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CONTRIBUTION OF ROOT STRUCTURAL AND FUNCTIONAL FEATURES TOWARDS SALINITY TOLERANCE IN *DIPLACHNE FUSCA* (L.) P. BEAUV. EX ROEM. & SCHULT. SUBSP. *FUSCA*

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Abstract

A study was conducted to evaluate structural and functional modifications in *Diplachne fusca* roots under varying regimes of salt stress. Differently adapted populations were collected from ecologically different regions in the Punjab region namely Pakka Anna (hypersaline dryland), Sahianwala (saline waterlogged area), Kalar Kahar (hypersaline salt marsh), Rahimyar Khan (saline sandy loam) and Treemu (saline seasonal inundation). The plants were grown in normal non-saline condition at the University of Agriculture, Faisalabad for 6 months to acclimatize them in the Faisalabad conditions. Three salt stress levels were maintained thereafter, i.e., 0, 200 and 400 mM NaCl for 90 days. All populations responded differently to increasing salt levels. The Pakka Anna population was found to be the most tolerant which relied mainly on increased K^+ uptake but maintained Ca^{2+} uptake in root. An increase in root cross-sectional area was also observed in this population due to increase in storage parenchyma and vascular tissues. This appeared to be crucial towards water conservation in this population. The Sahianwala population collected from saline waterlogged area ranked second in salt tolerance. The notable anatomical modifications in this population included increased vascular tissue and aerenchymatous formation for efficient translocation of nutrients and gaseous exchange especially under anaerobic conditions. It was concluded that differently adapted populations evolved distinctly in geographically isolated regions by development of specific root anatomical features.

Key words: Aerenchyma, Ion uptake, Halophyte, Kallar grass, Sclerification.

Introduction

Salinity is a severe threat for the plants survivorship at global scale (Horie *et al.*, 2012). More than 45 million ha land has been degraded by salinity worldwide that is mostly comprised of irrigated area. In Pakistan about 26% land is affected by salinity (Munns & Tester, 2008; Saleem *et al.*, 2011) mainly due to irrigation with poor quality water and salt deposition in soils (Sattar *et al.*, 2010). Presence of high amount of salts in irrigated lands is the main cause of low crop production in the arid and semi-arid regions worldwide (Araus *et al.*, 2010).

Plants response to salinity varies depending upon its severity and duration (Ashraf, 2004). Salinity significantly decreases growth and development of plants that can be related to increase in the Na^+ and Cl^- ions (Tavakkoli *et al.*, 2010). Salinity restricts plant's water uptake and affects the performance of growth regulators like enzymes and eventually causes the death of the plant exposed to high salt concentrations for prolonged time periods (Mahajan & Tuteja, 2005; Hasanuzzaman *et al.*, 2012).

Plant roots in saline environment tolerate salinity by extensively spreading roots (Hameed & Ashraf, 2008). In addition, roots develops thick sclerenchyma, suberized endodermal cells (Rossato *et al.*, 2015) and thickening in endodermis mostly on the inner tangential walls (YuJing & Yong, 2000; Robbins *et al.*, 2014). Aerenchyma development is one of the important strategy to tolerate salinity in grasses (Lacramioara *et al.*, 2015). Roots encounters high concentration of salts in its environment (Horie *et al.*, 2012) and aerenchyma is effectively involved in exchange of gases and helps to maintain oxygen level (Seago *et al.*, 2005). Among marshy halophytes, aerenchyma is one of the most adapted root characteristic and help to survive during flooding (Al-Hassan *et al.*, 2015).

Diplachne fusca (L.) P. Beauv. ex Roem. & Schult is a halophytic species present in Pakistan and have the best adaptive components against salinity (Aslam *et al.*, 1991). *D. fusca* is distributed widely in salt-affected areas of Pakistan, which is locally known as Kallar grass or salt grass (Zafar & Malik, 1984). It is a perennial species and grows well in saline waterlogged soils where other species cannot grow effectively (Ahmad, 2010). It has fibrous and robust root system that dissolves $CaCO_3$ in soil and deep roots helps in lowering water table of waterlogged soils (Haq & Khan, 1971). It is hypothesized that modifications in root structure and function act as a first line of defense in *D. fusca* and hence, this species can withstand hyper-saline environments. Thus, the present study focused to appraise the significance of root morpho-anatomical and physiological features of *Diplachne fusca* for salinity tolerance.

Materials and Methods

Differently adapted populations of kallar grass (*Diplachne fusca* (L.) P. Beauv. ex Roem. & Schult. subsp. *fusca*) were collected from salt-affected areas of the Punjab to evaluate structural and functional modifications in roots under varying salt levels. The study sites selected included: Pakka Anna (highly saline wasteland), Sahianwala (highly saline waterlogged), Kalar Kahar (hypersaline saltmarsh), Rahimyar Khan (saline desert) and Treemu (saline seasonal inundation). Ten mother plants were randomly selected from their respective sites and grown in normal non-saline conditions in Botanic Garden research area of University of Agriculture, Faisalabad. After acclimation in the Faisalabad conditions, 15 ramets of equal size were separated from mother plants and planted in plastic pots containing sand, peat and loam in equal quantities. Three salt-levels were then maintain for 90 days, viz. 0 (control), 200 and 400 mM NaCl.

For anatomical studies, plant samples were preserved in FAA (formalin acetic alcohol) solution. Free-hand sectioning was done to prepare permanent slide of root. The sections were dehydrated and stained following the double staining technique proposed by Ruzin (1999). Photographs were taken by a camera-equipped compound microscope (Nikon 104, Japan). Anatomical measurement were taken with ocular micrometer, which was calibrated with stage micrometer. For ionic content, roots were crushed and digested in H_2SO_4. The concentration of Na^+, Ca^{2+} and K^+ were measured by flame photometer (Jenway, PFP-7, Japan) and Cl^- content by chloride meter (Jenway, PCLM 3, Japan).

The experiment was set up in a completely randomized design with 2-factor factorial arrangements and three replicates. The data were subjected to analysis of variance (ANOVA) using Minitab (v.17). The significance of means values was also computed by the same software.

Results

Morphological parameters: Analysis of variance of the data showing F-ratios is presented in Table 1. Among morphological parameters, root length increased with the increase in salinity stress in all populations, except in Treemu population, where it decreased at 400 mM NaCl. Root fresh weight generally increased with the increase in salt stress to 200 mM NaCl and then decreased at 400 mM NaCl. Sahianwala population was the only exception, where consistent increase was noted with increasing salinity level. Root dry weight increased invariably at 200 mM salt level but decreased at the highest level (Fig. 1).

Physiological parameters: Among physiological parameters, root Na^+ increased in all populations, while K^+ decreased in Treemu, Kalar Kahar and Rahimyar Khan with increasing salinity. Root Na^+ increased up to 400 mM continuously in all the populations but root Ca^{2+} decreased in Pakka Anna at 400 mM NaCl only. In comparison, root K^+ decreased at 200 mM in Pakka Anna and Sahianwala populations and increased at higher salt levels. Root Ca^{2+} was the highest at 0 mM and Cl^- at 400 mM salt levels of all populations (Fig. 2).

Anatomical parameters: Considerable variation was observed in root anatomical characteristics along increasing salinity stress. Populations showed significant changes in dermal, aerenchymatous and vascular tissues. Root cross sectional area continuously increased up to 400 mM in Pakka Anna and Sahianwala populations whereas it decreased in other populations at 400 mM NaCl. Epidermal cell area increased only at high salt level (400 mM) in Sahianwala and Kalar Kahar but decreased in other populations. Cortical region thickness decreased at high salt (400 mM NaCl) in all populations and highest value was noted at 200 mM in Rahimyar Khan. A rapid increase was observed in cortical cell area up to 400 mM in Pakka Anna and Sahianwala as compared to other populations which showed relatively less increase in these root anatomical attributes. Aerenchymatous area showed an increasing trend up to 400 mM NaCl in Sahianwala and Treemu population only, while other populations showed a decreasing trend in aerenchymatous area at 400 mM. Endodermal cell area increased at 400 mM only in Treemu population but decreased in all other populations. The maximum endodermal cell area was noted in Sahianwala at 200 mM NaCl concentration. Vascular region thickness increased in Pakka Anna and Sahianwala populations. The root metaxylem area showed significantly highest value only in Sahianwala population than any other populations at 200 mM NaCl concentration. Phloem area increased in Pakka Anna, Sahianwala and Treemu at 400 mM NaCl but pith region thickness decreased at this salt concentration (400 mM) (Fig. 3).

Table 1. Analysis of variance showing F-ratio of *Diplachne fusca* population collected from sal-affected areas in the Punjab.

Characteristics	Habitats	Treatments	Interaction of Treatment x Habitat
Root length	2.20**	48.61**	0.80^{NS}
Root fresh weight	10.43**	23.32**	0.52^{NS}
Root dry weight	6.28**	29.77**	3.19**
Root Na^+	4.88**	125.16**	0.60^{NS}
Root Cl^-	7.28**	162.90**	1.49^{NS}
Root K^+	55.85**	51.30**	2.45**
Root Ca^{2+}	5.15**	25.93**	0.36^{NS}
Root cross-sectional area	844.50**	128.50**	88.26**
Epidermal cell area	339.83**	17.10**	36.58**
Cortical region thickness	171.35**	36.25**	35.60**
Cortical cell area	4.52**	5.05**	1.98^{NS}
Aerenchyma area	192.43**	85.26**	52.67**
Endodermal cell area	3.65**	2.30**	1.57^{NS}
Vascular region thickness	175.50**	11.03**	18.37**
Metaxylem area	123.01**	37.62**	44.63**
Phloem area	13.63**	14.55**	7.92**
Pith region thickness	645.71**	187.83**	65.16**

** = Significant at p>0.01, NS = Not significant

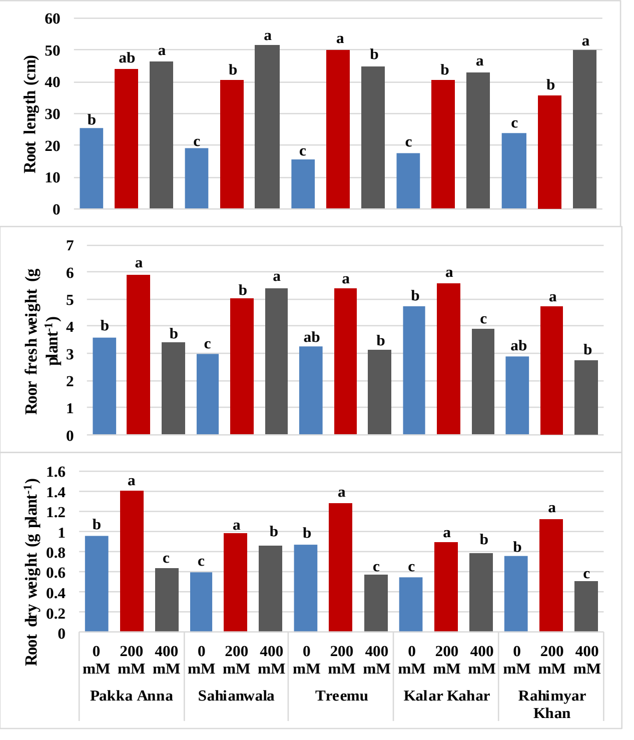

Fig. 1. Morphological parameters of *Diplachne fusca* (L.) P. Beauv. ex Roem. & Schult. subsp. *fusca* in salinity level of 0, 200 and 400 mM NaCl concentration.

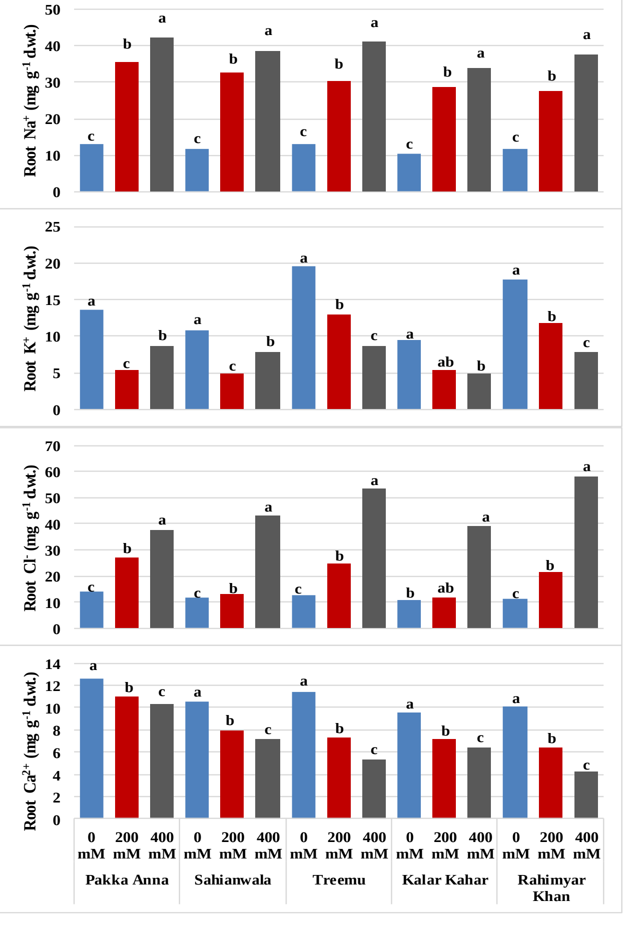

Fig. 2. Physiological parameters of *Diplachne fusca* (L.) P. Beauv. ex Roem. & Schult. subsp. *fusca* in salinity level of 0, 200 and 400 mM NaCl concentration.

Specific anatomical modifications: Salinity-induced sclerification was the most prominent feature of *D. fusca* populations, however, nature and intensity of sclerification was different in each population. Intensive sclerification was recorded in central vascular region of the Pakka Anna population, which increased significantly as salinity level increased. At the highest level, intensive sclerification was also recorded in the pith region. Sclerification was also observed in this population at outer cortical region under saline condition.

The Sahianwala population showed sclerification at vascular region under low salinity. As the salinity level increased, this population showed intensive extended sclerification in vascular, pith, endodermis, as well as in pericycle regions. Interestingly, the extent of sclerification was extended to inside epidermis when this population was exposed to the highest salt level at 400 mM. A similar trend noted in the Kalar Kahar population, but no sclerification recorded in the cortical region at any salinity level.

The Rahimyar Khan population showed a unique behavior upon exposure to salinity stress. The inner tangential wall of endodermis were found to be heavily sclerified, just alike to the walls of the pericycle region. A greatly reduction in vascular region was noted at the highest level, which was also heavily sclerified. Small patches of sclerenchyma were recorded in the least adapted population collected from Treemu at moderate salt level. However, this population showed intensive sclerification in entire stellar region, and also in the hypodermis region just inside epidermis at higher salinity levels (Fig. 4).

The study sites were not only ecological distinct regions but also varied in their extent of salt levels. Canal system in Pakistan was created before partition of Indian sub-continent, i.e., more than 100 years ago. As a result, many adjoining areas of this canal system like Sahianwala were heavily affected by waterlogging. Saltmarsh at Kalar Kahar is a permanent waterbody and included in RAMSAR site. Treemu is a seasonal inundation that receives flooding water seasonally during monsoon. When the differently adapted population were grown in controlled environments of Faisalabad, the pre-fixed genetic characters expressed. This might be a strong reason that all populations responded differently to varying salinity levels. It is expected that these differential behaviors have likely arose as a response to evolutionary pressures imposed on *D. fusca* populations in their native habitats.

Root growth generally increased in all populations along salinity gradient, which is the characteristic of most of halophytes including *Diplachne fusca*. Root length increased under high salinity in most populations of *D. fusca*. Increase in root length is a significant adaptation to tolerate salinity, as previously been reported in Bermuda grass (*Cynodon dactylon*) by Hameed *et al*., (2010), Hu *et al*., (2014) and Pessarakli (2015). In *D. fusca*, root fresh and dry weights, however, decreased under high salt concentration. Such reduction in root biomass has also been reported by Mane *et al*., (2011), Ceccoli *et al*., (2011), Hu *et al*., (2014) and Bazaz *et al*., (2015) under saline conditions.

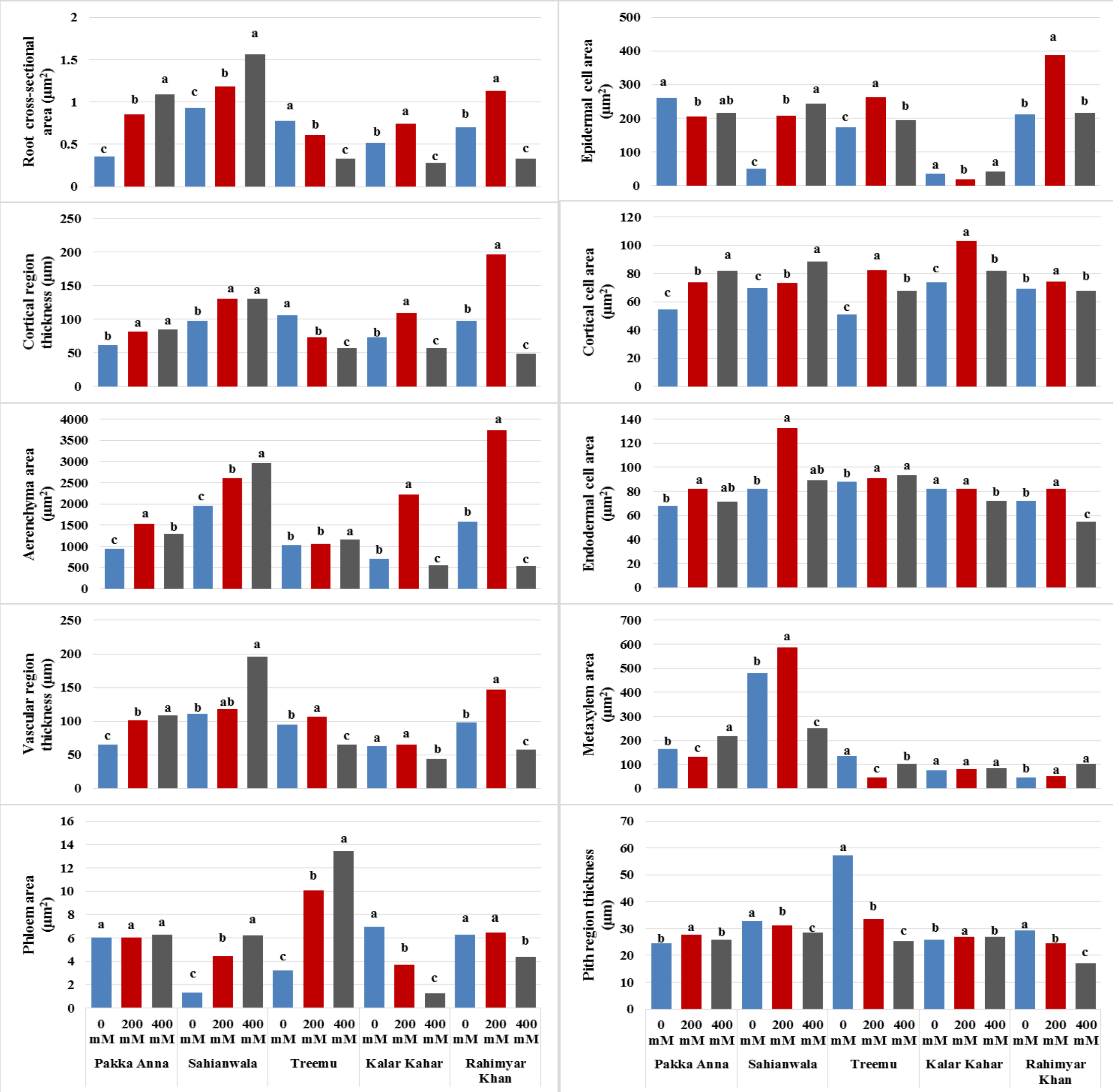

Fig. 3. Anatomical parameters of *Diplachne fusca* (L.) P. Beauv. ex Roem. & Schult. subsp. *fusca* in salinity level of 0, 200 and 400 NaCl concentration.

In the present study, the most tolerant Pakka Anna population showed high concentration of K^+, Na^+ and Cl^- along salinity gradient, while Ca^{2+} uptake decreased. Plants carry out different mechanisms to balance cellular homeostasis of Na^+ and K^+ under saline conditions. The main processes adapted by plants to adjust its metabolic activities are the discarding excessive Na^+, accompanied with increased accumulation of K^+ ions to maintain its growth properly (Ashraf *et al.*, 2010; Liu & Shi, 2010).

Discussion

D. fusca populations showed increased thickness in epidermal and endodermal tissues under salinity. Both layers are protective in nature and any increase will minimize water loss, and this may ensure better survival of a plant under stressful conditions. Sclerenchyma provides mechanical strength to delicate root tissue (Lu *et al.*, 2008). It plays a protective role as helps to reduce loss of water from the roots (Taleisnik *et al.*, 1999) and also limits radial flow of water (Bagniewska-Zadworna & Zenkteler, 2006).

Increase in the cortical thickness and cortical cell size of root is an adaptation for survival in salt stress environment (Grigore & Toma, 2007). Cortical thickness increased in most of populations of *D. fusca*. Cortical cells being parenchymatous in nature that involve in the storage of water, and hence, play a role in adjustment of osmotic stress due to saline conditions (Valenti *et al.*, 1992). Larger cortical cells with large central vacuoles can store more water than small-sized cells (Lynch *et al.*, 2014; Chimungu *et al.*, 2015) and this enable plants to endure high salinity (Hameed *et al.*, 2013; Alam *et al.*, 2015; Al-Hassan *et al.*, 2015; Paez-Garcia *et al.*, 2015).

Metaxylem size increased under different salinity levels in some of *D. fusca* populations. Wider vessels are more efficient in conduction of water and nutrients (Guo and Miao, 2010). In contrast, narrow vessels are less prone to damage when a plant is under severe osmotic stress (Hameed *et al*., 2009).

Aerenchyma is the most prominent feature of *Diplachne fusca* roots and this increased with increase in salt stress. Aerenchyma is responsible for the efficient exchange of gases throughout the plant (Evans, 2003; Malik *et al.*, 2003; Shimamura *et al*., 2010; Grigore *et al*., 2014), and also facilitates bulk movement of salts (Naz *et al.*, 2013; Lynch and Wojciechowski, 2015; Watson *et al.*, 2015).

The most tolerant population from Pakka Anna relied physiologically on increased K^+ uptake on highest salt level and stability in Ca^{2+} uptake, and this is an important phenomenon of most of halophytes to dilute harmful impacts of Na^+ and Cl^- ions. Anatomically stress tolerance in this ecotype can be related to increased root cross sectional area, which might be due to increased storage parenchyma (cortex) and vascular tissues. This may enhance water conservation considerably in the Pakka Anna population.

The Sahianwala population ranked second regarding salinity tolerance. It showed modifications like increased root area, which primarily due to increased vascular tissue and aerenchymatous formation. Aerenchyma is a characteristic of many aquatic halophytes that may increase survival of this halophyte in hypersaline-waterlogged environments. The Kalar Kahar population showed stability in many anatomical characteristics under high salinities, e.g., vascular region thickness, endodermal cell area, metaxylem area, and pith region thickness. This indicates high degree of tolerance of this population as it maintained conduction of mineral and nutrients along with storage in central pith region.

The Rahimyar Khan population showed stability in metaxylam area and cortical cell area indicating its unaltered conduction potential as well as more space for ion compartmentalization in cortical region. The least tolerant Treemu population showed stability in endodermal cell area and aerenchymatous area, but phloem area drastically increased in this population at high salinity.

Fig. 4. Root transverse sections of *Diplachne fusca* (L.) P. Beauv. ex Roem. & Schult. subsp. *fusca* in response to three salinity regimes.

Conclusion

It is concluded that differently adapted populations might have evolved differently in geographically isolated area for long enough time that specific anatomical feature have developed in these population. This indicates a significant change in genetic makeup of these populations as they adapt different strategies to cope with extremely high salinities.

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