PHOTOSYNTHETIC RESPONSE OF THREE AQUATIC SPECIES OF *SCHOENOPLECTUS* (REICHENB.) PALLA UNDER SALT STRESS

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Abstract: Physiological and anatomical modification in response to salt tolerance was investigated in three halophytic species of *Schoenoplectus* (Reichenb.) Palla. Plants collected from three different sites were grown in non-aerated hydroponic conditions. Four levels of NaCl (0, 100, 200 and 300 mM) were maintained in culture solution to investigate the response of growth and photosynthesis to salt stress. *Schoenoplectus triqueter* showed optimal growth at 100 mM NaCl and better photosynthetic response to salt stress than that recorded in the other two species (S. lacustris and S. juncoides). Net CO₂ assimilation rate of S. juncoides remained unaffected up to 200 mM NaCl, but at 300 mM NaCl it decreased drastically. By contrast, in S. triqueter, CO₂ assimilation was found to be stimulated at 100 and 200 mM NaCl, whereas stimulation of net CO₂ assimilation in S. lacustris was found only at 100 mM NaCl. Transpiration rate decreased in S. lacustris and S. triqueter with increasing salt stress, whereas in S. juncoides it increased at all salt levels. Stomatal conductance decreased in all three species at 300 mM. Water use efficiency in S. triqueter increased with an increase in external salt concentration. Of the three *Schoenoplectus* species, S. triqueter was more efficient in controlling stomatal movement. The dominant anatomical traits related to high salt tolerance in S. triqueter were found to be stomatal size, shape, density and orientation in distinct grooves; high water use efficiency, low transpiration rate, and low reduction in photosynthetic rate and chlorophyll pigments.

Key words: Photosynthesis, water use efficiency, chlorophyll, *Schoenoplectus* species, stomata, halophytes, Cyperaceae

INTRODUCTION

Sedges (Cyperaceae) have the potential to tolerate a variety of habitats, such as swamps, marshes, pools and ponds in either saline/brackish or freshwater conditions (Khan and Qaiser 2006). Although some species can tolerate highly saline and waterlogged areas (e.g. salt marshes and hyper saline wetlands), others can also tolerate dry-land saline/arid conditions (Bernhardt and Kropf 2006, Hameed et al. 2012).

Abiotic stresses like cold, drought, heat and especially salinity severely affect growth and metabolic processes of plants (Ashraf and Foolad 2007, Maheswari et al. 2012). This can be due to osmotic stress, ion imbalance, ionic toxicity, and nutritional imbalance (Upadhyay and Panda 2005, Munns and Tester 2008).

In addition to toxic effects, salt stress disturbs water relations of plants. Salinity–induced low water potential results in reduced turgor potential and water retention in many halophytic plants, such as *Sporobolus toclados* (Gulzar et al. 2005), *Solenostemma arghel* (Radwan et al. 2007), *Ipomoea pes-caprae* (Suarez 2011), and *Chenopodium quinoa* (Hariadi et al. 2011). High salt content in external growth medium strongly inhibits photosynthetic efficiency; the major causes are imbalance and toxicity of ions, stomatal regulation and the size and orientation of stomata (Naz et al. 2010a).

Growth and development of plants are generally restricted by salinity. A typical response of glycophytic plants is the reduction in growth and photosynthetic rates when subjected to saline conditions (Yang et al. 2009). Decreases in photosynthetic rate may be mainly due to stomatal regulation (Syvertsen and Levy 2005), and to some extent due to stomatal size and orientation (Naz et al. 2010b). Mechanisms of salinity tolerance relating to stomatal structure and function are based on stability in photosynthetic rate, transpirational rate, lower stomatal density and area, and sunken stomata (Liu et al., 2011; Saravanavel et al. 2011; Orsini et al. 2012; Eisa et al. 2012).

*Schoenoplectus* species dominate hyper-saline habitats in the Punjab region of Pakistan (Fig. 1, Kukkonen, 2001). The Kalar Kahar (Chakwal) has a relatively mild climate by comparison with Sahianwala and Jhang, with higher annual rainfall and lower maximum and higher minimum temperatures (Table 1). Jhang is semi-arid habitat with the Thal desert in close vicinity. Salt layers in the Salt Range (Chakwal) are generally deeper in the soil. Post Pleistocene deformations tilted the soil layers, and as a result the salt is within upper layers and is exposed in many places. Water from brine springs deposit salt along their route, with salt accumulating in lakes of the Salt Range (e.g., Kalar Kahar Lake). Runoff water during the rainy season is the other major source of dissolved salts from exposed salt rocks. Soil of the Sahianwala is heavily salt affected. Canal irrigation systems have caused waterlogging along the banks, and as a result highly saline waterlogged habitat suitable for Schoenoplectus species was formed. The SCARP (Salinity Control and Reclamation Programme) has been initiated by the Irrigation Department, in which large drains were constructed to carry saline waters from salt-affected soils. Jhang drain is an example of suitable drain habitat for *Schoenoplectus* species.
Given the high degree of tolerance of *Schoenoplectus* species to salinity, it was hypothesized that these species had some specific adaptive components relating to stomatal structure and function to survive successfully in extreme salinities. Thus, the major objective of the study was to examine the physiological and anatomical markers for salt tolerance and the aquatic response of halophytic plant species to salt stress.

**MATERIALS AND METHODS**

**Site Areas**

Three halophytic species of *Schoenoplectus* (Reichenb.) Palla were collected from salt-affected aquatic habitats in the Punjab region to investigate patterns of their growth, photosynthetic capacity and organic osmotica. Since two species, *S. lacustris* and *S. triqueter*, are endemic to specific habitats in the Punjab, we selected species rather than populations for the comparison, in order to identify adaptive features for future studies. *Schoenoplectus lacustris* (L.) Palla was collected from the hyper–saline waters of Kallar Kahar Lake in the Salt Range, *S. triqueter* (L.) Palla from a saline waterlogged habitat near Sahianwala (Faisalabad), and *S. juncoides* (Roxb.) Palla from a saline drain near Jhang city (Figs. 1 & 2).

**Sampling and Laboratory Analyses**

6 soil samples from the root rhizosphere (16 cm depth) were taken from each habitat to analyze physico-chemical characteristics (Table 1). For saturation percentage, pH and electrical conductivity of soil, the soil was dried at 70 °C and 200 g of soil were taken for preparing saturation paste. The saturation percentage was determined by the formula:

\[
SP\% = \frac{\text{Amount of water added (g)}}{\text{Mass of oven dried soil (g)}} \times \frac{100}{P_w/100}
\]

Where SP% is saturation percentage, \( P_w \) is known water content.

The soil extract was used to determine pH and electrical conductivity (ECE) using a pH/EC meter (WTW series InoLab pH/Cond 720). Sodium (Na\(^+\)) content was determined with a flame photometer (Jenway, PFP-7), and chloride (Cl\(^-\)) content with a digital chloride ion meter (Jenway, PCLM 3). Because each species inhabited a large area of habitat, 30 plants were randomly collected for each species from each site. Six sub-sites within each habitat were selected, starting at the bank with others each separated by a distance of 0.5 m perpendicular to the bank. Five mother plants for each replicate were selected from each sub-site. The average water depth of each sub-site was...
Table 1: Soil physico-chemical characteristics of the habitat of different Schoenoplectus (Reichenb.) Palla species from the Punjab

<table>
<thead>
<tr>
<th>Habitat</th>
<th>Sahianwala Faisalabad</th>
<th>Saline drain Jhang</th>
<th>Hyper saline lake Kalar Kahar</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plant species</td>
<td><em>Schoenoplectus triqueter</em></td>
<td><em>Schoenoplectus juncoides</em></td>
<td><em>Schoenoplectus lacustris</em></td>
</tr>
<tr>
<td>Coordinates</td>
<td>31 40 12.92 N, 73 12 22.81 E</td>
<td>31 17 04.27N, 72 24 45.17E</td>
<td>32 46 14.99 N, 72 42 20.87 E</td>
</tr>
<tr>
<td>Elevation (m ASL)</td>
<td>187.15</td>
<td>156.05</td>
<td>647.09</td>
</tr>
<tr>
<td>Annual rainfall (mm)</td>
<td>146.57</td>
<td>125.62</td>
<td>430.21</td>
</tr>
<tr>
<td>Maximum temperature (°C)</td>
<td>46.7 (Jun-Jun)</td>
<td>46.5 (Jun-Jul)</td>
<td>32.8 (Jun-Jul)</td>
</tr>
<tr>
<td>Minimum temperature (°C)</td>
<td>3.8 (Dec-Feb)</td>
<td>5.4 (Dec-Jan)</td>
<td>10.2 (Dec-Feb)</td>
</tr>
<tr>
<td>Saturation percentage</td>
<td>33.52</td>
<td>39.19</td>
<td>36.79</td>
</tr>
<tr>
<td>pH</td>
<td>8.17</td>
<td>7.12</td>
<td>7.71</td>
</tr>
<tr>
<td>ECe (dSm⁻¹)</td>
<td>47.63</td>
<td>25.67</td>
<td>37.63</td>
</tr>
<tr>
<td>Na⁺ (mg/kg⁻¹)</td>
<td>5853.32</td>
<td>3157.53</td>
<td>4039.15</td>
</tr>
<tr>
<td>Cl⁻ (mg/kg⁻¹)</td>
<td>2764.17</td>
<td>1726.27</td>
<td>2358.62</td>
</tr>
<tr>
<td>K⁺ (mg/kg⁻¹)</td>
<td>390.11</td>
<td>159.48</td>
<td>173.47</td>
</tr>
<tr>
<td>Ca²⁺ (mg/kg⁻¹)</td>
<td>263.52</td>
<td>97.32</td>
<td>144.84</td>
</tr>
<tr>
<td>Mg²⁺ (mg/kg⁻¹)</td>
<td>0.51</td>
<td>0.42</td>
<td>0.35</td>
</tr>
<tr>
<td>Source of salinity</td>
<td>Saline water-logged area</td>
<td>Saline water drain</td>
<td>Brine run-off water</td>
</tr>
</tbody>
</table>

ASL, above sea level; SCARP, Salinity Control and Reclamation Project.

Fig. 3: Morphological attributes of three *Schoenoplectus* (Reichenb.) Palla species grown under salt stress (n = 6)
different. All plants were grown in the Botanic Garden, University of Agriculture, Faisalabad, until their establishment in the local environment under non-saline waterlogged conditions. Twenty vegetative buds (ramets) of almost equal size were randomly detached from these mother plants for each replication and each treatment and grown in non-aerated flooded conditions. Fibre-glass containers (20 L capacity) were half-filled with sandy loam soil, and filled with water to create uniform conditions similar to the natural habitats of *Schoenoplectus* species. Since the natural habitat was predominantly affected by NaCl salinity, four salinity levels were maintained in a culture medium: control (no salt treatment), 100, 200, and 300 mM NaCl. The experiment was conducted during May to July under full sun-light growth conditions, with average day/night
temperatures of 38-41 °C and 24-26 °C, respectively, and photoperiod from 13 to 16 h, relative humidity ranged from 46.8-59.3 %. Under wild conditions, the main growth season is equivalent to the experimental period, in which all species produce inflorescences.

Morphological characters, plant height, root and shoot length, and fresh and dry weights of root and shoot were measured on completion of the experiment after 8 weeks of salinity treatment. Plants were uprooted from the containers and thoroughly washed with distilled water for the measurement of different morpho-anatomical and physiological characteristics.

Photosynthetic parameters including net CO₂ assimilation rate (A), transpiration (E), sub-stomatal CO₂ concentration (C₀), stomatal conductance (gₛ), and water use efficiency (WUE=ΔA/ΔE) of green succulent shoots, including scale leaves and leaf sheath, 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. 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).

For stomatal studies the photosynthetic stems were immersed in 70% alcohol solution for long-term preservation. A 2 cm piece of the stem, just above the small membranous scale leaves was selected for the study of size and orientation of stomata. Peels (epidermal strips) from the surface were isolated with the help of a razor, dehydrated with various ethanol grades, stained with safranin and fast green for lignified and suberinized tissues respectively.

Statistical analysis
The experiment was planned as a completely randomized design (CRD) with two factors (plant species and salinity levels) and six replications. ANOVA of the data was computed using MSStat Computer Programme (MStat Development Team, 1989). Standard error and LSD (Least Significant Difference) were calculated at p<0.05 level of significance for each variable (species as main factor, salinity levels as sub-factors), to test the differences among mean values (Steel et al., 1997).

RESULTS

Plant height in Schoenoplectus lacustris was not affected at moderate (100 mM) salt level, but at higher levels (200 and 300 mM) a significant increase in height was observed. In contrast, S. triqueter and S. juncoides showed an increase in height up to 200 mM NaCl, but a significant decrease was noted at the highest level (300 mM).

Root length in S. lacustris increased significantly at moderate salt level (100 mM), and decreased at higher levels (200 and 300 mM). In S. triqueter and S. juncoides, root length increased significantly with a not significant at 200 and 300 mM NaCl (Fig. 3). Shoot fresh weight in S. lacustris and S. juncoides decreased significantly with an increase in salt level of the growth medium. In contrast, in S. triqueter, a moderate level of salt stress (100 mM NaCl) increased shoot weight, but caused reduction in shoot weight at higher salt levels (Fig. 3). In S. lacustris, root fresh weight decreased at the highest salt level (300 mM NaCl). Root weight in S. triqueter was not affected at moderate salt level (100 mM NaCl), but higher salt levels resulted in a significant decrease. In S. juncoides the root weight increased significantly by the addition of salt to the growth medium, but the differences in root weights at moderate or higher salt levels were not significant (Fig. 3).

Shoot dry weight in S. lacustris and S. juncoides decreased significantly with an increase in salt level of the growth medium. In contrast, in S. triqueter, a moderate level of salts (100 mM) increased shoot dry weight, but higher salt levels decreased weight (Fig. 3). The root dry weight in S. triqueter was not affected at moderate salt levels (100 mM), but higher salt levels resulted in a significant decrease. In S. lacustris, root dry weight decreased only at 300 mM NaCl. In S. juncoides, root dry weight increased significantly and consistently with increase in external salt levels (Fig. 3).

Photosynthetic parameters like net CO₂ assimilation rate (A) and stomatal conductance (gₛ) generally decreased with an increase in salt level of the medium, except at moderate salt level (100 mM), where both attributes were affected significantly. The net CO₂ assimilation rate in all species was severely affected at the highest salt level (300 mM NaCl). Transpiration rate, by contrast, significantly decreased with increase in salt level in S. lacustris and S. triqueter, but increased significantly in S. juncoides.

Stomatal conductance decreased in all three species at varying levels of NaCl, however, the largest decrease was observed in S. lacustris. S. triqueter and S. juncoides showed a decrease in stomatal conductance at higher salt levels. Sub-stomatal CO₂ concentration in all three species was the least affected due to increasing salinity among photosynthetic parameters. In S. juncoides, this parameter decreased significantly, but only at higher salt levels (200 and 300 mM NaCl). Water use efficiency generally increased significantly in all Schoenoplectus species with the increase in external salt levels (Fig. 4).

Concentration of chlorophyll pigments (Chl a, b, and carotenoids) decreased significantly in all species with an increase in salt level of the growth medium. Chlorophyll b increased at 100 mM level only in S. lacustris and S. triqueter, but the difference between the two species was not significant (Fig. 4).

Stomatal density of the photosynthetic stem was not affected by moderate salt levels (100 and 200 mM
NaCl), but at the highest level the density increased significantly in *S. lacustris*. In *S. triqueter*, 100 and 200 mM NaCl did not affect stomatal density, but at the highest level (300 mM), it decreased significantly. In *S. juncoides*, stomatal density increased significantly at 100 mM NaCl, but decreased significantly at higher salt levels.

Stomatal area decreased significantly in *S. lacustris* and *S. juncoides* with an increase in salt level of the culture medium, but by contrast it increased significantly in *S. triqueter* (Fig. 5). The shapes of stomatal complexes (stomata and its subsidiaries) changed significantly in all species with increasing external salt dose (Fig. 5). In *S. lacustris*, an oval–shaped (broadest near the base) stomatal complex was recorded at 0 mM NaCl, but at 100 mM level, it changed to elliptic (broadest near the mid); at 200 mM it was broadly oval and at 300 mM, more or less rectangular. In *S. triqueter* stomatal complex shape was elliptic up to moderate salt level (100 mM NaCl), but it changed to oval or broadly oval at higher salt levels (200 and 300 mM). In *S. juncoides*, the shape of stomatal complex was more or less elliptic, but it changed to rectangular at high salt levels (200 and 300 mM NaCl). The transverse section of the stem showed orientation of the stomatal complex of different *Schoenoplectus* species (Fig. 6). Specific grooves/depressions appeared in all three species with increase in external salinity level, but being

![Fig. 5: Surface view of epidermis of three *Schoenoplectus* (Reichenb.) Palla species grown under salt stress, scale bar is the same for all photographs](image-url)
DISCUSSION

Halophytes are generally salinity indicator species that show high tolerance to salt-affected sites (Colmer et al. 2009). They can dominate a specific area by competition as other species cannot tolerate high concentration of salts, hence halophytes can completely colonize the area (Silvestri et al. 2005). Specific structural and functional adaptations have developed during the evolutionary history of the halophytes, which allow them to cope with high salt concentration (Hameed et al. 2009). The most important is succulence, the fleshy parts of leaves or stem contain a high proportion of water; hence they impose a dilution effect on salt concentration inside the plant body (Hameed et al. 2013). Another phenomenon in root filtering that prevents salts from entering in roots (Munns 2005). Last but not least, salt exclusion via salt glands/hairs or micro-hairs is a characteristics of several halophytic species (Flowers et al. 2010).

Halophytic plants can be used for the investigation of some specific adaptive mechanisms relating to structure and function that can counteract saline regimes. Salt stress-induced stimulation of growth under moderate salinities has been reported in many

Fig. 6: Transverse section of stem of of three *Schoenoplectus* (Reichenb.) Palla species grown under salt stress, arrows show the position of stomata, scale bar is the same for all photographs.
halophytic plants, e.g., *Atriplex hortensis* (Sai Kachout et al. 2009); *Kalidium foliatum* (Jia et al. 2011); *Agropyron elongatum, Kochia prostrata* and *Puccinellia distans* (Akhzari et al. 2012). Such stimulated growth may be a consequence of increased tissue water content, and/or utilization of toxic Na⁺ in metabolic processes by the halophytes (Prado et al. 2000; Khan et al. 2005; Chen et al. 2007, Arzani 2008, Conde et al. 2011).

On the basis of fresh and dry biomass production in *Schoenoplectus* species in the present study, *S. triqueter* can be rated as the most tolerant, as its fresh and dry weights increased at 100 and 200 mM NaCl, but decreased at the highest level (300 mM). In contrast, *S. juncoides* and *S. lacustris* were relatively sensitive to salinity based on shoot growth. Salt stress reduces shoot and root elongation and biomass, this could be due to reduction in water uptake by the plant (Bahrami and Razmjoo 2012), and may cause disturbance in the plant's water relations (Munns 2005). Better water relations were observed in *S. triqueter*, a species better adapted to high salt levels when compared with the other species.

Photosynthetic parameters were severely affected by salinity in all three *Schoenoplectus* species in the present study, which ultimately resulted in reduced plant biomass. Reduction in plant biomass is a general phenomenon in both glycophytes and halophytes, as reported in many plant species, e.g., *Pisum sativum* (Pandolfi et al. 2012), *Zea mays* (Ge et al. 2012) and *Chenopodium quinoa* (Eisa et al. 2012). Inhibition in photosynthesis due to salt induction may coincide with a decrease in transpiration rate, which may contribute to a positive water balance in many halophytic species (Rabhi et al. 2012, Eisa et al. 2012). *Schoenoplectus triqueter* showed a unique behaviour i.e. it showed relatively less affected photosynthetic rate and markedly reduced transpiration which indicate a better degree of salt tolerance via well managed water balance inside the plant body, under limited salt-induced moisture availability.

Enhanced water use efficiency at moderate salt level might be an advantage, bestowing long-term survival of plants under stress conditions (Ge et al. 2012; Edwards et al. 2012). Water use efficiency was improved at 300 mM NaCl level in all *Schoenoplectus* species, but *S. triqueter* and *S. lacustris* showed higher values, again confirming their better degree of tolerance to salinity.

Salt-induced reduction in chlorophyll content is believed to be an adaptive mechanism to cope with high salinities, because it may decrease the photosynthetic electron transport that can lead to the production of reactive oxygen species (Wang et al. 2003, Christian 2005). However, a decrease in chlorophyll content (Chl a, b and carotenoids) may correlate with reduction in total photosynthetic surface area in terms of growth reduction (Geissler et al. 2009, Naz et al. 2010b, Eisa et al. 2012). Although all *Schoenoplectus* species studied showed a significant reduction in chlorophyll content, *S. triqueter* was relatively less affected than the other two species, hence it was better adapted to high salinities.

Anatomical adaptations can be important to investigate salt tolerance markers in relation to stomatal functioning (Munns and Tester 2008). Under changing environmental conditions, stomata have the ability to control and adjust photosynthesis and transpiration in plants (Yan et al. 2012). Under limited water or salt stress conditions, stomatal size might be important to improve water use efficiency (Varone et al. 2012). To counteract high salts, reduction in transpiration rate and stomatal conductance decreased via adjustment in stomatal size and density. Stomatal regulation can play a critical role in water conservation under salt-induced moisture deficit conditions (Francini et al. 2007, Naz et al. 2010b, Saravanavel et al. 2011).

A reduction in stomatal density and increase in stomatal area, particularly at high salinities, may significantly contribute to the stomatal regulation and ultimately stomatal conductance and transpiration rate in *S. triqueter*. In contrast, smaller stomatal area in the other two species may correlate to better control of stomatal conductance (Varone et al. 2012), but at the same time higher density may not be very beneficial as it provides more space for transpirational water loss.

Stomatal shape may also contribute significantly to stomatal regulation. Size and shape of subsidiary cells can regulate efficient opening and closing of stomata, which is critical in controlling transpiration rate under unfavourable climatic conditions (Pillitteri and Torri 2012). Broadly oval subsidiary cells in *S. triqueter* therefore must be more efficient in controlling stomatal movement than thin rectangular shaped cells. Stomatal orientation also plays an important role in minimizing water loss through plant surfaces (Hetherington and Woodward 2003; Kim et al., 2010). Stomata located in grooves are protected from direct contact with the external environment. Moreover, wind movement is lower just above stomatal apertures, which may have a positive impact on transpirational rate by minimizing transpirational water loss (Beerling and Woodward 1997; Nilson and Assmann 2007).

In conclusion, a high degree of salt tolerance *S. triqueter* can be related to stomatal size, shape, and density, orientation of stomata in grooves, high water use efficiency, low transpiration rate, and low reduction in photosynthetic rate and chlorophyll content. Understanding of structural and functional adaptations in halophytic species can be of extreme value in the development of salt resistant or salt-tolerant crops.

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