Türkiye Tarımsal Araştırmalar Dergisi
dergipark.org.tr/tutad

Research Article

Turk J Agric Res
2022, 9(2): 175-184
© TÜTAD
ISSN: 2148-2306
e-ISSN: 2528-858X
doi: 10.19159/tutad.1068258

Assessment of Variability for Nutritional Traits of Burr Medic (*Medicago polymorpha* L.) Genotypes with Different Phenology

Ferat UZUN[1*], **Nuh OCAK**[2]

[1]*Ondokuz Mayıs University, Faculty of Agriculture, Department of Field Crops, Samsun, TÜRKİYE*
[2]*Ondokuz Mayıs University, Faculty of Agriculture, Department of Animal Science, Samsun, TÜRKİYE*

Received: 04.02.2022 **Accepted:** 25.07.2022

ORCID ID (By author order)
orcid.org/0000-0001-7389-5835 orcid.org/0000-0001-7393-1373

*Corresponding Author: fuzun@omu.edu.tr

Abstract: The objective of this study was to determine compatible genotypes for both grazing and ley farming systems concerning nutritional traits among burr medic (*Medicago polymorpha* L.) genotypes with different flowering times. Therefore, the variability for nutritional traits of early- (n=13), medium- (n=12) and late-flowering (n=19) genotypes from a breeding study carried out during the 2016-2018 years was assessed using one-way ANOVA and chemometric techniques such as principal component (PCA) and cluster (CA) analyses. Except for the acid detergent protein, calcium and magnesium contents, there were significant differences in the nutritional traits among the genotypes with different flowering times. The medium-flowering genotype had a significant advantage over especially early-flowering genotype in crude protein, acid detergent fiber, metabolizable energy, and relative feed value. There were significantly mutual correlations between most of the studied traits. Consequently, considerable amounts of variability were determined among the genotypes for all the traits under consideration. The 44 genotypes formed three clusters, in which cluster sizes ranged from 3 to 29 accessions per cluster. The PCA 1 and 2 had the highest eigenvalues of 6.44 and 1.35, describing 63.27% and 15.91% of the total variance, respectively. The PCA and CA results indicate that medium- and late-flowering genotypes had the best nutritional traits due to probably high photosynthetic capacity in the conditions of the present study.

Keywords: Annual alfalfa, cluster analysis, forage quality, nutritive value, plant breeding, principal component analysis

1. Introduction

Many grassland or rangeland areas of the world face the challenges such as requirements for arable land and bioenergy, competition between food and feed production, unconscious grazing, the need to preserve biodiversity and maintain ecosystem services and adapt to a changing climate (Lüscher et al., 2014; Uzun et al., 2017). Production in these lands should need to keep pace with requirements for optimum yields (meat and milk) from domestic ruminant (cattle, sheep, and goat) production systems and overcome these challenges (Lüscher et al., 2014). In recent decades, there has, therefore, been an increased awareness of the potential of legumes (*Fabaceae*), including annual medic species to contribute to ruminant production in these lands (Addis et al., 2005; Lüscher et al., 2014; Aydın et al., 2015; Stagnari et al., 2017; Cui et al., 2021). The forage legumes for rangelands have both high nutritional value and ability to fix atmospheric nitrogen (N) biologically. Moreover, desirable annual and perennial legumes offer essential opportunities for sustainable rangeland-based animal production. These forages contribute to animal production by increasing the yield and nutritional value of rangeland and field crops and raising the conversion efficiency of herbage to animal protein (Lüscher et al., 2014; Uzun and Ocak, 2019; Aydin et al., 2020). Researchers have to improve the quality and yield of legumes using advances in plant breeding to increase their contributions to both grazing and ley farming systems. Genotypes developed from populations collected in local areas supply more advantages than introduced standard genotypic materials for breeding studies due to their high adaptation capability (Seker et al., 2014).

Burr medic (*Medicago polymorpha* L.) is one of the self-generating legumes in the pastures or rangelands and a nutritious and palatable forage species (Muir et al., 2003; Addis et al., 2005; Cui et al., 2021). This species is an essential component of Mediterranean grazing systems (Del Pozo et al., 2002; Aydın et al., 2015) due to its beneficial efficacy concerning persistence, forage production and quality, and animal response (Rochon et al., 2004; Graziano et al., 2010; Aydın et al., 2015). Burr medic is the most frequent annual legume in a large area receiving from 30 to 1300 mm of annual rainfall in the Mediterranean zone (Del Pozo et al., 2002). Rochon et al. (2004) reported that rotationally grazed pasture with burr medic had higher productivity under heavy than low grazing intensity. Desirable annual legumes, including burr medic, reduce overgrazing pressure on rangelands (Nazari-Dashlibrown, 2004). Also, a ley farming system with a cereal crop and burr medic-pasture rotation can create biological and economic synergies between crops and livestock enterprises in terms of long-term productivity (Nazari-Dashlibrown, 2004; Crews et al., 2016). Therefore, burr medic is a critical ecological plant in various farming systems worldwide (Del Pozo et al., 2002; Cui et al., 2021).

The nutritional value and the voluntary intake of burr medic species influence livestock production. Some studies (Shirmardi et al., 2003; Aydin et al., 2007, Aydın et al., 2015) investigated the nutrient contents and feeding value of this forage harvested at different phenological stages such as early growth, early and complete flowering, or seeding. In these studies, the crude protein (CP), crude cellulose, acid detergent fiber (ADF), neutral detergent fiber (NDF), ash, calcium (Ca), and metabolisable energy (ME) burr medic forages have ranged from 13.7 to 24.4%, 15.67 to 29.2%, 22.3 to 43.7%, 31.7 to 66.2%, 14.5 to 17.67%, 1.18 to 1.32% and 9.2 to 11.8 MJ kg^{-1} dry matter (DM). Also, Aydın et al. (2015) noted the digestible DM (DDM, 66.14%), DM intake [DMI, 2.97% of body weight (BW)], and relative feed values (RFV, 152.9) of burr medic hay harvested at 50% flowering stage. These results indicate that the feeding value of burr medic forage is affected by different phenological stages.

The studies mentioned above encourage range managers and farmers to reduce costs associated with inorganic-N fertilizers and purchased forages and increase their reliance on burr medic-based rangeland and ley farming. Among burr medic (*M. polymorpha* L.) genotypes with different flowering times, there can be compatible genotypes to graze and ley farming systems or for multiple uses. It is prudent to choose a combination of annual or perennial forage species with different flowering times for sustainable forage crops and rangeland-based animal productions. A few studies investigated burr medic production and quality through breeding, agronomic practices, and genetics (Aydın et al., 2015; Cui et al., 2021). However, there is no knowledge about how nutritional traits distribute and varied (i.e. diversity) among early- (EF), medium- (MF), and late-flowering (LF) burr medic genotypes. For the low-cost and low-input rangeland and ley farming systems of the countries like Türkiye, compatible genotypes in agro-morphological and nutritional traits must be selected among the promising burr medic populations collected from the natural rangelands. Therefore, the objective of this study was to assess variability for the nutritional traits among EF, MF, and LF burr medic genotypes.

2. Materials and Methods

This study was carried out during the 2016-2018 years at the experimental field of the Agricultural Faculty, Ondokuz Mayıs University, located in the northern part of Türkiye (41°21' N, 36°15' E, elevation 140 m a.s.l.) under rainfed condition. The mean annual temperature and rainfall for long-term were 14.6 °C (ranged from 7.2 °C in winter to 23.2 °C in summer) and 717.9 mm (Anonymous, 2018) and classified as semi-humid climate according to Thornthwaite climate classification (Anonymous, 2016).

The soil structure of the trial area was clay, lime-free and slightly salty and neutral pH and insufficient in phosphorus (P) and rich in potassium (K) and organic matter.

In the first year (2016-2017) of the study, to determine the agro-morphological traits of 2025 burr medic populations collected from the Middle Black Sea Region of Türkiye were bred based on the traditional plant breeding method. Then, 45 seeds from each of 44 genotypes with the best agro-morphological traits selected from these populations were prepared separately for sowing, as explained by Uzun and Aydin (2004) and Aydın et al. (2015) and individually planted into plug trays (1 seed in each tray) containing peat in September 2017. After emergence, seedlings in trays were irrigated and fertilized to grow stronger. An 11.4 g diammonium phosphate (DAP), which delivers 2.05 g N and 5.23 g P_2O_5 per tray, was applied dissolving in water. All seedlings (20 seedlings per genotype) that reached a plant height of 8-10 cm were manually planted in progeny rows. A 16.7 kg DAP, which delivers 0.3 kg N and 1.8 kg P_2O_5 per hectare of this area, was applied as basal dose. The progeny rows were 16 m in length with 80 cm row to row (20 seedling per row) and 80 cm plant to plant spacing (0.64 m^2 per seedling). All other recommended management practices were followed according to the traditional plant breeding method (Acquaah, 2012) to raise a healthy plant. To determine the flowering time, days from seed sowing in trays to first inflorescence opening was counted for each genotype. Then, the genotypes were classified into three groups named as Early flowering (EF, n= 13, Genotype numbers: 1, 2, 8, 13, 17, 24, 25, 26, 28, 31, 39, 40 and 43), Medium flowering (MF, n= 12, Genotype numbers: 3, 5, 9, 11, 12, 29, 30, 33, 36, 37, 42 and 44) and Late flowering (LF, n= 19, Genotype numbers: 4, 6, 7, 10, 14, 15, 16, 18, 19, 20, 21, 22, 23, 27, 32, 34, 35, 38 and 41) genotypes based on the standard deviation of the mean of days to the first inflorescence opening (Table 1).

In 2018, agro-morphological traits (plant height and length, central leaflet length and width, etc.) of the genotypes were measured and recorded (Anonymous, 1991; Lermi and Palta, 2014). After determining the fresh and dry weights of each plant whose blooming time is determined and harvested, oven-dried subsamples were ground into a 1 mm sieve to analyze and calculate some hereafter nutritional traits. Their CP, NDF, ADF, ADP, Ca, P, K and magnesium (Mg) contents were determined using Foss near-infrared reflectance spectroscopy (Model 6500 Win ISI II v1.5 device) using the IC-0904FE calibration program. Then, the DDM, DMI, ME and RFV of each genotype were estimated according to the following empirical Equations 1-4 (Moore and Undersander, 2002), respectively.

$$\text{DMI (\% of BW)} = 120/\text{NDF\%} \quad (1)$$

$$\text{DDM (\%)} = 88.9 - (0.779 \times \text{ADF\%}) \quad (2)$$

$$\text{ME (MJ kg}^{-1}\text{ DM)} = 0.17 \times \text{DDM\%} - 2.0 \quad (3)$$

$$\text{RFV} = (\text{DDM} \times \text{DMI})/1.29 \quad (4)$$

To determine the association effect of floristic composition of a pasture and rangelands on tetany risk, it needs to be calculated the tetany risk ratio of each plant species. Therefore, the tetany risk ratio and Ca to P ratio are calculated on an equivalent weight basis using [K/(Ca + Mg)] and Ca/P formulas, respectively (Uzun et al., 2017).

The normal distribution and homoscedasticity of variances were analyzed using data obtained from single plants. Cluster Analysis (CA) and Principal Component Analysis (PCA) as the multivariate analysis was used to indicate the sources of variation differentiating the burr medic genotypes in terms of the nutritional traits and identifying the associations between the genotypes, respectively. All nutritional traits were used for CA to determine the variability among the genotypes and their groupings. Euclidean distances as similarity measures and the unweighted pair-group method were used for dendrogram with the Average Linkage. Before PCA was conducted to determine the relatedness (correlation and causal relationships) of nutritional traits, the suitability of data for factor analysis was assumed using the Kaiser-Meyer-Olkin (KMO) test and Bartlett's test (KMO: 0.717; χ^2: 2223.1, $p<0.001$). The first (PC1) and second (PC2) principal component axes scores were plotted to aid the visualization of group differences within burr medic genotypes. The degree of association between quantitative traits was analyzed using Pearson’s correlation coefficients. The nutritional traits of EF, MF, and LF burr medic genotypes were subjected to a one-way analysis of variance (ANOVA). The data from a single plant of each genotype were considered to be the experimental unit. For all analyses, significance was deemed at $p<0.05$. In this study, all analyses were performed using the IBM SPSS software package (SPSS v21.0: IBM Corp.).

3. Results and Discussion

Except for the ADP, Ca, and Mg contents of burr medic, the nutritional traits differed among the genotypes with different flowering times. The MF genotypes had higher CP and DDM contents than the EF genotypes ($p<0.05$). The ADF content and Ca/P ratio of burr medic were higher in the EF genotypes, whereas the K and P contents and the tetany risk ratio of burr medic were lower in the EF genotypes than the MF and LF genotypes ($p<0.05$). The NDF content of the EF genotypes were higher than those of the MF genotypes ($p<0.05$). The MF genotypes had higher DMI, RFV, and ME than the EF and LF genotypes ($p<0.05$) (Table 2). Nutritional traits of the studied genotypes were within the ranges reported for different burr medic cultivars (Muir et al., 2003;

Table 1. Classification of burr medic (*M. polymorpha* L.) genotypes based on days from seed sowing in trays to the first inflorescence opening

Genotypes	n	Mean days	Standard deviation	Minimum days	Maximum days
Early flowering	13	185.3	1.23	184	187
Medium flowering	12	195.1	0.86	194	196
Late flowering	19	199.4	1.12	198	201

Table 2. Nutritional traits among early (EF)-, medium (MF)- and late-flowering (LF) burr medic (*M. polymorpha* L.) genotypes*

	EF (n= 12)	MF (n= 13)	LF (n= 19)	SEM
Analyzed traits, g kg^{-1} DM				
Crude protein	203.5^{b}	221.9^{a}	214.9^{ab}	3.31
Acid detergent fiber	183.2^{a}	143.8^{b}	165.6^{b}	4.64
Neutral detergent fiber	284.5^{a}	241.6^{b}	263.2^{ab}	4.70
Acid detergent protein	3.5	2.9	3.3	0.12
Potassium (K)	3.06^{b}	3.34^{a}	3.42^{a}	0.050
Calcium (Ca)	11.90	11.51	11.81	0.118
Magnesium (Mg)	2.71	2.63	2.61	0.024
Phosphorus (P)	3.92^{b}	4.13^{a}	4.12^{a}	0.037
Calculated traits				
Digestible DM, %	74.6^{b}	77.7^{a}	76.0^{ab}	0.36
DM intake, % of BW	4.3^{b}	5.0^{a}	4.6^{b}	0.08
Relative feed value	248.7^{b}	301.3^{a}	271.4^{b}	5.91
Metabolizable energy, Mcal kg^{-1}	10.7^{b}	11.2^{a}	10.9^{b}	0.09
Ca/ P	3.04^{a}	2.79^{b}	2.87^{b}	0.03
K/(Ca + Mg)	0.09^{b}	0.11^{a}	0.11^{a}	0.002

*: BW: Bodyweight, ^{a,b}: Means that have no superscript in common are significantly different from each other ($p< 0.05$), SEM: Standard error of the mean

Shirmardi et al., 2003; Aydin et al., 2007, Aydın et al., 2015). Also, our results supported the idea that burr medic is a forage known for its high levels of nutrients (Cui et al., 2021) and thus, represents a valuable resource for grazing sheep and multiple uses (Ewing, 1986). The RFV, ME, and CP of the forage species are the major limiting nutritive properties for livestock since low forages in these properties are less digestible and palatable (Uzun et al., 2017). Improvement of forage quality in terms of nutritional traits might be more complex because environmental effects (Amini et al., 2018) such as the local temperatures and photoperiod influence the flowering and maturation dates (Iannucci et al., 2008). These impacts may be more effective for burr medic due to the high photosynthetic capacity of this species (Cui et al., 2021). Our results indicate that, in general, the MF genotype was more effective in nutritional traits because the growth of early and late types trend to come across to lower and higher temperatures, respectively. Burr medic with autumn–spring cycles can be essential components of sustainable agricultural systems and practices, and thus, contributed importantly to forage resources (Iannucci et al., 2008).

Correlation and causal relationships among 14 nutritional traits were determined using 44 burr medic genotypes. There were significant and strong correlations between most of the pairwise characteristics like NDF, ADF, DMI, DDM, RFV, and ME. Other traits showed significant positive correlations, including CP, DMI, DDM, RFV, and ME or P and K. Also, negative correlations were observed for the pairwise CP, ADF, ADP, NDF, DMI, DDM, RFV, and ME (Table 3). The degree (strong or weak), direction (positive or negative), and magnitude (significant, more significant, or very significant) of mutual association among the studied nutritional traits can be helpful in the indirect selection of desirable characteristics and simultaneous selection of several traits during the plant breeding (Tyagi and Khan, 2010). The highest positive or negative correlations between most of the pairwise traits that were analyzed and calculated may play an essential role in clarifying associations among the nutritional traits of the genotypes (Muir et al., 2003; Aydin et al., 2019). The significant negative mutual correlation between CP, NDF, and ADF is high-quality indicators of forages (Singh et al., 2017; Aydin et al., 2019) because of reflecting a lower proportion of structural cell-wall components, which are less digestible than cell contents (Lüscher et al., 2014). Moreover, the high DDM content of MF genotypes is due to the low cell wall concentration (Aydin et al., 2007).

The 44 burr medic genotypes formed three clusters, in which cluster sizes ranged from 3 to 29 accessions per cluster. Therefore, CA, which used nutritional traits distance amounts, indicated a remarkable sharpness dendrogram from burr medic genotypes. Cluster I consisted of 12 accessions in which three, two, and seven were from the EF, MF, and LF genotypes, respectively. Cluster II consisted of only three accessions, each of which was from three genotype groups. Cluster III was the largest group consisting of 29 accessions in which nine were from the EF and MF genotypes, respectively and 11 were from the LF genotypes. Cluster I, Cluster II and Cluster III had five, two, and nine subgroups, respectively. There was a significant difference between the subgroups in all traits, indicating a high diversity of genotypes

Table 3. Pearson’s correlation coefficients among 14 nutritional traits in burr medic (*M. polymorpha* L.) genotypes (two-tailed test)

Traits	CP	ADF	NDF	ADP	K	Ca	Mg	P	DDM	DMI	RFV	ME	Ca/P	K/(Ca+Mg)
CP	1.00													
ADF	-0.79***	1.00												
NDF	-0.70**	0.94***	1.00											
ADP	-0.37*	0.69**	0.63**	1.00										
K	0.64**	-0.56**	-0.65**	-0.11^{ns}	1.00									
Ca	0.47*	-0.03^{ns}	0.06^{ns}	0.11^{ns}	0.21^{ns}	1.00								
Mg	-0.12^{ns}	0.29^{ns}	0.33*	0.15^{ns}	-0.60**	0.24^{ns}	1.00							
P	0.86***	-0.66**	-0.67**	-0.13^{ns}	0.87***	0.36*	-0.46*	1.00						
DDM	0.79***	-0.99***	-0.94***	-0.69**	0.56**	0.03^{ns}	-0.29^{ns}	0.66**	1.00					
DMI	0.71***	-0.95***	-0.99***	-0.61**	0.63**	-0.06^{ns}	-0.30^{ns}	0.67**	0.95***	1.00				
RFV	0.73***	-0.97***	-0.98***	-0.62**	0.62**	-0.04^{ns}	-0.29^{ns}	0.67**	0.97***	0.99***	1.00			
ME	0.79**	-0.99***	-0.94***	-0.69**	0.57**	0.03^{ns}	-0.29^{ns}	0.66**	0.99***	0.95***	0.97***	1.00		
Ca/P	-0.23^{ns}	0.52**	0.62**	0.21^{ns}	-0.52**	0.63**	0.61**	-0.50*	-0.52**	-0.61**	-0.60**	-0.52**	1.00	
K/(Ca+Mg)	0.37*	-0.52**	-0.65**	-0.16^{ns}	0.88***	-0.27^{ns}	-0.78***	0.67**	0.52**	0.62**	0.60**	0.52**	-0.81***	1.00

CP: Crude protein, ADF: Acid detergent fiber, NDF: Neutral detergent fiber, ADP: Acid detergent protein, K: Potassium, Ca: Calcium, Mg: Magnesium, P: Phosphorus, DDM: Digestible dry matter, DMI: Dry matter intake, RFV: Relative feed value, ME: Metabolizable energy, ns: Nonsignificant; *: $p< 0.05$, **: $p< 0.01$, ***: $p< 0.001$

among the Clusters (data not shown). Cluster I had the highest values for all nutritional traits, whereas those of Cluster II had the highest values for the calculated nutritional traits (Figure 1). The differences between Cluster II and Cluster III for CP, NDF, and ADP were not significant. Therefore, the clustering and variance analysis results indicated that considerable diversity exists due to significant differences for most of the quantitative traits among the accessions, which has great significance to the plant breeder (Seker et al., 2014).

The most significant principal components (PCs) generated from nutritional traits and their statistical loadings are illustrated in Figure 2 and Figure 3. The first and second PCs (PC1 and PC2) had the highest eigenvalues of 6.44 and 1.35, describing 63.27% and 15.91% of the total variance, respectively, and accounted for 79.18% of the variability in the data set from 44 burr medic genotypes (Figure 2). The loadings corresponding to the PCs indicate that the three groups had high contributions. That is, from the PCA, three main nutritional traits may be identified based on natural groupings in the PC2 versus PC1 plot. Group 1 was composed of nutritional traits with positive loadings for PC1 and PC2 [CP (0.783 and 0.443), P (0.793 and 0.015), DDM (0.953 and 0.211), DMI (0.960 and 0.081), RFV (0.964 and 0.114) and ME (0.953 and 0.211)]. Group 2 included nutritional traits with positive loadings for PC1 and negative loadings for PC2 [K (0.793 and -0.015) and K/(Ca + Mg) (0.735 and -0.597)]. Group 3 was composed of nutritional traits with negative loadings for PC1 [ADF (-0.953 and -0.211), NDF (-0.964 and -0.059), ADP (-0.582 and -0.261), Ca (-0.004 and 0.724), Mg (-0.469 and 0.662) and Ca/P ratio (-0.661 and 0.662)]. Based on the correlation matrix loadings (≥ 0.75 and positive factor loadings) of the variables, CP, P, DDM, DMI, RFV, and ME contributed most strongly to PC1, while only K contributed strongly to PC1.

Figure 3 depicts the score plots of nutritional traits in the burr medic genotypes generated from comparing the groups based on PC1 and PC2. The nutritional traits of Group 1 and Group 2 were strongly related to the LF (seven and seven genotypes, respectively) and MF (five and three genotypes, respectively) genotypes but weakly related to the EF genotypes (one and two genotypes, respectively). However, Group 3 was strongly related to the EF (10 genotypes) but weakly related to the MF (four genotypes) and LF genotypes (five genotypes). The position of burr medic genotypes in the score plot was consistent with the correlation and CA results Also, it demonstrated that PC1 described the calculated traits (except for CP and P) distribution between the studied genotypes. The MF and LF genotypes were characterized by high values for CP, DDM, DMI, ME, and RFV, while the EF genotypes were characterized by high Ca/P and Mg values. However, the LF was somewhat in the middle and less tightly clustered than either of the other groups. Therefore, PC1 separated the MF genotypes from the EF genotypes. PC2 did not enable the EL samples to be separated from the CI and MIX treatments, which were associated with nutritional traits from groups 1 and 2, respectively. As reported previously (Walczak et al. 2017; Corrales-Retana et al. 2021; Kop-Bozbay et al. 2021), the results of PCA showed the maximum contribution of the nutritional traits with positive loadings, i.e. in Group 1, but formed two distinct groups (Group 1 and Group 2) with slight overlapping between genotypes (Kop-Bozbay et al. 2021). The results of multivariate analysis (PCA and CA) revealed that genotypes from these two groups (1 and 2) and clusters (I and III) could be used further in the improvement of this species as promising genotypes. There is a mutual relationship between the ADF and NDF contents and the estimated nutritional traits (DDM, DMI, RFV, and ME) based on correlation analysis. Therefore, these traits were in opposite quadrants in PCA, indicating a negative association with the ADF and NDF contents.

Cui et al. (2021) noted that the nutrient contents of plants are dependent on especially their photosynthetic capacity rather than the agricultural processes and practices. This argument may explain why PCA and CA grouped the genotypes differently with different flowering duration and the mutual correlations between the nutritional traits modified by flowering duration. As such, the burr medic genotypes had high diversity in the nutritional traits. The studied genotypes had the best nutritional traits coupled with medium and late-flowering compared with early flowering, as Muir et al. (2003) also revealed. These results indicate that assessing nutritional traits was an essential step in describing and categorizing genotypes and can, also, of great interest for sustainable production in many Mediterranean rangeland and ley farming systems (Nazari-Dashlibrown, 2004; Rochon et al., 2004; Iannucci et al., 2008). Indeed, burr medic genotypes with autumn-spring cycles can constitute the most productive part of forage resources by covering grazing area or crop field, if even at a relatively short duration (Del Pozo et al., 2002; Iannucci et al., 2008; Crews et al., 2016). Annual reseeding medics provide an abundance of fresh forage with

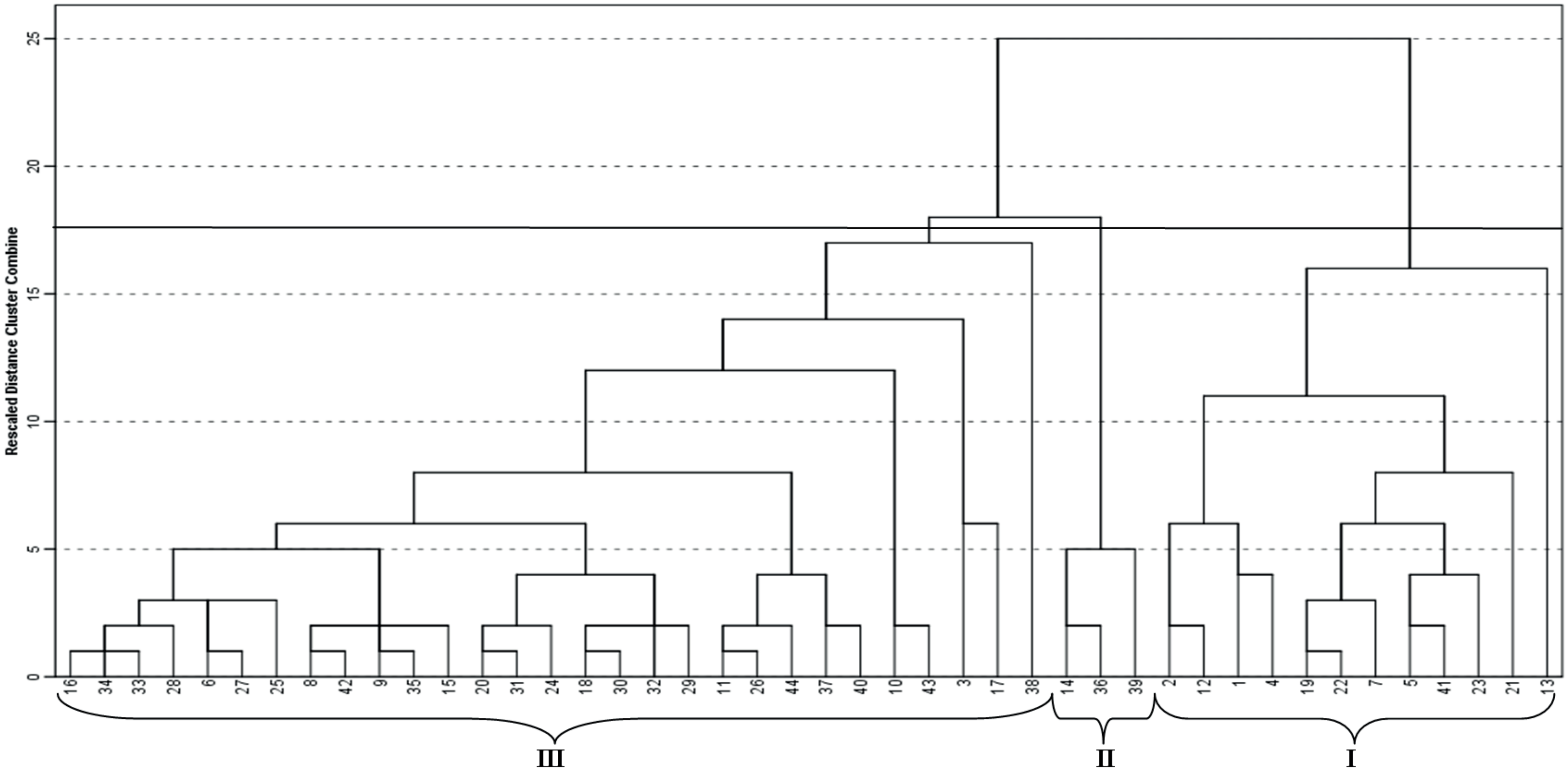

Figure 1. Dendrogram of burr medic (*M. polymorpha* L.) genotypes based on Average Linkage of 14 nutritional traits*

*: Early-flowering burr medic genotypes (1, 2, 8, 13, 17, 24, 25, 26, 28, 31, 39, 40 and 43), Medium-flowering burr medic genotypes (3, 5, 9, 11, 12, 29, 30, 33, 36, 37, 42 and 44), Late-flowering burr medic genotypes (4, 6, 7, 10, 14, 15, 16, 18, 19, 20, 21, 22, 23, 27, 32, 34, 35, 38 and 41)

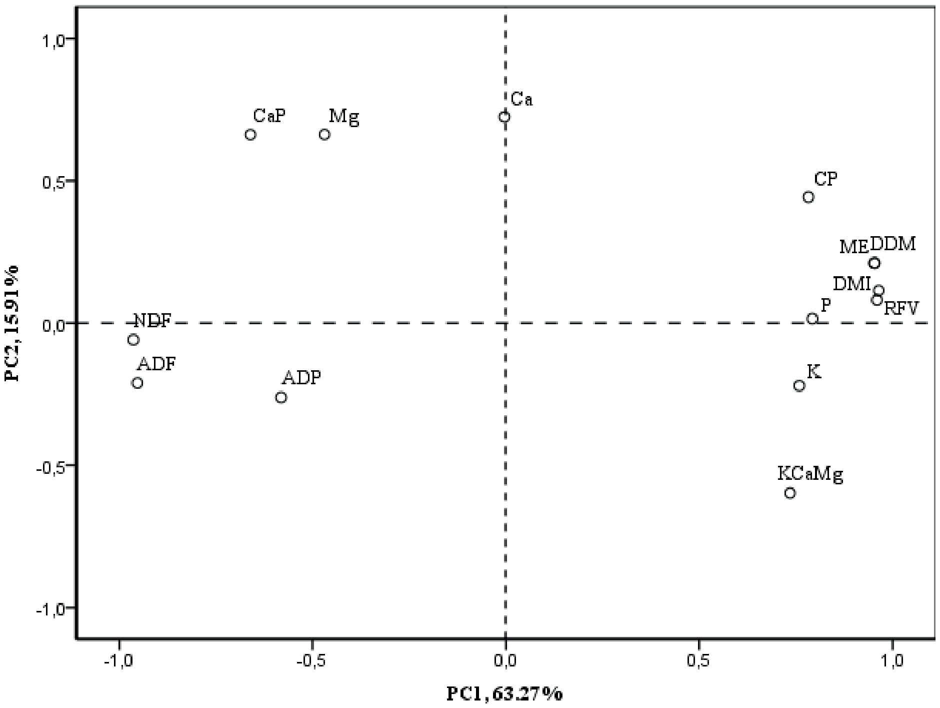

Figure 2. Scatter diagram based on loading scores of principal components (PC1 and PC2) for the nutritional traits (defined as nutrient content and forage quality indicator) of the investigated burr medic (*M. polymorpha* L.) genotypes with different flowering duration

CP: Crude protein, ADF: Acid detergent fiber, NDF: Neutral detergent fiber, ADP: Acid detergent protein, K: Potassium, Ca: Calcium, Mg: Magnesium, P: Phosphorus, DDM: Digestible dry matter, DMI: Dry matter intake, RFV: Relative feed value, ME: Metabolizable energy, CaP: Ca/P ratio, KCaMg: Tetany risk ratio (K/Ca+M

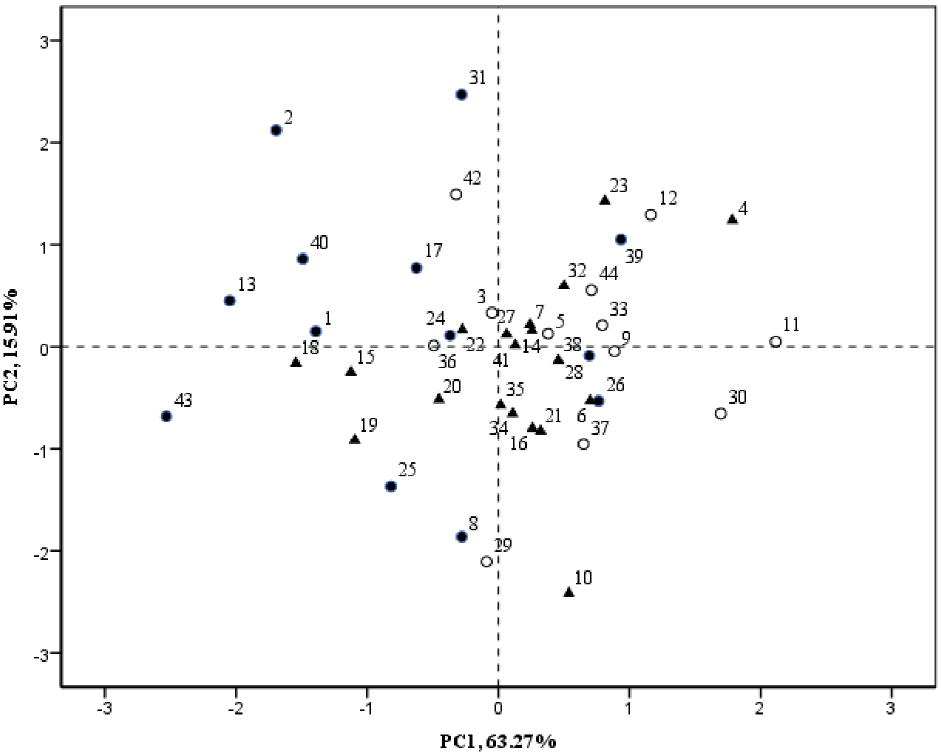

Figure 3. Diagram based on principal component (PCI and PC2) scores of investigated burr medic (*M. polymorpha* L.) genotypes

●: Early-flowering genotypes, ○: Medium-flowering genotypes, ▲: Late-flowering genotypes high nutritive value during the spring, summer, and fall seasons (Uzun and Ocak, 2019) and thus diminish the negative implications of grazing animals on desirable perennial species (Aydin et al., 2020). Furthermore, the reported results and suggested genotypes based on their nutritional relationships can be further utilized in breeding programs.

4. Conclusions

In conclusion, the nutritional traits differed among the genotypes with different flowering duration. Except for the ADP, Ca, and Mg contents of burr medic and the medium- and late-maturing burr medic genotypes had the best nutritional traits due to probably high photosynthetic capacity in our study conditions. Present diversity in the nutritional traits of the burr medic genotypes could be used further in the improvement of this species as promising genotypes. These results indicate that assessing nutritional traits was a crucial step in describing and categorizing genotypes and also can be of great interest for sustainable production in many Mediterranean rangeland and ley farming systems. In addition, the MF genotypes (5, 12, 33 and 44) may be a valuable source for further selection and improvement in nutritional value.

Declaration of Author Contributions

Conceptualization, Material, Methodology, Investigation, Project Administration, Funding Acquisition, Writing-Review & Editing, *F. UZUN*; Formal Analysis, Visualization, Writing-Review & Editing, *N. OCAK*. All authors declare that they have seen/read and approved the final version of the article ready for publication.

Funding

The research project was funded through the Scientific Research Fund of Ondokuz Mayıs University (Project Number: PYO.ZRT.1904. 17.011).

Declaration of Conflicts of Interest

All authors declare that there is no conflict of interest related to this article.

References

Acquaah, G., 2012. Principles of Plant Genetics and Breeding. John Wiley & Sons Ltd, Chichester.

Addis, M., Cabiddu, A., Pinna, G., Decandia, M., Piredda, G., Pirsi, A., Molle, G., 2005. Milk and cheese fatty acid composition in sheep fed Mediterranean forages with reference to conjugated linoleic acid cis-9, trans-11. *Journal of Dairy Science*, 88(10): 3443-3454.

Amini, F., Masoomi, P., Ramshini, H., Norouzian, M.A., 2018. Genetic diversity among tall fescue ecotypes using agro-morphological traits, nutritional values and AFLP molecular markers. *Iranian Journal of Genetics and Plant Breeding*, 7(2): 54-64.

Anonymous, 1991. Descriptors for Annual *Medicago*/Descripteurs pour *Medicago* Annuelles. International Board for Plant Genetic Resources, Via delle Sette Chiese 142-00145 Rome, Italy.

Anonymous, 2016. Climate of Türkiye According to Thornthwaite Climate Classification. Turkish State Meteorological Service, (https://www.mgm.gov.tr/FILES/iklim/iklim_siniflandirmalari/Thornthwaite.pdf), (Accessed: 19.01.2022). (In Turkish).

Anonymous, 2018. Samsun Province Climate Data. Turkish State Meteorological Service, (https://www.mgm.gov.tr/veridegerlendirme/il-ve-ilceler-istatistik.aspx?m=SAMSUN), (Accessed: 19.01.2018). (In Turkish).

Aydın, İ., Uzun, F., Algan, D., 2015. Yield and nutritive values of some wild annual medic species collected from different geographical location. *Anadolu Journal of Agricultural Science*, 30(3): 275-280. (In Turkish).

Aydin, I., Algan, D., Pak, B., Ocak, N., 2019. Similarity analysis with respect to some quality indicators and quality categories based on relative forage quality ranges of desirable rangeland forages. *Fresenius Environmental Bulletin*, 28(8): 5926-5936.

Aydin, İ., Pak, B., Algan, D., Ocak, N., 2020. Floristic patterns and qualities of forage species from mountainous rangeland in the Middle Black Sea Region of Turkey. *Turkish Journal of Agriculture-Food Science and Technology*, 8(3): 733-740.

Aydin, R., Kamalak, A., Canbolat, O., 2007. Effect of maturity on the potential nutritive value of burr medic (*Medicago polymorpha*) hay. *Journal of Biological Sciences*, 7(2): 300-304.

Corrales-Retana, L., Ciucci, F., Conte, G., Casarosa, L., Mele, M., Serra, A., 2021. Profile of fatty acid lipid fractions of omega-3 fatty acid-enriched table eggs. *Journal of Animal Physiology and Animal Nutrition*, 105(2): 326-335.

Crews, T.E., Blesh, J., Culman, S.W., Hayes, R.C., Jensen, E.S., Mack, M.C., Peobles, M.B., Schipanski, M.E., 2016. Going where no grains have gone before: From early to mid-succession. *Agriculture, Ecosystems and Environment*, 223: 223-238.

Cui, J., Lu, Z., Wang, T., Chen, G., Mostafa, S., Ren, H., Liu, S., Fu, C., Wang, L., Zhu, Y., Lu, J., Chen, X., Wei, Z., Jin, B., 2021. The genome of *Medicago polymorpha* provides insights into its edibility and nutritional value as a vegetable and forage legume. *Horticulture Research*, 8(1): 1-12.

Del Pozo, A., Ovalle, C., Aronson, J., Avendano, J., 2002. Ecotypic differentiation in *Medicago polymorpha* L. along an environmental gradient in central Chile. II. Winter growth as related to phenology and temperature regime. *Plant Ecology*, 160(1): 53-59.

Ewing, M.A.B., 1986. Serena and Circle Valley medic establishment. *Journal of the Department of Agriculture*, 27(4): 107-112.

Graziano, D., Di Giorgio, G., Ruisi, P., Amato, G., Giambalvo, D., 2010. Variation in pheno-morphological and agronomic traits among burr medic (*Medicago polymorpha* L.) populations collected in Sicily, Italy. *Crop and Pasture Science*, 61(1): 59-69.

Iannucci, A., Terribile, M.R., Martiniello, P., 2008. Effects of temperature and photoperiod on flowering time of forage legumes in a Mediterranean environment. *Field Crops Research*, 106(2): 156-162.

Kop-Bozbay, C., Akdag, A., Bozkurt-Kiraz, A., Gore, M., Kurt, O., Ocak, N., 2021. Laying performance, egg quality characteristics, and egg yolk fatty acids profile in layer hens housed with free access to chicory- and/or white clover-vegetated or non-vegetated areas. *Animals*, 11(6): 1708-1716.

Lermi, A.G., Palta, Ş., 2014. Investigation of some plant characteristics of *Medicago polymorpha* L. in ecology of Bartin province. *Çanakkale Onsekiz Mart University Journal of Agricultural Faculty*, 2(2): 141-149. (In Turkish).

Lüscher, A., Mueller-Harvey, I., Soussana, J.F., Rees, R.M., Peyraud, J.L., 2014. Potential of legume-based grassland-livestock systems in Europe: a review. *Grass and Forage Science*, 69(2): 206-228.

Moore, J.E., Undersander, D.J., 2002. Relative forage quality: An alternative to relative feed value and quality index. *Proceedings 13th Annual Florida Ruminant Nutrition Symposium*, January 10-11, University of Florida, USA, pp. 16-32.

Muir, J.P., Ocumpaugh, W.R., Read, J.C., 2003. Spring forage yield and nutritive value of Texas black medic accessions. *Agronomy Journal*, 95(4): 908-912.

Nazari-Dashlibrown, P., 2004. Reduction of overgrazing pressure on rangelands by grazing annual medics. *Cahiers Options Méditerranéennes*, 62: 443-446.

Rochon, J.J., Doyle, C.J., Greef, J.M., Hopkins, A., Molle, G., Sitzia, M., Scholefield, D., Smith, C.J., 2004. Grazing legumes in Europe: a review of their status, management, benefits, research needs and future prospects. *Grass and Forage Science*, 59(3): 197-214.

Seker, H., Yazici, A., Uysal, P., 2014. Analysis of variability, heritability, and genetic advance in seed yield and related traits of orchardgrass (*Dactylis glomerata* L.) populations. *Turkish Journal of Agriculture and Forestry*, 38(5): 633-643.

Shirmardi, H., Bolfaji, F., Mesdaghi, M., Chamani, A., 2003. Determination of nutritional value of six species range plants in Yekkeh Chenar, Maraveh Tappeh area (Golestan province) (In Persian). *Journal of Agricultural Science and Natural Resources*, 10(1): 131-149.

Singh, S., Bhat, B.V., Shukla, G.P., Gaharana, D., Anele, U.Y., 2017. Nutritional evaluation of different varieties of sorghum stovers in sheep. *Animal Feed Science and Technology*, 227: 42-51.

Stagnari, F., Maggio, A., Galieni, A., Pisante, M., 2017. Multiple benefits of legumes for agriculture sustainability: an overview. *Chemical and Biological Technologies in Agriculture*, 4(1): 1-13.

Tyagi, S.D., Khan, M.H., 2010. Studies on genetic variability and interrelationship among the different traits in microsperma lentil (*Lens culinaris* Medic.). *Journal of Agricultural Biotechnology and Sustainable Development*, 2(1): 15-20.

Uzun, F., Aydin, I., 2004. Improving Germination Rate of *Medicago* and *Trifolium* Species. *Asian Journal of Plant Sciences*, 3(6): 714-717.

Uzun, F., Garipoğlu, A.V., Ocak, N., 2017. Water use efficiency, yield, and nutritional quality of maize and sorghum cultivars as influenced by irrigation in a shallow soil. *Anadolu Journal of Agricultural Sciences*, 32(3): 358-366.

Uzun, F., Ocak, N., 2019. Some vegetation characteristics of rangelands subjected to different grazing pressures with single- or multi-species of animals for a long time (A case of Zonguldak province, Turkey). *Anadolu Journal of Agricultural Science*, 34(3): 360-370.

Walczak, J., Bocian, S., Kowalkowski, T., Trziszka, T., Buszewski, B., 2017. Determination of omega fatty acid profiles in egg yolk by HILIC-LC-MS and GC-MS. *Food Analytical Methods*, 10(5): 1264-1272.

CITATION: Uzun, F., Ocak, N., 2022. Assessment of Variability for Nutritional Traits of Burr Medic (*Medicago polymorpha* L.) Genotypes with Different Phenology. *Turkish Journal of Agricultural Research*, 9(2): 175-184.