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Analysis of genotype by environment interaction in barley across various locations in Iran using the AMMI model

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ABSTRACT

Barley (*Hordeum vulgare*) is an important global grain valued for its versatility and adaptability. This study utilized additive main effects and multiplicative interactions (AMMI) analysis to assess the adaptability and yield stability of twenty barley genotypes across eight locations, aiming to compare genotypes and identify suitable candidates. Significant genotypic variation was found for grain yield, highlighting the potential for targeted improvement. The AMMI analysis revealed that genotype, environment (location), and genotype-environment interaction (GEI), along with the first four interaction principal component axes, accounted for 86% of the yield variation. Environmental effects contributed 75.89% of the total sum of squares, while genotypic effects accounted for only 4.46%, and GEI effects for 19.65%. Isfahan and Karaj showed the highest GE interaction, indicated by their elevated IPCA1 scores in the AMMI1 biplot, while Birjand, Neishabor, and Varamin had the lowest scores and minimal GE interaction. The biplots identified genotypes 4 and 12 as the most stable and high-yielding, making them suitable for future genetic improvement programs. Conversely, genotypes 5, 10, 14, and 17 exhibited below-average yields and high IPCA1 scores, indicating instability but adaptation to specific locations. Crossbreeding contrasting genotypes could be beneficial for developing mapping populations for stability and yield genome studies in barley.

***Key words*:** Additive main effects and multiplicative interactions, GE interaction, *Hordeum vulgare*, Interaction principal component axis (IPCA).

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INTRODUCTION

Barley (*Hordeum vulgare* L.) is recognized as one of the principal cereal crops globally, owing to its essential nutrients that render it a valuable food source for both humans and livestock (You and Izydorczyk, 2007; Hemadesh *et al.,* 2021). Among cereal crops, barley ranks fourth in terms of both production volume and area under cultivation, contributing approximately 50% of the caloric intake required worldwide. In less developed regions, particularly in Africa and Asia, barley can account for up to 70% of caloric consumption. Additionally, barley serves as a key crop within the local agricultural systems of Iran (Bannayan *et al.,* 2010; Barati *et al.,* 2023). It is one of the earliest domesticated food crops, dating back to the advent of civilization. Its remarkable tolerance to extreme environmental conditions makes barley particularly suitable for cultivation in higher latitudes, altitudes, and arid desert regions (Baik and Ullrich, 2008; Taherian *et al.,* 2022). Furthermore, barley is extensively cultivated in arid and semi-arid Mediterranean regions for forage purposes, and as a grain crop, it exhibits relatively high drought tolerance, enabling it to thrive with limited soil moisture (Hemadesh *et al.,* 2021).

Genotype×Environment interaction (G×E) arises from the differential responses of genotypes across various environmental conditions and constitutes a significant source of variation in crop performance. The presence of G×E interaction typically diminishes the correlation between genotypic and phenotypic values, introduces bias in the estimation of heritability, and results in reduced selection efficiency (Gauch, 2006; Oral *et al.,* 2018; Saeidnia *et al.,* 2023). Consequently, understanding the magnitude of G×E interactions is essential for the development of high-yielding cultivars that exhibit stable performance across a diverse range of environments (Amini *et al.,* 2013; Namdari *et al.,* 2022). To this end, plant breeders employ multi-environment trials to assess the relative performance of genotypes within target environments, thereby facilitating the development of high-yielding and stable cultivars (Yan and Tinker, 2006). Various analytical approaches, ranging from simple Analysis of Variance (ANOVA) to more sophisticated evaluations of genotype performance, have been utilized to investigate and interpret G×E interactions. These advanced methods include univariate linear regression models (Saeidnia *et al.,* 2017a; Saeidnia *et al.,* 2017b; Kumar *et al.,* 2023) as well as multivariate models such as the additive main effects and multiplicative interactions (AMMI) model (Zobel *et al.,* 1988) and the Genotype×GE (GGE) biplot (Yan, 2001). Among the multivariate techniques developed for the analysis of G×E interactions, the AMMI model is particularly effective in describing the adaptive responses of genotypes across different environments, as it accounts for a substantial portion of the G×E sum of squares (Ebdon and Gauch, 2002) and has been deemed superior to both joint linear regression models and principal component analysis (Saeidnia *et al.,* 2021). Ahmadi *et al.* (2012) conducted a study on bread wheat (*Triticum aestivum* L.) and reported that the application of AMMI and GGE biplots enhanced visual comparisons and facilitated the identification of superior genotypes for each specific set of target environments. The results of AMMI analyses are typically presented in common graphical formats known as biplots, which aid in elucidating the patterns of genotypic responses across various locations (Tarakanovas and Ruzgas, 2006).

An essential component of a breeding program is the selection of ecologically adapted and genetically diverse plant materials that are indigenous to the target environments (Johnson *et al.,* 2010). Understanding genotype-environment (GE) interactions can assist breeders in achieving this objective and can also contribute to reducing the costs associated with genotype evaluation by eliminating unnecessary testing locations (Gauch *et al.,* 2008). The primary aims of this study were to (i) visually assess the adaptability and grain yield stability of barley varieties across eight locations utilizing the AMMI method; and (ii) identify

barley varieties that exhibit similar response patterns across all environments while also demonstrating high grain yield, as determined by the selected AMMI model.

MATERIALS AND METHODS

Plant materials and field experiments

The genetic material utilized in this study comprised twenty barley genotypes (see Table 1), which were cultivated across eight locations in Iran (see Table 2). The experimental design was structured as a Randomized Complete Block Design with three replications. At each of the eight locations, the plots consisted of six rows, each measuring 500 cm in length, with a row spacing of 20 cm and an intra-row spacing of 10 cm. Cultural practices, including irrigation, fertilization, and weed control, were consistently implemented each year at all locations. To maintain weed-free plots, manual weed control was conducted as necessary throughout the three-year duration of the study, and no chemical herbicides or toxic fertilizers were employed to manage weed growth. Prior to sowing, seeds were treated with fungicide, and no additional pest control measures were undertaken. The soil was fertilized with 80 kg of nitrogen (N) per

Table 1. Nomenclature and pedigree of the barley genotypes under investigation.

Entry	Parents/Pedigree
1	Behrokh
2	Michailo/Dobrinya//Yousef
3	26216/4/Arar/3/Mari/Aths*2//M-Att-73-337-1/5/Nosrat
4	82S:510/3/Arinar/Aths//DS 29/4/Sahra
5	82S:510/3/Arinar/Aths//DS 29/4/Sahra
6	Kavir/Badia/3/Torsh//9cr.279-07/Bgs/4/Karoon/Kavir/5/Sahra
7	Bgs/Dajia//L.1242/3/(L.B.IRAN/Una8271//Gloria'S'/3/Alm/Una80//....)/4/Sahra
8	Anoidium//Alanda/Hamra-01/3/Lignee527/NK1272//JLB70-63/4/Nik
9	Anoidium//Alanda/Hamra-01/3/Lignee527/NK1272//JLB70-63/4/Nik
10	Arbayan/NK1272/4/Arar/3/Mari/Aths*2//M-Att-73-337-1/5/Yousef
11	Yousef/3 / Dasht//EBC(a)/Badia/4/Nik
12	CANELA/3/HEGEGS679.82/SHYRI//LAUREL/4/CERISE/SHYRI//…/5/MALOUH//Aths/Lignee686/6/Nik
13	Bgs/Dajia//L.1242/3/(L.B.IRAN/Una8271//Gloria'S'/3/Alm/Una80//....)/4/Sahra
14	ZBL-2640/Nosrat
15	Bgs/Dajia//L.1242/3/(L.B.IRAN/Una8271//Gloria'S'/3/Alm/Una80//....)/4/Yousef
16	Beecher/1-BC-80411//1-BC-80593/3/Nik
17	Ashar/Beecher/3/Lignee527/NK1272//JLB70-63
18	BREA/DL70//TOCTE/3/BREA/DL70//CABUYA/4/TRADITION
19	Bgs/Dajia//L.1242/3/(L.B.IRAN/Una8271//Gloria'S'/3/Alm/Una80//....)/4/Sahra
20	MB-97-3

Table 2. Geographical and climatic characteristics of experimental locations.

Location	Latitude	Longitude	Altitude (m)	Average temperature (°C)	Average rainfall (mm)
Karaj	35° 49′ E	50° 58′ N	1300	14.2	256
Neishabor	36° 22′ E	58° 82′ N	1250	13.9	247.4
Varamin	35° 32′ E	51° 65′ N	918	16.9	156
Isfahan	32° 39′ E	51° 40′ N	1765	15.9	140
Birjand	32° 88′ E	59° 22′ N	1491	16.7	129
Mashhad	36° 21′ E	59° 62′ N	1100	14.7	230
Shiraz	29° 46′ E	52° 44′ N	1590	18	150
Yazd	31° 54′ E	54° 16′ N	1215	18.9	55

hectare and 90 kg of phosphorus (P) per hectare before sowing, with an additional application of 70 kg of N per hectare during the early stem elongation stage. Grain yield was assessed for each plot in its entirety across all eight locations and was subsequently utilized for analysis.

Statistical analyses

Prior to conducting the analysis of variance (ANOVA), the Kolmogorov–Smirnov test and Bartlett's test were employed to assess the normality of data distribution and the homogeneity of residual variance, respectively. Following this, a combined analysis of variance was performed to investigate the differences among genotypes, environments, and their interactions, utilizing the Proc MIXED procedure in SAS version 9.4 (SAS Institute, Cary, NC, USA).

Stability analysis utilizing the AMMI model

Following the verification of significant genotype-environment (GE) interactions, an analysis of adaptability and phenotypic stability was conducted utilizing the AMMI method, as described by Zobel *et al.* (1988). The results obtained from the AMMI model analysis were interpreted based on the AMMI1 and AMMI2 biplot analyses. In this context, the main effect of variety was treated as a fixed effect, while the main effect of location was treated as a random effect. The AMMI analysis for the data collected on grain yield was performed using GEA-R version 4.1 (Pacheco *et al.,* 2015).

RESULTS

Analysis of variance and genetic analysis

The results from the combined analysis of variance revealed highly significant differences ($P<0.01$) among the various locations (Table 3). Additionally, the effect of genotype was found to be significant, indicating substantial genotypic variation among the selected genotypes concerning grain yield, with a wide range observed for each trait. The genotype-environment interaction (GEI) effect was also highly significant, accounting for 19.65% of the total variation in grain yield (GY) (Table 3). The significant GE interaction highlights the differential responses of genotypes to environmental variations. The presence of GEI complicates the selection process, as it diminishes the utility of genotypes by reducing the correlation between genotypic and phenotypic values (Saeidnia *et al.,* 2022). Consequently, there is a pressing need for stability analysis.

Stability analysis using AMMI model

The application of the AMMI model for the partitioning of GEI demonstrated that the first four terms of the AMMI model were statistically significant, as determined by an approximate F-statistic. The analysis conducted using the AMMI4 model accounted for 86% of the total sum of squares associated with GEI. Specifically, the first four interaction principal component axes (IPCA1 to IPCA4) were identified as highly significant factors, explaining 31.08%, 26.74%, 16.00%, and 12.18% of the GE sum of squares, respectively (Table 1). The results of the AMMI analysis indicated significant variations among environments (locations), genotypes, and the interaction effects between environment and genotype. These findings underscore the influence of environmental factors on GE interaction, highlight the genetic variability present among the genotypes, and suggest the potential for selecting stable genotypes. The model revealed that 75.89% of the total sum of squares could be attributed to environmental effects, while only 4.46% of the variations were due to genotypic effects, and 19.65% were attributable to GEI effects (Table 4). The substantial sum of squares associated with environmental factors suggests a high degree of diversity among the locations studied. Furthermore, the considerable GEI relative to genotypic effects implies the potential existence of distinct mega-environments, each characterized by different top-yielding genotypes (Yan, 2024).

The IPCA1 scores for both genotypes and environments (locations) are illustrated in relation to the main effects, specifically the genotype mean and environment mean (see Figure 1). According to the AMMI model, genotypes exhibiting high mean performance (exceeding the mean yield) and

Table 3. Combine analysis of variance for grain yield across twenty barley genotypes in various locations over two years (2020-2022).

Source of variation	df	Sum of square	Mean of square
Year (Y)	1	3.52	0.11^{ns}
Location (L)	7	1069	4.89**
Y×L	7	218.83	15.5**
E1	32	64.58	-
Genotype (G)	19	83.34	3.13**
G×L	133	209.5	1.12^{ns}
G×Y	19	16.79	0.63^{ns}
G×Y×L	133	186.61	2.67**
E2	608	320	-
Total	959	2172.6	-

**: Statistical significance at the 0.01 probability level, whereas n.s indicates not significant.

Table 4. Results of the additive main effects and multiplicative interaction (AMMI) analysis of variance for grain yield among twenty barley genotypes evaluated across eight distinct locations.

Source of variation	df	Sum of square	Mean of square	Total variation (%)
Environment (E)	7	775.62	110.80**	75.89
Genotype (G)	19	45.53	2.40**	4.46
G×E	133	200.86	1.51**	19.65
PC1	25	62.44	2.50**	31.09
PC2	23	53.70	2.33**	26.74
PC3	21	32.14	1.53**	16.00
PC4	19	24.47	1.29**	12.18
Noise	56	28.10	0.50^{ns}	13.99
Residual	320	196.34	0.61^{ns}	0.00

**: Statistical significance at the 0.01 probability level, whereas n.s indicates not significant.
df: Degree of freedom, SS: Sum of squares, MS: Mean squares, PC: Principal component.

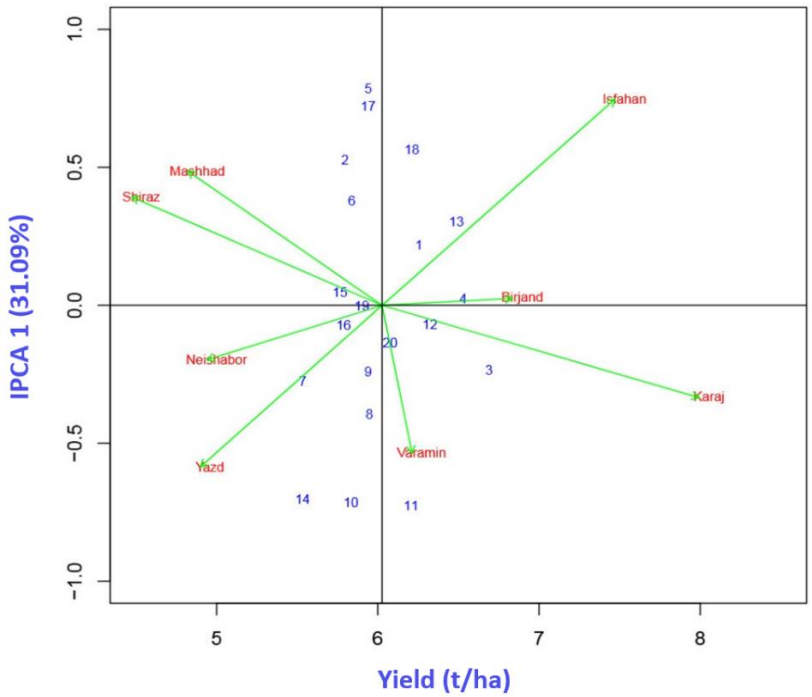

Figure 1. AMMI1 biplot displaying the main effects against the first principal component axis of interaction (IPCA1) for the grain yield of twenty genotypes of barley across eight locations.

IPCA scores approaching zero demonstrate general adaptability across all environments. Conversely, genotypes with high mean performance and substantial IPCA scores are indicative of specific adaptation to particular environments (Ebdon and Gauch, 2002). The AMMI1 graph reveals that, irrespective of the direction of the IPCA1 scores, several genotypes evaluated in this study—including genotypes 1, 3, 4, 12, 13, and 20—exhibited generally high yields with IPCA1 scores close to zero (refer to Figure 1 and Table 5). Consequently, these genotypes are characterized as generally stable and minimally influenced by GE interaction. Among these, genotypes 1, 4, and 13 demonstrated positive interactions with the environments of Birjand, Isfahan, Mashhad, and Shiraz, while genotypes 3, 12, and 20 positively interacted with the environments of Karaj, Neishabor, Varamin, and Yazd, as evidenced by their interaction scores sharing similar signs (Saeidnia *et al.,* 2017a; Saeidnia *et al.,* 2017b). In contrast, genotypes 5, 10, 14, and 17 exhibited grain yields below the grand mean and the highest IPCA1 scores, indicating that these genotypes are the most unstable, yet they are recognized as specifically adapted to certain locations. Additionally, genotypes 15, 16, and 19 displayed

relatively low IPCA1 scores and can be classified as moderately stable genotypes (Figure 1 and Table 5).

Among the environments examined in this study, Isfahan and Karaj exhibited the most significant contribution to the GE interaction, as evidenced by their attainment of the highest scores on the first principal component axis (IPCA1) and their positioning far from the origin in the AMMI1 biplot. Conversely, the locations of Birjand, Neishabor, and Varamin recorded the lowest IPCA1 scores, indicating a minimal contribution to the GE interaction (Figure 1). These environments demonstrated IPCA1 scores that were close to zero, resulting in a small and negligible interaction effect; thus, they were identified as the most stable locations. This finding suggests that all genotypes performed consistently well in these environments.

Figure 2 presents the AMMI-2 biplot, illustrating the first and second Interaction Principal Component Axes (IPCA1 and IPCA2) in relation to grain yield. This biplot facilitates a comparison of the relative magnitude and direction of the GE interaction as influenced by each genotype and environment. In this graphical representation, genotypes and environments positioned near the origin are deemed the most stable, indicating minimal contribution to the GE interaction. Conversely, those located further from the origin exhibit sensitivity and significant interaction effects. The AMMI-2 biplot accounts for 57.83% of the total sum of squares attributable to the GE interaction for grain yield (Figure 2). The analysis reveals that genotypes 4, 6, 7, 8, 12, 13, 16, and 19 are clustered near the origin, signifying their stability. Among these, genotypes 4 and 13 are identified as stable genotypes with commendable performance. In contrast, genotypes 1, 5, 10, 11, 14, and 17 are situated further from the origin, exhibiting pronounced interactive behavior. Consequently, these genotypes are classified as unstable and are recognized as being particularly adapted to specific environments (Figure 2).

Table 5. Name, mean grain yield (t. ha^{-1}), and IPCAs scores for twenty barley genotypes across eight locations analyzed for genotype×environment interaction in grain yield using the AMMI model.

Type	Name	Grain yield	IPCA1	IPCA2
Genotype	G1	6.254	0.219	1
Genotype	G2	5.834	-0.715	-0.092
Genotype	G3	6.207	-0.725	-0.114
Genotype	G4	6.324	-0.068	-0.195
Genotype	G5	6.487	0.304	-0.242
Genotype	G6	5.533	-0.702	0.599
Genotype	G7	5.765	0.047	0.516
Genotype	G8	5.788	-0.072	0.040
Genotype	G9	5.940	0.721	0.009
Genotype	G10	6.211	0.563	0.011
Genotype	G11	5.900	-0.002	-0.125
Genotype	G12	5.796	0.528	0.339
Genotype	G13	6.075	-0.137	-0.807
Genotype	G14	6.690	-0.234	-0.825
Genotype	G15	6.529	0.025	0.079
Genotype	G16	5.941	0.785	-0.272
Genotype	G17	5.838	0.377	-0.245
Genotype	G18	5.534	-0.276	-0.288
Genotype	G19	5.947	-0.395	0.209
Genotype	G20	5.939	-0.241	0.402
Location	Karaj	6.907	0.025	-0.073
Location	Neishabor	7.542	0.748	0.285
Location	Varamin	8.065	-0.335	-0.043
Location	Isfahan	4.892	0.487	-1
Location	Birjand	5.011	-0.197	0.185
Location	Mashhad	4.544	0.393	0.694
Location	Shiraz	6.283	-0.535	-0.045
Location	Yazd	4.970	-0.585	-0.002

AMMI: Additive main effects and multiplicative interaction, IPCA: Interaction principal component axis, IPCA1: First interaction principal component axis score, IPCA2: Second interaction principal component axis score.

DISCUSSION

The present study identified substantial genetic variation among barley genotypes concerning grain yield, indicating a significant genetic potential for enhancing this trait through targeted selection in breeding programs. This variation allows for the selection of genotypes exhibiting diverse grain yield values and stability. The pronounced genotype-environment (GE) interaction revealed that the relative performance of genotypes varied across different locations. Consequently, it was imperative to calculate phenotypic stability. The GE effect was approximately four times greater than the genetic (G) effect in the total variation, suggesting the potential existence of distinct mega-environments characterized by different top-yielding genotypes. This finding underscores the necessity for conducting stability analyses (Saeidnia *et al.,* 2022).

Accurate estimation of the magnitude and relative contributions of various components of genetic variance is essential for understanding the underlying gene action that governs traits of interest. The GEI presents a significant and complex challenge for plant breeders, geneticists, and agronomists involved in performance testing, as it can introduce biases in these estimates (Comstock and Moll, 1963). Such biases may result

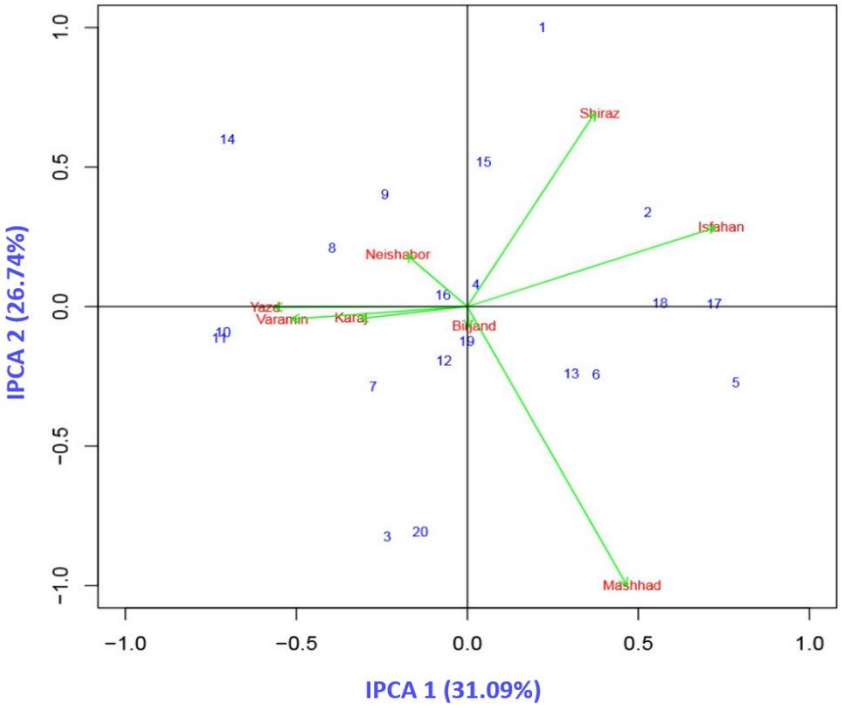

Figure 2. AMMI 2 biplot (IPCA1 vs. IPCA2) for the grain yield of twenty barley genotypes evaluated across eight locations (environments).

in inappropriate selections of breeding methods and hinder the development of new varieties (Bantayehu, 2010). Consequently, researchers in crop breeding are continually seeking genotypes with high yield potential and minimal GE interactions. Evaluating genotypes across multiple environments is beneficial for assessing their performance. Furthermore, conducting G×E analyses enhances the selection process for genotypes intended for specific target environments (Hassani *et al.,* 2018; Saeidnia *et al.,* 2021).

The AMMI analysis revealed that environmental factors were the predominant source of variability, highlighting significant differences among environmental means that contribute to variations in grain yield. A substantial portion of the total sum of squares was attributed to GEI, indicating pronounced differences in the genotypic responses of barley genotypes across various environments (Brar *et al.,* 2012). Consequently, it is pertinent to proceed with the estimation of phenotypic stability. In this study, the AMMI model illustrated the existence of GE interactions, which were further partitioned among the IPCA and residual noise. Through principal component analysis, it was demonstrated that a significant percentage of the GE sum of squares was accounted for by the first four interaction principal components. Specifically, these four IPCAs explained 86% of the variation in the total GEI sum of squares, aligning with the findings of Gauch (1993) and Purchase *et al.* (2000). However, this observation contrasts with the conclusions drawn by Yan (2024), who suggested that the most accurate AMMI model could be predicted using only the first two IPCAs. These findings underscore that the number of terms to be incorporated into an AMMI model cannot be predetermined without conducting a predictive assessment (Kaya *et al.,* 2002). Generally, factors such as the type of crop, the diversity of the germplasm, and the range of environmental conditions significantly influence the complexity of the optimal predictive model (Saeidnia *et al.,* 2023).

Differences in stability and adaptability to various environments can be qualitatively evaluated through the biplot graphical representation, which displays genotypes based on their principal component values (Vita *et al.,* 2010). Furthermore, the AMMI1 biplot serves as a diagnostic tool for identifying patterns of genotype-environment (GE) interaction. By employing pattern analysis within the AMMI model, genotypes and environments exhibiting similar responses are grouped together (Pourdad and Mohammadi, 2008). The findings from the AMMI2 biplot analysis categorized the varieties into three distinct groups. The first group, characterized by moderately high yield and stability, is considered highly desirable and comprises

genotypes 1, 3, 4, 12, 13, and 20, with genotypes 4 and 12 demonstrating greater stability than the others. The second group, which exhibits high yield but low stability, includes genotypes 5, 10, 14, and 17. The third group, identified as having low yield but high stability, which can be classified as moderately stable genotypes, consists of genotypes 15, 16, and 19. Consequently, the results of the AMMI2 biplot analysis indicate that among the twenty evaluated genotypes, only genotypes 4 and 12, which performed above the average yield, were broadly adapted, as evidenced by their IPCA scores being close to zero. Generally, environments with scores near zero exhibit minimal interaction across genotypes and provide limited discrimination among them (Anandan *et al.,* 2009). In the current study, this pattern was observed for the locations of Birjand, Neishabor, and Varamin, as they recorded lower scores and contributed minimally to the GE interaction.

In conclusion, the observed considerable and significant differences among the genotypes regarding grain yield reflect the genetic variability present among the entries. This variability suggests that it is feasible to identify the most desirable genotypes for each location, characterized by high stability and performance, through targeted selection and the AMMI model. Conversely, given the significance of the G×E interactions, the selection of superior genotypes for performance development should be conducted based on multi-environment trials. The first four interaction principal components collectively accounted for more than 86% of the total GEI for the grain yield of barley genotypes. Analysis of barley genotypes using the AMMI model revealed that certain genotypes (specifically, genotypes 4 and 12) exhibited both high yield and greater stability compared to others. In contrast, genotypes 5, 10, 14, and 17 demonstrated grain yields below the grand mean and the highest IPCA1 scores, indicating that these genotypes were the most unstable, yet they were identified as being particularly well-adapted to specific locations. Therefore, crosses between contrasting genotypes may represent a viable strategy for developing mapping populations aimed at genomic studies of stability and grain yield in this species. To validate the findings of this study and to enhance the understanding of genotype-environment interactions, future research should focus on AMMI analyses conducted across various locations over multiple years.

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