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Identification of high-yielding and stable barley genotypes for warm climates in Iran using the GGE biplot method

Ali Barati[1*], Alireza Pour-Aboughadareh[1], Hassan Zali[2], Ahmad Gholipour[3], Shirali Koohkan[4], Kamal Shahbazihomounlo[5], Ali Omrani[5], Akbar Marzooghian[6], Mehdi Jabbari[2], Omid Poodineh[4], Masoumeh Kheirgoo[3]

[1]Seed and Plant Improvement Institute, Agricultural Research, Education and Extension Organization (AREEO), Karaj, Iran.
[2]Crop and Horticultural Science Research Department, Fars Agricultural and Natural Resources Research and Education Center, Agricultural Research, Education and Extension Organization (AREEO), Darab, Iran.
[3]Crop and Horticultural Science Research Department, Golestan Agricultural and Natural Resources Research and Education Center, Agricultural Research, Education and Extension Organization (AREEO), Gonbad, Iran.
[4]Crop and Horticultural Science Research Department, Sistan Agricultural and Natural Resources Research and Education Center, Agricultural Research, Education and Extension Organization (AREEO), Zabol, Iran.
[5]Crop and Horticultural Science Research Department, Ardabil Agricultural and Natural Resources Research and Education Center, Agricultural Research, Education and Extension Organization (AREEO), Moghan, Iran.
[6]Crop and Horticultural Science Research Department, Khuzestan Agricultural and Natural Resources Research and Education Center, Agricultural Research, Education and Extension Organization (AREEO), Ahvaz, Iran.
[*]Corresponding author, 0009-0005-3783-3713. Email: abarati@spii.ir, barati32@yahoo.com.

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ABSTRACT

One of the primary objectives in the development of new crop varieties is to elucidate the genotype-by-environment interaction (GEI) effects observed in multi-environment trials (METs). Consequently, the primary aim of the present study was to identify superior barley genotypes in terms of grain yield and stability for potential application in the warm regions of Iran. To achieve this, a selection of genetic materials comprising 18 promising genotypes, in addition to a local cultivar (Oxin, serving as the reference check), was evaluated across five research stations: Darab, Ahvaz, Zabol, Moghan, and Gonbad, during the cropping seasons of 2019-2021. The findings indicated that the main effects of environment (E), genotypes (G), and their interactions (GEI) were highly significant. Mean comparisons revealed that the highest grain yields were recorded for genotypes G16, G8, G15, G1, and G9, respectively, when compared to other genotypes. The GGE biplot analysis demonstrated that the first two principal components accounted for 33% and 14.67% of the total variation in grain yield, respectively. Utilizing the polygon viewpoint of the GGE biplot, four mega-environments were identified within the warm climate of Iran. Based on these results, genotype G16 is recommended as a well-adapted genotype exhibiting high grain yield and stability in the target environments. Therefore, further comprehensive research on this genotype is warranted prior to its release for commercial cultivation.

Key words: GGE biplot, Mega-environment, Stability, Warm regions.

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INTRODUCTION

Barley (*Hordeum vulgare* L.) is extensively cultivated across diverse environmental conditions due to its superior tolerance to abiotic stresses compared to other crop species. It ranks as the fourth most widely cultivated cereal globally, following wheat, rice, and maize, and serves multiple purposes, including human consumption, animal fodder, and industrial applications, particularly in beer production. In Iran, barley is the second most cultivated crop after wheat, occupying a significant area during the 2021-2022 cropping seasons. The cultivated area for barley in Iran is estimated to be approximately 1.4 million hectares, yielding around 2.5 million tons (Anonymous, 2022). The regions dedicated to barley cultivation are categorized into three microclimates: cold, moderate, and warm. The warm microclimate is further subdivided into northern and southern zones, both of which are frequently subjected to various biotic and abiotic stresses. Consequently, the identification of barley genotypes exhibiting high performance and stability is a primary objective of the breeding program for barley in these regions.

One of the primary initiatives aimed at developing new crop varieties that are adapted to diverse environmental conditions is the analysis of genotype–environment interaction (GEI) (Ebem *et al.,* 2021; Linus *et al.,* 2023). For quantitative traits such as grain yield, a pronounced GEI can significantly impede the selection of superior genotypes, as it may compromise the accuracy of conclusions that would otherwise be valid (Anderson and Lee, 2014; Van Eeuwijk *et al.,* 2016). The presence of GEI results in variations in the genetic ranking of genotypes across different environments (Wodebo *et al.,* 2023). This phenomenon diminishes the correlation between genotypic and phenotypic values, thereby obstructing genetic advancement in plant breeding programs. Consequently, minimizing GEI remains a fundamental objective of any breeding initiative (Amelework *et al.,* 2023). Breeding programs frequently employ multi-environment trials (METs) to identify high-yielding and stable genotypes that exhibit broad adaptability during the final stages of cultivar development (Gerrano *et al.,* 2022). In these trials, the extent of GEI can be assessed using various analytical models, one of the most significant being genotype-by-environment interaction biplot analysis (GGE) (Yan and Tinker, 2006). This model offers enhanced insights into the aspects of superiority and stability when identifying both broad and specific adaptations (Yan and Kang, 2002). Within the GGE biplot framework, grain yield potential and stability are evaluated through average environment coordination (AEC), which is defined by the average principal component scores (IPCAs) across all test environments (Gerrano *et al.,* 2022). In this context, the GGE biplot model can be instrumental in (i) selecting high-performing genotypes for specific production environments, (ii) describing the discriminating ability and representativeness of test environments for genotype evaluation, (iii) elucidating the relationships among environments, and (iv) comparing and ranking genotypes based on average yield and stability (Yan and Tinker, 2006). Several studies have documented the successful application of this model in the selection of superior genotypes in barley (Vaezi *et al.,* 2017; Hilmarsson *et al.,* 2021; Ghazvini *et al.,* 2021; Pour-Aboughadareh *et al.,* 2023b) as well as in other crops such as wheat (Jedzura *et al.,* 2023), sunflower (Ghaffari *et al.,* 2021), safflower (Jamshidmoghaddam and Pourdad, 2013), lentil (Hossain *et al.,* 2023), cassava (Amelework *et al.,* 2023), and oat (Wedebo *et al.,* 2023). Breeding efforts in barley have focused on the development of new varieties that exhibit enhanced yield performance and stability across varying environmental conditions. Therefore, the objectives of the present study are (1) to analyze the GEI affecting grain yield in barley genotypes, (2) to assess the representativeness and discriminating ability of the test environments, and (3) to identify ideal genotypes with superior grain yield and stability for potential cultivation in the warm climate of Iran and for use in future breeding programs.

MATERIALS AND METHODS

A total of 18 promising barley genotypes (as detailed in Table 1), in addition to a local cultivar (cv. Oxin,

Table 1. Pedigrees of promising barley genotypes evaluated in the warm regions of Iran during the 2019-2021 cropping seasons.

Genotype	Pedigree
G1	Oxin (Reference genotype)
G2	(D-13)Bgs/Dajia//L.1242/3/(L.B.IRAN/Una8271//Gloria'S'/3/Alm/Una80//....)/4/Yousef
G3	Comp.Cr229//As46/Pro/3/Srs/4/Express/5/Yousef
G4	Yousef//Trompilo/L.Moghan
G5	Cr115/Por//Bc/3/Api/CM67/4/Giza120/5/H272/Bgs/3/Mzq/Gva//Alanda-01/6/Sahra
G6	Cr115/Por//Bc/3/Api/CM67/4/Giza120/5/H272/Bgs/3/Mzq/Gva//Alanda-01/6/Sahra
G7	Cr115/Por//Bc/3/Api/CM67/4/Giza120/5/H272/Bgs/3/Mzq/Gva//Alanda-01/6/Sahra
G8	Sahra*2/Torsh
G9	Yousef/3/Rhn-03//L.527/NK1272
G10	Yousef//Trompilo/L.Moghan
G11	Beecher/5/ Melusine/Aleli/3/Matico/Jet//Shyri/4/Arupo/K8755//Mora/3/Canela
G12	Lignee 527/NK1272//JLB 70-63/3/Rhn-03//Lignee527/As45
G13	Cr115/Por//Bc/3/Api/CM67/4/Giza120/5/H272/Bgs/3/Mzq/Gva//...Alanda-01/6/Sahra
G14	Cln-B/80.5138//Gloria-Bar/Copal/3/Aliso/4/Cabuya/5/Yousef
G15	Capul/Ciru
G16	Lignee 527/NK1272//JLB 70-63/3/Rhn-03//Lignee527/As45
G17	Cr115/Por//Bc/3/Api/CM67/4/Giza120/5/H272/Bgs/3/Mzq/Gva//...Alanda-01/6/Sahra
G18	Fajr30/Yousef
G19	WB-96-10

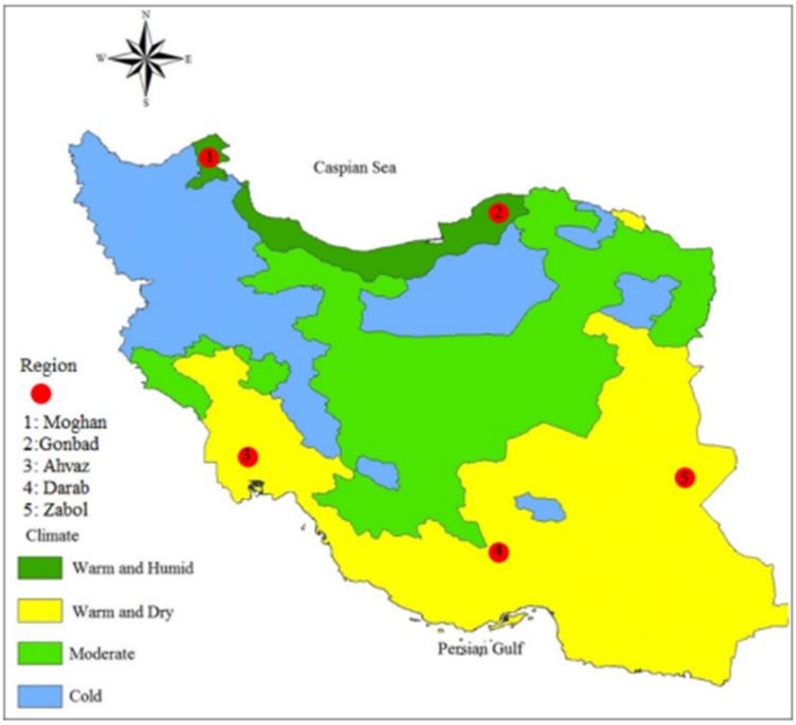

Figure 1. The geographical distribution of five specific environments within the warm climate of Iran.

serving as the reference genotype), were assessed across five agricultural research stations located in the warm regions of Iran, specifically Ahvaz, Darab, Zabol, Gonbad, and Moghan, during the cropping seasons of 2019 to 2021 (Figure 1). The experimental design employed at all research stations was a randomized complete block design with three replications. Each genotype was cultivated in six rows, each measuring 6 meters in length, with a spacing of 20 cm between rows. Sowing was conducted using an experimental planter (Wintersteiger, Ried, Austria), with a planting density of 300 seeds per square meter. Prior to sowing, basic fertilizers, specifically P2O5 and N, were applied at rates of 100 kg ha–1 and 32 kg ha–1, respectively. To manage broad-leaved and narrow-leaved weeds during the tillering stage, Granstar and Pumasuper herbicides were utilized. Irrigation was conducted once in the autumn and four times in the spring. Following the physiological maturity of the crops in each test environment, a combine harvester (Wintersteiger, Ried, Austria) was employed to harvest the experimental plots. Grain yield was measured for each genotype within the respective test environments. The experimental data collected from all environments were subjected to a combined analysis of variance. The least significant differences (LSD) method was utilized for the comparison of means. To evaluate the impact of genotype-environment interaction (GEI) on grain yield data, GGE biplot analysis was performed using the 'metan' package (Olivoto and Lucio, 2020) in R software (R Core Team, 2018).

RESULTS AND DISCUSSION

The results of the combined analysis of variance for grain yield data revealed significant effects attributable to genotypes (G), environments (E), and their interaction (GEI) (Table 2). These findings suggest notable differences in the genotypic responses of the examined barley varieties to varying environmental conditions in the warm regions of Iran. Consistent with our findings, previous studies have indicated that these two factors are primary sources of variation in barley and other crops under diverse environmental conditions (Farshadfar, 2008; Ghazvini *et al.,* 2021; Pour-Aboughadareh *et al.,* 2022, 2023a; Bakshi and Shahmoradi, 2023). The means comparison test indicated that genotypes G16, G8, G15, G1, and G9 exhibited higher grain yields compared to other genotypes, thereby identifying them as the most promising barley genotypes (Table 3).

Table 2. Combined ANOVA of grain yield in promising barley genotypes evaluated in the warm regions of Iran during the 2019-2021 cropping seasons.

Source of variation	df	SS	MS
Environment (E)	9	473.42	52.60**
Genotype (G)	18	15.08	0.84**
GE interaction	162	189.14	1.17**
Residual	378	149.41	0.39

**: Significant at 1% probability level.

Table 3. The average grain yield of the examined genotypes across various regions characterized by a warm climate in Iran during the cropping seasons of 2019 to 2021.

Genotype	Grain yield in environment (ton ha^{-1})										Total mean
	Ahvaz		Darab		Zabol		Gonbad		Moghan		
	1st (E1)	2nd (E2)	1st (E3)	2nd (E4)	1st (E5)	2nd (E6)	1st (E7)	2nd (E8)	1st (E9)	2nd (E10)	
G1	3.86	2.20	6.33	5.39	4.97	5.32	4.57	4.15	5.94	4.11	4.68
G2	4.74	3.08	6.22	4.45	3.06	6.54	3.68	3.89	4.97	4.91	4.55
G3	4.71	2.98	6.04	3.68	3.14	6.23	4.95	3.62	4.94	4.28	4.46
G4	5.66	2.98	5.85	3.65	4.06	5.38	4.26	3.11	4.06	4.25	4.32
G5	5.65	2.58	5.07	4.49	3.04	6.52	2.93	4.28	4.65	5.20	4.44
G6	4.48	2.13	5.38	4.20	4.80	5.43	3.55	3.95	5.00	4.51	4.34
G7	4.04	2.90	5.53	3.89	3.01	6.25	4.02	3.33	5.59	4.71	4.33
G8	5.27	2.75	5.98	3.76	4.09	4.92	5.28	4.66	5.75	5.19	4.77
G9	5.44	3.25	6.05	4.64	4.52	4.60	3.87	4.06	5.99	4.05	4.65
G10	4.98	2.83	5.30	4.51	4.14	5.91	3.81	3.81	4.50	5.62	4.54
G11	5.00	2.33	5.62	3.20	4.35	4.18	5.32	4.51	5.69	4.45	4.47
G12	5.08	2.35	7.04	3.44	3.25	5.77	3.38	4.53	3.85	5.06	4.38
G13	4.38	2.10	6.60	4.07	3.68	6.44	3.37	3.02	5.83	4.82	4.43
G14	5.30	3.11	6.36	4.45	3.25	5.88	4.12	3.31	5.66	4.04	4.55
G15	4.65	2.71	5.05	4.10	4.91	6.73	5.51	3.91	4.99	4.86	4.74
G16	5.06	2.71	6.86	3.73	5.39	5.46	4.21	4.22	5.83	5.92	4.94
G17	4.49	2.36	6.53	3.61	5.34	5.07	5.20	4.22	5.19	4.16	4.62
G18	5.27	3.30	6.48	4.04	3.24	4.69	4.59	3.99	5.79	5.01	4.64
G19	4.03	2.60	5.62	4.22	3.60	6.13	4.25	3.95	4.37	5.59	4.44

LSD 5%: 0.533
LSD 1%: 0.707

The GEI effect diminishes genetic improvement in plant breeding programs and complicates the selection of genotypes suitable for a wide range of environmental conditions (Perkins and Jinks, 1968). Previous analyses of GEI across various traits have demonstrated that barley exhibits significant sensitivity to environmental fluctuations (Ahakpaz *et al.,* 2014; Hilmarsson, 2021; Ghazvini *et al.,* 2022). Understanding the stability and adaptability of genotypes over multiple years and environments is essential for recommending genotypes that are appropriate for specific target environments. Therefore, it is imperative to investigate the GEI effect prior to the release of new high-yielding cultivars. This investigation can facilitate the selection of either widely adapted and stable genotypes for diverse environments or the optimal genotypes for particular target environments, thereby mitigating the adverse effects of GEI (Ghazvini *et al.,* 2024). In the current study, the results of the GGE biplot analysis revealed that the first two principal components (PCAs) accounted for 47.66% of the total variation in grain yield across different environments. One potential reason for the limited explanatory power of these two PCAs may be the increased number of test environments. Indeed, as the number of genotypes and test environments increases, the complexity of the GEI effect also escalates, which subsequently reduces the capacity of these components to account for total variation. Nevertheless, these components can still be utilized to elucidate changes in grain yield among the investigated genotypes. The polygon view of the GGE biplot is recognized as one of the most effective methods for determining the specific adaptation of a genotype within a target environment (Yan *et al.,* 2010). In this biplot, a polygon is formed by connecting the vertex genotypes with straight lines, while the remaining genotypes are situated within the polygon. As illustrated in Figure 2A, all environments were categorized into eight sectors. The environments of Ahvaz (E1 and E2) and E3 (Darab-first year) were grouped into one sector, constituting mega-environment I, with G14 identified as the best genotype within this sector. The second mega-environment (II) encompassed the second year for Gonbad (E8) and Moghan (E9), with G8 serving as the vertex genotype for these environments. The third mega-environment (III) was exclusively represented by E7 (Gonbad-first year), with G16 as the vertex genotype. The second year of Darab (E4) included genotypes G5, G15, and G18, which were situated in the fourth mega-environment. Environments E6 (Zabol-second year) and E10 (Moghan-second year) were classified into the fifth mega-environment, with genotype G4 as the vertex genotype for this sector. The E5 environment (Zabol-first year) was allocated to a separate sector without a designated vertex genotype. Prior studies by Pour-Aboughadareh *et al.* (2023a) and Ghazvini *et al.* (2021) identified four and five mega-environments for barley in the warm and cold climates of Iran, respectively.

Figure 2B illustrates the ranking pattern of the barley genotypes examined, based on their mean yield and stability across various environments. The

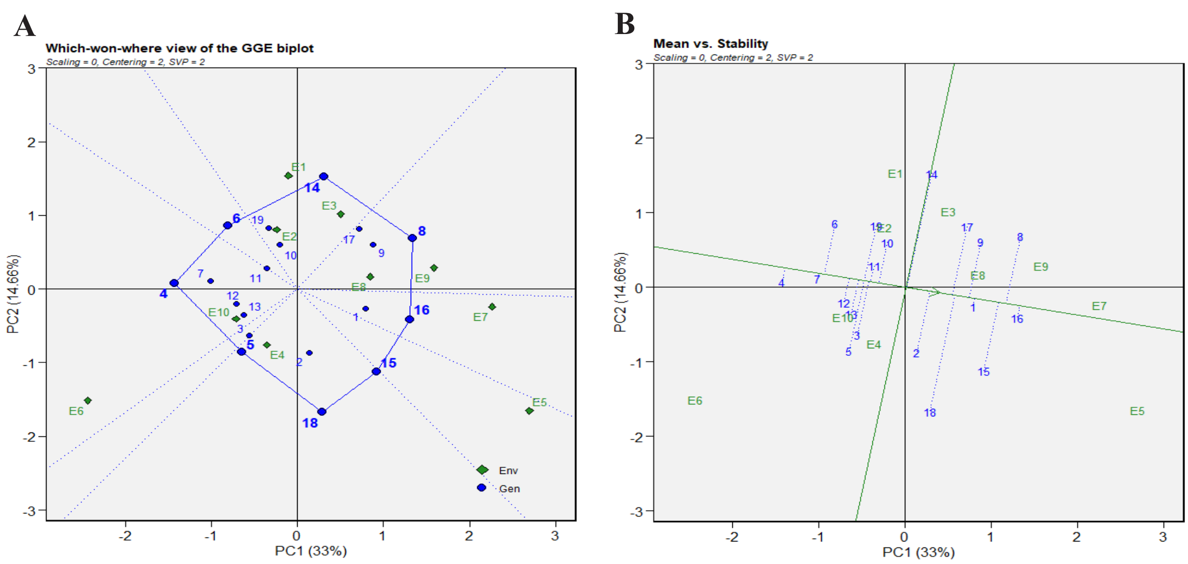

Figure 2. A: The graphical representation of the GGE 'which-won-where' biplot illustrating the winning genotypes for grain yield in each sector. **B:** Biplot for the simultaneous selection of grain yield and stability among the barley genotypes tested. For a comprehensive understanding of the environmental abbreviations, please refer to Table 3.

results indicate that genotypes G16, G8, G15, G1 (the reference genotype), and G9 exhibited the highest average grain yields in the tested environments. Genotypes G2 and G17 demonstrated performances that were closest to the grand mean value, as reflected in their positioning within the biplot. Notably, genotype G16, which achieved a high average grain yield, was identified as the most stable, while genotypes G18 and G14 exhibited significant instability across different environments. Conversely, certain genotypes, including G4, G7, and G12, despite having low average grain yields, displayed high levels of stability.

The METs (Multi-Environment Trials) can be employed to assess optimal environments for testing. Theoretically, an ideal environment is characterized by two key concepts: (i) discriminating ability and (ii) representative power (Yan *et al.,* 2000). Based on this theoretical framework, test environments can be categorized into three distinct types. Type I encompasses environments with short vectors that yield minimal information regarding genotypes; therefore, these environments should be excluded from future trials. Type II includes environments characterized by long vectors that form small angles with the average environment coordinate (AEC); such environments are deemed suitable for identifying high-yielding genotypes. Type III consists of environments with long vectors that create large angles with the AEC; these environments are advantageous for eliminating unstable genotypes. In the current study, the Zabol environments (E5 and E6), along with E7 (Gonbad-first year), which exhibited the longest vectors, demonstrated significant discriminating ability, categorizing them as Type III environments. Among the test environments, E5 (Zabol-first year), E7 (Gonbad-first year), and E9 (Moghan-first year) were classified as Type II environments. Consequently, these environments are recommended as ideal settings for the selection of high-yielding barley genotypes. Conversely, environments E3 and E4 (located in Darab) were identified as Type I environments; thus, this location should be excluded from adaptability and stability trials to minimize the costs associated with field evaluations (see Figure 3A).

A comparative analysis utilizing the GGE biplot was employed to identify the ideal genotypes, as illustrated in Figure 3B. The concept of the ideal genotype is hypothetically defined based on optimal productivity and stability, serving as a benchmark for the evaluation of the genotypes under investigation (Yan and Kang, 2002). Among the genotypes assessed, G16 emerged as the most favorable, followed by G1 (the reference genotype), G15, G8, and G9, all of which were situated near the average environment axis (AEA) and were thus selected as ideal genotypes. Notably, genotype G16 exhibited specific adaptability to the Gonbad environment in both years (E7 and E8) and to the Moghan environment in the first year (E9). The GGE biplot method is recognized as one of the most effective multivariate approaches for assessing both adaptability and stability across various crop species, as depicted in Figure 3B. Furthermore, numerous studies

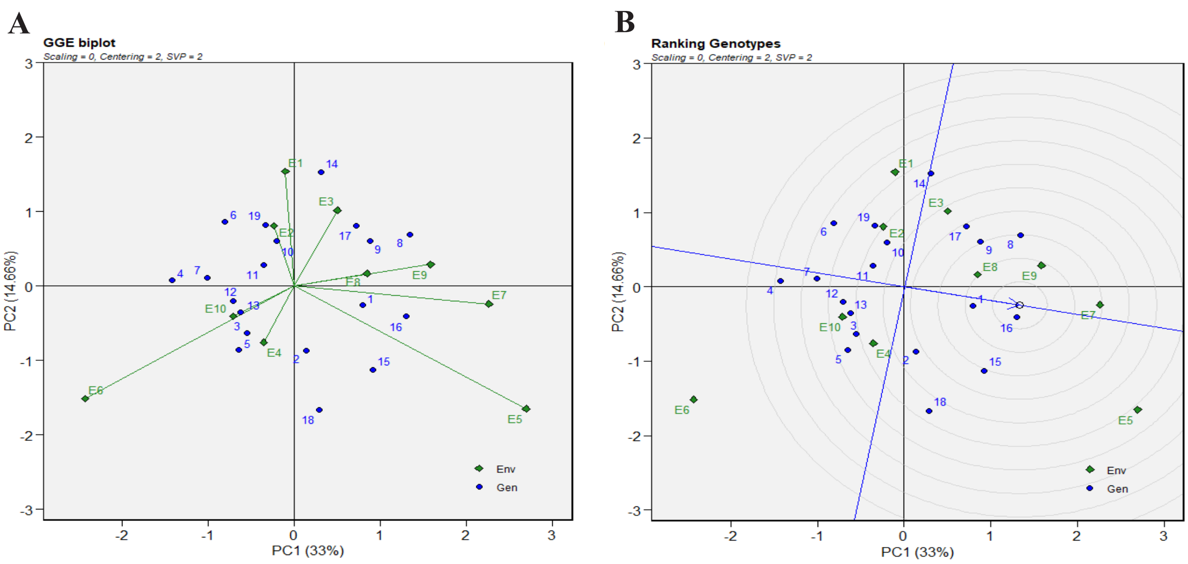

Figure 3. A: The graphical representation of the 'discriminating power and representativeness' of the GGE biplot. **B:** Comparison of promising barley genotypes against the 'ideal' genotype concerning grain yield and stability across four test locations. For a comprehensive understanding of the environmental abbreviations, please refer to Table 3.

have corroborated the high efficacy of this method in identifying superior barley genotypes in METs (Jalata, 2011; Ahmadi *et al.,* 2012; Mortazavian *et al.,* 2014; Kendal, 2016; Taheripourfard *et al.,* 2017; Vaezi *et al.,* 2019; Pour-Aboughadareh *et al.,* 2023b).

The findings of this study indicate that genotypes G16 and G8 exhibited the highest grain yields in the southern and northern regions of Iran, respectively (see Table 2). Furthermore, a comparative analysis of these two microclimates suggests that genotype G16 is a suitable candidate for cultivation in the warmer regions of Iran. Given the distribution of warm regions across the country, it is feasible to identify high-yielding and stable genotypes with general adaptability; however, such endeavors are often accompanied by various challenges. Therefore, the identification of high-yielding genotypes with specific adaptability, in addition to general adaptability, can be effectively achieved through data analysis derived from studies of this nature.

In Iran, barley breeding programs for warm regions are typically conducted at both northern and southern stations. Consequently, the identification of genotypes with general adaptability is infrequent. For example, several cultivars, including Nimrooz, Norooz, Zahak, Dasht, and Nobahar, have been introduced as superior cultivars specifically suited for the northern and southern regions of Iran's warm climate (Ghazvini and Yousefi, 1999; Ghazvini *et al.,* 2014; Ghazvini *et al.,* 2020). Recently, Ghazvini *et al.* (2019) and Barati *et al.* (2023) introduced two new cultivars, Oxin and Golchin, which exhibit high levels of stability and general adaptability for cultivation across various regions within Iran's warm climate. In conclusion, our findings indicate that among the genotypes investigated, G16 (Lignee 527/NK1272// JLB 70-63/3/Rhn-03//Lignee527/As45) demonstrates significant adaptability to diverse environments and achieves high grain yields in both northern and southern regions, making it a suitable candidate for testing in warm regions of Iran.

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