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Full Length Article

Structural and Functional Aspects of Salt Tolerance in Differently Adapted Ecotypes of *Aeluropus lagopoides* from Saline Desert Habitats

Nargis Naz[1], Sana Fatima[2], Mansoor Hameed[2*], Muhammad Ashraf[3], Mehwish Naseer[4], Farooq Ahmad[2] and Aneela Zahoor[2]

[1]*Department of Life Sciences, The Islamia University of Bahawalpur, Bahawalpur 63100, Pakistan*
[2]*Department of Botany, University of Agriculture, Faisalabad 38040, Pakistan*
[3]*Pakistan Science Foundation, Islamabad 44000, Pakistan*
[4]*Department of Botany, Government College Women University, Faisalabad 38040, Pakistan*
[*]For correspondence: hameedmansoor@yahoo.com; hameedmansoor@hotmail.com

Abstract

A study was conducted to investigate structural and functional modifications in three differently adapted ecotypes of *Aeluropus lagopoides* (Linn.) Trin. ex Thw. under saline conditions. Two ecotypes were collected from moderately saline Bailahwala Dahar (BD) and heavily salt-affected Ladam Sir (LS), respectively while one ecotype was selected from least saline Derawar Fort (DF), which was considered as a control. Vegetative shoots (ramets) of equal size were detached from the mother plant and grown in hydroponic culture solution. Four treatments were maintained during the experiment, viz. 0 (control), 100, 200 and 300 mM NaCl. Ecotype LS showed better growth in terms of morphological features like shoot length, root and shoot fresh and dry biomass, leaf area and number of tillers as well as physiological attributes like net assimilation rate, water use efficiency, turgor potential, root and shoot Ca^{2+} and accumulation of free proline, than those in ecotypes BD and LS under saline conditions. The root aerenchyma was more prominent in ecotype LS than that in the other ecotypes. In addition, ecotype LS depicted large cortical cells, thick endodermis barrier, high sclerification and large phloem area, all being responsible for efficient conduction of salts and water under saline stress. Structural and functional modifications for salinity tolerance in all three ecotypes were very specific and can be related to their survival under harsh environmental conditions and degree of salt tolerance. Anatomical modifications were the true representatives of habitat ecology of *A. lagapoides* ecotypes. It is concluded that long-term exposure of *A. lagopoides* ecotypes to their specific habitats imposed changes in their genetic makeup, which ensure successful survival in their respective habitat.

Keywords: Ions; Micro hairs; Salt tolerance; Sclerification; Stomatal density

Introduction

Water scarcity and soil salinity are the crucial factors which considerably decrease growth of most plants. High levels of salinity limit water uptake and increase accumulation of sodium in plants in addition to several other changes. Al these factors cause severe physiological and biochemical disorders in plants (Koyro *et al*., 2008; Munns and Tester, 2008).

Pakistan is characterized by arid and semi-arid regions with less than 60 cm annual rainfall. The low rainfall and scarcity of fresh water are responsible for the aridity of this region (Ashraf *et al*., 2006). Cholistan represents a hot arid sandy desert in southern Punjab spreading over 26,000 km^2 in Pakistan. This desert is characterized by high summer temperature (up to 52ºC) with low annual rainfall 100–200 mm (Arshad *et al*., 2009). Underground water is highly saline with a few exceptions. The water deficiency and increasing levels of salts accumulate in the upper soil surface in the desert due to high evapo-transpiration rates, which cause the twin menace, i.e., salinity and drought. However, the vegetation of the desert is quite diversified including a wide range of nutritious and stress tolerant species of grasses, herbs, shrubs, and trees.

The more tolerant and successful plant cultivars generally use more than one mechanism to cope high salinities (Cuartero *et al*., 2006) and the important one is osmotic adjustment achieved by the accumulation of inorganic and organic ions (Singh *et al*., 2010). This adjustment may occur at the cellular level by regulating ion fluxes through plasma membrane and may be at whole plant level by the accumulation of Na^+, K^+ and Cl^- (Shabala and Lew, 2002). The organic molecules which take part in osmotic adjustment include glycine betaine, carbohydrates, proline and free amino acids. These molecules not only play an important role in osmotic adjustment, but also play an osmoprotective role in higher plants, both glycophytes and halophytes. These organic molecules accumulate in plant

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cells under high osmotic stress and thus, prevent dehydration-induced cellular damage (Abdelmalek and Khaled, 2011).

Generally, halophytes combat two major problems, the first is ion toxicity, and the second physiological drought, i.e., limited water is available to them due to low water potential of the soil. In addition to several physio-biochemical attributes, anatomical structures are believed to significantly contribute to the plant survival, and these adaptive features have been fixed in halophytes or salt tolerant plants during long evolutionary process (Grigore and Toma, 2007).

The most important adaptive component against salt stress is the lignification (Kozlowski, 1997), which may improve resistance of plants against high levels of solutes, which is more prominent in roots as they are in direct contact with soil. Succulence is another adaptation, which is due to increase in size and volume of water storage tissues; however, this phenomenon is not common in halophytic/salt tolerant monocots (Munns and Tester, 2008). Another adaptation relating to salinity tolerance is stomatal size and orientation, which controls water loss through transpiration (Muhaidat *et al*., 2007).

Salt excretion is another effective mechanism of salt resistance in halophytic grasses such as *Aleuropus lagopoides* (Ahmed *et al*., 2012), *Chloris gayana* (Oi *et al*., 2013) and so many others. However, excretion of Na^+ and Cl^- along with some other ions like K^+, Ca^{2+} and Mg^{2+} has earlier been reported in *Aleuropus lagapoides* growing in natural habitats (Naz *et al*., 2009). They reported salt hairs, glandular hairs, trichomes and micro-hairs in this species which can effectively carry out salt excretion. Density of micro-hairs per unit significantly increased in *Aleuropus lagopoides* along with increasing salinity gradient which might have considerably contributed to salt excretion (Naz *et al*., 2013).

Aeluropus lagopoides (L.) Trin. ex Thw. is a halophytic perennial grass distributed in salt-affected habitats in Pakistan, such as coastal regions of Sindh and Balochistan and saline plains of the Punjab (Gulzar and Khan, 2001). This grass was found to be successfully adapted to highly salt-affected areas including saline waterlogged habitats and dryland salinities by developing specific morpho-anatomical and physiological modifications (Mohsenzadeh *et al*., 2006). In the previous papers Naz *et al*. (2009, 2013), explored *in situ* adaptive components of salinity tolerance, both physiological and anatomical, in five natural ecotypes of this grass. However, the environmental effect on adaptation of this grass to salinity stress could not be ignored, as it is well evident that phenotypic variation is the outcome of genetic and environmental influences. In contrast, in the present study, the focus was on examining structural and functional responses of three differently adapted ecotypes to salt stress under controlled environments, wherein the environmental effect was totally surmounted. The objectives were based on the hypothesis that each ecotype has adopted different structural and functional mechanism(s) for its successful survival under harsh climates. Thus, the present study was designed to explore the typical adaptive physio-anatomical components of salt tolerance in the three ecotypes originating from habitats with varying levels of salinity stress.

Materials and Methods

Three differently adapted ecotypes of *Aeluropus lagopoides* (L.) Trin. ex Thw., were collected from salt-affected habitats in the Cholistan desert, Pakistan to explore structural- and functional-based mechanism(s) of their adaptation under climatic adversaries. The sites selected were least saline DF, Derawar Fort (coordinates 29° 24’ 31.95 N, 71° 27’ 32.84 E, ECe 15.24 dS m^{-1}, Na^+ 3236.3 mg L^{-1}, Cl^- 1493.4 mg L^{-1}), moderately saline BD, Bailahwala Dahar (coordinates 29° 38’ 21.34 N, 70° 93’ 39.26 E, ECe 27.56 dS m^{-1}, Na^+ 4266.5 mg L^{-1}, Cl^- 1994.7 mg L^{-1}), and highly saline LS, Ladam Sir (coordinates 30° 53’ 26.47 N, 72° 64’ 25.05 E, ECe 39. 81 dS m^{-1}, Na^+ 5839.2 mg L^{-1}, Cl^- 2637.1 mg L^{-1}).

Stumps of twenty five mature plants of each ecotype of this grass were collected from each respective desert habitat and grown in the Botanical Garden, till their acclimatization. The soil adhering to the roots from each habitat was taken from a depth of 15 cm to analyze soil characteristics.

Twenty ramets of uniform size were randomly taken from the mother plants and grown for 12 weeks in aerated hydroponics using half-strength Hoagland’s culture solution keeping in view the low nutrient requirement of this species (Hoagland and Arnon, 1950). The hydroponic system was aerated for 12 h daily. Four salt levels i.e., 0, 100, 200 and 300 mM of NaCl were maintained throughout the experiment. The salinity was gradually increased by adding 20 mM daily in each treatment till the required level is achieved. Two-factorial completely randomized design (CRD) was used for the experiment with four replications, each replication contained 5 plants per treatment. Plants were carefully harvested from the hydroponic system after 8-weeks of growth under saline regimes and washed carefully for determining various morpho-anatomical and physiological characteristics.

For recording dry weights, plants were dried at 65°C in Memmert Lab-Oven. Morphological and growth characteristics such as plant height, root length, tillers and leaves, total leaf area and plant biomass were recorded. For anatomical characteristics, permanent slides of transverse sections were prepared following Ruzin (1999). The sections were dehydrated by a series of ethanol solutions, and stained with safranin (for lignified tissue) and fast green (for subrinized tissue), and then mounted on Canada balsam. Measurement of the sections was taken with the help of an ocular micrometer, and photographs with a camera-

equipped microscope (Nikon, Tokyo, Japan). Data for different dermal, mechanical, ground and vascular tissues were recorded.

Total amino acids were determined using the Moor and Stein (1948) method and total soluble proteins were estimated according to the Lowry *et al*. (1951) using UV–visible spectrophotometer (Hitachi 220, Japan). Total soluble sugars were determined using the method of Yemm and Willis (1954). Proline was determined using Bates *et al*. (1973) method.

Carotenoids and chlorophylls *a* and *b*, were estimated according to the method of Arnon (1949) using a spectrophotometer (Hitachi-220 Japan). The leaf water potential, osmotic potential and turgor potential were measured by a Scholander type pressure chamber and vapor pressure osmometer (Wescor 5500) using 3rd fully expanded leaf from the top.

Measurements of photosynthetic parameters like net CO_2 assimilation rate (*A*), transpiration rate (*E*), stomatal conductance (g_s), and sub-stomatal CO_2 concentration were recorded using LCA-4 ADC portable infrared gas analyzer (Analytical Development Company, Hoddesdon, England). Cations (Na^+, K^+, and Ca^{2+}) were recorded with a flame photometer (Jenway, PFP-7) and for Cl^-, a chloride meter (Jenway, PCLM 3) was used.

The data were subjected to statistical analysis using analysis of variance (ANOVA) in completely randomized design (CRD) with three replications.

Results

Morpho-agronomic Characteristics

All ecotypes of *Aeluropus lagopoides* from ecologically different habitats responded differently to increasing salt levels (Table 1). The Derawar Fort ecotype (DF) showed a significant and consistent reduction in many agro-morphic characteristics like shoot length, root length, root fresh weight, leaves and tillers per plants, and internodal length (Table 1). However, parameters like total leaf area, shoot fresh and dry weights and root dry weight increased significantly due to the induction of saline stress, and thereafter, all parameters decreased consistently and significantly.

In contrast Bailahwala Dahar ecotype, showed a significant increase in almost all agro-morphic characteristics at 100 and 200 mM NaCl levels (Table 1). However, the highest salt level resulted in a significant decrease in all parameters in this ecotype. The root length was the only parameter that showed a subsequent but significant decrease with increase in stress levels. In the LS (Ladam Sir) ecotype, all agro-morphic characteristics increased along with external salt level except root length, which showed a significant decrease under salt stress, but number of tillers per plant decreased only at 300 mM NaCl (Table 1).

Physiological Characteristics

Leaf water relations: A significant impact of increasing salt levels was recorded in all three ecotypes on leaf water relations, where a consistent increase in leaf water and osmotic potentials was recorded. In contrast, a significant decrease was recorded for leaf turgor potential in DF and BD ecotypes, but the LS ecotype showed a significant increase at 100 mM salt level, and thereafter, there was no further effect of higher salt levels on this parameter (Table 2).

Photosynthetic pigments: Chlorophyll pigments (Chl. *a* & *b*) consistently but significantly decreased in all three ecotypes under all levels of salt stress; however, the LS ecotype showed more chlorophyll content than the other two ecotypes at all salt regimes. Carotenoid content gradually but significantly increased in DF ecotype and a substantial and significant decrease was observed at the highest salt level (300 mM) (Table 2). The BD and LS ecotypes showed a significant decrease due to saline stress (100 mM NaCl), however, subsequent salt treatments had no effect on carotenoid content of these ecotypes (Table 2).

Gas exchange parameters: Water use efficiency increased significantly in all three ecotypes as the stress level increased. However, transpiration rate, stomatal conductance and sub-stomatal CO_2 decreased significantly (Table 2). Net CO_2 assimilation rate responded quite variably in *A. lagopoides* ecotypes as the stress level increased. For example, there was a consistent decrease in net CO_2 assimilation rate in DF ecotype along with salinity level. An increase up to 200 mM in BD ecotype, whereas a consistent increase in LS ecotype was recorded as salinity level increased.

Organic osmolytes: There was a gradual but significant increase in all organic osmolytes as salt level increased. The LS ecotype, however, accumulated relatively much higher concentration of these osmolytes. Accumulation of total free amino acids and total proteins was more prominent than that of proline or soluble sugars in all three ecotypes (Table 2).

Tissue ionic content: Ionic contents like Na^+, Ca^{2+} and Cl^- increased consistently in root, stem and leaves of all three *A. lagopoides* ecotypes with increasing salinity stress, whereas the concentration of K^+ decreased significantly (Table 3). The DF ecotype accumulated more Na^+ in stem and leaves than the other ecotypes, and the LS ecotype in roots. The LS ecotype accumulated significantly higher concentration of Ca^{2+} in root, stem and leaves compared with the other ecotypes. A consistent but significant decrease in K^+ concentration was recorded in DF and BD ecotypes as the salt level of growth medium increased. However, quite a different response was recorded in the LS ecotype, in which an increase was recorded in roots and a significant decrease in stem. Leaf K^+ increased significantly up to 200 mM salt level,

Table 1: Morpho-agronomic characteristics of differently adapted ecotypes of *Aeluropus lagopoides* (L.) Trin. ex Thw. from the Cholistan Desert under salt stress

Habitats	Drawar Fort				Bailahwala Dahar				Ladam Sir			
Salt levels	0 mM	100 mM	200 mM	300 mM	0 mM	100 mM	200 mM	300 mM	0 mM	100 mM	200 mM	300 mM
Morpho-agronomic characteristics												
Shoot length (cm)	43.51a	37.30b	32.07c	29.50c	40.06bc	43.22a	42.33b	38.97c	44.10d	46.27c	49.14a	48.05b
Root length (cm)	23.29a	21.51b	18.25c	14.11d	21.21a	18.06b	17.66c	14.60d	24.37a	22.50b	19.04c	17.56d
Number of tillers (plant^{-1})	17.76a	15.79b	15.79b	12.83c	18.75c	19.77b	20.72a	14.80d	20.72c	21.76b	22.69a	19.73d
Number of leaves (plant^{-1})	55.25a	50.32b	46.37c	42.43d	58.21b	61.17a	55.25d	56.24c	44.40d	53.28c	60.19b	62.16a
Total leaf area (cm^2)	115.78b	133.28a	107.39c	58.59d	197.09b	230.69a	189.51c	168.45d	132.21d	143.07c	164.77b	189.44a
Shoot fresh weight	13.32b	15.25a	12.04c	9.18d	12.73da	14.11b	14.90a	13.62c	11.15d	13.42c	15.09b	16.67a
Shoot dry weight (g plant^{-1})	4.54ab	5.03a	4.14b	3.06c	3.26d	4.24ab	4.74a	3.75c	4.14c	4.54b	5.23ab	5.82a
Root fresh weight (g plant^{-1})	9.18a	8.98ab	8.58b	8.19c	9.67bc	10.16a	9.97b	9.08c	8.68d	9.47c	10.16ab	10.75a
Root dry weight(g plant^{-1})	3.06ab	3.35a	2.86c	2.47d	2.66d	3.55ab	3.65a	3.16c	3.35d	3.85c	4.14ab	4.44a

Means with similar letters in each habitat are statistically non-significant at $p \leq 0.05$

Table 2: Physiological characteristics of differently adapted ecotypes of *Aeluropus lagopoides* (L.) Trin. ex Thw. From the Cholistan Desert under salt stress

Habitats	Drawar Fort				Bailahwala Dahar				Ladam Sir			
Salt levels	0 mM	100 mM	200 mM	300 mM	0 mM	100 mM	200 mM	300 mM	0 mM	100 mM	200 mM	300 mM
Leaf water relations												
Osmotic potential (-MPa)	1.38c	1.43b	1.56ab	1.66a	1.50c	1.55b	1.69ab	1.80a	1.34c	1.38bc	1.57b	1.89a
Water potential (-MPa)	0.56c	0.68bc	0.81b	1.20a	0.47c	0.57bc	0.68b	1.12a	0.43d	0.52c	0.62b	0.93a
Turgor potential (-MPa)	0.83a	0.75b	0.71b	0.46c	1.03a	0.98b	1.01ab	0.87	0.78b	0.91a	0.94a	0.92a
Organic osmolytes												
Total free amino acids (µg g^{-1})	1162.29d	1163.04c	1328.67b	1556.66a	1173.57d	1251.40c	1350.63b	1593.27a	1189.68a	1274.22c	1382.00b	1645.56a
Total soluble proteins (µg g^{-1})	733.49b	856.92a	857.59a	859.58a	880.19d	929.44c	1021.91b	1129.73a	1026.88d	1100.13c	1192.23b	1301.41a
Total soluble sugars (mg g^{-1})	20.65d	22.58c	25.81b	26.89a	22.73d	24.86c	28.42b	29.60a	23.09d	29.13c	30.67b	33.63a
Total proline (µg g^{-1})	131.62d	153.41c	222.39b	252.35a	171.68d	200.10c	290.08b	329.15a	256.81d	325.01c	442.22b	485.91a
Gas exchange parameters												
Net assimilation rate (µmol m^{-2}s^{-1})	14.80a	13.19b	12.88c	9.26d	12.65c	14.57a	13.27b	12.63c	12.05c	13.28b	15.12a	13.97b
Transpiration rate (mmol m^{-2}s^{-1})	3.12a	2.60b	1.50c	0.87d	3.15a	2.07b	1.37c	1.07d	3.24a	3.06a	2.36b	1.17c
Stomatal conductance (mmol m^{-2}s^{-1})	339.78a	266.19b	147.38c	98.26d	229.19a	184.18b	180.07c	147.38b	300.32a	295.79b	246.05c	187.06d
Substomatal CO_2 concentration (µmol mol^{-1})	279.65a	269.94b	218.94c	132.38d	272.38a	270.57b	223.04c	195.79d	275.28a	266.40b	259.49c	240.75d
Water use efficiency	4.74c	5.07c	8.58b	10.64a	4.01d	7.03c	9.68b	11.80a	3.72c	4.33c	6.40b	11.94a
Photosynthetic pigments												
Chlorophyll *a* (mg g^{-1})	1.63a	1.49ab	1.32b	1.18c	1.91a	1.74ab	1.55b	1.44c	2.23a	2.04ab	1.81b	1.68c
Chlorophyll *b* (mg g^{-1})	0.57a	0.52a	0.46b	0.33c	0.86a	0.78b	0.69bc	0.49d	0.88a	0.71b	0.65bc	0.55c
Carotenoids (mg g^{-1})	0.076c	0.096b	0.133a	0.041d	0.098a	0.059b	0.060b	0.061b	0.095a	0.057b	0.055b	0.054b

Means with similar letters in each habitat are statistically non-significant at $p \leq 0.05$

but thereafter it decreased (Table 3). Na^+/K^+ ratio (Fig. 1) in all three ecotypes responded similarly, i.e., increased in root, stem or leaves, particularly under highest salt level.

Excreted ions: All three ecotypes of *A. lagopoides* excreted Na^+ and Cl^- in bulk with increase in external salt levels, although the phenomenon was substantially higher in the LS ecotype as compared to other ecotypes (Table 3). Excretion of K^+ and Ca^{2+} was not as much as recorded in the case of Na^+ or Cl^- excretion. A consistent increase was recorded in K^+ and Ca^{2+} excretion in the DF and BD ecotypes with increase in salinity. The LS ecotype excreted these ions up to 200 mM salt level, and thereafter a significant decrease was recorded.

Anatomical Characteristics

Root anatomy: The DF ecotype generally possessed thicker root than that recorded in the other ecotypes, however, the root cross-sectional area increased significantly along with increasing salt levels (Table 4; Fig. 2). In the BD ecotype, only the highest salt level imposed a significant decrease.

Epidermis cell area gradually but significantly increased in the BD and LS ecotypes as the salt level increased, whereas the DF ecotype responded differently. Endodermal cell area, invariably increased in all ecotypes along with increasing external salt level (Table 4; Fig. 2).

Parenchymtous tissue (cortex and pith) responded quite differently to increasing salt levels. A significant and consistent increase in thickness of cortical region was recorded in the DF and LS ecotypes, but the pith cell area decreased as the salt level increased. In contrast, in the BD ecotype both cortical region and cortical cell area gradually and significantly increased along with salinity levels. The pith thickness increased significantly only in the DF ecotype with increase in salinity, but it decreased in the other two ecotypes. The pith cell area, however, increased in the BD ecotype as the salt level increased. This parameter, however, decreased in the DF and LS ecotypes (Table 4; Fig. 2).

Table 3: Ionic content and excreted ions of differently adapted ecotypes of *Aeluropus lagopoides* (L.) Trin. ex Thw. From the Cholistan Desert under salt stress

Habitats	Drawar Fort				Bailahwala Dahar				Ladam Sir			
Salt levels	0 mM	100 mM	200 mM	300 mM	0 mM	100 mM	200 mM	300 mM	0 mM	100 mM	200 mM	300 mM
Ionic content												
Root Na^+ (mg g^{-1})	21.08d	28.10c	34.25b	59.71a	24.86d	33.15c	40.40b	70.45a	29.84d	39.78c	48.48b	84.54a
Root K^+ (mg g^{-1})	11.83a	9.68b	8.60c	7.53d	13.02a	10.66b	9.47c	8.29d	9.66d	11.72b	13.68a	11.40c
Root Ca^{2+} (mg g^{-1})	6.99b	9.54a	8.07c	7.09d	8.08c	11.40a	9.32b	9.12bc	7.70c	8.13ab	8.88b	10.38a
Root Cl^- (mg g^{-1})	13.02d	15.70c	43.13b	65.60a	12.16d	13.25c	36.40b	55.37a	10.71d	11.67c	32.05b	48.75a
Stem Na^+ (mg g^{-1})	8.29d	14.80c	22.50b	36.70a	6.22d	16.87c	19.24b	27.53a	8.70c	23.62b	23.62b	28.67a
Stem K^+ (mg g^{-1})	14.88a	9.37b	6.87c	5.72c	15.39a	10.66b	7.10c	6.61d	16.16a	11.19b	7.46c	6.22d
Stem Ca^{2+} (mg g^{-1})	14.20c	19.25b	22.69a	22.15ab	11.98d	16.25b	19.15a	15.74c	15.63d	21.19c	24.98a	24.39ab
Stem Cl^- (mg g^{-1})	8.55d	9.57c	18.34b	26.19a	9.41d	10.54c	20.19b	28.83a	7.06c	7.90c	15.14b	21.62a
Leaf Na^+ (mg g^{-1})	12.83d	13.98c	14.88a	14.76ab	9.47c	10.77b	11.54a	10.39bc	11.42c	13.85b	16.16a	13.47b
Leaf K^+ (mg g^{-1})	11.84a	9.87b	8.60c	7.89d	9.47a	8.63a	7.10b	6.39c	7.03c	8.52b	9.95a	6.41d
Leaf Ca^{2+} (mg g^{-1})	8.39d	9.57c	11.25b	12.73a	9.14d	10.43c	12.26b	14.60a	9.73d	11.10c	13.05b	15.59a
Leaf Cl^- (mg g^{-1})	7.64d	8.55c	9.57b	11.64a	6.30c	7.06bc	7.90b	10.16a	5.43d	6.66c	7.20b	9.37a
Excreted ions cm^{-2}												
Na^+ (mg L^{-1})	27.62d	63.65c	132.21b	192.40a	28.61d	77.04c	138.13b	292.05a	39.47d	73.78c	245.68b	390.72a
K^+ (mg L^{-1})	10.85d	15.70c	17.22b	18.21a	17.76c	17.99c	19.38b	22.45a	22.69c	25.65d	27.93b	21.04a
Ca^{2+} (mg L^{-1})	6.99d	9.54a	10.84	11.07c	8.08c	11.39a	13..75b	14.76a	7.70c	10.76b	8.96b	6.15a
Cl^- (mg L^{-1})	17.57d	25.79c	37.92b	40.65a	15.79d	26.85c	33.84b	48.83a	20.72d	29.67c	38.64b	55.73a

Means with similar letters in each habitat are statistically non-significant at $p \leq 0.05$

Table 4: Root and stem anatomical characteristics of differently adapted ecotypes of *Aeluropus lagopoides* (L.) Trin. ex Thw. From the Cholistan Desert under salt stress

Habitats	Drawar Fort				Bailahwala Dahar				Ladam Sir			
Salt levels	0 mM	100 mM	200 mM	300 mM	0 mM	100 mM	200 mM	300 mM	0 mM	100 mM	200 mM	300 mM
Root anatomy												
Root thickness (µm)	20.72c	22.10ab	22.69a	21.11b	17.37ab	17.76a	17.56ab	16.18c	12.83d	15.00b	15.79a	14.80c
Epidermal cell area (µm^2)	711.82a	619.92b	412.77c	310.10d	413.52d	464.69c	588.18b	619.47a	412.78d	583.37c	619.55b	664.14a
Cortex region thickness (µm)	609.67d	794.67b	809.04a	775.19c	483.41d	601.86a	582.12b	522.98c	463.07d	483.42c	522.95b	642.35a
Cortical cell area (µm^2)	2788.37a	2509.48b	2168.69c	2088.32d	1239.25d	1301.22c	1339.29b	1394.06a	1734.9a	1301.22b	743.55c	712.67d
Pith thickness (µm^2)	153.46d	177.06c	207.21a	187.04b	187.45a	177.68b	171.30c	146.35d	256.54a	201.56b	197.64c	178.45d
Pith cell area (µm^2)	697.08a	596.04b	513.04c	464.72d	154.91c	332.65b	339.91b	354.23a	464.54a	309.81b	154.67c	142.38d
Sclerenchymatous thickness (µm)	493.37d	592.16c	599.83b	690.65a	394.73d	789.11c	880.38b	890.14a	493.22d	789.12c	885.43b	886.37a
Endodermal cell area (µm^2)	464.72d	619.62c	1012.18b	1161.80a	309.81d	464.72c	619.62b	659.63a	464.75d	494.12c	619.62b	664.14a
Metaxylem area (µm^2)	698.34d	6273.74a	5421.77b	4373.9c	4373.81a	3445.45b	3323.87c	2710.87d	1239.2b	1290.22a	1169.80c	1139.25c
Phloem area (µm^2)	619.63a	239.26d	465.56b	309.81c	509.88a	446.83b	154.91c	152.63c	464.82d	552.97c	777.58a	747.89b
Aerenchymatous area (µm^2)	47091.63b	91085.33a	29795.86c	18674.73d	3349.84d	24789.08b	24771.36b	20447.68c	2977.37d	20940.15c	29472.08b	37177.68a
Stem anatomy												
Stem area (mm^2)	13.62c	18.75a	16.38b	16.58b	19.34a	16.38b	15.17c	14.99d	19.17a	16.77b	16.34c	15.12d
Epidermal cell area (µm^2)	19.73d	25.63c	26.71b	29.64a	19.39d	21.56c	29.13b	39.42a	14.85c	19.29b	24.82a	19.37b
Sclerenchymatous thickness (µm)	104.34d	135.19c	204.98b	306.67a	98.35d	196.97c	394.65b	493.12a	197.84d	296.33c	345.18b	394.86a
Endodermal cell area (µm^2)	0.00	0.00	0.00	0.00	197.35d	216.48c	276.93b	296.84a	196.51d	219.76c	269.11b	296.43a
Cortical cell area (µm^2)	154.91a	92.49b	69.73c	54.82d	271.68a	123.52b	86.47c	69.80d	371.35a	247.88b	232.63c	92.49d
Vascular bundles area (µm^2)	12779.82d	22074.27a	20602.58b	19518.24c	155800.48b	193130.97a	26130.37d	32197.72c	20912.49d	19701.65c	19483.35a	19388.95a
Vascular bundle number	15.79a	13.81b	12.83c	11.84d	16.77	18.75c	20.72b	21.71a	14.80d	17.76c	21.71b	24.67a
Metaxylem area (µm^2)	929.46a	664.72b	619.91c	464.07d	309.81d	529.45c	827.76b	924.51a	1239.23a	909.81b	318.13c	77.45d
Phloem area (µm^2)	216.83a	162.65b	154.91c	122.26d	166.81c	348.07a	217.34b	139.42d	216.87d	387.02c	402.77b	417.72a

Means with similar letters in each habitat are statistically non-significant at $p \leq 0.05$

Sclerification in the outer cortical region invariably increased in all ecotypes along with salinity, but this increase was more prominent in the BD and LS ecotypes. Salinity resulted in formation of aerenchyma in the root cortex of the LS ecotype only, where a consistent and significant increase was recorded as the salt level increased. An increase in aerenchyma formation was also recorded in the DF ecotype but only at lower salt level, whereas there was a significant decrease in this parameter at higher levels. This parameter consistently and significantly decreased in the BD ecotype along with salinity (Table 4; Fig. 2).

Stem anatomy: The DF ecotype showed a significant increase in stem area at the lower level of NaCl, but in contrast, higher salt levels (200 and 300 mM) significantly decreased this parameter (Table 4; Fig. 3). Salt stress significantly affected stem area in the other two ecotypes, where a consistent and significant decrease was recorded as salinity increased.

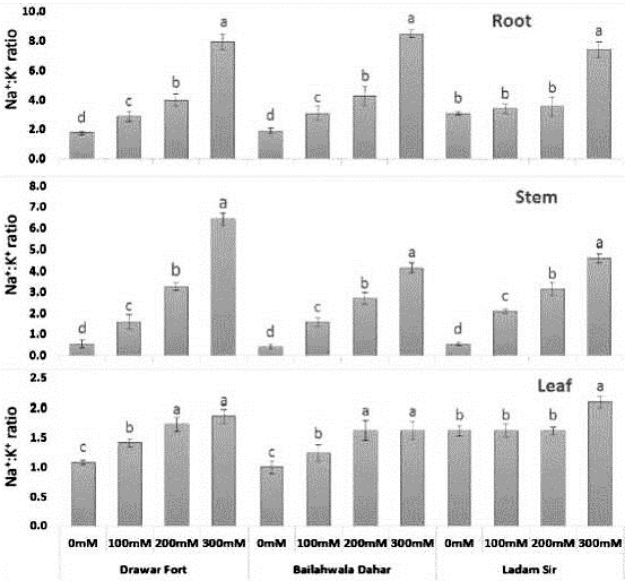

Fig. 1: Na^+: K^+ ratio of differently adapted ecotypes of *Aeluropus lagopoides* from the Cholistan Desert under salt stress

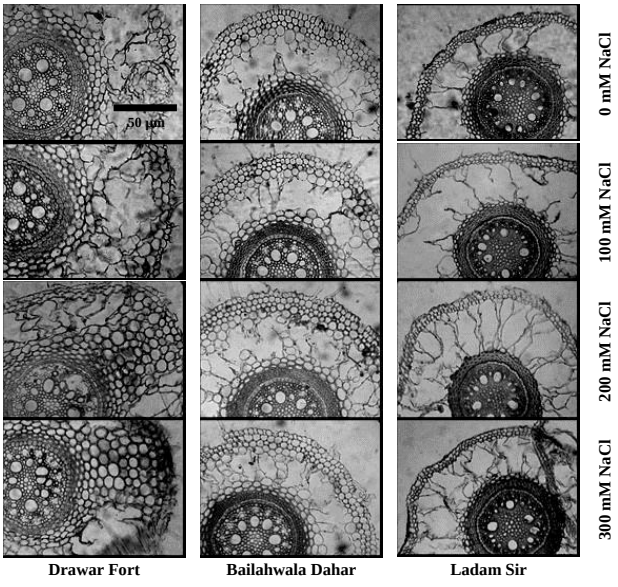

Fig. 2: Root transverse sections of differently adapted ecotypes of *Aeluropus lagopoides* from the Cholistan Desert under salt stress

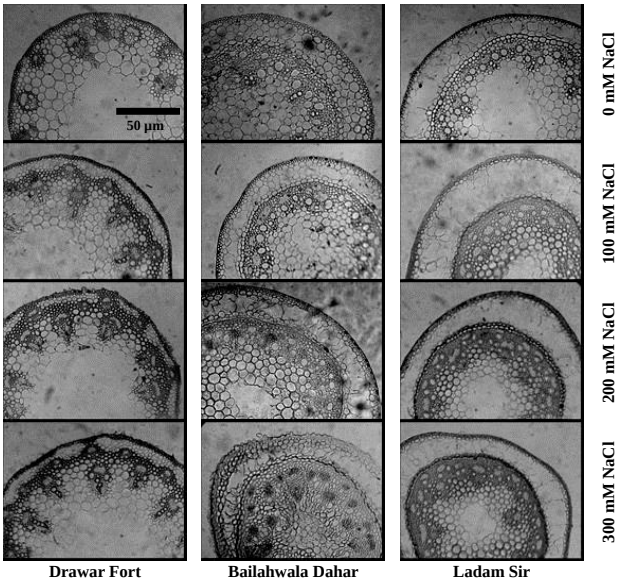

Fig. 3: Stem transverse sections of differently adapted ecotypes of *Aeluropus lagopoides* from the Cholistan Desert under salt stress

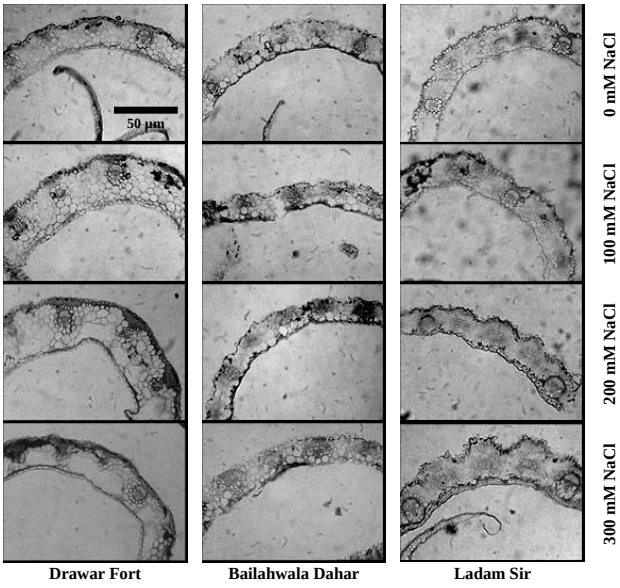

Fig. 4: Leaf sheath transverse sections of differently adapted ecotypes of *Aeluropus lagopoides* from the Cholistan Desert under salt stress

Epidermal cell area and sclrenchymatous area increased significantly in all three ecotypes however, the increase in epidermal cell area was more visible in the BD ecotype. In contrast, sclerification, particularly at higher salinity was significantly higher in the BD and LS ecotypes than that in the DF ecotype (Table 4; Fig. 3).

Endodermal layer was not recorded in the stem of the DF ecotype, whereas the BD and DF ecotypes had very specific endodermal layer. This layer separates the outer cortex from rest of the tissues, and this became more developed as the salt level increased. In contrast, the cortical cell area was severely affected due to salinity, where a significant decrease was recorded in all three ecotypes along the increasing levels of NaCl (Table 4; Fig. 3).

Table 5: Leaf anatomical characteristics of differently adapted ecotypes of *Aeluropus lagopoides* (L.) Trin. ex Thw. From the Cholistan Desert under salt stress

Habitat	Drawar Fort				Bailahwala Dahar				Ladam Sir			
Salt levels	0 mM	100 mM	200 mM	300 mM	0 mM	100 mM	200 mM	300 mM	0 mM	100 mM	200 mM	300 mM
Leaf blade anatomy												
Leaf thickness (µm)	236.41b	246.37a	217.67c	187.05d	296.31a	281.22b	253.77c	215.33d	291.66a	264.89b	221.44c	215.65d
Sclerenchymatous thickness (µm)	10.23d	17.33c	21.33b	29.45a	30.56d	34.77c	39.18b	42.49a	19.68d	29.56c	38.56b	44.56a
Adaxial epidermal cell area (µm^2)	38.04d	47.65c	59.33b	77.79a	37.43d	45.67c	59.43b	76.98a	36.34d	59.65c	74.55b	85.17a
Abaxial epidermal cell area (µm^2)	84.58b	93.67b	95.08b	157.36a	37.33d	78.65c	135.66a	152.88b	19.45d	37.87c	71.56b	158.33a
Bulliform cell area (µm^2)	372.28a	284.33b	150.56c	93.35d	2205.79a	1814.64b	1106.59c	1069.02d	432.62a	238.39b	91.44c	67.49d
Vascular bundle area (µm^2)	1549.33c	2788.43a	1592.67b	1239.89d	11628.04a	10223.65b	8514.22c	6970.16d	3253.67d	3711.36c	4137.50b	4957.57a
Metaxylem area (µm^2)	65.34d	67.23c	123.64b	158.76a	61.43d	65.67c	67.40b	73.01a	62.77d	105.84c	117.49b	161.59a
Phloem area (µm^2)	876.43c	1215.74a	1013.76b	781.61d	1947.95b	1523.64c	1116.57a	1097.64d	1083.65a	758.22b	659.60c	537.72d
Adaxial microhair density	34.23d	41.68c	43.64b	48.29a	32.38d	41.65c	46.25b	48.23a	29.28d	32.73c	53.46b	69.26a
Abaxial microhair density	19.28d	39.34c	58.32b	61.37a	37.54d	45.32c	59.22b	63.65a	43.87d	57.39c	68.54b	76.45a
Trichime density	3.54d	3.79c	3.93b	4.21a	2.59d	3.13c	3.62b	4.71a	3.97d	6.73c	8.10b	14.59a
Trichome length (µm)	15.23d	48.64c	65.63b	143.29a	18.49d	27.38c	53.29b	65.38a	121.08d	147.48c	169.70b	208.47a
Adaxial stomatal density	28.45a	25.17b	24.56c	23.90d	33.28a	30.31b	29.31c	28.53d	32.65a	30.40b	29.37c	28.20d
Abaxial stomatal density	25.39d	33.63c	37.49b	49.21a	36.37b	38.80a	33.15c	25.38d	39.05b	42.51a	37.19c	34.63d
Adaxial stomatal area(µm^2)	345.53a	321.05b	317.58b	314.38b	382.07b	397.17a	365.27c	338.32d	128.30b	142.39a	137.20b	134.16b
Abaxial stomatal area(µm^2)	314.64a	306.26b	283.74c	271.30d	370.17a	364.84b	351.49c	343.64d	276.29a	245.62b	212.43c	194.91d
Leaf sheath anatomy												
Leaf sheath thickness (µm)	187.39d	296.75b	325.11a	266.07c	177.65a	167.37b	158.43c	154.78d	173.52d	207.44c	214,06b	289.59a
Adaxial epidermal cell area (µm^2)	77.56d	154.14c	177.47b	309.56a	38.55d	73.01c	116.79b	155.46a	309.68a	152.38b	114.67c	78.09d
Abaxial epidermal cell area (µm^2)	154.56d	348.99b	461.76a	290.54c	74.31d	116.54c	135.36b	309.76a	154.34d	193.81c	348.54b	405.31a
Cortical cell area (µm^2)	929.87c	2326.31b	3795.50a	834.66d	159.80d	237.20b	278.41a	234.57c	185.29d	236.32c	1233.28a	948.54b
Sclerenchyma thickness (µm)	10.43d	21.69c	49.65b	58.05a	9.33d	18.87c	21.39b	29.19a	9.28d	17.38c	36.76b	42.60a
Microhair density	11.78c	16.43a	14.65b	10.57d	22.74d	26.87c	30.65b	36.65a	14.08d	30.64c	35.45b	42.96a
Vascular bundle area(µm^2)	5421.30d	8519.48a	8364.76b	7617.69c	9349.44a	5963.56b	4879.87c	3484.55d	6971.08d	9224.56c	11075.78b	13016.67a
Metaxylem area (µm^2)	38.54d	116.87c	387.90b	396.95a	382.47d	774.33c	789.56b	1540.65a	1546.57a	1443.65b	1363.75c	1248.54d
Phloem area (µm^2)	1626.43b	2198.65a	1557.27c	1425.65d	929.56a	697.47b	463.08c	308.43d	1236.87d	1384.65c	2163.76b	2891.54a

Means with similar letters in each habitat are statistically non-significant at p≤0.05

Vascular bundle area significantly increased in the DF ecotype along with salinity levels, but their number decreased significantly. In contrast, both vascular bundle area and vascular number increased consistently in the BD ecotype as the salt level increased. In the LS ecotype, a decrease in vascular bundle area was recorded along with salt level, but vascular number increased significantly. Metaxylem area consistently and significantly decreased in the DF and LS ecotypes along with salinity, but there was a significant increase in the BD ecotype. Phloem area, however, decreased in the DF ecotype, increased up to 200 mM in BD ecotype, and thereafter it decreased at 300 mM NaCl, It consistently and significantly increased in the LS ecotype with increase in the growth medium salt levels (Table 4; Fig. 3).

Leaf sheath anatomy: Increasing salt levels resulted in a significant decrease in leaf sheath thickness in the BD ecotype, whereas a marked increase was recorded in the other two ecotypes (Table 5; Fig. 4). Epidermal cell area generally increased significantly in all three ecotypes as the salt levels increased. However, the LS ecotype was a solitary case in which number of epidermal cells on the adaxial sheath surface decreased significantly.

Cortical cell area increased significantly in all three ecotypes with increase in salt regimes, however, the DF ecotype showed a significant decrease only at the highest salt level. Sclerification, in contrast, invariably increased in all ecotypes as the salt level increased.

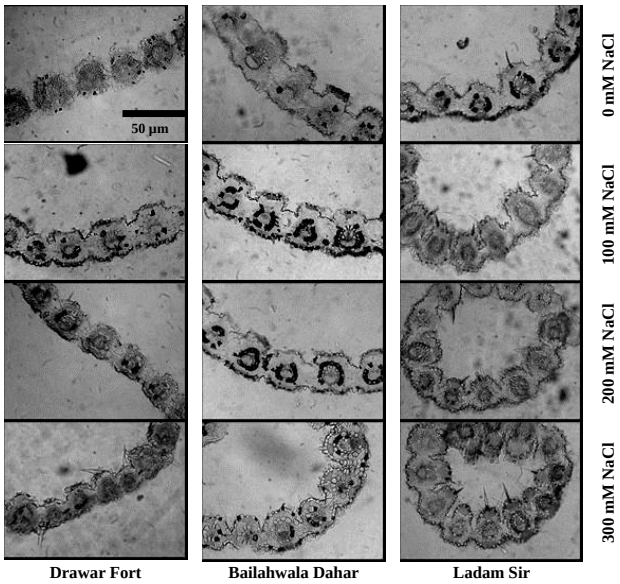

Fig. 5: Leaf blade transverse sections of differently adapted ecotypes of *Aeluropus lagopoides* from the Cholistan Desert under salt stress

The amount of sclerification, however, was considerably high in the DF ecotype, which was followed by that in the LS ecotype and the lowest being in the BD ecotype. The density of microhairs on leaf sheath surface was relatively

lower in the DF ecotype as compared to that in the BD or LS ecotypes, but it increased consistently along the salt levels with an exception of the DF ecotype, where its density significantly decreased particularly at the highest level (Table 5; Fig. 4).

Vascular bundle area increased in the LS and DF ecotypes as the salt level increased, whereas quite opposite response was recorded in the BD ecotype, where a significant decrease was recorded at varying external saline regimes. The size of the vascular bundles was the maximum in the LS ecotype, particularly at the higher salt levels. Metaxylem vessel area, in contrast, increased consistently and significantly in the DF and BD ecotypes along the increasing salt gradient, but it decreased in the LS ecotype. An increase was observed in the phloem area of the LS ecotype, and a decrease in the BD ecotype as the salt level increased. In the DF ecotype, this parameter showed a significant increase at the lower salt level, but the higher levels significantly reduced the phloem area (Table 5; Fig. 4).

Leaf blade anatomy: Leaf thickness generally decreased significantly in all three ecotypes as the salt levels increased. In the DF ecotype, lower salt level (100 mM NaCl) resulted in a significant increase, but higher levels adversely affected this parameter (Table 5; Fig. 5). Sclerification around vascular bundles increased significantly in all three ecotypes of *A. lagopoides* as the salt level was raised. The LS ecotypes showed significantly more sclerification, particularly at the higher salt levels than that in the other ecotypes. Epidermal cell area on both leaf surfaces in all three ecotypes increased consistently and significantly along external salinity gradient. Bulliform cells area decreased as salt level increased.

Vascular bundle area gradually but significantly increased in the LS ecotype along with salinity. This parameter decreased in the BD ecotype, whereas an increase in this parameter was noted in the ecotype up to 200 mM NaCl. The highest salt level resulted in a significant decrease in this parameter in the BD ecotype. Metaxylem vessel area increased in all three ecotypes as the salt level increased. The LS and DF ecotypes originally had wider vessels than those in the BD ecotype. Phloem area, however, decreased significantly along the salinity gradient in the BD and LS ecotypes, whereas this parameter increased up to 200 mM NaCl only (Table 5; Fig. 5).

The density of microhairs on the adaxial and abaxial leaf surfaces, as well as that of trichomes increased significantly and consistently with increase in external saline regimes. However, their density was considerably higher in the LS ecotype than that in the other ecotypes. Trichome length responded similarly, which consistently but significantly increased as the salt level increased. The size of trichomes was exceptionally high in the LS ecotype, whereas quite smaller trichomes were recorded in the BD ecotype (Table 5).

The density of stomata decreased significantly in all three ecotypes along with increasing external salt levels. The density on abaxial leaf surface significantly and gradually increased only in the DF ecotype, whereas, in the BD and LS ecotypes, the increase was recorded only at 100 mM NaCl, but the other salt levels significantly reduced the stomatal density. Stomatal area decreased significantly on abaxial leaf surface in all three ecotypes along with salinity. Adaxial stomatal decreased consistently in the DF ecotype, increased only at 100 mM NaCl in the BD ecotype, and increased consistently in the LS ecotype along the increasing salinity gradient (Table 5).

Specific Anatomical Modifications

All three differently adapted ecotypes showed varying structural response to increasing external salinity regimes At root level, the DF ecotype showed increased sclerification in the cortical parenchyma, but this sclerification was not complete i.e., a small portion of thin-walled paernchymatous cells were sclerified only on one side of the root. Moreover, parenchymatous cells in the cortex were more compact and intact with very little aerenchyma formation. However, disintegration of phloem tissue was quite visible on the sclerified side. In the BD ecotype, sclerification at the cortical region as well as outer cortical region increased with increasing external salt levels. This ecotype had large aerenchyma in the cortical region at all saline regimes. High proportion of aerenchyma was also recorded in the LS ecotype, whereas specific modifications in the roots were increased sclerification in the inner cortex and pith region.

Regarding stem anatomy, the DF ecotype showed intensive sclerification in the hypodermal region and epidermal layer under salinity stress. There was a distinct outer cortex, which was separated from the vascular tissue by a distinct endodermal layer. This layer consistently increased in size as the external salt level increased. The BD ecotype had increased sclerification in the epidermal layer, and at the highest salt level this structural feature became very prominent. The LS ecotype showed intensive sclerifiation in the epidermis and vascular region, particularly at higher levels.

Anatomical modifications in the leaf sheath of DF ecotype relied on extensive sclerification of abaxial epidermis and outside the vascular tissues. In addition, lysogenic aerenchyma was very prominent in the cortical parenchyma. The BD ecotype showed exceptionally large adaxial epidermis, however, there was no significant change in the sheath anatomy in this ecotype except the increased development of micro-hairs. The LS ecotype, in contrast, showed varying modifications with increase in salinity. The abaxial leaf surface became wavier with deep grooves and furrows. There was an intensive sclerification on the adaxial sheath surface and significantly increased density of micro-hairs.

A prominent structural change in leaf blade of the DF ecotype was the development of trichomes and micro-hairs with increasing external salt concentration, however, the size of trichomes at the highest salt level was exceptionally large. The BD ecotype also showed a significant increase in the density of microhairs and trichomes. Moreover, stomata and bulliform cells were in deep grooves. The leaves of the LS ecotype were more responsive to increasing salt stress. Sclerification on both sides of the vascular tissue increased significantly, stomata and bulliform cells deeply grooved, and the grooves were protected by a dense covering of long trichomes. Moreover, the density of microhairs also increased significantly and tight rolling of leaf rolling was visible in the LS ecotype at the highest salt level.

Discussion

Soil physico-chemical characteristics of the natural habitats of all three ecotypes were significantly different e.g., Derawar Fort (DF) was the least saline site, Bailahwala Dahar (BD) the moderate and Ladam Sir (LS) the highly saline site. As all these ecotypes evolved independently to different salt levels, these responded quite differently relating to their morpho-anatomical and physiological parameters.

The least tolerant DF ecotype showed stunted growth in terms of total biomass production. There was a significant increase in uptake of toxic ions (Na^+ and Cl^-) in root, stem and, which is a general phenomenon observed in many less tolerant or salt-sensitive plant species/cultivars (Colmer and Flowers, 2008; Naz *et al*., 2010). The high uptake of these toxic ions significantly retarded the uptake of K^+, but increased that of Ca^{2+}. Such type of antagonistic effect of these ions might have neutralized the toxic effect of Na^+ and Cl^- to some extent (Hariadi *et al*., 2011).

Prominent anatomical adaptations in the DF ecotype were increased stem area and epidermal cell area, sclerification in the hypodermal region and size of vascular bundles. All these modifications may improve water conservation in this ecotype, mainly due to better storage capacity, minimizing water loss, and better conduction of water. This may have been the major strategy of the DF ecotype to survive under saline arid conditions (Gulzar *et al*., 2003). Almost all leaf sheath anatomical characteristics were found to be increased in the DF ecotype. Leaf sheath covered the major portion of the stem in the ecotype, which could be a critical trait for water conservation by the plants (Naz *et al*., 2010). The density of salt excretory micro hairs also increased in the leaf sheath and leaf blade of the DF ecotype, which can excrete excessive salts out of the plant body.

The BD ecotype showed an increase in growth parameters moderate salt levels (200 mM). This showed better tolerance of this ecotype to salt stress than that of the DF ecotype. Increased height and biomass production has earlier been recorded in salt tolerant or halophytic species by several researchers, for example in *Plantago coronopus* (Koyro, 2006), *Atriplex hortensis* (Sai-Kachout *et al*., 2010) and *Salvadora persica* (Parida *et al*., 2016).

As was the case with DF ecotype, the BD ecotype showed high uptake of Na^+, Ca^{2+} and Cl^-, but restricted the uptake of K^+ in its underground as well as aerial organs. The main difference was the excretion of significantly higher quantities of toxic Na^+ and Cl^- from its leaf surface, which is again an indication of better tolerance of this ecotype. Maintenance of turgor was much better in the BD ecotype than that in the DF ecotype, and the accumulation of organic osmolytes, particularly total soluble proteins and proline were significantly higher. Moreover, net assimilation rate was higher and this may be the reason of its better growth than that of the DF ecotype.

Structural modifications in the BD ecotype at the root level were thicker epidermis, larger cortical cells, and high sclerification in the inner (just outside vascular tissue) and outer (hypodermal region) cortical regions. This will surely minimize water loss from the root epidermis (Rashid and Ahmed, 2011), better storage capacity in cortical parenchyma and mechanical strength to root tissue by increased sclerification (Jabnoune *et al*., 2009), which may also aid in preventing water loss (Hameed *et al*., 2012). In the stem of the BD ecotype, a significant increase was observed in the epidermal and endodermal cell area, sclerification around vascular tissue, and size of vascular tissue (number and area of vascular bundles, metaxylem area and phloem area). Increased sclerification, in particular, has been observed in many plant species to water scarcity (Lo *et al*., 2008), and especially under limited water availability, it provides mechanical strength to root tissue and hence, prevents soft tissue to collapse. This may again contribute significantly towards better degree of tolerance in the BD ecotype by conserving water in different tissues (Lo *et al*., 2008). One of the prominent features in the BD ecotype was the formation of endodermis just outside the vascular tissue, and this is even more beneficial when the hot, dry and highly saline desert environments cause degradation of external soft tissues, as was recorded in some previous studies (Naz *et al*., 2013).

The leaf sheath characteristics generally increased in the BD ecotype under saline stress, but sheath thickness, vascular bundle area and phloem area decreased significantly. However, a decrease in vascular areas may be little disadvantageous in the BD ecotype. The exceptionally large epidermal cells on the adaxial sheath surface is anyhow capable of protecting stem tissues, as well as may contribute towards storing additional moisture (Maricle *et al*., 2009).

Leaf thickness in the BD ecotype decreased significantly under salt stress, and this enabled the leaf to role tightly in the presence of deep-seated bulliform cells, which were in deep grooves. Rolling of leaves can effectively minimize water loss through leaf surface, and is an effective response of grass leaves to water deficit

conditions. In addition, sclerification on both sides of vascular bundles and epidermal cell area on the adaxial and abaxial leaf surfaces significantly increased, and this is of great ecological significance to survive under hot dry saline desert conditions (Gielwanowska *et al*., 2005). The increased density and size of trichomes in that case may provide additional benefit to a plant to cope with environmental stresses like drought and salinity. Increased density of micro-hairs with increasing salt level may be one of the most effective mechanisms for salinity tolerance in this salt excretory halophyte (Saqib *et al*., 2005).

High salt stress up to 300 mM NaCl appeared to be most suitable for the growth and development of the LS ecotype, which is ranked as most tolerant of all three ecotypes. Only root length was affected, while all other characters increased with increasing salt levels. . Structural and functional modifications in the LS ecotype strongly sported its high degree of salt tolerance and the most successful survival under highly saline and hot desert conditions. Among tissue ionic contents, only stem K^+ decreased due to salt stress, whereas an increase in Na^+, K^+ and Cl^- in the other plant parts was observed in this ecotype with increasing external salt levels. The increased uptake of K^+ and Ca^{2+} may certainly neutralize the toxic effect Na^+ and Cl^- (Zhu, 2001). Besides this, a decreased excretion of K^+ and Ca^{2+} at the highest salt level and an increased excretion of Na^+ and Cl^- could be of immense importance for the maintenance of turgor as well as normal metabolic processes in the LS ecotypes. This was the only ecotype, which showed increased turgor potential under salt stress as reported by Khan *et al*. (2000) in halophytic succulent *Suaeda fruticosa*. The important factor of maintaining leaf turgor is the high accumulation of organic osmolytes, in particular total free amino acids, total soluble sugars and free proline. All these are characteristic biochemical features of salt tolerant/halophytic species (Murakeözy *et al*., 2003). Moreover, there was a significant increase in net assimilation rate as well as water use efficiency in this ecotype with increase in external saline regimes.

Some specific structural modifications in the roots of the LS ecotype were increased root thickness, epidermal cell area and cortical region thickness. Thicker roots are generally related to stress tolerance (Bahaji *et al*., 2002), and high proportion of parenchyma can also increase the water storage capacity of roots (Lechthaler *et al*., 2016). High proportion of aerenchyma in roots also relates to salt tolerance in different plant species (Fan *et al*., 2003; Colmer and Flowers, 2008). Aerenchyma is the characteristics feature of *A. lagopoides* in the roots even under dryland salinities (Naz *et al*., 2009). Intensive sclerification was recorded outside the endodermis at high salinities in addition to highly developed endodermal layer. This again could be vital for controlling radial flow of water and is of great ecological significance. Stems of the LS ecotype were with distinct endodermis outside the vascular region, as was recorded in the BD ecotype, but in the former case the endodermis was much more developed. In addition, vascular region was intensively sclerefied with thicker epidermis and wider phloem in the LS ecotype. All these modifications are important for water conservation in this highly salt tolerant ecotype.

Leaf sheath thickness increased significantly in the LS ecotype covering the major portion of stem, being highly beneficial; moreover, there was increased epidermal cell area on both surfaces, cortical cell area, sclerification and phloem area, which might have enabled this ecotype to withstand high salinity by considerably conserving water (Baum *et al*., 2000). Moreover, the LS ecotype was more efficient in salt excretion to sheath surface as the density of micro-hairs significantly increased.

In the leaves of the LS ecotype, sclerification on each side of the vascular bundles increased significantly and this could be crucial for minimizing water loss through leaf surface (YuJing *et al*., 2000). Bulliform cells were in deep grooves and each groove was protected by a dense covering of trichomes. With stomata inside the groove can minimize transpirational water loss in the LS ecotype. Moreover, thin fibrous leaves can easily roll, hence protecting the adaxial leaf surface completely (Premachandra *et al*., 1993). The micro-hair density is significantly higher in the LS ecotype as compared to that in the other ecotype and this is an indication of high degree of salt tolerance in the LS ecotype. The stomatal density and area also decreased in the LS ecotype with a few exceptions and this again can play an important role in minimizing transpirational water loss (Berger and Altmann, 2000).

Conclusion

The structural and functional modifications for salinity tolerance observed in all three ecotypes were very specific and truly genetically determined as the environmental impact on variation was totally masked during the experimentation and thus these adaptations can be related to the degree of resistance of the three ecotypes to extreme arid saline habitats. In particular, anatomical modifications were the true representative of the habitat ecology of the specific *A. lagapoides* ecotypes. Since these ecotypes are grown in controlled environments, only genetically fixed characteristics expressed themselves. It is, therefore, concluded that long-term exposure of *A. lagopoides* ecotypes to their specific habitats imposed changes in their genetic makeup, which ensure successful survival in their respective habitat.

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