



Comparative evaluation of rescuegrass (*Bromus catharticus*) lines for forage yield and quality under mediterranean climate conditions

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Received: 25 December 2025; Accepted: 20 March 2026

ABSTRACT

Developing high-yielding and quality forage crops adapted to Mediterranean conditions is essential to meet the increasing feed demand in the region. This research was conducted for two consecutive years in 2020–21 and 2021–22 under Mediterranean climatic conditions in Adana, Turkiye to determine the yield and quality of seven rescuegrass *Bromus catharticus* Vahl. breeding lines developed by selection breeding. The experiment was conducted in a randomized complete block design (RCBD) with three replications. Significant effects of year, line, and year × line interactions were observed for most traits. Plant height increased from 76.1 cm (establishment year) to 111.4 cm (second year), while tiller number rose from 8.0 to 19.3/plant. Line 17 exhibited the highest fresh forage yield (19.9 t/ha) and dry matter yield (6.8 t/ha) in the second year, significantly outperforming other lines. Forage quality analysis revealed that Lines 13 and 14 showed superior nutritional value, with crude protein contents of 13.68% and 13.92%, respectively, and lower fiber fractions. Acid detergent fiber ranged from 41.99% (Line 13) to 45.08% (Line 17), while relative feed value varied between 62.3 and 69.3. Strong negative correlations were observed between yield and quality parameters, indicating a typical yield-quality trade-off in forage breeding. These results demonstrated substantial genetic variation among rescuegrass lines and identify Line 17 for high-yield breeding objectives and Line 13 for quality-focused programmes adapted to Mediterranean conditions. Future studies should focus on multi-location trials and molecular characterisation to develop improved rescuegrass cultivars for Mediterranean conditions.

Keywords: Agronomic performance, Cool-season grass, Genetic variation, Nutritive value

Economically sustainable livestock production depends on sufficient feed with appropriate nutritional value. Feed inputs constitute 60–70% of production costs (Alcicek 2021), and supply challenges cause yield losses and increased costs. In countries like Turkiye with inadequate forage cultivation areas and poorly managed rangelands, this problem is critical (Hatipoglu *et al.* 2020). Thus, developing native and adaptable forage species is a strategic priority for economic and ecological sustainability (Demiroglu Topcu *et al.* 2024).

Rescuegrass *Bromus catharticus* Vahl. is a cool-season C3 grass that thrives in temperate climates with mild winters and moderate temperatures, exhibiting peak growth during autumn, winter, and early spring (Belesky *et al.* 2007). The species includes annual, biennial, or short-lived perennial forms with high forage yield, cool-season adaptability, and vigorous tillering under irrigation (Gomez-Miranda *et al.* 2021). Its rapid regrowth capacity after each defoliation enables multiple harvests within a single growing season, making it particularly well-suited to intensive cut-and-carry forage management systems under Mediterranean conditions (Scheneiter *et al.* 2008). Due to its rapid growth rate during

the cool season, strong adaptability to diverse environments, and ability to remain green after seed maturation, rescuegrass has gained increasing importance as a forage grass in temperate agricultural systems worldwide (Sun *et al.* 2021). Native to South America, it is valued as an alternative forage source in Mediterranean regions, particularly for intensive early spring production (Lauriault *et al.* 2005, Scheneiter *et al.* 2008). For forage crops, substantial biomass yield alone is insufficient; quality parameters such as crude protein, fibre fractions, total digestible nutrients, and relative feed value are imperative (Van Soest *et al.* 1991).

Climate change, increasing costs, and limited irrigable land underscore the need for climate-resilient, high-yielding cultivars (Yuan *et al.* 2024). Evaluating breeding lines under diverse conditions enables agronomic assessment and contributes to variety improvement programmes (Casler and Vogel 1999). Although several studies investigated *Bromus* spp. under diverse climates, research on rescuegrass breeding in Mediterranean regions like Turkiye remains limited, hindering development of high-yielding, high-quality cultivars adapted to the region.

The present study constituted a comprehensive evaluation of seven rescuegrass breeding lines, developed through selection breeding, with a focus on forage yield and

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quality. The objective was to identify lines that exhibited superior performance in both yield and quality traits, thereby contributing to the advancement of forage crop breeding programmes adapted to Mediterranean environmental conditions.

MATERIALS AND METHODS

Study site, soil and climate characteristics: The experiment was conducted for two consecutive years i.e. 2020–21 and 2021–22 at the research fields of the Faculty of Agriculture, Çukurova University, Adana (37°01'01"N, 35°21'40"E; at an elevation of 35 m amsl), Turkey. Soil analysis of samples collected from the trial site classified the soil texture as clay-loam, characterised by low organic matter content (1.11%), available phosphorus at 18.9 kg/ha, high levels of available potassium (789 kg/ha), and a slightly alkaline pH of 7.7.

The average temperatures recorded during the establishment year of the experiment (20.6°C) and the second year (20.0°C) exceeded the long-term average temperature of 19.2°C. Total precipitation measured 362.3 mm in the first year (2020–2021) and 771.0 mm in the second year (2021–2022) (Fig. 1), compared to a long-term average of 668.8 mm for the period 1990–2022 (Anonymous 2023).

Experimental design and establishment: The experiment was laid out in a randomised complete block design (RCBD) with three replications. The plant material consisted of seven rescuegrass lines (3, 9, 13, 14, 17, 20, and 23) developed through selection breeding by the Department of Field Crops, Faculty of Agriculture, Tekirdag Namik Kemal University, from accessions originally obtained from the Western Regional Plant Introduction Station (Pullman, Washington, USA) gene bank. These lines may exhibit annual, biennial, or short-lived perennial growth habits depending on environmental conditions. The lines were developed under the temperate sub-humid conditions of the Trakya region, which differs from the Mediterranean conditions of the experimental site in Adana.

The experimental area was prepared in early autumn following wheat harvest. Seeds were sown manually on November 11, 2020, at a depth of 3–4 cm, with a row spacing was 20 cm and a seeding rate of 30 kg/ha (Casler *et al.* 1998). At sowing, 50 kg/ha nitrogen (N) and 128 kg/ha phosphorus (P₂O₅) were applied as basal fertiliser. An additional 50 kg/ha N was applied in autumn 2021 (Anonymous 2019). Supplemental sprinkler irrigation was provided as needed throughout the growing seasons, with a total 458 mm applied in the first year and 108 mm in the second year. Weeds were controlled by hand throughout the experiment.

Plant harvest and data collection: Harvesting occurred at panicle emergence (70% of plants) at 5–6 cm height. Three harvests were conducted in year 1 (April 28, May 28 and June 29, 2021) and two in year 2 (May 19 and July 6, 2022). Ten plants were randomly selected from each line before first harvest in both years. Plant height and tiller number were measured (Anonymous 2019).

Border rows and 50 cm from plot ends were removed. Fresh forage was weighed immediately. Up to 500 g was sampled per plot, dried at 70°C for 48 h, equilibrated to room temperature, and weighed for dry matter. Fresh forage and dry matter yields per hectare were calculated for each line and harvest. Total yields were obtained by summing all harvests in both years. Dried samples were ground through 1 mm sieve for analyses. Crude protein (CP) was determined by Kjeldahl method (N × 6.25). Neutral detergent fibre (NDF) and Acid detergent fibre (ADF) were determined as per Van Soest (1963) using ANKOM220 Fiber Analyzer.

Total digestible nutrient (TDN) was calculated from Metabolizable energy (ME) values derived from ADF per Belesky *et al.* (2007):

$$\text{ME (MJ/kg DM)} = 15.3 - 0.153 \times \text{ADF} \quad (1)$$

$$\text{TDN (\%)} = [((\text{ME}/4.184)/0.82)/4.409] \times 100 \quad (2)$$

The relative feed value was calculated using equations 3, 4 and 5 (Moore and Undersander 2002).

$$\text{Digestible dry matter (DDM \%)} = 88.9 - (0.779 \times \text{ADF, \% of DM}) \quad (3)$$

$$\text{Dry matter intake (DMI \% of DMO)} = \frac{120}{\text{NDF}} \quad (4)$$

$$\text{Relative feed value (RFV)} = \frac{\text{DDM} \times \text{DMI}}{1.29} \quad (5)$$

Statistical analyses: Characteristics were analysed using

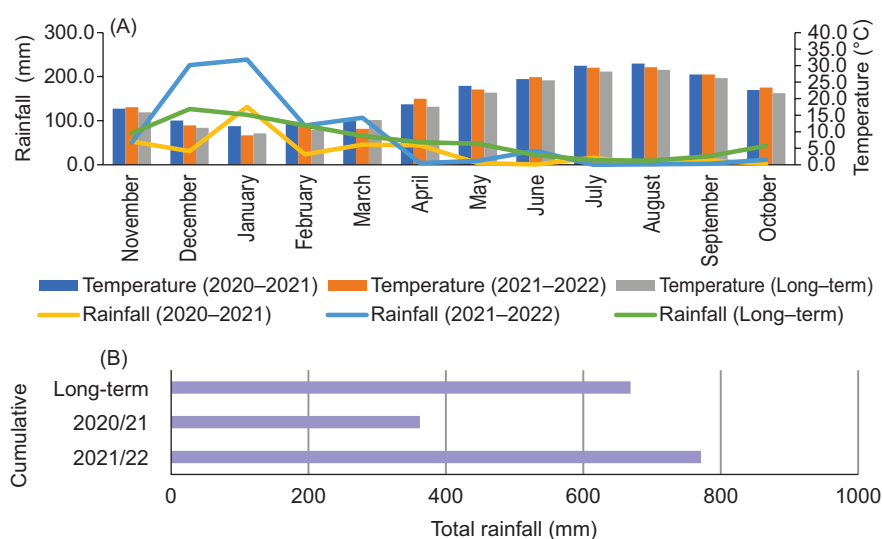


Fig. 1 Climatic characteristics of Adana, Turkiye, during the study period. (A) The diagram presents the temperature and precipitation averages for the 2020–21 and 2021–22 periods, as well as long-term averages; and (B) Cumulative precipitation for the periods 2020–21 and 2021–22, as well as the long-term average.

MSTAT-C (Michigan State University v.2.10) for split-plots in time (Steel *et al.* 1997).

$$X_{ijk} = \mu + a_i + b_j + \varepsilon_{ij} + c_k + (bc)_{jk} + \varepsilon_{ijk} \quad (6)$$

Where a_i , Block (replication); b_j , Main plot (lines); ε_{ij} , Error_a; c_k , Sub-plot (years); ε_{ijk} , Error_{ab}.

Statistically significant averages of the characteristics were compared by Duncan's test at the $p < 0.05$ level of significance. Pearson's correlation analysis was performed using the Metan package in the R statistical programme (R Core Team 2024), and heatmap was prepared with this package (Olivoto and Lucio 2020).

RESULTS AND DISCUSSION

The results of the analysis of variance revealed that the year and line factors had statistically significant effects on plant height, fresh forage yield, dry matter yield, crude protein (CP), and total digestible nutrient (TDN). Additionally, the line factor significantly affected acid detergent fibre (ADF), neutral detergent fibre (NDF), and relative fuel value (RFV) values, while the year factor had a significant effect on the number of tillers. Furthermore, the year \times line interaction had a significant influence on fresh forage yield and dry matter yield (Table 1).

The results revealed statistically significant differences among the lines and years ($p < 0.05$). Variations arising from both genetic diversity and environmental factors highlighted the presence of lines with high potential for utilization in breeding programmes and animal nutrition.

Agronomic traits and forage yield: In the two-year study, the average plant height was recorded as 76.1 cm in the establishment year and 111.4 cm in the second year. In addition, the average plant height of the different rescuegrass lines tested in the study ranged from 82.7 cm (line 9) to 107.8 cm (line 17) (Table 1). Although genetic structure determines plant height, light, temperature, and humidity are important environmental factors influencing the height of cool-season forage grasses (Wang *et al.* 2025). Belesky *et al.* (2007) reported that rescuegrass begins growth in early spring and exhibits better plant development in regions with mild winter conditions. Indeed, the higher plant height observed in the second year compared to the establishment year is because the December–May period (except April) of the second year was cooler than the same period of the establishment year (Turner *et al.* 2006). Moreover, plant height significantly varied depending on the lines evaluated in the study. The highest plant height was observed in lines 17 and 20, indicating that these lines tend to produce higher biomass compared to others (Table 1). Several researchers have emphasized that, in addition to light competition, genetic factors also contribute to variations in plant height in forage grasses (Buxton and Lentz 1993, Barre *et al.* 2015). In this context, the variation in plant height among lines can be explained by differences in their genetic makeup.

Tiller number/plant was higher in year 2 (19.3) than year 1 (8.0) (Table 1). Tiller number influences forage yield (Jatimliansky *et al.* 1997). Significant year but no

Table 1 Results of variance analysis and trait means for some morphological characteristics and forage yield traits of rescuegrass lines

	Plant height (cm)	Number of tiller/plant	Fresh forage yield (t/ha)	Dry matter yield (t/ha)
Lines				
3	92.8 b ⁺	13.8	12.9 c	4.1 c
9	82.7 d	11.8	17.1 ab	5.3 b
13	94.7 b	13.5	16.3 b	5.0 bc
14	83.9 cd	14.3	15.8 bc	4.9 bc
17	107.8 a	13.4	19.9 a	6.8 a
20	103.3 a	14.2	16.1 b	5.2 b
23	91.0 bc	14.5	17.2 ab	5.5 b
Year				
2020–2021	76.1 b ¹	8.0 b	6.8 b	2.4 b
2021–2022	111.4 a	19.3 a	26.1 a	8.1 a
Treatments ²				
Line	514.78**	4.89 ^{ns}	2599.98*	377.64**
Error _a	32.40	10.33	575.25	58.89
Year	13070.60**	1329.47**	389499.95**	33466.80**
Line \times Year	88.96 ^{ns}	10.55 ^{ns}	3234.81**	415.19**
Error _{ab}	46.12	8.81	396.36	49.42
CV (%)	7.24	19.74	12.10	13.40

⁺, Means followed by the same letters within a column are not significantly different according to Duncan's multiple test at the $p \leq 0.05$ significance level; ¹, Means followed by the same letters are not statistically different; *, $p \leq 0.05$; **, $p \leq 0.01$; ns, Non-significant ($p > 0.05$); ², Mean square.

line differences indicate environmental sensitivity. Frequent cutting reduces carbohydrates and increases axillary tillers (Scheneiter *et al.* 2008). Favourable spring conditions promote tillers. Higher year 2 tillers despite fewer harvests resulted from favourable spring temperatures.

Annual fresh forage yield, representing the cumulative yield of all harvests within each growing season, varied significantly among lines ($p \leq 0.05$). No significant differences among lines were observed in year 1, whereas line 17 produced significantly higher cumulative yield in year 2 (Fig. 2). The differences observed among lines in cumulative forage yield may be partly attributed to variations in regeneration potential following defoliation. Line 17, which produced the highest cumulative yield in year 2, likely possesses superior regrowth capacity after cutting, as rapid tiller production and root reserve mobilization are key determinants of yield recovery in cool-season grasses (Turner *et al.* 2006, Scheneiter *et al.* 2008). However, since regrowth rate was not directly measured in this study, further research is needed to quantify the regeneration potential of individual rescuegrass lines under Mediterranean conditions. Forage yield is the primary determinant of economic value in forage crops (Jefferson *et al.* 2005). In this study, both fresh forage

and dry matter yields increased approximately threefold in the second year compared to the establishment year. Despite three harvests being conducted in the establishment year and only two in the second year, annual forage yield increased under less frequent harvest regimes, as reported by Xia *et al.* (1994) for rescuegrass. Turner *et al.* (2007) noted that frequent harvesting reduces the concentration of water-soluble carbohydrates in stubble and roots, leading to yield losses during subsequent regrowth. Another study indicated that perennial forage grasses tend to allocate more resources to root development during the establishment year, producing only 10–60% of their potential yield in the first year under favourable soil moisture conditions (Miller 1984). Lauriault *et al.* (2005) reported that while the seasonal yield distribution of rescuegrass is relatively uniform, annual yield distribution varies considerably. Moreover, the more favourable temperatures observed during the second year compared to the establishment year likely contributed to the higher forage yield (Sanderson *et al.* 2002).

Significant year × line interaction for dry matter yield ($p \leq 0.01$) indicates year effects depended on lines. No line differences occurred in year 1, but line 17 had significantly higher yield in year 2 (Fig. 3). Line 17 had highest fresh forage (19.9 t/ha) and dry matter (6.8 t/ha) yields, showing superior biomass and resource efficiency. Lines exhibit yield variations (Abbott *et al.* 2012). Fig. 4 shows strong correlations among height, tillers, and yields, supporting yield-trait relationships (Sayar 2014).

In the second year of the study, due to high summer temperatures with a mean monthly temperature of 30°C in July (Fig. 1), the rescuegrass lines completely withdrew from vegetation, and the experiment was terminated in the autumn. This dormancy period is consistent with the known thermal limitations of rescue grass. The lines re-established in the second growing season through natural reseeding, which is a characteristic trait of the species, enabling the experiment to be conducted over two years. Ozduven *et al.* (2021) reported that rescuegrass lines grown under rainy conditions could not withstand summer heat and died back completely in the establishment year. Lauriault *et al.* (2005) reported that rescuegrass, which has a natural reseeding ability, does not provide satisfactory yields after the third year under different soil moisture regimes. Scheneiter and

Rosso (2005) reported that rescuegrass genetic material that can be used in breeding studies differ in terms of stand persistence. Scheneiter *et al.* (2008) stated that rescuegrass stands do not last longer than two years, which could be attributed to intra-species competition and ecological factors, or to harvest frequency, cutting height, and the species' natural reseeding ability. Jung *et al.* (1994) reported that autumn management, which allows reseeding opportunities at the end of summer, increases the stand persistence of rescuegrass. Matthew *et al.* (1999) noted that the persistence of rescuegrass depends on the formation of replacement tillers from buds at the base of harvested shoots. Although there is no consensus on why rescuegrass stands are not long-term persistent, the species' natural reseeding trait, harvest management, cutting frequency, environmental factors, and differences in the genetic structure of rescuegrass varieties used in experiments (Belesky and Fedders 1994, Xia *et al.* 1994, Rumball and Miller 2003) may explain the variation in stand duration observed among different rescuegrass genotypes.

Forage quality characteristics: Line 13 had lowest ADF (41.99%), statistically grouped with lines 3, 14, 23, and 9 (Table 2). Year and line factors significantly affected yield, traits, and quality. ADF/NDF showed line but no year differences. Lines 17 and 20 had highest ADF/NDF (lower digestibility); lines 13 and 3 lowest (superior digestibility). ADF/NDF affect digestibility and intake (Van Soest 1994). Variation reflects genetic differences (Hall *et al.* 1996).

Based on the two-year average values, the NDF content among different rescuegrass lines ranged from 75.35% (Line 23) to 80.50% (Line 17) (Table 2).

The average CP content was significantly lower in the second year (11.59%) compared to the establishment year (14.73%) ($p \leq 0.01$). Based on the two-year average values, line 14 exhibited the highest mean CP content (13.92%), followed by lines 13 (13.68%), 3 (13.49%), and 9 (13.39%), which were statistically grouped together (Table 2). CP is critical for nutrition. Year and line differences occurred; year 2 CP was lower. CP varies with genetics, harvest, environment, and soil. Higher temperatures cause earlier maturation, increasing lignin/cellulose and decreasing leaf-to-stem ratio (Linn and Martin 1989, Buxton 1995). Stem CP is half that of leaves (Minson 1990). Lower year 2 CP

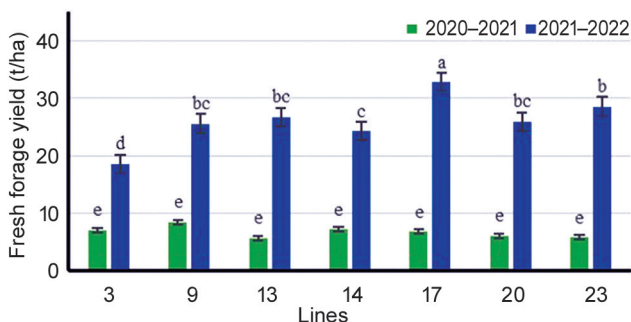


Fig. 2 Variation in fresh forage yield means depending on year and rescuegrass lines. Vertical bars represent the standard error of the mean.

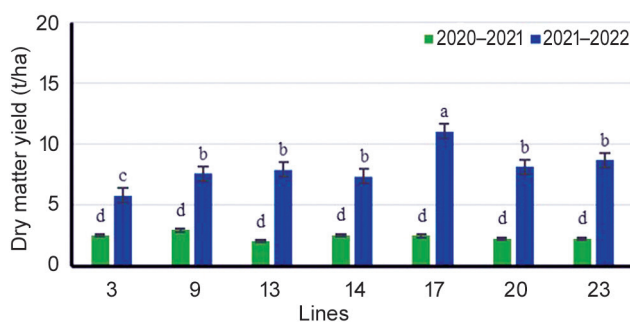


Fig. 3 Variation in dry matter yield means depending on year and rescuegrass lines. Vertical bars represent the standard error of the mean.

Table 2 Analysis of variance results and mean values of some quality characteristics of the rescuegrass lines

	ADF (DM%)	NDF (DM%)	CP (DM%)	TDN (DM%)	RFV
Lines					
3	42.15 c [†]	75.99 b	13.49 ab	58.51 a	68.71 a
9	42.70 bc	77.29 b	13.39 ab	57.96 ab	66.99 ab
13	41.99 c	75.51 b	13.68 a	58.67 a	69.31 a
14	42.19 c	75.90 b	13.92 a	58.48 a	68.74 a
17	45.08 a	80.50 a	12.69 bc	55.55 c	62.30 c
20	44.03 ab	80.34 a	12.35 c	56.61 bc	63.32 bc
23	42.28 c	75.35 b	12.60 bc	58.38 a	69.14 a
Year					
2020–2021	43.74	77.46	14.73 a ¹	56.90 b	65.93
2021–2022	43.49	77.07	11.59 b	58.57 a	67.93
Treatments ²					
Line	8.36**	30.14**	2.20**	8.55**	51.45**
Error _a	1.36	5.55	0.45	1.40	8.84
Year	18.47 ^{ns}	1.56 ^{ns}	103.58**	29.11**	42.05 ^{ns}
Line × Year	4.10 ^{ns}	2.67 ^{ns}	0.56 ^{ns}	1.13 ^{ns}	5.14 ^{ns}
Error _{ab}	4.70	5.36	0.61	1.74	9.79
CV (%)	3.04	3.00	5.94	2.29	4.67

[†], Means followed by the same letters within a column are not significantly different according to Duncan’s multiple test at the $p \leq 0.05$ significance level; ¹, Means followed by the same letters are not statistically different; *, $p \leq 0.05$; **, $p \leq 0.01$; ns, Non-significant ($p > 0.05$); ², Mean square. ADF, Acid detergent fibre; NDF, Neutral detergent fibre; CP, Crude protein; TDN, Total digestible nutrients; RFV, Relative feed value.

relates to negative tiller-CP correlation (Fig. 4). Lines 13 and 14 had highest CP; line 20 lowest. Values were below optimal (CP>15%) but above minimum (CP>7%) (Minson 1990, NRC 2001). High-yielding lines had lower quality (Putnam and Orloff 2016).

The average TDN value was significantly higher in the second year (58.57%) than in the establishment year (56.90%) ($p \leq 0.01$). The highest average TDN value (58.67%) was recorded in line 13, while lines 3 (58.51%), 14 (58.48%), 23 (58.38%), and 9 (57.96%) were statistically similar to line 13 in terms of TDN content (Table 2). TDN represents energy potential, inversely correlated with fiber, positively with CP. TDN was higher in year 2, reflecting climatic impacts (Belesky *et al.* 2006a). High TDN in lines 3, 13, 14, and 23 corresponded to low ADF and high CP, critical for animal performance. High TDN indicates carbohydrate richness (Belesky *et al.* 2006b).

According to the two-year averages, the RFV was significantly higher in line 13 (69.31) compared with other tested lines, except for lines 23 (69.14), 14 (68.74), 3 (68.71), and 9 (66.99) (Table 2). RFV varied among lines but not years. RFV evaluates quality from NDF/ADF, representing digestibility and intake. Line 23’s high RFV reflects low fibre and adequate CP (Rohweder *et al.* 1978).

The correlation analysis results showed that plant height exhibited significant positive correlations with fresh forage yield, dry matter yield and tiller number with correlation coefficients of 0.90, 0.91 and 0.87, respectively ($p < 0.001$)

(Fig. 4). Conversely, plant height showed significant negative correlations with crude protein, with correlation

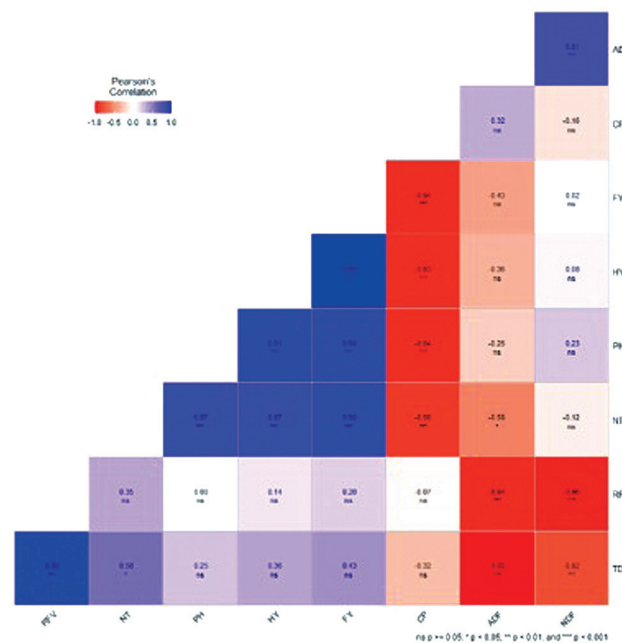


Fig. 4 Heatmap for correlation coefficients of forage yield and quality characters of rescuegrass lines. PH, Plant height; NT, Number of tiller; FY, Fresh forage yield; HY, Dry matter yield; ADF, Acid detergent fibre; NDF, Neutral detergent fibre; CP, Crude protein; TDN, Total digestible nutrients; RFV, Relative feed value.

coefficients of -0.94 ($p < 0.001$). Additionally, tiller number was positively correlated with fresh forage yield ($r = 0.90$), dry matter yield ($r = 0.87$) at $p < 0.001$, and with total digestible nutrients ($r = 0.58$) at $p < 0.05$. Tiller number also showed significant negative correlations with crude protein ($r = -0.90$), and acid detergent fibre ($r = -0.58$).

Fresh forage yield exhibited significant positive correlations with dry matter yield ($r = 0.99$, $p < 0.001$), which is an expected outcome given that dry matter yield is mathematically derived from fresh forage yield and dry matter percentage. More agronomically meaningful was the significant negative correlation between fresh forage yield and crude protein ($r = -0.94$, $p < 0.001$). Dry matter yield was significantly negatively correlated with crude protein ($r = -0.93$). ADF showed significant positive correlations with NDF ($r = 0.81$), and negative correlations with total digestible nutrients ($r = -1.00$), relative feed value ($r = -0.94$). NDF was significantly negatively correlated with relative feed value ($r = -0.97$), and total digestible nutrients ($r = -0.83$). Total digestible nutrients exhibited positive correlations with relative feed value ($r = 0.94$).

This study stated that selection breeding in rescuegrass produces lines with distinct performance characteristics suited for different agricultural objectives under Mediterranean climate conditions. High-yielding lines can address forage quantity demands, while quality-focused lines meet nutritional requirements for optimal livestock production. The observed genetic variation provides valuable germplasm for developing cultivars adapted to regional environmental challenges, though comprehensive multi-location evaluation remains essential for commercial recommendations.

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