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GGE biplot analysis of wheat-rye disomic addition lines

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Abstract

Chromosome addition lines have often been used to map the genes on donor chromosomes based on the presence/absence of the genes on the chromosomes added to the recipient genome. In this study a set of wheat-rye disomic addition lines (DALs) was used to locate QTLs controlling yield and stability on specific chromosome(s) in rye. Experiments were conducted using a randomized complete block design with three replications under three rainfed and irrigated conditions. The GGE [genotype plus genotype x environment (GE)] biplot methodology was used to analyze the grain yield data attempting to locate the chromosome(s) which probability involved in controlling genetic stability performance in rye. The results of combined ANOVA showed that the environment, genotype and GE interaction effects were found to be significant, indicating remarkable changes in ranking of genetic materials over the environments. According to GGE biplot analysis, two parents (Chinese spring vs. Imperial rye) were different in their adaptations and consequently yield and stability performance. The results also verified that it would be possible to determine contrasting DALs based on the stability and integrating yield with stability performance for improving wheat genetic materials. Ranking of the DALs based on the ideal genotype (high yield and stability performance) revealed that most of the genes involved in controlling high yield and stability performance are located on two chromosomes 7R and 5R in rye. It was also concluded that GGE biplot method can be used as efficient tool for identifying superior genetic materials in a multi-environment trials data.

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Introduction

Genetic materials such as alien additions, substitutions, translocations, deletions, monosomes, ditelosomes, and nullisomes are valuable genetic resources for both plant breeding and basic research (Szakács and Molnár-Láng, 2010). Alien chromosome addition lines have been developed for a variety of plant species and have been used for many purposes such as introducing valuable traits to the recipient species, mapping genes and markers on introgressed alien chromosomes, examining alien gene regulation, understanding meiotic pairing behavior and chromosome structure, and isolating individual chromosomes and genes of interest (Ananiev *et al*., 1997; Islam and Shepherd, 1990; Bass *et al.,* 2000; Muehlbauer *et al.,* 2000; Jin *et al.,* 2004).

Bread wheat (*Triticum aestivum* L.) addition lines have been produced with numerous species related to wheat, including rye (*Secale cereale*). Among these, the 'Chinese Spring' (CS)/'Imperial rye' wheat-rye disomic addition series (Driscoll and Sears, 1971) have been widely used all over the world to study the effect of individual rye chromosomes on quality parameters and resistance to biotic and abiotic stresses in the wheat genetic background, and to locate various genetic markers in rye, such as storage proteins, isozymes, and RFLP or RAPD loci (Gallego *et al.,* 1998; Taylor *et al.,* 1998; Jianzhong *et al.,* 2001; Aniol, 2004; Szakács and Molnár-Láng, 2010).

Wheat (*Triticum aestivum* L., 2n=42) is an important crop, but its ability to adapt in poor environment conditions, is inferior to some of wild grass species. Rye (*Secale cereale* L., 2n=14), one of its wild grass species, possess some good traits, which help its adaptation to poor soil conditions (Li, 1985; Li and Hao, 1990). Because rye and wheat, cross easily, a set of wheat–rye disomic addition lines were developed (Jianzhong *et al.,* 2001).

By growing disomic addition lines (DALs) under different growing conditions it may help to find genes useful for making wheat adaptable to unpredictable conditions. However, little is know about the study of genotype x environment (GE) interactions to determine the gene controlling stability performance in wheat-rye disomic addition lines.

The GE interactions have been studied regarding genotype stability in different species crops (Wricke, 1962; Finlay and Wilkinson, 1963; Eberhart and Russell, 1966; Becker and Leon, 1988; Lin and Binns, 1988; Gauch, 1992; Kang, 1993; Yan *et al.,* 2000; Fan *et al.,* 2007). Yan and Kang (2003) proposed using GGE Biplot Pattern Explorer (Yan *et al.,* 2000) to examine GE interaction with respect to stability analysis. A GGE biplot, which simultaneously displays the genotype main effect (G) and the GE effect of a multi-environment trials (MET) data (Yan *et al.,* 2000; Yan, 2001; Yan and Kang, 2003), can visually address many questions relative to genotype and test environment evaluation. On the basis of a single GGE biplot, genotypes can be evaluated for their performance in individual environments and across environments, mean performance and stability, and general or specific adaptations (Yan and Tinker, 2006).

Thus, the main objective of this study was to locate the genes controlling stability and yield performance in rye using the CS/'Imperial' disomic addition lines grown under different growing conditions by applying the GGE biplot approach.

Materials and methods

Plant materials

In this study a set of wheat-rye disomic addition lines (CS-IMP disomic addition lines, i.e., 1R to 7R) and their wheat (*Triticum arestivum* cv. Chinese Spring (2n=6X=42)) and rye (*secale cereale* cv. Imperial (2n=2X=14)) parents were used as experimental materials. The disomic addition line has a pair of homologous chromosomes of Imperial rye added to the genetic background of Chinese Spring wheat.

The genotypes were cultivated in the field of Campus of Agriculture and Natural Resources,, Razi

University, Kermanshah, Iran (47° 20´ N latitude, 34° 20´ E longitude and 1351.6 m altitude). Climate in the region is classified as semi-arid with mean annual rainfall of 378 mm. Minimum and maximum temperature at the research station were -27˚C and 44˚C, respectively. Each genotype was planted in 2 m rows and at 15 × 25 cm inter-plant and inter-row distances, respectively. Each plot consisted of 100 seeds (each row 50 seeds).

Statistical analysis

The grain yield data were subjected to stability analysis. Combined analysis of variance (ANOVA) was used to determine the effects of genotype, environment and GE interaction. The environments were considered as random effects and the genotypes as fixed factors.

GGE Biplot technique

The GGE biplot methodology (Yan *et al.,* 2000) was used to graphically analysis of GE interaction data attempting to identify the chromosomes of rye which carrying the genes controlling high yield and stability performance under different growing conditions.

To generate a GGE biplot (Yan *et al.,* 2000), the genotype-environment two-way table of yield was first environment- standardized; the environment-standardized table was then decomposed into principal components (PC) via singular value decomposition (SVD). The first two PCs (PC1 and PC2) were used to generate a GGE biplot, where as the rest were regarded as residuals (Yan and Tinker, 2006). All analyses were performed using the GGEbiplot software (Yan, 2001).

Results and discussion

Combined analysis of variance

The results of combined analysis of variance for grain yield data is given in Table 1. The main effects of environment (E), genotype (G), and GE interaction were found to be significant. The variance components for the E, G, and GE interaction giving an overall picture of the relative magnitudes of the genotype, environment and GE interaction variance terms. The E effect was the most important source of yield variation, accounting for 51.04.% of total sum of squares (TSS) followed by GE interaction and genotype effects which accounted for 25.94 and 2.52% of TSS, respectively (Table 1). The environment portion in MET data has been known to be the largest among all sources of variation, but it is regarded as irrelevant for genotype evaluation (Yan and Kang, 2003). This is the reason that the environment effect is removed from the observed phenotypic data, which helps concentrate on genotype and GE which are relevant for genotype evaluation (Yan and Kang, 2003; Fan *et al.,* 2007). The large GE interaction, relative to G effect, suggests the possible existence of different mega-environments with different top-yielding genotypes (Yan and Kang, 2003).

Table 1. Combined variance analysis of variance for yield across 3 environments

S.O.V	Df	Sum of squares	SS%	Mean of squares
Treatments	29	1170.7		
Genotypes	8	220.9	2.52	24.54**
Environments	2	646.1	51.04	323.04**
Interactions	16	303.7	25.94	16.87**
Pooled error	60	95.1		1.59

**: significant at 1% probability level

Partitioning of environment + (Gen × Env) interaction into environment (linear), Gen × Env (linear) and pooled deviation (Table 2) revealed that mean squares due to Gen × Env (linear) was significant, which revealed that the behavior of the genotypes is predictable over environments and this has resulted from the linear function of the environmental component. The mean square due to pooled deviation (non-linear) was non-significant, revealing that the non-liner component was not important for this trait which contributed to total Gen × Env interaction.

Table 2. Stability analysis of disomic addition lines over 3 different environments

S.O.V	Df	Sum of Square	Mean of Square
Total	29	1170.7	
Genotype	8	220.9	24.54**
Env. + (Gen. × Env.)	19	949.8	
Env. (linear)	1	646.1	466.1**
Gen. × Env. (linear)	8	293.65	32.63**
Pooled Dev.	10	10.05	1.005ns
G_1	1	0.02	
G_2	1	0.72	
G_3	1	0.52	
G_4	1	2.33	
G_5	1	1.91	
G_6	1	2.11	
G_7	1	1.87	
G_8	1	0.03	
G_9	1	0.54	
Pooled error	60	95.1	

ns and **: non-significant and significant at 1% probability level, respectively

Mean comparisons

The mean comparisons for wheat-rye disomic addition lines over the environments using the Duncan's test and some indices which directly obtained from GGE biplot analysis (Yan, 2001) are given in Table 3.

Table 3. Mean comparison, relative value, heritability adjusted relative value, superior index and heritability adjusted superior index for the genotypes tested over environments.

Code	Mean	RV%	HARV%	SI%	HASI%
1R(G1)	42.1ab	106	104	86	90
2R(G2)	23.9c	60	71	49	63
3R(G3)	42.7ab	107	105	87	91
4R(G4)	30.2bc	76	83	61	72
5R(G5)	45.2a	114	110	92	94
6R(G6)	34.6abc	87	91	77	78
7R(G7)	49.2a	124	117	100	100
ChS(G8)	43.5ab	109	106	88	91
RIM(G9)	46.7a	117	112	95	96

The mean values followed by common letters are not significant at 5% level of probability using Duncan's test. RV: Relative Value; HARV = Heritability Adjusted Relative Value; SI = Superior Index or Value Relative to Maximum; with 100 indicating the best; HASI = Heritability Adjusted Superior Index.

The 7R addition line had the highest mean yield followed by RIM (donor parent) and the 5R addition line. No significant difference was found between two parents. But the mean yield of addition lines ranged from 23.9 gr (for 2R) to 49.2 gr (for 7R), indicating a remarkable variation among the chromosomes of rye in the case of mean yield over the environments.

Genetic parameters

The highest percentage of relative value (RV%) was found for 7R (124%) while the lowest value was observed for 2R (60%), indicating that the RV% of 7R is about twice 2R (Table 3). According to heritability adjusted relative value (HARV%), the 7R had the highest value followed by RIM and 5R. The superior index (SI) was also calculated for wheat-rye disomic addition lines, where the 7R was the best. The heritability adjusted superior index (HASI) was recorded for 7R as the highest value. However, the HARV and HASI are recommended when evaluating genotypes across test environments (Yan, 2001).

Polygon view of biplot analysis

The polygon view of a GGE biplot explicitly displays the which-won-where pattern, and hence is a succinct summary of the GE pattern of a MET data set (Yan, 2001). It provides the best way for visualizing the interaction patterns between the genotypes and environments and to effectively interpret a biplot (Yan and Kang, 2003). The polygon is formed by connecting the markers of the genotypes that are furthest away from the biplot origin such 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). The polygon view of the GGE biplot indicates the best genotype(s) in each environment and groups of environments (Hunt, 2002). Fig. 1 is a polygon view of the GGE biplot which accounted for 88.62% (PC1=53.91%, PC2=34.65%) of the total GGE variation using environment-standardized model.

According to Fig. 1, the vertex genotypes were G2, G4, G5, G6 G7 and G8. These genotypes were the best or the poorest genotypes in some or all of the test environments since they had the longest distance

from the origin of the biplot. The G2, G4, G5 and G7 well performed in three environments (E1, E2 and E3), while the the other addition lines showed the lowest performance. The other vertex genotypes (G6, G8 and G10) without any environment in their sectors were not the highest yielding genotypes at any environment; thus, they were the poorest genotypes at all or some environments (Yan, 2001). The vertex genotype in each sector is the best genotype at environments whose markers fall into the respective sector (Yan *et al.,* 2000). Environments within the same sector share the same winning genotype, and environments in different sectors have different winning genotypes. Thus, the polygon view of a GGE biplot indicates the presence or absence of crossover GE interactions involving the most responsive genotypes, and is suggestive of the existence or absence of different mega-environments among the tested environments (Yan and Rajcan, 2002).

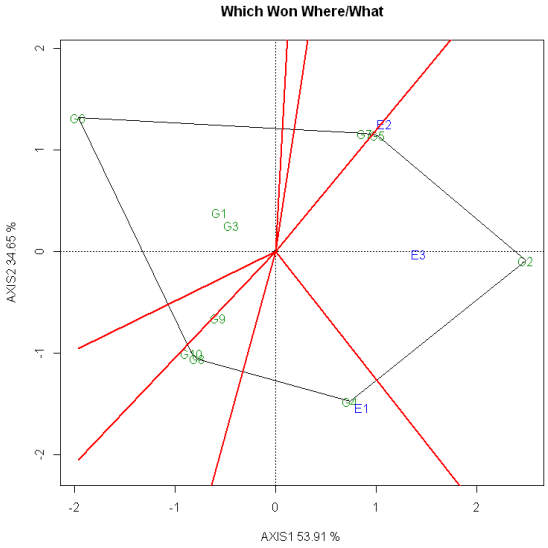

Fig. 1. Polygon views of the GGE biplot based on symmetrical scaling for the which-won-where pattern of genotypes and environments.

Ranking of disomic addition lines for both yield and stability performance

Fig. 2 shows the ranking of wheat-rye disomic addition lines and their parents for both mean yield and stability. The line passing through the biplot origin is called the average tester coordinate (ATC), which is defined by the average PC1 and PC2 scores of all environments. More close to concentric circles indicates higher mean yield. The line which passes through the origin and is perpendicular to the ATC with double arrows represents the stability of genotypes. Either direction away from the biplot origin, on this axis, indicates greater GE interaction and reduced stability (Yan, 2002).

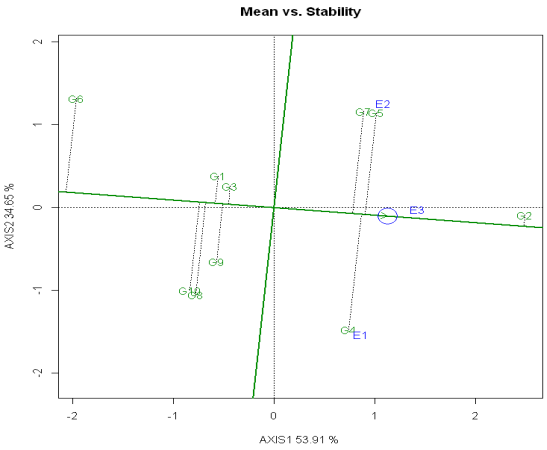

Fig. 2. Average environment coordination (AEC) views of the GGE-biplot based on environment-focused scaling for the means performance and stability of genotypes.

According to Fig. 2, genotypes with above-average means were from G5, G7, G4 and G2, while genotypes below-average means were from G3 and G1. However, the length of the average environment vector was sufficient to select genotypes based on yield mean performances. Genotypes with above-average means (G5, G7, G4 and G2) could be selected, whereas the rest were discarded. A longer projection to the ATC 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 G2 was more stable as well as high yielding. Conversely, G4, G5 and G7 were instable, but high yielding. The G1 and G3 were stable with low yield. It can be concluded that QTLs controlling yield and stability in Rye are located on chromosome 2R (G2).

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. 3). In Fig. 3 the genotypes are ranked relative to the ideal genotype. A genotype is more favorable if it is closer to the ideal genotype. Accordingly, addition line of G2 (2R) was more favorable than all the other genotypes, followed by G4 (4R), G5 (5R) and G7 (7R). The other genotypes were unfavorable because they were far away from the ideal genotype.

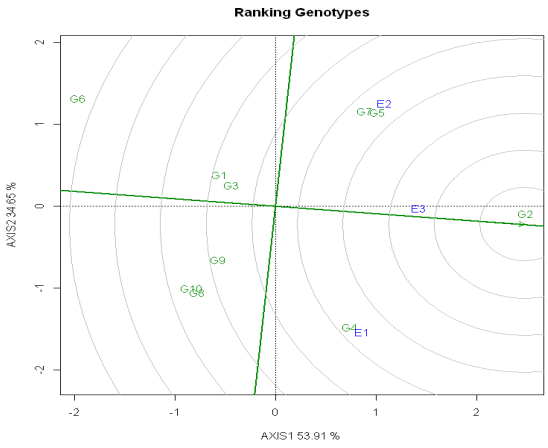

Fig. 3. GGE biplot based on genotype-focused scaling for comparison the genotypes with the ideal genotype.

Relationships among test environments

In GGE biplot, the correlation coefficient between any two environments is approximated by the cosine of the angle between their vectors. Acute angles indicates 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. According to Fig. 4, no relationship was found between the rainfed (E2) and irrigated environments (E2) (right angle) indicating that these two environments were independent in genotype rankings. The distance between two environments measures their dissimilarity in discriminating the genotypes. Thus, the presence of close associations among test environments 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 (Farshadfar *et al.*, 2011).

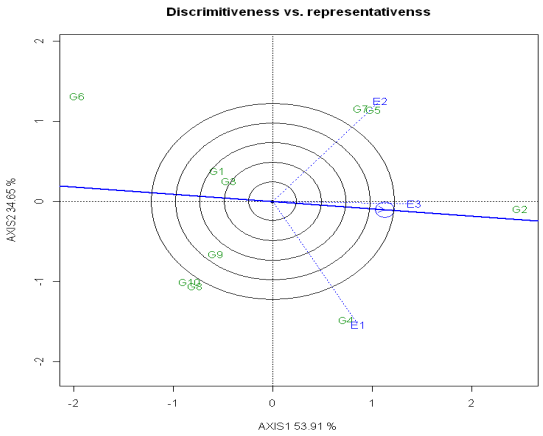

Fig. 4. Discriminating ability vs. representativeness of test environments

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