Genetic Variation of Advanced Bread Wheat Genotypes for Remobilization related Traits under irrigated and Rainfed conditions using GGE Biplot Method

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ABSTRACT: The accumulation of assimilate remobilization in 16 advanced bread wheat genotypes was investigated using in vitro and in vivo experiments in deputy of Kermanshah Sararood Dry Land Agricultural Research Institute during 2013-2014 cropping season. The experimental layout was conducted in a randomized complete block design with three replications under two complementary irrigation and rainfed conditions. In this study 24 morphophysiological traits were measured. 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 genotypes GGE biplot software was used. Results of combined analysis of variance showed significant differences for site, genotypes and genotype*site interaction in most of measured traits. The 16 advanced bread wheat genotypes were grouped into three clusters in irrigation condition and four cluster in rainfed condition. To wrap up the discussion, the ideal genotypes was G1 and G2 in both irrigation and rainfed condition as compared to the other genotypes.

Keywords: Wheat, Drought stress, Remobilization, Assimilate, GGEbiplot.

INTRODUCTION

World population is increasing at an alarming rate and important percentage of the needed food for this growing population is depended on agriculture. Wheat (Triticum aestivum L.) is one of the most important food crops in the world and so is a main crops as a sustained food (Cai et al. 2011). Different abiotic stresses decrease food productivity, so one of the purpose of all nations is reducing these damages to cope with the increasing food demands (Mahajan and Tuteja 2005). Stress is the result of abnormal physiological processes that influenced by one or a combination of biological and environmental factors (Levitt 1980). In the natural environments, plants often grow under various stresses such as drought, salt, low temperature, heat, flooding, oxidative stress and heavy metal toxicity which severely affect the growth, metabolism and yield of them. All these stress factors are threats for plants and inhibiting them from reaching to their full genetic potential and limit the crops productivity worldwide. Moreover, these stresses threat the stability of agricultural industry (Mahajan and Tuteja 2005).

Drought, cold and salinity are the most important among the other stresses which adversely affect plant growth and productivity (Cai et al. 2011). The environmental conditions in regions with Mediterranean climate are favorable for wheat growth until the anthesis but afterwards wheat is exposed to terminal heat (Zamani et al. 2014) and drought stress, so it is important to increase stress tolerant crops.

Pre-anthesis assimilation of stem reserves is one of the factors inhibiting against drought stress and reduces the effect of stress on yield. Mostly, drought stress increases the transfer of stem reserves if the plants are exposed to water stress (Yang and Zhang, 2006). Generally, water stress decreases the current photosynthesis and increases the contribution of remobilization during the grain filling. So, varieties with higher efficiency in remobilization of assimilates show more tolerance to drought stress and lower yield losses (Blum, 1998; Yang et al. 2000; Niu et al. 1998). Remobilizations of storage material from vegetative organs to grains compensate economic yield, particularly in drought stress conditions (Ramazanzadeh et al. 2012). Several factors and agents that have effect between sources and sink during the various stages of development, include genotype, planting date, rainfall and fertilization. In grains, accumulation dry matter amount in plants is more than the rate of consumption growth at during of period growth. Different sugars are stored in stem as excess photosynthetic and often are transferred from stem to seed in the next stage of growth usually begins two to three weeks after flowering (Sadras et al. 1993). Thus, two carbohydrate source involved in grain filling are current photosynthetic products that transmit directly to the seed and remobilization of stem storage materials.
Balance between translocation of assimilates from source to sink is so important and it is based on the build-up of assimilates in source on one hand and the capacity of sink on the other hand. Thus yield loss is results of imbalance between them. Higher fraction of compounds are remobilized to grains, when assimilate build-up decreases capability of source (Palta et al. 1994).

The accumulation of assimilate remobilization in wheat genotypes was investigated under limited irrigation by Moghaddam Khamseh et al. (2014). They report dry matter at flowering and maturity, the translocate dry matter, remobilization efficiency, and contribution of pre-anthesis assimilate to grain filling, spike harvest index and spike partitioning coefficient were affected by the limited irrigation. Dry matter at flowering and maturity, spike harvest index and contribution of pre-anthesis assimilates to grain filling were also influenced by irrigation* genotype effects.

Zamani et al. (2014) evaluated the ability of different wheat genotypes for accumulation and remobilization of stem water soluble carbohydrate under the heat stress conditions during the grain filling and expressed that there was significant variation among the genotypes for water soluble carbohydrate concentration and remobilization. Water soluble carbohydrate remobilization increased under heat stress (on average 60%). Strong association between maximum water soluble carbohydrate concentration in main stem and Water soluble carbohydrate remobilization was found.

Khakwani et al. (2012) have studied growth and yield response of wheat varieties to water stress at booting and anthesis stages of development and reported highly significant differences among means of wheat varieties in Leaf area, Relative water content, fresh weight, Plant height, Main spikes grain yield per plant, tillers grains yield per plant, Total grain yield per plant, biological yield per plant, main spike dry weight, straw dry weight, Harvest index, Stress Susceptibility Index, Tolerance, Mean Productivity, Geometric Mean Productivity and Stress Tolerance Index.

GGEbiplot (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, relationships among environments (Ma et al. 2004; Yan 2001), and in plant breeding it has been known as an innovative methodology in biplot graphical analysis (Frashadfar et al. 2012). GGEbiplot 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). This method was used for any bilateral databy other researchers (Yan 2002; Yan and Kang 2002; Dehghani et al. 2008; Asadi et al. 2013; Rahnejat and Farshadfar 2015).

MATERIALS AND METHODS

This study was done in in vitro and in vivo experiments in deputy of Kermanshah Sararood Dry Land Agricultural Research Institute at 2013-2014. In order to assess the 16 bread wheat advanced genotypes, this research was done conducted in two complementary irrigation and rainfed conditions in randomized complete block design (RCBD) with three replication in terms of assimilate remobilization ability in organs to grain and also spike photosynthesis ability. In complementary irrigation conditions of a farm, in spike appearance steps and grain filling, 30 mm irrigation was done. Remobilization ability was measured by two methods: sprayed with sodium chlorate and weight methods. Spike photosynthesis ability was also measured with sprayed by sodium chloride. In this research following traits was measured:

Assimilate remobilization from stem to grain (Sprayed method): Remobilization ability was measured using Papakosta and Gagianas (1991).

\[ \text{Rem} = \frac{[(\text{GWnsp} - \text{GWsp}) \times 100]}{\text{GWnsp}} \]

Rem: The ability of remobilization; GWsp: Mean weight of a grain at sprayed plants and GWnsp: Mean weight of a grain at not sprayed plants.

Assimilate remobilization from stem to grain (Weight method): This parameter was measured using Hagparast et al. (2006) method.

The spike photosynthesis ability: Damanafshan et al. (2013) method was used to measure of spike photosynthesis.

\[ \text{SPR} = \frac{[(\text{GWnsp} - \text{GWsp}) \times 100]}{\text{GWnsp}} \]

SPR: The ability of spike photosynthesis; GWsp: Weight mean of a grain per sprayed spikes; GWnsp: Weight mean of a grain per no sprayed spikes.

Primary growth rate: Mohammadi et al. (2012) method was used to measure primary growth rate as following equation:

\[ \text{EGV} = \frac{(\text{P2} - \text{P1})}{(\text{D})} \]

EGV, D, P1, and P2 are primary growth rate, number of days between two steps of plant height measuring, and plant height at the first and second times, respectively that are calculated based on cm per day.

Canopy temperature and leaf surface temperature: These traits were measured by using infrared thermometer.

In this study, morphological traits such as plant height, stem specific weight, peduncle length, extrusion peduncle length, length and width of flag leaf, and leaf surface temperature were measured.

Grain yield, spike yield, the number of grain in spike, biomass, the number of spike per area, and thousand grain weight per harvest time were measured. As well, days to maturity and spike per maturity time was recorded.

Leaf Relative Water Content (RWC): Leaf relative water content was measured in flowering stage. Turner and Kramer (1980) method was used to measure of leaf water content.
Leaf water content (%) = (fresh weight- dry weight)/(tumescent weight- dry weight) * 100

STORED WATER IN ISOLATED LEAVES:

Kelark method (1982) was used to calculate stored water percent in isolated leaves.

ELWR = (FW - ADW/FW) * 100

ELWR: stored water in isolated leaves; FW: primary weight leaf and ADW: second weight leaf (wilt leaf)

Statistical software. After normalization test, data was analyzed statistically. Analysis of variance was done on the obtained data to determine the effects of cultivar, trait and cultivar and trait interaction using the SAS 9.1 software. To show data graphically 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 GGE biplot ver 6.3 and to clustering of genotypes Spss ver 21 were used.

Plants materials. Seeds of 16 bread wheat advanced genotypes (Table 1) were obtained from "Research Center of Kermanshah- Iran".

Table 1: The codes, names, Variety/Line, Source and LAST. Ent. No of 16 advanced bread wheat genotypes.

<table>
<thead>
<tr>
<th>Variety/Line</th>
<th>LAST.Ent.No</th>
<th>Source</th>
<th>ENT.NO</th>
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<tr>
<td>RIJAW</td>
<td>RIJAW</td>
<td>G2</td>
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<tr>
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RESULT AND DISCUSSION

According to the results of combined analysis of variance (Table 2), site and genotypes were highly significant for most of traits measured except "stem weight specific", "water stored in the separated leaves" and "spike length" for site and "amount of dry matter", "primary growth rate", "canopy temperature", "flag leaf length", "peduncle length", "flag leaf length" and "flag leaf width" for genotypes. There was no statistical significant difference in genotype*site interaction for most of measured traits but was high statistical significant difference for 7 important traits measured as follow: "spike photosynthesis ability", "remobilization with spray method", "remobilization with weight method to stem", "remobilization with weight method to spike", "yields", "relative contribution stem reserves in yield" and "awn length". The significant interaction effects for these important traits and genotypes were showed that the rainfed has caused different reactions among the genotypes and it confirmed sufficient diversity among genotypes. Separate mean comparison result confirms diversity among genotypes. This variation reflects different mechanisms of the interior of plants and probably variation in gene activities.
Table 2: Combined analysis of variance (mean of squares) of 24 measured traits for 16 advanced bread wheat genotypes at dry and irrigation condition.

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<th>rem</th>
<th>SWS</th>
<th>TW</th>
<th>REWS</th>
<th>REWH</th>
<th>CTm</th>
<th>RWC</th>
<th>LTM</th>
<th>LWC</th>
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<td>111</td>
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<td>2.38</td>
<td>94.21</td>
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The "which is best for what" According of Figure 1a, the polygon view of the GGE biplot indicates the best genotype(s) in each trait and total traits at the irrigated condition. The polygon was formed by connecting the signs of the genotypes that are endmost away from the biplot origin and, all other genotypes are contained in the polygon. The lines that are perpendicular to sides of polygon or their extension are called rays (Ding et al., 2007; Yan, 2002). In figure 1a, ray 1 is perpendicular to the side that is connecting G1 and G2. Ray 2 is perpendicular to the side that is connected G1 and G8 and G10 and so on. These five rays divided the biplot into five sections, and all traits fall into four of them, so that the seventeen traits were placed inside one of them. The peak genotypes for each quadrant are the one that gives the highest amount for the traits that fall within that quadrant.

The highest amount in traits: awn length, extrusion length, water stored in the separated leaves, primary growth rate flag leaf length, flag leaf width, peduncle length, plant height, amount of transferred dry matter, remobilization efficiency, remobilization with weight method to stem, relative water content, relative contribution stem reserves in yield, stem weight specific, total weight, yield and yldP is G1; in remobilization with spray method and spike photosynthesis ability are G8 and G10; in amount of dry matter, spike length, leaf surface temperature and remobilization with weight method to spike is G13 and in leaf water content is G16. G1 genotype was obtained from cross of Kvz/YM71/3/Maya"S"//Bb/Iina line and Sardari and called Azar2. Azar 2 is resistant to disease, heat and cold stress. This genotype has good seed quality, extensive compatibility and high yield (Rostaei et al., 2000).
Because it has some very good characteristics, Azar2 is considered as the control genotype in a lot of research in Iran. Valizadeh and Salekzamani (2008), comprised different varieties of dry land wheat to moisture absorption, phosphorus and yield. They reported Azar 2 genotype has maximum moisture absorption, high phosphorus absorption, maximum yield and maximum harvest index. G16 and G13 genotypes were obtained from cross of Azar-2 * GENE BANK-3 and ATTILA2 * PBW65, respectively.

According Figure 1b, polygon shows "which genotype(s) is (are) best for what trait(s)" at the rainfed condition. In this polygon, six rays divided the biplot into six sections, and all traits are distributed in five of them, so that awn length, water stored in the separated leaves, primary growth rate, flag leaf length, flag leaf width, leaf water content, amount of transferred dry matter, remobilization efficiency, relative water content, relative contribution stem reserves in yield, total weight and yldP have the highest amount for G1 and in secondary importance G2.

Fig. 1. Polygon view of the GGE biplot show the “which is best for what” a: irrigated condition; b: rainfed condition for 16 a.
These traits had the lowest amount for G7. Extrusion length, remobilization with spray method, remobilization with weight method to stem and spike photosynthesis ability had the highest amount in G15. For G7, leaf surface temperature, relative water lost and remobilization with weight method to spike and for G11 canopy temperature, spike length and stem weight specific had the highest amount compared to other.

A. Interrelationship among traits

Fig. 2a and b prepare the synopsis of the interrelationships among the traits in irrigated and rainfed condition, respectively. The lines that junction the biplot's origin and the markers for the traits are called traits vectors. The angle between the vectors of two traits is related to the correlation coefficient between them. The cosines of the angle between the vectors of two traits are almost equal to the correlation coefficient between them (Ding et al., 2007; Yan, 2002). Based on the angles of traits vectors, the 24 traits are nearly grouped into three clusters in irrigated condition (Fig. 2a). To sum up, awn length, primary growth rate, relative water content, yield and yldP; extrusion length, remobilization with weight method to stem and relative contribution stem reserves in yield; amount of dry matter, leaf surface temperature, remobilization with weight method to spike and finally water stored in the separated leaves, flag leaf length, flag leaf width, peduncle length, plant height, remobilization efficiency, stem weight specific and total weight had high positive correlation with each other compared to the other traits. On the contrary, amount of dry matter, leaf surface temperature and, remobilization with weight method to spike had high negative correlation with other mentioned traits.

Based on correlation among traits in the rainfed condition (Fig. 2b), awn length, water stored in the separated leaves, primary growth rate, flag leaf length and relative water content; amount of dry matter, leaf surface temperature and remobilization with weight method to spike; peduncle length and plant height had the highest correlation with together, respectively. The correlation among traits is almost similar in tow irrigation and rainfed condition. Positive correlation between yield and other traits in both of the irrigation and rainfed condition is meant with increasing grain yield, other traits also increased. Since yield is controlled by quantitative genes, and genotype* environment interaction determines its amount, so different results in different condition is possible. Different factors can indirectly influence yield by effect on morphological traits, physiological traits, etc. The importance of different traits on yield was studied by many researchers and in the following some of them are mentioned. Taheri et al. (2013) expressed there was a positive and significant correlation between the grain yield and harvest index, biomass, spike length, and awn length in intensive stress conditions. Gupta et al. (2001) reported there was a direct positive relation between biological yield and grain yield. Drought stress at flowering-to-maturing stage, especially accompanied by high temperature, was shortened the Triticale grain filling period, and reduces the 1000 grain weight (Royo et al., 2000). Mohemmedi (1998) reported positive significant correlation between dry land wheat grain yield and 1000 grain weight, plant height, tiller no, length of last internodes, seed no per spike and harvest index are.
Drought stress disrupted the flowing photosynthesis and transfer of stored substances into grains, which can be cause of reduction of the number and weight of grains (Richards et al., 2002).

B. Interrelationship among genotypes

An important strategy for classifying germplasm, arrangement variability for a large number of samples, or analyzing genetic relationships among breeding materials is the use of established multivariate statistical algorithms. Multivariate analytical techniques include principal coordinate analysis (PCoA), cluster analysis and GGE biplot analysis are most commonly employed and reveal especially useful (Kelestanie et al., 2013).

Fig. 3a and b prepare the synopsis of the correlation among the traits in irrigated and rainfed condition respectively. The lines that junction the biplot's origin and angle between them follow the rules listed above. Based on the angles of genotypes vectors, the 16 bread wheat advanced genotypes are grouped into three clusters. First cluster include G14, G4, G15, G16, G12, G13, G5, G11 and G3. G7, G9, G6, G9, G10 and G8 placed in second cluster and third clusters include G1 and G2. Clustering was performed by using SPSS software in irrigated condition grouped all genotypes in three cluster (Figure 4a). First cluster include G8, G9, G10, G5, G12, G11, G7, G14, G6, G3, G4 and G15. Second cluster include G13 and G16 and G1 and G2 are placed in third cluster.

Figure 3b explained the correlation among genotypes in the rainfed condition. Based on the angles between genotypes vectors, the 16 advanced genotypes are grouped into four clusters. So that the genotypes were divided into the following clusters: first include G4, G15, G8, G9 and G12; second include G7, G13, G5, G10; third include G11, G3, G2, G6, G14, G16 and four include G1. Also, dendogram obtained from SPSS software grouped 16 advanced bread wheat cultivars into four group as following: first: G14, G16, G12, G4, G15, G8, G9; second: G7, G13; third: G6, G11, G3, G10 and G5 and four: G1 and G2. G3, G5 and G14 genotypes was obtained with cross of Azar-2 and TEU2 (Table 1). Based on clustering analysis (Fig. 3a and 3b; 4a and 4b), G3, G5 and G14 placed into first cluster in irrigation condition based on both of GGE biplot and SPSS analysis. G3 and G14; G5 and G14 placed into third cluster in rainfed condition based on GGE biplot analysis and SPSS analysis respectively. G6 and G15 placed in one group (Fig. 4a), these genotypes are result of cross of Azar-2 and pure line BW(38). ATTILA*2 and PBW65 are parents of G12 and G13 genotypes (Table 1), these tow genotypes placed inot first cluster in irrigation condition (Fig. 3a). Based on Table 1, one of the parents of G1, G3, G5, G6, G7, G14, G15 and G16 is Azar-2. G14, G15, G16, G5 and G3 (Fig. 3a); G5, G7, G14, G6, G3 and G15 (Fig. 4a) placed into first cluster in irrigation condition based on GGE biplot and SPSS analysis respectively.
Fig. 3: Polygon view relationship among cultivars, a: irrigated condition; b: rainfed condition for 16 advanced bread wheat genotypes.

Since GGEbiplot analysis is based on principal component analysis (PCA) and, PCA only use from the two first PCA of total variation, so biplot of the two first PCA that shows arrangement of genotypes in the two dimensional tolpcan't totally verify the dendrogram obtained from the cluster analysis (Asadi, Kelestanie, Mirfakhraii, & Abasi, 2013). Because cluster analysis uses from total variation it's clustering has more statistical valuable. To wrap up the discussion, clustering based on the correlation among genotype and in other words, clustering based on the angles between genotypes vectors (Fig. 3a, b) approximately was confirmed by obtained dendrogram from SPSS software (Fig. 4a, b). Genotypes with more similarity were placed in one cluster or have high correlation as compared to other genotypes.

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Fig. 4. Dendrogram of 16 advanced bread wheat genotypes for assimilate remobilization to grain and spike photosynthesis rate at irrigation (a) and dry (b) condition obtained from SPSS software.
Fig. 5. Polygon view the average trait coordination for 16 advanced bread wheat genotypes evaluation. a: irrigated condition; b: rainfed condition.

Genotype means traits
A picture explains sometimes easier and more comfortable than many pages written. GGE biplot is a graphical method that explains boring and confusing data and tables in a figure.

The GGE biplot software to select the best genotypes for all 24 measured traits in this study used from only one clearly figures. Asadi et al. (2013) to select the best cultivar among 20 bread wheat cultivars for 7 physiological traits use from this method.

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Figure 5 shows the ranking of the 16 advanced bread wheat genotypes based on their means traits. The line that passes 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 broad selection, the ideal genotypes are those have high mean and response to particular trait. Based on Figure 5a the ideal genotypes are G1 and G2 and the most variable genotypes were G16, G8 and G10 in irrigation condition. In rainfed condition (Figure 5b) G1 and G2 were the ideal genotypes, and G2 have more change in all traits as compared to the other genotypes. Drought stress is one of the major limitations to crop production (Tester and Langridge, 2010). To develop improved cultivars with enhanced tolerance to drought stress, identification of osmotic stress related molecules and determination of their roles and locations in several physiological, biochemical, and gene regulatory networks is necessary (Budak et al., 2013). For many breeding programs, understanding of genetic diversity among genetic materials is too important to make genetic population with high level of genetic diversity and it can also help to run a breeding program well (Asadi et al., 2013). One of the primary methods to evaluate genetic diversity and QTL mapping is using morphphysiological markers. It has confirmed that the morphphysiological markers can discriminate between genotypes. In this study, relationship among 16 bread wheat advanced genotypes has been characterized by using morphphysiological markers. We wish, these result will be useful for breeding programs of bread wheat.

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Abbreviations. Awn length: AL; Amount of dry matter: CDM; Fiber content: CFr; Protein content: CPp; Starch content: CSTr; Canopy temperature: CTm; Extrusion length: EL; Water stored in the separated leaves: ELWR; Primary growth rate: GS; Harvest index: HI; Spike length: HL; Flag leaf length: LL; Leaf surface temperature: LTm; Flag leaf width: LW; Leaf water content: LWC; Relative water lost: LWL; Number of seeds per ten spike: NSP10S; Number of seeds per five spike: NSP5S; Peduncle length: PEDL; Plant height: PH; Amount of transferred dry matter: REDM; Remobilization efficiency: REE; Remobilization with spray method: rem; Remobilization with weight method to spike: REWH; Remobilization with weight method to stem: REWS; Relative water content: RWC; Spike photosynthesis ability: SPRs; Relative contribution stem reserves in yield: SPRy; Stem weight specific: SWS; Total weight: TW; Yield: YLD; Yldp: yldP

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