



Research article

Stability analysis of forage yield of Iranian and exotic alfalfa (*Medicago sativa* L.) genotypes using genotype plus genotype-by-environment interaction biplot method

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Abstract

Importance of the work: The stability of forage yield under variable climate conditions is increasingly important for sustainable alfalfa production under climate change scenarios.

Objectives: To assess the genotype $\times$ environment interaction for the prediction of differential genotype responses and to support the selection of superior, low-risk genotypes.

Materials and Methods: The stability was evaluated of the forage yield of nine Iranian and international alfalfa genotypes over 2 y (2021–2022) in a randomized complete block design, with three replications across four agricultural research stations in Iran (Karaj, Dezful, Yazd, Jiroft).

Results: Combined analysis of variance for dry forage yield and plant height revealed significant effects of environment, genotype and their interaction. Stability analyses using Shukla's stability variance and Lin and Binns' superiority coefficient identified the Omid genotype as an optimal combination of high dry forage yield with 21.34 t/ha and acceptable stability. Furthermore, the genotype plus genotype-by-environment biplot analysis showed that Omid and Baghdadi (located closest to the ideal genotype) combined superior yield with stability across environments. The commercial CUF101 genotype, despite a moderate forage yield (20.62 t/ha), had favorable stability under unpredictable environmental conditions and was recommended for stress-prone areas.

Main finding: These results show that the applied method was reliable for selecting and deploying stable, high-yielding alfalfa cultivars adapted to diverse climatic regions of Iran.

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Introduction

Alfalfa (*Medicago sativa* L.), also known as lucerne, ranks among the most major forage crops globally. The millennia-long history of alfalfa cultivation in Iran has led to the emergence of region-specific cultivars. These cultivars, through continuous cultivation and natural selection over the years, have achieved desirable quantitative and qualitative yields and considerable stability within their associated regions. Such cultivars are known as ecotypes (Mofidian et al., 2009).

Yield stability, defined as the ability of a crop to maintain consistent performance across environments and over time, is a key objective of sustainable agricultural production (Urruty et al., 2016). Increasing climate variability has intensified the importance of stability for adapting cropping systems to environmental stresses such as drought, frost and extreme temperatures (Tigheelaar et al., 2018; Hufnagel et al., 2020). Consequently, plant breeding efforts increasingly emphasize the development of cultivars that combine high yield with stable performance across diverse environments (Mühleisen et al., 2014; Singer et al., 2018).

Traditionally, in forage crops, productivity (commonly expressed as annual dry matter yield) has been the primary breeding target (Barnes et al., 2003). However, limited yield progress has been achieved in alfalfa over the past five decades, despite its wide ecological adaptability (Lamb et al., 2006; Avci et al., 2013). This constraint is largely attributed to its autotetraploid nature, substantial non-additive genetic variance and strong genotype-by-environment interaction (GEI) effects (Annicchiarico et al., 2010).

Yield performance is determined by genotype (G), environment (E) and their interaction (GEI), with GEI causing differential genotype responses across environments and complicating cultivar selection (Yan and Tinker, 2006; Baxevanos et al., 2021). Environmental variation among years and locations substantially affects alfalfa biomass yield and related traits, further limiting genetic gain and yield improvement (Seiam and Mohamed, 2020; Hakl et al., 2019).

Numerous statistical methods have been developed to analyze GEI and yield stability, reflecting the complex nature of these traits (Reckling et al., 2021). Among them, the genotype plus genotype-by-environment interaction (GGE) biplot approach has been applied widely in multi-environment trials because it effectively visualizes GEI patterns and facilitates the identification of high-yielding and stable genotypes across environments (Yan et al., 2000; Haile and Kebede, 2021).

Yield stability analysis is critical in alfalfa because the forage yield results from repeated harvests over multiple years, where GE interaction is substantial. A key gap is the limited evaluation of year-over-year yield stability, particularly when comparing Iranian and exotic cultivars. Therefore, the objective of the present study was to assess the yield performance and stability of diverse alfalfa genotypes using GGE biplot analysis across four major agro-ecological regions of Iran.

Materials and Methods

Plant material and experimental environments

The forage yield stability was evaluated of nine alfalfa genotypes (four foreign and five Iranian cultivars, as shown in Table 1). The foreign genotypes were introduced through the Value for Cultivation and Use testing program sent by the Seed and Plant Certification and Registration Institute, while the Iranian genotypes were selected from long-established and widely adapted varieties in warm regions, with the Omid genotype being a synthetic cultivar released in 2016, whereas the other Iranian genotypes were from native populations. Experiments were conducted at four agricultural research stations in Karaj, Safiabad (Dezful), Yazd and Jiroft, Iran, over two harvest years (2021–2022), resulting in eight location $\times$ year combinations, hereafter referred to as environments. Location, year and their interaction (location $\times$ year) were considered sources of environmental variation affecting forage yield. Geographic and year-specific climatic data consisting of mean annual air temperature (measured in degrees celsius), total annual rainfall (measured in millimeters) and mean relative humidity (RH, measured as a percentage) for each environment are in Table 2 and the soil physical and chemical properties are in Table 3. Specifically, Karaj (1,321 m above mean sea level, asl, 13–14°C, 323–383 mm, 49–50% RH) is a high-altitude, temperate site, Dezful (82 m asl, 25–26°C, 103–194 mm, 48–51% RH) is a low altitude, hot site, Yazd (1,209 m asl, 21–22°C, 61–120 mm, 30–32% RH) is a high-altitude, arid site and Jiroft (624 m asl, 26°C, 56–189 mm, 38–41% RH) is a low-altitude, warm site. These sites provided contrasting climates, enabling robust evaluation of genotype $\times$ environment interactions and yield stability (Table 2).

Table 1 Characteristics of alfalfa genotypes

Genotype number	Name	Origin	Type of cultivar	Fall dormancy score	Cultivation zone
1	Gea	Italy	Synthetic	7.6	Warm regions
2	Ionian	Cyprus	Synthetic	7.7	Warm regions
3	CUF101	USA	Synthetic	7.9	Warm regions
4	AGNM72	Greece	Synthetic	7.6	Warm regions
5	Omid	Iran	Synthetic	8.5	Warm regions
6	Bami	Iran	Population	8.6	Warm regions
7	Nikshahri	Iran	Population	9.9	Warm regions
8	Baghdadi	Iran	Population	8.8	Warm regions
9	Yazdi	Iran	Population	8.1	Warm regions

Table 2 Geographical location and climatic characteristics of experimental sites across 2021 and 2022 growing seasons

Environment code	Year	Location	Longitude (°E)	Latitude (°N)	Altitude (m asl)	Average temperature (°C)	Annual rainfall (mm)	Relative humidity (%)
1	2021	Karaj	50.99	35.83	1321	13.8	382.6	49
2	2022					13.1	322.1	50
3	2021	Dezful	48.32	32.22	82	26.3	103.4	48
4	2022					25.6	193.7	51
5	2021	Yazd	54.36	31.90	1209	21.8	60.7	30
6	2022					20.9	119.6	32
7	2021	Jiroft	57.85	28.54	624	26	189.3	41
8	2022					26	56.2	38

asl = above mean sea level.

Table 3 Physicochemical properties of soil at research stations

Research station	Texture	pH	EC (ds/m)	Organic matter (%)	Total N (%)	Available P (mg/kg)	Available K (mg/kg)
Karaj	Clay loam	7.2	2.2	0.58	0.06	12.6	256
Dezful	Silty clay loam	7.4	1.1	1.002	0.05	6.43	140
Yazd	Sandy loam	7.8	3.1	0.55	0.04	8.7	135
Jiroft	Sandy loam	7.9	1.8	0.1	0.11	6.2	180

EC = electrical conductivity; N= nitrogen; P = phosphorus; K = potassium.

Experimental design and crop management

The trials were arranged in a randomized complete block design with three replications at each location. Each plot consisted of four rows, 5 m long, with 50 cm row spacing (each plot area was 10 m²). Plots were established without border rows; measurements were taken after removing 0.5 m from both ends of each row to minimize border effects. Replications were spaced 1 m apart and seeds were sown at 25 kg/ha. The first year was considered the establishment period and data were collected during 2021 and 2022.

Agronomic management was standardized across locations. Phosphorus fertilizer (80–120 kg P₂O₅/ha) was applied before planting based on soil test recommendations; nitrogen was not applied after establishment. Irrigation was supplied according to regional alfalfa recommendations to avoid severe water stress. Weed control was conducted mechanically or manually and no major pest or disease outbreaks occurred during the study.

Data collection

Forage was harvested at the 10–20% flowering stage. The number of cuttings varied primarily by location and year (6–10 per year), with Karaj (6, 6 for 2012 and 2022, respectively), Dezful (8, 10), Yazd (10, 10) and Jiroft (8, 10). The cumulative annual yield was calculated by summing all cuttings. Dry forage yield and plant height were recorded for all cuttings. Plant height was measured from the crown to the tallest stem of five randomly selected plants per plot. Fresh forage samples were oven-dried at 70°C for 72 h to determine the dry matter content, while the dry forage yield was calculated on a kilogram per hectare basis. The annual cumulative dry forage yield and mean plant height across harvests were used for statistical and stability analyses, reflecting the perennial and multi-harvest nature of alfalfa production.

Statistical analysis

Combined analysis of variance (ANOVA) was performed using a split-plot-in-time model, with genotypes treated as a fixed factor and years considered as a random factor, whereas locations and replications were treated as random factors. Year was modeled as random because the two years represented a random sample of possible climatic conditions, allowing inference beyond the experimental period (Piepho et al., 2003). Prior to ANOVA, homogeneity of error variances was tested using Bartlett's test (Bartlett, 1937), residual normality and independence were assessed using residual plots and normal probability plots, which confirmed that the ANOVA assumptions were satisfied. Mean comparisons were conducted using Duncan's multiple range test at the 5% significance level. All analyses were performed using the IBM-SPSS software version 22 (IBM Corp.; Armonk, NY, USA). Stability analysis was conducted using the GGE biplot method, based on Equation 1 (Yan et al., 2000):

$$y_{ij} - \mu - B_j = \lambda_1 \xi_{i1} \eta_{j1} + \lambda_2 \xi_{i2} \eta_{j2} + \varepsilon_{ij} \quad (1)$$

where y_{ij} is the mean yield of the i^{th} genotype in the j^{th} environment, μ is the overall mean of all observations, B_j is the main effect of environment j , λ_1 and λ_2 are the singular values corresponding to the first and second principal components (PC1 and PC2), respectively, ξ_{i1} and ξ_{i2} represent the genotype i eigenvectors for PC1 and PC2, respectively, while η_{j1} and η_{j2} are the environment j eigenvectors for PC1 and PC2, respectively, and ε_{ij} is the residual error term.

This model was implemented using the GENESTAT software (VSN International, 2010) and the necessary biplots were generated. To further evaluate yield stability, Shukla's stability variance was calculated using Equation 2 (Shukla, 1972) and the superiority index of Lin and Binns was computed for all genotypes using Equation 3 (Lin and Binns, 1988):

$$\sigma^2 = \frac{p / (p-2) (q-1)) \sum_{j=1}^q (Y_{ij} - Y_i - Y_{.j} + Y_{..})^2}{(SS_{GXE} / (p-1)(p-2) (q-1))} \quad (2)$$

where σ^2 is Shukla's stability variance, Y_{ij} is the observed yield (dry forage yield) of genotype i in environment j , Y_i is the mean yield of genotype i across all environments, $Y_{.j}$ is the mean yield of all genotypes in environment j , $Y_{..}$ is the overall mean yield of all genotypes across all environments, p and q correspond to the number of genotypes and environments, respectively.

Lower values of this statistic indicate greater stability of the genotype.

$$P_i = 1/2n \sum_{j=1}^n (X_{ij} - M_j)^2 \quad (3)$$

where P_i is the superiority index of genotype i , X_{ij} is the yield of genotype i in environment j , M_j is the best (maximum) yield observed in environment j and n is the number of environments.

A smaller superiority statistic indicates that the genotype is closer to being superior and more stable. This statistic is the mean of the squared deviations of the genotype yield from the best yield recorded across all environments, effectively comparing the relative yield of the genotype to the top performers. Thus, having a high mean yield alone is not sufficient; proximity to the highest yield in each environment is also crucial.

Lower values of both statistics indicate greater stability; however, it is acknowledged that a genotype may exhibit high stability with a low mean yield, which is biologically expected and has important implications for breeding decisions.

Results and Discussion

Combined analysis of variance across locations and years

Table 4 presents the results of the combined analysis of variance for the traits studied across the four locations (Karaj, Dezful, Yazd, Jiroft) during the two years (2021, 2022). After confirming the homogeneity of experimental error variances, the combined was performed. The effects of location and year were highly significant ($p < 0.01$) for both plant height and dry forage yield. The significance of year and location effects indicate meaningful difference in the overall means of all genotypes across different environments and years for the traits studied. The effect of genotype on dry forage yield and plant height was highly significant ($p < 0.01$). The observed significant differences could be attributed to the diverse genetic backgrounds and origins of the genotypes evaluated, providing a basis for selecting superior genotypes. The highly significant ($p < 0.01$) genotype $\times$ location interaction for both traits indicated that the genotype ranking varied across environments, suggesting specific rather than general adaptation. Likewise, the highly significant ($p < 0.01$) genotype $\times$ year interaction demonstrated there were differential responses to annual climatic fluctuations, emphasizing the importance of yield stability over time.

Table 4 Combined analysis of variance for studied traits at four locations during 2021–2022.

S.O.V.	df	Plant height(cm)		Dry forage yield (t/ha)	
		F value	p value	F value	p value
Location	3	48.37	0.000	416.90	0.000
Replication (Location)	8	2.73	0.045	1.10	0.432
Genotype(G)	8	10.23	0.000	5.16	0.000
Genotype × Location	24	3.31	0.000	5.78	0.000
Genotype × Replication (Location)	64	1.97	0.004	2.16	0.001
Year	1	11.99	0.009	26.83	0.001
Year × Location	3	44.93	0.000	15.93	0.001
Year × Replication (Location)	8	2.10	0.049	6.19	0.000
Year × Genotype	8	3.06	0.006	4.16	0.000
Year × Location × Genotype	24	3.40	0.000	6.72	0.000
Residual	64	-	-	-	-

df = degrees of freedom.

Furthermore, the highly significant genotype × location × year interaction highlights the complexity of genotype responses, justifying the application of stability and adaptability analyses. Therefore, to ensure the reliable selection of superior genotypes with desirable general and specific adaptability, stability analysis was performed based on the two-way genotype × environment data. The comparison of the mean forage yield of the tested genotypes across all locations and years at the 5% significance level is presented in Table 4. Regarding plant height, genotypes 3, 5 and 8 were the tallest with mean heights of 68.8, 67.8 and 67.8 cm, respectively, while genotype 1 had the shortest height with a mean of 61.5 cm among the genotypes evaluated. In this study, other than CUF101 which was the tallest, the Iranian genotypes exhibited greater plant height than the other foreign genotypes (Table 5). The greater plant height observed in the Iranian

genotypes may be associated with their derivation from traditional landraces shaped by long-term selection within local production systems, in contrast to the foreign cultivars bred under different environmental and management priorities (Annicchiarico et al., 2011). The comparison of mean dry forage yield, considering the significant effects of genotype, genotype × location interaction and the three-way genotype × location × year interaction (Table 4), showed that genotypes 8, 5, 6 and 2, with mean yields of 21.44, 21.34, 21.20 and 21.10 t/ha respectively, all belonged to the same statistical group and did not differ significantly from each other. The observed yield variability, together with significant genotype × environment interactions, has direct agronomic implications, as it affects genotype recommendation and breeding strategies for either broad or specific adaptation. These results reflect contrasting climatic conditions and management regimes

Table 5 Comparisons (mean ± SE) of plant height and dry forage yield among genotypes and locations during 2021–2022.

Genotype number	Name	Plant height (cm)	Dry forage yield (t/ha)
1	Gea	61.5±1.35 ^d	18.68±2.17 ^d
2	Ionian	64.1±1.42 ^c	21.10±2.15 ^{abc}
3	CUF101	68.0±1.74 ^a	20.62±2.16 ^{bc}
4	AGNM72	63.3±1.27 ^c	20.10±2.26 ^{bcd}
5	Omid	67.8±1.96 ^{ab}	21.34±2.59 ^{ab}
6	Bami	67.3±1.96 ^{ab}	21.20±2.77 ^{ab}
7	Nikshahri	64.6±1.70 ^c	19.4±2.45 ^{cd}
8	Baghdadi	67.8±1.68 ^{ab}	21.44±2.52 ^a
9	Yazdi	66.3±1.65 ^b	20.45±2.94 ^{bc}
Mean		65.6±0.56	20.48±0.81
Location		Plant height (cm)	Dry forage yield (t/ha)
Karaj		55.5±0.46 ^c	10.66±0.25 ^c
Dezful		66.3±0.90 ^{ab}	20.25±0.46 ^b
Yazd		69.5±0.58 ^{ab}	28.93±0.91 ^a
Jiroft		71.2±0.98 ^a	22.09±0.38 ^b

Mean±SD within each column followed by different lowercase superscript letters differ significantly ($p < 0.05$), based on Duncan's test.

across locations. Additionally, genotype 1 resulted in the lowest dry forage yield (18.68 t/ha), as shown in Table 5. The comparison of means by experimental locations in Table 5 shows that the highest dry forage yield potential, based on the average of the tested genotypes, was observed in Yazd (28.93 t/ha), while the lowest was in Karaj (10.66 t/ha). The reduced yield in Karaj was likely due to its cooler climate, shorter growing season and fewer annual cuttings, which limit seasonal biomass accumulation compared with warmer regions. Similar yield reductions under cooler environments with limited cutting frequency have been reported for alfalfa (Annicchiarico et al., 2011). Among these four locations, the average plant height of the genotypes was highest in Jiroft (71.2 cm). The lower dry forage yield in Karaj could be attributed to climatic conditions and fewer cuttings compared to the warmer regions.

Stability indices

To evaluate the stability and superiority of alfalfa genotypes, two commonly used statistical indices (Shukla's stability variance and Lin and Binns' superiority coefficient) were employed. The application of these indices resulted in different genotype rankings (Table 6), a pattern that is consistent with previous multi-environment studies in alfalfa and other forage crops and reflects the distinct conceptual foundations of the two methods. Shukla's variance, as a statistical measure, quantifies the contribution of each genotype to the genotype $\times$ environment interaction ($G \times E$) using combined linear models. This index, based on ANOVA, calculates the interaction variance specific to each genotype and considers genotypes with lower values as more stable (Shukla, 1972). Accordingly, in the present study, genotype number 7, with a Shukla's stability variance of 1.00, was identified as the most stable genotype among the nine genotypes evaluated (Table 6; Fig. 1). Genotypes 4, 5 and 1 ranked next in stability, whereas genotype 9, with a value of 12.34, exhibited the most unstable yield. Fig. 1 graphically illustrates this pattern by positioning genotype 7 in the lower-left quadrant, characterized by minimal stability variance but moderate-to-high Pi values, thereby visually distinguishing stability from yield superiority. The high stability but relatively low yield of genotype 7 suggests that this genotype possesses conservative agronomic attributes, such as reduced plasticity in biomass production, limited responsiveness to favorable environments and a more uniform regrowth rate across cuts, which collectively reduce yield fluctuations across environments but also constrain maximum yield potential.

Similar findings have been reported by Sadeghi et al. (2013) and Abdi et al. (2021), who observed that genotypes with minimal $G \times E$ contribution were classified as stable despite not achieving the highest yields. This agreement confirmed that Shukla's index primarily captures yield consistency rather than productivity. Conversely, the Lin and Binns' superiority coefficient (Pi), a yield-based index, calculates the mean squared distance of each genotype yield from the best yield in each environment (Lin and Binns, 1988). In this analysis, genotypes with yields closer to the top genotypes in each environment have lower Pi values and are superior. In the present study, genotypes 5 and 8 had the lowest Pi values (3.77 and 4.18, respectively), ranking first and second in superiority (Table 6), whereas genotype 7, despite its high stability (according to Shukla's index), exhibited a relatively higher superiority coefficient (10.81), as shown in Table 6. In Fig. 1, genotypes 5 and 8 are in the region combining relatively low Pi values with moderate stability variance, indicating their favorable balance between yield performance and adaptability. Their high Pi rankings could be attributed to physiological and agronomic traits of these Iranian local ecotypes, including greater plant height, enhanced regrowth after cutting, higher leaf area index and increased photosynthetic efficiency under variable conditions (Farshadfar et al., 2023). Combined with responsiveness to favorable temperature and moisture, these traits enabled these genotypes to reach near-maximum yields across multiple environments. Consistent with regional studies in the Middle East and Central Asia, local ecotypes generally exhibit superior adaptation and productivity under stress-prone conditions (Kaya et al., 2020; Abdi et al., 2021). The discrepancy between the two indices could be attributed to the fundamental differences in their calculation nature. For example, Shukla's variance considers only the fluctuations of genotypes relative to the $G \times E$ interaction and does not account for absolute yield, whereas the superiority coefficient incorporates the actual genotype yield relative to the best performers in each environment. Genotypes with moderate but stable yield (such as genotype 7) may be rated favorably by Shukla's index but unfavorably by the superiority coefficient. Other studies have confirmed this pattern. For example, Abdi et al., (2021) reported that genotypes identified as stable by Shukla's index did not show the highest yield based on the superiority coefficient in alfalfa. Similarly, in the study by Sadeghi et al (2013) on alfalfa genotypes under drought conditions, it was found that Shukla's stability variance identifies genotypes with lower fluctuations as stable; however, these genotypes may have lower productivity compared to

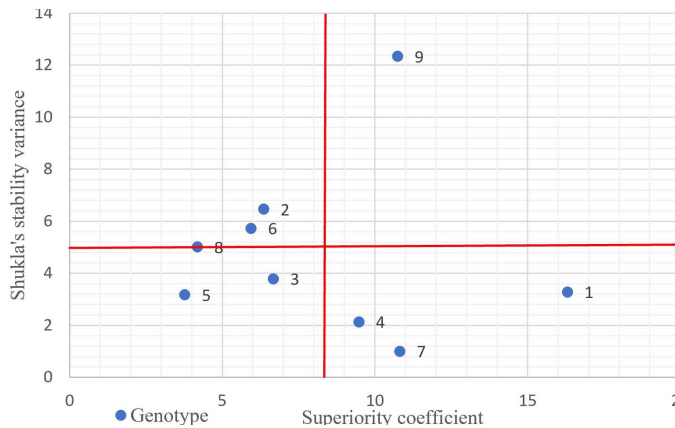


Fig. 1 Comparative analysis of Shukla's stability variance and Lin and Binns' superiority coefficient of alfalfa genotypes for dry forage yield.

highly variable but high-yielding genotypes. A similar study by Kaya et al. (2020) on Mediterranean alfalfa, under heat and drought stress, showed that selecting genotypes based solely on one index (Shukla's variance) may lead to the selection of genotypes with low yield but high stability, which are not necessarily suitable for economic exploitation. In addition, further investigation revealed that the superiority coefficient was more influenced by the mean yield in environments with a wide range of yield, as observed in the present study, where the annual dry forage yield varied from 9.11 to 34.46 t/ha across environments (Table 6), whereas Shukla's variance considers variability around the interaction pattern. This characteristic can be especially valuable in regions with severe climatic fluctuations and a need for predictable yield (such as rainfed systems) in decision-making. From a breeding and agronomic perspective, these findings reinforce other conclusions that no single stability index can adequately capture both yield performance and adaptability. Therefore, consistent with earlier studies, the combined use of both indices provides a more balanced basis for genotype selection. Based on this integrated interpretation, genotypes 5 and 8, which combined low P_i values with acceptable stability, can be recommended for cultivation in both marginal and moderately favorable environments (Fig. 1).

GGE-biplot analysis

Scatter plot

A scatter plot derived from modeling the G effect and GEI effect was constructed for the dry forage yield trait of alfalfa to identify the pattern of genotype classification

Table 6 Comparisons of dry forage yield across the studied environments during 2021-2022, including mean $\pm$ SE, superiority coefficient and Shukla's stability variance.

Genotype number	Name	Environment								Shukla's stability variance	Rank	Superiority coefficient	Rank
		Karaj				Yazd							
		2021	2022	2021	2022	2021	2022	2021	2022				
1	Gea	9.91±0.36	10.95±0.57	16.23±0.58	17.93±0.14	24.50±0.22	27.08±0.45	20.34±0.81	22.48±0.49	3.28	4	16.30	9
2	Ionian	11.49±1.14	10.39±0.93	22.71±0.87	25.10±0.45	28.56±0.62	25.84±0.40	23.47±0.36	21.23±0.51	6.46	8	6.35	4
3	CUF101	11.20±0.57	12.38±0.79	23.08±0.38	20.88±0.51	26.08±0.94	28.83±0.42	20.21±0.58	22.33±0.75	3.79	5	6.67	5
4	AGNM72	10.77±0.75	9.75±0.98	18.87±0.44	20.85±0.33	28.59±0.16	25.87±0.32	24.20±0.32	21.90±0.46	2.13	2	9.47	6
5	Omid	10.00±0.80	11.06±0.71	23.54±0.97	21.30±0.67	32.08±0.77	29.03±0.23	22.96±1.01	20.78±0.66	3.17	3	3.77	1
6	Bami	11.89±1.10	10.75±0.78	17.73±0.68	19.59±0.83	30.78±0.90	34.02±0.41	21.29±0.97	23.53±0.82	5.73	7	5.94	3
7	Nikshahri	10.07±0.21	9.11±0.50	17.43±1.16	19.27±0.64	26.39±1.18	29.16±0.94	23.06±0.51	20.86±0.44	1.00	1	10.81	8
8	Baghdadi	9.54±0.56	10.54±0.42	24.92±0.34	22.54±0.39	30.61±1.13	27.69±0.53	23.99±1.21	21.71±0.92	5.01	6	4.18	2
9	Yazdi	11.56±0.13	10.46±0.32	15.46±0.51	17.08±0.46	31.18±1.00	34.46±0.74	20.61±1.14	22.77±1.03	12.34	9	10.74	7

Calculation of Shukla's stability variance and Lin and Binns' superiority coefficient based on genotype by environment interaction for dry forage yield, respectively.

across environments (Fig. 2). PC1, explaining 56.13% of the variance, represented overall yield performance, while PC2, accounting for 27.71%, reflected yield stability across environments. Together, they accounted for over 83% of the total variance. This high explanatory power confirmed the GGE-biplot's ability to summarize both genotype performance and stability in the present study (Kumar et al., 2024; Sheikh et al., 2024).

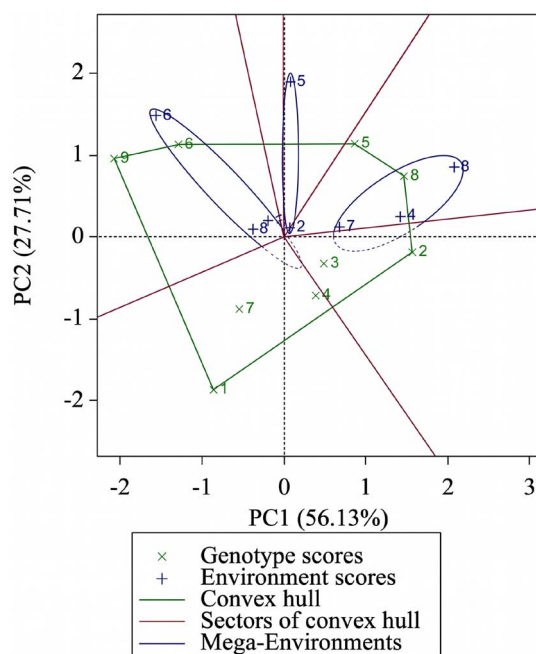


Fig. 2 Principal component (PCs, total = 83.84%) scatter plot of alfalfa genotypes and experimental environments to identify superior genotypes across mega-environments for dry forage yield in alfalfa.

A polygon was drawn by linking genotypes far from the origin such that all genotypes were enclosed within this polygon. Vertical red lines were sketched from the origin to each side of the polygon, dividing the genotypes into four sectors. Genotypes near the origin of the axes, such as genotype 3, exhibited moderate but stable yield across most environments, indicating low sensitivity to environmental variation. Conversely, genotypes at the corners of the polygon and distant from the center showed either the highest or lowest yield in specific environments and are often introduced as special candidates for targeted environments (Bakheit et al., 2022; Robins et al., 2024). The model classified the environments into three mega-environment groups, likely reflecting similar temperature, rainfall and cutting frequency in each group. For example, environments 3, 4 and 7, located in the upper-right section of the biplot,

formed a cluster with high-yielding conditions. In line with the observations of Berhanu et al. (2023) and Wodebo et al. (2024), such environmental classification enhanced the accuracy of selecting superior genotypes across different geographic regions. Genotypes 2, 5 and 8, positioned close to this cluster, exhibited high forage yield, possibly due to traits such as vigorous regrowth, good agronomical management and efficient nutrient utilization (Yang et al., 2009; Gonçalves et al., 2025; Lv et al., 2024). Genotype 6, at the vertex of a sector comprising environments 6, 8 and 1, demonstrated good adaptation to these specific environmental conditions. Genotype 1, positioned farthest from the center and at the vertex of an independent sector, demonstrated the lowest yield (18.64 t/ha; Table 4) and high instability, likely due to limited genetic adaptability and susceptibility to environmental stressors. Although genotype 7 exhibited acceptable stability within the polygon, it showed low yield and no specific adaptation, reflecting genetic constraints that limit performance under varying environments.

Overall, the scatter plot of the nine alfalfa genotypes evaluated across eight environments illustrated clear patterns of yield and stability, identifying specific candidates for targeted introduction and supporting practical breeding strategies.

Ranking plot

Fig. 3A illustrates the genotype ranking plot. The blue vectors represent the direction of genotype ranking based on mean dry forage yield and stability. The arrow corresponds to the average environment coordination (AEC) vector; genotypes positioned along this vector and farther from the origin are considered desirable for both yield and stability. In the present study, genotype 5 was closest to the tip of the AEC arrow and approximately 0.86 t/ha above the overall mean yield (Table 5), indicating its absolute superiority in dry forage yield and stability across diverse environments. Conversely, genotypes 6 and 9, positioned farther from the AEC axis, exhibited lower yield and greater instability, with yields of 21.20 t/ha and 20.45 t/ha, respectively. Among the test environments, environments 1, 2 and 7, located near the origin and the AEC axis, were representative and moderately discriminating, providing baseline information on genotype performance. In contrast, environments (such as environment 5) distant from the AEC axis, offer limited differentiation for ranking genotypes (Derbew et al., 2024; Kona et al., 2024).

The GEI analysis focusing on environment evaluation (Fig. 3B) demonstrated that environments 3 and 4, positioned along the AEC axis and farthest from the origin, had high discriminating

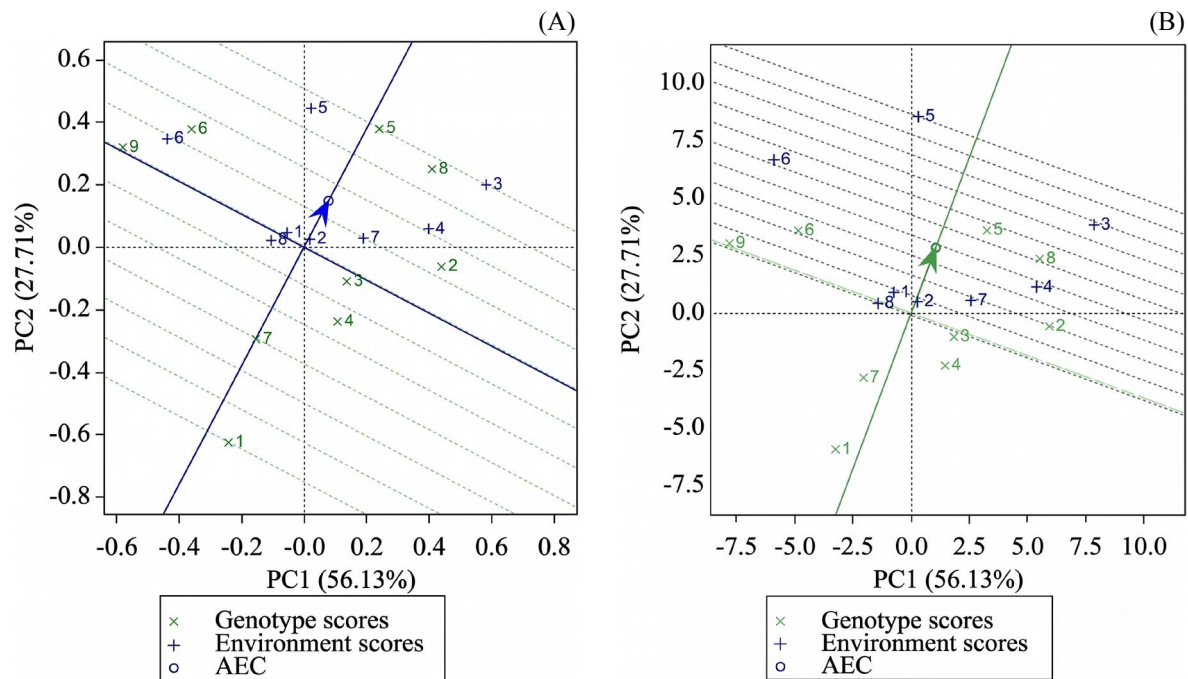


Fig. 3 Ranking biplot of principal components (PCs, total = 83.84%) for evaluating discriminative ability and selection efficiency of studied genotypes and environments for dry forage yield in alfalfa: (A) genotypes using Method = Environment; (B) environments using Method = Genotype, where AEC = Average Environment Coordination.

power, making them most suitable for selecting superior genotypes (Malosetti et al., 2013). In particular, environment 3, being the farthest from the origin, was the most informative for screening high-yielding genotypes. In contrast, environments 1 and 7, while low in discriminating ability, were representative of typical growth conditions and may be maintained in future trials to capture general environmental effects, though they are less useful for selection purposes (Ma et al., 2024).

These findings have practical implications for breeding programs. Genotype 5 is a strong candidate for release or targeted environments, while environments 3 and 4 should be prioritized as primary testing sites in future studies to maximize selection efficiency.

Ideal genotypes and environments

Identification of ideal genotypes and environments is a key advantage of graphical GGE-biplot analysis. Fig. 4 illustrates genotype comparison with the ideal genotype (Fig. 4A) and environment comparison with the ideal environment (Fig. 4B), based on combined G and GEI effects. The ideal genotype represents a conceptual benchmark combining high mean yield and stability (Yan and Tinker, 2006).

Genotypes located closer to the center of the concentric

AEC circles show superior performance, reflecting both high yield and stability. In the present study, genotypes 5 and 8 were closest to the ideal genotype position, indicating favorable performance across environments. Genotype 5, which also had a high mean dry forage yield in Table 5 (21.35 t/ha), was nearest to the ideal point, confirming its superiority in both yield and stability and supporting its recommendation for release and commercialization. In contrast, genotype 1 was positioned far from the ideal position and at a sharp angle relative to the AEC vector, indicating high yield variability and instability, consistent with its lowest mean yield (18.68 t/ha) reported in Table 5. Similar studies have demonstrated the effectiveness of this approach for identifying well-adapted, high-yielding alfalfa genotypes (Tian et al., 2023) and Greveniotis et al. (2024) emphasized that combining high yield with stability is a critical criterion for cultivar selection in breeding programs.

Environmental evaluation based on the ideal environment concept is presented in Fig. 4B. Vector length represents discriminating ability, whereas the angle with the AEC axis reflects representativeness of the target environment. Among the test environments, environments 5, 3 and 4 were positioned closest to the center of the concentric circles, indicating favorable combinations of discrimination and

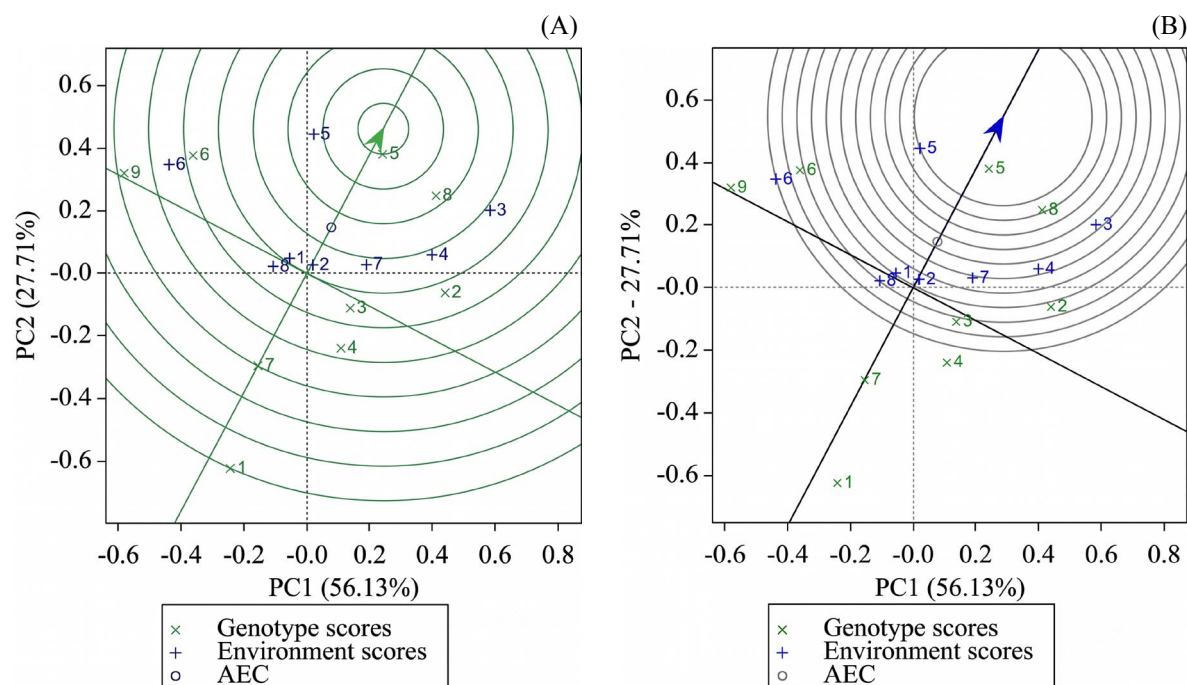


Fig. 4 Comparison biplot of principal components (PCs, total = 83.84%) for evaluating for dry forage yield in alfalfa of (A) genotypes and identification of ideal genotype; (B) relation among the environments, where AEC = Average Environment Coordination.

representativeness. Environment 5, located nearest the center, was identified as the ideal environment for genotype evaluation, while environments 3 and 4 also showed long vectors with small AEC angles, suggesting strong discriminating power and high relevance to target conditions.

Rather than relying on a single site, the combined use of environments 5, 3 and 4 is recommended for multi-location testing, as this strategy balances efficient genotype discrimination with representativeness of diverse growing conditions. In contrast, environment 6 had a large angle relative to the AEC axis, indicating poor alignment with genotype stability and limited usefulness for selection, although it may still capture specific stress-related responses. Similar conclusions have been reported by Yan et al. (2001) and Yan and Holland (2010), who noted that environments with long vectors and small angles relative to the AEC axis were most effective for indirect selection.

Overall, these results provide clear guidance for breeding programs. Genotype 5 is a strong candidate for release, while environments 5, 3 and 4 should be prioritized as primary testing locations to improve selection efficiency for yield and stability.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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