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REGULAR ARTICLE

Anatomical adaptations to salinity in cogon grass [*Imperata cylindrica* (L.) Raeuschel] from the Salt Range, Pakistan

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Abstract To examine anatomical adaptations in a potential forage grass, *Imperata cylindrica* (L.) Raeuschel, a population was collected from the natural salt-affected soils of the Salt Range, Pakistan. Using a hydroponic system, the degree of salt tolerance in terms of structural modifications in the Salt Range ecotype was compared with that in an ecotype collected from a normal non-saline habitat of the Faisalabad region. The Salt Range ecotype was superior to the Faisalabad ecotype in biomass production under saline conditions. High salt tolerance of the Salt Range ecotype was associated with increased succulence in root and leaf (mainly midrib), formation of aerenchyma in leaf sheath, increased vascular bundle area, metaxylem area and phloem area, highly developed bulliform cells on leaves and increased sclerification in root and leaf. Furthermore, both stomatal density and stomatal area were considerably reduced under high salinities in the Salt Range ecotype.

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Introduction

The Salt Range, which lies between the Thar desert and the Potohar Plateau in the Punjab province of Pakistan, is of distinct importance (McKerrow et al. 1992). Soil lying to the south and southeast of the Salt Range is heavily affected by salts (Qadir et al. 2005), mainly due to runoff water from exposed hills and brine springs. Grasses such as *Cynodon dactylon*, *Sporobolus arabicus*, *Imperata cylindrica* and *Aeluropus lagopoides* dominate the saline or saline arid habitats of the Salt Range (Chaudhry et al. 2001) and are thus presumed to be well adapted to high salinity. One of these grasses, *Imperata cylindrica* (L.) Raeuschel, was used in the present study to determine its anatomical adaptations to salinity stress, because this grass is capable of tolerating drought and salt (Matumura and Nakajima 1988; Santoso et al. 1997).

Many of the physiological phenomena associated with salinity tolerance are related to anatomical structures; for example, ion excretion via leaf salt glands in *Zoysia* was found to be associated with salinity tolerance, which in turn was related to leaf salt gland density (Marcum et al. 1998). Succulence, which is represented by increased water storage tissue, is an important feature of halophytes. However, salt-

secreting halophytes can reduce the salt content in their tissues and may not show evidence of succulence (Bell and O'Leary 2003; Grigore and Toma 2007).

Naturally adapted salt-tolerant plants can be used effectively for investigating adaptative mechanisms to counteract high levels of salts. Since genetically based variation in natural populations of plants has not been much investigated, it was felt valuable to examine the anatomical adaptations of *I. cylindrica* to salinity stress because of the potential value of such genetic resources (Munns et al. 2002; Ashraf 2004; Flowers and Colmer 2008) in improving the salinity tolerance of crop plants (Munns and Tester 2008).

Materials and methods

Plant material

An experiment was carried out at the Botanical Garden, Department of Botany, University of Agriculture, Faisalabad to investigate the anatomical adaptations of the salt-tolerant grass *Imperata cylindrica* (L.) Raeuschel against salt stress. A population was collected from the edges of a highly saline salt lake—Uchhali Lake—in the Salt Range, which is seasonally inundated so that the plants face diluted saline waters during the rainy season (coordinates 32°36′33.79″ N, 72°13′ 53.37″ E, soil pH 7.38, ECe 15.42 dS m^{-1}, Na^+ 2.904 g kg^{-1}, Cl^- 1.521 g kg^{-1}). Another ecotype of this grass was collected from a non-saline habitat on the edges of an agricultural field within the Faisalabad region (coordinates 31° 25′ 31.03″ N, 73° 03′ 53.39″ E, pH 6.70, ECe 2.92 dS m^{-1}, Na^+ 0.320 g kg^{-1}, Cl^- 0.330 g kg^{-1}).

Soil analysis

The soil taken from the root zone of the grass population from each habitat was analysed for physico-chemical characteristics according to the methods described in Handbook No. 60 (US Salinity Laboratory Staff 1954). The soil extract was used to determine the pH and ECe using a pH / electrical conductivity meter (WTW series InoLab pH/Cond 720; http://www.wtw.com/). Sodium (Na^+) was determined with a flame photometer (Jenway, PFP-7) and Cl^- with a chloride meter (Jenway, PCLM 3; http://www.jenway.com).

Methods

Twelve plants of both populations were grown in a normal non-saline soil for a period of 6 months in earthen pots filled with loam and sand in equal quantities. The plants were irrigated daily until established in the Faisalabad environments (average day and night temperatures 37±3 and 24±3 °C, respectively, photoperiod 11–12 h, relative humidity 45.9–58.6%.). Ramets, each with three tillers of uniform size, were detached from each plant and grown in half-strength Hoagland's nutrient solution (Hoagland and Amon 1950) for 8 weeks in hydroponics, and aerated with the help of air pumps for about 12 h daily. Three replicates were planted on thermopore sheets (National Thermopore Industries, Lahore, Pakistan), and five salinity levels were maintained after the establishment of the grass species, viz., control (no salinity treatment), 50, 100, 150 and 200 mM of NaCl in solution culture. Plants were carefully removed from the hydroponics after 60 days from the start of salt treatment for examination of morpho-anatomical characteristics.

For the anatomical studies, the thickest ramet of each replicate was selected. A piece 2 cm in length was taken from the base of fully expanded leaves for leaf anatomy, from the base of an internode of the main tiller for sheath anatomy, and the thickest adventitious root near the root/shoot junction for root anatomy. The material was fixed in formaldehyde acetic alcohol fixative (FAA, formaldehyde 10%, acetic acid 5%, ethanol 50% and distilled water 35%) for 48 h and subsequently transferred to 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 double-stained technique of safranine and fast green stains. Measurements were taken with a light microscope (Nikon SE, Anti-Mould, Tokyo, Japan), using an ocular micrometer, which was calibrated with a stage micrometer. Micrographs of stained sections were taken with a digital camera (Nikon FDX-35) on a stereo-microscope (Nikon 104, Japan).

Data for anatomical characteristics were recorded using all 12 plants and three replicates. The characteristics recorded during the investigation were leaf thickness, root area, dermal tissue, storage tissue, mechanical tissue, and vascular tissue.

Statistical analysis

The experiment was planned in a completely randomised design (CRD) with two factors (ecotypes and salinity levels) and three replicates. Analysis of variance of the data from each attribute was computed using the MSTAT Computer Program (MSTAT Development Team 1989). Standard error and LSD at 5% level of probability was calculated to test the differences among mean values (Steel et al. 1997).

Results

The Salt Range ecotype showed a progressive increase in root fresh and dry weights with increasing salt level (Fig. 1), whereas the reverse was true in that from the Faisalabad region. The ecotype from the Faisalabad region showed a substantial reduction in shoot dry weight with increase in external salt level (Fig. 1). At higher salt concentrations, shoot dry weight in the Salt Range ecotype was relatively less affected as compared to that in the Faisalabad ecotype; the Faisalabad ecotype did not survive at the highest salt level (200 mM NaCl).

Root anatomy

Root area was adversely affected by increased external salt levels in the Faisalabad ecotype (Table 1). However, in the Salt Range ecotype this parameter increased up to 150 mM NaCl, but further increase in salt level resulted in decreased root area. A consistent increase was recorded in sclerenchyma thickness in both the Faisalabad and the Salt Range ecotypes with increase in salt level, but the increase was relatively greater in the Salt Range ecotype (Table 1).

Increasing salinity generally decreased cortical cell area in both *I. cylindrica* ecotypes, the Salt Range ecotype being relatively less affected by increase in salt levels (Table 1). Endodermal thickness in both ecotypes of *I. cylindrica* increased with increasing salt levels although the increase in the Salt Range ecotype was slightly greater than its counterpart from the Faisalabad region (Table 1), particularly at the highest salt level (200 mM NaCl).

The Salt Range ecotype showed enhanced vascular region thickness and metaxylem area with increase in salt levels. The higher salt levels (100 and 150 mM NaCl) adversely affected these parameters in the Faisalabad ecotype (Table 1). Phloem area, on the contrary, did not show any consistent pattern. However, in the Salt Range ecotype, higher salt levels (150 and 200 mM NaCl) resulted in an increase in phloem area (Table 1). Pith cell area gradually decreased in the Faisalabad ecotype with increased salt level in the growth medium, but increased in the Salt Range ecotype (Table 1).

Leaf anatomy

Imperata cylindrica from the Faisalabad region showed an increment in midrib and lamina thicknesses with increasing salt level. The Salt Range ecotype had considerably thicker leaves than those recorded in the Faisalabad region (Table 2).

Midrib shape was quite different in the two ecotypes, being conical in the Faisalabad and round (and 2-fold thicker than in the Faisalabad ecotype, i.e. more succulent with large parenchyma) in the Salt Range. Leaf rolling was observed in both the ecotypes of *I. cylindrica*, but the Salt Range ecotype showed extensive leaf rolling, in particular at the higher salt levels (150 and 200 mM NaCl).

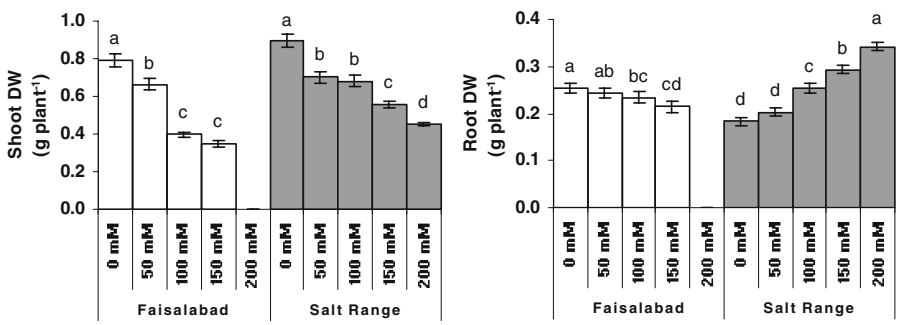

Fig. 1 Dry weights of root and shoot in two *Imperata cylindrica* ecotypes from the Salt Range and Faisalabad region subjected to different levels of salt (mean ± SE, $n=12$)

Table 1 Root anatomical characteristics in two *Imperata cylindrica* ecotypes from the Faisalabad (F) and Salt Range (SR) regions subjected to different levels of salt (mean ± SE, n= 12). Means for each characteristic in each row sharing similar letters are statistically non-significant

Characteristic	Ecotype	0 mM NaCl	50 mM NaCl	100 mM NaCl	150 mM NaCl	200 mM NaCl
Root area (mm^2)	F	2.41a±0.17	2.29b±0.19	1.67c±0.10	1.43d±0.12	–
	SR	1.74c±0.11	1.86c±0.15	2.30b±0.13	2.48a±0.11	1.59d±0.09
Sclerenchyma thickness (μm)	F	19.89c±1.38	20.74c±1.04	24.89b±0.87	33.88a±1.04	–
	SR	18.89e±1.04	24.89d±1.03	26.1c±1.04	32.84b±1.21	38.84a±0.52
Cortical cell area (μm^2)	F	1,653.28a± 136.99	1,561.05b± 54.79	1,345.05c± 125.57	1,061.01d± 54.79	–
	SR	1,591.17a ± 114.16	1,518.26a± 100.46	1,378.03b± 47.94	1,239.96c± 102.74	1,019.12d± 34.24
Endodermis thickness (μm)	F	78.81d±3.28	99.55c±4.15	102.96b±3.45	112.00a±5.53	–
	SR	72.31e±3.45	79.78d±2.07	88.03c±3.28	91.26b±3.80	132.74a±5.53
Vascular region thickness (μm)	F	82.96b±3.45	87.11a±3.63	74.66c±3.11	62.07d±3.45	
	SR	81.52e±3.80	87.09d±4.15	92.27c±3.45	99.50b±2.76	103.19a±4.15
Metaxylem area (μm^2)	F	661.31c±54.79	977.06a±114.16	771.53b±63.93	671.02bc±63.93	–
	SR	663.63d±113.02	753.59cd± 121.23	836.01c±118.76	1,258.05b± 132.52	1,653.28a± 123.61
Phloem area (μm^2)	F	220.44b±18.26	313.26a±34.24	275.55b±22.83	256.14b±21.87	
	SR	330.66c±27.39	306.06cd±17.12	275.55d±22.83	385.76b±31.96	401.69a±22.87
Pith cell area (μm^2)	F	182.27a±6.85	165.33b±13.69	165.33b±13.22	124.09c±10.27	–
	SR	124.07d±10.69	124.41d±10.27	155.21c±4.56	162.08b±6.85	182.47a±6.79

Both ecotypes of *I. cylindrica* had reduced adaxial epidermal thickness with increasing salinity (Table 2), but adaxial epidermis thickness in the Salt Range ecotype was considerably higher than that recorded in the Faisalabad ecotype. Epidermis thickness on the abaxial leaf surface increased with increase in salt level in both ecotypes up to 100 mM NaCl, and thereafter decreased with further increases in external salt level (Table 2). Salt hairs/glands were not seen in either ecotype of *I. cylindrica*.

Mesophyll cell area in both ecotypes increased at 50 mM NaCl. A further increase in salt level resulted in a gradual decrease in mesophyll cell area in both Faisalabad and Salt Range ecotypes (Table 2), the Salt Range ecotype being relatively less affected. An increase in the bundle sheath cell area was found in both ecotypes, but was more prominent in the Salt Range than in the Faisalabad ecotype.

Cortical cell area increased consistently in the Faisalabad ecotype with increase in the external salt level (Table 2). In the Salt Range ecotype, this parameter increased at 50 mM salt level, but with a further increase in salt level a gradual decrease in this characteristic was observed. Sclerenchyma thickness generally decreased with increase in salinity in the Faisalabad ecotype, but in contrast, it increased in the Salt Range ecotype (Table 2). Bulliform cell area generally increased with increase in salt level in both ecotypes, although its response to increasing salt levels was more pronounced in the Salt Range than the Faisalabad ecotype (Table 2).

Varying levels of NaCl resulted in a progressive increase in the area of vascular bundles and metaxylem area in the Salt Range ecotype (Table 2). However, there was a gradual decrease in these characteristics in the Faisalabad ecotype with increase in external salt level. Phloem area in both ecotypes of *I. cylindrica* increased with increase in salt level, but only slightly so in the Faisalabad ecotype (Table 2).

A steady increase in the stomatal density on the adaxial leaf surface with increased salt stress was observed in the ecotype from the Faisalabad region (Table 2). However, conversely, the Salt Range ecotype showed a consistent decline in this parameter.

Table 2 Leaf blade anatomical characteristics in two *Imperata cylindrica* ecotypes from Faisalabad (F) and Salt Range (SR) regions subjected to different levels of salt (mean ± SE, n=12). Means for each characteristic in each row sharing similar letters are statistically non-significant

Characteristic	Ecotype	0 mM NaCl	50 mM NaCl	100 mM NaCl	150 mM NaCl	200 mM NaCl
Midrib thickness (μm)	F	522.65c±21.78	680.27b±28.35	696.8b6±29.04	846.19a±31.12	—
	SR	1360.54b± 49.45	1,534.76a±50.83	1,300c.11± 50.49	1,219.51d± 56.71	1,186.32e± 63.97
Lamina thickness (μm)	F	224.43c±12.79	207.38d±11.06	265.57b±7.60	306.23a±11.75	–
	SR	306.22d±8.64	311.16d±10.72	325.31c±12.10	406.24a±12.79	341.13b±14.52
Adaxial epidermis thickness (μm)	F	8.26a±0.34	6.19b±0.25	4.13c±0.17	4.08c±0.34	–
	SR	20.64a±0.89	18.50b±0.72	16.51c±0.68	16.51c±0.68	12.38d±0.51
Abaxial epidermis thickness (μm)	F	14.50c±0.86	18.30b±0.34	20.75a±0.37	12.44d±0.51	
	SR	16.59d±0.51	18.96c±0.56	21.95a±0.69	20.74b±1.21	16.59d±0.86
Mesophyll thickness (μm)	F	165.33c±15.65	224.33a±22.83	185.00b±13.69	165.33c±11.69	–
	SR	233.48b±22.83	280.29a±27.39	275.55a±22.83	265.63a±25.32	224.72b±22.83
Bundle sheath cell area (μm^2)	F	110.22c±13.69	124.18c±18.26	165.33b±19.81	220.41a±22.83	
	SR	165.33d±9.13	220.44c±10.27	246.06bc±13.69	275.55ab±22.83	285.45a±22.83
Cortical cell area (μm^2)	F	2,961.74c± 254.20	3,102.29bc± 291.19	3,215.13b± 349.33	3,928.57a± 159.82	
	SR	5,101.51b± 671.26	5,818.28a± 150.69	4,723.19c± 267.13	4,621.66c± 465.77	3,959.74d± 410.97
Sclerenchyma thickness (μm)	F	49.78a±2.07	49.78d±2.07	38.18b±1.38	33.18c±2.24	
	SR	33.18d±1.38	41.48c±1.74	43.18c±2.35	51.48b±1.72	66.37a±2.76
Bulliform cell area (μm^2)	F	2,755.46d± 228.32	3,052.23c± 136.99	3,664.77b± 303.66	4,637.01a± 301.38	
	SR	4,308.84d± 108.45	5,621.14c± 465.77	5,510.93c± 456.64	6,872.14b± 511.44	8,817.48a± 730.62
Vascular bundle area (μm^2)	F	2,755.46c± 228.32	3,052.23c± 136.99	3,664.77b± 303.66	4,637.09a± 301.38	
	SR	4,308.84d± 108.45	5,621.14c± 465.77	5,510.93c± 456.64	6,872.33b± 511.44	8,817.48a± 730.62
Metaxylem area (μm^2)	F	275.55a±22.83	220.68b±17.12	206.29b±22.83	175.32c±18.26	
	SR	213.47d±39.95	275.18c±34.24	351.91b±34.24	413.72a±39.95	365.07b±34.24
Phloem area (μm^2)	F	743.97b±61.64	785.56b±83.09	840.29ab±88.21	930.73a±92.28	
	SR	661.31d±54.79	771.53d±63.93	1,102.19c± 91.32	1,343.28b± 61.64	1,570.37a± 163.25
Adaxial stomatal density	F	31.52d±1.31	34.57c±1.44	36.60b±1.52	41.68a±1.73	
	SR	53.88a±2.24	42.70b±1.78	33.55c±1.39	32.53c±1.35	28.47d±1.18
Abaxial stomatal density	F	30.50c±1.27	38.63a±1.61	32.53b±1.35	30.50c±1.27	
	SR	38.63a±1.61	30.50b±1.27	26.43c±1.10	25.52c±1.31	25.42c±1.06
Adaxial stomatal area (μm^2)	F	661.31a±54.79	482.21b±39.95	482.21b±39.95	482.21b±39.95	
	SR	482.21b±39.95	642.31a±54.79	661.31a±54.79	596.51b±47.94	578.65b±54.79
Abaxial stomatal area (μm^2)	F	1,096.78a± 90.88	767.75b±63.61	767.75b±63.61	658.07b±54.52	
	SR	767.75d±63.61	959.68b±79.52	1,096.78a± 90.88	987.10b±81.79	822.59c±68.16

Stomatal number on the abaxial surface in both ecotypes was generally reduced with increasing salinity, but the trend was somewhat inconsistent.

Stomatal area on the adaxial surface of the leaf in *I. cylindrica* from Faisalabad was little affected by salt levels in the growth medium (Table 2). On the other hand, stomatal area in the Salt Range ecotype was relatively inconsistent under varying salt levels. On the abaxial surface, stomatal area in the Faisalabad ecotype decreased consistently with increase in external salt level, but this character increased in the Salt Range ecotype only up to 100 mM NaCl level.

Leaf sheath

Leaf sheath thickness in the Faisalabad ecotype decreased gradually with increase in salt level of the growth medium, but the Salt Range ecotype showed increased thickness up to 150 mM NaCl (Table 3). Epidermal cell area on the adaxial surface decreased in the Faisalabad ecotype, but a consistent increase was recorded in the Salt Range ecotype in response to increasing salt levels (Table 3). A reduction in the epidermal cell area at the abaxial surface was recorded in both ecotypes.

Sclerenchyma was relatively more distinct in the Salt Range ecotype as compared to that in the Faisalabad ecotype (Table 3). In both cases, salt stress imposed a distinct increase in sclerenchyma thickness. A well developed aerenchyma was recorded in the leaf sheaths of both ecotypes (Table 3). The ecotype from Faisalabad showed a slight increase in aerenchyma area with increase in salt level, but this parameter was very much developed in the Salt Range ecotype, being the maximum at the highest salt level.

Vascular bundle area in the Faisalabad ecotype showed a gradual decrease with increase in salt level of the growth medium (Table 3), but in the Salt Range ecotype the area of vascular bundles increased up to 150 mM NaCl and thereafter decreased at the highest salt level. Metaxylem area in both ecotypes decreased with increase in salt level of the growth medium; however, the Salt Range ecotype was the more affected (Table 3). Both ecotypes showed a gradual decrease in phloem area (Table 3) with increasing salinity.

Table 3 Leaf sheath anatomical characteristics in two *Imperata cylindrica* ecotypes from Faisalabad (F) and Salt Range (SR) regions subjected to different levels of salt (mean ± SE, n=12). Means for each characteristic in each row sharing similar letters are statistically non-significant

Characteristic	Ecotype	0 mM NaCl	50 mM NaCl	100 mM NaCl	150 mM NaCl	200 mM NaCl
Sheath thickness (μm)	F	283.11a±9.33	257.18b±10.72	244.73c±10.20	228.58d±10.37	
	SR	199.62c±10.37	248.27d±9.85	285.6b±8.30	301.37a±10.72	257.18c±10.72
Adaxial epidermal cell area (μm^2)	F	123.39a±10.22	114.55b±6.81	101.35c±4.54	97.25c±4.54	
	SR	82.26d±6.81	94.84c±4.54	102.27bc±6.81	113.16ab±6.81	124.04a±4.54
Abaxial epidermal cell area (μm^2)	F	165.33a±13.69	165.33a±13.69	110.22b±9.13	110.22b±9.13	
	SR	206.66b±17.12	237.20a±11.41	210.74b±9.13	182.68c±6.85	165.33d±13.69
Sclerenchyma thickness (μm)	F	12.44d±0.51	14.45c±0.51	16.59b±0.69	18.30a±0.51	
	SR	14.30c±0.86	16.59d±0.51	18.44c±0.69	20.45b±0.69	24.25a±0.69
Aerenchyma area (μm^2)	F	2,077.09c± 201.13	3,015.06c± 226.03	5,223.46b± 267.13	6,479.59a± 205.48	
	SR	3,172.32c± 1029.72	7,753.17d± 770.58	9,299.92c± 393.85	12,427.85b± 511.44	13,116.06a± 1,086.80
Vascular bundle area (μm^2)	F	7,439.75b± 616.46	8,282.22a± 520.57	6,943.77b± 575.37	5,919.29c± 821.95	
	SR	5,455.48c± 794.55	7,233.09d± 599.34	9,589.39b± 452.07	10,580.98a± 876.75	8,183.72c±678.11
Metaxylem area (μm^2)	F	275.55a±22.83	234.34b±10.27	220.44b±18.26	220.44b±18.26	
	SR	245.01b±22.83	275.89a±13.69	195.36c±9.13	165.38d±10.27	110.67c±22.83
Phloem area (μm^2)	F	778.46a±47.94	806.37a±50.23	696.36c±71.92	596.84d±41.09	
	SR	791.74a±82.19	778.91a±47.94	744.37ab± 28.54	671.28b±63.93	563.78c±113.01

Discussion

The Salt Range population of *Imperata cylindrica* was growing in a saline soil with an ECe of 15.40 dS m^{-1}. The site was near the hyper-saline Ucchali Lake where diluted saline water is usually available after heavy rains. Soil of the Faisalabad ecotype was non-saline with an ECe of 2.92 dS m^{-1} and additional irrigation water available throughout the year.

On the basis of dry weights of root and shoot, the Salt Range ecotype can be regarded as more salt tolerant than the Faisalabad ecotype. In the Salt Range ecotype, shoot dry weight was less affected at the higher salinity levels (150 and 200 mM NaCl) than in the Faisalabad ecotype, which could not survive 200 mM NaCl. Remarkably, root dry weight increased in the Salt Range ecotype with increased salinity levels.

The ecotype of *I. cylindrica* from the Salt Range showed specific anatomical modifications such as increased thickness (succulence) of midribs and cortical parenchyma with larger cell area as compared to its counterpart from the Faisalabad region. These anatomical features may help in storing ions inside the plant body due to increased vacuolar volume, as neither ecotype has glands to remove salt from the leaves. Leaf succulence, as was recorded in the Salt Range ecotype, is an unusual phenomenon in monocots although more common in dicot species such as *Jaumea carnosa* (Omer and Schlesinger 1980), *Kandelia candel* (Hwang and Chen 1995), kidney bean (El-Araby and Hegazi 1999), grassland legumes (González et al. 2000) and halophytes (Flowers and Colmer 2008).

Bulliform cells play an important role in leaf rolling to avoid water loss during drought stress (Abernethy et al. 1998; Balsamo et al. 2006; Alvarez et al. 2008). The presence of greatly enlarged bulliform cells in the Salt Range ecotype is a significant adaptation against water loss under physiological drought conditions due to salt stress. Extensive leaf rolling was observed in the Salt Range ecotype; therefore, it can safely be referred to as an important adaptive defensive strategy against salt stress. Anton (1986) observed a similar phenomenon in some species of *Axonopus*.

Drought- and salt-tolerant species are generally equipped with a thick epidermis (Ristic and Jenks 2002) and this is perhaps the most effective mechanism against water loss through the leaf surface during limited moisture availability (Jenks and Ashworth 1999; YuJing et al. 2000). The epidermis on the adaxial surface of leaves from the salt-tolerant ecotype from the Salt Range originally was more than two-fold thicker compared to its counterpart from the Faisalabad region, indicating its better adaptability potential to prevent undue water loss under saline environments. This was also confirmed by Hajibagheri et al. (1983, 1984) in *Suaeda maritima* and Bray and Reid (2002) in *Phaseolus vulgaris* under salt stress.

Root aerenchyma is a characteristic feature of waterlogged plants. For example, Colmer and Flowers (2008) summarised reports of aerenchyma in halophytes under waterlogged conditions: aerenchyma has also been previously reported in *Imperata cylindrica* (Cheng and Chou 1997). The Faisalabad ecotype showed a considerable increase in aerenchyma formation with increased salinity level while, in the Salt Range ecotype, aerenchyma development increased up to moderate salt levels (50 and 100 mM NaCl), but at higher salt levels aerenchyma was transformed into parenchyma, and at the highest salt level this parenchyma was quite tightly packed. This may increase the area of storage tissue with increased vacuolar volume (succulence) for storing toxic ions, and hence represent an important strategy to cope with high salinities (Akhtar et al. 1998).

Increased sclerenchyma in the leaves and roots in the Salt Range ecotype under the highest salinity level may be of importance as it would provide rigidity to the organs. The Faisalabad ecotype also showed increased sclerification, but to a lesser extent. These results are in accordance with some reports of salt-induced sclerification in other plant species, e.g., *Spartina alterniflora* (Walsh 1990), *Kandelia candel* (Hwang and Chen 1995), cotton (Reinhardt and Rost 1995), *Puccinellia tenuiflora* (YuJing et al. 2000), and *Prosopis strombulifera* (Reinoso et al. 2004).

An increase in stomatal density and decrease in stomatal size under salinity has been reported in *Distichlis spicata* (Kemp and Cunningham 1981), wheat and barley (Gill and Dutt 1982), kenaf (Curtis and Läuchli 1987), and *Triticum aestivum* (Akram et al. 2002). The ecotype from the Salt Range seemed to be better adapted than the Faisalabad ecotype as stomatal density and area decreased, particularly at higher salt levels. This may be responsible for reducing water loss through leaf surfaces, and would therefore be critical under physiological drought.

Table 4 Specific anatomical adaptations in *Imperata cylindrica* ecotypes to cope with high saline environments

Characteristic	Faisalabad ecotype	Salt Range ecotype	Adaptive anatomical features in other species
Leaf midrib shape	Conical	Rounded	Change in leaf structure and hollow structure in midrib in population of *Imperata cylindrica* from high salinity habitat (Cheng and Chou 1997);
			Thicker leaves in wheat under salt stress (Hu et al. 2005)
Leaf and root sclerenchyma	Increased sclerification in roots below the epidermis	Increased sclerification in root cortex but relatively more than that in the Faisalabad ecotype	Lignification in outer root cortex under salinity in salt tolerant *Festuca rubra* ssp. *littoralis* (Baumeister and Merten 1981)
		Markedly increased sclerification in the root vascular region	Lignification in root vascular region of *Phaseolus vulgaris* under salinity (Cachorro et al. 1993).
		A significant increase in sclerification in the leaf midrib at both adaxial and abaxial surfaces	Sclerenchyma cells in leaf for avoidance of water loss in *Festuca nove-zelandiae* (Abernethy et al. 1998)
Root and leaf sheath aerenchyma	Aerenchyma formation in the roots with increase in salt level	Aerenchyma in the roots was transformed into compact parenchyma at higher salt levels	Aerenchyma formation in higher plant roots under salinity (Barrett-Lennard 2003)
	Increased aerenchyma in the leaf sheath with increase in external salt level	Increased aerenchyma in the leaf sheath but relatively more than in the Faisalabad ecotype	Reduced aerenchyma in *Salicornia europaea* roots under salt stress (Pearson and Havill 1988).
Root metaxylem	The area of root metaxylem decreased with increase in salt level	Increased root metaxylem area under saline conditions	Large metaxylem area under salinity in *Arabidopsis thaliana* (Baloch et al. 1998)
Leaf parenchyma	Disintegration of leaf parenchyma at higher salt levels	Parenchyma remained intact but parenchymatous cell area reduced due to salt stress	Increased leaf thickness due mainly to parenchyma in leaves of *Distichlis spicata* under salinity (Kemp and Cunningham 1981)
Leaf bulliform area	A slight increase in bulliform area due to salt	Remarkable increase in bulliform area at higher salinities	Well developed bulliform for extensive leaf rolling in *Festuca novae-zelandiae* under water stress (Aberenthy et al. 1998).
			High salinity resulted in well developed bulliform cells in *Deschampsia antarctica* (Gielwanowska et al. 2005).
Leaf succulence	Increased succulence with increase in salt level	Decreased leaf thickness but leaves much more thicker than those in the Faisalabad ecotype	Increased leaf succulence in salt tolerant genotypes of mulberry (Vijayan et al. 2008)

In conclusion, some specific anatomical adaptations in the Salt Range ecotype have developed due to the impact of natural selection on the population growing in this harsh environment (Table 4). Such adaptive traits in the salt-tolerant population from the Salt Range can be used for the identification of salt tolerance traits in other grass species. In addition, these anatomical adaptive features might be targets for incorporation into salt sensitive species through modern molecular and genetic engineering techniques.

References

Abernethy GA, Fountain DW, Mcmanus MT (1998) Observations on the leaf anatomy of *Festuca novae-zelandiae* and biochemical responses to a water deficit. N Z J Bot 36:113–123

Akhtar J, Gorham J, Qureshi RH et al (1998) Does tolerance of wheat to salinity and hypoxia correlate with root dehydrogenase activities or aerenchyma formation? Plant Soil 201:275–284. doi:10.1023/A:1004333318973

Akram M, Akhtar S, Javed IH et al (2002) Anatomical attributes of different wheat (*Triticum aestivum*) accessions/varieties to NaCl salinity. Int J Agric Biol 4:166–168

Alvarez JM, Rocha JF, Machado SR (2008) Bulliform cells in *Loudetiopsis chrysothrix* (Nees) Conert and *Tristachya leiostachya* Nees (Poaceae): structure in relation to function. Braz Arch Biol Technol 51:113–119. doi:10.1590/S1516-89132008000100014

Anton AM (1986) Contribucion al conocimiento de la anatomia foliar del genero *Axonopus* (Poaceae). Darwiniana 27:157–158

Ashraf M (2004) Some important physiological selection criteria for salt tolerance in plants. Flora 199:361–376

Baloch AH, Gates PJ, Baloch GM (1998) Anatomical changes brought about by salinity in stem, leaf and root of *Arabidopsis thaliana* (L.) *Heynh* (thale cress). Sarhad J Agric 14:131–142

Balsamo RA, Willigen CV, Bauer AM, Farrant J (2006) Drought tolerance of selected *Eragrostis* species correlates with leaf tensile properties. Ann Bot (Lond) 97:985–991. doi:10.1093/aob/mcl068

Barrett-Lennard EG (2003) The interaction between waterloggingand salinity in higher plants: causes, consequences and implications.Plant Soil 253:35–54

Baumeister W, Merten A (1981) Growth and root anatomy of two subspecies of *Festuca rubra* in response to sodium chloride salinization of the culture solution. Angew Bot 55:401–408

Bell HL, O'Leary JW (2003) Effects of salinity on growth and cation accumulation of *Sporobolus virginicus* (Poaceae). Am J Bot 90:1416–1424. doi:10.3732/ajb.90.10.1416

Bray S, Reid DM (2002) The effect of salinity and CO2 enrichment on the growth and anatomy of the second trifoliate leaf of *Phaseolus vulgaris*. Can J Bot 80:349–359. doi:10.1139/b02-018

Cachorro P, Ortiz A, Ros Barceló A, Cerdá A (1993) Lignin deposition in vascular tissues of *Phaseolus vulgaris* roots in response to salt stress and Ca^{2+} ions. Phyton 33:33–40

Chaudhry AA, Hameed M, Ahamd R et al (2001) Phyto-Sociologocal Studies in Chhumbi Surla Wildlife Sanctuary, Chakwal, Pakistan. I. Species diversity. Int J Agric Biol 3:363–368

Cheng KT, Chou CH (1997) Ecotypic variation of *Imperata cylindrica* populations in Taiwan: I. Morphological and molecular evidences. Bot Bull Acad Sinica (Taiwan) 38:215–223

Colmer TD, Flowers TJ (2008) Flooding tolerance in halophytes. New Phytol 179:964–974. doi:10.1111/j.1469-8137.2008.02483.x

Curtis PS, Läuchli A (1987) The effect of moderate salt stress on leaf anatomy in *Hibiscus cannabinus* (kenaf) and its relation to leaf area. Am J Bot 74:538–542. doi:10.2307/2443833

El-Araby MM, Hegazi AZ (1999) Growth responses and hormonal changes of kidney beans at different levels of soil salinization. J Union Arab Biol Cairo 7(B) Physiology & Algae 1999:327–343

Flowers TJ, Colmer TD (2008) Salinity tolerance in halophytes. New Phytol 179:945–963. doi:10.1111/j.1469-8137.2008.02531.x

Gielwanowska I, Szczuka E, Bednara J et al (2005) Anatomical features and ultrastructure of *Deschampsia antarctica* (Poaceae) leaves from different growing habitats. Ann Bot (Lond) 96:1109–1119. doi:10.1093/aob/mci262

Gill KS, Dutt SK (1982) Effect of salinity on stomatal number, size and opening in barley genotypes. Biol Plant 24:266–269. doi:10.1007/BF02879457

González LM, López RC, Fonseca I et al (2000) Growth, stomatal frequency, DM yield and accumulation of ions in nine species of grassland legumes grown under saline conditions. Pastos Forrajes 23:299–308

Grigore MN, Toma C (2007) Histo-anatomical strategies of Chenopodiaceae halophytes: Adaptive, ecological and evolutionary implications. WSEAS Trans Biol Biomed 4:204–218

Hajibagher MA, Hall JL, Flowers TJ (1983) The structure of the cuticle in relation to cuticular transpiration in leaves of the halophyte *Suaeda maritima* (L.) Dum. New Phytol 94:125–131. doi:10.1111/j.1469-8137.1983.tb02728.x

Hajibagher MA, Hall JL, Flowers TJ (1984) Stereological analysis of leaf cells of the halophyte *Suaeda maritima* (L.) Dum. J Exp Bot 35:1547–1557. doi:10.1093/jxb/35.10.1547

Hoagland DR, Amon DI (1950) The water culture method for growing plants without soil. In: Circular No. 347, Univ Calif Agric Exp Stn, Berkeley, CA, pp 1–39

Hu Y, Fromm J, Schmidhalter U (2005) Effect of salinity on tissue architecture in expanding wheat leaves. Planta 220: 838–848

Hwang YH, Chen SC (1995) Anatomical responses in *Kandelia candel* (L.) Druce seedlings growing in the presence of different concentrations of NaCl. Bot Bull Acad Sin 36:181–188

Jenks MA, Ashworth EN (1999) Plant epicuticular waxes: Function, production, and genetics. In: Janick J (ed) Horticultural reviews, vol 23. Wiley, New York, pp 1–68

Kemp PR, Cunningham GL (1981) Light, temperature and salinity effects on growth, leaf anatomy and photosynthesis of *Distichlis spicata* (L.) Greene. Am J Bot 68:507–516. doi:10.2307/2443026

Marcum KB, Anderson SJ, Engelke MC (1998) Salt gland ion secretion: a salinity tolerance mechanism among five zoysiagrass species. Crop Sci 38:806–810

Matumura M, Nakajima N (1988) Comparative ecology of intraspecific variants of the Chigaya, *Imperata cylindrica* var. *koenigii* (Alang-alang). III. Annual growth of the 3rd year communities originated from the seedlings. J Jpn Soc Grassl Sci 34:77–84

McKerrow WS, Scotese CR, Brasier MD (1992) Early Cambrian continental reconstructions. J Geol Soc 149:599–606. doi:10.1144/gsjgs.149.4.0599

MSTAT Development Team (1989) MSTAT user's guide: a microcomputer program for the design management and analysis of agronomic research experiments. Michigan State University, East Lansing, MI

Munns R, Tester M (2008) Mechanisms of salinity tolerance. Annu Rev Plant Biol 59:651–681. doi:10.1146/annurev.arplant.59.032607.092911

Munns R, Husain S, Rivelli AR et al (2002) Avenues for increasing salt tolerance of crops, and the role of physiologically based selection traits. Plant Soil 247:93–105. doi:10.1023/A:1021119414799

Omer L St, Schlesinger WH (1980) Field and greenhouse investigations of the effect of increasing salt stress on the anatomy of *Jaumea carnosa* (Asteraceae), a salt marsh species. Am J Bot 67:1455–1465. doi:10.2307/2442874

Pearson J, Havill DC (1988) The effect of hypoxia and sulphide on culture-grown wetland and non-wetland plants I: growth and nutrient uptake. J Exp Bot 39:363–374

Qadir M, Noble AD, Oster JD et al (2005) Driving forces for sodium removal during phytoremediation of calcareous sodic soils. Soil Use Manage 21:173–180. doi:10.1079/SUM2005312

Reinhardt DH, Rost TL (1995) Developmental changes of cotton root primary tissues induced by salinity. Int J Plant Sci 156:505–513. doi:10.1086/297273

Reinoso H, Sosa L, Ramírez L et al (2004) Salt-induced changes in the vegetative anatomy of *Prosopis strombulifera* (Leguminosae). Can J Bot 82:618–628. doi:10.1139/b04-040

Ristic Z, Jenks MA (2002) Leaf cuticle and water loss in maize lines differing in dehydration avoidance. J Plant Physiol 159:645–651. doi:10.1078/0176-1617-0743

Santoso D, Adiningsih S, Mutert E et al (1997) Soil fertility management for reclamation of Imperatai grasslands by smallholder agroforestry. Agrofor Syst 36:181–202. doi:10.1007/BF00142873

Steel RGD, Torrie JH, Dickie DA (1997) Principles and procedures of statistics-a biometric approach, 3rd edn. McGraw-Hill, Toronto

USDA Laboratory Staff (1954) Diagnosis and improvement of saline and alkali soils. US Government Printing Office, Washington, DC

Vijayan K, Chakraborti SP, Ercisli S, Ghosh PD (2008) NaCl induced morpho-biochemical and anatomical changes in mulberry (*Morus* spp.). Plant Growth Regul 56:61–69

Walsh GE (1990) Anatomy of the seed and seedling of *Spartina alterniflora* Lois. (Poaceae). Aquat Bot 38:177–193. doi:10.1016/0304-3770(90)90004-5

YuJing Z, Yong Z, ZiZhi H et al (2000) Studies on microscopic structure of *Puccinellia tenuiflora* stem under salinity stress. Grassl China 5:6–9