Analysis of genotype, environment and genotype × environment interaction in bread wheat in warm rainfed areas of Iran

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ABSTRACT

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Stable performance of new improved bread wheat (*Triticum aestivum* L.) genotypes in multi-environment trials is important in crop improvement programs. In such trials, genotype evaluation and mega-environment identification are the most important objectives. The yield stability of 18 bread wheat genotypes was investigated through genotype (G) and genotype × environment (GE) interaction using the GGE biplot technique. Field experiments were conducted in 12 warm rainfed environments in Iran to characterize G × E interactions for grain yield of bread wheat genotypes. A combined analysis of variance across locations showed that both main effects (environment and genotype) and GE interactions were highly significant. Principal component analysis was performed, and the first two principal components (PC1 and PC2) explained 45% and 26% of the total sum of squares, respectively; they were used to create GGE biplot diagrams. The polygon tool of the biplot suggested two bread wheat growing environments in warm dryland regions of Iran: a small one (Moghan) and a large one (Gachsaran, Gonbad and Khoramabad). Visualizing the mean and stability parameters of the genotypes in the biplot indicated that genotypes HAMAM-4 (G1) and CHEN/AEGILOPS SQUARROSA (TAUS)//BCN/3/VEE#7/4/PASTOR (G4) are adapted to warm rainfed areas of Iran. The vector view of the biplot showed that Gonbad, Khoramabad and Gachsaran were correlated, but had no association with Moghan. Finally, it was concluded that G1 and G4 showed high yield stability across environments and are, therefore, recommended for release in warm rainfed areas of Iran.

Key words: adaptability, drought, multi-environment trials, grain yield stability.

INTRODUCTION

Stable wheat production is a major concern in rainfed areas that are affected by different environmental factors. Growing adapted cultivars with high yield stability is an effective strategy for reducing environmental effects on wheat production in rainfed areas. To develop suitable cultivars, evaluation of improved genotypes is a critical phase in wheat breeding programs, because great numbers of genotypes need to be evaluated across locations over several years. Studying the response of genotypes under different conditions may significantly increase their productivity potential and performance (Kang, 2002). Differential responses of genotypes from one environment to another are known as genotype by environment (GE) interaction. Information on the structure of G × E interaction is very useful to plant breeders, because it can help select genotypes with wide and specific adaptation (Lin *et al*., 1986; Flores *et al*., 1998). There are several statistical procedures for analyzing multi-environment trials and identifying suitable adapted genotypes with high mean grain yield and yield stability.

Advances in computer science have made it possible to use multivariate statistical procedures of data analysis with fast and precise algorithms (Annicchiarico, 1997; Gauch *et al*., 2008). Although univariate statistical procedures can detect interactive effects of variables, many researchers

recommend using multivariate statistical procedures because datasets for multi-environment trials are derived from multiple environments (Kempton, 1984; Gauch, 2006). However, multiplicative G × E interaction is far too complex to be summarized by one or two stability parameters. Multivariate statistical approaches have been introduced to explore the multi-directional aspects of data and extract more information from G × E interaction components (Zobel *et al*., 1988; Gauch and Zobel, 1996; Gauch *et al*., 2008). Because these procedures are statistically more complex, a specialized statistical package is sometimes needed, and results lead to interpretations that are more consistent with the reality of the trials.

The biplot technique (Gabriel, 1971) was expanded by Kempton (1984) and Zobel *et al*. (1988) for use in analyzing grain yield stability. A biplot is a scatter plot that graphically displays a two-way dataset by both factors in such a way that relationships between these factors can mask the real performance of the individual genotypes. Recently, the extensive usefulness of biplot, as GGE model [genotype main effect (G) plus GE interaction effect] was proposed by Yan *et al*. (2001). The GGE biplot technique is a simple procedure which in recent years has gained popularity and is being used to analyze data from multi-environment trials. It has strongly captured the attention of plant breeders and has been successfully employed to determine relationships among genotypes, environments and G × E interaction effects (Yan *et al*., 2007; Yan *et al*.,

2010). The biplot GGE [based on the site regression (SREG) model] is an effective graphical tool for exploring G × E interaction and analyzing grain yield stability.

The GGE biplot procedure has been successfully employed to determine the relationship among genotypes, environments, mega-environments and suitable genotypes (high yielding, with good yield stability) all over the world (Dehghani *et al*., 2006; Sabaghnia *et al*., 2008a). GGE biplot analysis is also increasingly being used by researchers in Iran to analyze G × E interaction of multi-environment trials of different crops such as barley (*Hordeum vulgare* L.) (Dehghani *et al*., 2006); wheat (*Triticum aestivum* L.) (Mohammadi *et al.*, 2007); lentil (*Lens culinaris* Medik.) (Sabaghnia *et al*., 2008a); and maize (*Zea mays* L.) (Dehghani *et al*., 2009). Most of these authors reported that the GGE biplot analysis can be used efficiently for visualizing the "which-won-where" pattern of multi-environment trials and for mega-environment identification.

The objectives of this study were to analyze and interpret the main effects of genotype and environment, as well as G × E interaction effects on grain yield of 18 bread wheat genotypes in 12 different environments under rainfed conditions using the GGE biplot technique.

MATERIALS AND METHODS

Eighteen bread wheat genotypes (17 advanced breeding lines and cv. Kouhdasht as the improved check) were evaluated (Table 1) using a randomized

Table 1. Pedigree of the 18 bread wheat genotypes .

	Name/pedigree
G1	HAMAM-4 ICW92-0477-1AP-1AP-4AP-1AP-0AP
G2	ZEMAMRA-8 ICW91-0157-3AP-0TS-4AP-0TS-3AP-0L-0AP
G3	CHEN/AEGILOPSSQUARROSA(TAUS)//BCN/3/VEE#7/BOW/4/PASTOR CMSS93B01854T-040Y-8Y-010M-010Y-010M-8Y-0M-1KBY-0KBY-0M-0HTY
G4	CHEN/AEGILOPSSQUARROSA(TAUS)//BCN/3/VEE#7/BOW/4/PASTOR CMSS93B01854T-040Y-8Y-010M-010Y-010M-10Y-0M-4KBY-0KBY-0M-0HTY
G5	SERI/RAYON CRG2753.1-0B-099Y-099M-28Y-0B
G6	TJN//GHK"S"/BOW"S"/3/SHIR
G7	SITTA/CHIL/IRENA CMBW91MO3952T-0TOPY-6M-010SY-015M-010SY-9SY-0M-0SY-0AP
G8	PIGO/PASTOR CMSS95M01046S-0100M-050SY-050M-050SY-030M-5SY-0M-0SY-0AP
G9	BERKUT CMSS96M05638T-040Y-26M-010SY-010M-010SY-4M-0Y
G10	SERI*3//RL6010/4*YR/3/PASTOR/4/BAV92 CMSS96M05696T-040Y-14M-010SY-010M-010SY3M-0Y
G11	PASTOR/ /HXL7573/2*BAU CMSS97M00406S-0P20Y-97M-010Y
G12	CROC_1/AE.SQUARROSA(213)//PGO/3/BABAX CMSS97M00814S-030M-040SY-010M-010SY-19Y-0M
G13	BAVIACORA M 92 CM92066-J-0Y-0M-0Y-4M-0Y-0MEX
G14	GHK"S"BOW"S"//90 -ZHONG87
G15	KATILA-11
G16	NESTOR/3/HE1/3*CNO79//2*SERI CMSS92M00092S-015M-0Y-0Y-050M-25Y-2M-0Y
G17	SERI82/SHUHA "S" ICW89-0018-7AP-0AP-1AP-0TS-0AP
G18	KOOHDASHT

complete block design (RCBD) with four replications. The trials were conducted in the 2007, 2008 and 2009 cropping cycles at Gachsaran, Gonbad, Khoramabad and Moghan field stations in Iran (the locations' agro-ecological properties are shown in Table 2). An experimental drill was used to sow 1.05×7.00 m^2 plots, consisting of six rows with 17.5 cm row spacing. The trials were sown (300 seed m^{-2}) and managed following recommended agronomic practices; 40 kg N ha^{-1} and 70 kg P$_2$O$_5$ ha^{-1} were applied at planting and 40 kg N ha^{-1} were applied as top dressing at the stem elongation stage. Harvesting was done in 0.70×6 m plots using an experimental combine.

Table 2. Geographical and meteorological properties of four test locations.

Location	Longitude Latitude	Altitude (m)	Soil texture	Rainfall (mm)
Gachsaran	50° 50' E 30° 20' N	710	Silty clay loam	433.7
Gonbad	55° 12' E 37° 16' N	45	Silty clay loam	367.5
Khoramabad	23° 26' E 48° 17' N	1148	Silt-loam	433.1
Moghan	48° 03´ E 39° 01´ N	1100	Sandy-loam	271.2

Due to severe drought conditions in 2007, data from Moghan were not used and only data from 11 location × year combinations (environments) were analyzed. Homogeneity of residual variances was determined using Bartlett's homogeneity test, and results indicated the homogeneity of variances (χ^2=18.2, P<0.05). A combined analysis of variance was performed to determine the effect of genotype (G), environment (E) and G × E interaction on grain yield. In the GGE biplot technique, genotype (G) and G × E interaction are investigated together by separating the G+GE effect from the observed mean. The GGE2 model is:

$$Y_{ij} = \mu + e_j + \sum_{n=1}^{N} \lambda_n \gamma_{in} \delta_{jn} + \rho_{ij}$$

where Y_{ij} is the grain yield of the ith genotype in the jth environment; μ is the grand mean; e_j is the environment deviation from the grand mean; λ_n is the eigenvalue of the principal component (PC) analysis axis n; γ_{in} and δ_{jn} are the genotype and environment eigenvectors for axis n; n is the number of PCs retained in the model, and ρ_{ij} is the error term (Yan and Kang, 2002). The environment standardized model was used to generate the "which-won-where" biplot, and a non-standardized model was used to analyze the relationship between genotypes and environments. The biplots were generated using "GGE biplot" software (Yan, 2001).

The relationships among test locations were studied using Pearson's rank correlation.

RESULTS AND DISCUSSION

The combined analysis of variance revealed that effects of genotype (G), environment (E), and G × E interaction were highly significant (Table 3). Significant G × E interaction indicated that at least one bread wheat genotype behaved differently in at least one of the test environments; it implies genetic variability and the possibility of selecting genotypes with high grain yield and good yield stability. Therefore, it is possible to proceed to estimate phenotypic stability (Annicchiarico, 1997; Gauch *et al.*, 2008). Genotype × environment interaction makes it difficult to select the best performing, most suitable genotype with good yield stability because it reduces the efficiency of selection in plant breeding programs. The combined analysis of variance also showed a significant environment effect (Table 3). The contribution of environment to total (G + E + GE) variation was 96.31%, whereas G and G × E interaction contributed 0.62 and 3.07%, respectively. Using single year data, variance components for G, location (L) and GL interaction indicated the relative magnitudes of these sources of variation (Table 4). Location was always the most important source of variation for grain yield, accounting for 92.0-93.7% of the total variance, except in 2007, when it accounted for only 60.5%. Since G × L interaction is more important than G × Y interaction, mean grain yields across years were used for GGE biplot analysis.

Table 3. Combined analysis of grain yield for 18 bread wheat genotypes in 11 environments.

Sources of variation	df	Mean squares	% of G+E+GE
Environment (E)	10	161572682.5[**]	96.31
Rep./E	33	1271585.7	
Genotype (G)	17	609621.1[**]	0.62
G × E	170	302985.5[**]	3.07
Error	561	140808.5	

** and * Significant at the 0.01and 0.05 probability levels, respectively.
ns: Not significant.

The small contribution of G and G × E interaction effects to the environment for grain yield found in this study is similar to those reported in other crop adaptation studies in rainfed areas (Mohebodini *et al.*, 2006; Sabaghnia *et al.*, 2008b). Results of the analysis of variance of yearly data indicated large grain yield variation due to location, which is not in agreement with the results of genotype evaluation and mega-environment investigation (Gauch and Zobel, 1996; Yan *et al.*,

2007). This justified selecting GGE biplot analysis (Yan *et al*., 2000) as an appropriate model for analyzing multi-environment trial data in this study. When the GGE model was fitted, the first two PCs explained 71% (PC1 = 45% and PC2 = 26%) of the GGE sum of squares for the multi-environment trials (Fig. 1). The lowest mean yield was 2113.8 for G15 in Gachsaran, and the highest mean yield was 4139.9 for G1 in Khoramabad (Table 5). The highest yielding genotypes in Gachsaran were G1, G3 and G4, while the highest yielding genotypes in Khoramabad were G1, G16 and G17 (Table 5). Genotypes G1, G3 and G16, and genotypes G1, G11 and G12 were the superior genotypes in Gonbad and Moghan, respectively (Table 5).

Table 4. Genotype (G), location (L), and genotype × location (GL) variance terms for bread wheat multi-environmental trials.

| Year	Sources of variation	df	Mean squares	% of L+G+GL
2007	Location (L)	2	21454231.2**	60.5
	Rep. / L	9	1158486.3	
	Genotype (G)	17	689812.0*	16.5
	G × L	34	479704.1**	23.0
	Error	153	207922.5	
2008	Location (L)	3	50540898.0**	92.0
	Rep. / L	12	202419.6	
	Genotype (G)	17	268869.6**	2.8
	G × L	51	168784.4**	5.2
	Error	204	72178.2	
2009	Location (L)	3	101691284.8**	93.7
	Rep. / L	12	2425576.3	
	Genotype (G)	17	303391.1**	1.6
	G × L	51	303880.7**	4.8
	Error	204	159103.4	
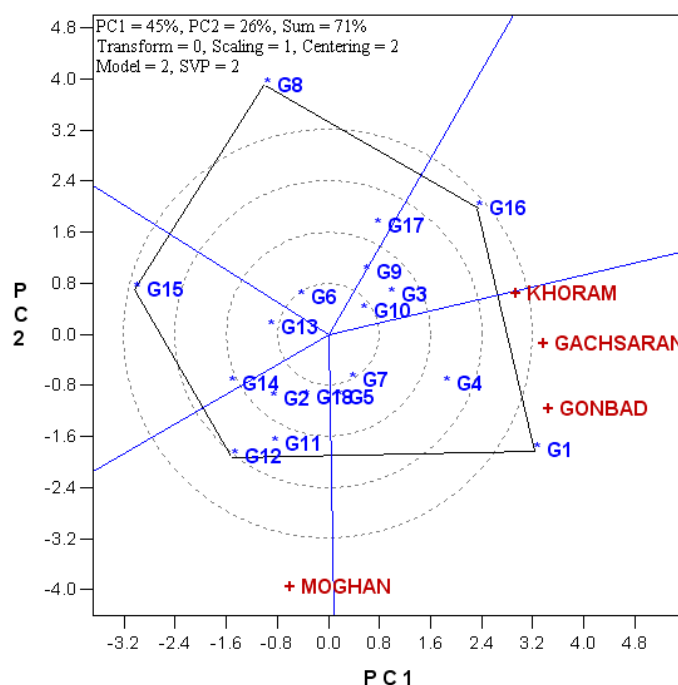
Fig. 1. Site regression (SREG) biplot for identifying winning genotypes and their respective environments.

The highly significant G × E interaction indicated that there was both additive (non-crossover) and non-additive (crossover) interaction in multi-environment trials (Table 3). When the phenotypic response of genotypes to different environments is not the same, G × E interaction is observed

(Annicchiarico, 1997). Plant breeders often have to select superior genotypes under changing conditions, and genotype performance is usually evaluated based on mean grain yield. However this is inadequate, because it does not fully indicate consistency of yield performance (Crossa *et al.,* 2010). Several statistical procedures have been proposed to identify the most suitable genotypes in the presence of G × E interaction. The G × E

interaction phenomenon has been investigated by statisticians, quantitative geneticists and plant breeders (Becker and Leon, 1988). Although quantitative geneticists are interested in estimating the magnitude of G × E interaction, plant breeders are interested in selecting the most suitable genotypes in the presence of G × E interaction (Freeman, 1973). However, statistical procedures for analyzing grain yield stability can be categorized

Table 5. Mean grain yield (kg ha-1) of bread wheat genotypes in different test locations.

	Gachsaran	Khoramabad	Gonbad	Moghan	Mean
G1	2613.1	4139.9	3406.6	2966.9	3281.6
G2	2373.8	3728.9	2877.3	2953.0	2983.3
G3	2507.3	3649.0	3258.8	2625.4	3010.1
G4	2816.9	3624.7	3238.0	2789.8	3117.3
G5	2273.4	3939.3	3071.0	2935.1	3054.7
G6	2174.6	3702.4	3168.1	2674.9	2930.0
G7	2347.9	3716.3	3229.9	2818.4	3028.1
G8	2280.2	3695.6	2844.3	2341.8	2790.5
G9	2327.1	3851.8	3154.8	2649.5	2995.8
G10	2450.3	3742.8	3126.0	2711.4	3007.3
G11	2131.6	3696.9	3163.8	2967.9	2990.1
G12	2183.0	3568.5	3058.9	2998.5	2952.3
G13	2243.5	3794.2	2912.8	2818.5	2942.3
G14	2130.3	3617.8	3029.7	2873.6	2921.9
G15	2113.8	3578.9	2651.3	2786.1	2782.5
G16	2375.2	4092.8	3347.0	2505.1	3080.0
G17	2402.1	4083.2	2916.6	2658.6	3015.1
G18	2302.8	3747.7	3064.3	2905.8	3005.3

into univariate, multivariate and nonparametric procedures (Lin *et al.,* 1986; Flores *et al.,* 1998; Dehghani *et al.,* 2010).

The genotypes that were farthest from the GGE biplot origin (G1, G8, G12, G15 and G16) formed the corners of a polygon when they were connected. Lines that started from the biplot origin and were perpendicular to the sides of the polygon produced five sectors (Fig. 1). These genotypes were superior in locations situated in their relative sectors. Thus genotype G1 was the best genotype in Gachsaran, Gonbad and Khoramabad (environment A), while genotype G12 was the best genotype in Moghan (environment B). Genotypes G8, G15 and G16 did not have the highest yield in any of the test locations (Fig. 1). Thus this figure suggests there are two groups of bread wheat growing environments among test locations. These results were verified based on the geographical properties, annual rainfall and other characteristics of the test locations. However, this environment pattern must be further verified through future multi-environment trials. The model outcome is worth using for the recommended purposes

applied to G × E modeling by Zobel *et al.* (1988) and Yan *et al.* (2000). The Moghan location in northwestern Iran has different climatic and geographic characteristics.

Genotype G1 was the best genotype in environment A (Gachsaran, Gonbad and Khoramabad), while genotype G12 was the best genotype in environment B (Moghan). Mohammadi *et al.* (2007) suggested the existence of four rainfed mega-environments for winter wheat in cold regions of Iran: I (Kermanshah and Maragheh); II (Shirvan and Zanjan); III (Sanandaj); and IV (Ardebil). Our study identified warm rainfed bread wheat environments, a finding that has implications for future bread wheat breeding programs operating under these conditions in Iran. Separating the target locations into different environments and deploying different genotypes in these environments is the best way to utilize the G × E interaction phenomenon. In the polygon view of the GGE biplot, locations in the same sector share the same winning genotype, and locations in different sectors have different winning genotypes. Thus, this biplot indicates the presence or

absence of crossover G × E interaction for the most responsive genotypes (Yan and Rajcan, 2002; Dehghani *et al.,* 2010).

Visualizing the mean grain yield and yield stability of the genotypes indicated that the mean yields of the studied genotypes ranked them in the following order: G1 > G4 > G16 > … > G8 > G15 (Fig. 2). Since the two axes of the mean location coordinate of the GGE biplot are orthogonal, projection of the genotypes on the perpendicular axis

must approximate the G × E associated with the genotypes (Yan *et al.*, 2000). The longer the projection of a genotype, regardless of direction, the greater the magnitude of G × E associated with the genotype, which provides an index of instability of the genotype across sites. Thus, the performance of G11, G12 and G16 was highly unstable, whereas genotypes G5, G6, G7, G13 and G18, followed by G1, G4 and G10, were highly stable (Fig. 2).

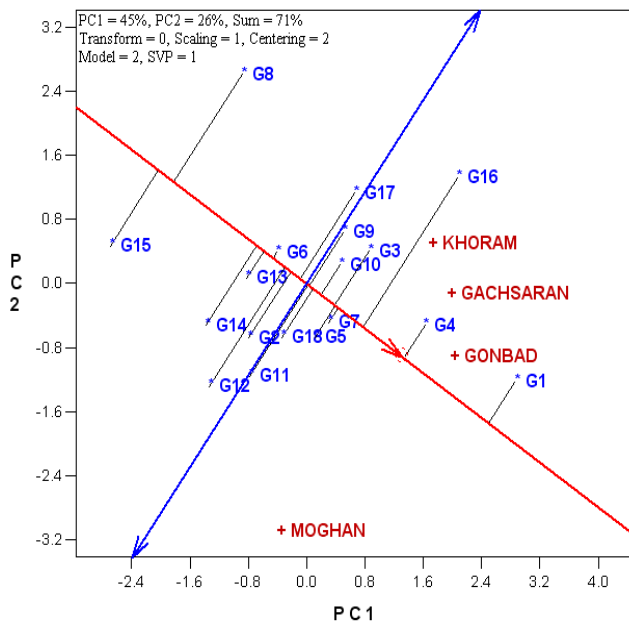Visualizing the mean yield and yield stability of

Fig. 2. Site regression (SREG) biplot for mean yield and yield stability of 18 bread wheat genotypes for specific genotype × environment interactions.

Fig. 3. Vector view of the site regression (SREG) biplot of the relationships among test locations.

the genotypes in the respective biplot indicated that G1 and G4 are suitable genotypes. Genotype G1 was the best performer in environment A. Also according to the vector view of the biplot, Gonbad, Khoramabad and Gachsaran were correlated with each other, but had no association with Moghan. It seems that these approaches are suitable for identifying bread wheat genotypes with high grain yield and good yield stability, and for determining the association among locations. Scores obtained from SREG analysis for the first two PCs (PC1 and PC2) represent mean yield and stability components that are comparable to the G main effect and an adaptability index such as linear regression coefficient (Goyal *et al.,* 2011). Yan and Kang (2002) reported a significant correlation between GGE distance (mean versus stability application of the biplot) and yield stability statistic (Kang, 1993; Dehghani *et al.,* 2010). Considering the high efficiency of Kang's (1993) yield stability statistic
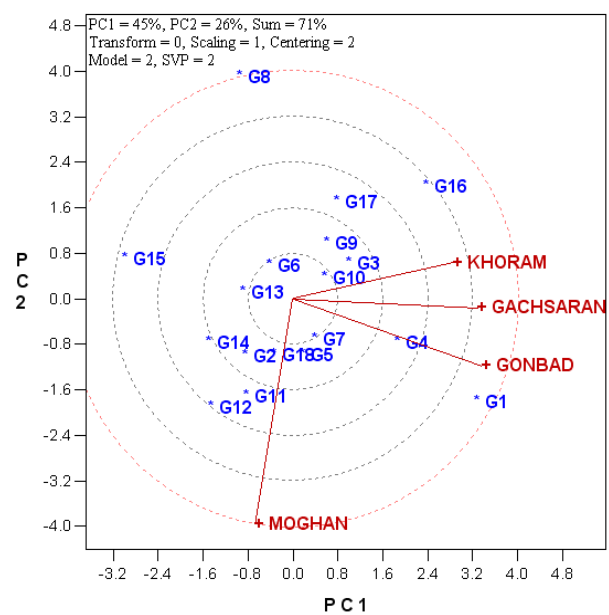
for simultaneously selecting mean yield and stability, identification of superior genotypes based on the ideal genotype and the ATC axis property could result in valid conclusions in G × E interaction studies.

A correlation study of test locations showed a relatively strong positive association between Khoramabad and Gachsaran, and between Gonbad and Gachsaran, and a relatively weak positive association between Khoramabad and Gonbad, as indicated by the acute angles between their vectors (Fig. 3). No association was observed between Moghan and other locations (Gachsaran, Gonbad and Khoramabad), as indicated by the near perpendicular vectors. Some of these correlations can be further verified from the original correlation coefficients, although some are not consistent with the original data (Table 6). For example; the biplot predicts Moghan has neither a positive or a negative correlation with the other locations, and

Table 6. Pearson's correlation coefficients and bootstrap resampling technique statistics (bias, mean and standard error) among four test locations.

Location 1	Location 2	Observed	Bootstrap statistics		
			Bias	Mean	SE
Gachsaran	Khoramabad	0.30	0.32	0.0254	0.28
	Gonbad	0.52	0.51	-0.0015	0.16
	Moghan	-0.10	-0.13	-0.0309	0.20
Khoramabad	Gonbad	0.37	0.32	-0.0435	0.27
	Moghan	-0.13	-0.13	-0.0042	0.27
Gonbad	Moghan	0.05	0.03	-0.0191	0.26

* Critical values of correlations at the P<0.05 and P<0.01 (df = 16) are 0.39 and 0.50, respectively.

the correlation coefficients in Table 6 confirm this conclusion. In contrast, positive correlation coefficients among Gachsaran, Gonbad and Khoramabad locations were not significant using Pearson's correlation and bootstrap re-sampling methods (Table 6). Such discrepancies are to be expected, because the GGE biplot method explained just 71% rather than 100% of the total variation in G+GE patterns. Yan *et al.* (2010) stated that since each measured datum contains some error, and as the GGE biplot model makes predictions based on the general pattern in the dataset, the predictions are probably more reliable than the individual data.

In conclusion, we found that genotypes G1 (3386.5 kg ha^{-1}) and G4 (3138.4 kg ha^{-1}) were adapted and suitable for Gachsaran, Gonbad and Khoramabad, whereas genotype G12 (3226.5 kg ha^{-1}) was adapted and suitable for Moghan; consequently, they are recommended for release as new bread wheat cultivars for these environments.

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