

## Physioanatomical adaptations in response to salt stress in *Sporobolus arabicus* (Poaceae) from the Salt Range, Pakistan

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**Abstract:** *Sporobolus arabicus* Boiss. is a perennial stoloniferous grass that prefers a saline or desert habitat. A natural population of *Sporobolus arabicus* growing in a salty area was compared with a normal nonsaline population. The salt-tolerant population showed better growth than its counterpart. Physioanatomical adaptations to saline environments in the salt-tolerant population were very specific. They included restricted toxic ion uptake, production of organic osmolytes, sclerification in roots, succulence in stem, and development of vesicular hairs on the leaves. These adaptations were not only for the excretion of toxic ions but also for the accumulation of inorganic ions in the parenchymatous tissue. The Salt Range population maintained osmotic and water potential, accumulated lower amounts of Na<sup>+</sup> and Cl<sup>-</sup> in the roots and shoots, and had higher organic osmolytes, increased sclerification in the roots, and succulence in the stems and leaves.

**Key words:** Ionic content, organic osmolytes, salt excretion, *Sporobolus*

### 1. Introduction

The ability of a plant to continue to grow in an extreme environment indicates a high degree of salt tolerance (Niknam & McComb, 2000). Generally, populations of different plant species inhabiting saline habitats are well adapted to these habitats, and the extent of their adaptation depends on the intensity of selection pressure (salinity level) of the habitat (Hameed & Ashraf, 2008). The mechanism that enables such plants to thrive on salt-affected soils is complex and involves many anatomical modifications and morphological, physiological, and biochemical processes (Jacoby, 1999).

Genus *Sporobolus* R.Br. (Poaceae) is known for high salt tolerance because its many species are known to inhabit saline habitats, and they show considerable tolerance to high salinity. Examples include *Sporobolus airoides* (Torr.) Torr. (Butler et al., 1974), *S. spicatus* (Vahl) Kunth (Ramadan, 2001), *S. arabicus* (Khan & Ungar, 2001), *S. virginicus* (L.) Kunth (Bell & O'Leary, 2003), and *S. ioclados* (Trin.) Nees (Gulzar et al., 2005). *Sporobolus arabicus* Boiss. species is a dominant component of vegetation in the Salt Range, where a large area of the foothill zone is affected by sodium chloride (Qadir et al., 2005).

Increased salt tolerance is needed in crops grown in the areas that are affected by salinity or at risk of salinisation

(Munns et al., 2002), and naturally adapted salt-tolerant plants provide excellent material for investigating the adaptation mechanisms used to tolerate high concentrations of salt (Flowers & Colmer, 2008; Hameed et al., 2009). The present study examines the structural and physiological adaptations of naturally adapted populations of *S. arabicus* in terms of ionic relations and osmoregulation.

### 2. Materials and methods

A hydroponic study was conducted to investigate the anatomical adaptive components, ionic relations, and osmotic adjustment under salt stress of a salt-tolerant population of the grass *Sporobolus arabicus*.

A population of *S. arabicus* was collected from the salt-affected habitat in the Salt Range, Pakistan, near the foothill region. The site is greatly impacted by salt deposition as a result of salts dissolving from the exposed hills. Another population of this grass (control) was collected from normal nonsaline habitats within the Faisalabad region.

Plants from both populations were grown in normal nonsaline soil for a period of 6 months (earthen pots, 23 cm) filled with loam and sand in equal quantities. The plants were kept under normal sunlight and irrigated daily with normal irrigation water until established in the

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Faisalabad environment. The ramets, each with 3 tillers of uniform size, were detached from each plant and grown in half-strength Hoagland's nutrient solution until their hydroponic establishment. Fibreglass containers of 25 L in capacity were used for hydroponics. The containers were aerated with air pumps for about 12 h daily and placed under transparent plastic covering to protect them from rainfall. The ramets were planted on a Thermopore sheet, and after the establishment of plants 5 salt levels were maintained for 8 weeks: control (no salinity treatment) and 50, 100, 150, and 200 mM of NaCl in Hoagland's nutrient solution. The experiment was arranged in a completely randomised design with 2 factors (population and salinity level) and 12 replications. Plants were carefully uprooted from the hydroponics after the completion of the experiment and washed with distilled water for the data analysis of physiological parameters: leaf water relations, organic osmolytes, and inorganic ionic content, according to the methods of Hameed and Ashraf (2008).

Anatomy of root, stem, leaf sheath, and leaf blade was studied using the thickest ramet of each replicate. A piece 2 cm in length was taken from the leaf base of fully expanded leaves for leaf anatomy, from the base of the internode of the main tiller for stem and sheath anatomy, and from the thickest adventitious root near the root/shoot junction for root anatomy. Formaldehyde acetic alcohol solution (formaldehyde 10%, acetic acid 5%, ethanol 50%, and distilled water 35%) was used to fix the material for 48 h, and it was subsequently transferred to an acetic alcohol solution (acetic acid 25% and ethanol 75%) for long-term storage. Free-hand sections were prepared by a series of dehydrations in ethanol using the standard safranin and fast green double-stained technique (Ruzin, 1999). Measurements were taken with a light microscope (Nikon SE Anti-Mould, Japan) using an ocular micrometer calibrated with a stage micrometer. Micrographs of the stained sections were taken with a digital camera (Nikon FDX-35) on a stereomicroscope (Nikon 104, Japan). Data for anatomical characteristics were recorded using all 3 plants from each replication at random.

### 2.1. Statistical analysis

Analysis of variance of the data from each attribute was computed using the MSTAT computer program. The least significant difference test at a 5% probability level was used to test differences among mean values (Steel et al., 1997).

## 3. Results

### 3.1. Soil physicochemical characteristics

Soil from the *S. arabicus*-inhabited Salt Range was heavily salt-affected (32°76'23.45"N, 72°62'27.58"E; pH 8.12; electrical conductivity [ECe], 34.36 dS m<sup>-1</sup>; Ca<sup>2+</sup>, 692.18 mg kg<sup>-1</sup>; K<sup>+</sup>, 397.30 mg kg<sup>-1</sup>; Na<sup>+</sup>, 5314.62 mg kg<sup>-1</sup>; Cl<sup>-</sup>, 2467.28 mg kg<sup>-1</sup>); soil from the Faisalabad region was

normal nonsaline (31°28'25.25"N, 73°12'42.87"E; pH 7.86; ECe, 1.52 dS m<sup>-1</sup>; Ca<sup>2+</sup>, 13.78 mg kg<sup>-1</sup>; K<sup>+</sup>, 40.54 mg kg<sup>-1</sup>; Na<sup>+</sup>, 81.68 mg kg<sup>-1</sup>; Cl<sup>-</sup>, 312.20 mg kg<sup>-1</sup>).

### 3.2. Growth parameters

Shoot dry weight of the population from Faisalabad gradually decreased with increases in external salinity levels (Figure 1), but the Salt Range population showed stability in this character, and its dry weight even increased slightly under 50 mM of NaCl. Root dry weight gradually increased up to 100 mM and 150 mM of NaCl in the Faisalabad and Salt Range populations, respectively. Furthermore, a sharp decrease in dry weight in the Faisalabad population and a slight reduction in the Salt Range population were observed at the higher salt levels (150 and 200 mM of NaCl), although in the latter population the root dry weight at 200 mM NaCl was at the same level as in the control treatment.

### 3.3. Ionic contents

There was a consistent increase in leaf Na<sup>+</sup> in both populations with increases in the salt level of the rooting medium (Figure 1). However, the Faisalabad population accumulated significantly higher amounts of Na<sup>+</sup> in the leaves as compared to the Salt Range population at all external salt regimes. There was a sharp decrease in leaf K<sup>+</sup> in the Faisalabad population with the addition of NaCl to the leaf medium. However, in the Salt Range population, leaf K<sup>+</sup> did not decrease up to 100 mM NaCl, but thereafter a slight reduction in leaf K<sup>+</sup> was observed at higher salt levels (i.e. 150 and 200 mM NaCl). It is also interesting to note that at 50 mM NaCl there was a marked increase in leaf K<sup>+</sup> in the Salt Range population.

Root Na<sup>+</sup> increased in both populations with increases in external salt levels. However, the extent of Na<sup>+</sup> accumulation in roots in both populations was almost the same. Although root K<sup>+</sup> decreased consistently with increases in salt levels of the growth medium, the Faisalabad population had significantly higher K<sup>+</sup> in the roots than the Salt Range population.

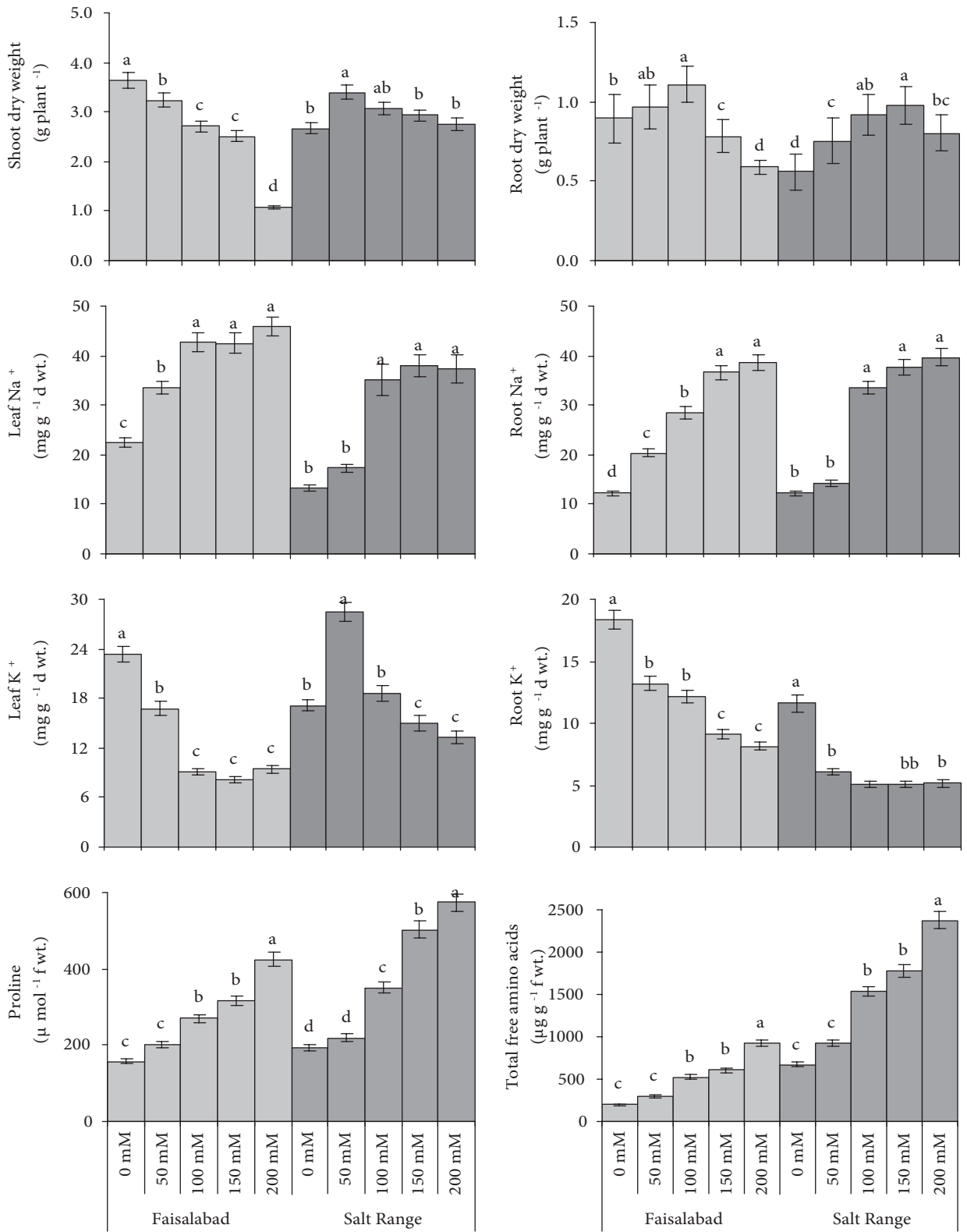
### 3.4. Organic osmolytes

Proline concentrations increased in both populations of *S. arabicus* with increases in salinity levels (Figure 1), but the Salt Range population responded more positively to increasing salt levels, as it accumulated significantly more proline than its counterpart from the Faisalabad region.

Induction of salt stress resulted in a significant increase in total free amino acids in both grass populations (Figure 1). However, the Salt Range population of *S. arabicus* showed markedly greater accumulation of free amino acids in the leaves at all salt levels as compared to the Faisalabad population.

### 3.5. Anatomical modifications

Extensive sclerification was observed in both populations in the cortical region below the epidermis (exodermis), but

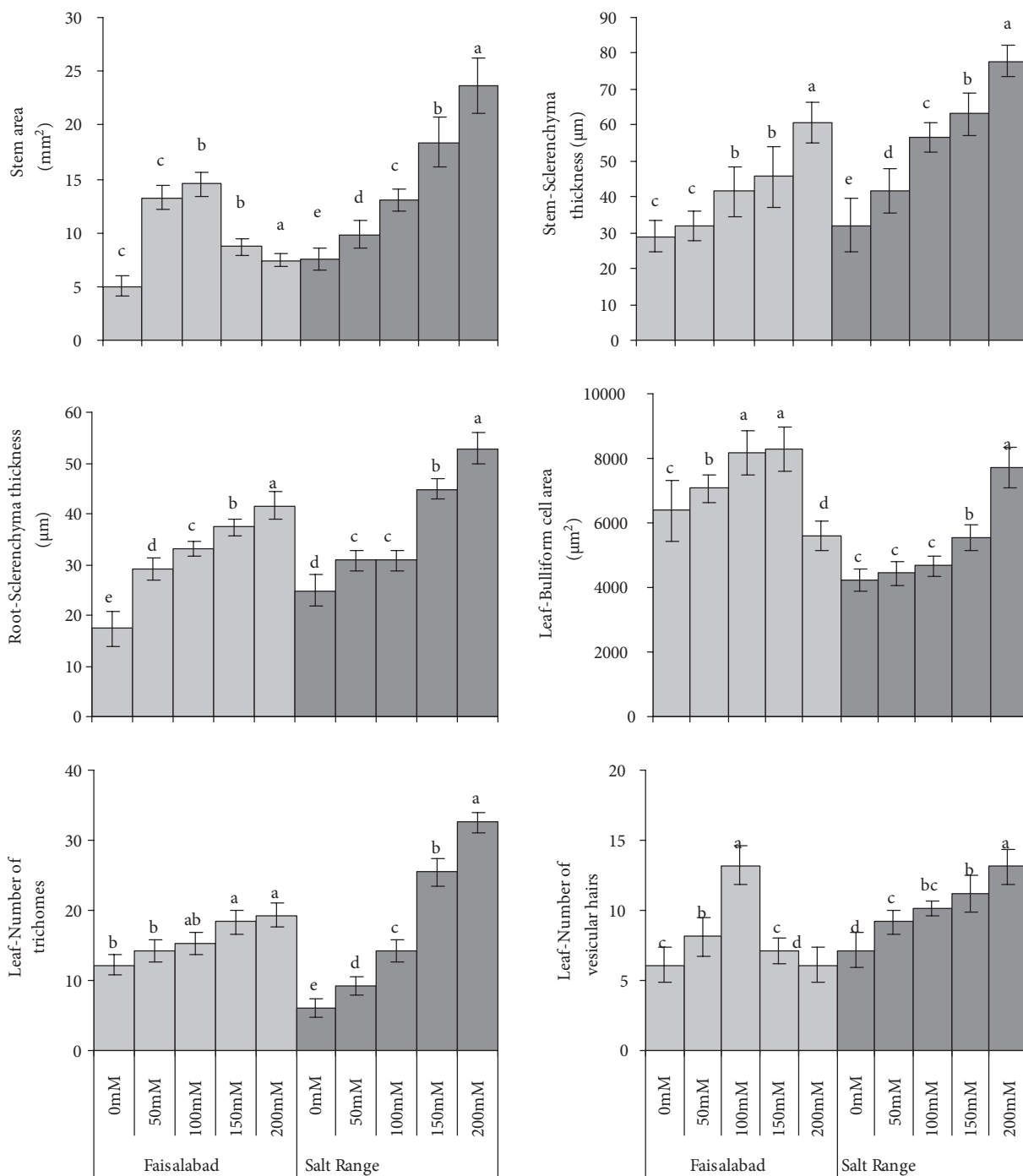


**Figure 1.** Some morphophysiological characteristics of *Sporobolus arabicus* ecotypes subjected to salt stress for 60 days (n = 12, means ± SE). Means sharing different letters are significant at P < 0.05. The letters indicate differences among concentrations of NaCl applications in each habitat.

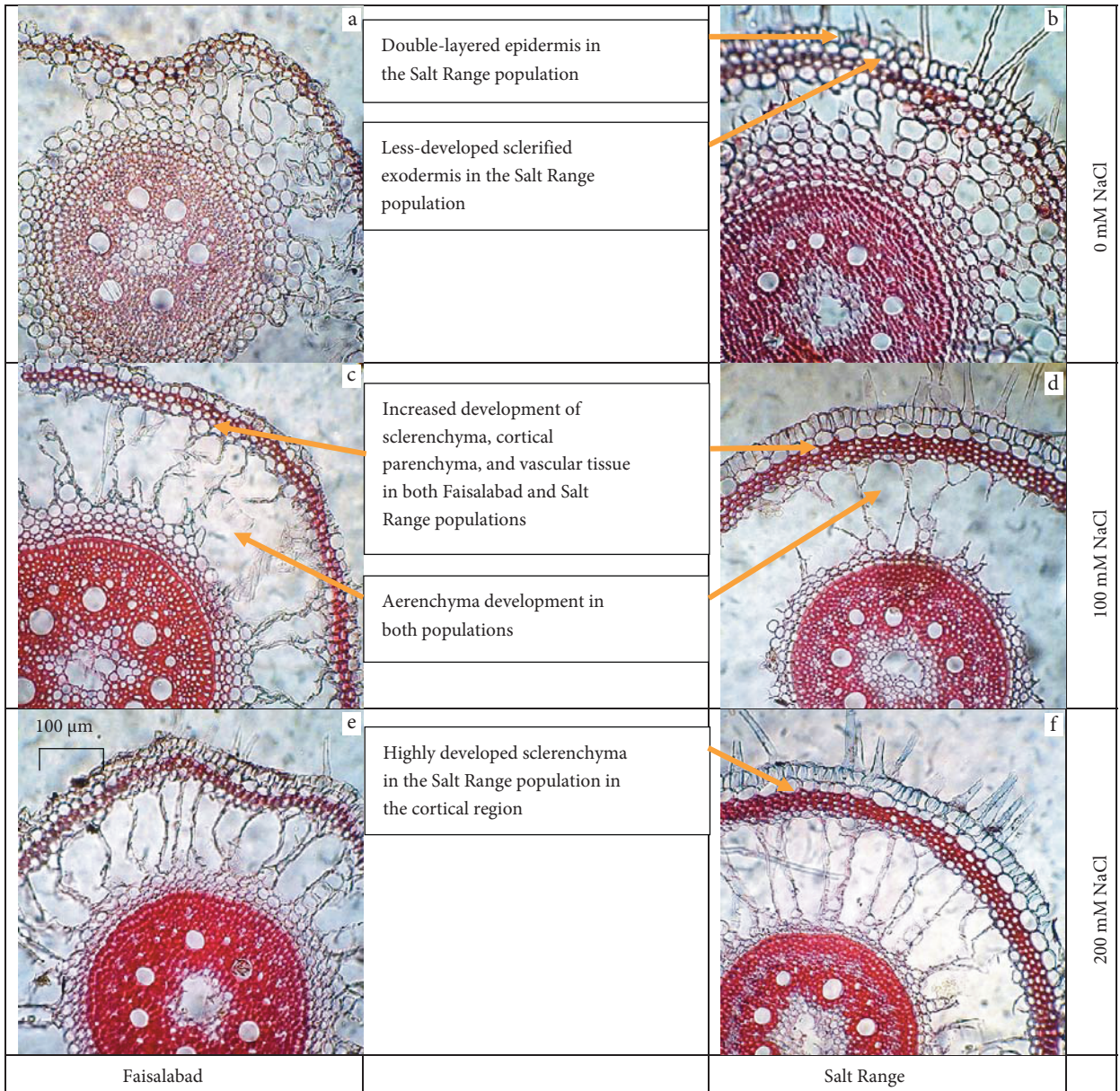
this sclerification was significantly higher in the Salt Range population, particularly under high salinities (Figures 2 and 3). In addition, salt stress resulted in the disintegration of root cortical parenchyma (aerenchyma formation) in both populations (Figure 2). Increased sclerification in

stems was also recorded in both *S. arabicus* populations (Figure 4).

Sclerification was recorded outside the vascular tissue of the leaf sheath in both *S. arabicus* populations (Figure 5). However, with increased salinity levels, the Faisalabad



**Figure 2.** Some root and leaf anatomical characteristics of *Sporobolus arabicus* ecotypes subjected to salt stress for 60 days (n = 12, means ± SE). Means sharing different letters are significant at P < 0.05. The letters indicate differences among concentrations of NaCl applications in each habitat.



**Figure 3.** Transverse sections of root of *Sporobolus arabicus* subjected to salt stress for 60 days. All figures are identical in scale and the scale bar is presented in Figure 3e.

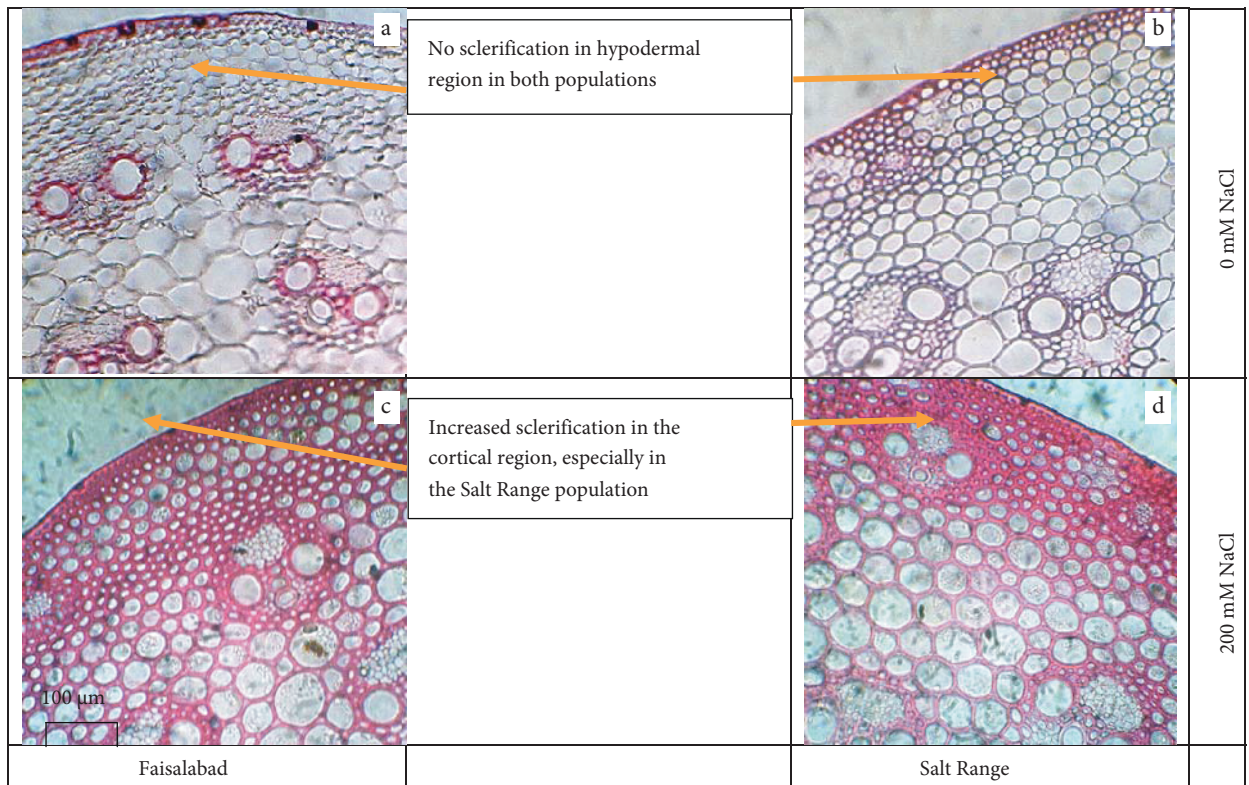
population showed extensive sclerification all over the abaxial surface of leaf sheath. The Salt Range population also showed intensive sclerification, but this was restricted to the epidermis of the abaxial side.

There was a progressive development of bulliform cells on the adaxial leaf surface in both populations with increases in salt levels (Figures 2 and 6). These cells were relatively broad and flattened at moderate salt levels and deeply seated at high salt levels. The density of vesicular hairs increased significantly in the Salt Range population with increases in external salt levels; however, in the

Faisalabad population the density only increased up to 100 mM NaCl (Figures 2 and 6). The Salt Range population also showed a significant increase in the density of trichomes with increases in salt levels of the growth medium (Figure 5).

#### 4. Discussion

Similar studies on the salt tolerance of Salt Range grasses that describe the structural and functional features of species other than *S. arabicus* have been published (e.g., Hameed & Ashraf, 2008; Hameed et al., 2008, 2009,



**Figure 4.** Transverse sections of stem of *Sporobolus arabicus* subjected to salt stress for 60 days. All figures are identical in scale and the scale bar is presented in Figure 4c.

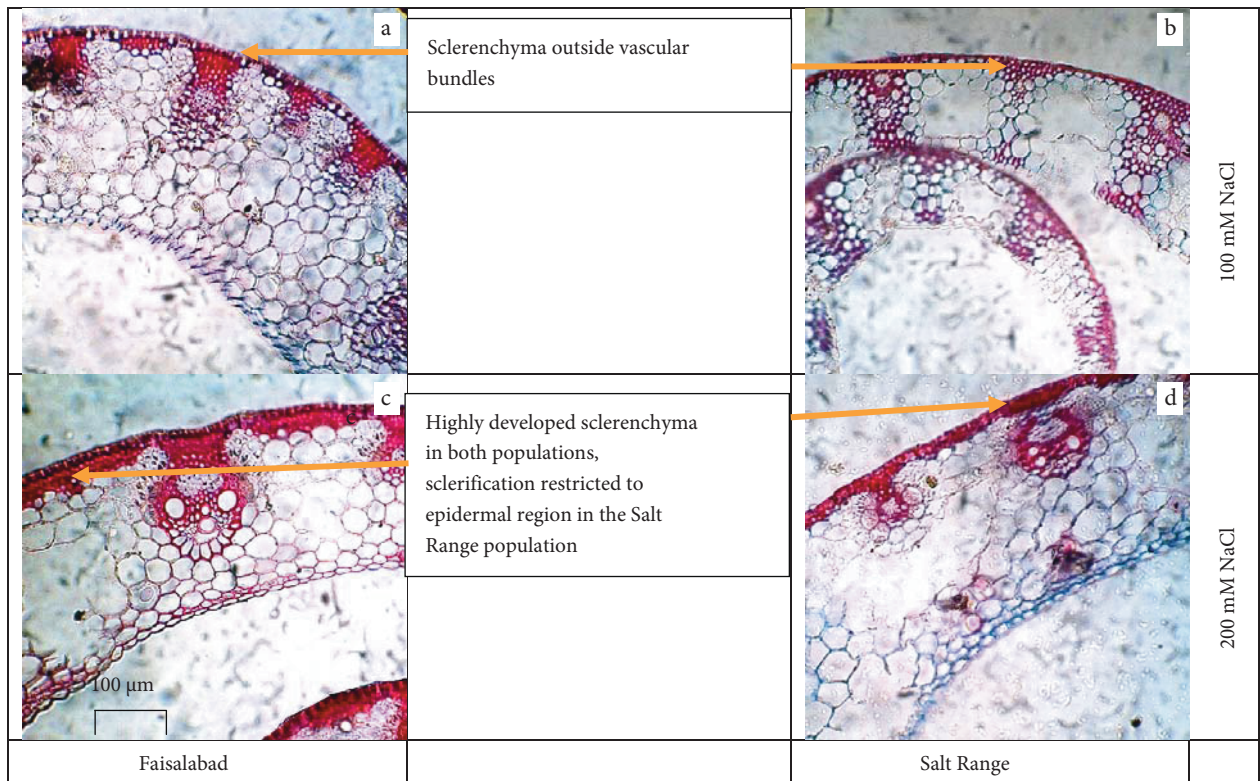
2010, 2011). In the present study, we have focused on the most tolerant among the Salt Range grasses, *S. arabicus*, which has very specific structural characteristics and physiological mechanisms for adaptation to high salinity levels.

Plant growth measured as shoot or root biomass was adversely affected in the Faisalabad population due to salt stress, whereas in the Salt Range population this biomass increased with the imposition of salt in the rooting medium. Stimulation of growth as observed in the Salt Range population demonstrates its marked adaptation to salt stress, and this is one of the prominent characteristics of most halophytes, e.g., *Sporobolus virginicus* (L.) Kunth (Bell & O'Leary, 2003), *Arthrocnemum macrostachyum* (L.) Moq. (Redondo-Gómez et al., 2010), *Aeluropus lagopoides* (L.) Thwaites (Naz et al., 2010), and *Chenopodium quinoa* Willd. (Eisa et al., 2012).

The Salt Range population of *S. arabicus* accumulated lower concentrations of  $\text{Na}^+$  in its leaves than its counterpart from Faisalabad at varying external salt levels, which is a feature of salt-tolerant species (Munns, 2002). In most plants, increased concentrations of  $\text{Na}^+$  in the aerial parts is generally associated with a concomitant reduction in  $\text{K}^+$  and/or  $\text{Ca}^{2+}$  concentrations (Munns & Tester, 2008). Leaf

and root  $\text{K}^+$  decreased considerably in both populations, but this reduction was more pronounced in the Faisalabad population, particularly at higher salt levels. Higher levels of leaf  $\text{K}^+$  and lower  $\text{Na}^+$  levels in the Salt Range population, compared to the Faisalabad ecotype, resulted in a higher  $\text{K}^+$ -to- $\text{Na}^+$  ratio. Maintenance of a high  $\text{K}^+$ -to- $\text{Na}^+$  ratio helps plants to thrive well under saline conditions (Ashraf, 2004).

A variety of organic osmolytes play an important role in plant salt tolerance (Touchette, 2007; Flowers & Colmer, 2008), and salt-tolerant and salt-sensitive plants mostly differ in their accumulation of these organic solutes. The Salt Range population accumulated significantly higher concentrations of osmolytes, particularly free amino acids and proline, than its counterpart from the Faisalabad region. It is generally known that proline plays a considerable role in osmotic adjustment (Yoshida et al., 1997). The higher proline accumulation in the salt-tolerant population could have been one of the important factors in the adaptation of the Salt Range population to salinity, as reported in *Simmondsia chinensis* (Tal et al., 1979) and *Triticum aestivum* (Tammam et al., 2008). In contrast, Ghars et al. (2008) linked salt tolerance with  $\text{Na}^+$  influx and  $\text{K}^+$  nutrition, but not proline accumulation, in *Arabidopsis thaliana*.



**Figure 5.** Transverse sections of leaf sheath of *Sporobolus arabicus* subjected to salt stress for 60 days. All figures are identical in scale and the scale bar is presented in Figure 5c.

Physiological modifications seemed to have played an important role in the maintenance of *S. arabicus* populations in the highly saline Salt Range habitat. However, specific anatomical modifications may also contribute significantly to increased salt tolerance in plants.

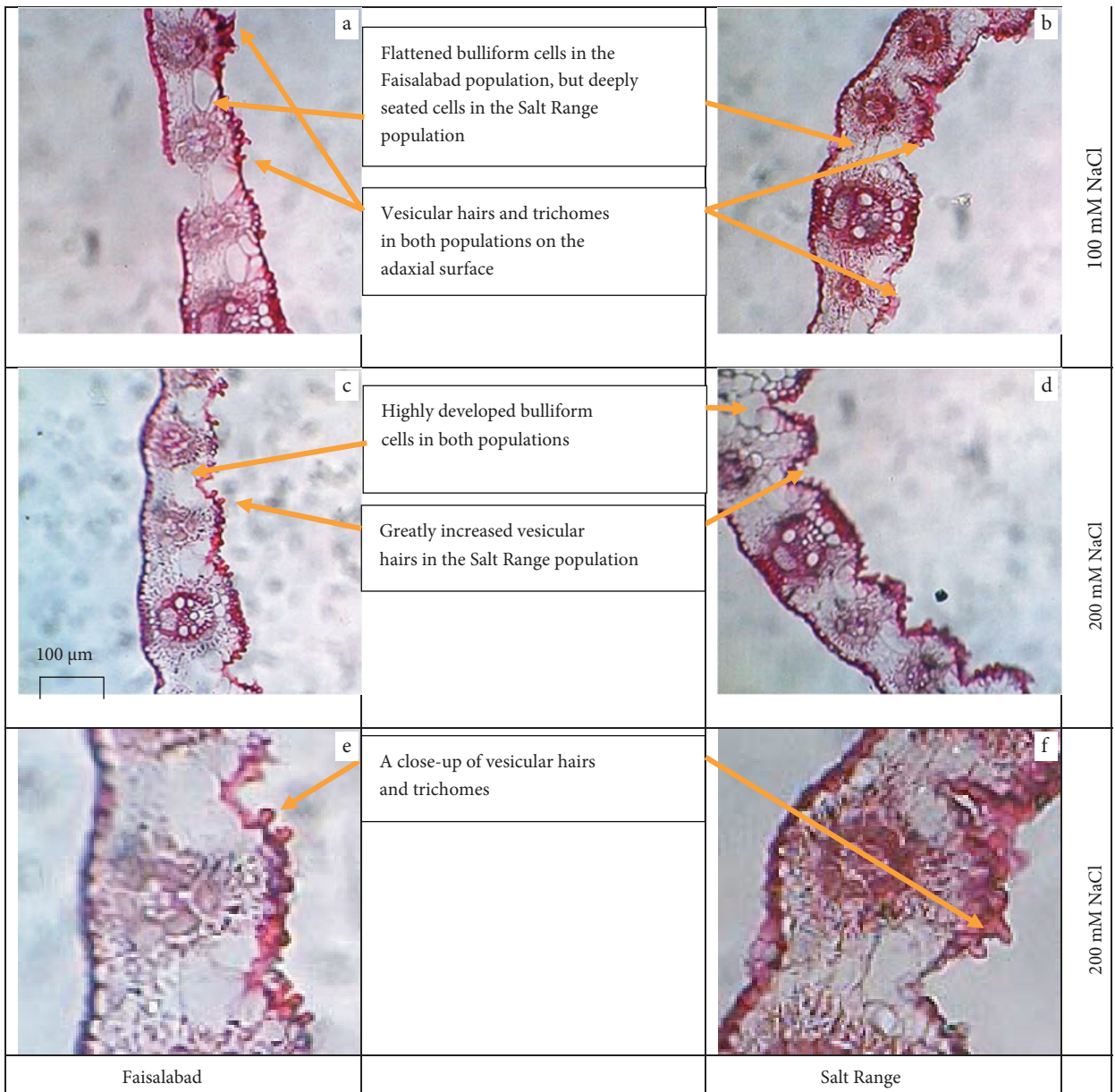
At the root level, development of aerenchyma with increases in salt levels is a unique feature. It is a typical characteristic of waterlogged plants (Cheng & Chou, 1997). Colmer and Flowers (2008) reported aerenchyma formation in halophytes. Increased aerenchyma in both populations may facilitate water and ion uptake through the roots, as reported in maize by Van der Weele et al. (1996).

In the Salt Range population, extensive sclerification below the root epidermis seemed to be critical to the physiological drought caused by salt stress. This is important for controlling the uptake and radial flow of water and solutes through the roots (Lux et al., 2004). Salt-induced sclerification in other plant species has also been reported, e.g., *Spartina alterniflora* Loisel. (Walsh, 1990) and *Imperata cylindrica* (L.) P.Beauv. (Hameed et al., 2009). In the Faisalabad population, sclerification was also recorded in the cortical region, but to a relatively lesser extent. In addition to cortical parenchyma, a double-layered epidermis may play a vital role in nutrient and

water transport through the roots in this species, which is a characteristic feature of salt-tolerant species (Ristic & Jenks, 2002). The epidermis was more developed under high salinities in this grass species, but especially in the Salt Range population.

At the stem level, succulence in terms of stem area is perhaps the most affected mechanism for the survival of *S. arabicus* under high salinities. Both populations showed increased stem area with increases in salt levels. In contrast, the Salt Range population had sclerification inside the epidermis of the stem, which may provide protection against water loss (Makbul et al., 2011).

Succulence in terms of leaf thickness seems to be the most important adaptive mechanism at the leaf level (Karlsons et al., 2011). The Faisalabad population was more responsive to salt stress, because a significant increase (more than 2-fold) in leaf midrib was recorded with the imposition of salt in the external medium. Leaf succulence is an unusual and rare phenomenon in monocots (Flowers & Colmer, 2008), but Hameed et al. (2009) reported distinct succulence in the leaves of a salt-tolerant grass, *Imperata cylindrica*. On the other hand, the Salt Range population originally had thicker leaves than the Faisalabad population, but, in fact, increasing salinity levels had a negligible impact on leaf succulence.



**Figure 6.** Transverse sections of leaf lamina of *Sporobolus arabicus* subjected to salt stress for 60 days. All figures are identical in scale and the scale bar is presented in Figure 6c.

Salt excretion through vesicular hairs is an important mechanism of salt tolerance in salt-excretory species (Naz et al., 2009). There are many reports of toxic  $\text{Na}^+$  excretion in highly salt-tolerant species via salt glands, e.g., in *Sporobolus spicatus* (Ramadan, 2001), *S. virginicus* (Bell & O'Leary, 2003), and *Cynodon* spp. (Marcum and Pessaraki, 2006). Salt stress induced the development of vesicular hairs in both populations of *S. arabicus*, but their density increased much more in the Salt Range population. At moderate salinities, trichome development was induced in both populations in addition to vesicular hairs. Adebooye

et al. (2012), in contrast, reported no change or reduced trichome density in *Trichosanthes cucumerina* under salinity stress.

At lower salinities the dumping of toxic ions is perhaps the major mechanism, as was indicated by leaf and stem succulence. More cortical parenchyma with larger cells (and, hence, larger vacuoles) may provide more space for storing toxic ions. Since the Salt Range population originally had succulent leaves, it may have spent its energy on growth and biomass production. In contrast, it was apparent that the Faisalabad population may have spent



more energy on tissue development than leaf succulence; for this reason, the decline in its biomass production under salt stress was greater.

The second mechanism is the excretion of toxic ions from the leaf surface. At moderate salt levels, both populations showed trichome development, which is vital for preventing water loss through leaf surfaces, i.e. desiccation tolerance due to physiological drought caused by salt stress. At high salinities, the mechanism shifted to salt excretion, and this was more pronounced in the Salt Range population.

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