

Identification of high-yielding genotypes for cold climate in Iran using the GGE Biplot method

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Abstract: The development of high-yielding and stable cultivars necessitates a comprehensive understanding of genotype-by-environment interaction (GEI) effects through multi-trial experiments (MTEs). In this study, the grain yield performance, stability, and adaptability of newly developed barley genotypes were assessed across eight locations in Iran over two cropping seasons (2020–2022) using GGE biplot analysis. Analysis of variance of the grain yield data revealed significant effects of genotype, environment, and their interaction. The GGE biplot analysis identified two mega-environments, with Ardabil, Arak, and Tabriz being the most discriminating and representative test sites. Based on the “which-won-where” and “mean vs. stability” views, genotypes G5, G17, and G18 were identified as high-yielding, stable, and ideal candidates. Genotype G17 exhibited specific adaptability to Hamedan, Miandood, and Karaj, whereas G5 and G18 were best adapted to Jolgeh Rokh, Mashhad, Tabriz, Ardabil, and Arak. These genotypes warrant further on-farm evaluation prior to their potential commercial release.

Keywords: Barley, combined analysis of variance, genotype-by-environment interaction, specific adaptability, yield stability

INTRODUCTION

Barley (*Hordeum vulgare* L.) is among the earliest domesticated cereals crops and is highly valued for its broad climatic adaptability. Its grains are rich in dietary fiber, vitamins, and minerals, contributing to digestive health and improved glycemic regulation in human diets. In addition to its nutritional value, barley serves as a major source of livestock feed and constitutes the primary raw material for malting in beer and whisky production, highlighting its industrial significant (Rahmati et al. 2024). Furthermore, its inherent tolerance to drought and low temperatures enhances its potential for sustainable cultivation in regions frequently exposed to environmental stress (Pour-Aboughadareh et al. 2023).

Evaluating promising breeding genotypes across multiple locations and cropping seasons is an effective approach to account for environmental variability within breeding programs (Malosetti et al. 2013). Regional yield trials, coupled

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with robust statistical methodologies, are crucial for identifying superior genotypes, clustering test sites based on yield performance, and precisely delineating target mega-environments in multi-environment trials (METs) (Yan and Tinker 2006). The genotype-by-environment (GE) interaction, which captures the differential performance of genotype across diverse environments, should be rigorously characterized to identify germplasm exhibiting either broad or specific adaptation.

The GGE biplot methodology integrates genotype main effects with GE interactions, projecting their multidimensional relationships onto a two-dimensional graphical space. This visualization facilitates the identification of “which-won-where” patterns, delineates mega-environments, and highlights genotypes with superior performance. Consequently, GGE biplot analysis has become a widely adopted tool in crop evaluation and plant breeding research (Vaezi et al. 2019, Kebede et al. 2023, Rahmati et al. 2024, Derbew et al. 2024, Mohammadi et al. 2025).

In many regions of Iran, particularly in high-altitude and frost-prone areas, severe winters, short growing seasons, and recurrent frost pose major challenges to crop productivity and yield stability. These harsh agroclimatic conditions constrain both yield potential and production reliability. Barley, characterized by its low input requirements and broad adaptability, has traditionally been cultivated in such marginal environments. Its capacity to withstand suboptimal conditions renders it a vital crop for food and feed production in regions where other cereals frequently fail. Although Iran’s annual barley output, approximately three million tons, remains modest compared that of leading global producers, its resilience underscores its indispensability in cold, arid, and semi-arid zones (Pour-Aboughadareh et al. 2023).

Yield-gap analyses indicate that frost damage, water scarcity, and other climatic stresses frequently prevent farmers from achieving maximum attainable yields, underscoring the necessity for improved agronomic practices and breeding strategies specifically adapted to cold climates (Rahimi et al. 2025). The application of advanced analytical methodologies, particularly the GGE biplot approach, has substantially enhanced the understanding of complex interactions between genotype performance and environmental factors. This has, in turn, guided breeding programs toward the development of cultivars with improved yield stability and adaptability for cold-climate barley production (Pour-Aboughadareh et al. 2023). In the present study, a MET was conducted to evaluate 18 promising barley genotypes for grain yield and stability. The GGE biplot analysis was employed to elucidate GE interactions and to identify high-yielding, stable adapted genotypes suitable for potential commercial release in Iran’s cold-climate regions and comparable agroecological zones.

MATERIAL AND METHODS

Plant materials and experimental layouts

Eighteen promising barley genotypes (Table S1), along with two local reference varieties, Jolge (G1) and Mahtab (G19), were evaluated through multi-environment trials (Table S1). Field experiments were conducted across eight research stations located in Iran’s cold-climate regions (Ardebil, Miandoab, Arak, Hamedan, Mashhad, Jolge Rokh, Tabriz, and Karaj) over two consecutive cropping seasons (2020-2021 and 2021-2022). Supplementary Table S2 provides additional meteorological data for each location. At each site, experiments were arranged in a randomized complete block design (RCBD) with three replications. Each experimental plot consisted of six rows, 6 m in length, with 20 cm inter-row spacing. Sowing was carried out using an experimental planter (Wintersteiger, Ried, Austria) at a seeding density of 400 seeds m⁻². Basic fertilizers (N and P₂O₅) were applied prior to sowing at rates of 32 and 100 kg ha⁻¹, respectively. During the growing season, five irrigations were applied at Zadoks’s growth stages 00, 32, 51, 75, and 85 (Zadoks et al. 1974). An additional 40 kg ha⁻¹ of N was top-dressed at stem elongation (ZGS 31). Following germination and early field establishment, cold-acclimated seedlings were sampled in early January 2020 for laboratory freeze testing. Freeze tolerance was assessed as the lethal temperature for 50% mortality (LT50). Plant crowns were placed in pots containing moist sand, preconditioned at -3 °C for 12 hours, and subsequently cooled at a rate of -2 °C h⁻¹ to -21 °C in a programmable freezer. At 2 °C interval between -3 °C and -21 °C, five plants per genotype and replication were removed for assessment. After freezing, crowns were replanted and maintained under controlled glasshouse conditions. LT50 values were determined based on regrowth observations 21 days post-treatment. At physiological maturity, plots were harvested using an experimental combine harvester (Wintersteiger, Ried, Austria), and grain yield was recorded for each genotype at all locations for subsequent statistical analysis.

Statistical analysis

Grain yield data from 20 barley genotypes evaluated across 16 environments (eight locations over two cropping years) were subjected to a combined analysis of variance (ANOVA). Genotypic performance across environments was further analyzed using the GGE biplot methodology as described by Yan (2001). Mega-environments (MEs) were delineated based on the “which-won-where” pattern derived from the GGE biplot. Genotypes mean performance and stability were assessed using the average environment coordinate (AEC) method, wherein the AEC represents a line passing through the biplot origin and the mean PC1 and PC2 scores of all environments (Yan 2001). The projection of each genotype onto this line indicates its mean performance, while its perpendicular distance from the AEC reflects stability (Yan et al. 2000). In the AEC view, both environments and genotypes were compared with an ideal genotype and ideal environment, represented by the center of the concentric circles within the biplot (Yan 2001). Test environments were further characterized according to the “discriminativeness vs. representativeness” pattern of the GGE biplot (Yan and Tinker 2006). All statistical analyses were conducted in R using the “metan” package (Olivoto and Lucio 2020) within the R software (R Core Team 2018).

RESULTS AND DISCUSSION

Combined analysis of variance and genotypic performance

Multi-environment trials (METs) are fundamental components of plant breeding programs, facilitating the evaluation of genotype performance across multiple years, locations, management practices, and their combinations. These trials enable breeders to better characterize genotypic responses to environmental variability and to formulate targeted recommendations for specific regions or stress conditions (Rahmati et al. 2024). In the present study, the effects of genotype (G), environment (E), and GEI were all highly statistically significant (Table S3). The relative contributions of these sources to the total sum of squares (TSS) are often used as indicators of their respective magnitudes (Kebede et al. 2023). Our results showed that the environmental effects accounted for the largest proportion of the total variation in grain yield (55.66%), whereas genotypic effects contributed only 3.91%. The GEI component was also substantial, explaining 18.60% of the total variation in grain yield (Table S3). The significant variation among genotypes reflects underlying genetic diversity, while the pronounced environmental variation indicates that genotype mean performance is strongly influenced by environmental conditions. As noted by Fehr (1993), this variability is often attributed to GEI effects, which can markedly alter genotype rankings across environments. These results align with previous studies that have reported significant differences among barley genotypes and a large proportion of yield variation explained by E and GEI effects (Vaezi et al. 2017, Ahakpaz et al. 2021, Pour-Aboughadareh et al. 2023, Ghazvini et al. 2024a, Rahmati et al. 2024). Mean grain yields for each genotype across environments are presented in Table S4. Grain yield exhibited wide variation, ranging from 4.8 tons ha⁻¹ in environment E11 to 10.4 tons ha⁻¹ in environment E12, with an overall mean of 7.1 tons ha⁻¹. Genotypic performance also varied notably: genotypes G10 recorded the lowest mean yields (6.57 tons ha⁻¹), whereas genotype G18 achieved the highest (7.62 tons ha⁻¹). Environments E4 and E12, with mean yields of 8.38- and 10.44-tons ha⁻¹, respectively, were the most productive, consistently supporting superior grain yields across all tested genotypes. The significant genotypes yield differences observed within specific environments, particularly at Tabriz (E7 and E15) and Hamedan (E4 and E12), suggest that these sites contributed substantially to the overall GEI and are therefore critical for assessing genotype performance.

For farmers, interpreting the implications of GEI necessitates a flexible, site-specific approach to crop management. To effectively address the challenges posed by GEI, plant breeders increasingly employ advanced mathematical methods and statistical modeling techniques to predict genotype performance across diverse environments. Among these analytical approaches, the GGE biplot has emerged as a particularly powerful tool for examining GEI effects on grain yield in barley and other crops species (Esan et al. 2023, Daba et al. 2023, Megerssa et al. 2024, Derbew et al. 2024, Barati et al. 2025).

Screening genotypes studied for cold tolerance

The analysis of LT50 data revealed significant variability in cold tolerance among the evaluated genotypes, as illustrated in Figure S1. Genotype G8 exhibited the highest level of cold tolerance, with an LT50 value of -15 °C, followed closely by genotype G6, which showed an LT50 of -14 °C. Several other genotypes, including G10, G16, G20, G1, and G19,

demonstrated comparable levels of cold tolerance, each with LT50 values of -13°C . These results identify genotype G8 as the most cold-tolerant among the tested materials, while the remaining genotypes showed intermediate levels of cold resilience.

Principal component analysis (PCA) was used to further decompose the GEI, revealing that six interaction principal components (IPCs) were statistically significant and collectively accounted for 82.97% of the GEI-associated variation in grain yield. The first two IPCAs explained 48.90% of this variation, with IPCA1 and IPCA2 contributing 26.54% and 22.36%, respectively (Figure 1A). Based on the two-year averaged data across eight test locations, the first two IPCAs explained 69.29% of the total GEI variation, with IPCA1 and IPCA2 accounting for 35.15% and 27.14%, respectively (Figure 1B).

Mean performance vs. stability pattern

Across 16 environments, genotypes G5, G17, G18, and G19 showed above-average grain yield, with G18 and G19 combining superior yield performance with the highest stability (Figure 2A). The two-year averages data similarly placed G5, G19, G18, G17, G7, G11, G4, G1, G20, G16, G13, and G6 above the overall mean yield. Within this group, G19, G5, and G18 were identified as the most stable and productive genotypes (Figure 2B). Accordingly, G18 and G19 are recommended for wide adaptation and broad cultivation, whereas G5 and G17, despite their high yield potential, appear more suitable for region-specific deployment.

Which-won-where pattern and mega-environment identification

In the 'which-won-where' view of the GGE biplot (Figure 3A), a convex hexagon was formed by connecting the genotypes positioned farthest from the biplot origin, thereby enclosing all other genotypes and test environments. The six genotypes located at the polygon vertices, G6, G8, G12, G16, G5, and G17, were identified as the highest-yielding genotypes within their respective sectors. Perpendicular rays drawn from the sides of the polygon divided the biplot into seven sectors, with environments distributed across four sectors and genotypes across seven. The first sector included environments E2 and E13, where G6 was identified as the winning genotype. The second sector included E15 environment, with G12 as the top genotype. The third sector encompassed environments E9 and E12, where G17 was identified as the superior genotype. The fourth sector, which comprised environments E1, E3, E4, E5, E6, E7, E8, E10, E11, E14, and E16, was dominated by genotypes G5, G7, and G16, indicating their broad adaptability and superior performance across multiple environments (Figure 3A). Based on the two-year average data, both genotypes and environments were

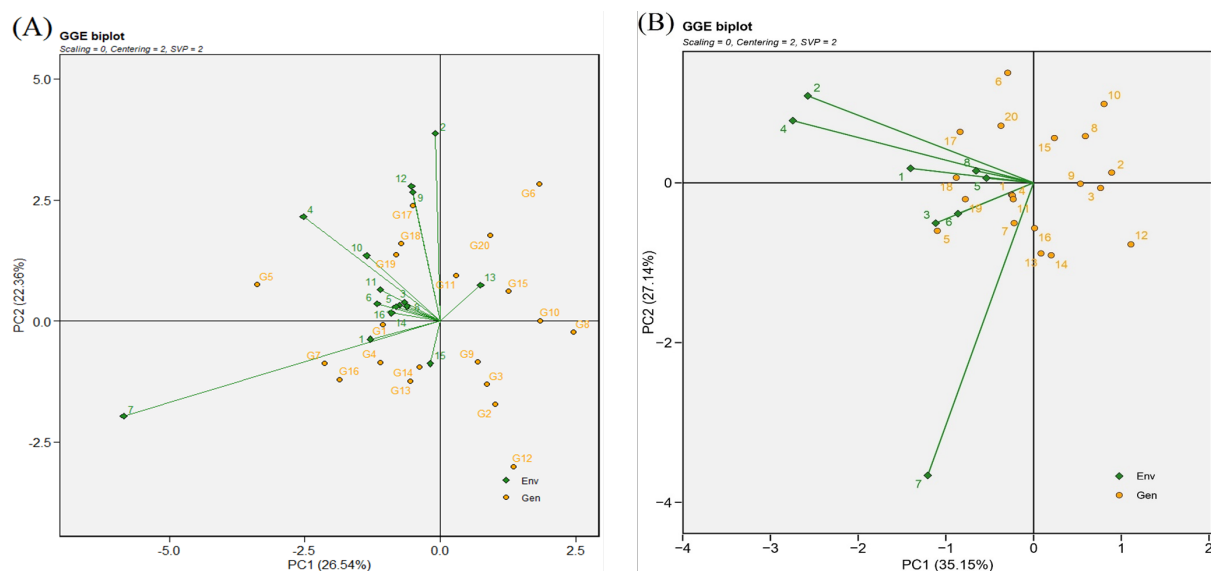


Figure 1. GGE biplot indicating the role of the investigated barley genotypes and test environments in the GEI effect. Panels A and B have drawn based on 16 test environments and 8 test locations over two cropping seasons, respectively.

distributed across seven sectors in the GGE biplot. The vertex genotypes identified were G6, G10, G12, G14, G13, G5, and G17. The polygon view of the GGE biplot further divided the test environments into two subregions, facilitating the delineation of mega-environments. The first mega-environment (ME-I) included environments E2 (Miandoab), E4

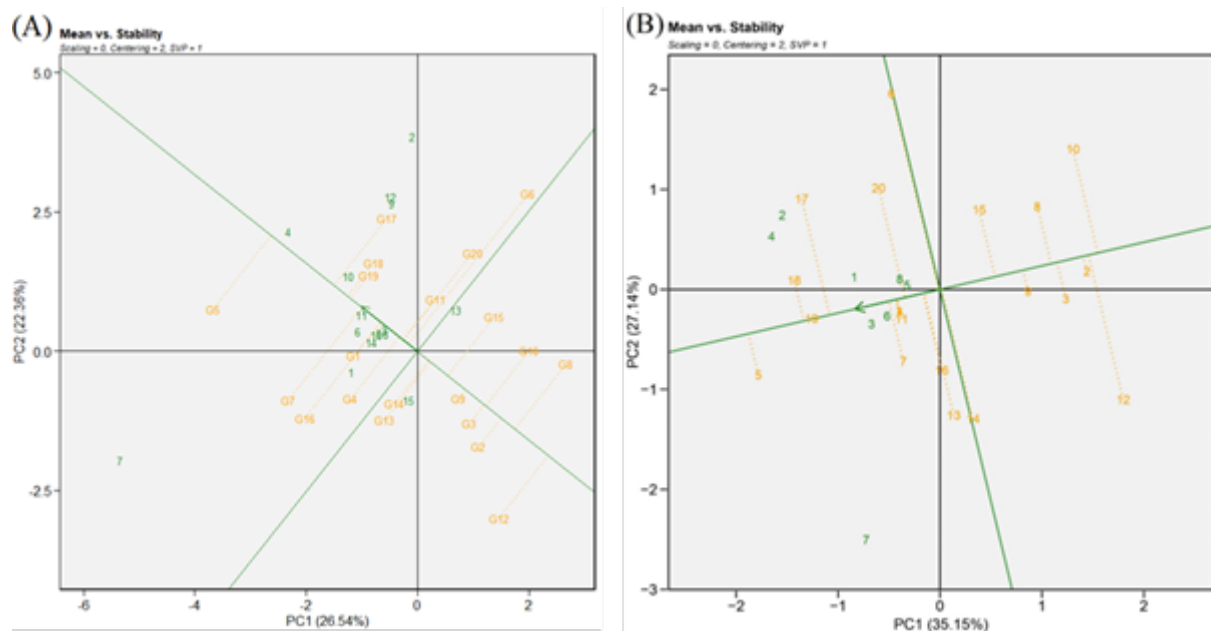


Figure 2. Biplot for simultaneous selection of grain yield and stability of barley genotypes tested based on 16 tested environments (A) and eight test locations over two cropping seasons, respectively.

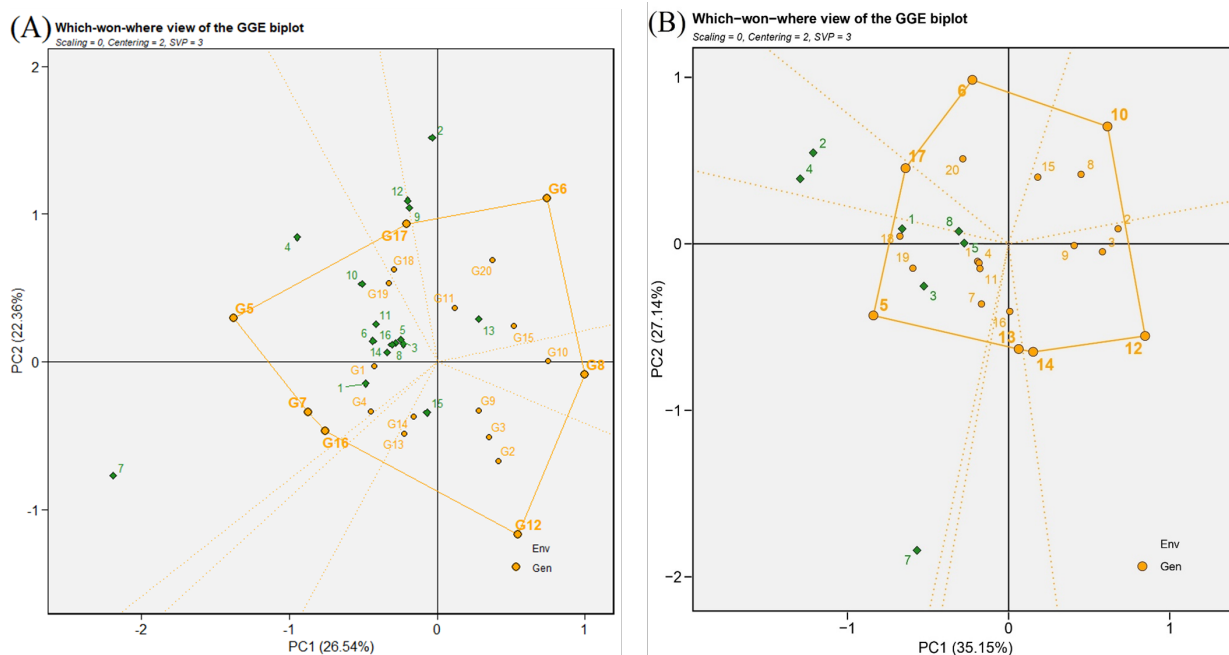


Figure 3. The view of the “which-won-where” patterns of test environments and barley genotypes based on 16 tested environments (A) and eight test locations over two cropping seasons (B), respectively.

(Hamedan), and E8 (Karaj), while the second (ME-II) comprised environments E1 (Ardebil), E3 (Arak), E5 (Mashhad), E6 (Tabriz), and E7 (Jolgeh Rokh). Genotype G17, located at the vertex of ME-I, and genotype G5, situated at the vertex of ME-II, were identified as the most representative and high-performing genotypes within their respective mega-environments. Additionally, genotypes G18 and G19 were positioned near ME-II and exhibited the highest grain yields in this region. In contrast, vertex genotypes G6, G10, G12, G13, and G14 did not achieve top yields in any test environment, indicating limited adaptability and inferior performance under the evaluated conditions (Figure 3B).

Discriminateness power vs. representativeness ability pattern

The ‘discriminateness power vs. representativeness ability’ view of the GGE biplot classified the test environments into three distinct types based on vector length and the angle between each environment vector and the average environment coordinate (AEC) axis (Yan et al. 2007). Type I environments, characterized by short vectors, contribute minimally to genotype discrimination and are therefore unsuitable for reliable genotype evaluation. Type II environments, distinguished by long vectors and small angles relative to the AEC axis, indicating both high discriminative power and strong representativeness; such environments are ideal for identifying high-yielding and stable genotypes. Type III environments, defined by long vectors and large angles to the AEC axis, display high discriminative capacity but poor representativeness, limiting their effectiveness in selection. Accordingly, E4 (first year-Hamedan) and E10 (second year-Miandoab) exhibit relatively small angles to the AEC axis and were classified as Type II, while E7 (first year-Tabriz), E2 (first year-Miandoab), E9 (second year-Ardebil), and E12 (second year-Hamedan) were identified as Type III environments (Figure 4A). The remaining environments were grouped as Type I environments (Figure 3A). Based on the two-year averaged data, the eight test locations were further classified as follows: Mashhad (E5) and Karaj (E8) as Type I; Ardebil (E1), Arak (E3), and Jolgeh Rokh (E6) as Type II; and Miandoab (E2), Hamedan (E4), and Tabriz (E7) as Type II environments (Figure 4B).

Ranking genotypes relative to the ideal genotype

In GGE biplot analysis, the ideal genotype represents a hypothetical cultivar that combines the highest mean yield performance with absolute stability across all test environments, depicted graphically at the center of a concentric-circle plot. The proximity of a genotype to this ideal point, reflects its overall desirability in terms of productivity and adaptability, providing a basis for identifying broadly adapted cultivars that minimize yield fluctuations under variable environmental

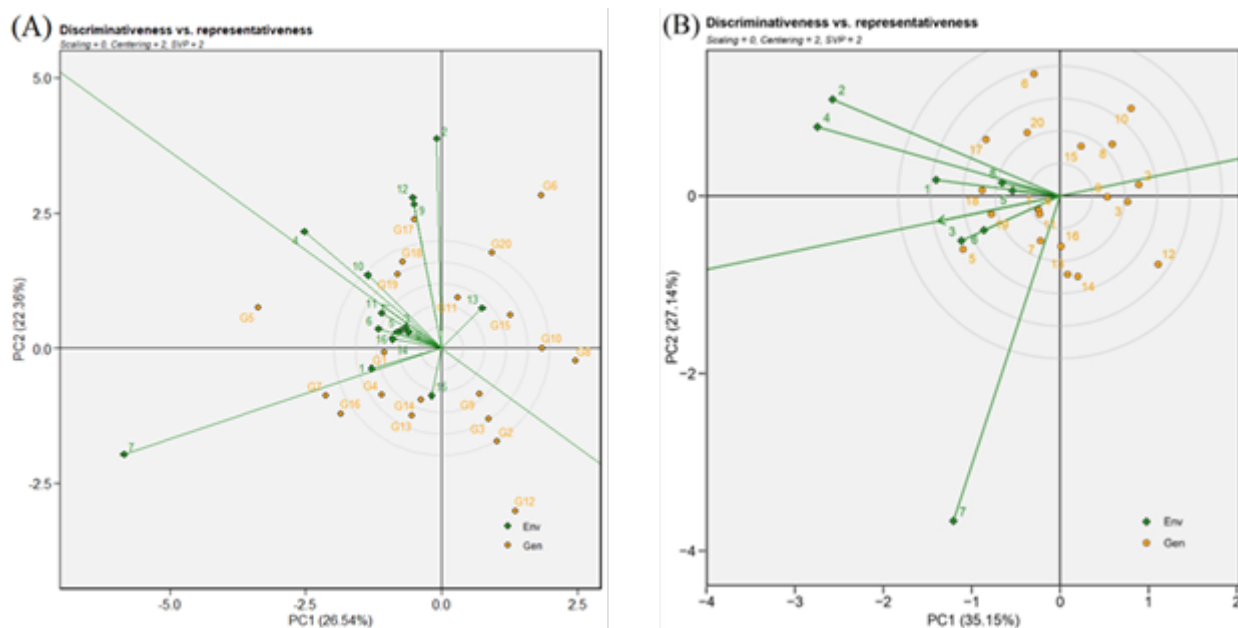


Figure 4. The view of the ‘discriminating power and representativeness’ patterns of 16 test environments (A) and eight test locations over two cropping seasons (B), respectively.

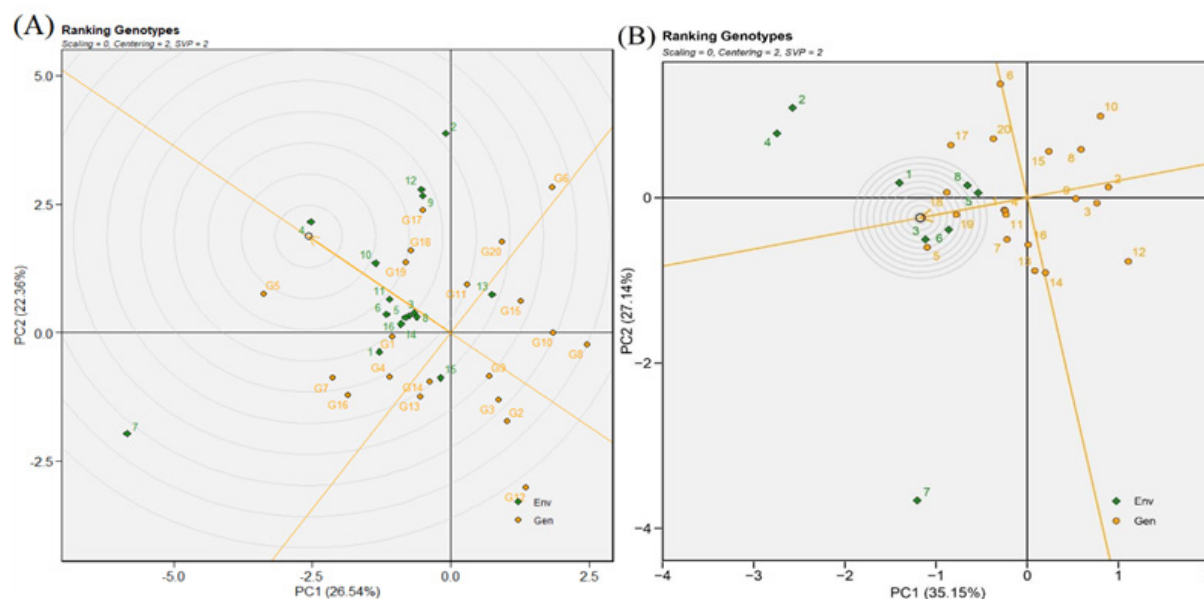


Figure 5. Comparison of promising barley genotypes in terms of grain yield and stability based on 16 tested environments (A) and eight test locations over two cropping seasons (B), respectively.

conditions (Yan and Tinker 2006). According to this criterion, the genotypes were ranked as follows: G5 > G19 > G18 > G17 > G1 > G7 > G11 > G4 > G16 > G20 > G14 > G13 > G15 > G9 > G6 > G3 > G10 > G2 > G8 > G12 (Figure 5A). Several environments were identified as ideal test locations, including E4 (first year-Hamedan), E10 (second year-Miandoab), E11 (second year-Arak), E6 (first year-Jolge Rokh), E9 (second year-Arak), and E12 (second year-Hamedan) (Figure 5A). Based on the two-year average data, genotype G5, closely followed by G18 and G19, was identified as the most ideal genotype, whereas Arak (E3) emerged as the most representative testing environment (Figure 5B). These results align with previous studies confirming the effectiveness of the GGE biplot approach for identifying ideal genotypes in METs (Vaezi et al. 2019, Bocianowski et al. 2019, Pour-Aboughadareh et al. 2023, Daba et al. 2023, Megerssa et al. 2024).

Our results indicated that genotypes G5, G17, and G18 are strong candidates for broad cultivation in Iran's cold-climate regions, as they combine high grain yield with exceptional yield stability. Genotypes G5 and G17 are sister lines derived from the pedigree 'Bereke-54/3/Rhn-03//L.527/NK1272'. 'Bereke-54' is a facultative barley cultivar recognized for its favorable agronomic traits and superior cold performance (Mussabaev et al. 2018, Ghazvini et al. 2024b), whereas the 'Rhn-03//L.527/NK1272' lineage contributed drought-tolerant germplasm that has been incorporated into successful cultivars such as Goharan (Nikkah et al. 2018). The combination of these parental traits likely underlies the superior adaptability of G5 and G17. Genotype G18, derived from the cross 'Bahtim7DL/79-W40762//DeirAlla106/3/Bahman', also showed outstanding agronomic performance under cold environments. The inclusion of Bahman in G18's pedigree, a cultivar valued in Iranian breeding programs for its adaptability, cold tolerance, and stable grain quality and is cultivated across the cold regions for more than 20 years. The complex pedigree of G18 confers broad genetic diversity, which likely enhances its stress tolerance, resource-use efficiency, and consistent performance across variable climatic conditions (Ghazvini et al. 2017). We recommend further on-farm validation trials for G5, G17, and G18 at larger scale. Based on MET results, G5 and G18 demonstrate specific adaptation to Jolge Rokh, Mashhad, Arak, Tabriz, and Ardebil, whereas G17 is particularly well adapted to Hamedan, Karaj, and Miandoab. Targeted deployments of these genotypes could maximize productivity and yield stability across Iran's diverse agro-climatic zones.

CONCLUSION

In this study, GGE biplot analysis was employed to investigate GEIs for grain yield among newly developed barley genotypes evaluated across eight cold-climate regions of Iran. The results highlight the effectiveness of GGE biplots

methodology in barley breeding programs by revealing substantial yield variation among genotypes, identifying two distinct mega-environments within Iran's cold zones, and recognizing Ardabil, Arak, and Tabriz as the most discriminating and representative test locations. Overall, G5, G17, and G18 were identified as high-yielding and stable candidates, and their favorable LT50 values further underscore their suitability for cultivation in cold-climate regions. These genotypes combine superior yield potential with strong stability across low-temperature environments, making them promising candidates for enhancing barley productivity in regions, where cold stress limits crop performance.

DATA AVAILABILITY

The datasets generated and/or analyzed during the current research are available from the corresponding author upon reasonable request.

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