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Physiological and molecular traits conferring salt tolerance in halophytic grasses

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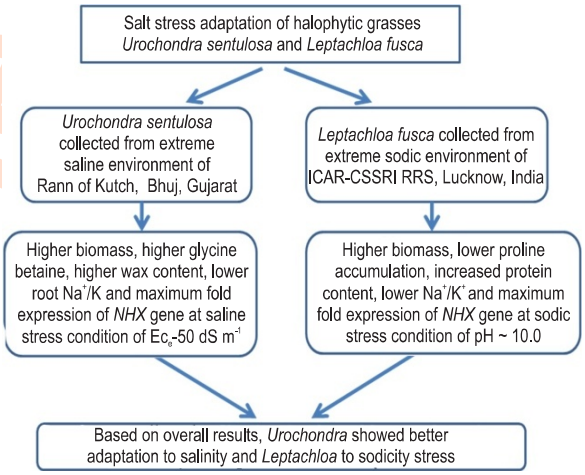
Abstract

Aim : This study was conducted to identify the physiological and molecular traits underpinning salt stress adaptation in halophytic grasses *Urochondra setulosa* and *Leptachloa fusca*.

Methodology : To assess the salt tolerance potential of *Urochondra setulosa* and *Leptachloa fusca*, the rooted cuttings and seeds were collected from Rann of Kutch, Bhuj, Gujarat and ICAR-CSSRI Regional Research Station, Lucknow, India, respectively using physiological, biochemical and molecular traits.



Results : Salt stress decreased the biomass production in both the species to varying extents. Leaf chlorophyll declined marginally (5-12%) in *Urochondra* and moderately (~28%) in *Leptachloa* under various salt treatments compared to controls. The values of ψ_w and ψ_s, i.e., –3.98 MPa and 760.5 mmol kg^{-1} were obtained under salinity stress of ECe ~ 50 dS m^{-1} in *Urochondra* whereas the values of ψ_w and ψ_s were – 3.63 MPa and 556 mmol kg^{-1} in *Leptachloa*. Osmoprotectant (proline, glycine betaine, total soluble sugar) and epi-cuticular wax content increased with increasing sodicity/salinity stresses in both grasss. The results showed that both halophytic grasses maintained lower Na^+/K^+ in their roots and which excludes the salt through the shoots portion. Expression of *NHX1* gene increased with an increase of not only sodic, but also saline stress in both the grasses.

Interpretation : The results demonstrate that *Urochondra* has a better adaption towards salinity and *Leptochloa* towards sodicity stress.

Key words : Gene expression, Halophytes, *Leptochloa fusca*, Salinity stress, *Urochondra setulosa*

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Introduction

Salinity is one of the major environmental stress adversely affecting agricultural production throughout the world, especially in the irrigated areas. Severity of problem is evidenced by the fact that nearly one fifth of the total global irrigated area (~ 45 Million ha) suffers from salinity and associated problems. Available evidence suggests that unabated salinization continues to impose substantial cost on productive farmlands in both irrigated and dry land areas across the globe and may attain epic proportions in the decades forward due to a steady expansion in irrigated area, use of salty waters in irrigation and climate change impacts (Sharma and Singh, 2017a; 2017b). Due to their glycophytic (salt sensitive) nature, most crop plants have weak ability to sustain excess salts in the growing medium and suffer from serious injuries when soil salinity exceeds their salt tolerance threshold (Flowers, 2004; Munns and Tester, 2008). In saline soils, plant growth is hampered initially by the osmotic stress and subsequently by salt specific effects. Most of the detrimental effects of salinity stress are ascribed to the excessive accumulation of Na^+ and Cl^- ions in plant tissues resulting in a number of physiological abnormalities suppressing plant growth and yield. Subsequent to entry inside the plant, Na^+ and Cl^- ions pose harmful effects, including disruption of cellular processes (James *et al.,* 2011).

In contrast to salt sensitive glycophytes, salt loving halophytes tolerate extreme levels of salinity. Halophytes employ following three strategies for reduced uptake and/or tolerance to the absorbed Na^+ and Cl^-: limiting Na^+ and Cl^- influx into the root cells; ion compartmentalization into vacuoles and increased $Na^+$and Cl^- efflux from the root cells (Blumwald *et al.,* 2000; Hasegawa *et al.,* 2000). These mechanisms of ion detoxification and cellular osmotic adjustment enable the halophytes to withstand persistently high salinity in the root zone. In order to adapt to salt-rich conditions, halophytes accumulate cytoplasm-compatible, organic and highly soluble compounds called osmo-protectants or osmolytes such as proline, glycine betaine and sugars etc., to maintain the osmotic pressure in cytoplasm for sufficient leaf turgidity and for protecting cell membranes, lipids and proteins from salt injury. The first family of cation/proton exchangers studied in the plants are *NHX1* transporters. There is conclusive evidence that *NHX1* plays a critical role in the vacuolar compartmentalization of Na^+ions, raising salt tolerance in plants (Rauf *et al.,* 2014).

In *Arabidopsis thaliana, AtNHX1* is identified as an important protein involved in salt tolerance and is suggested to modulate the compartmentalization of Na^+ions in vacuoles (Rodríguez-Rosales *et al.,* 2009). Subsequently, a large number of Na^+/H^+ antiporter homologs have been identified and isolated from different plant species including halophytes like *Atriplex gmelini* (Hamada *et al.,* 2001), *Suaeda salsa* (Li *et al.,* 2007), *Halostachys caspica* (Guan *et al.,* 2011), *Salicornia brachiata* (Jha *et al.,* 2011) and *Karelinia caspica* (Liu *et al.,* 2012). *Urochondra setulosa* and *Leptochloa fusca* are halophytes belonging to grass family Poaceae. These perennial grasses are widely grown in arid, salt-affected areas of Indo-Gangetic plains and elsewhere naturally that can serve as a source of animal fodder. This study also support the idea that Na^+/H^+ antiporter gene may be involved in Na^+ excretion and accumulation by these halophyte grasses, implying that *NHX1* gene from these grasses can be a good candidate gene for enhancing salinity and sodicity tolerance in other plant species, including major crops. The objective of the present study, therefore, was to assess the salt tolerance potential of *Urochondra setulosa* and *Leptachloa fusca,* using physiological, biochemical and molecular traits.

Materials and Methods

Experimental set up : The present investigation was conducted on *Urochondra setulosa* and *Leptochloa fusca* in a screen house at ICAR-CSSRI Experimental Farm, Karnal, India. The rooted cuttings and seeds of *U. setulosa* were collected from Rann of Kutch, Bhuj, Gujarat and those of *L. fusca* from Regional Research Station, ICAR-CSSRI, Lucknow, India. Using these propagules, these grasses were established in screening blocks filled with sandy and moist soil under natural conditions. After establishment, the plants were transferred to micro-plots (2.5m × 1.5m × 0.5m). Regular treatments were imposed at fortnight through saline/sodic water irrigation to achieve different stress levels of pH ~ 9.5, pH ~ 10, ECe ~ 30 dS m^{-1}, ECe ~ 40 dS m^{-1} and ECe ~ 50 dS m^{-1} in three replications along with control (pH ~ 7.8 and ECe ~ 0.83 dS m^{-1}). The micro-plots were irrigated at fortnightly intervals for maintaining the desired levels of salinity and sodicity. Screen house was covered with a high-quality polythene sheet to avoid rain water induced dilution so that uniform levels of salinity were maintained throughout the experiment.

Physiological and biochemical parameters: Data on different physiological and biochemical parameters were recorded after 12 weeks of salt treatment. Plants were gently removed from the soil after 24 weeks to measure the total biomass by weighing the whole plant. Chlorophyll content was estimated following the method of Hiscox and Israelstam (1979). Water potential (WP4C Dew-point Potentiometer) and osmotic potential (VAPRO 5600 Vapor Pressure Osmometer) were determined using the leaves and cell sap, respectively. To estimate the total soluble sugars, 100 mg of fresh leaf tissue was homogenized in 80 % ethanol and anthrone reagent, and readings were taken following the methodology of Yemm and Willis (1954). Total protein content was estimated by the method of Bradford method (1976).

Proline content, epicuticular wax and glycine betain were determined by the methods of Bates *et al.* (1973), Ebercon *et al.* (1977), and Grattan *et al.* (1996), respectively. Roots and leaves were sampled separately for measuring Na^+ and K^+ ion contents with flame photometer (Systronics Flame Photometer 128, Biozone India Scientific) after di-acid [HNO_3 : $HClO_4$ (3:1)] digestion of oven dried plant material. Chloride content in leaves was determined volumetrically.

Quantitative expression analysis: Total RNA was isolated from shoots of both plants by Trizol reagent of GCC Biotech according to manufacturer's instruction. The quality and concentration of extracted RNA were checked by agarose gel electrophoresis and Nanodrop spectrophotometer (DeNovix, DS-11+ spectrophotometer), respectively. Quantified RNA was used for *DNaseI* digestion to remove genomic DNA contaminations which was later reverse transcribed to cDNA using R2D 1st strand cDNA synthesis kit (GCC Biotech). The corresponding amount of cDNA was used as template among samples, and gene specific primers NHX1-F: TTCTGGATTGCTCAGTGCTT and NHX1-R: CAGCCAGCATGTAAGAGAGG were used to amplify the NHX1 and *Lf NHX1* in *U. setulosa* and *L. fusca*, respectively. A fragment of Actin was used as control having primer Actin-F (CGTACAACTCCATCATGAAG) and Actin-R (AGTGTTTGGATTGGTGGCTC). Real-Time PCR were carried out on CFX96 Real-Time PCR system (Bio-Rad) using the "Sso Fast Eva Green Supermix" (Bio-Rad). Each assay was performed in three replicates with no template control (NTC) and within each triplicate, values exceeding standard deviation >10% were discarded. To confirm product specificity, a melting curve analysis was performed.

Statistical analyses: The obtained data were analyzed statistically using 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

Salt stressed plants suffer from osmotic stress in the first phase and ionic stress in second phase during which salts (toxic ions) damage cell functions and structure, suppressing the growth and yield. Halophytes have evolved several complex strategies to avoid and/or tolerate salinity stress, making them interesting models for understanding the physiological and molecular mechanisms underlying salt tolerance. A suit of physiological and biochemical pathways including retention and/or acquisition of water, chloroplast protection, and maintenance of ion homeostasis, enable plants to cope up salinity stress (Kumar *et al.,* 2016). In this study, *U. setulosa* and *L. fusca* were selected based on their potential to remediate salt-affected soils while providing good amount of forage biomass. It was found that while salinity stress enhanced biomass production in *U. setulosa* (Table 1) and sodicity stress in *L. fusca* (Table 2), a reverse trend occurred under sodic and saline stress in these grasses, respectively.

The highest biomass was produced by *U. setulosa* at EC_e ~ 50 dS m^{-1} (38.35 g $plant^{-1}$) and *L. fusca* at pH ~ 10.0 (126 g $plant^{-1}$). In the current study, minor reduction in plant biomass in both saline and sodic treatments could be due to lower ionic load and/or slight decrease in leaf chlorophyll (Kumar *et al.,* 2018b). Being halophytic grasses, *U. setulosa* and *L. fusca* showed only marginal decrease in leaf chlorophyll content under different stress treatments. In *U. setulosa,* for example, pH ~ 10.0 and ECe ~ 50 dS m^{-1} led to a reduction of 5% and 12% in the total chlorophyll content, respectively. In comparison, *L. fusca* exhibited relatively higher reduction (~28%) in the same treatments (Table 1 and 2). Decrease in leaf chlorophyll content with increasing stress severity might be due suppression of enzymes responsible for synthesis of chlorophyll pigments (ALA synthase enzyme) or due to salt-induced increase in chlorophyllase activity or due to photo-inhibition or ROS formation (Singh *et al.,* 2016; Kumar *et al.,* 2018a). Plant–water relation under stress conditions explain how plants control or maintain optimal cell hydration because it has important implications for physiological and metabolic processes. Water potential (ψ_w) and osmotic potential (ψ_s) are physiological indices used to measure the extent of stress in plants; relative decline in ψ_w and ψ_s vary with tolerance threshold of plants (Pooja *et al.,* 2019). Increased salt concentrations in the root medium reduced leaf ψ_w and ψ_s in both grasses (Fig. 1).

Furthermore, it was also found that decline in leaf ψ_w and increased ψ_s were more prominent in saline stress than in sodic stress (Fig. 1). The minimum values of ψ_w and ψ_s at EC_e ~ 50 dS m^{-1} were –3.98 MPa and 760.5 mmol kg^{-1} in *U. setulosa* and– 3.63 MPa and 556 mmol kg^{-1} in *L. fusca,* respectively. Reduction in leaf ψ_w and ψ_s values might be due to osmotic stress induced reductions in water availability to the actively growing tissues. Decrease in ψ_w and ψ_s can be explained by reduced availability of water to growing tissues in saline and sodic soils. In order to overcome osmotic stress induced reduction in water absorption, plants tend to lower cellular osmotic potential by accumulating inorganic ions and compatible organic solutes.

Accumulation of compatible solutes in the cytoplasm is regarded as a key strategy for osmotic adjustment by the plants to endure salt stress. Proline, a major organic osmolyte, serves as an important source of carbon and nitrogen and may also act as a signalling regulatory molecule activating multiple responses for adaptation to salt stress (Kumar *et al.,* 2015; Lata *et al.,* 2017). Data pertaining to proline accumulation (Table 1 and 2) revealed that proline content increased linearly with an increase in both salinity and sodicity stresses. Significantly higher proline content was recorded at EC_e ~ 50 dS m^{-1} (7.20 mg g^{-1} in *U. setulosa* and 4.86 mg g^{-1} in *L. fusca*) than when these grasses were exposed to soil pH ~ 10.0 (5.37 mg g^{-1} in *U. setulosa* and 3.66 mg g^{-1} in *L. fusca*). In this experiment, increased accumulation of proline indicated the response of halophytic grasses for counteracting the adverse effects of toxic salt ions in cell vacuoles (Kumar *et al.,* 2016). Proteins, another class of organic osmolyte accumulating in salinized plants, likely release usable nitrogen after stress is relieved (Singh *et al.,* 1987) and may also contribute to osmotic adjustment (Lata *et al.,* 2017). While salinized plants commonly show altered protein levels due to increased synthesis or degradation, marked difference in protein content were not seen in both the grasses regardless of salt treatment (Table 1 and 2). In *U. setulosa,* protein content slightly increased to 14.22 mg g^{-1} at pH ~ 10.0 whereas it decreased with increasing salinity.

Table 1: Effect of sodicity and salinity on growth, physiological and biochemical attributes of *Urochondra setulosa*

Treatment		Biomass (g plant^{-1})	Chlorophyll content (mg g^{-1})	Proline content (mg g^{-1})	Protein content (mg g^{-1})	Total soluble sugars (mg g^{-1})	Glycine betaine (mg g^{-1})	Epicuticular wax load (mg g^{-1})	Shoot Cl^- (% DW)	Root Cl^- (% DW)
Control		33.55^{ab}	2.05^{a}	0.69^{e}	13.41^{b}	3.23^{d}	0.49^{e}	18.80^{d}	2.04^{c}	0.66^{d}
Sodic	pH ~ 9.5	32.75^{b}	1.99^{b}	3.43^{d}	14.12^{a}	4.08^{c}	0.97^{d}	19.50^{cd}	2.83b^{c}	0.69^{d}
Stress	pH ~ 10.0	28.10^{c}	1.94^{c}	5.37^{c}	14.22^{a}	4.99^{a}	1.19^{c}	22.10^{c}	3.01b^{c}	0.72^{d}
	ECe ~ 30 d Sm^{-1}	34.60^{a}	2.04^{a}	3.48^{d}	13.32^{b}	3.56^{d}	0.97^{d}	21.80^{c}	3.45^{b}	0.82^{c}
Saline	ECe ~ 40 d Sm^{-1}	35.25^{a}	1.88^{d}	5.68^{b}	12.59^{c}	4.33^{bc}	1.46^{b}	35.70^{b}	3.64^{b}	0.98^{b}
stress	ECe ~ 50 d Sm^{-1}	38.35^{a}	1.80^{e}	7.20^{a}	12.49^{c}	4.64^{ab}	1.79^{a}	39.00^{a}	5.93^{a}	1.29^{a}
General Mean		31.60	1.95	4.31	13.36	4.14	1.15	26.15	3.45	0.86
CV(%)		9.93	0.49	0.74	1.25	3.79	0.88	4.28	11.92	4.25
SE(d)		3.139	0.010	0.032	0.168	0.157	0.010	1.120	0.411	0.037
LSD at 5%		8.0699	0.0245	0.0816	0.4309	0.4033	0.026	2.8786	1.0573	0.0939

Means with at least one letter common are not statistically significant (p <0.05) using Duncan Multiple Range Test

Table 2 : Effect of sodicity and salinity on growth, physiological and biochemical attributes of *Leptochloa fusca*

Treatment		Biomass (g plant^{-1})	Chlorophyll content (mg g^{-1})	Proline content (mg g^{-1})	Protein content (mg g^{-1})	Total soluble sugars (mg g^{-1})	Glycine betaine (mg g^{-1})	Epicuticular wax load (mg g^{-1})	Shoot Cl^- (% DW)	Root Cl^- (% DW)
Control		115.10^{c}	2.24^{a}	0.86^{f}	15.80^{a}	2.71^{e}	0.51^{e}	33.00^{b}	2.83	0.36^{e}
Sodic	pH ~ 9.5	116.45^{b}	2.06^{b}	2.88^{d}	15.93^{a}	3.19^{cd}	1.11^{c}	41.00^{b}	2.81	0.39d^{e}
Stress	pH ~ 10.0	126.00^{a}	1.64^{d}	3.66^{b}	15.95^{a}	3.62^{c}	1.31^{a}	49.00^{ab}	2.83	0.4$_{d}$
	ECe ~ 30 d Sm^{-1}	109.75^{d}	2.03^{b}	1.60^{e}	15.84^{a}	3.08^{de}	0.74^{d}	37.00^{b}	2.98	0. 58^{c}
Saline	ECe ~ 40 d Sm^{-1}	97.00^{e}	1.94^{c}	3.40^{c}	15.81^{a}	4.07^{b}	1.22^{b}	41.00^{B}	3.32	0.71^{b}
stress	ECe ~ 50 d Sm^{-1}	76.25^{f}	1.61^{d}	4.86^{a}	15.40^{b}	5.50^{a}	1.38^{a}	63.00^{a}	3.81	0.8^{a}
General Mean		106.76	1.92	2.88	15.79	3.69	1.04	44.00	3.11	0.54
CV (%)		0.32	1.24	1.48	0.66	4.60	2.69	15.03	11.77	2.68
SE(d)		0.339	0.024	0.043	0.105	0.170	0.028	6.613	0.366	0.014
LSD at 5%		0.8711	0.0612	0.1098	0.2689	0.4372	0.072	17	NS	0.0372

Means with at least one letter common are not statistically significant (p <0.05) using Duncan Multiple Range Test

In *L. fusca,* no significant changes were observed in protein content. These proteins may either be synthesized *de novo* in response to salt stress or may be present constitutively at low concentrations and increase when plants are exposed to salt stress (Pareek *et al.,* 1997). Glycine betaine, another major compatible solute, increased in both saline and sodic treatments; although relatively a greater increase was found in saline conditions in both the grasses. In *U. setulosa,* GB content increased by 143 % in sodic (pH ~ 10.0) and 265 % in saline (EC_e~50 dS m^{-1}) stress as compared to control. A similar trend of increase was also found in *L. fusca* (Table 2). Glycine betaine helps in osmoregulation by stabilizing the oxygen-evolving activity of photosystem-II protein complexes at high salinity, and might also protect membranes, macromolecules, proteins (RuBisCo) and photosynthetic apparatus from oxidative stress (Sakamoto and Murata, 2002). Soluble sugar also play a key role in osmoregulation, controlling water potential and osmotic potential and act as a key component of source-sink partitioning between different organs in plant cells. Total soluble sugars significantly increased with an increase in salinity and sodicity of growing medium (Table 1 and 2). The maximum accumulation of soluble sugars in *U. setulosa* (4.9 mg g^{-1}) was recorded at pH ~ 10.0 (Table 1). However, soluble sugar accumulation was considerably higher (5.50 mg g^{-1}) in saline treatment (EC_e ~ 50 dS m^{-1}) than in sodic (3.62 mg g^{-1} at pH ~ 10.0) treatment in *L. fusca* (Table 2).

Plants produce and utilize sugars in various metabolic reactions; sugars also act as a carbon reservoir for non-photosynthesizing organs like roots, stems and flowers. Increased soluble sugar levels in salt stressed halophytic grasses in this experiment reflected a strategy by the plants to achieve balance between anabolic and catabolic processes (Garg and Singla, 2009). Salt-induced increase in soluble sugar content and

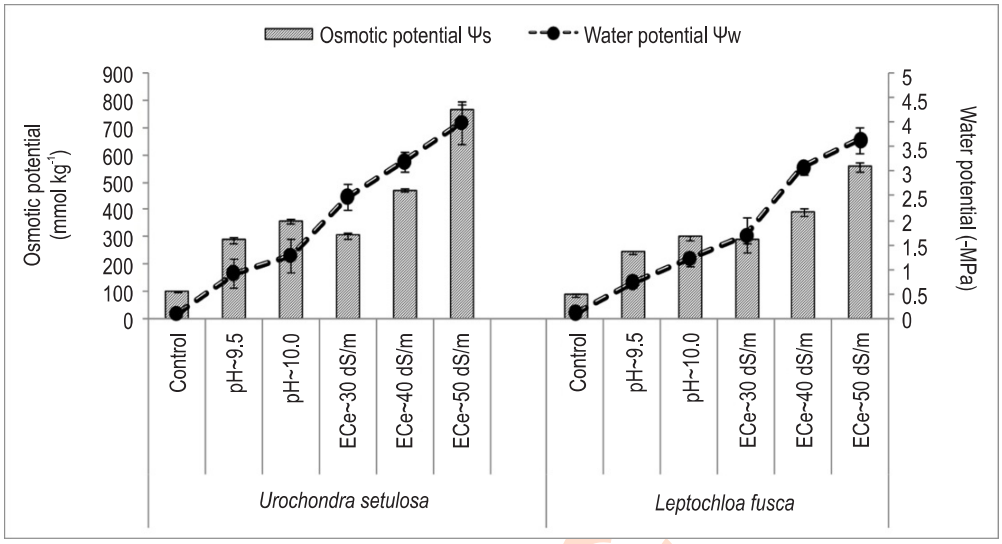

Fig. 1 : Osmotic and water potential in *Urochondra and Leptochloa* under different stress environments.

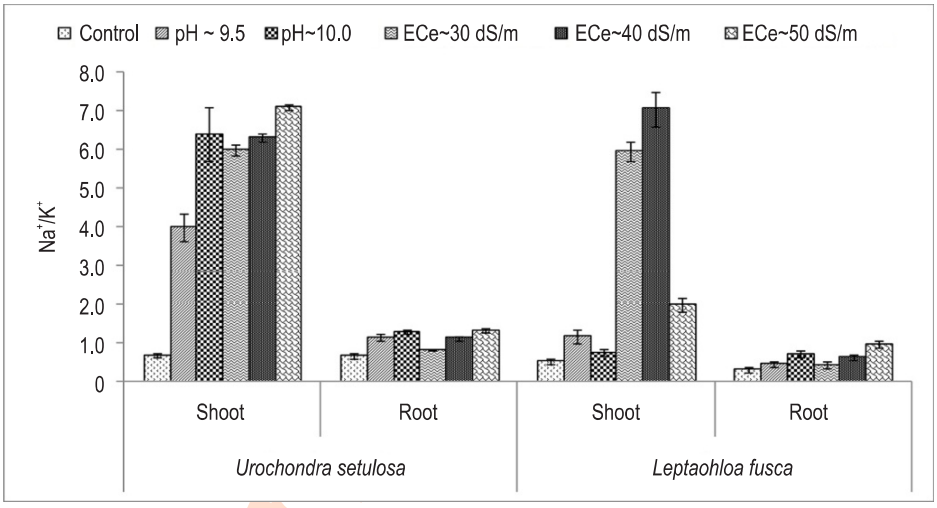

Fig. 2 : Na^+/K^+ in shoots and roots of *Urochondra* and *Leptochloa* under different stress environments.

a corresponding decrease in starch concentration has also been observed in other halophytes (Prado *et al.* 2000; Ashraf and Harris 2004). Epi cuticular wax, the outermost hydrophobic layer of aerial plant tissues, acts as a barrier to excessive non-stomatal transpiration (Yeats and Rose, 2013) and also play an important role in protecting the plants against various abiotic stresses. Data presented in Table 1 and 2 reflect the epi-cuticular wax content increased with increasing salinity and sodicity. However, significantly higher wax content was found in *L. fusca* than in *U. setulosa; U. setulosa* showed 1.16 and 2.07-fold increase at pH ~ 10.0 and Ec_e ~50 dS m^{-1}, respectively, whereas the corresponding increase were 1.48 and 1.91-fold in *L. fusca*. Epicuticular wax maintains equilibrium between the transpirational water loss and root water uptake by transpiration control and by regulating the exchange of gases and vapour (Riederer and Muller, 2008). It has also been reported that higher wax content is associated with

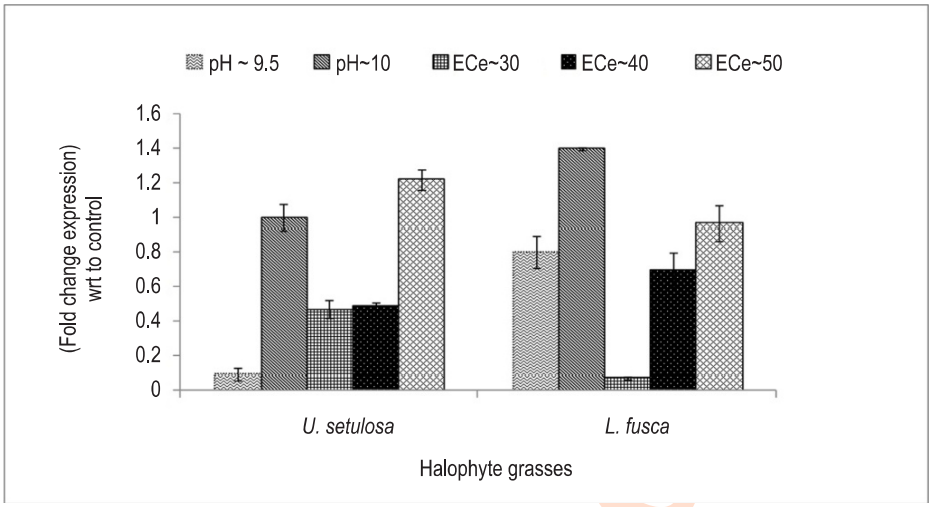

Fig. 3 : Fold-changes in the expression levels of NHX1 genes in *Urochondra setulosa* and *Leptochloa fusca* represented as log_{10} values.

lower transpiration rates enabling the halophytic grasses to survive in harsh saline conditions (Kumar *et al.,* 2016).

Excessive accumulation of Na^+ and Cl^- ions in leaves is considered to be harmful for normal plant metabolism and induces ionic imbalance, hampering the uptake of essential nutrients. Maintenance of lower leaf Na^+ concentration for favourable Na^+/K^+ ratio is an important aspect of salt stress tolerance (Kumar *et al.,* 2016; Kumar *et al.,* 2018b). Salt tolerant plants exhibit higher capacity for salt exclusion than their salt sensitive counterparts. In this experiment, both the halophytic grasses maintained lower Na^+/K^+ ratios in roots and also excluded salts from the foliage (Fig. 2). In salt-free soils, Na^+/K^+ ratio was 0.65 in both roots and shoots of *U. setulosa* whereas it was 0.29 in roots and 0.5 in shoots of *L. fusca.* With an increase in sodicity and salinity, the Na^+/K^+ ratio in both the grasses increased. The highest shoot Na^+/K^+ was recorded at pH ~ 10.0 (6.38) and EC_e ~ 50 dS m^{-1} (7.09) in *U. setulosa* and at pH ~ 9.5 (1.14) and EC_e ~ 40 dS m^{-1} (7.04) in *L. fusca* (Fig. 2).

Both the grass species tended to restrict the rise in Na^+/K^+ ratio by maintaining the internal Na^+ level by excreting excess Na^+ through salt glands and hairs. In this study, salt treatment increased Na^+/K^+ in roots and shoots in both the grasses, which might be due to a direct competition between K^+ and Na^+ ions at plasma membrane, inhibition of K^+ ions transport in xylem tissues and/or Na^+ ions induced K^+ efflux from the roots (Mann *et al.,* 2015). Both Na^+ and K^+ ions compete for entry into plant root cells and replacement of K^+ by Na^+ ions often leads to nutritional imbalance (Singh *et al.,* 2018). Increased accumulation of K^+ ions in shoots is possibly attributable to its enhanced selective uptake over Na^+ ions by the roots.

The accumulation pattern of Cl^- ions in shoots showed a trend similar to that of Na^+ ions only in saline treatments whereas no significant changes were detected under sodic stress. Like Na^+, Cl^- partitioning in roots indicated a tendency of these grasses for restricted Cl^- uptake with increasing salinity or sodicity. In sodic treatment, root Cl^- content increased by 9.09 % and 11.11 % in *U. setulosa* and *L. fusca,* respectively (Table 1 and 2). With the increase of salinity level, shoots of both the grasses accumulated more Cl^- ions than roots. While shoot Cl^- content increased by 48% at pH ~ 10.0 and 191 % at ECe ~ 50 dS m^{-1} in *U. setulosa* (Table 1), *L. fusca* showed non-significant difference for Cl^- content in sodic treatments but about 35% more shoot Cl^- in saline treatment than control. It was inferred from the results that these halophytes showed both succulence and Na^+ and Cl^- excretion via salt glands or bladders as adaptive traits under saline and sodic conditions.

Salinized plants also employ several molecular mechanisms to control the development and function of cells which depend on perception, integration and processing of signals. Genes such as *NHX1, P5CS, SOS1, SOS2, SOS3* etc., are considered as markers for salt tolerance in plants. In salt stressed plants, increased expression of these genes result in the maintenance of intracellular ion homeostasis. Halophytes have a well-developed mechanism for salt tolerance in comparison to glycophytes, which may be due to high competence of Na^+/H^+ antiporter genes and electro-gradient generating proton pumps (Khan, 2011). Real-time PCR performed using gene-specific primers indicated the expression profiles of *NHX1* gene in *U. setulosa* and *L. fusca.* Both stresses significantly affected the

expression of *NHX1* gene in both grasses.

Expression of NHX1 (given in fold change expression) was found to be up-regulated with an increase in sodic and saline stress as compared to control (Fig. 3). In *U. setulosa*, *NHX1* transcript level were abundant at ECe ~50 dSm^{-1}, *i.e.,* ↑ 16.5±3.4, compared with other treatments while in case of *L. fusca,* transcript level were abundant at pH ~ 10, *i.e.,* ↑ 24.86±1 which might be due to their inherent tolerance for saline and sodic environments, respectively. *NHX1* functions in intracellular sequestration of Na^+ and K^+ ions results in maintenance of lower Na^+/K^+ ratio. Earlier studies have also confirmed the presence of K^+/Na^+ transporter channels across the tonoplast mediated by Na^+/H^+ antiport activity, resulting in the compartmentalization of toxic ions in the vacuoles and K^+ homeostasis (Jeschke, 1984; Lan *et al.,* 2011). The ability of these grasses to survive in higher Na^+/K^+ ratio and higher *NHX1* expression clearly points towards higher salt tolerance capacity.

The results concluded that these halophytic plants tolerate excessive salinity by effective coordination between various physiological processes, metabolic pathways and protein or gene networks. In this study, an attempt was made to identify such adaptive mechanisms employed by these halophytic grasses to endure saline conditions. Based on overall results, *Urochondra* showed better adaptation to salinity and *Leptochloa* to sodicity stress.

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