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Ecophysiological adaptations and anti-nutritive status of sustainable cattle feed *Haloxylon stocksii* under saline conditions

Saman Ehsen, Zainul Abideen, Rabab Fatima Rizvi, Salman Gulzar, Irfan Aziz, Bilquees Gul, M. Ajmal Khan, Raziuddin Ansari*

Institute of Sustainable Halophyte Utilization, University of Karachi, Karachi, 75270, Pakistan

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ABSTRACT

Haloxylon stocksii is a perennial halophytic shrub found in saline arid habitats. Ecophysiology of this xerohalophyte was studied to better understand its adaptations to survive under saline conditions (0–300 mM NaCl). Growth parameters remained unaffected at 100 mM NaCl compared to non-saline control and declined at 300 mM; root biomass however, increased in 100 mM NaCl. Shoot water potential, osmotic potential and turgor decreased under saline conditions. Gas exchange parameters such as rate of photosynthesis, rate of transpiration and stomatal conductance decreased in 300 mM NaCl with a decline in intercellular carbon dioxide concentration suggesting stomatal limitation of photosynthesis. Water use efficiency increased significantly in 300 mM NaCl compared to 0 and 100 mM NaCl. Chlorophyll content decreased progressively with increasing salinity while chlorophyll a/b ratio remained unaffected. Potential photochemical quantum yield of PSII (Fv/Fm) and the effective quantum yield [Y (II)] were not affected by salinity; however, there was a constitutive increase in non-photochemical quenching under saline conditions (100 and 300 mM) and decrease in relative electron transport rate in 300 mM NaCl as a protective measure to reduce oxidative stress at cellular level. Total soluble sugars increased by ≈25% in 300 mM NaCl possibly indicating a lower energy demand for growth. Anti-nutritive secondary metabolites such as polyphenols, tannins, flavonoids, saponins and nitrates decreased under saline conditions except for some increase in oxalates in 100 mM NaCl. This study indicates that *Haloxylon stocksii* is a moderately salt tolerant plant with adaptations to down- regulate its photosynthetic machinery to avoid oxidative stress and could be a palatable animal feed.

1. Introduction

Soil salinity is rampant worldwide but the menace is more severe in arid and semiarid regions (Cude et al., 2018). Coupled with limited availability of fresh water, this degradation of arable lands has adversely affected crop yields of human and animal consumption (Koyro et al., 2011; Raiten and Aimone, 2017). Halophytes which have innate ability to resist salt stress can be used for saline land reclamation and / or grown commercially for various purposes without encroaching on agricultural lands (Khan et al., 2009; Hasanuzzaman et al., 2014). Some recent research has proved that many halophytes are nutrient rich palatable plants that could become ideal fodder for cattle (Koyro et al., 2014; Nikalje et al., 2018; Tlili et al., 2018; Toqeer et al., 2018).

Biomass production of salt resistant plants has a causal relationship with water relations, photosynthesis, mineral acquisition and oxidative stress (Abideen et al., 2014; Koyro et al., 2014; Moinuddin et al., 2014). Reduced stomatal conductance with increase in salt and drought stress results in higher water use efficiency (Flexas et al., 2004; Shoukat et al., 2018, 2019). According to Flexas et al. (2004), the initial effects of exposure to drought and salt stress occur by stomatal limitation (by reduced CO_2 availability in the leaf mesophyll) rather than by non-stomatal (biochemical) limitation of photosynthetic rates. Salt stress

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Abbreviations: Ci (μmol mol^{-1}), intercellular carbon dioxide concentration; DW (g plant^{-1}), dry weight; E (mmol H_2O m^{-2} s^{-1}), rate of transpiration; Fv/Fm, potential photochemical quantum yield of PSII; FW (g plant^{-1}), Fresh weight; Gs (mol H_2O m^{-2} s^{-1}), stomatal conductance; NPQ, non-photochemical quenching; Pn (μmol CO_2 m^{-2} s^{-1}), rate of photosynthesis; PPFD (μmol m^{-2} s^{-1}), photosynthetic photon flux density; R_D (μmol m^{-2} s^{-1}), rate of respiration; rETR (μmol m^{-2} s^{-1}), relative electron transport rate; SRL (cm g^{-1}), specific root length; SSL (cm g^{-1}), specific shoot length; TPC (%DW), total phenolic content; WUE (μmol CO_2 mmol^{-1} H_2O), water use efficiency; Y (II), effective photo-chemical quantum yield of PSII; Y (NO), yield of non-photochemical losses other than heat; Y (NPQ), yield of heat dissipation

* Corresponding author.

E-mail address: raziuddin@uok.edu.pk (R. Ansari).

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impairs photosynthetic ability of plants which alters the balance of electron generation (through electron transport chain) and neutralization (in photosynthesis and respiration) (Bose et al., 2017). In C_4 halophytes, PSII photochemistry is reported to be more resilient to salt stress than CO_2 fixation processes, with a balance between the light-harvesting processes and effective energy dissipating mechanisms (Maricle et al., 2007; Moinuddin et al., 2017).

Under saline conditions, halophytes tend to avoid oxidative damage by reducing chlorophyll content and accumulate secondary metabolites such as phenolic compounds resulting in decreased growth and photosynthesis (Ehsen et al., 2016, 2017; Qasim et al., 2017). These secondary metabolites have been reported to disturb palatability and digestion of livestock (El-Shaer, 2010; Ali et al., 2014; Abideen et al., 2015). For instance, in some *Haloxylon* species, phytochemicals such as 'haloxylase' showed significant inhibition of chymotrypsin enzyme (Ahmed et al., 2006). To the contrary salt resistant plants growing in saline areas may accumulate such compounds in lower quantities which are not harmful for animals (Ali et al., 2014). Owing to their sessile nature, considerable variation in environmental conditions could elicit production and accumulation of varying levels of secondary metabolites (Qasim et al., 2017).

Haloxylon stocksii (Boiss.) Benth. & Hooker, is a stem succulent perennial xero-halophyte of saline deserts of the Mediterranean and Central Asian region, in Afghanistan, India, Iran, Iraq, Syria and Turkey. It is distributed in Sindh and Baluchistan provinces of Pakistan (Jafri, 1966) with some populations existing up to the northern Himalayan mountains range of Pakistan (Stewart, 1972). In Karachi, *Haloxylon stocksii* occurs near the coastal and inland saline areas (Khan and Qaiser, 2006). According to Khan (1990) *H. stocksii* is a true halophyte which flourishes in salt deserts where salinity ranges between 19 and 34 dS m^{-1}. It can tolerate highly saline conditions by diluting salts in foliage through increased succulence and thus as a salt includer plant could be used for saline soil reclamation (Khassanov et al., 1994; Manousaki and Kalogerakis, 2011). It is a good browse for large ruminants and as silage for goats (El-Shaer, 2010; Rathore et al., 2012). Chemical analysis of its foliage shows 13.4% crude protein, 0.9% ether extract, 22.8% ash and 62.9% total carbohydrates, which constitutes 20.7% crude fibre (Rathore et al., 2012). Additional benefits include use as source of baking soda for making detergents and other carbonates for glass industry (Hammer et al., 1990; Rathore et al., 2012). Its seeds are reported to yield good quality edible oil with high poly-unsaturation (Weber et al., 2001; Munir et al., 2019).

Halophytes are reported to display a variety of physiological and biochemical strategies at the leaf and whole plant level under saline conditions, which allow them to occupy distinct ecological zones from the coastal toward inland habitats (Hassine et al., 2010; Gulzar et al., 2014; Rasool et al., 2019). The current study was designed to evaluate the ecophysiological adaptations of *Haloxylon stocksii* to survive in dry, sandy salt desert environments. The following hypotheses were tested: 1) Moderate salinity treatment will result in better growth by improved water relations and photosynthesis compared to non-saline conditions; 2) Under saline conditions, plants will protect their photosynthetic machinery by increase in non-photochemical quenching; and 3) *H. stocksii* will produce high levels of secondary metabolites under saline conditions.

2. Materials and methods

2.1. Plant material and culture conditions

Seeds of *Haloxylon stocksii* were collected in December 2013 from Karachi University campus, separated from inflorescences and allowed to germinate in plastic trays. Four week old seedlings of about 5 cm height were transplanted into plastic pots filled with sandy soil which were sub-irrigated (through holes drilled at the bottom of pots) with half strength Hoagland nutrient solution (Epstein, 1972). Seedlings were grown in an open netted greenhouse under ambient conditions (max. PPFD 600 μmol m^{-2} s^{-1}; temperature: 37 ± 3 °C; relative humidity: 50 ± 5%) for 28 days, when plants were about 5 cm tall. Plants ($n = 6$) were then subjected to 100 and 300 mM NaCl treatments (50 mM NaCl per day during 6 consecutive days) along with nutrient solution. Salinity treatments were selected based on preliminary experiment of salt tolerance. Once the maximum NaCl treatment was achieved, pots were flushed with respective treatment solution every week to maintain salinity for another 35 days. Water relations, gas exchange and chlorophyll fluorescence were measured and plants were harvested for growth parameters. For all chemical and physiological analyses, mature, fully developed photosynthetic branches from the first three nodes were used. Growth attributes such as specific shoot length (SSL) and specific root length (SRL) were calculated according to Panuccio et al. (2014) as follows:

$$\text{SSL (cm g}^{-1}\text{)} = \text{shoot length/shoot dry weight}$$

$$\text{SRL (cm g}^{-1}\text{)} = \text{root length/root dry weight}$$

2.2. Water relations

Shoot water potential was measured between 6:00 am and 9:00 am, after 35 days of achieving maximum salinity treatments, on excised photosynthetic branches from the 2nd node using the C-52 leaf sample chamber with the help of a psychrometer (Wescor, Logan Utah, U.S.A.). The sample chamber was equilibrated with a series of NaCl solutions (0–800 mM). Values in microvolts were converted to megapascals (MPa) units while NaCl dilutions were used to make a standard curve for calibration. Pressed sap of shoot material was used to determine osmotic potential with an osmometer (model 5520, Wescor Int., Logan Utah, USA). Shoot osmolality (mOsmol Kg^{-1}) readings were converted to pressure (MPa) units using the Vant-Hoff's equation (Guerrier, 1996).

2.3. Gas exchange

Steady state CO_2/H_2O leaf gas exchange readings were recorded by using the Li − COR 6400XT photosynthesis system (LI − COR Inc., Lincoln, NE, USA) at atmospheric CO_2 (400 μmol mol^{-1}) concentrations maintaining block temperature at 30 °C, VPD ≤ 2 kPa and PPFD of ≈700 μmol m^{-2} s^{-1} with a Philips halogen dichroic lamp as a light source. Readings were recorded on fully expanded photosynthetic branches, three days before plant harvest from 9 a.m. to 2 p.m. Rate of photosynthesis (Pn), stomatal conductance (Gs), intercellular CO_2 concentration (Ci), and rate of transpiration (E) were calculated using the internal Li − COR software. Water use efficiency was estimated as: WUE = Pn/E. Dark respiration rates were also measured after keeping the photosynthetic shoots in complete darkness for ≈30 min. Photosynthetic branches were allowed to equilibrate for up to 15 min inside the conifer sample chamber to obtain steady state gas exchange data. Gas exchange data were based on digital photographs of projected area calculated with the help of ImageJ 1.48v software of photosynthetic branches placed in the sample chamber.

2.4. Chlorophyll fluorescence

Chlorophyll fluorescence parameters were monitored on photosynthetic branches between 11:00 am and 1:00 pm one day before harvest on fully developed branches using PAM 2500, Walz, Germany (a portable modulated fluorimeter). Plants were completely darkened by black cloth for 30 min. Minimal fluorescence (Fo) was noted in dark-adapted branches followed by the application of modulated light (< 0.1 μmol photons m^{-2} s^{-1}), while the maximal fluorescence (Fm) was obtained by applying a saturating pulse of ≈10,000 μmol photons m^{-2} s^{-1} for 0.6 s (Baker and Rosenqvist, 2004). Fm and Fo were used to determine the potential photochemical quantum yield of fluorescence [Fv/Fm = (Fm-Fo)/Fm]. Steady-state (Fs) and maximal (Fm') fluorescence readings were taken in light-adapted branches. The effective photochemical quantum yield of fluorescence was calculated as [Y(II) = (Fm'-Fs)/Fm'] (Genty et al., 1989). Photochemical fluorescence quenching (qP) was estimated as (Fm'-F)/(Fm'-Fo') according to Schreiber et al., 1986 (as formulated by van Kooten and Snel, 1990). Non-photochemical fluorescence quenching parameters (NPQ) were calculated as NPQ = Fm′/(Fm′-1) (Bilger and Björkman, 1994). Quantum yield of non-photochemical energy conversion in PS II other than that caused by down-regulation of the light harvesting function Y (NO) = F/Fm and Quantum yield of non-photochemical energy conversion in PS II due to down-regulation of the light-harvesting function Y(NPQ) = (F/Fm') - (F/Fm) were determined (Genty et al., 1996) at a PPFD ≈ 700 μmol m^{-2} s^{-1} using the PAM light source. Relative electron transport rate (rETR) was calculated as rETR = PSII × PPFD × 0.5 × 0.84 (where PPFD = photosynthetic photon flux density incident on the leaf; 0.5 = factor that assumes equal light energy distribution between photosystems I and II), and 0.84 was assumed to represent the fraction representing leaf light absorbance (Genty et al., 1989).

2.5. Chlorophyll content

Chlorophyll was determined two days before harvest on the same or similar photosynthetic branches as those used for gas exchange and chlorophyll fluorescence measurements as in Knudson et al. (1977). About 50 mg of fresh branch was weighed and placed in sealed Eppendorf tubes containing 3 ml of 100% ethanol at ≈25 °C in complete darkness. The chlorophyll extracts were collected in a separate glass tube and replaced with ethanol daily for at least 3–4 consecutive days until the plant material became colorless and absorbance (A) was measured at 649 and 665 nm on a spectrophotometer (Model DU530, Beckman Coulter Inc., USA). Pigment concentrations were estimated as below (Lichtenthaler and Buschmann, 1987):

$$\text{Chla } (\mu\text{g/ml}) = 13.36\ A_{665} - 5.19\ A_{649}$$

$$\text{Chlb } (\mu\text{g/ml}) = 27.43\ A_{649} - 8.12\ A_{665}$$

$$\text{Total Chl } (\mu\text{g/ml}) = \text{Chla} + \text{Chlb}$$

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

2.6. Total soluble sugars

Total soluble sugars were determined on photosynthetic branches by the anthrone method (Ludwig and Goldberg, 1956). Hot water extracts were prepared with dried, finely ground branch material (0.5 g) added to 10 ml deionized water boiled on a water bath for 60 min. One ml of this diluted (1:20) and filtered extract was added to 2 ml anthrone reagent and allowed to boil for 11 min. The reaction was terminated by placing the extract in an ice bath and the absorbance recorded for all dilutions of standard and samples at 630 nm on a spectrophotometer using glucose as standard and DI water as reagent blank.

2.7. Determination of anti-nutrients

Anti-nutrient chemicals were analyzed on photosynthetic branches from the first three nodes, after drying in shade until constant weight. Phenolic contents (TPC) were determined with the help of Folin-Ciocalteu method (Singleton and Rossi, 1965). Flavonoids were analyzed according to Chang et al. (2002). Pearson's method was used to determine total tannins (Pearson, 1976). Total nitrate was analyzed according to Cataldo et al. (1975). Total saponins were determined according to Hiai et al. (1976), modified by Makkar et al. (2007). Total, water soluble and acid soluble shoot oxalates were analyzed following Karimi and Ungar (1986).

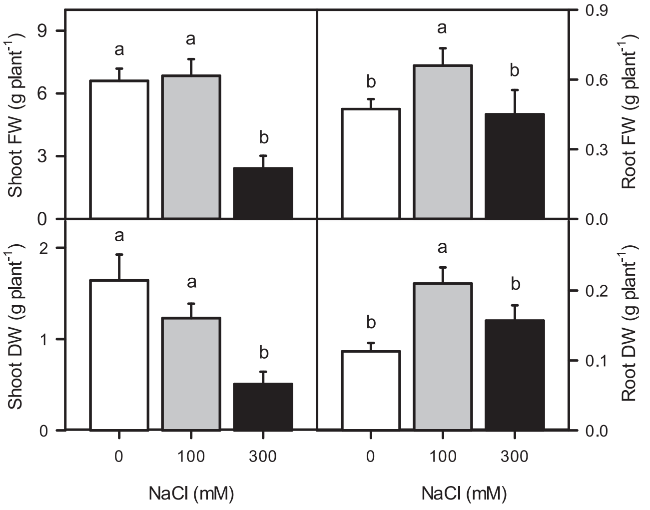

Fig. 1. Effect of 35 days of salinity (0, 100 and 300 mM NaCl) treatments on shoot and root fresh weight (FW) and dry weights (DW) of *Haloxylon stocksii*. Different letters above bars indicate significant differences between means ± S.E. (n = 3) at $P < 0.05$ (Bonferroni test).

2.8. Statistical analysis

SPSS software (SPSS for windows ver. 11.0) was used for one way analysis of variance (ANOVA) after subjecting the data to the Levene's homogeneity of variance test. Significant differences ($P < 0.05$) among means (± S.E.) were estimated by using the post-hoc Bonferroni test.

3. Results

A significant ($P < 0.05$) increase in root fresh and dry weight (Fig.1) and number of branches per plant in 100 mM NaCl compared to non-saline control (Fig. 2) was observed. Shoot fresh and dry weight, number of nodes and plant height remained unaffected in 100 mM NaCl compared to non-saline control but decreased in 300 mM NaCl (Figs. 1 and 2). Specific shoot length increased only in 300 mM NaCl while specific root length decreased under saline conditions (Fig. 2).

Leaf water, osmotic and turgor potential decreased significantly

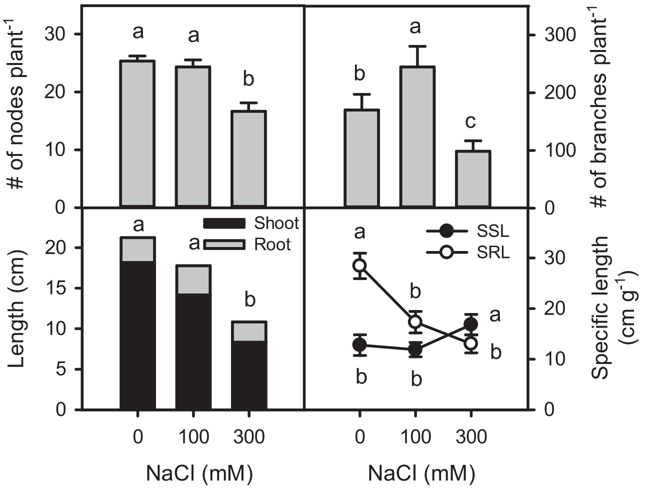

Fig. 2. Effect of 35 days of salinity (0, 100 and 300 mM NaCl) treatments on the number of number of nodes per plant, number of branches per plant, shoot and root length, specific shoot length (SSL; cm g^{-1}) and specific root length (SRL; cm g^{-1}) of *Haloxylon stocksii*. Different letters above bars indicate significant differences between means ± S.E. (n = 3) at $P < 0.05$ (Bonferroni test).

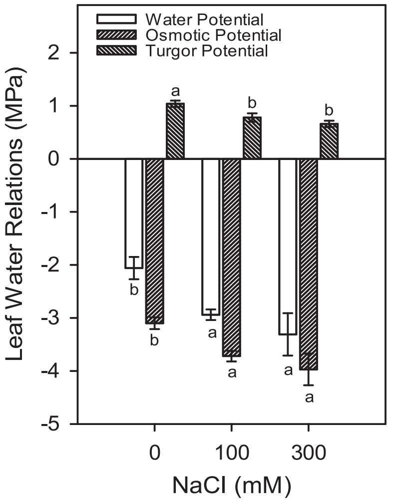

Fig. 3. Effect of 35 days of salinity (0, 100 and 300 mM NaCl) treatments on the shoot water and osmotic potential and turgor potential of *Haloxylon stocksii.* Different letters above bars indicate significant differences between means ± S.E. (n = 3) at $P < 0.05$ (Bonferroni test).

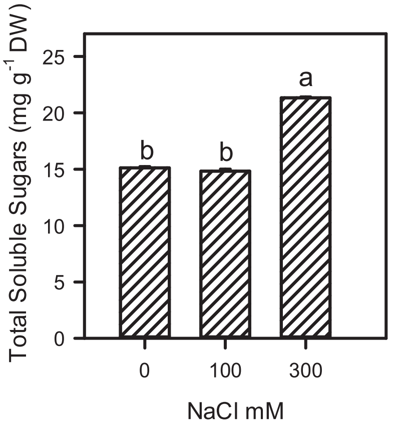

Fig. 4. Effect of 35 days of salinity (0, 100 and 300 mM NaCl) treatments on the total soluble sugar content of *Haloxylon stocksii* shoots. Different letters above bars indicate significant differences between means ± S.E. (n = 3) at $P < 0.05$ (Bonferroni test).

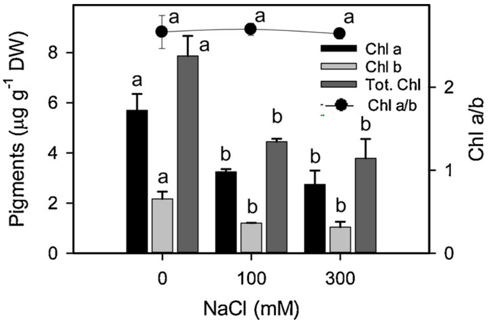

Fig. 5. Effect 35 days of salinity (0, 100 and 300 mM NaCl) treatments on photosynthetic pigments and chlorophyll a/b ratio of *Haloxylon stocksii.* Different letters above bars indicate significant differences between means ± S.E. (n = 3) at $P < 0.05$ (Bonferroni test).

Table 1

Gas-exchange parameters (Pn, photosynthesis; R, respiration; Gs, stomatal conductance; E, transpiration rate; Ci, intercellular concentration of CO_2), water use efficiency (WUE) and ratio of intercellular to ambient carbon dioxide concentration (Ci/Ca) of *Haloxylon stocksii* treated with 0, 100 and 300 mM NaCl for 35 days. Different letters with means ± SE (n = 3) indicate significant differences at $P < 0.05$ (Bonferroni test).

Gas exchange parameters	NaCl (mM) 0	 100	 300
Pn (µmol CO_2 m^{-2} s^{-1})	6.88 ± 0.20a	5.61 ± 0.77a	4.42 ± 0.49b
R (µmol CO_2 m^{-2} s^{-1})	3.27 ± 0.71a	3.56 ± 0.35a	2.67 ± 0.38b
Gs (mol H_2O m^{-2} s^{-1})	0.09 ± 0.01a	0.08 ± 0.02a	0.04 ± 0.01b
E (mmol H_2O m^{-2} s^{-1})	1.62 ± 0.02a	1.41 ± 0.24a	0.76 ± 0.09b
Ci (µmol mol^{-1})	252.61 ± 0.39a	261.62 ± 6.53a	181.35 ± 7.38b
WUE (µmol CO_2 $mmol^{-1}$ H_2O)	4.23 ± 0.06b	4.03 ± 0.14b	6.41 ± 0.24a
Ci/Ca	0.66 ± 0.01a	0.66 ± 0.01a	0.46 ± 0.01b

($P < 0.05$) under saline conditions (Fig. 3). Total soluble sugars increased significantly ($P < 0.05$) in 300 mM NaCl by 30% but remained unchanged in 100 mM compared to non-saline control (Fig. 4). Chlorophyll concentration was decreased ($P < 0.05$) in 100 and 300 mM NaCl compared to control while Chlorophyll a/b ratios remained unaffected (Fig. 5).

Significant variations were recorded for gas exchange characteristics of *Haloxylon stocksii* grown in saline conditions compared with control treatment during the experimental growth period. Gas exchange parameters *i.e.* Pn (net photosynthesis), Gs (stomatal conductance), Ci (intracellular CO_2 concentration), E (transpiration), remained similar in control and 100 mM NaCl but decreased by ca. 53% (Gs, E) in 300 mM ($P < 0.05$; Table 1). Water Use Efficiency (WUE) was increased significantly by 52% in 300 mM NaCl compared to other treatments. A 35% reduction in net photosynthesis, Ci and dark respiration (R) was found in plants exposed to 300 mM NaCl ($P < 0.05$). Fluorescence values indicated no change in potential photochemical quantum yield of PSII (Fv/Fm) in all treatments. Effective photochemical quantum yield [Y(II)], relative electron transport rate (rETR) and photochemical quenching (qP) decreased only in 300 mM NaCl (Table 2). Non-photochemical quenching (NPQ) increased by ca. 35% at 100 and 300 mM NaCl compared to control plants (Table 2). Y(NPQ) increased by 20% in 100 and 300 mM NaCl in comparison to control plants. Y(NO) was significantly decreased in 100 and 300 mM NaCl compared to control. Y (NPQ) and Y(NO) showed no significant difference between 100 and 300 mM NaCl (Table 2).

Phenol, flavonoids and tannin content decreased significantly in 100 and 300 mM NaCl treatments compared to non-saline control (Table 3). Nitrate concentration steadily decreased with increasing salinity. Saponin content was unaffected in 100 mM NaCl, but decreased significantly in 300 mM NaCl. Highest amount of total oxalate and acid soluble oxalate were recorded in 100 mM NaCl but remained unchanged in 300 mM NaCl in comparison to control plants. Water

Table 2

Effect of 35 d of salinity (0, 100 and 300 mM NaCl) treatments on the potential photochemical quantum yield of PSII [Fv/Fm]; effective photo-chemical quantum yield of PSII [Y(II)]; relative electron transport rate [rETR]; photochemical quenching [qP]; non-photochemical quenching [NPQ]; Yield of heat dissipation [Y(NPQ)]; Yield of non-potochemical losses other than heat [Y(NO)] in photosynthetic branches of *Haloxylon stocksii*. Different letters with means ± SE (n = 4) indicate significant differences at $P < 0.05$ (Bonferroni test).

Chlorophyll Fluorescence Parameters	NaCl (mM)		
	0	100	300
Fv/Fm	0.68 ± 0.02a	0.67 ± 0.01a	0.69 ± 0.01a
rETR (µmol e^- m^{-2} s^{-1})	40.66 ± 3.48a	39.33 ± 2.90a	30.33 ± 3.66b
qP	0.29 ± 0.05a	0.28 ± 0.05a	0.17 ± 0.01b
NPQ	0.69 ± 0.01b	0.95 ± 0.08a	0.94 ± 0.09a
Y(II)	0.13 ± 0.01a	0.13 ± 0.01a	0.09 ± 0.01b
Y(NPQ)	0.35 ± 0.01b	0.42 ± 0.02a	0.43 ± 0.02a
Y(NO)	0.52 ± 0.01a	0.45 ± 0.01b	0.46 ± 0.01b

Table 3
Effect of 35 d of salinity (0, 100 and 300 mM NaCl) treatments on anti-nutritive contents (% dry weight basis) in photosynthetic shoots of *Haloxylon stocksii*. Different letters with means ± SE (n = 4) indicate significant differences at $P < 0.05$ (Bonferroni test).

Anti-nutrients (% DW)	NaCl (mM)		
	0	100	300
Phenols	2.07 ± 0.06a	1.43 ± 0.01b	1.25 ± 0.02c
Flavonoids	0.35 ± 0.01a	0.27 ± 0.01b	0.21 ± 0.01c
Tannins	2.11 ± 0.01a	1.43 ± 0.01b	1.15 ± 0.01c
Nitrates	0.35 ± 0.01a	0.27 ± 0.01b	0.21 ± 0.01c
Saponins	1.77 ± 0.03a	1.72 ± 0.02a	0.81 ± 0.02b
Total oxalates	2.94 ± 0.06b	3.91 ± 0.70a	2.81 ± 0.66b
Acid soluble oxalates	1.62 ± 0.18b	2.52 ± 0.64a	1.80 ± 0.72b
Water soluble oxalates	1.38 ± 0.15a	1.38 ± 0.06a	1.08 ± 0.21b

soluble oxalate decreased by 22% in 300 mM in comparison to control plants (Table 3).

4. Discussion

Haloxylon stocksii showed an increase in the number of branches and root growth at 100 mM NaCl as in some other moderately salt resistant species (Flowers and Colmer, 2008; Slama et al., 2017). Root growth stimulation under moderately saline conditions is reported for some salt resistant grasses as well (Khan et al., 2000; Belkheiri and Mulas, 2013). Optimum root growth was reported in moderate NaCl in *Phragmites karka* (Abideen et al., 2014), *Spartina maritima*, *Pennisetum clandestinum* and *Plantago coronopus* (Koyro, 2014). Some grasses like *Halopyrum mucronatum*, *Sporobolus ioclados*, *Aeluropus lagopoides*, *Sporobolus tremulus* and *Paspalidium paspalodes* showed relatively higher growth under non-saline conditions (Khan et al., 1999; Moinuddin et al., 2014). Extensive root growth could be beneficial for absorption of K$^+$ and storage of excessive Na$^+$ in the root of *H. stocksii* by preventing Na$^+$ transport into the xylem stream as reported for *Atriplex griffithii, Phragmites karka*, *Distichlis spicata*, *Sporobolous airoides*, *Cynodon dactylon, Zoysia japonica* (Khan et al., 2000; Munns and Tester, 2008; Abideen et al., 2014). Root growth was maintained at 300 mM NaCl, thereby limiting net Na$^+$ influx and preventing Na$^+$ translocation to the shoot as reported in *Haloxylon recurvum* (Keisham et al., 2018). Increasing salinity concentration significantly reduced the root and shoot K$^+$ content while Na$^+$ was substantially increased in *H. recurvum* with unchanged root growth (Khan et al., 2000). Increase in root biomass under moderate salinity treatment but not in shoot biomass is suggestive of higher sensitivity of roots over shoots which is a common feature of xero-halophytes for successful adaptation in arid and saline habitats (Gorai and Neffati, 2011). An increase in specific shoot length (SSL) along with a concomitant decrease in specific root length (SRL) could also be related to an increase in inorganic content in aboveground parts such as in other salt includers of family Amaranthaceae (Khan et al., 2000).

Haloxylon stocksii showed an abrupt decrease in shoot water and osmotic potentials at 100 mM NaCl which indicates its osmoregulator strategy (Aziz and Khan, 2000; Shoukat et al., 2018). Khan et al. (2000) suggested that *H. stockii* could accumulate considerable salts in its tissues counterbalanced by synthesis of glycine betaine, an organic solute (Slama et al., 2007). However, synthesis of organic solutes requires energy at the cost of reduced plant growth (Rhodes et al., 2002; Slama et al., 2007). Choline, glycinebetaine, proline and trigonelline and their derivatives are nitrogenous osmolytes (Munns, 2002; Munns and Tester, 2008) while soluble sugars have also been reported among organic osmolytes (Gil et al., 2013). The increase in total soluble sugars in *H. stocksii* under high salinity (300 mM NaCl) could be related to decreased sugar export from stressed leaves to sink tissues (Moinuddin et al., 2014; Shoukat et al., 2019) and may result in reduced energy supply in sinks under salt stress (Gil et al., 2013). Antioxidant activity of sugars is also reported under saline conditions (Hassine et al., 2008; Sami et al., 2016).

Salt and drought stress often decrease the rate of photosynthesis in plants (Chaves et al., 2009; Geissler et al., 2009). A rapid decline in stomatal conductance could be an indicator of water deficit stress avoidance such as in *Chromolaena odorata* (Naidoo and Naidoo, 2018). Plants respond to low soil water availability either through stomatal limitation (by restricting CO$_2$ availability), or by non-stomatal (biochemical) limitation of photosynthesis which could have damaging salt effects on photosynthetic machinery (Flexas et al., 2004; Pagter et al., 2009; Drake et al., 2017). In contrast, decrease in photosynthesis along with a concomitant decrease in stomatal conductance and intercellular CO$_2$ concentration indicated stomatal limitation of photosynthesis in *H. stocksii* at high salinity (300 mM NaCl). In this study, photosynthetic rates (Pn) were not affected under moderate (100 mM) salinity treatments indicating availability of energy resources for maintaining plant growth, whereas decrease (≈35%) in photosynthesis under 300 mM NaCl could be associated with energy starvation and reduced plant growth (Marschner, 1995).

Excess electron flow through the PS II that cannot be used in thylakoid electron transport is either neutralized by non-photochemical quenching (NPQ) or through alternative electron sinks such as Mehler reaction, photorespiration or cyclic electron transport (Takahashi and Murata, 2008). Plants appear to deal with stress by coordination of multiple physiological traits (such as water relations and photosynthetic efficiency) rather than individual traits which may in turn indicate adaptations to the natural habitat where they occur (Gleason et al., 2017). The potential quantum yield of PSII (Fv/Fm), an indicator of photo-inhibition, was not affected by salinity in two xero-halophytes, *Nitraria retusa* and *Atriplex halimus* (Boughalleb et al., 2009). Similar results were obtained in case of *H. stocksii* in this study where NPQ rose constitutively under saline conditions. Similar results were also obtained for three of the four sub-tropical halophytic grasses tested by Moinuddin et al. (2017). Non-photochemical quenching (NPQ) or heat dissipation through the xanthophyll cycle is a ubiquitous mean to get rid of the excess light energy absorbed by photosynthetic tissues and prevents photo-inhibition (Bilger and Bjorkman, 1994). In *H. stocksii*, at high salinity (300 mM NaCl) NPQ competes with other non-regulated photochemical quenching mechanisms indicated by the relative changes in yield of photochemistry, Y(II), the yield of non-photochemical quenching, Y(NPQ) and the yield of non-regulated (non-enzymatic) processes including fluorescence and heat dissipation, Y(NO) (Han et al., 2010; Moinuddin et al., 2017). In addition, decreases in rETR, Y (II) and pigment contents along with decreased growth of *H. stocksii* at high salinity could be linked with the reduction in net assimilation rate and oxidative injury.

Soil salinity is also known to enhance the synthesis of secondary metabolites in plants (Bandaranayake, 2002). For example, polyphenols (phenols, flavonoids and tannins) could help in improving antioxidant activity of plants under salt stress and also possess medicinal and food value at low to moderate levels in plants (Hamada et al., 2016). However, higher concentration of polyphenols could be harmful for foraging animals. Polyphenols in *H. stocksii* did not generally increase beyond acceptable limits for livestock even under high salinity with growth reduction. Animal feed intake is increased if tannins in plant parts are found in low doses (≈4.5%); however, an increase to ≈9% of dry weight reduced food consumption in lambs (Villalba et al., 2004). Barry and McNabb (1999) recommended 0.5–3% of tannins as the acceptable limit for animal fodder. In this study, *H. stocksii* appeared to accumulate low tannin content (between 1.2 and 2.1%) which supports its use as animal fodder. Low tannin content is known to reduce mineral chelation (*e.g.*, calcium, zinc, and iron) helping in bioavailability of these nutrients (Kruger et al., 2013). Relatively low to moderate saponin content of *H. stocksii* (≈0.80–1.7%; *i.e.*, < 2%) in this study, would prevent high blood cholesterol (Burns, 1978) and weight loss in animals (Bosler et al., 1997) as higher saponin levels are reported to increase hemolytic and foaming activity and develop bitter taste (Burns, 1978). Thus, the saponin content in *H. stocksii* is generally lower than in most varieties of *Medicago sativa* (alfalfa) that were reported to be in the range of 0.3–3.8% (Small et al., 1990). Nitrates in *H. stocksii* remained low ($< 0.35\%$ of dry weight) and within acceptable limits for forage intake in this experiment. High ($> 1.5\%$) nitrate content is reported to reduce dietary intake of energy rich food (Burritt and Provenza, 2000). Low ($\leq 2\%$) oxalate content is recommended (Njidda, 2010) which would otherwise influence calcium absorption in ruminants causing impaired kidney functions (Malcolm, 1988). In *H. stocksii*, the increase in oxalates ($\approx 3\%$ of dry weight) under moderate salinity treatment was also within acceptable range for fodder (Njidda, 2010) and even lower than common halophytic fodder plants (3–5%; El-Shaer, 2010). Furthermore, levels of toxic anti-nutrients such as saponins have been reported to decrease substantially in ensilaged alfalfa by up to 12–100% (Kalač et al., 1996).

In conclusion, our first hypothesis proved to be partially true whereby only root growth was improved while photosynthetic responses remained unaffected under moderate salinity treatment. The second hypothesis was true as plants employed constitutively higher NPQ to avoid photo-inhibition under saline conditions. The third hypothesis proved false as most of the anti-nutritive compounds decreased with increases in salinity. This study suggests that *H. stocksii* is a moderately drought and salt tolerant plant with low anti-nutritive factors, within acceptable range for animal intake. However, further studies on *H. stocksii* plants growing in diverse natural habitats would be required to better understand its adaptations to abiotic stresses as well as grazing pressure.

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References

Abideen, Z., Koyro, H.W., Huchzermeyer, B., Ahmed, M.Z., Gul, B., Khan, M.A., 2014. Moderate salinity stimulates growth and photosynthesis of *Phragmites karka* by water relations and tissue specific ion regulation. Environ. Exp. Bot. 105, 70–76.

Abideen, Z., Qasim, M., Rasheed, A., Adnan, M.Y., Gul, B., Khan, M.A., 2015. Antioxidant activity and polyphenol content of *Phragmites karka* under saline conditions. Pak. J. Bot. 47, 813–818.

Ahmed, E., Nawaz, S.A., Malik, A., Choudhary, M.I., 2006. Isolation and cholinesterase-inhibition studies of sterols from Haloxylon recurvum. Bioorg. Med. Chem. Lett. 16, 573–580.

Ali, H., Gul, B., Adnan, M.Y., Ahmed, M.Z., Aziz, I., Gulzar, S., Ansari, R., Khan, M.A., 2014. NPK mediated improvement in biomass production, photosynthesis and Na^+ regulation in *Panicum antidotale* under saline conditions. Pak. J. Bot. 6, 1975–1979.

Aziz, I., Khan, M.A., 2000. Physiological adaptations to seawater concentration in *Avicennia marina* from Indus delta, Pakistan. Pak. J. Bot. 32, 171–189.

Baker, N.R., Rosenqvist, E., 2004. Applications of chlorophyll fluorescence can improve crop production strategies: an examination of future possibilities. J. Exp. Bot. 55, 1607–1621.

Bandaranayake, W.M., 2002. Bioactivities, bioactive compounds and chemical constituents of mangrove plants. Wetl. Ecol. Manag. 10, 421–452.

Barry, T.N., McNabb, W.C., 1999. The implications of condensed tannins on the nutritive value of temperate forages fed to ruminants. Br. J. Nutr. 81, 263–272.

Belkheiri, O., Mulas, M., 2013. The effects of salt stress on growth, water relations and ion accumulation in two halophyte *Atriplex* species. Environ. Exp. Bot. 86, 17–28.

Bilger, W., Björkman, O., 1994. Relationships among violaxanthin deepoxidation, thylakoid membrane conformation, and non-photochemical chlorophyll fluorescence quenching in leaves of cotton (*Gossypium hirsutum*). Planta 193, 238–246.

Bose, J., Munns, R., Shabala, S., Gilliham, M., Pogson, B., Tyerman, S.D., 2017. Chloroplast function and ion regulation in plants growing on saline soils: lessons from halophytes. J. Exp. Bot. 68, 3129–3143.

Bosler, D.A., Blümmel, M., Bullerdieck, P., Makkar, H.P.S., Becker, K., 1997. Influence of a saponin containing feed additive on mass development and carcass evaluation of growing lambs. Proc. Soc. Nutr. Physiol. 6, 46.

Boughalleb, F., Denden, M., Tiba, B.B., 2009. Photosystem II photochemistry and physiological parameters of three fodder shrubs, *Nitraria retusa*, *Atriplex halimus* and *Medicago arborea* under salt stress. Acta Physiol. Plant. 31, 463–476.

Burns, J.C., 1978. Anti-quality factors as related to forage quality. J. Dairy Sci. 6, 1809–1820.

Burritt, E.A., Provenza, F.D., 2000. Role of toxins in intake of varied diets by sheep. J. Chem. Ecol. 26, 1991–2005.

Cataldo, D.A., Haroon, M., Schrader, L.E., Young, V.L., 1975. Rapid colorimetric determination of nitrate in plant tissue by nitration of salicylic acid. Commun. Soil Sci. Plant Anal. 6, 71–80.

Chang, C., Yang, M., Wen, H., Chern, J., 2002. Estimation of total flavonoids content in Propolis by two complementary colorimetric methods. J. Food Drug Anal. 10, 178–182.

Chaves, M.M., Flexas, J., Pinheiro, C., 2009. Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell. Ann. Bot. 103, 551–560.

Cude, S.M., Ankeny, M.D., Norton, J.B., Kelleners, T.J., Strom, C.F., 2018. Capillary barriers improve reclamation in drastically disturbed semiarid shrub land. Arid Land Res. Manag. 32, 259–276.

Drake, J.E., Power, S.A., Duursma, R.A., Medlyn, B.E., Aspinwall, M.J., Choat, B., Creek, D., Eamus, D., Maier, C., Pfautsch, S., Smith, R.A., 2017. Stomatal and non-stomatal limitations of photosynthesis for four tree species under drought: a comparison of model formulations. Agric. For. Meteorol. 247, 454–466.

Ehsen, S., Qasim, M., Abideen, Z., Rizvi, R.F., Gul, B., Ansari, R., Khan, M.A., 2016. Secondary metabolites as anti-nutritional factors in locally used halophytic forage/fodder. Pak. J. Bot. 48, 629–636.

Ehsen, S., Rizvi, R.F., Abideen, Z., Aziz, I., Gulzar, S., Gul, B., Khan, M.A., Ansari, R., 2017. Physiochemical responses of *Zaleya pentandra* (l.) Jeffrey to NaCl treatments. Pak. J. Bot. 49, 801–808.

El-Shaer, H.M., 2010. Halophytes and salt-tolerant plants as potential forage for ruminants in the Near East region. Small Rumin. Res. 91, 3–12.

Epstein, E., 1972. Mineral Nutrition of Plants: Principles and Perspectives. John Wiley and Sons, New York.

Flexas, J., Bota, J., Loreto, F., Cornic, G., Sharkey, T.D., 2004. Diffusive and metabolic limitations to photosynthesis under drought and salinity in C_3 plants. Plant Biol. 6, 269–279.

Flowers, T.J., Colmer, T.D., 2008. Salinity tolerance in halophytes. New Phytol. 179, 945–963.

Geissler, N., Hussin, S., Koyro, H.W., 2009. Elevated atmospheric CO_2 concentration ameliorates effects of NaCl salinity on photosynthesis and leaf structure of *Aster tripolium*. J. Exp. Bot. 60, 137–151.

Genty, B., Briantais, J.M., Baker, N.R., 1989. The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochim. et Biophy. Acta (BBA)-General Subjects 990, 87–92.

Gil, R., Boscaiu, M., Lull, C., Bautista, I., Lidón, A., Vicente, O., 2013. Are soluble carbohydrates ecologically relevant for salt tolerance in halophytes? Funct. Plant Biol. 40, 805–818.

Gleason, S.M., Wiggans, D.R., Bliss, C.A., Comas, L.H., Cooper, M., DeJonge, K.C., Young, J.S., Zhang, H., 2017. Coordinated decline in photosynthesis and hydraulic conductance during drought stress in Zea mays. Flora 227, 1–9.

Gorai, M., Neffati, M., 2011. Osmotic adjustment, water relations and growth attributes of the xero-halophyte *Reaumuria vermiculata* L. (Tamaricaceae) in response to salt stress. Acta Physiol. Plant. 33, 1425–1433.

Guerrier, G., 1996. Fluxes of Na^+, K^+ and Cl^- and osmotic adjustment in *Lycopersicon pimpinellifolium* and *L. Esculentum* during short and long-term exposures to NaCl. Physiol. Plant. 97, 583–591.

Gulzar, S., Hameed, A., Ahmed, M.Z., Khan, M.A., et al., 2014. Is soil heterogeneity the major factor influencing vegetation zonation at Karachi coast? In: Khan, M.A. (Ed.), Sabkha Ecosystems: Volume IV: Cash Crop Halophyte and Biodiversity Conservation, Tasks for Vegetation Science 47. 14, Springer, Dordrecht. https://doi.org/10.1007/978-94-007-7411-7.

Hamada Abdelgawad, G.Z., Hegab, M.M., Pandey, R., Asard, H., Abuelsoud, W., 2016. High salinity induces different oxidative stress and antioxidant responses in maize seedlings organs. Front. Plant Sci. 7, 1–12.

Hammer, K., Pignone, D., Cifarelli, S., Perrino, P., 1990. Barilla (*Salsola soda*, Chenopodiaceae). Econ. Bot. 44, 410–412.

Han, W., Xu, X.W., Li, L., Lei, J.Q., Li, S.Y., 2010. Chlorophyll *a* Fluorescence responses of *Haloxylon ammodendron* seedlings subjected to progressive saline stress in the Tarim desert highway ecological shelterbelt. Photosynthetica 48, 635–640.

Hasanuzzaman, M., Nahar, K., Alam, M.M., Bhowmik, P.C., Hossain, M.A., Rahman, M.M., Prasad, M.N.V., Ozturk, M., Fujita, M., 2014. Potential use of halophytes to remediate saline soils. Bio. Med. Res. 1–13.

Hassine, A.B., Bouzid, S., Lutts, S., 2010. Does habitat of *Atriplex halimus* L. Affect plant strategy for osmotic adjustment? Acta Physiol. Plant. 32, 325–331.

Hassine, A.B., Ghanem, M.E., Bouzid, S., Lutts, S., 2008. An inland and a coastal population of the Mediterranean xero-halophyte species *Atriplex halimus* L. Differ in their ability to accumulate proline and glycinebetaine in response to salinity and water stress. J. Exp. Bot. 59, 1315–1326.

Hiai, S., Oura, H., Nakajima, T., 1976. Color reaction of some sapogenins and saponins with vanillin and sulfuric acid. Planta Med. 29, 116–122.

Jafri, S.S.H., 1966. Flora of Karachi. Nizamsons, Karachi.

Kalač, P., Price, K.R., Fenwick, G.R., 1996. Changes in saponin content and composition during the ensilage of alfalfa (*Medicago sativa* L.). Food Chem. 56, 377–380.

Karimi, S.H., Ungar, I.A., 1986. Oxalate and inorganic ion concentrations in *Atriplex triangularis* Willd. Organs in response to salinity, light level, and aeration. Bot. Gaz. 147, 65–70.

Keisham, M., Mukherjee, S., Bhatla, S.C., 2018. Mechanisms of sodium transport in plants – progresses and challenges. Int. J. Mol. Sci. 19, 647.

Khan, M.A., 1990. The relationship of seed bank to vegetation in a saline desert community. In: Sen, D.N., Mohammed, S. (Eds.), Marvel of Seeds. Proceeding of International Seed Symposium, Jodhpur, India, pp 87-92.

Khan, M.A., Qaiser, M., 2006. Halophytes of Pakistan: characteristics, distribution and potential economic usages. In: Khan, M.A., Benno, B., Kust, G.S., Barth, H.J. (Eds.), Sabkha Ecosystems. Tasks for Vegetation Science Volume 42. Springer, Dordrecht, pp. 129–153.

Khan, M.A., Ungar, I.A., Showalter, A.M., 1999. Effects of salinity on growth, ion content, and osmotic relations in *Halopyrum mucronatum* (L.) Stapf. J. Plant Nutr. 22, 191–204.

Khan, M.A., Ungar, I.A., Showalter, A.M., 2000. Effects of salinity on growth, water relations and ion accumulation of the subtropical perennial halophyte *Atriplex griffithii*var.*stocksii*. Ann. Bot. 85, 225–232.

Khan, M.A., Ansari, R., Ali, H., Gul, B., Nielsen, B.L., 2009. *Panicum turgidum*, a potentially sustainable cattle feed alternative to maize for saline areas. Agric. Ecosyst. Environ. 129, 542–546.

Khassanov, O.K., Rachimova, T., Tadzhiev, S.F., 1994. Biological characteristics of newly cultivated fodder plants for pastures of the arid zone in Uzbekistan. Genet. Resour. Crop Evol. 41, 125–131.

Knudson, L.L., Tibbitts, T.W., Edwards, G.E., 1977. Measurement of ozone injury by determination of leaf chlorophyll concentration. Plant Physiol. 60, 606–608.

Koyro, H.W., Khan, M.A., Lieth, H., 2011. Halophytic crops: a resource for the future to reduce the water crisis? Emir. J. Food Agric. 23, 1.

Koyro, H.W., Lieth, H., Gul, B., Ansari, R., Huchzermeyer, B., Abideen, Z., Hussain, T., Khan, M.A., Khan, M.A., 2014. Importance of the diversity within the halophytes to agriculture and land management in arid and semiarid countries. In: In: Böer, B., Öztürk, M., Al Abdessalaam, T.Z., Clüsener-Godt, M., Gul, B. (Eds.), Sabkha Ecosystems: Tasks for Vegetation Science, vol. 47. Springer, Dordrecht, pp. 175–198. https://doi.org/10.1007/978-94-007-7411-7_12.

Kruger, J., Taylor, J.R., Du, X., De Moura, F.F., Lönnerdal, B., Oelofse, A., 2013. Effect of phytate reduction of sorghum, through genetic modification, on iron and zinc availability as assessed by an in vitro dialysability bioaccessibility assay, Caco-2 cell uptake assay, and suckling rat pup absorption model. Food Chem. 141, 1019–1025.

Lichtenthaler, K., Buschmann, C., 1987. Chlorophylls and carotenoids: measurement and characterization by UV VIS spectroscopy. Current Protocols in Food Analytical Chemistry. John Wiley and Sons Inc, New York, pp. 1–8.

Ludwig, T.G., Goldberg, H.J.V., 1956. The anthrone method for the determination of carbohydrates in foods and in oral rinsing. J. Dental Res. 35, 90–94.

Makkar, H.P.S., Francis, G., Becker, K., 2007. Bioactivity of phytochemicals in some lesser-known plants and their effects and potential applications in livestock and aquaculture production systems. Animal 1, 1371–1391.

Malcolm, C.V., Clarke, A.J., D'Antuono, M.F., Swann, T.C., 1988. Effect of plant spacing and soil conditions on the growth of five *Atriplex* species. Agric. Ecosyst. Environ. 21, 265–279.

Manousaki, E., Kalogerakis, N., 2011. Halophytes an emerging trend in phytoremediation. Int. J. Phytoremediation 13, 959–969.

Maricle, B.R., Cobos, D.R., Campbell, C.S., 2007. Biophysical and morphological leaf adaptations to drought and salinity in salt marsh grasses. Environ. Exp. Bot. 60, 458–467.

Marschner, H., 1995. Mineral Nutrition of Higher Plants. Academic Press (U.K), London.

Moinuddin, M., Gulzar, S., Ahmed, M.Z., Gul, B., Koyro, H.W., Khan, M.A., 2014. Excreting and non-excreting grasses exhibit different salt resistance strategies. AoB Plant 6https://doi.org/10.1093/aobpla/plu038. plu038.

Moinuddin, M., Gulzar, S., Hameed, A., Gul, B., Khan, M.A., Edwards, G.E., 2017. Differences in photosynthetic syndromes of four halophytic marsh grasses in Pakistan. Photosynth. Res. 131, 51–64.

Munir, N., Abideen, Z., Sharif, N., 2019. Development of halophytes as energy feedstock by applying genetic manipulations. Front. Life Sci. https://doi.org/10.1080/21553769.2019.1595745.

Munns, R., 2002. Comparative physiology of salt and water stress. Plant Cell Environ. 25, 239–250.

Munns, R., Tester, M., 2008. Mechanisms of salinity tolerance. Annu. Rev. Plant Biol. 59, 651–681.

Naidoo, G., Naidoo, K.K., 2018. Drought stress effects on gas exchange and water relations of the invasive weed Chromolaena odorata. Flora 248, 1–9.

Nikalje, G.C., Srivastava, A.K., Pandey, G.K., Suprasanna, P., 2018. Halophytes in biosaline agriculture: mechanism, utilization, and value addition. Land Degrad. Dev. 29, 1081–1095.

Njidda, A.A., 2010. Chemical composition, fibre fraction and anti-nutritional substances of semi-arid browse forages of North-Eastern Nigeria. Nig. J. Basic Appl. Sci. 18, 181–188.

Pagter, M., Bragato, C., Malagol, M., Brix, H., 2009. Osmotic and ionic effects of NaCl and Na_2SO_4 salinity on *Phragmites australis*. Aquat. Bot. 90, 43–51.

Panuccio, M.R., Jacobsen, S.E., Akhtar, S.S., Muscolo, A., 2014. Effect of saline water on seed germination and early seedling growth of the halophyte quinoa. AoB Plants 6https://doi.org/10.1093/aobpla/plu047. plu047.

Pearson, D., 1976. The Chemical Analysis of Food, 4th ed. Churchill Living Edndiburgh London, pp. 78–80.

Qasim, M., Abideen, Z., Adnan, M.Y., Gulzar, S., Gul, B., Rasheed, M., Khan, M.A., 2017. Antioxidant properties, phenolic composition, bioactive compounds and nutritive value of medicinal halophytes commonly used as herbal teas. S. Afr. J. Bot. 110, 240–250.

Raiten, D.J., Aimone, A.M., 2017. The intersection of climate/environment, food, nutrition and health: crisis and opportunity. Curr. Opin. Biotechnol. 44, 52–62.

Rasool, S.G., Siddiqui, H., Hameed, A., Hussain, T., Aziz, I., Khan, M.A., Gulzar, S., 2019. Diurnal and seasonal changes in water and ion relations of coastal halophytes. In: Gul, B., Böer, B., Khan, M.A., Cluesener-Godt, M., Hameed, A. (Eds.), Sabkha Ecosystems: Volume VI: Asia/Pacific. Springer, Dordrecht, pp. 511. https://doi.org/10.1007/978-3-030-04417-6.

Rathore, V.S., Singh, J.P., Roy, M.M., 2012. *Haloxylon stocksii* (Boiss.) Benth & Hooker, a promising halophyte: distribution, cultivation and utilization. Genet. Resour. Crop Evol. 59, 1213–1221.

Rhodes, D., Nadolska-Orczyk, A., Rich, P.J., 2002. Salinity, osmolytes and compatible solutes. In: Läuchli, A., Lüttge, U. (Eds.), Salinity: Environment– Plant–Molecules. Kluwer, Dordrecht, Netherlands, pp. 181–204.

Sami, F., Yusuf, M., Faizan, M., Faraz, A., Hayat, S., 2016. Role of sugars under abiotic stress. Plant Physiol. Biochem. 109, 54–61.

Schreiber, U., Schliwa, U., Bilger, W., 1986. Continuous recording of photochemical and non-photochemical chlorophyll fluorescence quenching with a new type of modulated fluorometer. Photosynth. Res. 10, 51–62.

Shoukat, E., Aziz, I., Ahmed, M.Z., Abideen, Z., Khan, M.A., 2018. Growth patterns of *Phragmites karka* under saline conditions depend on the bulk elastic modulus. Crop Pasture Sci. 69, 535–545.

Shoukat, E., Abideen, Z., Ahmed, M.Z., Gulzar, S., Nielsen, B.L., 2019. Changes in growth and photosynthesis linked with intensity and duration of salinity in Phragmites karka. Environ. Exp. Bot. 162, 504–514.

Singleton, V.L., Rossi, J.A., 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Enol. Viticult. 16, 144–158.

Slama, I., Rabet, R.M., Ksouri, R., Talbi, O., Debez, A., Abdelly, C., 2017. Effects of salt treatment on growth, lipid membrane peroxidation, polyphenol content, and antioxidant activities in leaves of *Sesuvium portulacastrum* L. Arid Land Res. Manage. 31, 404–417.

Slama, I., Ghnaya, T., Messedi, D., Hessini, K., Labidi, N., Savoure, A., Abdelly, C., 2007. Effect of sodium chloride on the response of the halophyte species *Sesuvium portulacastrum* grown in mannitol-induced water stress. J. Plant Res. 120, 291–299.

Small, E., Jurzysta, M., Nozzolillo, C., 1990. The evolution of hemolytic saponin content in wild and cultivated Alfalfa (*Medicago sativa*, Fabaceae). Econ. Bot. 44, 226–235.

Stewart, R.R., 1972. An annotated catalogue of the vascular plants of West Pakistan and Kashmir. In: Nasir, E., Ali, S.I. (Eds.), Flora of West Pakistan. Fakhri Press, Karachi 1028 pp.

Takahashi, S., Murata, N., 2008. How do environmental stresses accelerate photoinhibition? Trends Plant Sci. 13, 178–182.

Tlili, A., Tarhouni, M., Cerdà, A., Louhaichi, M., Neffati, M., 2018. Comparing yield and growth characteristics of four pastoral plant species under two salinity soil levels. Land Degrad. Dev. 29, 3104–3111.

Toqeer, S., Qasim, M., Abideen, Z., Gul, B., Rasheed, M., Khan, M.A., 2018. Chemical composition and antioxidant activity of seeds of various halophytic grasses. J. Am. Oil Chem. Soc. 95, 1285–1295.

Van Kooten, O., Snel, J.F.H., 1990. The use of chlorophyll fluorescence nomenclature in plant stress physiology. Photosynth. Res. 25, 147–150.

Villalba, J.J., Provenza, F.D., Han, G.D., 2004. Experience influences diet mixing by herbivores: implications for plant biochemical diversity. Oikos 107, 100–109.

Weber, D.J., Gul, B., Khan, M.A., Williams, T., Wayman, P., Warner, S., 2001. Composition of vegetable oil from seeds of native halophytic shrubs. USDA Forest Service Proceedings RMRS-P-21. 2001.