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Full Length Research Paper

Biodiversity in red clover (T*rifolium pratense* L.) collected from Turkey. II: Nutritional values

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The objective of this study was to compare 47 red clover (*Trifolium pratense* L.) populations and one cultivar as a control in terms of nutritional values for animal feeding in the second production year (2010). The experiment was established in Samsun-Turkey. They were evaluated for crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), total digestible nutrients (TDN), dry matter intake (DMI), digestible dry matter (DDM), relative feed value (RFV), crude ash, Ca, K, Mg, P contents, Ca:P and K:(Ca+Mg) ratios in dry matter. There was big variability in all nutritional values among the populations. These values were determined as 127.9 to 208.4, 367.0 to 612.8, 503.3 to 756.1, 379.2 to 594.6, 15.9 to 23.9, 411.6 to 603.1, 50.6 to 112.0, 78.4 to 127.1, 15.5 to 22.2, 9.93 to 25.05, 3.08 to 4.58, 1.67 to 3.56, 4.53 to 11.45 and 0.46 to 1.29 g kg^{-1}, respectively.

Key words: Acid detergent fiber, crude protein, forage quality, red clover.

INTRODUCTION

Red clover (*Trifolium pratense* L.) is commonly grown throughout the world. It is an important perennial forage legume because it is used as hay or as pasture in crop rotations (Farnham and George, 1993) and has high productivity and protein content (Murray et al., 2007). It is especially suitable for cattle breeding, because it has quality and nutritive hay (Acikgoz, 2001).

The possibility to accurately predict the nutritive value of forage crops is a prerequisite for designing rations and directing forage crops breeding (Kruse et al., 2008). Nutrient deficiencies in low quality roughages affect microbial growth and fermentation in the rumen and result in an overall low animal productivity (Assefa and Ledin, 2001). Quality forage promotes good health and better performance (Barney Harris, 1992). Therefore, choosing superior yield and quality forage genotypes is very important for animal health as well as high milk and meat production (Tavlas et al., 2009).

Improvement of the quality of the major forage legumes requires that there is sufficient genetic variability when it comes to the main quality parameters (protein, fiber) and the levels of antinutritional and toxic substances in order to enable the development of new, higher-quality varieties. The latest efforts in forage legume breeding should encompass certain specific traits depending on the species in order to completely fulfill the needs for safe feed in animal husbandry (Vasiljevic et al., 2009). At present, the quality-orientated breeding of clover focuses on improving the content and quality of proteins, delaying the lignification process as plants mature, or increasing the share of carotens and decreasing the concentration of anti-nutritional compounds, for example isoflavones (Zuk-Golaszewska et al., 2010). Red clover is abounded in flora of Black Sea Region (Deveci, 2012). Additionally, it is reported that large variation existed among the red clover genotypes in terms of morpho-agronomic properties and salt tolerance (Onal Asci, 2011a,b)

The objective of this present research was to investigate chemical composition and nutritive value of red clover genotypes and to determine the similarity and differences in respect to nutritional variation of red clover accessions which naturally grown in Black Sea region.

MATERIALS AND METHODS

In this study, 48 red clover (*Trifolium pratense* L.) genotypes were taken under investigation. Among the genotypes, 47 populations were collected from 20 different locations in Black Sea Region in 2008 (Table 1 and Figure 2), and cultivar Start was used as control.

The scarified seeds were sown in seed trays and then seedlings

Table 1. Geographic distribution of naturalized populations of red clover in Black Sea Region, Turkey.

Geographical origin		Genotype number
Province	**District**	
Sinop	Erfelek	1-4
	Boyabat	5
Samsun	Bafra	6-8
	Merkez	9-10
	Tekkeköy	11
	Salıpazarı	12-15
	Kavak	16-17
	Ladik	18-20
	Vezirköprü	21-23
Ordu	Unye	24
	Merkez	25-26
	Gulyali	27-28
	Ikizce	29-33
	Akkus	34
	Kumru	35-36
	Korgan	37
	Aybasti	38-39
	Gurgentepe	40-42
	Golkoy	43
Trabzon	Caykara	44-46
	Unknown	47
	Start cv.	48

were transplanted into field at 70 cm row spacing with 50 cm plant spacing within the rows at the end of the March, 2009 in Samsun, Turkey (41°21´ N, 36°15´ E, 195 m).

The experiment was established in a clay soil with pH 7.1, in saturation extract in H_2O, organic matter content was 3.08%, available P content was 30.0 mg kg^{-1} and available K content was 116.3 mg kg^{-1}, $CaCO_3$ content was 1.34% and Mg content was 0.10%. The experimental area had the typical Mediterranean climatic conditions (Figure 1).

Plants were harvested for hay at 50% flowering stage (Onal Asci, 2009) in the second year of this study (in the summer of 2010). In term of the harvest time for hay, genotypes were divided into three groups between 16th May and 17th July.

Five different plants for each genotype were dried at 70°C during 48 h and then the samples were ground to pass through a 1 mm screen (Hoy et al., 2002). Crude ash content was determined by ashing at 550°C for 6 h (AOAC, 1990). Dry matter, crude protein, acid detergent fiber (ADF), neutral detergent fiber (NDF) and Ca, K, Mg and P contents of samples were determined using near infrared reflectance spectroscopy (NIRS) (Hoy et al., 2002; Poblaciones et al., 2008). NIRS was calibrated using software program coded IC-0904FE.

Dry matter intake (DMI), digestible dry matter (DDM), total digestible nutrients (TDN) and relative feed value (RFV) of plants were calculated as follows:

TDN = 73.5 + 0.62(%CP) – 0.71 (%ADF) (Anonymus, 2010).

DMI = 120 / NDF%dry matter basis (Horrocks and Valentine, 1999).

DDM = 88.9 - (0.779XADF%dry matter basis) (Horrocks and Valentine, 1999).

RFV = (DDM% x DMI %) / 1.29 (Tavlas et al., 2009).

Quality standards of legume hays are given in Table 2 (Basaran et al., 2011; Canbolat et al., 2006).

The basic statistics (minimum, maximum, mean, standart error) were done using SPSS 10.0 program. Hierarchical cluster analysis was carried using the Ward method in JMP 5.1 for Windows. Both chemical and quality results were reported on a dry matter basis.

RESULTS AND DISCUSSION

High forage yield is very important for producers but for livestock enterprises it is also important to produce high quality forages. Crude protein content of forage is one of the most important criteria for forage quality evaluation (Caballero et al., 1995; Assefa and Ledin, 2001). There was a big variability amongst the red clover populations in terms of crude protein content of hay (Table 3) similar to Leto et al. (2004) and Tavlas et al. (2009). The amount of crude protein in red clover populations varied from 127.9 to 208.4 g kg^{-1}. Crude protein (CP) content of hay in investigated populations were more than twice or thrice needed ratios which is approximately 7% for ruminants (Meen, 2001). Although only crude protein content of the population 42 was equal to the red clover commonly used in beef cattle diets issued by NRC (2001) (208 g kg^{-1}), crude protein content of four populations was higher than cultivar Start used as a control (Figure 3a). Additionally, population 22, 32, 40 and 42 and Start were considered as prime according to crude protein content which was >19% (Basaran et al., 2011; Canbolat et al., 2006).

Nutritive value of forages depends on their dry matter digestibility (Canbolat et al., 2006), thus, the other important quality characteristics for forages are the concentrations of NDF and ADF (Caballero et al., 1995; Assefa and Ledin, 2001). ADF is closely related to any indigestibility of forages and is the major factor when calculating energy content of feeds. The greater the ADF, the less digestible the feed and the less energy it will contain. NDF gives the best estimate of the total fiber content of a feed and is closely related to feed intake. As NDF values increase, total feed intake decreases. Large variation in terms of ADF and NDF were also determined amongst the populations (Table 3). While maximum ADF and NDF values were obtained from population 35, cultivar Start had minimum values (Figures 3 b and c). Additionally, Start and population 46 were considered as good according to ADF and NDF content (Basaran et al., 2011; Canbolat et al., 2006). There was a relationship between harvest time and fiber content. The latest harvested populations contained much ADF (> 500 g kg^{-1}) (Figure 3b). This conclusion is consistent with the findings of Vasiljevic et al. (2008) who reported that ADF and NDF content of red clover has higher levels in the summer due to increased temperatures and accelerated

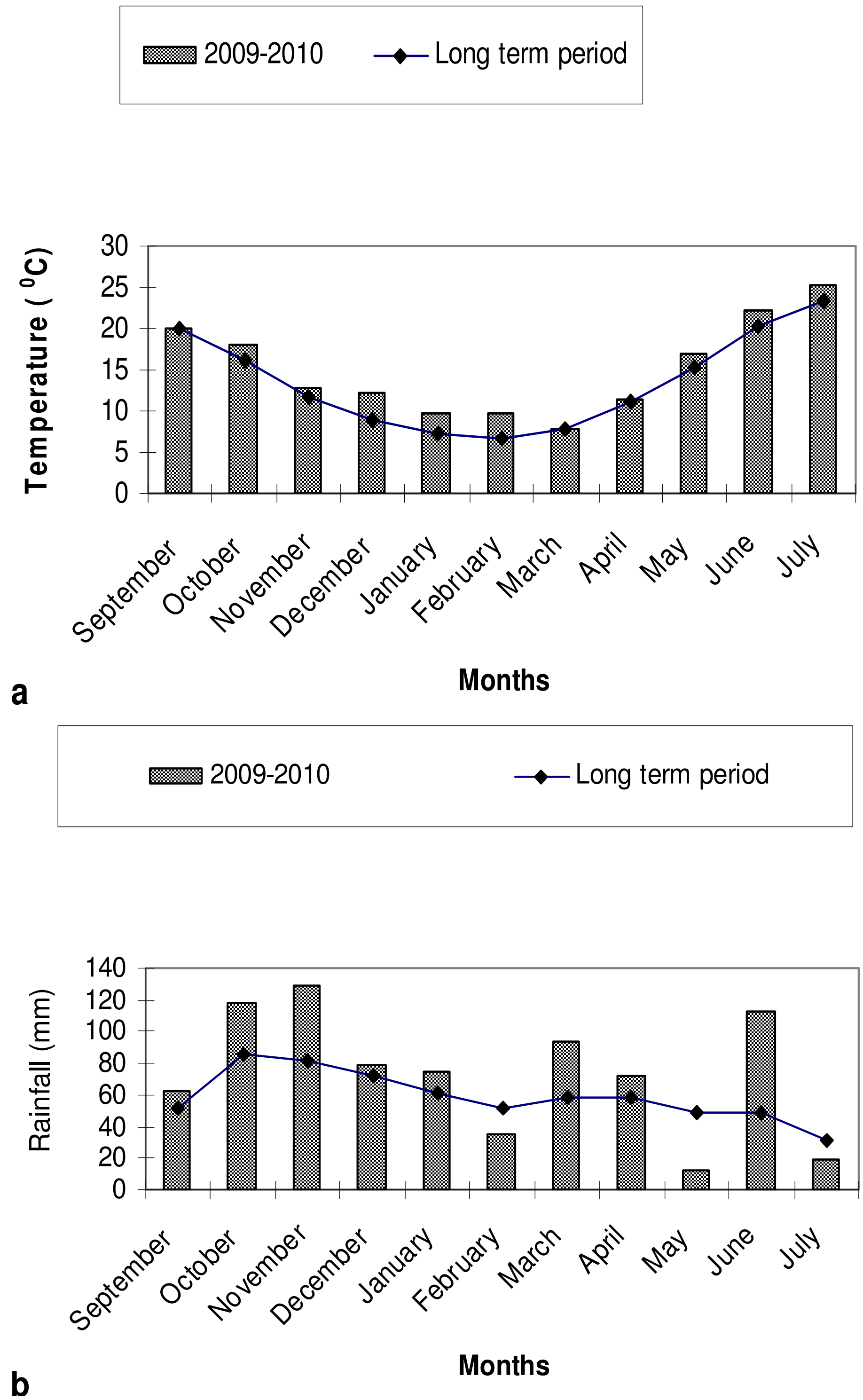

Figure 1. Climatic diagrams of Samsun (a) monthly means of temperature (b) monthly means of precipitation.

plant ageing. Similar to NDF(%) and other quality measures, maturity and harvest timing have the greatest influence on fibre digestibility. The NDF in leaves is more digestible than the NDF in stems. Lignification of the xylem tissue in mature stems significantly decreases fibre digestibility. As plants mature the leaf-to-stem ratio declines and so does the fibre digestibility (Anon, 2011). Growing conditions, such as temperature and moisture will also

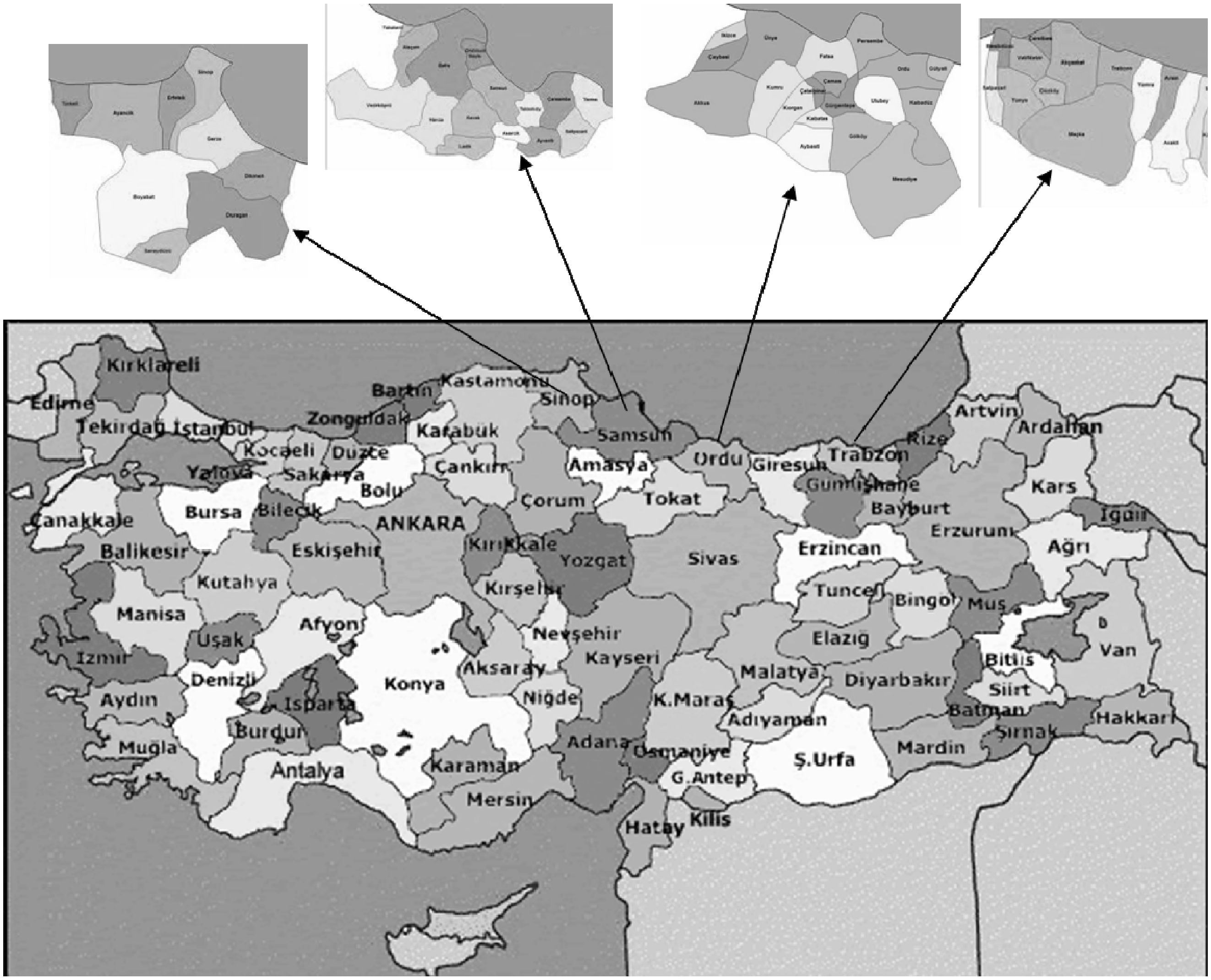

Figure 2. Geographic distribution of naturalized populations of red clover (*Trifolium pratense* L.) in Central-Black Sea Region Turkey.

affect lignification and NDF digestibility (Anon, 2011). Temperature usually has greater influence on forage quality than other environmental factors. Although increasing temperature normally hastens maturity, the primary effect may be through its effect on the leaf/stem ratio with high temperatures promoting stem over leaf growth. Digestibility of both leaves and stems is lowered by warm temperatures because of resulting high cell-wall (estimated by NDF) and low soluble sugar concentrations. Additionally, the NDF of forages grown under warm temperatures is usually less digestible than that of forages grown under cool temperatures (Buxton, 1995). The last harvested populations were exposed to higher temperature during growing period than the first harvested populations (Figure 1)

The TDN refers to the nutrients that are available for livestock and is related to the ADF concentration of the forage. As ADF increases there is a decline in TDN which means that animals are not able to utilize the nutrients that are present in the forage. There were large variations among the populations regarding their TDN value (Table 3). Tavlas et al. (2009) showed that TDN value has significant differences among red clover genotypes. On the average, the lowest acceptable level of TDN was 64% for high-producing cows and 57% for dry cows (Ward, 2011). This present results indicate that Start and population 46 has much TDN than the acceptable level of TDN for dry cow (Figure 3d).

The NDF is used to predict DMI and is negatively correlated with DMI, which means that when NDF is high, the quality and the DMI are low (Horrocks and Vallentine, 1999). Since DMI value was calculated from NDF, the observed differences were reflection of previously described NDF differences.

The RFV is an index that is used to predict the intake and energy value of the forages and it is derived from the

Table 2. Legume, grass and legume-grass mixture quality standards.

Quality standard	CP, % of DM	ADF, % of DM	NDF, % of DM	RFV
Prime	>19	< 31	< 40	> 151
1 (Premium)	17 - 19	31 - 40	40 - 46	151 - 125
2 (Good)	14 - 16	36 - 40	47 - 53	124 - 103
3 (Fair)	11 - 13	41 - 42	54 - 60	102 - 87
4 (Poor)	8 - 10	43 - 45	61 - 65	86 - 75
5 (Reject)	< 8	> 45	> 65	< 75

CP, Crude protein; ADF, acid detergent fiber; NDF, neutral detergent fiber; DM, dry matter;RFV, relative feed value.

Table 3. Some nutritional properties of genotypes.

Feature	N	Mean ± Std. error	Minimum	Maximum	CV (%)
Crude protein (g kg^{-1})	48	169.38 ± 2.74	127.9	208.4	11.2
ADF (g kg^{-1})	48	471.48 ± 8.60	367.0	612.8	12.6
NDF (g kg^{-1})	48	611.50 ± 10.41	503.9	756.1	11.8
TDN (g kg^{-1})	48	505.27 ± 6.70	379.2	594.6	9.2
DMI (g kg^{-1})	48	19.91 ± 0.32	15.9	23.9	11.1
DDM (g kg^{-1})	48	521.72 ± 6.70	411.6	603.1	8.9
RFV	48	81.34 ± 2.26	50.6	112.0	19.2
Crude ash (g kg^{-1})	48	97.80 ± 1.49	78.4	127.1	10.6
Ca (g kg^{-1})	48	17.88 ± 0.26	15.5	22.2	10.0
K (g kg^{-1})	48	18.68 ± 0.56	9.9	25.1	20.6
Mg (g kg^{-1})	48	3.66 ± 0.05	3.1	4.6	10.1
P (g kg^{-1})	48	2.79 ± 0.07	1.7	3.6	16.5
Ca:P	48	6.76 ± 0.25	4.5	11.5	26.0
K/(Ca + Mg)	48	0.88 ± 0.03	0.5	1.3	25.7

N, Red clover (Trifolium pratense L.) genotypes.

DDM and DMI. RFV is a widely accepted forage quality index in the marketing of hays in USA (Canbolat et al., 2006). Forage with an RFV value >151, 150 to 125, 124 to 103, 102 to 87, 86 to75 and <75 are considered as prime, premium, good, fair, poor and reject, respectively (Basaran et al., 2011; Aydin et al., 2010; Canbolat et al., 2006). Start and population 46 were higher in RFV value than the other red clover populations (Figure 4c).

Ash content is the inorganic part of the crops and gives idea about the total elements such as Ca, Mg, K, Fe, Zn etc (Tongel and Ayan, 2010). Minerals are important in animal feed because they have different roles in animal metabolism. Therefore, a balanced nutrient intake, including minerals, is required for optimal animal performance (Dasci et al., 2010). There was big variability among populations regarding their mineral content (Table 3). Ash content of the populations varied from 78.8 to 127.1 g kg^{-1}. It was in harmony with the data which were found by Onal Asci (2009) but lower than results of Drobna (2009). Besides the cultivar differences, soil and climatic factors might affect the ash contents (Acikgoz, 2001). Additionally, populations 37 had much mineral than cultivar Start (Figure 5a). Ca, K, Mg, and P content of populations ranged from 15.5 to 22.2 g kg^{-1}, 9.93 to 25.05 g kg^{-1}, 3.08 to 4.58 g kg^{-1} and 1.67 to 3.56 g kg^{-1}, respectively (Figures 5b, c, d and 6a). While populations 47 had much Ca content than cultivar Start, populations 6 had much K content than Start. Both Ca and K content of populations were higher than suggested value (3.0 and 8.0 g kg^{-1}, respectively) for ruminants (Tajeda et al., 1985). Although K content of the population was lower than Mouat and Anderson (1974) findings, most of populations had much Ca than their reports. It was reported that forages for cattle and sheep should contain P between 1.7 and 3.9 g kg^{-1} (NRC, 1996), 1.6 and 3.8 g kg^{-1} (NRC, 1985), respectively. P content of hay was close to recommended values (Figure 6a). It was reported that Mg concentration in leaf tissue of red clover was 3 g kg^{-1} (Gibson, 2008). In this present study, hay contained higher amounts of Mg than that reported by Gibson (2008). This difference could be due to hay structure including stem, leaf, flower and different ecological conditions.

Mineral imbalances, deficiencies or excess and low bioavailability of essential minerals result in negative economic impacts when animal performance and health are compromised (Van Soest, 1983). Results of this study

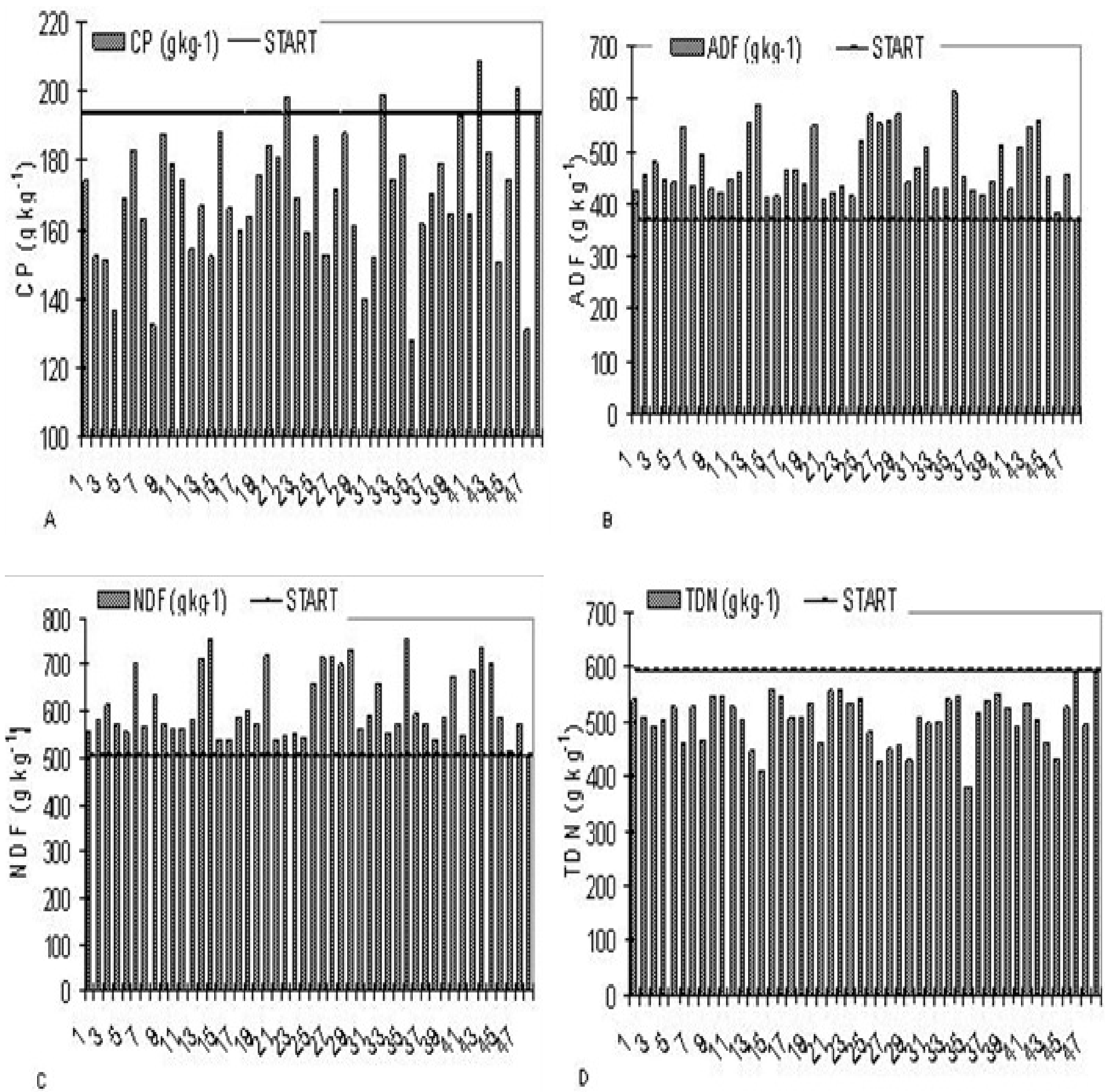

Figure 3. Some quality characters of red clover. Crude protein (A), ADF (B), NDF (C) and TDN (D). ADF, acid detergent fiber; NDF, neutral detergent fiber; TDN, total digestible nutrients.

eflect the existence of variation among the populations regarding their Ca:P and K:(Ca+Mg) ratios. Calcium is associated with phosphorous metabolism in animals (Madibela and Modiakgotla, 2004). In this current study the Ca:P ratio was between 4.53 to 11.45 for the populations. This results are lower than Parissi and Koukokura's (2009) report (Ca: P ratio of red clover was 11.8). Although the generally recommended ratio of Ca:P is 2:1, ranges in dietary 1:1 and 7:1 resulted in similar performance of ruminant livestock (Buxton and Fales, 1994) without unfavourable effects if adequate vitamin D is available (Barnes et al., 1990). These results indicate that milk fever can be seen in livestock when they are fed with hay of populations 18 (Figure 6b). Legumes usually are good sources of calcium but not phosphorus (Schroeder, 2004). Low P and high Ca content resulted in this wide Ca:P ratios (Ramirez-Orduna et al., 2005, Basaran et al., 2011). It is recomended that K:(Ca+Mg)

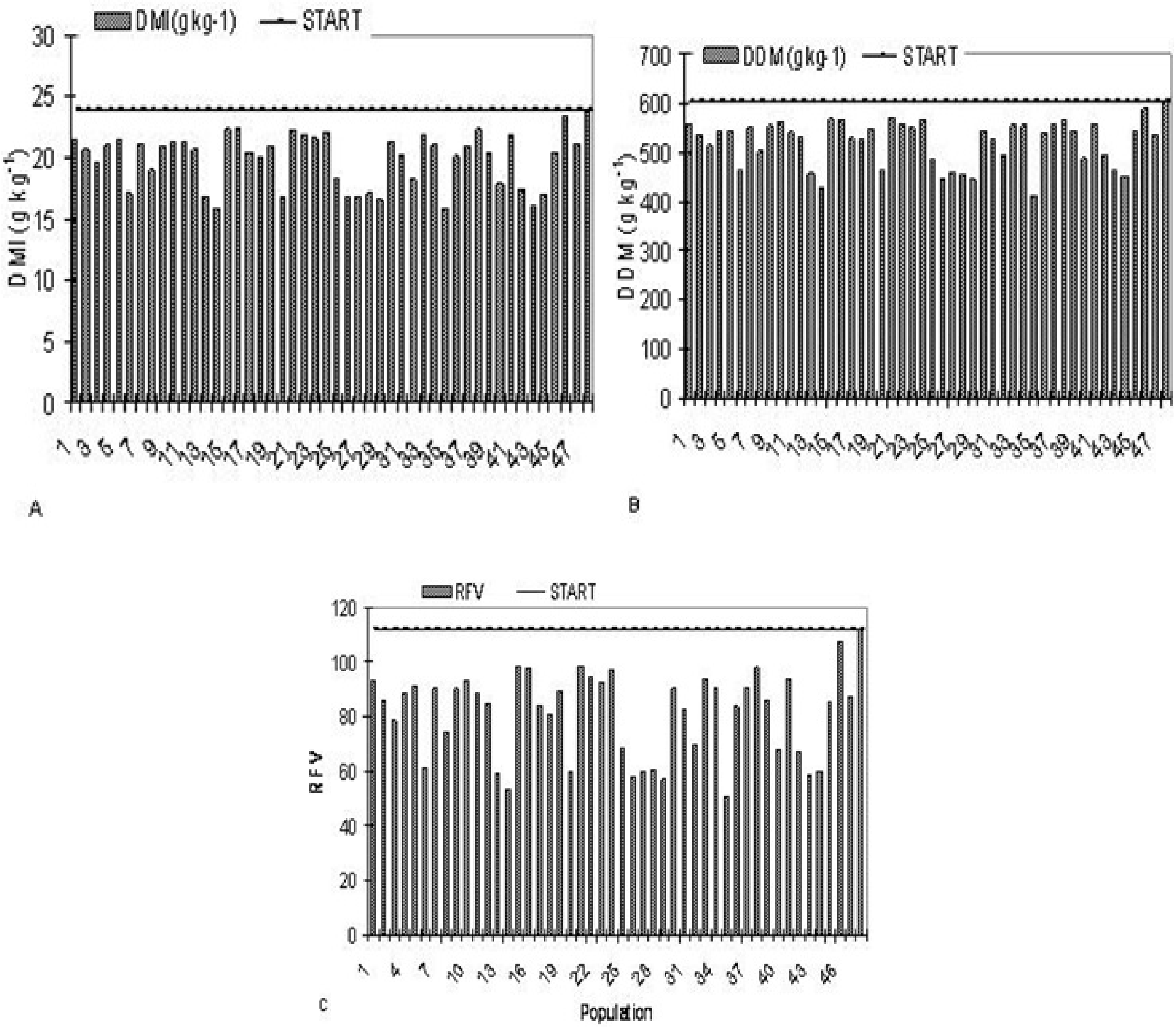

Figure 4. Some quality characters of red clover. DMI (A), DDM (B) and RFV (C). DMI, Dry matter intake; DDM, digestible dry matter; RFV, relative feed value.

ratio of forages should be below 2.20 (Kidambi et al., 1989). K:(Ca+Mg) ratio of all populations were lower than the critical value (2.20) causing tetany (Figure 6c). This result is similar to Mouat and Anderson (1974) reports.

The cluster analysis has different populations on the basis of similarity and thus provides a hierarchical classification (Sozen and Bozoglu, 2007). The statistics of the cluster analysis, based on the 14 nutritional properties, allowed the identification of five basic groups with variation between four and 19 (Figure 7). According to the cluster analysis, populations 1 and 10 seemed to be close to each others. While Cluster A was divided into four subgroups that included 19 genotypes collected from 11 different locations, Cluster C and E contained four genotypes. It was noted that there was statistically significant differences among and within the genotypes concerning locations. Onal Asci (2011b) reported that the statistics of the cluster analysis, based on the ten morpho-agronomic traits, allowed the identification of eight basic groups in the same populations.

The determined nutritional values on red clover populations showed that there was a significant genetic diversity

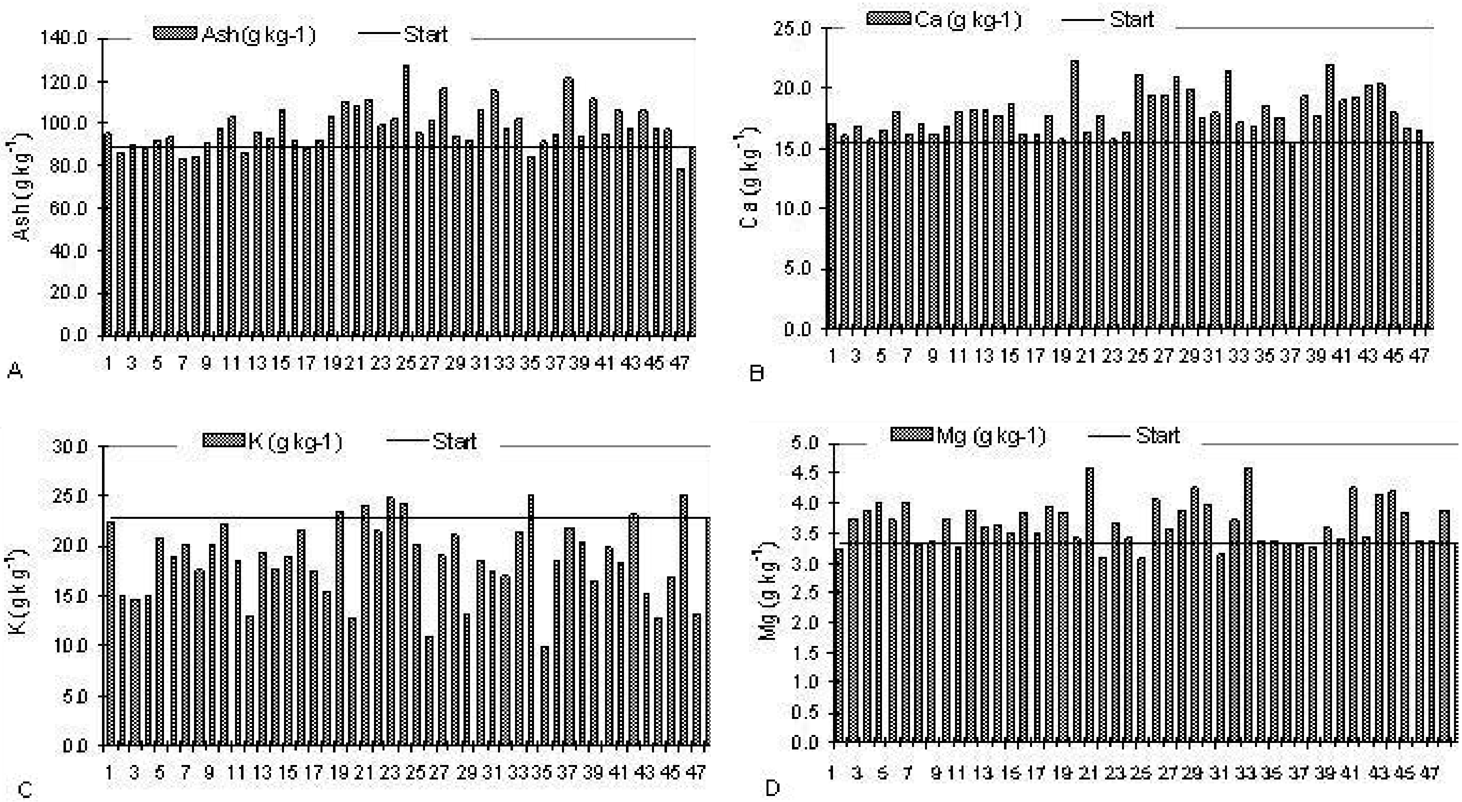

Figure 5. Some quality characters of red clover. Ash (A), Ca (B), K (C) and Mg (D).

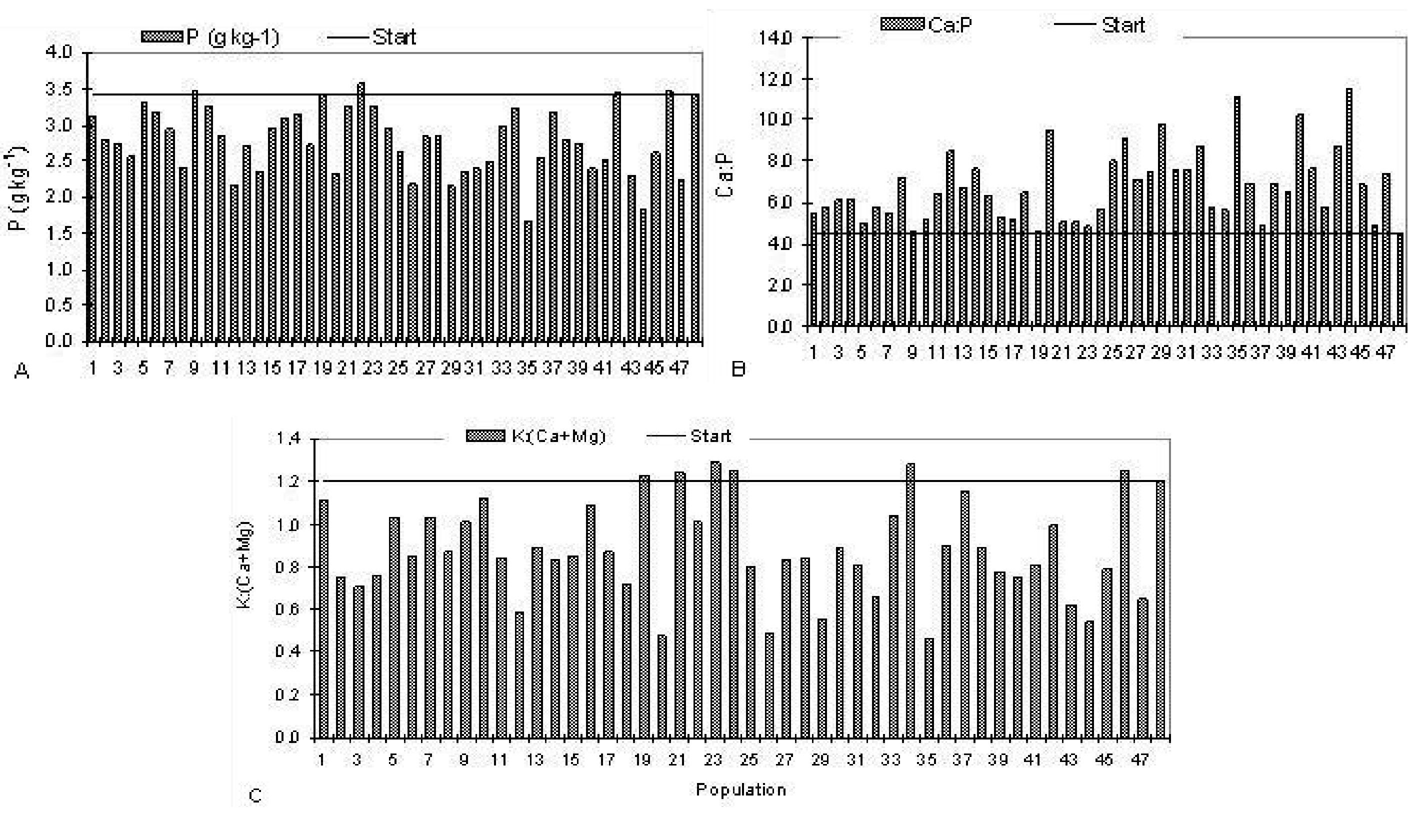

Figure 6. Some quality characters of red clover. P (A), Ca:P (B) and K: (Ca + Mg) (C).

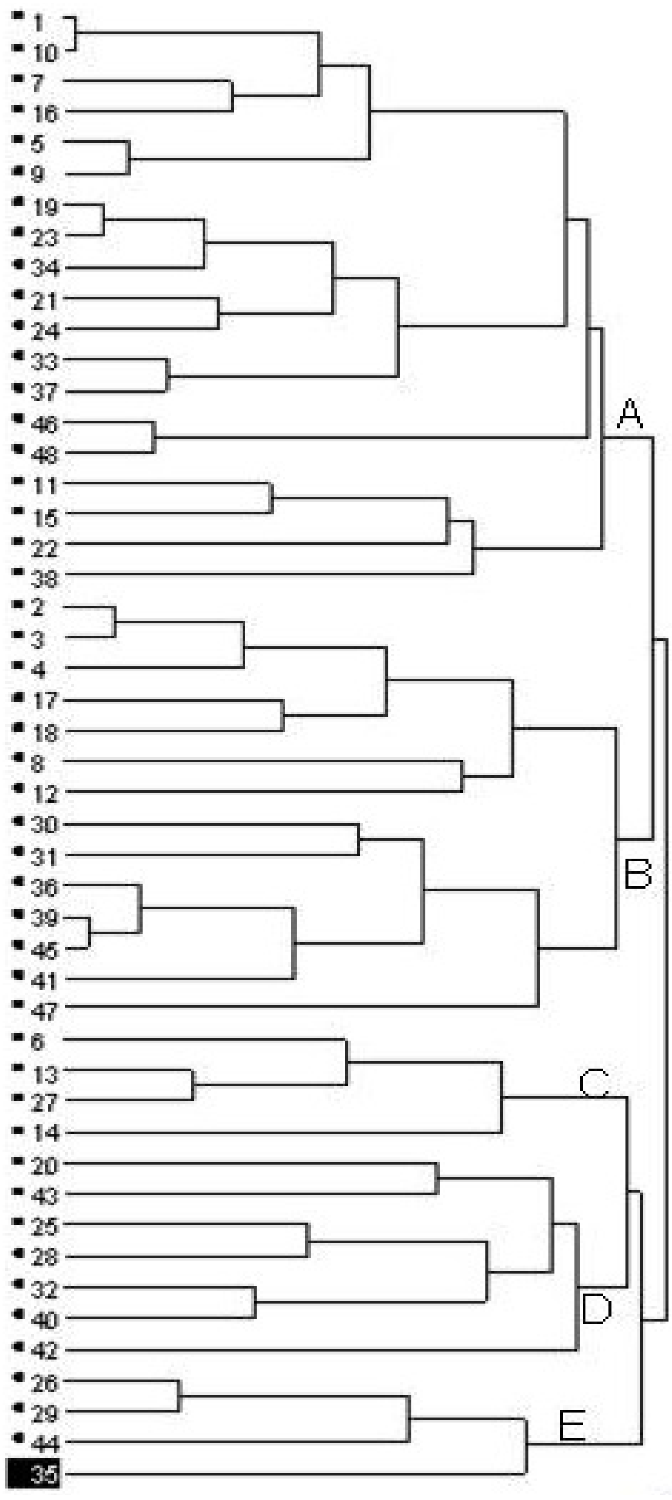

Figure 7. Dendrogram of 48 red clover cultivar/populations based on the 14 nutritional properties.

among 48 red clover genotypes. In this case, they can be used for releasing of new cultivars by breeders. While population 46 is hopeful to improve new cultivar which has lower ADF and NDF content, population 22, 32, 40 and 42 are hopeful to improve new cultivars which have high crude protein content.

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