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Identifying superior barley (*Hordeum vulgare* L.) genotypes using GGE-biplot across warm and moderate environments under irrigated conditions in Iran

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ABSTRACT

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Multi-environmental trials (METs) are essential to study genotype by environment interaction (GEI) for effective line (s) selection and cultivar recommendations in breeding programs. Twenty promising barley genotypes were studied across 12 environments in a randomized complete block design with three replications in six different warm and moderate sites in Iran across two cropping seasons (2014-2015 and 2015-2016) using the GGE-biplot method. The combined analysis of variance revealed that environment accounted for 49.3% of the total variation while G and GE-interaction (GEI) explained 4.4% and 25.1%, respectively. Three genotypes, G6, G8, and G18, were superior in terms of high performance and stability. The 12 test environments were grouped into three different mega- environments (Mega-1, 2 and 3). Genotypes G16, G20 and G9 were identified as the best winning genotypes in Mega-1, 2 and 3, respectively. Based on the GGE-biplot, genotype G3 was found to be the ideal genotype and environments (E8 and E9) were found to be the ideal environments. GGE-biplot provided a reasonable visual examination of the relationships among the test environments, the genotypes, and the GEI. Overall, genotype G3 was better than the other genotypes and is recommended for the warm sites of Iran.

Keywords: Barley, GE interaction, GGE-biplot, Grain yield, Multi-environmental trials

INTRODUCTION

Barley (*Hordeum vulgare* L.) is the second most important cereal crop in Iran and accounts for about 22% of the total cereal area harvest (Anonymous, 2017). Barley, with a wider geographic range than almost every other crop species, is more productive and its yield is less variable than wheat and most other small grains. Therefore, it is widely used by farmers with limited and poor resources in less favorable climate and soil conditions such as those found in Iran (Abdipur and Vaezi, 2014; Mehari *et al*., 2014). However, grain yield as a typical quantitative trait has low heritability and climate change (e.g., temperature and rainfall) has a strong impact on it. On average each year more than 10% of the area under barley cultivation in Iran cannot be harvested because of low performance due to low rainfall (Anonymous, 2017). Therefore, the development of new barley genotypes that can perform satisfactorily in a wide range of environments, especially in years with low rainfall, is very important. Genotype performance in each test environment is a result of genotype main effect (G), an environment main effect (E) and genotype × environment interaction (GEI) (Yan and Kang, 2003). Although a high proportion of the total yield variation can be accounted for by E, it is only G and GEI that are relevant to cultivar evaluation and mega-environment classification (Yan *et al*., 2000; Yan, 2002; Yan and Rajcan 2002; Kaya *et al*., 2006). GEI, through minimizing the association between phenotype and genotype, decreases the genetic progress in plant breeding programs (Comstock and Moll, 1963). Therefore, GE interaction must be either avoided by selecting

widely adapted and stable genotype for use across the wide range of environments or exploited by selecting superior genotype for each specific target environment (Ceccarelli, 1989). Multi-environment trials (METs) are the best tool for estimating G and GE effects (Yan and Kang, 2003; Yan and Tinker, 2006). In METs, a number of promising genotypes along with local check cultivar (s) are tested for adaptability over a number of sites and years. To reveal patterns of GEI in METs, numerous statistical methods have been developed such as joint regression (Finlay and Wilkinson, 1963; Eberhart and Russel, 1966; Perkins and Jinks, 1968), rank-based (nonparametric) methods (Huehn, 1990), additive main effects and multiplicative interaction (AMMI) (Gauch, 1992) and type B genetic correlation (Burdon, 1977; Yamada, 1962). These methods are commonly used to analyze METs data and GEI in different cereals such as rice (Balakrishnan *et al*., 2016; Akter, *et al*., 2014), bread wheat (Hagos and Abay, 2013), durum wheat (Haddad *et al*., 2016), maize (Zhang *et al*., 2011; Dehghani *et al*., 2009) and barley (Abdipur and Vaezi, 2014). Yan (1999) and Yan *et al*. (2000) proposed a relatively new method known as GGE-biplot for the graphical display of GEI patterns of MET data that uses some of the functions of above-mentioned methods simultaneously and has many advantages over them. GGE-biplot has two concepts. First, although the measured yield is the combined effect of G, E, and GEI, only G and GEI are relevant to and must be considered simultaneously in genotype evaluation, thus it is named GGE. Second, as to the graphical display of the GGE of MET data, the biplot technique developed by Gabriel (1971) was employed; hence it is named GGE-biplot. A GGE-biplot is portrayed by using the first two principal components (PC1 and PC2) and allows a visual examination of the relationships among the test environments, G, and GEI. When a GGE-biplot is used properly, it is better than any other method (e.g., "which-won-where" pattern) for mega-environments delineation and genotype evaluation, whereby specific genotypes can be introduced to specific mega-environments, genotype evaluation (the mean performance and stability), and environmental evaluation (the power to discriminate among genotypes in target environments). It is also very effective in test environment evaluation. GGE-biplots are simpler to construct than other methods such as AMMI graphs. Different views of the same GGE biplot can be used to address all three aspects of MET data analysis whereas a different graph has to be constructed in AMMI analysis to address each aspect. GGE-biplots are more informative than graphs made with other techniques (e.g., AMMI graphs) because their inner-product property allows information on the performance of each genotype in each environment to be preserved (Yan, 2011). The ability of GGE-biplot to mega-environment analysis. These abilities have led many researchers to use this technique in different crops (Zhang *et al*., 2016; Teodoro *et al*., 2015; Lakew *et al*., 2014; Xu *et al*., 2014; Naroul Rad *et al*., 2013; Mohammadi and Amri, 2012). Some studies have been done to understand GEI for effective barley line selection under rainfed conditions (Mohammadi *et al*., 2015; Mortazavian *et al*., 2014; Ahmadi *et al*., 2012; Mohammadi *et al*., 2012; Mohammadi *et al*., 2011; Mohammadi *et al*., 2009) or cold site (Koocheki *et al*., 2012). However, insufficient information exists with regard to the nature and magnitude of GEI patterns on barley genotypes in the warm locations of Iran under irrigation conditions, especially with new promising genotypes. Therefore, this study was initiated to evaluate the performance and quantify the magnitude of GEI stability of barley genotypes for grain yield across warm testing environments under irrigated conditions.

MATERIALS AND METHODS

Plant materials, testing locations and planting

A total of 18 improved barley genotypes along with checks, i.e., genotype G1 (Nimroz cultivar) and G20, were evaluated during two cropping seasons (2014-15 and 2015-16) at six different sites, including Ahvaz, Moghan, Darab, Gorgan, Zabol and Varamin, which represent the broad range of popular locations for barley cultivation in Iran. Details of agro-climatic characteristics of test environments are given in Table 1. Each trial was a randomized complete block design with three replicates. Sowing was done in 1.2 m × 6 m plots (7.2 m^2), consisting of six rows with 20 cm row spacing. The seeding rate was about 350 seeds m^{-2}. Fertilizers were applied based on soil tests at each station. Usually, fertilizer application was 32 kg N ha^{-1} and 100 kg P_2O_5 at planting (ZGS 00) and 40 kg N ha^{-1} at stem elongation (ZGS 31) and before heading (ZGS 40) stages (Zadoks *et al*., 1974). No disease was observed during the growth period and weeds were controlled using herbicides (Granstar and Puma Extra). Irrigation of barley genotypes was carried out during the growing season based on environmental conventions.

Table 1. Agro-climatic characteristics of testing environments

Location	Year	Mean yield (ton ha^{-1})	Latitude & Longitude	Altitude (m)	Rainfall (mm)
Ahvaz	2014-15	4.11	31.3183°N, 48.6706° E	17	0.92
	2015-16	3.68			0.45
Mugan	2014-15	4.41	54.2492° N, 6.9683° W	1353	0.42
	2015-16	3.16			1.14
Darab	2014-15	2.47	28.7560° N, 54.5547° E	1168	0.79
	2015-16	3.84			0.90
Gorgan	2014-15	3.94	36.8456°N, 54.4393° E	129	0.95
	2015-16	4.22			1.20
Zabol	2014-15	4.49	31.0324° N, 61.4902° E	483	0.30
	2015-16	4.51			0.45
Varamin	2014-15	5.50	35.3252° N, 51.6472° E	922	0.24
	2015-16	4.36			0.29

Data collection and statistical analysis

Grain yield data in kg plot-1 was taken from an area of 6 m^2 and converted in tons per hectare at 12% moisture content. Data normality test, homogeneity test of variances and analysis of variance were performed using system analysis software (SAS, 2010). Pearson correlation coefficients among testing environments were computed using the PROC CORR SAS (SAS, 2010). To present an ideal graphical display of the relationships among the genotypes and test environments, GGE-biplot software (Yan, 2001) was used. GGE-biplot software uses a model based on singular value decomposition (SVD) of the first two principal components (Yan, 2002) as follows:

$$Y_{ij} - \mu - \beta_j = \lambda_1 \xi_{i1} \eta_{j1} + \lambda_2 \xi_{i2} \eta_{j2} + \varepsilon_{ij}$$

where: *Yij* is the yield mean of *i*th genotype in *j*th environment, μ is the grand mean, β*j* is the main effect of environment *j*, μ+β*j* is the mean yield across all genotypes in environment *j*, $\lambda 1$ and $\lambda 2$ are the singular values (SV) for the first and second principal component (PC1 and PC2), respectively, $\xi i1$ and $\xi i2$ are the eigenvectors of hybrid *i* for PC1 and PC2, respectively, η1*j* and η2*j* are the eigenvectors of environment *j* for PC1 and PC2, respectively, and ε*ij* is the residual associated with genotype *i* in environment *j*.

RESULTS AND DISCUSSION

The analysis of variance showed significant differences ($p<0.01$) for G, E and GEI among evaluated barley genotypes. This result indicated that yield barley genotypes were significantly influenced by the environment that accounted for the largest part, namely 49.3% of the total yield variation, whereas G and GEI accounted for 4.4% and 25.1% of the variation, respectively (Table 2). The high contribution of E compared with G and GEI in explaining the variance has been reported in other studies (Mesapogu *et al*., 2016; Mortazavian *et al*., 2014; Jalata, 2011). GEI is composed of five significant components (IPCA) along with their contribution of sum of square (SS) with decreasing importance (Table 2). The first two explained about 61.17% variance of GEI.

The magnitude of GEI compared to G (almost six times) revealed that there was a

Table 2. Combined analysis of variance for grain yield of barley genotypes across 12 environments in Iran

Source of variation	Degree of freedom	Sum of square	Mean square	Explained variance (%)
Genotypes (G)	19	26.158	1.376**	3.44
Environment (E)	11	374.847	34.077**	49.29
Replication (E)	24	38.188	1.591**	5.02
G×E	209	190.782	0.913**	25.09
IPCA1	29	73.521	2.535**	38.53
IPCA2	27	43.207	1.6**	22.64
IPCA3	25	17.387	0.695**	9.13
IPCA4	23	16.077	0.699**	8.42
IPCA5	21	12.999	0.619**	6.81
Residual	84	27.591	0.328	14.47
Error	456	130.462	0.286	17.16

Repeatability (R^2) = 0.78, Broad sense heritability (H^2) =0.61, Coefficient of variation (%) = 13.15, Mean= 4.06 t ha^{-1}

differential yield performance among barley genotypes across testing environments and suggested the possible existence of different mega- environments. It is well known that the yield ranking of genotypes across environments may be a mixture of crossover and non-crossover types of GEI (Solonechnyi *et al*., 2015; Abdipur and Vaezi, 2014; Jalata, 2011; Kaya *et al*., 2006). As shown in Table 3, there is an inconsistent yield ranking for genotypes across environments and different genotypes performed maximum grain yields in different environments including genotypes G13 (in E3), G20 (in E5), G9 (in E6), G3 (in E8), G1 and G18 (in E9), G12 and G16 (in E10),

Table 3. Genotype and environment code, mean grin yield (t ha^{-1}) of 20 barley genotypes across 12 test environments in Iran

Genotype Code	Name,/Pedigree	Year 2014 E1	E2	E3	E4	E5	E6	2015 E7	E8	E9	E10	E11	E12	Mean Yield (t ha^{-1})
G1	NIMROZ	4.01	2.64	4.23	3.59	5.49	4.77	3.78	3.90	5.34	4.74	4.12	4.78	4.28 (cdef)*
G2	Kmk//Rbr/Wa2196-68/3/EBC(A)/4/ICNB93-369	3.78	1.80	4.19	3.62	4.93	6.10	3.86	3.69	4.48	3.92	2.88	3.87	3.93 (bcde)
G3	WI2219//Mza/DL71/3/WI2198/Emir/4/ICNB93-328	4.51	2.97	4.78	4.23	4.11	4.91	3.98	4.70	4.98	4.36	3.28	5.00	4.32 (abc)
G4	73M4-30/Rihane-03	4.22	1.55	4.44	4.35	6.09	5.50	3.74	3.83	4.24	4.25	2.52	4.02	4.06 (abcde)
G5	Rihane-03/3/Rihane//Aths/BC	4.20	2.82	4.59	3.88	4.52	6.25	3.69	4.03	4.19	3.21	2.55	3.81	3.98 (abcd)
G6	Johoob/4/Post//Copal"s"/Gloria"s"/3/Kavir	4.37	3.40	4.14	4.02	5.99	5.04	3.71	3.47	4.03	4.59	3.93	3.94	4.22 (f)
G7	L.527/1-BC-80100	4.22	2.17	4.30	4.19	4.57	4.61	3.72	4.04	4.08	4.28	2.73	4.22	3.93 (abcd)
G8	Kavir/Badia//1-BC-80073	4.12	3.02	4.35	3.89	4.51	6.84	3.81	3.92	4.49	4.35	3.24	4.50	4.25 (ab)
G9	Teran 78/1-BC-80411	3.98	1.96	5.02	3.85	4.75	7.07	3.67	3.60	4.74	3.27	2.68	3.84	4.04 (abcd)
G10	Ashar/5/L.527/Chn-01//Gostoe/4/Rhn-08/3/Deir Alla106//DL71/Strain205	4.20	2.51	4.56	3.94	4.68	5.33	3.45	4.52	4.82	3.54	2.40	4.66	4.05 (cdef)
G11	LB. Iran/Una 8271//Gloria"S"/3/Kavir/4/Arigashar	3.95	2.20	4.02	3.69	5.38	5.58	4.12	4.00	4.71	4.44	3.40	4.20	4.14 (bcde)
G12	73M4-30/Rihane-03	3.88	1.62	4.53	4.17	3.19	5.31	3.37	3.40	4.21	4.88	2.88	4.69	3.85 (abcd)
G13	Johoob/4/Post//Copal"s"/Gloria"s"/3/Kavir	4.59	3.00	5.98	4.48	3.61	5.92	3.39	3.53	5.11	4.07	3.72	4.78	4.35 (ef)
G14	Johoob/4/LB.Iran/una8271//Gloria"s"/3/L.Moghan	3.85	2.09	4.61	3.81	2.07	6.00	4.14	3.50	4.01	4.19	3.21	3.85	3.78 (abcd)
G15	Johoob/4/LB.Iran/una8271//Gloria"s"/3/L.Moghan	3.84	2.07	4.60	3.62	2.53	4.99	4.09	3.67	4.54	4.18	3.55	4.06	3.81 (f)
G16	Hml/Bichy	3.92	3.42	3.94	3.66	2.90	5.02	3.18	3.83	4.34	4.90	3.49	5.20	3.98 (a)
G17	Shuyler/3/ M.Rnb86.80/NB 2905//L.527/4/ICNB93-328	3.94	2.20	4.18	3.85	4.62	4.87	3.41	3.19	3.75	4.09	3.03	3.65	3.73 (def)
G18	LB.Iran/Una 8271//Gloria"S"/3/Kavir/4/Arigashar	3.89	2.67	4.42	3.54	4.52	5.73	3.34	4.16	5.50	4.26	3.32	5.26	4.22 (abcd)
G19	CIRU/M111	4.21	3.38	3.88	3.93	4.25	4.83	3.49	4.06	4.02	4.62	3.23	4.66	4.05 (def)
G20	(GOB/ALELI//CANELA/3/ARUPO*2/JET/4/ARUPO/K8755//MORA) EBYT-W-90-15	4.58	2.07	5.08	4.63	6.30	5.35	3.80	3.88	4.74	4.40	3.18	4.32	4.36 (ef)
Mean		4.11	2.48	4.49	3.95	4.45	5.50	3.69	3.85	4.52	4.23	3.17	4.36	4.06

Skwness= 1.07; Kurtosis= 1.46; Kolmogorov-Smirnov= 0.247$^{n.s}$; Shapiro-Wilk= 0.421$^{n.s}$
Bartlett's test Chi-square=11.237$^{n.s}$

E1: Ahvaz-01, E2: Moghan-01, E3: Darab-01, E4: Gorgan-01, E5: Zabol-01, E6: Varamin-01, E7: Ahvaz-02, E8: Moghan-02, E9: Darab-02, E10: Gorgan-02, E11: Zabol-02, E12: Varamin-02 (01 and 02: 2014-2015 and 2015-2016 cropping seasons, respectively).

***Means followed by the same letter are not significantly different at the 5% probability level (Least Significant Different (LSD) value (α=5%)).**

n.s: Not significant at 5% level.

G1 and G6 (in E11) (Table 2). This result shows the presence of possible crossover GEI as described by Baker (1988), Crossa (1990), Yan and Hunt (2001) and Kaya *et al*. (2006). This crossover GEI suggests the existence of a different mega-environment in which different winning genotypes can be selected. The above results are consistent with crossover GEI reports for barley genotypes in other studies (Solonechnyi *et al*., 2015; Mehari *et al*., 2014; Jalata, 2011; Dehghani *et al*., 2006; Jackson *et al*., 1993; Van Oosterom *et al*., 1993; Ceccarelli and Grando, 1991; Ceccarelli, 1989). However, in other environments, three or more genotypes exhibited maximum performance. On the other hand, some genotypes had maximum yield in more than one environment, including genotype G1 in E9 and E11, G3 in E1, E7, E8 and E12, G6 in E2 and E11, G12 in E2, E10 and E12, G13 in E1, E3 and E4, G18 in E9 and E12, and G20 in E1, E4 and E5 (Table 3). This suggests that another possible known GEI exists, i.e., a non-crossover GEI.

GGE was partitioned through GGE-biplot and the first two PCs explained 46% (PC1= 24.6% and PC2= 21.4%) of the total GGE variation (Fig. 1). A GGE-biplot based on genotype focused scaling is a useful tool to graphically visualize the locations of genotypes on a biplot. A high correlation (r= 0.914) (data not shown) is obtained between genotype PC1 scores and genotype main effects for the dataset so that the yielding ability, genotype stability, discriminating and representativeness of environments can be efficiently visualized on the graph (Yan 1999; Yan *et al*., 2000).

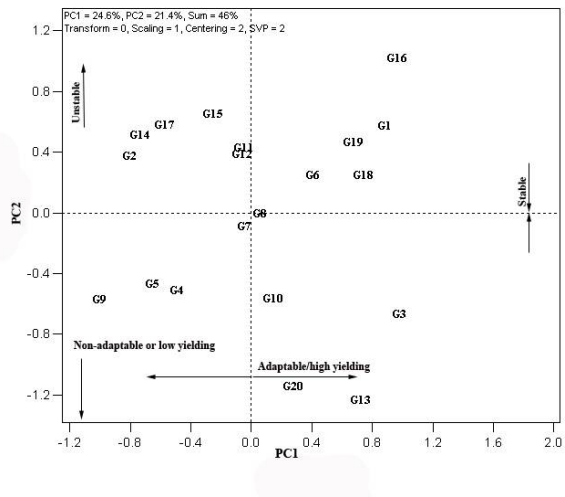

Fig. 1. GGE-biplot based on genotype focused scaling for barley genotypes

Accordingly, genotypes with PC1 scores greater than zero were high yielding (except genotypes G20 and G13) while genotypes with PC1 scores less than zero were identified as lower yielding or non-adaptable (except for genotypes G7, G11, G12 and G17) (Table 3 and Figure 1). However, some inconsistencies were observed because the biplot did not explain 100% GGE variation (Yan, 2002). On the other hand, PC2 is associated with genotypic stability or instability on the biplot graph and the high yielding genotypes can be divided into stable and unstable groups. Based on this, genotypes G8, G6 and G18 were high yielding as well as stable, since their absolute PC2 score is near zero. Whereas, the other group consisted of five high yielding but unstable genotypes: G19, G1, G16, G10 and G3 had larger absolute PC2 scores (Figure 1).

Identification and classification of mega-environments and winning genotypes

Determining the possible existence of different mega-environments in the target environment and winning genotype in each mega-environment is necessary (Gauch and Zobel,1997; Yan *et al*., 2000, 2001). The best way to visualize the interaction patterns between genotypes and environments is the polygon view of a biplot (Yan and Kang, 2003). For this purpose, the polygon is portrayed by connecting the markers of genotypes that are further away from the biplot origin such that all genotypes are contained in the polygon (Kaya *et al*., 2006). This polygon view shows the presence or absence of crossover GEI which is helpful in estimating the possible existence of different mega-environments which is critical (Yan and Rajcan, 2002; Yan and Tinker, 2006). Based on this, the genotypes on vertices of the polygon are either the best or poorest in one or more testing environment and the vertex genotype in each sector is the best genotype for all environments in the relevant sector because sites within the same sector share the same winning genotype (Yan 1999, 2002; Yan *et al*., 2000). As shown in Figure 2, eight lines/rays that are drawn perpendicular to the sides of the polygon divide it into 8 sectors; out of these sectors, environments fall into only four of them. Three environments (E2, E10, and E11) are located in sector 1 and the winning genotype for these environments which is located in the vertex was genotype G16. Sector 2 is defined by rays 2 and 3 and contained only E12. Five environments (E1, E3, E4, E5, E8 and E9) are located in sector 3 that is delineated by rays 3 and 4. Selecting only one

winning genotype in this section is difficult and three genotypes, G3, G13, and G20, simultaneously have the qualification of a winning genotype. In sectors 5 and 7, genotype G9 and G14 were recognized as the winning genotypes, respectively. However, none of the genotypes were in the remaining three sectors i.e., 4, 6 and 8.

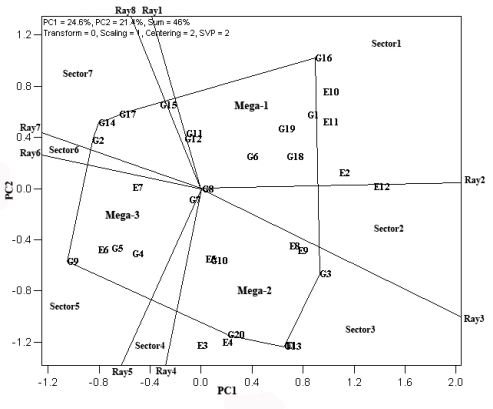

Fig. 2. Polygon views of the GGE-biplot based on symmetrical scaling for the which won where pattern of genotypes and environments

According to the mega-environment definition by Gauch and Zobel (1997), it appears that there are three possible mega-environments. For this purpose, E12 in sector 2 was intentionally combined with E2, E11 and E10 in sector 1 to constitute Mega-1 as these environments were similar to each other (Fig. 2, Table 3) and also had a strong positive correlation (Figure 2, Table 4). Thus, the first mega-environment (Mega-1) was comprised of four environments (E2, E10, E11, and E12) which are were located in sectors 1 and 2 with the genotype P being the best winner in these environments. The second mega-environment (Mega-2), with six environments (El, E3, E4, E5, E8, and E9), was large compared to Mega-1. As previously mentioned, three genotypes (G3, G13, and G20) had the same qualification as winning genotypes.

The third mega-environment (Mega-3) was comprised of two environments (E6 and E7) that were contained in sector 5 and G9 was the winning genotype. However, some genotypes, G2, G14, G15 and G17, were located in sector 7 for which there is no environment. GGE-biplot has already been used by many researchers to

Table 4. Correlation coefficients among 12 test environments in Iran

	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12
E1	**1**											
E2	**0.368**	**1**										
E3	**0.517***	**-0.129**	**1**									
E4	**0.820****	**-0.100**	**0.584****	**1**								
E5	**0.395**	**-0.068**	**-0.115**	**0.265**	**1**							
E6	**-0.147**	**-0.158**	**0.358**	**-0.091**	**-0.016**	**1**						
E7	**-0.049**	**-0.311**	**-0.013**	**-0.074**	**0.050**	**0.126**	**1**					
E8	**0.325**	**0.290**	**-0.075**	**0.024**	**0.162**	**-0.144**	**0.101**	**1**				
E9	**0.147**	**0.097**	**0.406**	**-0.091**	**0.145**	**0.143**	**-0.021**	**0.454***	**1**			
E10	**-0.021**	**0.220**	**-0.380**	**0.040**	**-0.070**	**-0.581****	**-0.095**	**-0.109**	**-0.043**	**1**		
E11	**0.079**	**0.502***	**-0.023**	**-0.195**	**-0.069**	**-0.301**	**0.103**	**-0.192**	**0.314**	**0.603****	**1**	
E12	**0.168**	**0.475***	**0.044**	**0.001**	**-0.185**	**-0.264**	**-0.462***	**0.509***	**0.602****	**0.482***	**0.328**	**1**

***,**: significant at 5% and 1% probability level, respectively.**

mega-environment analysis in barley plants (Jalata, 2001; Solonechnyi *et al*., 2015; Dogan *et al*., 2016; Kendal *et al*., 2016).

Evaluation of relation among test environments

To estimate the pattern of environments, a GGE-biplot that depends on environment-focused scaling was portrayed (Figure 3). Environment PC1 and PC2 scores had both negative and positive scores indicating that there was a difference in the rankings of yield performance among genotypes across environments. This inconsistency indicates the presence of crossover GEI. Similar findings have been reported by Mortazavian *et al*. (2014) and Zhang *et al*. (2016). However, environment PC1 scores in this study showed GEI components against reports (Mohammadi *et al*., 2015; Yan and Hunt, 2001; Yan *et al*., 2000) that indicated PC1 for non-cross, and also PC2 scores showed GEI components against several reports (Solonechnyi *et al*., 2015; Jalata, 2011) that indicated non-crossing over for PC2.

Although the correlation between the environment can be used to determine the relationship between them (Table 4), the vector view of GGE-biplot (Figure 3) gives a succinct view of the correlation among the environments. A GGE-biplot based on environment focused scaling for environments was portrayed to visualize the relationship between environments. For this purpose, lines were drawn to connect the test environments to the biplot

origin, known as environment vectors, and the cosine of the angle between the two environments was used to approximate the relationship between them (Kaya *et al*., 2006; Yan and Tinker, 2006). Based on this, all environments which are found in each quadrant (II, III or IV) were positively correlated with each other as the angle between them was less than 90° (i.e., an acute angle) which was also true for environments in quadrants II, III and IV. Regardless of the environment within

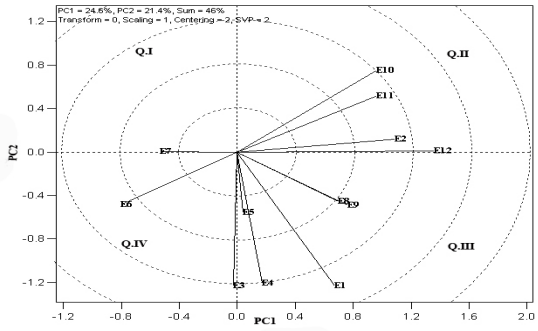

Fig. 3. GGE-biplot based on genotype focused scaling for test environments

a quarter, there was also a positive relationship among environments from different quarters. For instance, E10, E11, E2 and E12 of quadrant II were positively correlated with E8 and E9 of quadrant III and there was also positive relationship between E2 and E1, E12 and E1, E4 and E5, E6 and E5 and E4, E6 and E7. On the other hand, the angle between E11 and E1, E2 and E4 or E5, E12 and E3, E1 and E6, E3 and E7 were nearly 90°; hence the correlation between them was close to 0. This no association was also shown in Table 4.

However, there were some discrepancies between these two methods. Based on GGE-biplot (Figure 3), there was a very close correlation between E4 and E5, but the actual correlation was not significant (Table 4). Furthermore, E5 and E3 showed a positive correlation in Figure 3, but these environments showed a negative correlation in Table 4. However, GGE-biplot is not 100% efficient and these inconsistencies are to be expected (Yan, 2002; Kaya *et al*., 2006; Jalata, 2011). Based on Figure 3, there was a negative correlation between E7 and all the remaining environments (except with E6 and E5) and there also existed a negative correlation between E10 and all the remaining environments (except with E2, E4, and E11) as the angle between them was greater than 90° (i.e., an obtuse angle). Such a wide obtuse angle represents strong negative correlations among environments and suggests a high crossover GEI (Yan and Tinker, 2006).

It is well understood that testing environments with a close association reveals similar information about the genotype, therefore, this information could be obtained from fewer test environments and could reduce testing costs where resources are limited (Jalata, 2011; Yan and Tinker, 2006; Kaya *et al*., 2006). The phenotypic correlation between environments may be used to study indirect response to the selection when there are no correlations of error effects among testing environments (Cooper and Delacy, 1994). Hence, indirect selection may be carried out for the same character if measured on the same genotypes in different environments. Test environment E12 was significantly positively correlated with E2, E8, E9 and E10 environments. On the other hand, E2 and E10 were significantly positively correlated with E11. Furthermore, E3 or E4 were correlated well together and also with the E1 environment. Beside this, E6 and E7 were also significantly positively correlated (Table 4). This suggests that indirect selection could be effective for grain yield in testing environments showing a significant positive correlation. The existence of a significant correlation between environments showed that the obtained information was similar enough that testing environments may be reduced to minimize cost without significantly affecting the validity of information.

Discriminating ability and representativeness of test environments

It is well understood that the discriminating power and the representativeness view of GGE-biplot is an important measure of testing environments (Dehghani *et al*., 2006). Therefore, a GGE-biplot was drawn and the length of concentric circles on the biplot were used to visualize the length of the environment vectors which is proportional to standard deviation within the respective environments on the biplot and also shows the discriminating ability of the environments (Yan, Tinker, 2006). As shown in Figure 4, E1 (Ahvaz-01) and E12 (Varamin-02) with long vectors were the most discriminating, while E5 (Gorgan-01) and E7

(Ahvaz-02) were the least discriminating environments. On the other hand, test environments which are consistently non-discriminating provide little information on the genotype differences (Jalata, 2011; Yan and Tinker, 2006) and/or the performances of all genotypes in the testing environment were uniform.

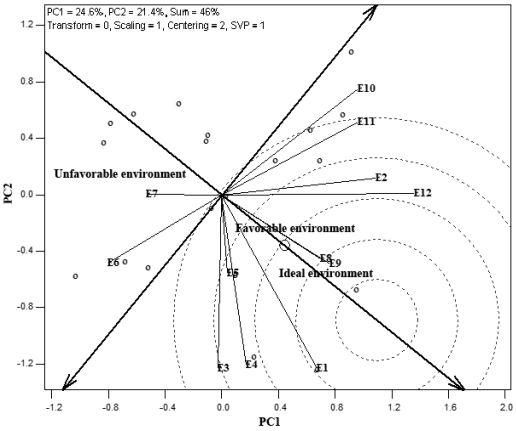

Fig. 4. GGE-biplot based on discriminative ability and representativeness view of test environments to select ideal environment

However, a GGE-biplot based on the average environment axis (AEA) (Yan *et al*., 2001) can help to get a better view of the discriminating power and representativeness of a testing environment. The AEA line passes through the average environment and biplot origin. The average environment has the average coordinates of all test environments and was represented by a small circle. According to Yan and Tinker (2006), a test environment that has a smaller angle with the AEA is more representative of other test environments. Based on this, E2 (Moghan-01) was the most representative environment, whereas E6 (Varamin-01) and E7 (Ahvaz-02) with a large deviation from AEA were the least representative. Zhang *et al*., (2016); Jalata (2011); Yan and Tinker (2006) showed good test environments for selecting generally adapted genotypes that are both discriminating and representative. Accordingly, E8 (Moghan-02) and E9 (Darab-02) were two desirable test environments for selecting widely adapted genotypes. On the other hand, testing environments that are discriminating but non-representative, such as E6 (Varamin-01), are useful for selecting specifically adapted genotypes if the target environment is divided in to mega-environments (Yan and Tinker, 2006). However, some environments, such as E7 (Ahvaz-02) and E5 (Zabol-02), have very short vectors and are known as non-discriminating testing environments (Jalata, 2011; Yan and Tinker, 2006). These environments are less useful, and that may be due to unfavorable rainfall conditions such as reduced rainfall and increasing temperature, especially at the end of the growing season (Table 2).

Although the ideal test environment should be both highly discriminating and most representative, compared to other environments (e.g., in the center of concentric circles). Under natural conditions such environment does not exist but could be used as a reference (Kaya *et al*., 2006; Yan and Tinker, 2006). However, the favorable test environments must have large PC1 scores (more discriminating genotypes) and near zero PC2 scores (more representative of an average environment) (Yan *et al*., 2001). Accordingly, both E8 (Moghan-02) and E9 (Darab-02) were the ideal test environments where the best genotypes could be most easily identified (Fig 5). Based on Figure 5, the concentric circles following the ideal test environment (except E6 and E7) were favorable environments. For instance, E1 (Ahvaz-01) was more favorable than E4 (Gorgan-01) and E12 (Varamin-02). This may be due to better yielding conditions at Ahvaz-01 such as an increase in rainfall during flowering and grain filling (Table 2).

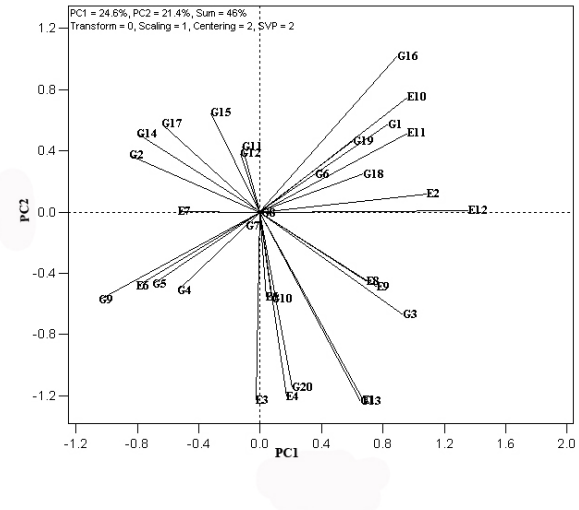

Fig. 5. GGE-biplot view to show the performance each barley genotype in each test environment

The relationship between barley genotypes and testing environments

Both the genotype and environmental vectors were portrayed on a biplot and the performance of each genotype in each environment was determined according to the angle between its vector and the environment's vector (Fig. 6). As shown in Fig. 6, different barley genotypes showed different responses in different environments. Based on this, genotypes G1, G6, G16 and G19 in E10, G2, G14, G17, G15, G11 and G12 in E7, and G7, G4, G5 and G9 in E6 performed well than in others, because the angle

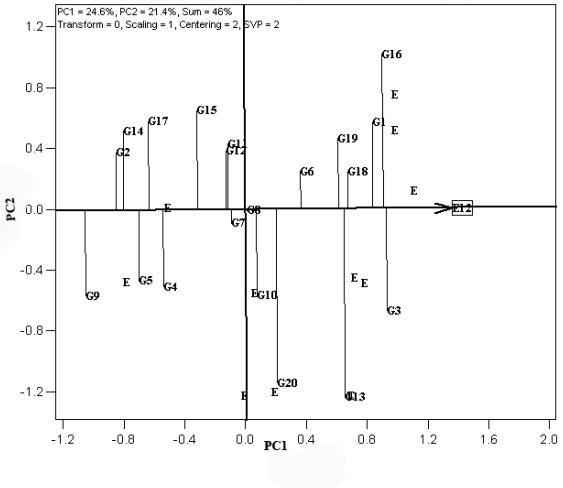

Fig. 6. Ranking genotypes based on performance in E12

between its vector and the environment's vector is less than 90°. On the other hand, genotypes G3 (in E8 and E9), G18 (in E11), G13 (in E1) and G20 (in E4) performed specifically better than in others. However, genotypes G11, G12 or G15, with an angle of about 90°, had poor or near average performances in most of the environments. Genotype G8 had a very short vector located nearer to the biplot origin than other genotypes. Such a genotype has an average value in each of the environments and also has a minimum contribution to both G and GE interaction. On the other hand, genotype G16 with the longest vector is the best genotype, while genotype G14 with the longest vector is the poorest or the most unstable. These findings are consistent with Yan and Tinker (2006).

Ranking genotypes based on performance in a specific environment (E12)

To visualize ranking genotypes based on their performance in an environment, a line which is called the axis for this environment, is drawn that passes through the biplot origin and the environment (E12 in this study) and along it is the ranking of genotypes (Yan and Tinker, 2006; Jalata, 2011). As shown in Figure 7, genotypes from G7 to G9 below the perpendicular line to the axis had a lower than average yield in E12. On the other hand, genotypes G3 to G20 had higher than average yield in E12, while genotype G10 showed a nearly average yield performance. Based on Fig. 7, genotype G3 was the highest yield in E12 followed by G16, in contrast to this, genotype G9 gave an inferior yield. This large difference in response among genotypes is mainly due to genotypic and genotypic and environmental interaction.

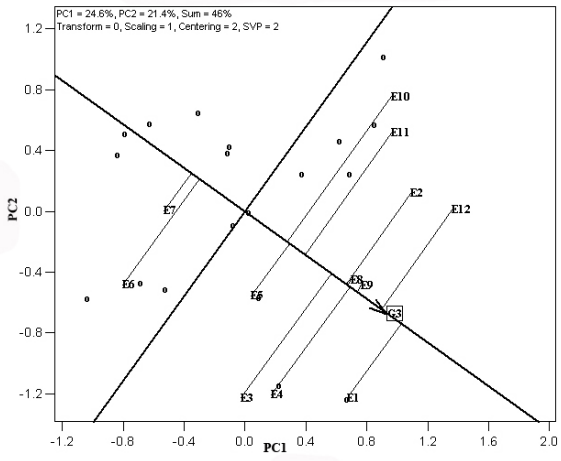

Fig. 7. Ranking environments based on the performance of genotype G3

Ranking environments based on the performance of a specific genotype

To visualize specific adaptations of a genotype across testing environments and the ranking of the test environments relative to the performance of that genotype, a line is drawn to pass through the biplot origin and genotype G3 as genotype and environments are ranked along that axis. As shown in Fig. 8, genotype G3 in all environments except E6 and E7 (which had a nearly average) had a higher than average performance. However, it performed better in E8 and E9 environments than the other remaining genotypes.

Determine the mean performance and stability of barley genotypes

It is well known that stability is meaningful only when associated with high mean

performance (Jalata, 2011; Yan and Tinker, 2006; Dehghani *et al*., 2006). Thus, to evaluate barley genotypes based on both mean

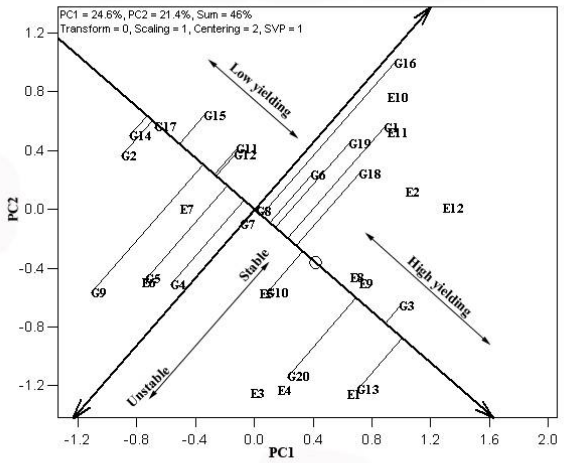

Fig. 8. Average environment coordination (AEC) views of the GGE-biplot based on environment focused scaling for the mean performance and stability of barley genotypes

performance and stability across environments, a GGE-biplot with an average environment coordination (AEC) view was drawn. As seen in Fig. 9, the AEC line is abscissa which points to a higher mean yield across testing environments or to a greater genotype main effect and the AEC ordinate is indicated by double arrows in either direction away from the biplot origin indicating greater GEI effect and reduced stability (Kaya *et al*., 2006; Yan and Tinker, 2006). Thus, the AEC ordinate separates genotypes with above average means (e.g., genotypes G13 to G16), from those with below average means (e.g., genotypes G7 to G14 except for G7) (Fig. 9). It should be noted that the length of the average environment vector relative to the biplot is the measure of the relative importance of the genotype main effect and GEI such that the longer the vector is, the more important the genotype main effect is, indicating the a more meaningful selection based on mean performance (Kaya *et al*., 2006). Accordingly, genotypes G3, G13, and G20 had the highest mean yield across environments, whereas, genotypes G14, G2 and G17 were the lowest yielding genotypes across test environments. On the other hand, genotypes G9 and G8 with the longest and shortest genotype vector were highly unstable (poorly stable) and the most stable, respectively. Therefore, genotype with above average mean performance (e.g., G3, G13, and G20) could be selected for future breeding whereas the remaining genotypes may be discarded.

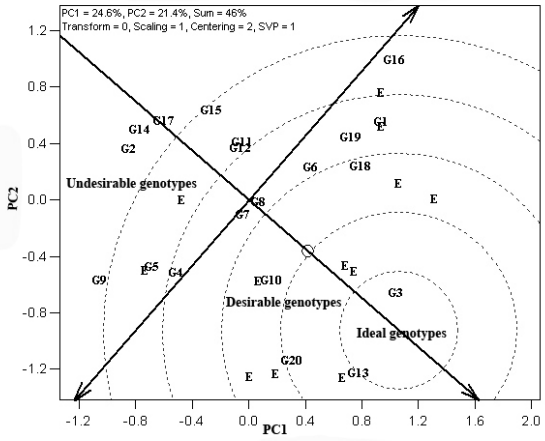

Fig. 9. Comparison of barley genotypes with ideal genotype (G3)

However, stability without high mean performance is meaningless and high yielding and stable genotypes should have large PC1 scores but near zero absolute PC2 scores and such genotypes are more easily identified at locations with large PC1 scores but near zero PC2 scores (Jalata, 2011; Yan and Tinker, 2006; Yan *et al*., 2000).

Fig. 9 presents a ranking of genotypes based on mean and stability to find an ideal barley genotype. An ideal genotype is on the positive direction and has a vector length equal to the longest vectors of the genotypes on the positive side of AEA with the largest vector length of high yielding genotypes and indicated by an arrow pointed to it (Jalata, 2011; Kaya *et al*., 2006; Yan and Tinker, 2006). Based on this, genotype G3 which was located in the center of concentric circles was an ideal genotype and genotypes located closer to the ideal genotype (G13) are more desirable than the others. On the other hand, the farthest genotypes from the center of concentric circles (e.g., genotypes G17, G14 and G2) were undesirable.

Comparison ideal genotype with other genotypes

To visualize the comparison of two genotypes, a

GGE-biplot was drawn by connecting their markers with a straight line and drawing a perpendicular line/equality line that passes through the biplot origin (Fig. 10). Genotypes with better performances were located on its side of the equality line (Jalata, 2011; Yan and Tinker, 2006; Yan *et al*., 2000). As it can be seen in Fig. 10, the performance of an ideal genotype (G3) was better than genotype G9 in almost all test environments except E6 and E7 while genotype G9 had the best performance in E6 and E7. The difference between the two genotypes by environment indicates the presence of crossover interaction. This difference may be due to different reasons including the difference in rainfall, temperature, maturity, disease and etc.

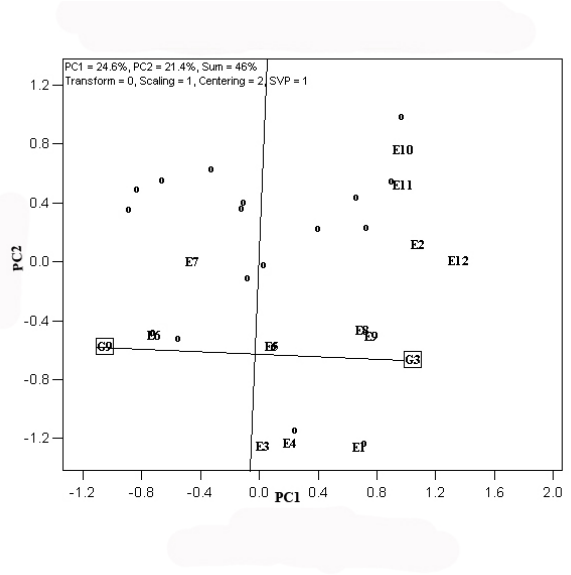

Fig. 10. Comparing two barley genotypes (G3 and G9) across test environments

CONCLUSIONS

The yield variation and yielding pattern of barley genotypes were highly varied within small geographical locations in Iran. Therefore, assessing yield performance and stability for effective breeding line selection and cultivar recommendation across different testing environments is vital. The results of the present study indicated barley grain yield performances were highly influenced by environment followed by GEI (that effect was about six times greater than genotype effect) and genotype. Barley genotypes indicated crossover GEI across the environments and there were desirable genotypes in terms of high mean yield in testing environments. Test environments were divided into three possible mega-environments (Mega-1, 2 and 3). Genotypes G16 (in Mega-1) and G9 (in Mega-3), as the best winning genotypes, showed (104.4% and 121.77%) and (125.76% and 117.51%) overall yield advantage over check genotypes (G1 and G20), respectively. This indicates how much specific adaptation is more important than wide adaptation in barley growing areas of Iran. Despite the superiority of genotypes G3 and G13 to G1, none of the genotypes G3 and G13 did not lead to another check genotype namely G20 in mega-2, and this genotype showed 107.03% overall yield advantage over both genotypes G3 and G13, respectively. This superiority shows the correct choice of genotype G20 as a genotype with high yield performance and stability in Mega-2.

Therefore, the barley breeding program in the Seed and Plant Improvement Research Institute of Iran should consider these three different mega-environments separately to maximize yield potential of barley through the exploitation of positive GEI. However, [further METs] by considering necessary environmental and biological variables [may be needed] to better clearly identify the magnitude of GEI and the causes of GEI.

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