PHYSIOCHEMICAL RESPONSES OF ZALEYA PENTANDRA (L.)
JEFFREY TO NAACL TREATMENTS

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Abstract

Zaleya pentandra is a moderately salt resistant xero-halophyte, used locally as cattle fodder and as a source of medicine for various ailments. The present study deals with the effect of salinity on growth, leaf water relations, photosynthesis and anti-nutritive chemicals of this plant. Plants were grown in plastic pots containing sandy loam soil irrigated with Hoagland’s nutrient solution under various salt (0, 75 and 150 mM NaCl) treatments in an open netted green house. Shoot and root length and biomass, number of leaves and nodes, remained unchanged at 75 mM NaCl treatment compared to non-saline controls. Shoot dry weight decreased by about 70% at 150 mM NaCl treatment, while root dry weight remained unaffected by salinity. Leaf osmotic potential also was unaffected at 75 mM NaCl but decreased at 150 mM NaCl. Leaf water potential decreased progressively with increasing salinity treatments. Calculated turgor pressure decreased with increase in salinity. Rate of photosynthesis was unaffected under 75 mM NaCl treatment but decreased by about 50% at high NaCl treatment (i.e. 150 mM NaCl). Similar trends were observed for stomatal conductance and rate of transpiration with concomitant increase in water use efficiency (WUE) at 150 mM NaCl. There was no change in the intrinsic photochemical efficiency of PSII (Fv/Fm) (no photo-inhibition) under saline conditions. However, the effective photochemical efficiency of PSII (Fv/Fm’) was generally low particularly at 150 mM NaCl. Among anti-nutrient chemicals, saponin and nitrate decreased significantly under saline conditions, tannins increased whereas, oxalates, phenols and flavonoids were unaffected. However, all these chemicals were within acceptable limits for cattle feed except for oxalates, which were marginally higher.

Key words: Antioxidants, Fodder, Growth, Photosynthesis, Water relations.

Introduction

Zaleya pentandra (L.) C. Jeffrey, (locally called ‘Wahoo’ in Sindhi) is a perennial xero-halophyte growing on coastal and near-coastal sandy salt flats of Africa, India, Iran and Pakistan (Hedge et al., 1990) but may also be found up to 1600 m above sea level (Khan & Quaiser, 2006). It could be used as fodder for cattle and camels when rains are scanty however, it grows luxuriantly after monsoon rains (Bhatti et al., 2001). In rural Sindhi, it is used to treat stomach complaints, respiratory tract infection, cough, and snake bites (Afzal et al., 2013; Bhatti et al., 2001; Khan et al., 2006; Qasim et al., 2010). Zaleya pentandra is also reported to be effective against gonorrhea and respiratory tract infections due to the presence of some steroids (Afzal et al., 2013). Its saponin and potash contents are useful for soap manufacturing and burnt leaves provide vegetable salt in saline and arid parts of Africa (Burkili, 1985).

Halophytes usually survive under saline conditions either by salt exclusion or salt dilution by increasing succulence, to achieve osmotic adjustment (Khan et al., 2009; Munns & Tester, 2008). Sub-tropical plants of dry saline habitats grow and photosynthesize actively after monsoon rains when temperature and soil salinity are reduced (Khan et al., 2000). However, photosynthetic efficiency under stressful conditions requires close coordination between photosynthetic carbon assimilation and photochemical reactions of PSII (Bellasio et al., 2016). Most plants respond to physiological drought by minimizing stomatal conductance for conserving water to maintain high water use efficiency (Larcher, 2003), along with reduced growth (Aziz & Khan, 2003). Similarly, halophytes appear to minimize water loss by stomatal regulation and protect PSII by energy dissipating mechanism (Vercampt et al., 2016). Salinity affects photosynthetic activity by disturbing the balance between electron generation through the photosynthetic electron transport and alternate electron sinks such as Mehler reaction and non-photochemical quenching at PSII (Moinuddin et al., 2017; Bellasio et al., 2016).

Plants produce a variety of secondary metabolites such as flavones, phenols and tannins to cope with abiotic stresses and as a deterrent for herbivores (Qasim et al., 2010; Wahid & Ghazanfar, 2006; Taiz & Zeiger, 2010; Swingle et al., 1996). Enhanced synthesis of secondary metabolites under stressful conditions is believed to protect the cellular structures from oxidative damage (Buchanan et al., 2000; Qasim et al., 2016), in addition to osmotic advantage for plants (Chalker-Scott, 1999; Winkel-Shirley, 2002; Close & McArthur, 2002). Some secondary metabolites such as flavonoids are known to protect the photosynthetic machinery from damaging effects of high light intensities, while others provide defense against herbivores and pathogens (Harborne & Williams, 2000; Taiz & Zeiger, 2010).

The aim of this study was to determine the effects of NaCl on growth, water relations, photosynthesis and accumulation of anti-nutritive chemicals in Zaleya pentandra.

Materials and Methods

Plant material and culture conditions: Seeds of Zaleya pentandra were collected in August 2013 from University of Karachi and seedlings were raised for six weeks in plastic pots (26 cm high x 20 cm dia.) filled with sandy loam soil. Pots were sub-irrigated with Hoagland’s nutrient solution (Epstein, 1972) poured into 2 L plastic trays placed below pots with 0, 75 and 150 mM NaCl solutions. These pots were kept in a netted greenhouse (Max. PPFD = 500 μmol m⁻² s⁻¹). Sodium chloride (@25
mM NaCl per day) was provided with nutrient solution and plants were harvested after 45 days of salinity treatment. Plants were separated into root, stem and leaves and weighed for fresh and oven dry biomass (60°C for 48 h). Total plant length, root and shoot length, number of leaves and nodes were also recorded. The plant material was subsequently subjected to chemical analysis. The following derived biomass ratios were also estimated:

\[
\text{Specific root length (SRL)} = \frac{\text{Root length}}{\text{Root dry weight}}
\]
\[
\text{Specific shoot length (SSL)} = \frac{\text{Shoot length}}{\text{Shoot dry weight}}
\]

**Water relations:** Water potential was measured on 5 mm dia. leaf discs from the second node leaves using a C-52 sample chamber connected to a thermocouple psychrometer (Wescor, Logan Utah, U.S.A.). Microvolt readings were converted to mega-pascal (MPa) units using a standard curve of NaCl solutions (0-800 mM). Leaf-pressed sap was used to determine osmolality (mOsmol Kg\(^{-1}\)) with the help of a vapor pressure osmometer (model 5520, Wescor Int., Logan Utah, USA). Osmotic potential was calculated from leaf osmolality by using the Vant-Hoff’s equation (Guerrier, 1996).

**Gas exchange:** After 6 weeks of salinity treatment, steady state CO₂/H₂O gas exchange parameters were determined with the help of a Li-COR 6400XT portable photosynthesis system (LI-COR Inc., Lincoln, NE, USA) using the confier chamber. Photosynthetic branches placed in the sample chamber for logging steady state gas exchange readings. Sample chamber conditions were maintained as follows: [CO₂] = 400 µmol mol\(^{-1}\); block temperature = 30°C; and PPFD = 700 µmol m\(^{-2}\) s\(^{-1}\) using a Philips halogen dichroic lamp. Dark respiration rate was also measured following each gas exchange measurement by turning off the light source and covering the chamber with a black cloth.

**Chlorophyll fluorescence:** Chlorophyll fluorescence was measured on photosynthetic shoots (one measurement per plant) using a fluorometer (PAM 2500, Walz, Germany) on the same branch as used for gas exchange measurements. Plants were dark-adapted for 30 min by completely covering photosynthetic branches with a dark cloth. The minimal fluorescence (Fo) was measured on dark-adapted tissues, while the maximal fluorescence (Fm) value was obtained by imposing a 0.6 s saturating pulse (10,000 µmol photons m\(^{-2}\)s\(^{-1}\)). Fo and Fm were used to calculate the intrinsic photochemical quantum yield of PSII (Fv/Fm = (Fm-Fo)/Fm). The minimal fluorescence level in light-adapted leaves (Fo) was estimated following the method of Baker & Rosenqvist (2004). Effective photochemical quantum yield of PSII was calculated as Fm' - Fs/Fm'. The quantum yield of non-light induced fluorescence quenching (Y(NO)) and quantum yield of light induced (Zeaxanthin-dependent) non-photochemical fluorescence quenching (YNPQ) were determined as described by Kramer et al. (2004) at a PPFD ~700 µmol m\(^{-2}\) s\(^{-1}\) using the PAM light source.

**Water soluble sugars:** Water soluble sugars in photosynthetic shoots were estimated according to Ludwig & Goldberg (1956). Oven dried, powdered plant material (0.5 g) was mixed in 10 ml deionized water and boiled in a water bath for 1 h. The extract was filtered and stored in a refrigerator at 4°C. The hot water extract (2 ml) was mixed with 2 ml anthrone reagent and boiled for 11 min. The reaction was abruptly terminated in an ice bath. Absorbance was recorded at 630 nm on a UV/VIS spectrophotometer (DU530 Beckman Coulter Inc., USA) with glucose as standard and de-ionized water as reagent blank.

**Chlorophyll content:** Chlorophyll was determined by the method of Knudsen et al. (1977). Fresh leaf material was weighed and immediately immersed in 100% ethanol at room temperature in the absence of light. The extracts were replaced with pure ethanol and collected on a daily basis in a separate glass tube for 3-4 days until the shoots were colorless. Pigment concentrations were estimated according to Lichtenthaler (1987).

Chla (µg/ml) = 13.36 A\(_{665}\) - 5.19 A\(_{649}\)
Chlb (µg/ml) = 27.43 A\(_{649}\) - 8.12 A\(_{665}\)
Tot. Chl (µg/ml) = Chla + Chlb

where Chla, Chlb and Tot. Chl represent chlorophyll a, b and total chlorophyll, respectively.

**Determination of antinutrients:** Total phenolic content (TPC) was estimated using the Folin-Ciocalteu colorimetric method (Singleton & Rossi, 1965). The aluminum chloride colorimetric method was used (Chang et al., 2002) to quantify flavonoids in plant samples. Pearson’s method (1920) was used to determine total tannins in plant samples. Total nitrates were investigated by the method of Cataldo et al. (1975). Total saponin content was determined by using Hui et al. (1976) with some modifications (Makkar et al., 2007). Oxalates were determined according to Karimi & Ungar (1986).

**Statistical analyses:** SPSS Statistics for Window, ver. 20.0 (Anon., 2011) software was used to perform statistical analyses. Significant differences (p<0.05) among means (± S.E) are represented by Bonferroni (Post-Hoc test). Graphs were plotted using SigmaPlot version 11.0 (Systat Software Inc., San Jose, CA, USA).

**Results**

**Growth parameters:** The study showed that high salinity (150 mM NaCl) reduced plant fresh biomass in comparison with control treatments. Root dry weight of Z. pentandra was unaffected by salinity treatments although root length decreased (F = 9.9; p<0.05) by about 40% at 75 mM NaCl (Fig. 1; Table 1). Shoot dry weight was less influenced by low (75 mM NaCl) salinity but decreased significantly (F = 56.40; p<0.05) at 150 mM NaCl treatment while shoot length showed progressive decrease with increasing salinity treatments. Shoot growth appeared to be influenced more by increasing salinity than root growth (Table 1). Plants maintained similar shoot to root biomass ratios in the low salinity treatment but decreased (~50%) significantly (F= 56.4; p<0.05) at high salinity (150 mM NaCl). With an increase in salinity treatments from 0 to 150 mM NaCl, specific root length (SRL) increased at 75 mM NaCl while specific shoot length (SSL) decreased at 150 mM NaCl (Table 1).
PHYSIOCHEMICAL RESPONSES OF ZALEYA PENTANDRA C. JEFFREY TO NaCl TREATMENTS

Fig. 1. Zaleya pentandra plants grown at (0, 75 and 150 mM NaCl) for 45 d.

Water relations: Leaf water ($\psi_w$), osmotic potential ($\psi_s$) and turgor pressure ($\psi_p$) of Zaleya pentandra. Different letters with means ± SE indicate significant differences at $p<0.05$ (Bonferroni test).

Water relations: Leaf water ($F=19.8; p<0.05$) and osmotic potential progressively decreased ($F=22.6; p<0.05$) with increases in NaCl concentration (Fig. 2). Turgor potential also decreased with increases in salinity however, the values at both salinity treatments (75 & 150 mM) were at par, when compared statistically (Fig. 2).

Total soluble sugars: Total soluble sugars (TSS) also showed decreasing trend under both salinity treatments, however the reduction was statistically non-significant at 75 mM NaCl and significant at 150 mM NaCl treatment. The relative decrease at 150 mM NaCl treatment was 35% compared to control (Fig. 3).

Chlorophyll content and photosynthetic $\text{CO}_2$ exchange: One-way analysis of variance (ANOVA) indicated significant variation in chlorophyll a ($F=5.27; p<0.05$) and total chlorophyll ($F=5.13; p<0.05$) content (Fig. 4). However, among the gas exchange parameters rate of photosynthesis ($A_o$), respiration, stomatal conductance ($G_s$), transpiration ($E$) remained unchanged in low (75 mM NaCl) but decrease significantly ($F=1.18; p<0.05$) whereas, intercellular $\text{CO}_2$ concentration ($C_i$) and intrinsic water use efficiency (WUE) increased at 150 mM NaCl salinity treatment (Table 2).

Chlorophyll fluorescence parameters: The intrinsic photochemical efficiency ($Fv/Fm$) was unaffected by salinity treatments, however, the electron transport rate (ETR), photochemical quenching ($q_P$) and effective photochemical quantum yield ($Y(II)$) decreased significantly only at the high (150 mM NaCl) salinity treatment (Table 3). The yield of non-photochemical quenching (YNPQ) increased at 150 mM NaCl whereas, the yield of non-regulated processes (YN0) other than heat dissipation remained unchanged (Fig. 5).
Table 1. Effect of 45 d of NaCl (0, 75 and 150 mM) treatments on growth parameters of *Zaleya pentandra*. Different letters with means ± SE indicate significant differences at *p*<0.05 (Bonferroni).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>0</th>
<th>75</th>
<th>150</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shoot length (cm)</td>
<td>52.92 ± 1.85c</td>
<td>44.45 ± 3.81b</td>
<td>19.64 ± 2.46a</td>
</tr>
<tr>
<td>Root length (cm)</td>
<td>10.58 ± 1.12b</td>
<td>5.72 ± 0.64a</td>
<td>6.69 ± 0.30a</td>
</tr>
<tr>
<td>Root FW (g)</td>
<td>0.56 ± 0.03b</td>
<td>0.62 ± 0.12b</td>
<td>0.34 ± 0.10a</td>
</tr>
<tr>
<td>Root DW (g)</td>
<td>0.16 ± 0.01a</td>
<td>0.16 ± 0.01a</td>
<td>0.14 ± 0.07a</td>
</tr>
<tr>
<td>Shoot FW (g)</td>
<td>4.88 ± 0.53b</td>
<td>3.56 ± 0.63b</td>
<td>1.05 ± 0.28a</td>
</tr>
<tr>
<td>Shoot DW (g)</td>
<td>1.06 ± 0.06b</td>
<td>0.78 ± 0.08b</td>
<td>0.21 ± 0.05a</td>
</tr>
<tr>
<td>Shoot/root (DW)</td>
<td>6.75 ± 0.09b</td>
<td>5.03 ± 0.65b</td>
<td>2.64 ± 1.50a</td>
</tr>
<tr>
<td>No. of leaves</td>
<td>302 ± 60.40c</td>
<td>208 ± 37.01b</td>
<td>54.00 ± 8.66a</td>
</tr>
<tr>
<td>No. of nodes</td>
<td>86.67 ± 15.01b</td>
<td>75.01 ± 16.02b</td>
<td>21.33 ± 3.84a</td>
</tr>
<tr>
<td>SRL</td>
<td>0.015 ± 0.01a</td>
<td>0.028 ± 0.01b</td>
<td>0.02 ± 0.01a</td>
</tr>
<tr>
<td>SSL</td>
<td>0.02 ± 0.01b</td>
<td>0.018 ± 0.01b</td>
<td>0.01 ± 0.00a</td>
</tr>
</tbody>
</table>

SRL = Specific root length; SSL = Specific shoot length

Table 2. Gas-exchange parameters (*A*<sub>N</sub>, photosynthesis; *Gs*, stomatal conductance; *Ci*, intercellular CO<sub>2</sub> concentration; *E*, rate of transpiration; *R<sub>D</sub>*), rate of dark respiration) and water use efficiency (WUE; *A*<sub>N</sub>/E) of *Zaleya pentandra* treated with 0, 75 and 150 mM NaCl for 35 d. Different letters with means ± SE indicate significant differences at *p*<0.05 (Bonferroni test).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>0</th>
<th>75</th>
<th>150</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A</em>&lt;sub&gt;N&lt;/sub&gt; (µmol CO&lt;sub&gt;2&lt;/sub&gt; m&lt;sup&gt;-2&lt;/sup&gt;s&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>6.21 ± 1.27a</td>
<td>5.47 ± 1.22a</td>
<td>3.79 ± 0.46b</td>
</tr>
<tr>
<td><em>R&lt;sub&gt;D&lt;/sub&gt;</em> (µmol CO&lt;sub&gt;2&lt;/sub&gt; m&lt;sup&gt;-2&lt;/sup&gt;s&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>0.54 ± 0.01a</td>
<td>1.28 ± 0.02b</td>
<td>1.36 ± 0.02b</td>
</tr>
<tr>
<td><em>Gs</em> (mol H&lt;sub&gt;2&lt;/sub&gt;O m&lt;sup&gt;-2&lt;/sup&gt;s&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>0.08 ± 0.01a</td>
<td>0.07 ± 0.01a</td>
<td>0.03 ± 0.01b</td>
</tr>
<tr>
<td><em>E</em> (mmol H&lt;sub&gt;2&lt;/sub&gt;O m&lt;sup&gt;-2&lt;/sup&gt;s&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>1.05 ± 0.08a</td>
<td>1.13 ± 0.05a</td>
<td>0.57 ± 0.03b</td>
</tr>
<tr>
<td><em>Ci</em> (µmol CO&lt;sub&gt;2&lt;/sub&gt; mol&lt;sup&gt;-1&lt;/sup&gt;air)</td>
<td>263 ± 12.56a</td>
<td>258 ± 16.16a</td>
<td>161 ± 7.3b</td>
</tr>
<tr>
<td>WUE (µmol CO&lt;sub&gt;2&lt;/sub&gt;mmol&lt;sup&gt;-1&lt;/sup&gt;H&lt;sub&gt;2&lt;/sub&gt;O)</td>
<td>5.77 ± 0.78a</td>
<td>4.74 ± 0.82a</td>
<td>6.52 ± 0.44a</td>
</tr>
</tbody>
</table>

Fig. 5. Effect of NaCl (0, 75, and 150 mM) treatments on the relative yields of photochemistry, *Y*(II); light induced non-photochemical quenching, *Y*(NPQ) and non-light induced fluorescence quenching *Y*(NO) in *Zaleya pentandra* leaves.
Table 3. Effect of 45 day of NaCl (0, 75, 150 mM) treatments on the potential photochemical quantum yield of PSII $\text{Fv}/\text{Fm}$; effective photo-chemical quantum yield of PSII $\text{Y(II)}$; relative electron transport rate $\text{rETR}$; photochemical quenching $\text{qP}$; non-photochemical quenching $\text{NPQ}$; in photosynthetic shoots of Zaleya pentandra. Different letters with means ± SE indicate significant differences at $p<0.05$ (Bonferroni test).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>NaCl (mM)</th>
<th>0</th>
<th>75</th>
<th>150</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{Fv}/\text{Fm}$</td>
<td>0.66 ± 0.03a</td>
<td>0.65 ± 0.03a</td>
<td>0.62 ± 0.01a</td>
<td></td>
</tr>
<tr>
<td>$\text{Y(II)}$</td>
<td>0.17 ± 0.01a</td>
<td>0.14 ± 0.01a</td>
<td>0.08 ± 0.01b</td>
<td></td>
</tr>
<tr>
<td>$\text{qP}$</td>
<td>0.47 ± 0.01a</td>
<td>0.35 ± 0.02a</td>
<td>0.26 ± 0.02b</td>
<td></td>
</tr>
<tr>
<td>$\text{rETR}$</td>
<td>54.66 ± 1.20a</td>
<td>45.66 ± 4.17a</td>
<td>26.33 ± 2.96b</td>
<td></td>
</tr>
<tr>
<td>$\text{NPQ}$</td>
<td>1.08 ± 0.05a</td>
<td>1.02 ± 0.08a</td>
<td>1.25 ± 0.06b</td>
<td></td>
</tr>
</tbody>
</table>

Table 5. Acceptable range of antinutritive chemicals in animal feed.

<table>
<thead>
<tr>
<th>Chemical composition</th>
<th>Acceptable range</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tannins</td>
<td>4-10%</td>
<td>Villalba et al., 2004</td>
</tr>
<tr>
<td>Nitrates</td>
<td>0.05-1.2%</td>
<td>Villalba et al., 2004</td>
</tr>
<tr>
<td>Saponin</td>
<td>2-6%</td>
<td>Khokhar &amp; Chauhan, 1986; Shi et al., 2004</td>
</tr>
<tr>
<td>Oxalates</td>
<td>2%</td>
<td>Njidda, 2010</td>
</tr>
</tbody>
</table>

Chemical analysis: Total flavonoids and total oxalates were unaffected with increasing salinity treatments. Phenol, tannins and nitrates remained unaffected at 75 mM NaCl but decreased substantially ($p<0.05$) at 150 mM NaCl compared to non-saline control. Saponins decreased ($p<0.05$) progressively with increase in salinity treatments (Table 4). Acid soluble oxalate increased ($p<0.05$) while the water-soluble oxalates decreased ($p<0.05$) transiently at 75 mM NaCl compared to the non-saline controls (Table 4).

Ecophysiological investigations of the salt resistance mechanisms of halophytic plants with potential as fodder could be beneficial in identification of key traits leading towards their efficient utilization (Moinuddin et al., 2014; Qasim et al., 2010). Salt resistant plants could be grown on saline, degraded lands as a source of fodder and/or chemicals with commercial importance. In this experiment, growth of Z. pentandra was unaffected by moderate salinity. Decreased growth of Z. pentandra under higher NaCl treatment (150 mM NaCl) seemed to occur as a result of reduced water uptake and lower photosynthetic rates (Abdeen et al., 2014; Flowers & Colmer, 2008). Z. pentandra grown in moderate salinity (75 mM NaCl) treatment showed visible adjustment by producing longer roots (SRL), possibly to tap less saline water from deeper soil layers (Eissen-Stat, 1992) and to avoid root zone salt toxicity (Alvarez et al., 2012).

Plants suffering from physiological drought usually minimize their growth at the cost of osmotic adjustment (Gorai & Neffati, 2011; Khan et al., 2000). A progressive decrease in water potential of Z. pentandra with the increases in salinity indicated an ‘osmoconformor’ strategy (Khan et al., 2000) which allowed it to maintain sufficient hydration (Hussein et al., 2013). Lower osmotic potentials in Z. pentandra at 150 mM could result from salt accumulation in leaves for osmotic adjustment (Munns, 2002).

Table 4. Effect of 45 d of NaCl (0, 75 and 150 mM) treatments on leaf anti-nutritive chemicals of Zaleya pentandra. Different letters with means ± SE indicate significant differences at $p<0.05$ (Bonferroni).

<table>
<thead>
<tr>
<th>Chemical analysis (% DW)</th>
<th>NaCl (mM)</th>
<th>0</th>
<th>75</th>
<th>150</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenol</td>
<td>1.24 ± 0.01b</td>
<td>1.23 ± 0.01b</td>
<td>1.38 ± 0.03a</td>
<td></td>
</tr>
<tr>
<td>Flavonoid</td>
<td>0.37 ± 0.01a</td>
<td>0.41 ± 0.01a</td>
<td>0.40 ± 0.01a</td>
<td></td>
</tr>
<tr>
<td>Tannin</td>
<td>1.56 ± 0.01b</td>
<td>1.65 ± 0.03b</td>
<td>1.96 ± 0.02a</td>
<td></td>
</tr>
<tr>
<td>Nitrate</td>
<td>0.84 ± 0.13a</td>
<td>1.09 ± 0.05a</td>
<td>0.46 ± 0.03b</td>
<td></td>
</tr>
<tr>
<td>Saponin</td>
<td>2.50 ± 0.10a</td>
<td>1.91 ± 0.07b</td>
<td>1.30 ± 0.05c</td>
<td></td>
</tr>
<tr>
<td>Total oxalate</td>
<td>2.82 ± 0.26a</td>
<td>3.0 ± 0.36a</td>
<td>3.01 ± 0.39a</td>
<td></td>
</tr>
<tr>
<td>Acid soluble oxalate</td>
<td>0.96 ± 0.12b</td>
<td>1.74 ± 0.33a</td>
<td>1.08 ± 0.35b</td>
<td></td>
</tr>
<tr>
<td>Water soluble oxalate</td>
<td>1.86 ± 0.15a</td>
<td>1.26 ± 0.10b</td>
<td>1.92 ± 0.24a</td>
<td></td>
</tr>
</tbody>
</table>

Discussion

At the cellular level, halophytes tend to sequester excess salts in the vacuoles (Shahala & Mackay, 2011) and counter balance their toxic effects by synthesizing organic solutes (Munns & Tester, 2008; Slama et al., 2007). As a result, plant growth is reduced due to the high energy cost of synthesizing organic compounds such as choline, glycinebetaine, proline and polyols (Rhodes et al., 2002; Slama et al., 2007). Low molecular weight carbohydrates could also act as chaperones or ROS scavengers (Gil et al., 2013). However, a 35% reduction of total soluble sugars in Z. pentandra under saline conditions appeared to be a result of reduced photosynthetic rates.

Under saline conditions, lower availability of water could induce stomatal closure to conserve water (higher WUE: Koyro, 2006; Moshelion et al., 2015) leading to restricted CO$_2$ availability at the carboxylation sites or may cause non-stomatal (biochemical) limitations of the photosynthetic machinery (Geissler et al., 2015; Sánchez et al., 2016). Photosynthetic CO$_2$ fixation is sensitive to drought or salt stress above the threshold for a particular species (Geissler et al., 2015; Hussin et al., 2013). In this study, photosynthetic rates, stomatal conductance ($G_s$) and intercellular CO$_2$ concentrations ($C_i$) were unaffected at low salinity while at the higher salinity treatment, lower $C_i$ and $G_s$ suggested stomatal limitation of photosynthesis in Z. pentandra (Munns, 2002). An increase in dark respiration under saline conditions also indicates energy
expenditure on osmotic adjustment at the cost of plant growth (Jacoby et al., 2011).

Little change in potential photochemical quantum yield (Fv/Fm) showed no damage to PS-II in Z. pentandra under saline conditions however, lower effective photochemical quantum efficiency Y(II) at 150 mM NaCl could suggest down regulation of linear electron flow to avoid oxidative burst (Boughalleb et al., 2009). Lower Y(II) values at high salinity were also reflected by lower electron transport rates (eETR) and photochemical quenching (qP) due to reduced photosynthetic efficiency (Pagter et al., 2009) not related to PS-II damage (Miamaiti et al., 2014). Z. pentandra appeared to increase non-photochemical quenching (NPQ) through heat dissipation at the highest salinity indicating the role of xanthophyll cycle. The distribution of light energy also indicates greater role of Y(NPQ) rather than non-regulated processes of dissipating excess light energy Y(NO) with decrease in yield of photochemistry Y(II).

A well protected PSII can also be corroborated with the biosynthesis of soluble organic compounds (carbohydrates, proline) and secondary metabolites (phenols and flavonoids). Secondary metabolite production has been linked with improved antioxidant activity in plants under stress (Abideen et al., 2015; Ahmed et al., 2015). Halophytes showed higher polyphenol contents with increasing salinity as a possible defense mechanism against salt toxicity (Ben Amor et al., 2006; Bendaly et al., 2016; Daly et al., 2009). A slight increase in total phenols and tannins under the higher salt treatment in Z. pentandra could possibly help in stabilizing the oxygen-evolving complex and protect photosystem II from damaging effects of ROS (Geisser et al., 2015; Hafsi et al., 2016).

The concentration of plant secondary metabolites needs to be considered while formulating diets consisting of salt resistant species as forage for small ruminants (See Table 5). Phenols, tannins and flavonoids could have medicinal value at low concentrations however; higher concentrations could be detrimental for livestock (Ksouri et al., 2007). Animals usually prefer low tannins in feeds (<4.5%) whereas, concentrations exceeding 9% could result in lower food intake in lambs (Barry and McNabb, 1999; Villalba et al., 2004). Similarly, >3% saponins could result in hemolytic activity and foaming (Burns, 1978). At >1.5% concentration, nitrates could reduce dietary intake of energy rich feed (Burritt & Provenza, 2000) and oxalates at >2% of plant biomass may interfere with calcium absorption leading to impaired renal functions (Malcolm et al., 1988; Njidda, 2010).

In general, polyphenols were within acceptable range for cattle feed in Z. pentandra grown under saline and non-saline conditions (see Table 5). Amaranthus sp. widely used as fodder in Egypt and the Near East region contained 3-5% of total oxalates (El-Shaer, 2010). Z. pentandra also had somewhat higher oxalate content than the acceptable range, but remained less than 3% under saline conditions.

This study suggests that Zaleya pentandra is a facultative xero-halophyte which produces moderate levels of secondary metabolites under saline conditions, within acceptable limits reported for forage and fodder. However, further field studies are needed to evaluate its fodder value.

Acknowledgements

This study was supported by the Higher Education Commission of Pakistan grant awarded to RA and in part through the HEC Indigenous Ph.D. Scholarship to Ms. Saman Ehlsen.

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(Received for publication 10 March 2016)