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Short Communications

Adaptive mechanism of stress tolerance in *Urochondra* (grass halophyte) using roots study

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Plant roots provide support in plant positioning and nutrition uptake and are the primary site exposed in response to salinity (Rajaei *et al*. 2009). Adaptation to salinity stress is controlled by cascades of events at the physiological, biochemical and molecular level. During salt stress, initially plants experience osmotic stress (as soon as salt concentration in the root zone begins to rise) which inhibits water uptake, cell expansion and later on specific ion effects that commence with the accumulation of injurious concentrations of ions such as Na^+ and Cl^- in the plant cells. As a result, several defense mechanisms are triggered to re-establish homeostasis, i.e. the accumulation or depletion of certain metabolites, resulting in the imbalance in the levels of a relatively small set of cellular proteins, which could increase, decrease, appear, or disappear due to salt stress (Kong *et al*. 2005) and ultimately enable the plants to survive. Differential expression of proteins is an essential part of plant response to environmental stress as well as for adaptation to environmental conditions. Salinity has a dual effect on protein profile pattern in the plants. Salt stress generally leads to decreased protein biosynthesis and also commences synthesis of preferential specific stress proteins necessary for tolerating the effect of salinity (Kumar *et al*. 2015). Stress induced proteins probably increase resistance towards damage caused by potentially deleterious ions and salts by increasing the ability of the cell to compartmentalize them in the vacuole (Lata *et al*. 2017). Salinity not only exerts its depressive effect quantitatively but also qualitatively on the proteins. So, identification of salt stress-induced protein-level changes is thus a very important approach for understanding the molecular mechanism of response to salt.

The present investigation was conducted on *Urochondra setulosa* (grass halophyte) grown in screen house of the Division of Crop Improvement, CSSRI, Karnal. The planting material of *Urochondra* (root cuttings and seed) was collected from Rann of Kutch, Bhuj, Gujarat. The plants were established in screening blocks (2.5 m × 1.5 m × 0.5 m) filled with sandy loam soil in a screen house under natural conditions. Treatments were imposed regularly through saline/sodic water irrigation to achieve different stress levels of pH ~ 9.5, pH ~ 10, ECe ~ 30 dS/m, ECe ~ 40 dS/m and ECe ~ 50 dS/m in three replications along with control (pH ~ 7.8 and ECe ~ 0.83 dS/m). The screen house is covered with a high quality polythene sheet to avoid rain water and maintain the desired stress level in the screening blocks. After 60 days of imposition of stress treatments, plants were uprooted and roots samples were collected in 3 replicates to measure proline content (Bates *et al*. 1973), protein content (Bradford *et al*. 1976), Na^+, K^+ and protein profile expression (Laemmli 1970) in *Urochondra*. For differential expression of proteins, phenol extraction method was used to extract the protein from root tissue followed by methanol/ammonium acetate precipitation (Hurkman and Tanaka 1986) and resolved on Sodium Dodecyl Sulphate – Poly Acrylamide Gel Electrophoresis (SDS-PAGE, 10 %), after quantification on Nanodrop (Denovix, DS-11+). The data were analyzed statistically using randomized block design (Version 9.3, SAS Institute Inc., Cary, NC, USA).

It was observed that being a halophytic plant, *Urochondra* accumulated higher Na^+ content (% DW) in their roots under stress conditions and salinity stress caused higher accumulation (69.74% increase than control) at ECe ~ 50 dS/m in comparison to sodic stress (8.33% increase than control) at highest level of sodic stress, i.e. pH ~ 10.0 (Fig 1).

Excessive accumulation of Na^+ in the roots has been considered harmful for normal metabolism of plants, and tolerant plants have the capacity of successful salt removal (Kumar *et al*. 2016). Potassium (K^+) plays an important role in resistance to salinity by maintaining cell osmotic

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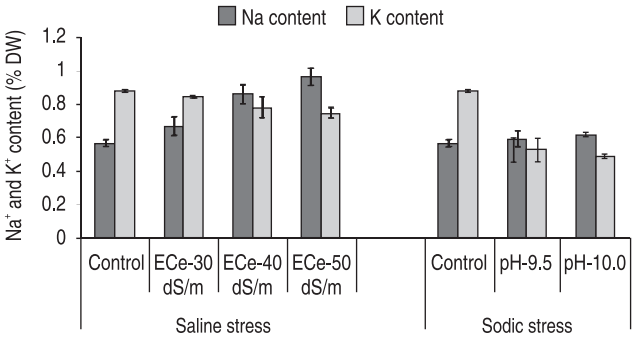

Fig 1 Effect of salinity and sodicity stresses on root Na^+ and K^+ content (% DW) of halophytic grass.

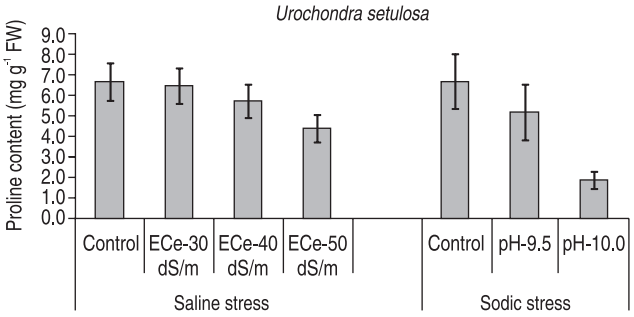

Fig 3 Effect of salinity and sodicity stresses on root protein (mg/g FW) of halophytic grass.

potential. From literature, it was found that there is a strong positive correlation between the ability to retain K^+ and overall plant salt tolerance (Sun *et al*. 2015). No significant decline was seen in K^+ content up to ECe ~ 40 dS/m (11.36% reduction). Further increase in salinity level caused reduction in K^+ content whereas sodic stress significantly caused reduction in K^+ content, i.e. 46.2% reduction at pH ~ 10.0 (Fig 1). The decrease in K^+ content, in roots is generally due to direct competition between K^+ and Na^+ at plasma membrane which causes inhibition of Na^+ on K^+ transport process in xylem tissues and/or Na^+ induced K^+ efflux from the roots (Mann *et al*. 2015). Kuiper (1984) also reported that plants can accumulate Na^+ at the expense of K^+ and Ca^{2+} under salt stress.

Proline accumulation is an important physiological index for plant response to salt stresses and also acts as signalling regulatory molecule which activates multiple responses for adaptation process (Lata *et al*. 2017). Decreased rate of protein synthesis or increased proteolysis leads to accumulation of proline under stress environment. From the present study, it was noted that salinity, induced higher proline accumulation in roots of *Urochondra* than sodicity. At salinity level of ECe ~ 50 dS/m, roots accumulated 4.57 folds higher proline, whereas roots under pH ~ 10.0 showed 3.11 fold higher accumulation than the control roots (Fig 2). Such modifications in response to salt stress appear to be associated with the enhanced ability of halophytic grass to tolerate such stressful conditions. The higher concentrations of proline under stress are helpful

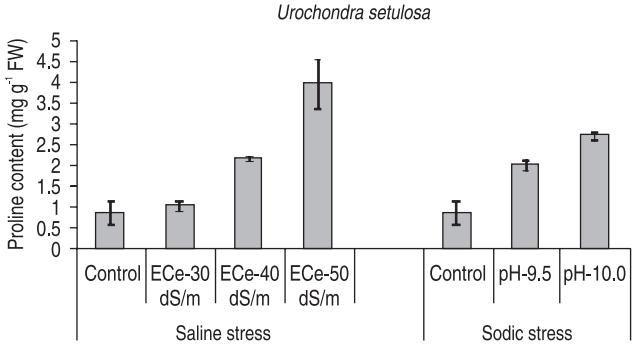

Fig 2 Effect of salinity and sodicity stresses on root proline (mg/g FW) of halophytic grass.

to plant as proline participates in osmotic adjustments. Besides the role as osmolyte, proline can also confer enzyme protection and increases membrane stability (Khatkar and Kuhad 2000, Kumar *et al*. 2016, Lata *et al*. 2017).

Alteration of protein synthesis or its degradation is one of the fundamental metabolic processes that plays role in osmotic adjustment (Lata *et al*. 2017) and also provides a storage form of nitrogen which could be reutilized when stress is over (Singh *et al*. 1987). Mean protein content in roots was 5.04 mg/g FW and similar to K^+, protein content decreased with increasing stress level but higher decrease was observed under sodicity stress. Salinity stress caused 34.63% reduction in protein content in roots of *Urochondra* in comparison to control roots (Fig 3). Significantly higher reduction of 72% was noted under sodicity stress of pH ~ 10.0. This decrease in protein content in response to salt stress might be due to higher proteolysis or result of protein denaturation (Kumar *et al*. 2015). These results showed that sodic stress had more damaging effect on *Urochondra* than salinity stress which is further examined through differential protein profile expression using SDS-PAGE. Under salinity/ sodicity stresses, protein profiling showed qualitative and quantitative differences in polypeptide resolution that indicate their cellular and molecular adaptive mechanism in *Urochondra* roots.

In control roots, a total of 26 polypeptide bands were expressed ranging from 12.43 kDa to 81.3 kDa. Salinity stress of ECe ~ 30 dS/m induced the expression of 4 new polypeptide bands of molecular weight (MW) 19.05 kDa, 24.65 kDa, 84.9 kDa and 97.2 kDa with depletion of one polypeptide, i.e. 15.21 kDa. Further increase in salinity also induced 3 new polypeptides (16.25 kDa, 114.65 kDa and 134.5 kDa) but the total number of polypeptide bands remains same as that was present at ECe ~ 30 dS/m because increased salinity also caused disappearance of 3 polypeptides (22.48, 26.91 and 28.33 kDa). This appearance and disappearance of polypeptide bands suggested that polypeptide expression might be related to the genotypic stress tolerance or it may be due to the negative consequence of salinity on protein/gene synthetic machinery (Rani *et al*. 2007). Likewise at ECe ~ 50 dS/m, *Urochondra* roots showed de novo synthesis of 2 polypeptide bands of MW

17.75 kDa and 18.66 kDa that might be critical for plant to adapt under highly stressful conditions (Amzallag and Lerner 1994). The altered and enhanced expression of proteins might be responsible for the survival and growth of *Urochondra* under high salinity stress that affects the functional capabilities of grass halophyte (Lata *et al*. 2017). Under sodic stress conditions of pH ~ 9.5, there was appearance of 2 new polypeptide bands of MW 17.75 kDa and 18.66 kDa, whereas 3 polypeptides of 14.17, 25.12 and 26.91 kDa disappeared. Interestingly, it was noted that the similar polypeptides also appeared at high salinity condition of ECe ~ 50 dS/m which suggested the tolerance limits of grass halophyte to sodic stress. Further increase in sodic condition leads to disappearance of 4 polypeptide bands (15.21, 21.35, 22.48 and 26.32 kDa). Disappearance of polypeptides under high sodic condition might be due to their involvement in tolerance process or related to the breakdown of polypeptides during stress (Kumar *et al*. 2015). But high sodicity also induced appearance of 2 polypeptide bands of 15.81 kDa and 16.25 kDa with a total of 23 polypeptide bands. The synthesis and upregulation of new polypeptides at higher stress may be to impart tolerance. Such changes in metabolism under stress conditions might lead to the accumulation or depletion of certain metabolites, alterations in enzyme activities, changes in protein synthesis machinery, and synthesis of new sets of proteins which are specifically associated with particular type of stresses (Bhagwat and Apte 1989, Blinda *et al*. 1997).

Plants cannot avoid abiotic stresses, instead they have evolved the ability to adapt or compensate for stressful conditions by altering physiological and developmental processes to maintain growth and reproduction. Under stressed conditions, this complex interactive system adjusts homeostatically to minimize the negative impacts of stress and maintain metabolic equilibrium. The present study described the tolerance mechanism of *Urochondra setulosa* (grass halophyte) by maintaining root Na^+ and K^+ content, increased osmolyte accumulation and differential expression of protein.

SUMMARY

An experiment was conducted on *Urochondra setulosa* (grass halophyte) to explore its survival mechanism under stress conditions. For this, different treatments of salinity/sodicity (pH ~ 9.5, pH ~ 10, ECe ~ 30 dS/m, ECe ~ 40 dS/m and ECe ~ 50 dS/m) were created in micro-plots. Roots are the primary structure that first senses the negative effects of salt stress. So, roots were selected to study the tolerance mechanism. Salinity stress caused higher Na^+ accumulation and less reduction in K^+ content in comparison to sodic stress. Roots accumulated 4.57 folds higher proline at ECe ~ 50 dS/m, whereas under pH ~ 10.0, 3.11 fold higher accumulations than the control roots were observed. Higher reduction in protein content was observed under sodicity stress than salinity stress. In control roots, a total of 26 polypeptide bands were expressed ranging from 12.43 kDa to 81.3 kDa. Under high salinity stress, number of polypeptide bands increased to 31 at ECe ~ 50 dS/m that might be responsible for their survival and growth while sodic stress led to disappearance of more number of polypeptides with a total number of 23 polypeptides at pH ~ 10.0. Interestingly, it was also found that sodic stress had higher damaging affect on *Urochondra* metabolism in comparison to salinity stress which makes it salinity tolerant grass.

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