



RESEARCH PAPER

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Evaluation of phenotypic stability in canola (*Brassica napus*) using GGE-biplot

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Key words: Canola, genotype × environment interaction, GGE biplot, stability.

<http://dx.doi.org/10.12692/ijb/6.1.350-356>

Article published on January 10, 2015

Abstract

In order to explore the effect of genotype, environment and genotype × environment interaction (GEI) on grain yield of 15 canola genotypes in four different locations, a randomized complete block design with 3 replications in each location was laid out on 2013-2014 growing season. GGE biplot graphical method was used to visualize any relationships among treatments and environments. The results of analysis of variance showed highly significant effects among genotypes, environments (locations) and genotypes × environment interactions indicating the presence of genetic diversity and possible detection of phenotypic stability in genotypes. The first 2 principal components (PC1 and PC2) that explained 65.11% and 19.64% of GGE sum of squares (SS), were used to create a 2-dimensional GGE biplot. The graphics obtained from GGE biplot for clustering of the environments revealed three clusters: First group included E2 and E1, second group covered E4 and third cluster included E3. The graphics also showed the best genotype for each environment and ranked all genotypes based on all environments too. Collective analysis of the biplots suggested two canola mega-environments. The first mega-environment contained environments: E3 with genotype G10, genotype G7 gave the highest performance in environment E1, E2 and E4. Genotypes G7, G9 and G14 exhibited the highest mean yield and stability, while genotypes G1, G8 and G5 displayed the poorest mean yield and stability. On the other hand, E4 was the best representative of the overall environments and the most powerful to discriminate genotypes performance.

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Introduction

Oil seeds are the second source of world food after cereals and according to FAO statistics, rapeseed is the third source of vegetable oil after soybean and palm (FAO, 2010). The oilseed *Brassica* species especially rapeseed (*B. napus* L.) have important role in oilseed production because of their wide adaptation to different diverse climatic conditions (Downey and Rimer, 1993). Seed yield of canola is a quantitative trait, which is largely influenced by the different environmental effects and hence in most of the cases it has low heritability (Rameeh, 2010).

Multi-environment trials (MET) are conducted to evaluate yield stability performance of genetic materials under varying environmental conditions (Yan *et al.*, 2000; Yan and Rajcan, 2002). A genotype grown in different environments will frequently show significant fluctuations in yield performance. These changes are influenced by the different environmental conditions and are referred to as genotype-by-environment (GE) interaction (Allard and Bradshaw, 1964). However, GE interaction reduces the genetic progress in plant breeding programs through minimizing the association between phenotypic and genotypic values (Comstock and Moll, 1963). Hence, GE interaction must be either exploited by selecting superior genotype for each specific target environment or avoided by selecting widely adapted and stable genotype across wide range of environments (Ceccarelli, 1989).

The detection of GEI in trials has led to the development of procedures that are generically called stability analyses. The numerous stability statistics available to the plant breeder and to the production agronomist provide different strategies and approaches of dealing with GEI. Stability is an important concept for plant breeders interested in analyzing GEI data (Farshadfar *et al.*, 2012).

GGE biplot analysis was recently developed to use some of time function of these methods jointly. The biplot method originated with Gabriel (1971), and its use was subsequently expanded by Kempton (1984)

and Zobel *et al.* (1988). The extensive usefulness of GGE biplot, where G = genotype effect and GE = genotype-by environment effect, has only recently been elucidated. The GGE biplot is a multi-faceted tool in quantitative genetic analyses and plant breeding. In addition to dissecting GEI, GGE Biplot helps analyze genotype-by-trait data, genotype-by-marker data and diallel cross data (Yan, 2001). These aspects make GGE biplot a most comprehensive tool in quantitative genetics and plant breeding.

GGE biplot (Yan, 2001), is a graphical method to display the information in a bilateral table and was used to visualize any crossover treatment - environment interactions, relationships among treatments and relationships among environments (Ma *et al.*, 2004) and in plant breeding it has been known as an innovative methodology in biplot graphical analysis. GGE biplot analysis is an effective method which is based on principal component analysis (PCA) and also it is increasingly being used in agriculture for G×E interaction data analysis (Asadi *et al.*, 2013).

The biplot technique was used to display the GGE of METs data, referred to as a GGE biplot (Yan, 2001; Yan *et al.*, 2000). GGE biplot is an effective tool for: 1) megaenvironment analysis ("which-won-where" pattern), whereby specific genotypes can be recommended to specific mega-environments; 2) genotype evaluation (the mean performance and stability), and 3) environmental evaluation (the power to discriminate among genotypes in target environments) (Yan and Kang, 2002).

The objectives of this study were to interpret G main effect and GE interaction of 15 rapeseed genotypes over 4 environments; (2) application of the GGE biplot technique to examine the possible existence of different mega environments (3) visual assessment of yield variation across environments based on the GGE biplot and (4) application of this method to determine discriminating ability and representativeness of the environments.

Materials and methods

Plant genetic materials

This experiment was carried out in four different locations of Kermanshah, Iran during 2013-2014 growing season. A set of 15 canola genotypes selected from advanced experiments of research stations were used as experimental material (Table 1). Experimental layout was a randomized complete block design with 3 replications in each location. Each plot consisted of 4 rows of 6 meter length. Data on seed yield were taken from the middle two rows of each plot. At harvest seed yield was determined for each genotype at each test environments.

Statistical analysis

Analysis of variance was done to determine the effects of genotype, environment and genotype \times environment interaction using the SAS 9.1 software. The graphic representation of data for “which is best for what”, “ranking of genotypes based on yield and stability”, “examining the genotypes and environments”, “relation among environments” and ranking environments based on genotypes a GGE biplot ver 6.3 was used. The first two components resulted from principal components were used to obtain a biplot by GGE biplot software (Yan, 2001).

Results and discussion

Combined analysis of variance exhibited significant differences among environments (E), genotypes (G) and G \times E interaction (Table 2) indicating the presence of genetic diversity and possible detection of phenotypic stability in genotypes. The first and second interaction principal component analysis (IPCA1 and IPC2) explained 65.11% and 19.64% of the G \times E variation, respectively. GGE stands for genotype main effect (G) plus genotype by environment interaction (GE) and the GGE concept is based on the understanding that genotype main effect (G) and genotype by environment interaction (GE) are the two sources of variation that are relevant to genotype evaluation and that they must be considered simultaneously, not alone or separately, for appropriate genotype evaluation (Yan, 2002).

The “which is best for where”

Visualization of the “which-won-where” pattern of MET data is important for studying the possible existence of different mega-environments in a region (Gauch and Zobel, 1997; Yan *et al.*, 2000, 2001). The polygon view of a biplot is the best way to visualize the interaction patterns between genotypes and environments and to effectively interpret a biplot (Yan and Kang, 2003).

Table 1. Genotype code and name of 15 canola genotypes.

No.	Code	Name
1	G1	GK Helena
2	G2	GK olivia
3	G3	Antol
4	G4	GKH1103
5	G5	Billy
6	G6	Liliane
7	G7	GKH 305
8	G8	Lioness
9	G9	Modena
10	G10	Okapi
11	G11	Opera
12	G12	Slm046
13	G13	Talaye
14	G14	Zarfam
15	G15	Oase

The polygon view of the GGE biplot (Fig. 1) indicates the best genotype(s) in each environment and total environments. The polygon was established by connecting the markers of the genotypes that are endmost away from the biplot origin that all other genotypes are contain in the polygon. The rays are lines that are perpendicular to the sides of the polygon or their extension (Yan, 2002; Ding *et al.*, 2007). In Figure 1, ray 1 is perpendicular to the side that connects G10 and G1, ray 2 is perpendicular to the side that connects G1 and G5, and so on. These five rays divided the biplot into five sections, and all environments fall into two of them, so that the three environments fall into one of them. The peak genotypes for each quadrant are the one that gave the highest amount of yield for the environments that fall within that quadrant. The highest amount of yield in environment E3 was belonged to G10 and in E1, E2 and E4 was attributed to G7. The other vertex

genotypes, G₁ and G₂ are the poorest in all environments. Biplot can be used to visualize interaction among genotypes and trait.

Ranking of genotypes based on yield and stability

Yield performance and stability of genotypes were evaluated by an average environment coordination (AEC) method (Yan, 2001; Yan and Hunt, 2002; Yan, 2002). In this method, an average environment is defined by the average PC₁ and PC₂ scores of all environments, represented by a small circle (Fig. 2). A line is then drawn to pass through this average environment and the biplot origin; this line is called the average environment axis and serves as the abscissa of the AEC. The ordinate of the AEC is the line that passes through the origin and is perpendicular to the AEC abscissa (Fig. 2). Unlike the AEC abscissa, which has one direction, with the arrow pointing to greater genotype main effect, the AEC ordinate is indicated by double arrows, and either direction away from the biplot origin indicates greater GEI effect and reduced stability. The AEC ordinate

separates genotypes with below-average means from those with above-average means. Furthermore, the average yield of genotypes is approximated by the projections of their markers to the AEC abscissa. The length of the average environment vector (the distance from biplot origin and the average environment marker), relative to the biplot size, is a measure of the relative importance of genotype main effect vs. GEI. The longer it is, the more important is the genotype main effect, and the more meaningful the selection based on mean performance. For this study, the length of the average environment vector was sufficient to select genotypes based on yield mean performances. Genotypes with above-average means could be selected (genotypes 7, 14, 9, 2, 15, 10, 6, 13 and 12), whereas the rest were discarded. On the other hand, genotypic stability is quite crucial, in addition to genotype yield mean. A longer projection to the AEC ordinate, regardless of the direction, represents a greater tendency of the GEI of a genotype, which means it is more variable and less stable across environments or vice versa.

Table 2. Analysis of variance for grain yield of 15 canola genotypes in four environments.

Source	df	SS	MS	F	F prob
Treatments	59	133226375	2258074	60.03	0.000
Genotypes	14	37522360	2680169	71.25	0.000
Environments	3	34201261	11400420	261.24	0.000
Block	8	349121	43640	1.16	0.3297
Interactions	42	61502753	1464351	38.93	0.000
IPCA	16	41944656	2621541	69.69	0.000
IPCA	14	18530384	1323599	35.19	0.000
IPCA	12	1027713	85643	2.28	0.01259
Residuals	0	0			
Error	112	4213127	37617		
Total	179	137788623	769769		

The ranking of 15 genotypes based on their mean grain yield and yield stability for 4 environments is shown in Figure 2. It has been reported that when PC₁ in a GGE biplot approximates the G (mean performance), PC₂ must approximate the G × E associated with each genotype, which is a measure of instability (Yan *et al.*, 2000; Yan, 2002).

The line passing through the biplot origin and the environmental average is indicated by circles and is

known as the average environment coordinate (AEC) axis, which is defined by the average PC₁ and PC₂ scores for all environments. Projection of genotype markers onto this axis should, therefore, approximate the mean yield of the genotypes. Thus, genotypes 7, 14, 9 and 2 had higher grain yield. The line which passes through the origin but is perpendicular to the AEC represents the status of the genotypes stability. A position in either direction away from the biplot origin, on this axis, indicates greater G × E interaction

and reduced stability (Yan, 2002). Therefore, genotypes 5, 10, 1 and 3 showed a more variable and less stable performance than the other genotypes. Genotypes 9, 14 and 2 were more stable than the others (Fig. 3). Genotypes 9, 14 and 2 revealed simultaneously high grain yield and stability.

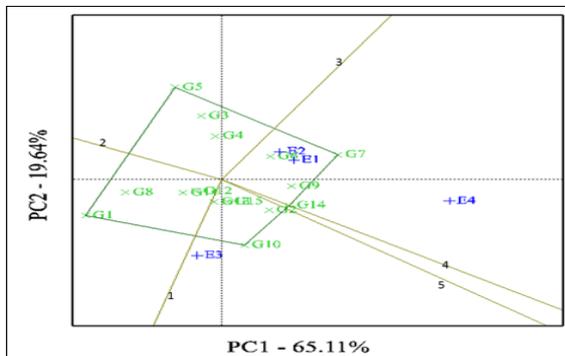


Fig. 1. Polygon views of the GGE biplot based on symmetrical scaling for the which-won-where pattern of genotypes and environments.

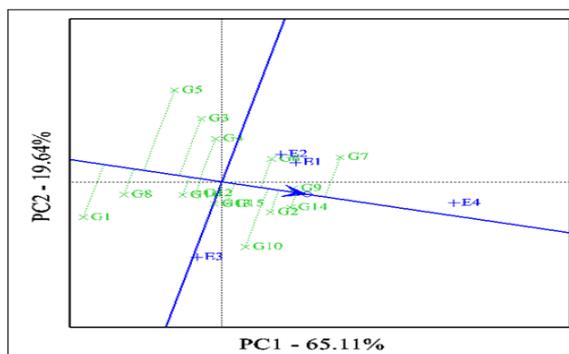


Fig. 2. Average environment coordination (AEC) views of the GGE-biplot based on environment-focused scaling for the means performance and stability of genotypes.

Ranking genotypes and environments relative to the ideal genotype and environment

An ideal genotype should 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 highyielding genotypes and with zero GEI, as represented by an arrow pointing to it (Fig. 3). 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. Because the units of both PC1 and PC2 for the genotypes are the original unit of yield in the genotype-focused scaling (Fig. 3), the units of the AEC abscissa (mean yield) and ordinate (stability) should also be the original unit of yield. The unit of the distance between genotypes and the ideal genotype, in turn, is the original unit of yield as well. Therefore, the ranking based on the genotype-focused scaling assumes that stability and mean yield are equally important (Yan, 2002). Figure 3 revealed that 2, 9 and 14 which fell into the center of concentric circles, were ideal genotypes in terms of higher yielding ability and stability, compared with the rest of the genotypes.

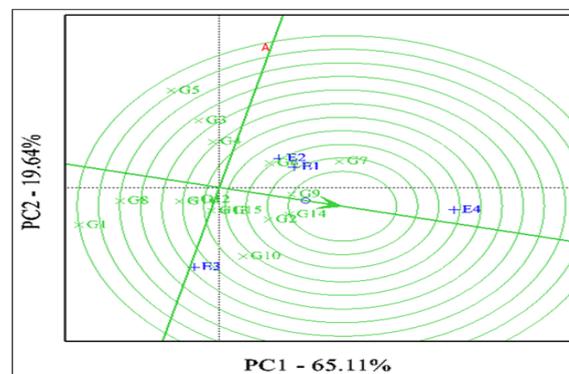


Fig. 3. GGE biplot based on genotype-focused scaling for comparison the genotypes with the ideal genotype

The ideal test environment should have large PC1 scores (more power to discriminate genotypes in terms of the genotypic main effect) and small (absolute) PC2 scores (more representative of the overall environments). Such an ideal environment is represented by an arrow pointing to it (Fig. 5). Although such an ideal environment may not exist in reality, it can be used as a reference for genotype selection in the MEYTs. An environment is more desirable if it is located closer to the ideal environment. Thus, using the ideal environment as the center, concentric

circles were drawn to help visualize the distance between each environment and the ideal environment (Yan *et al.*, 2000; Yan and Rajcan, 2002). Figure 5 indicated that E4 which fell into the center of concentric circles, was an ideal test environment in

terms of being the most representative of the overall environments and the most powerful to discriminate genotypes.

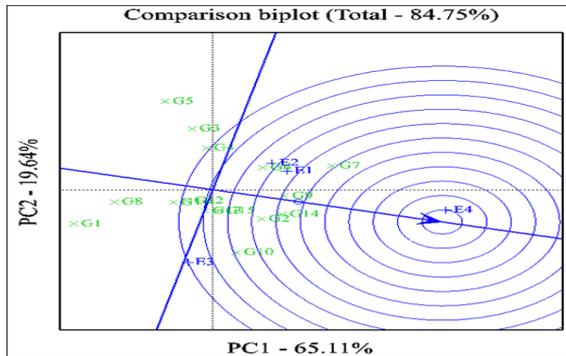


Fig. 4. GGE-biplot based on environment-focused scaling for comparison the environments with the ideal environment.

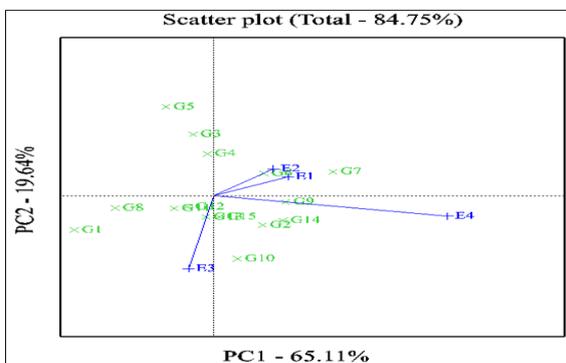


Fig. 4. Location's vector to determine the relation among regions.

Relationship among test environments

Fig. 5 provides the summary of the interrelationships among the test environments. The lines that connect the biplot origin and the markers for the environments are called environment vectors. The angle between the vectors of two environments is related to the correlation coefficient between them. The cosine of the angle between the vectors of two environments approximates the correlation coefficient between them (Kroonenberg, 1995; Yan, 2002). Acute angles indicate a positive correlation, obtuse angles a negative correlation and right angles no correlation (Yan and Kang, 2003). A short vector may indicate that the test environment is not related to other environments. Figure 5 showed the relation among environments. The angle existing between the axes of two environments showed the correlation of two environments. Therefore, the tested

environments were divided into three groups based on biplot technique. The first group consisted E2 and E1, the second group had E4 while the third group had E3.

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