



Research article

Analysis of genotype-by-environment interaction effect in barely genotypes using AMMI and GGE biplot methods

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ABSTRACT

Genotype-by-environment interaction (GEI) analysis play a key role in any breeding program involving the development of new varieties for cultivation across various environments or in a specific region. The additive main effects and multiplicative interaction (AMMI) method and the GGE biplot are the two main statistical tools that have emerged to analyze GEI in multi-environment trials (METs). The main goal of the present study was to identify the best-performing and stable barley genotypes for the warm regions of Iran. For this purpose, 18 new advanced barley genotypes were investigated in five warm locations in Iran during two cropping seasons (2021–2023). In all experiments, test genotypes were evaluated in a randomized complete block design (RCBD) with three replications. Based on results, grain yield was significantly dependent on environments (E), genotypes (G), and GEI. The GEI effect was further divided into three principal component axes (IPCA). The AMMI method identified genotypes G3, G9, G10, and G14 as ideal genotypes due to their low IPCA scores and high performances. In the GGE biplot analysis, the initial two PCAs accounted for 49.36 % of the total variation of grain yield, including both G and GEI effects. Based on averaged two-year data, genotypes G3, G4, G10, and G14 showed particular adaptability in the Zabol and Moghan regions. Moreover, the ranking of test environments showed good discriminatory and representative abilities for the Zabol and Moghan regions, so these environments constituted a mega-environment in Iran's warm climate. The genotype ranking indicated G3, G10 and G14 genotypes as the superior genotypes with the highest grain yield and stability in different test environments. Moreover, these results were confirmed by the results obtained by WAASB and WAASBY biplots. In conclusion, genotypes G3, G10 and G14 can be suggested for commercial usage and cultivation in various regions in Iran's warm climate.

1. Introduction

Barley (*Hordeum vulgare* L.) is a cereal crop plant and one of the oldest edible crops, having been domesticated nearly 11,000 years

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ago. This cereal is best adapted to production in different climates due to its high level of adaptability and tolerance to various environmental stresses [1]. Global barley production in 2021–2022 was estimated at about 145 million tons, ranking fourth after wheat, rice and maize [2]. This cereal is a well-adapted and significant crop in Iran, and its average annual grain production and harvested area are ~3 million tons per hectares and over 2 million hectares in 2021, respectively [2]. Barley's seed has versatile uses in the feed and food industry due to the presence of various essential minerals such as calcium and phosphorus, moderate amounts of protein, small amounts of vitamins, and dietary fiber. Moreover, the seed's high carbohydrate content has made it used in bread, stews, soups, and other foods. Consequently, these healthy benefits make barley considered an ideal source of feed and food for animals and humans [3]. Since it is cultivated in a wide range of regions of the country with different environmental conditions, this heterogeneity of environments will lead to occurrence of the GEI [4–7]. The GEI hinders the selection of high-yielding and stable genotypes, and introduces ideal genotypes for specific regions [8]. Therefore, to reduce the dramatic and negative impact of GEI on the efficiency of breeding programs, a comprehensive knowledge of the GEI can provide ideal opportunities for breeders and agronomists to increase the accuracy of selection in their breeding programs.

Numerous statistical models and methods have been proposed and developed to decipher GEI effects in a MET. Among them, the additive main effects and multiplicative interaction (AMMI) [9] and genotype (G) + genotype × environment (GGE) biplot [10] are two useful methods to analyze the impact of GEI effects. Both statistical methods are based on principal components analysis (PCA) that provides a particular way to assess the relationships between environments, genotypes, and genotypes with environments. Moreover, both methods can display a graphical view of the results, which in turn results in a better understanding of the response of test genotypes in different environments [11]. However, the main disparities between these methods can be attributed to some additional features of the GGE biplot model, such as the ranking of genotypes and environments, the “which-won-where” pattern, mean vs. stability, as well as, discriminatory power and representativeness ability of test environments. Nevertheless, both methods are complementary and allow breeders to comprehend GEI effects and identify best genotypes as well as the suitable environments. Therefore, deciphering GEI effects is one of the primary goals in determining stability and adaptation of genotypes [12]. To date, these methods have widely used for identification of high-yielding and stable genotypes in METs, and many studies have also reported their usefulness [4,12–22]. For example, Vaezi et al. [13] used the AMMI and GGE biplot method in identification of superior barley genotypes for semi-tropical regions of Iran. In a study conducted by Pour-Aboughadareh et al. [20], several promising genotypes of barley were identified superior genotypes in the cold regions of Iran. Another important advantage of GGE biplot analysis is the determination of mega-environments. Indeed, this objective helps breeders and agronomists improve breeding programs through the management of resource use, time, efficiency of selection, etc. Previously, several studies reported this method's suitable efficiency in identifying ideal and mega-environments in barley and other crop plants [17,18,20].

Table 1

The passport of studied genotypes of barley and test environments.

Code	Pedigree				
G1	Oxin [Reference]				
G2	Golchin [Reference]				
G3	Rojo/3/LB.IRAN/Una8271//Gloria"S"/Com"S"/4/Briges				
G4	Rojo/3/LB.IRAN/Una8271//Gloria"S"/Com"S"/4/Rihane-03				
G5	Zarjau/80-5151//OK84817ICBH94-0402-0AP-0AP-17AP-0AP-12AP-11AP-0AP-0TR-0 TR-0AREC				
G6	Zarjau/80-5151//OK84817ICBH94-0402-0AP-0AP-17AP-0AP-12AP-16AP-0AP-0TR-0 TR-0AREC				
G7	Lignee 527/NK1272//JLB 70-63/3/Rhn-03//Lignee527/As45				
G8	KAROON/KAVIR//Rhodes'S'//Tb/Chzo/3/Gloria'S'/4/Sahra/5/Yousef				
G9	Anoidium/Arbayan-01/3/Lignee527/NK1272//JLB70-63/4/Beecher				
G10	Anoidium/Arbayan-01/3/Lignee527/NK1272//JLB70-63/4/Bgs/Dujia//L.1242				
G11	Bgs/Dajia//L.1242/3/(L.B.IRAN/Una8271//Gloria'S'/3/Alm/Una80// ...)/4/Nosrat/5/Rhn-03//L.527/NK1272				
G12	CIRU/TOCTE				
G13	Courlis/Rhn-03//Jonoob				
G14	Zahak/4/Bgs/Dajia//L.1242/3/L.B.IRAN/Una8271//Gloria'S'/3/Alm/Una80// ...)/4/Rojo ...				
G15	Rojo/3/LB.IRAN/Una8271//Gloria"S"/Com"S"/4/Gloria'S'/Copal'S'//As46/Aths/3/Rhn-03				
G16	Rojo/3/LB.IRAN/Una8271//Gloria"S"/Com"S"/4/Anoidium/Arbayan-01/3/Lignee527/ ...				
G17	Merzaga(Orge077)/Alanda-01 ICB98-0908-0AP-13AP-0AP-3TR-10AP-0AP-0TR-0 TR				
G18	PENCO/CHEVRON-BAR/6/P.STO/3/LBIRAN,UNA80//LIGNEE640/4/BLLU/5/PETUNIA 1				
G19	(Salt-4)LB.Iran/Una 8271//Gloria"S"/Come"s"-11M/3/Kavir/4/Karoon				
G20	W-98-10				
No.	Environments				
	Location	Year	Coordinates	Geographical position	Climate condition
1	Ahvaz	2021–2022	31° 19' 13" N 48° 40' 09" E	South	Warm and dry
2		2022–2023			
3	Darab	2021–2022	28° 45' 07" N 54° 32' 40" E	South	Warm and dry
4		2022–2023			
5	Gonbad	2021–2022	31° 01' 43" N 61° 30' 04" E	Northeast	Warm and humid
6		2022–2023			
7	Zabol	2021–2022	37° 15' 00" N 55° 10' 02" E	Southeast	Warm and dry
8		2022–2023			
9	Moghan	2021–2022	39° 38' 54" N 47° 55' 03" E	Northwest	Warm and humid
10		2022–2023			

In addition to aforementioned methods, the best linear unbiased predictions (BLUP) is also used for GEI analysis in METs. The BLUP model predicted the performance of genotypes in a mixed model with high efficiency [23]. In recent years, Olivoto et al. [24] introduced two models to overcome the weakness of the AMMI model and integrate its functions with the BLUP model. Indeed, the weighted average of absolute scores from the SDV of the BLUP matrix for the GEI effects generated by the linear mixed-effect model (WAASB) and the WAASB plus response variable (WAASBY) help researchers make the better decisions when the selection should take into account different weights of average performance and stability. In these methods, test genotypes are grouped into four quadrants and clusters, and evaluated for adaptability concepts. Previously, Ahakpaz et al. [14] evaluated the response of several barley genotypes to rainy conditions using WAASB and WAASBY and noted the high efficiency of these indices in identifying superior genotypes in terms of performance and stability. In addition, a study by Pour-Aboughadareh et al. [19] identified several superior barley genotypes using the WAASB index for cultivation in warm regions of Iran. Rajput et al. [25] used the WAASB index to select stable sources of soybean resistance in India. In the present study, MET was carried out on 18 newly promising barley genotypes to evaluate grain yield and stability. The experimental data were subjected to analyze the GEI effect using AMMI and GGE biplot methods to identify high-yielding and stable genotypes. Moreover, identifying ideal environments was another objective of the present study.

2. Materials and methods

2.1. Plant materials and field trials

In the present study, a set of barley genotypes including 18 newly promising genotypes along with two local varieties as reference controls (cv. Golchin and cv. Oxin) were evaluated in a MET. The pedigree of the genetic materials is shown in Table 1. High yield and stability are two main characteristics of the reference genotypes. In addition, these genotypes have general adaptability in the warm regions of Iran. Moreover, all test genotypes were selected based on their grain yield and other agronomic characteristics during various breeding cycles such as preliminary barley screening nursery (PBSN), preliminary regional barley yield trial (PBYT), and advanced regional barley yield trial (ABYT).

Experiments were conducted in five geographical regions in Iran's warm climate, which included Ahvaz (E1 and E2), Darab (E3 and E4), Gonbad (E5 and E6), Zabol (E7 and E8) and Moghan (E9 and E10) for two consecutive crop years (2021–2022 and 2022–2023) (Table 1). Among these regions, Moghan and Gonbad are located in the northern part of the country and have warm and humid conditions, while Darab, Zabol and Ahvaz are located in the southern part of Iran and have warm and dry conditions. Additional weather information is shown in Supplementary Table S1. The same seed source was used for all experiments, which consisted of three replications arranged in a randomized complete block design. Seeding density was set as 350 seeds per square meter (150 kg ha^{-1}), and planting was done using a small planter (Wintersteiger, Ried, Austria). At all locations, seed sowing was done on 10–15 November. The number of irrigations was one time in autumn. In spring season, five irrigations were done in the different growth and development stages such as stem elongation (ZGS 31), booting (ZGS 41), anthesis (ZGS 69), mild development (ZGS 71), and dough development (ZGS 83). Experimental plots consisted of six rows with 5 m long and 0.2-m spacing between rows. Cultural practices representative of each region were used. Fertilizers such as nitrogen (N) and phosphorus (P_2O_5) at 32 and 100 kg ha^{-1} , respectively, were applied to all experimental sites before planting. In stem elongation phase, 40 kg ha^{-1} of N was applied. Pumasuper and Granstar herbicides were used to control narrow- and broadleaf weeds at the tillering stage. At the harvest time, all experimental plots were harvested using a small grain harvester (Wintersteiger, Ried, Austria) after removing border effects. The net plot size for each genotype was estimated as 6 m^2 . Grain yield data were determined based on tons ha^{-1} for each.

2.2. Statistical analysis

The performance of the studied genotypes in five regions and two years was subjected to the AMMI analysis based on the following model [26]:

$$\mu_{ij} = \mu + \alpha_i + \tau_j + \sum_{k=1}^p \lambda_k \alpha_{ik} t_{jk} + \rho_{ij} + \varepsilon_{ij}$$

where, α_i is the additive effects of genotype, τ_j is the environmental mean, λ_k is the singular value for the k th interaction principal component axis (IPCA), t_{jk} is the j th element of the k th eigenvector, and α_{ik} is the i th element of the k th eigenvector. A residual ρ_{ij} remains if not all p IPCA are used, where $p \leq \min(g-1; e-1)$. Moreover, the combined analysis of variance (ANOVA) was computed based on grain yield data across investigated genotypes. The GGE biplot method was used to study the following patterns: (1) discriminativeness power and representativeness ability of test environments, (2) 'which-won-where' pattern of GGE, (3) ranking of genotypes tested across all environments and ranking test environments. To combine the features of AMMI and BLUP methods, the WAASB and WAASBY indices were calculated. All statistical analyses were computed in R software [27] using packages 'metan' [28].

3. Results

3.1. Combined analysis of variance (ANOVA) and the AMMI model

The result of combined ANOVA showed the significant effect for environments (E), genotypes (G), and their interaction (GEI) (Table 2). The coefficient of variation (CV) for grain yield across test environments was estimated as 14.22 %, suggesting a high level of

yield variability among test genotypes in response to environmental conditions. The result of AMMI analysis based on grain yield data showed a significant influence of E, G, and GEI (Table 3). Among the sources of variation, the largest proportion of the total variation was belonged to the E (87.40 %), whereas GEI and G accounted for 10.47 % and 2.13 %, respectively. Moreover, the GEI effect was further divided into three significant interaction principal component axes (IPCA). The proportion of variation in IPCA1, IPCA2 and IPCA3 were 31.83 %, 24.80 % and 12.36 %, respectively. The average grain yield of the tested genotypes in different test environments ranged from 4.13 (corresponding to G18) to 5.03 tons ha⁻¹ (corresponding to G14) with an average of 4.56 tons ha⁻¹ (Supplementary Table S2). All genotypes except G6, G8, G12, G18, G19, and G20 showed a grain yield equal to or higher than the average. The environmental means varied between 1.69 (environments E7 (Zabol-first year)) and 5.61 (E4 (Darab-second year)) tons ha⁻¹. The AMMI-1 biplot showed the distribution pattern of the studied genotypes based on IPCA1 scores and grain yield (Fig. 1A). Based on this biplot, genotypes G1, G3, G4, G5, G9, G10, G14 and G17 showed higher grain yield compared to the grand mean (4.56 tons ha⁻¹). Of these, G9, G10, and G17 showed IPCA1 scores closer to zero; therefore, they were selected as superior genotypes with the most stability rate.

The AMMI 2 biplot (Fig. 1B) showed the importance of the first two IPCA scores in clarifying the complexity of the GEI effect and determining genotypes adaptation. Based on this biplot, genotypes and environments close to the beginning of the biplot indicate the lowest effects on the GEI, and vice versa. Accordingly, environments E2 (Ahvaz-second year), E4 (Darab-second year), E9 (Moghan-first year), and E10 (Moghan-second year) showed a significant impact on GEI. On the other hand, genotypes G8, G11, G17 and G19 with the lowest IPCA scores showed broad stability in all testing environments. The recommended four top-ranked genotypes using the AMMI model in different test environments are shown in Table 3. Based on results, genotype G14 ranked first in environments Zabol (E7 and E8), E1 (Ahvaz-first year), and E9 (Moghan-first year), as well as appeared in six of the ten environments. Genotype G9 ranked first in environment E6 (Gonbad-second year) and five of the ten environments in the top four ranking. Other results are shown in Table 4.

3.2. GGE biplot analysis

The results of the GGE biplot analysis are summarized in four biplots (Figs. 2–5). The viewpoint of ‘Discriminateness power vs. Representativeness ability’ classified test environments into three types. The main discriminator parameters are determined by the length of the environment vector and the angle between the environment vectors with the average environment coordinate axis (AEC) [10]. Environments with short vectors are placed in Type I; these environments provide little information about differences between genotypes and are therefore not suitable for genotype evaluation. Type II environments are discriminated from other environments by long vectors and small angles to the AEC axis. These environments are suitable for identifying high-yielding and stable genotypes. Type III environments have long vectors and large angles to the AEC axis; therefore, like Type I environments, these environments have low efficiency in evaluating genotypes. Accordingly, environments E9 and E10 (Moghan) and E8 (Zabol-second year) have slightly smaller angles to the AEC axis than other environments and have been characterized as Type II. Type I environments included Darab (E3 and E4), Gonbad (E5 and E6) and E7 (Zabol-first year) (Fig. 2A). Other environments were grouped as Type III environments. In addition, based on averaged data, Zabol (E4) and Moghan (E5) showed good discriminatory potential and representative ability (Fig. 2B). The GGE analysis scoring polygon based on ten test environments showed that all environments were divided into four main sectors. The first sector included Zabol (E7 and E8), E1 (Ahvaz-first year) and E9 (Moghan-first year) environments with G14 as the top genotype. The second sector included E3 (Darab-first year) with G9 as apex genotype. The Gonbad environments (E5 and E6) along with E4 (Darab-second year) were placed in the third sector, and the winning genotype for these environments was G7. Environments E2 (Ahvaz-second year) and E10 (Moghan-second year), along with genotypes G15 and G16, were grouped in the fourth sector. Three main sectors were identified based on the averaged data from the two years. The first sector consisted of the Ahvaz region, with the G15 genotype showing specific adaptability for it, while the second sector included the Zabol (E4) and Moghan (E5) regions with the winning G14 genotype. The third sector included Darab and Gonbad environments without any winning genotypes (Fig. 3B). In general, genotypes G14 and G15 revealed specific adaptability in the Zabol/Moghan and Ahvaz locations, respectively.

Fig. 4A shows the “mean vs. stability” view of the GGE biplot. According to this biplot, genotypes G14, G4, G15, G13, G3, G10, G20 and G17 showed the highest average grain yield in different test environments. Of these, G15 and G20 showed yields similar (4.48 and 4.45 tons ha⁻¹, respectively) to the grand mean (4.56 tons ha⁻¹). Moreover, genotypes G4 followed by G14, G3 and G15 with high

Table 2

The results of AMMI model for grain yield in promising genotypes of barley across different regions in the warm climate of Iran during 2021–2023 years.

Source of variation	df	SS	MS	F	Probability	TSS (%)
Environment (E)	9	1033.8	114.86	273.14	**	
Replication (E)	20	24.1	1.20	2.86	**	
Genotype (G)	19	25.2	1.33	3.16	**	
G × E Interaction	171	123.8	0.724	1.72	**	
Residual	380	159.8	0.42			
CV	14.22 %					

* and ** Significant at $P < 0.01$, respectively. SOV, df, SS, MS, and TTS indicate source of variation, degree of freedom, sum of square, mean sum of square, and the total sum of square, respectively.

Table 3

The results of AMMI model for the studied genotypes of barley across different environments in the warm regions of Iran.

S.O.V	df	SS	MS	F	Probability	TSS (%)
Total	599	1366.7	2.28			
Treatments	199	1182.9	5.94	14.13		
Genotypes	19	25.2	1.33	3.16	**	2.13
Environments	9	1033.8	114.87	95.51	**	87.40
Block	20	24.1	1.2	2.86		
Interactions	171	123.8	0.72	1.72	**	10.47
IPCA1	27	39.4	1.46	3.47	**	31.83
IPCA2	25	30.7	1.23	2.92	**	24.80
IPCA3	23	15.3	0.66	1.58	*	12.36
Residuals	96	38.5	0.4	0.95		
Error	380	159.8	0.42			

* and ** significant at $P < 0.05$ and $P < 0.01$, respectively. SOV, df, SS, MS, and TTS indicate source of variation, degree of freedom, sum of square, mean sum of square, and the total sum of square, respectively.

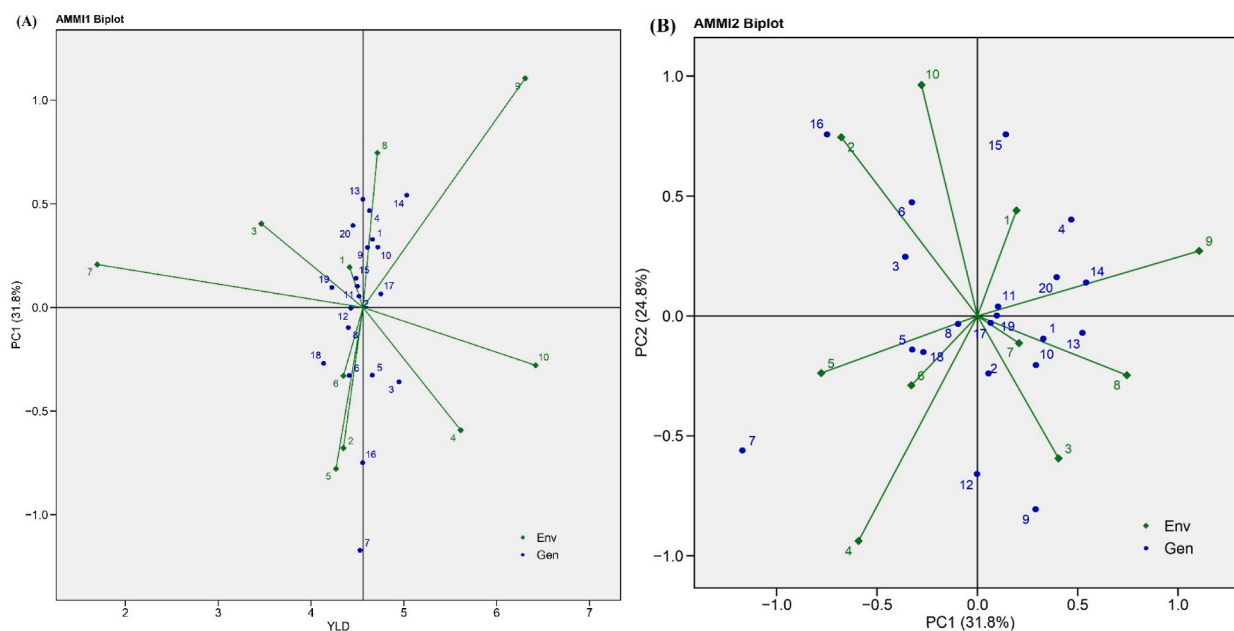


Fig. 1. The AMMI1 (A) and AMMI2 (B) biplots indicating the GEI for the studied genotypes of barley across ten test environments in the warm climate of Iran.

Table 4

First four ranked genotypes identified using the AMMI model.

Environments	Legend	Grain yield	Rank 1	Rank 2	Rank 3	Rank 4
Ahvaz	E1	4.416	G14	G16	G9	G5
	E2	4.349	G3	G16	G7	G15
Darab	E3	3.464	G1	G3	G10	G12
	E4	5.613	G7	G9	G12	G5
Gonbad	E5	4.269	G7	G3	G16	G5
	E6	4.35	G9	G7	G14	G5
Zabol	E7	1.698	G14	G9	G10	G17
	E8	4.715	G14	G9	G13	G10
Moghan	E9	6.308	G14	G4	G1	G13
	E10	6.42	G16	G3	G15	G14

grain yields showed the greatest stability due to their position in the biplot (Fig. 4A). The result of averaging two-year data confirmed this result. As shown in Fig. 4B, genotypes G14, G10, G13 and G3 with high grain yield and the smallest distance from to AEC axis were identified as stable genotypes. As a result of the ideal viewpoint of GGE biplot, the ranking of genotypes was in order of genotype G14 >

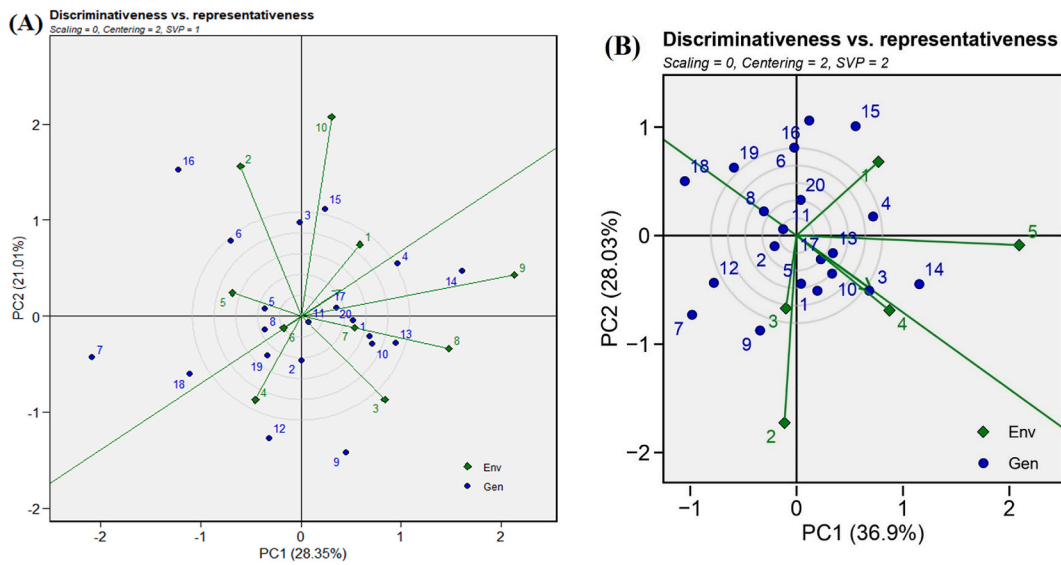


Fig. 2. A view of the ‘discriminating power and representativeness’ of the GGE biplot based on ten test environments (A) and averaged two-year data (B).

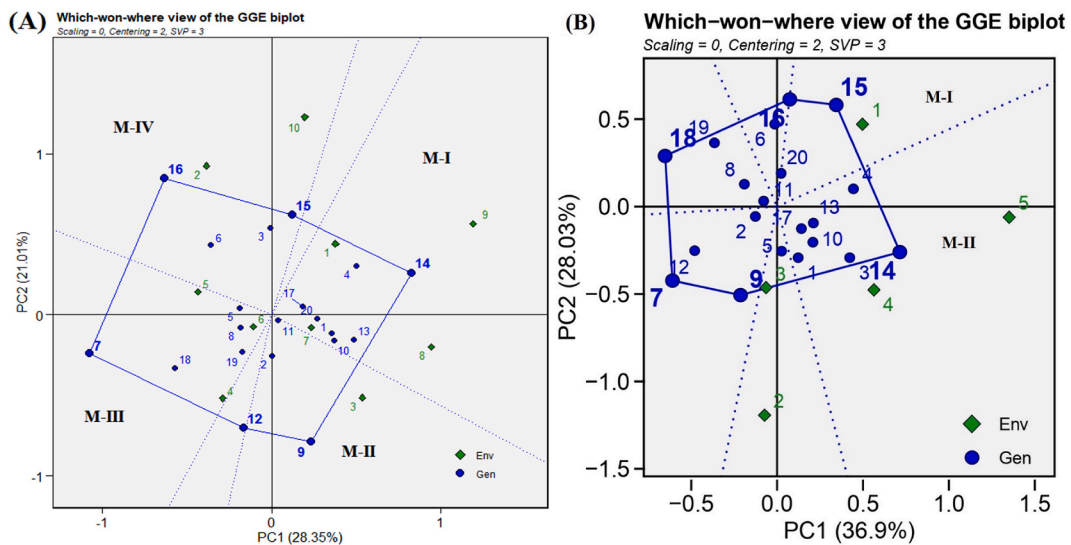


Fig. 3. View of the ‘which-won-where’ biplot of winning genotypes for grain yield in each sector based on ten test environments (A) and averaged two-year data (B).

G4 > G13 > G2 > G15 > G17 > G1 > other genotypes. Besides, test environments E1 (Ahvaz-first year), Zabol (E 7 and E8) and Moghan (E9 and E10) were identified as ideal environments. Based on the averaged data, genotype G14 was identified as ideal genotype. Moreover, the Zabol region was also identified as an ideal environment than other regions (Fig. 5B).

3.3. Genotype ranking using WAASB and WAASBY indices

To better illustrate the ranking pattern of the genotypes studied, the WAASB index was determined. The most important application of this index relates to the creation of a biplot using grain yield compared to the WAASB index. In this biplot, all entries were divided into four quadrants (Fig. 6A). The first quadrant (I) consisted of the most volatile genotypes with lower grain yield than the main average. In other words, these genotypes have the highest contribution to GEI. In addition, environments with high discrimination ability are located in this quadrant. The second quadrant (II) consists of unstable but highly productive genotypes. Moreover, environments with good genotype discrimination ability are distributed in this quadrant. Genotypes with low productivity but wide adaptation are placed in the third quadrant (III). Moreover, environments existing in this quadrant have low discrimination ability. The

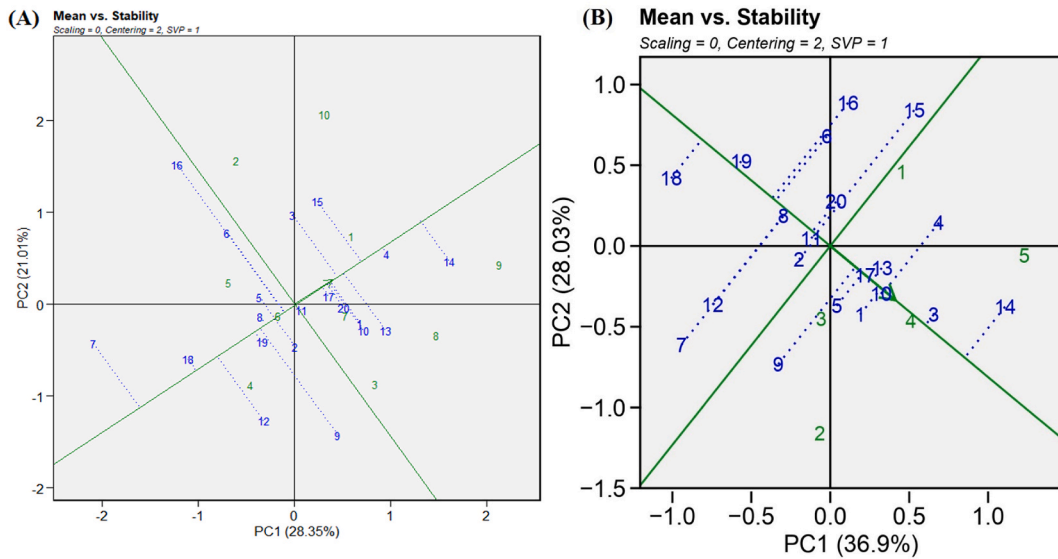


Fig. 4. Biplot for simultaneous selection of grain yield and stability of barley genotypes tested based on ten test environments (A) and averaged two-year data (B).

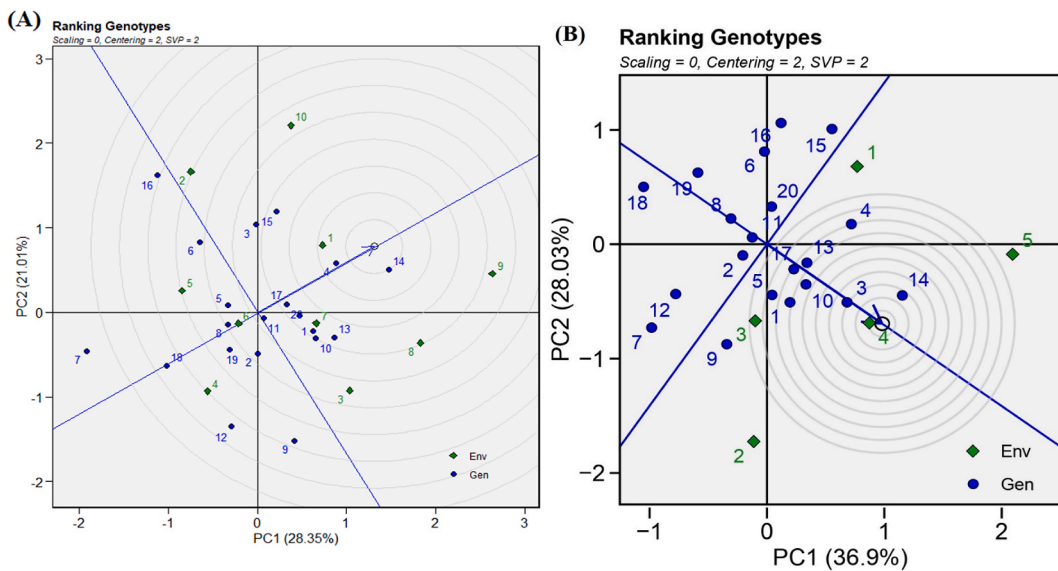


Fig. 5. Comparison of promising barley genotypes in terms of grain yield and stability based on ten test environments (A) and averaged two-year data (B).

fourth quadrant (IV) is more important, and genotypes belonging to this section show above-average productivity and high stability due to lower WAASB (wide adaptation) values. The environments in this quadrant can be considered productive, but with low discriminatory abilities. Based on the results, genotype G7 along with environments Ahvaz (E1 and E2), E3 (Darab-first year), and E5 (Gonbad-first year) were in the first quadrant (I). The second quadrant (II) consisted of genotypes G4, G9 and G14 with environments Moghan (E9 and E10), E4 (Darab-second year) and E8 (Zabol-second year). Genotypes G2, G6, G8, G11, G12, G15, G18, G19 and G20 along with environments E6 (Gonbad - second year) and E7 (Zabol - first year) were placed in the third quadrant (III). The fourth quadrant (IV) consisted of genotypes G1, G3, G5, G10 and G17. Of the genotypes studied, G16 and G13 were located on the border of quadrants I-II and III-IV, respectively.

Based on the averaged data, the Ahvaz (E1), Darab (E2), Gonbad (E3), and Zabol (E4) regions, along with genotypes G7, G15 and G18, were placed in quadrant I; genotypes G4, G9 and G14, along with the Moghan region (E5), were placed in quadrant II; genotypes G2, G6, G8, G11, G12, G13, G19 and G20 were placed in quadrant III; and genotypes G1, G3, G5, G10 and G17 were placed in quadrant IV (Fig. 6B). Moreover, genotypes G13 and G16 were located on the border of quadrants I-II and III-IV, respectively. The WAASBY heat

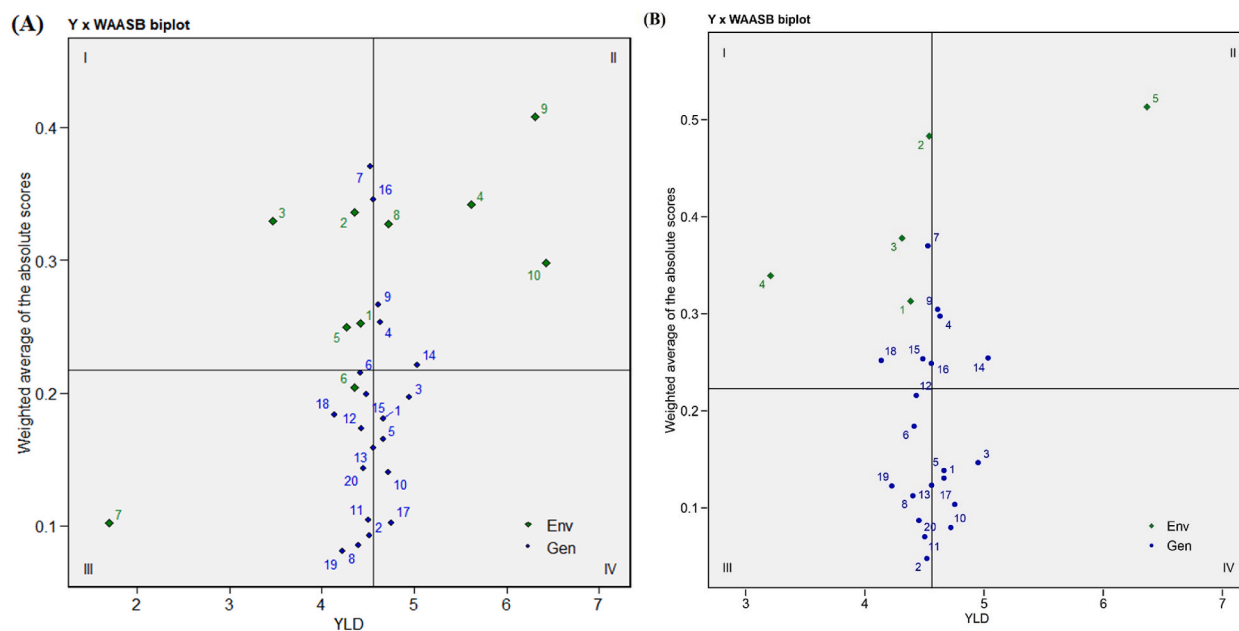


Fig. 6. The biplot was generated based on grain yield \times WAASB parameters for identification of high-yielding and stable genotypes of barley based on the ten test environments (A) and averaged two-year data (B).

map graph grouped barley genotypes based on WAASB index and grain yield with different weights (Fig. 7A). The right and left sides of the chart assign more weight to grain yield and stability, respectively. Accordingly, the first group (highlighted in green) consisted of genotypes G1, G3, G4, G5, G10, G14 and G17, which have suitable conditions in terms of grain yield and stability, especially G3, G5 and G14. The second group (highlighted in red) consisted of genotypes G2, G9, G11, G12 and G13, which had moderate stability but high yield, especially G9. Genotypes G6, G8, G15, G18 and G20 with weekly stability and low productivity were in the third group (highlighted in blue). The fourth group included genotypes G7, G16 and G19, which showed different ranges of productivity and stability. Among them, genotype G19 showed acceptable grain yield but low stability, while G16 with grain yield equal to the average showed relative stability. A heat map plot rendered from the averaged data showed a different clustering pattern. Genotypes G1, G3, G5, G6, G8 and G17 were placed in the first group. The second group consisted of genotypes G2, G9, G10, G11, G13 and G19. The third group included genotypes G7, G12, G15, G16, G18, and G20. Genotypes G4 and G14 were placed in the fourth group (Fig. 7B).

4. Discussion

In this research work, 18 newly bred barley genotypes were tested for grain yield across the 10 test environments (a combination of five locations and two years) in the warm climate in Iran. The results of the combined analysis of variance and AMMI analysis showed a significant difference E, G, and GEI (Tables 2 and 3). Moreover, the effect of E explained the greatest variation compared to G (87.40 % vs. 2.13 %) and GEI (87.40 % vs. 10.47 %) (Table 2). One of the main reasons for this finding may be the high variability in environmental conditions and differences between regions [21]. Indeed, as mentioned earlier, two test regions (Moghan and Gonbad) have warm and humid conditions, while other regions have warm and dry conditions. Therefore, these environmental differences may explain a large part of the total grain yield variability in the genotypes tested. Similarly, some researches have shown a large portion of E effect on grain yield in barley and other crops [17,18,21,22,24]. The GEI effect was further divided into three IPCAs, indicating the magnitude of the effect, which in turn suggests that tested genotype responded differently under environmental conditions [29,30]. Gauch and Zobel [31] found that when the first two components of the interaction (IPCA) account for much of the variation in grain yield, they are sufficient to identify the better genotypes. In addition, Habtegebriel [30] showed that IPCA1 scores of genotypes can be used as a useful tool to assess yield stability. Moreover, the AMMI-1 and AMMI-2 biplots were served to evaluating of the general adaptability of genotypes under study. Accordingly, genotypes G14 and G17 with the lowest IPCA scores and highest grain yield than the average and reference genotypes [G1 and G2] were identified as superior genotypes in the test environments. Moreover, genotype G14 was identified as the best genotype in most test environments, suggesting the broad adaptability of this genotype.

The results of the GGE biplot method were summarized in four biplots. One of these biplots is the discrimination and representativeness of the test environments that helps breeders to remove additional environments that provide little information about GEI and costs management. Moreover, identifying ideal environments for seed multiplication and other breeding tasks is another advantages of this biplot [32]. In this context, the test environments are divided into three types (I–III), and among them, Type II environments are very important for breeders. In this study, the Moghan and Zabol regions were identified as Type II environments. Moghan is located in the northwestern part of Iran, where warm and humid weather conditions prevail. Moreover, this region can be

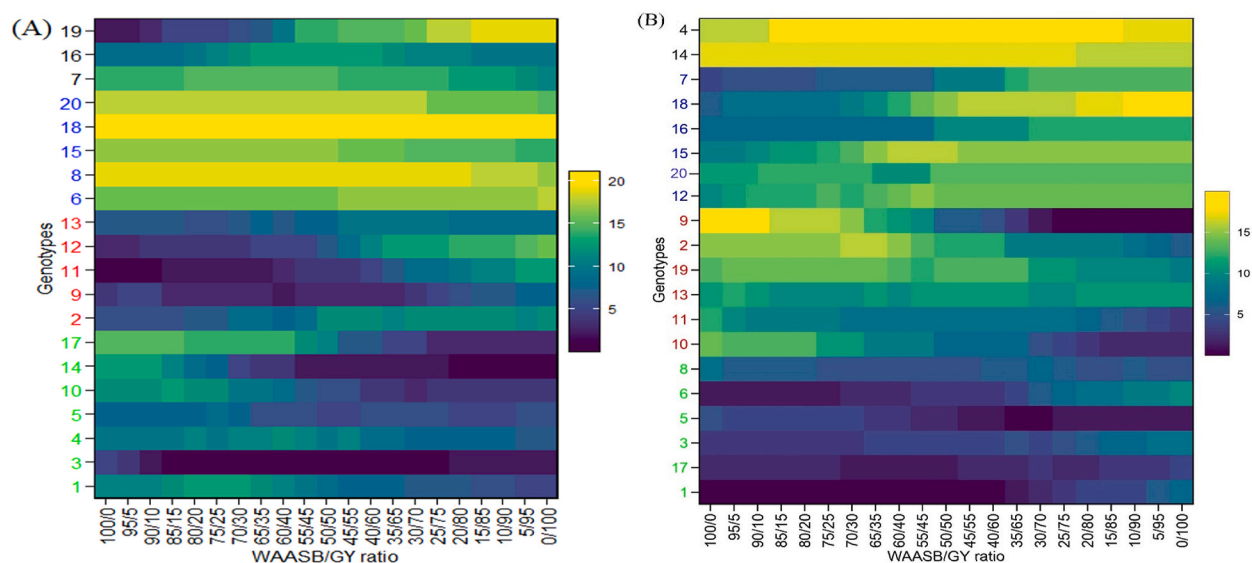


Fig. 7. Heatmap revealing the rank of 20 investigated genotypes of barley based on different weights for the grain yield and stability. Panels A and B have been rendered based on the ten environment data and averaged two-year data, respectively. The first, second, third, and fourth groups have been highlighted in green, red, blue, and black colors, respectively.

identified as an ideal location for various breeding activities, such as a place for multiplication, an ideal target environment for testing new varieties for their full yield potential, and testing for pests and diseases [33]. The Zabol region, on the other hand, with its warm and dry weather conditions, is a suitable site to test genotypes in response to water shortage stress in the final phase of growth, and even thermal stress. As shown in Fig. 2, the position of these regions in the biplot, especially Moghan, reveals their proportional contribution to the GEI effect, which was further supported by their great average compared to other environments. In addition to this biplot view, the “who-won-where” pattern also revealed comparable results. As we expected, the Moghan and Zabol regions were grouped in the same part of polygon, and most importantly, the G14 genotype was identified as the top genotype in the formed mega-environment. Considering the grain yield of other genotypes, G1 [reference genotype], G3, G4, G7, G10 and G13 revealed good adaptability in these regions (Fig. 3).

Using Fig. 4, the stability of genotypes was determined by their distance from the AEC line; thus, the higher the distance indicates greater instability and vice versa [10]. Our results showed that G4 genotypes, followed by G14, G3 and G15 genotypes with high grain yield showed the highest stability due to their position in the biplot. GEI analysis in MET is one of the most important steps in any plant breeding program, especially when breeders emphasize the development and introduction of new varieties for target regions. In this regard, simultaneous assessment of average yield and stability of genotypes creates conditions for breeders to avoid significant commercial losses [18]. Among the best genotypes identified, G14, G10 and G3 with the highest grain yield and the shortest distance from the AEC axis, especially G3 and G10, can be recommended as stable genotypes in the five test regions (Fig. 4). Ideal genotypes were identified by their proximity to concentric circles based on grain yield. Genotypes that were very far from the arrowhead indicated poor yield. In this biplot (Fig. 5), ideal genotypes are always placed in the innermost circle and closer to the arrowhead in the center of the circular ring [34,35]. In the present study, genotypes G1, G3, G4, G10, G13, G14 and G17 were identified as ideal genotypes, and among them, genotype G14 with placement in the innermost circle was singled out as a superior genotype to others. Similar results were obtained by Bocianowski et al. [5], Linus et al. [11], Vaezi et al. [13], Oladosu et al. [34], Khan et al. [35], Kendal [36], Enyew et al. [37], and Daba et al. [38], who reported the high efficiency of the GGE biplot method in MET experiments.

In order to achieve a realistic decision in identifying high-yielding and stable genotypes, two new proposed indices, WAASB and WAASBY, were used. The WAASB index provides a two-dimensional graph and classifies test environments and genotypes into four quadrants. In fact, this clustering pattern helps breeders identify favorable environments that make a major contribution to the GEI effect, as well as the status of test genotypes in terms of average performance and stability. Based on this pattern, genotypes and environments are classified into the following four groups: (i) low-productive and unstable genotypes together with environments with high discrimination ability, (ii) high-productive and most unstable genotypes together with environments with good discrimination ability, (iii) low-productive and widely adapted genotypes together with environments with low discrimination ability, and (iv) high-productive and stable genotypes together with environments with low discrimination ability [24]. Our results based on averaging two years of data revealed that the Moghan region can be considered a discriminatory region with high discriminatory ability in Iran’s warm climate. Moreover, genotypes G4, G9 and G14 were identified as the most productive compared to other genotypes. However, genotype G14 with a lower WAASB value than G4 and G9 showed a high level of stability in all test environments (Fig. 6). Moreover, a rendered heat map based on WAASBY index and grain yield ratio confirmed the above results (Fig. 7). This method provides weights for average yield and stability to better prioritize genotype productivity over stability and vice versa [24]. As shown in Fig. 7, the three genotypes G3, G10 and G14 showed better weights for stability and average productivity. As shown by these methods, G3, G10 and

G14 can be recommended as high-producing barley genotypes with the best stability in the five regions of Iran.

5. Conclusion

In the current research, we used AMMI and GGE biplot methods to investigate the effect of GEI on grain yield in a set of new barley development genotypes in five regions of Iran. Our results reveal the potential of these methods as useful statistical tools in barley breeding programs. Ultimately, our findings revealed that G3, G10 and G14 genotypes with interesting average yield and stability in different regions across in the warm climate of Iran. Moreover, genotypes G14 and G15 revealed specific adaptability in the Zabol/Moghan and Ahvaz locations, respectively. Hence, these genotypes can be considered as the superior genotypes for further investigation in the on-farm evaluation before marketing. Moreover, these genotypes can also be used as suitable parental lines in future hybridization programs to improve the genetic background of barley. Furthermore, the Zabol and Moghan regions with high representativeness potential and the discriminating power were identified as ideal environments.

Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

Salim Rahmati: Writing – original draft, Methodology, Investigation. **Reza Azizi-Nezhad:** Software. **Alireza Pour-Aboughadareh:** Writing – review & editing, Software, Methodology, Investigation, Conceptualization. **Alireza Etminan:** Conceptualization. **Lia Shooshtari:** Software.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.heliyon.2024.e38131>.

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