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SALINITY INFLUENCE ON SURVIVAL, GROWTH AND NUTRIENT DISTRIBUTION IN DIFFERENT PARTS OF *MILLETTIA PINNATA* SEEDLINGS

SUMMARY

Millettia pinnata is a mangrove associate tree species in the Sundarbans and commonly used for soil stabilization along the embankment and riverbank of coastal area. This study examined the effect of different salinity levels on seedling survival, growth, nutrients (Nitrogen, Phosphorus, Potassium) and Sodium distribution in different parts (leaf, stem and root) as well as total chlorophyll and proline concentration in the leaves of *Millettia pinnata* seedlings. Survival and growth study was carried out in different saline treatments from 0 to 35 ppt and from 0 ppt to 25 ppt respectively at 5 ppt interval for six months. Highest survival (100%) was observed at 0 to 10 ppt salinity and then gradually decreased to 10% at 25 ppt salinity. No survival was observed at 30 and 35 ppt salinity. Biomass increment showed significantly negative correlation (r=-0.84) with salinity levels. Oven dried biomass increment was the highest (6.97 g/month) at 0 ppt salinity and gradually decreased to 0.75 g/month at 15 ppt salinity. Nitrogen and potassium concentration in different seedling parts and phosphorus in leaves showed significant negative correlation with salinity levels. However, sodium concentration in different parts showed significant positive correlation with salinity levels. Total chlorophyll concentration was the highest (0.62 mg/g) in the leaves of seedlings grown at 0 ppt and decreased to 0.29 mg/g at 25 ppt salinity. Highest proline concentration (6.88 µmoles/g) was measured at 25 ppt salinity and lowest (1.52 µmoles/g) at 0 ppt salinity.

Keywords: Salinity, Growth, *Millettia pinnata*, Nutrient, Chlorophyll, Proline

INTRODUCTION

Salinity appears to be a critical environmental factor in the mangrove ecosystem which regulates species composition and diversity in the Sundarbans (Pethick, 2011). Salinity affects the availability of nutrients to plants which may

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influence metabolism and osmoregulation of plant cell (Taiz and Zeiger, 2006). Moreover, it regulates absorption and transportation of nutrients within different plant parts (Munns and Tester, 2008; Mahmood et al. 2014). Salt has a limiting effect on photochemistry of plants, negatively affects photosynthesis process by inhibiting chlorophyll synthesis, thereby reducing growth (Munns and Termaat, 1986). To cope with saline environment, mangrove species accumulate some compatible solute in their cytoplasm in order to maintain high osmotic potential (Parida et al. 2004). Among different compatible solutes, proline has been found to occur frequently in salt tolerant species (Popp et al. 1985). Salt tolerance varies with the species (Hossain et al. 2001; Ball, 2002). Mangrove associates can tolerate less salt compared to true mangroves (Tomlinson, 1986; Paliyavuth, 2001; Youssef, 2007).

Millettia pinnata is a fast growing multipurpose mangrove associate tree species (Allen and Allen, 1981) and found to occur in slight saline to moderate saline areas in the coastal regions of Bangladesh (Mahmood, 2015). Presently, this species has drawn much attention for its nitrogen fixing capacity, good coppicing ability and for the ability to tolerate water logging (Misra and Singh, 1987). It is also a potential source of biodiesel now a days (Shrinivasa, 2001; Sharma and Singh, 2008). Salt intrusion from the sea and reduced flow of fresh water from the upstream might have significant influence on plant community in the coastal regions of Bangladesh. Therefore, selection of suitable plant species are of great importance for plantation on the salt affected marginal land and embankment of the coastal areas. Considering the ecological and economic significance of *M. pinnata*, it may appear as a suitable species for plantation in the salt affected areas. However, scientific information on survival and growth of this species at different salinity levels is still scanty. Therefore, present study was designed to evaluate the influence of salinity on survival and growth, nutrients (Nitrogen, Phosphorus and Potassium), Sodium distribution pattern in different parts (leaf, stem and root), as well as chlorophyll and proline concentration in the leaf of *Millettia pinnata* seedlings.

MATERIAL AND METHODS

Description of the study area.

Sundarbans is located between 21° 30' and 22° 30' N latitudes and between 89° 00' and 89° 55' E longitudes. It is a unique habitat for a wide diversity of flora and fauna (Karim, 1995) and has been declared as world's heritage site by UNESCO in 1997 (Basar, 2012). Based on the level of soil salinity, Sundarbans is divided into less saline (LS), moderate saline (MS) and strong saline (SS) zones having salinity <2 dS/m, 2-4 dS/m and >4 dS/m respectively (Siddiqi, 2001). Rainfall is strongly seasonal (from May to October) with 87% of the mean annual rainfall (1500 mm). Temperature ranges from 18.50 to 35.20 in summer and from 12.20 to 28.80 in winter. Soil is silty to sandy clay loam, as well as bulk density, particle density and porosity vary from 1.18 to1.27 g/cc, 2.31 to 2.52 g/cc and 46–52%, respectively. Soil pH is 7.8 (Siddiqi, 2001).

Seed collection and raising of seedlings

Mature seeds of M. pinnata were collected from mother trees in the Moderate saline zone of the Sundarbans. Collected seeds were sorted manually and defective seeds were discarded. Seeds were reddish brown in color. Its average length and width were 1.5 to 2 cm and 0.5 to 1 cm respectively. Seeds were sown on germination bed (5 m ×1.5 m) filled with 30 cm thick layer of coarse sand. Seedlings were raised under fresh water condition.

Experiment setup

A total of 160 polyethylene terephthalate (PET) bottles (20 cm height and 9 cm diameter of each) were taken. Three month old seedling was planted in each bottle filled with coarse sand. Twenty pet bottles with seedlings were kept in a plastic box (100 cm × 20 cm × 30 cm). Therefore, a total eight boxes were prepared. Eight treatments of salinity (0 to 35 ppt at 5 ppt interval) were applied randomly to the boxes. Sixteen liters of full strength modified Hogland solution was given in each box as a source of nutrient. Salinity in each treatment level was increased gradually at weekly interval following Mahmood et al. (2014). Nutrient solution was changed weekly. Salinity and water level of each treatment were checked and corrected at 24 hours interval. This experiment was conducted for 6 months in the glass house of forest nursery of Khulna University. Number of survived seedlings in each treatment was counted at the end of the experiment.

Growth study

A total of 82 polyethylene terephthalate (PET) bottles (20 cm height and 9 cm diameter were taken. Three month old seedlings were used for this experiment. Initial collar diameter, height and green biomass of each seedling were measured and recorded. One seedling was planted in each pet bottle filled with pre-gravel. Initial green biomasses of ten seedlings were measured individually. Then, it was dried in an oven at 80°C for 4 days to calculate green to oven-dried weight conversion ratio. No seedlings were survived at high saline treatments (30 to 35 ppt) during survival study. So, this experiment was set up with salinity ranging from 0 to 25 ppt salinity at 5 ppt interval with three replications. Each replication contained four pet bottles with seedlings kept in a plastic box (55 cm × 30 cm × 20 cm). Thus, 18 boxes were prepared. Eight litter of modified Hogland nutrient solution was added to each box to nourish the seedlings. Six levels of saline treatments (0 to 25 ppt at 5 ppt interval with three replications was applied to the seedlings. Initially the salinity of the nutrient solution was maintained zero to prevent the seedlings from sudden stress of salinity. Salinity level of each treatment was increased gradually following Mahmood et al. (2014). Distilled water was used for 0 ppt treatment level. The nutrient solution was replaced weekly. Salinity and water level of each plastic box were checked and corrected at 24 hours interval. The pH of each treatment was maintained 8. Salinity of the treatment solution and that inside the pet bottle were cheeked and found similar for both the cases. This experiment was conducted for six month in the glass house of forest nursery of Khulna University. At the end of the experiment, seedlings were harvested. Their height,

collar diameter, and green biomass were measured and recorded salinity treatment wise. Growth in term of height and diameter increment as well as oven-dried biomass was measured at the end of experiment.

Nutrients: nitrogen (N), phosphorus (P), potassium (K) and sodium (Na) in seedling parts. Subsamples (100 g of each) of seedling parts (leaf, stem and root) were collected from the harvested seedlings of each treatment and dried in oven at 80°C for four days. Each oven dried sample was processed and acid digested following Allen (1989). Concentration of nitrogen and phosphorus in the sample extract was measured by using colorimetric method following Baethgen and Alley (1989) and Timothy et al. (1984) respectively in UV-VIS Spectrophotometer (HITACHI-2900U). Potassium and sodium concentrations in sample extract were measured by Flame Photometer (PFP7, Jenway LTD, England).

Chlorophyll and proline in leaf

At the end of the experiment, 3 fresh leaves from each saline treatment were taken randomly for the measurement of total chlorophyll. The leaves were cut into disks (0.83 cm diameter) using a disk cutter. Total chlorophyll was extracted from the said leaf disk following Dimethyl Sulphoxide (DMSO) method (Hiscox and Israelstam, 1979). Proline in leaf samples were measured following Bates et al. (1973).

Statistical analysis

Seedling survival percentage values of all treatments were transformed to arcsine and compared among saline treatments by one-way Analysis of Variance (ANOVA) followed by Duncan Multiple Range Test (DMRT) at $p<0.05$ by using SAS (6.12) Statistical software. Height, collar diameter, oven-dried biomass increment, chlorophyll and proline concentration of seedlings were compared among salinity treatments by one-way ANOVA followed by DMRT using the above Statistical software. Nitrogen, phosphorus, potassium and sodium concentration in different parts of seedlings were compared among the salinity treatments and also among different plant parts by using two-way ANOVA followed by DMRT using the above Statistical software. Moreover, correlations among all studied parameters were evaluated using SPSS-20 statistical software.

RESULTS AND DISCUSSION

Survival of seedlings.

Survival percentage of seedlings varied significantly among the saline treatments (Figure 1a). Survival of seedlings was the highest (100%) at 0 to 10 ppt salinity. It decreased from 85% to 10% among 15 ppt and 25 ppt salinity. No seedlings survival was observed at 30 ppt and 35 ppt salinity. Survival percentage showed strong negative correlation with salinity levels (Table 2).

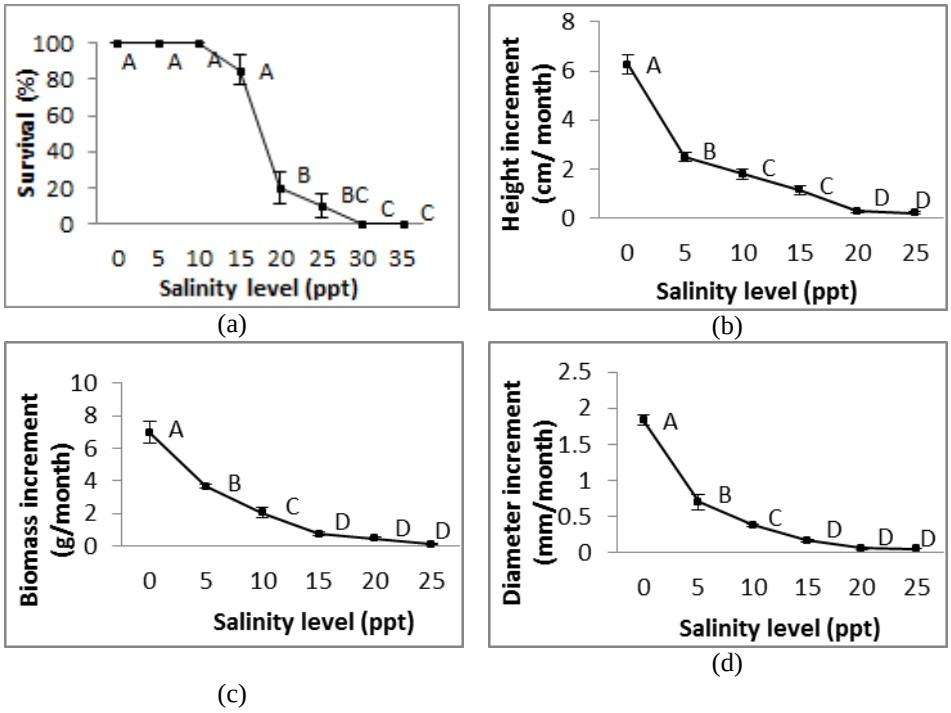


Figure 1: Salinity influence on *Millettia pinnata* (a) Survival of seedling (b) Height increment (c) Collar diameter increment (d) Oven-dried biomass increment. Similar alphabet along the line are not significantly ($p>0.05$) different. Vertical bars indicate standard error.

Growth of seedlings

Height, collar diameter, and oven-dried biomass increment separately varied significantly among salinity treatments (Figure 1b, 1c, 1d). Highest increment in height (6.26 cm/month), collar diameter (1.83 mm/month), and oven-dried biomass (6.97 g/month) were found at 0 ppt salinity whereas lowest increment in height (0.22 cm/month), collar diameter (0.06 mm/month), and oven-dried biomass (0.12 g/month) was measured at 25 ppt salinity. Sharp decrease in growth parameters were observed at 5 ppt salinity. Strong negative correlation was observed between salinity levels and height, collar diameter as well as between salinity levels and oven-dried biomass (Table 2).

Nutrients (N, P and K) and Na distribution in seedling parts

Significant variation in N, P, K and Na concentration was observed in different parts of seedlings of M. pinnata with different salinity levels. Comparatively higher concentration of N (2.63 to 2.10%), P (0.70 to 0.64%) and K (1.68 to 1.24%) was observed in leaves than other parts of seedlings at different salinity levels. On the other hand, higher Na concentration was found in roots (1.97 to 0.73%) than other parts of seedling at different salinity levels (Table 1).

Table 1: Concentrations (%) (Mean±Standard Deviation) of nitrogen (N), phosphorus (P), potassium (K) and sodium (Na) in different parts *Millettia pinnata* seedlings.

	Nitrogen			Phosphorus			Potassium			Sodium		
Salinity level (ppt)	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem	Leaf
0	1.35±0.04	1.10±0.10	2.63±0.21	0.63±0.08	0.54±0.03	0.70±0.11	1.13±0.02	0.67±0.0	1.68±0.07	0.73±0.02	0.32±0.01	0.34±0.03
5	1.35±0.04	1.12±0.13	2.64±0.22	0.61±0.08	0.53±0.03	0.69±0.16	0.62±0.02	0.54±0.07	1.58±0.12	1.14±0.06	0.54±0.01	1.12±0.01
10	1.33±0.15	0.94±0.07	2.56±0.11	0.62±0.08	0.53±0.08	0.65±0.10	0.59±0.02	0.46±0.04	1.53±0.07	1.33±0.03	0.83±0.04	1.14±0.02
15	1.26±0.03	0.83±0.06	2.20±0.04	0.61±0.03	0.53±0.11	0.64± 0.08	0.58±0.02	0.41±0.05	1.40±0.06	1.35±0.06	1.05±0.01	1.32±0.02
20	1.27±0.06	0.8±0.1	2.19±0.05	0.60±0.13	0.53±0.03	0.64±0.11	0.56±0.01	0.35±0.01	1.30±0.07	1.42±0.01	1.10±0.02	1.35±0.09
25	1.25±0.13	0.8±0.1	2.1±0.1	0.60±0.05	0.53±0.03	0.64±0.08	0.46±0.09	0.32±0.03	1.24±0.08	1.92±0.20	1.16±0.01	1.51±0.06

Table 2: Correlation matrix of all studied parameters.

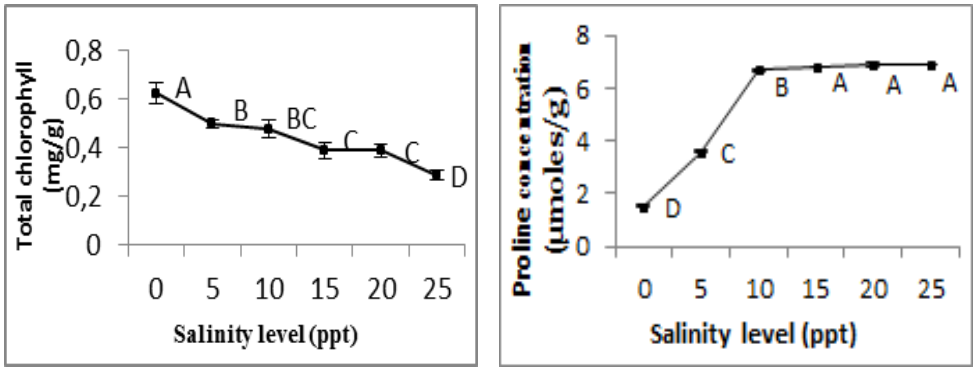
		Salinity	Survival	Biomass	Diameter	Height	N (Root)	N (Leaf)	N (Stem)	P (Root)	P (Leaf)	P (Stem)	K (Root)	K (Leaf)	K (Stem)	Na (Root)	Na (Leaf)	Na (Stem)	Chlorophyll	Proline
Salinity	r	1																		
	Sig																			
	N	160																		
Survival	r	-0.84																		
	Sig	0.00																		
	N	160																		
Biomass	r	-0.84	0.48																	
	Sig	0.00	0.00																	
	N	61	61																	
Diameter	r	-0.83	0.43	0.89																
	Sig	0.00	0.00	0.00																
	N	61	61	61																
Height	r	-0.84	0.47	0.87	0.89															
	Sig	0.00	0.00	0.00	0.00															
	N	61	61	61	61															
N (Root)	r	-0.47	0.22	0.40	0.43	0.40														
	Sig	0.05	0.39	0.10	0.08	0.10														
	N	18	18	18	18	18														
N (Leaf)	r	-0.87	0.61	0.76	0.75	0.65	0.45													
	Sig	0.00	0.01	0.00	0.00	0.00	0.06													
	N	18	18	18	18	18	18													
N (Stem)	r	-0.80	0.61	0.66	0.76	0.76	0.45	0.69												
	Sig	0.00	0.01	0.00	0.00	0.00	0.06	0.00												
	N	18	18	18	18	18	18	18												
P (Root)	r	-0.39	0.61	0.39	0.37	0.25	-0.22	0.47	0.23											
	Sig	0.11	0.01	0.11	0.14	0.31	0.38	0.05	0.37											
	N	18	18	18	18	18	18	18	18											

P (Leaf)	r	-0.59	0.46	0.67	0.56	0.55	0.23	0.60	0.38	0.44										
	Sig	0.01	0.06	0.00	0.02	0.02	0.36	0.01	0.12	0.07										
	N	18	18	18	18	18	18	18	18	18										
P (Stem)	r	-0.17	-0.01	0.22	0.25	0.21	0.29	0.26	0.30	-0.08	0.12									
	Sig	0.51	0.96	0.37	0.32	0.40	0.25	0.30	0.23	0.75	0.64									
	N	18	18	18	18	18	18	18	18	18	18									
K (Root)	r	-0.79	0.45	0.90	0.87	0.88	0.29	0.58	0.59	0.27	0.56	0.26								
	Sig	0.00	0.06	0.00	0.00	0.00	0.24	0.01	0.01	0.27	0.02	0.29								
	N	18	18	18	18	18	18	18	18	18	18	18								
K (Leaf)	r	-0.91	0.74	0.72	0.80	0.72	0.46	0.82	0.69	0.43	0.63	0.17	0.68							
	Sig	0.00	0.00	0.00	0.00	0.00	0.05	0.00	0.00	0.07	0.01	0.51	0.00							
	N	18	18	18	18	18	18	18	18	18	18	18	18							
K (Stem)	r	-0.94	0.64	0.90	0.88	0.92	0.41	0.78	0.72	0.36	0.71	0.20	0.86	0.88						
	Sig	0.00	0.01	0.00	0.00	0.00	0.09	0.00	0.00	0.14	0.00	0.43	0.00	0.00						
	N	18	18	18	18	18	18	18	18	18	18	18	18	18						
Na (Root	r	0.92	-0.69	-0.83	-0.83	-0.85	-0.40	-0.72	-0.67	-0.39	-0.55	-0.22	-0.83	-0.83	-0.89					
	Sig	0.00	0.00	0.00	0.00	0.00	0.10	0.00	0.00	0.12	0.02	0.38	0.00	0.00	0.00					
	N	18	18	18	18	18	18	18	18	18	18	18	18	18	18					
Na (Leaf)	r	0.86	-0.54	-0.91	-0.90	-0.91	-0.40	-0.66	-0.66	-0.34	-0.58	-0.20	-0.96	-0.79	-0.91	0.89				
	Sig	0.00	0.02	0.00	0.00	0.00	0.10	0.00	0.00	0.17	0.01	0.43	0.00	0.00	0.00	0.00				
	N	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18				
Na (Stem)	r	0.96	-0.59	-0.92	-0.94	-0.90	-0.50	-0.85	-0.83	-0.31	-0.63	-0.19	-0.82	-0.87	-0.94	0.87	0.89			
	Sig	0.00	0.01	0.00	0.00	0.00	0.03	0.00	0.00	0.21	0.01	0.46	0.00	0.00	0.00	0.00	0.00			
	N	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18			
Chlorophyll	r	-0.89	0.65	0.87	0.85	0.77	0.34	0.88	0.64	0.58	0.62	0.15	0.80	0.81	0.85	-0.87	-0.86	-0.87		
	Sig	0.00	0.00	0.00	0.00	0.00	0.17	0.00	0.00	0.01	0.01	0.54	0.00	0.00	0.00	0.00	0.00	0.00		
	N	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18		
Proline	r	0.86	-0.43	-0.93	-0.94	-0.91	-0.41	-0.70	-0.77	-0.25	-0.65	-0.21	-0.87	-0.76	-0.90	0.82	0.89	0.95	-0.79	
	Sig	0.00	0.07	0.00	0.00	0.00	0.09	0.00	0.00	0.32	0.00	0.41	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
	N	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	

N and K concentration in different parts (roots, leaves and roots) and P concentration in leaves showed significantly negative correlation with salinity levels. However, P concentration in roots and stems showed no significant correlation with salinity. Sodium concentration in different parts of seedlings showed significant positive correlation with salinity (Table 2).

Total chlorophyll and proline concentration in leaves

Total chlorophyll concentration of leaves varied significantly among salinity treatments (Figure 2a).



(a) (b)

Figure 2: Salinity influence on (a) Total chlorophyll concentration (b) Proline concentration in leaves of *Millettia pinnata* seedling. Similar alphabet along the line are not significantly ($p>0.05$) different, Vertical bars indicate standard error.

Highest concentration (0.62 mg/g) of total chlorophyll was measured at 0 ppt, and it decreased to 0.29 mg/g at 25 ppt salinity. Total chlorophyll concentration showed strong negative correlation with salinity levels (Table 2). Proline concentration in leaves varied significantly among salinity treatments, lowest proline concentration (1.52 µmoles/g) was measured at 0 ppt salinity which increased up to 6.67 µmoles/g at 10 ppt salinity (Figure 2b). However, no significant increase in proline concentration was observed at high salinity (from 15 to 25 ppt). Proline concentration showed strong positive correlation with salinity levels (Table 2).

Salinity gradient is recognized as a potential stressor and a critical environmental factor that regulates growth, height, survival, and zonation patterns in mangroves (Lin and Sternberg, 1992; Mahmood et al. 2014). Mangroves usually invest a large proportion of its photosynthetic product for the surviving in high saline environments (Lopez-Hoffman et al. 2006). Nucleic acid and protein synthesis are two early processes of seedling growth. However, these processes are suppressed by salinity (Bewely and Black, 1985). Moreover, high soil salinity affects plant growth by creating low water potential, ion toxicities, nutrient deficiencies or a combination of these factors (Khan et al. 2000). Similarly, these factors may be responsible for the survival of mangrove seedling

at high salinity (Mahmood et al. 2014). These could be the reason for comparatively high survival and growth of *M. pinnata* seedlings at low salinity level. Higher concentration of Na+ may disrupt nutrient transport which restricts plant growth (Grant et al. 1987). In this study, salinity did not show any significant implications with N and P concentration in different parts of seedlings except leaves. However, it showed significant influence on K+ and Na+ concentration at different parts of seedlings. Gorham et al. 1986 observed a drastic reduction of leaf $NO3^{-}$ when plants were grown in saline condition. However, there was no strong evidence on the relationship between N concentration in other parts of plants and salinity levels (Munns and Termaat, 1986). Influence of salinity on P concentration in plant parts is variable and depends on the species and experimental conditions (Champagnol, 1979). Salinity reduced the concentration of K+ in plant parts (Ratthert, 1982), and the excess NaCl leads to loss of potassium due to membrane depolarization by Na+ (Cramer et al. 1985). This could be the reason for antagonistic relation between K+ and Na+ concentration in different parts of seedlings of M. pinnata. According to Singh (1990) and Singh and Yadav (1999), P. pinnata failed to grow at ECe 32.5 dS/m and K contents did not exhibit any definite relationship with increasing salinity under 31 months experiment of 9 month old seedlings. However, we found that in case of seedlings survived up to 25 ppt salinity, K concentration decreased with increased salinity.High concentration of sodium can cause ionic imbalance (Rathert et al. 1981) that influences the osmotic adjustment (Marschner et al.1986) and enzyme inhibition in plants (Flowers et al. 1977; Greenway and Osmond, 1972).

Total chlorophyll concentration depends on the biological process and development stages of plants and concentration of salt (Khan, 2003; Iqbal et al. 2006; Mahmood et al. 2014). Decrease in chlorophyll content at high saline environment results in rapid maturing of leaves (Yeo et al. 1991). Furthermore, decrease in chlorophyll content at higher salinity might be due to changes in the lipid-protein ratio of pigment-protein complexes or increased chlorophyllase activity (Iyengar and Reddy, 1996). Salt stress reduces the photosynthetic capability of plant (Dubey, 1997; Jamil et al. 2007; Bayuelo-Jimenez et al. 2002). The inhibition in photosynthesis under saline condition can be explained by the decline in chlorophyll content (Delfine et al. 1999; Jamil and Rha, 2007). In our study, the concentration of total chlorophyll decreased with increasing salinity levels. Low concentration of chlorophyll in plant leaves results lower uptake of CO2, which ultimately reduces photosynthesis (Francios and Mass, 1993; XinWen et al. 2008).

In another way, plants synthesize proline under arid and salinity stress conditions in order to protect themselves and to regulate their physiological process (Edreva, 1998). Genotypes with a high proline accumulation and chlorophyll content, high K/Na ratio and low Na+ and Cl^{-} accumulation are more tolerant to salt (Mane et al. 2011).

CONCLUSION

Millettia pinnata produced comparatively low proline and chlorophyll which make it less salt tolerant than other dominant mangrove species in the Sundarbans. This species can survive and grow up to 25 ppt salinity, but 0 to 5 ppt salinity appeared to be a favorable range of salinity for its growth and development.

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