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STRUCTURAL AND FUNCTIONAL ASPECTS OF IONIC RELATION IN ROOTS OF *TYPHA DOMINGENSIS* PERS. ECOTYPES UNDER SALT STRESS

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Abstract

An investigation was carried out to evaluate ionic relation of roots on the basis of anatomical and physiological responses in differently adapted ecotypes of *Typha domingensis* Pers. under salinity stress. Six ecotypes were collected from ecologically different habitats in the Punjab namely Jahlar, Sheikhupura, Sahianwala, Gatwala, Treemu and Knotti. Plants were grown in non-aerated waterlogged conditions and subjected to different salt levels (0, 100, 200 and 300 mM of NaCl). The Sahianwala ecotype showed better performance under salt stress on the basis of structural modifications like thicker root, larger root aerenchymatous cavities and cortical cell area, and functional response like high K^+ uptake and reduced uptake of Cl^-. This ecotype also showed low reduction in root and shoot fresh weight as compared to all other ecotypes. The Knotti ecotype was the second best that relied on exceedingly large aerenchymatous cavities and reduced Cl^- uptake for salt tolerance. The sensitive ecotypes from Sheikhupure and Treemu showed a significant decline in growth and most of the anatomical characteristics, in particular the root-cross-sectional area. Overall it was found that all ecotypes of *Typha domingensis* Pers. responded differently to salt stress, which indicates a heterogeneity on the root adaptive components.

Key words: *Typha*, Ecotypes root anatomy, Ionic content.

Introduction

Wetlands are threatened due to increasing level of salinity throughout the world. Two basic responses to salinization are the graded response of species richness and secondly exhibiting special diversity. Salinity is lethal to wetland biodiversity and disturbs many physiological, structural and ecological functioning, ultimately severely affecting the life cycle, food availability and strength of plants in their habitats. Concentration of the dissolved salts in the wetland refers to salinity and high level of salt resulted the biodiversity loss in wetlands (Davis *et al*., 2003).

Wetland plants are found throughout the world and are the most dominant part of the ecosystem. These plants have a crucial role in the maintenance of wetland. Wetlands show diverse collection of species with a variety of adaptations, physiological tolerance and other different strategies which enable them to tolerate in different stressed conditions (Jackson & Clomer, 2005). The degree of salt tolerance in halophytes depends on their capacity to vacuolize the toxic ions and produce compatible solutes in the cytoplasm of the cell (Glenn & Brown, 1999). It is well documented that under high salinity conditions halophytes can accumulate the Na^+ in vacuoles and these accumulated ions can be used as osmotica in the cell (Jaleel *et al*., 2008).

Anatomical structures modifications is an adaptive mechanism for plants (Grigore & Toma, 2007). Many environmental factors negatively alter the root system (Malamy, 2005). The most common after effects are the modification of cell wall (Minic & Jouanin, 2006) and decline in parenchymatous size (Dolatabadian *et al*., 2011). The most imperative response is the sclerification in cortical parenchyma and other tissues and thickening of endodermis (Abulfaith, 2003). Development and formation root aerenchyma of the cortical cell can also be associated with salinity stress in halophytes of aquatic condition (Evans, 2003; Nazemi *et al*., 2015).

Formation of sclerenchyma in the cortex of the root gives strength to these metabolically active tissues and large aerenchyma finally play a significant role in preventing collapse in aquatic plants. Decreased water loss through surface of root can also be correlated to the thickening of sclerenchyma, endodermis and large metaxylem vessels (Cholewa & Griffith, 2004; Kulkarni, 2015).

Typha domingensis Pers. is a dominating species of fresh water and salt wetlands, which is well adapted to hyper-saline and metal polluted waters. The present study was conducted to evaluate structural and functional response in differently adapted populations of *T. domingensis* to high salinities.

Material and Method

Selection of the differentially adapted populations: Six differentially adapted ecotypes of *Typha domingensis* Pers. were collected from different habitats in the Punjab region, which were likely to be tolerant to salinity stress. Gatwala (ECe = 3.12) and Treemu (ECe = 2.52) ecotypes were collected from fresh waters of canal and rivers, respectively, which were rated as the normal ecotypes. Ecotypes from hyper-saline Jahlar Lake (ECe = 16.57) and saline water-logged at Knotti Garden (ECe = 34.47) in the Salt Range, and from saline wetland near Sahianwala (ECe = 18.85) were expected to be adapted to high salinities. Water bodies near Sheikhupura, (ECe = 2.54) were polluted with industrial wastes, but salinity level was relatively low.

Experimentation: Six ecotypes of *Typha* were studied for their degree of tolerance to salinity stress. Various physiological and morpho-anatomical characteristics were evaluated against this salt stress. All these ecotypes were acclimated in the research area of Botanic Garden, University of Agriculture, Faisalabad for a period of six month in non-aerated standing water. The vegetative buds of equal size were detached from each mother plant and were grown in plastic tubs (capacity 20 L). Plastic tubs were half filled with a mixture of sand and clayey-loam soils in 1:1 ratio, and then filled with 10 L irrigation water. The experiment was conducted during May to July, 2011 at normal growth conditions under full sunlight; the average temperatures were 38-41°C and 24-26°C, respectively and 13-16 hours was photoperiod range. The range of relative humidity was 46.8 to 59.3%.

Anatomical studies: Adventitious root were randomly selected for the anatomical studies. Near root-shoot junction, a 2 cm piece was selected and immediately preserved in FAA solution containing v/v 5% formalin, 10% acetic acid, 50% ethanol and 35% distilled water. Permanent slides were prepared by the technique of free-hand sectioning using safranin and fast green for staining lignified and parenchymatous tissues, respectively. Ocular micrometer was used to record the microscopic data, and digital photographs were taken by camera-equipped compound microscope.

Statistical design: The experiment was designed in 2-factorial completely randomized design with six replications. The data was subjected to analysis of variance (ANOVA) and means were compared by least significant range (LSD) test.

Results

A gradual and significant reduction in shoot fresh weight was observed with increased salt levels of the medium in all ecotypes of the *T. domingensis* except that collected from Sahianwala and Knotti. A significant decrease in the Sahianwala ecotype was recorded by induction of salts, but higher treatments had no effect on fresh biomass (Fig. 1). The ecotype from Knotti showed a significant decline in shoot fresh biomass, but only up to 200 mM salt level. A significant reduction in root fresh weight in ecotypes from Sheikhupura, Jahlar, Treemu and Gatwala. The Sahianwala and Knotti ecotypes showed a consistent decrease only at 100 mM salt level, but higher levels had no impact on fresh weight of root.

Root Na^+ content showed a significant increase in all ecotypes of *Typha* commensurate with increasing the salinity levels. The ecotypes from Jahlar and Treemu accumulated higher concentration of Na^+ content in roots compared to other ecotypes. A significant reduction in root K^+ was observed in ecotypes from Jahlar, Sahianwala and Gatwala. The ecotypes from Sheikhupura, Treemu and Knotti revealed a significant decrease in root K^+, but only at 300 mM NaCl level (Fig. 2).

Root N significantly reduced in the ecotypes from Jahlar and Sheikhupura as the salt level increased in the growth medium. Plants from Treemu and Gatwala revealed a significant decrease up to 200 mM NaCl, but at high salinity level did not affect this parameter. The ecotype from Knotti showed a significant reduction by the induction of salinity (100 mM), but higher levels resulted in a no change (Fig. 2).

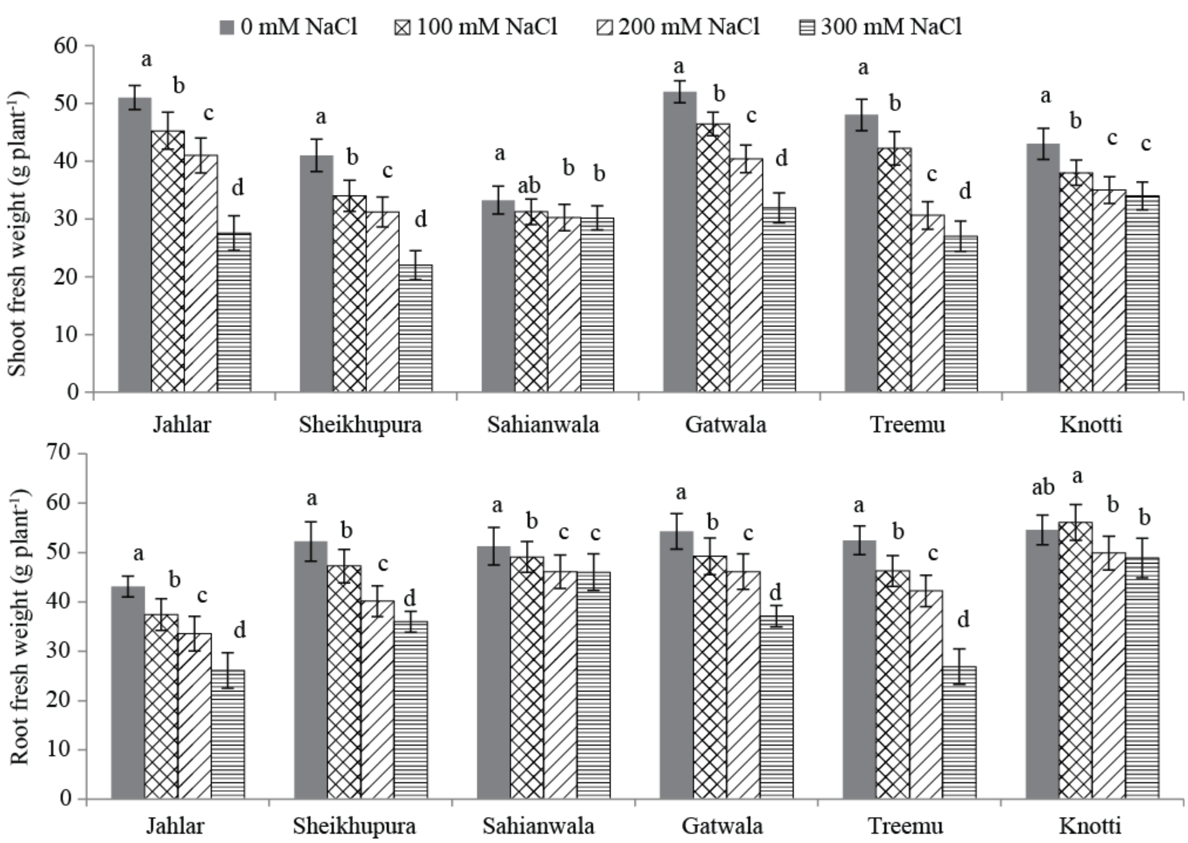

Fig. 1. Root and shoot fresh weight of six ecotypes of *Typha domingensis* Pers. under different levels of salt stress.

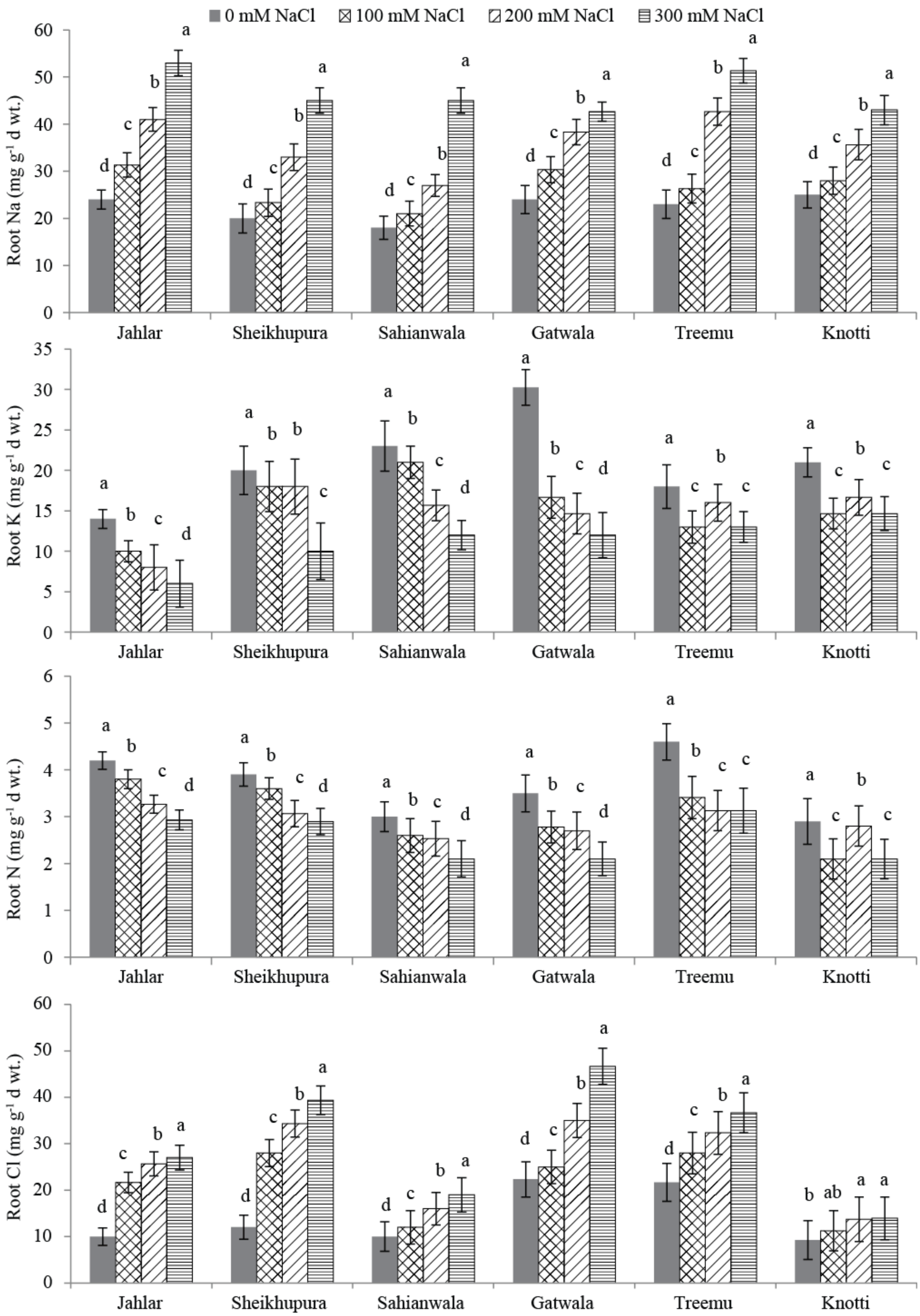

Fig. 2. Root ionic content of six ecotypes of *Typha domingensis* Pers. under different levels of salt stress.

A significant increase in root Cl^- content was observed in five ecotypes of *Typha* in response to salt stress, which were from Jahlar, Sheikhupura, Sahianwala, Gatwala and Treemu. Low salinity imposed no effect on root Cl^- in the ecotype from Knotti. The highest level (300 mM NaCl), however, had a significant g in root Cl^- (Fig. 2).

Root cross-sectional area showed a significant increase in the ecotypes from Jahlar and Gatwala up to 100 mM NaCl level, but at higher salinity levels (200 and 300 mM) had non-significant impact on value related to this parameter. The ecotype from Sahianwala showed a significant increase as salinity level of the growth medium increased. The Sheikhupura ecotype showed significant reduction, but only at higher salt levels (200 and 300 mM NaCl). In contrast, Knotti ecotype showed an increase at 100 mM NaCl but afterwards, had no impact on this parameter (Fig. 3, Plate 1).

A significant decrease was noticed in the ecotypes from Sahianwala, Gatwala and Treemu in root epidermal cell area commensurate with high stress level. In contrast, a significant reduction was recorded in the Jahlar and Sheikhupura region plants, but only up to 200 mM NaCl level. The ecotype from Knotti was not affected at low salt concentrations, however high salt level (300 mM NaCl) showed a significant reduction (Fig. 3, Plate 1).

Ecotypes from Jahlar, Sahianwala, Gatwala and Treemu showed a significant increase in root epidermal thickness in response to increasing salinity levels. Increasing salt levels had no impact on the Knotti and Sheikhupura ecotypes. There was a significant increase in root aerenchyma area in ecotypes from Jahlar, Sheikhupura, Sahianwala, Gatwala and Treemu along with increasing salt levels. The ecotype from Knotti showed a significant increase in this parameter, but only at the highest level (200 mM NaCl) (Fig. 3, Plate 1).

A significant reduction was recorded in root endodermal cell area of three ecotypes i.e., from Jahlar, Sheikhupura and Treemu, those from Sahianwala, Gatwala and Knotti showed a significant reduction only at 300 mM NaCl level (Fig. 3, Plate 1). Root vascular region thickness revealed a significant reduction in ecotypes from Sahianwala and Gatwala. The Sheikhupura and Treemu ecotypes were not affected regarding this parameter at 100 mM NaCl, but at higher salt levels (200 mM NaCl and 300 mM NaCl) resulted in a significant reduction in vascular region thickness. In ecotypes from Jahlar and Knotti, vascular region thickness was not affected by increasing salt levels (Fig. 4, Plate 1).

A significant decrease in metaxylem area of root was observed in ecotypes from Sahianwala, Gatwala, Treemu and Knotti along with increasing salt levels. The Jahlar and Sheikhupura site plants showed significant decrease in metaxylem area, but only at 300 mM salt level (Fig. 4, Plate 1).

A significant increase was recorded in root cortical cell area in the ecotypes from Sheikhupura, Sahianwala, Gatwala and Treemu in response to increasing levels of salt stress. The Jahlar and Knotti ecotypes showed a significant decrease up to 200 mM NaCl level, but the highest level had no effect. A significant decrease in phloem area was observed in almost all ecotypes along with increasing salt levels (Fig. 4, Plate 1).

Discussion

All ecotypes of *Typha domingensis* showed good tolerance to salt stress, but the ecotypes from Sahianwala and Knotti were found to be better adapted than the other ecotypes. The third best ecotype was that from Jahlar. All three ecotypes were collected from salt-affected habitats, either from saline wetlands of the Punjab plains or hyper-saline salt lakes in the Salt Range.

Shoot and root fresh biomass invariably decreased in all ecotypes, however, those from salt-affected habitats showed relatively less reduction in response to salt stress. The best performer was from Sahianwala, followed by the Knotti and Jahlar ecotypes. Plant biomass is a good indicator of stress tolerance that may even increase at low salinities in most of the halophytes (Khan *et al*., 2000; Munns, 2002; Al-Garni, 2006).

Deeper and more robust root system is generally related to stress tolerance, in particular water scarcity (Maiti, 2012; Munns *et al.*, 2012). However, reduction in root length is a general response of plants to salinity stress (West *et al*., 2004). *Typha domingensis* is a species of aquatic wetlands, and therefore deeper root system may not contribute significantly. However, the robust root system, as was recorded in the Sahianwala and Knotti ecotypes under severe stress can be related to high degree of tolerance.

There is generally increase in Na^+ uptake under salt stress (Nawaz *et al*., 2013). All the ecotypes responded similarly for this parameter in response to salt stress, indicating the universal feature of this species. The *T. domingensis* ecotypes seem to be more specific towards the uptake of Cl^-. The more tolerant ecotypes from Sahianwala and Knotti accumulate significantly lower Cl^- than all other ecotypes, which is an indication of salt tolerance. The restricted uptake of Cl^- has also been recorded by Wahome *et al*. (2001) in salt tolerant *Rosa* species.

All ecotypes of *Typha* showed a marked reduction in root K^+ and N under salt stress as was also recorded by Sairam *et al*. (2002) in wheat and Parida & Das, (2005) in several plant species. The response is, however more or less similar in all the ecotypes. This indicates low contribution of K^+ and N in stress tolerance in this particular species. A significant reduction in root K^+ has been reported by early researchers in a number of plant species e.g., Fionn *et al*. (2013) in *Arbidopsis thaliana*.

Many root anatomical characters negatively alter in response to increasing salinity stress, such as vascular bundle area, epidermal cell area, phloem area, and metaxylem area. The Sahianwala area plants had relatively thicker roots as compared to that observed in all other ecotypes, and there was a significantly increase in value related to this parameter as the salt level increased. Root thickness is generally due to the presence of storage parenchyma (Nawaz *et al.*, 2014), and therefore, root can be related to stress tolerance having more space to conserve water. This has also been reported by several authors (Jeong *et al.*, 2010; Renault *et al.* (2013).

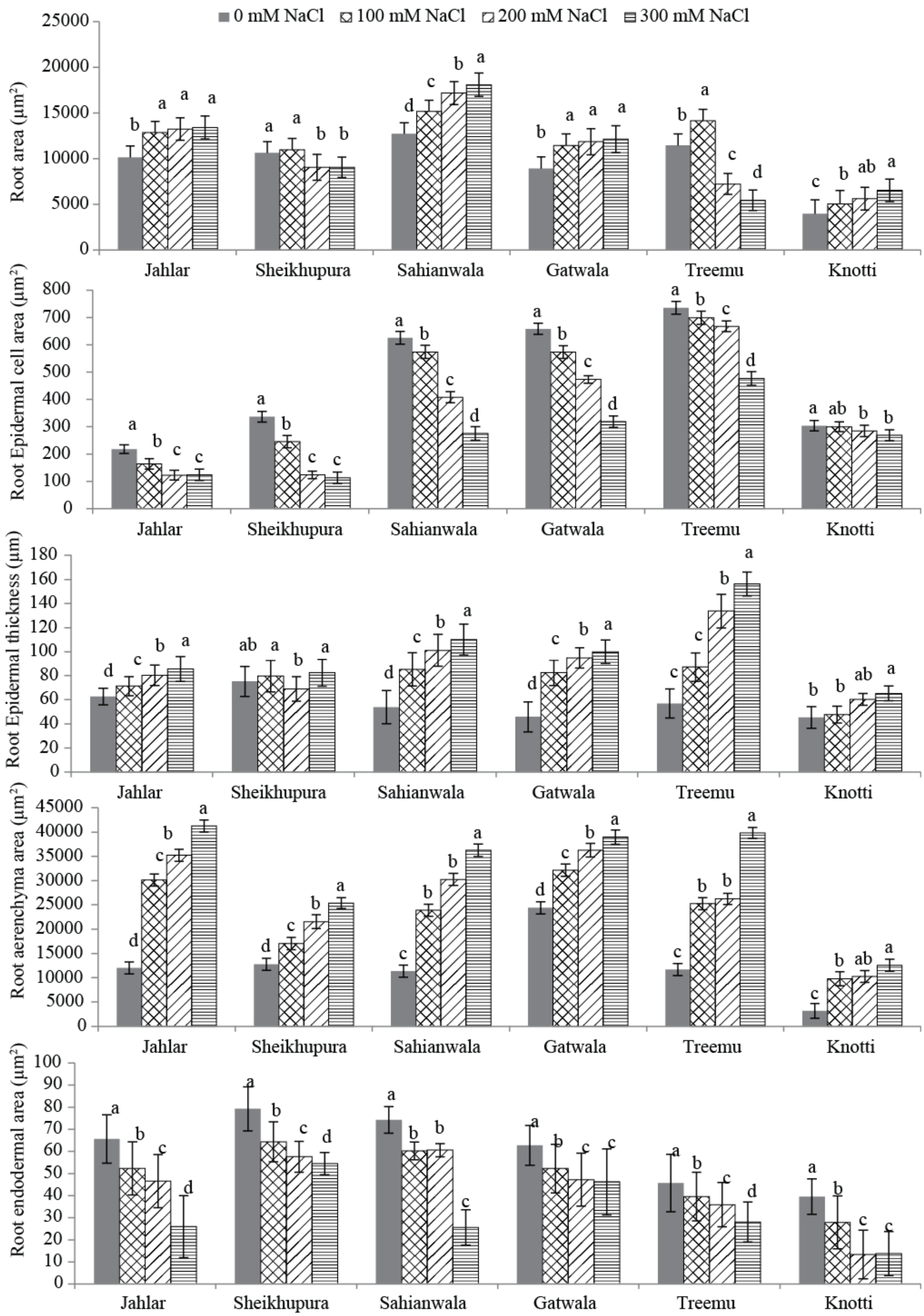

Fig. 3. Root anatomical characteristics of six ecotypes of *Typha domingensis* Pers. under different levels of salt stress.

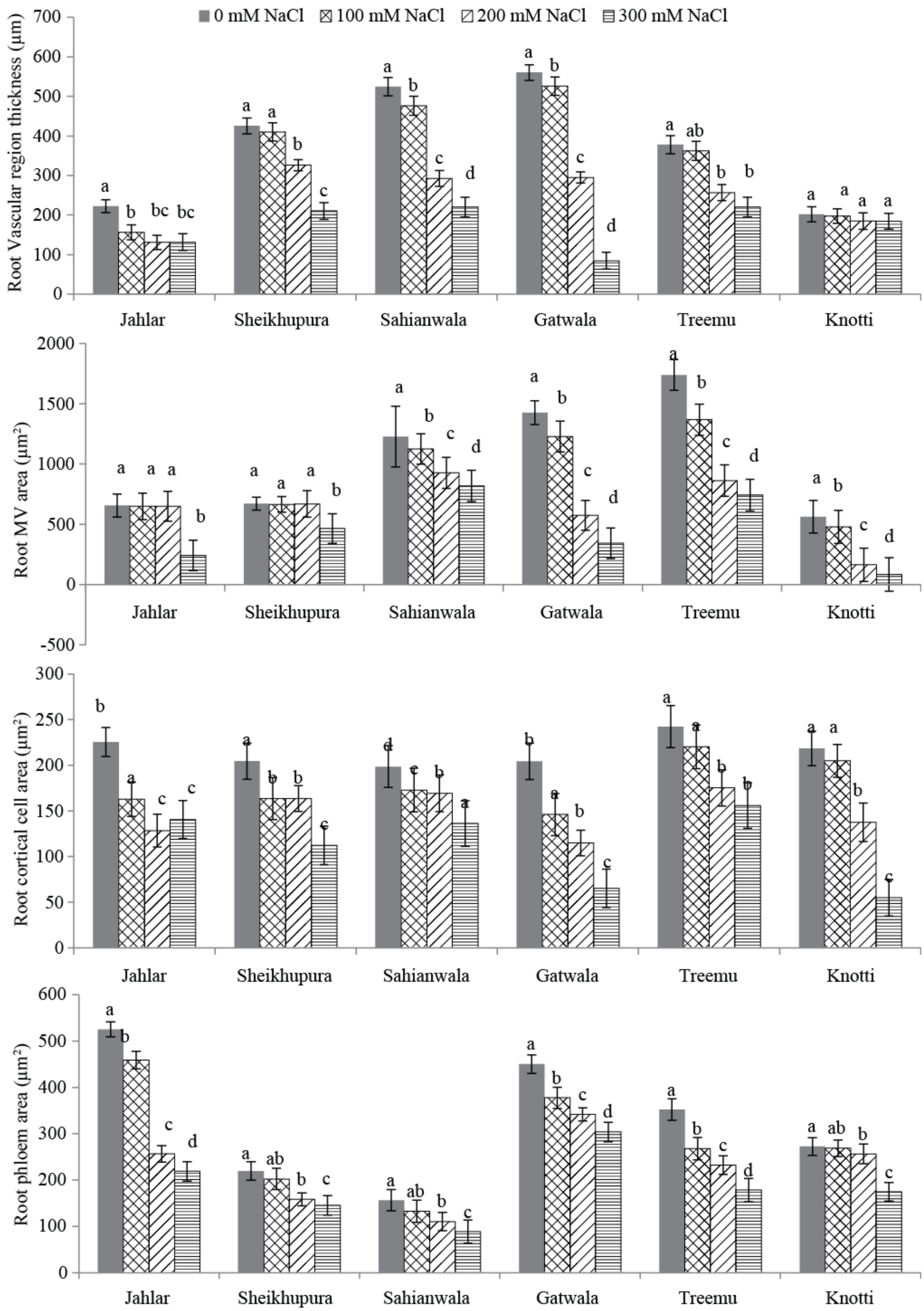

Fig. 4. Root anatomical characteristics of six ecotypes of *Typha domingensis* Pers. under different levels of salt stress.

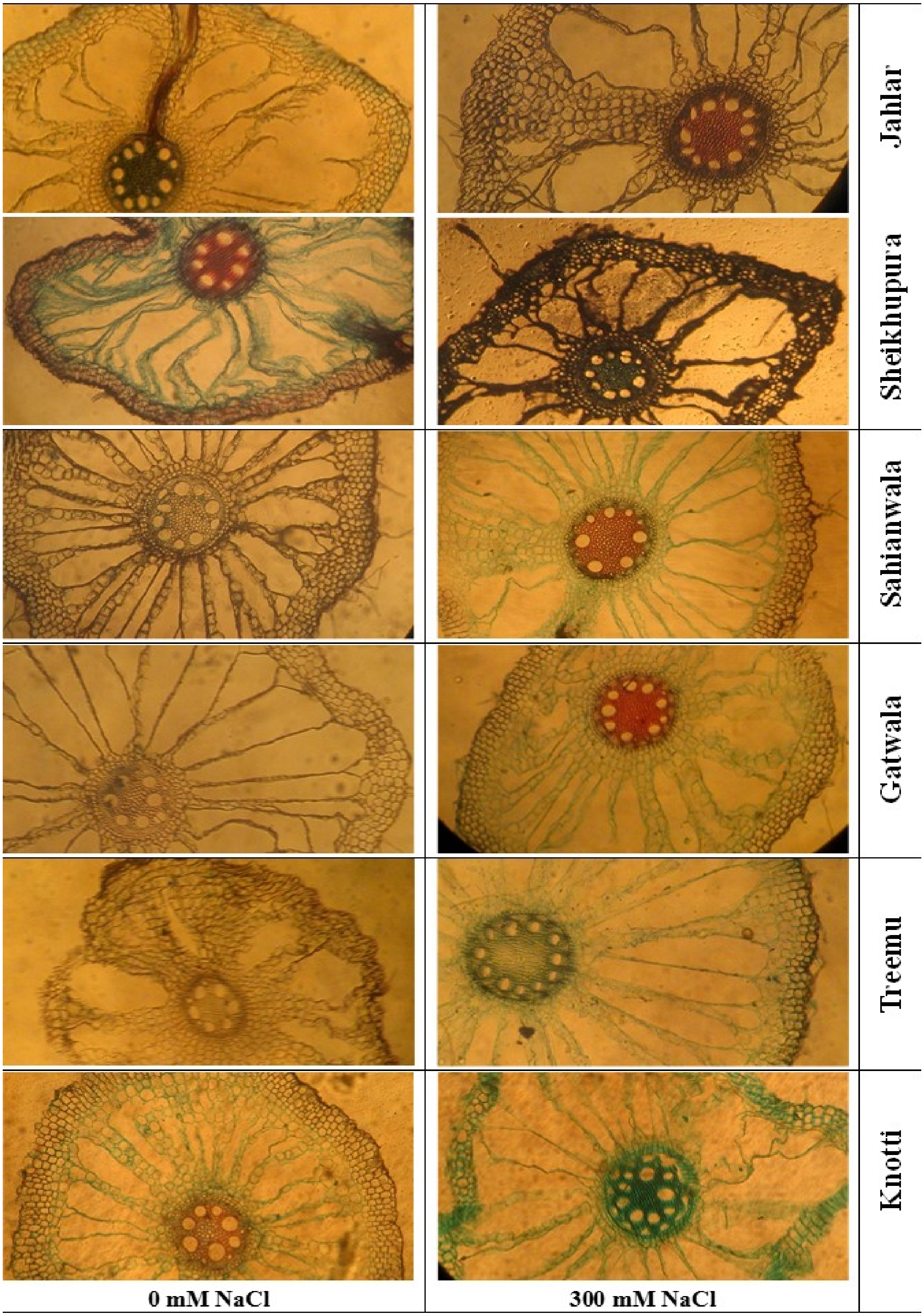

Plate 1. Root transverse section of six ecotypes of *Typha domingensis* Pers. under different levels of salt stress.

Large parenchymatous cells must have larger vacuoles, and certainly have more space to store water storage. This phenomenon may be critical in terrestrial plants where water availability is significantly reduced. Cortical region in *T. domingensis* is composed of large aerenchymatous cavities, which is an adaptation to waterlogged conditions (Takahashi *et al*., 2014). Aerenchyma formation is a common mechanism in most of the halophytes (Alam *et al.,* 2011; Alamri *et al.,* 2013), which is extremely useful in gaseous transport under anaerobic conditions like waterlogging (Suralta & Yamauchi, 2008). It has also been reported that few air cavities are filled with water, and this may help in bulk movement of salts, and therefore, can play a decisive role in the survival of a species in extreme salinities (Van der Weele *et al*., 1996). In present case, aerenchyma invariably increased along with salinity level. Moreover, the Knotti ecotype showed exceedingly large cavities than the other ecotypes, which may enable this ecotype to survive in hyper-saline lake environments.

Endodermal thickness, cortical cell area and phloem area invariably decreased in response to salinity stress, which is a general impact of salinity on internal structure of root (Munns, 2002; Tavakkoli *et al*., 2010). Metaxylem area and vascular region thickness increased on in the Jahlar ecotype, which is from hyper-saline water of Jahlar Lake. Efficient conducting in such harsh climates might be of great ecological significance to cope with high salinities.

The most tolerant ecotype from Sahianwala showed very specific structural and functional modifications for salt tolerance. These includes stable fresh biomass, low Cl^- uptake by roots, increased root area and epidermal thickness, increased aerenchyma, large vascular bundle region and wide metaxylem vessels. The second best ecotypes from Knotti relied on less affected root K^+ and N content, low Cl^- uptake, stable root cross-sectional area and less affected root epidermal cell area. The most sensitive ecotypes from Sheikhupure showed a marked decrease in fresh biomass, increased root Cl^- uptake, decreased root area and epidermal cell area and less aerenchyma formation.

It is concluded from the present studies that there are variations in all ecotypes for salinity tolerance, which is based not only on structural response, but also physiological mechanism is involved. Strategy for salt tolerance adopted by each population is specific, which may relate to their adaptation to a specific habitats and environmental conditions. The most tolerant ecotype from Sahianwala showed high K^+ uptake, large vascular bundle area and cortical cell area. Uptake of K^+ is an important strategy of halophytic species. Structural modifications in the Knotti ecotype are very specific, in particular, presence of exceedingly large aerenchymatous cavities. It is found that environmental heterogeneity played a critical role in genetic variation in this species.

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