Indian Journal of Agricultural Sciences **92** (9): 1051–1055, September 2022/Article
https://doi.org/10.56093/ijas.v92i8.91277

Mineral nutrient analysis of three halophytic grasses under sodic and saline stress conditions

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Received: 2 July 2019; Accepted: 24 June 2022

ABSTRACT

Present study was carried out to assess the effects of soil salinity/sodicity on mineral nutrient status of *Urochondra setulosa*, *Leptochloa fusca* and *Sporobolus marginatus* at ICAR- Central Soil Salinity Research Institute, Karnal, Haryana during 2016–19. Treatments of salinity/sodicity (*p*H ~ 9.5, *p*H ~ 10, ECe ~ 30 dS/m, ECe ~ 40 dS/m and ECe ~ 50 dS/m) were created in microplots (2.5 m × 1.5 m × 0.5 m) using saline/sodic water. Na^+ and Cl^- content (% DW) significantly increased with increasing sodicity/salinity stress condition in all three grass halophytes, whereas K^+ content decreased. These grass halophytic species showed relatively less reduction in Ca, Mg and Fe contents up to sodic stress of *p*H ~ 9.5 and salinity level of ECe ~ 40 dS/m. Zn, Cu and Mn content decreased with increasing stress conditions but higher decrease was observed under sodic stress. The Na^+/K^+ and Na^+/Ca^{2+} ratio was considered as indicators for measuring salt tolerance in plants. Na^+/K^+ ratio increased with increasing stress condition in all the three grasses but *Leptachloa* maintained their Na^+/K^+ near *p*H 1.0 under sodic stress condition and also maintained their Na^+/Ca^{2+} below 1.0 up to *p*H ~ 9.5 and ECe ~ 40 dS/m. Higher sodic stress of *p*H~10.0 caused significant increase in Na^+/Ca^{2+} in *Urochondra* and *Sporobolus,* whereas under highest salinity level, *Leptachloa* showed highest value for Na^+/Ca^{2+}. Changes in the accumulation patterns of nutrient in response to salinity is an important aspect and study showed highest positive correlation between Ca - Mg & Zn and negative between Na - Ca and K.

Keywords: *Leptachloa*, Nutrients, *Sporobolus, Urochondra*

Out of 17 essential nutrient elements plants uptake C, H and O from the air and water while other elements like N, P, K, Ca, Mg, Fe, Zn, Cu, Mn, S, Mo, B and Co from the soil. These nutrient elements are taken by plants in the form of ions from the root zone (Pessarakli *et al.* 2015). Salt affected soils adversely modified the growth of most crop plants by the presence of soluble salts, with or without high amounts of exchangeable sodium (Meena *et al.* 2018). The main problem in salt affected soils is nutrient deficiency because the availability, absorption and transport of nutrients within the plant are affected by salt stress (Munns and Tester 2008). Nutrients have several functions in plant growth and metabolism and their availability is generally affected by soil physical and chemical characteristics such as structure, texture, moisture content, temperature, *p*H and available nutrient content (Meena *et al.* 2017). Under salt stress conditions, presence of excess Na^+ and Cl^- in the root zone interfere with the normal physiological processes and ultimately leads to plant death.

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One of the deleterious effects of the high stress levels (either salinity or sodicity) is manifested as nutrient imbalance like high soil Na^+ concentrations reduce the amounts of available K^+, Mg^{++} and Ca^{++} for plants resulting in Na^+ toxicity on one hand and deficiencies of essential cations on the other (Kumar *et al.* 2018a). Although most of the plant species tend to adjust osmotically (i.e. lowering the water potential) in saline soils to prevent the loss of turgour (Kumar *et al.* 2018b and Lata *et al.* 2019) but, ionic effect is long-term and continuous because it depends on the intracellular salt ion levels which increase with the duration of salinity stress (Mann *et al.* 2019). So, we have evaluated three halophytic grasses differing in their tolerance towards salinity/sodicity i.e. *Urochondra setulaosa*, *Leptochloa fusca* and *Sporobolus marginatus* to study the status of nutrients in plants under variable salt stress.

MATERIALS AND METHODS

Seeds and root slips of halophytic grasses (*Urochondra setulosa* and *Sporobolus marginatus*) were collected from Regional Research Station, ICAR-Central Arid Zone Research Institute, Bhuj and *Leptochloa fusca* from Regional Research Station, ICAR-Central Soil Salinity Research Institute, Lucknow. These grasses were established through root cuttings in porcelain pots of 20 kg capacity filled with sandy loam soil in a screen house. After establishment, these grasses were transferred to micro-plots (2.5 m × 1.5 m × 0.5 m) having different stress conditions, i.e. control, *p*H ~ 9.5, *p*H ~ 10, ECe ~ 30 dS/m, ECe ~ 40 dS/m and ECe ~ 50 dS/m under natural environment at ICAR-CSSRI, Karnal during 2016–19. Stress treatments were given through saline/sodic irrigation water supplemented with 3:1 of Cl^- dominated salts for salinity and $NaHCO_3^-$ for sodicity. Regularly, ECe and *p*H were checked and desired stress levels were maintained.

After 120 days of establishment, plants were harvested for nutrient uptake analysis in three replications. Harvested samples were oven-dried for 72 h at 60°C or till attaining constant weight. Acid-digestion of dried leaf samples was done with 10 ml of HNO_3:$HClO_4$ (3:1) for all nutrients except chloride. This acid digested mixture was heated on hot plate till a clear solution was formed and 0.5–1 ml remains were left. This clear solution was diluted with 50 ml DDW, filtered with Whatman filter paper no. 42. Na^+ and K^+ contents were measured with the flame photometer (PFP7, Jenway, Bibby Scientifc, UK). Determination of Ca, Mg, Fe, Zn, Cu and Mn was done with AAS (Zeenit 700 P, Analytical Zena, Germany). Chloride content of leaves was determined by the modified method of Chhabra (1973). Data analyzed statistically using RBD (Version 9.3, SAS Institute Inc., Cary, NC, USA) and least significant difference test was applied at 5% probability level to compare the mean differences.

RESULTS AND DISCUSSION

In the present study, we have described the comparative mineral nutrient analysis in three halophytic grasses, i.e. *Urochondra setulosa*, *Leptochloa fusca* and *Sporobolus marginatus* in saline and alkaline stresses. In general, nutrients have several specialized functions in plant growth and metabolism (Taiz and Zeiger 2009). Nutrient deficiency as well as ion toxicity and osmotic stress are factors attributed to the deleterious effect of salinity on plant growth and productivity. Excessive accumulation of Na^+ in the leaves/shoots has been considered highly harmful for normal metabolism of plants, and tolerant plants have the capacity of successful salt removal. Both Na^+ and K^+ ions compete for entry into plant root cells and the replacement of K^+ by Na^+ often leads to nutritional imbalances (Singh *et al.* 2018). Maintenance of low leaf Na^+ concentration and higher K^+/Na^+ ratio is an important aspect of stress tolerance (Kumar *et al.* 2016). Results showed that Na^+ and Cl^- content (% DW) significantly increased with increasing sodicity or salinity stress condition in all grass halophytes, whereas K^+ content decreased. In *Urochondra*, Na^+ content increased by 5.4 folds at highest level of sodic stress, i.e. *p*H ~ 10.0 and 7.58 folds increase at salinity stress of ECe ~ 50 dS/m, whereas no significant decrease was found for K^+. Sodic stress caused reduction in K^+ content, i.e. 19.7% reduction under *p*H ~ 10.0 while the content was increased under saline stress upto ECe ~ 40 dS/m, i.e. 47% increase in comparison to control treatment (Table 1). Further increase in salinity caused reduction in K^+ content.

Similar results were obtained for Cl^- content as that of Na^+ in *Urochondra*. Maximum accumulation of Cl^- (5.93%) was found at salinity stress of ECe ~ 50 dS/m. Being a sodicity tolerant grass, *Leptachloa* accumulate less Na^+ in their leaves under sodic stress (2.23% DW at *p*H ~ 10.0) as compared to salinity stress (7.0% DW at ECe ~ 50 dS/m) and this grass also maintained or showed less reduction (13.49%) in K^+ content under sodic stress than salinity stress (55.35% reduction). In case of Cl^-, the content was increased with increasing stresses but the effect was non-significant (Table 1). The sodicity and salinity tolerant grass *Sporobolus* also showed similar increase and decrease in Na^+ and K^+ content as that was observed in *Leptachloa* (Table 1). Cl^- content increased with increasing stress, but under higher salinity level (ECe ~ 50 dS/m) the content was reduced in *Sporobolus*. Ionic effect is continuous and long-term effect of cumulative nature that increases intracellular salt ion levels with the duration and intensity of salinity stress. It has been observed that Na^+ competes with K^+ uptake through Na^+/K^+ co-transporters and may also block K^+ specific transporters

Table 1 Effect of sodicity and salinity stresses on Na^+, K^+ and Cl^- contents in halophytic grasses

Treatment		*Urochondra setulosa*			*Leptachloa fusca*			*Sporobolus marginatus*		
		Na^+ content	K^+ content	Cl^- content	Na^+ content	K^+ content	Cl^- content	Na^+ content	K^+ content	Cl^- content
Control		0.62^E	0.66^B	2.04^C	1.08^E	2.15^A	2.83	1.28^D	1.59^A	2.74^D
Sodic stress	*p*H ~ 9.5	2.42^D	0.59^{CD}	3.01^{BC}	1.35^E	1.95^B	3.81	2.35^C	1.05^B	3.10^{CD}
	*p*H ~ 10.0	3.35^C	0.53^D	3.45^B	2.23^D	1.86^B	2.83	3.10^B	1.01^B	3.36^{BC}
Saline stress	ECe ~ 30 dS/m	3.25^C	0.61^{BC}	2.83^{BC}	3.28^C	1.65^C	2.48	2.63^C	1.00^B	3.63^{AB}
	ECe ~ 40 dS/m	3.72^B	0.97^A	3.45^B	5.73^B	1.00^D	2.92	3.25^B	0.83^C	3.81^A
	ECe ~ 50 dS/m	4.70^A	0.54^D	5.93^A	7.00^A	0.96^D	3.81	4.33^A	0.63^D	1.95^E
General mean		3.01	0.65	3.45	3.44	1.59	3.11	2.82	1.02	3.10
SE(d)		0.088	0.027	0.411	0.209	0.038	0.366	0.117	0.063	0.171
LSD P=(0.05)		0.2257	0.0691	1.0573	0.5382	0.0978	NS	0.301	0.1614	0.4396

Means with at least one letter common are not statistically significant (P<0.05) using DUNCAN's multiple range test.

of root cells under salinity (Mann *et al.* 2015). Although, sodium is not considered an essential element but plants can accumulate Na^+ at the expense of Ca^{2+} and K^+ under salt stress. From the available literature, it was found that Cl^- can be toxic to plants at high concentrations, with critical concentrations for toxicity estimated to be 4–7 mg/g for sensitive species and 15–50 mg/g for tolerant species (Xu *et al.* 2000). Under salt stress conditions, Na^+ is the dominant toxic ion that interferes with K^+ uptake and disrupts the gaseous exchange through efficient stomatal regulation and necrosis whereas Cl^- accumulation in tissues leads to chlorotic toxicity symptoms due to chlorophyll degradation (Kumar *et al.* 2016).

Results (Table 2) showed that all these grass halophytic species showed relatively less reduction in Ca, Mg and Fe contents (mg/g) up to sodic stress level of *p*H ~ 9.5 and salinity level of EC_e ~ 40 dS/m. Beyond these levels, these halophytes showed significant decrease in Ca, Mg and Fe contents. On the basis of mean values across all the treatments, 4.28 mg/g Ca, 3.03 mg/g Mg and 0.97 mg/g Fe contents was obtained in *Urochondra*; 5.0 mg/g Ca, 3.68 mg/g Mg and 1.01 mg/g Fe in *Leptachloa* and 4.28 mg/g Ca, 3.05 mg/g Mg and 0.89 mg/g Fe contents in *Sporobolus*, respectively. Calcium helps in maintaining the structural and functional integrity of the plasma membrane by restricting the toxic effect of Na^+. It also acts as a secondary messenger in the regulation of signal transduction pathways for the response to abiotic stress and in the promotion of K^+/Na^+ selectivity (Rengel 1992). Particularly for calcium content, there was mean reduction of 27.92% in *Urochondra*, 36.55% in *Leptachloa* and 24.65% in *Sporobolus* in comparison to their respective control (Table 2). Reduction in the uptake of Ca^{2+} under stress environment results mainly due to enhanced Na^+ which induced reduction in the binding of Ca^{2+} to the plasma membrane and higher efflux of Ca^{2+} (Rengel 1992 and Singh *et al.* 2018). Being a part of chlorophyll molecules, magnesium plays an important role in photosynthesis reactions and activation of several enzyme systems. *Leptachloa* had higher mean Mg content (3.68 mg/g) than *Urochondra* and *Sporobolus* but the percent reduction was found minimum in *Urochondra* (20.66%). All these grasses showed statistically non-significant effect of sodicity and salinity stresses on Fe content (Table 2) and on mean basis, the uptake of Fe in leaves tissue was 0.97 mg/g in *Urochondra*, 1.01 mg/g in *Leptachloa* and 0.89 mg/g in *Sporobolus*. Sodicity and salinity might affect iron absorption which could cause Fe deficiency in young leaves (Rabhi *et al.* 2007). Reduction in Fe uptake mainly occurs due to high *p*H or by the presence of high bicarbonate concentrations which can inhibit Fe uptake

Table 2 Effect of sodicity and salinity stresses on Ca, Mg, Fe, Zn, Cu and Mn contents in halophytic grasses

Treatment/Trait	Control	Sodic stress		Saline stress			LSD (P = 0.05)
		*p*H ~ 9.5	*p*H ~ 10.0	ECe ~ 30 dS/m	ECe ~ 40 dS/m	ECe ~ 50 dS/m	
Urochondra setulosa							
Ca (mg/g)	5.49^{A}	4.83^{AB}	2.58^{C}	5.24^{A}	4.41^{AB}	3.12^{BC}	1.799
Mg (mg/g)	3.79^{A}	3.34^{AB}	1.69^{C}	3.65^{A}	2.99^{AB}	2.69^{B}	0.806
Fe (mg/g)	1.40	0.82	0.78	1.08	0.94	0.78	NS
Zn (µg/g)	43.49^{A}	37.80^{B}	32.34^{C}	42.59^{A}	40.77^{AB}	34.60^{C}	3.086
Cu (µg/g)	36.58^{A}	29.41^{B}	17.25^{D}	25.38^{BC}	23.41^{C}	21.82^{CD}	4.640
Mn (µg/g)	89.83^{A}	71.63^{B}	69.80^{BC}	72.65^{B}	65.07^{CD}	61.08^{D}	4.916
Leptachloa fusca							
Ca (mg/g)	7.88^{A}	5.68^{B}	3.91^{C}	5.46^{B}	3.94^{C}	3.12^{C}	1.130
Mg (mg/g)	5.57^{A}	4.02^{B}	3.90^{B}	4.47^{B}	2.40^{C}	1.74^{C}	0.919
Fe (mg/g)	1.26	1.15	0.82	1.05	0.93	0.86	NS
Zn (µg/g)	50.30^{A}	45.93^{AB}	42.09^{BC}	47.83^{AB}	36.33^{CD}	33.88^{D}	6.586
Cu (µg/g)	40.37	32.62	31.29	36.60	35.48	33.54	NS
Mn (µg/g)	90.73^{A}	87.90^{A}	76.78^{BC}	88.70^{A}	81.03^{B}	70.75^{C}	6.691
Sporobolus marginatus							
Ca (mg/g)	5.68	4.08	2.99	5.06	4.19	3.71	NS
Mg (mg/g)	4.29^{A}	3.67^{AB}	2.32^{D}	3.41^{BC}	2.66^{CD}	1.98^{D}	0.815
Fe (mg/g)	1.05	0.95	0.70	0.96	0.90	0.80	NS
Zn (µg/g)	46.50^{A}	41.12^{B}	29.19^{C}	41.60^{B}	37.85^{B}	30.50^{C}	4.504
Cu (µg/g)	37.05	34.79	27.49	35.51	31.62	30.68	NS
Mn (µg/g)	80.28^{A}	78.23^{AB}	74.90^{ABC}	72.33^{BCD}	68.50^{CD}	67.60^{D}	6.489

Means with at least one letter common are not statistically significant (P<0.05) using DUNCAN's multiple range test.

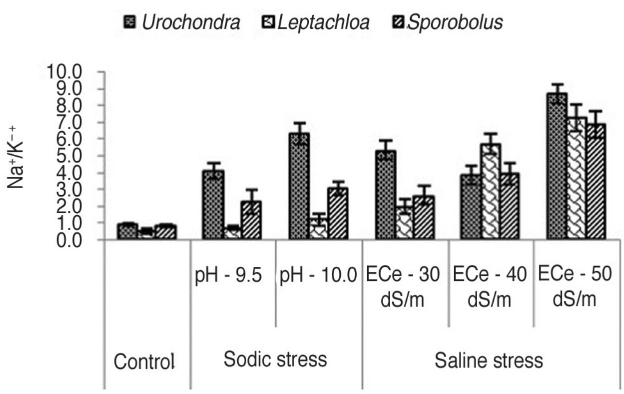

Fig 1 Effect of sodicity and salinity on Na^+/K^+ in halophytic grasses.

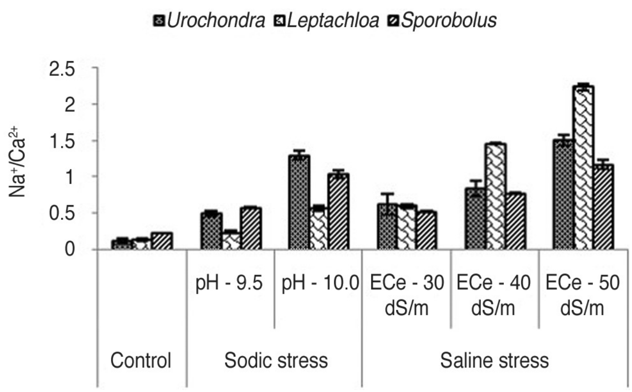

Fig 2 Effect of sodicity and salinity on Na^+/Ca^{2+} in halophytic grasses.

mechanisms (Lucena 2006). In the present study, Ca, Mg and Fe concentrations were reduced with increasing sodicity and salinity (Table 2). Salt stress mainly affects physiological mechanism of plants due to excessive accumulation of Na and Cl which resulted into ionic imbalance and reduced uptake of other mineral nutrients, such as K, Ca and Mg (Grattan and Grieve 1994 and Hasegawa *et al.* 2000).

The changes in Zn, Cu and Mn content under stress environment mainly depend on the plant types, tissue, salinity and micronutrient concentration in soils (Hu and Schmidhalter 2001). Results showed that Zn, Cu and Mn content decreased with increasing stress conditions but higher decrease was observed under sodic stress. Zinc acts as structural stabilizer for DNA-binding proteins, helps in the synthesis of chlorophyll, IAA, and important component of GDH, CAT and SOD enzymes (Aravind and Prasad 2004). The largest decrease in leaf Zn content (37.23%) was detected in *Sporobolus,* whereas the lowest reduction was noted in *Leptachloa* (16.0%) under sodic stress (Table 2). Copper act as an essential cofactors for many enzymes and also involved in many physiological processes, i.e. electron transport, mitochondrial respiration and cell wall metabolism (Marschner 1986). In case of Cu, higher decrease was observed in *Urochondra* under sodic conditions, whereas *Leptachloa* showed lowest reduction (Table 2). *Urochondra* accumulated 71.68 µg/g Mn, 82.65 µg/g Mn in *Leptachloa* and 73.64 µg/g Mn in *Sporobolus* on mean basis. However, the reduction in Mn content was lowest in *Sporobolus,* i.e. 6.7 and 15.79% under sodic stress of *p*H ~ 10.0 and salinity stress of ECe ~ 50 dS/m followed by *Leptachloa* (15.38 and 22.02%) and highest in *Urochondra* (22.3 and 32.0% reduction), respectively in comparison to control (Table 2).

The Na^+/K^+ ratio is considered as one of the most important indicator of the plant response to salt stress and maintenance of low leaf Na^+ concentration and low Na^+/K^+ is an important aspect of stress tolerance (Shabala and Cuin 2008). Present study showed that the ratio increased with increasing stress condition in all the three grasses but *Leptachloa* maintained their Na^+/K^+ near to 1.0 under sodic stress condition which showed that this grass accumulate less Na^+ or maintained their K^+ in the leaves tissue under sodic stress (Fig 1).

In addition to Na^+/K^+, Na^+/Ca^{2+} ratio could also be considered as indicator by measuring salt tolerance in plants (Koksal *et al.* 2016 and Kumar *et al.* 2018c, 2021). Here it was found that Na^+/Ca^{2+} increased with increasing stress condition and higher increase in ratio was observed under salinity stress (Fig 2). These three grasses maintained their Na^+/Ca^{2+} below 1.0 up to *p*H ~ 9.5 and ECe ~ 40 dS/m. Higher sodic stress of *p*H ~ 10.0 caused significant increase in Na^+/Ca^{2+} in *Urochondra* and *Sporobolus,* whereas at highest salinity level, *Leptachloa* showed maximum or highest value for Na^+/Ca^{2+}. Increase in the Na^+/Ca^{2+} ratio under stress conditions caused the deterioration of membrane permeability and the excessive uptake of the other salt ions, especially Na^+. Among all these parameters studied, highest significant positive correlation was found between Ca and Mg (r = 0.816**) and Ca and Zn (r = 0.799**) while highest negative correlation was observed between Na and Ca (r = - 0.419*) and Na and K (r = - 0.411*) for all the three halophytic grasses.

Halophytes rely heavily on the use of inorganic ions (Na^+, Cl^- and K^+) to maintain shoot osmotic and turgor pressure under saline conditions. In the present study also, the grass halophytes attempted to maintain a high K^+/Na^+ ratio in the cytosol to survive in saline or alkaline stress but Na^+/Ca^{2+} increased with increasing stress condition and higher increase in ratio was observed under salinity stress than alkaline conditions. The level of mineral nutrients was maintained by *Urochondra* under high salt stress along with Na/K ratio while in alkaline conditions of *p*H 9.0; *Leptachloa* balanced the Na^+/K^+ ratio. *Sporobolus* adapted to both types of stresses. Consequently, these insights uncover molecular and genetic interconnections between abiotic stress tolerance and ion accumulation and in this way point to the fundamental importance of the signaling integration for plant biology.

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