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Chromosomal localization of the genes controlling phenotypic stability in rye using GGE-biplot

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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 [Chinese spring-Imperial (CS-IMP)] disomic addition lines (DALs) was used to locate the genes controlling yield stability on specific chromosome(s) in rye. Experiments were conducted using a randomized complete block design with three replications under both rainfed and irrigated conditions for three cropping seasons. The GGE [genotype plus genotype x environment (GE)] biplot methodology was used to analyze the grain yield data. The results of combined ANOVA showed significant (P<0.01) environment, genotype and GE interaction indicating remarkable changes in ranking of genetic materials over the environments. According to GGE biplot analysis, two parents (Chinese spring vs. Imperial) were different in their yield adaptation. 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) revealed that most of the genes involved in controlling high yield and stability are located on two chromosomes 7R and 5R in rye.

Key words: Wheat- rye disomic addition lines, GGE biplot, genotype × environment interaction, gene location.

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 [21]. 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 [1, 3, 10, 13, 19].

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' wheat-rye disomic addition series [5] 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 [2, 11, 12, 21, 22].

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 [15, 16]. Because rye and wheat cross easily, a set of wheat–rye disomic addition lines were developed [12, 30].

By growing the 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 known about the study of genotype × 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 [4, 6, 7, 8, 9, 14 17, 23, 27, 30]. Yan and Kang [26] proposed using GGE Biplot Pattern Explorer [27] 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 [24, 26, 27] 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 [29]. This methodology has already been recommended for analyzing GE interaction in MET data to identify stable and high yielding genetic materials in different crop species [7, 18, 20, 27, 29].

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

To locate QTLs controlling yield and yield stability, 7 disomic addition lines (1R to 7R) of *Secale cereale cv.* Imperial (2n=2x=14) into the genetic background of Chinese Spring (CS= recipient) wheat (2n=6x=42) and Rye variety Imperial (RIM = donor) together with Rye variety Lovaspatonai (RLO) were used in 6 rainfed and irrigated conditions in the College of Agriculture, Razi University, 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 experimental design for each environment was a complete randomized block design with three replications. The plots consisted of 2m and at 15×25 cm inter-plant and inter-row distances, respectively. Each plot consisted of 100 seeds (each row 50 seeds). At the time of harvesting 5 single plants were selected randomly and grain yield was measured.

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.

The GGE biplot methodology [27] was used to graphically analysis the 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 [27], the genotype-environment two-way table of yield was first environment- standardized and then the environment-standardized table was 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 [29]. All analyses were performed using the GGE-biplot software [24].

RESULTS AND DISCUSSION

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 gave 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, accounted for 64.3% of total sum of squares (TSS) followed by GE interaction and genotype effects which accounted for 14.4 and 10.2% 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 [26]. This is the reason that the environment effect is removed from the observed phenotypic data, which helps to concentrate on genotype and GE that are relevant for genotype evaluation [7, 26]. The large GE interaction, relative to G effect, suggests the possible existence of different mega-environments with different top-yielding genotypes [26].

Descriptive of some univariate statistics in GGE biplot analysis

The mean comparisons for wheat-rye disomic addition lines over the environments using Duncan's multiple range test and some indices which directly obtained from GGE biplot analysis [24] are given in Table 2.

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 case of mean yield over environments.

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 than 2R (Table 2).

According to heritability adjusted relative value (HARV%), 7R had the highest value followed by RIM and R5.

The superior index (SI) was also calculated for wheat-rye disomic addition lines, where 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 [24].

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 [24]. It provides the best way for visualizing the interaction patterns between the genotypes and environments and to effectively interpret a biplot [26]. 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 included in the polygon. The rays are lines that are perpendicular to the sides of the polygon or their extension [25]. The polygon view of the GGE biplot indicates the best genotype(s) in each environment and groups of environments [26]. Fig. 1 is a polygon view of the GGE biplot which accounted for 76.4% (PC1=50.5%, PC2=25.9%) of the total GGE variation using environment-standardized model.

According to Fig. 1, the vertex genotypes were RIM, 7R, 1R, 2R and 4R. 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 RIM followed by the addition lines 7Rand 5R well performed in three (YS1, YS2, YP3) out of six environments, while the 1R followed by 3R and CS showed the highest performance in the other three environments (YS3, YP1, YP2). The other vertex genotypes (i.e., 2R and 4R) 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 [24]. The vertex genotype in each sector is the best genotype at environments whose markers fall into the respective sector [27]. 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 [28].

Ranking of wheat-rye 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 [25].

According to Fig. 2, genotypes with above-average means were from 3R to RIM, while genotypes below-average means were from 2R to 4R. However, the length of the average environment vector was sufficient to select genotypes based on yield mean performances. Genotypes with above-average means (i.e. from 3R to RIM) 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, genotypes 7R and 5R were more stable as well as high yielding. Conversely, RIM and ChS were more variable, but high yielding. 1R and 3R with average yield performance were more instable.

An ideal genotype have the highest mean performance and will 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 [24]. 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 7R was more favorable than all the other genotypes, followed by 5R. Ranking of other addition lines based on the ideal genotype was RIM > ChS > 1R > 3R. The other genotypes were unfavorable because they were far away from the ideal genotype.

Ranking of wheat-rye disomic addition lines relative to parents

In Fig. 4, the distance of disomic addition lines are evaluated relative to donor parent (RIM) as reference. A genotype is more similar to donor parent if it is located closer to the concentric circles where the donor parent is. Thus, using the donor as the center, concentric circles were drawn to help visualize the distance between each disomic addition line to its donor parent. Accordingly, the 7R and 5R addition lines were more similar to donor parent in the case of yield production. In contrast, the addition lines 2R followed by 6R and 4R were far away from the donor parent. In other word these addition lines are carrying chromosome which don't have the genes controlling yield production. Similarly, Fig. 5 shows the distance of disomic addition lines relative to recipient parent (ChS). According to Fig. 5, the addition lines 7R, 5R, 3R and 1R were located closer to the concentric circles where the recipient parent is.

Comparison the performance of parents (RIM vs. ChS) across environments

The performance of two genotypes can be easily compared on the GGE biplot [27]. To compare two genotypes (i.e., RIM and ChS) first connect their markers by a straight line; then draw a perpendicular line that passes through the biplot origin. This perpendicular divides the environments into two groups. Each of these two genotypes yielding better than the other will locate at environments with markers on its side of the perpendicular, and vice versa [26].

According to Fig. 6, RIM well performed at environments YS2, YP3 and YS1, while ChS yielded better at the other three environments. Thus, the two parents are adapted to environmental groups and generally they are differed in their adaptations.

Evaluation the performance of wheat-rye disomic addition lines at a specific environment

Fig. 7 shows ranking the relative performance of wheat-rye disomic addition lines at the environment YP1 with the highest yielding production. A line was drawn that passed through the biplot's origin and the YP1 marker to make a YP1-axis, and then another line was perpendicularly drawn from each genotype toward the YP1-axis. The genotypes were ranked on the basis of their projections onto the YP1-axis, with rank increasing in the direction toward the positive end [27]. In environment YP1, the addition line 7R had the highest yield followed by 1R, 3R, ChS (recipient parent), RIM (donor parent), 5R, 6R, 4R and 2R. The line, which passed through the biplot's origin and was perpendicular to the YP1-axis, separated genotypes 7R, 1R, 3R, ChS, RIM and 5R that had higher yield than average yield from genotypes 2R, 4R and 6R that had lower yield than average yield.

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 [26]. A short vector may indicate that the test environment is not related to other environments. According to Fig. 8, no relationship was found between the rainfed and irrigated environments at each cropping season indicating the environment at each cropping seasons were independent in genotype rankings.

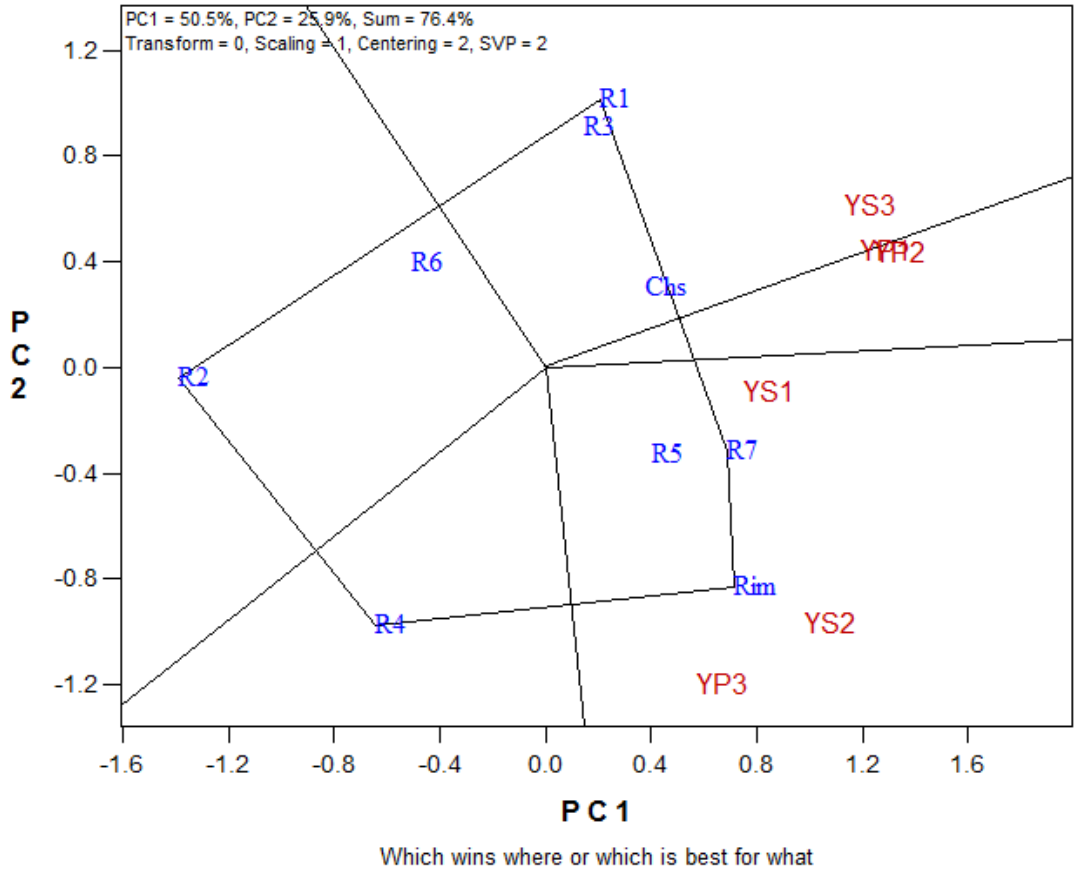

Figure 1. Polygon view of GGE biplot based on the yield data of wheat-rye disomic addition lines over six environments.

YP1, YP2 and YP3 are the normal environments in the first, second and third cropping seasons, while YS1, YS2 and YS3 are the rainfed environments in the first, second and third cropping seasons which the trails conducted. The 1R to 7R are the complete set of wheat-rye disomic addition lines and RIM and ChS are the donor and recipient parents, respectively.

Table 1. Combined analysis of variance for yield data of wheat-rye disomic addition lines tested across six environments

Source	Df	MS	%TSS
Environment (E)	5	12881.6**	64.4
Rep/E	12	179.9	2.2
Genotype (G)	8	1272.1**	10.2
G x E	40	359.3**	14.4
Error	96	92.5	8.9
Total	161		

*** Significant at 1% level of probability; %SST: Percentage relative to total sum of squares*

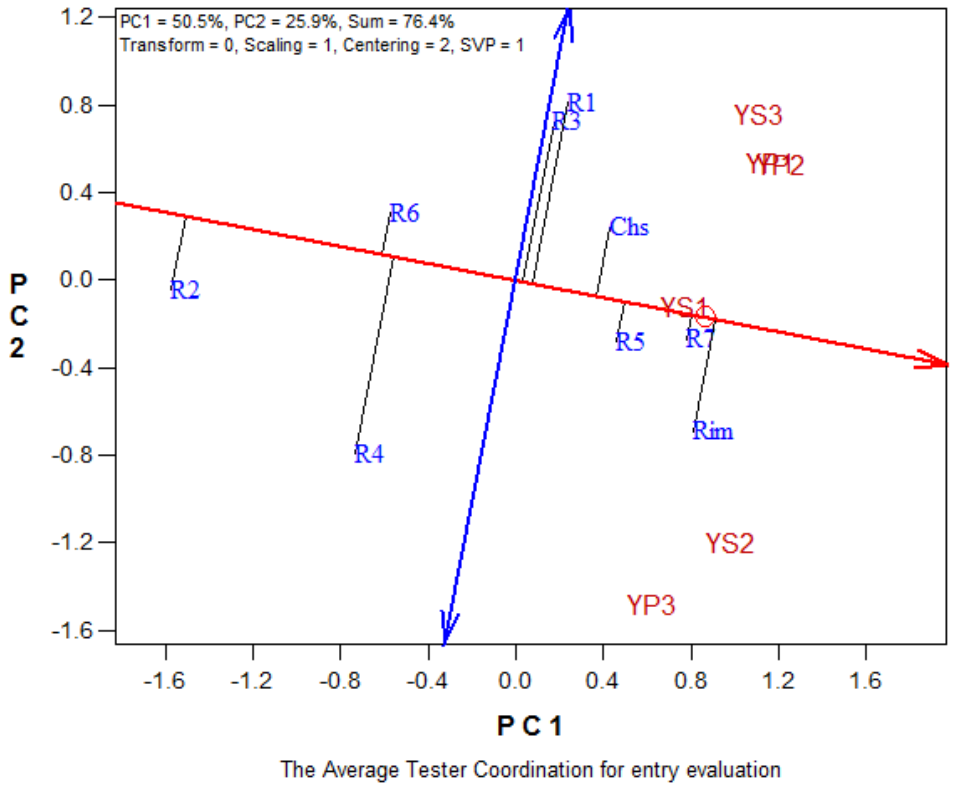

Figure 2. Average tester coordination (ATC) views of the GGE biplot for evaluating genotypes for both mean yield and stability performance.

YP1, YP2 and YP3 are the normal environments in the first, second and third cropping seasons, while YS1, YS2 and YS3 are the rainfed environments in the first, second and third cropping seasons which the trails conducted. The 1R to 7R are the complete set of wheat-rye disomic addition lines and RIM and ChS are the donor and recipient parents, respectively.

Table 2. 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%
R1	42.1ab	106	104	86	90
R2	23.9c	60	71	49	63
R3	42.7ab	107	105	87	91
R4	30.2bc	76	83	61	72
R5	45.2a	114	110	92	94
R6	34.6abc	87	91	77	78
R7	49.2a	124	117	100	100
ChS	43.5ab	109	106	88	91
RIM	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.

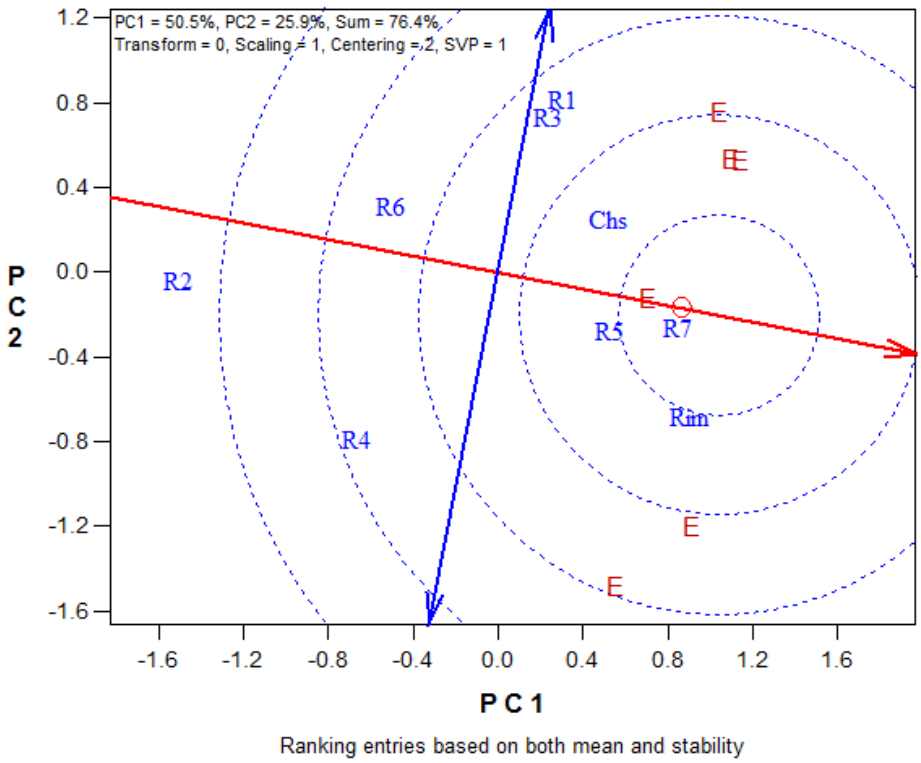

Figure 3. GGE biplot for ranking of genotypes relative to an ideal genotype. The 1R to 7R are the complete set of wheat-rye disomic addition lines and RIM and ChS are the donor and recipient parents, respectively.

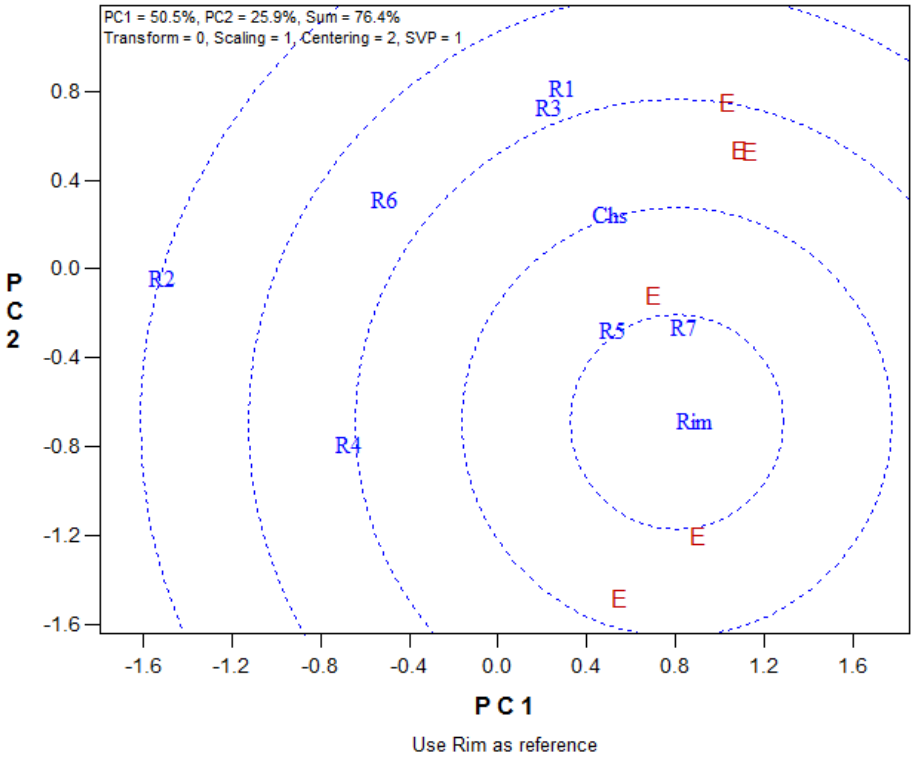

Figure 4. GGE biplot which shows the distance of wheat-rye disomic addition lines relative to donor parent (RIM). The 1R to 7R are the complete set of wheat-rye disomic addition lines and RIM and ChS are the donor and recipient parents, respectively.

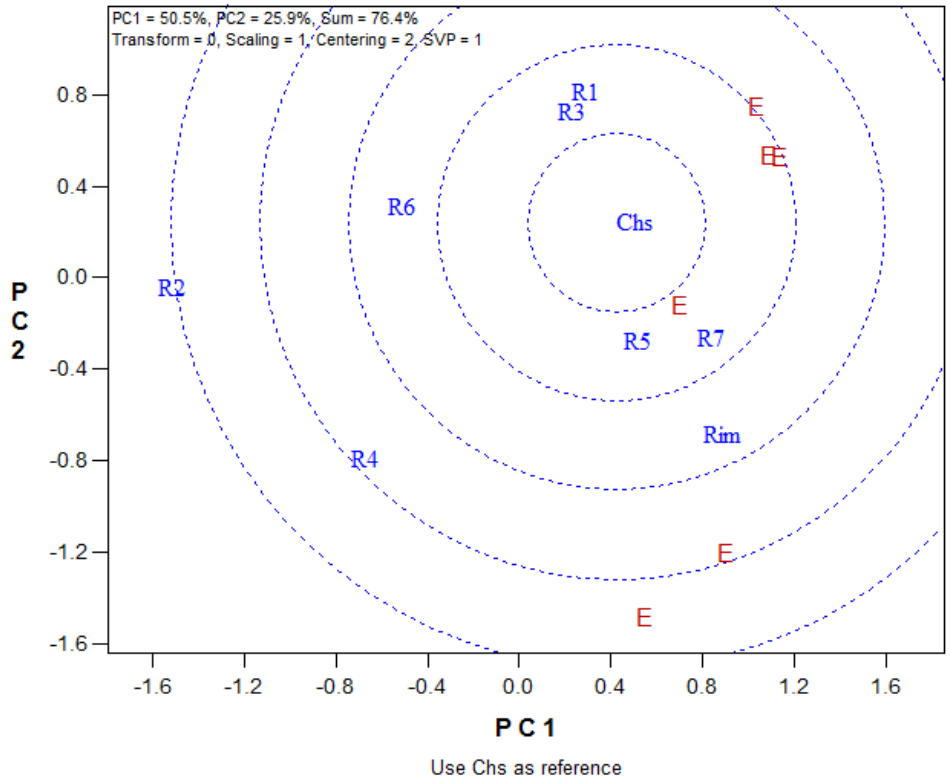

Figure 5. GGE biplot which shows the distance of wheat-rye disomic addition lines relative to recipiant parent (ChS). The 1Rto 7R are the complete set of wheat-rye disomic addition lines and RIM and ChS are the donor and recipient parents, respectively.

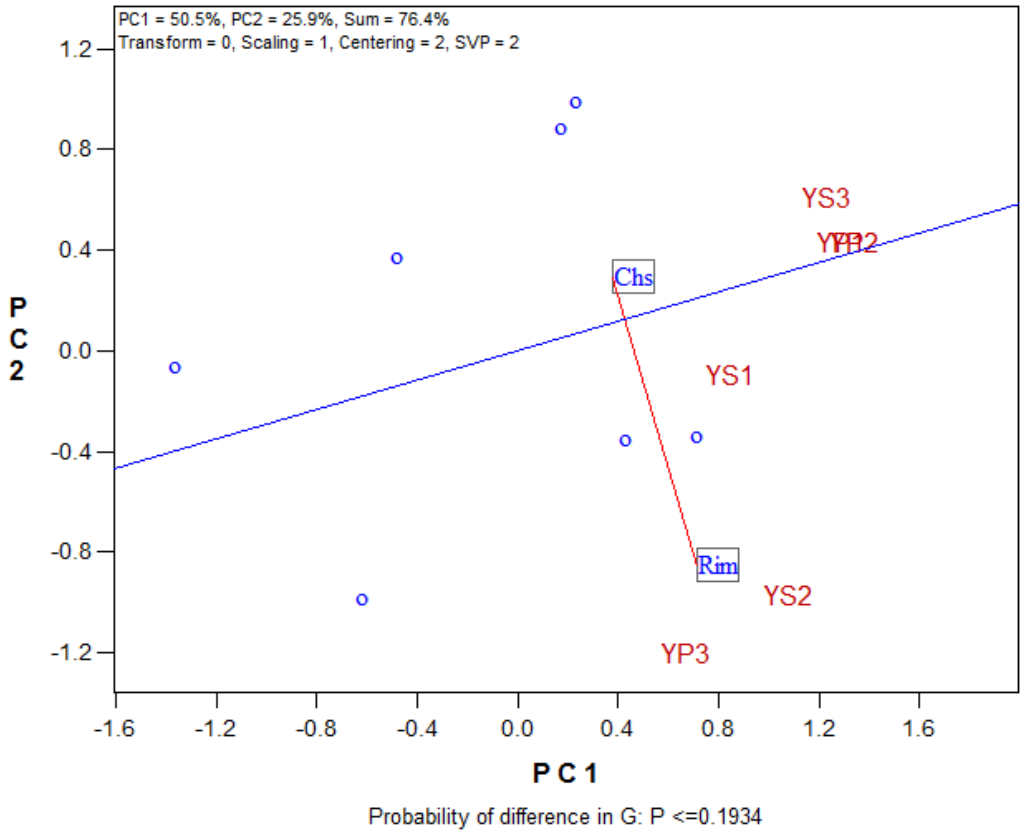

Figure 6. GGE biplot which compares the two parents (RIM vs. ChS) for their yield potential over environments.

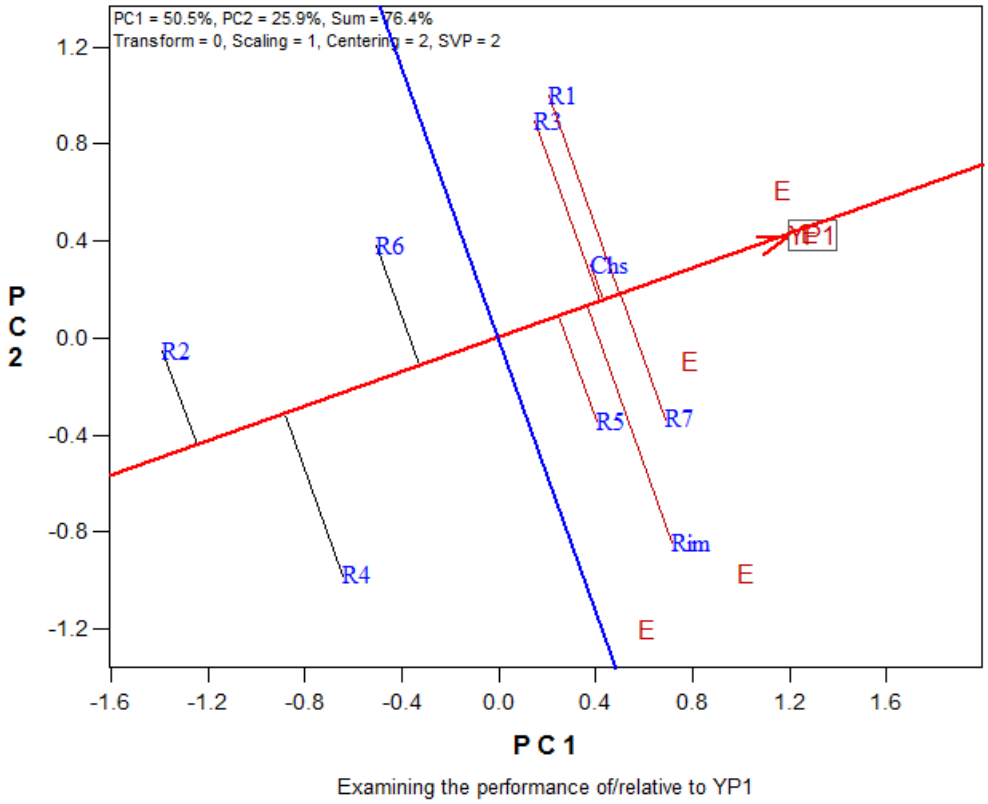

Figure 7. Ranking of genotypes based on the highest yielding environment (YP1)

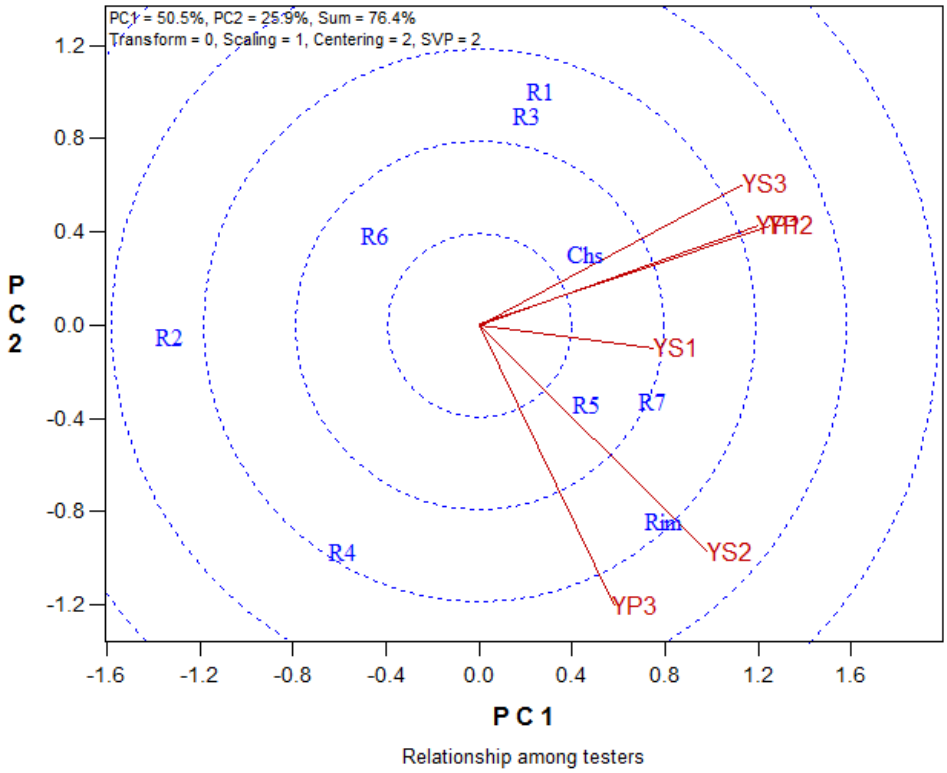

Figure 8. GGE biplot which shows relationships among test environments

CONCLUSION

Wide hybridization and combining chromosome engineering was widely used in creating new materials and breeding new cultivars with various desirable characters of wild species expressed in wheat. In this study, a set of wheat-rye disomic addition lines was used to locate the genes controlling stability performance in rye under different growing conditions. The results showed that the grain yield of wheat-rye disomic addition lines was significantly influenced by environment effects. The results also verified that it would be possible to determine contrasting dsomic addition lines based on the stability and integrating yield with stability performance for improving wheat genetic materials. Thus, the results presented in this report show that there is a clear opportunity to continue to breed wheat with high yield and high-yield stability. The present finding allow to recommend the GGE biplot model for analyzing GE interactions and effectiveness of this approach in identifying the addition lines which have good stability performance relative to recipient parent. In conclusion, our results showed that the effects of adding different rye chromosomes in the genome of Chinese spring for the stability performance were different. According to the results, most of the genes controlling stability performance in rye are probability located at least on two different rye chromosomes (7R and 5R).

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