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## GGEbiplot analysis of genotype $\times$ environment interaction in *Agropyron intermedium*

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### Abstract

In order to identify genotypes of *Agropyron intermedium* with high forage yield and stability an experiment was carried out in the Research station of Kermanshah Iran. The 11 accessions were sown in a randomized complete block design with three replications under rainfed and irrigated conditions during 2013-21-014 cropping seasons. Combined analysis of variance indicated high significant differences for location, genotype and G  $\times$  E interaction (GEI) at 1% level of probability. Mean comparisons over environments introduced G<sub>4</sub>, G<sub>3</sub> and G<sub>5</sub> with maximum forage yield over rainfed and irrigated conditions. Minimum forage yield was attributed to genotype G<sub>1</sub>. GGEbiplot analysis exhibited that the first two principal components (PCA) resulted from GEI and genotype effect justified 99.37% of total variance in the data set. The four environments under investigation fell into two apparent groups: irrigated and rainfed. The presence of close associations among irrigated (E<sub>1</sub> and E<sub>3</sub>) and rainfed (E<sub>2</sub> and E<sub>4</sub>) conditions suggests that the same information about the genotypes could be obtained from fewer test environments, and hence the potential to reduce testing cost. The which-won-where pattern of GGEbiplot introduced genotypes G<sub>3</sub> and G<sub>4</sub> as stable with high forage yield for rainfed condition, while G<sub>5</sub> was stable with high yield for irrigated condition. According to the comparison of the genotypes with the Ideal genotype accessions G<sub>4</sub>, G<sub>3</sub> and G<sub>9</sub> were more favorable than all the other genotypes.

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## Introduction

*Agropyron* with high forage yield and wide stability in different climate especially drought and Salt tolerance is one of the most important forage crops (Sutka *et al.*, 1995). Since, there is high variation within and among different species of *Agropyron*, so selection response for improving important traits is high (Arghavani *et al.*, 2010). *Agropyron* has been applied in wide hybridization specially to transfer alien genes into cultivated wheat (Farshadfar, 2012; Xu, and Conner, 1994).

In crop breeding programs, genotypes are evaluated in multi-environment trials (METs) for testing their performance across environments and selecting the best genotypes in specific environments. Genotype  $\times$  environment (GE) interaction is an important issue faced by plant breeders in crop breeding programs. A significant GE interaction for a quantitative trait such as grain yield can seriously limit progress in selection. Variance due to GE interaction is an important component of the variance of phenotypic means in selection experiments (Hallauer *et al.*, 2010).

GEI affects breeding progress because it complicates the demonstration of superiority of any genotype across environments and the selection of superior genotypes (Magari and Kang, 1993; Ebdon and Gauch, 2002). Another undesirable effect of GEI includes low correlation between phenotypic and genotypic values, thereby reducing progress from selection. This leads to bias in the estimation of heritability and in the prediction of genetic advance (Comstock and Moll, 1963). Therefore, the magnitude and nature of GEI determine the features of a selection and testing program.

Yield data from regional performance trials, or more generally, multi-environment trials (MET), are usually quite large, and it is difficult to understand the general pattern of the data without some kind of graphical presentation. The biplot technique provides a powerful solution to this problem. A biplot that displays the GGE of a MET data, referred to as a GGE

Biplot (graphical method), is an ideal tool for MET data analysis (Yan, 2001; Yan and Hunt, 2001).

The GGE biplot analysis of these data showed that ideal test environments could discriminate superior performing from poor ones, and identify the target areas. GGE biplot analysis was recently developed to simultaneously use some of the functions of stability methods. In phenotypic variation, E explains most of the variation, and G and  $G \times E$  are usually small (Yan, 2002). However, only G and  $G \times E$  interaction are relevant to cultivar evaluation, particularly when  $G \times E$  interaction is determined as repeatable (Hammer and Cooper, 1996). Hence, Yan *et al.* (2000) deliberately put the two together and referred to the combination as GGE. Following the proposal of Gabriel (1971), the biplot technique was also used to display the GGE of MET data, and is referred to as a GGE biplot (Yan, 2001; Yan *et al.*, 2000). The GGE biplot is in fact a data visualization tool that graphically displays  $G \times E$  interaction in a two-way table (Yan *et al.*, 2000). The GGE biplot is an effective tool for the following applications: 1) Mega-environment analysis (e.g.; “which won - where” pattern), whereby specific genotypes can be recommended for specific mega-environments (Yan and Kang, 2003). 2) Genotype evaluation (mean performance and stability), and 3) Environmental evaluation (to discriminate among genotypes in target environments). GGE biplot analysis is increasingly being used in  $G \times E$  interaction studies in plant breeding research (Butron *et al.*, 2004; Dehghani *et al.*, 2006; Kaya *et al.*, 2006; Samonte *et al.*, 2005; Yan and Tinker, 2005).

The objectives of this study were (i) to interpret G main effect and GE interaction obtained by combined analysis of yield performances of 11 *Agropyron intermedium* over 4 environments (ii) application of the GGE biplot technique to identify stable and high yielding genotypes.

## Materials and methods

In order to evaluate phenotypic stability of forage

yield 11 accessions of *Agropyron intermedium* were prepared from gene bank of Research Institute of Forests and Rangelands, Tehran, Iran (Table 1).

The experiment was carried out in the Research station of Kermanshah Iran (47° 20' N latitude, 34° 20' E longitude and 1351.6 m altitude). Climate in the region is classified as semiarid with mean annual rainfall of 378 mm. Minimum and maximum temperature at the research station were -27°C and 44°C, respectively.

The genotypes were sown in a randomized complete block design with three replications under rainfed and irrigated conditions during 2013-21-014 cropping seasons. Each replication consisted of 11 genotypes with 2 m length and 1 m wide and the distance between two plots was 75 cm. Single seeds were planted in 4 rows with 25 cm distance. Each plot consisted of 3 rows with 1 m in length and 20-cm row spacing. Data on forage yield were taken from all rows of each plot. At harvest forage yield was determined for each genotype at each test environments.

*Statistical analysis*

Analysis of variance on grain yield was conducted by Genstat software to determine the effect of environment (E), genotype (G) and GE interaction. Coefficients between pairs of locations were computed via SAS 9.2 software. The first two components resulted from principal components were used to obtain a biplot by GGE biplot software (Yan, 2001). The basic model for a GGE Biplot is:

$$Y_{ij} - \mu - \beta_j = \sum_{i=1}^k \lambda_i \xi_{il} \eta_{lj} + \varepsilon_{ij}$$

(1)

Where  $Y_{ij}$  = the mean yield of genotype  $i(=1,2,\dots,n)$  in environment  $j(=1,2,\dots,m)$ ,  $\mu$  = the grand mean,  $\beta_j$  = the main effect of environment  $j$ ,  $(\mu + \beta_j)$  being the mean yield of environment  $j$ ,  $\lambda_i$  = the singular value (SV) of  $l$ th principal component (PC), the square of

which is the sum of squares explained by  $PC_l(l=1,2,\dots,k$  with  $k \leq \min(m,n)$  and  $k=2$  for a two-dimensional biplot),  $\xi_{il}$  = the eigenvector of genotype  $i$  for  $PC_l$ ,  $\eta_{lj}$  = the eigenvector of environment  $j$  for  $PC_l$ ,  $\varepsilon_{ij}$  = the residual associated with genotype  $i$  in environment  $j$ . To generate a biplot that can be used in visual analysis of MET data, the SVs have to be partitioned into the genotype and environment eigenvector so that the model (1) can be written in the

form of  $Y_{ij} - \mu - \beta_j = \sum_{i=1}^k g_{il} e_{lj} + \varepsilon_{ij}$  where  $g_{il}$  and

$e_{lj}$  are called  $PC_l$  scores for genotype  $i$  and environment  $j$ , respectively. In a biplot, genotype  $i$  is displayed as a point defined by all  $g_{il}$  values, and environment  $j$  is displayed as a point defined by all  $e_{lj}$  values ( $l=1$  and  $2$  for a two-dimensional biplot) (Yan and Kang, 2003).

**Results and discussion**

*Combined analysis of variance and mean comparisons*

Combined analysis of variance indicated high significant differences for location, genotype and  $G \times E$  interaction (GEI) at 1% level of probability (Table 2). But maximum contribution of variance was observed for location (70.34%). In the multienvironment experiment the contribution of environment (location and year) is more than  $G$  and GEI (Farshadfar *et al.*, 2012). Farshadfar (2012) reported that in the *Agropyron species* different water potential in the irrigated and rainfed conditions accounted for maximum contribution of location. Significant difference between the genotypes indicating that selection for forage yield is desirable for introduction of high yielding accessions. Significant GEI with 6.62% of contribution in the total sum of squares (SS) exhibiting that we can proceed and calculate phenotypic stability in the genotypes under investigation. Least contribution of year effect in the total SS (0.02%) revealed that the effect of year on the forage yield is low.

Mean comparisons over environments introduced G4 (5284g), G3 (5079g) and G5 (5043g) with maximum forage yield over rainfed and irrigated conditions. Minimum forage yield was attributed to genotype one (G1=3708g).

**Table 1.** Numbers and codes of the *agropyron intermedium* accessions investigated.

Accessions codes	Numbers
890-2	1
890-4	2
890-5	3
890-6	4
890-7	5
890-9	6
890-10	7
890-11	8
89013	9
890-14	10

**Table 2.** Combined analysis of variance.

S.O.V	D.F	SS	% of TSS	MS
Location (L)	1	202898725	70.34	202898725**
Error 1	4	2305838	0.80	576460
Genotype (G)	10	31884997	11.05	3188500**
G×L	10	19094158	6.62	1909416**
Error 2	40	26558028	9.21	663951
Year (Y)	1	66286	0.02	66286 <sup>ns</sup>
Y×L	1	55884	0.02	55884 <sup>ns</sup>
Y×G	10	114008	0.04	11401 <sup>ns</sup>
Y×G×L	10	381308	0.13	38131 <sup>ns</sup>
Error 3	44	5091701	1.77	115720

\*\* : Significant at 1% probability level; ns: non-significant.

*Relationships among test environments*

GGE biplot, which was based on environment focused scaling, was portrayed to estimate the pattern of environments (Fig. 1). The vector view of the GGE biplot (Fig. 1) provides a summary of the interrelationships among the environments. The lines that connect the test environments to the biplot origin are called environment vectors. The cosine of the angle between the vectors of two environments approximates the correlation between them. For example, E1 and E3 (irrigated conditions) and E2 and E4 (rainfed conditions) were positively correlated (an

890-15	11
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*GGEbiplot analysis of phenotypic stability*

The GGE biplot 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 twodimensional biplot so that the interaction between each genotype and each environment can be visualized (Yan and Hunt , 2001). GGEbiplot was employed to identify stable genotypes. The first two principal components (PCA) resulted from GEI and genotype effect justified 99.37% of total variance in the data set. PCA1 and PCA2 explained 81.75% and 17.62% of variability respectively.

acute angle). But irrigated (E1 and E3) and rainfed (E2 and E4) conditions were not correlated (a right angle) indicating the environmental diversity and independent in genotype rankings.

The distance between two environments measures their dissimilarity in discriminating the genotypes. Thus, the four environments fell into two apparent groups: irrigated and rainfed. The presence of close associations among irrigated (E1 and E3) and rainfed (E2 and E4) conditions suggests that the same information about the genotypes could be obtained

from fewer test environments, and hence the potential to reduce testing cost. If two test environments are closely correlated consistently

across years, one of them can be dropped without loss of much information about the genotypes.

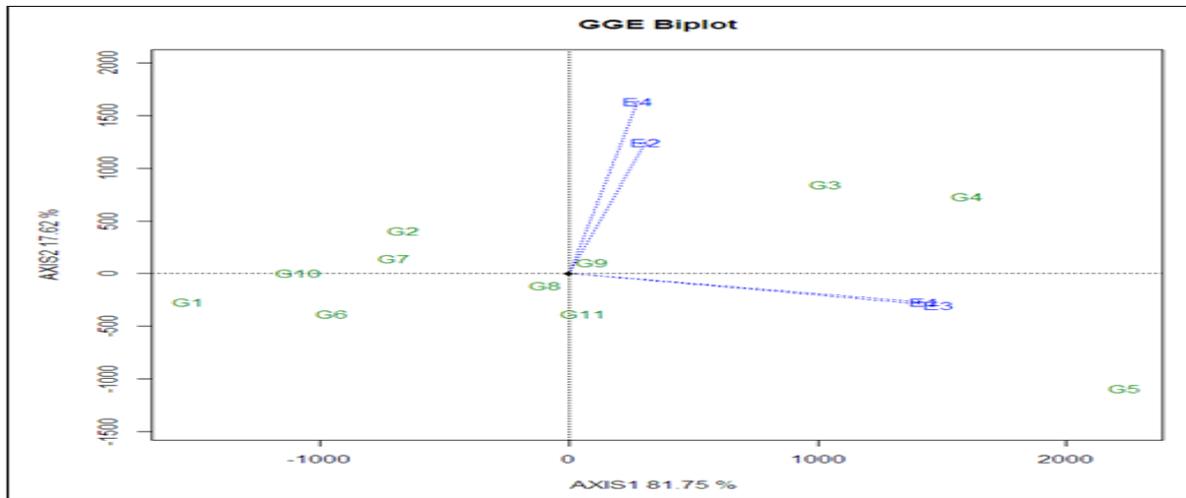


Fig. 1. GGE biplot based on relationships among test environments.

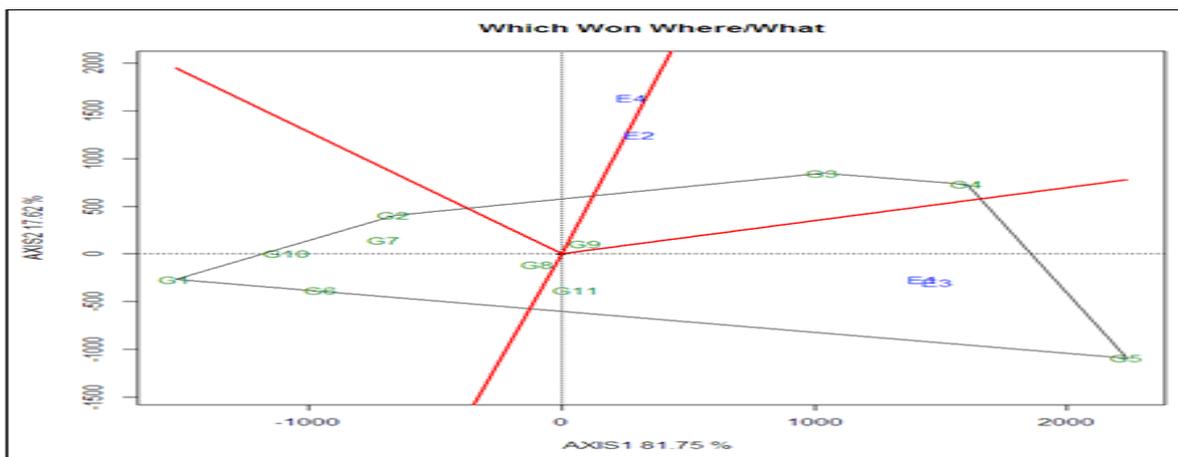


Fig. 2. Polygon views of the GGE biplot based on symmetrical scaling for the which-won-where pattern of genotypes and environments.

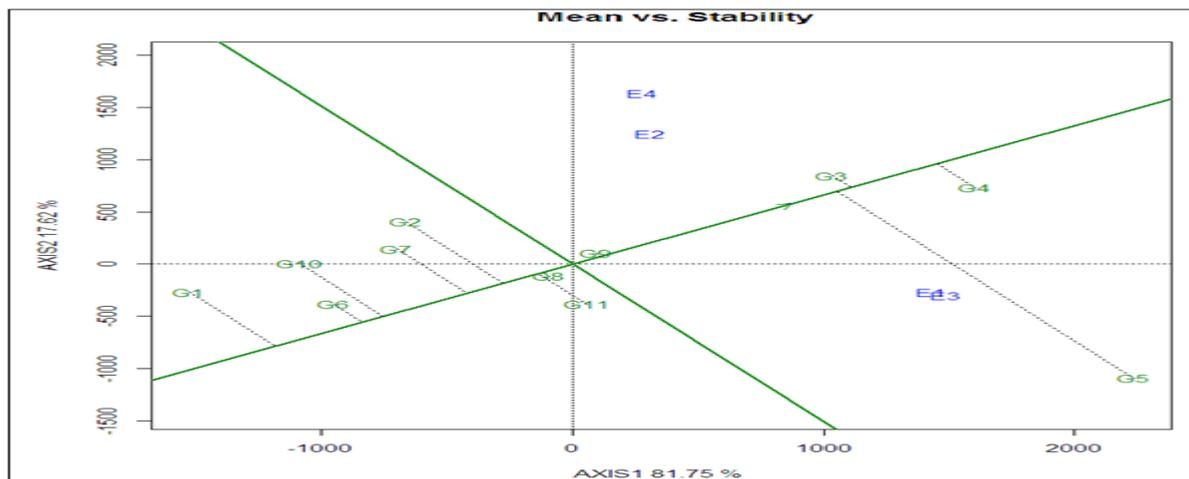
*Which Won Where Pattern*

One of the most attractive features of a GGE biplot is its ability to show the which-won-where pattern of a genotype by environment dataset (Fig. 2). Many researchers find this use of a biplot intriguing, as it graphically addresses important concepts such as crossover GE, mega environment differentiation, specific adaptation, etc (Yan and Tinker, 2006). The polygon is formed by connecting the markers of the genotypes that are further away from the biplot origin such that all other genotypes are contained in the polygon. Genotypes located on the vertices of the

polygon performed either the best or the poorest in one or more environments since they had the longest distance from the origin of biplot. The perpendicular lines are equality lines between adjacent genotypes on the polygon, which facilitate visual comparison of them. These lines divide the biplot into 4 sectors, and the environments fall into 2 of them (Fig. 2). An interesting feature of this view of a GGE biplot is that the vertex genotype(s) for each sector has higher (some times the highest) yield than the others in all environments that fall in the sector (Yan, 2002). Thus, E2 and E4, fell into sector 1 and the vertex

genotypes for this sector were G3 and G4, suggesting that they are stable with high forage yield and adapted with rainfed conditions. Similarly, E1 and E3, fell into sector 2 and the vertex genotype for this sector was G5, suggesting that the higher-yielding genotype with adaptability for irrigated condition was

G5. Sector 3 included G1, G2, G6, G7, G8 and G10 which showed no specific adaptation to any environment indicating their low yield in both conditions. Sector 4 with rainfed environment contained no genotypes.



**Fig. 3.** Average environment coordination (AEC) views of the GGE-biplot based on environment-focused scaling for the means performance and stability of genotypes.

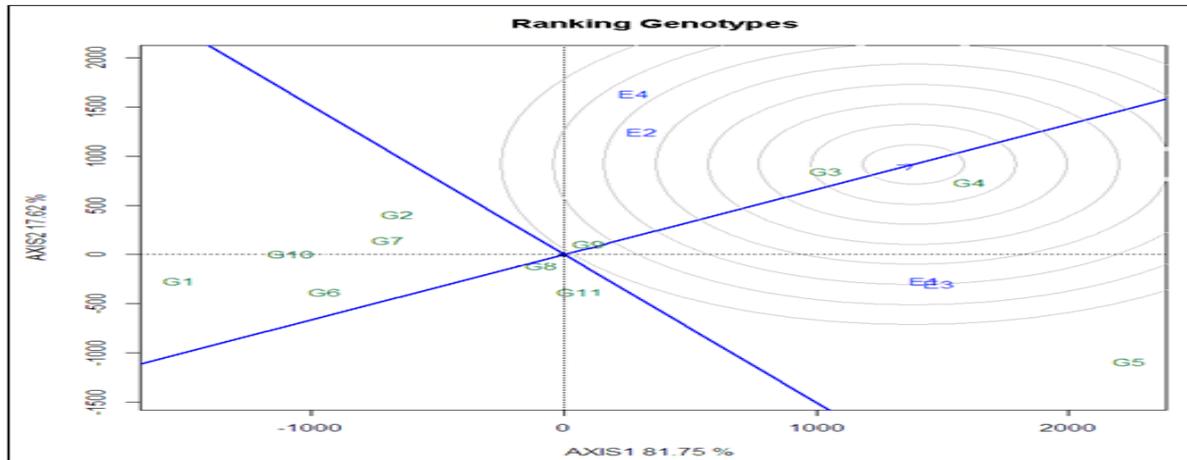
Performance and stability of genotypes were visualized graphically through the GGE biplot (Fig. 3). In Figure 3 X-axis is an indicator of forage mean yield, while Y-axis exhibits stability of genotypes. Therefore it is possible to identify simultaneously genotypes with high yield with stability. It is to be mentioned that in GGEbiplot the effect of G + GEI are considered simultaneously and not separated from each other, that, s why the line has an ascending order. If the contribution of G and GEI in the variance is equal, then the horizontal and central line will be parallel to X-axis, but here the contribution of GEI is less than G and this is the cause of line ascending order, to avoid confounding effect of G and GEI, and proportion of variance of PC1(which is usually the effect of genotype) go for GGI and therefore the effect G and GEI separate from each other (Yan *et al.*, 2009a). Yan *et al.* (2009b) reported that the ideal genotype has high PC1(high yield) and low PC2 (high stability). However yield and stability of accessions can be evaluated by average environment coordination (AEC) method (Yan, 2001; 2002). In

Fig. 3 the line with single arrow head is the AEC (average environment coordinate) abscissa. AEC abscissa passes through the biplot origin and marker for average environment and points towards higher mean values. The average environment has average PC1 and PC2 scores across environments (Yan, 2001).

The perpendicular lines to the AEC passing through the biplot origin are referred to as AEC ordinate. The greater the absolute length of the projection of a genotype indicates more instability. Furthermore, the average yield of genotypes is approximated by the projections of their markers to the AEC abscissa (Yan and Kang, 2003). According to Fig. 3, genotypes with above average means were from G9, G3 and G4, while genotypes below-average means were from G8 to G1. However, the length of the average environment vector was sufficient to select genotypes based on yield mean performances. Genotypes with above-average means (G9, G3, G4 and G5) could be selected, whereas the rest were discarded. A longer projection to the AEC ordinate, regardless of the direction,

represents a greater tendency of the GE interaction of a genotype, which means it is more variable and less stable across environments or vice versa. For

instance, genotype G3 was more stable as well as high yielding followed by G4. Conversely, G5 was unstable, but high yielding.



**Fig. 4.** GGE biplot based on genotype-focused scaling for comparison the genotypes with the ideal genotype.

*Comparison of the Genotypes with the Ideal Genotype*

An ideal genotype have the highest mean performance and be absolutely stable (i.e., perform the best in all environments). Such an ideal genotype is defined by having the greatest vector length of the high-yielding genotypes and with zero GE, as represented by the small circle with an arrow pointing to it (Yan, 2001). Although such an ideal genotype may not exist in reality, it can be used as a reference for genotype evaluation. A genotype is more desirable if it is located closer to the ideal genotype. Thus, using the ideal genotype as the center, concentric circles were drawn to help visualize the distance between each genotype and the ideal genotype (Fig. 4). In Fig. 4 the genotypes are ranked relative to the ideal genotype. A genotype is more favorable if it is closer to the ideal genotype. Accordingly, genotypes G4, G3 and G9 were more favorable than all the other genotypes. The other genotypes were unfavorable because they were far away from the ideal genotype.

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