Analysis of genotype and genotype × environment interaction in durum wheat in warm rainfed areas of Iran

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ABSTRACT

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The objective of this investigation was to evaluate the magnitude of G × E interaction effects on durum wheat grain yield and to identify superior genotypes adapted to the test environments. Twenty improved durum wheat genotypes were tested in five locations over three growing seasons. Combined ANOVA indicated that the effect of year (Y) was significant and that of the location (L) was not, but that their interaction (Y × L) was highly significant. The main effect of genotype was also significant, as was the genotype × year interaction (G × Y); genotype × location interaction (GL) was not significant, but three-way interactions (G × Y × L) were highly significant. Clustering of genotypes based on intercept and slope parameters of the linear regression model produced three distinct groups, while using only line slopes for clustering produced no groups at all. The coefficient of determination of the linear regression model ranged from 0.84 (G10) to 0.98 (G2); therefore, it can be concluded that this clustering method was somewhat useful for this data set. According to the dendrogram of clustering based on G and G × E interaction of ANOVA, there were 15 genotypic groups, while according to the dendrogram of clustering based on G × E interaction of ANOVA, there were 12 genotypic groups. Considering all clustering methods and mean grain yield, genotypes G8 (2590 kg ha-1) and G13 (2592 kg ha-1) were superior and thus can be recommended as candidates for release in warm rainfed areas of Iran.

Keywords: cluster analysis, grain yield, grain yield stability, linear regression model

INTRODUCTION

Durum wheat (*Triticum durum* Desf.) is the most important cereal crop for making macaroni and other types of foods (Anonymous, 2002). Globally, durum wheat is grown on about 14 million hectares, or 6% of the world's total wheat growing area (USDA, 2009). Durum wheat production in Iran is relatively smaller than bread wheat production, and many companies have to import the semolina they need for making macaroni products (Karimizadeh *et al.*, 2012).

In recent years, several durum wheat breeding programs have been established with the purpose of improving grain yield potential using germplasm from the International Maize and Improvement Center (CIMMYT) and the International Center for Agricultural Research in Dry Areas (ICARDA). However, it is essential to identify which of the new improved genotypes are adapted to the conditions and production practices prevailing in Iran's durum wheat growing regions. The effects of climate and soil factors are very important when improving new

durum wheat genotypes for increased grain yield (Rharrabti *et al.*, 2003). Thus, plant breeders aiming to increase grain yield should try to select genotypes adapted to diverse environmental conditions.

Genotype × environment (G × E) interaction is an important issue in the improvement of breeding materials because it reduces grain yield stability in different environments (Löffler *et al.*, 2005). The complexity of G × E interaction, particularly in environments prone to abiotic stress, can reduce grain yield heritability. In most multi-environmental trials, G × E interaction impedes plant breeding progress for broad as well as specific adaptation (Dreccer *et al.*, 2008). The effectiveness of evaluating new improved genotypes is influenced by an understanding of G × E interaction and the degree to which the test locations are represented in multi-environment trials (Podlich and Cooper, 1998).

Several statistical procedures have been developed for modeling G × E interaction and identifying the most stable genotypes across different test environments. Parametric univariate

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methods such as the joint linear regression model (Finlay and Wilkinson, 1963) are the methods most commonly used for identifying superior genotypes; however, new stability analysis methods such as multivariate methods or nonparametric statistics are also applied (Sabaghnia *et al*., 2006). Multivariate methods have three main purposes: summarizing information, eliminating "noise" from the data sets and revealing the structure of the data sets (Crossa *et al*., 1990; Gauch, 1992). Multivariate methods can also be used for determining grain yield stability and identifying genotypic groups possessing desirable traits (Lin *et al*., 1986). Lin and Thompson (1975), Lin (1982) and Lin and Butler (1990) proposed a special cluster analysis for analyzing two-way G × E interaction data. Cluster analysis can identify differences among genotypes for the breeder via classification of genotypes (Karimizadeh *et al.*, 2006; Sabaghnia *et al.,* 2012).

Lin and Thompson (1975) used the deviation mean square from the linear regression model as the dissimilarity index for genotype grouping. Lin

(1982) used the G × E interaction mean square as the dissimilarity index by slightly adjusting the distance coefficient. Lin and Butler (1990) introduced a new dissimilarity index according to the linear regression model which uses only the genotypic effect for genotype classification. Lin and Butler (1990) also proposed another new dissimilarity index based on the mean square of G × E interaction. Determining a cut-off point is critical because it reduces the risk of a type II error. Special F-tests for stopping the clustering procedure in each method were defined by Lin and Butler (1990). The objective of the present research was to use cluster analysis (i.e., four clustering methods) for grouping as well as studying G × E interaction in durum wheat in warm rainfed areas of Iran.

MATERIALS AND METHODS

The dataset analyzed in the present investigation was obtained from durum wheat multi-environmental trials conducted at five locations in the 2008 to 2010 cropping cycles (Table 1).

Table 1. Geographical characteristics of test locations.

Location	Longitude/ latitude	Altitude (m)	Soil texture	Soil type†	Rainfall (mm)
Gachsaran	50° 50´ E 30° 20´ N	710	silty clay loam	Regosols	460.8
Gonbad	55° 12´ E 37° 16´ N	45	silty clay loam	Regosols	367.5
Khorramabad	33° 39´ E 48° 28´ N	1125	silty-loam	Regosols	433.1
Ilam	46° 36´ E 33° 47´ N	975	clay-loam	Regosols	502.6
Moghan	47° 88´ E 39° 39´ N	100	sandy-loam	Cambisols	271.2

† Based on the FAO soil classification system (FAO, 1990).

Twenty new improved durum wheat genotypes or breeding lines were obtained from the ICARDA and CIMMYT durum wheat breeding programs (Table 2). A randomized complete block design replicated four times was used in each environment (year × location combination). Each plot consisted

of six rows, 7 m long with between and within row spacing of 17.5 and 5 cm, respectively. In all environments, 50 kg N ha^{-1} and 70 kg P$_2$O$_5$ ha^{-1} were applied at planting and 40 kg N ha^{-1} at stem elongation. Appropriate pesticides and herbicides were used to control insects and diseases as well as

Table 2. The cross name/pedigree and origin of 20 durum wheat genotypes used in this study.

No.	Code	Cross name / Pedigree	Origin
1	G1	BCR//MEMO/GOO/3/STJ7	ICARDA
2	G2	Altar84/STN/Wdz-2	CIMMYT
3	G3	DON-Md 81-36	ICARDA
4	G4	Stj3//Bcr/Lks4 ICD94-0994-Cabl-10AP-0AP-2AP-0AP	ICARDA
5	G5	OUASERL-1 ICD96-0758-C-2AP-0AP-5AP-0AP	ICARDA
6	G6	GA//2*CHEN/ALTAR84	CIMMYT
7	G7	AGAR1/5/SHEA/STK//BIT3/KYP/4/CHAH88	ICARDA
8	G8	OSSL-1/4/MRBSH/3/RABI//GS/CR /5/HNA ICD96-0744-C-1AP-0AP-3AP-0AP-3AP-AP-2AP-0AP	ICARDA
9	G9	DA-6 BLACK AWNS/3/BCR//MEMO/ GOO ICD96-0058-C-0AP-2AP-0AP-9AP-AP-3AP-0AP-3AP-AP	ICARDA
10	G10	D86135/ACO89//PORRON_ 4/3/ SNITAN CDSS96Y00582S-1M-0Y-0M-0Y-0B-3Y-0B	CIMMYT
11	G11	DUKEM/3/RUFF/FGO//YAV79/6/CGEN/ALTAR 84/4/… CDSS97Y00407S-9Y-0M-0Y-0B-0B-1Y-0M	CIMMYT
12	G12	SHAG_26/SNITAN CDSS96Y00415S-1Y-0M-0Y-1B-0Y-0B-0B	CIMMYT
13	G13	GEDIZ/FGO/ /GTA/3/SRN_1/4/TOTUS/ 5ENTE/… DSS97Y00835S-0T0PM-4Y-0M-0Y-0B-0B-3Y-0BLR-4Y-0B	CIMMYT
14	G14	CMH82A.1062/3/GGOVZ394//SBA81/PLC/4/AAZ-1/… CDSS99Y00643S-0M-0Y-16Y-0M-0Y-0B	CIMMYT
15	G15	SOOTY-9/RASCON-37/3/SOOTY-9/TARRO-1//AJAIA-2 CDSS97Y00565-8Y-0M-0Y-0B-0B-1Y-0M	CIMMYT
16	G16	LLARETA INIA/3/STOT//ALTAR 84/ALD /4/… CDSS99B01149T-0TOPY-0M-0Y-48Y-0M-0Y-0B	CIMMYT
17	G17	ALTAR84/STN/WDZ-2	CIMMYT
18	G18	GREEN-14//YAV-10/AUK	CIMMYT
19	G19	Seimareh	ICARDA
20	G20	Dehdasht	ICARDA

weeds. To remove border effects, the four middle rows in each plot were harvested for grain yield assessment; yield was adjusted for 10% seed moisture before converting it to kg ha^{-1} for statistical analysis.

Analyses of variance were performed for the data set in each environment, and the Anderson-Darling normality test and Bartlett's test for homogeneity of variances were also applied. A combined analysis of variance using SAS software was performed on the data set to partition out environment (E), genotype (G) and G × E interaction. Genotype was regarded as a fixed effect, while environment was regarded as a random effect.

The four cluster analysis methods used were based on: (1) the intercept and slope of the linear regression model (Lin and Thompson, 1975); (2) the similarity of G × E interaction (Lin, 1982); (3) the slope of the linear regression model (Lin and Butler, 1990); and (4) the similarity of the G effect and G × E interaction (Lin and Butler, 1990). Details of these clustering procedures are given in Lin and Butler (1990). The statistical package Sl16 (Lin *et al.*, 1992) was used for all four methods of cluster analysis.

RESULTS AND DISCUSSION

Effects of all sources of variation except location and G × L were significant (Table 3); the GE interaction was highly significant, which indicated that the studied genotypes showed complicated GE interactions.

Table 3. Combined analysis of variance for grain yield of durum wheat genotypes.

Source of variation	DF	MS	% of (G, E, GE)
Year (Y)	2	206213572.0*	35.72
Location (L)	4	94220057.1ns	32.64
Y × L	8	34610128.9**	23.98
Replication/ YL	45	618760.2	
Genotype (G)	19	680166.4*	1.12
G × Y	76	341113.3*	2.25
G × L	38	314535.9ns	1.04
G × Y× L	152	248317.9**	3.27
Residual	855	102927.8	

** and *: Significant at the 0.01 and 0.05 probability levels, respectively.
ns: Not significant.

The relatively large magnitude of G × E interaction implies more dissimilar plant genetic systems that control different physiological processes and yield stability (Cooper *et al.*, 2001). The expression of grain yield as a quantitative trait is the result of genotypic, environmental and G × E interaction effects (Huehn and Leon, 1985). The relative contribution of G × E interaction effects found in this investigation was similar to those reported in other studies in rainfed areas, which

makes it difficult to select the most suitable and superior genotypes (Mohebodini *et al.*, 2006; Sabaghnia *et al.*, 2008; Karimizadeh *et al.*, 2012).

The results of the joint linear regression model (Finlay and Wilkinson, 1963) are presented in Table 4. The pooled error estimate was used to perform an F-test for stopping the clustering process and determining the cutoff point. In this step, genotype G14 was grouped in a cluster containing other genotypes; there was a significant difference among

Table 4. Linear regression parameters and regression analysis of variance statistics.

Genotype	Mean grain yield	Intercept	Slope	SS Total	SS Reg.†	SS Res.‡	R²
G1	2478	2478	0.93	12138511	11439507	53770	94.2
G2	2491	2491	1.10	16500838	16238339	20192	98.4
G3	2430	2430	0.97	13340894	12485447	65803	93.6
G4	2578	2578	1.02	15106204	13847153	96850	91.7
G5	2357	2357	0.90	11420976	10894806	40475	95.4
G6	2491	2491	1.02	14478755	13952549	40477	96.4
G7	2505	2505	0.93	12124159	11546523	44434	95.2
G8	2590	2590	1.02	14573921	13906970	51304	95.4
G9	2566	2566	0.94	12235683	11801765	33378	96.5
G10	2582	2582	0.95	14365200	12083463	175518	84.1
G11	2246	2246	0.82	9703344	8944903	58341	92.2
G12	2476	2476	1.06	16988457	15103351	145008	88.9
G13	2592	2593	0.94	12366662	11861964	38823	95.9
G14	2694	2694	1.15	18584055	17604686	75336	94.7
G15	2575	2575	1.02	14464020	13780076	52610	95.3
G16	2532	2532	1.04	15155977	14461684	53407	95.4
G17	2454	2454	1.04	15123056	14480149	49454	95.7
G18	2313	2313	1.06	16426798	14884496	118639	90.6
G19	2587	2587	1.12	17292560	16601293	53174	96.0
G20	2537	2537	0.96	13059946	12263520	61263	93.9

†Linear regression model sum of squares.
‡ Residual sum of squares.

them due to G and GE sources in the linear regression model or to the intercept and slope parameters. The positions of the genotypes and the cut-off point are given in Fig. 1. According to this dendrogram, there were three different genotypic groups. For improving the effectiveness of this method, it has been indicated that most of the variation among genotypes is included in the between-group component (Lin and Thompson, 1975). The coefficient of determination (R^2) of the linear regression model ranged from 0.84 (G10) to 0.98 (G2) (Table 3). Genotypes with high coefficient of determination (R^2) values can be evaluated adequately via the joint linear regression model and the response of the genotypes to different environments is predictable (Crossa, 1990). Regarding high R^2 values, it can be concluded that this clustering method is useful to some extent for

this data set.

Most of the suitable genotypes (with high slopes) were clustered in major groups, indicating their specific adaptation to favorable conditions (Fig. 1). Genotypes G11 and G14 were the most unadapted and adapted genotypes, respectively; they had specific adaptation to poor and rich environments, as evidenced by their low and high line slopes, respectively. Clustering genotypes based on similarity of linear regression model parameters (intercept and slope) indicated considerable variation among genotypes. This may be due to the different origins, pedigrees and breeding procedures of these improved genotypes. Brandle and Brule-Bable (1991) reported that this clustering method may be a suitable tool for selecting the most stable as well as the highest yielding genotypes.
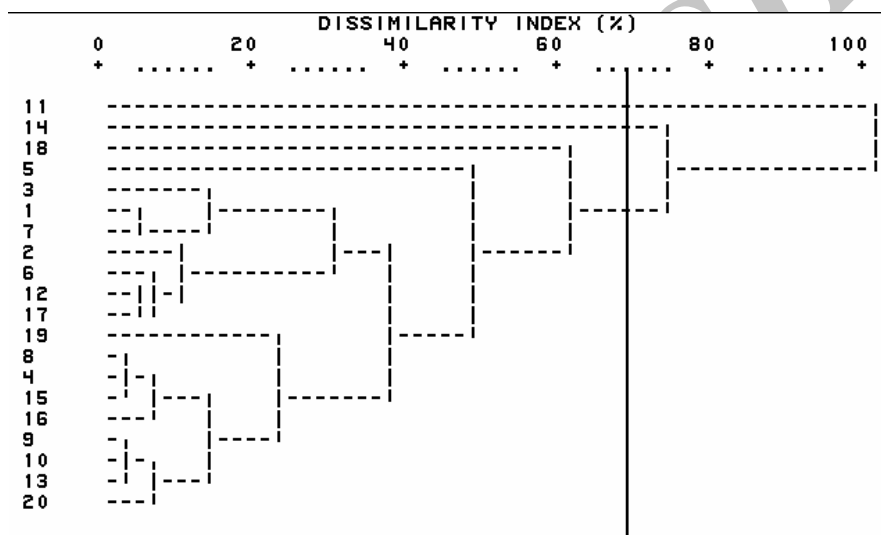

Fig. 1. Dendrogram of dissimilarity indices based on line slope and intercept parameters of the regression model for durum wheat genotypes.

Similar to the method of Lin and Thompson (1975), the line slope of the joint linear regression model (Finlay and Wilkinson, 1963) was used for clustering durum wheat genotypes (Lin and Butler, 1990). The F-test statistic was not significant in clustering cycles and there was no significant difference among durum wheat genotypes based on lines slopes. According to Yue *et al*. (1997), the variation of the estimates of the regression coefficient is usually so small that classifying genotypes for stability and adaptability is difficult. The clustering cycles are summarized in Fig. 2

Although Lin and Butler (1990) proposed this clustering method for improving the effectiveness of the previous clustering method (clustering based on both intercept and slope), it could not distinguish genotypic variations using linear slopes.

Karimizadeh *et al*. (2006) reported that there was good agreement between these two clustering methods when studying multi-environment trials of different maize hybrids. The G × E interaction in the linear regression model was partitioned into heterogeneity (randomized variation) and residual components. The heterogeneity component was not significant, indicating that the contribution of non-random effects was greater than that of random effects in G × E interaction. When the line slopes are identical for all genotypes, this component is distributed as χ^2 and is independent of environmental effects. Therefore, considering the high R^2 values, the model is suitable and the GE interaction partitioning provides a method for testing systematic GE interaction.
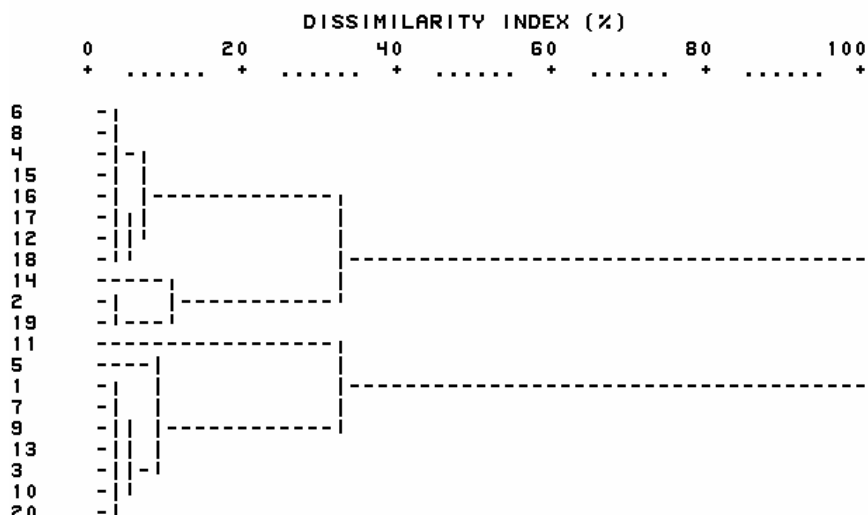
Fig. 2. Dendrogram of dissimilarity indices based on the line slope of the regression model for durum wheat genotypes.

In multi-environment trials, E usually explains most of the total grain yield variation, while G and G × E interaction are small (Yan and Kang, 2003). Lin and Butler (1990) proposed a dissimilarity index using G and G × E interaction simultaneously in terms of distance adjusted for these effects in ANOVA.

The F-test statistic was significant in cycle 6, where the dissimilarity index was 30652.8. In this step, genotype G15 was grouped in a cluster containing genotypes G8 and G9. Thus there was a significant difference between these clusters based on G and G × E sources. It should be mentioned that 20% of the pooled error in the combined ANOVA

table was used for obtaining the cutoff point in the dendrogram (Robert, 1997).

According to the dendrogram in Fig. 3, there were 15 genotypic groups: genotypes G3, G4, G5, G10, G11, G12, G14, G15, G18, G19, and G20 as individual groups, while G7 and G13; G8 and G9; G1 and G17; and G2, G6 and G16 were composite groups. This grouping pattern showed high variation among the studied durum wheat genotypes considering both genotypic main effects and GE interaction. This clustering procedure, which uses both G and G × E interaction sources, can be useful for identifying the most stable genotype according to Type I stability (Lin *et al*., 1986). Successful
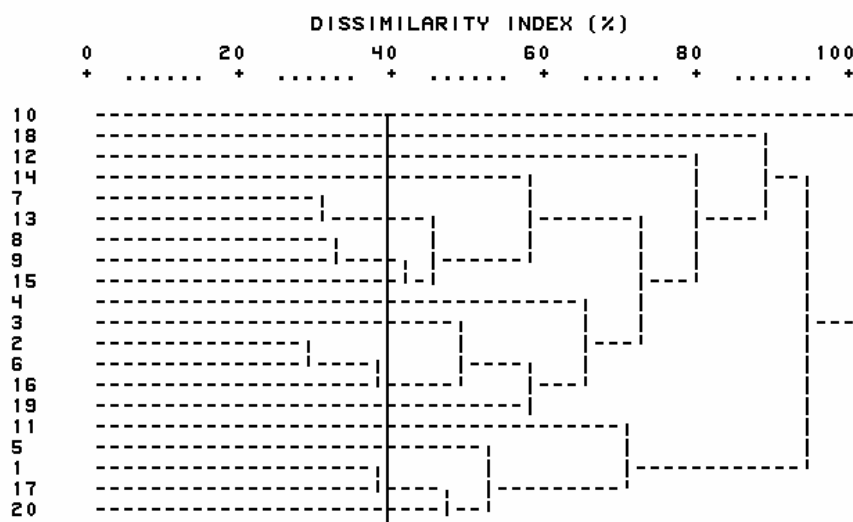

Fig. 3. Dendrogram of dissimilarity indices based on G and G × E interaction of the ANOVA model for durum wheat genotypes.

applications of type I stability have been reported in multi-environment trials of different crops in semi-arid regions (Mohebodini *et al*., 2006; Dehghani *et al*., 2008), while the other stability types (Types 2

and 3) are very popular among plant breeders for assessing yield stability. Finally, considering both mean grain yield and stability performance, genotypes G8 and G13 may be regarded as the

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superior genotypes according to joint linear regression-based on clustering procedures.

The dissimilarity index of Lin (1982) is identified in terms of distance adjusted for the effects of genotypes and is equivalent to error term or within group MS of G × E interaction of ANOVA. In the present study, the optimized number of genotypes must be informative for G × E interaction interpretation. Similar to method 3, the cut-off point was fixed at 20% of the pooled error in combined ANOVA (Robert, 1997); therefore, G × E interaction within clusters must be less than 20% of the total variation. The F-test statistic was significant in cycle 8, where the dissimilarity index was 31846.9; in this step, genotypes G2 and G6 were grouped together in a cluster containing genotypes G8 and G9, and there was a significant difference between these clusters based on G × E interaction. Visualization of this clustering method indicated that there were 12 genotypic groups including genotypes G4, G10, G11, G12, G14, G18 and G19 as individual groups, while G1 and G17; G3 and G16; G4, G5 and G20; G7, G13 and G15; and G2, G6, G8 and G9 were composite groups (Fig. 4).
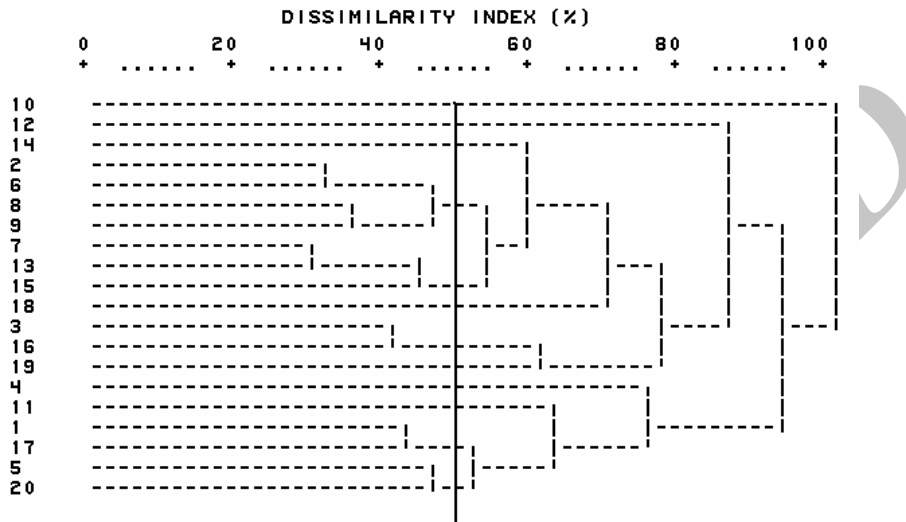

Fig. 4. Dendrogram of dissimilarity indices based on G × E interaction in the ANOVA model for the studied durum wheat genotypes.

Lin (1982) reported this clustering method as an analytical tool for studying data sets from multi-environment trials, which provides a logical base for comparing genotypes within clusters based on their average effect. The most prominent finding according to Fig. 4 is: genotypes G2, G6, G8 and G9 as well as genotypes G7, G13 and G15 had relatively moderate mean grain yield and high stability and were grouped in the same cluster, while other more stable or high yielding genotypes were clustered individually or merged within clusters based on their average effect. Clustering the durum wheat genotypes based on similarity of G × E interaction revealed considerable variation among genotypes. Karimizadeh *et al*. (2006) showed that clustering maize hybrids using both ANOVA-based methods (G × E or G × E and G) produced similar results.

Significant effects of genotype, environment and G × E interaction were observed, but the contributions of environment (92.3%) to total variation were higher than those of genotype (1.1%) and G × E interaction (6.6%). It has been reported that environment explains 80% or more of the total

variation, while G and G × E interaction are relevant to genotype evaluation (Yan and Tinker, 2005). However, ANOVA was relatively uninformative in explaining G × E interaction when analyzing the durum wheat data set. In the multi-environment yield trials, genotypic main effects provided the only relevant information, while G × E interaction effects were absent or ignored. However, differences among genotypes were widely observed in the test environments in the presence of G × E interaction as large as those reported in other studies (Annicchiarico, 1997).

G × E interaction may also be useful for genotype selection, indicating positive interaction with the test environment (Ceccarelli, 1996). In the present investigation, multivariate techniques were used as part of cluster analysis. The clustering method is an effective analysis that graphically displays how various genotypes were differentiated from G and G × E sources. This analysis described the properties of a group of various genotypes and gave a reasonable and useful interpretation of the data set. In general, these results will be useful to plant breeders when grouping durum wheat genotypes according to similarity

indexes that illustrate G or G + GE effects, as presented in Figs. 1 to 4.

This study showed that various clustering methods were very effective for studying G × E interaction patterns and grouping genotypes. As a general rule, the linear regression model is effective when 50% of the total sum of squares is accounted for by linear G × E interaction (Hayward *et al.*, 1993). However, the R^2 values for the studied durum wheat genotypes were high (more than 50%); therefore, methods 1 and 2 (based on the linear regression model) can be regarded as the most suitable methods. Other methods (based on G × E or on G and G × E) are also useful for understanding G × E interaction. These methods showed that G × E interaction was an important source of grain yield variation and the differential response of genotypes is clearly reflected in correspondent dendrograms. Although several statistical methods for estimating G × E interaction have been developed over the last decades, it was only recently that G × E interaction has been investigated using clustering methods (Robert, 1997; Karimizadeh *et al.*, 2006). In practice, most plant breeders usually characterize their improved genotypes based on the performance of local checks or commercial cultivars. This method has some limitations when statistically analyzing G × E interactions within multi-environment trials. In contrast, clustering methods used for studying multi-environment trials allow the selection of superior genotypes.

The joint linear regression model is also used to explore G × E interaction in multi-environment trials. Brandle and Brule-Bable (1991) and Lin and Lin (1994) indicated that cluster analysis based on a regression model is able to distinguish similarities and dissimilarities among genotypes. Although most linear regression models follow the Type II stability concept, but study of multi-environment trials through G × E interaction implies Type I stability concept; however, Type II and Type III stability concepts are popular among most plant breeders.

In contrast, all the clustering methods used in this investigation enable plant breeders to group a data set into homogeneous subsets and find the G × E interaction structure. Clustering results showed that there were distinct genotypic groups of durum wheat genotypes based on G × E interaction and G + GE interaction. Of further interest was the fact that the improved genotypes in some groups were either more stable with lower mean grain yield or higher yielding with lower stability, indicating that cluster analysis was successful in identifying inherent variations among genotypes.

It was concluded that: (1) genotypes G8 (2590 kg

ha⁻¹) and G13 (2592 kg ha⁻¹) were the superior genotypes based on the four clustering methods, mean grain yield and stability performance, and can thus be recommended as suitable candidates for release in warm rainfed areas of Iran; and (2) the magnitude of G× E interaction and changes in the ranking of genotypes across test environments suggest that some genotypes are specifically adapted to homogeneously grouped test environments.

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