20 days of salt stress.

The mean RGR over the salinity levels for exposure times varied from 0.03 (20 days) to 0.13 (5 days). Among the exposure times, 5 days of salt exposure gave the highest RGR followed by 10, 15, and 20 days, whereas among the salinity levels, RGR varied significantly between 0.11 (control) and 0.03 (16 dSm^{-1}). The mean of STI for different exposure times varied from 91.65% (5 days) to 63.07% (20 days) and for salinity levels ranged from 61.17% (16 dSm^{-1}) to 100% (control). Overall, 12 dSm^{-1} salt concentration and 15 days salt exposure were the best conditions with respect to RGR and STI.

The slope of the salt tolerance index showed 9.24% decrease per exposure time (5 days), and 9.58% decrease per salinity level (Figure 3). This might be due to the effect of high salinity level and long exposure time. Based on the relative growth rate, two linear functions with different slopes in this region were identified. In the first region, the slope was 39 mg decrease per salt exposure time (5 days) between 5 and 10 days exposure time, whereas after 15 days, the relative growth rate reduction per exposure time was 48 mg (Figure 4).

A separate analysis of variance based on different exposure times showed that STI and RGR were significantly affected by salinity levels in each exposure time. According to the result of STI at 5 and 10 days after salinity stress, no significant differences were detected between the accessions; however, the accessions showed significant differences ($P \leq 0.01$) at 15 and 20 days after salt exposure time (Table 4). RGR among the accessions for all exposure times were significantly different, as shown in Table 3. Furthermore, the results revealed that the STI at 15 and 20 days after salinity stress was significantly affected by the interaction of SL × AC. Most accessions of *A. paniculata* were able to tolerate up to 12 dSm^{-1}, which could be maintained for more than 15 days at seedling stage. At 16 dSm^{-1}, the leaves began to fall after 10 days.

DISCUSSION

Soil salinity is one of the major abiotic stress factors that limits the distribution and productivity of crops in arid and semiarid regions of the world. Shoot and root growth inhibitions are common responses to salinity, and plant

Table 1. Plant height, leaves number, root length, and total dry weight of six accessions of *A. paniculata* before applying salinity treatments.

Accession	PH		NL		RL		TDW	
	Mean ± SD	CV (%)	Mean ± SD	CV (%)	Mean ± SD	CV (%)	Mean ± SD	CV (%)
11179	9.2 ± 1.01	11.0	5.3 ± 0.41	7.8	4.1 ± 0.34	8.3	0.10 ± 0.02	20.1
11216	9.5 ± 0.90	9.7	4.9 ± 0.34	6.9	4.3 ± 0.40	9.4	0.10 ± 0.01	8.6
11241	8.9 ± 1.03	11.6	4.1 ± 0.62	15.3	4.4 ± 0.47	10.7	0.10 ± 0.01	12.6
11249	9.2 ± 1.01	11.0	4.9 ± 0.56	11.4	4.4 ± 0.48	11.0	0.09 ± 0.01	15.6
11264	8.3 ± 0.70	8.5	4.1 ± 0.42	10.3	3.6 ± 0.47	13.1	0.09 ± 0.01	14.1
11329	8.0 ± 0.00	0.0	3.6 ± 0.37	10.4	4.1 ± 0.28	6.8	0.09 ± 0.00	10.6

PH: plant height (cm), NL: number of leaves, RL: root length (cm), TDW: total dry weight (g). The seedlings were grown for 30 days (six to eight leaf stages). The data are mean values of at least three independent measurements. SD: standard deviation, CV: variation coefficient.

Table 2. Variance analysis of the effects of different exposure times and salinity levels on the measured characteristics of six accessions of *A. paniculata.*

Source	df	Mean square					
		STI	RGR	NL	PH	RL	TDW
Exposure time (ET)	3	14094.07**	0.14**	2.92**	2.54**	0.89**	1.54**
Salinity level (SL)	4	16539.46**	0.04**	13.47**	9.20**	5.61**	0.33**
Accession (AC)	5	251.15**	0.00**	0.84**	2.87**	0.41**	0.08**
ET × SL	12	1237.72**	0.01**	1.05**	0.22**	0.60**	0.07**
ET × AC	15	106.30**	0.01**	0.11**	0.03**	0.13**	0.01**
SL × AC	20	28.09**	0.00ns	0.08**	0.04**	0.11**	0.00**
ET × SL × AC	60	11.41*	0.00^{ns}	0.0^{5**}	0.01^{ns}	0.04**	0.00^{ns}

**, * and ns, refer to 1%, 5% and not significant, respectively. STI: salt tolerance index, RGR: relative growth rate, NL: number of leaf, PH: plant height, RL: root length and TDW: total dry weight.

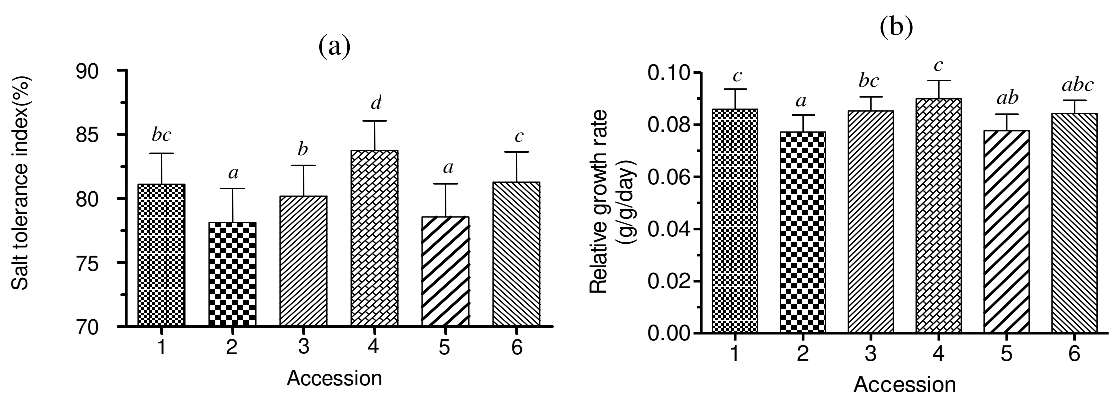

Figure 1. Salt tolerance index (a) and relative growth rate (b) of six accessions of *A. paniculata* under different salinity levels of NaCl (control, 4, 8, 12, and 16 dSm^{-1}) and various exposure times (5, 10, 15, and 20 days). Mean values ± standard error (SE) are from three independent replicates. Values superscripted by different letters are significantly different (P ≤ 0.01). The accessions were: (1) 11179, (2) 11216, (3) 11241, (4) 11249, (5) 11264, and (6) 11329.

growth is one of the most important agricultural indices of salt stress tolerance as indicated by different studies (Munns, 2002; Ruiz et al., 2005). In order to determine salt stress tolerance of six accessions of *A. paniculata*,growth parameters like plant height, number of leaves, root length, fresh and dry weight of shoot and root, salt tolerance index, and relative growth rate were evaluated under different concentrations of NaCl and various exposure times. Root and shoot lengths, as well as fresh and dry weights of shoot and root of all

Table 3. Effects of salinity levels and exposure times on some morphological traits of *A. paniculata*.

ET	NL	PH	LR	FW	TDW	SL	NL	PH	LR	FW	TDW
0	8.8 ± 0.1^{a}	4.5 ± 0.1^{a}	4.2 ± 0.1^{c}	0.6 ± 0.01^{a}	0.09 ± 0.0^{a}	0	15.0 ± 0.4^{e}	9.3 ± 0.3^{e}	5.4 ± 0.2^{e}	2.4 ± 0.18^{e}	0.40 ± 0.03^{e}
5	10.0 ± 0.2^{b}	5.7 ± 0.2^{b}	4.6 ± 0.1^{d}	1.2 ± 0.05^{d}	0.18 ± 0.0^{b}	4	12.8 ± 0.3^{d}	7.8 ± 0.2^{d}	4.8 ± 0.1^{d}	1.7 ± 0.12^{d}	0.34 ± 0.02^{d}
10	10.3 ± 0.2^{bc}	6.2 ± 0.2^{c}	3.7 ± 0.1^{a}	0.9 ± 0.07^{b}	0.28 ± 0.0^{c}	8	10.4 ± 0.2^{c}	6.3 ± 0.2^{c}	3.8 ± 0.1^{c}	0.8 ± 0.05^{c}	0.31 ± 0.02^{c}
15	10.8 ± 0.4^{c}	6.9 ± 0.2^{d}	3.9 ± 0.1^{b}	1.2 ± 0.11^{c}	0.43 ± 0.01^{d}	12	9.4 ± 0.2^{b}	5.5 ± 0.1^{b}	3.5 ± 0.1^{b}	0.5 ± 0.04^{b}	0.26 ± 0.01^{b}
20	13.1 ± 0.5^{d}	7.9 ± 0.3^{e}	4.4 ± 0.2^{c}	2.2 ± 0.22^{e}	0.54 ± 0.02^{e}	16	7.6 ± 0.2^{a}	4.6 ± 0.1^{a}	3.1 ± 0.1^{a}	0.5 ± 0.04^{a}	0.21 ± 0.01^{a}

ET: Exposure times (days), SL: Salinity levels (dSm^{-1}), NL: number of leaf, PH: plant height (cm), RL: root length (cm), FW: Fresh weight (g) and TDW: total dry weight (g). Different letters indicate significant differences between the values of pairs of treatments within columns at $P \leq 0.01$.

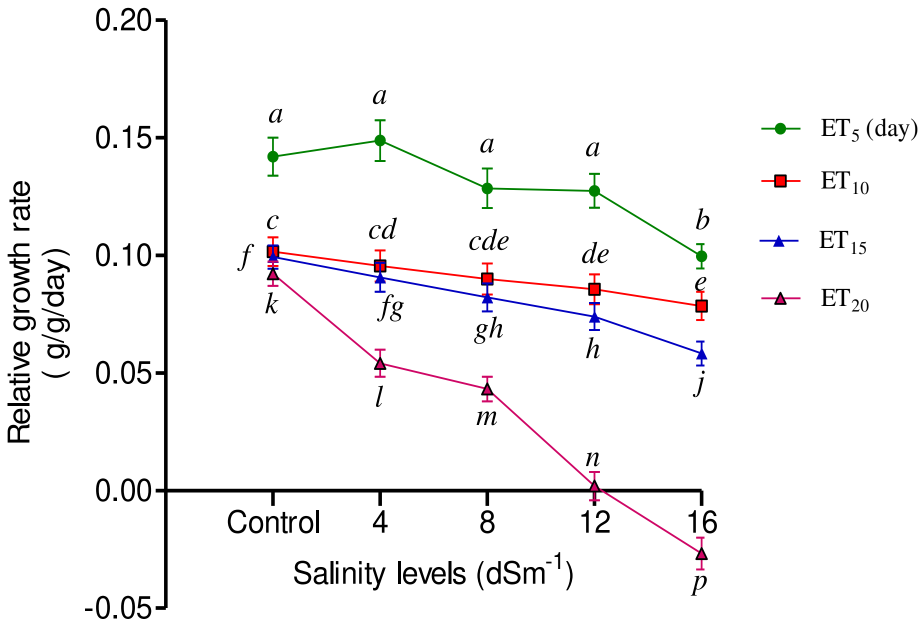

Figure 2. Interaction effect of salinity levels and exposure times on relative growth rates of six accessions of *A. paniculata* .The five days exposure time showed the highest RGR, while 20 days seedlings could not tolerate 12 and 16 dSm^{-1} salinity levels and RGR was the lowest. Vertical bars represent standard error (SE) for three samples.

accessions were affected and decreased under different salinity levels and exposure times.

Biomass differences among plant species under salinity conditions are important in determining salt-tolerance of plants. In agreement with the reports of Wenqing and Peng (2000) and Munns (2002), the findings of this study indicated that exposure time and salinity level were two important factors affecting the plant growth and development under salinity condition. Our results also matched up well with the findings of Rajpar et al. (2007), who showed that salt stress inhibited the growth of *A. paniculata* seedlings. This may be due to the negative effects of salt stress on water absorption and other biochemical processes (Parida and Das, 2005). The inhibition of plant growth and development under salt stress condition could be due to either osmotic reduction in the water surrounding the root system or excessive accumulation of ions known as specific ion effect (Ashraf and Sarwar, 2002; Munns and James, 2003; Taffouo et al., 2004). Under low level of salinity, the salt ions facilitated the plant growth as indicated by the increase in certain measured morphological traits, such as shoot and root lengths, and number of leaves in the seedlings similar to the results of Rajpar et al. (2007), while under extreme levels of salinity (12 and 16 dSm^{-1}), the growth was inhibited. In salt-tolerant accessions, the dry weight was less affected by salinity which was also observed previously in other salt-tolerant or moderately salt-tolerant accessions of certain plant species, such as sugar beet and cotton (Greenway and Munns, 1980), because of the fact that dry weight is always used to establish salt tolerant indices in plants. In *A. paniculata* seedlings, the slope of the salt tolerance index showed approximately 9% decrease in dry weight per exposure time and salinity

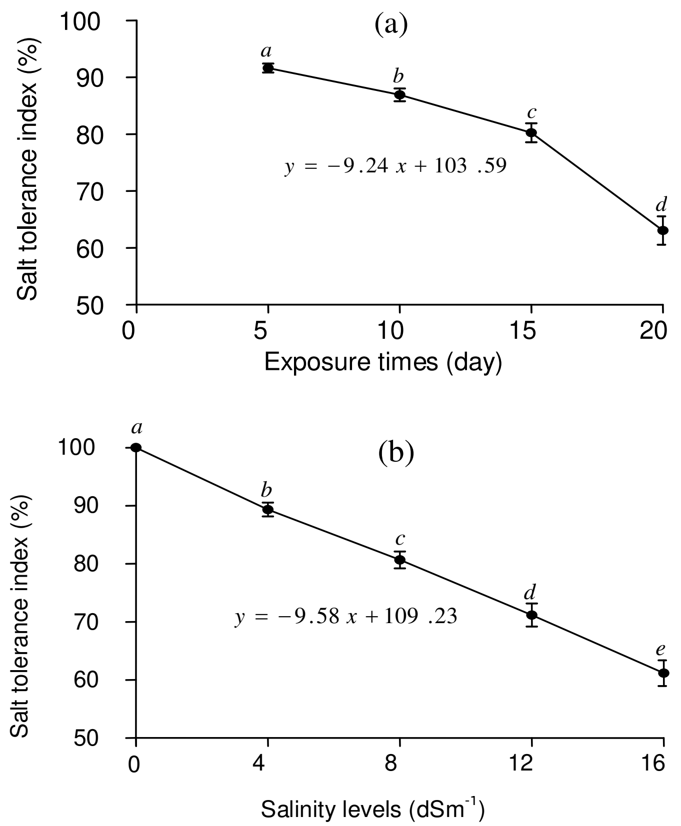

Figure 3. The effects of exposure times (a) and salinity levels (b) on trend of salt tolerance index of *A. paniculata.* Increasing the exposure times and salinity levels led to decrease in STI and the highest reduction was observed at 20 days of exposure time and the salinity level of 16 dSm^{-1}. Vertical bars represent standard error (SE) for three samples.

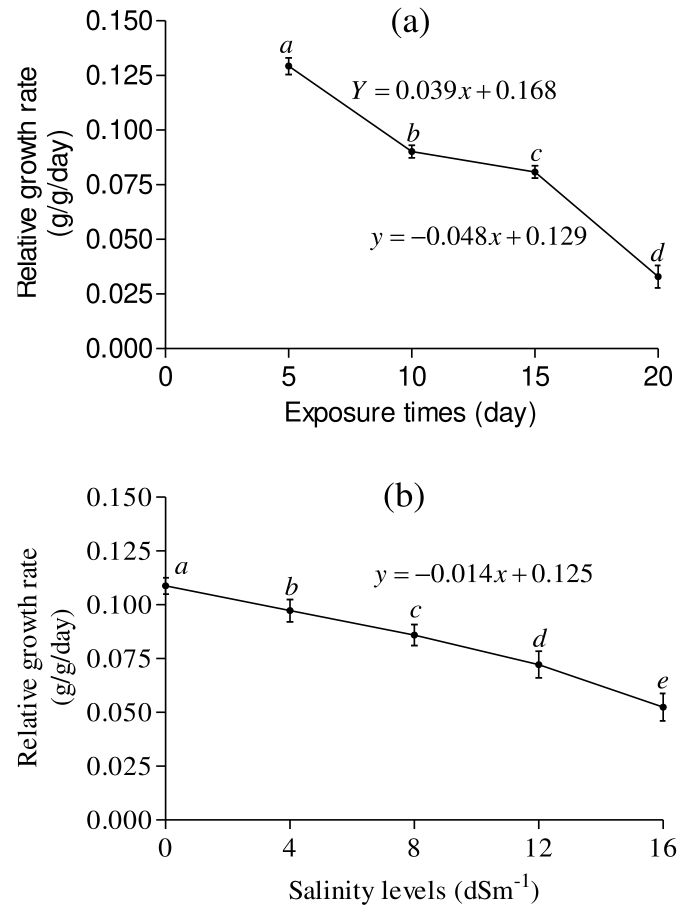

Figure 4. The effects of exposure times (a) and salinity levels (b) on the relative growth rate of *A. paniculata*. Increasing the exposure times and salinity levels led to decrease in RGR and the highest reduction was at 20 days exposure time and the salinity level of 16 dSm^{-1}. Vertical bars represent standard error (SE) for three samples.

level. This may be due to the effect of high salinity level and long exposure time. Based on the relative growth rate, the yield decline after the tolerance threshold is represented by two different linear slopes. In the initial period, the seedlings were in the process of adapting to salinity stress due to osmotic reduction of water surrounding the root system, but in the second stage (15 days of exposure time), the seedlings were unable to withstand the salinity stress due to the accumulation of sodium ion.

Most accessions of *A. paniculata* were able to tolerate up to 12 dSm^{-1} of maximum salinity (NaCl) for more than 15 days at seedling stage. With a prolonged exposure to salt stress, the plant growth was poor at the extreme level (12 dSm^{-1}), but better at low levels. An increase of total dry weight is usually documented as a general response to salinity and may improve salinity tolerance by restricting the flux of toxic ions to the shoot and consequently, delaying the onset of the tolerance threshold. The overall results suggested 12 dSm^{-1} salinity and 15 days of stress as the maximum salinity level and exposure time for *A. paniculata* at the seedling stage.

Conclusions

The salinity level and exposure time significantly affected the morphological traits, relative growth rate and salt tolerance in *A. paniculata* seedlings. The seedling response in terms of STI was similar in the early stage of exposure time, but increasing the exposure time significantly reduced the growth rate. In view of the decreasing patterns of all the growth indices as the level of salinity increased, it could be concluded that *A. paniculata* cannot be classified as a salt tolerant plant. Based on the plant response to salinity, *A. paniculata* accessions can stand up to a maximum of 12 dSm^{-1} salinity level for more than 15 days at the seedling stage.

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Table 4. Variance analysis of the effects of salinity levels on the measured characteristics of six accessions of *A. paniculata* at different exposure times separately.

Source	Mean square of STI				Mean square of RGR			
	5 days	10 days	15 days	20 days	5 days	10 days	15 days	20 days
Salinity(SL)	1052.6**	2190.4**	4633.1**	12376.5**	0.006**	0.001**	0.004**	0.04**
Accession(AC)	14.3 ^{ns}	30.4 ^{ns}	443.8**	81.6**	0.003**	0.008**	0.005**	0.01**
SL × AC	7.9 ^{ns}	10.8 ^{ns}	34.9**	8.7**	0.001^{ns}	0.00 ^{ns}	0.00 ^{ns}	0.00 ^{ns}

** and ns, refer to 1% and not significant, respectively.

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