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AMMI and AMMI based analysis of phenotypic stability in wheat-agropyron disomic addition lines

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Abstract

In order to identify QTLs controlling yield stability in Agropyron using AMMI and AMMI based stability statistics an experiment was conducted in three environments. Combined analysis showed significant genotype \times environment interaction (GEI) indicating the presence of genetic variation and possible chromosomal localization of QTLs controlling adaptation in agropyron. AMMI analysis exhibited that the two multiplicative axis terms explained 71.35% and 28.75% of GEI sum of squares, respectively. According to biplot analysis G1(E1) and G2 (E2) (adaptive group 1) exhibited specific adaptability for irrigated environment. Genotypes G5 (E5) and G7 (E7) (adaptive group 2) revealed specific adaptation for rainfed environments E2 and E3. The accessions G3 (E3), G6 (E6) and G8 (E8) (adaptive group 3) on the IPCA= 0 showed stability and general adaptability with grain yield close to mean yield and negligible interaction. AMMI1 (IPCA1) and AMMI2 (IPCA2) biplot introduced G7 (E7) and G4 (E4) with high grain yield and specific adaptability for environments E3 and E2 (stress conditions), G1 (E1) and G2 (E2) with low grain yield and specific adaptation for irrigated environment (E1). G5 (E5) and G6 (E6) were discriminated as stable genotypes with high and average yield, respectively. It is concluded that QTLs controlling specific adaptation in agropyron are distributed on chromosomes E1 and E2 (irrigated conditions) and chromosomes E5 and E7 (rainfed conditions), while QTLs monitoring stability and general adaptability are mainly located on chromosome E3, E5 with average grain yield and E6 with high grain yield. AMMI based stability statistics were positively correlated (an acute angle), and associated with grain yield except AMGE_i (right angle). It is concluded that all of the AMMI based measures except AMGE_i discriminate stable entries with high grain yield at the same manner.

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Introduction

The process of identification of stable genotype is difficult because of $G \times E$ interaction. Although the plant breeders have observed genetic differences for adaptability, they have been unable to fully exploit these differences in breeding stable genotypes. This has been largely due to the problem of defining and measuring phenotypic stability. Various attempts were made to characterize the behaviors of genotypes in response to varying environments.

Because of the complex nature of $G \times E$ interaction, little information is available on the chromosomal location of the genes conditioning $G \times E$ interaction and related physiological traits affecting adaptability (Farshadfar and Sutka, 2003).

Disomic alien addition lines (DAALs), in which single pairs of homologous chromosomes from a related species are added to the wheat complement, are worthwhile material to identify alien chromosomes carrying useful genes controlling phenotypic stability and form the starting point for the cytogenetic transfer of alien genetic material to wheat (Gale and Miller, 1987; Szakacs and Molnar, 2010).

Numerous methods have been developed to expose patterns of GE interaction. Among these, the additive main effects and multiplicative interaction (AMMI) model is a powerful multivariate method for multi-environmental trials (Romagosa and Fox, 1993). This technique, also called FANOVA (Gollob, 1968), incorporates both additive and multiplicative components into an integrated, powerful least squares analysis (Gauch, 1992; Voltas *et al.*, 1999). Plots showing both the genotypes and the environments simultaneously can be of great assistance in this respect, and are called biplots (Gabriel, 1971; Rubio *et al.*, 2004).

The AMMI method is used for three main purposes. The first is model diagnoses, AMMI is more appropriate in the initial statistical analysis of yield trials, because it provides an analytical tool of

diagnosing other models as sub cases when these are better for particular data sets. Secondly, AMMI clarifies the $G \times E$ interaction and it summarizes patterns and relationships of genotypes and environments. The third use is to improve the accuracy of yield estimates. Gains have been obtained in the accuracy of yield estimates that are equivalent to increasing the number of replicates by a factor of two to five (Gauch, 1992).

Some methods are based on the additive main effects and multiplicative interaction model, e.g., AMMI stability value (ASV) (Purchase *et al.*, 2000), parameter of (Annicchiarico, 1997) (Dai), distance of IPC point with origin in space (Dzi), stability statistic based on the first IPC axes (FPi), stability statistic based on the first two IPC axes (Bi), stability statistic based on fitted AMMI model (FAi), Wrick's ecovalance in term of AMMI (Wi(ammi)), modified AMMI stability value (MASV), sums of the absolute value of the IPC scores (SIPC), sum across environments of the GEI modeled by AMMI (AMGE), averages of the square eigenvector values (EV), absolute value of the sum across environments (AV(AMGE)), and absolute value the relative contribution IPCs to the interaction (Zai) (Dehghani *et al.*, 2010). Different AMMI stability parameters reflect various aspects of GE interaction and so introduce different genotypes as the most stable or unstable candidates. It seems plausible that yield stability estimated from AMMI and various stability statistics derived from AMMI model could be more repeatable than other stability statistics (Sneller *et al.*, 1997; Dehghani *et al.*, 2010).

Rozgard and Farshadfar (2014) used AMMI and AMMI based parameters for locating the genes controlling adaptation in rye using wheat-rye disomic addition lines. Zali *et al.* (2012) screening stable genotype in chickpea using AMMI and AMMI based stability statistics.

The present paper is an attempt to (i) analyze stability and examine the genotype \times environment interaction

for grain yield of wheat-agropyron disomic addition lines (ii) to locate the genes controlling stability and yield performance in agropyron and (iii) screening AMMI based stability statistics.

Materials and methods

Plant genetic materials and experimental design

In order to identify QTLs controlling yield stability in Agropyron an experiment was conducted in three environments. The experiment was laid out with eight disomic addition lines (DALs) of *Agropyron elongatum* (2n=2x=14) into the genetic background of Chinese Spring (CS) wheat (2n=6x=42) in a randomized complete blocks design with three replications. The DALs were named as: 1E to 7E indicating addition of chromosomes 1E to 7E from *Agropyron elongatum* into the genom of CS, respectively.

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). The environments were considered as random factors, while genotypes as fixed factors.

Irrigation was manipulated to create three different environments: (i) a fully irrigated control treatment, (ii) a mid-season water stress treatment where the crop was under progressive stress form approximately floral initiation (pre-anthesis) to flowering (post-anthesis) and rewatered thereafter until maturity and (iii) terminal stress, where irrigation was terminated at grain filling, and continuing until maturity.

The grain yield data were recorded for each genotype at each environment. Combined analysis of variance

for grain yield data was performed to determine the effects of environment (E), genotype (G), and GEI. The mean values of genotypes at each experiment were used to analyze yield stability.

Statistical analysis

The grain yield data were subjected to combined analysis of variance, mean comparison using Duncan's multiple range test (DMRT), cluster analysis and following biometrical analysis by statistical software's SPSS and EXCEL. The IRRISTAT software was used for AMMI analysis. AMMI analysis is a combination of analysis of variance and multiplication effect analysis. Briefly, analysis of variance is used to partition variance into three components: genotype deviations from the grand mean, environment deviations from the grand mean, and GE deviations from the grand mean. Subsequently, multiplication effect analysis is used to partition GE deviations into different interaction principal component axes(IPCA), which can be tested for statistical significance through analysis of variance (ANOVA). The AMMI analysis is interpreted by plotting the IPCAs of GE in various types of biplots.

AMMI based stability statistics

AMMI based stability parameters were calculated according to the following methods.

$$SIPC_i = \sum_{k=1}^N |\lambda_k^{0.5} \gamma_{ik}| \quad (\text{Sneller et al., 1997})$$

$$EV_i = \sum_{k=1}^N \frac{\gamma_{ik}^2}{N} \quad [21]$$

$$Da_i = \sqrt{\sum_{k=1}^N (\lambda_k \gamma_{tk})^2} \quad (\text{Annicchiarico, 1997})$$

$$FP_i = \lambda_1^2 \gamma_{i1}^2 \quad [22]$$

$$AV_{(AMGE)} = \sum_{j=1}^E \sum_{k=1}^N |\lambda_k \gamma_{ik} \delta_{jk}| \quad (\text{Sneller et al., 1997})$$

$$Za_i = \sum_{k=1}^N |\delta_k \gamma_{ik}| \quad (\text{Sneller et al., 1997})$$

All genotypes ranked in this manner, and the ranks of yield and stability variance summed for each genotype. Spearman's coefficient of rank correlation was calculated on the ranks to measure the relationship between the statistics. PCA based on the

rank spearman correlation matrix used for better understand the relationships among the yield stability statistics. All statistical analyses performed using the STATISTICA and GENSTAT softwares.

Results and discussion

Combined analysis of variance

Combined analysis of disomic addition lines tested in different environments showed that genotypes grain yield was significantly ($P < 0.01$) affected by environments (E), genotypes (G) and genotype \times environment interaction (GEI) (Table 1) indicating the presence of genetic variation and possible chromosomal localization of QTLs controlling adaptation in agropyron.

Table 1. AMMI analysis of adaptation in wheat-agropyron disomic addition lines 3 environments.

S.O.V	Df	Sum of square	SS%	Mean of squares
Total	71	620.6		
Treatments	23	568.3	91.57	24.71**
Genotypes	7	33.9	5.97	4.84**
Environments	2	408.8	71.93	204.40**
Interactions	14	125.6	22.10	8.97**
IPCA ₁	8	89.6	71.35	11.19**
IPCA ₂	6	36.1	28.75	6.02**
Residuals	0	0	0	0
Pooled error	48	52.3	8.43	1.09

** : significant at 1% probability level

Significant interactions were resulted from the changes in the relative ranking of the genotypes or changes in the magnitudes of differences between genotypes from one environment to another. The significant $G \times E$ effect demonstrated different responses of genotypes to the variation in environmental conditions indicating the necessity of testing genotypes at different environments (Mortazavian *et al.*, 2014).

71.93% of the total sum of squares was justified by environmental fluctuations exhibiting that the environments were diverse, with large differences among environmental means causing most of the variation in grain yield. In multi environmental trial

(MET), environment explains 80% or higher of the total yield variation (Yan, 2002). Only a small portion (5.97%) of the total sum of squares was attributed to genotypic effects. GEI significantly explained 22.10% of the treatments variation in grain yield. The magnitude of the GEI sum of squares was about 4 times larger than that of genotypes, indicating sizeable differences in genotypic response across environments. High percentage of E and $G \times E$ interaction out of total variations of genotypes grain yield, implicates the low efficiency of indirect selection to improve potential yield, ignoring the GEI effect (Mortazavian *et al.*, 2014). As GEI was significant therefore we can further proceed and calculate phenotypic stability (Farshadfar, 2008).

AMMI model and biplot analysis

In AMMI model, principal component analysis is based on the matrix of deviation from additivity or residual, while pattern analysis employs both classification and ordination techniques. In this respect both the results of AMMI analysis, the genotype and environment will be grouped based on their similar responses (Gauch, 1992; Pourdad and Mohammadi, 2008). GEI was further partitioned by principal component analysis (Table 1). Ordination technique using an approximate F-statistic (Gollob, 1968) revealed high significant differences for IPC1 and IPC2. The Gollob's test most often retains the multiplicative axis terms of little practical relevance that is, axis with a low proportion of explained GE variation. In this study, the two multiplicative axis terms explained 71.35% and 28.75% of GEI sum of squares, respectively.

The first two interaction principal components (IPC1 and IPC2) retained by Gollob's F-test accounted for 100% of GE interaction. Corrected grain yield can be obtained by AMMI1 and AMMI2 for each environment and used as a selection criteria in breeding programs. In general the importance of AMMI model is in reduction of the noise even if principal components do not cover much of the GESS [Gauch, 1992; Gauch and Zobel, 1996].

The AMMI model used in this research exhibited a more complex interaction which required a maximum of two PC axes to account for considerable amount of variation in the GEI. Also two first IPCs for each genotype over all environments are given in Table 2.

The IPCA scores of genotypes in the AMMI analysis are an indication of stability or adaptability over environments [Purchase *et al.*, 2000; Gauch and Zobel, 1996].

Table 2. Mean yield and measures of stability from AMMI model for disomic addition lines.

Gen. no.	IPCA ₁	IPCA ₂	EV ₁	SIPC ₁	Da ₁	Dz ₁	AV _(AMGE)	FP _i	B _i	Za ₁	AMGE	ASV	Mean
G ₁	-1.388	-0.482	0.210	1.87	3.37	0.65	6.40	10.53	11.33	0.50	-0.003	6.40	4.98
G ₂	-0.804	-0.290	0.071	1.09	1.96	0.38	3.74	3.53	3.82	0.29	-0.002	3.74	4.85
G ₃	-0.051	0.760	0.083	0.81	1.42	0.41	2.47	0.01	2.02	0.13	-0.0001	2.47	3.87
G ₄	0.827	-1.142	0.251	1.97	2.87	0.71	6.38	3.74	8.26	0.43	0.002	6.38	4.87
G ₅	1.281	-0.097	0.151	1.38	3.00	0.55	4.86	8.96	9.00	0.41	0.003	4.86	4.10
G ₆	0.082	-0.255	0.010	0.34	0.51	0.14	1.06	0.04	0.26	0.06	0.0002	1.06	3.01
G ₇	0.554	0.927	0.152	1.48	2.16	0.55	4.76	1.68	4.66	0.31	0.001	4.76	3.23
G ₈	-0.500	0.579	0.071	1.08	1.59	0.38	3.52	1.37	2.53	0.24	-0.001	3.52	4.03

IPCA_i: Interaction principal component, EV₁: Averages of the square eigenvector values, SIPC₁: Sums of the absolute value of the IPC scores, Da₁: Parameter of Annicchiarico (1997), Dz₁: Distance of IPC point with origin in space, AMGE₃: Sum across environments of the GEI modeled by AMMI, AV_(AMGE): Absolute value of the sum of the environments, ASV: AMMI stability value, FP_i: Stability statistic based on the first IPC axes of the first IPC axes, B_i: Stability statistic based on the first IPC axes of the first two IPC axes and Za₁: Absolute value the relative contribution IPCs to the interaction.

27]. The greater the IPCA scores, the more specific adapted is a genotype to certain environments. The more the IPCA scores approximate to zero, the more stable or adapted the genotype is over all the environments sampled.

Biplot analysis

To have a better discussion on the biplots resulted from the AMMI analysis we must consider the following points (Kempton, 1984; Kroonenberg, 1995):

- (i) The center of biplot shows the mean of a genotype or an environment.
- (ii) A long distance of a genotype (or an environment) from the center of biplot indicates a large interaction with that genotype (or environment).
- (iii) The long length of a genotype on the environmental vector reveals more deviation from the mean and vice versa.

(iv) The angle between the vectors of a genotype and an environments shows that the interaction is positive or negative.

To investigate the main effects and interactions, AMMI1 biplot was constructed for yield. In Fig. 1, AMMI1 biplot of additive main effects or mean yield are shown along the abscissa and the ordinate represents the first IPCA or multiplicative interaction. The interpretation of a biplot assay is that if main effects have IPCA score close to zero, it indicates negligible interaction effects and when a genotype and an environment have the same sign on the IPCA axis, their interaction is positive; if different, their interaction is negative. Biplot space of Fig. 1 is divided into 4 sections from low yielding environments in sections 1 (up left) and 4 (low left) to high yielding environments in sections 2 (up right) and 3 (low right). It is clear from the Biplot of Fig. 1 that the points for environment are more scattered than the points for genotypes indicating that variability due to environments is higher than that due to genotypes

difference which is in complete agreement of ANOVA (Table 1). On the biplot, the points for the generally adapted genotypes would be at right hand side of grand mean levels (this suggests high mean performance) and close to the line showing $IPCA=0$ and (this suggests negligible or no $G \times E$ Interaction).

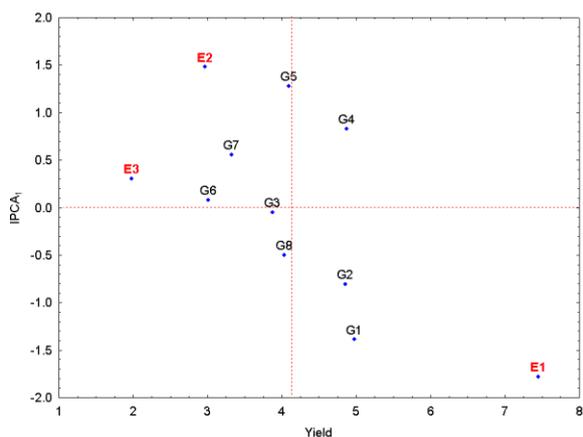


Fig. 1. Biplot of the first interaction principal component axis (IPCA1) versus mean yield for disomic addition lines.

According to the AMMI model, the genotypes which are characterized by means greater than grand mean and the IPCA score nearly zero are considered as generally adaptable to all environment. However, the genotype with high mean performance and with large value of IPCA score are consider as having specific adaptability to the environments.

According to Fig. 1: G1(E1) and G2 (E2) (adaptive group 1) exhibited specific adaptability for irrigated environment. As the genotypes and environments of first adaptive group have the same sign on the IPCA axis, their interaction is positive. Genotypes G5 (E5) and G7 (E7) (adaptive group 2) revealed specific adaptation for rainfed environments E2 and E3 with grain yield equal and less than mean yield and positive interaction, respectively. The accessions G3 (E3), G6 (E6) and G8 (E8) (adaptive group 3) on the $IPCA=0$ showed stability and general adaptability with grain yield close to mean yield and negligible interaction.

AMMI1 (IPCA1) and AMMI2 (IPCA2) biplot (Fig. 2) introduced G7 (E7) and G4 (E4) with high grain yield and specific adaptability for environments E3 and E2 (stress conditions), G1 (E1) and G2 (E2) with low grain yield and specific adaptation for irrigated environment (E1).

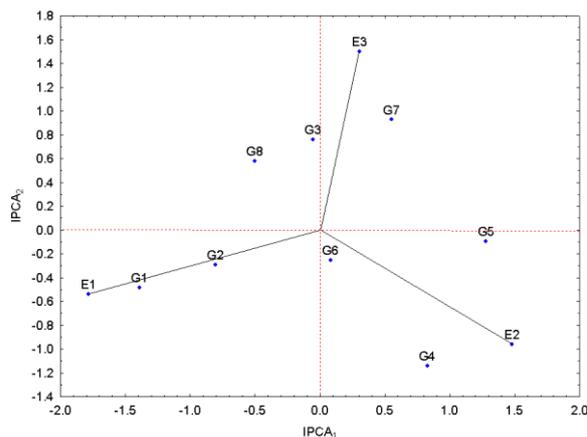


Fig. 2. Biplot of the first interaction principal component axis (IPCA1) versus the second interaction principal component axis (IPCA2) for disomic addition lines.

G5 (E5) and G6 (E6) were discriminated as stable genotypes with high and average yield, respectively.

It is concluded that QTLs controlling specific adaptation in agropyron are distributed on chromosomes E1 and E2 (irrigated conditions) and chromosomes E5 and E7 (rainfed conditions), while QTLs monitoring stability and general adaptability are mainly located on chromosome E3, E5 with average grain yield and E6 with high grain yield. Therefore the genes on chromosome E3, E5 and E6 in agropyron are recommended to be used for simultaneous improvement of yield and stability in wheat through chromosome engineering.

AMMI Analysis was also conducted and the stability of genotypes was predicted on the basis of mean performance and the magnitude of IPCA1 scores in soybean (Zobel *et al.*, 1988), maize and wheat (Crossa, 1990), sorghum (Zavala-Garcia *et al.*, 1992),

barley (Romagosa and Fox, 1993) and chickpea (Zali *et al.*, 2011).

Identification of environments

Environment that appears almost in a perpendicular line have similar means and those that fall almost in a horizontal line have similar interaction pattern. AMMI1 biplot (Fig. 1) thus exhibited that

environment differed in main effect and interactions. The environments E2 and E3 had similar main effect and interaction with genotypes. The ranking in such environments is likely to be quite variable, thus making it complex to produce variety recommendations. Further the environment E1 was the highest yielding and the highest interacting, hence is the most suitable only for the specifically adapted genotypes.

Table 3. Ranks of mean yield and AMMI based stability statistics in disomic addition lines.

Gen. no.	EV ₁	SIPC ₁	Da ₁	Dz ₁	AV _(AMGE)	FP _i	B _i	Za ₁	AMGE	ASV	Mean
G ₁	7	7	8	7	8	8	8	8	1	8	8
G ₂	3	4	4	3	4	5	4	4	2	5	6
G ₃	4	2	2	4	2	1	2	2	4	2	3
G ₄	8	8	6	8	7	6	6	7	7	6	7
G ₅	5	5	7	5	6	7	7	6	8	7	5
G ₆	1	1	1	1	1	2	1	1	5	1	1
G ₇	6	6	5	6	5	4	5	5	6	4	2
G ₈	2	3	3	2	3	3	3	3	3	3	4

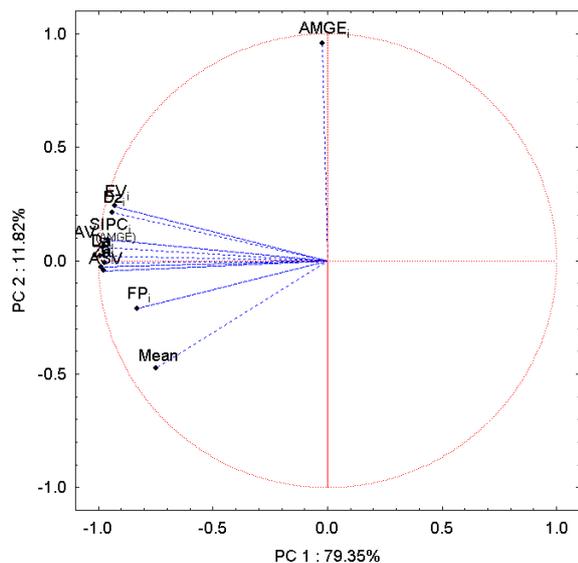


Fig. 3. Biplot of AMMI based stability statistics and mean yield in disomic addition lines across 3 environments.

AMMI based stability statistics

Various measures of AMMI based stability statistics and the mean yield for each genotype over all environments are given in Table 2. Genotypic rank differences over environments indicated the presence of crossover GEI. This was confirmed by the significant effect of the GEI in the analysis of variance (Table 1) and indicated the need to assess the

response of the genotypes to environmental variation. The genotypes were ranked with respect to their stability with each of the measures of stability from AMMI model such that lesser the value of the rank more is the stability. The stability rank orders displayed by these measures of stability from AMMI model presented in Table 3. According to the rank 1 of all stability measures and mean grain yield genotype G6 (E6) was identified with high grain yield with stability followed by G3, therefore most of the QTLs controlling yield and yield stability are located on chromosome E6 and E3 of Agropyron.

Screening AMMI based stability parameters

To better understand the relationships, similarities and dissimilarities among the AMMI based stability statistics, principal component analysis (PCA) was used based on the rank correlation matrix. The main advantage of using PCA over cluster analysis is that each statistics can be assigned to one group only (Khodadadi *et al.*, 2011). The relationships among different indices are graphically displayed in a biplot of PCA1 and PCA2 (Fig. 3). The PCA1 and PCA2 axes which justify 91.17% of total variation, mainly distinguish the stability indices in different groups.

One interesting interpretation of biplot is that the cosine of the angle between the vectors of two indices approximates the correlation coefficient between them. The cosine of the angles does not precisely translate into correlation coefficients, since the biplot does not explain all of the variation in a data set. Nevertheless, the angles are informative enough to allow a whole picture about the interrelationships among the AMMI based stability statistics (Yan and Kang, 2003). As the

cosine of the angle between the vectors of two stability indices approximates the correlation between them therefore, all the AMMI based stability statistics were positively correlated (an acute angle), and associated with grain yield except AMGE_i (right angle). It is concluded that all of the AMMI based measures except AMGE_i discriminate stable entries with high grain yield at the same manner.

Table 4. Spearman’s rank correlation for mean yield and measures of stability from AMMI model in disomic addition lines.

	EV ₁	SIPC ₁	Da ₁	AMGE ₁	Dz ₁	ASV	FP _i	B _i	AV _(AMGE)	Za ₁
SIPC ₁	0.93**									
Da ₁	0.81*	0.88**								
AMGE ₁	0.24	0.17	0.10							
Dz ₁	1.00	0.93**	0.81*	0.24						
ASV	0.74*	0.83*	0.98**	0.00	0.74*					
FP _i	0.67	0.81*	0.95**	0.03	0.67	0.98**				
B _i	0.81*	0.88**	1.00	0.10	0.81*	0.98**	0.95**			
AV _(AMGE)	0.88**	0.95**	0.98**	0.07	0.88**	0.95**	0.92**	0.98**		
Za ₁	0.88**	0.95**	0.98**	0.07	0.88**	0.95**	0.93**	0.98**	1.00	
Mean	0.62	0.71*	0.76*	-0.28	0.62	0.86**	0.81*	0.76*	0.81*	0.81*

* and **: significant at 5% and 1% probability level, respectively

Conclusion

It is apparent that the phenotype of crop plants is a joint contribution of both genes as well as environment. The genotype-environment interaction reduces association between phenotypic and genotypic values and leads to bias in the estimates of gene effects and combining ability for various characters sensitive to environmental variations. Such traits are less amenable to selection. The process of identification of stable genotype is difficult because of G × E interaction. One of the most critical question is whether stability is genetic? If stability is non-genetic, it is not heritable and thus selection for such a parameter is fruitless. Various authors have proved that stability indices are genetic and hence heritable. If stability is heritable, the next step in the genetic analysis is identification of the chromosomal location of the genes controlling stability. Various techniques (biometrical, cytogenetic and molecular) have been used to locate the genes monitoring quantitative traits

among which cytogenetic methods (monosomic, disomic, substitution and disomic addition analysis) have been widely used. Because of the complex nature of phenotypic stability, very little information is available on the chromosomal location of the genes conditioning adaptation. Disomic addition lines in which a single pair of chromosomes from related species is added to the full chromosome complement of the recipient, can be used to identify chromosomes carrying the genes controlling adaptation and phenotypic stability and form the starting point for gene transfer and genetic improvement of genotypic stability. Numerous methods for multi-environment trials data have been developed to expose patterns of GE interaction. Among these, the additive main effects and multiplicative interaction (AMMI) model is a powerful multivariate method for multi-environmental trials. AMMI analysis exhibited that QTLs controlling specific adaptation in agropyron are distributed on chromosomes E1and E2 for irrigated

conditions and chromosomes E5 and E7 for rainfed conditions, while QTLs monitoring stability and general adaptability are mainly located on chromosome E3, E5 with average grain yield and E6 with high grain yield. Therefore the genes on chromosome E3, E5 and E6 in agropyron are recommended to be used for simultaneous improvement of yield and stability in wheat through chromosome engineering. AMMI based stability statistics were positively correlated, and associated with grain yield except AMGEi. It is concluded that all of the AMMI based measures except AMGEi discriminate stable entries with high grain yield at the same manner.

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