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Yield stability and forage quality of *Festulolium* cultivars across six environments in Central Anatolia: an AMMI and GGE biplot approach

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Abstract

Background Genotype × environment interaction (GEI) is a key determinant of cultivar performance in perennial forage systems, yet stability data for *Festulolium* under semi-arid continental climates remain scarce. This three-year study (2022–2024) evaluated yield stability, forage quality, and adaptability of five *Festulolium* cultivars grown under irrigation at two contrasting locations (Ankara, Kayseri) in Central Anatolia, Türkiye.

Methods A randomized complete block design with four replications was used, and stability was assessed through Additive Main Effects and Multiplicative Interaction (AMMI), Genotype plus Genotype × Environment (GGE) biplot, Eberhart–Russell regression, AMMI Stability Value (ASV), and Genotype Selection Index (GSI). Plant height, crude protein, acid detergent fiber, neutral detergent fiber, Ca, K, Mg, P, fresh forage yield, dry matter yield (DMY), and crude protein yield were determined.

Results Environment was by far the dominant source of variation (~ 96% of the treatment sum of squares for the yield traits); the pooled genotype × environment interaction was not statistically significant for the yield traits but AMMI concentrated it in a significant first interaction principal component (IPCA1), whereas for the forage-quality traits (crude protein, ADF, NDF) GEI was statistically significant. GGE biplot analysis identified Achillis as the highest-yielding cultivar, whereas Perseus (ASV = 22.7) and Lofa (ASV = 31.8) showed the lowest interaction with the environment. Eberhart–Russell regression revealed broad adaptability for Lofa (bi = 1.07) and Perseus (bi = 1.03), while Achillis (bi = 1.13) responded strongly to favorable environments. Achillis and Lofa are recommended on the basis of their combined mean dry matter yield and ASV-based stability. Most macromineral concentrations met ruminant requirements, with K reaching 4.30% at Kayseri; Mg, however, declined to ~ 0.09% by the third year at Kayseri, falling below the commonly accepted adequacy threshold and indicating a possible need for supplementation. These patterns reflect strong environmental control over forage mineral composition.

Conclusions Achillis and Lofa are recommended for irrigated *Festulolium* production in Central Anatolia.

Keywords *Festulolium*, AMMI analysis, GGE biplot, Genotype × environment interaction, Yield stability, Forage quality, Eberhart–Russell, Central Anatolia

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Background

Animal-based protein intake, a fundamental pillar of healthy and balanced nutrition, depends on the development of livestock production and the reduction of the roughage deficit. Bridging this gap is possible through the improvement of forage areas and the enhancement of forage production [1]. Changing climatic conditions necessitate the adoption of novel methods and environmentally adapted cultivars to sustain and increase agricultural productivity [2].

One of the consequences of climate change is the deterioration of pasture vegetation and grassland quality, leading to significant adverse effects on forage production for livestock [3]. In the coming decades, severe summer droughts and irregular precipitation distribution are expected to intensify [2]. Therefore, the development of cultivars suitable for future cultivation is of paramount importance. Under the increasingly dry conditions of semi-arid regions, drought-tolerant perennial forage grasses are central to sustaining forage supply, motivating the search for cultivars that combine productivity with stability.

Lolium perenne L. occupies a dominant position among European forage grasses owing to its high regrowth capacity, rapid establishment, tolerance to frequent cutting, and high nutritive value [4]. Species within the genus *Festuca* generally exhibit superior stress tolerance. Because *Festuca* and *Lolium* are closely related, they can be successfully hybridized [5, 6]. Intergeneric hybridization was initiated over 100 years ago and has resulted in nearly one hundred cultivars [2].

Festulolium hybrids combine the high yield, quality, palatability, digestibility, and rapid growth of *Lolium* with the winter hardiness, drought tolerance, and disease resistance of *Festuca* [7–10]. *Festulolium* is valued for its rapid germination, weed suppression, excellent winter hardiness, high regrowth capacity, good digestibility, and high protein content [11–13]. Studies by Staniak and Harasim [14] and Staniak [15] demonstrated that drought stress affects yield more than nutritive value in *Festulolium*. For the semi-arid continental conditions of Central Anatolia, summer drought tolerance under irrigation and adequate winter survival are both relevant; in the present trials winter survival governed persistence and regrowth, whereas summer performance depended on irrigation.

Multi-environment trials are essential for evaluating cultivar performance under diverse conditions. The genotype × environment interaction (GEI) is a critical factor in plant breeding, as it can mask the true genetic potential of genotypes and complicate cultivar selection [16]. The Additive Main effects and Multiplicative Interaction (AMMI) model partitions the GEI into principal components and provides a powerful tool for identifying stable and adapted genotypes [17]. The GGE biplot methodology further facilitates visual assessment of cultivar performance, mega-environment identification, and cultivar recommendation [18, 19]. Eberhart and Russell [20] stability parameters, including regression coefficient (bi) and deviation from regression (S^2di), remain widely used for characterizing cultivar adaptability. These approaches are complementary rather than redundant: AMMI partitions the interaction into interpretable axes and, with the AMMI Stability Value (ASV), quantifies each genotype's contribution to it; the GGE biplot visualizes which-won-where patterns and mega-environment structure; the Eberhart–Russell regression characterizes the type of adaptation (general versus specific) through bi and S^2di; and the Genotype Selection Index (GSI) combines yield and stability into a single ranking. Their joint use therefore provides a more complete and cross-validated assessment of stability and adaptation than any single method. Despite this methodological progress and the promise of *Festulolium* for semi-arid regions, its yield stability and adaptation under the continental, semi-arid conditions of Central Anatolia have not yet been assessed in a multi-environment framework, and it remains unknown whether the available cultivars are broadly adapted or environment-specific in this region.

The objective of this study was to evaluate five *Festulolium* cultivars for herbage yield, forage quality, and yield stability using AMMI analysis, GGE biplot analysis, and Eberhart–Russell stability parameters across six environments (two locations × three years) under irrigated conditions in Central Anatolia. Specifically, the study aimed to identify broadly adapted versus environment-specific cultivars suitable for irrigated forage systems in semi-arid regions. We hypothesized that environmental variation would dominate total variation, that the cultivars would differ in stability and adaptation, and that different cultivars would be favored at the two locations, reflecting location-specific adaptation within Central Anatolia.

Methods

Plant material and experimental sites

Five *Lolium*-type *Festulolium* cultivars (Achillis, Lofa, Hostyn, Perseus, and Perun) were evaluated at Ankara and Kayseri in Central Anatolia, Türkiye, over three consecutive years (2022–2024). The Ankara experimental soil was non-saline, clay-loam, slightly alkaline, moderately calcareous, low in phosphorus and organic matter, and rich in potassium. The Kayseri soil was non-saline, sandy-loam, slightly alkaline, non-calcareous, and low in phosphorus and organic matter. Long-term and experimental-year climate data are presented in Table 1. All five cultivars are ryegrass-type (*Lolium*-type) × *Festulolium* cultivars bred and maintained by DLF Seeds, with breeding based in the Czech Republic. Achillis, Hostyn and Perun are derived from Italian ryegrass × meadow fescue

Table 1 Climate data for Ankara and Kayseri trial locations during 2022–2024 and the long term*

Months	Monthly total precipitation				Monthly mean temperature (°C)				Monthly mean relative humidity (%)			
	Long term mean	2022	2023	2024	Long term mean	2022	2023	2024	Long term mean	2022	2023	2024
Ankara												
January	40	36.2	3.9	49.1	0.3	0.9	3.9	4.1	77.2	72.6	66.9	76.6
February	34.9	41.5	20.3	13.3	1.8	4	2.7	7	72.5	72.6	62.4	64
March	39.6	30.7	66	25.8	5.8	2.3	8.2	8.5	64.4	59.5	68.2	62.2
April	41.7	3.2	36.4	34.5	11.3	14.1	11	16.9	58.2	43.2	62.5	46.6
May	52	12.8	100.3	104	16.1	16.6	15.8	15.7	56.7	49.6	62.4	61.1
June	36.9	100	93.9	61.7	20	20.9	19.9	24.7	51.9	58.7	64.7	42.7
July	14.1	20.5	1.1	20.8	23.4	23.1	25	25.6	44.1	47.5	40.4	43.6
August	12.6	25.7	0	5.2	23.5	26.2	28.3	25.7	42.8	46	32.9	40.3
September	17.8	3.4	27.7	9.1	19	20.5	21	21.2	47.7	42.8	45.7	49.8
October	26.8	14.3	2	2.1	13.2	13.7	15.9	14.1	58.4	60.7	51.4	52
November	31.6	9.5	73.5	17.6	7.3	10	10.7	7.6	69.6	61.7	73.1	66.1
December	44.3	22.3	23.9	48.4	2.6	6.3	6.9	4.6	77.7	78.9	80.3	81.4
Mean/Total	392.3	320.1	449	391.6	12	13	14	15	60.1	57.8	59.2	57.2
Kayseri												
January	35.2	14.8	2.6	30.4	−1.7	−1	1.4	2.2	76.6	62.7	70.2	74.7
February	36.5	5	3.3	7.7	0.1	1.5	−1.4	4.9	74	70.8	68.3	62.9
March	41.8	32.3	22.4	42.4	4.7	3.8	6.6	5.6	68.3	75.3	67.6	68.4
April	52.1	23.1	37	17.4	10.6	13.4	9.5	14.3	62.3	46	64.5	54.4
May	51.8	72.1	20.9	67.3	15	13.8	13.5	13.6	61.2	60.7	61.8	66.1
June	39.5	82.9	42.7	14.1	19	19.9	17.7	22.2	55.8	54.5	65.3	44.5
July	10.5	0.6	73.4	23.7	22.2	21.6	21.7	22.9	49.5	46.9	43.9	49.9
August	8.8	0	7.5	8.5	22	25.1	24.8	23.2	49.2	40.9	35.6	42.7
September	15	0	27.3	5.5	17.3	18.5	18.1	18.2	54.1	71.8	47.5	65.5
October	28	17.9	15.4	0.5	11.8	12.4	12.9	12.1	63.6	66.3	60.6	65.5
November	32.4	13.4	12.5	11	5.5	8	8.2	7.5	71.7	72.5	67.6	65
December	37.4	16.9	9	15.5	0.6	3.9	5.1	3.5	77.1	79.75	71.5	70
Mean/Total	389	279	274	244	10.6	11.7	11.5	12.5	63.6	62.3	60.4	60.8

* General Directorate of Meteorology, Ministry of Environment, Urbanization and Climate Change of the Republic of Türkiye

(*Lolium multiflorum* × *Festuca pratensis*), Perseus from the reciprocal meadow fescue × Italian ryegrass cross, and Lofa from Italian ryegrass × tall fescue (*L. multiflorum* × *F. arundinacea*); all are tetraploid (2n = 4x = 28). Because the relative contributions of the *Lolium* and *Festuca* genomes are cultivar-specific and not fully quantified for these cultivars, only the parental cross is reported here [21].

Experimental design

The experiment was established in autumn 2021 using a randomized complete block design with four replications at both locations. Seeds were hand-sown on 20 October 2021 at 2–3 cm depth in six rows per plot. Row length was 5 m with 25 cm inter-row spacing and 50 cm between blocks. Three harvests were taken in 2022 and 2023 at both locations. In 2024, three harvests were obtained at Kayseri and two at Ankara. The third (autumn) harvest could not be obtained at Ankara in 2024 because higher summer and early-autumn temperatures at this site (June–September means ~ 2.5–3 °C above Kayseri; Table 1), together with low mid-summer rainfall, accelerated senescence and limited post-second-cut regrowth, so the stand did not reach a harvestable third cut; at the cooler Kayseri site, sufficient autumn regrowth was obtained for three harvests. As a result, the 2024 annual cumulative FFY, DMY and CPY at Ankara were based on two harvests rather than three. The Ankara-2024 environment mean is therefore expected to be somewhat lower than it would have been with a third harvest, and this environment enters the AMMI and GGE analyses with a correspondingly reduced mean. Because all cultivars in Ankara-2024 were harvested on the same two occasions, however, the genotype ranking within that environment is unaffected; the missing harvest influences the position of the environment rather than the relative performance of the cultivars, and is acknowledged as a source of environmental rather than genotypic variation. Fertilization consisted of 100 kg ha^{-1} P_2O_5 and 50 kg ha^{-1} N at sowing, with 50 kg ha^{-1} N after each harvest. The trial area was irrigated by sprinkler. Before planting, soil samples were taken from the experimental fields to determine soil-moisture constants, including field capacity and wilting point, available water-holding capacity (AWC = FC – WP) and bulk density (γs). Soil moisture content was monitored throughout the growing season using tensiometers installed and calibrated in the plots, and irrigation was applied to restore the soil to field capacity whenever 50% of the available water-holding capacity had been depleted. The gross depth of irrigation water required to return the soil to field capacity was calculated following Doorenbos and Pruitt [22] as $d = [(FC - CWC)/100] \times \gamma s \times D/Ea$, where d is the gross irrigation depth (mm), FC the field capacity (%), CWC the current soil water content (%), γs the bulk density (g cm^{-3}), D the soil depth (mm) and Ea the application efficiency, taken as 75% for the sprinkler system. Irrigation was scheduled from soil-moisture status independently of the number of cuts and was applied identically at both locations, with approximately six irrigations per growing season at each site. At Ankara in 2024, irrigation was continued after the second cut in an attempt to obtain a third cut, but the stand did not regrow owing to early stand ageing, so no third cut was obtained despite continued irrigation. The average annual gross irrigation applied to *Festulolium* was approximately 490 mm at Kayseri and 700 mm at Ankara under the prevailing climatic conditions of each site. This soil-moisture-based scheduling approach follows the same principle as sensor-driven irrigation scheduling systems that maintain soil water close to field capacity [23]. Plots were established as pure stands at a seeding rate of 25 kg ha^{-1}.

Measurements

Fresh forage yield (FFY) was determined at the 10% heading stage. Dry matter yield (DMY) was determined by drying 500 g samples at 70 °C for 48 h. Crude protein yield (CPY) was calculated as DMY × (CP/100). Dried samples were ground in a laboratory mill to pass a 1 mm sieve. Crude protein (CP), ADF, NDF and the mineral concentrations (Ca, K, Mg, P) were all determined by near-infrared reflectance spectroscopy (NIRS) using a FOSS NIRSystems Model 6500 instrument with WinISI II v1.5 software and the manufacturer-developed IC0904FE calibration package [24]. Because a proprietary, manufacturer-supplied commercial calibration was used, the corresponding calibration and validation statistics (e.g., R^2, SEC and SEP) reside with the provider and were not available to the authors. Crude protein values from this commercial calibration were validated against the Kjeldahl reference method on the first-cut samples of the first year at both locations ($n = 40$). The two methods were strongly correlated (NIRS = 1.02 × Kjeldahl – 0.54; $R^2 = 0.91$, $r = 0.95$): the regression slope close to unity indicates negligible proportional bias, whereas the small negative intercept reflects a minor systematic offset, with NIRS underestimating crude protein by approximately 0.4% relative to Kjeldahl. Given this strong and consistent agreement, the NIRS values were retained and the remaining cuts and years were analysed by NIRS alone.

Statistical and stability analyses

Analysis of variance (ANOVA) for individual locations and the combined data across locations were performed using JMP Pro (Version 17, SAS Institute Inc., Cary, NC, USA). Means were compared using Tukey's Honestly Significant Difference (HSD) test at $P \le 0.05$. In the combined analysis, the six location × year combinations were treated as environments and, consistent with the

fixed-effects basis of the AMMI and GGE models, genotypes and environments were regarded as fixed effects, while replications (blocks) within each environment were treated as random. The genotype × environment interaction was the term of primary interest and was partitioned into interaction principal component axes by AMMI. The yield traits (FFY, DMY and CPY) were expressed as annual cumulative values per location × year environment for the combined and stability analyses, whereas the forage quality and mineral traits, which were determined at each harvest, were analysed with harvest included as an additional factor (location × year × harvest), together with genotype. Each location × year was analysed as an independent environment; the correlation among the three annual observations from the same perennial plots was not explicitly modelled, which is acknowledged as an assumption of the analysis. Prior to the combined analysis, Bartlett's test of homogeneity of error variances was performed. The test indicated significant heterogeneity of error variances among environments (for DMY, $\chi^2 = 56.9$, df = 5, $P < 0.001$), as expected given the large differences in productivity among the six environments. Because the experimental design was fully balanced (four replications in each environment) and the AMMI and GGE analyses applied here are primarily descriptive multiplicative procedures, the combined analysis was retained, following the procedures for the combined analysis of multi-environment data described by McIntosh [25].

Six environments were defined as factorial combinations of two locations and three years (Ankara-Y1, Ankara-Y2, Ankara-Y3, Kayseri-Y1, Kayseri-Y2, Kayseri-Y3).

Genotype × environment interaction (GEI) was assessed using the Additive Main effects and Multiplicative Interaction (AMMI) model [16, 17], and the Genotype plus Genotype × Environment interaction (GGE) biplot methodology [18, 19]. Both AMMI and GGE biplot analyses were performed using the "metan" package [26] in R software (Version 4.3.2; R Core Team [27]). GGE biplots were constructed based on singular value decomposition (SVD) of the environment-centered data matrix with symmetric scaling (Scaling = 0, Centering = 2, SVP = 2).

The AMMI Stability Value (ASV) was calculated following Purchase et al. [28] as:

$$\mathrm{ASV} = \sqrt{\lfloor (\mathrm{SS}_{\mathrm{IPCA1}}/\mathrm{SS}_{\mathrm{IPCA2}} \times \mathrm{IPCA1\ score})^2 + (\mathrm{IPCA2\ score}^2) \rfloor}$$

where lower ASV indicates greater genotype stability across environments.

Eberhart and Russell [20] stability parameters, including regression coefficient (b_i), deviation from regression (S^2d_i), and coefficient of determination (R^2), were calculated for each cultivar across environments. A cultivar with $b_i = 1.0$ is considered broadly adapted, $b_i > 1.0$ indicates above-average responsiveness to favorable environments, and $b_i < 1.0$ indicates specific adaptation to less favorable environments.

The Genotype Selection Index (GSI) was calculated following Farshadfar [29] as GSI = Mean Rank + ASV Rank, where a lower GSI value indicates a desirable combination of high yield and stability.

Results

Plant height

Plant height varied with location, year and harvest. Averaged across all environments and harvests, cultivar means were similar (Hostyn 64.2 cm, Achillis 63.8 cm, Perun 62.7 cm, Perseus 59.6 cm, Lofa 59.1 cm); the dominant effect was location, with plants substantially taller at Ankara (79.8 cm) than at Kayseri (45.9 cm). Tall single values (up to ~ 105 cm for Achillis at Ankara) occurred only in the most favourable environment–harvest combinations (Fig. 1).

Crude protein content

Protein content ranged from 7.52% to 12.69% across all environments and showed a declining trend over the years at both locations. At Ankara, Hostyn and Perun generally recorded the highest protein values. At Kayseri, Achillis showed the highest value in year 2 (11.87%) (Fig. 2). During the first two years of the study, protein content increased with successive harvests at both locations.

ADF and NDF

All ADF values generally ranged between 24 and 34%, and remained below the 35% acceptability threshold (Fig. 3). ADF values generally decreased in the third harvests of years 1 and 2. NDF values ranged between 48 and 64% and rose sharply in year 3, particularly at Kayseri (exceeding 64%). At Ankara, NDF values remained more stable across the three years, with the highest NDF concentrations observed in the first harvests and lower values in subsequent harvests, consistent with the typical pattern of decreasing fiber content following early-season cuts.

Mineral content (Ca, K, Mg, and P)

Mineral concentrations varied substantially between locations, years, and harvests. Calcium (Ca) content ranged from approximately 0.22% to 0.78%, with Ankara generally exhibiting higher values than Kayseri, particularly during the first year. Potassium (K) content was consistently higher at Kayseri (up to 4.30%) than at Ankara (2.65–3.83%), but declined sharply in the third year at Kayseri. Magnesium (Mg) followed a similar declining

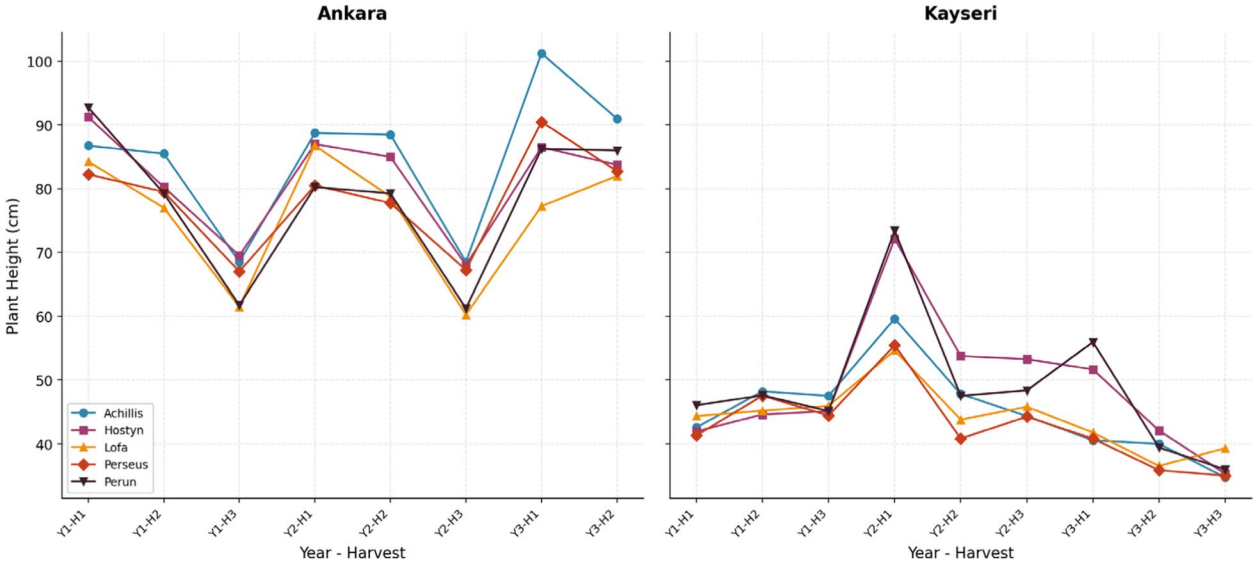


Fig. 1 Plant height (cm) of five Festulolium cultivars across harvests at Ankara and Kayseri (Y: Year, H: Harvest)

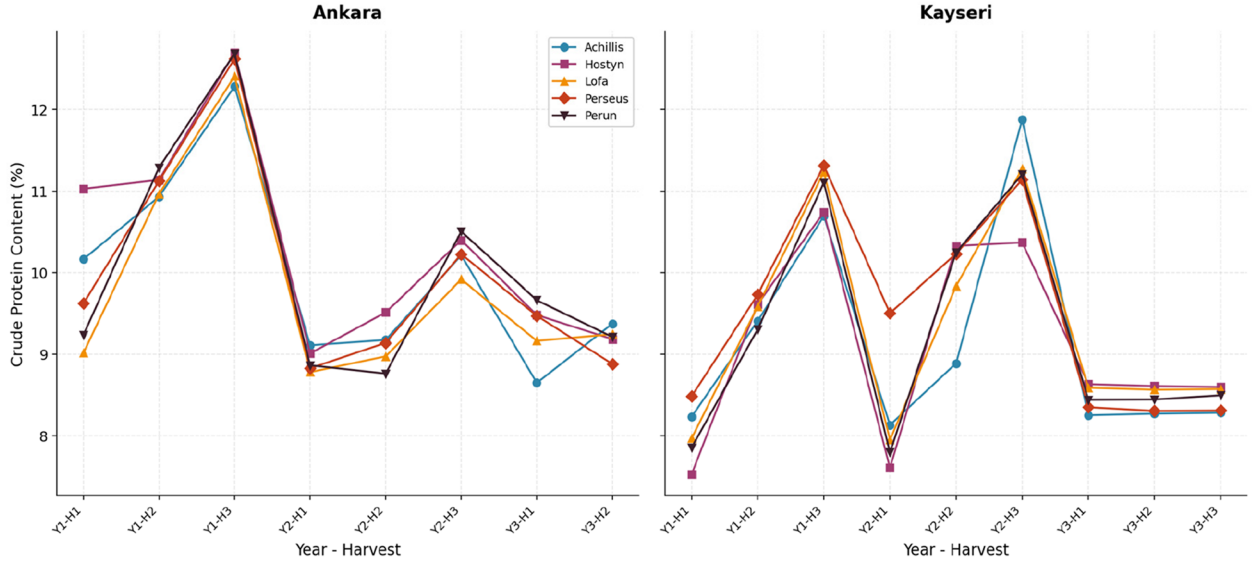


Fig. 2 Crude protein content (%) across harvests at Ankara and Kayseri (Y: Year, H: Harvest)

pattern in Kayseri, dropping to approximately 0.09% in the third year, while Ankara values remained in the 0.13–0.30% range throughout the trial. Phosphorus (P) content was the most stable parameter, ranging from 0.35% to 0.50% across both locations and all harvests, with values generally decreasing in the third year. Among cultivars, Perun and Perseus often showed slightly higher Ca and Mg values at Ankara, whereas all five cultivars exhibited similar patterns in Kayseri, indicating a stronger environmental than genotypic effect on mineral composition (Fig. 4). Combined ANOVA confirmed that environment dominated all minerals (Ca 96.0%, K 84.1%, Mg 95.2%, P 96.8% of treatment sum of squares); the genotype × environment interaction was not significant for Ca ($P = 0.443$), Mg (0.489), P (0.234) or K (0.074), indicating that mineral composition was environmentally controlled rather than cultivar-specific.

Yield performance

Annual DMY ranged from 5,454 to 20,815 kg ha^{-1} at Ankara and from 8,399 to 13,097 kg ha^{-1} at Kayseri (Fig. 5). The highest annual FFY, DMY and CPY were obtained in year 2 at Ankara location for all cultivars (Figs. 5, 6 and 7). The mean annual DMY across all cultivars at the Ankara location was highest in the second year (Fig. 8). In Ankara, based on cumulative annual yield, Lofa ranked first in year 1, Achillis in year 2, and Perun in year 3. In Kayseri, Achillis ranked first in year 1

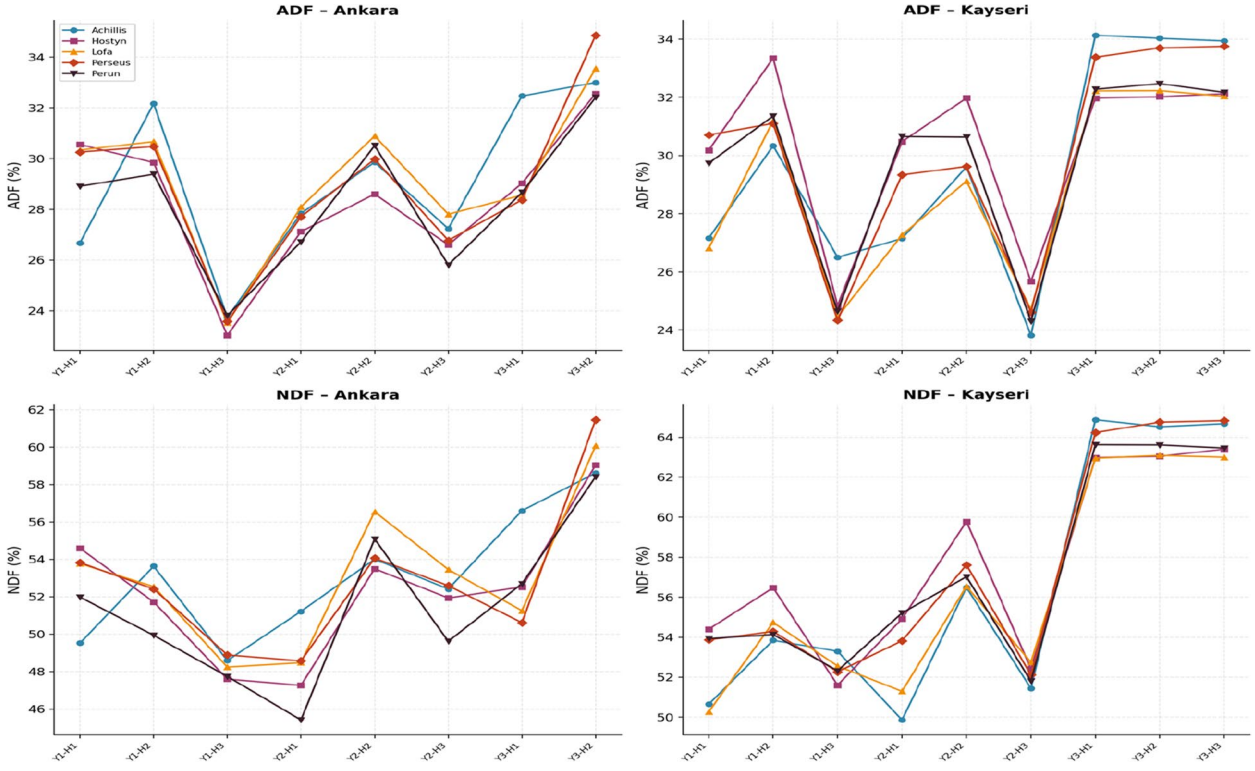


Fig. 3 ADF and NDF content (%) across harvests at both locations (Y: Year, H: Harvest)

and Hostyn in year 2, while Perun showed superior performance in year 3. Across the six environments, Achillis recorded the highest mean DMY (12,278 kg ha^{-1}), 8.7% higher than the lowest-yielding cultivar, Perseus (11,300 kg ha^{-1}).

AMMI Stability Analysis

The AMMI analysis partitioned the GEI into interaction principal component axes. For DMY, the first two axes together explained 86.2% of the GEI sum of squares (IPCA1 = 62.4%, Gollob F = 2.26, $P = 0.033$; IPCA2 = 23.8%, $P = 0.345$), with IPCA1 capturing the majority of the structured interaction. This pattern is statistically coherent: because AMMI concentrates the structured portion of the interaction into the first multiplicative axis, IPCA1 can be significant even when the pooled GEI term is not, as the pooled F-test is diluted by the unstructured (noise) component of the interaction. The AMMI Stability Value (ASV) ranked cultivars from most to least stable: Perseus (22.7) > Lofa (31.8) > Perun (41.5) > Hostyn (52.8) > Achillis (54.5). Because the yield and stability rankings were almost perfectly inverse, the Genotype Selection Index (GSI) did not differentiate the cultivars (all GSI = 6); cultivar recommendation was therefore based primarily on mean dry matter yield together with ASV-based stability rather than on GSI alone (Table 2). In this framework, stability is defined by the proximity of a genotype's IPCA1 score to the origin (least interaction), summarized quantitatively by the ASV, whereas the GSI integrates ASV rank with mean-yield rank to balance stability and productivity. The AMMI partitioning of the interaction, with the significance of each axis, is summarized in Table 3.

AMMI biplots

The AMMI1 biplot (mean performance vs. IPCA1) for DMY revealed the relative positions of genotypes and environments along the yield and interaction axes. Genotypes positioned near the horizontal zero line of IPCA1 with high mean values represent stable, high-yielding cultivars. Achillis showed the highest mean yield but with moderate IPCA1 deviation, while Perun demonstrated moderate yield with intermediate stability (Fig. 9).

A similar pattern was observed for crude protein yield, with Achillis showing the highest mean CPY and similar IPCA1 deviation across environments (Fig. 10).

The AMMI2 biplot (IPCA1 vs. IPCA2) displayed the interaction patterns between genotypes and environments. Genotypes closer to the origin showed less interaction with environments, indicating broader stability. Because the AMMI2 biplot depicts interaction patterns rather than mean yield, environments are described here by their interaction behaviour, while yield ranking of environments is derived separately from environment mean yields and AMMI1 main effects; Ankara-Y3

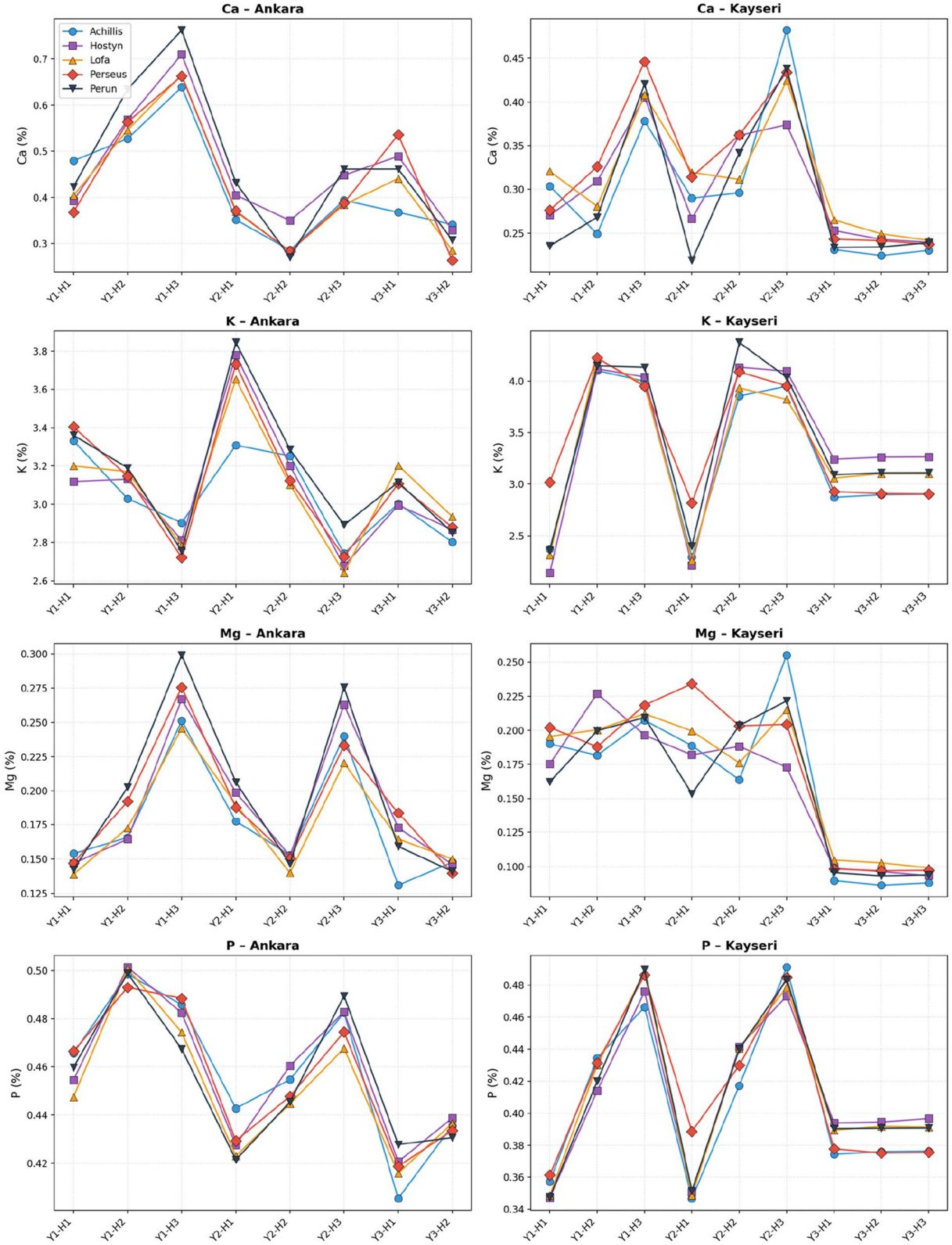


Fig. 4 Ca, K, Mg and P content (%) of Festulolium cultivars across harvests at Ankara and Kayseri (Y: Year, H: Harvest)

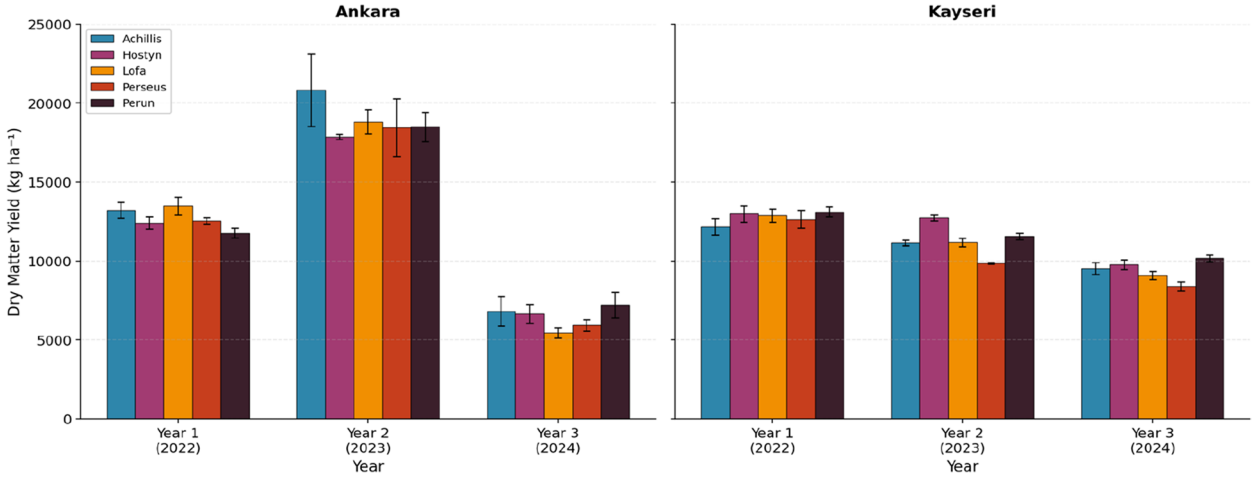


Fig. 5 Annual cumulative dry matter yield (kg ha^{-1}) at Ankara and Kayseri. Error bars: ± SE

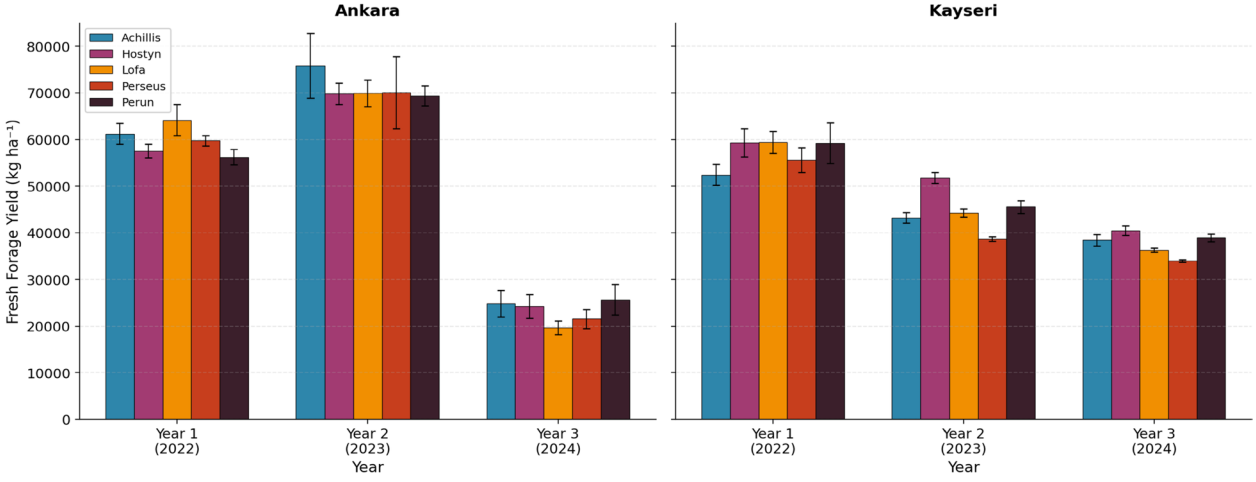


Fig. 6 Annual cumulative fresh forage yield (kg ha^{-1}) at both locations. Error bars: ± SE

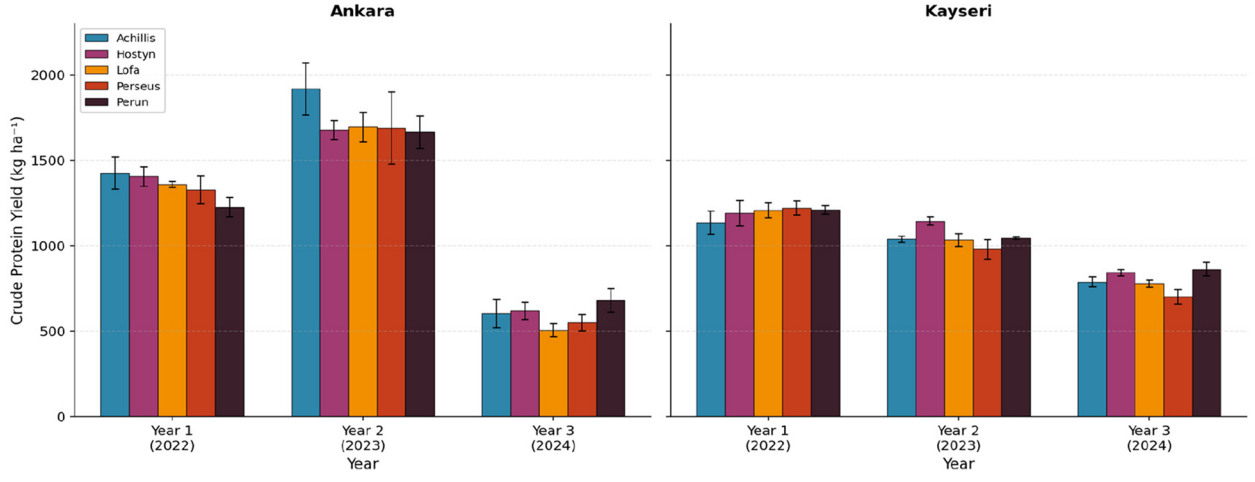


Fig. 7 Annual cumulative crude protein yield (kg ha^{-1}) at both locations. Error bars: ± SE

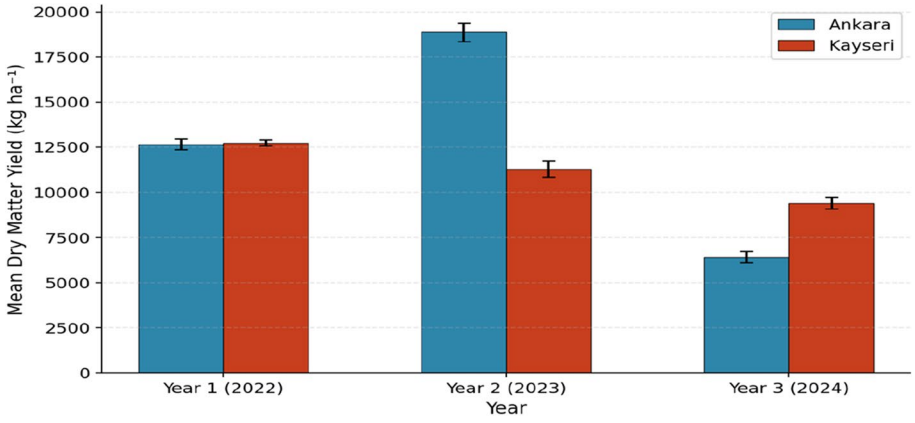


Fig. 8 Mean annual DMY by location across all cultivars. Error bars: ± SE

Table 2 Stability parameters and selection indices for dry matter yield (kg ha^{-1}) of five *Festulolium* cultivars across six environments

Genotype	Mean	bi	S^2di	R^2	CV(%)	ASV	ASV Rank	Mean Rank	GSI
Achillis	12,278	1.13	158,101	0.98	38.6	54.5	5	1	6
Hostyn	12,063	0.88	91,335	0.97	30.9	52.8	4	2	6
Lofa	11,809	1.07	− 132,699	0.99	38.1	31.8	2	4	6
Perseus	11,300	1.03	− 115,023	0.99	38.3	22.7	1	5	6
Perun	12,047	0.89	− 111,984	0.98	31.0	41.5	3	3	6

Table 3 AMMI analysis of variance for dry matter yield, partitioning the genotype × environment interaction (GEI) into interaction principal component axes (IPCA)

Source	df	SS	MS	F	p	% of GEI
GEI	20	49,405,736	—	—	—	100
IPCA1	8	30,835,472	3,854,434	2.26	0.033	62.4
IPCA2	6	11,734,104	1,955,684	1.15	0.345	23.8
Residual	6	6,836,160	1,139,360	—	—	13.8
Error	72	—	1,707,372	—	—	—

and Kayseri-Y3 showed the largest interaction signals (Fig. 11).

GGE biplot analysis

The GGE biplot for DMY displayed the genotype and genotype × environment effects simultaneously. The first two principal components together explained 83.6% of the GGE variation (PC1 = 50.0%, PC2 = 33.6%). The biplot revealed cultivar–environment associations, with Achillis and Lofa associated more with the Ankara environments and Perun and Hostyn with the Kayseri environments, indicating differential cultivar adaptation between the two locations. These which-won-where/mega-environment patterns are regarded as preliminary and require confirmation across additional environments. The GGE biplot for crude protein yield revealed comparable associations, indicating that the interaction patterns for DMY were largely consistent with those for CPY (Figs. 12, 13).

Eberhart–Russell stability analysis

The Eberhart–Russell regression analysis showed that all cultivars had high R^2 values (≥ 0.97), indicating that the linear regression model adequately explained yield variation across environments. For DMY, Achillis had the highest regression coefficient (bi = 1.13), indicating above-average responsiveness to favorable environments. Hostyn (bi = 0.88) and Perun (bi = 0.89) had regression coefficients below unity, suggesting better adaptation to less favorable environments. Lofa (bi = 1.07) and Perseus (bi = 1.03) had values close to unity, indicating broad adaptability (Fig. 14). In addition to R^2, the deviation from regression (S^2di) was examined; no cultivar showed a significant S^2di, indicating that all conformed adequately to the linear Eberhart–Russell model, so stability was inferred from bi and S^2di jointly rather than from R^2 alone.

ASV and Genotype Selection Index

The ASV ranking for yield traits consistently identified Perseus and Lofa as the most stable cultivars (lowest ASV), while Achillis and Hostyn showed the least stability. However, stability alone is insufficient for cultivar recommendation, and the most stable cultivars were also the lowest-yielding. Because yield and stability ranks were

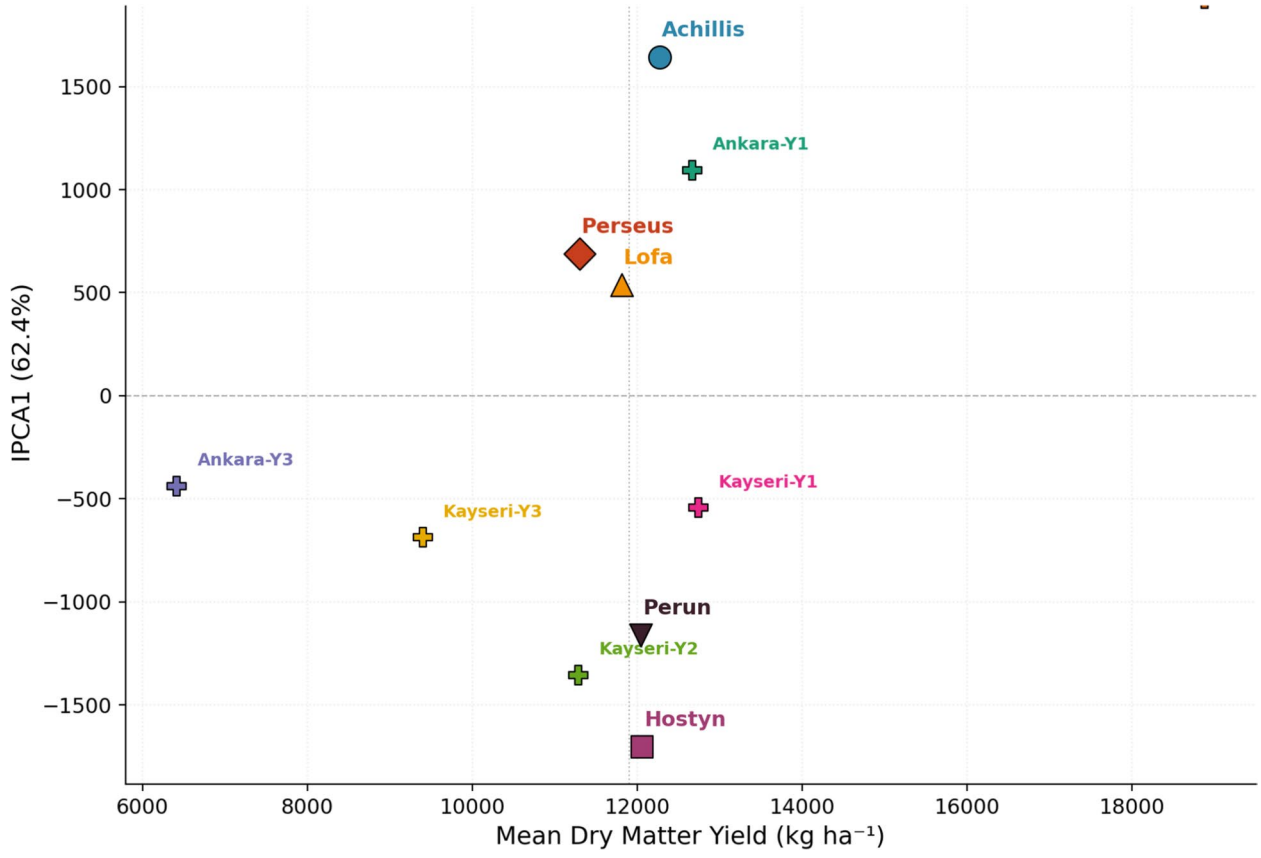


Fig. 9 AMMI1 biplot (Mean vs. IPCA1) for dry matter yield of Festulolium cultivars. Colored circles: genotypes; plus signs: environments

almost perfectly inverse, the GSI did not separate the cultivars (all GSI = 6); the recommendation therefore rests on mean yield together with ASV-based stability, favouring Achillis (highest yield) and Lofa (good stability with acceptable yield) (Figs. 15, 16).

Discussion

Genotype × Environment ınteraction and stability

The results showed that genotype × environment interaction was significant for the forage-quality traits (crude protein, ADF and NDF) but, for the yield traits, the pooled interaction was not significant (AMMI concentrated it in a significant IPCA1), underscoring the importance of multi-environment testing for *Festulolium* cultivar selection. Similar findings were reported by Kemešytė et al. [30], who found significant environmental effects on total dry matter yield in Lithuanian *Festulolium* trials. The AMMI analysis effectively partitioned the interaction into interpretable components, revealing that environmental variation (location and year effects) was the dominant source of variation, consistent with the general pattern observed in forage grass trials [19]. Biologically, the location-driven divergence indicates that cultivar performance was shaped mainly by the contrasting moisture and soil regimes of the two sites rather than by intrinsic genotype ranking: the most stable cultivars (Perseus, Lofa) buffered yield against environmental fluctuation, whereas the most responsive cultivar (Achillis) exploited favourable conditions.

The GGE biplot analysis provided a powerful visualization of cultivar-environment relationships. The differential association of Achillis with Ankara environments and Perun/Hostyn with Kayseri environments indicates differential cultivar responses between the two locations; the existence of distinct sub-optimal mega-environments within Central Anatolia, however, remains tentative. This pattern is consistent with the contrasting soil (clay-loam vs. sandy-loam) and climate (higher vs. lower precipitation) characteristics of the two locations. Gauch [16] emphasized that such mega-environment patterns are critical for targeted cultivar deployment in forage production systems.

Cultivar stability and adaptability

The Eberhart–Russell analysis revealed distinct adaptability patterns among cultivars. Achillis (bi = 1.13) can be classified as a cultivar responsive to favorable environments, performing exceptionally well at Ankara but showing reduced advantage under the more challenging Kayseri conditions. Hostyn (bi = 0.88) and Perun

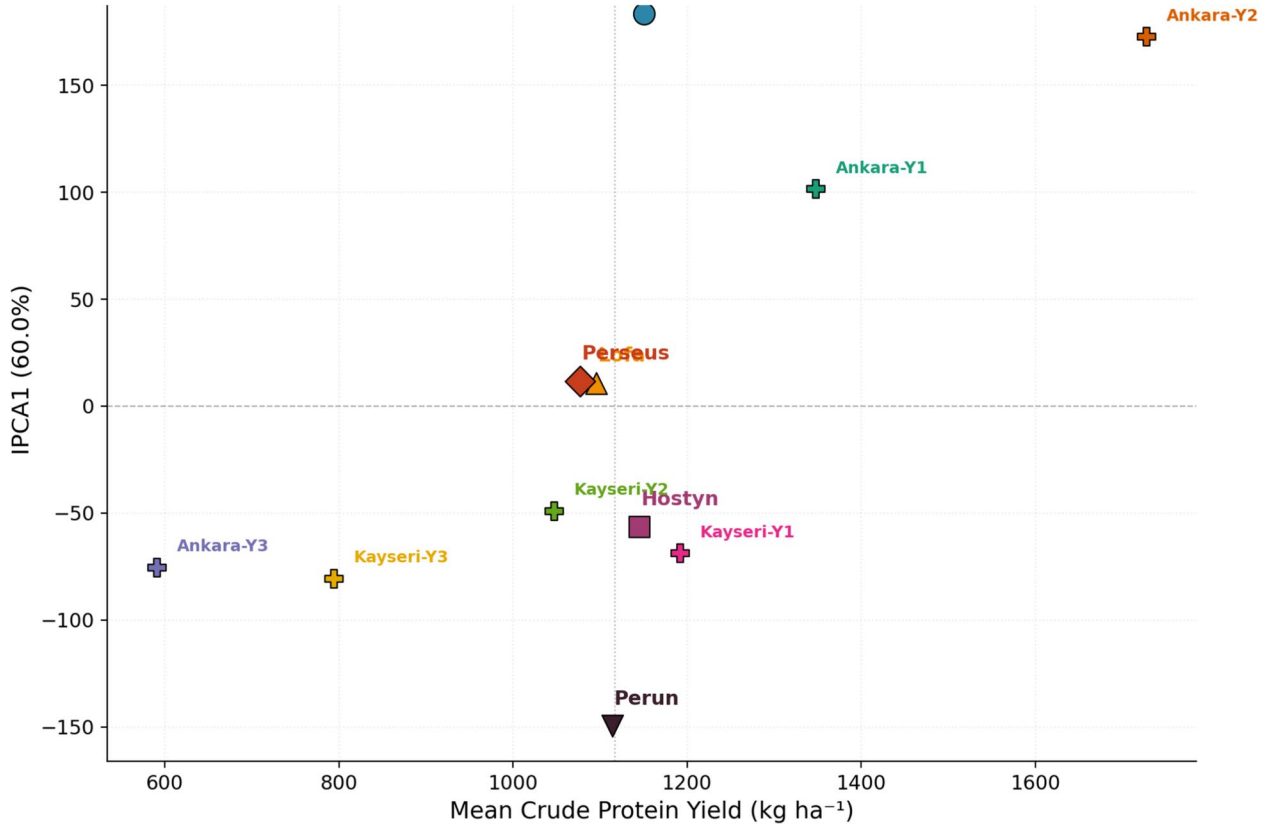


Fig. 10 AMMI1 biplot (Mean vs. IPCA1) for crude protein yield

(bi = 0.89) demonstrated below-average responsiveness, suggesting specific adaptation to less favorable environments — a valuable characteristic for sustainable production under variable climatic conditions [20].

The ASV analysis ranked Perseus and Lofa as the most stable cultivars; however, their mean yields were among the lowest. This inverse relationship between stability and mean performance is a common observation in multi-environment trials [28]. In the present dataset the GSI (ASV rank + mean-yield rank) did not differentiate the cultivars, because the yield and stability rankings were almost perfectly inverse, producing similar GSI values; cultivar recommendation was therefore based primarily on mean dry matter yield together with ASV-based stability — Achillis for its superior yield and Lofa for its combination of good stability with acceptable yield, whereas Perseus, although most stable, ranked lowest in yield and is not prioritised for general deployment. Notably, the AMMI and GGE analyses were concordant: both identified Lofa and Perseus as the most stable cultivars and distinguished the Ankara and Kayseri environments as separate groups, which strengthens confidence in the stability assessment.

Quality parameters

The decrease in crude protein content and the increase in fiber components (ADF and NDF) observed over the years at both locations can be attributed to morphological and physiological changes associated with stand aging. In particular, the pronounced increase in fiber content in the third year at the Kayseri location suggests that environmental conditions further accelerated this process. As stand age progresses, significant structural changes occur in the plant, including a reduction in the formation of new tillers and an increase in the proportion of older tillers. Consequently, leaf renewal becomes limited, senescence processes in aging leaves are accelerated, and the amount of photosynthetically active tissue declines. As a result of these morphological changes, the leaf-to-stem ratio decreases, and the plant shifts toward a structure richer in structural carbohydrates and more fibrous in nature. The accumulation of structural carbohydrates leads to an increase in ADF and NDF values, while simultaneously causing a decline in crude protein content [31–33].

The higher crude protein concentrations observed in the third harvests (particularly in the first and second years) are most likely attributable to the morphological characteristics of plant tissues formed during early post-cut regrowth. Following defoliation, newly developed

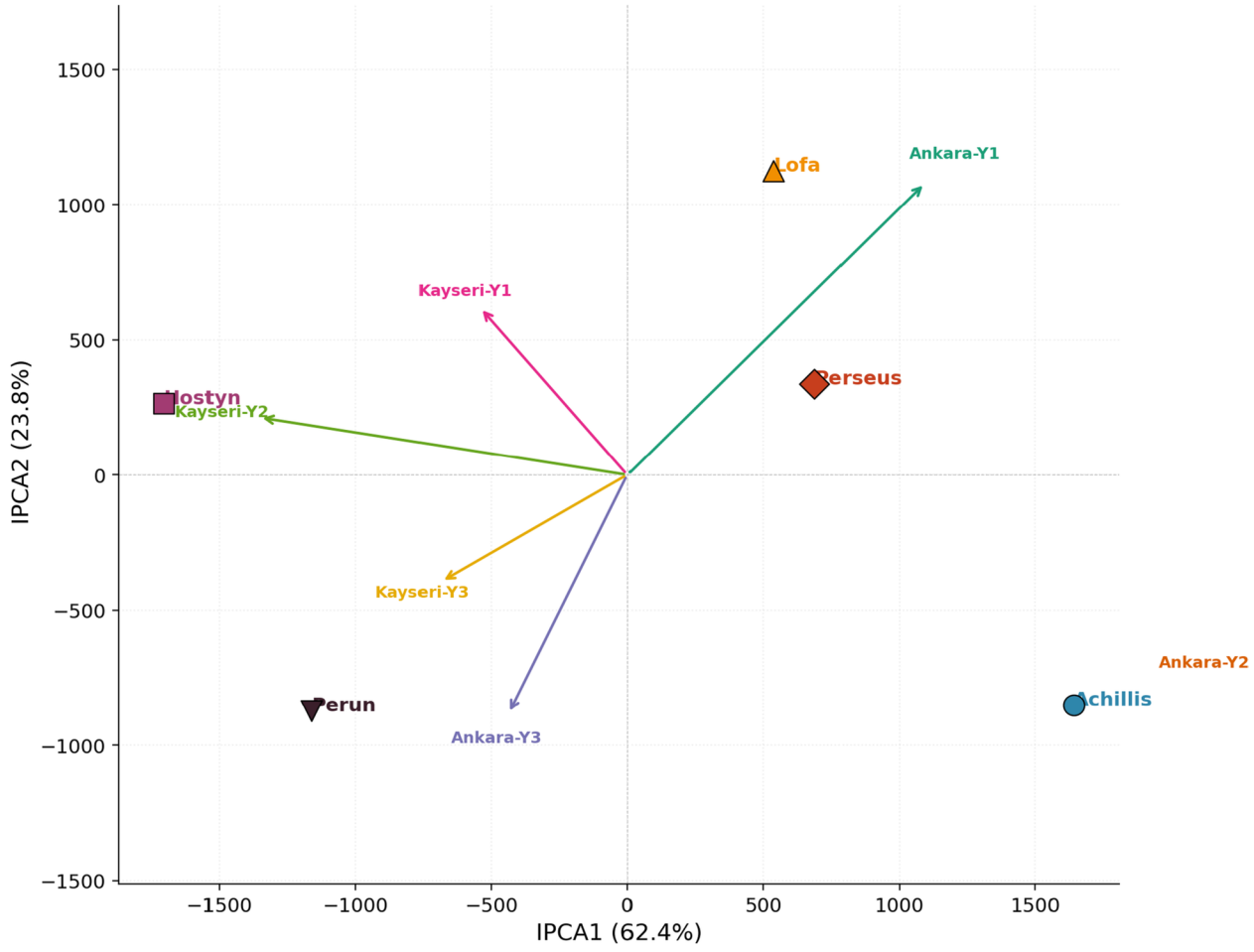


Fig. 11 AMMI2 biplot (IPCA1 vs. IPCA2) for dry matter yield. Arrows represent environments; markers represent genotypes

shoots are typically younger, more vegetative, and characterized by a higher leaf proportion, which contributes to increased crude protein content. Indeed, in perennial cool-season grasses, regrowth periods—especially those with shorter growth intervals—are reported to be associated with higher protein concentrations [12, 34]. Similarly, shorter regrowth intervals allow plants to remain physiologically younger and less lignified, which are key factors contributing to higher protein levels [35]. In addition, Elgersma and Søegaard [36] reported that as the growth period extends, cell wall components increase, leading to a decline in crude protein concentration. In the present study, ADF values showed a marked decrease in the third harvests at both locations, particularly in the first and second years. The observed variation in ADF can be attributed to changes in plant morphology and regrowth dynamics, with lower values associated with periods dominated by younger, less lignified tissues [35, 37].

The mineral composition results revealed that environment (location × year × harvest) had a stronger influence on Ca, K, Mg, and P concentrations than cultivar identity, a pattern consistent with previous reports for ryegrass and related forage grasses [38]. Calcium concentrations (0.22–0.78%) generally met or exceeded the 0.30% threshold considered adequate for lactating dairy cattle [39, 40], with Ankara values consistently higher than Kayseri, likely reflecting differences in soil Ca availability and uptake efficiency under the contrasting climatic conditions of the two sites. Phosphorus values (0.35–0.50%) were within the 0.30–0.50% range typically reported for cool-season grasses and were sufficient to meet maintenance requirements of grazing ruminants [39], although values declined toward the third year, mirroring the general decline in nutritive quality with stand age.

Potassium concentrations were notably higher at Kayseri (up to 4.30%) than at Ankara, likely reflecting differences in soil K availability and uptake dynamics between the two locations. Elevated dietary K is known to interfere with Mg absorption in ruminants, and the marked decline of Mg at Kayseri in the third year (approximately 0.09%) was particularly notable, falling below the 0.20% threshold commonly cited as adequate for ruminant nutrition [40, 41]. When K, Ca, and Mg values were

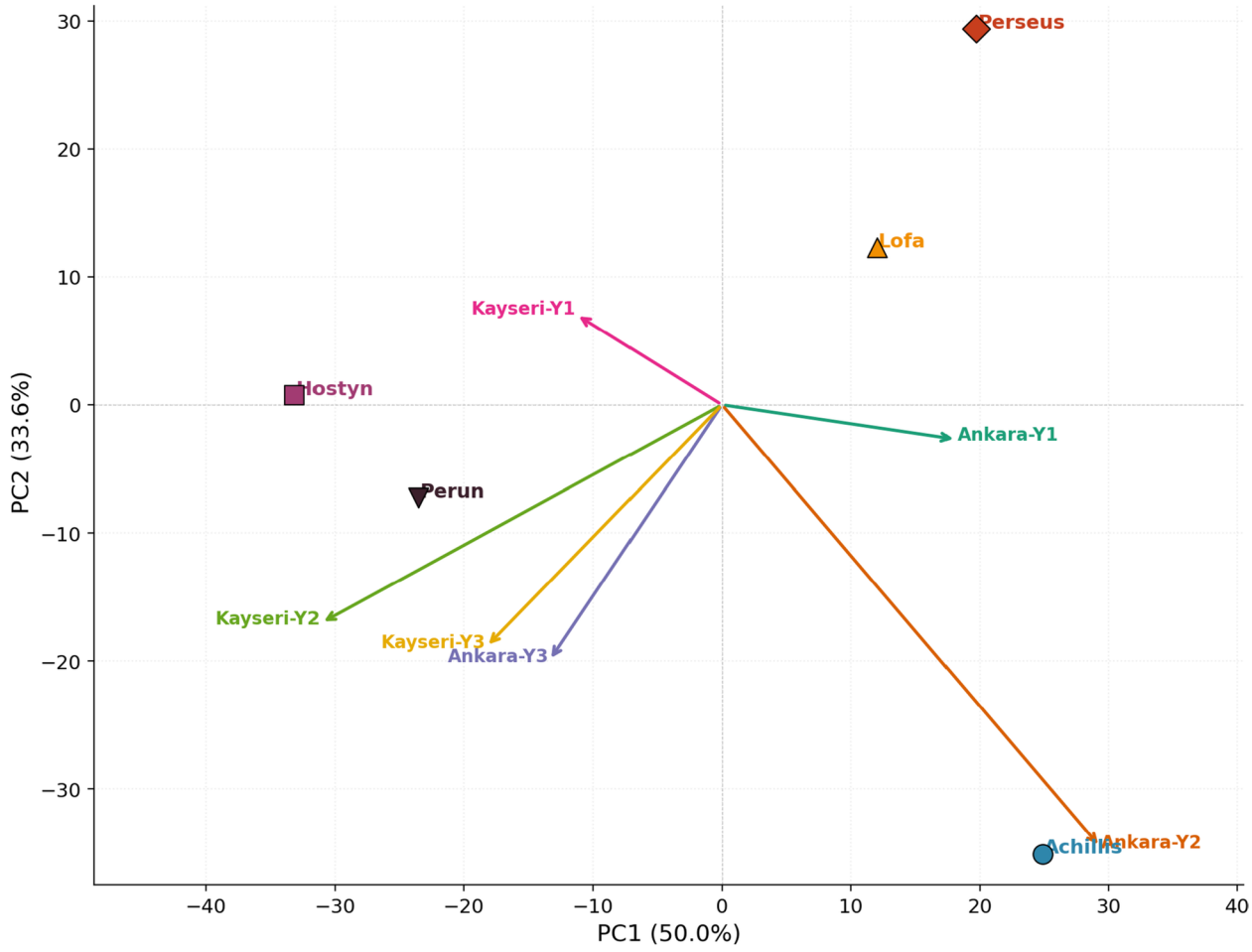


Fig. 12 GGE biplot for dry matter yield. Markers: genotypes; arrows: environment vectors

considered jointly, herbage harvested under such conditions would warrant Mg supplementation if used as a sole feed for lactating ruminants [42]. Among cultivars, the relatively narrow range of variation indicates that genotypic selection for improved mineral composition in *Festulolium* would require targeted breeding efforts, as has been demonstrated for Italian ryegrass cultivars bred specifically for higher Mg uptake [38, 43].

Yield performance across environments

In *Festulolium* cultivars, DMY, FFY and CPY were generally similar at both locations in the first year, which may be attributed to the establishment phase during which plants had not yet developed distinct responses to environmental conditions. The highest yields were obtained in the second year at Ankara (DMY up to 20,815 kg ha^{-1}), reflecting full stand establishment, improved root system development, and more efficient utilization of environmental resources [12, 30]. However, a marked decline was observed at Ankara in the third year (down to 5,454 kg ha^{-1}), whereas Kayseri exhibited a more gradual but consistent decreasing trend across the three years (13,097 to 8,399 kg ha^{-1}). This contrast suggests that Ankara, while supporting higher peak productivity under favorable years, may be more sensitive to year-to-year fluctuations, whereas Kayseri provides a comparatively more stable but lower-yielding environment. The progressive yield decline at both locations is consistent with the general pattern reported in multi-year *Festulolium* studies, where stand age and accumulated abiotic stress typically reduce productivity after the second production year [30]. Climatic factors, particularly temperature and precipitation, are widely recognized as key determinants of forage yield and stand persistence in cool-season grasses [12, 44], and winter hardiness has been highlighted as a critical trait differentiating stable from less persistent *Festulolium* types [12]. Differences in micro-climatic conditions between Ankara and Kayseri, together with stand-age effects, likely contributed to the divergent yield trajectories observed in this study. Environmental ranking referred to here is based on environment mean yields and the Eberhart–Russell environmental index, rather than implied from the biplots. The contribution of winter hardiness to the observed yield

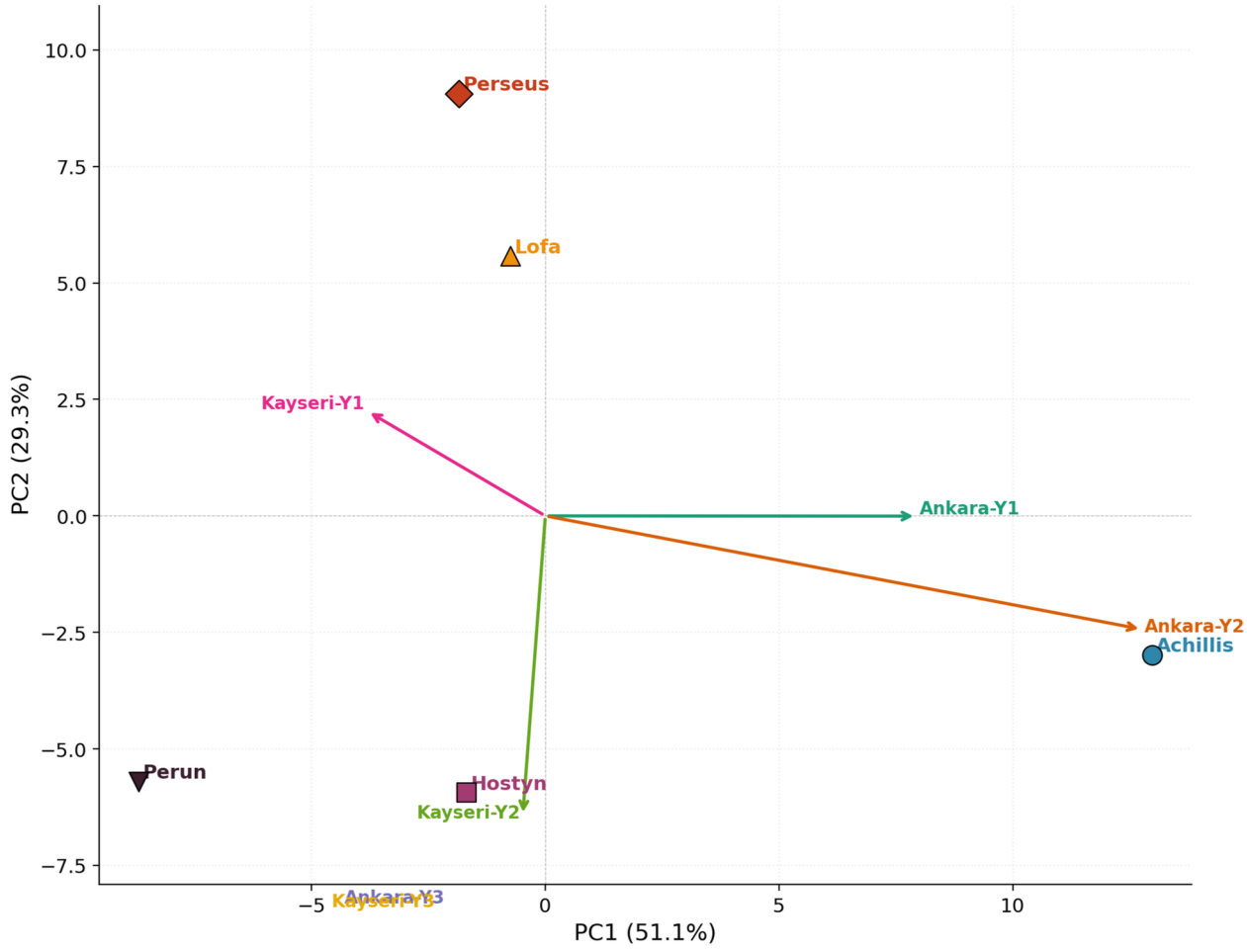


Fig. 13 GGE biplot for crude protein yield

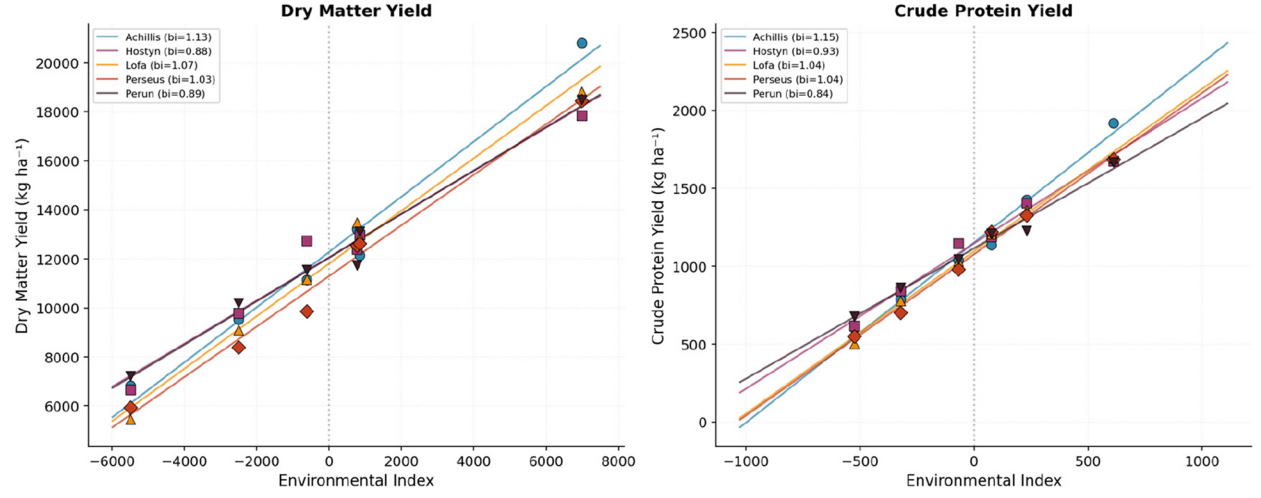


Fig. 14 Eberhart–Russell regression of Festulolium cultivars across environments. bi = regression coefficient

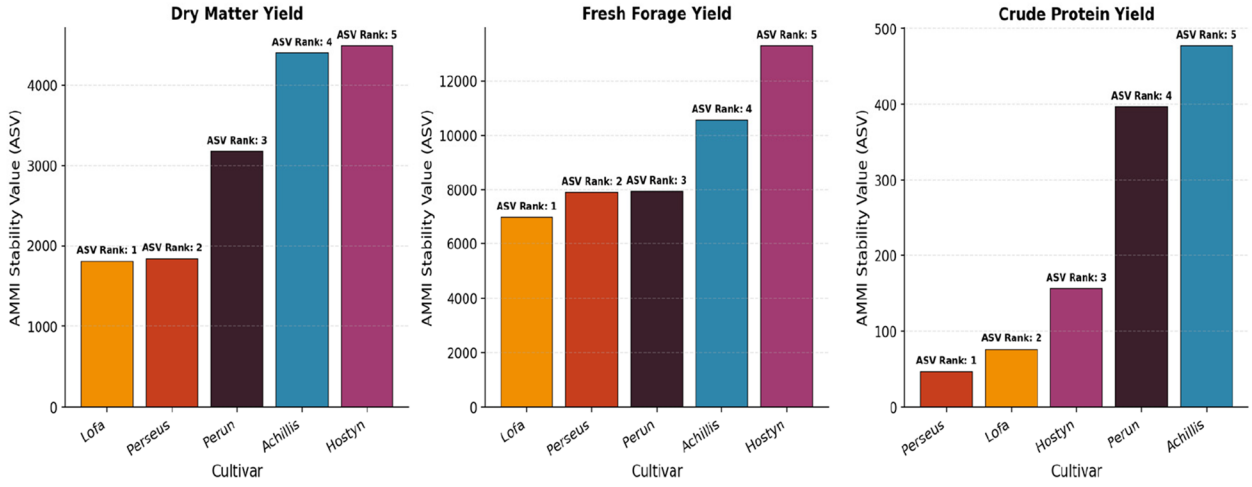


Fig. 15 AMMI Stability Values (ASV) of Festulolium cultivars for three yield traits. Lower ASV indicates greater stability

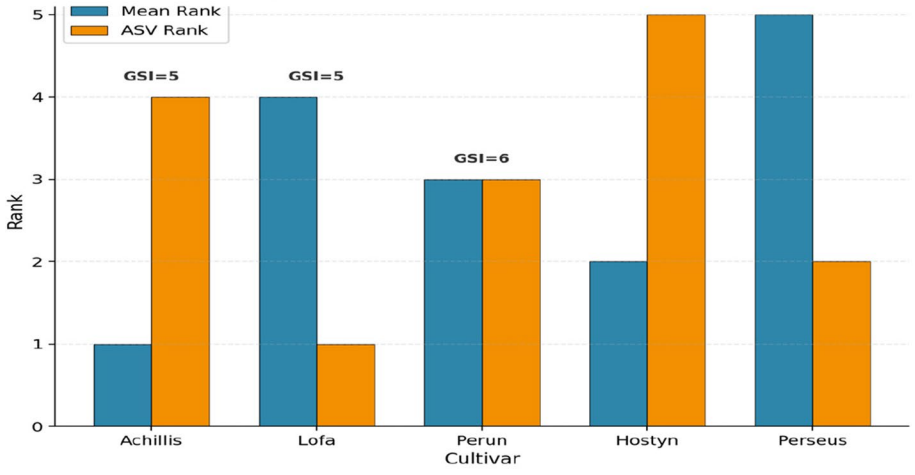


Fig. 16 Genotype Selection Index (GSI) for dry matter yield. GSI = Mean Rank + ASV Rank; lower GSI is more desirable

decline is proposed here as a hypothesis to be tested in future studies rather than demonstrated.

Limitations

Several limitations should be considered when interpreting these results. First, the relatively small number of cultivars (five) limits the resolution of the stability analyses, so the cultivar rankings should be regarded as indicative rather than definitive. Second, the study was conducted at only two locations over three years; consequently, distinct sub-optimal mega-environments within Central Anatolia could not be delineated, and the location-specific recommendations remain preliminary. Third, error variances were heterogeneous among environments (Bartlett's test, $P < 0.001$); although the balanced design and the descriptive nature of the AMMI and GGE procedures justify the pooled analysis, this heterogeneity is acknowledged. Finally, several mechanistic interpretations could not be verified because the relevant traits were not measured: the year-to-year quality trends are descriptive and not all supported by formal interaction tests or post-hoc comparisons; the stand-aging and morphological explanations for the protein and fibre changes are putative, as tiller density, leaf-to-stem ratio and senescence were not recorded; the higher calcium concentration at Ankara is plausible but unconfirmed, as no soil-chemical, irrigation-water or plant-uptake data were collected; and the contribution of winter hardiness to the yield decline could not be assessed, as winter survival and tiller-persistence were not recorded. In addition, the 2024 comparison at Ankara was based on two rather than three harvests, which lowers the Ankara-2024 environment mean but does not affect the within-environment cultivar ranking. These constraints should be addressed in future multi-environment trials with a broader cultivar panel.

Conclusions

This study showed that environment was the predominant source of variation in the yield and quality of *Festulolium* cultivars across six environments in Central Anatolia. The combined application of AMMI, GGE biplot, and Eberhart–Russell analyses provided complementary insights into cultivar stability and adaptability: Genotype × environment interaction was significant for the forage-quality traits (crude protein, ADF, NDF) and, for yield, was captured by a significant first AMMI axis, although the pooled interaction term did not reach significance. The AMMI and GGE analyses nonetheless remain informative for dry matter yield, because they partition this interaction into multiplicative axes and thereby recover a structured, significant first component (IPCA1) that the pooled F-test—diluted by the unstructured portion of the interaction—does not detect.

Among the cultivars, Achillis was the highest-yielding and most responsive to favourable environments and is recommended for high-input irrigated systems, whereas Lofa combined high stability (ASV = 31.8) with broad adaptability (bi = 1.07) and acceptable yield and is recommended alongside Achillis for general use. Perun, with intermediate stability and adaptation to less favourable environments (bi = 0.89), is a strategic alternative, while Perseus and Hostyn are secondary options. Because the GSI did not differentiate the cultivars in this dataset, these recommendations rest on mean yield combined with ASV-based stability.

These findings illustrate the value of AMMI and GGE biplot methodologies for *Festulolium* cultivar evaluation. However, as the study was limited to two locations over three years, the location-specific recommendations should be regarded as preliminary, and additional environments and genotypes are required for robust environmental classification and mega-environment delineation.

Abbreviations

ADF	Acid detergent fiber
AMMI	Additive Main Effects and Multiplicative Interaction
ANOVA	Analysis of variance
ASV	AMMI Stability Value
CP	Crude protein
CPY	Crude protein yield
DMY	Dry matter yield
FFY	Fresh forage yield
GEI	Genotype × environment interaction
GGE	Genotype plus Genotype × Environment interaction
GSI	Genotype Selection Index
HSD	Honestly Significant Difference
IPCA	Interaction Principal Component Axis
NDF	Neutral detergent fiber
NIRS	Near-infrared reflectance spectroscopy
RCBD	Randomized complete block design
SVD	Singular value decomposition

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Authors' contributions

Authors' contributions C. T. K.: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Software, Validation, Visualization, Writing – original draft. S. Ç. Ö.: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Software, Validation, Visualization. O. O.: Conceptualization, Formal analysis, Investigation, Methodology, Resources, Software, Validation, Visualization. Ç. Ş.: Investigation, Methodology, Resources. S. U.: Conceptualization, Formal analysis, Investigation, Methodology, Resources, Validation, Visualization. C. S.: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Software, Validation, Visualization, Project administration, Writing – review & editing.

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Data availability

All data supporting the findings of this study are available within the paper and its Supplementary Information. Microsatellite primer sequences are provided in Supplementary Tables, along with original reference describing the microsatellites used in this study.

Declarations

Ethics approval and consent to participate

Not applicable. This study did not involve human participants, human data, human tissue, or animals.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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