PHOTOSYNTHETIC RESPONSE IN BUTTONWOOD (*CONOCARPUS ERECTUS* L.) TO SALT STRESS

MEHWISH NASEER[1], MANSOOR HAMEED[2*], ANEELA ZAHOOR[2],
FAROOQ AHMAD[2], SANA FATIMA[2] AND MUHAMMAD SAJID AQEEL AHMAD[2]
KHAWAJA SHAFIQUE AHMAD[3] AND MISHAL IFTIKHAR[4]

[1]*Department of Botany, Government College Women University, Faisalabad, Pakistan*
[2]*Department of Botany, University of Agriculture, Faisalabad, Pakistan*
[3]*Department of Botany, University of Poonch, Rawalakot, Pakistan*
[4]*Department of Botany, University of Sargodha, Sargodha, Pakistan*
Corresponding author's e-mail address: hameedmansoor@yahoo.com

Abstract

Structural and functional aspects of photosynthesis in *Conocarpus erectus* L., a species native to salt marshes of North America and now cultivated throughout the world for its tolerance against a variety of environmental stresses were investigated under high salinity. This species showed better growth at salt level up to 300 mM, whereas extreme salt stress (400 mM NaCl) resulted in a decrease in growth and other physio-anatomical parameters relating photosynthetic activity. Anatomical characters such as leaf and epidermal thickness, proportion of mesophyll tissue, large cortical cells, efficient conducting tissue (phloem and metaxylem area) and stomatal size as well as density contributed towards salinity tolerance and normal photosynthetic activity in *C. erectus*.

Key words: Buttonwood, Gas exchange parameters, Leaf anatomy, Salinity tolerance, Stomata.

Introduction

Conocarpus erectus L. (buttonwood) is an evergreen shrub or tree that belongs to family Combretaceae and native to Florida's mangrove forest ecosystem in North America. Its native habitat is moist and mostly grows on shorelines in tropical and subtropical areas around the world (Hegazy *et al*., 2008). It is present on the rock lands of the Florida Keys, edges of salt flats and hammocks, borders of brackish and marshes, and sometimes on the disturbed or destroyed parts of South Florida. It is a highly tolerant plant even it can withstand severe desert heat and summer temperature may be greater than 47°C. Since the nutrient status of *C. erectus* is very low so it grows very well in the soils that have very low fertility (El-Mahrouk *et al*., 2010). Mostly, it is planted in parks, streets and yards as ornamental and the potted plants are used to make bonsai (Abohassan *et al*., 2010). The wood is hard and persistent, and is used to make house, posts, railroad ties, charcoal and fuel. The bark consists of 16.5-18.5% tannin, and used in tanning different materials. Leaves are useful in preparing some folk medicines, which are tonic for diarrhea, catarrh, fever, conjunctivitis, anemia and diabetes (Al-Humaid & Moftah, 2007). It is an important source of food and protection for wildlife and prevents erosion (Al-Humaid, 2005).

C. erectus can endure different stresses like salinity, drought, heat, water logging, light frosts, diseases and pests (Hegazy *et al*., 2008). Accumulation of salt is less in *C. erectus* as compared with other plants so it is more efficient in preventing salts from going into the xylem sap (Lopez-Portillo *et al*., 2005). Low salinities increase growth and development in this species, which mainly depend on ion regulation, leaf succulence, and the presence of salt glands in leaf lamina (El-Mahrouk *et al*., 2010; Asif *et al*., 2014). It has also been reported that *C. erectus* can tolerate high salinities more easily than drought stress as its growth is less affected under high salinities than under mild drought (El-Juhany & Aref, 2005). A closely related *C. lancifolius* is also reported to be tolerant to mild salinity and severe droughts, which depends on proline accumulation to regulate phytosynthetic capacity and electron transport rate (Redha *et al*., 2012). This species shows high percentage of survival under high salinities at early growth stages (Shirazi *et al*., 2006).

Abiotic stresses like heat, drought, water logging and particularly salt stress affect not only the plant growth but also its metabolic processes (Ashraf & Foolad, 2007; Maheshwari *et al*., 2012). Different plants respond to salinity in different ways such as changes water balance and its uptake, transpiration, metabolic pathways, exchange of gases, leaves optical properties, ion uptake, respiration and photosynthesis, morpho-anatomical characteristics and balance of hormones (Khatoon *et al*., 2000; Ibrahim *et al*., 2007).

Plants growing in saline conditions enable them to survive by doing various physiological and anatomical adaptations. Among physiological adaptations ion homeostasis is an earlier response which involves uptake of specific ions (Flowers & Colmer, 2008) and maintenance of turgor by osmotic adjustments (Abou-Leila *et al*., 2012). Anatomical characteristics involved in salinity tolerance are related to changes in stomatal length and width (Cavusoglu *et al*., 2008), stomata number and cell number of epidermis (Cavusoglu *et al*., 2007a), distance between vascular bundles (Cavusoglu *et al*., 2007b) and leaf thickness. Significant anatomical characteristics which plays an important role in salt tolerance include stomatal shape, size, density and orientation (Naz *et al*., 2010), increased thickness (succulence) in leaf, root and stem (Hameed *et al*., 2009), increased vascular bundle area (Ali *et al*., 2009), high water use efficiency, low transpiration rate, and low reduction in photosynthetic rate and chlorophyll pigments (Batool *et al*., 2013).

Anatomical studies are important to understand the plants adaptations to any stress environment. Leaf anatomy of *C. erectus* was studied to understand its anatomical adaptations to salinity stress. As leaves are more prone to respond to external environments, it is hypothesized that *C. erectus* must have adapted specific leaf anatomical features which stabilize photosynthetic response, as this species is capable of growing well in a variety of environmental stresses. It is hypothesized that leaf anatomy may play a critical role in photosynthetic activity in this species. The present study was, therefore, conducted to relate gas exchange parameters and photosynthetic pigments with leaf anatomical modifications under salt stress.

Materials and Methods

A pot experiment was conducted to assess the photosynthetic response of *Conocarpus erectus* L., to different salt levels, with six replicates and five treatments were laid out in completely randomized design (CRD). Seedlings (two-week old) were taken from the local nursery and then grown in plastic pots filled with pure sand, and irrigated with Hoagland's solution (Hoagland & Arnon, 1950). Salinity was slowly increased by adding 25 mM salt solution every day. Five salt levels, i.e., 0, 100, 200, 300 and 400 mM were maintained for three months and after the completion of the experiment, gas-exchange parameters were recorded. The plants were then uprooted from the pots and they were washed with double-distilled water for the measurement of chlorophyll pigments and other morpho-anatomical characteristics.

Morphological characteristics: Morphological characters such as leaves per plant, leaf fresh and dry weights, and total leaf area were recorded.

Chlorophyll pigments: Chlorophyll *a*, *b* and carotenoids were determined according to the method of Arnon (1949). Fresh leaves were extracted overnight in 80% acetone at 0-4°C. The extracts were centrifuged at 10,000 x *g* for 5 min. The absorbance of the supernatant was read at 645, 663 and 480 nm on a UV-Visible spectrophotometer (Hitachi-220 Japan).

Photosynthetic parameters: These including net assimilation rate (*Pn*), transpiration (*E*), sub-stomatal CO_2 concentration (*C_i*), stomatal conductance (*g_s*), and water use efficiency (WUE=*A/E*) of shoots were measured using LCA-4 ADC portable infrared gas analyzer (Analytical Development Company, Hoddesdon, England). Measurements were performed between 9 a.m. and 11 a.m. at ambient temperatures ranging from 22°C to 27°C.

Anatomical characteristics: Leaves were collected for the stomatal and anatomical studies and fixed in FAA (v/v formalin 5%, acetic acid 10%, ethanol 50%, and distilled water 35%) for 36 h and later on it was transferred to acetic alcohol (v/v ethanol 75% and acetic acid 25%) for long term storage. A 2-cm piece from the leaf base along with midrib was selected for leaf anatomy. Anatomical characteristics of leaf dermal, parenchymatous and vascular tissues were recorded. For stomatal studies the

leaves were immersed in 70% alcohol solution for long-term preservation. Epidermis was peeled by using razor, dehydrated with various ethanol grades, stained with safranin and mounted in Canada Balsam for microscopic examination following Ruzin (1999). Data for anatomical characteristics were recorded with a micro-meter, which was calibrated with stage micrometer, and photographed with a camera-equipped compound microscope.

Statistical analysis: Data will be analyzed using the Fisher's analysis of variance technique under two factors completely randomized design (CRD) and treatment means will be compared by least significant difference (LSD) test at 5% probability level (Steel *et al.*, 1997). Data was also subjected to multivariate RDA (redundancy analysis) to correlate morpho-anatomical and physiological characteristics along salinity gradient using CONACO (v. 4.5 for Windows). Response curves of all the characteristics were drawn on ordination Axis 1 using Generalized Linear Model (GLM).

Results

Morphological characteristics: There was a significant increase in number of leaves per plant with increase in salt levels up to 300 mM, but it decreased significantly at the highest salt level (400 mM). A significant increase in total leaf area per plant was recorded at 200 mM salinity level, but higher salt levels resulted in a significant decrease in this parameter. Both fresh and dry weights of leaves were not affected by lower salt level (100 mM), however, 200 and 300 mM salt levels showed a significant increase. A significant decrease was recorded at highest stress level of 400 mM (Table 1).

Chlorophyll pigments: Chlorophyll *a* contents were increased strongly and significantly at 100 mM salt level and after that with further increase in salt level it decreased significantly up to highest salt level (400 mM). Similar to chlorophyll *a*, chlorophyll *b* contents were also largest at 100 mM salt level and it decreased significantly with further exposure to salt stress. A significant increase in carotenoids contents was observed up to 200 mM salt level and it decreased significantly then with further increase in salt levels (Table 1).

Photosynthetic parameters: A significant increase in net assimilation rate was recorded at 100 mM salt level, but further increase in salt levels resulted in significant decline in this parameter. Transpiration rate also was increased significantly at 100 mM NaCl, but, it decreased significantly with further rise in salt stress, however, at higher salt levels differences in transpiration rate were non-significant. Stomatal conductance was decreased significantly at 100 mM NaCl but at higher salt levels (300 and 400 mM NaCl) it varied non-significantly. A significant reduction in sub-stomatal CO_2 concentration was recorded with the induction of salt to growth medium. Water use efficiency increased significantly with the increase in salt level up to 200 mM but, after that a significant decrease was noted (Table 1).

Table 1. Morpho-physiological and leaf anatomical characteristics of *Conocarpus erectus* L. under salt stress (*n*=6).

	0 mM	100 mM	200 mM	300 mM	400 mM
Morphological characteristics					
Number of leaves per plant	30.67b±3.84	34.42c±0.57	34.33c±1.20	45.71d±3.17	25.83a±6.56
Leaf area (cm^2)	17.14b±3.15	16.96b±1.89	21.87d±2.85	19.55c±3.19	13.13a±0.65
Fresh weight of leaves (g/plant)	18.21a±3.08	18.91a±2.26	28.48b±5,82	34.84c±3.61	21.27a±6.63
Dry weight of leaves (g/plant)	6.51a±0.92	7.23a±0.72	10.51b±1.05	14.41c±1.19	9 .31b±1.02
Chlorophyll pigments					
Chlorophyll *a* (mg/g fr.wt.)	1.15a±0.14	1.78e±0.17	1.67d±0.12	1.40c±0.11	1.29b±0.13
Chlorophyll *b* (mg/g fr.wt.)	0.55a±0.18	1.09d±0.24	0.91c±0.21	0.72b±0.17	0.52a±0.10
Carotenoids (mg/g fr.wt.)	0.43a±0.01	0.71c±0.01	0.76c±0.01	0.52b±0.02	0.42a±0.03
Photosynthtic parameters					
Net assimilation rate (*Pn*, μmol/m^2 s)	9.23c±0.74	13.54e±0.94	12.67d±0.63	8.21b±0.83	7.18a±1.09
Transpiration rate (*E*, mmol/m^2 s)	4.73c±0.52	4.96d±0.61	3.37b±0.68	3.14a±0.33	3.11a±0.25
Stomatal conductance (*g$_s$*, mol/m^2 s)	0.47c±0.06	0.42b±0.04	0.41b±0.05	0.37a±0.06	0.36a±0.06
Sub-stomatal CO_2 concentration (*Ci*, μmol/mol)	250.72e±20.39	243.74d ±32.15	236.86c±46.93	222.19 b ±17.55	207.16 a ±25.71
Water use efficiency (*A/E*)	1.95a±0.03	2.73c±0.15	3.76d±0.07	2.61c±0.06	2.31b±0.05
Leaf anatomical characteristics					
Lamina thickness (μm)	505.16a±62.37	490.56a±31.93	546.41b±32.99	931.49d±67.18	838.15c±72.05
Palisade tissue thickness (μm)	157.68a±22.90	154.73a±18.16	192.68b±20.84	207.36c±31.63	151.84a±10.16
Spongy tissue thickness (μm)	297.84a±52.86	300.76a±29.74	405.88b±56.52	557.72c±60.26	645.37d±61.53
Abaxial epidermal cell area (μm^2)	663.23e±125.52	643.13d±12.55	625.93c±22.38	602.38b±12.06	401.99a±6.97
Adaxial epidermal cell area (μm^2)	964.77c±13.93	982.30d±11.19	1024.99e±20.74	884.39b±20.98	462.25a±5.32
Cortical region thickness (μm)	537.22a±17.76	575.24b±20.95	563.56ab±12.95	870.16c±13.45	589.84b±12.74
Cortical cell area (μm^2)	2773.51a±303.48	2793.61a±368.96	3175.47b±540.79	3147.04b±365.94	4622.52c±122.5
Vascular bundle area (mm^2)	0.18a±0.01	0.25b±0.02	0.32c±0.01	0.28b±0.03	0.26b±0.02
Phloem area (μm^2)	73436.63a±1113.38	82469.07b±1115.43	81588.53b±1213.45	84452.40b±1513.35	94566.53c±1612.66
Metaxylem area (μm^2)	803.97b±131.79	964.01c±139.24	1065.19d±230.78	824.43b±197.54	683.32a±175.21
Abaxial stomatal density	61.37a±5.77	73.75b±4.35	82.66c±6.63	79.55c±5.77	59.38a±5.71
Adaxial stomatal density	36.25b±4.23	39.86b±5.77	46.18c±3.45	49.76c±4.32	26.84a±4.24
Abaxial stomatal area (μm^2)	3447.12a±104.94	3758.31b±279.21	3959.29c±459.63	4642.62e±243.68	4361.25d±140.68
Adaxial stomatal area (μm^2)	4240.67a±535.15	4384.79b±245.45	4723.02c±315.87	4786.75c±303.36	7235.26d±417.18

Means with similar letters in each row are statistically non-significant at p≤0.05

Anatomical characteristics: A significant increase in lamina thickness was noted at 300 mM salt level, but at the highest level (400 mM), it was decreased significantly. Like lamina thickness, palisade tissue thickness was increased significantly at 300 mM and decreased at 400 mM salt level. Salt level 100 mM did not affect spongy tissue thickness, whereas a significant increase in this parameter was recorded with further increase in salt level of the medium (Fig. 1, Table 1).

Salt stress resulted in a significant and consistent decrease in abaxial epidermal cell area. In contrast, adaxial epidermal cell area was increased significantly up to 200 mM salt level, thereafter a significant decrease was noted with further increase in salt levels. Cortical region thickness increased gradually and significantly up to 300 mM salt level, but the highest level (400 mM NaCl) resulted in a significant decrease. Cortical cell area, on the other hand, was not affected by the induction of salt in growth medium, but the highest levels resulted in a significant increase in this parameter. Differences were, however, not significant among 200 and 300 mM salt levels in relation to cortical cell area (Fig. 1, Table 1).

Vascular bundle area increased significantly with the rise in salt levels up to 200 mM, but at higher salt levels, a significant decrease in this parameter was recorded. A significant increase was recorded in phloem area by the induction of salt in growth medium. There was no further change in this parameter up to 300 mM salt level, but at the highest level (400 mM NaCl), a significant increase

was recorded. Metaxylem area increased significantly up to 200 mM salt level, but higher salt levels resulted in a significant decrease (Fig. 1, Table 1).

Stomatal density on abaxial leaf surface increased significantly up to 200 mM salt level, but 300 mM level resulted in no further change. The highest level (400 mM NaCl), however, showed a significant decrease in this parameter. Stomata density on adaxial surface was not changed by the induction of salt to growth medium (100 mM NaCl), but it increased significantly with further increase in salt levels up to 300 mM. The highest salt level (400 mM), however, resulted in a significant decrease in this parameter. Abaxial stomatal area increased significantly with increase in salinity level up to 300 mM salt level, and thereafter a significant decrease was observed. In contrast, a gradual and significant increase in adaxial stomatal area was noted with increase in salt level of the growth medium (Fig. 2, Table 1).

RDA (redundancy analysis): Two photosynthetic parameters, i.e., net assimilation rate and transpiration rate were strongly associated with 0 mM salt level, however, a number of parameters like chlorophyll *a* & *b*, carotenoids, stomatal conductance, sub-stomatal CO_2 concentration and water use efficiency showed weak association with this level. Number of leaves was the only parameter among all morpho-physiological characteristics that is influenced by 300 mM salt level (Fig. 3).
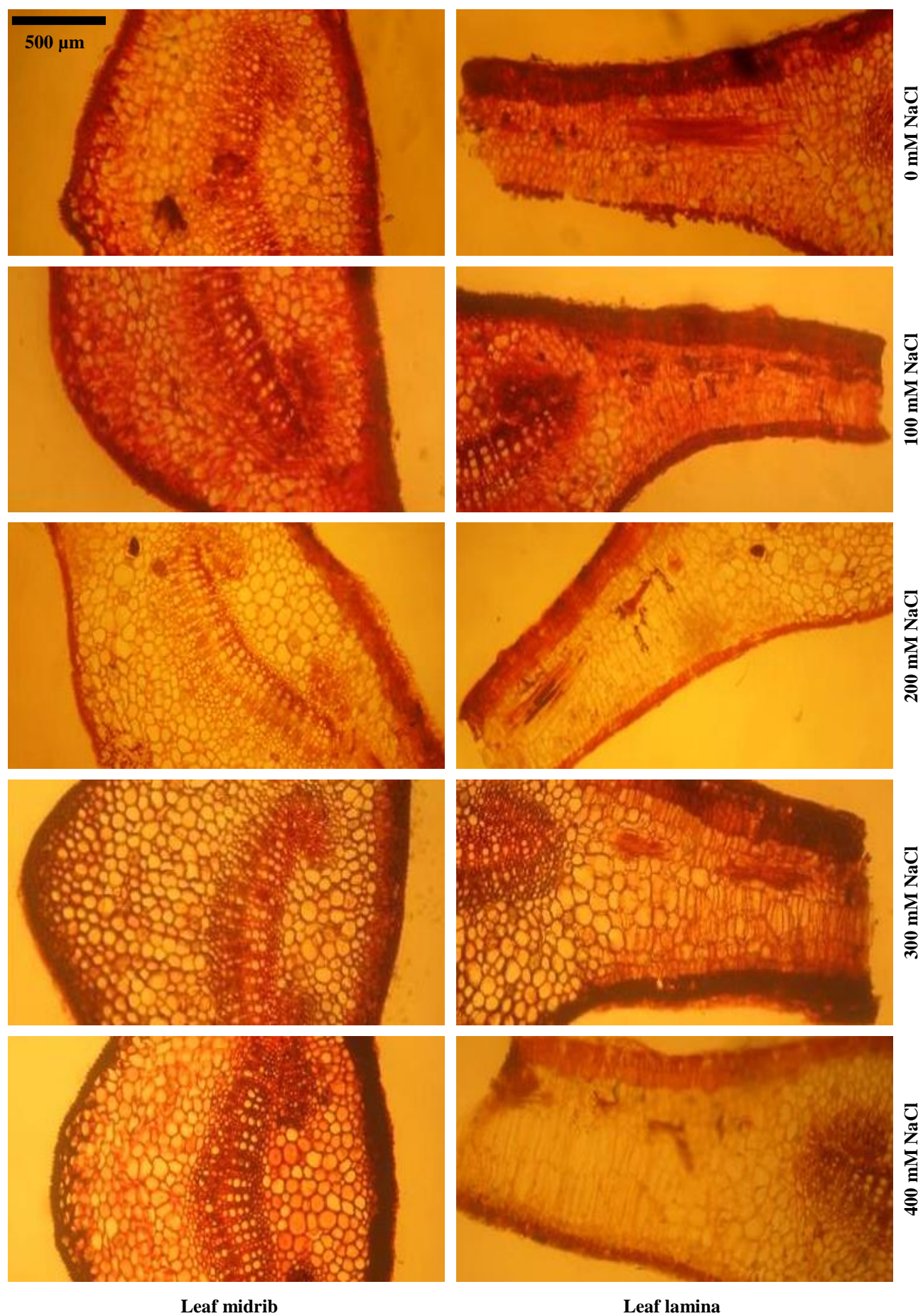
Leaf midrib

Leaf lamina

Fig. 1. Transverse section of leaf of *Conocarpus erectus* L. under salt stress.
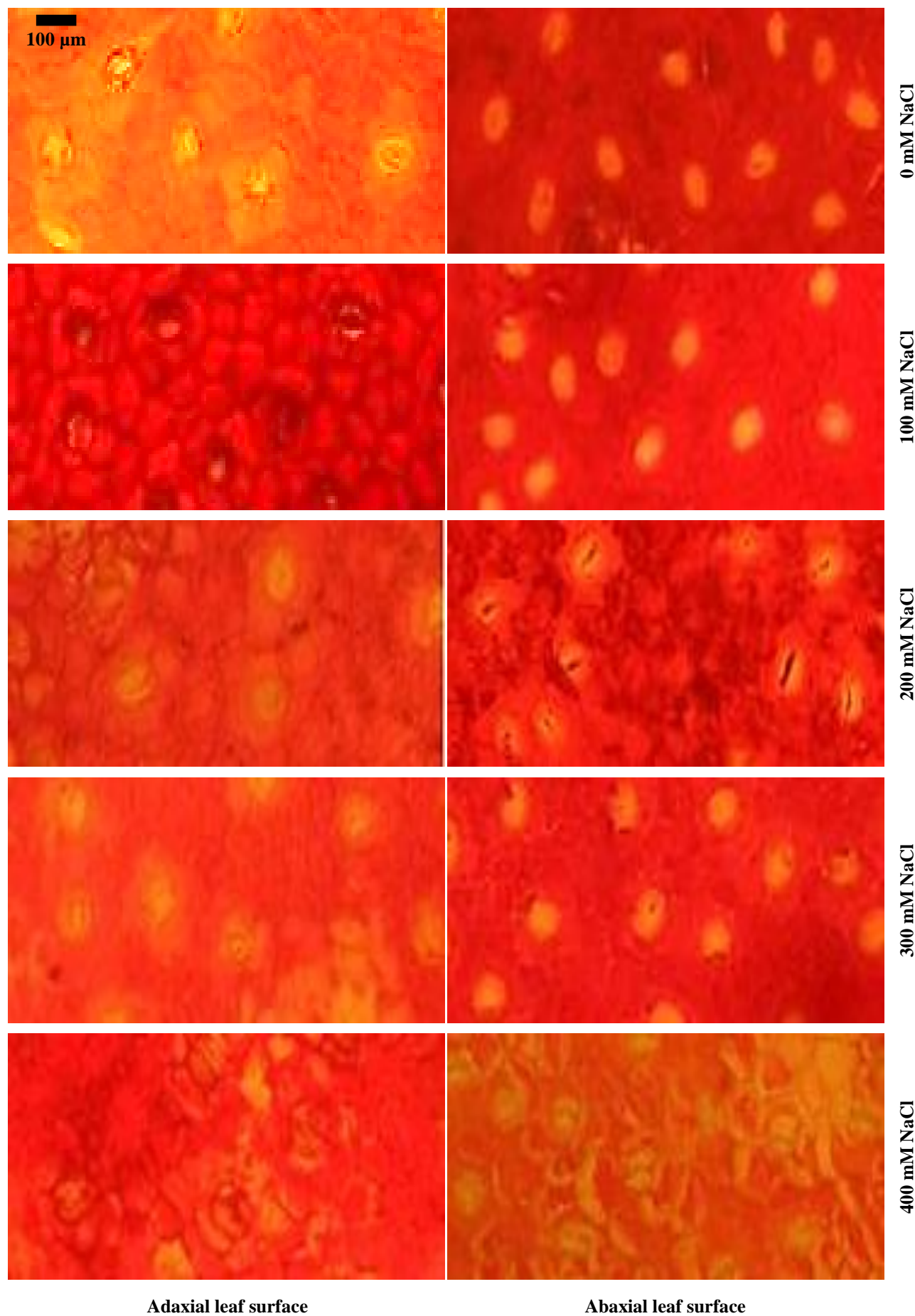
Adaxial leaf surface   **Abaxial leaf surface**

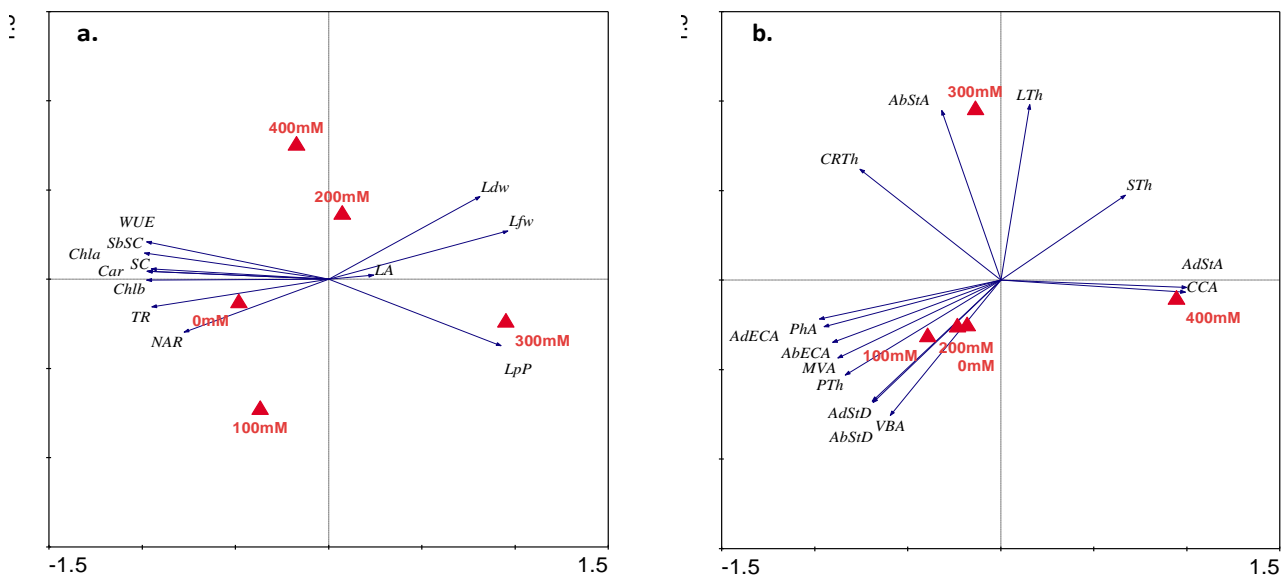Fig. 2. Leaf epidermis of *Conocarpus erectus* L. under salt stress.

Fig. 3. RDA ordination biplot of morpho-physiological (a.) and anatomical (b.) characteristics of *Conocarpus erectus* L. under salt stress. LpP: Number of leaves per plant, LA: Leaf area, Lfw: Fresh weight of leaves, Ldw: Dry weight of leaves, Chl*a*: Chlorophyll *a*, Chl*b*: Chlorophyll *b*, Car: Carotenoids, NAR: Net assimilation rate, TR; Transpiration rate, SC; Stomatal conductance, SbSc: Sub-stomatal CO$_2$ concentration, WUE: Water use efficiency, Lth: Lamina thickness, PTh: Palisade tissue thickness, STh: Spongy tissue thickness, AbECA: Abaxial epidermal cell area, AdECA: Adaxial epidermal cell area, CRTh: Cortical region thickness, CCA: Cortical cell area, VBA: Vascular bundle area, PhA: Phloem area, MVA: Metaxylem area, AbStD: Abaxial stomatal density, AdStD: Adaxial stomatal density, AbStA: Abaxial stomatal area, AdStA: Adaxial stomatal area

A strong relationship was recorded for vascular bundle area, abaxial and adaxial stomatal density, palisade tissue thickness and metaxylem area with no or low salinities, i.e., up to 200 mM salt level. Parameters like epidermal cell area on adaxial and abaxial leaf surfaces and phloem area weakly related to 0, 100 and 200 mM salt levels. Abaxial stomatal area and lamina thickness weakly associated with 300 mM NaCl level, whereas cortical cell area and adaxial stomatal area strongly with 400 mM level (Fig. 3).

GLM model of salinity gradient: Morpho-anatomical and physiological characteristics response to salinity gradient is presented in GLM model (Fig. 4). Among morphological parameters, leaf area and number of leaves per plant sharply decreased along salinity gradient from low salinity to high salinity. A slight decrease was recorded for fresh weight of leaves, however, dry weight of leaves showed an increasing trend with the increase in salt levels. A decreasing trend in the all chlorophyll pigment contents was recorded with the increase in NaCl concentration however, this decrease was more pronounced for chlorophyll *b* and carotenoids but, not for chlorophyll *a*. Net assimilation rate and transpiration rate were severely affected with increase in salinity levels from low to high. A similar trend was recorded for sub-stomatal CO$_2$ concentration and water use efficiency, however, these were not much affected as compared to net assimilation and transpiration rates. Stomatal conductance, in contrast, was not affected with salinity gradient.

Among leaf anatomical characteristics, a decline in the curve for abaxial and adaxial epidermal cell area, metaxylem area and palisade tissue thickness was noted along salinity gradient, while this decrease was more conspicuous for abaxial and adaxial epidermal cell area. An increase was recorded for lamina thickness, spongy tissue thickness, phloem area and cortical cell area with increase in salinity level however, lamina thickness, spongy tissue thickness and cortical cell area showed noticeable increase as compared to phloem area. Cortical region thickness and vascular bundle area were not changed with increasing NaCl salt concentrations. Both abaxial and adaxial stomatal density was sharply decreased along salinity gradient, whereas adaxial stomatal area increased with increase in salinity levels.

Discussion

Earlier studies have shown that *C. erectus* can tolerate high salinities (Passioura *et al*., 1992; Asif *et al*., 2014) as well as other environmental stresses like drought (Lopez-Portillo *et al*., 2005; Al-Humaid & Moftah 2007), high temperature (El-Juhany & Aref, 2005) and water logging (Ellison & Farnsworth, 1997; Hegazy *et al*., 2008; Martin *et al*., 2011). However, the degree of tolerance is stronger for salinity and waterlogging than any other stress (Parida & Jha, 2010). In the present studies, *C. erectus* showed better growth at high salinities than under normal growth conditions. Extreme salt stress, however, resulted in a decrease in growth and other physio-anatomical processes relating to photosynthetic activity. This species is also known as button mangrove, and so it can tolerate estuarine habitats like other mangrove species do (Charles *et al*., 1997; Saha *et al*., 2011). It has developed some specific mechanisms which enable it to photosynthesize and survive in waterlogged saline.
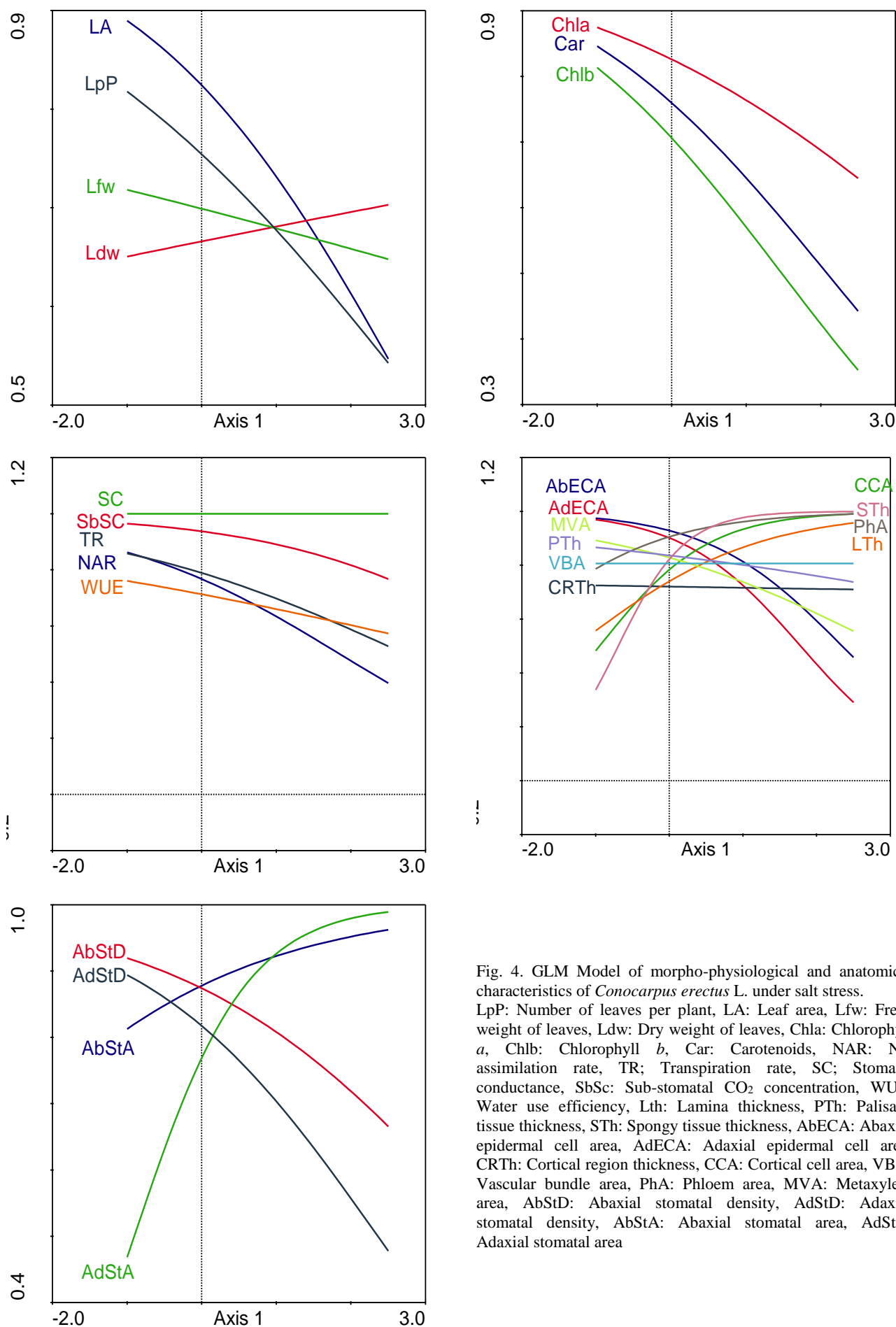
Fig. 4. GLM Model of morpho-physiological and anatomical characteristics of *Conocarpus erectus* L. under salt stress.
LpP: Number of leaves per plant, LA: Leaf area, Lfw: Fresh weight of leaves, Ldw: Dry weight of leaves, Chla: Chlorophyll *a*, Chlb: Chlorophyll *b*, Car: Carotenoids, NAR: Net assimilation rate, TR; Transpiration rate, SC; Stomatal conductance, SbSc: Sub-stomatal CO_2 concentration, WUE: Water use efficiency, Lth: Lamina thickness, PTh: Palisade tissue thickness, STh: Spongy tissue thickness, AbECA: Abaxial epidermal cell area, AdECA: Adaxial epidermal cell area, CRTh: Cortical region thickness, CCA: Cortical cell area, VBA: Vascular bundle area, PhA: Phloem area, MVA: Metaxylem area, AbStD: Abaxial stomatal density, AdStD: Adaxial stomatal density, AbStA: Abaxial stomatal area, AdStA: Adaxial stomatal area

One of the most prominent impacts of salt stress on less tolerant or glycophytic plants are reduction in growth and biomass production, which might be a defensive tactics of plants in response to adverse growth condition (Yang *et al.*, 2009). Plants might spend vital energy for survival rather than normal vegetative growth (Lesica & Crone, 2007). Halophytes in general, variably responded to high salinities, as they may promote growth (Xianzhao *et al.*, 2013), increase succulence (Hameed *et al.*, 2013), stomatal regulation (Naz *et al.*, 2010) high water use efficiency (Batool *et al.*, 2013), and more importantly utilization of toxic ions in metabolic processes (Bose *et al.*, 2013).

In general, photosynthetic efficiency depends upon leaf area per plant (Evan & Poorter, 2001), leaf angle (Posada *et al.*, 2012), amount of radiation received (Ort *et al.*, 2011), light intensity (Neri *et al.*, 2003), stomata size, density and orientation (Naz *et al.*, 2010), stomatal regulation (Hameed *et al.*, 2010), and more importantly the proportion of mesophyll tissue present in a leaf (Hameed *et al.*, 2012). All these can singly or in combination may alter gas exchange parameters, in particular, net assimilation rate. However, the response may differ greatly in different species or even ecotypes of a same species (Righetti *et al.*, 2007). In the present study, we evaluate structural and functional aspect of photosynthesis in a halophytic tree species, which can also survive in a multiple abiotic stresses.

Structural and functional aspects of adaptive mechanism to salinity tolerance are very specific, and vary from species to species, within species or even plant to plant. While considering photosynthetic activity, response of chlorophyll pigments to salinity stress is of prime importance. Amount of all chlorophyll pigments, i.e., Chlorophyll *a* & *b* and carotenoids generally enhanced in the present studies, and this may ultimately lead to increased photosynthetic rate (Saravanavel *et al.*, 2011), which again an indication of high degree of tolerance of *C. erectus*. However, a reduction in chlorophyll contents has earlier been reported in a number of species including halophytes (Ali *et al.*, 2004 in rice; Jaleel *et al.*, 2008 in *Catharanthus roseus*; Sai-Kachout *et al.*, 2009 in *Atriplex hortensis*).

Among structural modifications, increased leaf thickness with rise in salt levels is beneficial. It may provide more space for water storage, and therefore, critically important in survival of a plant species under limited moisture availability (Brouillette *et al.*, 2006; Donovan *et al.*, 2007). Leaf thickness has been related with salinity tolerance by Ishida *et al.* (2005) in *Macaranga gigantean* and Dolatabadian *et al.* (2011) in Soybean.

Adaptation to saline environment is also found to be associated with increased proportion of mesophyll tissue (palisade and spongy) per leaf, which may ensure normal exchange of gases and photosynthetic activities under harsh climates (Ashraf & Harris, 2013). Large and developed mesophyll cells are reported to be an indication of adaptation for saline habitats (Arafa *et al.*, 2009; Hameed *et al.*, 2013).

A unique response of *C. erectus* to salinity stress is the decrease in thickness of epidermal layer on both leaf surfaces under high salinities. Generally increased epidermis thickness along with thick cuticle is related to reduction in evaporational loss through leaf surface (Hameed *et al.*, 2010; Zhuang *et al.*, 2011), but a decrease in our case is an indication that epidermal thickness may not matter the degree of tolerance. However, the shiny glaucous surface on adaxial side may contribute to some extent, and this have to be investigated.

Another crucial modification in leaves is the increase in cortical region thickness and its cellular area along with increasing salinity levels. Similar increase has also been reported in many salt tolerant species (Rich *et al.*, 2008; Batool & Hameed, 2013), and this storage parenchyma may improve the capacity to store extra water, which can be critical in the survival (Kong & Li, 2008; Ogburn & Edwards, 2010).

Vascular tissues are critically important in conduction of water and solutes, especially under extreme salinity. The increased size of vascular tissues, such as metaxylem and phloem in our case is an indication of high degree of tolerance of *C. erectus* by increasing conduction efficiency, as reported by (Sperry, 2003; Lopez-Portillo *et al.*, 2005).There is an agreement with many earlier investigations by a number of researchers e.g., Hameed *et al.* (2009) in *Imperata cylindrical*, Rewald *et al.* (2012) in *Citrus* and Batool *et al.* (2013) in *Schoenoplectus* spp.

Stomata play a decisive role in adjusting not only respiration but also photosynthesis (Yan *et al.*, 2012) during environmental stresses like salinity, and this improves water use efficiency in low water potential induced by salinity stress (Orsini *et al.*, 2012). In present study, stomatal density and area increased with the increase in salt levels, but the density decreased at higher levels. However, stomatal regulation along with stomatal size, density and orientation may contribute significantly. The variation in stomata exists in species related to stomatal density and size. A decrease in stomatal area and density has been reported by many earlier researchers, e.g., Walsh (1990) in *Spartina alterniflora*, Botti *et al.* (1998) in Jojoba species, Bray & Reid (2002) in *Phaseolus vulgaris* and Gielwanowska *et al.* (2005) in *Deschampsia antarctica*. In our studies, regulation of stomatal complex seems to be more important.

Morpho-physiological characteristics in *C. erectus* generally not influenced by salinity as most of them are associated with 0 mM salt level. As salinity increased, leaf dry weight was slightly influenced, but at higher level, there was a clear association of leaf per plant. This might indicate the high degree of tolerance it is directly related to increased photosynthetic capacity of a species (Munns *et al.*, 2006). Anatomical parameters are associated with 0 or low salinity levels, but as the salinity level increased, leaf thickness and abaxial stomatal area showed a clear-cut relationship. The highest salt level, however, was strongly associated with cortical cell area and stomata area on adaxial leaf surface. Both these parameters may be directly involved in water conservation by storing additional water and minimizing water loss, hence may ensure the successful survival of *C. erectus* in high salinities.

Conclusion

Salinity tolerance and normal photosynthetic activity can be related to leaf thickness, amount of mesophyll per leaf, thick epidermal layer, large proportion of storage parenchyma, efficient conducting tissue and stomatal size and density. All these may significantly contribute to increased tolerance in *C. erectus*, normal growth and survival, and normal photosynthesis under high salinities.

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