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**ARAŞTIRMA MAKALESİ** **RESEARCH ARTICLE**

# Comparison of Grass Pea (*Lathyrus sativus* L.) Genotypes in Forage Yield, Nutrient Composition and Phytotrapic Traits*

Mürdümük (*Lathyrus sativus* L.) Genotiplerinin Kaba Yem Verimi, Besin İçeriği ve Fitoterapik Özellikler Bakımından Karşılaştırılması

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## Abstract

The grass pea (*Lathyrus sativus* L.), belonging to the legume family, has a short vegetative period and is highly resistant to drought. Also, it is an important forage crop for animal feeding and health, as well as for the yield and quality of animal products. This study aimed to determine the hay yield and quality of a total of 12 grass pea genotypes, including 9 populations (1603, 2006, 2401, 4301, 4403, 5001, 6408, 6410, and S3) and 3 registered varieties (GAP Mavisi, İptaş, Karadağ). The experiment was conducted with three repetitions according to a randomized complete block design in Bilecik during the vegetation periods of 2022 and 2023. Hay yield, crude protein ratio, plant height, acid detergent fiber, neutral detergent fiber, crude ash, mineral contents (K, P, Ca, and Mg), condensed tannin, total flavonoid content, total phenolic content, free radical scavenging activity (DPPH), total alkaloid, and ODAP content (N-oxalyl-L-alpha, beta-diamino propionic acid) were determined in this study. According to two-year results, hay yield ranged between 3.00-4.55 t $ha^{-1}$. The highest crude protein rate was observed in populations 1603 (19.44%), 2006 (20.00%), 2401 (19.82%), and 6410 (19.28%), which were part of the same statistical group. The populations of 2006 (2.20 mg $g^{-1}$), 2401 (2.00 mg $g^{-1}$), 5001 (1.32 mg $g^{-1}$), and 6408 (1.77 mg $g^{-1}$) were at the desired level in terms of ODAP content. The K, P, Ca, and Mg contents of the genotypes varied between 2.75-3.11%, 0.44-0.49%, 0.91-0.99%, and 0.15-0.21%, respectively. As a result; GAP Mavisi come into prominence however, no statistical difference was found among the genotypes in terms of hay yield. Among the genotypes were detected significant differences in terms of quality traits, and this situation helped to determine the genotype/genotypes that stand out in the ecology of the region. Accordingly, the 2401 population showed superior performance in terms of forage quality compared to the other genotypes in the Bilecik ecology. Besides, it was determined that the 2006, 5001, and 6408 populations show promising results in terms of the examined traits.

**Keywords:** Grass pea, Forage crop, Secondary metabolite, Global warming, Animal health

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*This study was summarized from the Mertcan SEZER's MSc thesis.

# Öz

Baklagiller familyasında yer alan mürdümük (*Lathyrus sativus* L.) kısa vejetasyon süresine sahip olup, kuraklığa karşı oldukça dayanıklıdır. Mürdümük ayrıca, hayvan besleme ve sağlığı ile hayvansal ürünlerin verim ve kalitesi için önemli bir yem bitkisidir. Bu çalışmada; 9 populasyon (1603, 2006, 2401, 4301, 4403, 5001, 6408, 6410 ve S3) ve 3 tescilli (GAP Mavisi, İptaş, Karadağ) çeşit olmak üzere toplam 12 mürdümük genotipinin kuru ot veriminin ve kalitesinin belirlenmesi amaçlanmıştır. Deneme, Bilecik ekolojik koşullarında 2022 ve 2023 vejetasyon dönemlerinde 3 tekrarlamalı olarak ve tesadüf blokları deneme desenine göre yürütülmüştür. Çalışmada; kuru ot verimi, ham protein oranı, bitki boyu, asit deterjanda çözünmeyen lif, nötr deterjan çözünmeyen lif, ham kül, besin maddeleri (K, P, Ca ve Mg), kondanse tanen, toplam flavonoid, toplam fenolik, radikal kovucu aktivite (DPPH), toplam alkaloit ve ODAP (N-oksalil-L-alfa, beta-diamino propiyonik asit) içerikleri belirlenmiştir. İki yıllık sonuçlara göre, genotiplerin kuru ot verimi 3.00-4.55 t $ha^{-1}$ arasında değişmiştir. En yüksek ham protein oranı aynı istatistiksel grupta yer alan 1603 (%19.44), 2006 (%20.00), 2401 (%19.82) ve 6410 (%19.28) popülasyonlarında gözlenmiştir. ODAP içeriği bakımından 2006 (2.20 mg $g^{-1}$), 2401 (2.00 mg $g^{-1}$), 5001 (1.32 mg $g^{-1}$) ve 6408 (1.77 mg $g^{-1}$) popülasyonları istenilen düzeyde bulunmuştur. Genotiplerin K, P, Ca ve Mg içerikleri sırasıyla %2.75-3.11, %0.44-0.49, %0.91-0.99% ve %0.15-0.21 arasında değişmiştir. Sonuç olarak; kuru ot verimi bakımından GAP Mavisi ön plana çıkmış ancak, genotipler arasında istatistiksel açıdan bir farklılık tespit edilmemiştir. Kalite özellikleri bakımından ise genotipler arasında önemli farklılıklar tespit edilmiş olup, bu durum bölge ekolojisinde öne çıkan genotip/genotiplerin belirlenmesine yardımcı olmuştur. Buna göre; Bilecik ekolojisinde 2401 popülasyonu yem kalitesi açısından diğer genotiplerden daha üstün performans sergilemiştir. Ayrıca, 2006, 5001 ve 6408 populasyonlarının da incelenen özellikler bakımından ümit var oldukları belirlenmiştir.


**Keywords:** Mürdümük, Yem bitkisi, Sekonder metabolit, Küresel ısınma, Hayvan sağlığı

## 1. Introduction

Biodiversity is an indispensable element of human life, especially food. It is estimated that 20% of biodiversity will be lost by 2030, both directly and indirectly, especially with the increase in population and industrialization. This threat is more serious in regions where industrialization and urbanization are most intense. The protection of plant genetic resources has become an important problem in the world today (Gürlük, 2021). These materials, which are fully adapted to the ecological conditions of the region in which they grow, are of great importance for breeding studies to be carried out today and in the future. Turkey has a very important position in the world in terms of plant genetic resources due to both its geographical structure and the different ecological regions it has. The fact that one third of the approximately 9000 species in its flora are endemic increases this importance even more.

The grass pea genus *(Lathyrus*) is a member of the legume family (*Fabaceae*) and includes 160 non-perennial and perennial species (Plitmann et al.,1995). The regions where the Lathyrus genus exhibits species and variety richness are the Mediterranean basin, Asia Minor, and the warm areas of North America and South America. The species of the Lathyrus genus that are of economic significance worldwide are *L. sativus*, *L. cicera*, and *L. Ochrus*, as well as the lesser-known *L. sylvestris*, *L. latifolius*, and *L. tinginatus*.

The most widely cultivated grass pea species worldwide is *L. sativus*. The plant is used in animal feeding 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 among the primary drought-tolerant cultivated plants. 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 (Lambein et al., 2019). 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. Additionally, as a legume plant, cultivation of grass pea provides a significant amount of nitrogen in the soil (Sayar and Han, 2015).

Although grass pea has many superior features agriculturally, the development and spreading of its cultivation have been limited due to a neurotoxic amino acid it has, namely ß- N-oxalyl-L-α, ß-diamino propionic acid (ODAP). The ODAP consists of two isomerous structures, namely α –ODAP and ß –ODAP (Chase et al., 1985). α –ODAP corresponds to almost 5% of the total ODAP content of the grain and has no toxic effect. ß-ODAP causes Lathyrism disease, which results in irreversible paralysis in the legs (Hanbury et al., 2000). Lathyrism disease is seen both in humans and animals and is caused by intense consumption of grass pea grains for 3 to 4 months (Mehta et al.,1994).

Studies have revealed that the secondary metabolites found in plants are highly significant for rumen health and animal yield (Rochfort et al., 2008; Patra et al., 2006; Ağamirzaoğlu et al., 2024). Dohi et al. (1997) reported an increase in forage consumption in animals fed on crops with phenolic compounds, with a positive effect on yield. The antioxidant and antimicrobial effects of these compounds were stated by Santos Neto et al. (2009). On the other hand, flavonoids and phenolic compounds were found to be effective in taking such nutritional deficiencies as ruminal fermentation, bloating, and acidosis under control (de Paula et al., 2016). Kowalczyk et al. (2013) reported that the use of external antibiotic supplements in animal feeding was prohibited in 2006 and thus secondary metabolites could function as an alternative.

It is reported that it is in the digestive systems of ruminants where 25% of global warming is generated (Lascano and Cardenas, 2010). By attaching to protease and substrate proteins, condensed tannins found in the structure of legumes create a structure that is hard to break up in the rumen (pH=6-7). After going through the rumen without being broken up, it is broken up again at the abomasum, where the pH value falls to the level of 2.3-3.5, boosting digestion and the absorption of essential amino acids in the small intestine (Archimède et al., 2011). As a result, secondary components, such as condensed tannins, reduce the methane produced in the digestive system and released to the environment. Under the effect of tannins, nitrogen is directed to the feces instead of urine after passing through the small intestines without breaking up. Then it quickly turns into ammonia and nitrogen oxide and passes to the atmosphere while the N in feces turns into soil organic matter (Martin et al., 2016). Condensed tannins also have an anthelmintic effect, reducing endozoa in animals and increasing yield.

## 2. Materials and Methods

In this study, a total of 12 grass pea (*Lathyrus sativus* L.) genotypes were used. This genotype includes 3 registered varieties (GAP Mavisi, İptaş, and Karadağ). The 9 local populations were collected by Prof. Dr. Uğur BAŞARAN from different provinces of Turkey, and this information on populations was given by Basaran et al. (2016) (*Table 1*). The genotypes were then tested under the same ecological conditions. The trial was held under the ecological conditions of Bilecik for two years in 2022 and 2023.

***Table 1. Origin 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 (Gap International Agricultural Research and Training Center) | | | |
| **İptaş** | Registered variety (Gaziosmanpaşa University, Agriculture Faculty, Field Crop Department) | | | |
| **Karadağ** | Registered variety (Gaziosmanpaşa University, Agriculture Faculty, Field Crop Department) | | | |

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

The temperature and precipitation values of the experiment region for long-term and the years 2022 and 2023 are given in *Table 2*. The average temperature was 10.3 °C for long-term years, and 9.9 °C and 10.6 °C for 2022 and 2023, respectively. While the provinces' total precipitation values for long years (273.6 mm) and for 2022 (291.2 mm) were close to each other, this value was 307.6 mm in 2023, the second year of the experiment (*Table 2*).

***Table 2. Meteorological data of experiment area in the longterm and experimental years**** 

| **Months** | **Temperature (°C)** | | | **Precipitation (mm)** | | |
|---|---|---|---|---|---|---|
| | **Long-term** | **2022** | **2023** | **Long-term** | **2022** | **2023** |
| **January** | 2.7 | 1.8 | 5.9 | 48.0 | 57.9 | 17.0 |
| **February** | 4.1 | 4.8 | 3.7 | 44.4 | 66.9 | 33.6 |
| **March** | 7.2 | 2.4 | 8.1 | 48.7 | 27.3 | 74.7 |
| **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*** | ***10.3*** | ***9.9*** | ***10.6*** | ***273.6*** | ***291.2*** | ***307.6*** |

* Tukish State Meterogical Service.

The study was established on 01.04.2022 in the first year and on 03.04.2023 in the second year. The experiment was set up in the randomized complete blocks design with 3 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. No irrigation was applied in the trial. Harvest

(08.06.2022 and 13.06.2023) was done after the edge effect of the plots (0.5m from the top and bottom of each plot) was removed and right in the flowering period of the plants.

### *2.1. Plant height*

Determined by measuring the distance from the soil level to the top growth point in the 5 plants selected from the middle row in each repetition.

### *2.2. Hay yield*

The 500 gr of plant sample taken in the harvesting time was dried in a drying oven at 60 °C until it reached constant weight and weighed. Then the values obtained were proportioned to the fresh yield to calculate the hay yield.

### *2.3. 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, ADF, NDF, potassium (K), phosphorus (P), calcium (Ca), and magnesium (Mg) contents of the samples were measured on the Near Infrared Reflectance Spectroscopy (NIRS) (Foss 6500) device using the IC-0904FE suite program (Hoy et al., 2002). These analyses were conducted at the Field Crops Department Laboratory of Faculty of Agriculture, Yozgat Bozok University.

### *2.4 Crude ash content*

The 2 gr of ground samples were weighed and burned in the incinerator at 550 °C for 4 hours, then the remaining amount was calculated as ash content (Kacar, 1972).

### *2.5. Condensed tannin*

The 0.01 gr of ground samples was weighed, and 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 hour, 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 formula below was used in the calculation of the condensed tannin content.

Absorbance (550 nm x 156,5 x dilution factor)/ Dry weight (%).

### *2.6. Total flavonoid content*

Quercetin stock solution was prepared in a 200 m $L^{-1}$ concentration and five different concentrations were obtained from it by way of dilution. Plant extracts (1 ml) were mixed with the same amount of 2% $AlCl_3$, kept in room conditions for 10 minutes, and read at the absorbance value of 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 adapted per the Folin-Ciocalteu Reactive (FCR) method by Singleton et al. (1999). 0.2 ml of sample solutions were taken and first 9 ml of distilled water, then 0.2 ml of Folin-Ciocalteu was added and left to rest for 3 minutes for the experiment. Lastly, 0.6 ml of sodium carbonate ($Na_2CO_3$) (20%) was added, and a total volume of 10 ml was reached. After being incubated in the dark at room temperature for 2 hours, 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. 0.1 mg $ml^{-1}$ of gallic acid was prepared as the main stock was prepared and seven different concentrations were obtained by way of dilution. Pure water was added for 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 gallic acid (GAE $g^{-1}$) extract.

### *2.8. Free radical scavenging capacity (DPPH)*

Free radical activity was determined by using the well-known radical 2,2-difenil-1-pikrilhidrazil (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. After incubation in the dark at room temperature for 30 minutes, it was read in the spectrophotometer device at the absorbance value of 517 nm. Ascorbic acid and butylated hydroxytoluene (BHT) was used as a standard. Sample dissolver was added to the test tube for control in the same amount as the extract solution and each trial was done in three repetitions. The DPPH radical scavenging percentage was calculated per the formula below.

$$\text{DPPH radical scavenging activity\%} = [(\text{Acontrol} - \text{Aextract})/\text{Acontrol}] \times 100. \qquad \text{(Eq. 1)}$$

### *2.9. Total alkaloid analysis*

The total alkaloid content of the samples was determined using a modification of the 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 reached. The powder mixture was added 1 ml of KOH (150.4 g $l^{-1}$) and mixed until a homogenous texture was achieved. The mixture was taken into a centrifuge tube, added 6 ml of chloroform, and was centrifuged 3000 g for 5 minutes. 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). 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 $g^{-1}$ per the following formula.

$$\text{TA} = 0.248 * \text{V}/\text{ sample weight (g)} \qquad \text{(Eq. 2)}$$

### *2.10. ODAP analysis*

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

### *2.11. Data analysis*

The obtained data was analyzed per the randomized complete blocks design using the MSTAT-C program and the Duncan test was used in the comparison of the differences among group averages. Descriptive statistics, analysis, principal component analysis and hierarchical clustering (JMP-22) were employed to identify patterns and relationships among the grass pea genotypes

## 3. Result and Discussion

Plant height and hay yield values of different grass pea genotypes are shown in *Table 3*. While the impact of the genotypes on separate and combined years in terms of both values was not significant, there was a difference significant at the probability level of 1% between the years. Plant height and hay yield varied between 41.85 - 49.68 cm and 3.00-4.55 t $ha^{-1}$, respectively. Both plant height and hay yield were higher in the second year (*Table 3*). Annual precipitation and precipitation distribution are significant factors in arid climate conditions. The reason why the plant height and hay yield values of the grass pea genotypes were higher in the second year was because the total precipitation was high in 2023 (*Table 2*). Such that, this was clearly observed in field conditions, with plants having higher plant height and a more exuberant 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 do the plant height and biomass yield. Kökten et al. (2018) reported a plant height between 23.0-44.1 cm for grass peas under the ecological conditions of Elazığ province while Sayar et al. (2013) reported that hay yield in grass pea genotypes ranged from 4.63-7.12 t $ha^{-1}$, Atıs and Acıkalın (2020) reported an average hay yield value of 3.76 t $ha^{-1}$. Although the results of the study match those of other 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.

***Table 3. Plant height and hay yield of grass pea genotypes***

| Genotypes | Plant height (cm) | | | Hay yield (t ha$^{-1}$) | | |
|---|---|---|---|---|---|---|
| | 2022 | 2023 | Average | 2022 | 2023 | Average |
| **1603** | 38.13 | 53.52 | 45.83 | 3.09 | 4.50 | 3.80 |
| **2006** | 37.73 | 51.44 | 44.59 | 2.68 | 4.18 | 3.43 |
| **2401** | 38.73 | 54.10 | 46.42 | 2.83 | 5.60 | 4.21 |
| **4301** | 34.67 | 49.04 | 41.85 | 2.66 | 3.35 | 3.00 |
| **4403** | 37.93 | 52.50 | 45.22 | 2.42 | 3.66 | 3.04 |
| **5001** | 37.27 | 52.46 | 44.86 | 2.69 | 5.67 | 4.18 |
| **6408** | 36.40 | 52.36 | 44.38 | 2.85 | 4.63 | 3.74 |
| **6410** | 39.13 | 48.58 | 43.86 | 3.35 | 3.70 | 3.53 |
| **S3** | 36.73 | 52.92 | 44.83 | 2.64 | 3.61 | 3.13 |
| **GAP Mavisi** | 40.93 | 58.43 | 49.68 | 3.83 | 5.26 | 4.55 |
| **İptaş** | 38.07 | 51.77 | 44.92 | 2.62 | 4.58 | 3.60 |
| **Karadağ** | 39.13 | 52.40 | 45.77 | 3.27 | 3.62 | 3.44 |
| **Average** | ***37.91$^{B**}$*** | ***52.46$^{A}$*** | | ***2.91$^{B**}$*** | ***4.36$^{A}$*** | |
| **Mean square error** | ***20.310*** | ***10.035*** | ***15.172*** | ***4073.649*** | ***11693.697*** | ***7883.673*** |
| **Coefficient of variation (%)** | ***11.89*** | ***6.04*** | ***8.62*** | ***21.93*** | ***24.78*** | ***24.41*** |

** is 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' being the highest and 'b' the lowest.

***Table 4. Crude protein ratio and protein yield of grass pea genotypes***

| Genotypes | Crude protein ratio (%) | | | Protein yield (t ha$^{-1}$) | | |
|---|---|---|---|---|---|---|
| | 2022** | 2023** | Average** | 2022 | 2023 | Average |
| **1603** | 18.84$^{ab}$ | 20.04$^{ab}$ | 19.44$^{abc}$ | 0.58 | 0.89 | 0.74 |
| **2006** | 19.81$^{a}$ | 20.20$^{ab}$ | 20.00$^{a}$ | 0.53 | 0.84 | 0.69 |
| **2401** | 18.66$^{abc}$ | 20.97$^{a}$ | 19.82$^{ab}$ | 0.53 | 0.63 | 0.85 |
| **4301** | 17.50$^{bcd}$ | 18.67$^{cd}$ | 18.09$^{de}$ | 0.46 | 0.74 | 0.54 |
| **4403** | 17.26$^{d}$ | 20.35$^{ab}$ | 18.80$^{cd}$ | 0.42 | 1.14 | 0.58 |
| **5001** | 17.91$^{bcd}$ | 20.10$^{ab}$ | 19.01$^{bcd}$ | 0.48 | 0.91 | 0.81 |
| **6408** | 18.22$^{bcd}$ | 19.69$^{bc}$ | 18.96$^{bcd}$ | 0.52 | 0.74 | 0.71 |
| **6410** | 18.56$^{a-d}$ | 20.00$^{ab}$ | 19.28$^{abc}$ | 0.62 | 0.73 | 0.68 |
| **S3** | 17.36$^{cd}$ | 20.07$^{ab}$ | 18.71$^{cd}$ | 0.46 | 1.17 | 0.59 |
| **GAP Mavisi** | 17.52$^{bcd}$ | 17.60$^{e}$ | 17.56$^{e}$ | 0.67 | 0.93 | 0.80 |
| **İptaş** | 18.11$^{bcd}$ | 18.20$^{de}$ | 18.15$^{de}$ | 0.47 | 0.82 | 0.65 |
| **Karadağ** | 17.78$^{bcd}$ | 20.53$^{ab}$ | 19.15$^{abc}$ | 0.58 | 0.74 | 0.66 |
| **Average** | ***18.13$^{B**}$*** | ***19.70$^{A}$*** | | ***0.53$^{B**}$*** | ***0.86$^{A}$*** | |
| **Mean square error** | ***0.485*** | ***0.366*** | ***0.425*** | ***126.101*** | ***392.693*** | ***259.397*** |
| **Coefficient of variation (%)** | ***3.84*** | ***3.07*** | ***3.45*** | ***21.32*** | ***23.13*** | ***23.28*** |

** is 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' being the highest and 'e' the lowest.

*Table 4* reveals that the impact of genotypes on crude protein ratio was significant ($p \leq 0.01$) in separate and combined years but, insignificant on protein yield. There was a significant difference at the probability level of 1% between the years in terms of both values. The highest crude protein rate was observed in populations 1603 (19.44%), 2006 (20.00%), 2401 (19.82%), 6410 (19.28%) and variety of Karadağ (19.15%), which were part of the same statistical group, while the lowest value belonged to the Gap Mavisi variety, with 17.56%. The crude protein rate was determined as 18.13% in the first year and 19.70% in the second year. The protein yield of the genotypes were ranged from 0.54 and 0.85 t ha$^{-1}$. Average protein yield values for 2022 and 2023 were determined as 0.53 t ha$^{-1}$ and 0.86 t ha$^{-1}$, respectively (*Table 4*). The fact that the precipitation rate was higher in the second year led to a more exuberant habitus growth by the plants, which was also reflected in the protein rate. The observations made on the experiment plot revealed this even more clearly. As a matter of fact, vegetative part growth was more intense in plants in 2023, which, in turn, led to a higher crude protein rate (*Table 4*). On the other hand, protein yield, which is the multiplication of hay yield by crude protein rate, was also higher in 2023. Gülümser et al. (2023) stated the crude protein rate and protein yield for grass pea as 20.62% and 0.51 t ha$^{-1}$, respectively.

***Table 5. ADF and NDF ratios of grass pea genotypes***

| Genotypes | Acid detergent fiber (%) | | | Neutral detergent fiber (%) | | |
|---|---|---|---|---|---|---|
| | 2022** | 2023** | Average** | 2022** | 2023** | Average** |
| **1603** | 34.64$^{de}$ | 30.58$^{cd}$ | 32.61$^{d}$ | 47.22$^{e}$ | 41.50$^{d}$ | 44.36$^{e}$ |
| **2006** | 33.66$^{e}$ | 31.21$^{bcd}$ | 32.43$^{d}$ | 47.07$^{e}$ | 43.56$^{bc}$ | 45.32$^{de}$ |
| **2401** | 35.30$^{cde}$ | 31.80$^{bc}$ | 33.55$^{bcd}$ | 49.16$^{de}$ | 43.41$^{bc}$ | 46.28$^{bcd}$ |
| **4301** | 36.48$^{a-d}$ | 35.26$^{a}$ | 35.87$^{a}$ | 47.47$^{e}$ | 48.68$^{a}$ | 48.07$^{a}$ |
| **4403** | 36.83$^{a-d}$ | 31.27$^{bcd}$ | 34.05$^{bcd}$ | 51.01$^{a-d}$ | 41.83$^{cd}$ | 46.42$^{bcd}$ |
| **5001** | 35.58$^{b-d}$ | 31.77$^{bc}$ | 33.67$^{bcd}$ | 52.23$^{abc}$ | 42.27$^{bcd}$ | 47.25$^{abc}$ |
| **6408** | 35.32$^{cde}$ | 32.37$^{bc}$ | 33.85$^{bcd}$ | 49.94$^{b-e}$ | 43.61$^{bc}$ | 46.78$^{a-d}$ |
| **6410** | 35.01$^{de}$ | 30.59$^{cd}$ | 32.80$^{cd}$ | 49.46$^{cde}$ | 42.08$^{cd}$ | 45.77$^{cde}$ |
| **S3** | 38.56$^{a}$ | 32.84$^{b}$ | 35.70$^{a}$ | 52.56$^{ab}$ | 44.10$^{b}$ | 48.33$^{a}$ |
| **GAP Mavisi** | 37.45$^{abc}$ | 31.88$^{bc}$ | 34.67$^{ab}$ | 49.55$^{cde}$ | 42.29$^{bcd}$ | 45.92$^{b-e}$ |
| **İptaş** | 38.00$^{a}$ | 30.69$^{cd}$ | 34.34$^{abc}$ | 53.02$^{a}$ | 42.03$^{cd}$ | 47.53$^{ab}$ |
| **Karadağ** | 37.61$^{ab}$ | 29.73$^{d}$ | 33.67$^{bcd}$ | 51.66$^{a-d}$ | 40.80$^{d}$ | 46.23$^{bcd}$ |
| **Average** | ***36.20$^{A**}$*** | ***31.67$^{B}$*** | | ***50.03$^{A**}$*** | ***43.01$^{B}$*** | |
| **Mean square error** | ***0.975*** | ***1.362*** | ***1.168*** | ***0.936*** | ***2.337*** | ***1.637*** |
| **Coefficient of variation (%)** | ***3.12*** | ***3.22*** | ***3.19*** | ***2.25*** | ***3.06*** | ***2.75*** |

** is 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' being the highest and 'e' the lowest


| | 2022 | 2023 | Means |
|---|---|---|---|
| Average (%) | 8.54 | 9.15 | |
| Mean square error | 0.740 | 0.932 | 0.836 |
| Coefficient of variation (%) | 10.08 | 10.55 | 10.34 |

***Figure 1. Crude ash ratio of grass pea genotypes***

** is 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' being the highest and 'e' the lowest.

The ADF and NDF values, the impact of the genotypes on separate and combined years and the difference between the years were significant at the probability level of 1%. The ADF and NDF contents of the genotypes varied between 32.43-35.87% and 44.36-48.33%, respectively. The average ADF and NDF rates were determined as 36.20-50.03%, respectively, for 2022, and 31.67-43.01%, respectively, for 2023 (*Table 5*). ADF and NDF signify the fiber content in forage crops. High fiber content in forage crops means difficulty in digestion. Therefore, forage crops should have low ADF and NDF rates. It was seen that some populations have lower levels of ADF and NDF than the varieties. This results from genetic differences and environmental conditions and is promising for populations. In addition, the fact that the first year was drier than the second caused stress in plants, leading to an increase in cellulose and lignin. Başaran et al. (2011) reported that the ADF and NDF rates of the grass pea plant varied between 28.80-34.40% and 33.42-45.01%, respectively. The fact that the experiments were conducted in different ecologies, the delays in harvest times, the varieties used, and whether the trials were conducted in summer or winter can be cited among the causes of the differences observed in the research results.

*Figure 1* features the crude ash content values of the grass pea genotypes for combined years. The impact of the genotypes on crude ash content in separate and combined years was significant ($p \leq 0.01$) while there was no statistical difference between the years. The highest crude ash content was identified in the populations 2401 (9.10%), 4403 (10.14%), 5001 (9.78%), and 6408 (9.11%), and the varieties İptaş (9.11%) and Karadağ (10.34%) while the lowest value was observed in population S3, with 7.32%. Crude ash, the quality factor in forage crops, constitutes the baseline data in trace element analyses. There are antagonistic and synergistic effects between the crude ash rate and yield. Accordingly, the populations S3 and 4301, which had low ash rates, also had low hay yield values in this study. Therefore, increasing the crude ash rate is among the primary goals in the amendment of forage crops (Geren et al., 2004). The fact that some populations had crude ash content at the level of the involved varieties (Figure 1) is significant for the development of variety improvements.

***Table 6. Potassium and phosphorus content of grass pea genotypes***

| Genotypes | Potassium (%) | | | Phosphorus (%) | | |
|---|---|---|---|---|---|---|
| | 2022* | 2023** | Average | 2022 | 2023 | Average* |
| **1603** | $2.75^{bc}$ | $2.85^{bc}$ | 2.80 | 0.42 | 0.52 | $0.47^{b}$ |
| **2006** | $2.87^{bc}$ | $2.90^{b}$ | 2.89 | 0.44 | 0.54 | $0.49^{a}$ |
| **2401** | $2.84^{bc}$ | $2.68^{cd}$ | 2.76 | 0.44 | 0.52 | $0.48^{ab}$ |
| **4301** | $2.78^{bc}$ | $3.08^{a}$ | 2.93 | 0.36 | 0.52 | $0.44^{c}$ |
| **4403** | $3.11^{ab}$ | $2.80^{bcd}$ | 2.96 | 0.43 | 0.55 | $0.49^{a}$ |
| **5001** | $3.42^{a}$ | $2.80^{bcd}$ | 3.11 | 0.43 | 0.55 | $0.49^{a}$ |
| **6408** | $2.96^{bc}$ | $2.65^{d}$ | 2.81 | 0.42 | 0.54 | $0.48^{ab}$ |
| **6410** | $3.03^{bc}$ | $2.72^{bcd}$ | 2.88 | 0.42 | 0.55 | $0.49^{a}$ |
| **S3** | $3.06^{bc}$ | $2.7^{bcd}$ | 2.89 | 0.41 | 0.54 | $0.48^{ab}$ |
| **GAP Mavisi** | $2.73^{c}$ | $2.77^{bcd}$ | 2.75 | 0.42 | 0.51 | $0.46^{b}$ |
| **İptaş** | $2.95^{bc}$ | $2.79^{bcd}$ | 2.87 | 0.42 | 0.55 | $0.49^{a}$ |
| **Karadağ** | $2.82^{bc}$ | $2.87^{bc}$ | 2.85 | 0.43 | 0.55 | $0.49^{a}$ |
| **Average** | ***2.94*** | ***2.80*** | | ***$0.42^{B**}$*** | ***$0.54^{A}$*** | |
| **Mean square error** | ***0.036*** | ***0.010*** | ***0.023*** | ***0.002*** | ***0.001*** | ***0.002*** |
| **Coefficient of variation (%)** | ***6.47*** | ***3.48*** | ***5.27*** | ***3.80*** | ***2.52*** | ***3.40*** |

* is significant at $p \leq 0.05$, ** is 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' being the highest and 'd' the lowest

The effect of the genotypes on K was very significant in separate years ($p \leq 0.05$ and $p \leq 0.01$), but not significant in the combined year. The effect of the genotypes on P was not significant in separate years, and also significant ($p \leq 0.05$) in the combined years. There was a significant difference ($p \leq 0.05$) in Ca among the genotypes in 2023, but no difference was observed in 2022 and the combined years (*Table 6* and *7*). The effect of the genotypes on Mg was not significant in separate years, but it was significant in combined years. Besides, the second year showed higher values compared to the first year in terms of all macro elements. The K, P, Ca, and Mg contents of the genotypes in combined years varied between 2.75-3.11%, 0.44-0.49%, 0.91-0.99%, and 0.15-0.21%, respectively. Macronutrients are the elements most needed by the animals. These elements are highly critical for animal health, with a considerable impact on yield and quality. Lack of or excessive phosphorus and calcium lead to rachitism in animals while potassium ensures acid-base balance in the body and magnesium contributes to bone structure (Dua and Care, 1999; Gürsoy and Macit, 2017). For animal health and quality, it is desired for forage crops to have 0.3-0.8% K, 0.1-0.2% Ca, 0.1-0.2% Mg, and 0.2% P (Mayland and Hankins, 2001). In the study, macronutrient contents in all genotypes were higher than these values (*Table 6* and *7*). Also; it was determined that K and P contents of cited by Başbağ et al (2012) in grass pea genotypes fully coply with the research findings, but their Mg and Ca contents findings were found to be higher than the research findings.

***Table 7. Calcium and magnesium content of grass pea genotypes***

| Genotypes | Calcium (%) | | | Magnesium (%) | | |
|---|---|---|---|---|---|---|
| | 2022 | 2023* | Average | 2022 | 2023 | Average* |
| **1603** | 0.92 | 1.00$^{bc}$ | 0.96 | 0.17 | 0.16 | 0.17$^{bc}$ |
| **2006** | 0.89 | 1.06$^{ab}$ | 0.98 | 0.20 | 0.17 | 0.19$^{b}$ |
| **2401** | 0.90 | 1.03$^{ab}$ | 0.97 | 0.16 | 0.14 | 0.15$^{d}$ |
| **4301** | 0.85 | 1.12$^{a}$ | 0.99 | 0.23 | 0.18 | 0.21$^{a}$ |
| **4403** | 0.90 | 0.92$^{c}$ | 0.91 | 0.19 | 0.16 | 0.17$^{bc}$ |
| **5001** | 0.86 | 0.99$^{bc}$ | 0.92 | 0.18 | 0.14 | 0.16$^{cd}$ |
| **6408** | 0.90 | 1.06$^{ab}$ | 0.98 | 0.20 | 0.16 | 0.18$^{b}$ |
| **6410** | 0.93 | 1.05$^{ab}$ | 0.99 | 0.21 | 0.17 | 0.19$^{b}$ |
| **S3** | 0.89 | 1.05$^{ab}$ | 0.97 | 0.21 | 0.15 | 0.18$^{b}$ |
| **GAP Mavisi** | 0.93 | 1.06$^{ab}$ | 0.99 | 0.17 | 0.17 | 0.17$^{bc}$ |
| **İptaş** | 0.88 | 0.97$^{bc}$ | 0.93 | 0.20 | 0.15 | 0.17$^{bc}$ |
| **Karadağ** | 0.94 | 0.97$^{bc}$ | 0.95 | 0.18 | 0.17 | 0.17$^{bc}$ |
| **Average** | ***0.90$^{B**}$*** | ***1.02$^{A}$*** | | ***0.16$^{B**}$*** | ***0.19$^{A}$*** | |
| **Mean square error** | ***0.003*** | ***0.003*** | ***0.003*** | ***0.002*** | ***0.001*** | ***0.002*** |
| **Coefficient of variation (%)** | ***5.48*** | ***5.82*** | ***5.64*** | ***9.95*** | ***7.47*** | ***9.07*** |

* is significant at $p \leq 0.05$, ** is 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' being the highest and 'd' the lowest

*Table 8* and *9* shows the secondary metabolite contents of grass pea genotypes. The impact of the genotypes on separate and combined years and the difference between the years were significant at the probability level of 1% in terms of total flavonoid and phenolic contents values. The total flavonoid and phenolic contents of the genotypes in combined years varied between 47.02-62.65 mg QE $g^{-1}$ and 48.99-66.79 mg GA $g^{-1}$, respectively (*Table 8*). The impact of the genotypes on DPPH was very significant in both years and combined years ($p \leq 0.01$) while their impact on condensed tannin was very significant in the first year and combined years ($p \leq 0.01$) and insignificant in the second year. In terms of both values, the difference between the years was at a significance level of 1% (*Table 9*). The highest DPPH value was identified in the Karadağ variety with 65.21% and the lowest was seen in population 1603 with 47.25%. The average DPPH content was 65.26% in the first year and 46.02% in the second. The condensed tannin content of the grass pea genotypes varied between 1.03-1.37% and the average condensed tannin content was determined as 1.32% in 2022 and 1.01% in 2023 (*Table 9*).

Secondary compounds in plants (total flavonoid, total phenolic, DPPH, tannin, ODAP, alkaloid, etc.) are the energy sources that allow them to maintain their lives. Plants continue their growth by synthesizing these substances under stress conditions. The secondary compound contents of the genotypes in this study were higher in 2022 because it was a drier period and they were stressed. On the other hand, the secondary compounds in plants have different functions. Total phenolic, total flavonoid, and DPPH content show phytotherapeutic traits, leading to healthier animals and higher yield and quality in animal products (Kuhnen et al., 2014). The fact that these compounds were high in some populations is promising in terms of variety improvements. Condensed tannins in the plants are important for reducing the methane gas released from ruminants, which causes global warming. Therefore, including forage crops rich in condensed tannin 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 condensed tannins 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 condensed tannin contents of all the genotypes in the study were lower than the critical level (*Table 9*).

*Table 8. Total flavonoid and total phenolic contents of grass pea genotypes*

| Genotypes | Total flavonoid (mg QE $g^{-1}$) | | | Total phenolic (mg GA $g^{-1}$) | | |
|---|---|---|---|---|---|---|
| | 2022** | 2023** | Average** | 2022** | 2023** | Average** |
| 1603 | $54.98^{d}$ | $39.05^{d}$ | $47.02^{e}$ | $57.96^{g}$ | $44.65^{a-d}$ | $51.31^{ef}$ |
| 2006 | $60.18^{bcd}$ | $45.06^{cd}$ | $52.62^{cd}$ | $70.02^{cde}$ | $47.91^{abc}$ | $58.97^{bc}$ |
| 2401 | $67.13^{ab}$ | $48.22^{bc}$ | $57.67^{abc}$ | $85.97^{a}$ | $47.62^{abc}$ | $66.79^{a}$ |
| 4301 | $59.49^{bcd}$ | $50.11^{abc}$ | $54.80^{bc}$ | $71.49^{cde}$ | $50.99^{a}$ | $61.24^{bc}$ |
| 4403 | $61.46^{bcd}$ | $44.01^{cd}$ | $52.74^{cd}$ | $67.64^{c-f}$ | $49.20^{ab}$ | $58.42^{bcd}$ |
| 5001 | $65.79^{abc}$ | $48.89^{bc}$ | $57.34^{abc}$ | $75.15^{bc}$ | $40.75^{de}$ | $57.95^{bcd}$ |
| 6408 | $59.35^{bcd}$ | $53.46^{ab}$ | $56.40^{bc}$ | $64.65^{efg}$ | $43.45^{bcd}$ | $54.05^{de}$ |
| 6410 | $67.83^{ab}$ | $40.60^{d}$ | $54.22^{bcd}$ | $73.30^{bcd}$ | $44.79^{a-d}$ | $59.04^{bc}$ |
| S3 | $56.37^{cd}$ | $40.82^{d}$ | $48.59^{de}$ | $61.20^{fg}$ | $36.79^{e}$ | $48.99^{f}$ |
| GAP Mavisi | $63.11^{bcd}$ | $56.80^{a}$ | $59.96^{ab}$ | $74.41^{bc}$ | $48.43^{abc}$ | $61.42^{bc}$ |
| İptaş | $62.65^{bcd}$ | $53.50^{ab}$ | $58.08^{abc}$ | $65.11^{d-g}$ | $48.73^{abc}$ | $56.92^{cd}$ |
| Karadağ | $75.15^{a}$ | $50.14^{abc}$ | $62.65^{a}$ | $80.77^{ab}$ | $42.71^{cd}$ | $61.74^{b}$ |
| Average | $62.79^{A**}$ | $47.56^{B}$ | | $70.64^{A**}$ | $45.50^{B}$ | |
| Mean square error | 28.043 | 13.390 | 20.716 | 20.089 | 11.010 | 15.550 |
| Coefficient of variation (%) | 8.43 | 7.69 | 8.25 | 6.35 | 7.29 | 6.79 |

* $p \leq 0.05$, ** is 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' being the highest and e the lowest.

*Table 9. Total DPPH and condensed tannin contents of grass pea genotypes*

| Genotypes | DPPH (%) | | | Condensed tannin (%) | | |
|---|---|---|---|---|---|---|
| | 2022** | 2023** | Average** | 2022** | 2023 | Average** |
| 1603 | $61.64^{d}$ | $32.87^{f}$ | $47.25^{e}$ | $1.05^{c}$ | 1.05 | $1.05^{bc}$ |
| 2006 | $65.63^{bc}$ | $48.87^{b}$ | $57.24^{c}$ | $1.60^{ab}$ | 1.14 | $1.37^{a}$ |
| 2401 | $68.28^{a}$ | $38.24^{e}$ | $53.26^{d}$ | $1.11^{c}$ | 0.95 | $1.03^{c}$ |
| 4301 | $65.60^{bc}$ | $52.09^{b}$ | $58.85^{bc}$ | $1.64^{ab}$ | 1.15 | $1.40^{a}$ |
| 4403 | $65.05^{bc}$ | $41.68^{cde}$ | $53.36^{d}$ | $1.26^{bc}$ | 0.91 | $1.09^{bc}$ |
| 5001 | $68.19^{a}$ | $48.68^{b}$ | $58.43^{bc}$ | $1.13^{c}$ | 1.02 | $1.08^{bc}$ |
| 6408 | $63.91^{c}$ | $44.75^{c}$ | $54.33^{d}$ | $1.29^{abc}$ | 0.95 | $1.12^{bc}$ |
| 6410 | $64.81^{bc}$ | $50.65^{b}$ | $57.73^{bc}$ | $1.27^{bc}$ | 0.90 | $1.08^{bc}$ |
| S3 | $65.76^{bc}$ | $40.14^{de}$ | $52.95^{d}$ | $1.30^{abc}$ | 0.94 | $1.12^{bc}$ |
| GAP Mavisi | $66.72^{ab}$ | $52.60^{b}$ | $59.66^{b}$ | $1.68^{a}$ | 1.05 | $1.36^{a}$ |
| İptaş | $61.54^{d}$ | $42.70^{cd}$ | $52.12^{d}$ | $0.98^{c}$ | 1.08 | $1.03^{c}$ |
| Karadağ | $66.03^{bc}$ | $59.00^{a}$ | $62.51^{a}$ | $1.52^{ab}$ | 0.99 | $1.25^{ab}$ |
| Average | $65.26^{A**}$ | $46.02^{B}$ | | $1.32^{A**}$ | $1.01^{B}$ | |
| Mean square error | 1.365 | 4.523 | 2.944 | 0.041 | 0.010 | 0.026 |
| Coefficient of variation (%) | 1.79 | 4.62 | 3.08 | 15.37 | 10.04 | 13.76 |

** is 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' being the highest and 'e' the lowest. DPPH: Free radical scavenging capacity.

The impact of the genotypes on ODAP was significant ($p \leq 0.01$) in both separate and combined years, while their impact on total alkaloid content was significant ($p \leq 0.01$) in the second year and not significant in the first year and combined years. In terms of both values, the difference between the years was at a significance level of 1% (*Table 10*). The highest ODAP was identified in the Karadağ variety with 3.05 mg $g^{-1}$ and the population 1603 with 2.83 mg $g^{-1}$, while the lowest was identified in the population 5001 with 1.32 mg $g^{-1}$. The total alkaloid content of the grass pea genotypes varied between 2.41-2.77 g 100$g^{-1}$ (*Table 10*). 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 10*), 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, which has a direct negative impact on the nervous system and is thus undesirable. Thus, developing grass pea varieties with low or no ODAP content enables substantial advantages in spreading grass pea cultivation. Depending on genetic and environmental factors, the ODAP content varies between 0.2-7.2 mg $g^{-1}$ (Deshpande and Campbell, 1992), and for safe consumption, this value needs to be below 2.0 mg g-1'dan (Abd El Moneim et al., 1999). The populations 2401, 5001, and 6408 in this study were below this level. This is of significance for animal health and yield, as well as new variety amendment.

***Table 10. ODAP and total alkaloid contents of grass pea genotypes***

| Genotypes | ODAP (mg $g^{-1}$) | | | Total alkaloid (g $100g^{-1}$) | | |
|---|---|---|---|---|---|---|
| | 2022** | 2023** | Average** | 2022 | 2023* | Average |
| **1603** | $2.84^{ab}$ | $2.82^{a}$ | $2.83^{ab}$ | 3.53 | $1.30^{cd}$ | 2.41 |
| **2006** | $2.87^{ab}$ | $1.53^{de}$ | $2.20^{cd}$ | 3.39 | $1.99^{a}$ | 2.69 |
| **2401** | $2.02^{bc}$ | $1.97^{cd}$ | $2.00^{de}$ | 3.61 | $1.57^{a-d}$ | 2.59 |
| **4301** | $2.78^{ab}$ | $2.37^{abc}$ | $2.58^{bc}$ | 3.45 | $1.82^{abc}$ | 2.63 |
| **4403** | $2.64^{ab}$ | $2.28^{bc}$ | $2.46^{bcd}$ | 3.68 | $1.56^{a-d}$ | 2.62 |
| **5001** | $1.42^{c}$ | $1.22^{ef}$ | $1.32^{f}$ | 3.60 | $1.43^{bcd}$ | 2.51 |
| **6408** | $2.56^{ab}$ | $0.98^{f}$ | $1.77^{e}$ | 3.55 | $1.60^{a-d}$ | 2.58 |
| **6410** | $2.83^{ab}$ | $1.94^{cd}$ | $2.39^{bcd}$ | 3.71 | $1.56^{a-d}$ | 2.63 |
| **S3** | $2.58^{ab}$ | $1.98^{cd}$ | $2.28^{cd}$ | 3.63 | $1.60^{a-d}$ | 2.61 |
| **GAP Mavisi** | $2.50^{ab}$ | $2.44^{ab}$ | $2.35^{cd}$ | 3.56 | $1.98^{ab}$ | 2.77 |
| **İptaş** | $2.63^{ab}$ | $2.51^{ab}$ | $2.57^{bc}$ | 3.38 | $1.21^{d}$ | 2.29 |
| **Karadağ** | $3.31^{a}$ | $2.80^{a}$ | $3.05^{a}$ | 3.60 | $1.85^{ab}$ | 2.73 |
| **Average** | ***$2.58^{A**}$*** | ***$2.07^{B}$*** | | ***$3.56^{A**}$*** | ***$1.62^{B}$*** | |
| **Mean square error** | ***0.191*** | ***0.063*** | ***0.127*** | ***0.032*** | ***0.081*** | ***0.056*** |
| **Coefficient of variation (%)** | ***16.94*** | ***12.08*** | ***9.07*** | ***5.04*** | ***17.51*** | ***9.18*** |

* is significant at $p \leq 0.05$, ** is 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' being the highest and 'e' the lowest. DPPH: Free radical scavenging capacity.

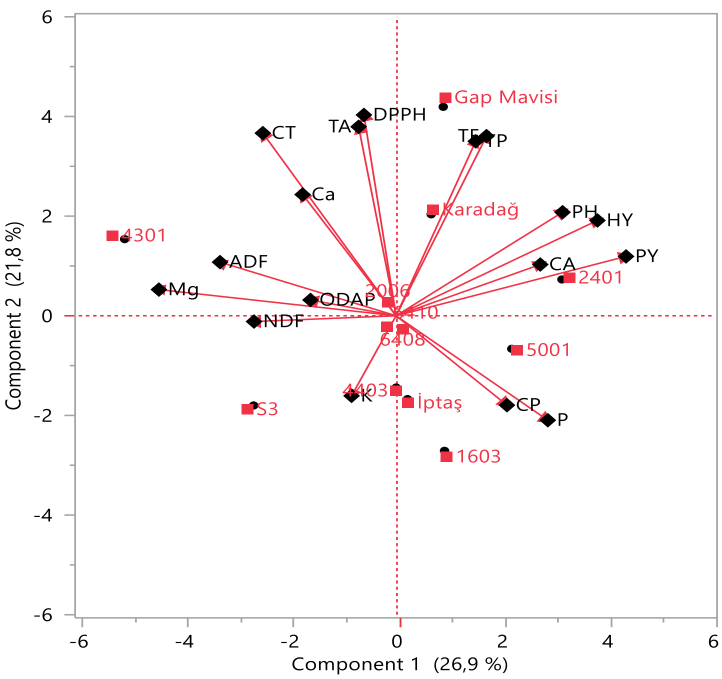


***Figure 2. Pricipal component analysis of grass pea genotypes***

(CP: Crude protein ratio; PH: Plant height; ADF: Acid detergent fibe; NDF: Neutral detergent fiber; HY: Hay yield; 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-diamino propionic acid)

It has been reported that the principal component analysis (PCA) has effective for evaluating the phenotypic diversity in addition to identifying genetically distant clusters of genotypes and selecting important traits contributing to the total variation in the genotypes. The PCA allows natural grouping of the genotypes and is 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; Sing et al., 2020). The relationships between the genotypes and the examined traits in this study can be easily distinguished thanks to the PCA graphic (Figure 1). Sharifi et al. (2018) reported that principal component analyses (PCA) have been used to compare genotypes on the basis of multiple traits and to identify genotypes or groups that are particularly good in certain aspects, and that can be candidates for future breeding. The biplot graphic analyses of the 29 chicory genotypes are present in Figure 2. The yield and quality results of the present study revealed that the first principal component (PCA 1) and the second (PCA 2), respectively, exhibited 26.90% and 21.80% (total 48.70%). It is seen that some populations are better than varieties in various properties. For example, in terms of the undesired ODAP content, the populations 2006, 5001, and 6408 exhibited superior performance in comparison to the varieties. Also, the population of 2401 showed good results in terms of forage yield and quality (*Figure 2*). According to the PCA graphic results, it is seen that especially the 2401 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.

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 the hierarchical clustering structure (Wilkinson and Friendly, 2009; Barua et al., 2022). The heat map of the 17 properties examined in the study through a total of 12 grass pea genotypes, including 3 registered and 9 different populations, per 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 the 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. According to the heat map, four major genotype groups were created in terms of the properties examined. The first group featured 1603, 2006, 6408, and 6410 genotypes, the second consisted of 2401, 5001, 4403, İptaş, and Karadağ, the third consisted of GAP Mavisi variety, and fourth consit of 4301 and S3. In the graph, the third and fourth groups are particularly noteworthy. While the third group (GAP Mavisi variety) is highlighted by HY, the fourth group (populations of 4301 and S3) is highlighted by ADF and NDF. These genotypes exhibited high values. On the other hand, wo major groups were created in terms of properties, with the first one consisting of PH, HY, PY, CA, TF, TP, CP, P, ADF and the second consisting of NDF, K, Ca, Mg, DPPH, TA, CT and ODAP (*Figure 3*).

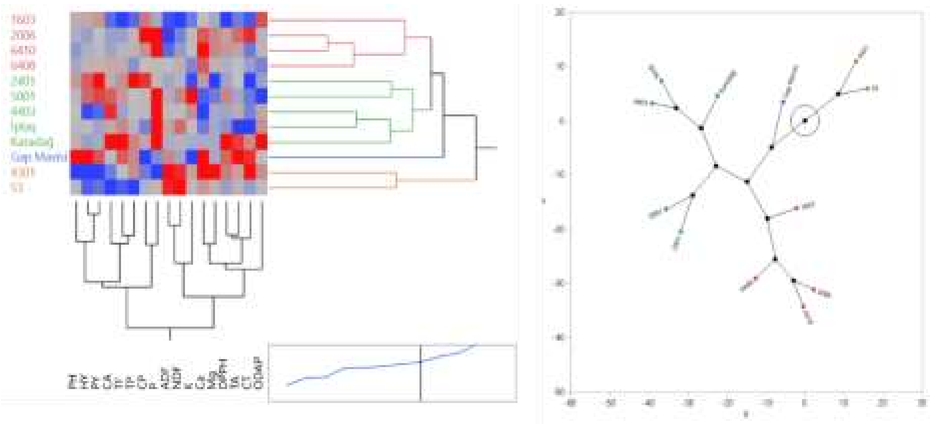

***Figure 3**. Heat map and hierarchical cluster analysis of grass pea genotypes*

(CP: Crude protein ratio; PH: Plant height; ADF: Acid detergent fibe; NDF: Neutral detergent fiber; HY: Hay yield; 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-diamino propionic acid)

## 4. Conclusion

As a result of this study conducted under precipitation-dependent conditions to determine the roughage and some quality features of 12 grass pea genotypes as a summer crop for two years (in the growing seasons of 2022 and 2023), significant differences were identified among the genotypes. Precipitation differences between the years affected both the genotypes and the properties examined. According to the average data from two years, GAP Mavisi come into prominence in terms of hay yield; however, no statistical difference was found among the genotypes in terms of hay yield. For this reason, th quality features 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 forage yield quality, the populations 2401 exhibited a superior performance. Besides, the populations of 2006, 5001, and 6408 can be evaluated as genetic material.

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**Ethical Statement**

There is no need to obtain permission from the ethics committee for this study.

**Conflicts of Interest**

We declare that there is no conflict of interest between us as the article authors.

**Authorship Contribution Statement**

Concept: Sezer, M., Gülümser, E., Collection or Processing: Sezer, M., Gülümser, E., Literature Search: Sezer, M., Gülümser, E., Writing, Review and Editing: Gülümser, E.

## References


Abd El Moneim, A. M., Van Dorrestein, B., Baum, M. and Mulugeta, W. (1999). Role of ICARDA in Improving the Nutritional Quality and Yield Potential of Grass Pea *(Lathyrus sativus*) for Subsistence Farmers in Developing Countries. *Improving Human Nutrition Through Agriculture: The Role of International Agricultural Research Workshop*, 5–7, October, Los Banos, Philippines.

Adams, M. W. (1995). An estimate of homogeneity in crop plants with special reference to genetic vulnerability in dry season. *Euphytica*, 26: 665-679.

Ağamirzaoğlu, M., Valizadeh, N. and Rahimi, A. (2024). Comparison of secondary metabolites and essential oil content of some origanum species. *Journal of Tekirdag Agricultural Faculty*, 21(5), 1075-1090.

Archimède, H., Eugène, M., Marie-Magdeleine, C., Boval, M., Martin, C., Morgavi, D., Lecomte, P. and Doreau, M. (2011). Comparison of methane production between temperate and tropical forages: A quantitative review. *Animal Feed Science and Technology*, 166-167: 59-64.

Arıcı, R. Ç. (2023). Determination of performance of some grasspea (*Lathyrus sativus* L.) genotypes in semiarid climate conditions. *Turkish Journal of Agricultural and Natural Sciences*, 10(4): 984–992. https://doi.org/10.30910/turkjans.1246539

Arvouet-Grand, A., Vennat, B., Pourrat, A. and Legret, P. (1994). Standardisation d`un extrait de propolis et identification des principaux constituants. *Journal de Pharmacie de Belgique*, 49: 462-468.

Atıs, I. and Acıkalın, S. (2020). Yield, quality and competition properties of grass pea and wheat grown as pure and binary mixture in different plant densities. *Turkish Journal of Field Crops*, 25(1): 18-25. https://doi.org/10.17557/tjfc.737476

Barry, T. N. (1987). Secondary Compounds of Forages. In: Nutrition of Herbivores. Ed(s): Hacker, J.B. and Ternouth, J.H., Academic Press, Sydney, Australia.

Barua, H., Saha, S. R., Ivy, N. A., Rasul, G. and Islam, A. A. (2022). Genetic divergence of guava (*Psidium guajava* L.) genotypes in Bangladesh: Guava Genotypes in Bangladesh. *SAARC Journal of Agriculture*, 20(1): 15-28. https://doi.org/10.3329/sja.v20i1.60618

Başaran, U., Mut, H., Aşci, Ö. Ö., Acar, Z. and Ayan, İ. (2011). Variability in forage quality of Turkish grass pea *(Lathyrus sativus* L.) landraces. *Turkish Journal of Field Crops*, 16(1): 9-14.

Basaran, U., Mut, H., Gulumser, E. and Dogrusoz, M. C. (2016). Evaluation of Turkish grass pea (*Lathyrus sativus* L.) collections for its agronomic characters with a special reference to ODAP content. *Legume Research-An International Journal*, 39(6): 876-882. https://doi.org/10.18805/lr.v0iOF.3547

Başbağ, M., Aydın, A., Çaçan, E., Sayar, M. S. (2012). Determination of quality characters of some grass pea taxa (*Lathyrus* spp.) collected in natural areas of Southeastren Anatolia Region of Turkey. *Reserach Journal of Agricultural Sciences*, (2): 111-114.

Bate-Smith, E. C. (1975). Phytochemistry of proanthocyanidins. *Phytochemistry*, 14: 1107-1113. https://doi.org/10.1016/0031-9422(75)85197-1

Cartea, M. E., Picoagea, A., Soengas, P. and Ordás, A. (2002). Morphological characterization of kale populations from Northwestern Spain. *Euphytica*, 129: 25-32.

Chase, R. A., Pearson, S., Nunn, P. B. and Lantos, P. L. (1985). Comparative toxicities of and B-N-Oxalyl-L-A, B- iaminopropionic acids to rat spinal cord. *Neuroscience Letters*, 55(1): 89-94. https://doi.org/10.1016/0304-3940(85)90317-9

de Paula, E. M., Samensari, R. B., Machado, E., Pereira, L. M., Maia, F. J., Yoshimura, E. H. and Zeoula, L. M. (2016). Effects of phenolic compounds on ruminal protozoa population, ruminal fermentation, and digestion in water buffaloes. *Livestock Science*, 185: 136-141. https://doi.org/10.1590/S1516-35982014000400006

Deshpande, S. S. and Campbell, C. G. (1992). Genotype variation in BOAA, condensed tannins, phenolics and enzyme inhibitors of grass pea (*Lathyrus sativus*). *Canadian Journal of Plant Science*, 72(4): 1037-1047. https://doi.org/10.4141/cjps92-130

Dohi, H., Yamada, A. and Fukukawa, T. (1997). Intake stimulants in perennial ryegrass (*Lolium perenne* L.) fed to sheep. *Journal of Dairy Science*, 80: 2083–2086. https://doi.org/10.3168/jds.S0022-0302(97)76153-8

Dua, K. and Care, A. D. (1999). The role of phosphate on the rates of mineral absorbtion from the forestomatch of sheep. *The Veterinary Journal*, 157: 51-55. https://doi.org/10.1053/tvjl.1998.0259

Geren, H., Avcıoğlu, R. and Soya, H. (2004). Investigations on the herbage performances of some common vetch (*Vicia sativa*) cultivars under Bornova conditions. *ANADOLU Journal of Aegean Agricultural Research Institute*, 14(2): 35-38.

Gezer, K., Duru, M. E., Kivrak, I., Turkoglu, A., Mercan, N., Turkoglu, H. and Gulcan, S. (2006). Free-radical scavenging capacity and antimicrobial activity of wild edible mushroom from Turkey. *African journal of Biotechnology*, 5(20). 1924-1928.

Gülümser, E., Yıldırım, İ., Kardeş, Y. M. and Başaran, U. (2023). Determination of roughage yield and qualıty of grass pea (*Lathyrus sativus* L.) and cmmon vetch (*Vicia sativa* L.) in Bilecik ecological conditions. *MAS Journal of Applied Sciences*, 8(Special issue): 938–942. https://doi.org/10.5281/zenodo.10003589

Gürlük, S. (2021). On the use of shadow price of biodiversity in natural resource management: The Case of Uludağ national park. *Journal of Tekirdag Agricultural Faculty*, 18(2): 234-246.

Gürsoy, E. and Macit, M. (2017). Determination of mineral contents of some legume and cereal forages grown as naturally in pastures of Erzurum province. *Alınteri Journal of Agricultural Sciences*, 32(1): 1-9 https://doi.org/10.28955/alinterizbd.279756

Hanbury, C. D., White, C. L., Mullan, B. P. and Siddique, K. H. M. (2000). Are view of the potential of *Lathyrus sativus* L. and *Lathyrus cicera* L. grain for use as animal feed. *Animal Feed Science and Technology,* 10546: 1–27. https://doi.org/10.1016/S0377-8401(00)00186-3

Hoy, W. K., Smith, P. A. and Sweetland, S. R. (2002). The development of the organizational climate index for high schools: its measure and relationship to faculty trust*. High School Journal*, 86(2): 38-49.

INEN (2005). Ecuadorian Technical Standard for Legumes – Debittered Chocho Grain. Published by the Ecuadorian Institute for Standardization, Quito. (Grano desamargado de chocho Norma Tecnica Ecuatoriana Leguminosas Grano desamargado de chocho. Instituto Ecuatoriano de Normalizacion, Quito). (In Spanish)

Kacar, B. (1972). Chemical Analysis of Plants and Soil. (2nd Edition) Ankara University Faculty of Agriculture Publications, Ankara, Türkiye.

Kökten, K., Özdemir, S., Kardeş, Y. M. and Kaplan, M. (2018). Biplot analysis for herbage yield and quality attributes of different grasspea (*Lathyrus sativus* L.) genotypes. *Fresenius Environmental Bulletin*, 27(9): 6079-6086.

Kowalczyk, E., Patyra, E. and Kwiatek, K. (2013). Organic acids and their ımportance in animal husbandry. *Medycyna Weterynaryjna*, 69(5): 269-273.

Kuhnen, S., Moacyr, J. R., Mayer, J. K., Navarro, B. B., Trevisan, R., Honorato, L. A. and Pinheiro Machado Filho, L. C. (2014). Phenolic content and ferric reducing–antioxidant power of cow's milk produced in different pasture-based production systems in southern Brazil. *Journal of the Science of Food and Agriculture,* 94(15): 3110-3117.

Kumar, R. and Singh, M. (1984). Tannins: Their adverse role in ruminant nutrition. *Journal of Agricultural and Food Chemistry*, 32: 447-453.

Lambein, F., Travella, S., Kuo, Y. H., Van Montagu, M. and Heijde, M. (2019). Grass pea (*Lathyrus sativus* L.): orphan crop, nutraceutical or just plain food? *Planta,* 250: 821-838. https://doi.org/10.1007/s00425-018-03084-0

Lascano, C. E. and Cárdenas, E. (2010). Alternatives for methane emission mitigation in livestock systems*. Revista Brasileira de Zootecnia*, 39: 175-182. https://doi.org/10.1590/S1516-35982010001300020

Martin, C., Copani, G. and Niderkorn, V. (2016). Impacts of forage legumes on intake, digestion and methane emissions in ruminants. *Legume Research,* 12: 8-10.

Mayland, H. F. and Hankins, J. L. (2001). Mineral Imbalances and Animal Health: A Management Puzzle. In: Anti-Quality Factors in Rangeland and Pastureland Forages. Ed(s): Launchhbaugh, K., Universty of Idaho, Moskow, Russia.

Mehta, S. L., Ali, K. and Barna, K. S. (1994). Somaclonal variation in a Food Legume – *Lathyrus sativus*. J*ournal of Plant Biochemistry and Biotechnology*, 3: 73–77.

Patra, A. K., Kamra, D. N. and Agarwal, N. (2006). Effect of plant extracts on in vitro methanogenesis, enzyme activities and fermentation of feed in rumen liquor of buffalo. *Animal Feed Science and Technology*, 128 (3-4): 276–291. https://doi.org/10.1016/j.anifeedsci.2005.11.001

Plitmann, U., Gabay, R. and Cohen, O. (1995). Innovations in the Tribe *Vicieae* (*Fabaceae*) from Israel. *Israel Journal of Plant Sciences*, 43: 249-258. https://doi.org/10.1080/07929978.1995.10676609

Rao, S. L. N. (1978). A sensitive and specific colorimetric method for determination of a,bdiaminopropionic acid and *Lathyrus sativus* neurotoxin. *Analytical Biochemistry*, 86(2): 386-396. https://doi.org/10.1016/0003-2697(78)90762-5

Rochfort, S., Parker, A. J. and Dunshea, F. R. (2008). Plant bioactives for ruminant health and productivity. *Phytochemistry*, 69(2): 299–322. https://doi.org/10.1016/j.phytochem.2007.08.017

Santos Neto, T. M., Mota, R. A., Silva, L. B. G., Viana, D. A., Lima-Filho, J. L., Sarubbo, L. A., ... and Porto, A. L. F. (2009). Susceptibility of *Staphylococcus* spp. isolated from milk of goats with mastitis to antibiotics and green propolis extracts. *Letters in Drug Design & Discovery,* 6(1): 63-68. https://doi.org/10.2174/157018009787158599

Sayar, M. S. and Han, Y. (2015). Determination of seed yield and yield components of grasspea (*Lathyrus sativus* L.) lines and evaluations using GGE biplot analysis method. *Journal of Agricultural Sciences*, 21(1): 78-92.

Sayar, M. S., Han, Y., Seydoşoğlu, M. and Başbağ, M. (2013). Determination of forage yield, its affecting components, and relationships among traits of some grass pea (*Lathyrus sativus* L.) lines in Diyarbakır ecological conditions. *X$^{th}$ Turkey Field Crops Congress*, 10-13 September, Volume III: 56-64, Konya, Türkiye.

Sharifi, P. and Ebadi, A. A. (2018). Relationships of rice yield and quality based on genotypes by trait (GT) biplot. *Anais da Academia Brasileira de Ciências*, 90(1): 343-356.

Sing, K. S., Suneetha, Y., Sandeep Raja, D. and Srinivas, T. (2020). Principal component analysis for yield and quality traits of coloured rice (*Oryza sativa* L.). *The Pharma Innovation Journal*, 9(7): 456-462.

Singleton, V. L., Orthofer, R. and Lamuela-Raventos, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Methods Enzymol*, 299: 152-178. https://doi.org/10.1016/S0076-6879(99)99017-1

Undi, M., Wittenberg, K., McGeough, E. J. and Ominski, K. H. (2016). Impact of forage legumes on greenhouse gas output and carbon footprint of meat and milk. *Legume society*, (12): 26-28.

Wilkinson, L. and Friendly, M. (2009). The history of the cluster heat map*. American Statistical*, 63(2): 179-184. https://doi.org/10.1198/tas.2009.0033