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

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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-arid regions 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 (Triticum aestivum 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 $1 \times 6.5$ 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 *Triticum aestivum* L.

<table>
<thead>
<tr>
<th>Number of genotypes</th>
<th>Name of cultivar</th>
<th>Origin of cultivar</th>
<th>The date of introducing</th>
<th>pedigree</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>khorzra</td>
<td>Gorgan</td>
<td>1973</td>
<td>(P4160(F3)*Nre69)Lr64</td>
</tr>
<tr>
<td>2</td>
<td>Tajan</td>
<td>Simit</td>
<td>1995</td>
<td>Bow<code>s7/Nks</code>s^~</td>
</tr>
<tr>
<td>3</td>
<td>Naz</td>
<td>Simit</td>
<td>1978</td>
<td>Jupateco 73</td>
</tr>
<tr>
<td>4</td>
<td>Alborz</td>
<td>Simit</td>
<td>1978</td>
<td>Fn-Md*k117/Cofin2</td>
</tr>
<tr>
<td>5</td>
<td>Hyrmand</td>
<td>Zabol</td>
<td>1991</td>
<td>Byt/4/Jar/Cfn/Sr70/Jup`s^~</td>
</tr>
<tr>
<td>6</td>
<td>Shiromi</td>
<td>Simit</td>
<td>1997</td>
<td>Attila</td>
</tr>
<tr>
<td>7</td>
<td>Golestan</td>
<td>Simit</td>
<td>1986</td>
<td>Alondra`s^~</td>
</tr>
<tr>
<td>8</td>
<td>Inia 66</td>
<td>Simit</td>
<td>1968</td>
<td>Lr64/Sn64</td>
</tr>
<tr>
<td>9</td>
<td>pastor</td>
<td>Simit</td>
<td>1997</td>
<td>Pastour</td>
</tr>
<tr>
<td>10</td>
<td>Arta</td>
<td>Simit</td>
<td>2007</td>
<td>(HD2206/Hork//Bul…</td>
</tr>
<tr>
<td>11</td>
<td>Darya</td>
<td>Simit</td>
<td>2007</td>
<td>SHA4/CHIL…</td>
</tr>
<tr>
<td>12</td>
<td>Moghan 3</td>
<td>Simit</td>
<td>2007</td>
<td>Luan/3/V763.23/V879.c8//Pvn</td>
</tr>
<tr>
<td>13</td>
<td>Morvarid</td>
<td>Simit</td>
<td>2009</td>
<td>MILAN/SHA7</td>
</tr>
<tr>
<td>14</td>
<td>Atrak</td>
<td>Simit</td>
<td>1995</td>
<td>Kauz`s^~</td>
</tr>
<tr>
<td>15</td>
<td>Falat</td>
<td>Simit</td>
<td>1990</td>
<td>Kvz/Buho`s^~//Kal/Bb=Seri82</td>
</tr>
<tr>
<td>16</td>
<td>Rasol</td>
<td>Simit</td>
<td>1992</td>
<td>Veery<code>s^~=Kvz/Buho</code>s^~//Kal/Bb</td>
</tr>
<tr>
<td>17</td>
<td>kohdasht</td>
<td>Ecarda</td>
<td>2000</td>
<td>TR8010200</td>
</tr>
<tr>
<td>18</td>
<td>Gondad</td>
<td>Gorgan</td>
<td>2011</td>
<td>ATRAK/WANG-SHUI-BAI</td>
</tr>
<tr>
<td>19</td>
<td>N-80-19</td>
<td>Simit</td>
<td>2005</td>
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</tr>
<tr>
<td>20</td>
<td>N-87-19</td>
<td>Gorgan</td>
<td>2002</td>
<td>MILAN CM75118/KA…</td>
</tr>
<tr>
<td>21</td>
<td>N-87-20</td>
<td>Gorgan</td>
<td>2003</td>
<td>SABUF/7/ALTAR…</td>
</tr>
<tr>
<td>22</td>
<td>Zagros</td>
<td>Simit</td>
<td>1995</td>
<td>TAN<code>s^~//V</code>s^~</td>
</tr>
<tr>
<td>23</td>
<td>Line A</td>
<td>Simit</td>
<td>2005</td>
<td>IRANA/BABAX/PASTOR</td>
</tr>
<tr>
<td>24</td>
<td>Karim</td>
<td>Ecarda</td>
<td>2011</td>
<td>HAMAM4</td>
</tr>
<tr>
<td>25</td>
<td>N-87-21</td>
<td>Simit</td>
<td>2012</td>
<td>BABAX//PASTOR</td>
</tr>
<tr>
<td>26</td>
<td>N-87-22</td>
<td>Simit</td>
<td>2013</td>
<td>SH1/CHV2</td>
</tr>
</tbody>
</table>

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 xylesms, 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](image)

**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.
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 genotypes respectively (Fig 8; B, Fig 9; I).
the xylem vessel diameter in wheat leaves is under the effect of genotype, water stress and their interaction indicating that small 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–Poiseuille equation has been shown to be proportional to the observed values (Altus et al. 1985). Decreasing xylem diameter may play a role in adaptation 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).

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 (Djanaguiraman 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 (Figure 10; 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 (Figure 11; X).

![Figure 6](image6.png)

Figure 6. comparing between widths of midrib in several genotypes

![Figure 7](image7.png)

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: G2, C: G3, D: G4, E: G5, 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 10. Cross-section of genotypes of *Triticum aestivum* L. leaf (m), M: G13, 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 12. Cross-section of genotype of *Triticum aestivum* L. leaf (μm), Y: genotype 25, Z: genotype 26.
CONCLUSIONS
In general, anatomical comparison between 26 wheat 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.

REFERENCES


