



## Research article

# Integrated physiological and molecular approaches to study salt-induced adaptation in halophytes collected from extremely saline lands

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## Abstract

Diverse halophytes were collected across the extremely salt-affected lands of Haryana to explore their salt potential. Higher levels of Na<sup>+</sup> and Cl<sup>-</sup> were observed in leaves (3.20 and 4.55%) than in stem (0.78 and 1.09%) and root (0.81 and 0.80 %). Interestingly, these plants also accumulated higher concentrations of K<sup>+</sup> for their survival. Similarly, Ca<sup>2+</sup> accumulation was higher in leaves (1.57%) than in stems (1.15%) and roots (1.18%). The salt tolerance potential of these halophytes was assessed by examining the ratio of accumulation of toxic ions, namely Na/K, Na/Ca, and Na+Cl to K+Ca, resulting in their characterization and ranking from highly salt tolerant to least tolerant. The utilization of this ionic accumulation index has the potential to serve as a criterion for the elimination of surplus toxic ions from soil. The halophytes employed adaptive mechanisms by synthesizing osmolytes, such as proline, soluble sugars, and soluble proteins, in both leaves and roots. The upregulation of genes P5CS and NHX1, responsible for proline synthesis and membrane transportation, further confirmed the halophytes' ability to tolerate high salinity. The findings of these studies indicated the potential for domesticating salt-tolerant halophytes in salt-affected soils.

**Keywords:** Halophytes, Ion accumulation, Osmolytes, Salinity

## Introduction

In the era of climate change, decreased precipitation and increased evapotranspiration, along with the soil salinity and poor groundwater resources, affect morphological, anatomical, and physiological traits/strategies, which ultimately reduce the yield and productivity of crops (Soni *et al.*, 2020; 2021; Dhansu *et al.*, 2022; Souguir *et al.*, 2019; Louhaichi and Hassan, 2023). Globally, around 1000 million hectares of land affected by salinity pose a risk to agricultural processes and restrict crop production. In India, the combination of salinity and water logging has resulted in the conversion of nearly 20% of irrigated lands into barren areas, rendering them uncultivable in extreme cases, particularly in arid and semi-arid regions. These twin problems of excess salts in soil and water logging are threatening the sustainability of agricultural production in large parts of Haryana state in the country. As per a report of the Haryana Kisan Aayog, more than 50,000 hectares are affected by soil salinity and water logging, having shallow water table depth of less than 1.5 meters. Salinity in waterlogged areas is 35 to 40 ds/m, although

the normal limit is 4 ds/m. Most of the water-logged saline soils are found in the central inland depression basin of the state, including Rohtak, Hisar, Jhajjar, Bhiwani and Palwal districts.

One of the sustainable goals for enhanced food security and livelihood includes efficient management of these salt-affected soils. Given the escalating issue of soil salinization and water scarcity, there is now a significant focus on investigating the tolerance mechanisms of halophytes, which are naturally salt-tolerant plants (Mangalassery *et al.*, 2017). Halophytes are a group of plants that have the ability to survive and flourish in challenging saline environments by undergoing various physiological, biochemical, and molecular adaptations. These alterations may include the biosynthesis of certain osmolytes or differentiation of toxic ions across various cellular compartments, thus maintaining ion homeostasis (Kumar *et al.*, 2016, 2018, 2019; Mann *et al.*, 2019a). The mechanism of salt tolerance is a complex and multigenic process mainly governed by ion homeostasis through transport and cellular location of Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup> and Cl<sup>-</sup> in

addition to the various other adaptive features. A lot of information is available for salt tolerance mechanism of model plant species *Arabidopsis thaliana* (Borsani et al., 2001) and *Thellungiella halophila* but these two plant species are short-lived and could survive at lower levels of salinity. Hence, the real *in situ* picture of true halophytes need to be elucidated for better salt adaptive strategies. Although many halophytes, including Suaeda (Guo et al., 2019), *Salicornia brachiata* (Jha et al., 2009), *Urochondra setulosa* and *Dichanthium annulatum* (Mann et al., 2021), *Sporobolus marginatus* (Mann et al., 2019b), Salvador, *Aegilops tauschii* (Mansouri et al., 2019), Amaranthus and Chenopodium species and *Atriplex* etc (Kumar et al., 2021) have been explored for their salt tolerance mechanisms but still a single adaptive strategy through physiological and biochemical interactions and the gene network analysis at high salt concentrations need to be explored. Transcriptomics studies on salt-related pathways/genes in halophytes are being explored now for the development of climate-smart crops through genetic engineering. Differentially expressed genes in *S. salsa* at 30% NaCl (Guo et al., 2019) and in *Beta vulgaris* at 200 and 400 mM NaCl (Lv et al., 2018) also identify the salt tolerance mechanism in leaves and roots. A major gene for Na<sup>+</sup> ion exclusion, SOS2, was found to be involved in salt tolerance in mutated *Arabidopsis* plants (Ohta et al., 2003). *S. alterniflora* maintains the salt tolerance ability by regulating the uptake and accumulation of Na<sup>+</sup>, K<sup>+</sup> and Cl<sup>-</sup> through up-regulation of selective stress-related transporters (Bedre et al., 2016). In view of these facts, this study was planned to assess the salt tolerance potential of the collected halophytes based on the key traits for ion partitioning and osmolyte accumulation and the corresponding gene expression.

## Materials and Methods

**Experimental site and plant materials:** A survey was carried out during 2017-18 in the saline lands of district Hisar, Jhajjar and Rohtak in Haryana state of India. Finally, after considering the salinity levels, four sites representing high salinity were selected for study- (1) Barren field of ICAR-CIRB, Hisar (2) Village Dhandlan, Jhajjar (3) Village Dighal, Jhajjar and (4) Village Chuliyana, Rohtak. These sites turned into wastelands due to the non-cultivation of crops in lieu of high salinity. Soil samples were collected from these sites at three different soil depths viz. 0 to 15, 15 to 30 and 30 to 45 cm in three replicates and further analysis was done at ICAR-CSSRI, Karnal. Soil samples were air-dried and passed through a 2 mm sieve in the laboratory for undertaking various analyses. Soil ECe (EC of saturation extract) and pHs (pH of saturation extract) were determined using EC-meter and pH meter. A total of 16 halophytic plant species were collected as *Heliotropium ramossimum*, *Withania somnifera*, *Aerva tomentosa*, *Atriplex amnicola*, *Desmostchya*

*bipinnata*, *Portulaca portucalustrum*, *Atriplex nummularia*, *Atriplex lentiformis*, *Tamarix aphylla*, *Arundo donax*, *Suaeda fruticosa*, *Suaeda nudiflora*, *Dysphania ambrosioides*, *Aelurolopus lagopoides*, and *Dichanthium annulatum* from different salinity sites across Rohtak, Jhajjar and Hisar district of Haryana. In addition to these, germplasm of four other halophytic plants i.e. *Urochondra setulosa* and *Sporobolus marginatus*, taken as highly salt tolerant check plants as per our previous reports which were originally collected from extreme saline-sodic Kachhch plains, Rann of Kachhch, Bhuj, Gujarat and *Leptachloa fusca*, from ICAR-CSSRI, Regional Research Station, Lucknow and *Chenopodium quinoa* from ICAR-CSSRI repository, Karnal were also included in the study. One set of collected halophytes were also established in porcelain pots under control conditions (without salinity) at ICAR-CSSRI, Karnal.

**Ions and osmolytes analyses:** The ionic contents of Na<sup>+</sup>, K<sup>+</sup>, and Ca<sup>2+</sup> were determined using oven-dried and acid-digested samples of leaves, stems, and roots separately (Arora et al., 2018). The Na<sup>+</sup> and K<sup>+</sup> contents were measured with a flame photometer (Flame Photometer 128, Systronics) and Ca<sup>2+</sup> with an atomic absorption spectrophotometer (Z Express 8000, Spectrum Technology) using a standard solution. The ionic contents, Na<sup>+</sup>, K<sup>+</sup> and Ca<sup>2+</sup> were expressed in percent (%) value after standard calculations. Chloride content (Cl<sup>-</sup>) of leaf, stem and root tissue was determined volumetrically by a modified method of Chhabra (1973). The major osmolytes involved in salt tolerance, like proline, total soluble sugars and total soluble proteins, were assessed in leaves as well as roots. Proline content (mg g<sup>-1</sup> fresh weight) was estimated by the method of Bates et al. (1973). Bradford reagent (Bradford, 1976) was used for the estimation of total soluble proteins (mg g<sup>-1</sup> FW) at 595 nm. Total soluble sugars (mg g<sup>-1</sup> FW) were measured at 625 nm using anthrone reagent by the method of Yemm and Willis (1954). The salt tolerance potential of these halophytes was calculated based on the ratio of accumulation of toxic ions, i.e., Na+Cl, against beneficial ions K+Ca (Kumar et al., 2021) and were characterized as highly tolerant to moderately salt tolerant.

**Real time PCR analysis:** Fresh leaf tissue (~80 mg each) was used for the isolation of total RNA using RNA-Xpress™ Reagent (Hi-Media) according to manufacturer's instruction and treated with RNase-free DNase I (Thermo Scientific) to remove residual DNA. RNA was reverse transcribed using iScript™ cDNA Synthesis Kit (Bio-rad) according to the manufacturer's instructions. The reaction was carried out in 20 µL mixture by adding 1-µg of template RNA, 1-µL reverse transcriptase and 4 µL of reaction buffer (containing oligo-dT) in 0.2 mL centrifuge tubes. The volume was made up to 10 µL with DEPC-treated water and heated the mixture at 65°C for

5 minutes followed by quick chill on ice. The tubes were incubated at 25°C for 10 minutes and 46°C for 30 minutes and finally reaction was terminated by heating at 95°C for 1-minute. cDNA was synthesized in Thermal Cycler (Biometra TAdvanced, Analytik Jena) with initial priming at 25°C for 5 minutes, followed by reverse transcription at 46°C for 20 minutes. The reaction was terminated at 1°C for one minute and cDNA was stored in the refrigerator. The gene-specific primers (Table 1) were designed in-house using the Primer Quest Tool (<https://eu.idtdna.com/Primerquest/Home/Index>) from the available sequences of halophytes *D. annulatum* (Accession No. PRJNA665324) and *U. setulosa* (Accession No. PRJNA561259) for the major osmolyte proline (P5CR) and the membrane Na<sup>+</sup>/H<sup>+</sup> transporter (NHX1).

Real-Time quantitative expression analysis was done as per the manufacturer's protocol of Sso Advanced Universal SYBR Green Supermix (Bio-Rad) in three technical replicates with three biological replicates. 100 ng cDNA was taken as a template, 1x Green Supermix was mixed with 500 mM forward and reversed gene-specific primers and total volume of 20 µL was made up with NFW (nuclease-free water). Quantitative reaction was carried out in real time-PCR (Bio-Rad CFX-96™) following polymerase activation and DNA denaturation at 95°C for 20 to 30 sec. Further amplification of 35 to 40 cycles included denaturation at 95°C for 5 seconds, annealing, extension and plate reading at 60°C for 15 to 30 seconds. The actin gene was used as a housekeeping gene for normalization. Quantitative gene expression was calculated by applying the 2<sup>-ΔΔCt</sup> method (Livak and Schmittgen 2001). Out of 19 halophytic species collected, five contrasting grass halophytic species, i.e., *Arundo donax*, *Aeluropus lagopoides*, *Urochondra setulosa*, *Sporobolus marginatus* and *Leptachloa fusca* were selected based on the salt tolerance potential for real time PCR analysis.

**Statistical analysis:** All the data were subjected to variance analysis using the SAS (Version 9.3, SAS Institute Inc., Cary, NC, USA). TUKEY's Honestly significant difference was applied at 5% probability level to compare the mean differences.

## Results and Discussion

**Ion (Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup> and Cl<sup>-</sup>) concentrations:** In the majority of salt-affected soils, the dominating cation was

Na<sup>+</sup> with Cl<sup>-</sup> as the dominating anion. Salt tolerance is mainly associated with the reduced Na<sup>+</sup> and Cl<sup>-</sup> loading into the xylem, as both these ions are metabolically toxic to the plants and present in high concentrations in saline soils (Tavakkoli *et al.*, 2011). In addition to this, higher Na<sup>+</sup> alters the concentration of other ions, particularly K<sup>+</sup>, Ca<sup>2+</sup> and NO<sub>3</sub><sup>-</sup> which results in ion imbalance (Kumar *et al.*, 2016). Keeping in mind the behavior of Na<sup>+</sup> and Cl<sup>-</sup>, partitioning of Na<sup>+</sup>, K<sup>+</sup> and Cl<sup>-</sup> ions was estimated in leaves, stems and roots (Table 2). Na<sup>+</sup> was the most notorious cation which significantly varied among different halophytic species and different plant parts. It was observed that leaves had a higher mean accumulation of Na<sup>+</sup> (3.20% of dry weight (DW)) in comparison to stem (0.78% DW) and root (0.81% DW). In leaves, highest Na<sup>+</sup> was accumulated in *S. nudiflora* (5.44% DW) than the check *U. setulosa*, *D. ambrosioides*, *A. nummularia*, and *S. fruticosa*, whereas *H. ramosimum*, *W. somnifera* and *A. tomentosa* accumulated the lowest (1.07% - 1.17% DW). All the three *Atriplex* species, i.e. *A. amnicola*, *A. nummularia* and *A. lentiformis* also showed good Na<sup>+</sup> hyper-accumulator abilities and accumulated 3.48, 4.93, and 3.07% Na<sup>+</sup>, respectively (Table 2). In the stem portion, *T. aphylla* accumulated the highest Na<sup>+</sup> (1.92% DW) followed by *S. fruticosa*, *A. lentiformis* and *L. Fusca*, while *A. tomentosa* accumulated the lowest (0.16% DW). In the case of roots, *A. nummularia* accumulated the highest Na<sup>+</sup> (1.73% DW) followed by *A. lentiformis* and *S. fruticosa*, whereas the lowest accumulation was noted in *A. donax* (0.29% DW) and *D. ambrosioides* (0.31% DW), which were statistically at par followed by *A. tomentosa* (0.34% DW). The mature leaves of *Atriplex* species accumulated more Na ions than roots and shoot and roots tended to maintain the lowest Na<sup>+</sup> concentration (Devi *et al.*, 2016). Sodium metabolism in plants is very tricky since, on the one hand, Na<sup>+</sup> is required for maintaining turgor and osmotic potential, and on the other hand, excess Na<sup>+</sup> is harmful (Kumar *et al.*, 2018; 2019). Overall, it was found that most of these halophytic plants translocated Na<sup>+</sup> from the root to the leaf, which is an important strategy under salt stress (Maathuis *et al.*, 2014; Mann *et al.*, 2019b). This showed that these plants, particularly hyper-accumulator plants, could achieve the balance between intake of inorganic osmoticum and efficient sequestration of potentially harmful ions by effective cellular Na<sup>+</sup> partitioning (Maathuis, 2014). The quantitative expression of the Na<sup>+</sup>/H<sup>+</sup> transporter (Fig 1) also showed the highest expression in *S. marginatus*

**Table 1.** List of primer sequences for real time quantification

| Gene         | Forward primer         | Reverse primer         | Amplicon length |
|--------------|------------------------|------------------------|-----------------|
| <i>Actin</i> | CGTACAACTCCATCATGAAG   | GAGCCACCAATCCAAACACT   | 237 bp          |
| P5CR         | ATTGGTCTGGTCAACGACG    | ATCTCTCCCAAGCACATGAC   | 91 bp           |
| <i>NHX1</i>  | GGCATAATGTGACAGAGAGCTC | GGCATCCATCCCAACATATAGG | 104 bp          |

**Table 2.** Na<sup>+</sup>, K<sup>+</sup> and Ca<sup>2+</sup> ion partitioning in different plant parts of halophytes collected from saline lands of Haryana

| Halophytes/traits                | Na <sup>+</sup> content (% DW) |                    |                    | Cl <sup>-</sup> content (% DW) |                    |                    | K <sup>+</sup> content (% DW) |                    |                     | Ca <sup>2+</sup> content (% DW) |                      |                    |
|----------------------------------|--------------------------------|--------------------|--------------------|--------------------------------|--------------------|--------------------|-------------------------------|--------------------|---------------------|---------------------------------|----------------------|--------------------|
|                                  | Leaf                           | Stem               | Root               | Leaf                           | Stem               | Root               | Leaf                          | Stem               | Root                | Leaf                            | Stem                 | Root               |
| <i>Heliotropium ramosissimum</i> | 1.08 <sup>k</sup>              | 0.98 <sup>cd</sup> | 0.67 <sup>i</sup>  | 6.10 <sup>cd</sup>             | 0.82 <sup>j</sup>  | 0.73 <sup>fg</sup> | 1.34 <sup>ij</sup>            | 0.55 <sup>h</sup>  | 0.95 <sup>efg</sup> | 1.12 <sup>gh</sup>              | 0.35 <sup>ijkl</sup> | 0.51 <sup>g</sup>  |
| <i>Withania somnifera</i>        | 1.07 <sup>k</sup>              | 0.63 <sup>f</sup>  | 0.74 <sup>gh</sup> | 3.02 <sup>h</sup>              | 0.74 <sup>ij</sup> | 0.18 <sup>m</sup>  | 1.68 <sup>i</sup>             | 0.37 <sup>ij</sup> | 0.70 <sup>fg</sup>  | 1.05 <sup>gh</sup>              | 0.21 <sup>m</sup>    | 0.48 <sup>g</sup>  |
| <i>Suaeda nudiflora</i>          | 5.44 <sup>a</sup>              | 1.01 <sup>cd</sup> | 0.99 <sup>d</sup>  | 6.59 <sup>abc</sup>            | 0.69 <sup>jk</sup> | 1.01 <sup>e</sup>  | 8.74 <sup>b</sup>             | 2.14 <sup>d</sup>  | 4.17 <sup>a</sup>   | 1.56 <sup>a</sup>               | 0.68 <sup>de</sup>   | 1.04 <sup>b</sup>  |
| <i>Aeluropus logopoides</i>      | 2.39 <sup>h</sup>              | 0.18 <sup>h</sup>  | 0.80 <sup>fg</sup> | 5.72 <sup>de</sup>             | 1.06 <sup>gh</sup> | 1.13 <sup>d</sup>  | 3.11 <sup>h</sup>             | 1.94 <sup>e</sup>  | 2.20 <sup>bcd</sup> | 1.48 <sup>ab</sup>              | 0.42 <sup>hijk</sup> | 0.91 <sup>de</sup> |
| <i>Suaeda fruticosa</i>          | 4.69 <sup>c</sup>              | 1.32 <sup>b</sup>  | 1.37 <sup>c</sup>  | 6.39 <sup>bc</sup>             | 1.91 <sup>a</sup>  | 1.39 <sup>a</sup>  | 10.59 <sup>a</sup>            | 3.49 <sup>a</sup>  | 2.38 <sup>bc</sup>  | 1.32 <sup>cde</sup>             | 0.81 <sup>bc</sup>   | 0.86 <sup>de</sup> |
| <i>Aerva tomentosa</i>           | 1.17 <sup>k</sup>              | 0.16 <sup>h</sup>  | 0.34 <sup>l</sup>  | 5.30 <sup>ef</sup>             | 0.98 <sup>h</sup>  | 0.43 <sup>k</sup>  | 1.08 <sup>j</sup>             | 0.33 <sup>j</sup>  | 0.41 <sup>g</sup>   | 1.17 <sup>efg</sup>             | 0.38 <sup>ijkl</sup> | 0.67 <sup>f</sup>  |
| <i>Atriplex amnicola</i>         | 3.48 <sup>f</sup>              | 0.98 <sup>cd</sup> | 0.82 <sup>f</sup>  | 5.36 <sup>ef</sup>             | 1.78 <sup>b</sup>  | 1.26 <sup>b</sup>  | 6.56 <sup>cd</sup>            | 3.40 <sup>a</sup>  | 1.68 <sup>cde</sup> | 1.35 <sup>bcd</sup>             | 0.57 <sup>ef</sup>   | 0.94 <sup>cd</sup> |
| <i>Atriplex nummularia</i>       | 4.93 <sup>b</sup>              | 0.76 <sup>e</sup>  | 1.73 <sup>a</sup>  | 4.49 <sup>g</sup>              | 0.82 <sup>j</sup>  | 1.15 <sup>d</sup>  | 5.92 <sup>e</sup>             | 2.50 <sup>c</sup>  | 2.49 <sup>bc</sup>  | 1.21 <sup>def</sup>             | 0.49 <sup>fgh</sup>  | 1.03 <sup>bc</sup> |
| <i>Atriplex lentiformis</i>      | 3.07 <sup>g</sup>              | 1.04 <sup>c</sup>  | 1.66 <sup>b</sup>  | 5.29 <sup>ef</sup>             | 1.09 <sup>fg</sup> | 1.22 <sup>bc</sup> | 6.93 <sup>c</sup>             | 2.94 <sup>b</sup>  | 1.07 <sup>efg</sup> | 1.49 <sup>ab</sup>              | 0.54 <sup>fg</sup>   | 0.84 <sup>de</sup> |
| <i>Arundo donax</i>              | 4.47 <sup>d</sup>              | 0.56 <sup>f</sup>  | 0.29 <sup>l</sup>  | 3.30 <sup>h</sup>              | 0.49 <sup>j</sup>  | 0.24 <sup>l</sup>  | 6.40 <sup>d</sup>             | 1.28 <sup>f</sup>  | 2.55 <sup>b</sup>   | 0.98 <sup>hi</sup>              | 0.87 <sup>b</sup>    | 0.81 <sup>e</sup>  |
| <i>Tamarix aphylla</i>           | 3.82 <sup>e</sup>              | 1.92 <sup>a</sup>  | 0.89 <sup>e</sup>  | 7.13 <sup>a</sup>              | 1.61 <sup>c</sup>  | 1.20 <sup>c</sup>  | 8.58 <sup>b</sup>             | 2.31 <sup>d</sup>  | 1.35 <sup>def</sup> | 1.13 <sup>fg</sup>              | 0.76 <sup>cd</sup>   | 0.91 <sup>de</sup> |
| <i>Dysphania ambrosioides</i>    | 5.07 <sup>b</sup>              | 0.42 <sup>g</sup>  | 0.31 <sup>l</sup>  | 6.83 <sup>ab</sup>             | 1.50 <sup>d</sup>  | 0.51 <sup>j</sup>  | 6.48 <sup>cd</sup>            | 1.26 <sup>f</sup>  | 2.50 <sup>bc</sup>  | 0.89 <sup>j</sup>               | 0.48 <sup>fghi</sup> | 0.68 <sup>f</sup>  |
| <i>Portulaca portulacastrum</i>  | 1.62 <sup>j</sup>              | 0.33 <sup>g</sup>  | 0.53 <sup>j</sup>  | 1.76 <sup>k</sup>              | 0.19 <sup>n</sup>  | 0.47 <sup>jk</sup> | 1.24 <sup>ij</sup>            | 0.29 <sup>j</sup>  | 1.00 <sup>efg</sup> | 0.37 <sup>j</sup>               | 0.29 <sup>lm</sup>   | 0.34 <sup>h</sup>  |
| <i>Desmostachya bipinnata</i>    | 1.76 <sup>j</sup>              | 0.57 <sup>f</sup>  | 0.69 <sup>hi</sup> | 1.79 <sup>jn</sup>             | 0.40 <sup>m</sup>  | 0.28 <sup>l</sup>  | 1.49 <sup>ij</sup>            | 0.54 <sup>hi</sup> | 1.04 <sup>efg</sup> | 1.08 <sup>fgh</sup>             | 0.42 <sup>hijk</sup> | 0.43 <sup>gh</sup> |
| <i>Sporobolus marginatus</i>     | 2.30 <sup>h</sup>              | 0.79 <sup>e</sup>  | 0.69 <sup>hi</sup> | 2.12 <sup>jk</sup>             | 1.88 <sup>a</sup>  | 0.55 <sup>i</sup>  | 4.10 <sup>g</sup>             | 0.31 <sup>j</sup>  | 1.13 <sup>efg</sup> | 1.47 <sup>ab</sup>              | 0.45 <sup>fghi</sup> | 1.05 <sup>ab</sup> |
| <i>Leptochloa fusca</i>          | 3.93 <sup>e</sup>              | 1.04 <sup>c</sup>  | 0.83 <sup>f</sup>  | 2.37 <sup>ij</sup>             | 0.61 <sup>k</sup>  | 0.63 <sup>h</sup>  | 3.90 <sup>g</sup>             | 0.92 <sup>g</sup>  | 1.04 <sup>efg</sup> | 1.39 <sup>bc</sup>              | 0.72 <sup>cd</sup>   | 1.15 <sup>a</sup>  |
| <i>Urochondra setulosa</i>       | 5.42 <sup>a</sup>              | 0.62 <sup>f</sup>  | 0.78 <sup>fg</sup> | 4.81 <sup>fg</sup>             | 1.61 <sup>c</sup>  | 0.71 <sup>g</sup>  | 8.41 <sup>b</sup>             | 0.64 <sup>h</sup>  | 2.23 <sup>bc</sup>  | 1.45 <sup>abc</sup>             | 0.68 <sup>de</sup>   | 1.09 <sup>ab</sup> |
| <i>Dicanthium annulatum</i>      | 3.10 <sup>g</sup>              | 0.92 <sup>d</sup>  | 0.42 <sup>k</sup>  | 2.74 <sup>hi</sup>             | 1.16 <sup>f</sup>  | 0.76 <sup>f</sup>  | 1.14 <sup>j</sup>             | 0.35 <sup>j</sup>  | 0.68 <sup>fg</sup>  | 1.20 <sup>ef</sup>              | 0.31 <sup>klm</sup>  | 0.85 <sup>de</sup> |
| <i>Chenopodium quinoa</i>        | 2.03 <sup>i</sup>              | 0.62 <sup>f</sup>  | 0.94 <sup>de</sup> | 5.28 <sup>ef</sup>             | 1.32 <sup>e</sup>  | 1.39 <sup>a</sup>  | 5.43 <sup>f</sup>             | 1.25 <sup>f</sup>  | 1.02 <sup>efg</sup> | 1.57 <sup>a</sup>               | 1.18 <sup>a</sup>    | 0.62 <sup>f</sup>  |
| General mean                     | 3.20                           | 0.78               | 0.81               | 4.55                           | 1.09               | 0.80               | 4.90                          | 1.41               | 1.61                | 1.23                            | 0.56                 | 0.80               |
| CV (%)                           | 1.61                           | 4.20               | 2.27               | 4.21                           | 2.64               | 1.87               | 3.00                          | 4.01               | 17.46               | 7.34                            | 12.18                | 7.64               |
| SE(d)                            | 0.042                          | 0.027              | 0.015              | 0.156                          | 0.023              | 0.012              | 0.120                         | 0.046              | 0.229               | 0.073                           | 0.056                | 0.050              |
| HSD (P<0.05)                     | 0.1594                         | 0.1013             | 0.0571             | 0.592                          | 0.0886             | 0.0462             | 0.4548                        | 0.1748             | 0.868               | 0.149                           | 0.2102               | 0.1013             |

Means with at least one letter common are not statistically significant (P≤0.05) using Tukey's HSD Test

followed by *U. setulosa*, *Aeluropus* and *L. fusca* with the lowest expression in *A. donax*. The fold change values of *NHX1* represent higher activity of membrane transporter  $\text{Na}^+/\text{H}^+$  which controls the accumulation of Na. Higher *NHX* activity lowers the Na/K ratio. *NHX* proteins are ubiquitous membrane transporters for the sequestration of additional  $\text{Na}^+$  in vacuoles or the removal of excess  $\text{Na}^+$  from the cells (Himabindu *et al.*, 2016). The expression of the gene encoding  $\text{Na}^+/\text{H}^+$  exchanger was up-regulated along with the salinity stress in *D. annulatum* and *U. setulosa* (Mann *et al.*, 2019; 2021). Similarly, the Arabidopsis ABC transporter, AtABCG36, was involved in adaptation to salinity stress by reducing the shoot sodium content (Kim *et al.*, 2010).  $\text{Na}^+$  pumping into the vacuole against the electrochemical gradient for ion homeostasis through  $\text{Na}^+/\text{H}^+$  transporter has also been reported in halophytic plant *Mesembryanthemum crystallinum* L (Barkla *et al.*, 1995).

Besides  $\text{Na}^+$  toxicity,  $\text{Cl}^-$  is another important micronutrient that helps in cell turgor and pH homeostasis act as a co-factor in photosynthesis, maintaining potential across the membranes and catalysts for some enzymes (Tavakkoli *et al.*, 2011, Kumar *et al.*, 2019). In our studies, it was observed that  $\text{Cl}^-$  accumulation was higher in leaves (4.55% DW) as compared to stem (1.09% DW) and roots (0.80% DW), although few reports depicted more  $\text{Cl}^-$  accumulation in shoot parts as in *Atriplex* species. The highest amount of  $\text{Cl}^-$  was seen in leaves of *T. aphylla* (7.13% DW) followed by *D. ambrosioides* (6.83% DW) and *S. nudiflora* (6.59% DW), whereas *P. portucalustrum* restricted  $\text{Cl}^-$  accumulation (1.76% DW) to the minimum level in comparison to other halophytic plants (Table 2). This showed that the halophytic plants developed adaptive strategies to compartmentalize  $\text{Na}^+$  and  $\text{Cl}^-$  in leaves and their proportionate accumulation in vacuoles protected the cytosol from harmful effects. The range of  $\text{Cl}^-$  accumulation varied significantly from 0.19 to 1.91% in the stem, whereas roots of these halophytic plants restrict  $\text{Cl}^-$  accumulation or might transport  $\text{Cl}^-$  to the leaves portion via transpiration stream. The highest accumulation of 1.39% on dry weight basis was recorded

in *S. fruticosa* and *C. quinoa* followed by *A. amnicola* (1.26%) and *A. lentiformis* (1.22%). *W. somnifera*, *A. donax*, *D. bippinnata*, *A. tomentosa* and *P. portucalustrum* showed less than 0.5%  $\text{Cl}^-$  accumulation in their root tissues.  $\text{K}^+$  is an essential macronutrient (4–6% of its dry matter) that is involved in most of the regulatory processes such as protein synthesis, stomata opening-closing, phloem sugar loading and also acts as organic osmolyte, along with its inhibitory uptake by excess  $\text{Na}^+$  leading to growth impairment in plants under salt stress (Kumar *et al.*, 2018; Wu *et al.*, 2018).

Results depicted that these halophytic plants had accumulated a sufficient amount of  $\text{K}^+$  in their plant parts, i.e., 4.90% in leaves, 1.41% in stems and 1.61% in roots on a dry weight basis. Significantly higher  $\text{K}^+$  (2.9 times) was found in leaves as compared to stems and roots. In leaves, maximum accumulation was noted in *S. fruticosa* (10.59% DW) followed by *T. aphylla* (8.58% DW) even more than *U. setulosa* (8.41% DW). The lowest leaf  $\text{K}^+$  was seen in *D. annulatum* (1.14% DW) and *P. portucalustrum* (1.24% DW). Interestingly, it was also observed that halophytes that had higher leaf  $\text{Na}^+$  also retained higher  $\text{K}^+$  as well. Stems of *S. fruticosa* (3.49% DW), *A. amnicola* (3.40% DW) and *A. lentiformis* (2.94% DW) had higher  $\text{K}^+$  in comparison to other studied halophytic plants. Root  $\text{K}^+$  ranged from 0.41 to 4.17% in these halophytes and maximum root  $\text{K}^+$  was recorded in *S. nudiflora* while minimum in *A. tomentosa*. Interestingly, it was also noted that besides accumulating higher concentrations of  $\text{Na}^+$ , these plants also accumulated higher  $\text{K}^+$  and *A. amnicola*, *Suaeda* species, *A. donax*, *A. logopoides*, *C. quinoa* and *U. setulosa* had the lowest  $\text{Na}^+/\text{K}^+$  in all the plants parts. Our findings also supported the long-distance transport of K in these halophytes, as suggested earlier by Wu *et al.* (2018), where the lowest K levels were observed in roots and highest in young leaves of halophytes. It was observed that these halophytes developed the salt tolerance strategy through the retention of  $\text{K}^+$  in root, stem and leaves. Calcium ( $\text{Ca}^{2+}$ ) is the most important secondary messenger in many signal transduction pathways and also maintains membrane integrity. Significant variations were noted for  $\text{Ca}^{2+}$  accumulation in our results and it was observed that *C. quinoa* had the highest  $\text{Ca}^{2+}$  in its leaf and root tissue (1.57% DW and 1.18% DW), whereas *L. fusca* (1.15% DW) in stem tissue. Among different plant parts,  $\text{Ca}^{2+}$  content varied from 0.37 to 1.57% in leaves, 0.34 to 1.15% in stem and 0.21 to 1.18% in roots. Increasing water salinity increases  $\text{Ca}^{2+}$  ions in the shoots and leaves of *Atriplex* compared to roots. The  $\text{Ca}^{2+}$  ameliorates the inhibition of cell wall binding of  $\text{Na}^+$  and plasma membrane, leading to reduced leakiness of membranes as well as enhanced cell division (Al-Khateeb, 2006).

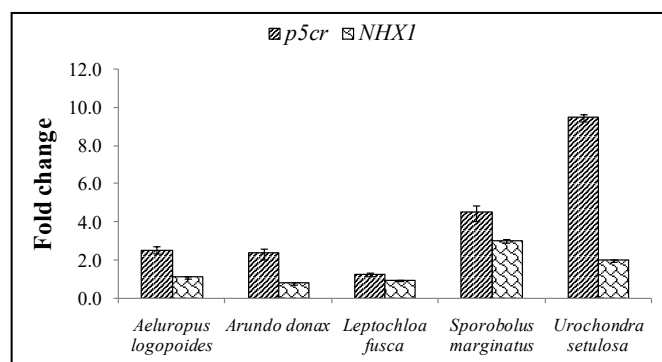


Fig 1. Quantitative gene expression under salinity in different halophytes

**Osmolytes (proline, total soluble sugars and total soluble proteins):** Osmolytes are the metabolites that maintain the osmotic potential, protein folding, detoxify

the free radicals and hence, protect various enzymes and macromolecules (Kumar et al., 2018b; Mann et al., 2019a). Among these, proline is a widely known osmolyte that could serve as a source of C and N and might act as a signaling molecule that could act as a sink for excess reductants, providing the  $\text{NAD}^+$  and  $\text{NADP}^+$  necessary for photosynthesis and respiration processes (Mann et al., 2015). A significant accumulation of proline in leaves and root tissues was observed in these halophytes. The amount of proline accumulated in leaves ranged from 1.69 (*A. nummularia*) to 5.67  $\text{mg g}^{-1}$  (*C. quinoa*), with a mean value of 2.63  $\text{mg g}^{-1}$  (Table 3). Only two halophytes, *Chenopodium quinoa* and *Aerva tomentosa* had higher proline in leaves than the check plants of *U. setulosa*. In the case of roots, the highest proline was accumulated in *A. tomentosa* (2.68  $\text{mg g}^{-1}$ ), followed by *A. lagopoides* (2.32  $\text{mg g}^{-1}$ ), whereas *L. fusca*, *D. annulatum* and *D. bippinnata* showed the lowest accumulation. Similar to  $\text{Na}^+$  and  $\text{Cl}^-$ , proline is also accumulated more in leaves than roots to protect the plant from the injurious effect of these ions by

shielding membrane integrity and increasing structural stability of ion transporters and these variations in proline accumulation might be due to various factors, such as the environmental factors, habitats, stress intensity and duration, which in turn enables the survival of these halophytic plants in their natural saline habitats (Al Hassan et al., 2016). Accumulated proline also played an adaptive role in regulating osmotic balance in the cytoplasm and reduction of  $\text{K}^+$  loss by up-regulating and down-regulating genes responsible for proline synthesis and degradation simultaneously (Al Hassan et al., 2016; Lata et al., 2017; 2019).

The quantitative expression analysis also showed that the gene responsible for the synthesis of proline i.e. *P5CR* (Pyrroline-5-carboxylate reductase) in leaf tissue, was found to be up-regulated in all the halophytes. Among 5 halophytic grasses, the maximum expression of *P5CR* was found in case of *U. setulosa* and the least expression was found in the case of *L. fusca* (Fig 1). Pyrroline-5-carboxylate (P5C) is an intermediate product

**Table 3.** Assessment of compatible osmolytes ( $\text{mg g}^{-1}$  FW) in leaves and roots of halophytic plant

| Halophytes/Traits                | Proline content     |                   | Total soluble sugars |                   | Total soluble proteins |                    |
|----------------------------------|---------------------|-------------------|----------------------|-------------------|------------------------|--------------------|
|                                  | Leaves              | Roots             | Leaves               | Roots             | Leaves                 | Roots              |
| <i>Heliotropium ramosissimum</i> | 1.91 <sup>gh</sup>  | 1.99 <sup>e</sup> | 3.29 <sup>i</sup>    | 2.12 <sup>k</sup> | 8.53 <sup>a</sup>      | 1.48 <sup>k</sup>  |
| <i>Withania somnifera</i>        | 2.24 <sup>ef</sup>  | 2.17 <sup>c</sup> | 2.50 <sup>k</sup>    | 2.90 <sup>g</sup> | 7.08 <sup>f</sup>      | 1.63 <sup>j</sup>  |
| <i>Suaeda nudiflora</i>          | 1.89 <sup>ghi</sup> | 1.11 <sup>i</sup> | 7.41 <sup>a</sup>    | 3.84 <sup>d</sup> | 7.84 <sup>c</sup>      | 3.00 <sup>f</sup>  |
| <i>Aelurolopus lagopoides</i>    | 4.22 <sup>c</sup>   | 2.32 <sup>b</sup> | 4.23 <sup>g</sup>    | 5.12 <sup>a</sup> | 7.44 <sup>d</sup>      | 3.42 <sup>e</sup>  |
| <i>Suaeda fruticosa</i>          | 1.73 <sup>kl</sup>  | 1.30 <sup>g</sup> | 6.19 <sup>d</sup>    | 3.44 <sup>e</sup> | 7.26 <sup>e</sup>      | 3.07 <sup>f</sup>  |
| <i>Aerva tomentosa</i>           | 5.51 <sup>b</sup>   | 2.68 <sup>a</sup> | 2.30 <sup>l</sup>    | 1.29 <sup>l</sup> | 4.43 <sup>k</sup>      | 4.72 <sup>b</sup>  |
| <i>Atriplex amnicola</i>         | 1.96 <sup>g</sup>   | 1.39 <sup>f</sup> | 3.25 <sup>ij</sup>   | 4.55 <sup>b</sup> | 6.72 <sup>g</sup>      | 5.55 <sup>a</sup>  |
| <i>Atriplex nummularia</i>       | 1.69 <sup>l</sup>   | 1.20 <sup>h</sup> | 3.36 <sup>i</sup>    | 3.20 <sup>f</sup> | 8.02 <sup>b</sup>      | 4.40 <sup>c</sup>  |
| <i>Atriplex lentiformis</i>      | 1.96 <sup>gh</sup>  | 1.14 <sup>i</sup> | 3.57 <sup>h</sup>    | 4.13 <sup>c</sup> | 6.16 <sup>h</sup>      | 2.03 <sup>hi</sup> |
| <i>Arundo donax</i>              | 1.90 <sup>gh</sup>  | 1.14 <sup>i</sup> | 2.27 <sup>l</sup>    | 1.30 <sup>l</sup> | 5.17 <sup>j</sup>      | 1.47 <sup>k</sup>  |
| <i>Tamarix aphylla</i>           | 2.27 <sup>e</sup>   | 1.29 <sup>g</sup> | 4.48 <sup>f</sup>    | 3.44 <sup>e</sup> | 6.07 <sup>h</sup>      | 3.10 <sup>f</sup>  |
| <i>Dysphania ambrosioides</i>    | 1.80 <sup>ijk</sup> | 1.98 <sup>e</sup> | 7.07 <sup>b</sup>    | 2.19 <sup>k</sup> | 7.55 <sup>d</sup>      | 2.99 <sup>f</sup>  |
| <i>Portulaca portucalustrum</i>  | 3.33 <sup>d</sup>   | 1.42 <sup>f</sup> | 6.40 <sup>c</sup>    | 2.43 <sup>i</sup> | 7.60 <sup>d</sup>      | 2.72 <sup>g</sup>  |
| <i>Desmostachya bippinnata</i>   | 1.91 <sup>gh</sup>  | 1.03 <sup>j</sup> | 6.35 <sup>c</sup>    | 3.23 <sup>f</sup> | 6.91 <sup>f</sup>      | 2.09 <sup>h</sup>  |
| <i>Sporobolus marginatus</i>     | 2.15 <sup>f</sup>   | 1.13 <sup>i</sup> | 3.13 <sup>j</sup>    | 3.30 <sup>f</sup> | 4.17 <sup>l</sup>      | 1.93 <sup>i</sup>  |
| <i>Leptochloa fusca</i>          | 1.79 <sup>jk</sup>  | 1.01 <sup>j</sup> | 5.17 <sup>e</sup>    | 2.30 <sup>j</sup> | 6.97 <sup>f</sup>      | 3.48 <sup>e</sup>  |
| <i>Urochondra setulosa</i>       | 4.21 <sup>c</sup>   | 2.09 <sup>d</sup> | 3.35 <sup>i</sup>    | 2.92 <sup>g</sup> | 5.19 <sup>j</sup>      | 1.15 <sup>l</sup>  |
| <i>Dicanthium annulatum</i>      | 1.87 <sup>hij</sup> | 1.02 <sup>j</sup> | 2.53 <sup>k</sup>    | 3.23 <sup>f</sup> | 5.41 <sup>i</sup>      | 1.39 <sup>k</sup>  |
| <i>Chenopodium quinoa</i>        | 5.67 <sup>a</sup>   | 2.18 <sup>c</sup> | 3.12 <sup>j</sup>    | 2.67 <sup>h</sup> | 5.31 <sup>ij</sup>     | 3.73 <sup>d</sup>  |
| General mean                     | 2.63                | 1.56              | 4.21                 | 3.03              | 6.52                   | 2.81               |
| CV (%)                           | 2.15                | 1.83              | 1.95                 | 2.00              | 1.62                   | 2.62               |
| SE(d)                            | 0.046               | 0.023             | 0.067                | 0.050             | 0.086                  | 0.060              |
| LSD ( $p < 0.05$ )               | 0.0938              | 0.0471            | 0.136                | 0.1005            | 0.1752                 | 0.1219             |

Means with at least one letter common are not statistically significant ( $p \leq 0.05$ ) using Tukey's HSD Test

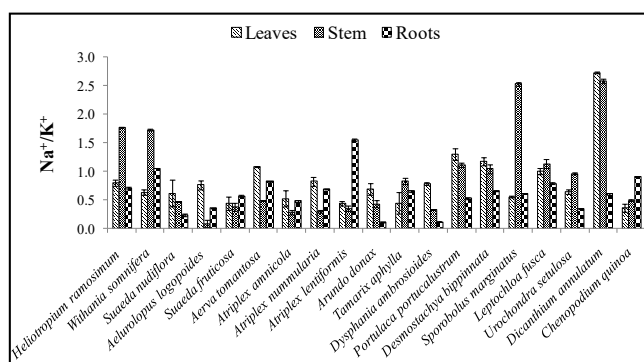
of both proline biosynthesis and catabolism. Thus, higher expression of this gene with increasing salt levels showed the increased synthesis of proline. Mann *et al.* (2021) also reported the upregulation of transcripts for  $\Delta$ -1-pyrroline-5-carboxylate synthase, Betaine aldehyde dehydrogenase and Trehalose 6-phosphate phosphatase, indicating the protective role of proline, glycine betaine and trehalose with increasing salt concentration in halophytes. In *Lycium humile*, proline accumulation and antioxidant capacity, together with the development of a large leaf water-storage parenchyma that allowed  $\text{Na}^+$  accumulation acted as an efficient osmotic adjustment system at pulsed salt treatments of 750 and 1000 mM NaCl (Palchetti *et al.*, 2021). Transgenic lines of rice constitutively overexpressing ornithine- $\delta$ -aminotransferase (OAT) produced higher levels of proline, suggesting its role in salt tolerance (You *et al.*, 2012).

Soluble sugars are the major carbon source of energy that also maintain homeostasis and act as regulatory messengers for gene expression (Peng *et al.*, 2016). Total soluble sugar (TSS) content varied significantly ( $p \leq 0.01$ ) in leaves and roots of studied halophytic species. The soluble sugars accumulated more in leaves ( $4.21 \text{ mg g}^{-1}$ ) than in roots ( $3.03 \text{ mg g}^{-1}$ ). In leaves, TSS ranged from 2.27 to  $7.41 \text{ mg g}^{-1}$ , whereas in roots 1.29 to  $5.12 \text{ mg g}^{-1}$  (Table 3), helped in buffering the redox potential of cell, maintaining storage reserves and protecting the cellular structures, which were driven by the up-regulation of genes of sugar synthesis and transport (Theerawitaya *et al.*, 2015). Soluble proteins play a crucial role in osmotic adjustment by acting as a storage form of nitrogen that could be used during recovery from stress conditions and also involved in redox metabolism, osmolyte metabolism, defense-related proteins and gene expression (Kosová *et al.*, 2013; Pooja *et al.*, 2017; 2019). Significant variability was noted for protein accumulation among different halophytes in leaves and roots. Leaves accumulated  $6.52 \text{ mg g}^{-1}$  proteins on a mean basis, with maximum accumulation in *H. ramosissimum* followed by *A. nummularia*. Roots showed lesser protein accumulation than leaves and found that *A. amnicola* had the highest protein ( $5.55 \text{ mg g}^{-1}$ ) and *A. donax* had the lowest ( $1.47 \text{ mg g}^{-1}$ ). This differential accumulation of proteins in leaves and roots under higher salinity showed adaptive defense mechanisms, which were triggered to re-establish homeostasis through synthesis or degradation of some important metabolites and cellular proteins with a differential status of appearance or disappearance due to salt stress and ultimately enable the plants to survive. Such changes in the metabolism under stress conditions might lead to stress adaptation, which was ensured by the alterations in enzyme activities, and changes in protein synthesis machinery that resulted in the restructuring of the relevant signaling pathways (Shinozaki and Yamaguchi-Shinozaki 2007; Pooja *et al.*, 2019).

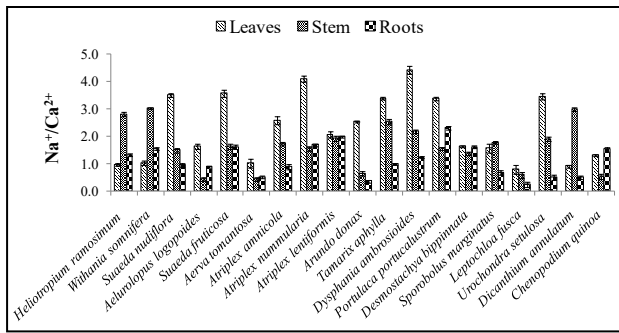
### Salt tolerance potential based on ionic ratios:

Sodium, generally, is not considered a major element but plants accumulate  $\text{Na}^+$  in exchange for  $\text{Ca}^{2+}$  and  $\text{K}^+$  under salt stress and hence,  $\text{Na}^+/\text{K}^+$  and  $\text{Na}^+/\text{Ca}^{2+}$  ratios are considered the most important indicators to study the response of plants grown under salt stress (Koksal *et al.*, 2016). In the present study, it was observed that most of the halophytic plants maintained low  $\text{Na}^+/\text{K}^+$  in leaves, stem and root tissues (Fig 2). This showed that besides accumulating a higher concentration of  $\text{Na}^+$ , these plants also accumulated a higher concentration of  $\text{K}^+$  as well for their survival. *A. amnicola*, *Suaeda* species, *A. donax*, *A. lagopoides*, *C. quinoa* and *U. setulosa* had the lowest  $\text{Na}^+/\text{K}^+$  in all the plant parts and maximum  $\text{Na}^+/\text{K}^+$  was observed in the leaf and stem of *D. annulatum* (2.73 and 2.58). To cope with the detrimental effects of salt-induced ionic stress, these halophytic plants maintained a low tissue  $\text{Na}^+/\text{K}^+$  ratio, which could be considered their key characteristic feature (Kumar *et al.*, 2016). *L. fusca*, *A. tomentosa* and *C. quinoa* had the lowest  $\text{Na}^+/\text{Ca}^{2+}$  ratio in leaf, stem and root tissues (Fig 3). It is interesting to note that leaves had relatively higher  $\text{Na}^+/\text{Ca}^{2+}$  ratio in all the studied halophytes except *H. ramosissimum*, *W. somnifera* and *D. annulatum*, where stem and root tissue had higher  $\text{Na}^+/\text{Ca}^{2+}$  than leaf tissue (Fig 3).  $\text{Na}^+/\text{Ca}^{2+}$  ratio, other important trait that characterized salt tolerance potential because  $\text{Ca}^{2+}$  restricted the excessive uptake of the other salt ions, especially  $\text{Na}^+$  and enhanced the membrane stability (Villora *et al.*, 2000).

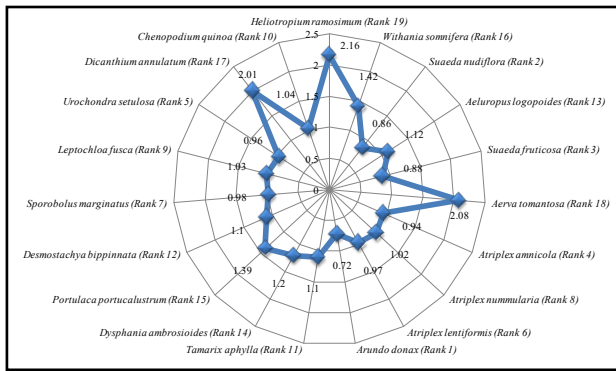
Salt-induced ionic stress leads to such variability for  $\text{Na}^+/\text{Ca}^{2+}$  ratio in different halophytes because  $\text{Ca}^{2+}$  significantly generates ion selective movement for  $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{Cl}^-$  respectively and hence negotiates the toxicity of salinity (Al-Khateeb, 2006). In the present study, differential salt tolerance potential was calculated based on the accumulation of  $\text{Na}^+$  and  $\text{Cl}^-$  in the whole plant over the accumulation of  $\text{K}^+ + \text{Ca}^{2+}$  and characterized the halophytic plants as highly tolerant having the lowest ratio (Fig 4). The studied halophytes were ranked in the following order from highest to moderate salt tolerance



**Fig 2.**  $\text{Na}^+/\text{K}^+$  in different plant parts of halophytes collected from waste saline lands of Haryana



**Fig 3.**  $\text{Na}^+/\text{Ca}^{2+}$  in different plant parts of halophytes collected from waste saline lands of Haryana



**Fig 4.** Characterization of halophytes based on salt tolerance potential  $[(\text{Na}+\text{Cl})/(\text{K}+\text{Ca})]$

as *A. donax*  $\geq$  *S. nudiflora*  $\geq$  *S. frutescens*  $\geq$  *A. amnicola*  $\geq$  *U. setulosa*  $\geq$  *A. lentiformis*  $\geq$  *S. marginatus*  $\geq$  *A. nummularia*  $\geq$  *L. fusca*  $\geq$  *C. quinoa*  $\geq$  *T. aphylla*  $\geq$  *D. ambrosioides*  $>$  *A. lagopoides*  $>$  *D. bipinnata*  $>$  *P. portulacastrum*  $>$  *W. somnifera*  $>$  *D. annulatum*  $=$  *A. tomentosa*  $=$  *H. ramosissimum*. Owing to the complexities of assessing plant performance under natural conditions, halophytic plants maintained turgor by sequestering  $\text{Na}^+$  and  $\text{Cl}^-$  and accumulated compatible solutes for osmotic adjustment because soil salinity is spatially heterogeneous even within the root zone (Shabala and Mackay, 2011).

## Conclusion

This study helped to identify the salt tolerance potential of selected halophytes through their salt-loading capacity to survive in extreme saline lands. Hence, firstly it is recommended that salt-affected soils and poor-quality irrigation water resources be judiciously utilized for growing highly salt-tolerant halophytic species as an ideal option for rehabilitation of the existing degraded agricultural lands. Secondly, owing to the adaptability of these plants to saline habitats, further understanding of adaptive mechanisms is essential for their possible utilization in advanced crop development programs through modern tools to impart better resources for salt-affected regions.

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## References

- Al Hassan, M., M. Del Pilar López-gresa, M. Boscaiu and O. Vicente. 2016. Stress tolerance mechanisms in *Juncus*: responses to salinity and drought in three *Juncus* species adapted to different natural environments. *Functional Plant Biology* 43: 949-960.
- Arora, N. K., A. Datta, S. K. Chaudhari, R. K. Yadav and P. C. Sharma. 2018. Soil, water and plant analysis manual. ICAR-CSSRI, Karnal. pp. 1-138.
- Bates, L., R. Waldren and I. Teare. 1973. Rapid determination of free proline for water-stress studies. *Plant and soil* 39: 205-207.
- 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* 72: 248-254.
- Bedre, R., V. R. Mangu, S. Srivastava, L. E. Sanchez and N. Baisakh. 2016. Transcriptome analysis of smooth cordgrass (*Spartina alterniflora* Loisel), a monocot halophyte, reveals candidate genes involved in its adaptation to salinity. *BMC Genomics* 17: 1-18.
- Borsani, O., V. Valpuesta and M. A. Botella. 2001. Evidence for a role of salicylic acid in the oxidative damage generated by NaCl and osmotic stress in *Arabidopsis* seedlings. *Plant Physiology* 126: 1024-1030.
- Chhabra, R. 1973. Kinetics of absorption of chloride and phosphorus, their interaction and effect on growth and composition of tomato plants. PhD Thesis, KUL, Belgium.
- Dhansu, P., R. Kumar, A. Kumar, K. Vengavasi, A. K. Raja, S. Vasantha, M. R. Meena, N. Kulshreshtha and S. K. Pandey. 2022. Differential physiological traits, ion homeostasis and cane yield of sub-tropical sugarcane varieties in response to long-term salinity stress. *Sustainability* 14: 13246. <https://doi.org/10.3390/su142013246>.
- Guo, S. M., Y. Tan, H. J. Chu, M. X. Sun and J. C. Xing. 2019. Transcriptome sequencing revealed molecular mechanisms underlying tolerance of *Suaeda salsa* to saline stress. *PloS One* 14: 0219979.
- Himabindu, Y., T. Chakradhar, M. C. Reddy, A. Kanygin, K. E. Redding and T. Chandrasekhar. 2016. Salt-tolerant genes from halophytes are potential key players of salt tolerance in glycophytes. *Environmental and Experimental Botany* 124: 39-63.
- Jha, B., P. K. Agarwal, P. S. Reddy, S. Lal, S. K. Sopory and M. K. Reddy. 2009. Identification of salt-induced genes from *Salicornia brachiata*, an extreme halophyte through expressed sequence tags analysis. *Genes and Genetic Systems*

- 84: 111-120.
- Khateeb, S. A. 2006. Effect of calcium/sodium ratio on growth and ion relations of alfalfa (*Medicago sativa* L.) seedling grown under saline condition. *Journal of Agronomy* 5: 175-181.
- Kosová, K., I. T. Prášil and P. Vítámvás. 2013. Protein contribution to plant salinity response and tolerance acquisition. *International Journal of Molecular Sciences* 14: 6757-6789.
- Kim, D. Y., J. Y. Jin, S. Alejandro, E. Martinoia and Y. Lee. 2010. Overexpression of AtABCG36 improves drought and salt stress resistance in *Arabidopsis*. *Physiologia Plantarum* 139: 170-180.
- Kumar, A., A. Kumar, P. Kumar, C. Lata and S. Kumar. 2018a. Effect of individual and interactive alkalinity and salinity on physiological, biochemical and nutritional traits of Marvel grass. *Indian Journal of Experimental Biology* 56: 573-581.
- Kumar, A., A. Kumar, C. Lata and S. Kumar. 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.
- Kumar, A., A. Kumar, C. Lata, S. Kumar, S. Mangalassery, J. P. Singh, A. K. Mishra and D. Dayal. 2018b. Effect of salinity and alkalinity on response of halophytic grasses *Sporobolus marginatus* and *Urochondra setulosa*. *Indian Journal of Experimental Biology* 56: 573-581.
- Kumar, A., A. Mann, A. Kumar and S. Devi. 2018. Potential and role of halophyte crops in saline environments. In: S.K. Gupta, M. R. Goyal and A. Singh (eds). *Engineering Practices for Management of Soil Salinity*, CRC Press. pp. 379-416.
- Kumar, A., A. Mann, A. Kumar, N. Kumar and B. L. Meena. 2021. Physiological response of diverse halophytes to high salinity through ionic accumulation and ROS scavenging. *International Journal of Phytoremediation* 23:1041-1051.
- Kumar, A., A. Mann, C. Lata, N. Kumar and P. C. Sharma. 2019. Salinity-induced Physiological and Molecular Responses of Halophytes. In: J. C. Dagar, R.K. Yadav and P. C. Sharma (eds). *Research Developments in Saline Agriculture*, Springer. pp. 331-356.
- Lata, C., A. Kumar, S. K. Sharma, J. Singh, S. Sheokand, Pooja, A. Mann and B. Rani. 2017. Tolerance to combined boron and salt stress in wheat varieties: Biochemical and molecular analyses. *Indian Journal of Experimental Biology* 55(5): 321-328.
- Lata, C., S. Soni, N. Kumar, A. Kumar, Pooja, A. Mann and S. Rani. 2019. Adaptive mechanism of stress tolerance in *Urochondra* (grass halophyte) using roots study. *Indian Journal of Agricultural Sciences* 89: 1050-1053.
- Livak, K. J. and T. D. Schmittgen. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the  $2^{-\Delta\Delta CT}$  method. *Methods* 25: 402-408.
- Louhaichi, M. and Sawsan Hassan. 2023. Evaluation of selected fodder species for alley cropping systems. *Range Management and Agroforestry* 44: 241-247.
- Lv, X., Y. Jin and Y. Wang. 2018. *De novo* transcriptome assembly and identification of salt-responsive genes in sugar beet M14. *Computational Biology and Chemistry* 75: 1-10.
- Maathuis, F. J. 2014. Sodium in plants: perception, signalling, and regulation of sodium fluxes. *Journal of Experimental Botany* 65: 849-858.
- Maathuis, F. J., I. Ahmad and J. Patishtan. 2014. Regulation of Na<sup>+</sup> fluxes in plants. *Frontiers in Plant Science* 5: 105882.
- Mangalassery, S., D. Dayal, A. Kumar, K. Bhatt, R. Nakar, A. Kumar, J. P. Singh and A. K. Misra. 2017. Pattern of salt accumulation and its impact on salinity tolerance in two halophyte grasses in extreme saline desert in India. *Indian Journal of Experimental Biology* 55: 542-548.
- Mann, A., S. K. Bishi, M. K. Mahatma and A. Kumar. 2015. Metabolomics and salt stress tolerance in plants. In: S.H. Wani and M. A. Hossain (eds). *Managing Salt Tolerance in Plants: Molecular and Genomic Perspectives*, CRC Press. pp. 251-266.
- Mann, A., A. Kumar, M. Saha, C. Lata and A. Kumar. 2019b. Stress induced changes in osmoprotectants, ionic relations, antioxidants activities and protein profiling characterize *Sporobolus marginatus* Hochst. ex A. Rich. salt tolerance mechanism. *Indian Journal of Experimental Biology* 57: 672-679.
- Mann, A., N. Kumar, C. Lata, A. Kumar, A. Kumar and B. L. Meena. 2019a. Functional annotation of differentially expressed genes under salt stress in *Dichanthium annulatum*. *Plant Physiology Reports* 24: 104-111.
- Mann, A., N. Kumar, A. Kumar, C. Lata, A. Kumar, B. L. Meena, D. Mishra, M. Grover, S. Gaba, C. Parameswaran and N. Mantri. 2021. *de novo* transcriptomic profiling of differentially expressed genes in grass halophyte *Urochondra setulosa* under high salinity. *Scientific Reports* 11: 5548. <https://doi.org/10.1038/s41598-021-85220-7>.
- Palchetti, M. V., M. Reginato, A. Llanes, J. Hornbacher, J. Papenbrock, G. E. Barboza, V. Luna and J. J. Cantero. 2021. New insights into the salt tolerance of the extreme halophytic species *Lycium humile* (Lycieae, Solanaceae). *Plant Physiology and Biochemistry* 63: 166-177.
- Peng, J., J. Liu, L. Zhang, J. Luo, H. Dong, Y. Ma, X. Zhao, B. Chen, N. Sui and Z. Zhou. 2016. Effects of soil salinity on sucrose metabolism in cotton leaves. *PLoS One* 11: e0156241.
- Pooja, A. S. Nandwal, M. Chand, A. Kumar, B. Rani, A. Kumari and N. Kulshrestha. 2017. Comparative evaluation of changes in protein profile of sugarcane varieties under different soil moisture regimes. *International Journal of Current Microbiology and Applied Sciences* 6: 1203-1210.
- Pooja, A. S. Nandwal, M. Chand, K. Singh, A. K. Mishra, A. Kumar, A. Kumari and B. Rani. 2019. Varietal variation in physiological and biochemical attributes of sugarcane varieties under difference soil moisture regimes. *Indian Journal of Experimental Biology* 52: 721-732.
- Rengasamy, P. 2010. Soil processes affecting crop production in salt-affected soils. *Functional Plant Biology* 37: 613-620.
- Shabala, S. and A. Mackay. 2011. Ion transport in halophytes.

- In: I. Turkan (ed). *Advances in Botanical Research*. Vol. 57. Academic Press. pp. 151-199.
- Shinozaki, K. and K. Yamaguchi-Shinozaki. 2007. Gene networks involved in drought stress response and tolerance. *Journal of Experimental Botany* 58: 221-227.
- Soni, S., A. Kumar, N. Sehwat, A. Kumar, N. Kumar, C. Lata and A. Mann. 2021. Effect of saline irrigation on plant water traits, photosynthesis and ionic balance in durum wheat genotypes. *Saudi Journal of Biological Sciences* 28: 2510-2517.
- Soni, S., A. Kumar, N. Sehwat, N. Kumar, G. Kaur, A. Kumar and A. Mann. 2020. Variability of durum wheat genotypes in terms of physio-biochemical traits against salinity stress. *Cereal Research Communications* 49: 45-54.
- Souguir, D., M. Zouari, G. Hörmann and M. Hachicha. 2019. Behavior of some plant species used as alternatives for salt-affected soil reclamation and treated wastewater valorization. *Range Management and Agroforestry* 40: 207-214.
- Tavakkoli, E., F. Fatehi, S. Coventry, P. Rengasamy and G. K. McDonald. 2011. Additive effects of Na<sup>+</sup> and Cl<sup>-</sup> ions on barley growth under salinity stress. *Journal of Experimental Botany* 62: 2189-2203.
- Theerawitaya, C., R. Tisarum, T. Samphumphuang, H. P. Singh, S. Cha-um, C. Kirdmanee and T. Takabe. 2015. Physio-biochemical and morphological characters of halophyte legume shrub, *Acacia ampliceps* seedlings in response to salt stress under greenhouse. *Frontiers in Plant Science* 6: 630.
- Víllora, G., D. A. Moreno, G. Pulgar and L. Romero. 2000. Yield improvement in zucchini under salt stress: determining micronutrient balance. *Scientia Horticulturae* 86: 175-183.
- Wu, H., X. Zhang, J. P. Giraldo and S. Shabala. 2018. It is not all about sodium: revealing tissue specificity and signalling roles of potassium in plant responses to salt stress. *Plant and Soil* 431: 1-17.
- Yemm, E. and A. Willis. 1954. The estimation of carbohydrates in plant extracts by anthrone. *Biochemical Journal* 57: 508.
- You, J., H. Hu and L. Xiong. 2012. An ornithine d-aminotransferase gene OsOAT confers drought and oxidative stress tolerance in rice. *Plant Science* 197: 59-69.