AMMI analysis of wheat substitution lines for detecting genes controlling adaptability

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ABSTRACT

To locate the genes controlling adaptability in bread wheat using AMMI (additive main effect and multiplicative interaction) model, twenty-one substitution lines derived from the parents Chinese Spring (recipient) and Chayan (donor) were used in a randomized complete block design with three replications in three different environments in the Agricultural College of Razi University, Kermanshah, Iran. The results of AMMI analysis indicated significant differences among all sources of variation except AMMI2. AMMI1 explained that 84% of the variability related to genotype × environment interaction. The chromosomes 7A, 2B, 3A, 7D and 4B had the highest grain yield, and chromosomes 4D, 2A, 4A and 5A lowest. AMMI stability value (ASV) ranked chromosomes 5B > 3B > 3A > 6A as the most stable, and 2A > 4A > 7A as unstable. AMMI1 biplot analysis revealed that Chromosomes 3B, 5B and 5D had high stability and medium mean grain yield, whereas the chromosome with both low yield and stability were 2A, 4A, 5A and 4D. The chromosomes 3A, 4B, 7D followed by 2B, were identified as chromosomes with both high yield and stability performance in different environments. Chromosomes 2A and 4A with large AMMI1 score also showed specific adaptability with environment E2, chromosomes 5A and 4D with environment E3, while chromosome 7A with environment E1. The findings indicated that most of the genes controlling adaptability were located on chromosomes 3A in A genome, 4B and 2B in B genome and 7D in D genome, and also chromosomes 2A, 4A, 5A and 4D carried the genes controlling specific adaptability to water stress condition, while the genes responsible for adaptation to irrigated condition were located on chromosome 7A.

Key words: Adaptability, AMMI model, Grain yield, Substitution lines**,** Wheat

INTRODUCTION

Among crop plant, wheat (*Triticum aestivum* L., 2n = 6x = 24, AABBDD) is a staple food for more than 35% of the world population and it is also the first grain crop in Iran (Golestani and Assad, 1998). Wheat is mainly grown on rainfed land about 35% of the area of developing countries consists of semi-arid environments in which available moisture constitutes a primary constrain on wheat production, so wheat often experiences drought stress conditions during its growth cycle. Thus, improvement of wheat

productive for drought tolerance is a major objective in plant breeding programs for rainfed conditions (Farshadfar *et al,* 2013a; Farshadfar *et al,* 2013b; Bayoumi *et al,* 2008). The genotype by environment interaction (GEI) is a major problem in the study of quantitative traits because it complicates the interpretation of genetic experiments and makes predictions difficult. Therefore, the first goal of plant breeders in a crop breeding program is the development of cultivars or genotypes which are stable or adapted to a wide range of diversified environments (Delacy *et al,* 1996; Yan, 2002; Farshadfar, 2011; Farshadfar *et al,* 2013c). Multi-environment trials (METs) are conducted to evaluate yield stability performance of genetic materials under varying environmental conditions. A genotype grown in different environments will frequently show significant fluctuations in yield performance (Shukla, 1972; Yan and Rajcan, 2002; Ahmadi *et al,* 2012). Numerous methods have been developed to reveal patterns of GE interaction, such as joint regression (Finlay and Wilkinson, 1963; Eberhart and Russel, 1966; Perkins and Jinks, 1968), additive main effects and multiplicative interaction (AMMI, Gauch, 1992) and type B genetic correlation (Burdon, 1977; Yamada, 1962). These methods are commonly used to analyze METs data and have also been applied on GE interaction in wheat (Farshadfar and Sutka, 2003; Mohammadi and Amri, 2008). The AMMI model combines the analysis of variance for the genotype and environment main effects with principal components analysis of the GEI interaction (Zobel *et al,* 1988; Gauch and Zobel, 1997). Purchase *et al*, (2000) developed the AMMI stability value (ASV) based on the AMMI model's IPCA1 and IPCA2 (interaction principal components axes 1 and 2, respectively) scores for each genotype. The ASV is comparable with the methods of Eberhart and Russell (1966) and Shukla (1972) stability methods. Genetic materials such as alien additions, substitutions, translocations, deletions, monosomes, ditelosomes, and nullisomes are valuable genetic resources for both plant breeding and basic research (Farshadfar, 1999). Substitution lines in which a single pair of chromosomes from donor parent is substituted with the similar chromosome of the recipient parent, can be used for many purposes: 1) to study the location of the individual chromosomes or genes and determine their effect in genotypes with different genetic backgrounds, 2) to improve the agronomic value of cultivated wheat varieties by incorporating a character and 3) to study the hybrid vigor (Morgan, 1991; Morgan and Tan, 1996; Farshadfar *et al,* 2012a). The objective of the present investigation was to identify the chromosome(s) most probably carrying the genes controlling adaptability in wheat using AMMI model and cluster analysis.

MATERIALS AND METHODS

Plant genetic material and experimental conditions

In order to locate QTLs controlling yield stability, twenty-one substitution lines (Table 1) derived from the wheat genotypes Chinese Spring and Chayan, were used in a randomized complete block design with three replications under three different environments (E1: normal condition; E2: water stress before anthesis; E3: water stress after anthesis) in the experimental farm of College of Agriculture, Razi University, Kermanshah, Iran (47° 20′ N latitude, 34° 20′ E longitude and 1351m altitude) during 2011-2012. Climate in this region is classified as semi-arid with mean annual rainfall of 478mm and mean annual temperature of 13.8°C. The plots consisted of 3m rows and at 15×30 cm inter-plant and inter-row distances, respectively. The parents were initially chosen for their differences in drought tolerance. The donor parent Chayan, is a drought-tolerant wheat variety, whereas the recipient parent Chinese Spring, is a drought-sensitive variety. These substitution lines were often used to locate genes for major agriculture characteristics. The seeds were kindly provided by Dr. M. Tahir, ICARDA, Syria. Grain yield was recorded under three different environments at physiological maturity stage. The physiological maturity stage was considered when 90% of seed changed color from green to yellowish and stopped photosynthetic activity.

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).

AMMI stability value (ASV)

The AMMI stability value (ASV) as described by Purchase *et al,* (2000) was calculated as follows:

$$ASV = \sqrt{\left[\frac{IPCA1_{sumofsquare}}{IPCA2_{sumofsquare}}\left(IPCA1_{score}\right)\right]^2 + \left(IPCA2_{score}\right)^2}$$

Where, SS_{IPC1}/SS_{IPC2} is the weight given to the IPC1 value by dividing the IPC1 sum of square on the IPC2 sum of square. The larger the IPCA (interaction principal component analysis) scores, either negative or positive, the more specifically adapted a genotype is to certain environments, smaller IPCA scores indicate a more stable genotype across environments.

RESULTS AND DISCUSSION

AMMI analysis of variance

The analysis of variance (ANOVA) and AMMI model of twenty-one wheat substitution lines for grain yield across three environments are presented in Table 2. The ANOVA resulted in highly significant differences (P<0.01) for genotypes (G) and environments (E), indicating the presence of genotypic variability, different responses of genotypes to water stress condition and possible localization of QTLs controlling yield and yield stability (Farshadfar, 1999; Farshadfar *et al,* 2011). The genotype (G) and environment (E) effects were accounted for 6. 5 and 64.2% of total sum of squares (TSS), respectively (Table 2). ANOVA of genotype × environment interaction (GEI) also revealed significant differences (P<0.01) for grain yield. As GEI was significant, it was possible to proceed and calculate phenotypic stability (Lin *et al,* 1986; Farshadfar and Sutka, 2003). The GEI was accounted for 11% of TSS and was greater than the G effect. Gauch and Zobel (1997) reported that E accounts for about 80% of the total variation, while G and GEI each account for about 10% in normal METs. The large E and GEI in this study suggest the possible existence of different mega-environments with different top-yielding genotypes (Yan and Kang, 2003). This result revealed that there was a differential yield performance among substitution lines across testing environments due to the presence of GEI. The presence of GEI complicates the selection process as GEI reduces the usefulness of genotypes by confounding their yield performance through minimizing the association between genotypic and phenotypic values (Crossa, 1990; Farshadfar *et al,* 2012b). The AMMI analysis partitioned the sum of squares (SS) of GEI into two terms of AMMI (interaction principal components axes, IPCA). In this study, the first and second multiplicative axis terms explained 86 and 14% of GEI sum of squares, respectively. The first IPCA was found to be significant. This suggested that the AMMI model with the first multiplicative term was adequate for evaluation of the grain yield variation explained by GEI in the present data set. The adequacy of the multiplicative terms containing the real structure of GEI was inspected by estimating the amount of noise

present in the interaction from the pooled error and comparing it with the sum of squares retained in consecutive AMMIn models (Voltas *et al,* 2002). The average grain yield of the substitution lines ranged from 2.93 g to 6.14 g in three environments (Table 3). The chromosomes 7A, 2B, 3A, 7D and 4B had the highest grain yield, which indicated that these chromosomes possibly carried genes for positive effects on yield, while chromosomes 4D, 2A, 4A and 5A indicated minimum grain yield, might carry genes with negative effects on yield. The reports of Cao *et al,* (2009), Morgan and Tan (1996), Bai *et al,* (2007) and Farshadfar *et al,* (2012b) also showed that chromosomes 7A, 4B and 2B might carry genes for adapting to stressful environments in breed wheat.

IPCA, Crossover and Non-Crossover GEI

The magnitude of the GEI sum of squares was about 2 times larger than that for genotypes (Table 2). It is very common for METs data to embody a mixture of crossover and non-crossover types of GEI (Farshadfar, 1999; Ahmadi *et al,* 2012). IPCA scores of genotype and environment also took both positive and negative values (Table 3). Consequently, a genotype that has large positive IPCA score with some environments most have negative interaction with some other environments. Thus, these scores presented a disproportionate genotype response (Yan and Hunt, 2001; Mohammadi *et al,* 2007), which was the major source of variation for any crossover (qualitative) interaction. This disproportionate genotype response is referred to as crossover GEI for convenience. Diversely, scores with the some sign or near zero represent a non – crossover (quantitative) GEI or a proportionate genotype response (Mohammadi and Amri, 2008, Farshadfar *et al,* 2011).

AMMI stability value (ASV)

Table 3 reflects IPCA scores and AMMI stability values (ASV). The AMMI model does not provide a quantitative stability measure, such a measure is essential in order to quantify and rank genotypes according to their yield stability, that is why ASV is proposed by Purchase *et al,* (2000). ASV is in effect the distance from the coordinate point to the origin in a two-dimensional scattergram of IPCA1 scores against IPCA2 scores. This statistical method can be used to evaluate stability after reduction of noise from the GEI effects. Since the IPCA1 score contributes more to GEI sum of square (Table 2), it has to be weighted by the proportional difference between IPCA1 and IPCA2 scores to compensate for the relative contribution of IPCA1 and IPCA2 total GE sum of squares. Chromosomes with least ASV and IPCA1 score are the most stable. From Table 3, chromosomes 5B, 5D, 3B, 3A and 6A showed the lowest scores in the IPCA1,and 2A, 4A, 7A and 1B the highest. The ASV ranked 5B > 3B > 3A > 6A as the most stable, and 2A > 4A > 7A as the most unstable. The findings indicated that most of the QTLs controlling phenotypic stability are located on chromosomes 5B, 3B and 3A in wheat, while chromosomes A2, A4 and 7A were undesirable for improvement of general adaptation. It can be concluded from Table 3 that chromosomes in genome B possibly carried genes with positive effects on yield and yield stability. The results are in accordance with the results of Farshadfar (1999), Zhang *et al,* (2000 and 2005), Bai *et al,* (2007) and Farshadfar *et al,* (2012b).

AMMI biplot analysis

Graphical representation of interaction using AMMI parameters is known as biplot. Biplot formulation of interaction will be successful only when significant proponing of GEI is concentrated in the first or first two IPCA. Fig. 1 depicts AMM1 biplot based on IPCA1. This biplot explained 84% of the variability relating to GEI. AMMI biplot was used to study the pattern of response of G, E, and GEI using main effect of means vs the IPCA1, and also used to identify genotypes with broad or specific adaptation to target environments for grain yield. The horizontal axis shows the grand means yield of all substitution lines, whereas vertical axis indicates the zero point for the IPCA1 scores. The chromosomes with IPCA1 scores close to zero expressed general adaptation whereas the larger scores depicted more specific adaptation to environments with IPCA1 scores of the same sign. Therefore, either direction away from the

biplot origin indicates greater GEI and reduced adaptability (Gauch, 1992). The genotypes on the right side of the line have yield performance greater than mean yield and the genotypes on the left side of this line had yields less than mean yield. Accordingly, chromosomes 3B, 5B and 5D can be considered as stable with medium grain yield. The chromosomes 2A, 4A, 7A and 1B have larger interaction effects, hence may be regarded as unstable. Yan (2002) stated that an ideal genotype should have the highest mean performance and be absolutely stable. According to AMMI1 biplot, chromosomes 3A, 4B, 7D followed by 2B, were identified as chromosomes with both high yield and stability performance in different environment. Zhang *et al,* (2005), Cao *et al,* (2009) and Farshadfar *et al,* (2012b) reported chromosomes 7A, 3A, 2B and 4B may carry genes related to yield stability in wheat. Chromosomes with low yield and low stability were 2A, 4A 5A and 4D. From Fig. 1, chromosomes 2A and 4A with large IPCA1 score showed specific adaptability with environment E2, chromosomes 5A and 4D with environment E3, while chromosome 7A with environment E1, indicating that these chromosomes were ideal for these environments with higher performance. Farshadfar and Sutka (2003) reported that most of the QTLs controlling adaptation in wheat were located on chromosomes 4A and 5A in A genome, 4B in B genome and 2D and 7D in D genome. They also showed that chromosomes 3A, 4A, 3D and 7D carried the genes controlling specific adaptation to rainfed condition and QTLs responsible for adaptation to irrigated condition were located on chromosomes 1A, 3D, 1D and 7D.

Cluster analysis

Cluster analysis is the task of assigning a set of objects into groups/clusters so that the objects in the same cluster are more similar to each other than to those in other clusters (Naroui Rad *et al,* 2012). Cluster analysis showed that the twenty-one substitution lines based on three environments divided into three groups with 8, 8 and 5 chromosomes, respectively (Fig. 2). As seen in dendrogram, chromosomes located in the first group demonstrated the high adaptability with medium grain yield (Table 3) in comparison with other chromosomes. Chromosomes with both high adaptability and grain yield were located in the second group. Thus, it is apparent this group identified as a superior group. Chromosomes with except 8A were in the third group had the lowest grain yield and adaptability. The highest distance or dissimilarity between chromosomes was observed for 1B and 3B while the highest similarity was obtained for 3B and 5B for adaptation. AMMI and Cluster analysis of substitution lines based on grain yield and their adaptability showed different chromosomes groups which were classified three groups. Group one is highly desirable which is high yield and high adaptability. The group with medium yield but high adaptability is desirable for specific selection, whereas low yield and low stability is possible for special breeding purposes, e.g. drought resistance selection. The classification is similar to the previous work of family behaviour plots using joint regression (Farshadfar *et al,* 2011; Finlay and Wilkinson, 1963).

Conclusion

The results of AMMI analysis of variance for grain yield data indicated that the differences among all sources of variation with except IPCA2 were highly significant (P<0.01), indicating the presence of genotypic variability, different responses of genotypes to testing environments and possible localization of QTLs controlling yield and yield stability. The chromosomes 7A, 2B, 3A, 7D and 4B had the highest grain yield, and chromosomes 4D, 2A, 4A and 5A lowest. Chromosomes 5B, 5D, 3B, 3A and 6A showed the lowest scores in the IPCA1,and 2A, 4A, 7A and 1B the highest. The ASV ranked 5B > 3B > 3A > 6A as stable, and 2A > 4A > 7A as the most unstable. AMMI1 biplot analysis revealed that Chromosomes 3B, 5B and 5D had high stability and medium mean grain yield, whereas the chromosome with low yield and stability were 2A, 4A, 5A and 4D. The chromosomes 3A, 4B, 7D followed by 2B, were identified as chromosomes with both high yield and stability performance in different environments. Chromosomes 2A and 4A with large IPCA1 score showed specific adaptability with environment E2, chromosomes 5A and 4D with environment E3, while chromosome 7A with environment E1, indicating that these

chromosomes were ideal for these environments with higher performance. The findings indicated that most of the QTLs controlling phenotypic adaptability are located on chromosomes 3A, 4B, and 7D followed by 2B in wheat.

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Table 1: Code of investigated wheat substitution lines

Substitution line	Code	Substitution line	Code	Substitution line	Code
1A	1	1B	8	1D	15
2A	2	2B	9	2D	16
3A	3	3B	10	3D	17
4A	4	4B	11	4D	18
5A	5	5B	12	5D	19
6A	6	6B	13	6D	20
7A	7	7B	14	7D	21

**Table 2:** AMMI analysis of twenty-one wheat substitution lines across three environments for grain yield.

S.O.V.	DF	SS	MS	T SS (%)	GEI SS (%)
Genotype (G)	20	132 .9	6 .65**	6 .5	
Environment (E)	2	1308 .7	654 .3**	64 .2	
GE interaction (GEI)	40	222 .8	5 .57**	11	
AMMI1(IPCA1)	21	192.4	9.19**		86
AMMI2(IPCA2)	19	30.4	1.6		14
Residual (noise)	124	345 .5	2 .78		

** : significant at 0.01 level of probability.

Table 3: First and second interaction principal components analysis (IPCA), AMMI stability value (ASV) and mean grain yield of twenty-one wheat substitution lines over three environments

Substitution line	IPCA1	IPCA2	AVS	Mean grain yield*
1A	0.43	-0.47	1.18	4 .47 abc
2A	-1.41	-0.47	3.58	2 .94 c
3A	0.19	0.10	0.50	5 .47 ab
4A	-1.31	-0.17	3.30	3 .46 bc
5A	-0.46	0.07	1.16	3 .47 bc
6A	0.20	-0.15	0.53	4 .16 abc
7A	1.11	-0.35	2.83	6 .14 a
1B	-0.95	0.17	2.41	4 .79 abc
2B	0.36	0.32	0.96	5 .44 ab
3B	0.09	0.38	0.44	4 .68 abc
4B	0.26	-0.12	0.65	5 .39 ab
5B	0.08	0.22	0.31	4 .81 abc
6B	0.42	-0.85	1.37	4 .91 abc
7B	0.34	0.36	0.93	4 .89 abc
1D	0.50	-0.23	1.29	5 .37 ab
2D	0.45	0.33	1.18	4 .57 abc
3D	-0.36	0.02	0.91	4 .33 abc
4D	-0.57	0.09	1.44	2 .93 c
5D	-0.07	0.80	0.82	4 .59 abc
6D	0.45	0.49	1.24	4 .99 abc
7D	0.23	-0.54	0.79	5 .57 ab

*:The mean values followed by common letters are not significant at 5% level of probability using Duncan's test.
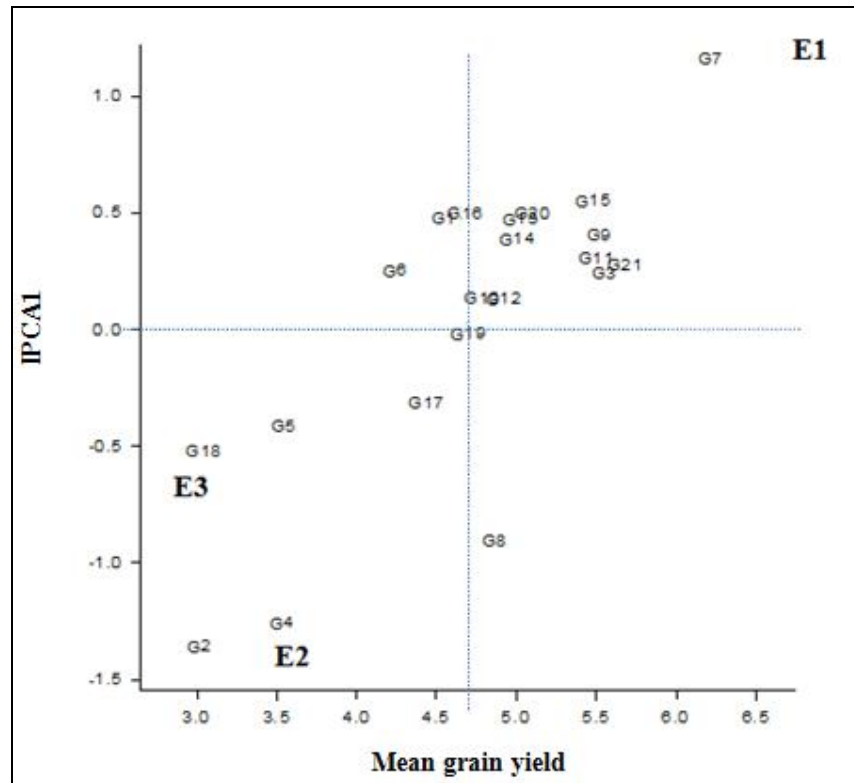
Figure 1: AMMI biplot for grain yield of twenty-one wheat substitution lines and three testing environments with X-axis plotting yield means from 2.93 6.14 g and Y-axis plotting IPCA1 from -1.41 to 1.11. Numbers 1 to 21 are the code of substitution lines from 1A to 7D, respectively (shown in Table 1). E1, E2 and E3 are normal condition, water stress before flowering stage and water stress after flowering stage, respectively.
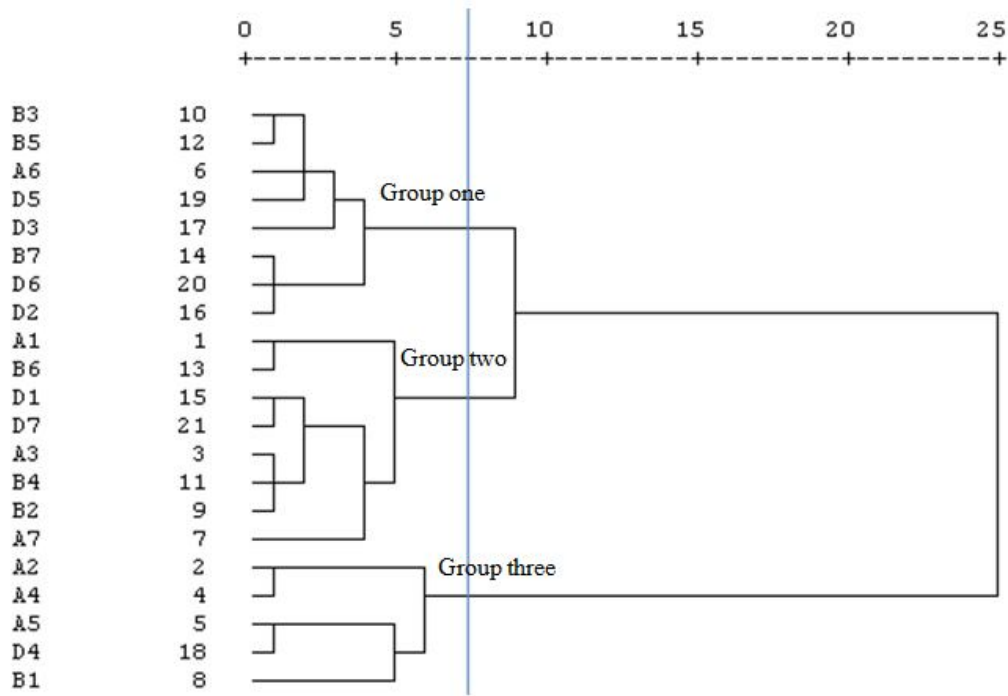
Figure 2: Dendrogram of cluster analysis for grain yield of twenty-one wheat substitution lines classified based on three testing environments.