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MURAT AZAK

ERDEM GÜLÜMSER

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Silage yield and quality of different grass pea (*Lathyrus sativus* L.) genotypes

Murat AZAK, Erdem GÜLÜMSER*

Department of Field Crops, Faculty of Agriculture and Natural Science, Bilecik Şeyh Edebali University, Bilecik, Turkiye

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Abstract: This study aimed to determine the silage yield and quality of different 12 grass pea (*Lathyrus sativus* L.) genotypes (Gap Mavisi, İptaş, Karadağ, 1603, 2006, 2401, 4301, 4403, 5001, 6408, 6410, and S3). The trial was established in Bilecik's ecological conditions in 2022 and 2023. It was carried out according to the randomized block design with four replications, and the plants were harvested at the full flowering stage. The harvested plants were chopped to 2 cm in size and ensiled in vacuum bags and left to ferment at 25 ± 2 °C for 45 days. The silage yield, pH, dry matter ratio, Flieg score, lactic acid (LA), acetic acid (AA), citric acid (CA), propionic acid (PA), succinic acid (SA), crude protein ratio (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), potassium (K), phosphorus (P), calcium (Ca), magnesium (Mg), condensed tannin (CT), total flavonoid (TF), total phenolic (TP), radical scavenger activity (DPPH), total alkaloid (TA), and N-oxalyl-L-alpha, beta-diaminopropionic acid (ODAP) contents were determined. The silage yields of the genotypes ranged from 9.01 to 13.64 t ha^{-1}. The highest Flieg score was determined in the 5001 population with 85.88. The CP, ADF, and NDF ratios of silages varied between 19.35% and 23.06%, 29.19% and 32.28%, and 36.72% and 43.81%. The highest LA was determined in the 5001 populations with 2.63%. The nutrient contents of all silages were at a level to meet the needs of the animals. The lowest ODAP content was determined in the 5001 (0.95 mg g^{-1}) and 6408 (0.90 mg g^{-1}) populations. As a result, the 5001 population exhibited superior performance to the other genotypes in terms of silage quality. This shows that the population in question is promising for the region and is also important in future breeding studies in terms of being evaluated as a material.

Key words: Grass pea, genotype, silage yield, quality

1. Introduction

The grass pea (*Lathyrus sativus* L.) is a member of the legume family (*Fabaceae*) and is used for feeding animals in the form of silage, hay, or grain, or for grazing. Since it is more advantageous than other legume forage crops due to its short vegetation period, the crop is also used as a green manure crop for improving soil structure and as a legume or vegetable in human nutrition. Its most important feature is drought tolerance. It can be cultivated in regions with a precipitation rate of 250 mm and is thus a primary drought-tolerant cultivated plant. On the other hand, it can also be successfully cultivated in submerged areas or areas where the annual precipitation rate is high. With its strong root system and high nitrogen-fixing capacity (67 kg ha^{-1}), it can be cultivated in many different soil types. It is more tolerant of grazing than many other legumes. As it can be cultivated under many different soil and climate conditions without the need for fertilizing or pesticide application and is resistant to stress factors, it is also important for sustainable agriculture and legume amendment. It is a highly suitable model crop, particularly to achieve an understanding of its drought tolerance mechanism and explore the genes associated with it (Tokarz et al., 2020).

In recent years, producers have preferred silage as a source of quality roughage. The main reason is that silage is a cheap feed source and it increases the yield and quality of animals fed with silage. On the other hand, nutritional losses occur in plants during the drying process. Since drying is not required during the silage production phase, these losses can be avoided (Kaymak et al., 2021).

The high buffering capacity and protein content found in legume plants prevent silage fermentation and silage pH cannot reach the desired level (Playne and McDonald, 1966). Another obstacle to fermentation is the low levels of soluble carbohydrates in legume forage plants (Owens et al., 1999). However, in recent years, the silage technology developed has helped overcome the difficulties with legumes in silage production. This means that animals are fed with higher-quality feed. Albrecht and Beauchemin (2003) reported that the soluble protein ratio in legume hay was 37.7%, while it was 55.80% in silage.

The aim of the present study was to determine the silage yield and quality of differences grass pea genotypes.

* Correspondence: erdem.gulumser@bilecik.edu.tr

2. Materials and methods

A total of 12 grass pea (*Lathyrus sativus* L.) genotypes were used. They included three registered varieties (Gap Mavisi, İptaş, and Karadağ). Information on the nine local populations is given in Table 1. The genotypes were tested under the same ecological conditions. The trial was held under the ecological conditions of Bilecik, Türkiye, for 2 years, in 2022 and 2023.

The soil analysis results of the regions where the trial plots were located revealed similar soil structure properties in the two areas. Accordingly, both soil structures were clayey-loamy, with medium values of lime (8.78% and 12.19%) and organic matter (2.43% and 2.74%) content in 2022 and 2023 (Table 2).

The temperature and precipitation values of the experiment region for long years and the 2022 and 2023 are given in Table 2. The average temperature was 15.8 °C for long years and 17.7 °C and 15.2 °C for 2022 and 2023, respectively. While the province's total precipitation values for long years (132.5 mm) and for 2022 (139.1 mm) were close to each other, this value was 182.3 mm in 2023, the second year of the experiment (Table 3).

The experiment was set up in the randomized blocks design with 4 repetitions. Trials were set up manually in 6 rows (plot area: 7.2 m^2) with a row spacing of 30 cm, row length of 4 m in plots, and 60 seeds per m^2. DAP fertilizer was applied during sowing with 80 kg of P_2O_5 per hectare (ha). No irrigation was applied in the trial. Plant materials harvested at the flowering stage was ensiled in 2 kg special jars and stored at 25 ± 2 °C under laboratory conditions for 45 days.

2.1. Silage yield

After the plants were harvested and weighed, the green forage yield was calculated as t ha^{-1} from fresh weight. Reducing the silage losses by 25% over the yields of green forage allowed the calculation of silage yield.

2.2. Dry matter and pH

The dry matter ratio (DM, %) was computed after silage samples were dried for 48 h at 105 °C in a hot-air oven. A digital pH meter was used to measure the pH of the silage samples. The Flieg score, a number calculated using pH and dry matter ratio (DM%), is a useful indicator of silage quantity:

[Flieg score = 220 + (2 × DM% – 15) – 40 × pH)]. Flieg scores of 81–100 indicate very good, 61–80 good, 41–60 medium, 21–40 poor, and 0–20 very poor silage quality.

2.3. Organic acids

A 20-g silage sample was taken from each jar and mixed with 100 L of distilled water for 30 min by an electric blender and then filtered. The pH of silage samples was determined using a digital pH meter. Organic acid analysis (lactic acid (LA), acetic acid (AA), butyric acid (BA), citric acid (CA), and succinic acid (SA)) of the silages

Table 1. Origins of the investigated grass pea genotypes.

Genotypes	Genotypes origin			
	City	**Town**	**Village**	**Altitude (m)**
1603	Bursa	Harmancık	Demirciler	719
2006	Denizli	Cal	Baklancakırlar	886
2401	Elazığ	Merkez	Uzuntarla	995
4301	Kütahya	Domaniç	-	-
4403	Malatya	Darende	Başdirek	1445
5001	Nevşehir	Kozaklı	Kalecik	1120
6408	Uşak	Ulubey	Kılsa	800
6410	Uşak	Ulubey	Kılsa	800
S3	ICARDA			
Gap Mavisi	Registered variety			
İptaş	Registered variety			
Karadağ	Registered variety			

Table 2. Soil properties of the experiment areas during the experimental years.

Properties	2022		2023	
	Results	Degree	Results	Degree
Structure	50.38	Clay-loam	58.30	Clay-loam
pH	7.91	Light alkali	7.98	Light alkali
Salinity (%)	0.019	Saltless	0.011	Saltless
$CaCO_3$ (%)	8.78	Middle	12.19	Middle
Organic matter (%)	2.43	Middle	2.74	Middle
P_2O_5 (kg ha^{-1})	17.70	Very little	19.90	Very little
K_2O (kg ha^{-1})	139.50	Little	240.80	Middle

Table 3. Meteorological data of the experiment area in the long term and experimental years.

Months	Temperature (°C)			Precipitation (mm)		
	Long-term	2022	2023	Long-term	2022	2023
April	11.5	13.3	11.3	41.9	24.8	55.6
May	16.2	16.8	14.6	47.7	19.0	67.6
June	19.9	20.3	19.7	42.9	95.3	59.1
Average/total	**15.8**	**17.7**	**15.2**	**132.5**	**139.1**	**182.3**

was performed on an HPLC (Shimadzu; Kyoto, Japan) auto sampler system model LC- 20AT equipped with four pumps and an SPDM20A diode array detector (DAD).

2.4. Crude protein ratio, acid detergent fiber, neutral detergent fiber, and mineral contents

After being dried at 60 °C until they reached constant weight, the plants were ground in a mill with a sieve diameter of 1 mm in the laboratory before analysis. Then the crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), potassium (K), phosphorus (P), calcium (Ca), and magnesium (Mg) contents of the samples were measured on a near infrared reflectance spectroscopy (NIRS) (Foss 6500) device using the IC-0904FE software suite.

2.5. Condensed tannin

First, 0.01-g ground samples were weighed and then 6 mL of tannin solution was added to them in a tube to be mixed in a vortex. After being soaked in boiling water for 1 h, the samples were taken out and kept at 100 °C for another hour. The reading was done after cooling at an absorbance value of 550 nm (Bate-Smith, 1975). The following formula was used in the calculation of the condensed tannin content:

Absorbance (550 nm × 156.5 × dilution factor) / dry weight (%).

2.6. Total flavonoid content

Quercetin stock solution was prepared in a 200 mg L^{-1} concentration and five different concentrations were obtained from it by dilution. Plant extracts (1 mL) were mixed with an equal volume of 2% $AlCl_3$, incubated at room temperature for 10 min, and the absorbance was measured at 415 nm. The same procedure was carried out for standard quercetin and the flavonoid contents of the samples were calculated as the equivalent of quercetin (mg QE g^{-1}) (Arvouet-Grand et al., 1994).

2.7. Total phenolic content

The total phenolic content of the extracts was determined by adapting the Folin–Ciocalteu reactive (FCR) method

reported by Singleton et al. (1999); 0.2-mL sample solutions were taken and first 9 mL of distilled water and then 0.2 mL of Folin–Ciocalteu reagent was added and left to rest for 3 min for the experiment. Lastly, 0.6 mL of sodium carbonate (Na_2CO_3) (20%) was added and a total volume of 10 mL was obtained. After being incubated in the dark at room temperature for 2 h, it was measured in the spectrophotometer at the absorbance value of 760 nm. In plotting the standard calibration curve, gallic acid dissolved in pure water was used. As the main stock 0.1 mg mL^{-1} gallic acid was prepared and seven different concentrations were obtained by dilution. Pure water was added for the control in the same amount as the sample solution (0.2 mL). Based on the gallic acid standard graph, the total phenolic matter content in all plant extracts was calculated as the equivalent of mg of gallic acid (GAE g^{-1}) extract.

2.8. Free radical scavenging capacity (DPPH)

Free radical activity was determined using the well-known radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical (Gezer et al., 2006). For the determination of DPPH radical scavenging activity, a concentration was prepared by solving 4 mg of DPPH in 100 mL of methanol. Dilutions were made from the extracts in concentrations different than the main stock. For each sample, 3.2 mL of DPPH radical and 200 µL of extract solutions of different concentrations were used. After incubation in the dark at room temperature for 30 min, it was read in the spectrophotometer at the absorbance value of 517 nm. Ascorbic acid and butylated hydroxytoluene (BHT) were used as standard. Sample dissolver was added to the test tube for the control in the same amount as the extract solution and each trial was done in three repetitions. The DPPH radical scavenging percentage was calculated using the following formula:

DPPH radical scavenging activity% = [(acontrol – aextract) / acontrol] × 100.

2.9. Total alkaloid

The total alkaloid content of the samples was determined using a modification of the Ecuadorian Service for Standardization (INEN) (2005) method. Accordingly, 1.2 g of Al_2O_3 was added to 0.2 g of the sample and was mixed until powder form was obtained. To the powder mixture was added 1 mL of KOH (150.4 g L^{-1}) and it was mixed until a homogenous texture was obtained. The mixture was put into a centrifuge tube, 6 mL of chloroform was added, and it was centrifuged 3000 × *g* for 5 min. The filtrate was filtered into a glass bottle. The chloroform, centrifuging, and filtrate collection procedure was repeated not less than 10 times. The extract was vaporized at 30 °C until no alkaloid was left in it (1 mL remained). Then 5 mL of NaOH (0.40 g L^{-1}) and 2 drops of methyl red indicator were added and titrated with sulfuric acid (0.01 mL) for alkaloid analysis. The total alkaloid content was calculated as 100 g^{-1} using the following formula:

TA = 0.248 × V / sample weight (g).

2.10. ODAP

ODAP analysis was performed using the o-phthalaldehyde (OPT) method reported for the plant by Rao (1978). OPT was prepared by mixing with o-phthalaldehyde reactive, borate buffer, mercaptoethanol, and diaminopropionic acid (DAP) was used as a standard. The powdered plant (2 g) samples were put in test tubes and 2 mL of pure water was added. After being kept in boiling water, the tubes were cooled down to room temperature and centrifuged. To the clear solution taken from the tube was added 0.2 mL of 3 N KOH and it was kept in boiling water for 30 min. After hydrolysis, 0.7 mL of water and 2 mL of OPT were added and a reading was performed in the spectrophotometer at 425 nm.

2.11. Data analysis

The data obtained were analyzed according to the randomized parcel design using the JMP 13 software suite, and Duncan's test was used to compare the differences among group averages. Descriptive statistics, principal component analysis (PCA), and hierarchical clustering were utilized to identify patterns and relationships among the grass pea genotypes. In the present study, silage yield was evaluated over 2 years, while quality traits were evaluated over a single year.

3. Results

The silage yield values of the different grass pea genotypes are shown in Figure 1. While the impact of the genotypes on individual and combined years in terms of both values was not insignificant, there was a significant difference at the probability level of 1% between the years. The silage yields of the genotypes ranged from 9.01 to 13.64 t ha^{-1}. Both plant height and hay yield were higher in the second year. While the average silage yield was determined as 8.73 t ha^{-1} in 2022, it was determined as 12.76 t ha^{-1} in 2023.

The pH, dry matter ratio (DM), Flieg score, LA, PA, AA, SA, and CA values of the genotypes are shown in Table 4. There was a significant ($p \leq 0.01$) difference between genotypes in terms of all traits except SA. The pH and DM ranged from 4.54 to 5.28 and from 22.64% to 31.24%, respectively. The highest Flieg score (85.88), LA (2.63%), and PA (1.85%) contents were determined in the 5001 populations. The AA, CA, and SA contents of grass pea genotypes ranged between 0.20% and 0.33%, 0.149% and 0.426%, and 0.019% and 0.043%, respectively.

The crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), calcium (Ca), potassium (K), phosphorus (P), and magnesium (Mg) ratios of the grass pea genotypes silages are shown in Table 5. There was a significant ($p \leq 0.01$) difference between the genotypes in terms of CP, ADF, NDF, and Ca but not in terms of the other traits. The CP, ADF, NDF, and Ca contents of grass pea genotypes ranged from 19.35% to 23.06%,

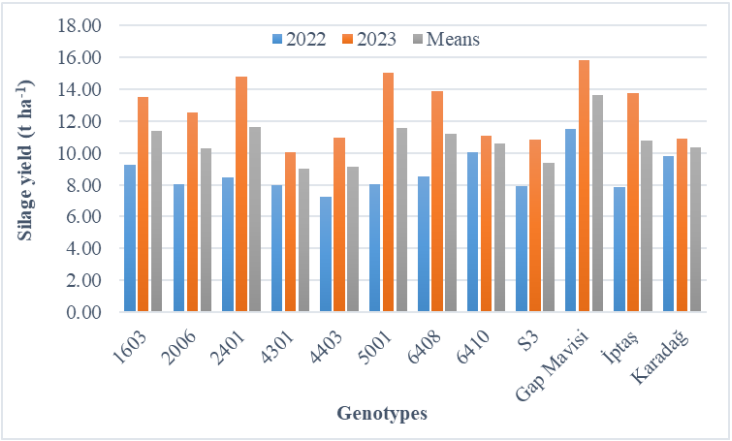

Figure 1. Silage yield of the grass pea genotypes over 2 years.

29.19% to 32.13%, 37.35% to 43.81%, and 0.85% to 1.03%, respectively (Table 5).

The total phenolic (TP), total flavonoid (TF), radical scavenger activity (DPPH), condensed tannin (CT), total alkaloid (TA), and N-oxalyl-L-alpha, beta-diaminopropionic acid (ODAP) contents are shown in Table 6. There was a significant difference ($p \leq 0.05$ and $p \leq 0.01$) between the genotypes in terms of all traits except TF. Grass pea silages TF contents ranged from 18.53 to 23.43 mg QE g^{-1}. The highest CT content was determined in the 2401 population (1.74%), while the lowest was in the İptaş variety (0.85%). TP and DPPH contents ranged from 78.86 to 122.40 mg GA g^{-1} and from 21.59% to 56.80%, respectively. The lowest TA (1.09 g 100 g^{-1}) was determined in the İptaş variety, while the lowest ODAP content was in the 5001 (0.95 mg g^{-1}) and 6408 (0.90 mg g^{-1}) populations (Table 6).

It has been reported that PCA is effective for evaluating phenotypic diversity in addition to identifying genetically distant clusters of genotypes and selecting important traits contributing to the total variation in the genotypes (Kaplan et al., 2024). PCA allows natural grouping of the genotypes and is a precise indicator of differences among genotypes. The main advantage of using PCA is that each genotype can be assigned to one group only (Adams, 1995; Singh et al., 2020). The relationships between the genotypes and the examined traits in the present study can be easily distinguished thanks to the PCA graph (Figure 2).

A heat map or hierarchical cluster analysis is a two-dimensional data visualization approach indicating the data in the rows and columns of a data matrix along with a hierarchical clustering structure (Wilkinson and Friendly, 2009; Barua et al., 2022). The heat map of all the properties examined in the study across a total of 12 grass pea genotypes, including three registered and nine different populations, based on the clustering analysis, is shown in Figure 3. The differences and similarities among genotype groups can be identified through clustering analysis. On the other hand, clustering analysis is also used in demonstrating the taxonomic relations among genotypes (Cartea et al., 2002). A heat map based on clustering analysis shows how the color changes are grouped by tone or saturation or the changes within a group (Barua et al., 2022). The color distribution openly demonstrates the role played by the cultivation environment and the impact of genotypic differences in terms of the properties inspected in the grass pea genotypes. The positive or negative outcomes of the interaction among the genotypes and properties are clearly seen on this map. This can be associated with the genetic differences among the genotypes. Moreover, a wide variation was identified among grass pea genotypes and the examined properties based on the heat map in the clustering analysis.

4. Discussion

Annual precipitation and precipitation distribution are significant factors in arid climate conditions. The reason why the silage yield values of the grass pea genotypes were higher in the second year was because the total precipitation was high in 2023 (Table 2). This was clearly observed under field conditions, with the plants exhibiting a more vigorous habitus. Arıcı (2023) reported a high rate of germination in the grass pea plants cultivated under precipitation-dependent conditions and that as precipitation increases so does yield. Başaran et al. (2018) reported an average silage yield value of 0.70 t ha^{-1}. Although the results of the study match those of studies by other researchers, there are also some differences, which could be explained by the differences in the genotypes used, the conditions under which the experiment was conducted, and precipitation and temperature values.

The pH value in silage should be below 5.00 to prevent the proliferation of enterobacterial and clostridial spores, which have a negative effect on fermentation (Filya, 2001).

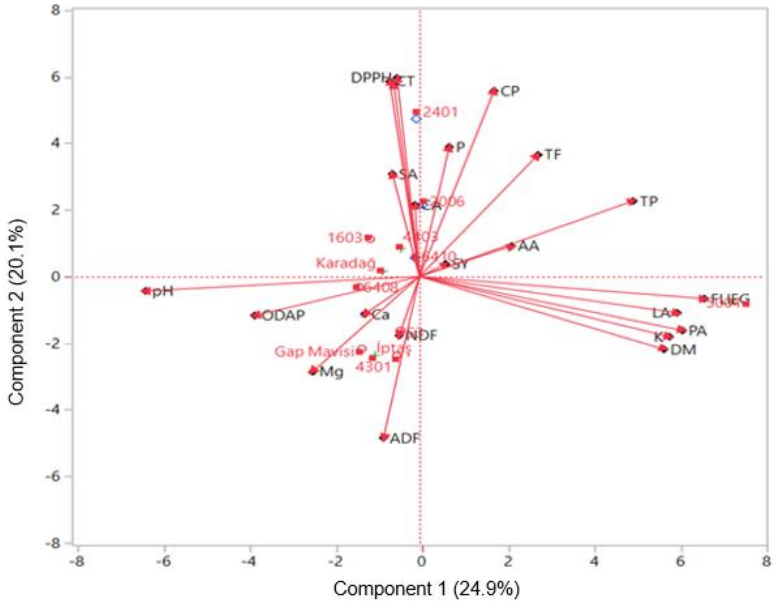

Figure 2. Principal component analysis of the grass pea genotypes.

SY: silage yield; DM: dry matter ratio; LA: lactic acid; PA: propionic acid; AA: acetic acid; CA: citric acid; SA: succinic acid; CP: crude protein ratio; ADF: acid detergent fiber; NDF: neutral detergent fiber; K: potassium; P: phosphorus; Ca: calcium; Mg: magnesium; CT: condensed tannin; TF: total flavonoid content; TP: total phenolic content, ASH: crude ash ratio, DPPH: radical scavenging activity; TA: total alkaloid content; ODAP: N-oxalyl-L-alpha, beta-diaminopropionic acid.

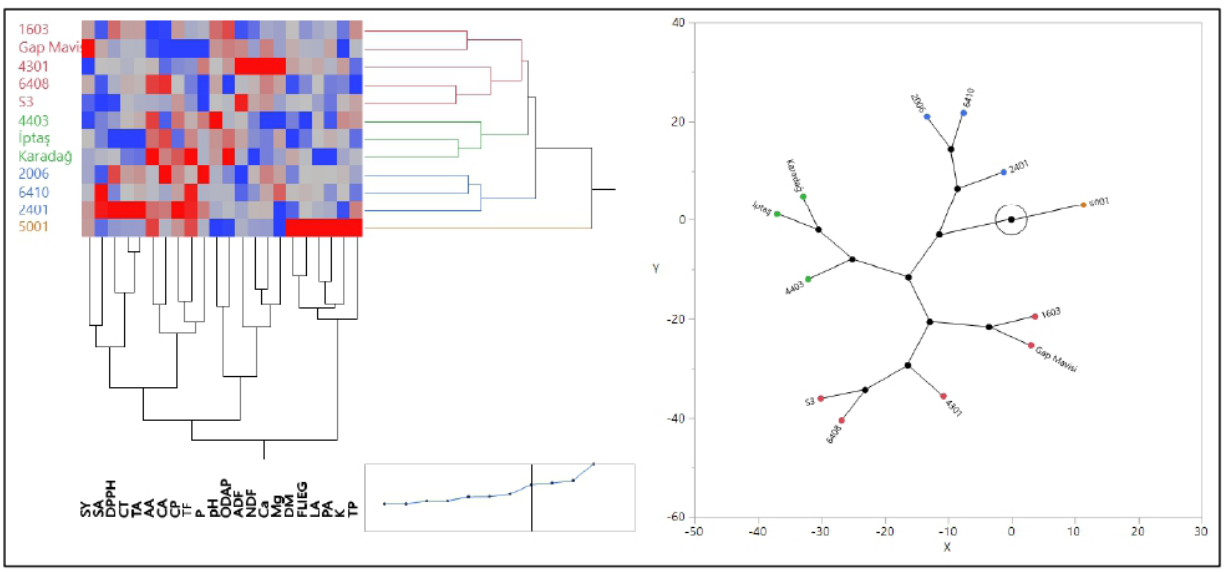

Figure 3. Heat map and hierarchical cluster analysis of the grass pea genotypes.

SY: silage yield; DM: dry matter ratio; LA: lactic acid; PA: propionic acid; AA: acetic acid; CA: citric acid; SA: succinic acid; CP: crude protein ratio; ADF: acid detergent fiber; NDF: neutral detergent fiber;; K: potassium; P: phosphorus; Ca: calcium; Mg: magnesium; CT: condensed tannin; TF: total flavonoid content; TP: total phenolic content, ASH: crude ash ratio, DPPH: radical scavenging activity; TA: total alkaloid content; ODAP: N-oxalyl-L-alpha, beta-diaminopropionic acid.

Table 4. Fermentation traits of the grass pea genotypes' silages.

Genotypes	pH**	DM**	FLIEG**	LA**	PA**	AA**	CA**	SA^{ns}
1603	5.16^{ab}	25.32^{bcd}	49.11^{bcd}	1.21^{b}	0.79$^{b–e}$	0.20^{b}	0.154^{d}	0.020
2006	4.94^{bc}	23.79^{bcd}	54.84^{bc}	1.44^{b}	0.84^{bcd}	0.28^{ab}	0.426^{a}	0.020
2401	4.92^{c}	23.21^{cd}	54.63^{bc}	1.27^{b}	0.76$^{b–e}$	0.31^{a}	0.347^{abc}	0.042
4301	5.01^{bc}	26.64^{b}	58.01^{b}	1.63^{b}	0.79$^{b–e}$	0.29^{a}	0.185^{cd}	0.026
4403	5.28^{a}	23.75^{bcd}	41.16^{d}	1.49^{b}	0.53^{de}	0.32^{a}	0.174^{cd}	0.022
5001	4.54^{d}	31.24^{a}	85.88^{a}	2.63^{a}	1.85^{a}	0.32^{a}	0.260$^{a–d}$	0.021
6408	5.07^{abc}	22.89^{d}	47.99^{bcd}	1.40^{b}	0.63^{de}	0.32^{a}	0.403^{ab}	0.020
6410	4.96^{bc}	25.44^{bcd}	57.61^{bc}	1.45^{b}	0.68^{cde}	0.28^{b}	0.245^{bcd}	0.043
S3	5.01^{bc}	25.08^{bcd}	54.76^{bc}	1.15^{b}	0.61^{de}	0.27^{ab}	0.243^{bcd}	0.019
Gap Mavisi	5.10^{abc}	26.07^{bc}	53.13^{bc}	1.28^{b}	1.06^{b}	0.21^{b}	0.149^{d}	0.029
İptaş	5.10^{abc}	22.64^{d}	46.15^{cd}	1.40^{b}	0.97^{bc}	0.31^{a}	0.385^{ab}	0.022
Karadağ	5.09^{abc}	26.40^{b}	54.07^{bc}	0.54^{c}	0.48^{e}	0.33^{a}	0.341^{abc}	0.025
Means	**5.16**	**25.32**	**54.78**	**1.49**	**0.83**	**0.29**	**0.276**	**0.026**

ns: not significant, **: significant at $p \leq 0.01$. Means in the same line with different letters differ significantly ($p < 0.05$). The different superscript letters in the table indicate the order of statistical significance, with 'a' representing the highest and 'e' the lowest. DM: dry matter ratio (%); LA: lactic acid (%); PA: propionic acid (%); AA: acetic acid (%); CA: citric acid (%); SA: succinic acid (%).

In the present study, pH values of the 2006 (4.94), 2401 (4.92), 5001 (4.54), and 6410 (4.96) populations were at the desired levels (Table 4). Panyasak and Tumwasorn (2013) stated that if the silage contains more than 40% dry matter, palatability decreases, while if the silage contains low dry matter content (<25%), most of the carbohydrate source may be leached. In the present study, the DM content of genotypes other than the 2006 (23.79%), 2401 (23.21%), 4403 (23.75%), and 6408 (22.89%) populations were within the desired range. Kilic (1986) indicated that a Flieg score between 81 and 100 was considered very good, between 61 and 80 was considered good, between 41 and 60 was considered medium, between 21 and 40 was considered poor, and between 0 and 20 was considered very poor silage quality, which was excluded from the experiment. The Flieg scores of the silage determined in the present study indicated medium, good, and very good quality silage. Başaran et al. (2018) reported the Flieg score of grass pea silage to be 61.80 (good silage quality).

LA prevents the development of yeasts, fungi, and aerobic bacteria that deteriorate the quality of silage while increasing milk yield in animals. In a high-quality silage, the desired LA is at least 2.0% (Alçiçek and Özkan, 1996). In the current study, only 5001 populations were at the desired level. Seydoşoğlu (2019) found the LA content of grass pea silage to be between 1.82% and 1.92%. There were differences between the findings of previous research and the results obtained from the current study due to variety, harvest time, and cultural processes applied. AA inhibits fermentation during silage and deteriorates the quality of the silage. In addition, high AA is an indication that the silage has taken in air. Accordingly, the AA content should be at most 0.8% for a good silage (Alçiçek and Özkan, 1996). The AA content of the grass pea silages varied between 0.20% and 0.33%, and all silages were below the critical value. Gulumser (2019) reported that the AA content of grass pea silage varied between 0.79% and 1.82%. Ünal et al. (2024) reported that PA is a short-chain fatty acid and prevents aerobic deterioration that may occur in silage by reducing mold and fungus development. Therefore, high PA content is important for silage quality. In the present study, the 5001 genotype stands out in terms

Table 5. Quality traits of the grass pea genotypes' silages.

Genotypes	CP**	ADF**	NDF**	Ca**	K^{ns}	P^{ns}	Mg^{ns}
1603	22.05^{ab}	29.35^{d}	37.35^{d}	0.92^{bc}	2.45	0.47	0.18
2006	22.22^{ab}	29.19^{d}	39.21^{bc}	0.98^{ab}	2.53	0.49	0.19
2401	23.06^{a}	30.20^{bcd}	39.07^{bc}	0.95^{ab}	2.42	0.48	0.17
4301	20.54^{cd}	32.28^{a}	43.81^{a}	1.03^{a}	2.57	0.48	0.21
4403	22.38^{ab}	30.64^{bcd}	37.65^{cd}	0.85^{c}	2.59	0.48	0.17
5001	22.11^{ab}	30.31^{bcd}	38.05^{bcd}	0.91^{bc}	2.72	0.47	0.17
6408	21.66^{bc}	30.46^{bcd}	39.25^{bc}	0.98^{ab}	2.46	0.46	0.19
6410	22.00^{ab}	29.52^{cd}	37.87^{cd}	0.96^{ab}	2.52	0.47	0.20
S3	22.08^{ab}	32.13^{a}	39.69^{b}	0.97^{ab}	2.53	0.46	0.18
Gap Mavisi	19.35^{e}	31.20^{ab}	38.06^{bcd}	0.98^{ab}	2.41	0.46	0.18
İptaş	20.02^{de}	30.91^{abc}	37.83^{cd}	0.89^{bc}	2.51	0.47	0.19
Karadağ	22.58^{ab}	30.30^{bcd}	36.72^{d}	0.89^{bc}	2.49	0.47	0.18
Means	**21.67**	**30.54**	**38.71**	**0.94**	**2.51**	**0.48**	**0.18**

ns: not significant, **: significant at $p \leq 0.01$. Means in the same line with different letters differ significantly ($p < 0.05$). The different superscript letters in the table indicate the order of statistical significance, with 'a' representing the highest and 'e' the lowest. CP: crude protein (%); ADF: acid detergent fiber (%); NDF: neutral detergent fiber (%); Ca: calcium (%); K: potassium; (%); P: phosphorus (%); Mg: magnesium (%).

of PA content (Table 4). SA helps to protect livestock from various diseases and contributes to the development of the animals' bodies. Furthermore, it supports the fermentation of silage (McDonald et al., 1991). CA has the function of stimulating rumen fermentation and improving animal performance (Kung Jr L et al., 1998).

Legumes have a high value in terms of CP content. This is important for the balanced nutrition of animals (Budak and Budak, 2014). Karadeniz et al. (2020) reported that the CP content of grass pea silage was 24.24%. ADF and NDF consist of lignin and cellulose. While ADF is an energy indicator in ruminant rations, NDF expresses the acceptability of feed by animals (Gül and Tekçe, 2014). High ADF and NDF levels prevent feed intake in animals due to energy density, while also causing a decrease in the yield and quality of animal products. Accordingly, ADF should be 30% and NDF should be 40% and below in feed (Ates, 2012). In the present study, the ADF and NDF rates of most genotypes were at the desired levels. K is a very important element in animal nutrition and provides acid–base balance in the body (Gürsoy and Macit, 2017), while P plays a role in the development of the bone structure, fertility, and quality of animal products (Dua and Care, 1999). Ca and Mg contribute to the development of the bone tissue and skeletal system of animals. In high-quality forage, K should be at least 0.8%, P 0.21%, Ca 0.18%, and Mg 0.20% (Kidambi et al., 1993). The nutrient elements determined in all silages were higher than the desired levels (Table 5). Gulumser (2019) reported that the K, P, Ca, and Mg contents of grass pea silage varied between 2.42% and 2.93%, 0.23% and 0.27%, 0.77% and 0.96%, and 0.32% and 0.35%, respectively.

Secondary metabolites (phenolics, flavonoids, and DPPH) exhibit antioxidant, antimicrobial, and antiallergic properties. These contents contribute to the healthy functioning of the rumen morphology of animals and help them exhibit resistance to different stress conditions (Robbins, 2003; Rochfort et al., 2008; Patra et al., 2016; Lee et al., 2017; Karaer et al., 2024). In addition, these substances increase the yield and quality of products obtained from animals. The CT in plants is important for reducing the methane gas released from ruminants, which causes global

Table 6. Phytotherapeutic traits of the grass pea genotypes' silages.

Genotypes	TF^{ns}	TP*	DPPH**	CT**	TA*	ODAP**
1603	20.93	105.37^{ab}	46.95^{b}	1.35^{b}	1.63^{ab}	2.61^{ab}
2006	21.71	91.48^{bc}	50.95^{b}	1.36^{b}	1.67^{ab}	1.41^{ef}
2401	23.24	107.56^{ab}	56.80^{a}	1.74^{a}	2.01^{a}	1.82^{cde}
4301	20.28	84.10^{bc}	34.51^{c}	1.18^{bc}	1.50^{bc}	2.18^{bcd}
4403	21.79	103.94^{abc}	30.31^{cd}	1.26^{b}	1.58^{ab}	1.77^{de}
5001	22.91	122.40^{a}	29.77^{cd}	1.10^{bc}	1.37^{bc}	0.95^{f}
6408	19.80	78.86^{c}	32.73^{cd}	1.30^{b}	1.55^{abc}	0.90^{f}
6410	23.30	85.49^{bc}	27.85^{d}	1.23^{b}	1.57^{ab}	1.79^{de}
S3	20.33	101.77^{abc}	22.36^{e}	1.20^{bc}	1.44^{bc}	1.82^{cde}
Gap Mavisi	18.53	94.71^{bc}	31.14^{cd}	1.19^{bc}	1.46^{bc}	2.34^{bc}
İptaş	22.28	84.03^{bc}	21.59^{e}	0.85^{c}	1.09^{c}	2.42^{ab}
Karadağ	23.43	94.76^{bc}	34.27^{c}	1.00^{bc}	1.23^{bc}	2.88^{a}
Means	**21.54**	**96.21**	**34.94**	**1.23**	**1.51**	**1.91**

ns: not significant, *: significant at $p \leq 0.05$; **: significant at $p \leq 0.01$. Means in the same line with different letters differ significantly ($p < 0.05$). The different superscript letters in the table indicate the order of statistical significance, with 'a' representing the highest and 'f' the lowest. TF: total flavonoid (mg GA g^{-1}); TP: total phenolic (mg GA g^{-1}); DPPH: radical scavenger activity (%), CT: condensed tannin (%); total alkaloid (g 100 g^{-1}); ODAP: N-oxalyl-L-alpha, beta-diaminopropionic acid (mg g^{-1}).

warming. Therefore, including forage crops rich in CT enhances animal yield and quality while also increasing carbon sequestration by reducing ammonia and nitrogen oxide emissions (Undi et al., 2016). Kumar and Singh (1984) and Barry (1987) reported that low levels of CT in plants (2.0%–3.0%) reduce ruminal protein degradation and high CT levels (<3.0%) adversely impact protein digestion, as well as microbial and enzyme activities. The CT contents of all the genotypes in the present study were lower than the critical level (Table 6). High alkaloid levels in forage crops lead to poisoning in animals. Therefore, low alkaloid content is desired in forage crops. Some grass pea populations have lower alkaloid levels than the varieties (Table 6), which is important for new variety improvements and animal health. The ODAP content is among the primary factors restricting the cultivation of the grass pea plant, which plays an important role in animal nutrition. ODAP is a free amino acid with a direct negative impact on the nervous system and is thus undesirable. El-Moneim et al. (1999) reported that the ODAP contents should be below 2.2 mg g^{-1} for safe consumption. The ODAP contents of the genotypes were at the desired level except for the varieties and 1603 population (Table 6).

The silage yield and quality results of the present study revealed that the first principal component (PCA 1) and the second (PCA 2) exhibited 24.90% and 20.10%, respectively (total 45.00%). It is seen that some populations are better than varieties in various properties. For example, the populations of 5001 exhibited superior performance in comparison to the varieties in terms of the undesired SY, FLIEG, LA, K, PA, and DM content. Moreover, the varieties showed better results in terms of silage quality (Figure 2). According to the PCA graph results, especially the 5001 population is valuable in terms of genetic resources. It is also predicted that the population in question can be evaluated as breeding material for developing new varieties.

According to the heat map, four major genotype groups were created in terms of the properties examined. The first group featured 1603, 4301, 6408, S3, and Gap Mavisi genotypes; the second consisted of 4403, İptaş, and Karadağ; the third consisted of 2006, 6410, and 2401; and the fourth group consisted of 5001. As seen in the graph, the 5001 population exhibited different variations compared to the other genotypes. This population was at the desired level in terms of silage quality and came to the

fore. It is possible to evaluate it as breeding material. On the other hand, three major groups were created in terms of silage yield and quality traits.

5. Conclusion

It was found that precipitation differences between the years affected both the genotypes and the properties examined. While no differences were identified among the genotypes in silage yield based on the average data of the two years, quality traits helped with the determination of the genotype for the ecology where the study was conducted. This will shed light on the amendment efforts to make populations into varieties. Accordingly, based on silage yield quality, the population 5001 exhibited a superior performance.

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Conflict of interest

MA and EG declare that they have no competing interests.

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