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Full Length Research Paper

Screening of superior chickpea genotypes for various environments of Iran using genotype plus genotype × environment (GGE) biplot analysis

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Superior crop cultivars must be identified through multi-environment trials (MET) and on the basis of multiple traits. The objective of this study was to explore the effect of genotype (G) and genotype × environment interaction (GE) on grain yield of 17 chickpea genotypes (*Cicer arietinum* L.) in six different research stations of Iran. GGE (G plus GE) biplot methodology was used to evaluate phenotypic stability in genotypes. A site regression (SREG) analysis to assess G × E interactions and to identify stable genotypes of chickpea was undertaken. These genotypes were developed by various breeders at different research institutes/stations of Iran and International Center for Agricultural Research in Dray Areas (ICARDA). Results indicated that the first two principal components explained 95% of the total GGE variation, with PC1 and PC2 explaining 73 and 22%, respectively. Genotypes Flip 93-93, Flip 94-123C and S 96002 had the highest mean yield and genotype Bivanij had the poorest mean yield. Thus the performance of genotype ILC 6142 was highly variable, whereas genotypes S 96003, Flip 93-48C and S 96027 were highly stable. Collective analysis of the biplots suggests four chickpea mega-environments in Iran. The first mega-environment contained locations Kermanshah, Gorgan and Ghachsaran, with genotype Flip 93-93 being the winner. Genotype Flip 85-57 × 12-071-1005 gave the highest performance in location Ilam and genotypes S 96032 and Bivanij gave the highest performance in locations Urmia. The Lorestan made up the other mega-environment with ILC 6142 as the winner.

Key words: *Cicer arietinum* L., genotype × environment interaction, site regression analysis, GGEBiplot.

INTRODUCTION

Legumes have been considered as a rich source of protein throughout the world and contain approximately three times more proteins than cereals. Chickpea (*Cicer arietinum* L.) is one of the top five important legumes on the basis of whole grain production (FAO, 2000). It is a staple food crop in many tropical and subtropical countries of Asia. Chickpea is the third most important pulse crop in the world, representing 14% of total world pulse production (Kelley et al., 2000). Chickpea is grown on 700,000 ha in Iran and ranks fourth in the world after India, Pakistan and Turkey. It is the most important legume of the country and grown on more than 64% of the total food legume area (FAO, 2001) . Iran is currently one of the world's largest net importers of agricultural products, importing about 30% of its requirements. Rapid population growth is expected to increase the demand for food. Iran is working towards increasing its agricultural efficiency. To increase its efficiency, the agricultural sector of Iran is attempting to

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Abbreviations: AMMI, additive main effect and multiplicative interaction effect; **E,** environment main effect; **G,** genotype main effect; **GE,** genotype × environment interaction; **GGE,** G plus GE; **MET,** multi - environment trials; **PC,** principal component; **SREG,** site regression models.

improve chickpea production with identification and introducing the stable and adaptive cultivars.

Chickpea varieties must show high performance for yield and other essential agronomic traits. Multi-environment trials (MET) play an important role in selecting the best cultivars (or agronomic practices) to be used in future years at different locations and in assessing a cultivar's stability across environments before its commercial release. When the performance of cultivars is compared across sites, several cultivar attributes are considered, of which grain yield is one of the most important. Cultivars grown in MET trials react differently to environmental changes. This differential response of cultivars from one environment to another is called genotype × environment (GE) interaction. GE interactions are an important issue facing plant breeders and agronomists. A significant GE interaction for a quantitative trial such as grain yield can seriously limit progress in selection. The study of the GE interaction may assist understanding of stability concept. Information on the structure and nature of GE interaction is particularly useful to breeders because it can help determine if they need to develop cultivars for all environments of interest or if they should develop specific cultivars for specific target environments.

Phenotypic stability has been extensively studied by biometricians who have developed numerous methods to analyze it (Eberhart and Rusell, 1966; Lin et al., 1988; Huhn, 1979; Kang and Pham, 1991) . Usually a large number of genotypes are tested over a number of site and year, and it is often difficult to determine the pattern of genotypic responses across environments without the help of graphical display of the data (Yan et al., 2001). The biplot technique (Gabriel, 1971) provides a powerful solution to this problem. Biplot analysis is a multivariate analytical technique that graphically displays the two-way data and allows visualization of the interrelationship among environments, and the interrelationship between genotypes and environments. Biplots are useful for summarizing and approximating patterns of response that exist in the original data (Gabriel, 1971). Two types of biplots, GE biplot (Zobel et al., 1988) and GGEbiplot (Yan et al., 2000), were used to visualize the genotype × environment two-way data but each had its unique functions. The "GE" biplot refers to graph of the genotype by environment interaction obtained from the additive main effects and multiplicative interactions (AMMI) model. The "GGE" refers to the genotype main effect (G) plus the GE interaction, which are the two sources of variation of the site regression (SREG) model (Burgueno et al., 2001). The measured yield of each cultivar in each test environment is a measure of environment main effect (E), genotype main effect, and GE interaction (Yan and Kang, 2003). Typically, E explains up to 80% or higher of the total yield variation, however it is G and GE that are relevant to cultivar evaluation (Yan, 2002). Yan et al. (2000) presented standard biplots of the site regression model to enhance its interpretation for selecting the best performing cultivars in subsets of sites. In analyzing Ontario winter wheat performance trial data, Yan and Hunt (2001) used a GGEbiplot constructed from the first two principal components (PC1 and PC2) derived from PC analysis of environment-centered yield data. GGEbiplot can be useful in two major aspects. The first is to display the which-won-where pattern of the data that may lead to identify high-yielding and stable cultivars and discriminating and representative test environments (Yan et al., 2001). A major challenge of plant breeding is finding the useful information within the quantities of data. The GGEbiplot graphically displays G and GE of a MET in a way that facilitates visual cultivar evaluation and mega- environment identification. The GGEbiplot software was chosen to facilitate the application of the GGEbiplot methodology in MET data analysis and the analyses of two-way data.

The objectives of this study were to (1) interpret G main effect and GE interaction obtained by SREG analysis of yield performances of 17 chickpea genotypes over sixteen environments; (2) use the GGEbiplot technique to examine the possible existence of different mega-environments in chickpea-growing regions in Iran; (*3*) visually assess how to vary yield performances across environments based on the GGEbiplot, and other objectives were to apply this method to determine discriminating ability and representativeness of the environments.

MATERIALS AND METHODS

Experimental design and plant materials

Data analyzed in this study were obtained from sets of chickpea yield trials conducted for three years (2002-2004) at six different research stations in Iran including Ghachsaran (GHA), Gorgan (GOR), Urmia (EUR), Ilam (ILA), Kermanshah (KER) and Lorestan (LOR). The detailed description of these test locations is given in Table 1. In each environment (year X location), 17 genotypes were tested. The genotypes were developed by various breeders at different research institutes/stations of Iran and International Center for Agricultural Research in Dray Areas (ICARDA). The names of genotypes, cods and origin of these genotypes are given in Table 2. At each environment a randomized complete block design with four replications was used. The trial fields were plowed with tractors usually from June to August and disc harrowed few days prior to planting time. The experimental plots consisted of four rows of 4 m length. Row to row and plant-to-plant distances was kept at 30 and 10 cm respectively at all the environments. Weeds were controlled by hand-weeding about two or three times, as required. Neither herbicides nor insecticides were used in any trials, as there was no need for them. Data on seed yield were taken from the middle two rows of each plot, leaving aside the guard rows on either side of a plot. Upon harvested seed yield was determined for each genotype at each test environments, the average was computed in accordance with the experimental design.

Data analysis

Analysis of variance was conducted by SAS (SAS Institute, 1996), to determine the effect of location (L), genotype (G) and GE interaction among these factors, on grain yield. Correlation coefficients between

Table 1. Agro-climatic characteristics of testing environments.

Environments		Mean	Latitude	Altitude m	Temp(˚C)[a]		Rainfall (mm)		Soil condition	
Location	Year	(Kg ha^{-1})	Longitude		Min	Max	PS[b]	GS[c]	Texture	Type[d]
Gorgan	2002	2026.8	36˚51 N	13.3	4.4	31.5	100.2	290.3	Sandy-	Cambisols
	2003	1998.4	54˚16 E		4.1	33.5	178	543	loam	
	2004	2616.5			3.8	34.2	135	425		
Kermanshah	2002	1249.0	34˚19 N	1322	3.8	38	121.2	358.6	Silt-loam	Cambisols
	2003	1157.5	47˚07 E		3	39.5	45	216		
	2004	1456.8			5.3	37	128.4	398.5		
Lorestan	2002	1115.1	23˚26 N	1147.7	5.6	38.2	155.2	499	Silt-loam	Regosols
	2003	957.6	48˚17 E		3.4	34.2	119.6	369.5		
	2004	1181.9			4	32				
							140.1	430.8		
Urmia	2002	1214.1	37˚27 N	1091	2.8	36	101	300.1	Sandy-	Cambisols
	2003	1283.8	57˚55 E		3.5	38.7	85.3	254	loam	
	2004	1376.3			4	35	71.4	233.7		
Ghachsaran	2003	2053.3	30˚10 N	669.5	6.4	39.1	145.2	487.5	Silt-loam	Regosols
	2004	2011.9	50˚50 E		5.3	39.2	180	575		
Ilam	2003	1904.0	33˚38 N	1363.4	5	32.1	183	564	Silt-loam	Cambisols
	2004	1834.0	46˚25 E		4.9	37.6	150.3	458		

[a] Temp(˚C) = Mean seasonal temperature; [b] PS = Preseasonal rainfall; [c] GS = Growing season; [d] Type = According to FAO system of soil classification.

Table 2. Genotype code, name and origin of 17 chickpea genotypes.

Genotype code	Name	Origin	Genotype code	Name	Origin
G1	S 96002	ICARDA	G10	Flip 93-48C	ICARDA
G2	S 95293	ICARDA	G11	Flip 94-60C	ICARDA
G3	S 96003	ICARDA	G12	Flip 94-30C	ICARDA
G4	S 96027	ICARDA	G13	ILC 482-205C	ICARDA
G5	S 96078	ICARDA	G14	Flip 94-123C	ICARDA
G6	S 96032	ICARDA	G15	Flip 85-57 × 12-071-1005	ICARDA
G7	S 96019	ICARDA	G16	Kurosh × 12-071	Iran
G8	Flip 93-93	ICARDA	G17	Bivanij	Iran
G9	ILC 6142	ICARDA			

pairs of environments were computed via SAS (SAS Institute, 1996). In addition, principal component axes (PCAs) were extracted and statistically tested by Gollob's (1968) F-test procedure (Vargas and Crossa, 2000). The first two components were used to obtain a biplot by GGEbiplot software (Yan 2001), which is a windows application that fully automates biplot analysis.

RESULTS AND DISCUSSION

In this investigation, partitioning and interpretation of the G main effect and GE interaction were based on SREG models. The measured yield of each genotype in each test environment is mixture of environmental main effect (E), genotype main effect (G), and interaction term of genotype and environment (GE), however it is G and GE that are relevant to cultivar evaluation (Yan, 2002). Yan et al. (2000) proposed a standard biplot of G + GE based on a SREG model referred to GGEbiplot. It was constructed using the first two principal components (PC1 and PC2) derived from subjecting the environment-centered data to singular-value decomposition.

Table 3. Site regression (SREG) analysis of variance for grain yield (kg ha^{-1}) of the genotypes across locations.

Source	Df	Mean square	F-test	Explained (%)
Model	101	272683.98	36.00	
location (L)	5	11108550.28	426.94	85.03**
Genotype (G)	16	744449.52	63.11	12.57**
G × L	80	189616.27	10.19	2.03**
Interaction PCA 1	21	985640	14.50	72.79**
Interaction PCA 2	19	334022	4.92	22.20**
Interaction PCA 3	17	36234	0.53	2.14
Interaction PCA 4	15	30902	0.45	1.60
Interaction PCA 5	13	22403	0.33	1.00
Residuals	86	96943.39		

** Significant at the 0.01 probability level.

Table 4. Genotype (G), location (L) and genotype by location (GL) variance terms for yield lentil multi-environmental trials, 2002 to 2004.

Year	Source	Df	Sum of squares	Explained (%)
	L	5	67127073	49.27
2002	G	16	31220720	22.92
	GL	80	37888656	27.81
	L	5	72782281	67.00
2003	G	16	8489371	7.81
	GL	80	27364623	25.19
	L	3	86027776	82.58
2004	G	16	6196491	5.95
	GL	48	11956078	11.48

The site regression analysis of variance of grain yield (kgha^{-1}) of the 17 genotypes tested in sixteen environments showed that 92.24% of the total sum of squares was attributable to location effects, only 6.18% to genotypic effects, and 1.57% to GE interaction effects (Table 3). The variance components for the location, genotype and genotype × location based on the yearly data are presented in Table 4 which gives an overall picture of the relative magnitudes of the genotype (G), location (L), and genotype × location interaction (GL) variance terms. Location was always the most important source of yield variation accounting for 49.27 to 82.58% of the total variance. The large yield variation due to L, which is irrelevant to cultivar evaluation and mega environment investigation (Gauch and Zobel, 1996), justifies selection of SREG procedures for analyzing the MET data.

Results from SREG analysis also showed that the first principal component axis (PC1) of the genotype main effect plus interaction captured 72.79% of the sum of squares in 21.87% of degrees of freedom. Similarly, the second principal component axis (PC2) explained a further 22.2% of the GGE sum of squares. Furthermore, PC1 and PC2 had sums of squares greater than that of genotypes. The mean squares for the PC1 and PC2 were significant at P = 0.01. An F-test at P = 0.01 suggested that two principal component axes of the interaction were significant for the model with 101 degrees of freedom.

The GGEbiplot graphically displays G plus GE of a MET in a way that facilitates visual cultivar evaluation and mega-environment identification (Yan et al., 2000). Only two PC (PC1 and PC2) are retained in the model because such a model tends to be the best model for extracting patterns and rejecting noise from the data. In addition, PC1 and PC2 can be readily displayed in a two-dimensional biplot so that the interaction between each genotype and each environment can be visualized (Yan and Hunt, 2002).

There are numerous ways to use a GGEbiplot, but the polygon view of the biplot is most relevant to the mega-environments identification. For this purpose, the genotypes that are connected with straight lines so that a polygon is formed with all other genotypes contained within the polygon (Figure 1A). The vertex genotypes in this investigation are Flip 93-93, ILC 6142, Bivanij, Kurosh × 12-071 and Flip 85-57 × 12-071-1005. These genotypes are the best or the poorest genotypes in some or all of the locations since they had the longest distance from the origin of biplot. There are five sectors in Figure 1A. The

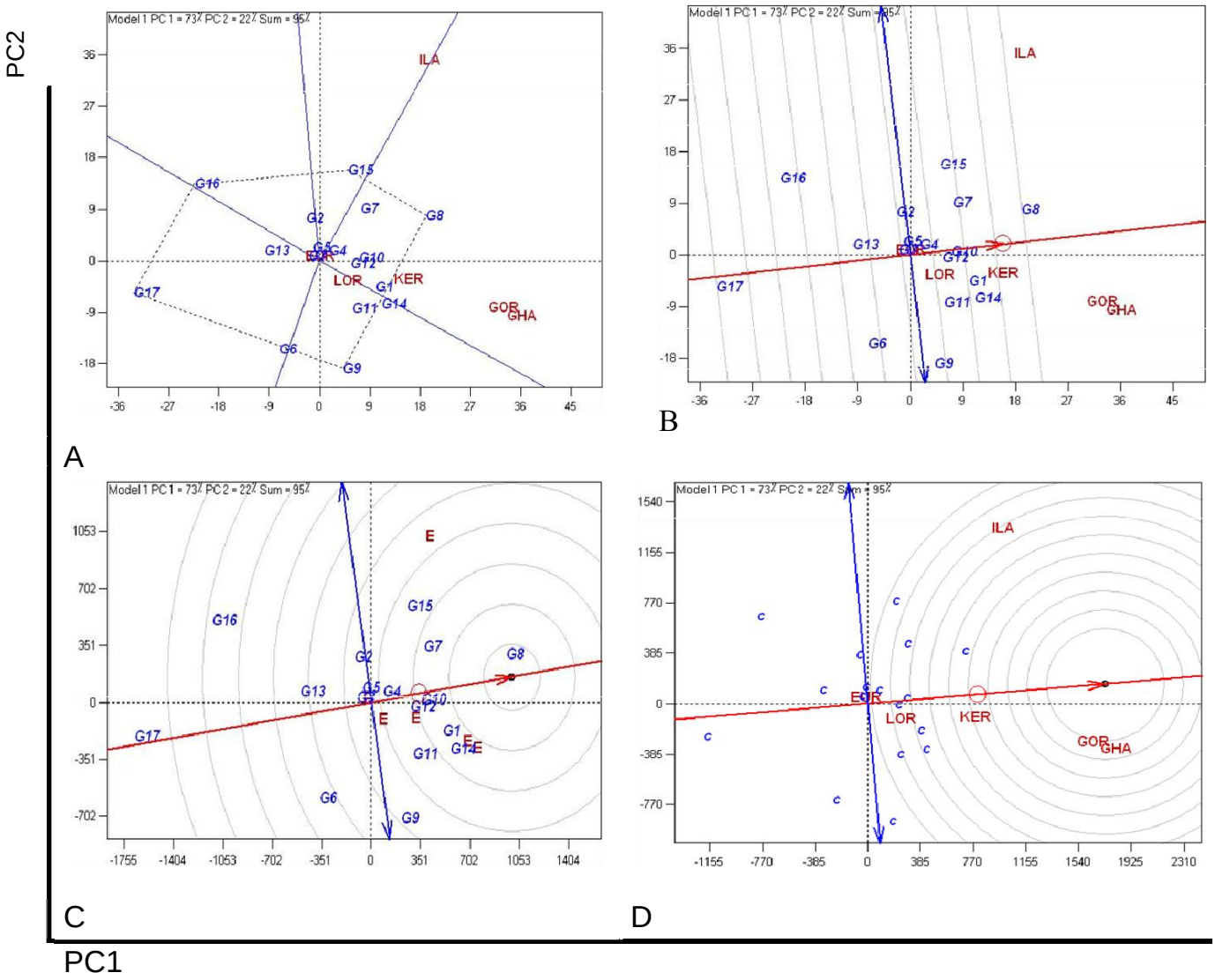

Figure 1. (A) Mega-environment and their winning genotypes, (B) Cultivars ranking based on both average yield and stability (C) Comparison of the locations with the *ideal* location based on both discrimininating ability and representativeness of the target location, (D) Comparison of the genotypes with the *ideal* genotype for both mean yield and stability.

vertex genotype for each sector is the one that gave highest yield for locations that fall within that sector. Therefore, the first mega-environment contained locations KER, GOR and GHA, with genotype Flip 93-93 being the winner. Genotype Flip 85-57 × 12-071-1005 gave the highest performance in location ILA and genotypes S 96032 and Bivanij gave the highest performance in locations EUR. Also genotype ILC 6142 gave the highest performance in locations LOR. Genotype Kurosh × 12-071 did not give the highest yield in any of the locations, that is it was the poorest genotype in all of locations. Another use of Figure 1A is that the locations are grouped based on the best genotypes and we have four groups of locations: ILA as a group, EUR as a group, KER, GOR and GHA and LOR as a group. Another application of the GGEbiplot geometry is to visually identify the mean performance and stability of genotypes. The mean yield of the genotypes can then be approximated by nominal yields of the genotypes in that mean location. In Figure 1B, genotypes Flip 93-93 and Flip 94- 123C had the highest mean yield and genotypes Bivanij and Kurosh × 12-071 had the poorest mean yield. Mean yields of the genotypes were in the following order: Flip 93-93>S 96019>Flip 94-123C>S 96002>Flip 93-48C>Flip 85-57 × 12-071-1005>Flip 94-30C>Flip 94-60C>S 96027> ILC 6142>S 96078>S 95293>S 96003>S 96032>ILC 482-205C>Kurosh × 12-071>Bivanij.

The performance of genotypes ILC 6142, Kurosh × 12-71 and Flip 85-57 × 12-071-1005 is highly variable (less stable), whereas genotypes S 96003, Flip 93-48C and S 96027 are highly stable. An ideal genotype is one that has

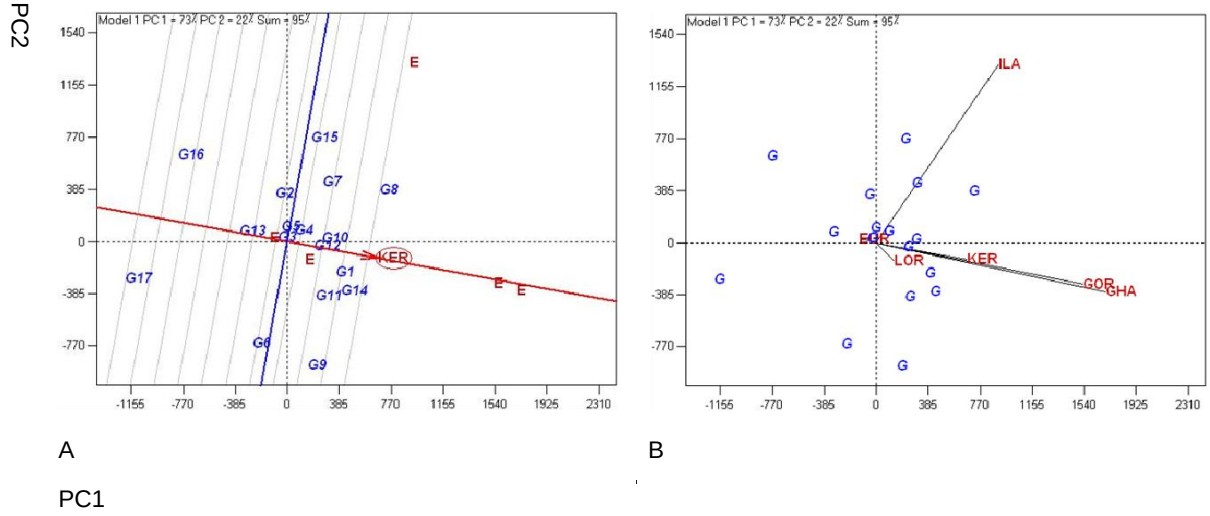

Figure 2. (A) The performance of different cultivars in a location (KER), (B) correlation between locations.

Table 5. Correlation coefficients among test locations.

Location	Urmia	Gorgan	Kermanshah	Lorestan	Ghachsaran
Gorgan	-0.36				
Kermanshah	-0.27	0.82**			
Lorestan	-0.15	0.47	0.62**		
Ghachsaran	-0.30	0.96**	0.84**	0.58*	
Ilam	-0.13	0.39	0.35	-0.12	0.38

both high mean yield and high stability. The center of the can centric circles in Figure 1C represents the position of an ideal genotype, which is defined by a projection on to the mean-location axis that equals the longest vector of the genotypes that had above-average mean yield and by a zero projection on to the perpendicular line (zero variability across environments). A genotype is more desirable if it is closer to the ideal genotype. Therefore genotypes Flip 93-93, Flip 93-48C and S 96002 are more desirable than other genotypes.

Discriminating ability is an important measure of a test location. A test location, lack of discriminating ability provides no information about the cultivars and, therefore the test location is useless. Another equally important measure of a test location is its representativeness of the target location. If a test location is not representative of the targets location, it is not only useless but also misleading since it may provide biased information about the tested cultivars. An ideal location should be highly differentiating of the genotypes and at the same time representative of the target location. The GGEbiplot way of measuring representativeness is to define an average location and use it as a reference or benchmark. The average location is indicated by small circle (Figure 1D). The ideal location, represented by the small circle with an arrow pointing to it, is the most discriminating of genotypes and yet representiveness of the other tests locations. Therefore Gorgan, Ghachsaran and Kermanshah were relatively desirable test locations, whereas Urmia, Lorestan and Ilam were relatively undesirable test locations.

Figure 2A illustrates graphic comparison of the relative performance of all genotypes at location Kermanshah. In this figure, genotypes Flip 93-93, Flip 94-123C and S 96002 had the highest at Kermanshah and genotypes Bivanij and Kurosh × 12-071 had the poorest yield.

The vector view of a GGEbiplot provides a succinct summary of the interrelationship among the environments (Yan, 2002). Figure 2B is referred to as the vector view of the GGEbiplot, in which the environments are connected with the biplot origin via lines. This view of the biplot helps understand the interrelationships among the environments. The cosine of the angle between the vectors of two environments approximates the correlation coefficient between them. Therefore, the most prominent relations by Figure 2B are: A near zero correlation among Ilam and Kermanshah, Gorgan, Ghachsaran and Lorestan as indicated by the near perpendicular vectors (r = cos90 = 0); a positive association among Kermanshah, Gorgan, Ghachsaran and Lorestan as indicated by acute angles. The correlation coefficients among the six test locations are presented in Table 5. The correlation coefficients among the locations indicate that the biplot currently shows

relationship among the location that had relatively large loading on both PC1 and PC2 (Table 5). The number of correlation coefficients increases quickly to an unmanageable level as more locations are involved. Such a vector view of a biplot can be used to identify different mega-environments, thus that test locations from different mega-environments should have large angles, hence low or negative correlations.

It is clear that the GGEbiplot method is an excellent tool for visual MET data analysis. Analysis of stability and identification of mega- environments on chickpea using this method has not been already reported. In addition, this study indicated the possibility of improving progress from selections under diverse location conditions by applying GGL biplot. Multivariate analysis such as SREG analysis is an important tool for breeders, geneticists, and agronomists for analysis of MET data. We agree that G and GE must be considered simultaneously in genotype evaluation and mega-environment analysis. Compared with conventional univariate methods of the MET data analysis, SREG procedures have some advantages. The most important advantage of these methods is graphical presentation of the MET data, which greatly enhances our ability to understand the patterns of the data. These methods have a usage in selecting superior genotypes and test environments for a given mega-environment. This useful application is available in SREG and AMMI models by aid of GGEbiplot software and AMMIWINS program, respectively, and these can improve the identification of mega-environments and favorable genotypes. These methods are important tools for selecting high yielding, stable genotype. In conclusion, we suggest use of the SREG analysis for identification of favorable genotypes and mega-environments in chickpea.

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