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Comparisons of the Grazing Type Population with Hay-Type Cultivars of Alfalfa (*Medicago sativa* L.) and Determination of Their Yield-Related Characteristics Under Rainfed Conditions

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ABSTRACT

Alfalfa (*Medicago sativa* L.) is one of the most important forage legume crops for increasing the quantity and quality of degraded rangelands. Although rangelands in Türkiye have a great diversity of species in terms of vegetation composition, no cultivar has been developed for grassland rehabilitation. For this reason, improvement for grazing type cultivars is one of the priorities of alfalfa breeding studies. In this alfalfa study, the advanced grazing type population (L-1739), and hay type cultivars (Bilensoy-80, Savaş, Gözlü, and Plato) were evaluated for agro-morphological, and quality traits under the rainfed condition in 2012-2014 and compared in terms of the measured traits. The L-1739 had the highest number of stems, with 22.54 stems per plant in two years. However, the hay-type cultivars had a higher plant height and stem diameter than L-1739. In addition, it produced similar quality but had a lower forage yield. The general yields were 4.46 and 1.37 t ha^{-1} for green and dry forage, respectively, as a two-year mean. The quality traits of all genotypes tested in the study were similar. As a result, the L-1739 should be used for rangeland rehabilitation by over-seeding and hay-type cultivars (except Gözlü) may be used for establishing artificial pasture on cropland under rainfed conditions. Moreover, hay yield was found to be strongly correlated with plant height and stem diameter. Similar traits and their degree of similarity were determined by the method of cluster analysis.

Keywords: alfalfa, grazing type, hay type, green forage, dry forage.

INTRODUCTION

Alfalfa is one of the most widely cultivated forage legumes in the world, mown as green forage and sown as pasture for its high nutritional quality for animal feed, high biomass production potential and high adaptability (Barnes and Sheaffer, 1995; Avcı et al., 2013; Shi et al., 2017; Huang et al., 2018). Alfalfa also has the largest cultivated area (643 592 hectares), ranking first among all forage crops in Turkiye (Anonymous, 2023). It is now the most commonly used species in the rotation of cropping systems under irrigated conditions (Shi et al., 2017).

In addition, it can be useful for revegetation of degraded rangelands and establishment of artificial pastures (Açıkgöz, 2021). It is extremely difficult to find domestic productions of the species that can be used to improve the rangelands of the country (Tan and Serin, 2009). For the above reasons, many varieties are needed and this goal can only be achieved through successful breeding studies.

Forage crop breeding has some difficulties such as having various use purposes, being perennial, weak seedling development, and taking a long time to develop varieties (Schitea et al., 2007; Sabanci and Tosun, 2009; Tan and Serin, 2009). Alfalfa breeding programs are paying attention to increasing yield potential, improving forage nutritive quality, and developing tolerance to harsh environmental (abiotic/biotic) stresses (Petcu et al., 2014; Tucak et al., 2014; Shi et al., 2017).

Alfalfa is a perennial species having high genetic complexity at individual and population levels due to its autotetraploidy and cross-pollinate (allogamy) traits, therefore differentiated a high level of heterozygosity, and strong inbreeding depression (Julier et al., 2000; Tucak et al., 2010; Shi et al., 2017; Açıkgöz, 2021).

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Recently, recurrent phenotypic selection is one of the most useful methods to improve alfalfa cultivars and aims to collect frequently desired genes in the population (Li and Brummer, 2012; Shi et al., 2017). The successful breeding and development of new materials with high yield and good forage quality depends on the genetic diversity and high variability of the main alfalfa breeding materials (Julier et al., 2000; Sabancı and Tosun, 2009; Živković et al., 2012).

Pasture or grazing-type alfalfa has a broad crown structure, a prostrate shape, and rhizomes (Açıkgöz, 2021). The same author notes that they are favoured for grazing and over-seeding degraded rangelands because they can withstand extremes of cold and heat, grazing and animal pressure. Some traits, such as nutritional value, resistance to early season drought, improved regrowth, grazing types developed for dry land, and the possibility of breeding a genotype with low bloat potential, show promise for the use of alfalfa in rangelands (Keuren and Matches, 1988; Berdahl et al., 1989; Ren et al., 2021). Since there are no grazing-type cultivars registered in Türkiye so far, they must be developed immediately to reseed degraded rangelands and establish artificial pastures. Thus, they can also prevent soil erosion, maintain soil structure and increase soil fertility.

In the previous alfalfa breeding study before this trial, mass selection (Şehirali and Özgen, 1988; Demir and Turgut, 1999) was applied over an 11-year period and an advanced grazing type population named L-1739 was developed for rainfed conditions. This alfalfa trial had three objectives, which were explained in turn: 1) to determine the potential performance of the study genotypes under rainfed conditions in semi-arid regions, 2) to compare hay-type genotypes with grazing-type population, and 3) to determine yield-related traits.

MATERIAL AND METHODS

The field experiment was conducted in a previously fallowed field in 2012, 2013 and 2014 at the Gölbaşı location of the Field Crops Central Research Institute in Ankara, Türkiye. The soil in Gölbaşı location was clay-loam, with pH slightly alkaline (8.04), poor organic matter (1.32), average phosphorus content (63.7 kg ha^{-1}), high potassium content (2074.6 kg ha^{-1}, very high lime content (27.86%) (Anonymous, 2012).

During the experimental years of 2012, 2013, and 2014, total precipitation, average temperatures, and average relative humidity were 460.0, 320.0, and 532.3 mm; 12.9, 12.8 and 13.4^{o}C; 59.4, 53.6, and 59.1% at Gölbaşı, respectively (Anonymous, 2014). Long-term average precipitation (2000-2011), temperatures, and relative humidity are 389.9 mm, and 12.4^{o}C, and 59.2%, respectively. The climatic data for the test years were compared with the long-term climate data. The total precipitation in 2012 and 2014 were higher than long-term data, but that in 2013 was the opposite. The average temperatures of the years of the experiment were higher than the long-term values. The average relatives of humidity in 2012 and 2014 were the same as the long-term average, but the second-year data was higher.

This trial was established in a randomized complete block design with 4 replications covering four hay-type alfalfa cultivars, namely Bilensoy-80 (Agriculture Research For Field Crops Institute, Ankara, Türkiye) Gözlü (The General Directorate of Agricultural Enterprises, Republic of Türkiye Ministry of Agriculture and Forestry), Savaş (Eastern Anatolia Agriculture Research Institute, Erzurum, Türkiye), Plato (Private Sector Kazak Agriculture, Ayaş, Ankara, Türkiye) and the grazing type advanced population, L-1739.

Seeds were sown by hand and the seed rate was 15 kg ha^{-1} in 13 April 2012. The parcel area was 1.6 m x 5.0 m = 8.0 m^{2}, with 8 rows separated by 20 cm. After seeding, 36 kg ha^{-1} N, and 92 kg ha^{-1} P_2O_5 fertilisers were applied in the soil and the top layer of the soil was pressed with a roller of ploughing. Weed control was done manually, if required. No data were collected in the seeding year, 2012. Data on morphological and agronomic traits were collected from 27 May to 07 June 2013 and from 02 June to 05 June 2014.

Statistical analysis

The ANOVA method was used to analyse for all measured data, with genotype being the principal factor for alfalfa cultivars and population in the Excel software program of Microsoft Office 2010 (Avcı, 2020). The significance of the major effects was estimated using the F test. Differences among averages of study materials were grouped by the Least Significant Difference (LSD) test at a 5% level of probability. The same software program also applied correlation analysis. Cluster Analysis was implemented in the Minitab 16 software.

Data collection

When genotypes reached 10% bloom, 10 plants were cut from each plot and measured plant morphological traits such as plant height (PH), stem diameter (SD) and stem number (SN) (Ünal and Eraç, 2000; Anonymous, 2001). To determine dry forage yield (DFY), 4.8 m^2 of each plot was twice harvested for green herbage in a year, weighed, and added together. These collected parcel data were converted into yield per hectare.

Fresh forage samples (weighed 500 g) from each plot were dried at 70°C for 48 h and re-weighed to determine dry matter content using a technique developed by Tekkanat and Soylu (2005) and convert green herbage to DFY.

These samples were also used to estimate quality traits [crude protein ratio (CP), digestible crude protein ratio (DCP), acid detergent fiber (ADF), and neutral detergent fiber (NDF)] as a percentage of dry forage by with near-infrared reflectance (NIR) (Kutlu, 2008). Relative feed value (RFV) was calculated with a formula using {[88.9 - (0.779 x ADF%)] x (120 / NDF%)} / 1.29 (Starkey et al., 1993).

RESULTS AND DISCUSSION

Morphological traits

In the Gölbaşi location, the year, genotype, and genotype x year interaction were all significant for PH (Table 1). The interaction has been significant because the values assigned to the varieties have changed over time. For instance, in 2013, Bilensoy genotype registered the highest value while L-1739 the lowest one; however, in 2014, due to a similar value, they were placed in the same LSD group. In other words, the L-1739 has always been the shortest, and Gözlü and Savaş have remained medium. The study results for plant height (Table 1) were comparable to those measured elsewhere as higher (Rosellini et al., 1991; Demiroğlu et al., 2008; Avcı et al., 2013; Tucak et al., 2014; Gökalp et al., 2017; Turan et al., 2017) or similar (Volenec et al., 1987; Chamble and Warren, 1990; Prosperi et al., 1996; Başbağ et al., 2009; Ünal et al., 2012).

There was a significant difference between years and among genotypes for stem diameter, but no for interaction (Table 1). Values for SD in this study were similar to those reported elsewhere (Ünal and Fırıncıoğlu, 2007; Demiroğlu et al., 2008; Başbağ et al., 2009; Ünal et al., 2012; Avcı et al., 2013); Gökalp et al. (2017), but higher than reported by Tucak et al. (2014).

Significant differences were measured for stem number among the genotypes, and between years, but there was no genotype x year interaction (Table 1). Lower SNs were obtained previously at this location by Demiroğlu et al. (2008) and higher SNs were reported by Turan et al. (2017). Differences in research results may be due to differences in growing conditions, management and genetic material.

Table 1. Means and results of statistical analysis of plant height (cm), stem diameter (mm), and stem number (number/plant) for a grazing type (L-1739) and four hay types of alfalfa at Gölbaşı, Türkiye, in 2013 and 2014

Genotypes	Plant Height			Stem Diameter			Stem Number		
	2013	2014	Ave.	2013	2014	Ave.	2013	2014	Ave.
L-1739	58.37 D	29.6 B	43.99 B	2.93 B	2.11	2.52 B	24.83 A	20.25 A	22.54 A
Bilensoy 80	74.57 A	32.15 B	53.36 A	3.43 AB	2.43	2.93 A	20.20 ABC	12.38 C	16.29 BC
Gözlü	70.95 AB	29.85 B	50.40 A	3.55 A	2.24	2.90 A	14.83 C	11.35 C	13.09 D
Savaş	67.75 BC	33.62 AB	50.69 A	3.18 AB	2.40	2.79 AB	21.38 AB	17.00 B	19.19 B
Plato	65.05 C	38.02 A	51.54 A	3.38 AB	2.70	3.04 A	17.63 BC	13.60 C	15.61 CD
Mean	67.34	32.65	50.00	3.29	2.37	2.83	19.77	14.92	17.34
$F_{(var)\ (0.05)}$	**	*	**	ns	ns	*	*	**	**
$LSD_{\ (0.05)}$	5.18	5.06	3.60	0.52	0.41	0.31	5.78	2.90	2.94
$F_{\ Year\ (0.05)}$			**			**			**
$F_{\ var*year\ \ (0.05)}$			**			ns			ns
CV (%)	4.99	10.05	7.02	10.27	11.11	10.62	18.96	12.60	16.51

*, ** Significant at 5 and 1% probability levels, respectively.

Forage yields

Significant differences were seemed for FFY among the genotypes, between years, and variety x year interaction (Table 2). Savaş cultivar had the highest FFY, moreover, Bilensoy-80 and Plato cultivars also possessed a yield close to it. The L-1739 produced the smallest FFY of all genotypes. Hay-type produced more forage than grazing-type in this experiment and previous studies (Brummer and Moore, 2000; Kallenbach et al., 2002). In this study, the first year had higher FFY than the second-year value. A similar result was found by Atış et al. (2019). This status may be explained by current agricultural practices, insufficient rainfall, and genetic structure.

Significant differences were observed for DFY among the genotypes and between years, but the year x variety interaction was not significant (Table 1). Among genotypes, Bilensoy-80 and Savaş had greater DFY than Gözlü and L-1739 with Plato being intermediate. This trial data (Table 1) was lower than some reports (Waddington and Steppuhn, 1995; Chedjerat et al., 2016) and variable compared to others (Boe et al., 2020). The difference in these results shows the effect of environmental factors and genetic structure on yield. Moreover, growing season rainfall and the soil's water-holding capacity are essential suppliers of rainfed alfalfa forage yield (Jia et al., 2006; Jun et al., 2014). There is a positive correlation between the amount of water used in the alfalfa crop and dry matter yield (Shewmaker et al., 2011; Rogers et al., 2016).

Table 2. Means and results of statistical analysis of the fresh and dry forage yields (t ha^{-1}) for a grazing type (L-1739) and four hay types of alfalfa at Gölbaşı, Türkiye, in 2013 and 2014

Genotypes	Fresh Forage Yield			Dry Forage Yield		
	2013	2014	Ave.	2013	2014	Ave.
L-1739	4.48 B	2.53 D	3.50 B	1.32 C	0.81 C	1.07 C
Bilensoy 80	6.06 A	3.79 BC	4.93 A	1.81 AB	1.25 AB	1.53 A
Gözlü	4.55 B	3.21 CD	3.88 B	1.44 C	1.08 B	1.26 BC
Savaş	5.89 A	4.25 AB	5.07 A	1.82 A	1.29 AB	1.56 A
Plato	5.01 AB	4.85 A	4.93 A	1.47 BC	1.45 A	1.46 AB
Mean	5.20	3.73	4.46	1.57	1.18	1.37
$F_{(var)\ (0.05)}$	*	**	**	*	**	**
$LSD_{\ (0.05)}$	1.11	0.76	0.65	0.34	0.22	0.20
$F_{\ Year\ (0.05)}$			**			**
$F_{\ var*year\ \ (0.05)}$			*			ns
CV (%)	13.86	13.33	14.31	14.28	12.26	14.47

*, ** Significant at 5 and 1% probability levels, respectively.

Quality traits

Hay quality traits are important selection criteria for determining high quality genotypes in breeding programs (Julier et al., 2000; Atış et al., 2019). While the year effect was significant for all quality variables, no significant differences were observed among genotypes and the genotype x year interactions (Table 3 and 4). The alfalfa quality values measured in this study compare well with the values reported elsewhere (Karslı et al., 2002; Şengül et al., 2003; Kır and Soya, 2008; Başbağ et al., 2009, 2020; Çaçan et al., 2012, 2020; Avcı et al., 2013; İnal, 2015; Yılmaz and Albayrak, 2016; Engin and Mut, 2017; Gökalp et al., 2017; Turan et al., 2017; Atış et al., 2019). When the results of all these studies are examined together, the results appear to be quite different. The variations in alfalfa quality are explained by climatic factors (year effect) and genotypic traits (Saruhan and Kuşvuran, 2011; Sabancı et al., 2013; Gökalp et al., 2017). Furthermore, environmental factors strongly affect CP (Yılmaz and Albayrak, 2016), especially drought stress reduces CP (Liu et al., 2018).

The RFV in dry forage crops over 151, between 150-125, 124-103, 102-87, 86-75, and less than 75 are classified as prime, premium, good, fair, poor, and rejected, respectively (Kiraz, 2011). The genotypes in this study are classed as good based on those index values (Table 4).

Table 3. Means and results of statistical analysis of the crude protein (%) and digestible crude protein ratios (%) for a grazing type (L-1739) and four hay types of alfalfa at Gölbaşı, Türkiye, in 2013 and 2014

Genotypes	Crude protein ratio			Digestible crude protein ratio		
	2013	2014	Ave.	2013	2014	Ave.
L-1739	19.44 A	23.42	21.43	13.85 AB	16.98	15.41
Bilensoy 80	17.81 B	23.65	20.73	12.75 C	16.98	14.87
Gözlü	18.58 AB	23.92	21.25	13.31 BC	17.31	15.31
Savaş	18.68 AB	24.01	21.35	13.35 BC	17.30	15.32
Plato	19.47 A	23.73	21.60	13.96 A	17.05	15.51
Mean	18.80	23.75	21.27	13.44	17.12	15.28
$F_{(var)\ (0.05)}$	*	ns	ns	**	ns	ns
$LSD_{(0.05)}$	0.92	0.78	4.19	0.61	0.53	3.01
$F_{Year\ (0.05)}$			**			**
$F_{var*year\ (0.05)}$			ns			ns
CV (%)	3.16	2.12	18.65	2.96	2.00	18.64

*, ** Significant at 5 and 1% probability levels, respectively.

Table 4. Means and results of statistical analysis of the acid detergent fiber (%) and neutral detergent fiber (%) rates, relative feeding values for a grazing type (L-1739) and four hay types of alfalfa at Gölbaşı, Türkiye, in 2013 and 2014

Genotypes	Acid detergent fiber			Neutral detergent fiber			Relative feeding value		
	2013	2014	Ave.	2013	2014	Ave.	2013	2014	Ave.
L-1739	42.40 BC	33.72	38.06	42.40 BC	33.72	38.06	42.4 BC	33.72	38.06
Bilensoy 80	44.31 A	32.76	38.53	44.31 A	32.76	38.53	44.31 A	32.76	38.53
Gözlü	41.72 BC	33.02	37.37	41.72 BC	33.02	37.37	41.72 BC	33.02	37.37
Savaş	43.21 AB	32.98	38.10	43.21 AB	32.98	38.10	43.21 AB	32.98	38.10
Plato	41.00 C	32.44	39.63	41.00 C	32.44	39.63	41.00 C	32.44	39.63
Mean	42.52	32.98	38.34	42.52	32.98	38.34	42.52	32.98	38.34
$F_{(var)\ (0.05)}$	*	ns	ns	*	ns	ns	*	ns	ns
$LSD_{(0.05)}$	1.84	1.29	3.82	1.84	1.29	3.82	1.84	1.29	3.82
$F_{Year\ (0.05)}$			**			**			**
$F_{var*year\ (0.05)}$			ns			ns			ns
CV (%)	2.80	2.53	9.71	2.80	2.53	9.71	2.80	2.53	9.71

*, ** Significant at 5 and 1% probability levels, respectively.

Correlation coefficients

At the beginning of breeding studies, it is important to know the characteristics that are closely related to yield and to choose plants with these traits. For this reason, the Pearson correlation was performed to ascertain the relationships among the measured characteristics (Table 5). There were determined significant relationships between DFY and all other measured traits except SN, CP and DCP (Table 5). High negative correlation were seen between DFY and RFV. In a previous study, a high correlation was obtained between DFY and traits such as PH, FLW, FLL, SD, FFY (Başbağ et al., 2009), which supports our study results. In addition, Strbanovic et al. (2015) also found a high correlation between yield and PH. Considering this information, the phenotypic selection at the initiation of a breeding program should take into account morphology and quality traits. Therefore, in order to reach high yield populations, more precise and successful applications will be made and appropriate selection criteria will be established. According to the findings of this study, PH and SD are appropriate selection markers for alfalfa throughout the early stages of breeding programs.

Table 5. The correlation coefficients among the ten traits measured alfalfa genotypes in this study

	PH	SD	SN	CP	DCP	ADF	NDF	RFV	FFY	DFY
PH	1	0.892**	0.403**	-0.435**	-0.449**	0.783**	0.718**	-0.886**	0.649**	0.642**
SD		1	0.205	-0.367*	-0.378*	0.647**	0.595**	-0.731**	0.593**	0.572**
SN			1	-0.385*	-0.39*	0.343*	0.269	-0.429**	0.158	0.107
CP				1	0.999**	0.098	0.23	0.128	-0.23	-0.143
DCP					1	0.084	0.216	0.142	-0.243	-0.157
ADF						1	0.987**	-0.97**	0.501**	0.536**
NDF							1	-0.934**	0.457**	0.506**
RFV								1	-0.536**	-0.551**
FFY									1	0.971**
DFY										1

*, ** Significant at 5 and 1% probability levels, respectively

Cluster analysis (CA)

In breeding programs, it is important to understand the similarities between agro-morphological traits associated with high forage yield and excellent quality.

The correct phenotype selection in many individual plants of the nursery plot is a key factor in the success of breeding programs during the initial breeding period. For this reason, CA was implemented under the tested traits. According to the CA results, a high similarity relationship was found between the following binary traits: CP and DCP; ADF and NDF; FFY and DFY; pH and SD (Table 6). In addition, similarity levels of PH with ADF and SN were secondarily high. The smallest and largest distances existed between CP and DCP and between PH and DCP, respectively. In the same analysis, the ten traits measured were divided into six clusters (groups) (Figure 1). Cluster 1 had two traits, PH and SD. Cluster 2 owned only one trait as SN. Cluster 3 possessed CP and DCP. Cluster 4 included ADF and NDF. Cluster 5 contained one trait, RFV. Cluster 6 had FFY and DFY. As a result, in the early stages of alfalfa breeding programs, we should select taller and thicker plants. Thus, breeding objectives can be easily achieved by developing genotypes with high-yield potential.

Table 6. The level values of similarity and distance examined by cluster analysis (average linkage) related to agro-morphological values and quality traits of alfalfa genotypes

Step	Traits	Traits	Similarity level (%)	Distance level
1	CP	DCP	99.973	0.000
2	ADF	NDF	99.347	0.013
3	FFY	DFY	98.544	0.029
4	PH	SD	94.603	0.107
5	PH	ADF	84.295	0.314
6	PH	FFY	77.852	0.442
7	PH	SN	62.372	0.752
8	CP	RFV	56.761	0.864
9	PH	DCP	31.940	1.361

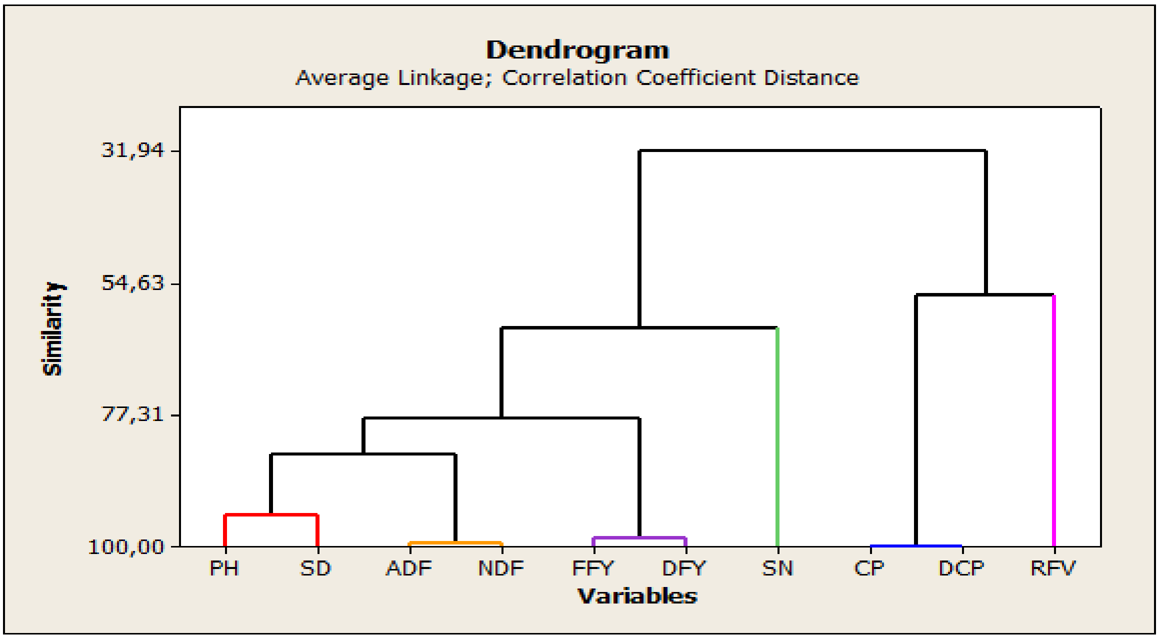

Figure 1. Dendrogram revealed by cluster analysis based on agro-morphological values and quality traits of alfalfa genotypes

CONCLUSIONS

In this study, Bilensoy-80 and Plato cultivars had the highest plant height and thickest stem diameter, respectively. In addition, the L-1739 possessed the highest stem number. Compared to the L-1739 with other genotypes, it had thinner and more shoots and gave similar quality, but produced a lower forage yield. In addition, our results showed a high correlation between yield and plant height, as well as stem diameter, therefore, these traits are suitable selection indicators to reach high-yielding populations in the initial stages of alfalfa breeding programs. In the cluster analysis result, high similarity levels were observed between crude protein and digestible crude protein; acid detergent fiber and neutral detergent fiber; fresh forage yield and dry forage yield; plant height and stem diameter. To sum up, the L-1739 should be used for rangeland rehabilitation, and other genotypes (except Gözlü) can also be used to make artificial pasture stands under rainfed conditions. The findings of this study will be useful as they are planned and pioneered for future grazing-type studies. Future grazing experiments should assess the L-1739 population's ability to persist and endure at various grazing densities.

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