Genetic Variation of the 20 Bread Wheat Cultivars under Chilling Stress by Using GGE Biplot Analysis

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Abstract

Chilling stress in cereal crops is one major form of cold stresses that be appeared in some regions of Iran every year; and a significant part of all cold losses is just peculiar to this stress. For this purpose study of genetic diversity of 20 bread wheat cultivars using by artificial chilling stress pattern was performed. Experimental material at reproductive stage were treated in four levels of temperature (+8 (control), +2, 0 and -2 degree of Celsius) and then physiological characteristics, electrolyte leakage, chlorophyll, proline and fructan, were assessed. GGE biplot is a graphical method to display the information in a bilateral table and were used to visualize any relationships among treatments. The result of variance analysis showed high significant interaction effects between cultivars and each of temperature level for all of characteristics and for this reason there is desirable genetic diversity between cultivars. The graphics obtained from GGE biplot for clustering of these cultivars reveal four clusters: First group includes Orum and Second group covering Navid. Third cluster includes Pishgam, Crasshahi, Line A, Omid and Alvand and fourth group covering other 12 cultivars. The graphics also showed the best cultivar for each trait and also ranked all cultivars based on all traits. By crosses between these cultivars therefore the effective quantitative alleles are distinguishable and usable to the chilling stress tolerance, and marker assisted selection programs.

Keywords: Chilling Stress; Chlorophyll; Electrolyte Leakage; Fructan; GGE Biplot; Proline; Wheat

Introduction

World population is increasing at an alarming rate And important percentage of the food needs for this growing population relative to agriculture. Wheat (Triticum aestivum L.) is one of the main crops as a sustained food (Cai et al., 2011). Food productivity is declining due to the effect of different abiotic stresses, so lessen these damages is purpose of all nations to cope with the increasing food requirements (Mahajan and Tuteja, 2005). Stress is the result of abnormal physiological processes that Influence by one or a combination of biological and environmental factors (Levitt, 1980). Plant growth and productivity is facing by different forms of abiotic and biotic stress factors. Plants are frequently exposed to a collection of stress conditions such as low temperature, salt, drought, flooding, heat, oxidative stress and heavy metal toxicity. All these stress factors are threats for plants and intercept them from reaching their full genetic potential and limit the crop productivity worldwide. Abiotic stress actually is the main reason of crop failure worldwide. These stresses, threaten the stability of agricultural industry (Mahajan and Tuteja, 2005). Cold, salinity and drought are important among the other stresses, which adversely affect plant growth and productivity, so it is important to extension stress tolerant
crops (Cai et al., 2011). Three types of cold stress have been identified that include: Frost, Freezing and Chilling Stress (Levitt, 1980).

A considerable problem in several areas of the world is post-head-emergence spring radiative frost damage of winter cereals. The problem happens in regions that main growing season restrict to the late winter and spring by drought and heat of summer that daytime temperatures are ideal for growth, but night temperatures can down to destructive levels. Farmers usually in order to minimize frost risk, delay tillage to stalling head emergence but this practice confront crops to increasing temperatures and dwindling water supplies late in the season, decreasing yield potentials as heading is delayed (Frederiks et al., 2011).

Low temperatures may be damage to wheat at all stages of crop development. However, capability increases with increasing development of the crop. Contrary, the hazard of damaging frosts occurring reduces as spring progresses. Physiological characteristics which lead to greater frost tolerance include glaucosity, absence of awns low water content and high fructan content (Whaley et al., 2004). Each plant has its especially set of temperature requirements, that are optimum for its suitable growth and development which this set of temperature conditions may be stressful for another plant. When plants those are native to warm habitat exposed to low non-freezing temperatures, display symptoms of injury, that are appear from 48 to 72 h after stress induced. This time is different from plant to plant and also depends upon the sensitivity of a plant to cold stress (Mahajan and Tuteja, 2005).

Cold stress may cause various seedling injuries, delayed heading, yield reduction due to spikelet sterility (Andaya and Mackill, 2003) and chilling stress also reduced leaf expansion, wilting, chlorosis (yellowing of leaves) and may lead to necrosis (death of tissue) and strongly disturbed the reproductive development of plants (Mahajan and Tuteja, 2005). Low temperature stress induce significant changes in biochemistry and physiology of plants (Berova et al., 2002). Usually many physiological processes and photosynthesis is sensitive to cold stress, that is a main proof for the decline of growth and productivity decrease of plants under low temperature (Liang et al., 2007).

Cell membrane leakage tests have been extensively used to identify the level of plant tolerance to various stresses. This technique is pretty easy, repeatable and quick and needs cheap equipment and it is proper for the analysis of large numbers of samples and it also has been used to quantify damage in various abiotic stress situations such as low temperatures (Arvin and Donnelly, 2008). Environmental stress with changes in cell membrane and increased cell permeability reduces the cell’s ability to control rate of ion movement in and out of cells and cause damage in great texture (Farkhondeh et al., 2012). Cell membrane damage is one of the effective ways of measuring cell membrane leakage. Free radicals production can increase with cold stress and stimulate the formation of covalent bonds form of lipid radicals that rigidifying the membrane chilling can disrupt major (Jing et al., 2009).

One of the most important chloroplast components for photosynthesis is relative chlorophyll content, and it has a positive relationship with photosynthetic rate (Guo et al., 2008). Chlorophyll contents decreased under low temperature damage (Liang et al., 2007). Chlorophyll a has been approved as a fast, non-invasive, and inexpensive method to discover stress effects in plants (Martínez-Peníalver et al., 2011) Environmental stresses increases amount of proline in many plant species. Proline is involved in cellular osmoregulation and it has many protective effects. Plants by rising in amount of proline levels were reported to exhibit increased tolerance to abiotic stresses (Cvikrová et al., 2012). Most of plant for counteract of abiotic stress agglomerate osmolytes including proline. Proline application resist an enhancement in methylglyoxal and lipid peroxidation during cold stress (Kumar and Yadav, 2008). Amounts of free proline increased in stress. High levels of free proline have protective effect on cells membrane (Farkhondeh et al., 2012).

Fructans there are in many economically important cereals and grasses such as wheat (Triticum aestivum) and it is involved in protection of osmotic potentials, membrane stability, cold hardiness and freezing tolerance. Freezing tolerance in wheat has affirmative correlated with high fructan contents (Rao et al., 2011) and also it have been known as one of the primary stored forms of energy in 15% of higher plants, it also can fuel quick regrowth in grasses, maintain plants against cold and drought stress through membrane stabilization and adjust osmosis during flower opening (Li et al., 2013). Fructan plays role in the resistance of plants to drought and cold stress (Asega and de Carvalho, 2004). Fructan content have a very important role in effects to abiotic stresses like chilling, freezing, drought and salinity in wheat (Valluru and Van den Ende, 2008).

Materials and Methods

This survey performed in 2011 at the Faculty of agriculture, Tarbiat Modares University. In this study an experiment with 20 varieties of wheat with four treated cold levels of stress (8 (control), +2, 0, -2 Celsius) in
factorial arrangement in randomized completely design implemented. and then electrolyte leakage, chlorophyll (a, b, ab and cartenoids), proline and fructan, were assessed.

**Plant material and chilling treatment**


First, seeds were surface sterilized in 5% sodium hypochlorite solution for 5 min, then rinsed with sterilized water. After wards seeds were cultivated on wet filter paper in Petri dishes and placed in germinator whit 20 °C for 4 days. When the seedlings reached in 2 cm, in order to vernalization, they were transferred for seven weeks to 3±1°C, then the seedlings were Transplanting in pots and transferred in growth chamber under 16 h of day (25 °C) and 8 h of night (18 °C) whit 300 µmol/m²/s of light intensity. When the plant wheat reached in early of reproductive stages (heading and flowering Zydky code 50 to 68) (ZADOKS et al., 1974), they for one week transferred to growth chamber under 16 hours of day (16 °C) and 8 h of night (8 °C) or temperature control, then to start stress, temperature reduced 2 °C per one hours from 8 °C and the pots were placed for 2 h under stress in each of stress temperature levels (2, 0 and -2 °C) and after stress, temperature up to 8 °C with ramp 2 °C per hours and sampling was done after 24 h.

**Measuring the stability of the cytoplasmic membrane**

Electrolyte leakage is used to study the change in membrane structure and permeability this test determines the degree of cell membrane injury caused by chilling stress based on electrolyte leakage from the cells. For measurement stability of the cytoplasmic membrane, 150 mg flag leaf separated from each variety and split inside falcon 50 cc, then 20 cc water twice distilled added to each falcon and placed over shaker at room temperature for 15 h. After this time electric conduction was read from samples by metrum conduction planter. Then the samples transmitted inside anobutore at temperature of 80 °C for 45min. After getting the sample in room temperature, final electric conduction of samples was read. By using the formula $\text{EL} \% = \frac{c_t}{c_{tot}} \times 100$ determined electrolyte osmosis percent. In this formula $c_t$ is the electric conduction at first time and $c_{tot}$ is the final electric conduction (Bertin et al., 1996)

**Measuring the Determination of chlorophyll concentration and stability**

To determining the density and stability of leaf chlorophyll, separated 50 mg of flag leaf and split inside falcon 15cc, then hemogenized with 99.8% of methanol. Chlorophyll concentration was read by using of spectrophotometer planter (shimadzu-UV-160A) in wavelength of 470, 652.4 and 665.2 nm. Chlorophyll a, b and all of them and cartenoid obtained according to µg/ml by following formula (Lichtenthaler, 1987).

\[
C_a = 16.72 A_{665} - 9.16 A_{652} \quad , \quad C_{a+b} = 1.44 A_{665} - 24.93 A_{652} \quad C_b = 34.09 A_{652} - 15.28 A_{665} \\
C_c = 1000 A_{470} - 1.63C_a - 104.96C_b / 221
\]

**Measuring the quantity of proline**

For identifying the proline content, separated and pulverized 0.2 gr from wheat leaf in mortar and homogenized with 10 ml acid sulphosalislic 3.3% for 10 min. The solution filtered with wattmen and 2ml reagent ninhydrin (1.25g ninhydrin +30ml acid glacial +20ml acid phosphoric 6M) and 2ml acid acetic added to 2ml provided plant essence. The obtained solution placed for 1h at temperature 100°C benmary. After getting the sample in room temperature, added to each tubes 4 ml of Toluene. After shaking tubes severely, two separate phases was created and the upper phase was read at a wavelength of 518 nm by spectrophotometer planter.

**Measuring the quantity of fructan**

In order to measuring the content of fructan, 0.2 gr from wheat leaf prepared and homogenized with 3 ml of 50 mM sodiumphosphate in mortar. The solution filtered with wattmen paper, then 1ml of this mirtuered solution added to 5ml antron 0.02% and acid solphoric 70%. After 15 min, the solution was placed in benmary at 100°C for 7.5 min. Then left the samples in room temperature, samples absorption was read at 625nm wavelengths and Standard of inulin with concentrations of 0-30 (µg m l⁻¹) was used (Jermyn, 1956).
Biplot analysis

Biplot analysis (Yan et al., 2000), is a graphical method to display the information in a bilateral table and were used to visualize any crossover treatment _ environment interactions, relationships among treatments, and relationships among environments (Ma et al., 2004) and in plant breeding it has been known as an innovative methodology in biplot graphical analysis (Frashadfar et al., 2012). GGE biplot analysis is an effective method which is based on principal component analysis (PCA) and also it is increasingly being used in agriculture for G*E interaction data analysis (Ding et al., 2007).

Statistical software

After normalization test, data was analyzed statistically. Analysis of variance was done for obtained data to determine the effects of cultivar, trait and cultivar and trait interaction using the SAS 9.1 software. To show graphically data to “which is best for what”, “interrelationship among cultivars”, “cultivar means trait”, “examining the cultivars and traits” and ranking trait based on cultivar and reciprocally used by GGE biplot ver 6.3.

Results and discussion

The analysis of variance showed that chilling stress, varieties and interactive effects of cold stress and Varieties have significant differences at the 1% level in all of physiological treats (Table 1). The significant interaction effects were showed that the chilling stress has caused different reactions among the Varieties and it confirmed sufficient diversity among varieties. Separate mean comparison result confirms diversity among varieties. This variation reflects different mechanisms of the interior of plants and probably variation in gene activities.

Table 1: Analysis of variance (mean of squares) of total chlorophyll content (C.a+ C.b), chlorophyll a (C. a), b (C. b), carotenoid (Car), electrolyte leakage (E.l), proline and fructan

<table>
<thead>
<tr>
<th>S.O.V</th>
<th>df</th>
<th>C. a</th>
<th>C. b</th>
<th>C.a+ C.b</th>
<th>Car.</th>
<th>E.l</th>
<th>proline</th>
<th>fructan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cultivar</td>
<td>19</td>
<td>12.55**</td>
<td>35.64**</td>
<td>31.17**</td>
<td>7.91**</td>
<td>0.13**</td>
<td>1369.2**</td>
<td>0.082**</td>
</tr>
<tr>
<td>Chilling Stress</td>
<td>3</td>
<td>247.76**</td>
<td>47.87**</td>
<td>77.91**</td>
<td>61.9**</td>
<td>0.40**</td>
<td>3387.69**</td>
<td>0.087**</td>
</tr>
<tr>
<td>Cultivar * Chilling Stress</td>
<td>57</td>
<td>18.55**</td>
<td>10.68**</td>
<td>16.7**</td>
<td>5.79**</td>
<td>0.12**</td>
<td>924.63**</td>
<td>0.067**</td>
</tr>
<tr>
<td>error</td>
<td>160</td>
<td>5.90</td>
<td>7.30</td>
<td>8.02</td>
<td>2.48</td>
<td>0.012</td>
<td>288.80</td>
<td>0.034</td>
</tr>
<tr>
<td>CV%</td>
<td></td>
<td>24.34</td>
<td>25.30</td>
<td>19.7</td>
<td>17.45</td>
<td>8.19</td>
<td>17.29</td>
<td>20.12</td>
</tr>
</tbody>
</table>

* Significant at 1% level

The “which is best for what”

The polygon view of the GGE biplot (shown in figure 1) indicates the best cultivar(s) in each trait and total of traits. The polygon was established by connecting the markers of the cultivars that are endmost away from the biplot origin that all other cultivars are contain in the polygon. The rays are lines that are perpendicular to the sides of the polygon or their extension (Ding et al., 2007; Yan, 2002). In figure 1, ray 1 and 2 is perpendicular to the side that connects Bam and Orum, ray 3 is perpendicular to the side that connects Orum and Navid, and so on. These six rays divided the biplot into six sections, and all traits fall into four of them, so that the four traits fall into one of them. The peak cultivars for each quadrant are the one that gave the highest amount for the traits that fall within that quadrant. The highest amount in traits cell membrane leakage, proline, chlorophyll a and carotenoid is Orum, in total chlorophyll is Navid and in fructan is Shiraz respectively. The other vertex cultivar, Bam, Aflak and Darab 2 are poorest in all traits. Biplot can be used to visualize Interaction among cultivars and trait. The visualizing graphic of cultivar’s means and their traits show different genotypes which were classified into four groups.
Interrelationship among cultivars

Figure 2 prepare the synopsis of the interrelationships among the cultivars. The lines that junction the biplot’s origin and the markers for the cultivars are called cultivar vectors. The angle between the vectors of two cultivars is related to the correlation coefficient between them. The cosine of the angle between the vectors of two cultivars almost is equal to the correlation coefficient between them (Ding et al., 2007; Yan, 2002). Based on the angles of cultivar vectors, the 20 cultivar are grouped into four. First group includes Orum and Second group covering Navid. Third cluster includes Pishgam, Crasshahi, Line A, Omid and Alvand and fourth group covering other 12 cultivars (figure 2). This clustering approximately confirmed obtained dendrogram from SPSS software. So that 20 cultivar of bread wheat in this dendrogram divided to four clusters and these clusters approximately approved GGE Biplot grouping (figure 3).

Cultivar means trait

Figure 4 shows the ranking of 20 cultivars based on their means traits. The line that pass through the biplot center is called “the average trait axis” that is defined by the average of PC1 and PC2 scores of all traits. More close to concentric circles show higher mean. The line that passes through the center and is vertical to the trait axis with two side arrows represents the variability of cultivar (defined as A line). Both directions away from the biplot center, on this axis, indicate more interaction among each cultivar and traits and increase variation (Ding et al., 2007; Yan, 2002). For a specific selection, the ideal cultivars are those have high mean and response to particular trait. In first group of traits, total chlorophyll and proline, the mean trait of each cultivar was in the following order: the highest mean belong to Darab 2, then Sivand and so on. In second group, chlorophyll a, carotenoid and cell membrane leakage, the mean trait of cultivars were in the following order: the highest were Mv17, then Shiraz, and so on. In third group, Fructan, the highest mean trait of cultivar were Orum, then Omid, and so on and in fourth group, the best cultivar was Navid.

Examining the cultivars and traits

Figure 5a shows the performance of different cultivars in cell membrane leakage. A line that passes through the biplot center and labeled CML is the cell membrane leakage axis. The cultivars are ranked according to their projections on to the cell membrane leakage axis. The line passing through the biplot origin and perpendicular to the cell membrane leakage axis separates genotypes. Genotypes that have higher mean were placed at the right side of line A, e.g., cultivars Shiraz, Kavir and Gascogen and genotypes that have lower mean were placed at the left side of the line A e.g., Orum, Omid and Line A. And also figure 5b shows that in the trait of Fructan Above the mean cultivars ranking are the traits in right side of line A, e.g., cultivars Shiraz, Kavir and Gascogen. Cultivars Orum, Omid and Bam in the left side of line A are below the mean genotypes ranking.

Figure 6 shows the results of on the basis of Shiraz cultivar for all traits. The line passes through the biplot center and labeled Shiraz is the cultivar Shiraz axis. The traits are ranked along the cultivar Shiraz axis arranged for left to right direction. Thus, the relative mean of cultivar Shiraz in different traits follows the order as, chlorophyll a> carotenoid> cell membrane leakage> chlorophyll b> proline> total chlorophyll> fructan. The vertical line (labeled A) to the Shiraz cultivar axis separates traits with low and high amount from left to right respectively.
Figure 1: Polygon view of the GGE biplot shows the “which is best for what”

Figure 2: Polygon view relationship among cultivars
Figure 3: Dendrogram of 20 bread wheat cultivar obtained from SPSS software

Figure 4: Polygon view the average trait coordination for cultivar evaluation
**Figure 5a:** Polygon view examining the performance of/relative to cell membrane leakage

**Figure 5b:** Polygon view examining the performance of/relative to Fructan
**Figure 6**: Polygon view examining the performance of/relative to Shiraz
References


