ORIGINAL ARTICLE

The modulation of various physiochemical changes in *Bruguiera cylindrica* (L.) Blume affected by high concentrations of NaCl

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Abstract

Bruguiera cylindrica is a major mangrove species in the tropical mangrove ecosystems and it grows in a wide range of salinities without any special features for the excretion of excess salt. Therefore, the adaptation of this mangrove to salinity could be at the physiological and biochemical level. The 3-month-old healthy plantlets of *B. cylindrica*, raised from propagules were treated with 0 mM, 400 mM, 500 mM and 600 mM NaCl for 20 days under hydroponic culture conditions provided with full strength Hoagland medium. The modulation of various physiochemical changes in *B. cylindrica*, such as chlorophyll *a* fluorescence, total chlorophyll content, dry weight, fresh weight and water content, Na^+ accumulation, oxidation and antioxidation (enzymatic and non-enzymatic) features were studied. Total chlorophyll content showed very minute decrease at 500 mM and 600 mM NaCl treatment for 20 days and the water content percentage was decreased both in leaf and root tissues with increasing concentration. A significant increase of Na^+ content of plants from 84.505 mM/plant dry weight in the absence of NaCl to 543.38 mM/plant dry weight in plants treated with 600 mM NaCl was recorded. The malondialdehyde and the metabolites content associated with stress tolerance (amino acid, total phenols and proline) showed an increasing pattern with increasing NaCl concentration as compared to the control in both leaf and root tissues but the increase recorded in plantlets subjected to 500 mM was much less, indicating the tolerance potential of this species towards 500 mM NaCl. The significant decrease of sugar content was found only in 600 mM NaCl on 20 days of treatment, showing that the process of sugar synthesis was negatively affected but the same process remains less affected at 500 mM NaCl. A slight reduction in ascorbate and glutathione content and very less increase in carotenoid content were observed at 500 mM and 600 mM NaCl stress. Antioxidant enzymes (APX, GPX, SOD and CAT) showed an enhanced activity in all the treatments and the increased activity was more significant in 600 mM treated plants. The result establishes that *B. cylindrica* tolerates high NaCl concentration, to the extent of 500 mM NaCl without any major inhibition on photosynthesis and metabolite accumulation. Understanding the modulation of various physiological and biochemical changes of *B. cylindrica* at high levels of NaCl will help us to know the physiochemical basis of tolerance strategy of this species towards high NaCl.

Keywords *Bruguiera cylindrica* · Mangroves · Metabolic regulation · Chlorophyll *a* fluorescence · NaCl stress

Introduction

Intertidal forest areas are mainly inhabited by mangrove plants (Tomlinson 1986). These mangrove plants include woody trees and shrubs and they are the connecting line between the ocean and land mass of the tropical coastlines (Saenger 2002; Spalding 2010; Parida and Jha 2010). At the ecosystem level, mangrove forests have significant potential in terms of its various uses and economic value (Barbier et al. 2011). Mangroves are always of great interest to biologist due to its high productivity, diversity and at the same time it has got several social and economic importance (Salem and Mercer 2012).

Mangrove forests occupy an inter-tidal habitat, where sediment accumulation along with subsurface processes results in the elevation of sediments (Gilman et al. 2008). The tidal inundation from bay water influences the sediment salinities of these regions (Schile et al. 2014). With

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increasing distance from the bay and with increasing elevation, salinity gradients generally increase linearly (Veenklaas et al. 2015). Moreover, the evapotranspiration in marsh areas further enhances the soil salinities (Passioura et al. 1992; Callaway et al. 2007). However, salt marshes are one of the most biologically productive ecosystems on the Earth, providing sources of organic matter and nutrients (Kennish 2001). Mangroves in the inter-tidal regions are exposed to high salinity due to evaporation of water and withdrawal of the sea due to tide. The water is swept off and salinity concentration increases (Parida et al. 2002; Perri et al. 2017). Although mangroves are tolerant towards salinity, high level variation in salinity is intolerable and deleterious to mangroves (Lovelock and Feller 2003).

The plants exposed to high NaCl concentration have developed various biological responses (Parida and Das 2005). The salinity tolerance potential of mangroves is mainly the sum effects of its varied capacities such as (1) effective ion compartmentation, (2) osmo regulation, (3) selective uptake of ions and its transport for compartmentation, (4) regulating the movement of toxic ions to the shoot regions and (5) capacity to regulate salt influx (Munns 2002; Parida and Jha 2010). Depending on their salt—tolerance potential, halophytes are classified as obligate or facultative, facultative halophytes are also able to grow in fresh water, but differ from non-halophytes in positively responding to enhancement of salinity with increased growth, up to an optimum level. Obligate halophytes have optimal growth under ranges of salinity similar or greater to those of facultative halophytes but are not able to survive under fresh water conditions (Krauss and Ball 2013). Mangroves are generally classified into three major groups based on salt eliminating mechanisms such as (1) salt excluders, (2) salt secretors and (3) salt accumulators (Parida and Jha 2010). Some mangroves (e.g. *Rhizophora* spp., *Ceriops* spp., *Bruguiera* spp., etc.) have adapted the mechanisms for eliminating excess salt by ultrafiltration in root cell membranes (Khan and Aziz 2001; Wang et al. 2002). Salt secreting mangroves (*Acanthus* spp., *Avicennia marina* and *A. officinalis*) regulate the internal level of salt by expelling the excess salt through glands present on the leaves (Meher-Homji 1988; Selvam 2003). Salt accumulating type mangroves (species of *Lumnitzera* and *Excoecaria*), accumulates high level of salts in the cells and tissue and at the same time avoid toxicity due to these salts by sequestering toxic ions into the vacuoles of leaves, translocate the ions out of leaves by cuticular transpiration and as a last step these plants shed the leaves as (Tomlinson 1986; Aziz and Khan 2001b). *Bruguiera cylindrica* L. is a mangrove belonging to Rhizophoraceae that grows along the Indian seacoast. It shows no morphological structures to secrete salt (Hanagata et al. 1999) and the adaptation of this mangrove to salinity is at the physiological level. *Bruguiera cylindrica* is a facultative mangrove and are able to grow in fresh water and at the same time facultative mangroves are tolerant towards high levels of salinity (Atreya et al. 2009).

The success of the mangroves to establish and survive under harsh conditions is by their special morphological, anatomical, physiological and biochemical features (Ball and Farquhar 1984; Parida and Jha 2010). Mangrove trees survive the harsh environment with specialized adaptations such as salt-excreting leaves and viviparous nature of the propagules (Ball and Pidsley 1995). The viviparous condition, which is generally found in the genus *Bruguiera*, is suggested to have great adaptive significance to survive in the intertidal region by avoiding high salinity at germination stage (Henkel 1979). Mangroves develop diverse mechanisms associated with anatomic characteristics where, *Bruguiera* species are prominent with sunken stomata beneath the epidermis (Miller et al. 1975) and also single-layered hypodermal cells (Saenger 1982).

An important technique used to evaluate the photochemical performance of plants under high salinity conditions is the Chl *a* fluorescence (Krause and Weis 1991). Chl *a* fluorescence provides information on the relationship between structure and function of photosystem-II (PS-II), reaction center (RC), and core complexes in plants subjected to salt stress (Yamane et al. 2000; Misra et al. 2001). Also the importance of studying metabolites associated with stress tolerance in plants turns to be significant, while assessing the stress tolerance potential of the same. Moreover, plants have generally efficient complex enzymatic and non-enzymatic antioxidant defense systems in order to avoid the stress injury (Kasote et al. 2015). Mangroves with high levels of antioxidants, have greater resistance to this oxidative damage (Parida et al. 2004b). The activities of the antioxidative enzymes such as catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (GPX) and superoxide dismutase (SOD) increases under high salinity and there exists a direct correlation between these enzyme activities and salt tolerance potential of mangroves (Takemura et al. 2000; Parida et al. 2004b).

Salinity is one of the important reasons that harmfully affects plant growth and metabolism particularly in the arid and semi-arid regions of the world. This environmental issue is getting more attention throughout the world due to intensification of agriculture and global climate change (Kathiresan 2003). Identification and characterization of salt-tolerant plants and analyzing the mechanisms imparting salt tolerance is being thoroughly investigated today (Flowers and Colmer 2008). In this aspect, mangroves growing under the influence of soil water salinity seem to be promising candidates for conducting a detailed study of the above mentioned aspect. Understanding the mechanisms of salt tolerance in mangroves and identification of salt tolerant genes from mangroves will lead to effective means to breed or genetically engineer salt tolerant crops. Moreover,

this study provides base-line information for exploiting the genetic basis of high salt tolerance potential of *B. cylindrica*.

Materials and methods

Experimental setup

Mature propagules of *B. cylindrica* L. were collected from the mangrove ecosystem of Murikkumpadam, Kochi, Kerala. These propagules were washed under running tap water and propagated in soil/mud collected from the regions of the mangrove habitats. They were housed in a greenhouse under controlled conditions of temperature (32 ± 2 °C), light intensity (250 ± 75 µmol m^{-2}s^{-1}) and humidity ($60 \pm 5\%$) and during this period the plantlets were provided with tap water. The 3-month-old healthy plantlets having 6–8 leaves (3–4 nodes) were selected for different treatments. The plantlets were carefully removed from the soil and placed in a water-filled plastic container. The plantlets were washed to remove any soil particles sticking to the roots and finally rinsed in distilled water. The plantlets were then placed in the glass bottles (15 cm $\times$ 7 cm) filled with Hoagland's nutrient medium (300 ml). The healthy plantlets of *B. cylindrica* were then subjected to varying concentrations of NaCl (0 mM, 400 mM, 500 mM, 600 mM NaCl) for 20 days under hydroponic culture conditions provided with full-strength Hoagland medium. The second and third pairs of leaves from the tip of the shoots were harvested for the study at different intervals (0 day, 5 days, 10 days, 15 days and 20 days). Plants grown in Hoagland's nutrient medium were designated as control and those in the same medium containing various concentrations of NaCl as the treated plants.

Physiological studies

Dry weight and water content

For dry weight measurements, the fresh weight of leaves and roots was recorded using an electronic weighing balance, and the weighed samples were dried at 100 °C and followed by 60 °C. After 48 h the dried samples were kept in a desiccator, allowing it to cool and then finally weighed. The samples were reweighed as described above at regular intervals, until the weights became constant.

Water content (g) = fresh weight − dry weight.

Total chlorophyll and carotenoid content

Leaf tissue of 0.5 g was homogenized in cold acetone (80%). The homogenates were centrifuged at 4 °C in the dark at 5000 rpm for 10 min, and absorbance of the acetone extracts were measured at 750 nm, 663 nm, 645 nm, and 470 nm.

Total chlorophyll and carotenoid content were calculated according to the method of Arnon (1949).

Chl *a* fluorescence parameters

Chl *a* fluorescence transients were measured with the Plant Efficiency Analyzer (Handy PEA, Hansatech Ltd., Norfolk, UK), is a portable fluorometer having high resolutions (Strasser et al. 2004). All measurements were performed on the upper surfaces of the fully expanded leaves following a dark adaptation period of 20 min by attaching light exclusion clips to the leaf surface before the measurements. Maximal fluorescence was induced by a 1 s pulse of continuous light (650 nm, 3000 µmol m^{-2} s^{-1}) with a data acquisition rate of 10 µs for the first 2 ms and at 1 ms thereafter. Chl *a* fluorescence signals were analyzed with the Biolyzer HP3 software (Laboratory of Bioenergetics, University of Geneva, Switzerland). Various fluorescence parameters like the area over the curve, maximal fluorescence (F_m), the photochemical efficiency of PS-II (F_v/F_m), the relative variable fluorescence at *j* step (V_j), Performance index (PI), ABS/CS$_m$ (number of photons absorbed by a PS-II cross section), electron transport per cross section (ET$_o$/CS$_m$) were measured in relative units (RU) and the phenomenological leaf model was deduced in response to NaCl stress in *B. cylindrica*.

Biochemical estimations

Estimation of malondialdehyde (MDA)

MDA was extracted and estimated according to the method of Heath and Packer (1968). MDA concentration was calculated using its molar extinction coefficient of 155 mM l^{-1} cm^{-1}.

Total soluble sugar content

Total soluble sugar content was extracted from leaf samples by 80% ethanol and estimated following the method of Dubois et al. (1956). Standard curve was plotted with D-glucose as standard.

Proline content

Free proline content was extracted from leaf using 3% sulphosalicylic acid and estimated following the method of Bates et al. (1973) using L-proline as standard.

Total phenolics content

Total phenolics content were extracted using 80% ethanol and estimated according to the procedures described earlier by Folin and Denis (1915) using Folin–Ciocalteau reagent (0.5 ml

of 1 N). A standard curve was prepared using different concentrations of catechol.

Amino acid content

Free amino acids were extracted from leaf samples using 70% ethanol and estimation was carried out following the method of Moore and Stein (1948) using ninhydrin reagent. Total free amino acids were calculated from a standard curve prepared with glycine.

Enzymatic antioxidant mechanisms

Enzyme extracts preparation and assay of enzyme activity Fresh leaf tissues (0.5 g) were weighed and homogenized in 5 ml of ice-cold 50 mM potassium phosphate buffer (pH 7.0) using a prechilled mortar and pestle. The homogenized extract was filtered through muslin cloth and centrifuged at 10,000*g* for 15 min at 4 °C. The supernatants were collected and used for the enzyme assay (Yin et al. 2009).

Catalase (CAT, EC 1.11.1.6) Activity was determined by the method of Kar and Mishra (1976). The activity of CAT was determined as a decrease in absorbance at 240 nm for 1 min following the decomposition of H_2O_2. One unit of the enzyme was defined as μ moles H_2O_2 decomposed per min per mg protein.

Superoxide dismutase (SOD, EC 1.15.1.1) Activity was assayed by method of Giannopolitis and Ries (1977). SOD activity was conceded for monitoring the ability of SOD to inhibit the photochemical reduction of nitroblue tetrazolium (NBT). The formazan accumulation in different tubes was quantified using UV–VIS spectrophotometer (Systronics 2201) by recording the absorbance of the developed blue colour at 560 nm against the blank. Results were expressed as units SOD mg^{-1} protein^{-1}.

Guaiacol peroxidase (GPX, EC 1.11.1.7) Guaiacol peroxidase activity was measured according to Gaspar et al. (1975). The increase in absorbance due to oxidation of guaiacol was measured at 420 nm using a UV-double beam spectrophotometer (Systronics 2201) for 3 min at intervals of 30 s.

Ascorbate peroxidase (APX, EC 1.11.1.11) Activity was assayed as described by Nakano and Asada (1981). The absorbance was read at 290 nm. One unit of the enzyme was defined as μ moles of ascorbate oxidized per minute per mg protein.

Non-enzymatic antioxidant mechanisms

Ascorbate and glutathione content

Ascorbate and glutathione content was extracted from leaf using 5% TCA and measured by the method of Chen and Wang (2002).

Determination of Na$^+$ content

Samples were prepared according to the method of Allan (1969). Plant tissues were weighed and dried in a hot air oven at 60 °C. The dried samples were digested by refluxing in a mixture of nitric acid and perchloric acid in the ratio of 10:4 until the solution became colourless using Kjeldahl's flask heated in a heating mantle. After the digestion was completed, the solution was filtered and further transferred to standard flask and the volume was made up to 50 ml and stored in screw-capped containers. The samples were analyzed using flame photometer (systronics, serial number 968) and the standard used was NaCl (5 ppm and 100 ppm).

Statistical analysis

Analysis of statistical results was carried out with Duncan's multiple range tests at 5% probability level. Data were subjected to one-way ANOVA using the SPSS software 16.0. The data is an average observation from three independent experiments, each with three replicates. The data represent mean $\pm$ standard error.

Results

Physiological studies

Dry weight and water content

Fresh weight, dry weight and water content of leaf and root were decreased when treatment was done with 600 mM NaCl as compared to control plants. DW was decreased by 50%, 46% and 55% in leaves and 26%, 34% and 54% in root on treatment with 400 mM, 500 mM and 600 mM NaCl, respectively as compared with the control and also water content was decreased by 48%, 78% and 105% in leaf and 25%, 57% and 85% in roots on treatment with 400 mM, 500 mM and 600 mM, respectively as compared with the control plant (Table 1).

Total chlorophyll content

The total chlorophyll content in leaves was showing a decreasing pattern upon increasing concentration of NaCl, i.e., 13%, 16% and 24% reduction in the treatments of 400 mM, 500 mM and 600 mM NaCl, respectively on 20 days of treatment. But there was no significant change in total chlorophyll content in 100 mM, 200 mM and 300 mM

Table 1 Dry weight, fresh weight and water content (g) of root and leaf tissues of *B. cylindrica* L. subjected to various concentrations of NaCl (0 mM, 400 mM, 500 mM and 600 mM)

Days	NaCl (mM)	Fresh weight (g)		Dry weight (g)		Water content (g)	
		Leaf	Root	Leaf	Root	Leaf	Root
0	Control	3.45 ± 0.127^a	1.25 ± 0.080^a	0.84 ± 0.012^a	0.25 ± 0.009^a	2.60 ± 0.131^a	0.99 ± 0.214^b
	400	3.12 ± 0.135^a	1.23 ± 0.070^a	0.67 ± 0.012^b	0.21 ± 0.124^b	2.44 ± 0.145^a	1.02 ± 0.561^b
	500	2.69 ± 0.112^b	1.03 ± 0.080^b	0.43 ± 0.011^c	0.31 ± 0.214^a	2.26 ± 0.137^b	0.72 ± 0.216^c
	600	2.54 ± 0.144^b	1.42 ± 0.097^a	0.45 ± 0.024^c	0.21 ± 0.098^b	2.09 ± 0.134^b	1.20 ± 0.089^a
5	Control	3.05 ± 0.173^a	1.07 ± 0.083^b	0.54 ± 0.014^a	0.20 ± 0.007^b	2.51 ± 0.122^a	0.86 ± 0.084^b
	400	2.28 ± 0.118^b	1.04 ± 0.072^b	0.44 ± 0.009^b	0.22 ± 0.124^b	1.84 ± 0.046^c	0.82 ± 0.008^b
	500	2.70 ± 0.126^b	1.71 ± 0.091^a	0.45 ± 0.014^b	0.33 ± 0.134^a	2.25 ± 0.214^b	1.37 ± 0.123^a
	600	2.69 ± 0.138^b	1.36 ± 0.073^a	0.52 ± 0.021^a	0.27 ± 0.245^a	2.17 ± 0.678^b	1.09 ± 0.145^b
10	Control	1.84 ± 0.167^b	1.77 ± 0.074^a	0.35 ± 0.254^b	0.35 ± 0.219^a	1.48 ± 0.234^b	1.42 ± 0.314^a
	400	2.21 ± 0.214^a	1.09 ± 0.085^b	0.44 ± 0.312^a	0.25 ± 0.012^b	1.76 ± 0.214^a	0.83 ± 0.009^b
	500	1.90 ± 0.347^b	0.60 ± 0.045^c	0.39 ± 0.245^b	0.15 ± 0.012^c	1.50 ± 0.165^b	0.44 ± 0.011^d
	600	1.71 ± 0.423^b	0.84 ± 0.024^b	0.42 ± 0.465^a	0.21 ± 0.021^b	1.28 ± 0.214^c	0.62 ± 0.007^c
15	Control	2.06 ± 0.498^c	1.51 ± 0.100^b	0.48 ± 0.021^b	0.29 ± 0.054^b	1.57 ± 0.314^a	1.21 ± 0.025^a
	400	2.48 ± 0.278^b	0.92 ± 0.097^c	0.52 ± 0.001^b	0.22 ± 0.014^b	1.95 ± 0.245^a	0.70 ± 0.054^c
	500	1.59 ± 0.467^d	1.53 ± 0.104^b	0.36 ± 0.006^c	0.37 ± 0.021^a	1.23 ± 0.314^b	1.16 ± 0.241^b
	600	2.87 ± 0.345^a	1.66 ± 0.121^a	1.64 ± 0.004^a	0.40 ± 0.031^a	1.23 ± 0.216^b	1.26 ± 0.314^a
20	Control	4.31 ± 0.257^a	1.63 ± 0.131^a	1.81 ± 0.014^a	0.35 ± 0.012^a	2.53 ± 0.356^a	1.27 ± 0.142^a
	400	2.27 ± 0.231^b	1.24 ± 0.234^b	0.75 ± 0.089^b	0.26 ± 0.009^b	1.52 ± 0.179^b	0.98 ± 0.086^b
	500	1.96 ± 0.467^b	0.98 ± 0.118^c	0.63 ± 0.013^b	0.26 ± 0.008^b	1.33 ± 0.213^b	0.71 ± 0.045^c
	600	1.49 ± 0.344^c	0.87 ± 0.210^d	0.36 ± 0.212^c	0.21 ± 0.021^c	1.13 ± 0.189^c	0.66 ± 0.670^d

Values are the mean $\pm$ SE of three independent experiments ($n = 9$)

Different letters inside the table indicate the significant difference at $p \leq 0.05$ according to one way ANOVA

NaCl treated plants as compared to control and the reduction recorded was less than 10% (Fig. 1).

Chl *a* fluorescence parameters

Chlorophyll *a* fluorescence was analysed to study the impact of high NaCl stress on photosystem II functioning. The area over the fluorescence curve was decreased by 59% in 600 mM NaCl treatment, while 400 mM and 500 mM NaCl treatment showed a decrease of only 8% and 35%, respectively as compared to the control plants on 20 days of treatment (Table 2). Performance index (PI) showed a decrease of 61% and 77% in plants treated with 500 mM and 600 mM NaCl concentration as compared to the control plants, while treatment with 400 mM NaCl concentration showed only a reduction of 14% as compared to the control plants. Maximum quantum yield of primary photochemistry of PS-II (F_v/F_m), measured in plants treated with 0 mM, 400 mM, 500 mM and 600 mM NaCl concentrations showed that there was a decrease of Fv/Fm with increasing NaCl concentration. The decrease was found significant in plants subjected to 600 mM NaCl on 20 days of treatment. The control plants showed the maximum F_v/F_m value of 0.855 which was reduced up to 0.751 in 600 mM NaCl treated plants. Plants treated with 400 mM and 500 mM were not significantly exhibiting any signs of photochemical inhibition as indicated by the F_v/F_m value of 0.837 and 0.820, respectively. The value of F_m decreased from the control plants (4064) to an extend of 4008, 3616 and 3608 respectively in plants treated with 400 mM, 500 mM and 600 mM of NaCl (Table 2). Relative variable fluorescence at *j* step (V_j) in 600 mM NaCl treated plants increased 35% as compared to the control plants, where as 400 mM and 500 mM NaCl treatments showed an increase of only 5% and 15%, respectively.

The Chl *a* fluorescence parameters were also analyzed by means of dynamic energy pipeline leaf model. The Chl *a* fluorescence parameter ABS/CSm (number of photons absorbed by cross section), was showing a decreasing pattern upon increasing NaCl concentrations (0 mM, 400 mM, 500 mM and 600 mM). ABS/CSm value decreased by 1%, 11% and 12% in the treatments of 400 mM, 500 mM and 600 mM NaCl, respectively on 20 days over the control plants. Similarly a decrease in the electron transport per cross section (ET_o/CS_m) due to inactivation of reaction center complexes was 8%, 23% and 69% after the treatment with 400 mM, 500 mM and 600 mM NaCl concentrations over the control plants (Fig. 2).
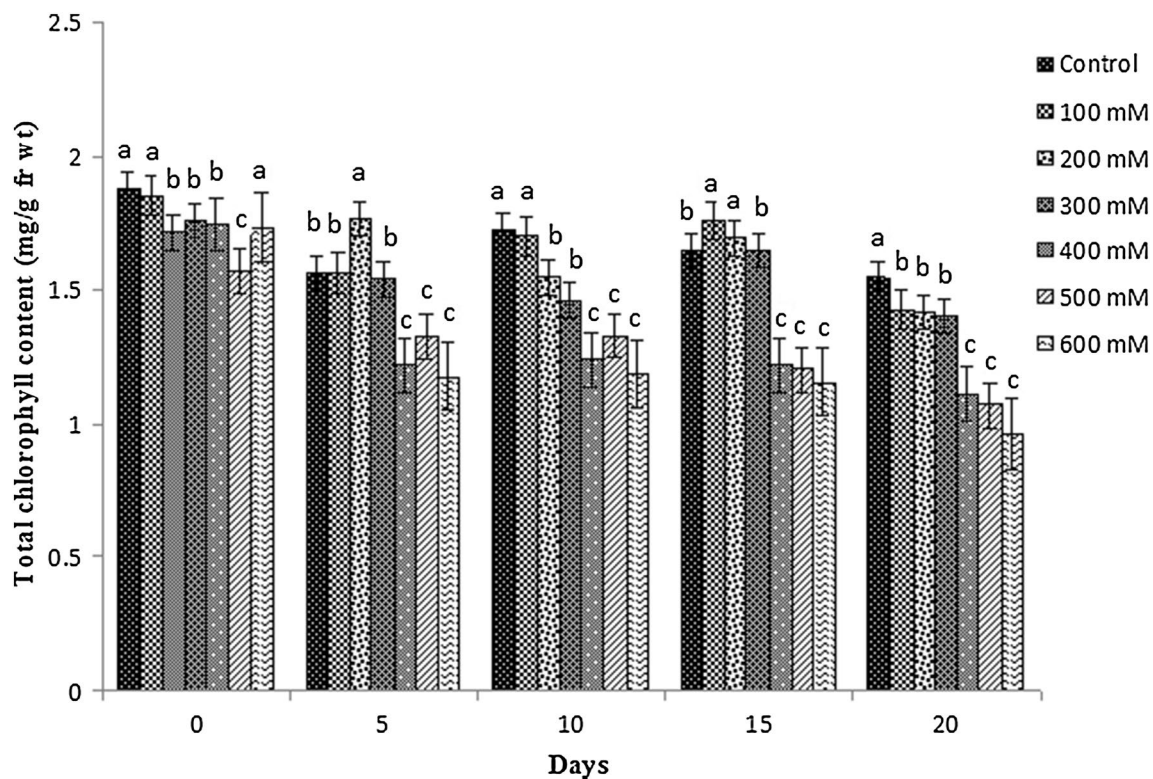
Fig. 1 Total chlorophyll content of *B. cylindrica* L. subjected to 0 mM, 100 mM, 200 mM, 300 mM, 400 mM, 500 mM and 600 mM NaCl. Values are the mean ± SE of three independent experiments ($n = 6$). Different letters on top of the bar indicate significant difference at $p \leq 0.05$ according to one-way ANOVA

Table 2 Changes in the Chl *a* fluorescence parameters (Area, F_m, F_v/F_m, V_j and PI in RU) of *Bruguiera cylindrica* leaves exposed to different concentration of NaCl (0 mM, 400 mM, 500 mM and 600 mM)

NaCl (mM)	Area	F_o	F_m	F_v/F_m	V_j	PI
0	39117 ± 1865.02[a]	587 ± 0.220[c]	4064 ± 200.1[a]	0.855 ± 0.03[a]	0.6871 ± 0.032[c]	5.3553 ± 0.254[a]
400	35630 ± 1654.2[b]	653 ± 0.321[b]	4008 ± 198.2[b]	0.837 ± 0.03[b]	0.7282 ± 0.031[c]	4.5877 ± 0.22[b]
500	27041 ± 1135.3[c]	649 ± 0.224[b]	3616 ± 178.5[c]	0.820 ± 029[b]	0.803 ± 0.041[b]	2.0879 ± 0.102[c]
600	15741 ± 435.01[d]	898 ± 0.332[a]	3608 ± 179[c]	0.751 ± 0.028[c]	0.9343 ± 0.045[a]	1.2257 ± 0.010[d]

Values are the mean ± SE of three independent experiments ($n = 9$)

Different letters inside the table indicate the significant difference at $p \leq 0.05$ according to one way ANOVA

Malondialdehyde (MDA)

The MDA content was found to be increased upon increasing NaCl concentration in both leaf and root tissues. The MDA content showed an increase of 6%, 22% and 125% in leaf and 7%, 13% and 33% increase was observed in root when treated with 400 mM, 500 mM and 600 mM NaCl respectively over the control plants on 20 days of treatment (Fig. 3a, b).

Metabolites

Soluble sugars content decreased in both leaf and root tissues of *B. cylindrica* when exposed to increasing concentration of NaCl (0 mM, 400 mM, 500 mM and 600 mM NaCl). On initial days of treatment (5 days and 10 days), the total soluble sugar increased in leaf tissue of plants subjected to 500 mM NaCl concentration. On 20 days of treatment there was a gradual decrease of total soluble sugar in 400 mM,

Fig. 2 Energy pipeline leaf model of phenomenological fluxes (per cross section-CS_m) of *Bruguiera cylindrica* leaves exposed to various concentrations of NaCl (0 mM, 400 mM, 500 mM and 600 mM). The values of each parameter can be seen in relative changes in width of each arrow. Active RCs are shown as open circles and inactive RCs are closed circles
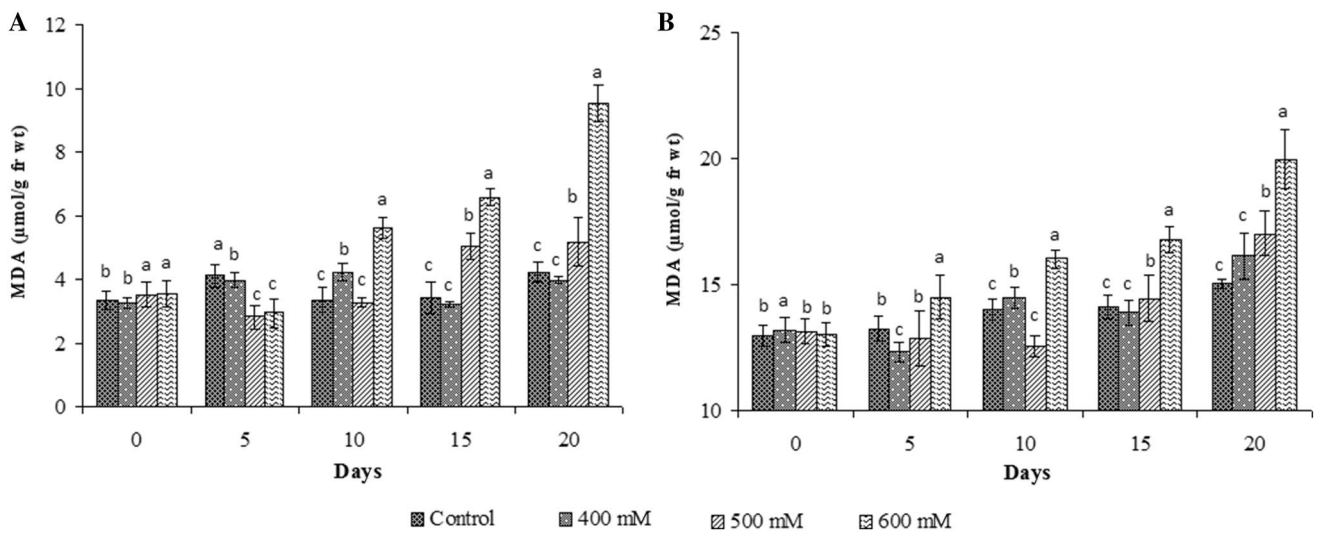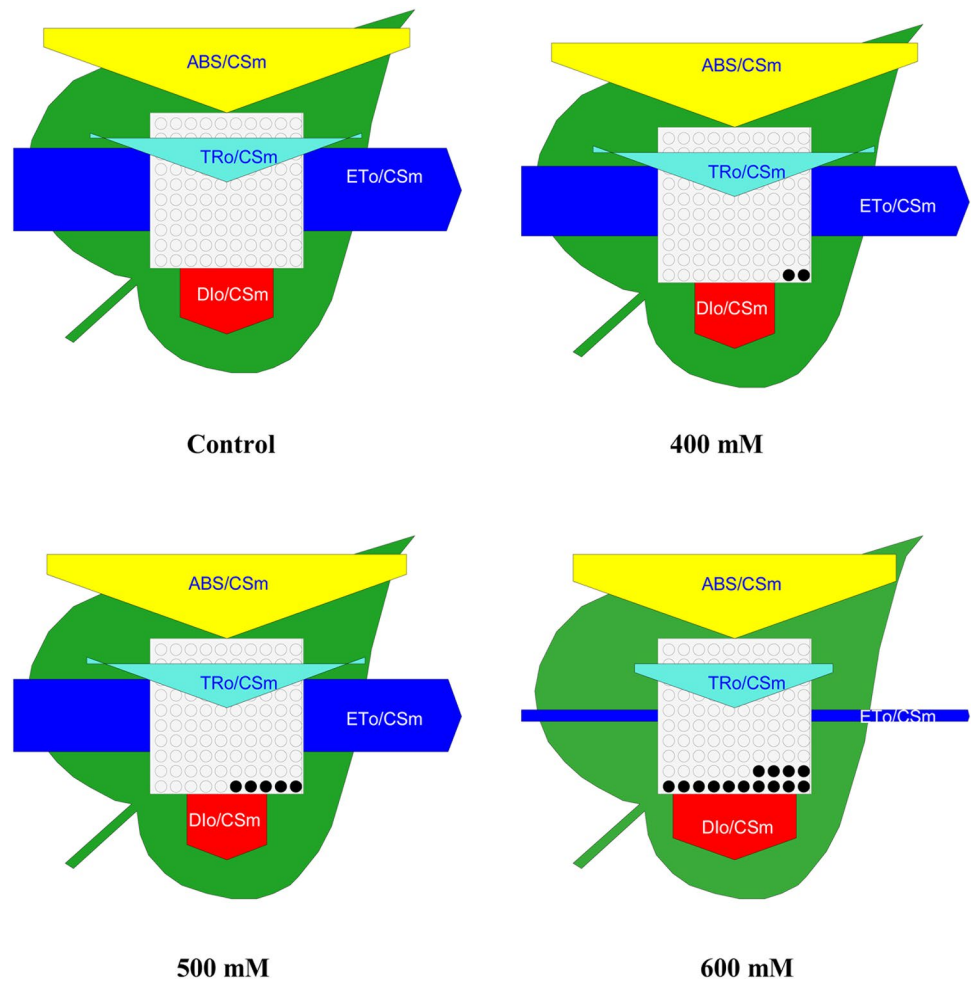

Control

400 mM

500 mM

600 mM

Fig. 3 MDA content (μmol/g FW), in the leaves (**a**) and roots (**b**) in *B. cylindrica* L. subjected to various concentration of NaCl. Values are the mean ± SE of three independent experiments ($n=6$). Different letters on top of the bar indicate significant difference at $p \le 0.05$ according to one-way ANOVA

500 mM and 600 mM NaCl concentration in both leaf and root tissues, viz. 9%, 30% and 52% in leaf and 6%, 47% and 54% in root respectively over the control plants (Fig. 4a, b). Proline content in leaf and root increased upon increasing the NaCl concentration on 15 days and 20 days of treatment. Proline content was found to increase to the extent of 39%, 58% and 124% with 400 mM, 500 mM and 600 mM NaCl treatment in leaf tissue and in root the increase was about 12%, 39% and 93% on 20 days over the control plants (Fig. 4c, d).

The total phenolics content was found to be increasing upon increasing NaCl concentrations in both leaf and root tissues of *B. cylindrica*. There was a gradual increase of 1%, 7% and 42% in leaf and 3%, 10% and 22% in root tissue when treated with 400 mM, 500 mM and 600 mM NaCl concentrations on 20 days of treatment over the control plants (Fig. 5a, b). The highest amino acids content was recorded on 20 days in both leaf and root tissues, subjected to treatment with 600 mM NaCl concentration and the increase was 84% and 65% higher in leaf and root respectively on

20 days over the control plants. In the case of 400 mM and 500 mM NaCl, the increase in amino acid content on 20 days was 12%, 47% and 7%, 17% in leaf and root respectively (Fig. 5c, d).

Enzymatic antioxidants

Among the four enzymes, APX activity was enhanced significantly by four and sixfold in leaf and three and fourfold in root in 500 mM and 600 mM NaCl treated plants on 20 days (Fig. 6a, b). On 20 days, GPX activity increased by two and threefold in leaf and double and twofold in root on treatment with 500 mM and 600 mM NaCl, respectively (Fig. 6c, d). SOD activity in *B. cylindrica* increased by two and fourfold in leaf and double and twofold in roots, on 20 days of exposure to 500 mM and 600 mM NaCl over the control plants (Fig. 6e, f). At higher NaCl concentrations (500 mM and 600 mM), an increase of 50% and onefold in catalase activity was found in leaf and root, respectively and the maximum increase in activity was on 20 days of treatment (Fig. 6g, h).
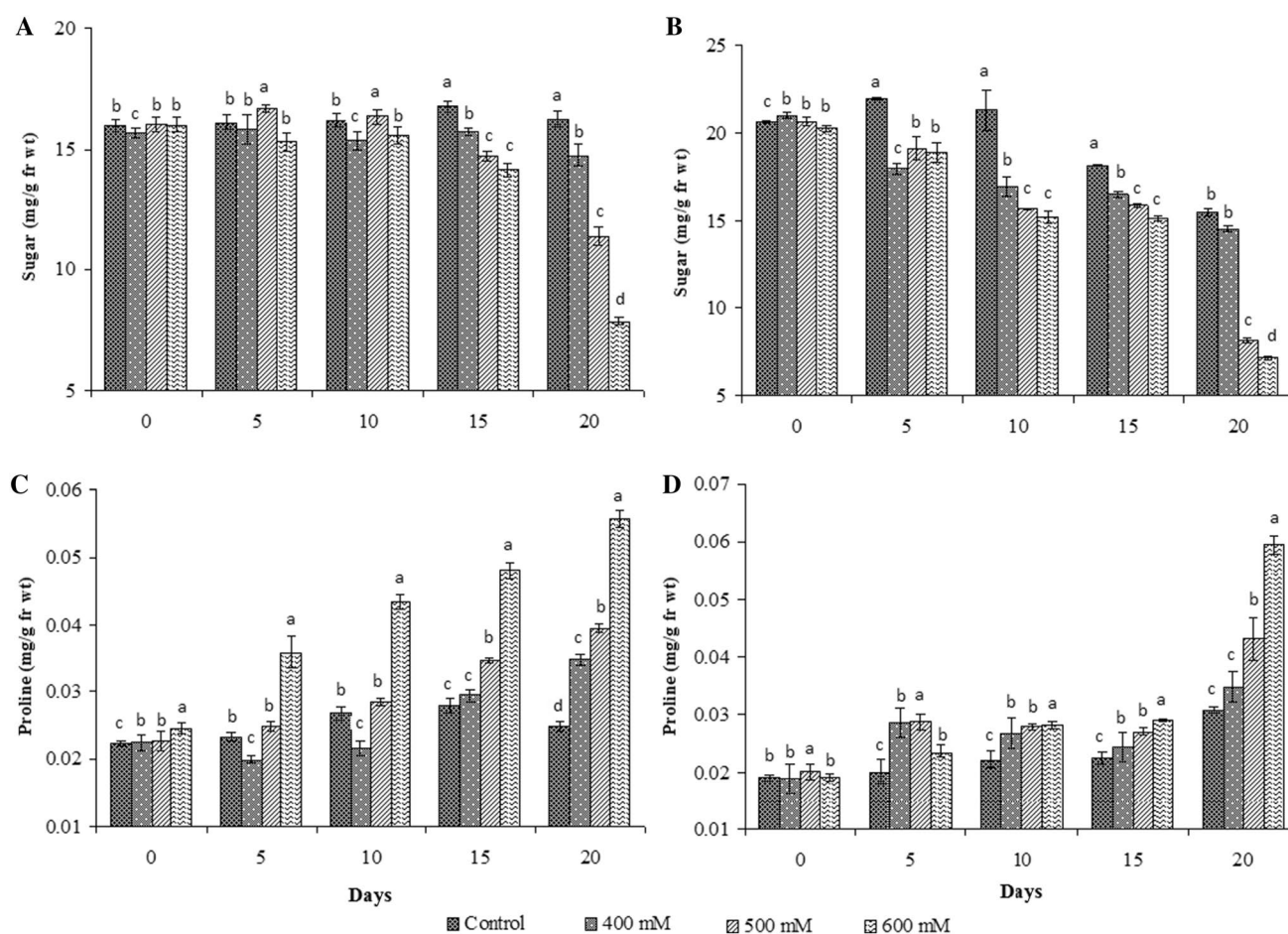

Fig. 4 Total sugar content (leaf **a** and root **b**) and proline content (leaf **c** and root **d**) (mg/g FW), in *B. cylindrica* L. subjected to various concentrations of NaCl. Values are the mean ± SE of three independent experiments (*n* = 6). Different letters on top of the bar indicate significant difference at *p* ≤ 0.05 according to one-way ANOVA
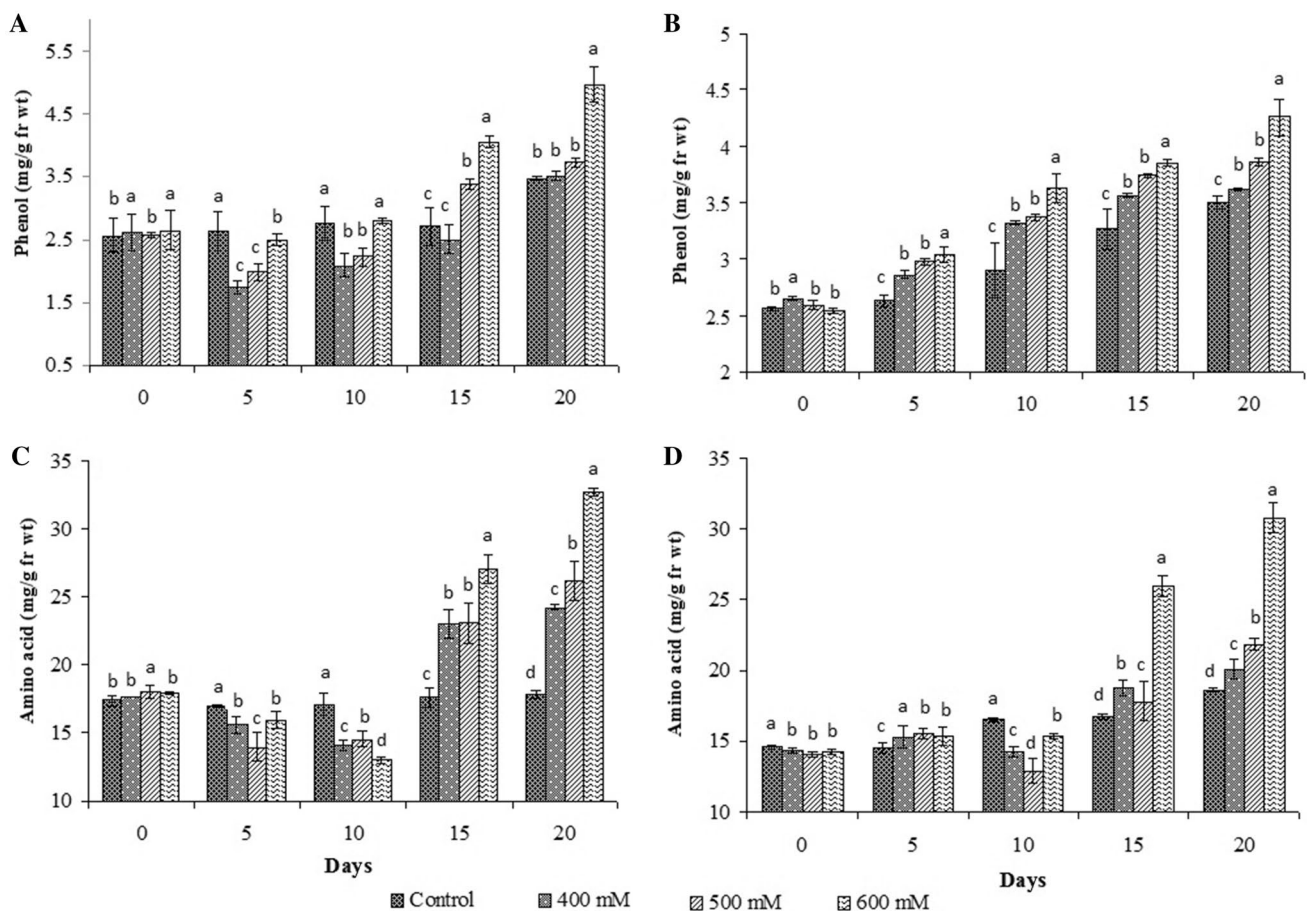
Fig. 5 Phenol content (leaf **a** and root **b**) and amino acid content (leaf **c** and root **d**) (mg/g FW), in *B. cylindrica* L. subjected to various concentrations of NaCl. Values are the mean ± SE of three independent experiments ($n = 6$). Different letters on top of the bar indicate significant difference at $p \leq 0.05$ according to one-way ANOVA

Non-enzymatic antioxidants

Ascorbate and glutathione content showed a decreasing trend with increasing NaCl treatments both in leaf and root as compared to control plants. On 20 days of NaCl treatment (600 mM), the ascorbate content decreased significantly as compared to the control plants. The decrease was to the extent of 19%, 67% and 68% in leaf (Fig. 7a) and 28%, 53% and 61% in root of plants subjected to 400 mM, 500 mM and 600 mM NaCl, respectively as compared to the control plants (Fig. 7b). Treatment with 400 mM, 500 mM and 600 mM NaCl showed a decrease in glutathione content to the extent of 1%, 42% and 63% in leaf and 2%, 53% and 65% in root respectively on 20 days of treatment (Fig. 7c, d). In contrast with these results the carotenoid content was showing an increasing pattern upon increasing concentration of NaCl, i.e., an increase of 6%, 8% and 21% in 400 mM, 500 mM and 600 mM NaCl treated plants on 20 days of treatment as compared to control plants (Table 3).

Na⁺ accumulation

After 20 days of NaCl treatment, a significant increase of Na⁺ accumulation was found when plants treated with higher concentrations. The Na⁺ content of plants when treated without NaCl shows 84.5057 mM/plant dry weight and 299.70 mM, 353.33 mM and 543.38 mM/plant dry weight accumulation were observed when plants treated with 400 mM, 500 mM and 600 mM NaCl concentrations (Table 4).

Discussion

Bruguiera cylindrica L. is a facultative mangrove species belonging to rhizophoraceae, therefore it can grow normally in non saline condition (Atreya et al. 2009). As the propagules were raised in soil/mud from the mangrove habitat, the propagules grew well and got acclimatized in 90 days period. In this period watering was done with fresh water,
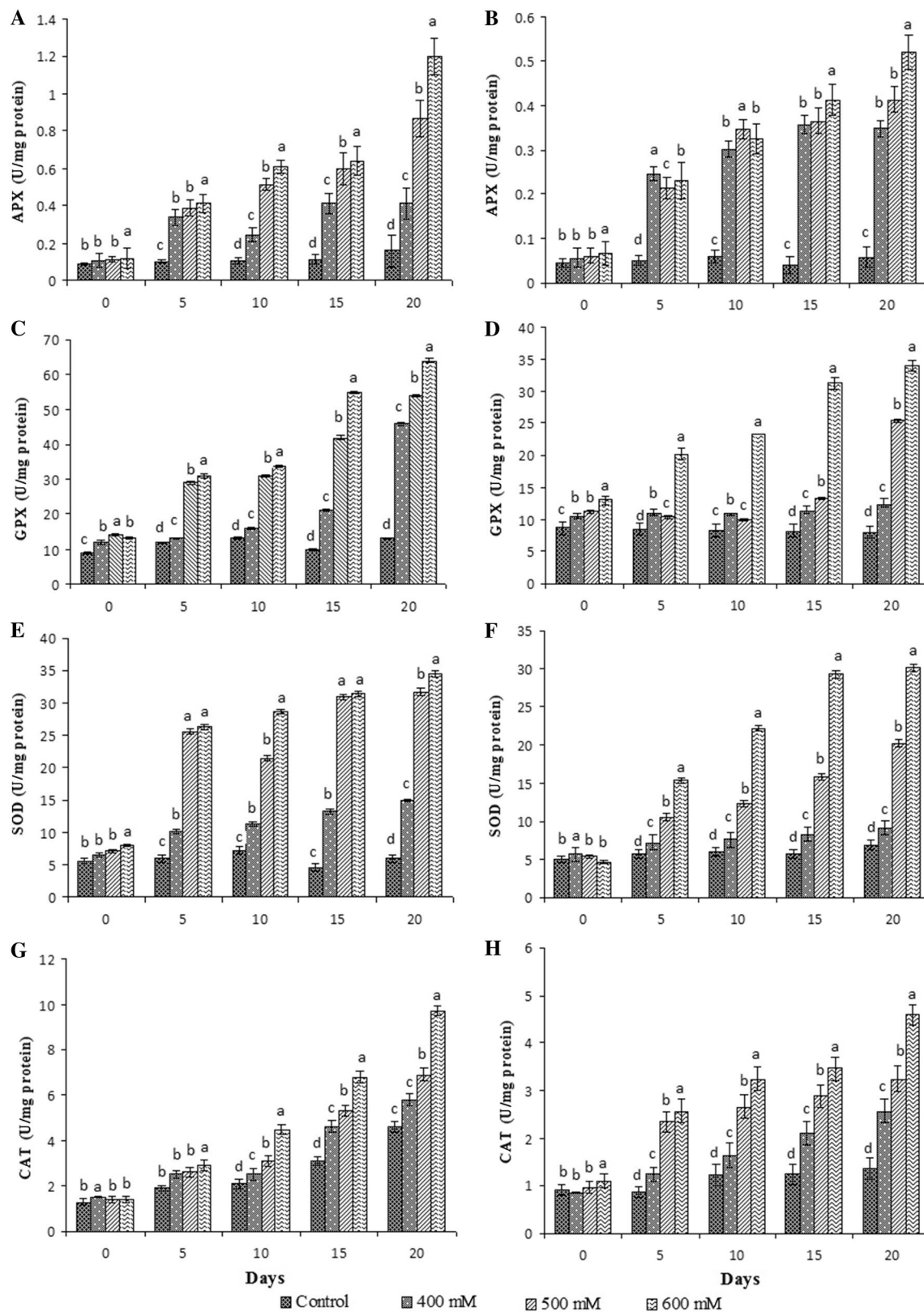
Fig. 6 Ascorbate peroxidase (APX) (leaf **a** and root **b**), guaiacol peroxidase (GPX) (leaf **c** and root **d**) superoxide dismutase (SOD) (leaf **e** and root **f**), catalase (CAT) (leaf **g** and root **h**) in *B. cylindrica* L. subjected to various concentrations of NaCl. Values are the mean ± SE of three independent experiments ($n = 6$). Different letters on top of the bar indicate significant difference at $p \leq 0.05$ according to one-way ANOVA
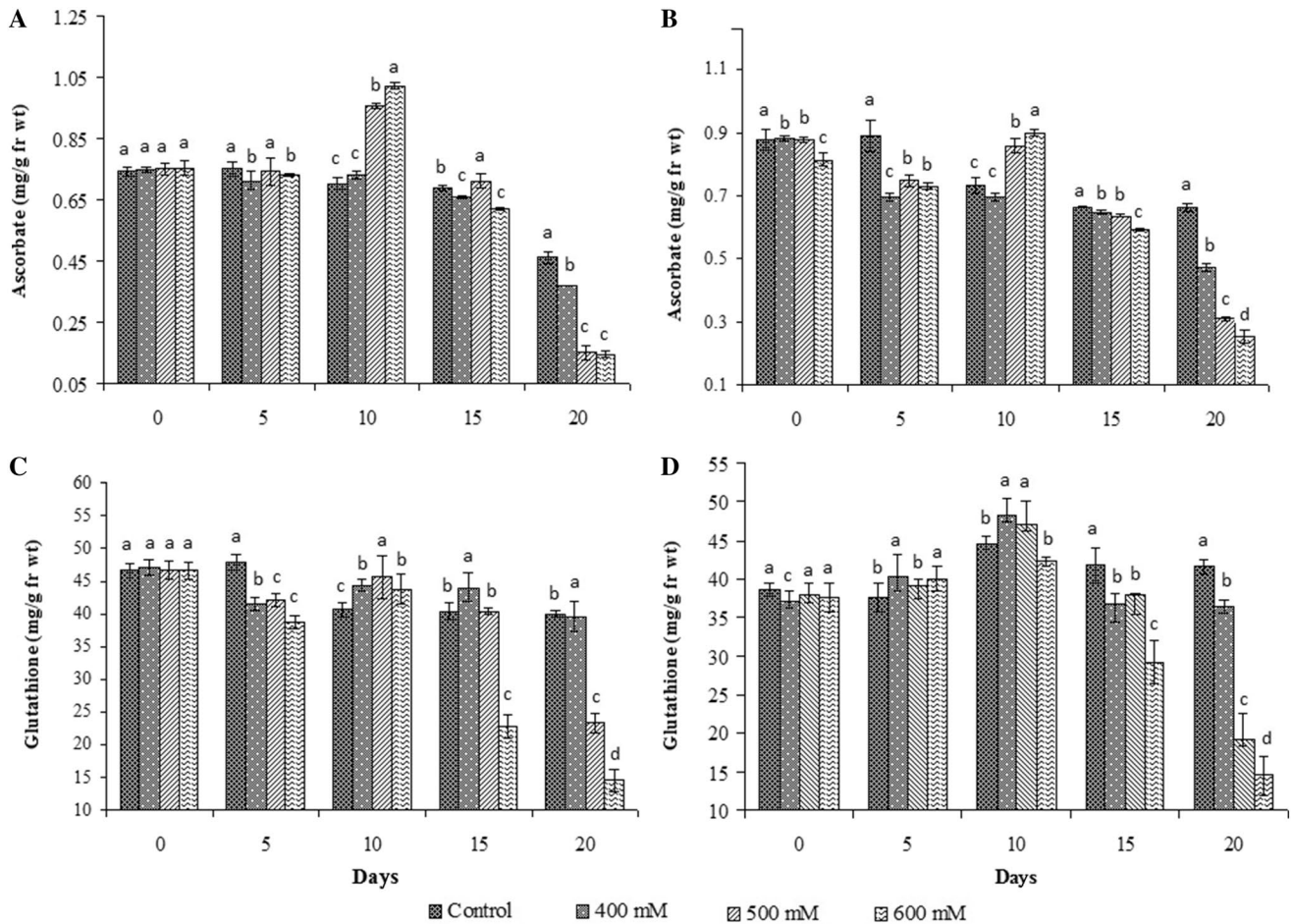
Fig. 7 Ascorbate content (leaf **a** and root **b**) and glutathione content (leaf **c** and root **d**) (mg/g FW), in *B. cylindrica* L. subjected to various concentrations of NaCl. Values are the mean ± SE of three independ-ent experiments ($n=6$). Different letters on top of the bar indicate significant difference at $p \leq 0.05$ according to one-way ANOVA

Table 3 Carotenoid content (mg/g FW), in the leaves of *B. cylindrica* L. subjected to various concentrations of NaCl (0 mM, 400 mM, 500 mM and 600 mM)

Days	Control	400 mM	500 mM	600 mM
0	2.05 ± 0.106^a	2.04 ± 0.112^a	2.03 ± 0.113^a	2.04 ± 0.109^a
5	2.01 ± 0.101^b	2.07 ± 0.114^b	2.04 ± 0.110^b	2.17 ± 0.125^a
10	2.23 ± 0.123^b	2.28 ± 0.123^b	2.46 ± 0.102^a	2.36 ± 0.145^{ab}
15	2.31 ± 0.156^a	2.45 ± 0.156^a	1.82 ± 0.098^c	2.07 ± 0.158^b
20	2.18 ± 0.110^c	2.19 ± 0.164^c	2.35 ± 0.102^b	2.64 ± 0.167^a

Values are the mean ± SE of three independent experiments ($n=9$)

Different letters inside the table indicate the significant difference at $p \leq 0.05$ according to one way ANOVA

Table 4 Na+content of *B. cylindrica* L. subjected to various concentrations of NaCl (0 mM, 100 mM, 200 mM, 300 mM, 400 mM, 500 mM and 600 mM) on 20 days of treatment

NaCl (mM)	Na+ (mM/plant)
Control	84.50 ± 16.54
100	194.08 ± 22.41
200	230.13 ± 24.32
300	255.20 ± 20.98
400	299.70 ± 24.38
500	353.33 ± 31.21
600	543.38 ± 46.25

Values are the mean ± SE of three independent experiments ($n=9$)

which almost washed off the salinity present in the soil/mud. *Bruguiera cylindrica* plantlets exhibited normal growth with formation of 6–8 leaves in 90 days period (data not shown). As being a facultative mangrove, it could very well adjust with the non saline condition.

Decreased dry weight and water content of leaf and root tissues of *B. cylindrica* was observed and the maximum decrease was observed in root tissue than in leaf during high NaCl treatment (600 mM), may be due to the carbon allocation from the shoots to the roots and the consequent

increase of the dry matter at the expense of the water content, and this may help the plant to tolerate the high salinity stress (Clough and Sim 1989; Barr 2013). At higher salinity *Aeluropus lagopoides* showed distinguishing variation in fresh and dry plant biomass indicating its higher tolerance. Thus mangrove species with maximum dry weight percentage at higher salinity are more tolerant (Rao et al. 2005). Generally high salinity brings about a decrease in chlorophyll content due to changes in the lipid protein ratio of pigment–protein complexes or increased chlorophyllase activity (Iyengar and Reddy 1996). Even some of the halophytes such as *Aegiceras corniculatum* could not tolerate 250 mM NaCl over a 30 days treatment period as indicated by significantly decreased chlorophyll content (Parida et al. 2004a). In *Ceriops roxburghiana* the optimal salt concentration for the overall better performance of the seedlings was 300 mM NaCl, the total chlorophyll content decreased significantly even when treated with 300 mM NaCl concentration (Natarajan and Chellappan 2004). However, there are reports of certain halophytes showing enhanced tolerance towards salinity such as in the case of *Suaeda salsa*, under high salinity and high light conditions no significant changes in pigments were observed and *S. salsa* showed high resistance to salinity stress as well as to photoinhibition, when treated with 400 mM NaCl and exposed to full sunlight (Lu et al. 2002). The loss of chlorophyll is often used as an indicator of the cellular component of salt stress (Singh and Dubey 1995), and at the same time the loss of pigments could also be an adaptive mechanism to prevent the damage to photosynthesis by reducing the possibility of photo inhibition of photosystem II (Maslova and Popova 1993). In *B. cylindrica* even after the treatment of the plantlets with 500 mM NaCl only 16% reduction in chlorophyll content was recorded and even under treatment with 600 mM NaCl the reduction was only 24%. The reduction of chlorophyll content in *B. cylindrica* when treated with 100–400 mM NaCl was very meagre, less than 10%. This clearly demonstrates the very less impact of these NaCl concentrations (100–400 mM) on the photosynthetic pigment of this mangrove plant. There are fairly no earlier reports on such a high tolerance of halophytes towards NaCl as indicated from the reduction of chlorophyll content.

Chlorophyll *a* fluorescence is very useful to study various fundamental aspects of photosynthesis. When plants previously adapted to darkness are illuminated, the resulting chlorophyll *a* fluorescence kinetics shows the efficiency of photochemical reactions (Kalaji et al. 2011). The OJIP transient correspond to the successive reduction of electron transport pool of PS-II (Govindjee 1995).Various Chl *a* fluorescence parameters like F_v/F_m, F_m, area over curve, V_j and PI are indicators found to be more consistent for exploring the effect of changes in PS-II activity (Mehta et al. 2010). Area over the fluorescence induction curve between F_o and F_m

is proportional to the pool size of the electron acceptor Q_A on the reducing side of PS-II. The area will be dramatically reduced when the electron transfer from reaction center to quinone pool is blocked (Strasser et al. 2000). The decrease in area over the fluorescence curve was much less in *B. cylindrica* subjected to 500 mM indicating its tolerance potential. Salt stress is known to inhibit the electron transfer rates at the donor side of PS-II to greater extend (Mehta et al. 2010). Performance index (PI) gives a total picture regarding the functioning of reaction centers (RC), the energy flux reaching the PS-II reaction centers and utilized for electron transport (Mehta et al. 2010). PI is an indicator of sample vitality and although this parameter decreases in *B. cylindrica* with increase in NaCl concentration to which it is exposed to, the loss was much less in 500 mM treatment. Variable fluorescence (V_j) gives an indication of the deleterious effects of salt stress on electron transport process especially at the acceptor side of PS-II. V_j represents ($F_j - F_o/F_m - F_o$, where F_j is the fluorescence at *j* step i.e. at 2 ms) relative variable fluorescence at 2 ms (Fedina et al. 2003). Increased value of V_j under high NaCl stress negatively influence the electron transport at the acceptor side of PS-II (Faseela and Puthur 2017) and also the electron transport from the RCs to the plastoquinone pool is seen to be disrupted (Strasser et al. 2000). There was no significant increase of V_j observed in *B. cylindrica* even at high NaCl concentration (600 mM NaCl).

According to Bjorkman and Demmings (1987), healthy plants typically may attain the maximum F_v/F_m value between 0.78 and 0.84, below which it is considered to be stressed, and our results shows that *B. cylindrica* subjected to 600 mM NaCl had F_v/F_m value of 0.751 at 600 mM and 0.820 in 500 mM treated plants on 20 days of treatment. It indicates that even under extreme salinity, *B. cylindrica* can tolerate high NaCl with a minor decrease in F_v/F_m value. Potential quantum yield (F_v/F_m) is a widely used metric for photosystem II performance and has been used as an indicator of plant growth in some species during adverse environmental conditions (Cavender-Bares and Bazzaz 2004; Sui and Han 2014). In *S. salsa* there is no changes in the maximal efficiency of PS-II photochemistry (F_v/F_m) was observed in both control and salt-stressed plants suggesting that salt stress did not impart any negative effects on PS-II primary photochemistry (Lu et al. 2002). The initial (F_o) and the maximum (F_m) values of the Chl *a* fluorescence are widely used to determine the maximum quantum yield of the primary photochemistry of PS-II ($\Phi_{po} = Fv/Fm$) (Waldhoff et al. 2002). In our study the F_m value decreased in *B. cylindrica* on 20 days at 600 mM NaCl treatment, which indicate the accumulation of inactive PS-II reaction centers (Kalaji et al. 2011). The inhibition of electron transport at the donor side of the PS-II and decrease in the pool size of Q_A is shown up by decrease in the value of F_m (Neubauer and Schreiber 1987). The initial fluorescence (F_o) increased in

B. cylindrica on 20 days at 600 mM NaCl concentration, and no significant increase was found in 400 mM and 500 mM NaCl concentrations. The increase in F_o can attributed to the inactivation of PS-II reaction centers (Melis 1985; Krause and Weis 1991) and also associated with the disconnection of PS-II reaction center from the antenna complexes (Srivastava et al. 1997). It was further proposed by Yamane et al. (1997) that the F_o increase caused by high saline exposure can be due to electrons fed from reduced stromal components into the PQ pool.

The Chl *a* fluorescence derived parameters are visualized by means of dynamic energy pipeline leaf model, which deals with the phenomenological energy fluxes (per cross-section) (Congming and Vonshak 1999). Dissipation (DI_o/CS_m) refers to the loss of absorbed energy through heat, fluorescence and energy transfer to other systems (Strasser et al. 2004). Electron transport in a PS-II cross section (ET_o/CS_m) refers the reoxidation of reduced Q_A via electron transport over a cross section of active and inactive RCs (Force et al. 2003). The number of active RCs in PS-II cross section is indicated as open circles and inactive RCs as closed circles. A significant increase of the former parameter (DI_o/CS_m) and the decrease in the latter parameter (ET_o/CS_m) was observed only in *B. cylindrica* subjected to 600 mM NaCl concentration, but 400 mM and 500 mM NaCl treatment, did not impart any major negative effect on photochemistry of this plant.

The increased level of MDA is routinely used as an index of lipid peroxidation under stress conditions and it indicates the membrane damage due to lipid peroxidation by the effect of ROS generated under different environmental stress conditions. Even some mangroves also could not tolerate the NaCl concentration above 400 mM as indicated by the increased rate of MDA content. MDA content was increased in the root of *Kandelia candel* upon 300 mM NaCl treatment, along with the subsequent increase in the activity of various antioxidant enzymes. In 450 mM of NaCl solution, the root of *K. candel* was damaged and coinciding with it, the MDA content had reached the maximum (Wang et al. 2014). Increased rate of lipid peroxidation was observed in *B. gymnorrhiza* exposed to 400 mM NaCl and above which the cellular membrane gets completely damaged as a result of high salinity (Zhang et al. 2004). The increased MDA content recorded in *A. ilicifolius* treated with 400 mM NaCl indicates that the rate of membrane lipid peroxidation aggravated upon increase in NaCl concentration (Shackira and Puthur 2016). But in *B. cylindrica* even 500 mM NaCl treated plantlets showed only 25% increase in MDA content, which is yet another proof for the greater tolerance potential of this species.

The accumulation of compatible solutes is a basic strategy of the plants for its protection and survival during abiotic stress condition (Sruthi et al. 2017). On 5 days and 10 days of treatment, the total soluble sugar increased in leaf tissue of plants subjected to 500 mM NaCl concentration and the significant decrease of sugar content was found only in plant subjected to 600 mM NaCl on 20 days, showing that the process of sugar synthesis was negatively affected in the latter concentration. This was a clear indication that *B. cylindrica* can tolerate high NaCl concentration in the initial period of stress and also sugar accumulation contributed significantly towards maintaining the osmoticum of the cell sap. But in some halophytes like *A. corniculatum*, total sugar content decreased at 250 mM of NaCl and the decrease was 1.9-fold during 30 days of treatment. In *Excoecaria agallocha* total sugar content decreased on exposure to 300 mM of NaCl (Sozharajan and Natarajan 2015). A significant decrease in the total sugar content at 400 mM NaCl, the maximum NaCl tolerance level was reported in *B. gymnorrhiza* by Takemura at al. (2000). Compatible solutes are synthesized in living organisms in response to osmotic stress. Out of various compatible solutes, sugars can contribute significantly towards osmotic stress tolerance by ensuring membrane integrity (Manchanda and Garg 2008), acting as osmoprotectants, maintaining cell turgidity and protecting membranes against increased salinity (Cooper and Farrant 2002). Accumulation of sugars would contribute greatly towards osmotic adjustment than proline. Moreover, sugars act as source of energy required for adaptive or defensive responses to stresses (Gebre et al. 1997).

Generally proline act as an osmoprotectant, also protecting plants from stress through different courses, such as detoxification of reactive oxygen species, protection of membrane integrity, protect cellular components from dehydration injury and stabilization of enzymes/proteins (Hayat et al. 2012). A prominent increase of proline content was observed in *B. cylindrica* subjected to high NaCl concentrations (500 mM and 600 mM) indicates that this metabolites has a greater role in imparting stress tolerance to *B. cylindrica* probably through osmotic adjustment. Enhanced accumulation of proline in response to increasing salt concentrations (NaCl 300–400 mM) has been reported in halophytes like *Thellungiella halophila, Mesembryanthemum crystallinum*, and *B. parviflora* making it an important adaptive mechanism towards high salinity (Sanada et al. 1995; Parida et al. 2002; Kant et al. 2006). Increased levels of proline accumulation in salt-stressed calli of *Suaeda nudiflora* suggested that besides its role as an osmolyte, proline also protected the callus cells from oxidative damage caused by free radicals during salt stress (Cherian and Reddy 2003). Our observation is consistent with the previous reports, that proline accumulation occurs in plant with increasing conductivity and salinity of the growth medium, illustrating a linear relationship (Demiral and Turkan 2006).

Muthukumarasamy et al. (2000) reported that an increase in polyphenols in plant tissue can take care of the ionic

stress caused by NaCl. The enhancement in phenol content is supposed to be an adaptive mechanism, towards various kinds of stresses and the exact mechanism of its interference in stress alleviation is not fully elucidated (Agastian et al. 2000). The lesser extend of increase in phenolics content in *B. cylindrica*, indicates that the plant is not exposed to extreme stress condition. Enhanced level of phenol was observed in *B. parviflora* when treated with 400 mM and was associated with reducing the oxidative stress induced damage to cells (Parida et al. 2002). The amino acid content in *B. cylindrica* increased significantly only in 600 mM NaCl treatment but in 500 mM the accumulation was very less as compared to other halophytes. Vicente et al. (2004), reported that the leaves of a halophyte *Plantago crassifolia* accumulated greater content of free amino acids during an extended period of 300 mM NaCl exposure. The increased content of amino acids and polyphenols recorded in *B. cylindrica* upon NaCl treatment indicates that this species possessed an effective NaCl stress tolerance mechanism. Although this species proves tolerance to high NaCl concentration, other mangrove species, like *A. corniculatum* are not adaptable to high NaCl levels (500 mM) in hydroponic culture (Parida et al. 2002).

Plants have developed antioxidant mechanisms to eliminate or reduce ROS, which are efficient at different levels of stress-induced deterioration. Generally, environmental stresses increase the production of superoxide, depending on the species, the stress period, intensity and the age of plants (Parida et al. 2004a). NaCl tolerance is strongly related to the efficiency of antioxidant enzymes, SOD, CAT and GPX in scavenging reactive oxygen species (Thatoi et al. 2014). Plants are equipped with non enzymatic antioxidants (ascorbate, glutathione and carotenoids) which detoxify active oxygen species in multiple redox reactions, thereby contributing towards protection against oxidative stress and maintaining redox homeostasis (Liu et al. 2017). In *A. ilicifolius*, increased activity of SOD, CAT, GPX and APX was shown and the increased activity was more significant in 400 mM treated plants. A decrease in activity of antioxidant enzymes was shown in plants treated with 600 mM, indicating that *A. ilicifolius* exhibits a tolerance potential towards NaCl upto a level of 400 mM, beyond which it turns to be intolerable (Shackira and Puthur 2016). But in *B. cylindrica* there was a significant increase in the production of antioxidant enzyme at 400 mM, 500 mM and 600 mM NaCl treated plants on 20 days indicates the tolerance capacity of this species to extreme NaCl concentration. And the significant accumulation of APX enzyme in *B. cylindrica* leaf tissue induced by NaCl stress suggests the possibility of using this enzyme as a potential biomarker to identify the salt tolerance potential of this as well as the related species. The increased activity of SOD indicates that it catalyzes the disproportion reaction of two superoxide radicals generated from the NaCl treatment, to generate O_2 and H_2O_2. As the rate of O_2 and H_2O_2 radicals increases by SOD pathway, the plant switch on the GPX and CAT activity in order to remove these deleterious free radicals from the cells. Coinciding with our results, a sudden increase in SOD activity has been recorded in two mangroves, *B. gymnorrhiza* and *B. parviflora* during NaCl stress (Takemura et al. 2000; Parida et al. 2004a). Similarly, catalase activity was increased under NaCl stress in *B. gymnorrhiza* (Takemura et al. 2000). Cherian et al. (1999) have reported that GPX activity was increased in root and shoot tissues in *Avicennia marina* and Parida et al. (2004a) have reported an enhancement of APX content in *B. parviflora* subjected to NaCl stress. Increase in activity of APX could be either by the activation of preexisting APX or by the synthesis of fresh APX by the salt treatment (Lee et al. 2001).

Non enzymatic antioxidants like ascorbate, glutathione content in *B. cylindrica* decreased significantly only in 600 mM NaCl stress but the decrease of the same was very less in 500 mM indicating that the synthesis process of these antioxidant metabolites was not negatively affected at the latter concentrations of NaCl. Ascorbate is a ubiquitous soluble antioxidant in photosynthetic organisms and it is important reducing substrate for H_2O_2 detoxification (Nakano and Asada 1981). In *A. ilicifolius* about threefold decrease of ascorbate content was observed in leaf tissue, when subjected to 400 mM NaCl and it is likely due to its involvement in reducing H_2O_2 to H_2O, catalyzed by ascorbate peroxidase (APX) (Shackira and Puthur 2016). Prolonged treatment with high NaCl level caused a lowering of ascorbate level in *B. parviflora* (Parida et al. 2004a). Less decrease in glutathione content was observed with increasing NaCl concentration (600 mM) in *B. cylindrica*. But that was not a case with a true mangrove, *B. parviflora*, subjected to increased levels of NaCl (400 mM) under hydroponic culture, resulted in decrease of glutathione (GSH + GSSG) content (Parida et al. 2004a). The decline in glutathione contents may be due reduced rates of GSH synthesis, increased rates of degradation and GSH transport to other cell compartments or plant organs (Herschbach et al. 1998; Tausz et al. 2004).

The increase of carotenoid content in *B. cylindrica* with increase in NaCl concentration is a clear indication that carotenoids play a significant role in antioxidation and stabilization of thylakoid membranes. In *B. gymnorrhiza*, significant interaction was seen between salinity levels and carotenoid content, i.e., a significant increase in carotenoid content with increasing NaCl concentration (400 mM) (Ye et al. 2002). Carotenoids are lipid soluble pigments functioning as antioxidants and it has multiple functions in plant metabolism including oxidative stress tolerance. Mild and moderate saline stress produce an increase in carotenoids levels as a tolerance mechanisms. Carotenoids are pigments with several functions in plants, besides their direct role in photosynthesis, it has an active role in oxidative stress tolerance (Gill and Tuteja 2010). Carotenoids play important

role in PS-I assembly and the stability of light harvesting complex proteins as well as thylakoid membrane stabilization (Niyogiet al. 2001).

Many of the reports with regard to maximum NaCl tolerance in mangroves cultured hydroponically, have shown that the tolerance limit have not exceeded 400 mM of NaCl. In the case of *A. corniculatum*, it could tolerate maximum NaCl concentration of 250 mM for 30 days and at 300 mM, the leaves began to fall within a short period of culturing. The salt secreting mangrove *A. corniculatum* can be sustained and propagated under low salinity conditions (Parida et al. 2004a). Under hydroponic-culture conditions, seedlings of *B. parviflora* could tolerate NaCl up to 400 mM and when the plants were treated with 500 mM NaCl, indication of metabolic inhibition was shown up (Parida et al. 2002). Physiological and biochemical responses induced by salt stress (400 mM NaCl) were studied in laboratory-grown young plants of the mangrove, *B. gymnorrhiza*, it was observed that the plants could not tolerate 400 mM NaCl as indicated by its metabolic inhibition (Takemura et al. 2000). *Bruguiera cylindrica*, which has no specific salt excretion mechanisms, belongs to the classes of facultative mangrove is found to tolerate high NaCl concentration to the extent of 500 mM NaCl without any major inhibition on photosynthesis and metabolic accumulation.

Significant increase of Na^+ accumulation was found in *B. cylindrica* when treated with higher NaCl concentration (600 mM) on 20 days of treatment, it indicates that the Na^+ concentration in different mangrove species vary remarkably and it indicates their salt tolerance potential (Jayatissa et al. 2005; Kodikara 2009). The salt tolerance potential of a plant can be due to the plant's capacity to tolerate the effects of excess salt in the growing medium (Taiz and Zeiger 1991). The ionic stress impacts become predominant in *B. cylindrica* only above 500 mM NaCl and at this stage the ability to control Na^+ transport into the cell is lost and accordingly the ionic effect dominates the osmotic effect. Salt accumulation is carried out by two phases in leaves of the mangroves *B. cylindrica, Avicennia rumphiana* and *A. marina* (Cram et al. 2002). The first phase is rapid osmotic phase, in which leaf salt content increases as it grows from bud to maturity and the second phase is a slower ionic phase which leads to changes in ion concentration and/or in leaf thickening, which accelerates senescence of mature leaves. The osmotic stress has a greater effect on growth rates than the ionic stress (Munns and Tester 2008).

Conclusion

Salinity is the most important abiotic stress, negatively affecting plant growth and threatening global crop production. Mangroves tolerate high salinity by excluding potentially harmful salts as in *B. cylindrica*. Even though 500 mM of NaCl was found to impart inhibition on the accumulation of many of the metabolic process of the mangroves, it was not found to impart any significant negative effects on photochemistry, increasing salinity caused no additional reduction of F_v/F_m indicating that photochemical process in *B. cylindrica* has special protection even under high NaCl concentration (500 mM). The steadiness of PS-II activity even at high salinity stress suggests that it is an important strategy for *B. cylindrica* to grow in very high saline soil. The steadiness of PS-II activity as compared to PS-I in this mangrove species on exposure to higher concentrations of NaCl have to be explored further. *Bruguiera cylindrica* exhibits high antioxidant enzyme activities even at 500 mM of NaCl, some antioxidative features exhibited better functionality even at 600 mM, preventing the toxic accumulation of reactive oxygen species. Our results showed that *B. cylindrica* on exposure to high salt concentration enhanced the activities of various antioxidant enzymes and clearly showed a differential extent of alterations of APX, GPX, SOD and CAT activities on NaCl exposure. Further, the results indicate that the antioxidative enzymes such as APX could serve as salt specific marker(s) for studying salt tolerance and variance in salinity tolerance among the mangroves. As *B. cylindrica* is well adapted for higher NaCl concentrations, it seems to be a suitable candidate for the afforestation and ecological restoration of coastal areas and mangrove habitats affected with high sediment salinity. Moreover, a clear understanding regarding the modulation of various physiochemical changes occurring in *B. cylindrica* at high levels of NaCl will help us to know the physiochemical basis of salt tolerance in mangroves. Moreover, identification of salt tolerant genes from this mangrove will lead to effective means of molecular breeding of salt tolerant crops for enhanced salt tolerance.

Author contribution statement SP was responsible for the design of experiments, data analysis, and drafting the manuscript. JTP took care of the study origin and design, and edited the manuscript.

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