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Variation in Leaf Anatomy in Wheat Germplasm from Varying Drought-Hit Habitats

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ABSTRACT

Leaf anatomical changes in four biotypes of wheat and one approved variety, Barani 83, were investigated under two regimes of water, i.e., irrigated and stressed. Thickness of leaf, cuticle, epidermis, hypodermis, and number of stomata generally increased under water stress while the number of hair and stomatal length decreased. Accession Pak 15800 showed the maximum increase in epidermis and hypodermis thickness and produced the maximum values of leaf thickness, mesophyll thickness and number of hair under water stress; whereas, stomatal number remained stable and vascular bundle area was decreased. It was followed by Pak 1794 and Pak 15797. Susceptible entry, Pak 15720, showed lower values of the characters under stress conditions. Huge disintegration of mesophyll was noted in Barani 83 and Pak 15794. On the basis of all the anatomical characteristics, Pak 15800 was regarded as the best entry of all the accessions. Leaf thickness, leaf hairiness, increase in size of protective and mechanical tissues and mesophyll integrity seem to be good selection criteria for drought tolerance in wheat.

Key Words: Wheat; Germplasm; Drought; Leaf anatomy

INTRODUCTION

Wheat has been grown in the low moisture and rainfed areas since ancient times, but inadequate moisture availability hampers its productivity to a great extent (Dubetz & Bole, 1973; Khannachopra *et al.*, 1994; Hameed *et al.*, 1995). It is important to find a better wheat germplasm capable of drought tolerance. Leaf anatomical characteristics are considered the true indicators of stress influence (Jones *et al.*, 1980; Rojas *et al.*, 1983; Santacruz & Cock, 1984; Nayeem, 1989; Venora & Calcagno, 1991; Aberentthy *et al.*, 1998). Number of epidermal cells decreases progressively with the increase in water stress, but number of stomata decreases slightly (McCree & Davis, 1974). Aberentthy *et al.* (1998) observed stomata only on adaxial surface in native tussock grass (*Festuca novae*). Drought resistant wheat genotypes had greater stomatal frequency than susceptible genotypes in drought conditions, and drought susceptible genotypes had higher frequency than drought resistant in irrigated conditions (Nayeem, 1989).

Xerophytic plants usually have thick cuticles (Martin & Juniper, 1974). Increased cuticle deposition has been reported in maize under water stress (Ilahi, 1982). In contrast, epidermis layer is reported to be thinner in drought tolerant genotypes of cotton (Bhatt & Andal, 1979), but Rojas *et al.* (1983) reported thicker external wall in drought resistant sugarcane and Jones *et al.* (1980) smaller epidermal cells in *Lolium perenne* due to water stress. However, Grace and Russell (1977) reported higher epidermal appendages due to drought in *Lolium perenne*.

Drought is associated with reduction in leaf thickness (Santacruz & Cock, 1984) but thicker leaf blade is related with greater degree of xeromorphy in *Glycine* spp. (Petrova, 1988) and durum wheat (Venora & Calcagno, 1991). Mesophyll cells are more prone to damage than bundle sheath cells by water stress (Giles *et al.*, 1974). Drought susceptible winter wheat has relatively larger vascular tissue but greater amount of sclerenchyma in drought hardy variety (Ridley & Todd, 1966).

Xeromorphy is related to greater amount of mechanical tissues, shorter distance between vascular bundles and smaller chlorenchyma cells (Kuptsov *et al.*, 1981), also with greater number of stomata on upper leaf surface (Maimistov, 1981). Rojas *et al.* (1983) reported thicker external epidermal wall and cuticle, greater number of sclerenchymatous fibres, more abundant parenchyma and lower number of stomata in drought resistant sugarcane.

Considering such a scientific pursuit of paramount importance, it was intended to hunt the xeric adaptations out of the wheat germplasm, which was collected from dry cultivated fields of Baluchistan, Pakistan. The present study was planned to investigate the response of xeric germplasm from varying water stressed habitats under simulated water stress conditions, especially focusing on leaf anatomical adaptations.

MATERIALS AND METHODS

The seeds of four land races of wheat (*Triticum aestivum* L.) were collected from hotter and drier parts of Baluchistan Province of Pakistan and that of an approved drought resistant variety, Barani 83 from Ayub Agricultural Research Institute (AARI), Faisalabad (Table I). The materials were grown in a field of Botany Department, University of Agriculture, Faisalabad under two regimes of water *viz.* irrigated receiving 40 cm of water (four irrigations after sowing each of 5 cm at tillering, booting, anthesis and grain filling stages, plus 20 cm rain) and stressed (rainfed, receiving 20 cm rain and no irrigation after sowing). The experiment was conducted in randomized complete block design with three replications.

Table I. Details of wheat material used in the experiment

Entry name	Details of collecting site
Pak 15720	48 km from Basima towards Kharan, Baluchistan
Pak 15794	7 km east of Sibi, Baluchistan
Pak 15797	13 km east of Sibi, Baluchistan
Pak 15800	34 km from Sibi on road to Quetta, Baluchistan
Barani 83	Ayub Agricultural Research Institute, Faisalabad

For the anatomical studies basal portion of about 2 cm of the flag leaf after ear emergence was fixed in formaline acetic acid (FAA) solution comprising of 50% alcohol, 10% formaline, 5% acetic acid and 35% distilled water. The material was then kept in acetic alcohol solution in 3:1 ratio of acetic acid and alcohol for long-term preservation. Leaf thickness, cuticle thickness, epidermal thickness, hypodermal thickness, mesophyll thickness, mesophyll disruption, vascular bundle area, hair/trichome, number and length of stomatal apparatus per unit area were studied during the investigation.

RESULTS AND DISCUSSION

Leaf anatomical characteristics generally showed increasing trend under moisture stress. However, the response of all the entries was variable. The thickest leaves were found in Pak 15800 under both regimes of water, i.e., irrigated and stressed measuring 500.0 and 604.2 µm respectively (Table II), while Pak 15720 had the minimum values of this character; 400.0 µm under irrigation and 483.3 µm under stress. All the entries increased their thickness under drought, the maximum increase was noted in Pak 15797 (22.03%) as compared to well-irrigated condition. It was closely followed by Pak 15797 and Pak 15720; whereas, Pak 15794 showed somewhat stability.

Cuticle thickness was increased under water stress except in Barani 83 (Fig. 1). The maximum increase was seen in Pak 15794 (72.22%). Pak 15800 produced the thickest cuticle under stress (6.4 µm), indicating its potential to resist drought. Epidermis thickness presented very similar pattern as that of cuticle, but Barani 83, showed a decrease of 24% under stress conditions. The maximum increase was noted both in Pak 15800 and Pak 15720 (13.45%) with a thickest epidermis layer under water deficit environments (13.5 µm).

Hypodermis increased among the entries except Pak 15720 under drought stress and the maximum increase was seen in Pak 15800 (45.67%) closely followed by Pak 15794 (43.53%). Pak 15794 had the thickest hypodermis region under favorable moisture conditions (68.2 µm) while Pak 19797 under drought (92.0 µm). The thinnest of this was recorded in Pak 15720 under both regimes of water, 62.0 µm under irrigation and 56.8 µm under drought.

Mesophyll thickness was reduced in only Pak 15800 while others showed increases, maximally in Pak 15794 (28.82%). Pak 15800 had the thickest mesophyll in both the regimes of water, measuring 80.7 µm under normal irrigation and 72.7 µm under stressed condition, even this reduced one was the thickest among others. High mesophyll disruption was noted in Barani 83 and Pak 15797, while low in Pak 15720 due to water stress. However, Pak 15800 and Pak 15794 had their mesophyll tissue undamaged under stress.

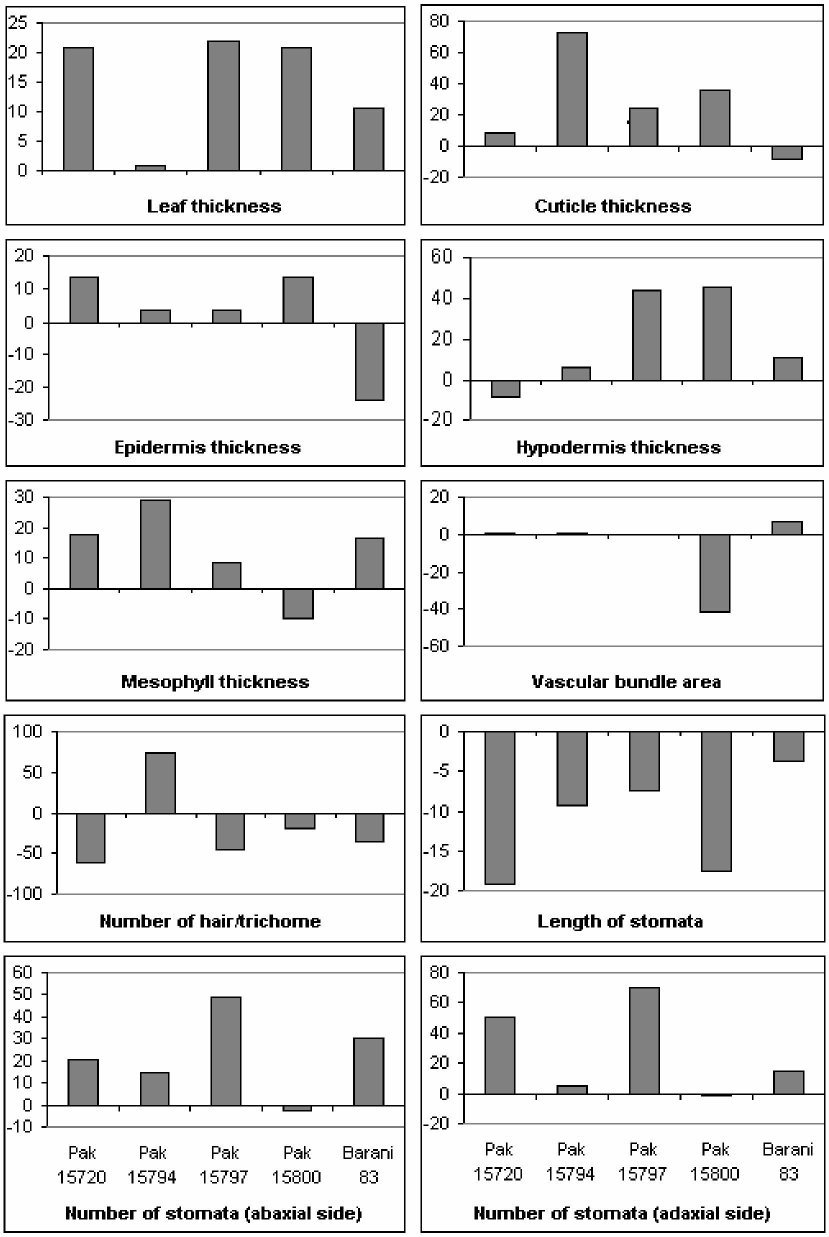
Maximum increase in the area of vascular bundles was found in Barani 83 (12.60%). However, Pak 15800 showed a considerable reduction in vascular bundle area under stress (66.88%). In other cases, it remained somewhat stable, Pak 15720 and Pak 15794 showed slightly increased area due to water stress, whereas, Pak 15797 had a slight reduction.

Except Pak 15794, all the other entries showed a decrease in number of hair/trichomes under stress. Pak 15800 possessed the second highest under irrigation and the highest numbers under drought, proving its capability of drought tolerance, however, Pak 15794 showed an increase while Pak 15720 (62.07% decrease) was the worst.

Number of stomata per unit area was increased under water stress except Pak 15800, where a slight decrease was seen on both leaf surfaces. Pak 15800 also showed much lower stomatal number on adaxial side under drought, which helps it in checking transpiration under adverse conditions. Pak 15797 showed the maximum increase on both leaf surfaces, 48.99% on abaxial side and 70.75% on adaxial side. Stomatal length was invariably reduced under drought condition in all the entries where its maximum decrease was recorded in Pak 15720 (19.18%) followed by Pak 15800 (17.45%). Its minimal was in Barani 83 (3.70%).

All the entries studied here are either collected from arid/semi-arid regions of the country or approved drought tolerant stuff, that might be the reason for the enhancement of all the characters due to water stress, however, the response of each entry is specific for particular character. Increased leaf thickness in these entries seems to be associated with drought tolerance. Pak 15800 with the thickest leaves in both water regimes can be regarded it as the best drought tolerant in view of the fact that thick leaves are known to be associated with drought resistance (Petrova, 1988; Venora & Calcagno, 1991). Water stress, in general, coupled with the reduction of leaf thickness (Cock, 1984), but here all the entries got enhancement in leaf thickness under adverse moisture conditions, which indicates their drought loving nature.

Fig. 1. Per cent increase or decrease due to per cent water stress in wheat germplasm from Baluchistan



Cuticle plays a very important role in checking undue water loss from leaf surface that normally get increased under limited moisture supply e.g. maize (Ilahi, 1982), sugarcane (Rojas *et al,* 1983) and durum wheat (Venora & Calcagno, 1991). Barani 83 was the singular case where water stress resulted in a reduction in cuticle thickness (Fig. 1), indicating its poor adaptation to drought. The minimum of this character in Pak 15720 under irrigated condition may not attract the attention of research workers and plant breeders but its enormous increase under drought indicates its fabulous capability to minimize undue water loss and withstand severe moisture stress condition. On the contrary, Pak 15800 can be regarded as equally good with the thickest deposition of cuticle under inadequate moisture supply and reasonably thick cuticle under normal irrigation.

Epidermis thickness presents more or less same pattern of increase due to water stress, where only Barani 83 was adversely affected. However, this increased epidermis thickness was much less as compared to that in cuticle. These contrasting results are not in agreement with some previous reports (Bhatt & Andal, 1979). Reduction under water stress was reported by McCree and Davis (1974) in grain sorghum and Jones *et al.* (1980) in rye grass. Nevertheless, Barani 83 followed the similar pattern.

Well-developed hypodermis is supposed to play an important role in drought resistance (Ridley & Todd, 1966; Kuptsov *et al.*, 1981; Rojas *et al.*, 1983; Petrova, 1988). Here again, tolerant entries (Pak 15800 and Pak 15797) showed a marked increase under insufficient water supply. Pak 15720 was the singular case that got reduction.

The thickest mesophyll tissue in Pak 15800 under both regimes of water makes it more likeable stuff for plant breeders and geneticists, besides it got reduction under water stress. Persistent mesophyll cells in Pak 19800 and Pak 19794 again specify them more resistant entries, as it is assumed that mesophyll cells are more prone to damage due to water deficit (Giles *et al.,* 1974 in maize). Mesophyll disruption was high in Pak 15797 and Barani 83, while low in Pak 15720, all of them can be regarded as less tolerant.

Vascular bundle area approximately remained stable due to water stress, though Pak 15800 got massive reduction (68.88%) under stress. Smaller vascular bundles conceivably more efficient in water and nutrient uptake, as it is assumed that larger vascular bundles are the characteristics of drought susceptible wheat entries (Ridley & Todd, 1966). However, prominent vascular bundles are reported drought resistant durum wheat (Venora & Calcagno, 1991).

Epidermal appendages, i.e., hair or trichomes, are thought to be related with drought tolerance because they often increase under drought (Grace & Russell, 1977) and also prevent undue water loss (Martin & Juniper, 1974). All the entries under study got reduction here, but the intensity of reduction varies considerably. Pak 15800 depicted the

Table II. Leaf anatomical characteristics of wheat germplasm under two regimes of water

Characteristics	Treatment	Pak 15720	Pak 15794	Pak 15797	Pak 15800	Barani 83
Leaf thickness (µm)	Irrigated	400.0	475.0	491.7	500.0	475.0
	Stressed	483.3	497.2	600.0	604.2	525.0
Cuticle thickness (µm)	Irrigated	5.7	3.6	4.2	4.7	5.7
	Stressed	6.2	6.2	5.2	6.4	5.2
Epidermis thickness (µm)	Irrigated	11.9	12.9	12.4	11.9	15.0
	Stressed	13.5	13.4	12.9	13.5	11.4
Hypodermis thickness (µm)	Irrigated	62.0	68.2	64.1	58.9	64.4
	Stressed	56.8	72.2	92.1	85.8	71.3
Mesophyll thickness (µm)	Irrigated	54.3	51.0	60.0	80.7	61.0
	Stressed	64.0	65.7	65.0	72.7	71.0
Mesophyll disruption due to stress		Low	None	High	None	High
Vascular bundle area	Irrigated	15.2	14.8	17.7	15.4	12.7
(x 1/1000 μm^2)	Stressed	15.4	15.0	17.5	5.1	14.3
Number of hair/trichomes	Irrigated	8.7	2.7	3.7	8.3	6.7
	Stressed	3.3	4.7	2.0	6.7	4.3
Stomata number (abaxial)	Irrigated	118.7	118.7	114.3	129.7	119.0
	Stressed	143.3	136.3	170.3	126.3	155.3
Stomata number (adaxial)	Irrigated	68.7	96.0	78.3	80.7	94.7
	Stressed	103.3	100.7	133.7	79.7	108.3
Stomata length (µm)	Irrigated	21.9	21.4	21.8	21.2	18.9
	Stressed	17.7	19.4	20.2	17.5	18.2

minimum reduction and also had the maximum hairiness in both moisture regimes, hence it can safely be concluded as the best entry for arid/semi-arid regions. The susceptible entries were Pak 15794 and Pak 15720 in relation to this character.

Stomatal density plays a very important role in drought resistance that generally increases with increasing water stress (Jones *et al.*, 1980; Maimistov, 1981; Nayeem, 1989). However, some workers reported a stability or decrease in the stomatal number (McCree & Davis, 1974; Rojas *et al.*, 1983). All the entries here possessed lower stomatal number on adaxial leaf surface than on abaxial surface. Pak 15800 again showed a slight reduction in stomatal number on both leaf surfaces, but minimum values under water stress. This may give it additional assistance to withstand under adverse environmental conditions as it has an ability to curtail water loss when there is limited moisture availability. In addition, it also had the minimum of stomatal length under water deficit, giving it extra aid in more efficient opening and closing of stomata according to environmental conditions. Reduction in stomatal length due to water stress was observed in all the entries, as it is predicted (Grace & Russell, 1977).

On the basis of several leaf anatomical characteristics, Pak 15800 can safely be categorized as the best drought tolerant entry showing a considerable increase in its leaf thickness, protective layers (cuticle and epidermis) and mechanical layer (hypodermis) under drought conditions. These adaptive components help this entry in preventing water loss under water deficit environments, indicating its potential to survive well under adverse condition of water deficit conditions. Increased mechanical tissue, enhanced mesophyll layer and increased number of hair/trichomes again prove it as the most tolerant wheat entry against drought. Reduction in stomatal length and number under drought helps it to prevent water loss thus making Pak 15800 the best entry for low precipitation areas. Both Pak 15797 and Pak 15794 showed fair potential for drought resistance regarding some leaf characteristics, rendering them reasonable stuff for drought prone areas. However, the performance of Barani 83 and Pak 15720 was much lower than average.

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