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Evaluation of grain yield stability of barley genotypes using additive main effects and multiplicative interaction model

Elnaz Ramzi[1], Ali Asghari[1*], Asghar Ebadi[*1] Omid Sofalian[1], Asghar Mehraban[2]

[1]Faculty of Agriculture and Natural Resources, University of Mohaghegh Ardabili, Ardabil, Iran.
[2]Moghan Agricultural Research Center, Moghan, Iran.
*Corresponding authors; ali_asgharii@yahoo.com; asghar_ebadi@yahoo.com

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Abstract

Objective: Barley as one of the most important cereals is crucial to supplying the required energy for both humans and animals. Grain yield is strongly influenced by environmental conditions and breeders often determine the stability of high-yielding genotypes across various locations before recommending a cultivar for release.

Methods: For this research, 18 barley genotypes were tested at four different research stations including Gachsaran, Moghan, Khorramabad, and Gonbad in Iran. The additive main effects and multiplicative interaction (AMMI) model was used to identify the stable genotypes.

Results: Partitioning of the GE interaction indicated that the first interaction principal component axes (IPCA) captured 35.9% of the interaction sum of squares. However, the five IPCAs accounted for 87.4% of the total interaction. Moreover, 13 AMMI-based stability statistics were calculated. Using cluster analysis, AMMI stability indices were divided into four groups. This analysis showed that most indices do not correlate with the grain yield. AMMI indices were placed in separate groups with large distances from the grain yield. However, the yield stability index (YSI) showed the highest correlation with grain yield, and based on that, G13, G18, and G11 genotypes were recognized as superior genotypes. G13 and G18 genotypes had an acceptable performance in two years and four locations, so they may be regarded as genotypes with both high yield and stability in these environments. However, by using the AMMI2 mega-environmental analysis to select suitable cultivars for each mega-environment, the target region was divided into three sub-regions, and the winner genotypes of each sub-region were identified. The first mega-environment consisted of the areas Gonbad and Ghachsaran, where genotype G14 was the winner; the second mega-environment consisted of the Khorramabad area, where genotype G2 was the winner, and the third mega-environment included only the Moghan area, where genotype G13 was the winner.

Conclusion: In conclusion, the AMMI2 mega-environmental analysis identified three mega environments and the best genotypes specifically adapted to these environments. Since two test areas Gonbad and Ghachsaran were located in the first mega-environment, it seems that the performance ranking of the test genotypes in these areas will be almost the same, and in coming years, the test can be performed in only one of these areas.

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Introduction

Barley (*Hordeum vulgare* L.) is widely cultivated and used in Iran and the world. Barley is the fourth most important grain product in the world after wheat, rice, and maize, and its grains are used as animal feed and forage, malt beverage, human food, soil amendment, and have medicinal value (El-Hashash and El-Absy 2019). According to FAO (2022), barley cultivated area in Iran during the 2022 growing season was about 1.65 million hectares and harvested production was estimated at three million tons.

Achieving high-yielding and stable barley varieties is considered one of the most important goals for breeding this crop. The crop performance is strongly influenced by environmental conditions so broad changes in their performance are observed both between years in a location and between locations in a year (Pacheco *et al.* 2005). The different response of genotypes from one environment to another is called genotype × environment (GE) interaction. GE interaction makes it difficult to compare genotypes over a range of years or locations and plays a key role in developing strategies for crop improvement (Yan and Hunt 1998). Depending on whether GE interactions are controlled or not, it can lead to gains or losses in breeding programs (Yan and Kang 2003). To investigate the GE interaction, varieties should be grown in a wide range of environments and the ultimate goal of the plant breeding programs is the selection of high-yielding and high-compatible genotypes for these environments. However, it is difficult and almost impossible to find genotypes that can be highly adapted to all environmental conditions. Therefore, plant breeders manage GE interaction by selecting genotypes with specific adaptations.

Various statistical methods including univariate, multivariate, and nonparametric have been developed to determine the grain yield stability to explain the information included in the GE interaction data matrix (Finlay and Wilkinson 1963; Shukla 1972; Lin and Binns 1988; Fox *et al.* 1990; Kang and Pham 1991). Each of them reflected different aspects of stability and no single method can adequately explain cultivar performance across environments (Annicchiarico 1997). The response level of genotypes in a given environment can be better predicted using multiplicative models such as the additive main effects and multiplicative interaction model (AMMI) (Gauch 1988;

Gauch and Zobel 1988, 1989). Recently, due to higher efficiency, the tendency of most researchers to use multivariate methods has increased. AMMI is a popular extension of the analysis of variance and principal component analysis (PCA) for studying the GE interaction. This model is a hybrid analysis that incorporates both the additive and multiplicative components of the two-way data structure. AMMI extracts genotype and environment main effects and uses principal component axes to explain patterns in the GE interaction matrix (Romagosa and Fox 1993; Kanouni *et al.* 2023). Various stability indices have been introduced based on the AMMI model, including averages of the squared eigenvector values (Zobel 1994), AMMI's stability values (ASV) (Purchase 1997), the sum of the absolute values of the IPC scores (SIPC) (Sneller *et al.* 1997), AMMI stability index (ASI) (Jambhulkar *et al.* 2017), D index (Annicchiarico 1997), and AMMI based stability parameter (ASTAB) (Rao and Prabhakaran 2005). The use of the AMMI stability parameters permits the evaluation of yield stability after reducing the noise from the GE interaction effects (Sabaghnia *et al.* 2012). Also, AMMI allows the determination of mega-environments which are defined as a subset of locations with homogeneous environmental conditions, where the performance of certain genotypes is more similar through the years (Kandus *et al.* 2010). The identification of mega-environments is important for the following reasons. The first reason is that marginal environments show a high GE interaction effect because they are often affected by various stresses. Therefore, genotypes that win in highly favorable environments may rank poorly in these conditions. On the other hand, within favorable environments, conventional management procedures in the past have produced a rather coherent environment easily targeted by plant breeders (Gauch and Zobel 1997). In general, most plant breeders feel that instead of selecting one genotype for all environments, they should select genotypes suited to the conditions of mega-environments.

This study was conducted to understand the extent and causes of GE interactions using the AMMI methodology in barley production areas of Iran.

Materials and Methods

Experiments

The data in this study were obtained from the sets of barley yield trials conducted for three years (2017- 2019) in different areas of Iran. In each year, 16 genotypes along with two check cultivars (Mahoor and Khorram) were tested at four different research stations. All the selected research stations are located in warm climates including Gachsaran, Moghan, Khorramabad, and Gonbad. The names, pedigree, and origin of studied genotypes are given in Table 1. At each environment, a randomized complete block design with four replications was used. The experimental plots consisted

of six rows with 6 m long and 0.2 m spacing between rows, which resulted in a plot area of 7.2 m^2 and the seeding rate was 350 seeds per m^2 for each genotype. The seeds were planted with an experimental planter (Wintersteiger). Weeds were controlled by a chemical herbicide (Granstar). At the harvest time, grain yield was determined for each genotype at each plot after removing marginal plants in each test environment.

Table 1. Genotype code, name, or pedigree of 18 studied barley genotypes tested in four locations in Iran.

Genotype code	Name or pedigree of the genotype
G1	Mahoor as check
G2	Khorram as check
G3	HART-BAR/CANELA//MSEL CBSS01Y00777T-Z-0Y-10M-0M-1M-0Y(PRBYT2010-11-44)
G4	CANELA/CHERI CBSS01Y00007S-0Y-6M-0M-1M-0Y(PRBYT2010-11 -28)
G5	Moroc9-75//WI2291/WI2269 ICB93-1132-0AP-31AP-0AP-5TR-8AP-0AP(PRBYT2010-11-81)
G6	6B89.2027/5/ATACO/BERMEJO//HIGO/3/CLNB/80.5138//GLORIABAR/COPAL/4/CHEVRONBAR/6/LEGACY CBSS01Y00858T-B-0Y-9M-0M-2M-0Y(PRBYT2010-11-31)
G7	Moroc9-75//WI2291/WI2269 ICB93-1132-0AP-31AP-0AP-6TR-38AP-0AP(PRBYT2010-11-85)
G8	MNS1//CALI92/ROBUST CBSS01Y00154S-0Y-10M-0M-3M-0Y(PRBYT2010-11-33)
G9	Moroc9-75//WI2291/WI2269 ICB93-1132-0AP-31AP-0AP-5TR-20AP-0AP(PRBYT2010-11-82)
G10	MSEL//CLI18/E.QUEBRACHO CBSS01Y00023S-0Y-10M-0M-1M-0Y(PRBYT2010-11-26)
G11	Giza127/4/Gloria'S'/Saida//Mtn'S'/EH165/3/LBIran/Una80//Lignee640 ICB97-0488-0AP-21AP-6TR-0AP(PRBYT2010-11-20)
G12	MSEL//CLI18/E.QUEBRACHO CBSS01Y00023S-0Y-10M-0M-1M-0Y(PRBYT2010-11-42)
G13	WI2291//Apm/PI000046/3/Hml-02/4/Arda/Moroc9-75 ICB01-0006-0AP-28AP-0AP(PRBYT2010-11-99)
G14	WI2291/4/7028/2759/3/6982//Ds/Apro/5/Zanbaka/3/ER/Apm//Lignee131I CB94-0590-0AP-9A-0AP-0AP-14AP-0AP-9AP-0AP(PRBYT2010-11-93)
G15	Moroc9-75//WI2291/WI2269 ICB93-1132-0AP-33AP-0AP-18TR-41AP-0AP(PRBYT2010-11-88)
G16	Moroc9-75//WI2291/WI2269 ICB93-1132-0AP-31AP-0AP-6TR-46AP-0AP(PRBYT2010-11-86)
G17	Moroc9-75//WI2291/WI2269 ICB93-1132-0AP-13AP-0AP-19AP-0AP(PRBYT2010-11-122)
G18	Hml-02//WI2291/Bgs ICB83-1554-1AP-1AP-6AP-0AP-23AP-0AP-13AP-0AP(PRBYT2010-11-121)

Statistical analysis

Analysis of variance was carried out for each test environment. Homogeneity of residual variances was verified by Bartlett's homogeneity test, before carrying out the combined analysis of variance. The AMMI model was used to study the GE interaction as follows:

$$Y_{ij} = \mu + \alpha_i + \beta_j + \sum_N \lambda_n \gamma_{in} \delta_{jn} + \rho_{ij} + \varepsilon_{ij}$$

Where Y_{ij} is the observed mean yield of ith genotype in the environment j, μ the grand mean, α_i the genotype main effect, β_j the environment main effect, λ_n the eigenvalue of the interaction principal component analysis (IPCA), γ_{in} and δ_{jn} are the genotype and environment scores for the

IPCA axis, ρ_{ij} interaction residual, N the number of IPCA retained in the model, and ε_{ij} the random error term.

MATMODEL software (Gauch 2007) and the associated program, AMMIWINS, were used for the AMMI and mega-environment analyses. AMMIWINS identifies each mega-environment by its winning genotypes, counts its number of wins, and calculates the average expected yield over those environments included in that mega-environment.

Thirteen stability statistics derived from the AMMI analyses were used. The $SIPC_1$, $SIPC_v$, and $SIPC_f$ are sums of the absolute value of the IPC scores: $\sum_{n=1}^{N} \lambda_n^{0.5} \gamma_{in}$ for the *i*th genotype (for $SIPC_1$, N was one; for $SIPC_v$, N was the number of IPC that were retained in the AMMI model via cross-validation test; for $SIPC_f$, N was the number of IPCs that were retained in the AMMI model via Gollob's F test); EV_1, EV_v, and EV_f, which were suggested by Zobel (1994), are averages of the squared eigenvector values: $\sum_{n=1}^{N} \frac{\gamma_{in}^2}{N}$ (for EV_1, N was one; for EV_v, N was the number of IPC that were retained in the AMMI model via cross-validation test; for EV_f, N was the number of IPCs that were retained in the AMMI model via Gollob's F test). AMMI's stability values (ASV) were calculated using the following formula (Purchase 1997):

$$ASV = \left(\frac{SS_{IPCA1}}{SS_{IPCA2}}(IPCA1)^2(IPCA2)^2\right)^{0.5}$$

Where, $SSIPCA_1$ and $SSIPCA_2$ are the sums of squares of the interaction of first and second IPC, respectively. AMMI stability index (ASI) has been used to get a quantitative value for the stability analysis by Jambhulkar *et al.* (2017), which helps in the interpretation of the results:

$$ASI = ([PC_1^2 \times \theta_1^2] + [PC_2^2 \times \theta_2^2])^{0.5}$$

θ_1, θ_2, and θ_n are percentage sum of squares explained by the 1st, 2nd, and nth IPC, respectively.

Modified AMMI stability index (MASI) and Modified AMMI stability value (MASV) are the extensions of ASV and ASI as implied by Ajay *et al.* (2018, 2019):

$$MASV = \left(\sum_{n-1}^{N-1}\left(\frac{SSIPC_n}{SSIPC_{n+1}}\right) \times (PC_n)^2 + (PC_N)^2\right)^{0.5}$$

$$MASI = \left(\sum_{n=1}^{N} PC_n^2 \times \theta_n^2\right)^{0.5}$$

Rao and Prabhakaran (2005) used the ASTAB index with the following formula:

$$ASTAB = \sum_{n=1}^{N} \lambda_n \gamma_{in}^2$$

Annicchiarico (1997) used the D index that measured as the unsquared Euclidean distance from the n =1, 2, …, N PC axes included in the AMMI model:

$$D = \left(\sum_{n=1}^{N} (\lambda_n \gamma_{in})^2 \right)^{0.5}$$

Zali *et al.* (2012) used the sum across environments of the absolute value of GEI modeled by AMMI (AVAMGE):

$$AVAMGE = \sum_{i=1}^{N} \sum_{j=1}^{M} \left| \lambda_n \lambda \gamma_{in} \delta_{jn} \right|$$

Zali *et al.* (2012) also used the absolute value of the relative contribution of IPCs to the interaction (Za):

$$Za = \sum_{i=1}^{N} |\theta_n \gamma_{in}|$$

The yield stability index was calculated using the equation YSI = RASV + RY (Farshadfar 2008). The terms RASV and RY in this context stand for the genotype mean yield ranking across environments and AMMI stability value ranking, respectively.

Statistical packages for the combined analysis of variance, AMMI analysis, and twelve AMMI stability indices were calculated using RStudio, R version 4.3.2 by using 'Agricolae' and 'Metan' packages.

Results and Discussion

An annual mean yield of the 18 investigated barley genotypes across 12 environments (a combination of four locations and three years) is shown in Table 2. Bartlett's test which was performed to assess homogeneity of variances before combined analysis, showed non-significance ($p > 0.05$) Chi squares statistic, indicating homogeneity of variances. The combined analysis of variance for grain yield showed highly significant differences among genotypes, environments, and their interactions (Table 3). This analysis showed that 54% of the total sum of squares was attributable to environmental effects (E), 3.4% to genotypic effects (G), and 15.4% to GE interaction. This result shows that genotypes performance is greatly influenced by the environment. A large sum of squares for environments indicated that the environments were diverse. The significant GE interaction for yield confirms the differential response of genotypes to environments. A significant large-scale GE interaction could

mean that there are at least two target breeding environments with different climatic conditions in a region, instead of one (Pour-Aboughadareh *et al.* 2022). Since the GE interaction contributes more to the overall variance, there is a greater likelihood that certain cultivars performed better in a particular environment.

Two basic approaches have been used to determine the optimal number of multiplicative terms to be retained in the GE interaction component. The result of full cross-validation across 1000 randomizations of the data is shown in Table 3. The full cross-validation yielded minimum root mean square prediction differences (RMSPD) at the first component, although the RMSPD values for the other four components were very similar in size and any could be chosen to represent the optimum number of components for the model. Also, partitioning of GE interaction indicated that the AMMI-5 model described the GE interaction patterns for yield using the first five IPCA scores based on F Gollob's. Results from the AMMI analysis also showed that the first PC axis (IPCA1) of the interaction captured 35.9% of the interaction sum of squares. Similarly, the second PC axis (IPCA2) explained a further 19.4% of the GE interaction sum of squares. The five IPCAs accounted for 87.4% of the total interaction.

The results for average grain yield and the estimated AMMI-based stability parameters for 18 tested genotypes are shown in Table 4. Genotype G13 showed the best average grain yield in a total

Table 2. Annual mean yield (kg.ha^{-1}) of 18 barley genotypes tested in four locations in Iran.

Genotype code	Cropping season											
	2016-2017				2017-2018				2018-2019			
	E1	E2	E3	E4	E1	E2	E3	E4	E1	E2	E3	E4
G1	1062	2306	2830	2017	1611	2207	2309	3255	2161	2481	3213	2591
G2	1473	2548	3608	2031	1695	2289	2875	3194	1918	2416	2274	2201
G3	1105	2012	2653	2132	1479	1997	2566	2792	1854	2579	2611	2489
G4	1119	2078	2306	1927	1307	2374	2303	3289	1494	2676	2494	2681
G5	1321	2173	3378	1627	1629	1809	2713	3230	1938	2618	2560	2285
G6	1234	2135	2431	1562	1475	2114	2471	2508	1716	2124	2053	2083
G7	1174	2170	2153	1784	1416	2217	2925	3072	1658	2461	2372	2223
G8	1428	1833	2731	1623	1859	2016	2188	3087	2290	2318	2405	2397
G9	1334	2247	3166	1267	1916	2009	2529	3367	2499	2809	1627	2149
G10	1051	2223	2302	1865	1390	1868	2446	2502	1734	2560	2199	1806
G11	1200	1973	2807	1386	1826	2046	2838	2633	2451	2568	2873	2389
G12	1266	2243	2381	1802	1718	2067	2623	2986	2170	2461	2559	2792
G13	1312	2350	3171	2151	1897	2557	2733	3086	2481	2398	2466	2504
G14	1581	2081	1988	1902	1985	1709	2175	3228	2390	2543	2637	3241
G15	1104	2209	2689	2132	1637	1832	2390	2872	2171	2376	2321	2443
G16	1155	2437	2618	1964	1588	1974	2176	2553	2020	2366	2200	2045
G17	1309	2177	2670	1280	1786	2162	3014	2743	2264	2579	1735	1870
G18	1351	2108	2656	2097	1650	2056	2861	3395	1949	2649	2597	2413

E1: Gachsaran; E2: Moghan; E3: Khorramabad and E4: Gonbad.

of 12 test environments, followed by genotypes G2, G1, and G18, while G6, G10, and G16 showed the lowest performance. According to the minimum values of EV_1 and EV*v* parameters (these two parameters were similar and thus, only EV_1 was calculated), genotypes G8, G10, and G11 were the most stable genotypes (Table 4). Genotypes G12, G3, G6, and G13 were the most stable genotypes based on EV_f parameter. Genotypes that are introduced by EV as stable genotypes often indicate low mean yield across test environments. However, G10 and G6 had a high average performance among the investigated genotypes and they can be considered as the most favorable genotypes. According to the $SIPC_1$ statistic, G10, G8, and G11 were the most stable genotypes. Based on the $SIPC_f$ statistic, which uses the sum of the absolute value of eigenvalues of the remaining components, G15 was the most stable genotype, followed by G3, G12, and G6. Genotypes G14, G9, and G17 were ranked as the least stable based on both $SIPC_1$ and $SIPC_f$ scores. According to the ASV parameter, which measures the distance from the genotype coordinate point to the origin in a two-dimensional scatter diagram of IPCA2 against IPCA1 scores, genotypes with the lowest ASV values are considered the most stable. Based on the ASV, the most stable genotypes for grain yield were G15, G16, and G6 (Table 4). G14 and G9 were ranked the least stable because they had the highest ASV values. The

Table 3. Additive main effects and multiplicative interactions (AMMI) analysis of variance for grain yield (kg.ha^{-1}) of the 18 barley genotypes tested in 12 environments.

SOV	df	Mean squares	Gollob's F	RMSPD†	Explained (%)
Environment (E)	11	16.561 **			54.0
Year (Y)	2	11.170			12.3
Location (L)	3	32.710			53.9
L × Y	6	10.287 **			33.9
Replication/E	36	0.550			
Genotype (G)	17	0.675 **			3.4
G × E	187	0.278 **			15.4
IPCA 1	27	0.691	4.977 **	0.417††	35.9
IPCA 2	25	0.403	2.903 **	0.420	19.4
IPCA 3	23	0.297	2.141 **	0.424	13.1
IPCA 4	21	0.254	1.826 *	0.426	10.2
IPCA 5	19	0.240	1.729 *	0.425	8.8
G × Y	34	0.330 *			21.6
G × L	51	0.379 **			37.1
IPCA 1	19	0.153	3.041 **	0.269	44.9
IPCA 2	17	0.140	2.793 **	0.267††	36.9
G × L × Y	102	0.210 **			41.3
Pooled error	648	0.142			27.2

* and **: Significant at the 0.05 and 0.01 probability levels, respectively.
†RMSPD: The root mean square prediction differences, predicted by MATMODEL software with repeating 1000 times splitting data.
††**:** The selected model with a minimum root mean square predictive difference.

ranking of genotypes in terms of stability by the ASI index was almost similar to ASV. Therefore, genotypes G16, G15, and G6 were the most stable genotypes.

Genotype G6, followed by G13, G3, G12, and G18, showed higher stability compared to other tested genotypes based on the ASTAB. According to AVAMGE, G15, G12, G6, and G13 were the most stable genotypes. Based on the distance between the IPCA points and the origin in space (D index), genotypes G6, G15, G13, and G18 showed the greatest stability compared to the other genotypes. The MASV and MASI indices identified genotypes G11 and G6 as superior genotypes and G14, G9, and G17 were ranked the least stable because they had the highest MASV and MASI values. The YSI index was used to exploit both yield and stability at the same time, and the most stable genotypes with the highest grain yield were G13, G18, and G11, which had the lowest YSI values.

Table 4. Average yields ($kg.ha^{-1}$) and AMMI stability parameter estimates of 18 barley genotypes tested in 12 environments.

Genotype	Yield	ASTAB	ASI	ASV	YSI	AVAMGE	D	EV_1	EV_f	MASI	MASV	$SIPC_1$	$SIPC_f$	ZA
G1	2337	416.7	4.43	22.84	16	2283.7	818.1	0.067	0.055	4.86	30.70	12.04	38.76	0.19
G2	2377	519.7	6.00	30.96	17	2422.6	955.5	0.109	0.063	6.16	36.52	15.33	46.72	0.24
G3	2189	133.0	3.03	15.63	21	1310.5	484.2	0.025	0.016	3.07	18.50	7.29	22.06	0.12
G4	2171	556.0	5.27	27.20	27	2631.4	935.7	0.088	0.076	5.56	35.07	13.76	45.29	0.22
G5	2273	417.1	3.00	15.47	16	1935.3	756.2	0.031	0.064	3.68	27.02	8.24	42.04	0.18
G6	1992	120.6	1.47	7.57	21	1170.6	406.0	0.006	0.018	1.85	14.56	3.52	23.50	0.10
G7	2135	441.8	2.34	12.05	22	1987.1	774.3	0.007	0.067	3.25	27.57	3.99	41.35	0.17
G8	2181	216.0	2.41	12.42	21	1696.9	569.8	0.001	0.029	2.63	20.86	1.18	23.93	0.10
G9	2243	900.6	8.06	41.56	26.00	3437.5	1258.1	0.196	0.109	8.19	48.16	20.60	54.45	0.29
G10	1995	234.5	1.80	9.28	23	1653.1	562.3	0.001	0.036	2.29	20.01	1.16	30.99	0.12
G11	2249	705.2	1.69	8.73	12	2570.5	904.5	0.001	0.123	2.94	29.99	1.66	48.92	0.17
G12	2255	134.6	3.37	17.39	19.00	1168.5	500.5	0.037	0.015	3.43	19.39	8.99	22.46	0.12
G13	2425	127.6	1.87	9.66	8	1212.4	420.0	0.012	0.020	2.15	15.02	5.15	23.88	0.10
G14	2288	1020.3	9.06	46.74	23	3673.9	1371.5	0.233	0.115	9.09	52.84	22.43	56.58	0.32
G15	2181	146.6	1.16	6.01	13	1082.2	417.8	0.005	0.025	1.63	13.84	3.24	21.78	0.08
G16	2091	349.8	1.02	5.28	17	1780.3	627.0	0.002	0.063	1.96	20.21	1.98	31.80	0.11
G17	2132	689.8	7.04	36.33	31	3252.7	1106.4	0.172	0.083	7.35	42.50	19.27	49.81	0.26
G18	2315	146.4	1.76	9.09	9	1254.6	441.9	0.007	0.023	2.03	15.65	3.99	25.49	0.11

ASTAB: AMMI-based stability parameter; ASI: AMMI stability index; ASV: AMMI's stability values; YSI: Yield stability index; AVAMGE: Sum across environments of the absolute value of GEI modeled by AMMI; D: The index that was measured as the unsquared Euclidean distance; EV_1, EV_f: Averages of the squared eigenvector values; MASI: Modified AMMI stability index; MASV: Modified AMMI stability value; $SIPC_1$, $SIPC_f$: Sums of the absolute value of the IPC scores; Za: Absolute values of the relative contribution of IPCs to the interaction.

Relationship between AMMI-based stability indices and grain yield

To better understand the relationships of different AMMI indices with grain yield, a hierarchical cluster analysis based on unweighted values of 13 AMMI stability indices and average yield, was executed (Figure 1). Spearman's coefficient of correlation was used as a similarity measure required in the unweighted pair-group average method. The dendrogram showing the hierarchical classification of stability methods is illustrated in Figure 1. The 13 AMMI stability indices were classified into four groups (Figure 1). The grouping of indices showed that most of them do not correlate with the grain yield. AMMI indices were placed in separate groups with a large distance from the grain yield.

We found those EV_1 and $SIPC_1$ parameters that use only one significant PC, clustered together (Group 1), indicating that the two measures were similar in their power of classifying genotypes

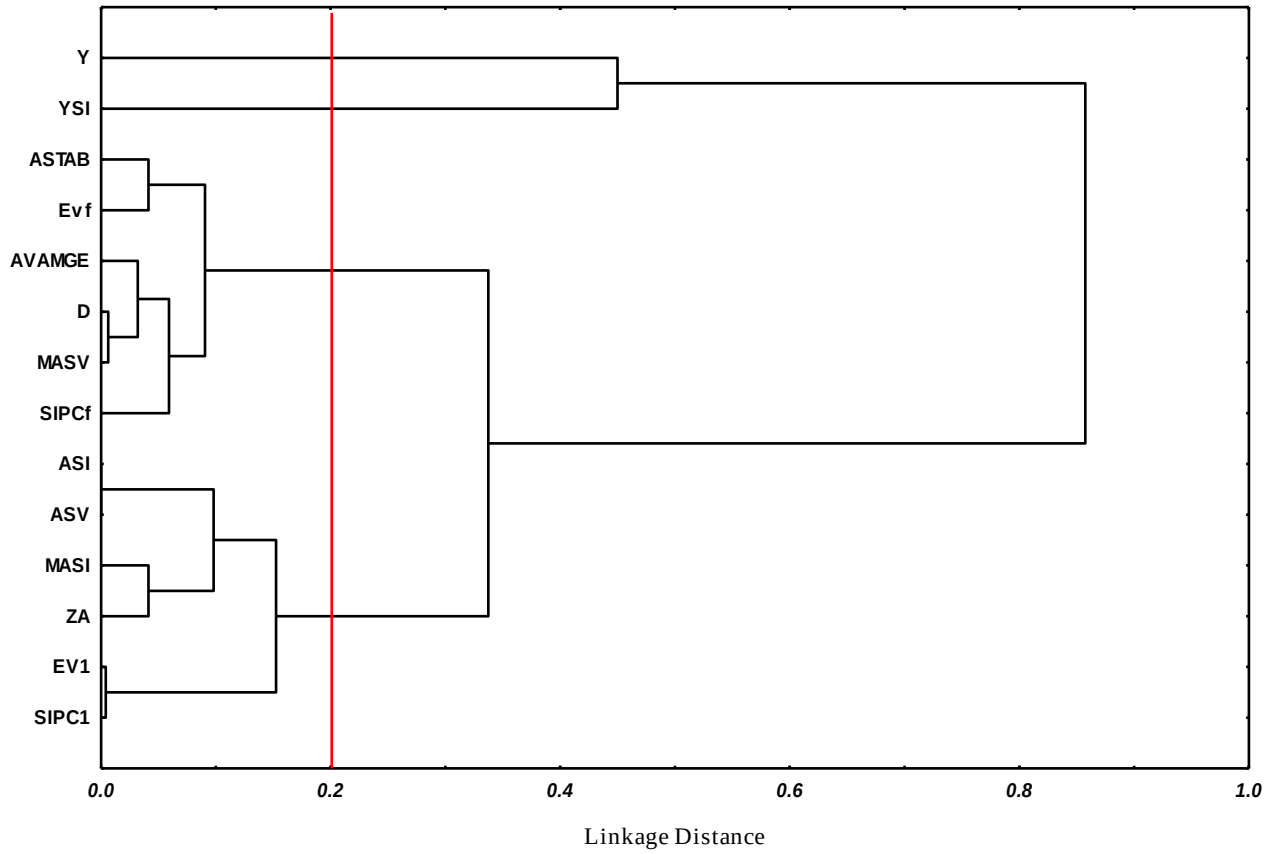

Figure 1. Dendrogram showing the hierarchical classification of average yield and 12 AMMI stability indices based on Spearman's correlation coefficients for 18 barley genotypes.
Y: Yield; YSI: Yield stability index; ASTAB: AMMI-based stability parameter; EV1, EVf: Averages of the squared eigenvector values; AVAMGE: Sum across environments of the absolute value of GEI modeled by AMMI; D: Index measured as the unsquared Euclidean distance; MASV: Modified AMMI stability value; SIPC1, SIPCf: Sums of the absolute value of the IPC scores; ASI: AMMI stability index; ASV: AMMI's stability values; MASI: Modified AMMI stability index; Za: Absolute values of the relative contribution of IPCs to the interaction.

according to their stability under different environmental conditions. According to these methods, the most stable genotypes were G8, G10, and G11.

Group 2 included ASI, ASV, MASI, and Za. The first two indices used the first two main components (IPCA1 and IPCA2) along with their sum of squares to estimate the stability of genotypes and had almost similar results in the grouping of genotypes. When more than two main components are significant in the ASI methods, part of the information in the data becomes unusable. To solve this problem, Ajai *et al.* (2018) used a modified version that would cover all available IPCAs. The MASI and Za use more significant components than ASV and ASI, and considering that 5 IPCA were significant in our experiment, their results can be more reliable. According to these methods, the most stable genotypes were G16, G15, and G6.

Group 3 included AVAMGE, D, MASV, $SIPC_f$, EV_f, and ASTAB which used maximum significant PCs. These indices which were not generally associated with yield, were evaluated independently for the grain yield. Based on these indices, the most stable genotypes for the grain yield were G15, G12, G13, G6, and G3.

Group 4 included YSI, which was the closest index to the grain yield, and based on this index, G13, G18, and G11 genotypes were chosen as the superior genotypes.

Mega-environment analysis

For a long time, most breeders used the term stability to denote a genotype that always showed consistent performance under all environmental conditions. This idea of stability corresponds to the concept of homeostasis, which is widely used in quantitative genetics and may be considered as biological (static) concept of stability (Becker and Leon 1988). Biological stability is not acceptable to most plant breeders who prefer the agronomic concept of stability (Becker 1981; Mortazavian and Azizi-nia 2014). In the static or biological concept, the performance of a genotype remains unchanged regardless of the environmental conditions and stable genotypes do not react to the improvement or environmental conditions (Lin *et al*. 1986). Therefore, according to these parameters, stable genotypes are recommended for locations where growing conditions are unfavorable. This concept of stability is useful for quality traits, disease resistance, or stress characteristics (Cheshkova *et al*. 2020). In developing countries such as Iran, due to limited access to agricultural inputs, the need for stable genotypes that can cope with environmental changes increases. In this situation where the availability of agricultural inputs is not guaranteed, the selection of genotypes for different locations should be done with greater precision. To meet the need of different areas for compatible cultivars, especially areas with high fertility, we used AMMI2 mega-environmental analysis to select suitable

cultivars for each mega-environment. Because the environmental factor in this analysis is a combination of locations and years, it is not helpful when recommendations of cultivars to specific locations are required. Therefore, we used the set of cultivar × location means averaged across years. Partitioning of the genotype by location (GL) interaction indicated that the AMMI2 model described the GL interaction pattern for yield using the first two IPCA scores based on Gollob's F test. The two principal components explained only 81.8% of the GL interaction sum of squares (Table 3). The first mega-environment consisted of the areas Gonbad and Ghachsaran, where the genotype G14 was the winner (in terms of yield and stability). The second mega-environment consisted of the Khorramabad area, where genotype G2 was the winner. The tertiary mega-environment represented the Moghan area, where genotype G13 was the winner (Table 5). If two or more test areas are located in the same mega-environment, it means that they have no crossover interactions with each other. Therefore, the performance ranking of the test genotypes in these areas will be almost the same, and in the coming years, the test can be performed in only one of these areas.

Table 5. AMMI2 mega-environments and their winning genotypes for the 18 barley genotypes grown in four locations at three years.

AMMI mega-environments	Winner genotypes	Expected values for yield (t.ha^{-1})
Mega-environment 1	G14	
Ghachsaran		1.986
Gonbad		2.791
Mega-environment 2	G2	
Khorramabad		2.919
Mega-environment 3	G13	
Moghan		2.435

Conclusion

The barley genotypes investigated in this study exhibited wide variability for grain yield. AMMI stability indices were classified into four different groups using cluster analysis, and each of them introduced their own superior genotypes. The distinguishing characteristic of each group was the number of components that the indices used to evaluate the genotypes. The AMMI procedure used in this study indicated a more complex interaction which required five PC axes to account for a considerable amount of variation due to GE interaction. Therefore, it was clear the parameters that use all five IPCAs, were better than those parameters that used only the first IPCA. Finally, based on grain yield and all AMMI stability indices, the genotypes G13 and 18 had good yield performance and stability in several environments and can be a candidate for introduction as a new cultivar. When the contribution of the GE interaction to the total variance is higher, the genotypes are more likely to

have evolved for a specific environment. Therefore, the target region was divided into different sub-regions (mega-environments) which were relatively uniform in terms of genotypic responses and the winner genotypes of each sub-region were identified. Based on the mega-environmental analysis, the genotype G13, which is recognized as the superior genotype based on the stability indicators, is only recommended for the Moghan area. While the genotype G2 was recommended for Khorramabad and G14 for Gonbad and Gachsaran.

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Ethical considerations

The authors avoided data fabrication and falsification.

Conflict of interest

The authors declare that they have no conflict of interest with any organization concerning the subject of the manuscript.

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