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ANATOMICAL STUDIES OF DROUGHT TOLERANCE RELATED TRAITS OF 26 WHEAT VARIETIS IN IRAN

Maryam ABBASI[1]*, Elham FAGHANI[2], Habibollah SOGHI[2], Ali HOSSEIN KHANI[3]

[1]Department of Biology, College of Basic Sciences, Central Tehran Branch, Islamic Azad University, Tehran, Iran
[2]Golestan Agriculture and Natural Research and Education Center, Iran
[3]Department of Cellular and Molecular Biology, Young Researchers and Elite Club, Central Tehran Branch, Islamic Azad University, Tehran, Iran
*Corresponding author: Mar.Abasi@iauctb.ac.ir

ABSTRACT

Wheat (*Triticum aestivum* L.) is known as a drought semi-tolerant species. Reduction in wheat growth and yield are the most common responses to drought or salt stress mainly caused by an inhibition of leaf expansion and stem elongation. One of the important abiotic stress factors limiting wheat production in semi aridregions is drought. Recent climate changes such as temperature changes and decreasing rainfall in different regions of Iran have had significant impact on agro-ecosystems and have caused drought stress to become a severe limiting factor in wheat production. This research was conducted for evaluation of leaf anatomical and cytological traits of 26 wheat varieties in Golestan province (Iran) over 40 years in order to identify the most effective traits in determining maximum yield potential. The width and length of vascular bundles, diameter of meta xylem, distance between vascular bundles to upper and lower epidermis, fiber bundles diameter and width of midrib and lamina were measured. Finally, based on the anatomic results, wheat varieties with the highest adaptation ability to drought stress were identified and introduced.

Keywords: *waterless stress, wheat, anatomical study, Iran*.

INTRODUCTION

Wheat (*Triticumaestivum* L.) has been cultivated by many civilizations for over 9,000 years. It is a cereal grain belongs to Poaceae family, which has been known as semi-tolerant plants to drought. Wheat is believed to have originated in Mediterranean region, especially in Syria and Palestine and then spread out to the rest of the world (Jenkins, 1996).

Wheat is grown on more than 250,000,000 hectares and its world production is 500 million tons (FAO, 2016). The top wheat producer countries in the world are Denmark, Netherlands, France, Belgium and Germany. Iran is the eleventh most producer country in the world. FAO’s latest forecast for 2016 world cereal production stands at 2521 million tons, 0.2 percent lower than the 2015 estimate due to low rainfall and drought stress. Wheat plays a pivotal role in proving food for Iranians. The Iran's per capita use of wheat stood at 194 kilograms per year making it the seventh most consumer country in the world. Iran is an arid and semi-arid country located on world's desert belt. About 30 per cent of the total cropped area in the country is under wheat cultivation (Mohammad, 2006). Drought stress and high temperature during growing seasons are the most important limiting factors that influence the wheat production (Islamian, 2010). In these areas, farmers do not obtained optimal results in high need varieties to irrigation due to lack of adequate water in spring and/or lack of enough irrigation as a result of consumption of irrigation water for summer agricultures, consequently the wheat agriculture suffered from drought in end of season (Mahfuzi, 2009).

A large volume of researches in the world have been focusing on the effects of environmental conditions on wheat growth and development. Scientist are expecting to produce wheat cultivars for cultivation in arid lands (Mollasadeghi, 2011; Khan, 2015; Mousavi, 2008; Hamam, 2014). Studying on superior genotypes such as drought and cold tolerant genotypes is crucial to take advantages from highlands where water deficit stress is responsible for reducing leaf area and changing leaves cytological characteristics and finally yield loss (Richards, 2004).

of photosynthetic rates and high transpiration efficiencies (Evans *et al*. 1994). Cuticle thickness (Rojas *et al*. 1983), stomatal frequency (Rebetzke *et al*., 2010), length (Bohnert and Jensen, 1996), movement and sensitivity (Drake *et al*., 2013) are among anatomical characteristics which are believed to be useful for breeding water stress tolerant genotypes. Leaf morphological characters including leaf area (Zagdanska an Kozdo, 1994), shape (Reddy *et al*., 2004), duration (Verma*et al*., 2004) and developing behavior (Hu *et al*., 2000) Considering the world's population growth, global water shortages and drought stress in arid and semi-arid regions, the current study was aimed to highlight the role of anatomical and cytological characteristics of wheat cultivars in improving wheat tolerance against rough climate conditions.

For this purpose, 26 wheat cultivars (release over 40 years) were studied in terms of leaf anatomical and cytological characteristics to introduce the most compatible cultivars to be grown under stressful conditions.

MATERIALS AND METHODS

Physiological aspects

In this study, 26 wheat cultivars (Table), which have been released over 40 years, were evaluated in terms of physiological and anatomical aspects in Gorgan Agricultural Research Station (Iran). The field was prepared using moldboard plough and disk. Chemical fertilizers were applied according to the soil analysis results. The experimental design was a randomized complete block design with thee replicates. The plots were 1 × 6.5 m consist of 5 rows. The wheat seeds were sown in early January based on seed weight at 350 seeds per square meter. During growing season data were collected and finally crop was harvested in June.

Anatomical aspects

In order to study leaves anatomical chrematistics, leaf samples were fixed in alcohol-glycerin solution (1:1 v:v) for one month. In all cultivars, middle part of basal leaves was chosen to collect the samples. The Green methyl and Carmen stain were used for staining. Stained cross sections were studied under Olympus optical microscope equipped with digital camera and 40x lenses. The Measurement software was used to measure different parameters. The results were statistically analyzed using SAS software.

Table 1: name, origin and pedigree of genotypes of *Triticumaestivum* L.

Number of genotypes	Name of cultivar	Origin of cultivar	The date of introducing	pedigree
1	khazra	Gorgan	1973	(P4160(F3)*Nr69)LR64
2	Tajan	Simit	1995	Bow˝s˝/Nkt˝s˝
3	Naz	Simit	1978	Jupateco 73
4	Alborz	Simit	1978	Fn-Md*k117/Cofin2
5	Hyrmand	Zabol	1991	Byt/4/Jar//Cfn/Sr70/Jup˝s˝
6	Shirodi	Simit	1997	Attila
7	Golestan	Simit	1986	Alondra˝s˝
8	Inia 66	Simit	1968	Lr64/Sn64
9	pastor	Simit	1997	Pastour
10	Arta	Simit	2007	(HD2206/Hork//Bul…
11	Darya	Simit	2007	SHA4/CHIL…
12	Moghan 3	Simit	2007	Luan/3/V763.23/V879.c8//Pvn
13	Morvarid	Simit	2009	MILAN/SHA7
14	Atrak	Simit	1995	Kauz˝s˝
15	Falat	Simit	1990	Kvz/Buho˝s˝//Kal/Bb=Seri82
16	Rasol	Simit	1992	Veery˝s˝=Kvz/Buho˝s˝//Kal/Bb
17	kohdasht	Ecarda	2000	TR8010200
18	Gondad	Gorgan	2011	ATRAK/WANG-SHUI-BAI
19	N-80-19	Simit	2005	SW89.3064/STAR…
20	N-87-19	Gorgan	2002	MILAN CM75118/KA…
21	N-87-20	Gorgan	2003	SABUF/7/ALTAR…
22	Zagros	Simit	1995	TAN˝s˝/V˝s˝
23	Line A	Simit	2005	IRANA/BABAX//PASTOR
24	Karim	Ecarda	2011	HAMAM4
25	N-87-21	Simit	2012	BABAX//PASTOR
26	N-87-22	Simit	2013	SH1/CHV2

RESULT AND DISCUSSION

In this study two groups of traits were evaluated. The first group traits, which are equally found in all cultivars, included uni-cellular epidermis, mesophylla type, vascular bundles sheet, proto and meta xylems, proto and meta phloem, simple stomata in upper and lower epidermis and fiber in primary and secondary vascular bundles. The second group traits, which differ from cultivar to cultivar, included width of fiber in lower epidermis, length and width of vascular bundles, phloem width, phloem length, the maximum diameter of xylem, distance between vascular bundles and adaxial leaf surface, midrib widthand lamina width.

Fiber width

In all cultivars fiber was observed under epidermis of abaxial surface. There is no report stating fiber thickness change due to drought stress. It seems that increase in fiber accumulation not only increase leaf stability and conserve midrib, but also improve water holding capacity in parenchyma cells under drought stress conditions. As shown in Table 1, the maximum fiber width was observed in 14 and 16 genotypes. In addition, number 2 genotype showed the minimum fiber width compared with the other genotypes(Fig8& Fig 10; B, N,P)

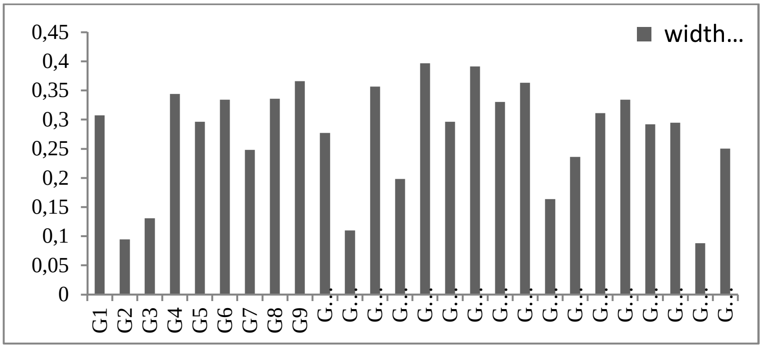

Figure 1: fiber width in different wheat genotypes

Width and length of central vascular bundles

Midrib anatomy consists of central large vascular bundles by large metaxylem vessels on either side of protoxylem. Phloem well developed. Bundle sheaths have extended to upper and lower epidermis. Vascular tissue system are completely surrounded by chlorenchymatous bundle sheath and involved in transportation of the different compounds. Changes in vascular bundles is known as a response to water deficit stress (Claudio and Andrea, 1998). There was significant difference between cultivars in terms of width of vascular bundles. The maximum and minimum width was observed in genotypes number 16 and number 2 (Fig 2), respectively (Fig8; B,Fig 10; P). Furthermore, significant difference was found between wheat cultivars in relation to length of vascular bundles. The maximum and minimum length was observed in genotypes number 16 and number 2, respectively (Fig 3) and (Fig8; B,Fig 10; P). The biggest vascular bundles were observed in number 16 genotype (Rasool) (Fig 8; P). Bigger vascular bundles improve leaf efficiency under drought stress conditions.

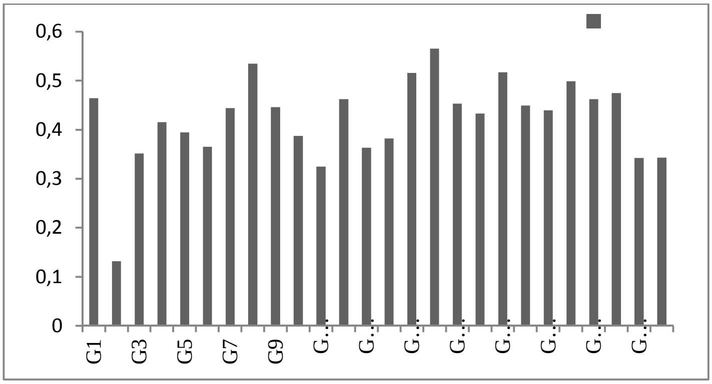

Figure 2. comparing between width of vascular bundle in several genotypes

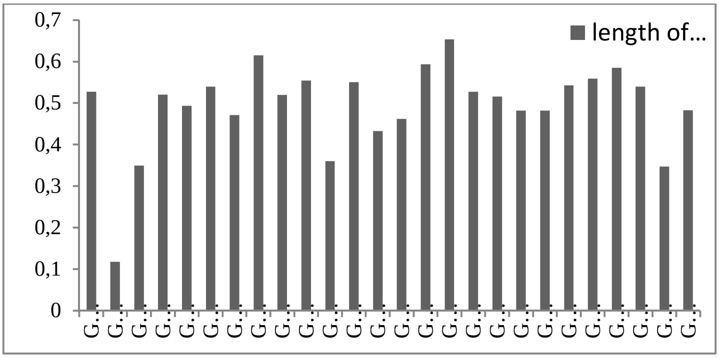

Figure 3. comparing between length of vascular bundle in several genotypes

Width of Phloem: has been shown the phloem diameter in wheat leaves that was affected by water stress condition were not changed in accordance with their corresponding grain size (Jafarian et al., 2012). This is in contrast with what is expected from the phloem sieve tubes diameters implying that there may be other limiting factors affecting grain size in these cultivars.In addition, significant difference was obtained when phloem width of different genotypes was compared. The maximum and minimum width was related to number 8 and number 2 genotypes, respectively (Fig 9; H, Fig 8; B). It should be noted that different phloem lengths were observed in different genotypes. According to Figure 4, the maximum and minimum phloem length was related to number 9 and number 2 genotypesrespectively (Fig8 ;B, Fig 9; I).

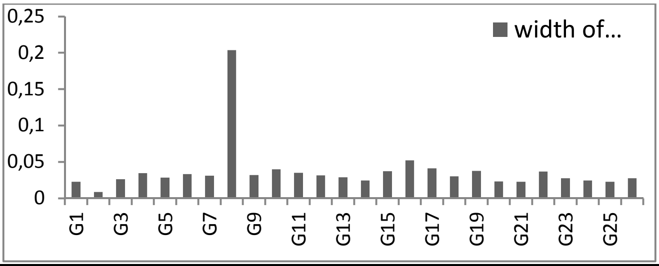

Figure 4. comparing between width of phloem in several genotypes

Xylem vessel diameter

the xylem vessel diameter in wheat leaves is under the effect of genotype, water stress and their interaction indicating thatsmall size vessels, if desired, could be selected for, depending on the water availability.The theoretical xylem hydraulic conductivity computed from the diameter of individual vessels using the Hagen–Poiseuilleequation has been shown to be proportional to the observedvalues (Altus *et al*. 1985). Decreasing xylem diameter may play a role inadaptation of plants to water stress condition since smaller diameter decreases the hydraulic conductivity of the xylem (Martre*et al*. 2001). In addition, significant difference was obtained when phloem width of different genotypes was compared. The maximum and minimum width was related to number 8 and number 2 genotypes, respectively (Fig 8; B, Fig 9; I).

It should be noted that different phloem lengths were observed in different genotypes. According to Figure 5, the maximum and minimum phloem length was related to number 9 and number 2 genotypes, respectively (Fig 9; I, Fig 8; B).

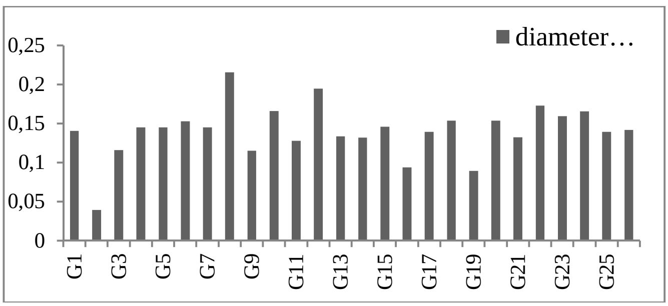

Figure 5. Comparing between xylem vessel diameter in several genotypes

Width of Lamina and Midrib

Environmental stresses were shown to change mesophyll cells dimensions in other crop plants. For example high temperature stress increased the thickness of palisade and spongy cell layers and lower epidermal cells in soybean leaves (Djanaguiramana*et al.* 2011) . In this study, there was significant difference between cultivars in relation to lamina width so that the maximum lamina width was observed in number 16 genotype (Rasool), the genotype that showed the maximum tolerant against water deficit stress (Figure 7) and (Fig 10; P). The number 15 genotype showed similar results. The minimum lamina width was related to number 18 genotype (Figure 7) and (Figure10; R). In case of midrib width, the number 16 genotype showed the widest midrib among other genotypes(Figure 6). By contrast, the narrowest midrib was related to number 25 genotype (Figure 6) and (Figure11; X).

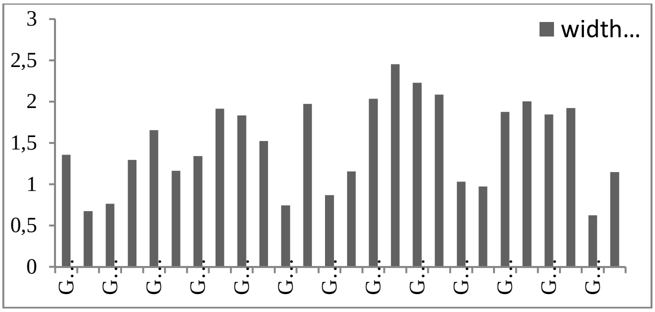

Figure 6. comparing between widths of midrib in several genotypes

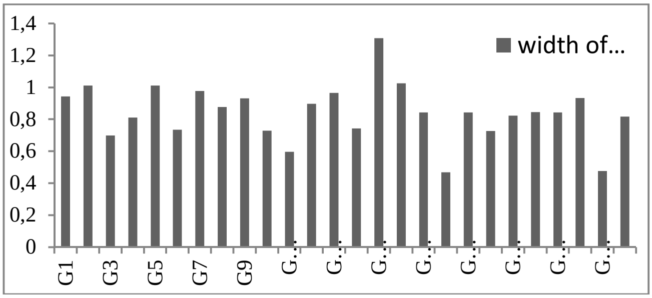

Figure 7. comparing between width of lamina in several genotypes

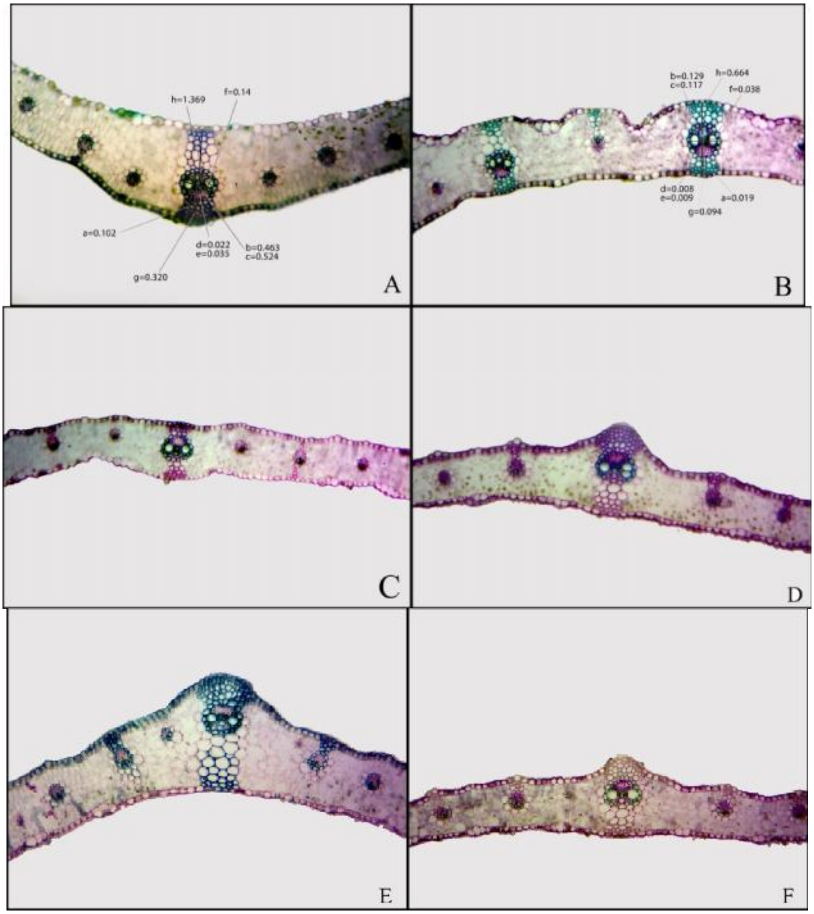

Figure 8. Cross- section of genotypes of *Triticum aestivum* L.leaf (μm), A : G1, B: G 2, C: G 3, D: G 4, E: G 5 , F: G6. **a**: width of fiber, **b**: width of vascular bundle, **c**: length of vascular bundle, **d**: width of phloem, **e**: length of phloem, **f**: diameter of xylem, **g**: distance between V.S. to epidermis, **h**: width of lamina.

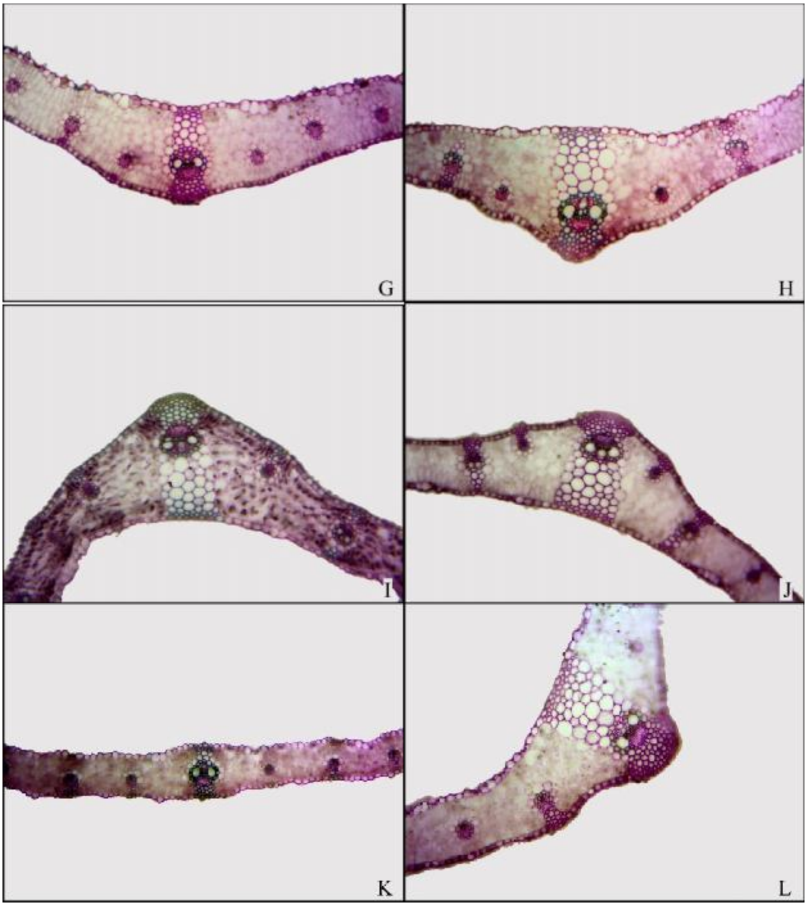

Figure 9. Cross- section of genotype of *Triticumaestivum* L. leaf (µm), G : genotype 7, H: genotype 8, I: genotype 9, J: genotype 10, K: genotype ,genotype 11, L: genotype 12.

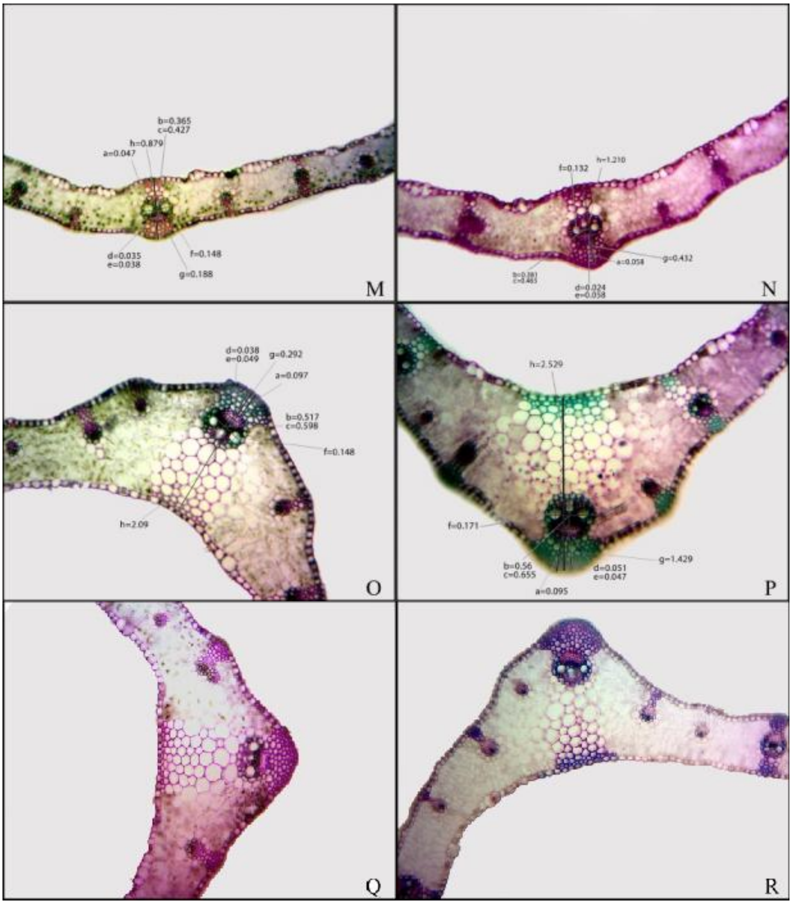

Figure 10. Cross- section of genotypes of *Triticum aestivum* L. leaf (µm), M : G 13, N: G14, O: G15, P: G16, Q: G17 , R: G18, **a**: width of fiber, **b**: width of vascular bundle, **c**: length of vascular bundle, **d**: width of phloem, **e**: length of phloem, **f**: diameter of xylem, **g**: distance between V.S. to epidermis, **h**: width of lamina.

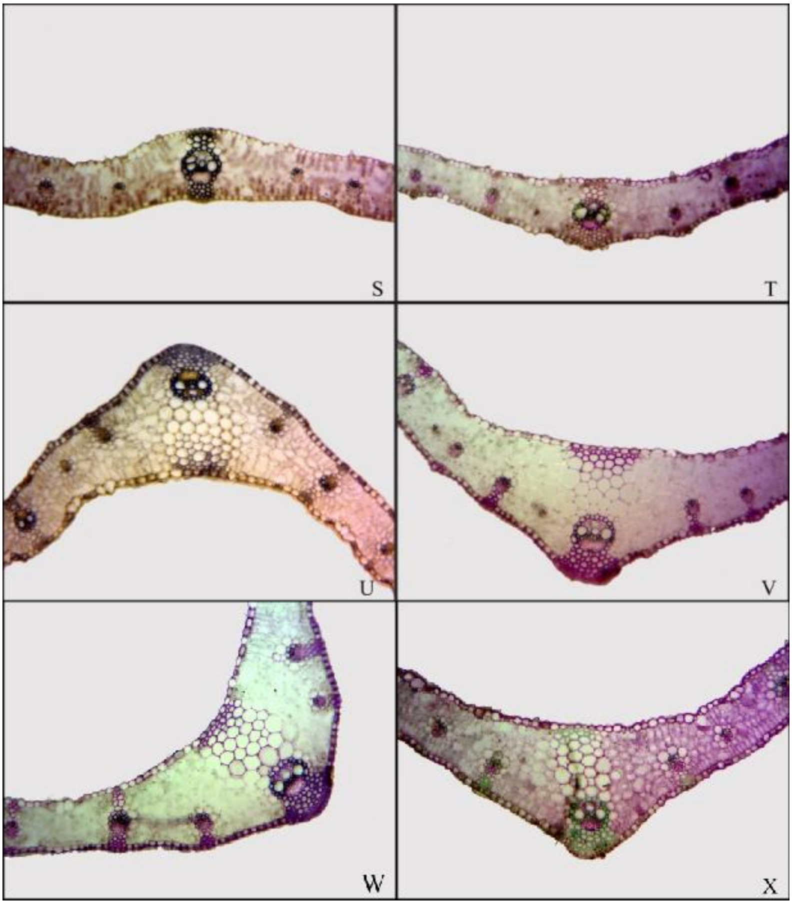

Figure 11. Cross- section of genotypes of *Triticumaestivum*L. leaf (µm), S : genotype 19, T: genotype 20, U: genotype 21, V: genotype 22, W: genotype 23 , X: genotype 24.

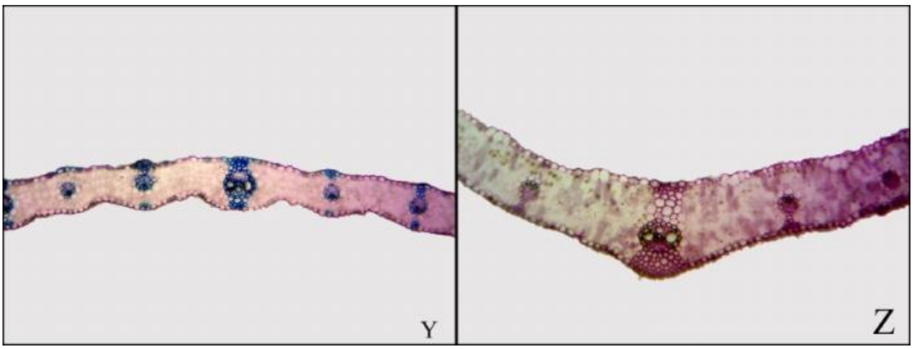

Figure 12. Cross-section of genotype of *Triticum aestivum* L. leaf (µm), Y: genotype 25, Z: genotype 26.

CONCLUSIONS

In general, anatomical comparison between 26wheat cultivars indicated that number 16 genotype (Rasool) has more complex vascular bundles and more evaluated sieve elements compared with the other cultivars. The Rasool genotype is originated from CIMMYT, which is produced by hybridization between previous generations with high level of fiber in abaxial surface of midrib and thick vascular bundles in the midribs. In addition, Rasool genotype is high temperature tolerant genotype. These characteristics help plants by reducing water loss under drought stress conditions. By contrast, number 2 genotype (Tajan) was less evaluated in terms of vascular bundles properties. In sum, Rasool genotype is highly recommended to be cultivated in central regions of Iran, where drought stress is the most limiting factor for wheat production in drylands.

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