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Determination of Feed Yield and Quality Parameters of Bermudagrass (*Cynodon dactylon* L. (Pers.)) Populations Collected from Natural Flora

Sedat Severoglu * and M. Kerim Gullap

Department of Field Crops, Faculty of Agriculture, Ataturk University, 25240 Erzurum, Turkey
* Correspondence: sedat.severoglu@atauni.edu.tr

Abstract: A two-year study (2020–2021) was conducted in the Erzurum province to investigate the feed quality parameters of 102 genotypes of Bermudagrass (*Cynodon dactylon*) collected from different locations in natural flora. Two control cultivars were also examined in this study, namely Coastal and Survivor. Parameters such as the fresh herbage weight, dry herbage weight, crude protein (CP) ratio, acid detergent fiber (ADF) ratio, neutral detergent fiber (NDF) ratio, and relative feed values (RFVs) were analyzed. In the first year of this study, Bermudagrass populations collected from natural flora were rooted in pots under greenhouse conditions. In the second year, the rooted plants were transplanted into experimental fields at the Atatürk University Plant Production Implementation and Research Center. The present genotypes generally had superior crude protein, ADF, NDF ratios, and relative feed values (RFVs) than the control cultivar. The fresh herbage weights ranged from 9.20 to 95.37 g per plant, while the dry herbage weights varied from 5.22 to 45.24 g per plant. The findings of this study showed that most of the genotypes collected from natural flora had superior feed quality parameters to the control genotypes.

Keywords: Bermudagrass; population; genotype; feed quality

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1. Introduction

As the world's population increases, the demand for food is growing, while the available agricultural land remains limited [1]. In this context, increasing crop production per unit area may play a vital role. For this purpose, in addition to revealing the right cultivation techniques, it is of great importance to develop new plant varieties with a superior yield and high quality. The natural flora is an important source of genes for plant breeding studies and forage crops play an important role in this respect.

Especially with regard to global warming, it is important to cultivate plants with high water use efficiency and high temperature resistance [2–4]. Indeed, as a result of global warming, daytime temperatures in the region during the summer months are recorded to be significantly higher than the optimal photosynthesis temperature range (20–25 °C) for cool-season plants [5,6]. In this regard, Bermudagrass (*Cynodon dactylon* L. Pers.), which is a typical C4 plant, is of significant importance. The most common photosynthetic system in plants is the three-carbon system, and plants that perform photosynthesis through this system are called C3 plants. Examples of such plants include wheat, barley, cotton, sugar beet, clover, and alfalfa. On the other hand, in hotter regions of the world, the photosynthetic system is the four-carbon system, and plants that utilize this system for photosynthesis are referred to as C4 plants. Examples of C4 plants include maize, sorghum, sugarcane, pearl millet, and Bermudagrass [7]. This plant, which is widely distributed in Turkey's flora, can grow in a wide range from coastal areas up to an altitude of 3000 m [8,9]. Bermudagrass (*Cynodon dactylon*) is of great importance in terms of forage production, pasture improvement, soil conservation, and landscaping due to its ability to produce

high-quality forage, suitability for mixed cropping, and its ability to cover a large area of the soil surface.

The primary objective in plant breeding is to obtain a more uniform plant population by disabling undesirable genes [10–12]. Due to the absence of selective breeding in natural ecotypes, a wide variation is observed in terms of yield and quality traits [13,14]. Indeed, a study conducted with button clover (*Medicago orbicularis* L.) collected from natural flora reported wide variation in terms of forage production per plant (ranging from a minimum of 134 g per plant to a maximum of 1655.0 g per plant) [15]. Similar results were obtained in the studies conducted by [16,17].

In C4 forage crops, it is expected that the quality of the forage would be lower compared to C3 plants due to their anatomical and biosynthetic structure [18]. However, while C3 plants experience a decline in forage quality during their summer dormancy period, reaching a value that cannot even meet the basic needs of animals for survival [19], C4 plants, due to their ability to stay green, can provide sufficient quality forage. In [20], it was reported that, under terrestrial climate conditions, due to the impact of global warming and the extension of the summer drought period, supplementary feeding costs would increase in pastures dominated by C3 plants during the summer months. The identification of suitable C4 plants that can remain green during the summer months and the development of genotypes with high yield and high forage quality among them can be effective in mitigating the severity of this problem. As a matter of fact, studies conducted by [21–23] reported wide variation in terms of quality within the plant materials collected from natural flora. The study of *Bituminaria bituminosa* (L.) C.H. Stirtion in [22] reported that the crude protein (CP) content ranged from 13.65% to 21.05%, the neutral detergent fiber (NDF) content ranged from 43.03% to 57.01%, and the acid detergent fiber (ADF) content ranged from 27.85% to 44.72%.

Commercial varieties generally do not adapt well to the eastern Anatolia region due to their low-altitude origin. However, the natural ecotypes of this plant, encountered in the region's natural flora, are expected to be cold-tolerant. In fact, the collected natural material in this study was successfully grown in Erzurum, one of the coldest provinces in the region. In this study, the forage production and basic quality characteristics of the material discussed are emphasized. Furthermore, forage yield and quality characteristics of wild Bermudagrass genotypes collected from natural flora were tried to be determined.

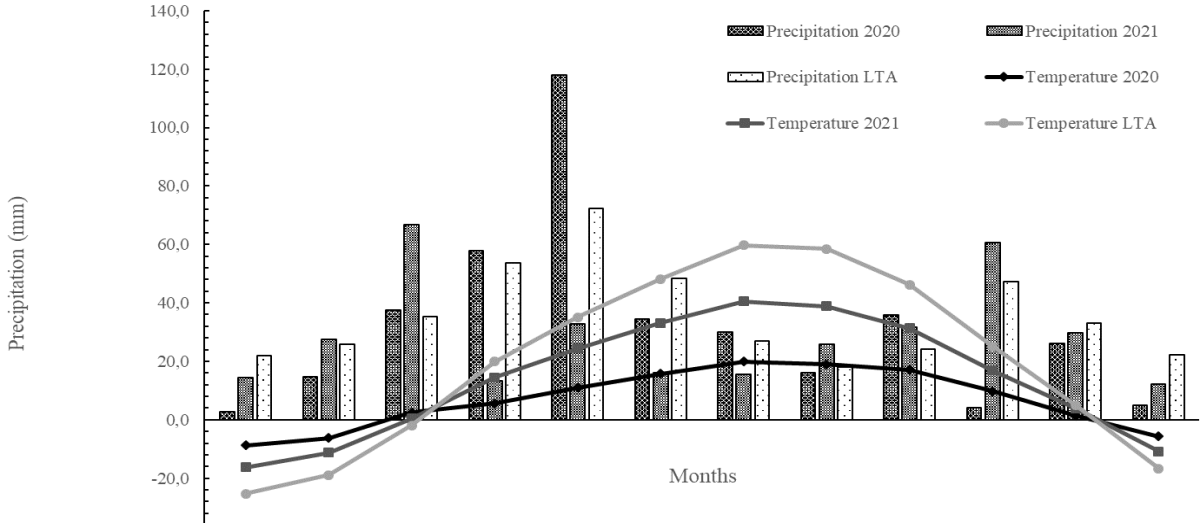
2. Materials and Methods

This study was carried out during a period of two years with materials collected from the natural flora (mainly pastures, in addition to meadows, forests, and field lands) of Agri, Ardahan, Bayburt Bingol, Erzincan, Erzurum, Igdir, Kars, and Mus provinces in the eastern Anatolia and northeastern Anatolia regions (Figure 1). The materials collected from natural flora in 2020 were grown for 2 years in the experimental fields of Ataturk University Plant Production Implementation and Research Center in 2020 and 2021. The relative feed values were calculated using the NDF (Neutral Detergent Fiber) and ADF (Acid Detergent Fiber) ratios. For each genotype, 10 plants were harvested separately, and their individual CP (Crude Protein), ADF, and NDF values were calculated and averaged. The relative feed values were calculated using the NDF (Neutral Detergent Fiber) and ADF (Acid Detergent Fiber) ratios. For each genotype, 10 plants were harvested separately, and their individual CP (Crude Protein), ADF, and NDF values were calculated and averaged.

The texture classes of the soil samples taken from the experimental area were determined using the Bouyoucos hydrometer method [24]. The soil structure of the experimental area was recorded as clay-loam [25]. The soils of the experimental area were unsaline with an average electrical conductivity of 2.23 dS m^{-1} [26], slightly alkaline with a pH value of 7.64 [27], limey with a lime content of 2.24% [27], low in organic matter with an organic matter content of 1.05% [27], low in available phosphorus with a value of 0.53 kg P_2O_5 ha^{-1} [27], and excessive in extractable potassium with a value of 15.31 kg K_2O ha^{-1} [28].

Figure 1. The location of the provinces where the Bermudagrass was collected in relation to the map of Turkey.

The average temperatures in 2020 (6.8 °C) and 2021 (7.1 °C) at which the experiments were conducted were higher than the long-term average (5.7 °C). The annual total precipitation in 2021 (346.3 mm) was lower than that in 2020 (382.7 mm) and the long-term average (429.9 mm). The second year of the study was both hotter and had less precipitation as compared to the first year and the long-term averages (Figure 2). Furthermore, the years in which the planting took place were particularly favorable in terms of temperature for the cultivation of Bermudagrass plants.



	January	February	March	April	May	June	July	August	September	October	November	December
Precipitation 2020	2,8	14,8	37,6	57,8	118,0	34,6	30,0	16,2	35,8	4,0	26,2	4,9
Precipitation 2021	14,3	27,6	66,8	13,4	32,8	16,0	15,4	25,8	31,6	60,6	29,8	12,2
Precipitation LTA	21,9	25,9	35,4	53,8	72,4	48,4	27,0	18,1	24,3	47,4	33,2	22,1
Temperature LTA	-9,1	-7,7	-2,4	5,4	10,7	14,9	19,2	19,5	14,8	8,2	1,1	-5,8
Temperature 2021	-7,4	-5,0	-2,0	8,9	13,4	17,5	20,6	20,0	14,2	7,1	2,5	-5,2
Temperature 2020	-8,8	-6,2	2,5	5,6	11,0	15,7	19,9	18,9	17,2	9,8	1,4	-5,7

* The long-term average represents the average over 92 years from 1929 to 2021

Figure 2. Climate data of Erzurum province for the years 2020 and 2021 and the long-term averages *.

A total of 102 genotypes were collected from the natural flora and two control cultivars (Coastal and Survivor) were also included in this study. The present study was carried out in three stages: plant collection, greenhouse experiments, and field experiments. The plants were collected from the natural flora, coordinates, and altitudes of collection points were recorded and soil samples were taken. Material collection was performed by removing the plants with the soil and random sampling method was used in material collection [29,30]. During the plant collection phase of this study, to represent the populations in the regions and keep the variation wide, a distance of at least 4–5 km was left between the collection points and the plants within an area of approximately 100 m^2 were considered as the population. The collection process was carried out at 100 points, and 10 plants were randomly taken from each point, only by looking at the phenotype of the plants and a total of 1000 plants belonging to at least 100 populations were collected.

Plants that were removed from their original place with soil were planted in pots prepared beforehand in the greenhouses within 24 h. As such, 10 plants were taken from each population by cutting their shoots with scissors and a plant collection of 1020 pots belonging to 102 populations was created. After the collected populations were rooted in the greenhouse environment, they were transplanted into experimental fields in the form of single-row columns on 27 April 2020. Plants from each population were planted in a row at 70 cm × 70 cm spacings. Field trials were conducted in augmented experimental design. Plants were randomly distributed into six blocks and control cultivars were repeated in each block. Following the planting process, irrigations were practiced and 1 kg N per hectare was applied to the experimental plots for better establishment [31]. The first stages of synthetic variety breeding, the principles of which were determined by [32], were carried out in this study.

At the beginning of flowering, 10 plants were harvested from each population by leaving 5 cm stubble height. The fresh herbage weight per plant was determined and plants were dried at 60 °C until it reached a constant weight and the dry herbage weight per plant was determined [33]. The dried samples were ground in a feed mill for quality analysis. The nitrogen ratio of the ground samples was determined by the Kjeldahl method in accordance with the principles specified by [34]. For the crude protein (CP) ratio, the nitrogen ratio was multiplied by the coefficient of 6.25, as recommended by [35]. The neutral detergent fiber (NDF) and acid detergent fiber (ADF) ratios were determined with the use of an ANKOM fiber analyzer device in accordance with the principles specified in [36]. Relative feed values greater than 100 indicate increased feed quality and values lower than 100 indicate reduced feed quality [37,38].

Statistical analyses were carried out with the use of R statistical analysis software [39] in accordance with augmented randomized complete blocks design (AugmentedRCBD) [40–42]. Significant means were compared with the use of the least significant difference (LSD) multiple comparison test. Results are presented as the mean and standard deviation (SD), unless otherwise stated, and differences were considered significant for $p < 0.001$.

After standardizing all data, the principal component (Biplot) and hierarchical clustering analyses were performed. In the hierarchical clustering analysis, statistically appropriate distance and linkage algorithms were selected. The "cophenetic" distance and "Wald" linkage algorithms were used in this analysis. Additionally, the Jaccard index was used to determine the optimal number of clusters. All statistical analyses regarding the traits examined in the study were performed using the "cluster", "factoextra", "dendextend", and "corrplot" packages in the R software [43].

3. Results and Discussion

In the combined analysis of the years, there were highly significant differences in the fresh herbage weight per plant values (Table 1). The average fresh herbage weight per plant was determined to be 37.55 g per plant, the highest value was obtained from genotype G47, and the lowest from genotype G42 (Table 1).

Table 1. Variance analysis for the FHW, DHW, CP, NDF, ADF, and RFV of the genotypes [1].

Source of Variation	DF	FHW	DHW	CP	NDF	ADF	RFV
Block (eliminating treatments)	5	0.00 ns	0.06 ns	0.00 ns	0.00 ns	0.00 ns	0.00 ns
Treatment: check	1	0.06 ns	2.29 ns	0.08 ns	0.02 ns	0.94 ns	0.07 ns
Treatment: test	101	3.64 ***	2.64 ***	0.14 ns	0.67 ns	0.75 ns	0.69 ns
Treatment (ignoring blocks)	103	3.91 ***	2.90 ***	0.15 ns	1.03 ns	1.11 ns	1.15 ns
Treatment: test vs. check	1	35.03 ***	29.73 ***	1.14 ns	38.88 ***	36.76 ***	49.08 ***

[1] DF: Degree of freedom, FHW: fresh herbage weight, DHW: dry herbage weight, CP: crude protein, NDF: neutral detergent fiber, ADF: acid detergent fiber, RFV: relative feed value, ns: non-significant, ***: $p \leq 0.001$.

In the conducted study, both the test genotypes and all genotypes showed statistically significant differences at a significance level of 1%. These differences may have arisen due to the genetic characteristics of the utilized genotypes and the distinct abiotic conditions (as presented in Table 1) of the regions where the genotypes were collected. These factors likely played a role in the observed variations. Furthermore, the significant climatic differences between the years may have had a significant impact on the formation of this difference. Because the plants were transplanted in April, which was the first year of establishment, these may have experienced significant problems during winter emergence in the second year due to low temperatures and precipitation. Indeed, the statements made by several researchers, such as [17,44–47], that the green forage yield varies depending on the plant species and genus, plant density per unit area, utilization method, maturity period, genetic characteristics, and environmental factors support our study. The fact that the test vs. check treatment was important in this study shows that the test genotypes are significantly different from the control genotypes. As a matter of fact, although many genotypes have numerically higher green forage yield values than control genotypes, according to the results of multiple comparison tests, the G47 genotype has a higher green forage yield value than the test genotypes. A wide variation in the traits in natural populations is expected, which is a desirable outcome for breeding programs. Indeed, [15], who conducted a similar study with *Medicago orbicularis* L. plant, highlighted wide variation in terms of green forage yield.

The dry herbage weights of the genotypes varied between 5.22 and 45.24 g per plant with the highest value from genotype G47, followed by genotypes G73 and G66, and the lowest value from genotype G49, followed by genotypes G42 and G93. Dry herbage weights of the Coastal and Survivor cultivars varied between 8.96 and 13.30 g per plant (Table 2). In this study, statistically significant differences were determined between the test genotypes and all genotypes, indicating that the observed differences, similarly to the green forage yield per plant, may be attributed to both genetic and ecological variations among the plants. Indeed, many researchers [48–51] stated that both ecological conditions and genetic variations among cultivars have a significant impact on the dry matter yield of plants. In addition, a test vs. check treatment was important in this study, which may have been due to the significant differences in the test genotypes in terms of dry matter yield compared to the control genotypes. Indeed, [52] conducted a similar study with *Medicago sativa* L. collected from natural vegetation and reported a wide variation in dry matter yield between 1143 kg ha^{-1} and 2183 kg ha^{-1}. The presence of numerous genotypes surpassing the control varieties in the study is a promising result.

Table 2. Coordinate, altitude, FHW, DHW, CP, NDF, ADF, and RFV values of the genotypes [1].

Genotype	Altitude (m)	FHW (g per plant)	DHW (g per plant)	CP (%)	NDF (%)	ADF (%)	RFV
G1	1768	45.26	22.10	11.81	61.15	28.85	101.96
G2	1671	31.79	14.13	11.46	61.34	29.31	100.85
G3	1410	37.51	17.46	10.53	62.64	29.94	97.52
G4	1424	18.50	11.87	11.86	63.39	28.14	98.30

Table 2. *Cont.*

Genotype	Altitude (m)	FHW (g per plant)	DHW (g per plant)	CP (%)	NDF (%)	ADF (%)	RFV
G5	1544	36.00	19.77	13.02	61.25	28.48	101.17
G6	836	23.02	11.58	10.92	63.19	31.22	95.32
G7	1522	26.56	17.07	12.44	63.46	31.37	94.48
G8	1663	44.21	23.67	11.62	63.43	30.32	95.94
G9	1265	17.01	11.14	11.48	59.94	27.86	104.17
G10	817	53.75	29.12	9.98	63.10	33.39	93.34
G11	1808	84.80	35.92	10.07	66.63	29.36	92.41
G12	971	51.82	24.85	11.37	62.83	26.41	101.60
G13	1564	14.24	9.51	11.39	61.51	30.50	98.38
G14	1564	13.65	10.28	11.16	59.22	30.83	101.77
G15	817	85.47	36.50	11.45	64.40	30.14	94.92
G16	1444	23.98	14.03	9.78	55.37	27.69	112.95
G17	1386	36.51	22.74	11.21	61.53	26.66	102.92
G18	1354	26.31	18.21	11.53	67.56	29.61	90.67
G19	1845	59.62	34.71	10.41	64.92	30.17	93.59
G20	1121	46.64	22.33	10.20	61.79	29.21	101.30
G21	1013	41.01	20.74	10.37	67.67	29.76	91.57
G22	1662	17.59	13.20	12.52	58.03	25.75	110.75
G23	1846	39.39	20.01	12.41	59.65	25.84	107.12
G24	1411	13.10	9.19	10.98	58.77	27.94	107.19
G25	1355	28.95	20.89	11.45	61.95	32.71	95.17
G26	1775	59.80	25.29	10.82	63.99	29.42	96.11
G27	1625	61.77	32.75	9.70	61.42	28.00	102.84
G28	1585	24.39	16.57	12.43	58.64	28.82	105.58
G29	1145	33.17	21.96	11.33	57.93	27.25	108.77
G30	1158	77.64	33.62	11.91	64.09	29.12	96.10
G31	1779	28.42	17.02	10.75	64.34	31.20	93.35
G32	1355	15.15	12.55	12.21	63.82	29.91	95.57
G33	1775	44.12	18.40	11.45	58.92	28.57	105.26
G34	1673	44.57	23.93	12.48	63.29	26.43	100.26
G35	1705	46.50	19.60	9.99	65.20	30.18	94.59
G36	1712	27.27	13.52	11.79	62.37	30.54	97.90
G37	1795	17.29	7.51	10.12	66.32	30.67	91.29
G38	1400	23.81	15.26	11.48	65.03	30.87	92.93
G39	1970	41.75	23.18	10.50	60.91	29.00	101.74
G40	1262	20.27	14.09	11.03	64.04	29.12	96.25
G41	1296	12.71	6.85	11.71	59.48	28.95	103.83
G42	1463	9.20	6.53	10.67	55.52	27.42	114.31
G43	824	46.71	19.32	11.05	65.68	31.22	91.54
G44	1907	40.48	17.93	10.21	63.39	30.33	87.18
G45	1228	30.61	13.18	10.45	63.36	32.00	94.96
G46	1187	47.17	19.63	12.12	62.30	29.02	99.20
G47	1408	95.37	45.24	12.02	61.26	28.28	102.28
G48	1588	38.67	18.94	11.53	61.73	29.18	100.89
G49	1320	13.19	5.22	12.86	59.33	26.19	107.50
G50	1536	44.46	17.71	12.11	59.52	29.56	103.32
G51	1536	71.88	28.73	11.93	61.87	28.79	100.78
G52	1759	47.51	24.03	10.72	65.79	31.73	91.19
G53	1680	59.02	28.06	10.62	65.08	31.12	93.35
G54	1686	56.26	24.20	9.07	61.87	29.40	100.60
G55	1805	38.63	22.55	10.80	65.30	31.66	91.91
G56	1476	28.67	18.68	13.25	61.54	29.14	101.39
G57	1008	46.08	18.77	10.46	64.80	33.41	90.86
G58	1592	32.72	21.43	11.20	58.98	29.86	103.66
G59	1163	35.70	18.30	11.09	59.70	25.80	107.61
G60	1413	26.40	17.26	11.34	60.91	29.94	100.33
G61	1158	19.70	8.11	9.44	58.34	23.09	113.29
G62	854	61.89	26.12	12.00	64.14	29.70	95.95
G63	1410	25.77	18.25	11.05	61.60	29.16	101.76
G64	1218	56.50	24.51	12.29	60.40	24.91	107.39
G65	1496	25.69	11.74	11.13	63.51	27.73	98.76
G66	1798	67.17	38.81	11.75	67.26	30.64	90.27
G67	1258	20.32	11.47	12.21	61.43	29.70	99.76
G68	1339	20.97	13.14	11.27	64.87	27.71	96.67
G69	1545	50.14	24.15	10.38	64.55	30.38	95.91
G70	1169	50.84	24.65	10.48	61.66	26.67	103.70

Table 2. *Cont.*

Genotype	Altitude (m)	FHW (g per plant)	DHW (g per plant)	CP (%)	NDF (%)	ADF (%)	RFV
G71	1713	36.57	23.33	11.09	62.66	29.21	98.36
G72	1866	90.39	42.00	11.93	67.27	31.08	89.73
G73	817	59.78	24.05	10.55	67.01	32.19	89.66
G74	1339	20.07	15.74	12.37	64.73	30.34	94.04
G75	1507	13.89	6.59	11.53	61.73	26.78	103.24
G76	1167	21.35	10.23	9.91	62.11	28.01	100.63
G77	1350	72.01	31.03	10.27	63.44	30.79	95.65
G78	1655	47.06	27.32	11.73	65.65	31.65	91.16
G79	1331	17.02	11.32	11.35	57.61	27.16	111.43
G80	1286	22.92	14.03	10.62	61.57	29.01	100.49
G81	1335	20.67	12.47	11.10	52.14	26.07	123.15
G82	839	64.14	28.10	12.57	62.79	30.22	97.42
G83	1386	32.12	18.07	10.87	63.93	27.00	98.97
G84	1860	24.19	16.10	11.76	63.02	27.90	99.83
G85	1838	26.51	15.11	11.01	62.69	31.59	95.75
G86	1355	28.03	18.15	12.08	62.18	26.78	101.94
G87	1549	31.54	19.51	13.38	64.77	31.00	93.50
G88	1035	48.66	23.37	10.98	62.58	27.34	100.58
G89	1158	29.10	18.60	12.29	62.57	26.00	102.33
G90	1163	39.36	21.04	11.68	60.33	26.90	106.38
G91	1734	37.76	21.81	12.45	63.37	29.09	97.90
G92	1661	25.61	13.46	10.06	55.86	26.21	115.53
G93	1434	10.56	6.58	12.65	60.24	29.98	101.32
G94	1176	15.90	8.75	10.36	57.52	26.47	111.07
G95	1130	22.60	8.85	10.77	56.44	27.91	112.33
G96	1302	25.76	12.83	11.74	63.85	28.64	97.82
G97	1654	29.03	12.14	12.78	59.59	28.69	104.30
G98	1624	25.77	11.03	9.96	65.47	30.98	93.63
G99	1743	70.70	31.00	10.38	65.38	29.40	94.11
G100	818	52.05	25.84	10.96	62.68	30.54	97.18
G101	1922	35.75	18.97	12.17	59.52	27.72	105.15
G102	1710	62.13	29.84	12.68	61.34	28.28	101.56
K1		19.04	8.96	11.89	55.60	24.35	117.08
K2		20.44	13.30	12.28	55.31	25.59	115.93
Mean		37.55	19.24	11.32	62.05	28.97	100.22
CV (%)		39.50	37.94	29.77	8.14	10.90	10.80
SD		19.14	8.13	0.90	3.01	1.98	6.79
LSD (control treatments)		16.25	8.04	3.87	5.72	3.57	12.55
LSD (test treatments in different blocks)		48.74	24.12	11.62	17.15	10.70	37.65
LSD (test treatments in the same block)		39.79	19.69	9.47	14.00	8.74	30.74
LSD (test treatment and control treatment)		37.22	18.42	8.87	13.10	8.17	28.76

[1] FHW: fresh herbage weight, DHW: dry herbage weight, CP: crude protein, NDF: neutral detergent fiber, ADF: acid detergent fiber, RFV: relative feed value.

The average crude protein ratio, an important indicator of nutritional value and digestibility [53], was identified to be 11.32% (Table 2). As the average of years, the lowest crude protein ratio was obtained from genotype G54, followed by genotypes G61 and G27. The highest crude protein ratio was obtained from genotype G87, followed by genotypes G56 and G5. The fact that the test genotypes were different compared to the control genotypes in this study was an important factor in the importance of a test vs. check treatment. Mathematically, while 15 genotypes have a higher crude protein content compared to the control genotypes, according to the multiple comparison test, the G87 genotype has a higher crude protein content compared to the other test genotypes. The emergence of such wide variations among the collected genotypes is considered a promising development for breeders. As a matter of fact, refs. [52,54], who conducted similar studies, determined that the crude protein ratio of alfalfa plants collected from natural flora showed a wide variation between 21.7 and 24.2% and 17.4 and 22.6%, respectively.

As the average over the years, the NDF ratios of the genotypes varied between 52.14 and 67.67% with the lowest value from genotype G81 and the highest value from genotype

G21 (Table 2). In this study, the test genotypes showed significant differences in terms of NDF ratio compared to the control genotypes, which led to the importance of the test vs. check treatment. These differences between the collected genotypes will provide an important opportunity for the development of new varieties to be used in breeding studies and will thus be preserved in our local varieties that are in danger of extinction. As a matter of fact, [23] and [55], who conducted similar studies with orchard grass and white sweet clover, reported significant variations in the NDF ratio.

As the average of the years, the ADF ratios of the genotypes varied between 23.09 and 33.41% with an average value of 28.97%. The lowest ADF ratio was recorded in genotype G61, followed by genotypes G64 and G22. The highest ADF ratio was seen in genotype G57, followed by genotypes G10 and G25. The ADF ratios of the Coastal and Survivor cultivars were recorded as 24.35% and 25.59%, respectively (Table 2). The ADF ratio, which consists of lignin and cellulose [56], has shown a significant difference in the test genotypes compared to the control genotypes. This difference observed between the collected genotypes and control cultivars made the test vs. control treatment important. It is expected that wild genotypes collected from natural flora exhibit significant variations in their genetic characteristics due to environmental conditions [57,58]. Indeed, many studies [55,59–63] have identified a wide variation in ADF content among the species collected from natural flora.

As the average over the years, the relative feed values (RFVs) of the genotypes varied between 87.18 and 123.15%, with an average value of 100.22%. The lowest RFV was obtained from genotype G44, followed by genotypes G73 and G72. The highest RFV was obtained from genotype G81, followed by genotypes G92 and G42. The relative feed value (RFV) was recorded as 117.08% for the Coastal cultivar and 115.93% for the Survivor cultivar. Only genotype G81 had a higher value than these cultivars (Table 2). The relative feed value (RFV) was used as an important feed quality indicator which was calculated by comparing the plants with alfalfa and a relative feed value of alfalfa was taken as 100 [64]. The feed quality is classified based on RFV as: <75: fifth class, 75–86: fourth class; 87–102: third class; 103–124: second class; 125–150: first class and >150: top quality [64–67]. Based on this classification of the present genotypes, 76 were in the third class, 26 in the second class and control cultivars were in the second-class quality group. It was seen that significant part of the present genotypes had a RFV of greater than the reference value of 100 (Table 2). The significant interaction between the test vs. check treatment in the conducted study indicates that there are significant differences between the test genotypes and control genotypes. Indeed, mathematically, many genotypes, except for G81, have a lower relative feed value compared to the control genotypes. However, according to the results of the multiple comparison test, the G44 genotype has a lower relative feed value compared to the test genotypes. As a result, it has been determined that there is wide variation among the collected genotypes, and this finding provides an opportunity to create valuable genetic material for further breeding studies. Indeed, many studies conducted on different plant species [68–72] have obtained wide variations.

Hierarchical clustering analysis was conducted with the use of the fresh herbage weight, dry herbage weight, crude protein, ADF, NDF, and RFV of 102 genotypes and control cultivars (Figure 3). The present genotypes were divided into two main clusters. The closeness and distance of genotypes from one another in terms of investigated parameters were determined and genotype G81 had the closest characteristics to the control cultivars.

The biplot graph showing the relationships between the genotypes and the investigated traits is presented in Figure 3. The biplot graph offers a visual assessment of the relationships between the investigated traits [73,74]. In present biplot, PC1 explains 56.90% of total variation and PC2 explains 20% of the total variation (i.e., the two principal components explain 76.90% of total variation) (Figure 4).

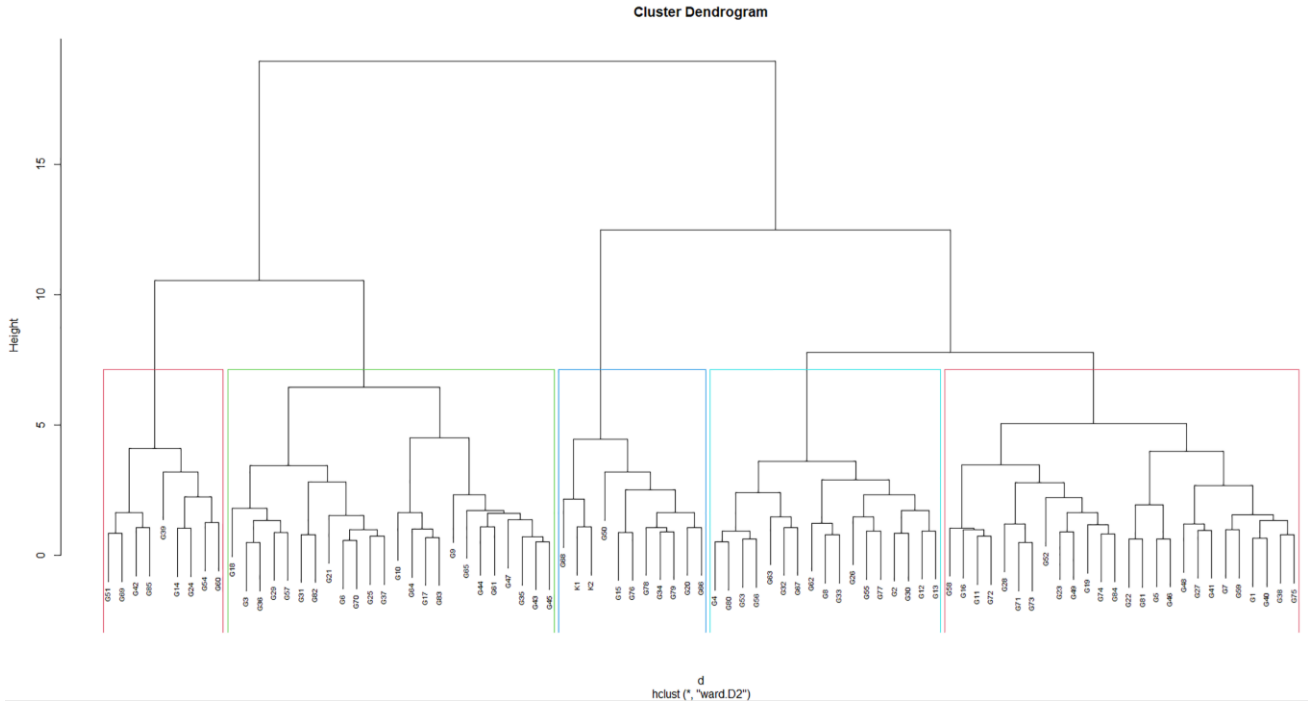


Figure 3. Hierarchical clustering analysis.

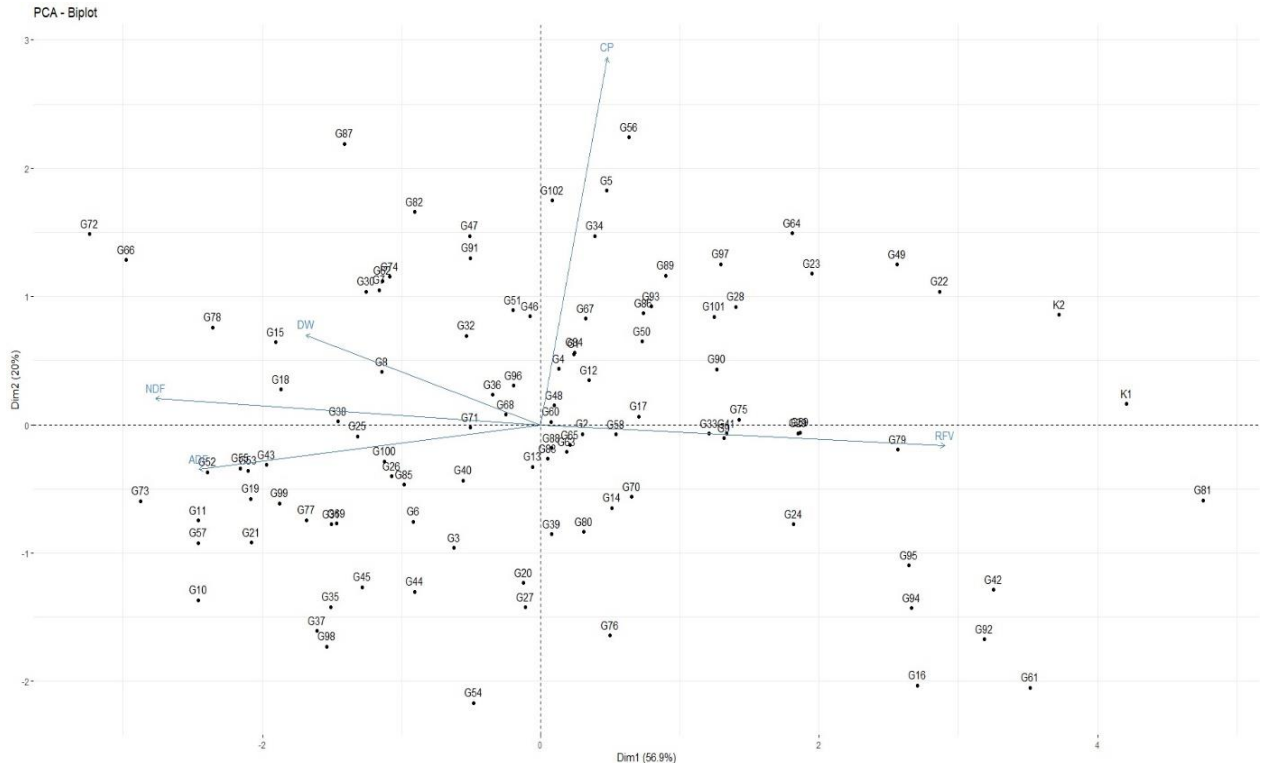


Figure 4. Biplot graph for the investigated parameters of the genotypes.

4. Conclusions

When the results were evaluated as a whole, the G1, G2, G3, G5, G7, G8, G10, G11, G12, G15, G17, G19, G20, G21, G22, G23, G26, G27, G28, G29, G30, G33, G34, G36, G39, G43, G44, G45, G46, G47 G48, G49. G50, G51, G52, G53, G54, G55, G56, G57, G58, G59, G62, G64, G66, G69, G70, G71, G72, G73, G74, G78, G82, G86, G87, G88, G89, G90, G91, G93, G97,

G99, G100, G101, and G102 genotypes stood out as being similar or superior to the control cultivars in terms of feed quality characteristics. According to the clustering analysis, genotype G81 demonstrated characteristics similar to the control cultivars. Therefore, these genotypes, especially their distant relatives, showing superior performances, are promising for the development of Bermudagrass cultivars suitable for cold high-altitude regions. In light of the findings obtained from this study, it has been decided to continue synthetic variety development studies on the considered material, and the research is ongoing.

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