

## Site Regression Biplot Analysis for Matching New Improved Lentil Genotypes into Target Environments

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Received: July 24, 2012 Accepted: October 27, 2013

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### Abstract

The evaluation of the yield stability of genotypes and environment is of prime concern to plant breeders. Therefore, a comprehensive analysis of the structure of the GE interaction is needed. The objective of this investigation was to evaluate the use of sites regression (SREG) GGE methodology to stratify the  $pe \times$  environment (GE) interaction in lentil. Yield data of 10 genotypes of lentil tested across 10 rain-fed environments during 2007 and 2008 growing seasons, using randomized complete block design with four replications, were analyzed. The location (L) explained 56 and 77% of the total (G + L + GL) variation for the first and second year, respectively. According to polygon view of biplot, Gonbad, Shirvan and Gachsaran with wining genotype G9, Ilam with wining genotype G5 and Kermanshah with wining genotype G8 were detected in the first year; and Gonbad and Ilam with wining genotype G5; Gachsaran with wining genotype G9; Kermanshah with wining genotype G2 and Shirvan with wining genotype G3 were detected in the second year. In the first year, genotypes G1 and G9 and in the second year genotypes G8 and G9 were the most favorable genotypes based on average tester coordinate biplot. Gachsaran location was more representative of the overall locations and more powerful to discriminate genotypes than the unfavorable ones. In conclusion, G9 (ILL6199) was found to be the most stable and higher yielding genotype which may be recommended for commercial release in semi-arid areas of Iran.

**Keywords:** GE interaction; GGE biplot analysis; Multi-environment trials

producing countries in the world and lentil is grown for human consumption as a rich source of protein. However, its average seed yield has remained very low ( $489 \text{ kg ha}^{-1}$ ) due to low yield potential of local cultivars and landraces (FAOSTAT 2010). Accordingly, new improved genotypes were released in recent years as cultivars for increasing the lentil production in Iran.

Targeting improved genotypes onto its growing locations is the ultimate interest of all plant breeding programs. These attempts which are known as multi-environment trials shows that the occurrence of genotype  $\times$  environment (GE)

### Introduction

Lentil (*Lens culinaris* Medik.) is an important crop with a high content of proteins, micronutrients and vitamins which are important in human food diet. It is a self-pollinating diploid food legume and is one of the most ancient domesticates (Sarker *et al.* 2009). In dry areas of Middle East, lentil is commonly grown in rotation with cereals to the decreasing demand of cereal crops for nitrogen fertilizers. Lentil ranks seventh among grain legumes, is grown on over 3.5 million hectares in Asia, Africa and North America with a production of over 3 million tons (Erskine 2009). Iran is one of the major lentil

important component of GE interaction and SREG model as a linear-bilinear model could be useful for assessing crossover interaction.

The SREG model is a multiplicative model which uses the genotype's main effects and GE interaction (G+GE) which are the two important sources of variation in cultivar selection (Yan *et al.* 2000). Graphic presentation of SREG model as GGE biplot is regarded as a powerful tool for effective interpretation of GE interaction in crop breeding programs (Yan *et al.* 2007). The GGE biplot graphically displays the two way data pattern and permits visualization on the interrelationships among genotypes, environments and their interaction. This procedure enables the plant breeder to know the yield performance of genotypes also in a specific environment. The GGE biplot procedure has been employed successfully in determining mega-environments as well as the most favorable genotypes of barley (Dehghani *et al.* 2006), lentil (Sabaghnia *et al.* 2008) and maize (Dehghani *et al.* 2009) in Iran.

The objectives of the present investigation were (i) to identify genotypes that combine high yields with stability across test environments through GGE biplot methodology and (ii) to determine the best test environments (representative and discriminating) of new improved lentil genotypes in Iran.

### Materials and Methods

Data for this investigation was obtained from nine improved lentil genotypes and a check cultivar (Gachsaran). These genotypes were from the ICARDA's (International Center for Agricultural Research in the Dry Areas) lentil improvement

interaction is inevitable (Annicchiario 2002). GE interaction refers to inconsistent yield performance of genotypes across locations and years. In the presence of GE interaction, mean yield is less predictable and cannot be interpreted based on genotypic or environmental effects alone (Ebdon and Gauch 2002). In order to identify the most stable genotypes, the GE interaction must be explored into stability analysis. Yield stability analysis has allowed plant breeders to identify widely adapted genotypes to use in crop improvement programs and has helped to improve recommendations to farmers (Kang 2002). Several environmental factors such as climatic conditions, biotic and abiotic stresses, soil properties, crop type, etc. may somewhat contribute to GE interaction and yield instability. (Flores *et al.* 1998).

Several statistical methods have been proposed for investigation of the GE interaction effect and exploiting its positive side in cultivar development process. Various aspects of these statistical methods to explain GE interaction and facilitate commercial cultivar release have been reviewed (Becker and Leon 1988; Flores *et al.* 1998). However, not all of them are always effective in analyzing the GE interaction of multi-environment dataset structure in plant breeding program (Sabaghnia *et al.* 2006). Also, these stability methods differ in the statistics used in the assessment, the statistical strategies employed, and the analyses. The sites regression (SREG) model (Crossa and Cornelius 1997) has been proposed as the useful tool for GE interaction studies and analyzing multi-environmental trials. The crossover type of interaction is the most

Analysis of variance was performed for individual environments to plot residuals and identify outliers. Primary statistical analyses such as normality test of the data using the Anderson-Darling normality test and homogeneity test of residual variances by Bartlett's homogeneity test were carried out. Statistical analysis of variance for SREG model was performed using the SAS codes program of Burgueno *et al.* (2001). To explore G plus GE variability in seed yield of lentil, we used the SREG model that is given by:

$$Y_{ij} = m + b_j + \sum_{n=1}^k \lambda_n x_{in} h_{in} + e_{ij}$$

where  $Y_{ij}$  is the mean of genotype  $i$  in environment  $j$ ;  $\mu$  is the grand mean;  $\beta_j$  is the environment  $j$  main effect;  $n$  is the singular value;  $\lambda_{in}$  and  $\zeta_{in}$  are, the singular vectors for genotypes and environments for  $n = 1, 2, \dots$ , respectively; and  $\varepsilon_{ij}$  is the residual effect. The GGE biplots were generated using the first two symmetrically scaled principal components (PC) for average tester coordinate and polygon view biplots. To visualize the correlations among locations, a vector view biplot was obtained. These graphic analyses were performed using the GGEbiplot software (Yan 2001). The results of this vector view biplot, were compared with Pearson's correlation coefficients.

program and their name, pedigree and origin of their parental lines are given in Table 1. These genotypes tested in 10 environments (five locations during two years), extracted from the Iran lentil performance trial programs. The trial locations were selected to sample climatic and edaphic conditions likely to be encountered in lentil growing areas and to vary in agro-climatic factors. Shirvan and Gonbad, in the north-east of Iran, are characterized by semi-arid conditions and have complex soil series of silty, clay and loam soil. Kermanshah and Ilam, in western Iran, have moderate rainfall and have silt loam soil. Gachsaran, in south western Iran, is relatively arid and has silt loam soil. The test locations varied in climatic conditions, as well as soil fertility, due to the localization ( $\hat{\cdot}$ ). The experimental design, at each environment (location  $\times$  year combination), was a randomized complete block design with four replicates. The seeds were planted according to local practice with planting rate of about 50 seeds  $m^{-2}$ . Plot size was 4  $m^2$ ; each plot consisted of four 4 m-long rows with 25 cm between rows. Appropriate pesticides were used to control insects, weeds and diseases, and appropriate fertilizers were applied for each environment. The harvested plot size was 1.75  $m^2$  (two 3.5<sup>m</sup> rows at the center of each plot). Mean grain yield was estimated for each genotype at each location  $\times$  year.

**Table 1. Origin of the 10 lentil genotypes which are studied in 10 environments**

Code	Name	Pedigree	Origin of parents	Mean yield (kg ha <sup>-1</sup> )
G1	FLIP 97-1L	ILL 5989 × ILL6199	ICARDA × ICARDA	1187
G2	FLIP 82-1L	Landrace	ICARDA	1145
G3	FLIP 92-15L	ILL 5588 × ILL5714	ICARDA × ICARDA	989
G4	FLIP 96-9L	ILL 6199 × ILL 6198	ICARDA × ICARDA	997
G5	FLIP 92-12L	ILL 5582 × ILL 707	Jordan × Cyprus	1168
G6	FLIP 96-4L	ILL 467 × ILL 45	Chile × Syria	1152
G7	ILL 7946	ILL 6209 × ILL5671	ICARDA × ICARDA	1107
G8	ILL 6037	ILL 4349 × ILL 4605	Canada × Argentina	1200
G9	ILL6199	ILL 5746 × ILL 975	ICARDA × Chile	1267
G10	Gachsaran	Cultivar	Iran	1002

**Table 2. Geographical properties and mean yield of the 10 lentil genotypes, studied in five locations**

Code	Location	Altitude (meter)	Longitude Latitude	Soil Texture	Rainfall (mm)
1	Gonbad	45	55° 12' E 37° 16' N	Silty Clay Loam	367
2	Kermanshah	1351	47° 19' E 34° 20' N	Clay Loam	455
3	Ilam	975	46° 36' E 33° 47' N	Clay Loam	350
4	Gachsaran	710	50° 50' E 30° 20' N	Silty Clay Loam	460
5	Shirvan	1131	58° 07' E 37° 19' N	Loam	267

indicated that the effects of genotype × location × year were highly significant (results were not shown). Therefore it seems that the GGE biplot analysis based on multiplicative model such as SREG model would be applied to multi-environment trials data because the GE interaction is significant, although its use should not be precluded when it is not significant.

According to Table 3, location was always the most important source of seed yield variation, accounting for 56 and 77% of the total variation

## Results and Discussion

In the combined analysis of variance for both years, the effects of locations and genotypes were significant (Table 3). Also the effect of genotype × location (GL) was significant. This indicates that there is at least one lentil genotype with a different behavior in at least one of the locations. Differential seed yield ranking across environments indicates the presence of crossover GE interaction. Also the results of combined analysis of variance across years and locations

methodology was employed to analyze the dataset.

The SREG analyses of variance for seed yield of 10 lentil genotypes across five locations are given in Table 4. The fitted GGL (G+GL) biplot model indicated that the first two PCs explained 89.37% (PC1 = 67.03% and PC2 = 26.26%) of variation for the first year and the first two PCs explained 93.29% (PC1 = 48.26% and PC2 = 41.11%) of variation for the second year. In this study F-test of Gollob (1968) was used to test the significance of PCs for the SREG model and indicated that first three PCs were significant and the magnitudes of the first two PCs were high. Therefore, GGL biplots can reflect data variations (Table 4). The relative contributions of the first two PCs to the total variation for seed yield of lentil found in this investigation were similar to those found in other crop adaptation studies in the rain-fed regions of Iran (Sabaghnia *et al.* 2008; Ebadi-Segherloo *et al.* 2010).

(L + G + GL). These results gave a general pattern of the relative magnitudes of the G, L, and GL variance components. According to Gauch and Zobel (1996), the unpredictable variance components such as year and location are irrelevant to genotype evaluation in multi-environment trials data analysis. Therefore, it can be stated that the use of SREG as the proper model for analyzing the MET data is logical. Also, based on Table 3, the GL interaction was larger than G term and so there are different mega-environments in lentil producing areas of Iran. Gauch and Zobel (1997) defined a mega-environment as a portion of a plant species' growing site with homogeneous conditions that causes similar yield performance of some genotypes. Regarding multi-directionality aspect of GE interaction, relative magnitudes of the L to the G and GL components and mega-environment identification for lentil, the GGL biplot

**Table 3. Genotype (G), location (L), and genotype  $\times$  location (GL) variance terms for lentil multi-environmental trials, 2007 and 2008**

Source of variation	df	MS (2007)	% of G+L+GL	MS (2008)	% of G+L+GL
Location (L)	4	18775046.59**	0.77	20652313.26**	0.56
Replication / L	15	137266.17		104043.77	
Genotype (G)	9	166870.18**	0.03	249740.97**	0.02
G $\times$ L	36	104822.11**	0.20	309919.00**	0.42
Error	135	39740.18		62687.77	

\*\* Significant at the 0.01 probability level

**Table 4. Analysis of variance for GGE biplot model in lentil performance trial yield data**

x	df	SS	%	%	MS	F
Year 2008						
GGE1	12	2546020.8	48.26	48.26	212168.4	13.37**
GGE2	10	2168539.0	41.11	89.37	216853.9	13.67**
GGE3	8	468923.0	8.89	98.26	58615.4	3.69*
GGE4	6	77346.0	1.47	99.72	12891.0	0.81
GGE5	4	14598.9	0.28	100.00	3649.7	0.23
Year 2009						
GGE1	12	8984531.1	67.03	67.03	748710.9	47.20**
GGE2	10	3520213.3	26.26	93.29	352021.3	22.19**
GGE3	8	739893.1	5.52	98.81	92486.6	5.83*
GGE4	6	123974.9	0.92	99.73	20662.5	1.30
GGE5	4	36140.3	0.27	100.00	9035.1	0.57

\*, \*\* Significant at the 0.05 and 0.01 probability levels, respectively

the GGL biplot polygon view is that each vertex genotype has higher yield than other genotypes in all locations that fall in the related sector (Yan 2002). The Kermanshah location fell into sector 3 and the vertex genotype for this sector was G8. In the second year, there were five rays in Figure 1B which divided the biplot into five sectors, and the locations fell into 4 of them. The Gonbad and Ilam locations fell into sector 1 and the vertex genotype for this sector was G5; the Gachsaran location fell into sector 2 with the vertex genotype G9; the Kermanshah location fell into sector 3 with the vertex genotype G2; and Shirvan location fell into sector 4 with the vertex genotype G3. A greater emphasis on stable performance (static concept) would not necessarily be harmful to farmers because they would expect high mean yield from the cultivated genotypes on their farms. Therefore, it seems that GGE

Polygon view of biplot has been used to identify “which-wins-where” patterns in multi-environmental trials data analysis. In this graph lines are drawn to connect the furthest genotypes in the biplot and then a line is drawn perpendicular to that side of the polygon so as to pass through the origin. The furthest genotype is the best performer in the location included in that sector. There are five rays in Figure 1A which divide the biplot into five sectors, and the locations fall into 3 of them in the first year. The Gonbad, Shirvan and Gachsaran locations fell into sector 1 and the vertex genotype for this sector was G9, suggesting that the most favorable genotype for these locations was G9. The location Ilam fell into sector 2 and the vertex genotype for this sector was G5. This genotype was better than the other genotypes which fell into sector 2 (genotypes G1 and G7). An interesting property of

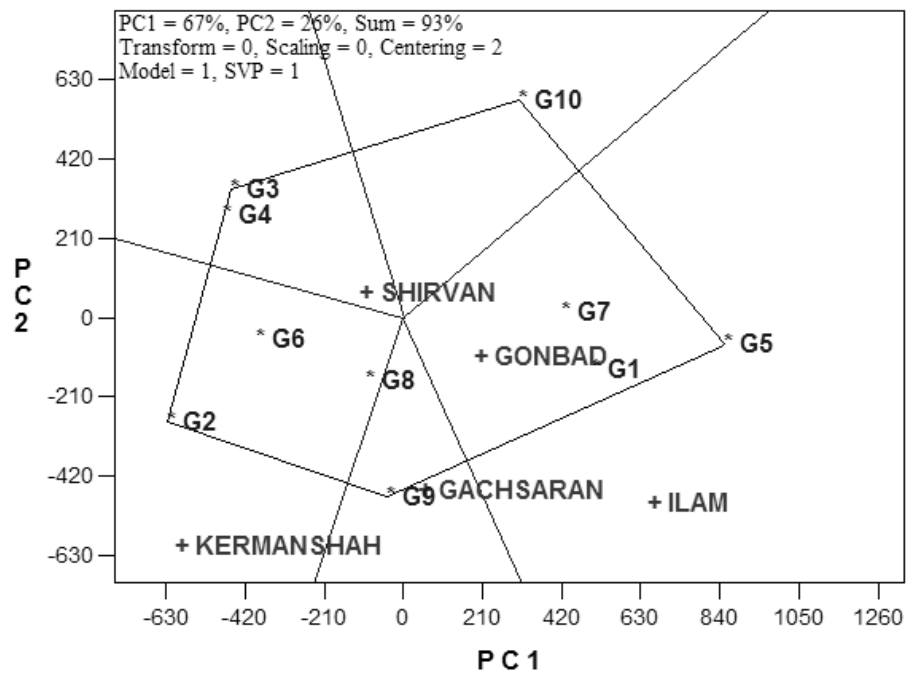
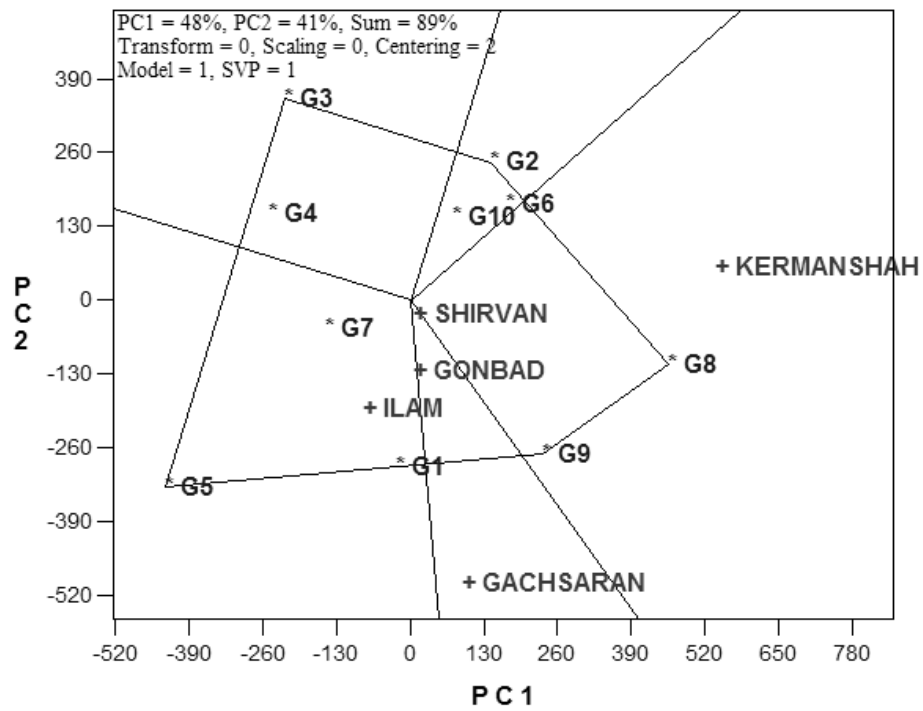


Figure 1. Site regression (SREG) biplot identification of winning genotypes and their mega-environments (A) up for first year and (B) down for second year

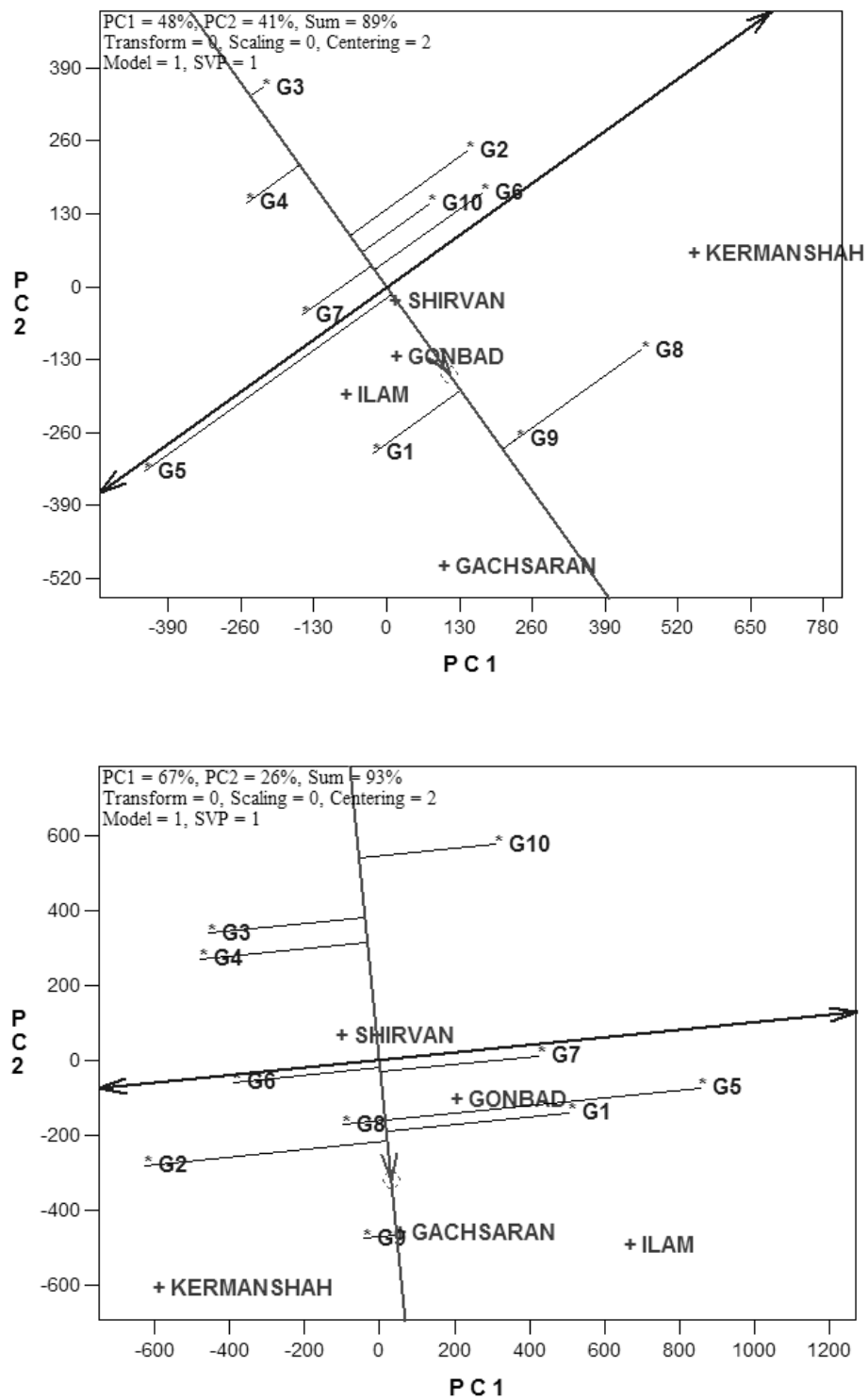
only genotype G9 is a good candidate for recommending as the most favorable genotype from both mean yield and stability aspects.

The vector view of a GGL biplot provides a summary of the interrelationships among the locations (Yan, 2002). Provided that the biplot explained an adequate amount ( $\geq 50\%$ ) of the total variation, the correlation coefficient between any two locations is reliable. The biplot in Figure 3A (the first year) and Figure 3B (the second year) explained 89.37 and 93.29% of the total variation, respectively, and so these biplots can be used for extracting interrelationships among the locations. The correlation coefficient between any two locations is estimated by the cosine of the angle between their vectors. Two locations are positively correlated if the angle between their vectors is  $< 90^\circ$ , negatively correlated if the angle is  $> 90^\circ$ , independent if the angle is  $90^\circ$ . Also, locations with longer vectors are more responsive to the genotypes; locations with shorter vectors are less responsive to the genotypes; and those located at the biplot origin are not responsive at all (Yan and Kang 2003).

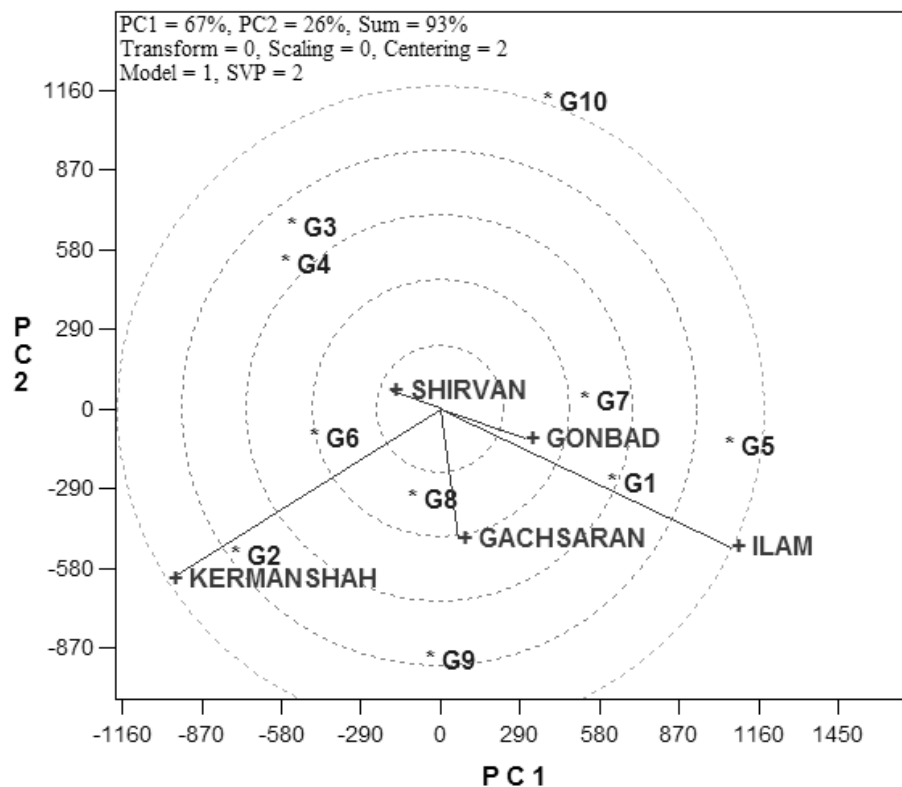
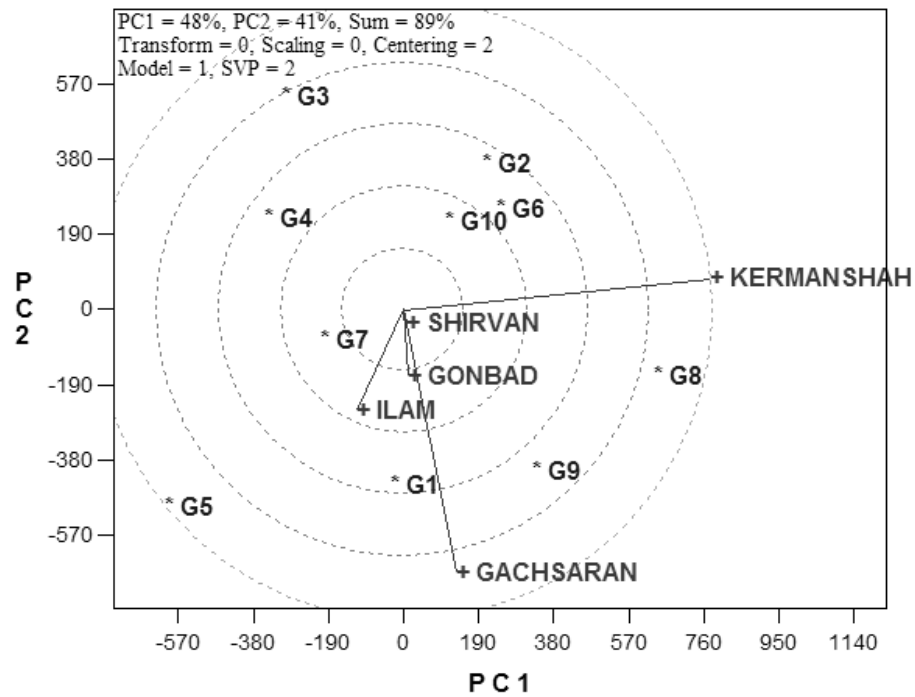
model is a suitable tool to achieve high mean yield genotypes which have acceptable stability (dynamic concept of stability). An inconsistent genotype performance across environments can provide additional information for the plant breeder and can help predict the variability expected among different regions (Kang and Pham 1991). Thus, GGE biplot provides a lot of flexibility in the hands of plant breeders for simultaneous selection for yield and stability.

In GGE biplot methodology, the yield and stability of the genotypes are examined by an average tester coordinate (ATC). The mean yield of the genotypes is estimated by their projections on the ATC x axis. The average location, as the virtual location, is shown by a circle and indicates the positive end of the ATC x axis. According to the ATC figure, the length of the average location vector was adequate to select genotypes based on mean yield. In the first year, genotypes with above average yield were selected (G1, G5, G8 and G9), whereas the remained genotypes were discarded (Fig. 2A). In contrast, G8 was the least stable genotype which had variable performance across test locations, while G1 and G9 were the most stable genotypes. The performance of genotypes G3, G4 and G7 close to ATC axe was stable, whereas some of them showed low mean yields (Fig. 2A). In the second year, genotypes with the above average yield were selected (G1, G2, G6, G7, G8 and G9), whereas the remained genotypes were discarded (Figure 2B). In contrast, most of them were unstable which had variable performance across test locations, while G8 and G9 were stable genotypes. Therefore it seems that





**Figure 2.** Site regression (SREG) biplot of mean and stability of 10 lentil genotypes for yield and specific genotype × environment interactions (A) up for first year and (B) down for second year



**Figure 3. Site regression (SREG) biplot for relationships between test locations (A) up for first year and (B) down for second year**

the length of the location vectors estimates the standard deviation within each location, which is a measure of their discriminating ability. Thus, Gachsaran and Kermanshah test locations were most discriminating locations for lentil yield performance. There is no doubt that multivariate methods are useful tools for analysis of multi-environment trials and visualization methods are useful to explore patterns of genotypes or locations. Simultaneous selection for both mean yield and stability of genotypes performance is an important consideration in breeding programs (Kang and Pham 1991; Kang 2002).

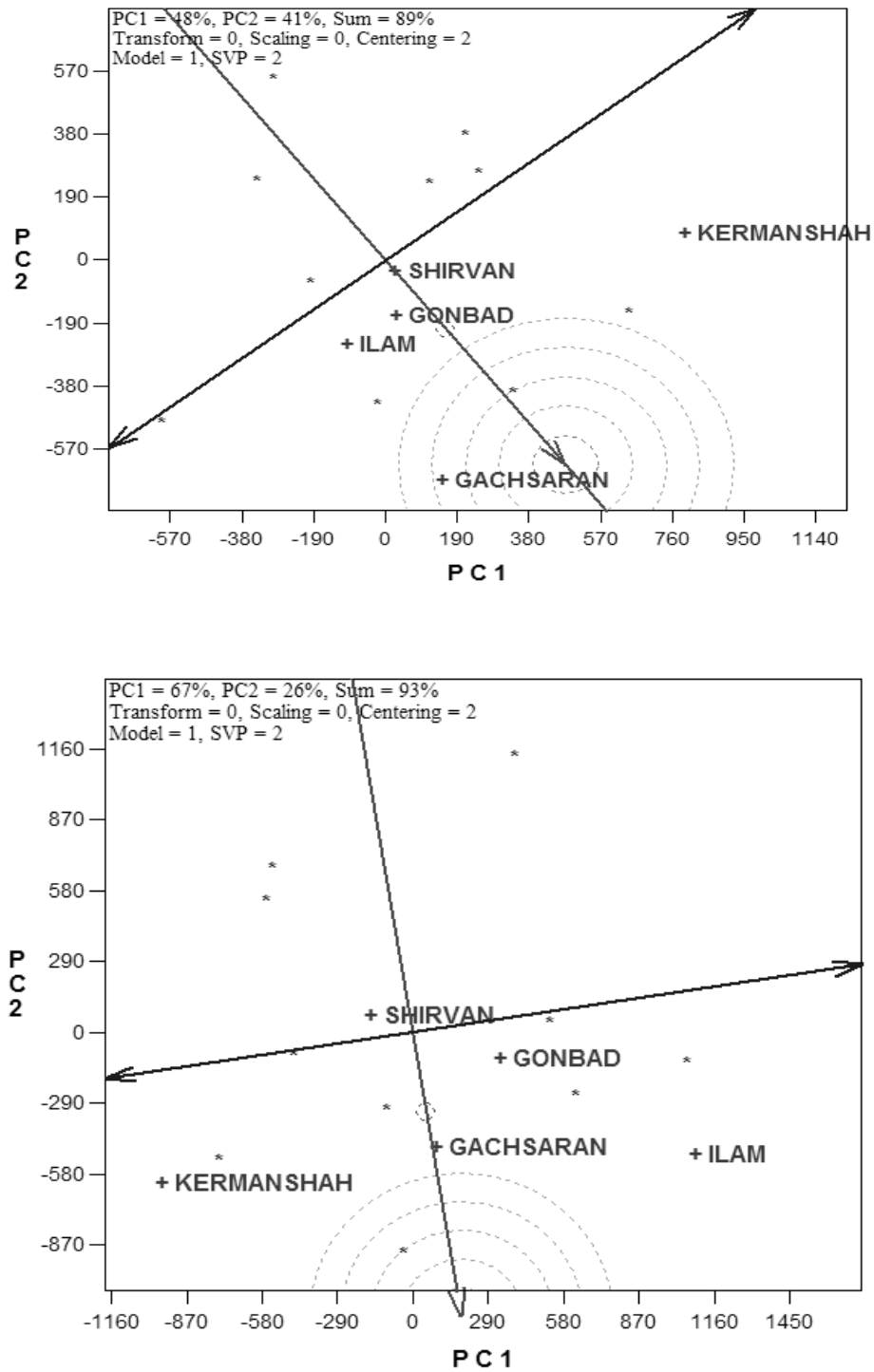
According to Yan (2002), discriminating ability and representativeness are the important properties of a test location which an ideal location should be highly differentiating of the tested genotypes and at the same time representative of the target locations. According to Figure 4A, location Gachsaran is more desirable test location than the other test locations in the first year. Similarly, based on Figure 4B, location Gachsaran is detected as the more desirable test location in the second year. Thus, genotype evaluation in Gachsaran maximizes the observed genotypic variation among genotypes for grain yield of lentil. The discriminating ability of a location can show the comparison of genotypes, but the presence of GE interaction complicates the identification of genotypes in the ideal test location (Yan *et al.* 2000). Usually non-additive or crossover GE interaction was observed in the most MET and it is essential to reveal the nature of GE interaction. GGE methodology is suitable tool to analyze these kinds of interactions

The most prominent relations based on Figure 3A were: (i) positive associations between Gonbad and Ilam locations; and between Gonbad and Gachsaran locations and (ii) a low or near-zero correlations between Gachsaran with Kermanshah locations in the first year. In the second year, (i) positive correlation between Gonbad and Ilam locations, (ii) a low or near-zero correlations between Gachsaran with Kermanshah locations, and between Gachsaran with Ilam locations, and (iii) a negative associations between Ilam and Kermanshah locations, were observed (Figure 3B). Although some of the above predictions can be verified from the Pearson's correlation coefficients but some others are not consistent with the original coefficients of correlation. Such discrepancies are seen because the GGL biplot method explained lower than 100% of the total variation. Although, all above conclusions have some errors but GGL biplot shows predictions on the general pattern of the whole dataset, the predictions are probably more reliable than the individual observations (Yan and Hunt 2002).

Locations with small angles (Gonbad and Ilam) between them were highly positively correlated across two years, and they provided similar information on genotypes, suggesting that these locations provide redundant information about genotypes. Obtaining similar information by using fewer test environments should reduce the cost of testing and increase breeding efficiency. Therefore, we can suggest that one of the two locations in each set be dropped to reduce the cost of testing. Also, in the vector view of the biplot,

main effect and absolute small PC2 scores in order to be more representative of the overall locations (Yan and Rajcan 2002).

partitioning them into their PCs. The test location should has large PC1 scores in order to discriminate genotypes in terms of the genotypic



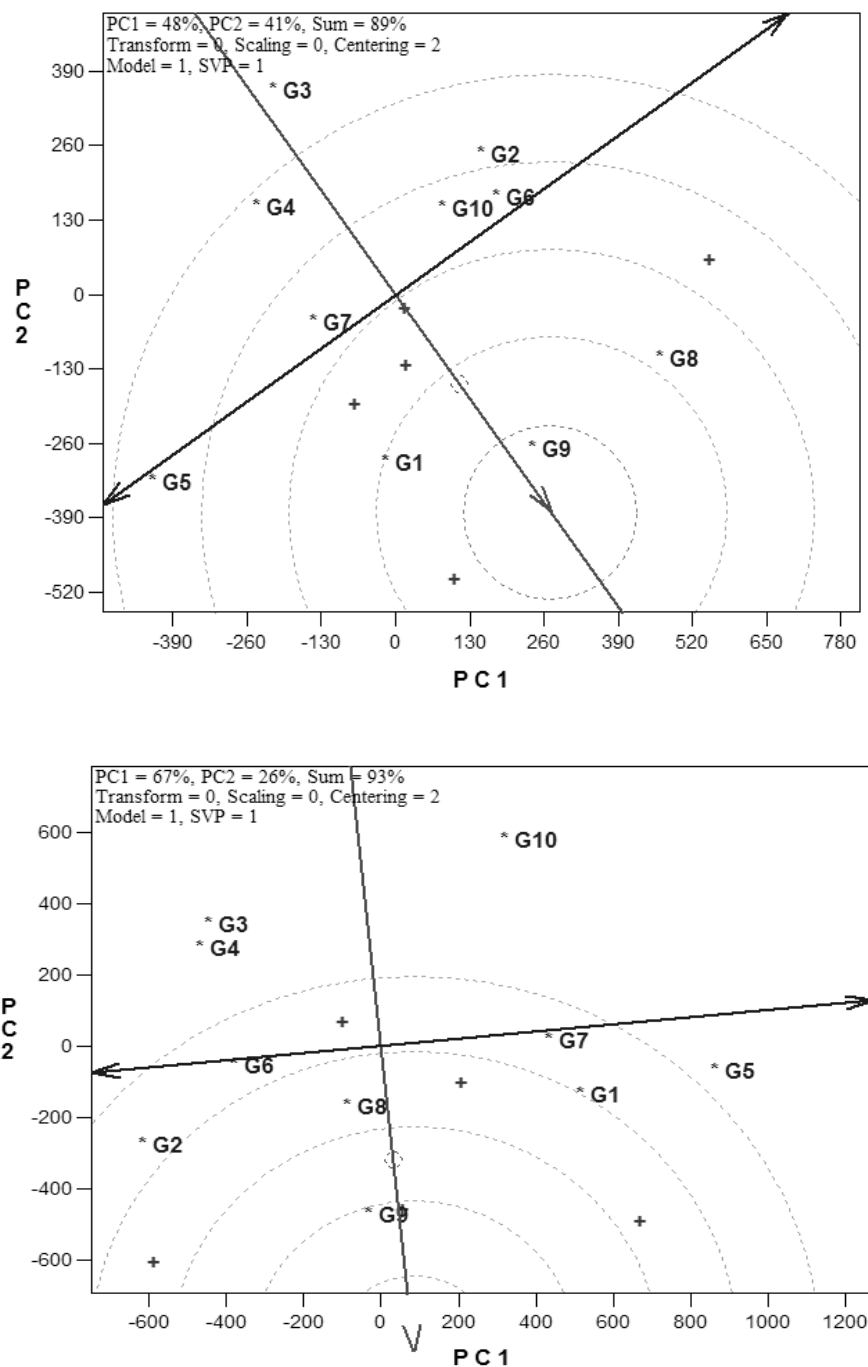
**Figure 4.** Site regression (SREG) biplot of ideal location and comparison of the location (A) up for first year and (B) down for second year

predictor of yield under similar low-stress target environments. Grain yield in the abiotic stress conditions was a poor predictor of yield in the target environments. Our findings are in agreement with those reported by Cooper *et al.* (1997), given that favorable location (Gachsaran) was more representative of the overall locations and more powerful to discriminate genotypes than the unfavorable ones.

### Conclusions

Our results indicated that the GGE biplot model is an excellent tool for visual MET data analysis. It has some advantages: graphical presentation, be more interpretative and facility of mega-environments identification. In conclusion, the following results can be summarized from the this investigation: (i) G9 (ILL6199) was found to be the most stable genotype as well as high mean yield performance and so is recommended for commercial release in semi-arid areas of Iran; and (ii) the GGE model was found to be useful in detecting the yield stability of the genotypes studied.

Another interesting application of GGE biplot procedure is to evaluate genotypes relative to an ideal genotype. This genotype as a virtual genotype is one that has both high mean yield and high stability. This genotype has large PC1 scores (high mean yield) and small (absolute) PC2 scores (high stability). A genotype is more favorable if it is closer to the ideal genotype position. Therefore, genotypes G9, G1 and G8 were more desirable than other lentil genotypes in the first year (Figure 5A) and genotypes G9, G8, G1 and G2 were more desirable than other lentil genotypes in the second year (Figure 5B). It seems that ideal genotype procedure of GGE biplot methodology is a proper tool for identifying high yielding genotypes as the most stable ones. This method can be considered similar to AMMI model which try to facilitate identifying more stable genotypes using AMMI procedure (Gauch and Zobel, 1996). In other words, ideal genotype procedure attempts to define the GE interaction by one parameter (distance from the ideal genotype) and summarize complex aspect of GE interaction using only one parameter. Cooper *et al.* (1997) suggested that yield under low-stress conditions was an effective



**Figure 5. Site regression (SREG) biplot of ideal genotype and comparison of the genotypes with the ideal genotype for the ten lentil genotypes which grown in five locations (A) up for first year and (B) down for second year**

available a time-limited version of GGE biplot. Contributions of the cooperators of the Iran's Dryland Agricultural Research Institute research stations are also gratefully acknowledged.

#### Acknowledgments

We wish to thank kindly Dr. Wei-Kai Yan (Eastern Cereal Oilseed Research Center of Agriculture and Agri-Food Canada) for making

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