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Quality and Mineral Elements Contents of *Trifolium* spp. Ecotypes Collected from Eastern Anatolia (Türkiye)

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HIGHLIGHTS

- In this study, plant identification and quality of pastures were measured from pastures in different provinces of the Southeast and comparisons were made.

Abstract

The flora of Turkey is rich plant species but there is less known about some species. Hence, there is need to determine species properties and their forage potentials for animal feeding. The objective of this study was determine to 14 different *Trifolium* species (*T. ambiguum, T. bullatum, T. campestre, T. cherleri, T. dasyurum, T. echinatum, T. grandiflorum, T. hirtum, T. nigrescens, T. pauciflorum, T. resupinatum, T. spumosum, T. stellatum* and *T. tomentosum*) that taken from natural habitats during the flowering period of the plants by cutting from the root collar of the plants from 12 different locations. The differences between *Trifolium* genotypes were found to be statistically very significant for all the examined traits. Crude protein (CP), Dry matter (DM), acid detergent insoluble fiber (ADF), neutral detergent insoluble fiber (NDF), acid detergent insoluble protein (ADP), digestible dry matter (DDM), dry matter intake (DMI), calcium (Ca), magnesium (Mg), phosphorus (P) and potassium (K) ratios and relative feed values (RFV), Ca/P and K/(Ca+Mg) values varied between 12.02-25.54%, 90.25-96.56%, 11.61-35.26%, 27.27-47.82%, 0.47-1.24%, 61.43-79.87%, 2.51-4.40%, 1.41-2.19%, 0.26-0.43%, 0.31-0.50%, 1.19-3.65%, 119.66-268.62, 3.24-6.09 and 0.56-1.97, respectively. Among the *Trifolium* species collected from different locations examined in the research, genotype number 25 (*Trifolium tomentosum*) stood out with high CP, DM, DDM, DMI and RFV values and low ADF and NDF ratios. Genotype number 17 (*Trifolium nigrescens* subsp. *petrisavii*) in terms of the same characteristics followed this genotype. While genotypes number 1 (*Trifolium ambiguum*), 16 (*Trifolium nigrescens* subsp. *petrisavii*) and 21 (*Trifolium resupinatum* var. *resupinatum*) stood out with high K values, genotypes number 25 (*Trifolium tomentosum*), 9 (*Trifolium cherleri*) and 21 (*Trifolium resupinatum* var. *resupinatum*) stood out with high Ca, Mg and P values, respectively. Among the *Trifolium* species examined, the best genotypes in terms of yield and quality were genotypes number 17 (*Trifolium nigrescens* subsp. *petrisavii*) and 25 (*Trifolium tomentosum*).

Keywords: *Trifolium* spp.; ecotype; herbage quality; mineral element content

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1. Introduction

One of the most significant obstacles to the development of grazing livestock is generally acknowledged to be undernutrition. Low levels of protein and energy are frequently the reason of cattle productivity that is not at its best. However, ruminants sometimes deteriorate in spite of an abundant feed supply (McDowell and Valle 2000). Reduced protein efficiency is typically the result of rapid and widespread ruminal breakdown of proteins in grass and legume forages. The rumen microbial community consumes amino acids for energy and releases ammonia through deamination when energy is limited but there is an oversupply of peptides and amino acids derived from plants. Therefore, forages with high water-soluble carbohydrates (WSC) may enhance the synchronization and balance of carbon (C) and nitrogen (N), boost rumen microbial protein production, and raise animal productivity. Cattle diets' protein degradability may be impacted by the species of forage chosen (Da Silva et al. 2014).

According to recent studies, dairy calves are frequently overfed protein; yet, milk and component yields can be maintained, and occasionally even enhanced, with lower protein consumption (Broderick 2018). Fiber often makes up over 25% of the total mixed rations and is a key component of diets offered to dairy ruminants. Fiber is essential for ruminant energy and rumen health maintenance. CP concentration has a negative connection with NDF, ADF, and lignin, which is consistent with multiple research (Du et al. 2016).

Low productivity and reproductive issues in grazing ruminants have long been attributed to mineral excesses or deficiencies in soils and forages. Worldwide, mineral deficiencies are frequently indicated by hair loss, skin conditions, non-infectious abortion, diarrhea, anemia, appetite loss, abnormalities of the bones, tetany, and low fertility. Calcium (Ca), chlorine (Cl), phosphorus (P), magnesium (Mg), potassium (K), sodium (Na), and sulfur (S) are the seven main minerals that forages provide. With the exception of Cl, each of these minerals has been shown to be inadequate for grazing animals under particular circumstances (McDowell and Valle 2000).

Lack of P is the most common mineral-element deficit in grazing animals worldwide. In tropical grazing zones, P insufficiency is more common than in temperate ones. Reproductive failure is the most detrimental economic effect of P shortage; P treatment significantly raises fertility levels in grazing cattle from throughout the globe. With the exception of cows that produce considerable amounts of milk or those that graze on acidic, sandy, or organic soils in humid regions where the herbage is primarily composed of fast-growing grasses devoid of legume species, Ca shortage is uncommon in grazing cattle. Ca deficiency is rarely observed in grazing beef cattle, especially during lactation, despite the fact that it can easily occur in young, growing animals and breastfeeding dairy cows fed local forages supplemented with concentrates (Fadlalla 2022).

The connection between magnesium and the dangerous metabolic disease grass tetany (hypomagnesaemia) makes it practically significant. Forage species and mineral composition, soil characteristics, fertilizer techniques, season, temperature, animal species, breed, and age all have an impact on grass tetany, a complex metabolic disease that affects ruminants. More severe cases may cause the affected cows to lose their appetite, walk stiffly, and reject the other cows in the herd. When older cattle graze grass or small-grain forages in chilly weather, such as in the early spring or an exceptionally rainy fall, grass tetany typically occurs. Non-specific symptoms of ruminant K insufficiency include sluggish development, decreased intake of feed and water, decreased feed efficiency, muscular weakness, and neurological problems. Very few cases of K insufficiency in ruminants that only graze forages have been confirmed. Other forage nutrient shortages are likely the primary cause of the lack of widespread K insufficiency, even when forages contain less than the necessary amount (McDowell and Valle 2000). Requirements of ruminants for Ca is 1.8-8.2 g kg^{-1} DM; for Mg is 1-2 g kg^{-1} DM; for P is 1.8-4.8 g kg^{-1} DM; for K is 6-8 g kg^{-1} DM (McDowell 1997).

Numerous factors, such as soil, plant species, maturation stages, yield, pasture management, and climate, interact to determine the concentration of mineral elements in plants from different parts of the world. For instance, the following factors influence the mineral content of forage: (i) plant maturity; (ii) species and species-specific variation; (iii) management practices like crop removal or grazing systems; (iv) fertilization, especially with K and N; and (v) soil and environmental conditions. Forage mineral concentrations are also influenced by interactions with other elements. The majority of naturally occurring mineral deficiencies in livestock are regionally specific and strongly correlate with the mineral content and properties of the soil. Young and alkaline geological formations are more abundant in most elements than are the older, more acid, coarse, sandy formations. However, availability factors, including soil pH, texture, moisture content and organic matter, are probably more often the limiting factors rather than soil mineral content. Animals' mineral intake is more influenced by the kind of plant and its amount of consumption than by the parent rock that produced the soil and supported the plants. The mineral content of several plant species growing in the same soil varies greatly. Legumes are often thought to be richer than grasses in a variety of mineral elements. The mineral value of most forages decreased as they became older, thus grazing cattle may not get enough K and P elements. P, K, Mg, Na, and Cl typically decrease as the plant ages (McDowell and Valle 2000).

In this study, 14 different *Trifolium* species (*T. ambiguum, T. bullatum, T. campestre, T. cherleri, T. dasyurum, T. echinatum, T. grandiflorum, T. hirtum, T. nigrescens, T. pauciflorum, T. resupinatum, T. spumosum, T. stellatum* and *T. tomentosum*) were taken from natural habitats during the flowering period of the plants by cutting from the root collar of the plants from 12 different locations in Batman, Diyarbakır, Şanlıurfa, Mardin, Siirt provinces of Türkiye, and determined to some properties such as crude protein (CP), acid detergent insoluble fiber (ADF), neutral detergent insoluble fiber (NDF), Ca, K, Mg and P ratio were measured and ADF, NDF, digestible dry matter (DDM), dry matter intake (DMI) ratio and relative forage values (RFV).

2. Materials and Methods

The materials of the research consist of 25 plant samples of 14 species of the genus *Trifolium* collected from different locations in the Southeastern Anatolia Region at 2023 (Table 1) (Figure 1).

Table 1. Locations, collection dates, and geographic coordinates of the plant samples

Location	Altitude (m)	Date
Batman-I	572	07.05.2023
Diyarbakır-I	887	10.05.2023
Diyarbakır-II	763	10.05.2023
Diyarbakır-III	920	10.05.2023
Diyarbakır-IV	1113	10.05.2023
Diyarbakır-V	885	10.05.2023
Diyarbakır-VI	667	13.05.2023
Diyarbakır-VII	652	15.05.2023
Şanlıurfa-I	1469	21.05.2023
Şanlıurfa-II	1329	21.05.2023
Mardin-I	1036	07.05.2023
Siirt-I	846	07.05.2023

2.1. Climatic Data of Locations

Mean temperature and total precipitation for all locations were in Tables 2. In Batman, the lowest temperature was recorded in January with 2.9 °C; the highest temperature was recorded in August with 31.6°C. The highest humidity was observed in December (85.6%), and the lowest humidity was observed in August (25.3%). Total precipitation was heavy in winter and spring (106.7 mm in November) and zero in summer. In Diyarbakır, the lowest temperature was recorded in February with 4.0°C; the highest temperature was recorded in August with 32.9°C. The highest humidity was recorded in December (84.4%), and the lowest humidity was recorded in July and August (23.3 and 23.6%, respectively). The highest rainfall was recorded in March with 131 mm, and there was almost no rainfall in the summer months (June and August). In Siirt, the lowest temperature was recorded in February with 3.6°C and the highest in August with 33.1°C. The highest humidity was recorded in December with 74.9% and the lowest in August with 18.7%. The highest rainfall was in November (115.6 mm) and almost none in the summer months. In Sanliurfa, the lowest temperature was recorded in February with 6.7 °C, and the highest temperature was recorded in August with 34.1 °C. The highest humidity was observed in December (71.3%), and the lowest humidity was observed in July (22.7%). The highest amount of rainfall was recorded in March with 255 mm (Table 2).

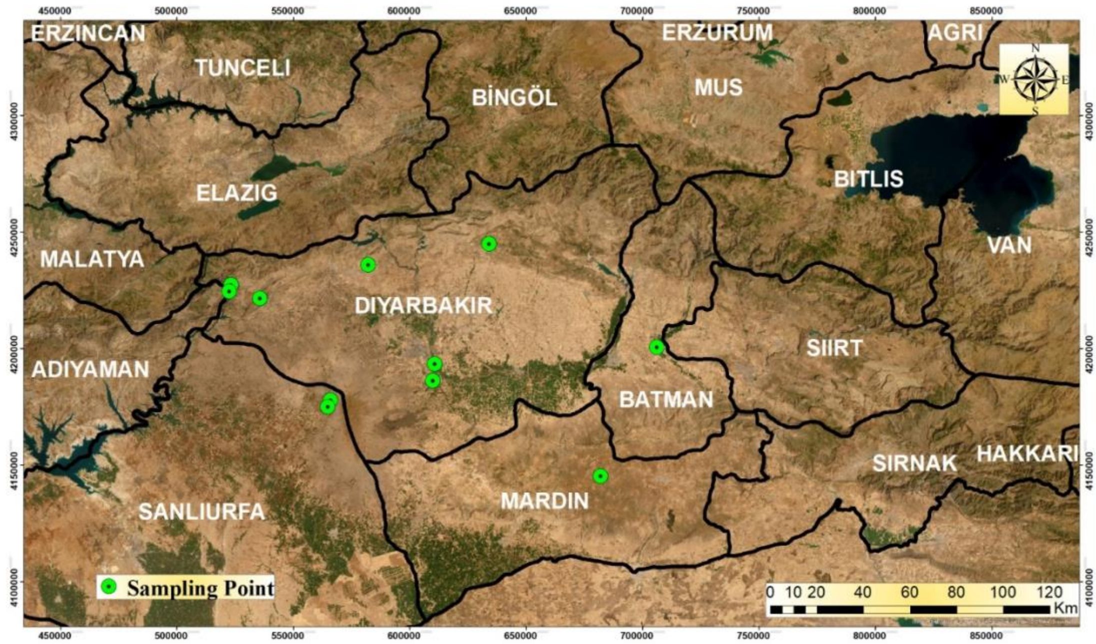

Figure 1. Location of work area

Table 2. Climatic data of Batman, Diyarbakır, Siirt, Şanlıurfa province

Months	Average Temperature (°C)				Total Precipitation (mm)			
	Batman	Diyarbakır	Siirt	Şanlıurfa	Batman	Diyarbakır	Siirt	Şanlıurfa
January	2.9	5.7	5.1	7.8	18.4	87.5	18.6	21.6
February	3.5	4.0	3.6	6.7	55.4	57.6	70.6	78.3
March	12.5	12.0	12.2	13.7	57.2	131.0	61.4	255.0
April	14.7	14.7	14.5	16.4	36	79.4	88.2	41.8
May	19.9	20.1	20.0	22.6	24.7	16.0	37.2	5.7
June	26.9	27.6	27.2	28.7	0.0	0.0	0.0	0.0
July	30.9	32.3	32.3	33.3	0.0	1.9	0.8	0.0
August	31.6	32.9	33.1	34.1	0.0	0.0	2.6	0.1
September	26.3	27.2	27.7	29.1	0.0	0.5	9.6	0.0
October	18.6	18.8	18.9	22.5	25.3	23.4	51.2	2.3
November	12.5	12.0	12.7	15.3	106.7	125.9	115.6	46.7
December	6.7	6.9	7.8	10.8	23.4	60.4	79.0	41.8

2.2. Measurements

Trifolium species were taken during the flowering period of the plants. Approximately 200 g plant samples from each species were cut and dried in a drying cabinet (Memmert ULM 800) at 70 °C and until a constant weight was achieved. The samples were ground in a laboratory-type mill (IKA, A11). It was sieved with a 1 mm diameter sample sieve (Retsch DIN-ISO 3310/2) and made ready for analysis.

The quality analyses of the species were analyzed at the Dicle University Science and Technology Application and Research Center laboratory with NIRS (Near Infrared Spectroscopy-Foss Model 6500) analyzer. In the analysis, crude protein (CP), acid detergent insoluble fiber (ADF), neutral detergent insoluble fiber (NDF), Ca, K, Mg and P values were measured. In addition, with the help of the determined ADF and NDF, digestible dry matter (DDM), dry matter intake (DMI) and relative forage values (RFV) were calculated and found. The following equations were used for calculations to validate the results of FT-NIR spectroscopy, randomly selected 20 samples were analyzed using the Dumas method for CP content, and using Ankom Fiber Analyzer for NDF and ADF contents. Dumas and Ankom Fiber Analyzer results had a significant correlation ($r \geq 0.9$, $P \leq 0.01$) with FT- NIR results. Therefore, FT-NIR results were used in the statistical analyses (Morrison 2003).

$$DDM\ (\%) = 88.9 - (0.779 \times ADF) \quad (1)$$

$$DMI\ (\%) = 120 / NDF \quad (2)$$

$$RFV = (DDM \times DMI) / 1.29 \quad (3)$$

2.3. Data analysis

Data analysis was realized using the Jump-Pro13 statistical package program using randomized completed parcel. Differences between means were compared using the Tukey HSD test. Principal component analysis (biplot) using the scatter plot model was performed using the Genstat 12th (Copyright 2011 VSN International Ltd) statistical package program (Genstat 2009).

3. Results and Discussion

The results showed that significant differences were found among the genotypes for all examined parameters ($P \leq 0.01$). The mean of the examined parameters were presented in Tables 6, 7 and 8.

The lowest CP ratio were measured in Siirt I (12.02%) and Diyarbakır V (12.27%) locations from genotypes number 9 and 10, respectively, and the highest CP ratio (between 23.11% and 25.54%) in Diyarbakır IV, Diyarbakır IV, Şanlıurfa I, Diyarbakır VII, Şanlıurfa I and Şanlıurfa II locations from genotypes number 17, 19, 20, 21, 22 and 25. DM ratio were lowest (between 90.89% and 91.17%) in Diyarbakır III, Batman I and Mardin II locations, from genotypes number 5, 7 and 14, and the highest DM rate (96.56%) in Şanlıurfa II location, from genotype number 25. The lowest ADF ratio (between 13.1% and 16.71%) were measured in Batman I, Diyarbakır I, Diyarbakır IV, Diyarbakır VII and Şanlıurfa I locations, from genotypes number 7, 16, 17, 21 and 22 and the highest ADF ratio (35.26% and 35.16%) were measured in Siirt I and Diyarbakır V locations, from genotypes number 9 and 10, respectively. *Trifolium cherleri, Trifolium angustifolium, Trifolium hybridum, Trifolium nigrescens, Trifolium lappaceum, Trifolium pilulare, Trifolium scabrum, Trifolium resupinatum, Trifolium spumosum* and *Trifolium tomentosum* were collected from ten different points in Hatay in a pasture by Ertekin (2021) to determine the chemical composition of collected clover species, and values of CA, CP, NDF, ADF, ADL, of species varied between 5.36-9.85%, 18.5-22.1%, 30.3-49.8%, 21.3-34.3%, 3.25-5.04%, respectively. It was determined that the values of DMD, DMI ratio, and RFV calculated for the nutritive values of these plants varied between 62.2-72.3%, 2.41-3.97%, and 116.2-222.2, respectively. On the other hand, *Trifolium alexandrinum*, *Trifolium spumosum*, *Trifolium resupinatum*, *Trifolium lappaceum*, *Trifolium campastre*, *Trifolium angustifolium* and *Trifolium purpureum* were collected from ten different points in Adana in a pasture by Kökten et al. (2011) to determine the chemical composition of collected clover species, and values of CP of species obtained 35.00%, 23.57%, 31.93%, 28.13%, 33.63%, 36.60%, 36.17%, respectively. While CP, ADF, NDF, DDM, DMI rates, and RFV in *Trifolium resupinatum* were obtained as 12.99%, 35.0%, 47.4%, 61.64%, 2.54%, 121.44, respectively, in Elazığ (Karadeniz and Kökten 2022), CP, ADF, NDF rates and RFV in *Trifolium repens* were obtained as 20.0%, 22.9%, 31.1%, 215, respectively, in Bingol (Cacan et al. 2024).

NDF ratio were lowest (between 27.27% and 30.95%) in Diyarbakır I, Diyarbakır IV and Şanlıurfa II locations, from genotypes number 16, 17 and 25, and the highest NDF ratio (43.98% and 47.82%) were measured in Diyarbakır VII and Diyarbakır V locations, from genotypes number 6 and 10. Pereira-Crespo et al. (2012) examined 316 samples of several clover species and concluded that the NDF concentrations ranged from 14.5 to 51.6%. The study's NDF contents fell within the ranges that Pereira-Crespo et al. (2012) reported.

The ADF results from our investigation are supported by Pereira-Crespo et al. (2012), who discovered that the ADF levels of several clover species ranged from 11.55 to 44.35%.

Table 6. Mean values of CP, DM, ADF and NDF

Location	Genotypes	CP	DM	ADF	NDF
Diyarbakır II	1. *Trifolium ambiguum*	17.49ıjk	91.75f	25.97cd	42.76bc
Sanlıurfa I	2-*Trifolium ambiguum*	22.76de	91.58fgh	19.24gh	39.17ef
Sanlıurfa I	3. *Trifolium bullatum*	20.21f	91.60fg	21.88efg	38.97efg
Sanlıurfa I	4. *Trifolium campestre*	18.36ghı	92.17de	23.15def	41.76cd
Diyarbakır III	5. *Trifolium campestre* subsp. *campestre* var. *campestre*	15.17l	91.17j	20.93fg	40.03de
Diyarbakır VII	6. *Trifolium campestre* subsp. *campestre* var. *campestre*	17.86hıj	92.37c	24.44de	43.98b
Batman I	7. *Trifolium campestre* subsp. *campestre* var. *campestre*	19.08fgh	90.89k	16.21ıj	37.08ghı
Sanlıurfa I	8. *Trifolium campestre* subsp. *campestre* var. *campestre*	22.29e	92.30cd	17.52hı	38.24e-h
Siirt I	9. *Trifolium cherleri*	12.02m	91.28ıj	35.26a	46.65a
Diyarbakır V	10. *Trifolium dasyurum*	12.27m	92.73b	35.16a	47.82a
Diyarbakır VII	11. *Trifolium echinatum*	22.72e	92.05e	22.14efg	38.77efg
Mardin I	12. *Trifolium grandiflorum*	18.67ghı	90.25l	21.65efg	37.23fgh
Diyarbakır III	13. *Trifolium hirtum*	16.45kl	92.05e	27.55bc	39.47e
Mardin II	14. *Trifolium hirtum*	18.53ghı	91.15j	23.53def	35.04ıj
Sanlıurfa I	15. *Trifolium nigrescens* subsp. *petrisavii*	19.34fg	91.41hı	19.54gh	35.15ıj
Diyarbakır I	16. *Trifolium nigrescens* subsp. *petrisavii*	22.50e	91.62fg	15.85ıjk	30.95l
Diyarbakır IV	17. *Trifolium nigrescens* subsp. *petrisavii*	24.11bc	91.64fg	13.10kl	27.27m
Diyarbakır VII	18. *Trifolium nigrescens* subsp. *petrisavii*	22.90cde	92.10e	21.41fg	37.00ghı
Diyarbakır IV	19. *Trifolium pauciflorum*	23.59b-e	92.40c	20.97fg	36.65hı
Sanlıurfa I	20. *Trifolium pauciflorum*	23.11cde	92.39c	21.79efg	39.57e
Diyarbakır VII	21. *Trifolium resupinatum* var. *resupinatum*	25.54a	92.46c	16.71hıj	33.20jk
Sanlıurfa I	22. *Trifolium resupinatum* var. *resupinatum*	24.07bcd	92.03e	14.49jkl	31.48kl
Diyarbakır VII	23. *Trifolium spumosum*	17.37ıjk	92.38c	29.10b	42.72bc
Diyarbakır IV	24. *Trifolium stellatum* var. *stellatum*	16.75jk	91.49gh	21.50fg	43.84b
Sanlıurfa II	25. *Trifolium tomentosum*	24.50ab	96.56a	11.60l	27.96m
	Mean	19.90	91.99	21.62	38.11
	CV(%)	**4.02**	**0.12**	**8.28**	**3.25**

Levels not connected by the same letter are significantly different.

Table 7. Mean values of ADP, DDM, DMI, RFV and K

Location	Genotypes	ADP	DDM	DMI	RFV	K
Diyarbakır II	1. *Trifolium ambiguum*	0.69hı	68.67ıj	2.81ı	149.41j	3.63a
Sanlıurfa I	2-*Trifolium ambiguum*	0.94c	73.91ef	3.09fg	177.71dfg	2.45ı
Sanlıurfa I	3. *Trifolium bullatum*	0.79fg	71.86fgh	3.08fg	171.59fgh	2.22j
Sanlıurfa I	4. *Trifolium campestre*	0.84ef	70.87ghı	2.88hı	158.60hıj	1.88l
Diyarbakır III	5. *Trifolium campestre* subsp. *campestre* var. *campestre*	0.66ı	72.59fg	3.02gh	170.49fgh	1.53o
Diyarbakır VII	6. *Trifolium campestre* subsp. *campestre* var. *campestre*	0.84ef	69.86hı	2.74ıj	148.43j	1.61n
Batman I	7. *Trifolium campestre* subsp. *campestre* var. *campestre*	0.56k	76.28cd	3.24ef	191.48de	1.80m
Sanlıurfa I	8. *Trifolium campestre* subsp. *campestre* var. *campestre*	0.94c	75.25de	3.14efg	183.29d-g	1.42p
Siirt I	9. *Trifolium cherleri*	0.68hı	61.43l	2.58jk	122.85k	1.19r
Diyarbakır V	10. *Trifolium dasyurum*	0.81efg	61.51l	2.51k	119.66k	1.34q
Diyarbakır VII	11. *Trifolium echinatum*	1.05b	71.65fgh	3.10fg	171.96fgh	2.68g
Mardin I	12. *Trifolium grandiflorum*	0.65ıj	72.03fgh	3.23ef	180.33d-g	2.16k
Diyarbakır III	13. *Trifolium hirtum*	0.73h	67.44jk	3.04gh	159.04hıj	2.80f
Mardin II	14. *Trifolium hirtum*	0.60jk	70.57ghı	3.44d	188.67de	2.55h
Sanlıurfa I	15. *Trifolium nigrescens* subsp. *petrisavii*	0.60jk	73.68ef	3.42d	195.21d	2.25j
Diyarbakır I	16. *Trifolium nigrescens* subsp. *petrisavii*	0.59k	76.55bcd	3.88b	230.34b	3.65a
Diyarbakır IV	17. *Trifolium nigrescens* subsp. *petrisavii*	0.47l	78.70ab	4.40a	268.62a	3.25c
Diyarbakır VII	18. *Trifolium nigrescens* subsp. *petrisavii*	0.86de	72.21fg	3.28de	184.17def	2.99e
Diyarbakır IV	19. *Trifolium pauciflorum*	0.90cd	72.56fg	3.28de	184.39def	2.77f
Sanlıurfa I	20. *Trifolium pauciflorum*	1.06b	71.93fgh	3.03gh	169.16ghı	2.59h
Diyarbakır VII	21. *Trifolium resupinatum* var. *resupinatum*	0.83ef	75.88cde	3.62c	212.76c	3.60a
Sanlıurfa I	22. *Trifolium resupinatum* var. *resupinatum*	0.81efg	77.61abc	3.82b	229.71b	3.43b
Diyarbakır VII	23. *Trifolium spumosum*	0.93c	66.23k	2.81ı	144.55j	3.11d
Diyarbakır IV	24. *Trifolium stellatum* var. *stellatum*	0.78g	72.15fg	2.78ı	154.89ıj	2.48ı
Sanlıurfa II	25. *Trifolium tomentosum*	1.24a	79.87a	4.29a	265.74a	2.68g
	Mean	0.79	72.05	3.22	181.32	2.48
	CV (%)	**3.79**	**1.92**	**3.11**	**5.00**	**1.20**

Levels not connected by same letter are significantly different.

The lowest ADP ratio (0.47% and 0.56%) were measured in Batman I and Diyarbakır IV locations, from genotypes number 7 and 17, and the highest ADP rate (1.24%) was measured in Şanlıurfa II location, from genotype number 25. DDM ratio were lowest (61.43% and 61.51%) in Siirt I and Diyarbakır V locations, from genotypes number 9 and 10, and the highest DDM ratio (between 75.88% and 79.87%) were measured in Batman I, Diyarbakır I, Diyarbakır IV, Diyarbakır VII and Şanlıurfa II locations, from genotypes number 7, 16, 17, 21 and 25. The lowest DMI ratio (between 2.51% and 2.81%) were measured in Diyarbakır II, Diyarbakır VII, Siirt I, Diyarbakır V and Diyarbakır VII locations, from genotypes number 1, 6, 9, 10, 23 and 24, and the highest DMI ratio (4.4% and 4.29%) were measured in Diyarbakır IV and Şanlıurfa II locations, from genotypes number 17 and 25, respectively. This study's DMD, DMI, and RFV values were comparable to those of Gürsoy and Macit's (2017) investigation of similar results.

RFV values were lowest (between 119.66 and 144.55) in Siirt I, Diyarbakır V and Diyarbakır VII locations, from genotypes number 9, 10 and 23, and the highest RFV values (268.62 and 265.74) were measured in Diyarbakır IV and Şanlıurfa II locations, from genotypes number 17 and 25. The lowest K rate (1.19%) was measured in Siirt I location, from genotype number 9, and the highest K ratio (between 3.6% and 3.65%) were measured in Diyarbakır II, Diyarbakır I and Diyarbakır VII locations, from genotypes number 1, 16 and 21. Around the world, forage crops constitute a vital source of animal feed and are integral to livestock husbandry and animal production systems. According to Lüscher et al. (2014), perennial forage legumes used as base feed make ideal fodder for a variety of livestock classes, such as dry dairy cows, dairy heifers, dairy beef, or beef cows. According to cultivar availability and seed production and marketing volumes, clover (*Trifolium* spp.) is the second most significant farmed perennial fodder legume in Europe, behind alfalfa (Hoekstra et al. 2018). This perennial plant has many benefits, including a high protein content and soil-improving qualities that improve livestock feed intake and lessen the need for synthetic nitrogen fertilizers (Tucak et al. 2023).

Table 8. Mean values of Ca, Mg, P, Ca/P and K/(Ca+Mg)

Location	Genotypes	Ca	Mg	P	Ca/P	K/(Ca+Mg)
Diyarbakır II	1. *Trifolium ambiguum*	1.58fgh	0.26k	0.44e	3.62ıj	1.97a
Sanlıurfa I	2-*Trifolium ambiguum*	1.78cd	0.36c	0.42f	4.20g	1.15j
Sanlıurfa I	3. *Trifolium bullatum*	1.70de	0.32e	0.40h	4.27fg	1.10jkl
Sanlıurfa I	4. *Trifolium campestre*	1.41k	0.28j	0.39ı	3.64ı	1.12jk
Diyarbakır III	5. *Trifolium campestre* subsp. *campestre* var. *campestre*	1.71de	0.24l	0.33m	5.19c	0.78no
Diyarbakır VII	6. *Trifolium campestre* subsp. *campestre* var. *campestre*	1.68e	0.29hı	0.34l	4.95d	0.82n
Batman I	7. *Trifolium campestre* subsp. *campestre* var. *campestre*	1.96b	0.30h	0.32n	6.09a	0.80n
Sanlıurfa I	8. *Trifolium campestre* subsp. *campestre* var. *campestre*	1.67ef	0.30h	0.37j	4.46ef	0.72o
Siirt I	9. *Trifolium cherleri*	1.70de	0.43a	0.31o	5.56b	0.56p
Diyarbakır V	10. *Trifolium dasyurum*	1.48jk	0.29h	0.32n	4.61e	0.75no
Diyarbakır VII	11. *Trifolium echinatum*	1.84c	0.31fg	0.41g	4.50e	1.25ı
Mardin I	12. *Trifolium grandiflorum*	1.84c	0.32ef	0.36k	5.04cd	1.00m
Diyarbakır III	13. *Trifolium hirtum*	1.70de	0.28j	0.38ıj	4.49e	1.41gh
Mardin II	14. *Trifolium hirtum*	1.65efg	0.32de	0.40h	4.16g	1.30ı
Sanlıurfa I	15. *Trifolium nigrescens* subsp. *petrisavii*	1.83c	0.27j	0.40h	4.59e	1.07kl
Diyarbakır I	16. *Trifolium nigrescens* subsp. *petrisavii*	1.64efg	0.28ıj	0.49b	3.36klm	1.89b
Diyarbakır IV	17. *Trifolium nigrescens* subsp. *petrisavii*	1.67e	0.28j	0.48b	3.46ı-l	1.67e
Diyarbakır VII	18. *Trifolium nigrescens* subsp. *petrisavii*	1.69e	0.33d	0.47c	3.57ıjk	1.48f
Diyarbakır IV	19. *Trifolium pauciflorum*	1.55hıj	0.35c	0.47c	3.33lm	1.46fg
Sanlıurfa I	20. *Trifolium pauciflorum*	1.55hıj	0.33d	0.45d	3.48ı-l	1.38h
Diyarbakır VII	21. *Trifolium resupinatum* var. *resupinatum*	1.63e-h	0.33d	0.50a	3.24m	1.83c
Sanlıurfa I	22. *Trifolium resupinatum* var. *resupinatum*	1.64efg	0.30gh	0.48b	3.41j-m	1.76d
Diyarbakır VII	23. *Trifolium spumosum*	1.49ıjk	0.33d	0.41g	3.59ıj	1.72de
Diyarbakır IV	24. *Trifolium stellatum* var. *stellatum*	1.57ghı	0.16m	0.40h	3.93h	1.43fgh
Sanlıurfa II	25. *Trifolium tomentosum*	2.19a	0.38b	0.45d	4.90d	1.04lm
	Mean	1.69	0.31	0.40	4.23	1.25
	CV (%)	**2.97**	**2.00**	**1.00**	**3.08**	**2.40**

Levels not connected by same letter are significantly different.

Kura clover (*Trifolium ambiguum*) has better CP and in vitro digestibility than alfalfa, but lower amounts of NDF, acid detergent fiber (ADF), and acid detergent lignin (ADL) (Seguin et al. 2002). Interest in using Kura clover in permanent pastures has increased as a result of these qualities. It is not advised to store Kura clover as hay because to its high leaf content (>85%), as field losses may be substantial (Peterson et al. 1994). Seguin and Mustafa (2003) determined quality of *Trifolium ambiguum* for two cultivars which had similar NDF (average of 355 g/kg of DM), ADF (281 g/kg of DM), EE (24 g/kg of DM), total carbohydrate (672 g/kg of DM), non-structural carbohydrate (354 g/kg of DM), TDN (650 g/kg of DM), and NEl (6.17 MJ/kg DM) contents.

The lowest Ca ratio (between 1.41% and 1.64%) were measured from genotypes number 4, 10, 19, 20, 21, 22, 23 and 24, and the highest Ca rate (2.19%) was measured from genotype number 25. For Mg ratio, the lowest value (0.16%) was measured in Diyarbakır IV location, from genotype number 24, and the highest value (0.43%) was measured in Siirt I location, from genotype number 9. The lowest P rate (0.31%) was measured in Siirt I location, from genotype number 9, and the highest P rate (0.5%) was measured in Diyarbakır VII location, from genotype number 21. Lee (2018) have reported that plant mineral matter contents vary significantly among forage species. K, Ca, Mg and P rates in *Trifolium repens* were obtained as 2.05%, 1.93%, 0.41%, 0.32%, respectively, in Bingol (Cacan et al. 2024).

Ca/P values were lowest (between 3.24% and 3.64%) at genotypes number 1, 4, 16, 17, 18, 19, 20, 21, 22 and 23, and the highest Ca/P value (6.09%) was measured from genotype number 7. The lowest K/(Ca+Mg) value (0.56%) was measured in Siirt I location, from genotype number 9, and the highest K/(Ca+Mg) value (1.97%) was measured in Diyarbakır II location, from genotypes number 1.

3.1. Biplot Analysis

The equality lines divided the biplot into sectors and the winning genotype for each sector was the one placed on the vertex.

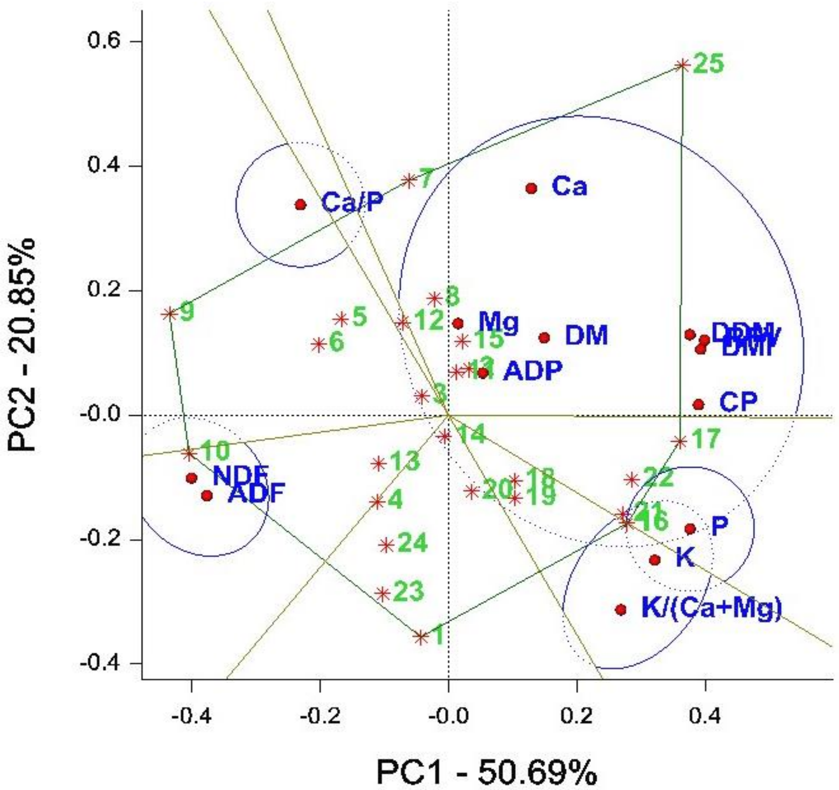

Figure 1. Polygons show suitable genotypes in each environment and show which genotype won where. Codes; **1.** *Trifolium ambiguum*, **2.** *Trifolium ambiguum*, **3.** *Trifolium bullatum*, **4.** *Trifolium campestre*, **5.** *Trifolium campestre* subsp. *campestre* var. *campestre*, **6.** *Trifolium campestre* subsp. *campestre* var. *campestre*, **7.** *Trifolium campestre* subsp. *campestre* var. *campestre*, **8.** *Trifolium campestre* subsp. *campestre* var. *campestre*, **9.** *Trifolium cherleri*, **10.** *Trifolium dasyurum*, **11.** *Trifolium echinatum*, **12.** *Trifolium grandiflorum*, **13.** *Trifolium hirtum*, **14.** *Trifolium hirtum*, **15.** *Trifolium nigrescens* subsp. *petrisavii*, **16.** *Trifolium nigrescens* subsp. *petrisavii*, **17.** *Trifolium nigrescens* subsp. *petrisavii*, **18.** *Trifolium nigrescens* subsp. *petrisavii*, **19.** *Trifolium paucifloru*, **20.** *Trifolium pauciflorum*, **21.** *Trifolium resupinatum* var. *resupinatum*, **22.** *Trifolium resupinatum* var. *resupinatum*, **23.** *Trifolium spumosu*, **24.** *Trifolium stellatum* var. *stellatum*, **25.** *Trifolium tomentosum*. Abbreviations: ADF; Acid detergent fiber, NDF; Nötral detergent fiber; ADP; Neutral detergent insoluble protein, CPY; Crude protein yield, DDM; Digestible dry matter, RFV; Relative feed value, DMI; Dry matter intake, K; Potassium, Mg; Magnesium, P; Phosphorus, Ca; Calcium, K; Potassium.

The studied environments were divided into seven sectors. The graph describes the 50.69 and 20.85% variation for PC1 and PC2, respectively. The total variation was 71.54%. The genotypes placed on the vertices of the polygon had the best or the poorest in examined parameters. Accordingly, genotypes number 16 and 21 had the best performance for P, K and K/Ca+Mg values. Genotype number 25 was the best for Ca, Mg, ADP, DM, DDM, DMI and RFV values. Genotype numbered 9 was the best for Ca/P ratio. Genotype number 10 was the best for ADF and NDF values. The parameters located on the left of the coordinate plane were negatively correlated with those located on the right, and the genotypes located in these areas are far from each other in terms of the traits in question.

4. Conclusions

Information about 14 different *Trifolium* species collected in the 12 different locations in Batman, Diyarbakır, Şanlıurfa, Mardin, Siirt provinces of Türkiye was determined. This study depicted that (i) genotypes number 17 and 25 were the most favorable genotypes according to CP, DM, DDM, DMI, ADF and NDF ratio and RFV values, (ii) genotypes number 1, 16 and 21 were the best genotypes for the K rate, (iii) genotypes number 25, 9 and 21 were the most stable genotypes according to Ca, Mg and P ratio.

When the obtained results are evaluated as a whole, it is suggested that more studies should be conducted on the genotypes number 17 and 25 from the examined *Trifolium* species and that studies should be conducted for their use in the improvement of meadows and pastures.

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