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Full Length Article

Anatomical Adaptations for Drought Tolerance in *Lasiurus scindicus* Henr in Punjab, Pakistan

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Abstract

Lasiurus scindicus Henrard is a drought tolerant grass frequently grown in arid to semiarid areas of Pakistan. However, information about the anatomical adaptations in *L. scindicus* under drought stress is not well known. Therefore, four naturally adapted populations of *L. scindicus* collected from Thal (TH) Salt Range (Kalar Kahar; KK), Chiniot (CN) and Cholistan (CH), Punjab, Pakistan were grown in pots at 40, 60, 80 and 100% water holding capacity (WHC) to examine their anatomical adaptations under drought stress. Moreover, half of soil saturation percentage was considered as 100% WHC and taken as control. Plant anatomical parameters like leaf thickness, epidermal thickness, sclerenchyma thickness, cortical cell area, metaxylem area and bulliform cell area were studied. In all populations from different ecological habitats, major anatomical modifications regarding leaves were reduced leaf lamina, well developed bulliform cells, and increased cuticle thickness under severe drought stress accompanied by thick epidermal layer for moisture conservation. Some specific root anatomical adaptations like increased lignification in pith and cortical cells, increased endodermal thickness, and size of xylem vessels were also observed under drought stress. Likewise, reduced stem, metaxylem and phloem area was also noted under drought stress, which played important role for increasing the plants capability to cope with drought stress. Due to these adaptations, *L. scindicus* plants markedly lowered transpiration rate and improved the additional storage capacity under drought conditions. Based on these anatomical adaptations observed in all populations under drought, these populations may be rated in order of drought tolerance as CH>TH>KK>CN. In conclusion, well-developed thick cutinized leaves, reduced metaxylem and phloem areas, and well developed bulliform cells are the major anatomical adaptations observed in *L. scindicus* to induce drought tolerance.

Keywords: Drought stress; Cuticle thickness; Anatomical adaptations; *Lasiurus scindicus*; Populations

Introduction

Drought is a major threat to all crops and has appeared as a major limiting factor for crop yield globally (Farooq *et al*., 2012). Intensive drought spell due to low rainfall poses a serious threat to vegetation in arid regions (Nawazish *et al*., 2006). Arid environments facing high radiations, water shortage and heat stress has become alarming threats for optimum plant growth (Araus *et al*., 2002). Plants face multiple harsh environmental stresses in arid regions such as salinity, drought and high temperature those affect their growth, metabolism and yield. However, plants exhibit different response to drought condition by making some modification in their physiological, structural and biochemical characteristics to survive under harsh natural conditions (Reddy *et al*., 2004; Hussain *et al*., 2018).

Anatomical modifications play major role in drought stress tolerance. It has been reported that thick epidermis, large sized bulliform cells, thick cuticle and increase in stomata density are major anatomical adaptations in drought stressed maize (*Zea mays* L.) (Ristic and Cass, 1999), common bean (*Phaseolus vulgaris*) (Silva *et al*., 1999) and tomato (*Solanum lycopersicum*) plants (Sam *et al*., 2000). Many structural adaptations such as reduced leaf area, increased cuticle thickness, xylem differentiation, pith compactness were reported to help in preventing water loss. Furthermore, thickness of sclerenchyma cell layers is an important strategy under water stress for water conservation, as reported in many plants against drought stress (Awasthi and Maurya, 1993).

Anatomical adaptations such as increase in leaf succulence (Hameed *et al*., 2009) and increase in sclerification in leaf tissues are very significant under drought stress in many grasses (Hameed *et al*., 2010). Major anatomical modifications, such as more developed and large size bulliform cells and an increase in metaxylem diameter were found in drought tolerant grasses (Vasellati *et al.*, 2001). These structural modifications play a vital role in water conservation under severe drought stress. Anatomical parameters that have been developed successfully against drought stress not only clarify the taxonomic status of plant but also help in assessing degree of tolerance and adaptation level to different abiotic stresses in different plant species or cultivars (Gilani *et al*., 2002). Moreover, increase in exodermis and endodermis in roots is a characteristic feature of drought tolerant plants, which plays a key role in preventing the collapse of the inner portion of the root and in controlling the desiccation of stelar tissues under drought stress (Naz *et al.,* 2013).

Size of bullifom cells is also one of major anatomical modification which plays a key role in maintaining turgor in drought stress condition. Increase in bulliform cell area was reported in water deficient conditions in maize (Alvarez *et al*., 2008). Leaves are generally thick and lignified and found cutinized under drought condition in maize. Increase in density of trichomes was also reported on leaf adaxial side in tomato (Perez-Estrada *et al*., 2000). Compact cylinder formation and curling of leaves under drought conditions was also reported in the leaves of *Festuca novae* (Abernethy *et al*., 1998).

Lasiurus scindicus is a drought tolerant, highly nutritive palatable grass. It is generally native to desert regions of Asia and Africa and locally known as gorkha or sewan grass in desert areas of Pakistan. Moreover, *L. scindicus* is a very tolerant grass for different abiotic stress especially drought stress and salinity stress. It grows well in different sandy plains of Thal and Cholistan region. It is one of the major sources of fodder and nutrition in Thal and Cholistan desert (Naz *et al*., 2009). However, information about the anatomical modification in *L. scindicus* under drought stress is very limited. Therefore, the present study was conducted to study the anatomical modifications under drought stress in *L. scindicus* populations which are differentially adapted to different environmental stresses under natural conditions.

Materials and Methods

Plant Material

Lasiurus scindicus (a drought tolerant grass) was selected for this study as it exhibits good growth in the semi-arid and arid regions under natural drought conditions. Four naturally adapted populations of *L. scindicus* were collected from Thal (TH) Salt Range (Kalar Kahar; KK), Chiniot (CN) and Cholistan (CH), Punjab, Pakistan.

Experiment Design

Four naturally adapted populations of *L. scindicus* collected from above mentioned sites of Punjab, Pakistan were grown in pots at 40, 60, 80 and 100% water holding capacity (WHC). Moreover, half of soil saturation percentage was considered as 100% WHC and taken as control. All populations collected were grown in pots in the Old Botanical Garden of University of Agriculture till establishment. Equal proportion of sand, clay and loam was used to fill the pots with equal weight of 8 kg dry soil in each pot. This experiment was performed in Completely Randomized Design with three repeats. After the establishment of seedlings, the plants were subjected to above mentioned drought stress levels, maintained at weight basis for three months.

Data Recorded

The fully-grown plants were uprooted with great care to collect samples for measuring different anatomical parameters. To examine anatomical variations under drought stress, pieces of root, stem and leaves were selected and cut (2 cm piece). They were fixed in Formalin acetic alcohol solution (formaldehyde solution 5%, acetic acid 10%, ethyl alcohol 50% and distilled water 35%) for 48 h. After fixation they were transferred to acetic alcohol (v/v acetic acid 25% and ethanol 75%) solution subsequently for good preservation. Free hand sectioning was performed to prepare permanent slides. Serial dehydration was done with ethanol and then sections were stained after dehydration using standard double-stained technique of fast green and safranine stains. Measurements for anatomical parameters was done with light micro-scope (Nikon 104, Japan) using an ocular micrometer, which was further calibrated with the help of stage micrometer. Photographs of sections were taken with digital camera (Nikon FDX-35). Root anatomical recorded were root area, epidermis thickness, sclerenchyma thickness, vascular tissue (xylem, phloem area) and pith thickness. Stem anatomical parameters were epidermis thickness, sclerenchyma thickness, cortical cell area, endodermis thickness and metaxylem cell area. Anatomical parameters regarding leaves were lamina thickness, bulliform cell area, metaxylem area, phloem area and sclerenchyma thickness.

Statistical Analysis

Data was analyzed by using statistical software minitab 17. Moreover, Microsoft excel program was used for graphical presentation of data along with ± SD (standard deviations) calculated using Microsoft excel program.

Results

Stem Anatomy

Stem anatomical attributes of *L. scindicus* were observed under drought stress. All populations showed a consistent decrease in stem area with gradual increase of drought stress levels. However, CH population showed maximum reduction in stem area at severe drought stress (Fig. 1 and 2).

All populations showed differential response in cortical cell area with induction of drought stress levels. Populations from CH and KK showed a differential response as there was increase in cortical cell area on moderate drought stress level while later on, they showed reduction

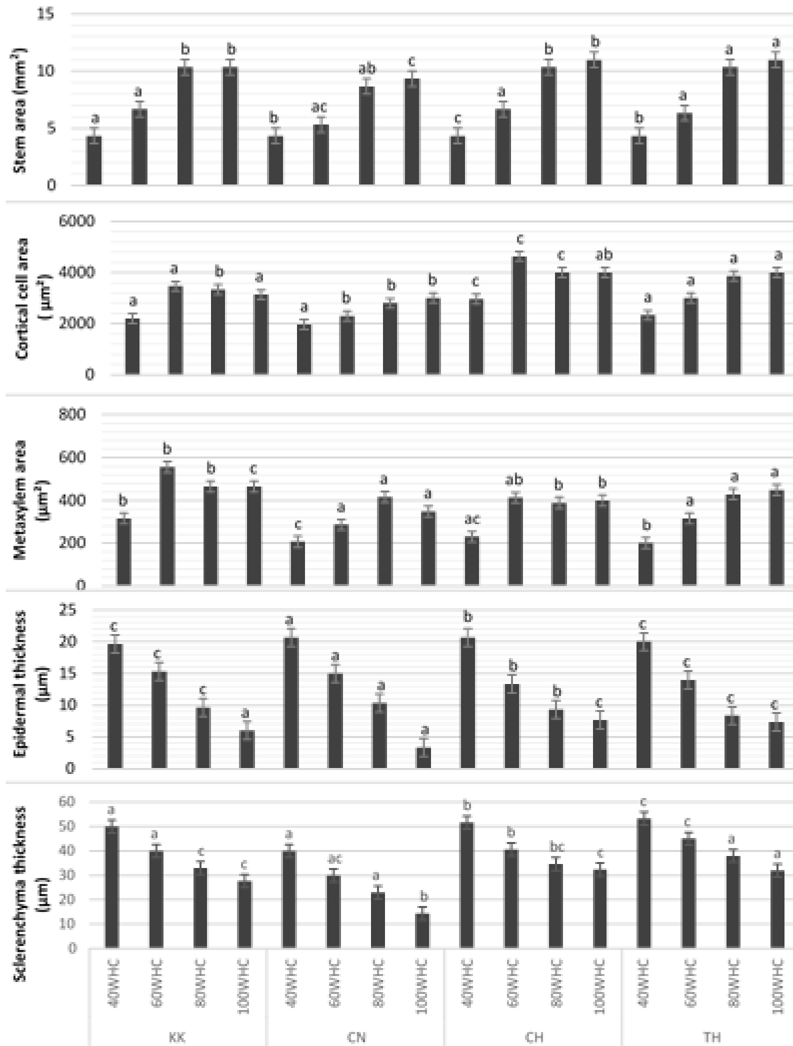

Fig. 1: Stem anatomical adaptations in *L. scindicus* populations grown under drought stress ±SD. Means with different letters were significantly different from each other at 5% probability level
KK = Kalar Kahar; CN = Chiniot; CH = Cholistan; TH = Thal

on severe drought stress level. Populations from CN and TH showed consistent decrease in cortical cell area with gradual increase in levels of drought stress (Fig. 1 and 2).

Metaxylem area of stem showed variable response in all populations. Populations from CN showed variable response as an increase in metaxylem area at low level of drought stress was observed, while metaxylem area decreased with subsequent increase in high drought stress levels. Populations from CH and KK showed a differential response as metaxylem area increased at 60% WHC while reduced at higher drought stress level (Fig. 1 and 2).

Epidermal thickness was found most stable anatomical parameter in response to drought stress. All four populations of *L. scindicus* showed increase in epidermal thickness with gradual increase in drought stress. All population were not much affected at moderate drought stress; however, they showed a significant increase in epidermal thickness at high drought stress. All populations of *L. scindicus* showed increase in sclerenchyma thickness with subsequent increase in drought stress levels, however more increase in sclerenchyma thickness was recorded in populations from KK, TH and CH than populations from CN (Fig. 1 and 2).

Root Anatomy

Root cross sectional area was one of the most variable characteristics, as it decreased in all collected populations. Populations from KK, CN and CH showed not much variation at low drought level (80% WHC) but a clear reduction in root cross sectional area was found at severe drought stress (60 and 40% WHC) except TH population, as it showed an increase in root area at low drought stress (80% WHC) later on, it also showed reduction in root area at high drought stress (Fig. 3 and 4).

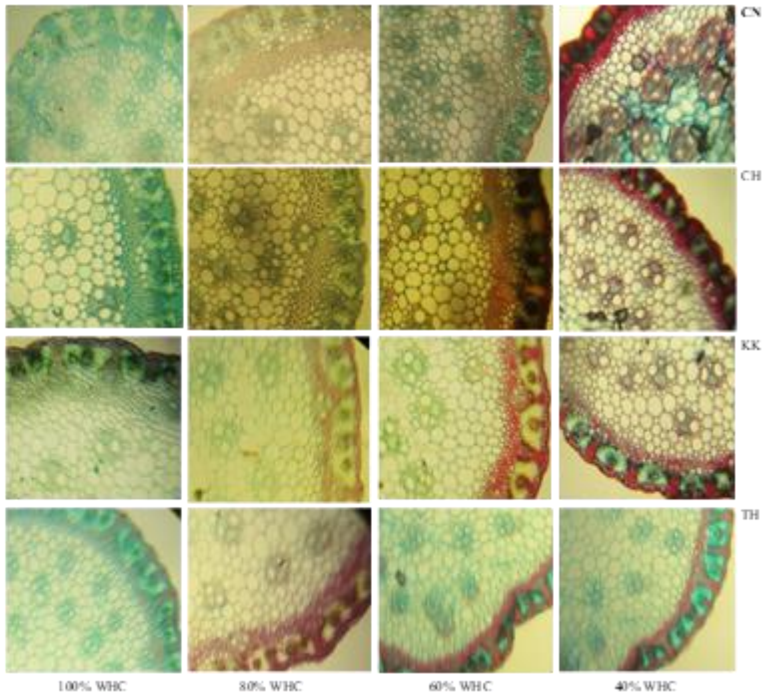

Fig. 2: Stem anatomy sections of *lasiurus scindicus* populations grown under drought stress

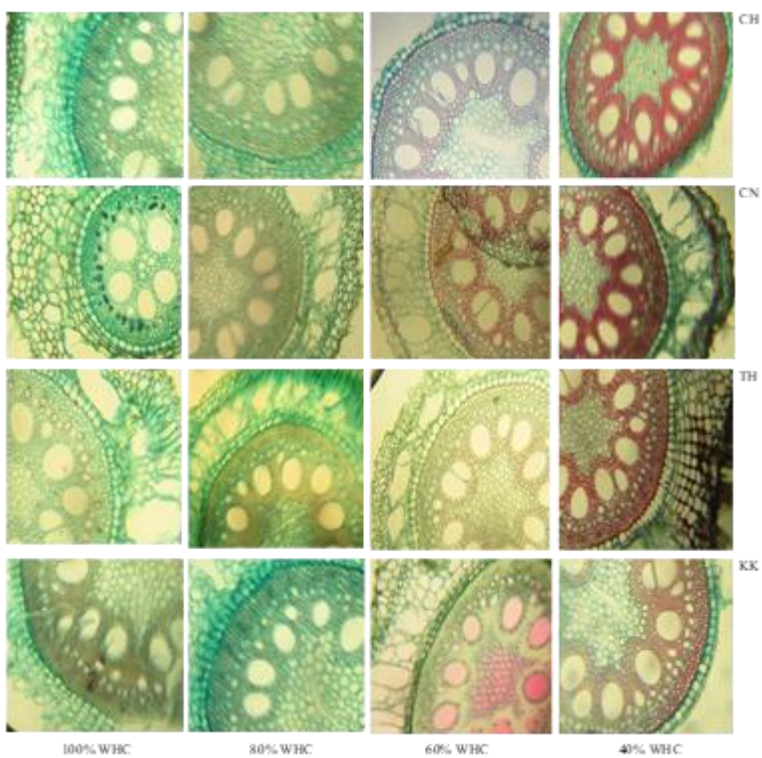

Fig. 3: Root anatomy sections of *lasiurus scindicus* populations grown under drought stress

Metaxylem area consistently decreased in all populations of *L. scindicus* with increase in drought severity. Populations from CH and TH showed much reduction in metaxylem area with subsequent increase in drought stress level than other populations (Fig. 3 and 4). A variable response regarding pith thickness was observed in all

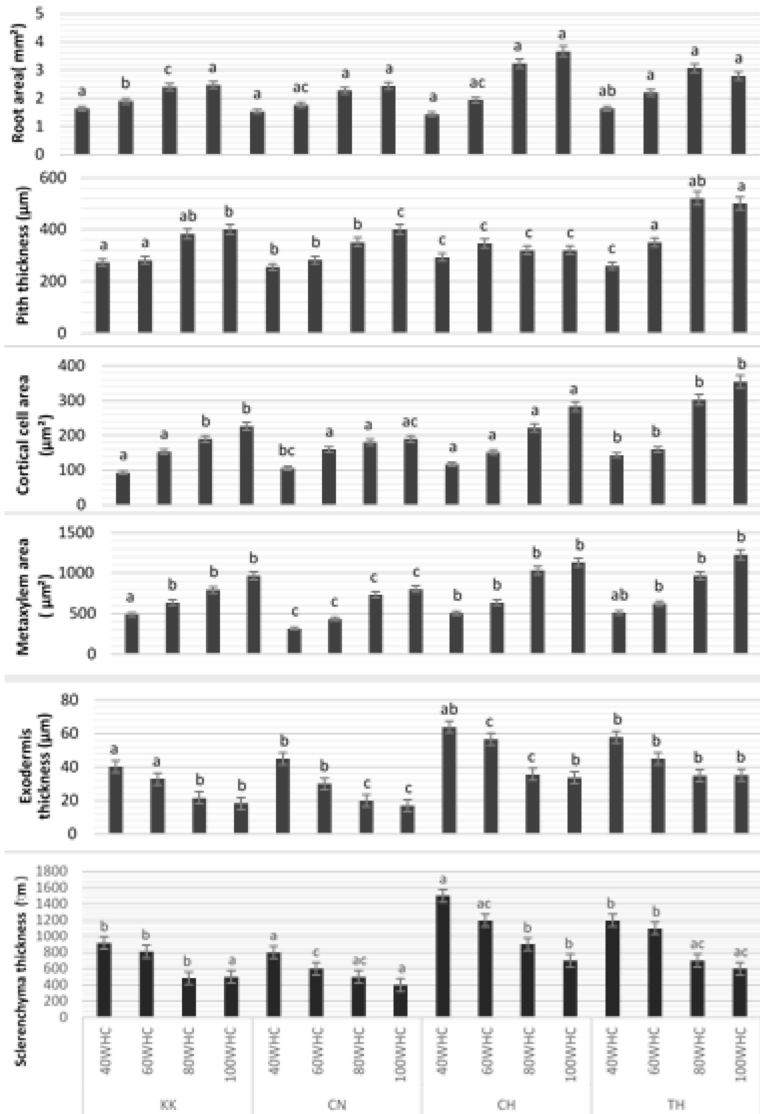

Fig. 4: Root anatomical adaptations in *L. scindicus* populations grown under drought stress ±SD. Means with different letters were significantly different from each other at 5% probability level
KK = Kalar Kahar; CN = Chiniot; CH = Cholistan; TH = Thal

populations. Populations from TH showed an increase in pith thickness at 80% WHC, while, later on reduction was observed in pith thickness at higher drought stress. Population from CH showed a variable response as increasing trend on 60% WHC was recorded while later reduction was found under highest drought stress level. Two populations from CN and KK exhibited decrease in pith thickness with gradual increase in levels of drought stress (Fig. 3 and 4)

A significant and consistent decrease in cortical cell area was recorded under drought stress in all populations of *L. scindicus*. Cortical cell area was more affected with subsequent increase in drought level in CN and KK populations however, TH and CH populations were least affected as these populations did not show much decrease in cortical cell area under mild drought stress level. Exodermis thickness gradually increased in all populations of *L. scindicus* however, TH and CH populations showed more increase in exodermis thickness than other two populations. Sclerenchyma thickness was most stable parameter as it consistently increased in all four populations with increase in drought intensity (Fig. 3 and 4).

Leaf Anatomy

All populations showed almost variable response in leaf anatomical adaptations under drought stress. Lamina thickness was not much affected at 80% WHC in three populations *i.e.*, CN, TH and CH, however, a reduction in lamina thickness was observed at 60 and 40% WHC (Fig. 5 and 6).

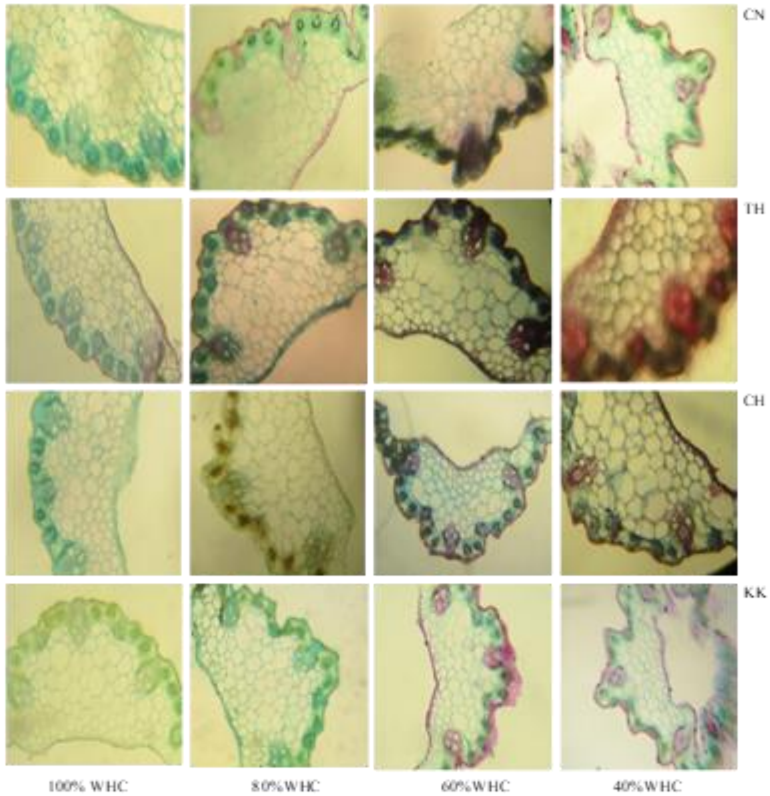

Fig. 5: Leaf anatomy sections of *lasiurus scindicus* under drought stress

Populations from KK showed slight increase in lamina thickness at low drought stress level; however, they also showed a decrease in lamina thickness with gradual increase in levels of drought stress. Populations from TH, CH and KK were adversely affected on higher drought stress levels (Fig. 5 and 6).

Metaxylem area was found variable in all populations under drought stress conditions. Metaxylem area in TH and CH populations gradually decreased with increase in drought levels. Populations from KK and CN exhibited a slight increase in metaxylem area at low level drought stress while later on reduction was observed at high drought levels (Fig. 5 and 6). Phloem area generally decreased with increase in drought level in all *L. scindicus* populations. Phloem area in TH and CH populations showed subsequent decrease with increase in drought levels. Populations from KK and CN exhibited differential response as increase in phloem area was observed at 80% WHC, while, a decline in phloem area was recorded at 60 and 40% WHC (Fig. 5 and 6).

Bulliform cell area showed a unique response in *L. scindicus* populations. A significant increase in bulliform area was also observed in CN and KK populations at low drought stress, where as a significant increase in bulliform area was noted at 60 and 40% WHC. Populations from CH and TH were not much affected at low drought stress while later on they showed a similar trend (Fig. 5 and 6). Sclerenchyma thickness increased consistently in all selected populations of *L. scindicus* with an increase in drought stress levels. Populations from TH, KK and CH showed general increase in sclerenchyma thickness on higher levels of drought stress while maximum increase in sclerenchyma thickness was observed in populations from TH with increase in drought level than other populations (Fig. 5 and 6).

Discussion

In the present study, anatomical parameters of *L. scindicus* under drought stress were examined because it is not reported earlier. Root thickness plays a key role in conservation of water to tolerate drought stress (Bahaji *et al*., 2002). Results revealed that all populations showed significant reduction in root cross sectional area under different levels of drought stress. As well as, root anatomical adaptations play a key role in developing tolerance against drought stress (Ahadiyat and Haarachchi, 2008). Cortical cells area is another crucial adaptation for drought tolerance. However, quite variable trend was recorded in the area of cortical cells along the increasing drought stress level. Well- developed cortical cell area helped in conservation of water that is vital modification for survival under drought conditions (Rashid *et al*., 2001).

In the present study, stem area is reduced with subsequent increase in level of drought stress, which has been reported under drought stress in many grasses (Basal, 2010).

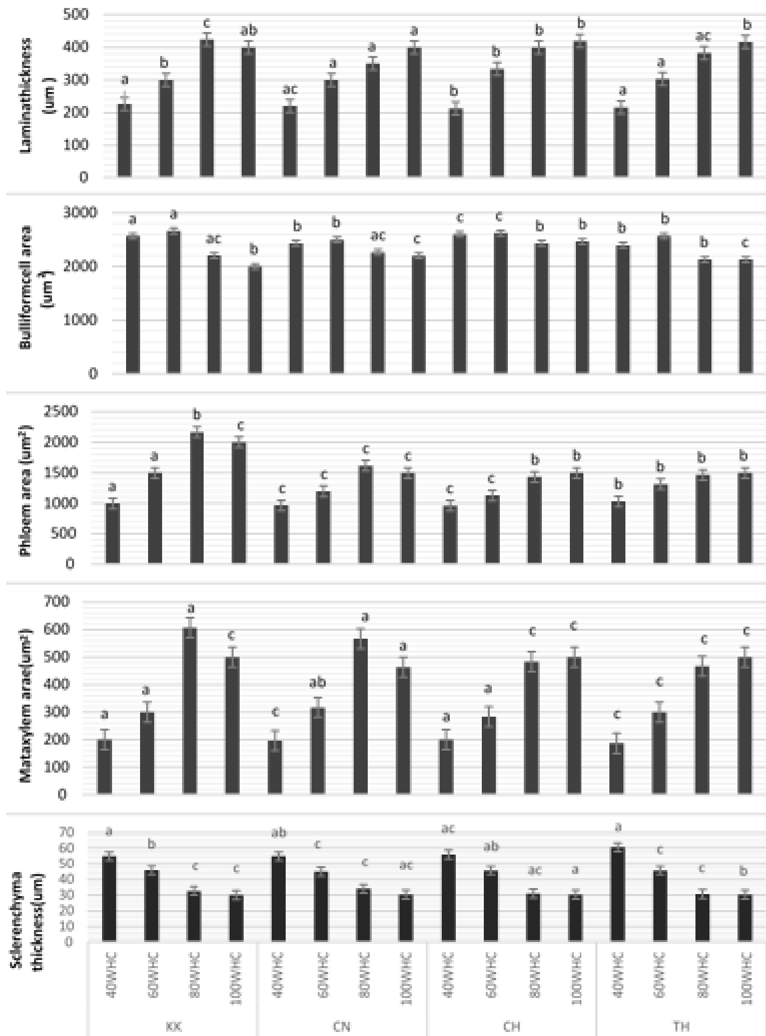

Fig. 6: Leaf anatomical adaptations in *L. scindicus* populations grown under drought stress ±SD. Means with different letters were significantly different from each other at 5% probability level
KK = Kalar Kahar; CN = Chiniot; CH = Cholistan; TH = Thal

However, there was an increase in sclerification in leaf vascular tissues *i.e*., metaxylem and phloem area as under drought stress leaf succulence increase to many folds (Wu *et al*., 2010). The anatomical modifications in stem such as reduced stem area, increased sclerification and, large size cortical cell resulted in increased and better uptake of water and minerals under drought stress (Ali *et al*., 2009) which helped *L. sindicus* to grow under suboptimal conditions.

Leaf anatomical adaptations had a significant relationship with drought stress tolerance. Increase in leaf sclerenchyma thickness and reduction in leaf cell area was reported under drought stress conditions in different grass species (YuJing *et al.*, 2000); as was observed in this study. Reduction in leaf lamina thickness was also observed in this study under drought conditions (Fig. 6). At leaf level, significant structural modifications in *L. sindicus* populations observed those include increased epidermal thickness, sclerenchyma thickness, reduced cortical area and increased bulliform cell area. All these may contribute to water conservation (Hameed *et al*., 2012).

There are specific anatomical modification *i.e*., sclerification and liginification in leaf and stem to tolerate drought conditions. Increase in lignification in epidermis, vascular tissues and dermal tissue thickness is reported with increase in severity of drought condition (Dolatabadian *et al*., 2011). Increased thickness in exodermis and endodermis was recorded under severe drought conditions in this study, as desert inhabitant populations shown more significant increase in exodermis and endodermis thickness. This characteristics feature is well known strategy of plant to face and survive under dry climatic condition and abiotic stresses (Boughalleb *et al*., 2009).

Bullifom cells play a key role in turgor maintenance under drought stress condition. Increase in bulliform cell size and their area is a prime anatomical adaptation to tolerate drought stress. Both these adaptations play key role for water conservation that helps plant in survival under drought conditions as was observed in present study (Fig. 6). Increase in size and area of bulliform cells was reported under drought stress in *Zea mays* (Alvarez *et al*., 2008). Large sized bulliform cells were found in all populations, especially in population of TH and CH regions, under severe drought stress. Increased bulliform cell area results in enhanced mechanical strength of leaf tissues in grasses and thus enable plants to cope water stress conditions. Leaf curling and rolling is one of major structural modification for survival under drought conditions. Leaf rolling maintains turgor potential of leaf that helps to cope water shortage condition. Large sized and more developed bulliform cells in grasses are involved in leaf curling under severe drought stress (Nawazish *et al.*, 2006).

These anatomical modifications such as increased sclerification, not only in the vascular region of root but also in the pith region may causes significant decrease in water loss from plant organs. More significant and intensive sclerification was also observed in vascular region and leaf tissues in this study. These anatomical adaptations contribute in enhancing rigidity in root tissues and also prevent water loss through stem and leaf surface (Shabala and Cuin, 2008). However, in the present investigations, stem and leaf shown more developed metaxylem and phloem area, increased sclerification on both sides of vascular tissues and intensive sclerification on the outer side of leaf sheath. The reduced and lignified vascular tissues and more developed protoxylem and metaxylem tissues were also observed in the present study. Metaxylem area was extremely reduced in all populations and may be a critical adaptation for drought tolerance enabling the plant to efficiently transport nutrients and water (Chen *et al*., 2006), nevertheless, reduced metaxylem vessels are less susceptible to cavitation and embolism and however it is found crucial adaptive component for developing tolerance for drought (Kondoh *et al*., 2006). These anatomical changes contribute to efficient water uptake and maintenance of turgor and moisture under harsh dry environmental conditions (Souza *et al*., 1999). The efficient and more developed vascular tissues can be regarded as critical anatomical modification for drought stressed desert regions.

On the whole, all populations showed significant structural modifications to cope with drought stress conditions. Population from CN exhibited thicker epidermis, thin leaves, developed bulliform cells and reduced metaxylem area, which are the significant structural adaptations under drought stress conditions. Population from KK developed efficient strategy for drought tolerance by reduced and fibrous leaves, smaller metaxylem and phloem vessels, intensive leaf sclerification and highly efficient large sized bulliform cells than other populations, however CH and TH populations developed thick leaves, large sized bulliform cells, reduced thicker lamina, extremely thick epidermis and reduced metaxylem area, than other three populations, which are the critical anatomical adaptations under unfavourable environmental conditions. Based on results of various anatomical adaptations shown by the all populations under drought stress, these may be ranked in order of drought tolerance as CH>TH>KK>CN.

Conclusion

Results of this study disclosed that well-developed thick cutinized leaves, reduced metaxylem and phloem areas and well developed bulliform cells were the major anatomical adaptations to induce drought tolerance in *L. scindicus*. The populations of *L. scindicus* in different locations was rated in order of drought tolerance as CH>TH>KK>CN. Furthermore, information obtained in this study can be used in future studies to understand drought tolerance mechanism in grasses.

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