Indian Journal of Agricultural Sciences **88** (8): 1296–1304, August 2018/Article

Effect of salinity and alkalinity on responses of halophytic grasses *Sporobolus marginatus* and *Urochondra setulosa*

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Received: 23 September 2017; Accepted: 26 April 2018

ABSTRACT

An experiment was conducted in micro-plots filled with sandy loam soil having 14% clay and 0.33% organic carbon to see the responses of halophytic grasses *Sporobolus marginatus* and *Urochondra setulosa* collected from the extreme saline-sodic Kachchh plains, Bhuj, Gujarat, India under alkalinity/salinity. Maximum photosynthetic rate was recorded in control treatment. Photosynthetic rate decreased at pH 9.0 + EC 20 dS/m (11.25 and 17.8 µmol CO_2/m^2/s) in *S. marginatus* and *U. setulosa* respectively, along with reduction in stomatal conductance and transpiration rate. In comparison to control, increased accumulation of total soluble sugars and proline may be due to increased osmotic adjustment for both halophytes. Similarly, at mixed stress of pH 9.0 with EC 20 dS/m, the epicuticular wax load increased in both *S. marginatus* and *U. setulosa* (24.0 and 40.0 mg/g). A positive correlation of stress was seen with Na^+ and Cl^- content while negative correlation with K^+ content. Na^+ content increased to about 3-7 fold at salinity level, EC 35 dS/m. Similarly, with treatment of mixed stress of pH 9.0 with saline level EC 20dS/m, 3-6 times increase in Na^+ with decrease in K^+ was observed in *S. marginatus* and *U. setulosa* respectively. These grass species maintained better gas exchange properties with higher osmolytes accumulations and balanced ionic relations under high stress conditions of salinity and alkalinity. These attributes might be providing physiological adaptable mechanisms for growth under salt affected environments.

Key words: Alkalinity, Ionic relations, Osmolytes, Salinity, *Sporobolus marginatus*, *Urochondra setulosa*

Salinity is a severe limitation to agricultural production in different parts of the world. Although traces of soluble salts are invariably found in almost all arable lands, certain geogenic and anthropogenic factors considerably accentuate the salinity problem resulting in partial to complete loss of soil productivity (Sharma and Singh 2015). Notwithstanding the fact that elevated salt levels have adversely impacted the settled agriculture since long, the twin menaces of waterlogging and salinity have attained alarming proportions in the last few decades; especially in irrigated regions. Globally, about 900 million hectares (M ha) constituting approximately 20% of the total agricultural land (Sharma and Singh 2015), are salt affected to varying extents. In India, salt affected soils occupy about 6.73 M ha area which is likely to substantially increase in the coming decades (Sharma and Singh 2015, FAO 2007). Excess salts in soil and water cause several morphological and physiological abnormalities in plants which eventually supress the plant growth. Depending on factors such as agro-climatic conditions, plant species, crop growth stage and the magnitude of salinity, such adverse effects include, *inter alia*, osmotic stress resulting in reduced water uptake and ionic imbalances causing impaired cell functioning (Sharma and Singh 2015, Singh *et al.* 2010).

These limitations render the saline lands either unsuitable or only partially suitable for the majority of crops suggesting the need to improve the crop salt tolerance. In absence of appropriate salt tolerant cultivars, salt-affected soils remain barren with severe repercussions for the national food security and biomass energy production. Halophytes are the plants capable of completing their life cycle under extreme saline conditions which is ascribed to mechanisms such as ion compartmentalization, osmotic adjustment, succulence, antioxidant systems, maintenance of redox status, and salt inclusion or excretion (Flower and Lauchli 1983). These physiological adaptations enable

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them to flourish even in highly saline habitats having up to seawater salinity (~50 dS/m). The economic biomass and yields of halophytes far exceed those of non-halophytes under similar salt concentrations. Consistent with these facts, a study was initiated with two halophytes, *Sporobolus marginatus* and *Urochondra setulosa* for studying their adaptation mechanisms to saline conditions.

Sporobolus marginatus is a variable tufted or stoloniferous perennial belonging to the family *Poaceae*. It naturally grows on dry grasslands of alkali and saline soils (*ushar* lands) of India and the Kafue flood plain in Zambia. *Sporobolus* plants are even seen soils having a pure salt crust. The roots are very thick and are covered with a soft felt of root hairs. *Sporobolus* is distributed from Northwest India to Baluchistan and tropical East Africa within altitude range of 500-1750 m. It is adapted to very dry conditions having extremely low (~300 mm) annual rainfall (www.fao.org).

Urochondra setulosa (Trin.) C.E. Hubbard (Poaceace) is distributed in the several coastal regions of Asia and Africa. It is a dominant species in the sand dunes, saline flats, salt marshes and saltwater creeks in the Indus delta and Hawkes Bay near Karachi. Cattle are often seen grazing on *Urochondra* plants suggesting its potential as a forage crop on saline soils. These observations imply that investigations to precisely delineate the salt tolerance mechanisms in such halophytes assume significance to harness their potential as potential forage crops in abandoned salt-affected lands. Continued cropping with such saline tolerant grasses could also improve soil quality in the long run.

MATERIALS AND METHODS

This experiment was conducted to understand the relative contribution of physiological mechanisms such as gas exchange attributes, chlorophyll fluorescence, osmoprotectants and ionic relations in imparting salt tolerance in *Sporobolus marginatus* (SM) and *Urochondra setulosa* (US). For this purpose, the seeds and root slips of SM and US collected from the extreme saline-sodic Kachchh plains, Bhuj, Gujarat, India were established in pots under controlled conditions in a glass house at ICAR-Central Soil Salinity Research Institute (CSSRI), Karnal (29°43`N, 76°58`E; 245 m above the mean sea level), Haryana, India. After establishment, these grasses were transferred to micro-plots (2.5m × 1.5m × 0.5m) filled with sandy loam soil having 14% clay and 0.33% organic carbon. Annual rainfall of the study site ranges between 700 and 800 mm. The experiment consisted of 9 treatments: two levels of alkalinity (T_1 – pH_2 9.5 and T_2 – pH_2 10.0), three of salinity (T_3 - EC_e 15 dS/m, T_4 - EC_e 25 dS/m, T_5 - EC_e 35/dS m) and three involving combined alkalinity/salinity (T_6 – pH_2 9.0 + EC_e 10 dS/m, T_7 – pH_2 9.0 + EC_e 15 dS/m, and T_8 – pH_2 9.0 + EC_e 20 dS/m) along with control (pH_2 7.1 and EC_e 0.63 dS/m). The net house was covered with a polythene sheet to ensure that rains did not leach the salts and the desired salinity levels are maintained. Salinity was induced by mixing the calculated amounts of NaCl, Na_2SO_4, $CaCl_2$ and $MgSO_4$ salts in 1:1:1:1 ratio by passing 3 pore volumes (700 L) of respective salinity water prepared using equal proportions of Cl^- and SO_4^{-2} of Na^+, Ca^{2+} and Mg^{2+}. Similarly, alkalinity was developed by mixing the desired amounts of $NaHCO_3$ on the basis of sodicity curve drawn earlier for these soils (Singh *et al.* 1992). Irrigation was done at ID/CPE ratio of 0.8 with a constant depth of 6 cm. The plants were harvested from 5 cm above the soil surface after 120 days of transplanting. At harvest, soil samples were also taken from 15-30 cm depth to measure the electrical conductivity (EC_e) and pH_2 in soil saturation extract (1:1 soil:water paste) using the conductivity meter and in 1:2 soil:water suspension using the pH meter, respectively.

Plant sampling was started at 30 days of after the imposition of stress treatments with two more samplings at an interval of one month. Net photosynthetic rate (P_N), transpiration (E), and stomatal conductance (gS) were measured with an infrared open gas exchange system. Same leaves were tagged for measuring the photochemical efficiency using the chlorophyll fluorescence meter. The maximum photochemical efficiency (*Fv/Fm*) and quantum photochemical yield [Y(II)] of photosystem II were determined using a portable pulse modulated fluorescence measurer (Junior PAM Chlorophyll Fluorometer, Germany) after adapting the leaves to dark for 25 min via special leaf clips. The readings were recorded after saturating 1 s light pulses to promote the closing of the photosystem II reaction center.

Osmoprotectants like total soluble sugars and proline were analysed along with protein content. Hundred mg of fresh leaf tissue was homogenized in 80 % (v/v) ethanol and used for the estimation of total soluble sugars using anthrone reagent (Yemm and Willis 1954). Total protein content was estimated using Bradford reagent (Bradford 1976), proline content using acid ninhydrin and sulphosalicylic acid (Bates et al. 1973) and epicuticular wax load using acidic $K_2Cr_2O_7$ (Ebercon *et al.* 1977). Na^+ and K^+ contents were measured with the flame photometer (PFP7, Jenway, Bibby Scientific, UK) after di-acid [HNO_3 : $HClO_4$ (3:1)] digestion of oven dried plant material. Chloride content of leaves was determined volumetrically by the modified method of Chhabra (1973).

All the data were subjected to variance analysis using the SAS (Version 9.3, SAS Institute Inc., Cary, NC, USA). Least significant difference test was applied at 5% probability level to compare the mean differences.

RESULTS AND DISCUSSION

Soil analysis after the removal of plants indicated marginal reductions in pH_2 and considerable decreases in EC_e of the micro-plot soil. Soil pH_2 reduced from 9.5 to 9.15 and from 10.0 to 9.6 in the alkali soils. In contrast, the corresponding reductions in salinity (EC_e) were greater; EC_e declined from 15.0 to 3.2; 25.0 to 6.4 and 35.0 to 14.4 dS/m^{-1}, respectively, in different treatments. A similar trend was noted in combined alkali/salinity treatments where soil

EC_e reduced from 10 to 6; 15 to 7.2; 20 to 9.2 dS/m while pH_2 reduced from 9 to 8.27, 8.33, 8.35, respectively.

Morpho-physiological responses

Growth analysis provides a reliable cue to understanding the plant responses to the environmental stresses. Analysis of variance of various growth parameters showed the effect of salinity and sodicity alone or in combination. Significant variability was observed in all the studied parameters under different treatments. Under sodic conditions, plant height increased in SM, i.e. by 12.83% at pH_2 9.5 and by 23.48% at pH_2 10.0 compared to control (Table 1). By comparison, plant height significantly decreased when SM plants were exposed to saline and combined stress treatments. For example, plant height was 38.06% less at EC_e 35 dS/m and 50.76% less at pH_2 9.0 + EC_e 20 dS/m than control. In case of US, plant height consistently decreased under both the stress conditions (Table 1). Total chlorophyll concentration is used as an index for evaluating the relative effects of stomatal and non-stomatal factors on P_N under stress conditions. Leaf chlorophyll levels invariably dropped both SM and US with increasing salinity/alkalinity in the experimental soil (Table 1). While plant biomass in SM increased by 9.47% at pH 9.5 and 1.17% at pH 10.0 over control, it reduced in saline as well as mixed stress treatments (Fig 1A). On the contrary, salt stress adversely affected plant biomass production in US (Fig 1B).

Gas exchange relations and chlorophyll fluorescence

In comparison to control plants, P_N declined in both the grasses and the maximum decreases of 63.2% and 37.8% occurred in SM and US, respectively, in

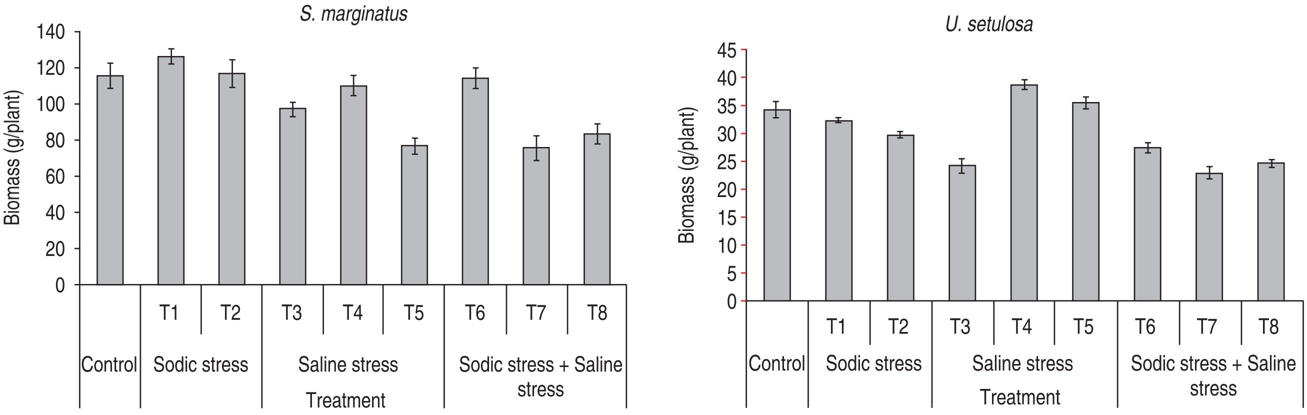

Fig 1 Effects of different salt environments on biomass (g/plant) in *Sporobolus marginatus* (A) and *Urochondra setulosa* (B).

Table 1 Biochemical characterization of *S. marginatus* and *U. setulosa* subjected to salt affected stress

Treatment/Trait	Analysis of variance among determined traits (Means sum of squares)											
	Biochemical analysis in *Sporobolus marginatus*						Biochemical analysis in *Urochondra setulosa*					
	Plant height	CC	TSS	Proline content	Protein content	EWL	Plant height	CC	TSS	Proline content	Protein content	EWL
Control	132.50^{c}	1.08^{A}	3.62^{de}	0.98^{h}	15.80^{c}	6.50^{e}	65.00^{a}	1.01^{A}	4.33^{cd}	0.68^{h}	16.52^{a}	16.50^{d}
pH 9.5	149.50^{b}	0.92^{B}	3.19^{ef}	2.95^{de}	15.93^{bc}	14.00^{d}	59.50^{b}	0.99^{B}	4.99^{b}	3.43^{f}	16.20^{a}	20.50^{cd}
pH 10.0	163.00^{a}	0.79^{DE}	3.08^{f}	4.70^{c}	15.95^{bc}	20.50^{ab}	45.50^{d}	0.96^{C}	3.23^{f}	5.37^{c}	16.00^{a}	24.50^{bcd}
EC 15 dS/m	107.00^{d}	0.83^{C}	2.71^{f}	1.58^{g}	15.40^{d}	16.50^{cd}	50.00^{c}	1.01^{A}	3.56^{ef}	3.47^{f}	14.78^{c}	18.50^{d}
EC 25 dS/m	90.50^{e}	0.79^{DE}	4.07^{cd}	2.88^{e}	15.84^{c}	17.50^{bcd}	44.25^{de}	0.94^{D}	4.08^{de}	5.68^{b}	14.99^{bc}	20.50^{cd}
EC 35 dS/m	81.75^{ef}	0.77^{E}	5.50^{a}	5.10^{a}	15.83^{c}	20.50^{ab}	40.50^{ef}	0.88^{F}	4.64^{bc}	7.20^{a}	15.37^{b}	31.50^{ab}
pH 9.0 + EC 10 dS/m	106.25^{d}	0.80^{D}	4.69^{b}	2.13^{f}	15.89^{bc}	19.00^{bc}	38.00^{fg}	0.91^{E}	4.89^{b}	2.11^{g}	14.45^{c}	21.00^{cd}
pH 9.0 + EC 15 dS/m	78.50^{f}	0.74^{F}	4.55^{bc}	3.00^{d}	16.10^{b}	20.00^{bc}	35.35^{g}	0.82^{G}	5.86^{a}	4.23^{e}	13.83^{d}	30.00^{abc}
pH 9.0 + EC 20 dS/m	65.00^{g}	0.72^{F}	4.20^{c}	4.90^{b}	17.02^{a}	24.00^{a}	25.10^{h}	0.76^{H}	5.66^{a}	4.66^{d}	12.46^{e}	40.00^{a}
General Mean	108.22	0.83	3.96	3.13	15.97	17.61	44.80	0.92	4.58	4.09	14.95	24.78
CV(%)	3.59	1.31	5.31	1.64	0.60	9.79	4.32	0.38	5.19	0.78	1.71	18.11
SE(d)	3.888	0.011	0.210	0.052	0.096	1.724	1.936	0.003	0.238	0.032	0.255	4.486
LSD (P=0.05)	8.9666	0.0249	0.4843	0.1188	0.2203	3.9756	4.4642	0.008	0.5488	0.0739	0.5884	10.345

Least significant difference test was applied at 5% probability level to compare the mean differences.

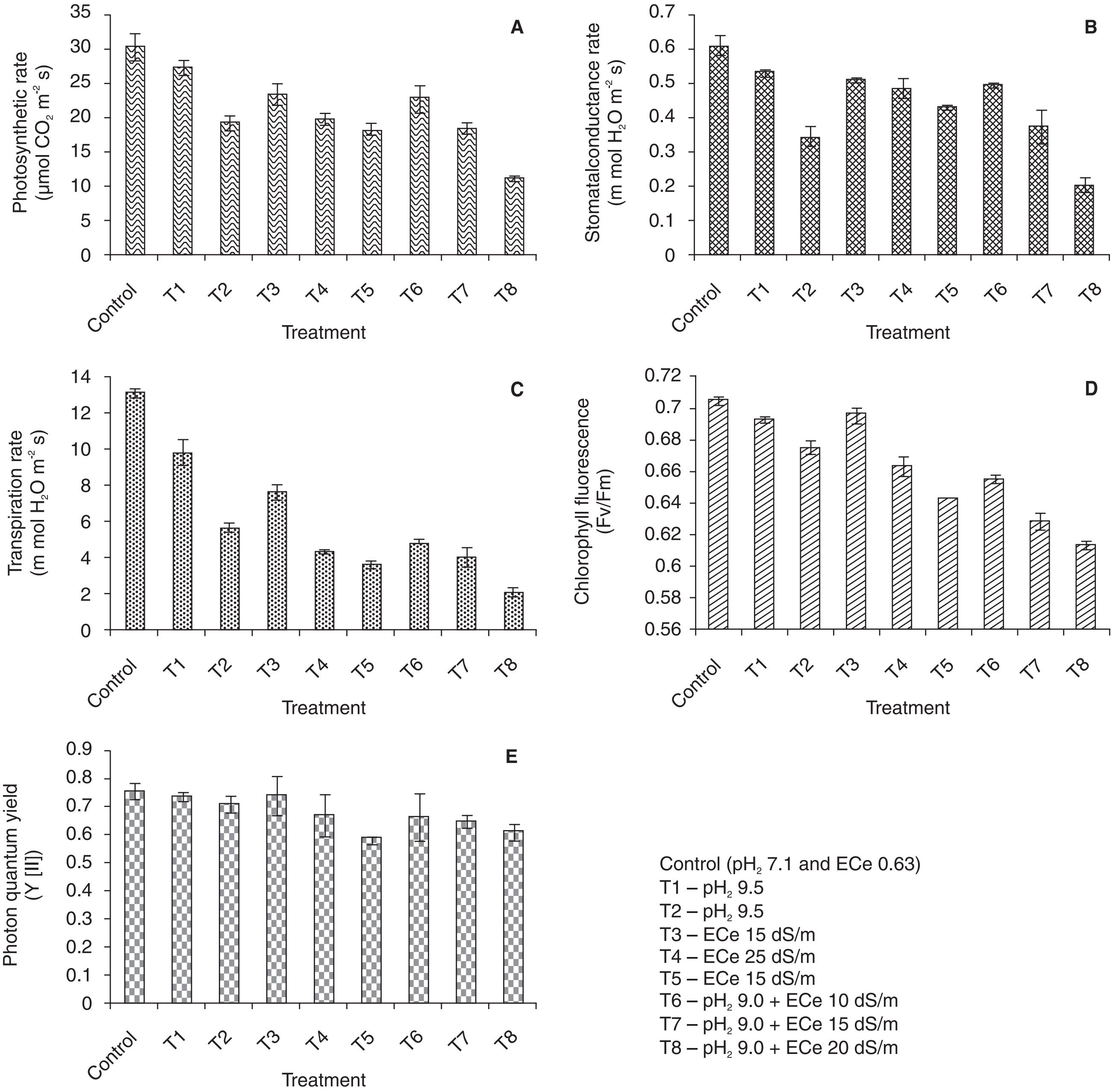

Fig 2 Effects of different salt environments on gas exchange attributes and chlorophyll fluorescence in *Sporobolus marginatus*.

soils having pH$_2$ 9.0 + EC$_e$ 20 dS/m (Fig 2A and 3A). Elevated salinity levels also decreased gS and E rates in both species. Stomatal conductance was nearly threefold higher in normal soils compared to the values noted in the combined stress treatment (pH 9.0 + EC 20 dS/m). While sodicity alone resulted in 43.18% and 29.0% reductions in gS, salinity suppressed gS by 55.07% and 66.41% in SM and US, respectively (Fig 2B and 3B). Decrease in E showed a similar trend in both the species (Figures 2C and 3C). Chlorophyll fluorescence measured in terms of Fv/Fm ratio also declined in the salt stressed plants (Fig 2D and 3D). Non-stressed control plants of SM and US had the highest Fv/Fm ratio (0.705 and 0.714, respectively) while those growing at pH$_2$ 10.0 had the Fv/Fm ratio of 0.675 and 0.657, respectively; at 35 dS/m salinity and combined stress treatment (pH$_2$ 9.0 + EC$_e$ 20 dS/m), Fv/Fm ratio in SM and US was 0.643 and 0.657, and 0.614 and 0.612, respectively. These data suggest that there were only nominal reductions in Fv/Fm ratio in the salinized plants of both species.

The maximum quantum yield of PSII also marginally decreased in the salt treated plants, *i.e.*, from 0.751 to 0.707 at pH$_2$ 10.0 (5.85 % reduction), to 0.579 at EC$_e$ 35 dS/m salinity (22.9 % reduction), to 0.607 at pH$_2$ 9.0 + EC$_e$ 20 dS/m (19.17% reduction) compared to the control plants in SM. Similarly, in US (Fig 2E and 3E), photon yield of PSII (0.616) under combined (pH$_2$ 9.0 + EC$_e$ 20 dS/m) stress treatment was 23.38% lower than the control values.

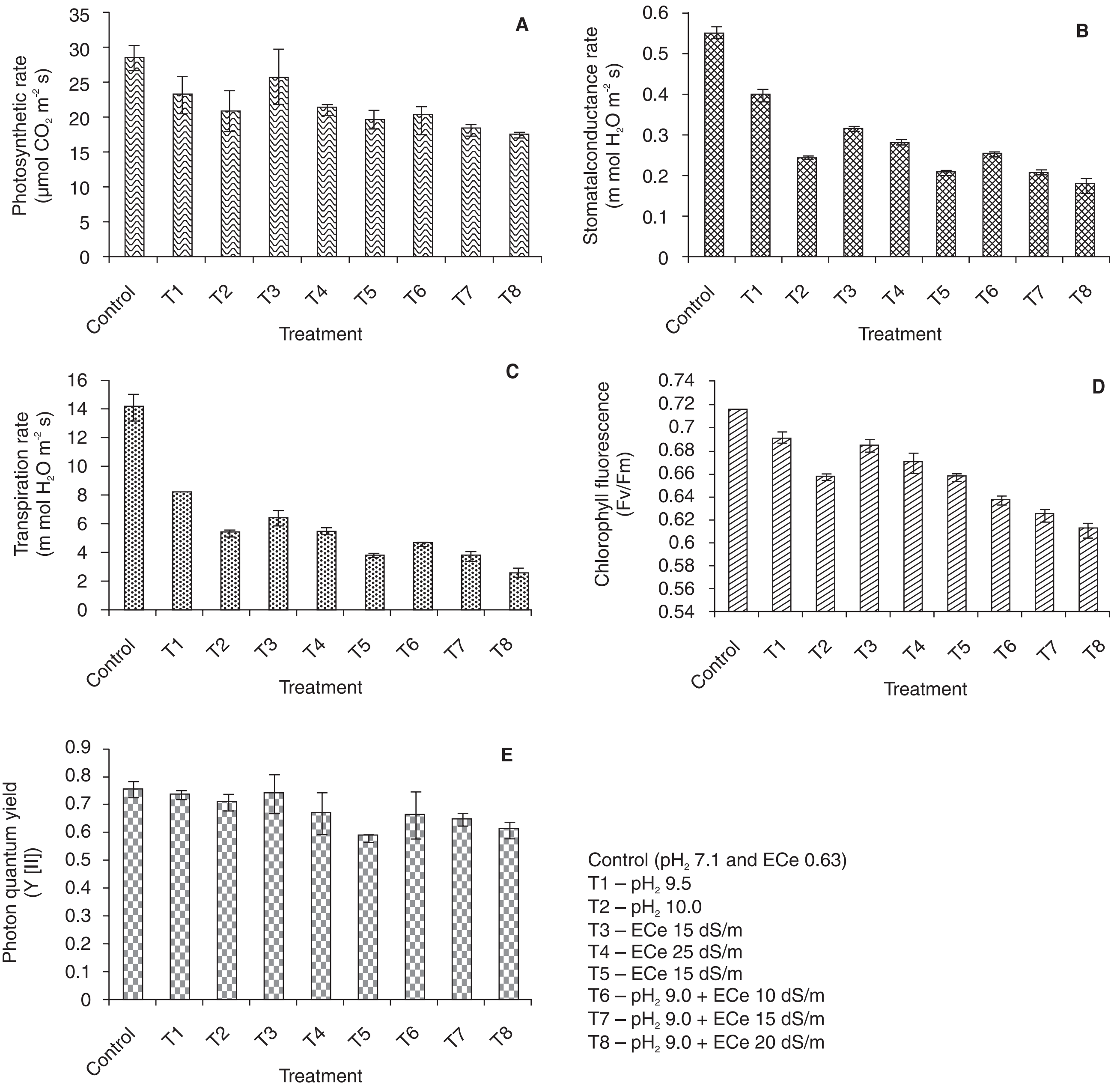

Fig 3 Effects of different salt environments on gas exchange attributes and chlorophyll fluorescence in *Urochondra setulosa*.

Osmo-protectants and ionic relations

Biochemical constituents such as total soluble sugars (TSS), protein content, proline content and epicuticular wax load (EWL) exhibited differential responses to variable stress conditions (Table 1). TSS amounts significantly increased with the increasing magnitude of stress, irrespective of the nature of stress (i.e., either salinity or sodicity) in comparison to control plants. Control plants of SM had TSS content of 3.62 mg/g DW which decreased to 3.19 mg/g at pH 9.5 and to 3.08 mg/g at pH 10.0 (Table 1). In contrast, TSS content significantly increased to 5.5 mg/g at EC_e 35 d/Sm salinity and to 4.2 mg/g at pH_2 9.0 + EC_e 20 dS/m. In US, the mean TSS content was 4.58 mg/g over all the treatments (Table 1) and the maximum accumulation of 5.66 mg/g was noted under combined stress treatment of pH_2 9.0 + EC_e 20 d/Sm.

Proline accumulation is an important physiological index for plant response to abiotic stresses. In this study, increased accumulation of proline probably helped counteract some of the adverse effects of toxic salt ions in the cell vacuoles. In SM, proline accumulation was 4.8, 5.2 and 5.0 times higher at pH_2 10.0, EC_e 35 dS/m and at pH_2 9.0 + EC_e 20 dS/m, respectively, while the corresponding increases in US were 7.9, 10.59 and 6.85 folds, respectively (Table 1). Protein accumulation plays an important role in cell survival under stress conditions by protecting and stabilizing the cell membranes. In US, protein content marginally decreased under different stress treatments. However, it increased with the increasing salinity or sodicity in SM (Table 1). EWL increased in both the halophytes across all treatments. In SM and US, EWL was 6.5 and 16.5 mg/g, respectively, under control conditions. Significant increases in EWL were observed under the combined stress (24.0 and 40.0 mg/g at pH_2 9.0 + EC_e 20 dS/m) than either sodicity (20.5 and 24.5 mg/g at pH_2 10.0) and salinity stress (20.5 and 31.5 mg/g at EC_e 35.0 dS/m) alone in SM and US, respectively.

Ionic relations

Na^+ partitioning (Root and Shoot): Salt stress significantly influenced the ionic concentrations as shown in Table 2. Na^+ distribution in roots and shoots of salinized plants indicated a tendency for restricted sodium uptake. In SM, higher Na^+ accumulation was observed in shoots (1.28 % DW) than in roots (0.73% DW). While roots restricted Na^+ uptake under sodic conditions (0.55% at pH 9.5 and 0.3% at pH 10.0), root concentrations of Na^+ significantly increased under salinity and the mixed stress treatments. Shoot Na^+ concentration was 3.1% at pH 10.0, 4.28% at EC 35 dS/m and 4.35% at pH 9.0 + EC 20 dS/m (Table 2). However in case of *U. setulosa,* the Na^+ concentration increased both in root and shoot under both the conditions but had lower accumulation in roots. Root Na^+ concentration was 0.57% DW under control conditions and increased to 0.62% DW at pH 10.0, 0.97% DW at EC 35 dS/m and 0.92% DW in the mixed stress treatment (under pH 9.0 + EC 20 dS/m). Shoot Na^+ levels increased by 4.93, 6.91 and 6.37 times at pH 10.0, salinity and mixed salinity sodicity, respectively, over control (Table 2).

*K^+ Partitioning (Root and Shoot)***:** A good supply of K^+ to plants can minimize injurious effects of high Na^+. K^+ levels gradually decreased while that of Na^+ considerably increased. Mean root K^+ concentrations were 0.85% DW in SM and 0.73% DW in US (Table 2). Under sodic conditions, root K^+ concentration decreased to 70.08% and 44.94% as compared to control (1.17% and 0.89%) in SM and US, respectively. SM plants accumulated sufficient amounts of K^+ in the shoots. Shoot Na^+ concentrations were 0.98%, 0.65% and 0.88% DW at pH 10.0, EC 35 dS/m and pH 9.0 + EC 20 dS/m in SM. In US plants, however, shoot K^+ concentration increased up to 25 dS/m i.e. 0.63% DW at EC 15 dS/m to 0.98% DW at EC 25 dS/m but declined to 0.6% DW at EC_e 35 dS/m. Combined saline-alkaline stress significantly reduced shoot K^+ concentration (Table 2).

Cl^- Concentration (Shoot): Prolonged exposure of plants to stress conditions often leads to higher Cl^- levels in the aerial parts; especially the leaves. Shoot Cl^- concentration increased with the increasing salt stress in both the halophytes and the maximum Cl^- accumulation was observed at pH_2 9.0 + EC_e 20 dS/m (4.4 % DW and 5.31 % DW) as compared to control (3.57 % DW and 2.04 % DW) in SM and US, respectively (Table 2).

Salt tolerance refers to the ability of plants to grow and complete the life cycle under elevated salt levels in the root zone. It is a complex trait and is determined by multiple biochemical pathways that facilitate retention and/or acquisition of water, protection of chloroplast functions and the maintenance of ion homeostasis. Ion toxicity and ionic imbalances caused by salinity disrupt several cellular functions and physiological processes (Duarte *et al.* 2013, Kumar *et al.* 2016, Lata *et al.* 2017), resulting in the slow and reduced plant growth. However, halophytes often show better growth at low-to-moderate salinities and are relatively less affected under extreme saline conditions. The decrease in chlorophyll content with increasing stress conditions implies a lower capacity of leaf tissues for light harvesting and production of reactive oxygen species which is mainly driven by excess energy absorption in the photosynthetic apparatus; this might be avoided by degrading the absorbing pigments (Kumar *et al.* 2016).

Photosynthesis is one of the main physiological processes affected by the salt stress, and the emission of chlorophyll fluorescence provides an indicator of the primary photochemistry of photosynthesis. Our results also revealed that gas exchange parameters reduced under stress conditions. Such perturbations in the gas exchange attributes can be ascribed to the decreased efficiency for light utilization, photoinhibition of photosystem (Herbinger *et al.* 2002) or might result due to increased production of reactive oxygen species (ROS). There exists a strong relationship between photosynthesis and stomatal conductance; stomatal closure tends to reduce the photosynthetic activity and transpiration rate that could be considered as an adaptive mechanism to cope up with the excessive salt. Stomatal closure and the consequent CO_2 deficit in the chloroplasts is the main cause of decreased photosynthesis under mild and moderate stresses (Chedila *et al.* 2007). Although reduced stomatal conductance imposed by high salinity restricts CO_2 diffusion, it might elevate the CO_2 partial pressure across the stomata that are utilized by leaves to maintain a consistently moderate rate of photosynthesis throughout the day, thus avoiding CO_2 starvation and photoinhibition. Photosystem II (PSII) is believed to be the most stress sensitive. The *F*v/*F*m ratio has been shown to be reliable stress indicator and the decline in Fv/Fm ratio under severe stress reflects a reduction in the ability of PSII to reduce the primary acceptor QA (Flexas *et al.* 2002). A decline in quantum yield of PSII was observed with increasing stress levels in our study which might be due to salt-induced stomatal closure.

Salinity is not inimical to all plants. The physiological basis of salt tolerance in halophytes has been intensively studied (Calatayud and Barreno 2001). Despite their polyphyletic origin, halophytes appear to have evolved the same basic method of osmotic adjustment: accumulation of inorganic salts (mainly NaCl) in the vacuole and the accumulation of compatible organic solutes in the cytoplasm. Halophyte vacuoles may have a modified lipid composition to prevent the leakage of Na^+ back to the cytoplasm (Koca *et al.* 2007). Although all of the halophytes exhibit better accumulation of salt, the level of total salt accumulation in the shoot is mostly species specific depending on different adaptive strategies. In this investigation, salt accumulation

Table 2 Ionic relations in *S. marginatus* and *U. setulosa* subjected to salt affected stress

Treatment/Trait	Analysis of variance among determined traits (Means sum of squares)									
	Ionic relations in *Sporobolus marginatus*					Ionic relations in *Urochondra setulosa*				
	Root Na^{+} (mmol/kg DW)	Shoot Na^{+} (mmol/kg DW)	Root K^{+} (mmol/kg DW)	Shoot K^{+} (mmol/kg DW)	Shoot Cl^{-} (mmol/kg DW)	Root Na^{+} (mmol/kg DW)	Shoot Na^{+} (mmol/kg DW)	Root K^{+} (mmol/kg DW)	Shoot K^{+} (mmol/kg DW)	Shoot Cl^{-} (mmol/kg DW)
Control	316.30^{G}	554.35^{E}	291.88^{A}	396.88^{A}	1004.23^{BC}	247.83^{E}	293.48^{G}	221.25^{A}	156.25^{C}	573.38^{E}
pH$_2$ 9.5	239.35^{H}	1010.87^{D}	200.38^{C}	256.25^{BC}	548.45^{F}	258.48^{E}	1054.35^{F}	132.50^{G}	143.75^{C}	847.61^{BCD}
pH$_2$ 10.0	129.35^{I}	1347.83^{C}	86.87^{E}	243.75^{BCDE}	847.61^{DE}	268.48^{DE}	1456.52^{E}	122.50^{H}	138.13^{C}	972.25^{B}
ECe 15 dS/m	346.30^{F}	1119.57^{D}	248.50^{B}	250.00^{BCD}	947.32^{CD}	291.30^{CD}	1413.04^{E}	211.75^{B}	135.63^{C}	972.25^{B}
ECe 25 dS/m	388.04^{D}	1391.30^{C}	206.75^{C}	212.50^{E}	772.82^{E}	375.22^{B}	1619.57^{CD}	196.13^{CD}	243.75^{B}	797.75^{CD}
ECe 35 dS/m	411.96^{B}	1858.70^{A}	173.75^{D}	162.50^{F}	1071.97^{B}	420.65^{A}	2043.48^{A}	187.50^{E}	150.00^{C}	1495.77^{A}
pH$_2$ 9.0 + ECe 10 dS/m	367.17^{E}	1130.43^{D}	275.13^{A}	268.75^{B}	947.32^{CD}	269.78^{DE}	1478.26^{DE}	201.63^{C}	281.25^{A}	922.39^{BC}
pH$_2$ 9.0 + ECe 15 dS/m	397.17^{C}	1684.78^{B}	234.38^{B}	231.25^{CDE}	1022.11^{BC}	308.26^{C}	1663.04^{C}	193.00^{DE}	143.75^{C}	747.89^{D}
pH$_2$ 9.0 + ECe 20 dS/m	453.26^{A}	1891.30^{A}	200.63^{C}	220.63^{DE}	1250.00^{A}	400.00^{AB}	1880.43^{B}	173.75^{F}	137.50^{C}	1420.99^{A}
General Mean	338.77	1332.13	213.14	249.17	934.65	315.56	1433.57	182.22	170.00	972.25
CV(%)	1.13	3.96	4.32	5.91	5.18	3.53	4.49	1.67	6.58	6.22
SE(d)	3.812	52.754	9.210	14.738	48.378	11.135	64.306	3.042	11.193	60.499
LSD (P=0.05)	8.7915	121.65	21.239	33.986	111.56	0.0591	0.3411	0.0281	0.1032	0.4952

Least significant difference test was applied at 5% probability level to compare the mean differences. Plant height (cm); CC, Chlorophyll concentration (mg/g); TSS, Total Soluble Sugars (mg/g DW); Proline content (mg/g FW); Protein content (mg/g FW); EWL, Epicuticular Wax Load (mg/g DW) patterns were different in two species, i.e. root Na^+ concentrations were less (0.78 % DW) as compared to shoot Na^+ concentrations (1.38% DW) in SM. However, in case of US, Na^+ concentration increased both in roots and shoots under both the conditions but accumulation was relatively lower in roots. Shoot Na^+ concentration increased by 4.93 fold under pH 10.0 which further increased under salinity (6.91 fold) and combined stress treatment (6.37 fold) over the respective control. Rabhi *et al.* (2010) reported that *Arthrocnemum indicum*, *Suaeda fruticosa,* and *Sesuvium portulacastrum* seedlings grown on a saline soil significantly reduced the soil salinity and electrical conductivity absorbing the soluble salts (mainly Na^+ ions). They also reported that *Sesuvium portulacastrum* was able to accumulate nearly 30% of Na^+ content in shoot over 170 d period. It was also found in the present investigation that soil salinity and alkalinity were reduced due to accumulation of salts in *Sporobolus* and *Urochondra*. Chaudhri *et al.* (1964) investigated the ability of *Suaeda fruticosa* to accumulate sodium and other salts and reported that the leaves of this plant contained 9.06% salt on a fresh weight basis. It was also seen in the present investigation that there was 9.47% increase in biomass accumulation at pH_2 9.5 and 1.17% increase at pH_2 10.0 in *Sporobolus* over control while in case of *Urochondra*, under saline condition, biomass increased up to EC_e 25 dS/m. Tissue-specific compartmentalisation appears to play an important role in most of the grasses (Bhatti *et al.* 1993) where the toxic ions like Na^+ and Cl^- are redistributed to the mature or senescing leaves and other organs. It is widely accepted that cell turgor is maintained by storage of Na^+ and Cl^- in vacuoles, with the solute potential of the cytosol adjusted by accumulation of K^+ and organic solutes. Ion accumulation in the shoot parts is possibly attributable to an enhanced selective ion uptake in favour of K^+ over Na^+ at the root level on one hand and a high transport capacity in favour of Na^+ versus K^+ from the root to the shoot on the other. It has also been observed in this study that mean root K^+ concentration decreased in *S. marginatus* and *U. setulosa* under saline conditions but sufficient amounts of K^+ accumulated in the shoots to alleviate the salt stress.

Plants also accumulate compatible solutes to withstand the stress conditions as such solutes play a key role in osmotic adjustment and osmoprotection (Patade *et al.* 2008). The higher total soluble sugar contents in both the grasses under extreme salinity levels could be regarded as an additional mechanism to prevent salt injury and it might also contribute towards the turgor maintenance (Burke *et al.* 2007). Under severe salinity stress, a low carbohydrate level could be due to either high respiration rate or a decrease in photosynthetic activity accompanied by reduction in the growth rate. The proteins that accumulate under stress conditions may provide a storage form of nitrogen that is re-utilized in post-stress recovery (Singh *et al.* 1987). Such modifications may lead to accumulation or depletion of certain metabolites resulting in an imbalance in the levels of a relatively small set of cellular proteins, which could increase, decrease, appear or disappear after stress treatment. Proline is a potent osmoregulator molecule and counteracts the adverse effects of toxic salt ions in the cell vacuoles, contributes to membrane stability and mitigates the effect of NaCl on cell disruption (Mansour 1998). The epicuticular waxes (ECW) are soluble waxes deposited on the outer surface and are composed of a mixture of chemical compounds such as hydrocarbons, alcohols and aldehydes which play a protective role when stomata are closed (Jenks and Ashworth 1999). When stress increases, stomata tend to close resulting in the higher deposition of ECW leading to decreased cuticular permeability for preventing the water loss (Blum 1988). Results of our study strengthen the earlier findings and reveal the differential behaviour of grass halophytes in terms of osmolytes accumulations and wax loading in response to the different salt stresses.

Sporobolus and *Urochondra* usually grow in salt-affected arid and dry areas. Both these halophytes varied in their growth, physiological and ion uptake responses under different salinity/sodicity levels. These salt tolerant perennial species showed several strategies for salt stress adaptation. These grasses are highly salt tolerant and could be cultivated using seawater irrigation. These halophytes are good candidate for landscape development and could also be used as forage and fodder. Future investigations are necessary to understand the correlations of different proteins, ions and genes imparting salt tolerance in these species. Molecular knowledge of response and tolerance mechanisms will pave the way for engineered plants that can tolerate salt stress and could be the basis for production of crops which can result in economic yield under salt-stress conditions.

ACKNOWLEDGEMENTS

Authors thankfully acknowledge the Director, ICAR-CSSRI for providing necessary facilities and Indian Council of Agricultural Research for financial help through National Agriculture Science Fund (NASF).

REFERENCES

Bates L S, Waldren R P and Teare I D. 1973. Rapid determination of free proline for water-stress studies. *Plant Soil* **39**: 205-7.

Bhatti A S, Steinert S, Sarwar G, Hilpert A and Jeschke W D. 1993. Ion distribution in relation to leaf age in *Leptochloa fusca* (L.). Kunth. (kallar grass). I. K., Na, Ca and Mg. *New Phytologist* **123**: 539–45.

Blum A. 1988. *Plant Breeding for Stress Environments*. pp. 1-223. CRC Press, Boca Raton, Florida, USA.

Bradford M M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. *Analytical Biochemistry* **7**: 248-54.

Burke J J. 2007. Evaluation of source leaf responses to water-deficit stresses in cotton using a novel stress bioassay. *Plant Physiology* **143**: 108–21.

Calatayud A and Barreno E. 2001. Chlorophyll a fluorescence, antioxidant enzymes and lipid peroxidation in tomato in response to ozone and benomyl. *Environmental Pollution* **115(2)**: 283-9.

Chaudhri I I, Shah B H, Naqvi N and Mallick I A. 1964.

Investigations on the role of *Suaeda fruticosa* Forsk in the reclamation of saline and alkaline soils in West Pakistan plains. *Plant Soil* **21**(1): 1–7.

Chedlia B A, Bechi B R and Boukhris M. 2007. Effect of water deficit on olive trees cv. Chemlali under field conditions in arid region in Tunisia. *Scientia Horticulture* **113**: 267-77.

Chhabra R. 1973. 'Kinetics of absorption of chloride and phosphorus, their interaction and effect on growth and composition of tomato plants'. Ph D thesis, KUL, Belgium.

Duarte B, Santos D, Marques J C and Cacador I. 2013. Eco physiological adaptations of two halophytes to salt stress: Photosynthesis, PS II photochemistry and anti-oxidant feedback – Implications for resilience in climate change. *Plant Physiology and Biochemistry* **67**: 178–88.

Ebercon A, Blum A and Jordan W R. 1977. A rapid colorimetric method for epicuticular wax content of sorghum leaves. *Crop Science* **17**: 179–80.

FAO. 2007. FAO Agristat. www.fao.org.

Flexas J and Medrano H. 2002. Drought-inhibition of photosynthesis in C-3 plants: Stomatal and nonstomatal limitation revisited. *Annals of Botany* **89**: 183-90.

Flowers T J and Lauchli A. 1983. Inorganic plant nutrition: V3. Sodium versus potassium: substitution and compartmentation. (*In*) *Encyclopedia of Plant Physiology*. pp 651–681. New Series, Springer-Verlag Berlin, Heidelberg.

Glenn E P, Brown J J and Blumwald E. 1999. Salt tolerance and crop potential of halophytes. *Critical Reviews in Plant Science* **18**(2): 227–55.

Herbinger K, Tausz M, Wonisch A, Soja G, Sorger A and Grill D. 2002. Complex interactive effects of drought and ozone stress on the antioxidant defence systems of two wheat cultivars. *Plant Physiology and Biochemistry* **40:** 691–6.

Jenks M A and Ashworth E N. 1999. Plant Epicuticular Waxes: Function Production and Genetics. (*In*) *Horticultural Reviews,* p: 23. Janick J (Ed).

Koca H, Bor M, Ozdemir F and T¨Urkan I. 2007. The effect of salt stress on lipid peroxidation, antioxidative enzymes and proline content of sesame cultivars. *Environmental and Experimental Botany* **60**(3): 344–51.

Kumar A, Kumar A, Lata C and Kumar S. 2016. Eco-physiological responses of *Aeluropus lagopoides* (grass halophyte) and *Suaeda nudiflora* (non-grass halophyte) under individual and interactive sodic and salt stress. *South African Journal of Botany* **105**: 36-44.

Lata C, Kumar A, Sharma S K, Singh J, Sheokand S, Pooja, Mann A and Rani B. 2017. Tolerance to combined boron and salt stress in wheat varieties: Biochemical and molecular characterization. *Indian Journal of Experimental Biology* **55:** 321-8.

Mansour M M F. 1998. Protection of plasma membrane of onion epidermal cells by glycinebetaine and proline against NaCl stress. *Plant Physiology and Biochemistry* **36**: 767-72.

Patade V Y, Suprasanna P and Bapat V A. 2008. Effects of salt stress in relation to osmotic adjustment on sugarcane (*Saccharum officinarum* L.) callus cultures. *Plant Growth Regulation* **55**: 169-73.

Rabhi M, Ferchichi S and Jouini J. 2010. Phytodesalination of a salt-affected soil with the halophyte *Sesuvium portulacastrum* L. to arrange in advance the requirements for the successful growth of a glycophytic crop. *Bioresource Technology* **101**(17): 6822–8.

Sharma D K and Singh A. 2015. Salinity research in India-achievements, challenges and future prospects. *Water Energy International* **58**(6): 35-45.

Singh G, Bundela D S, Sethi M, Lal K and Kamra S K. 2010. Remote sensing and geographic information system for appraisal of salt-affected soils in India. *Journal of Environment and Quality* **39**: 5-15.

Singh N K, Bracken C A, Hasegawa P M, Handa A K, Buckel S, Hermodson M A, Pfankoch F, Regnier F E and Bressan R A. 1987. Characterization of osmotin: a thaumatin-like protein associated with osmotic adjustment in plant cells. *Plant Physiology* **85**: 529–36.

Singh R B, Minhas P S, Chauhan C P S and Gupta R K. 1992. Effect of high salinity and SAR water on salinization, sodication and yield of pearl millet – wheat system. *Agriculture Water Management* **21**: 93-105.

Yemm E W and Willis A J. 1954. The estimation of carbohydrates in plant extracts by anthrone. *Biochemical Journal* **57**: 508-14.