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Full Length Research Paper

Biodiversity in red clover (*Trifolium pratense* L.) collected from Turkey. I: Morpho-agronomic properties

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Sustainibility of plant production, may only be provided by preservation of wild plant species and local cultivars. Preservation of these genetic resources in Turkey, which has a rich genetic diversity, is essential for breeding program to improve new cultivars. The objective of this study was to compare 47 red clover (*Trifolium pratense* L.) populations and one cultivar as a control in terms of morpho-agronomic characters in the second production year (2010) in Samsun-Turkey. Using cluster analysis procedure, populations were classified into eight groups, based on morpho-agronomic properties. Results show that there was a high diversity among the populations and some populations could be used in breeding programs.

Key words: Cluster analysis, morphology, red clover.

INTRODUCTION

There is a big gap in animal feeding in Turkey regarding roughage. Having various ecological conditions, Anatolia has the potential for many forage crops used in animal feeding to be grown successfully. Although these forage crops occupy about 25 to 30% of sowing area in developed countries, it is only 4% in Turkey (TurkStat, 2009). Suitable forage species and cultivars should be released to increase high quality forage production and to improve the pastures in Turkey. The most important source for this aim is the local populations which have already existed in the natural flora and have been adapted to ecological conditions of the region (Acar et al., 2001; Ayan et al., 2006). Mediterranean region and its border countries are one of the main gene centers of *Trifolium* genus.

Turkey is the richest Mediterranean country for clover species with over 100 species in its natural flora (Zohary and Heler, 1984).

Red clover is a valuable forage legume for temperate regions, and grown alone or in a mixture with grasses, because it is adapted to a wide range of soil types, pH, environmental and management conditions (Bowley et al., 1984; Taylor and Quesenberry, 1996) and gives good yields in areas not suitable for growing alfalfa owing to problems of soil acidity or excessive soil moisture (Smith et al., 1985). Furthermore, in association with *Rhizobium* bacteria, it has the ability to fix nitrogen from 125 to 220 kg ha^{-1} $year^{-1}$ (LaRue and Paterson, 1981). These properties added to high yield (in a two to three year pastures), good quality and suitability for conservation make red clover an attractive option for forage production (Frame, 1990). Red clover is clearly an important plant for the Black Sea region due to the fact that it has a light compensation point at 6 % of daylight (Taylor and Smith, 1995). Altough this advantage makes red clover an excellent small seeded legume to Northern part of Turkey which has low light compensation and short daylight period, currently, there are 2 registered red clover cultivars bred in Turkey in the 'national cultivar' list (Anonymous, 2011). Consequently, there is a need to improve new red clover cultivars for Turkey.

For a successful breeding program, genetic diversity and variability play a vital role. Population genetic diversity is a prerequisite for an effective plant breeding program (Arslanoglu et al., 2011). The conservation and sustainable use of plant genetic resources require accurate identification of their accession (Arif et al., 2010). Morphological characterization is the first step in description and classification of genetic resources (Smith and Smith, 1989). When various sets of cultivars or populations of red clover were analysed, large genetic diversity were determined among and within populations using morphological traits and molecular markers (Greene et al., 2004; Dias et al., 2008).

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

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

The aims of this research were (1) to investigate some plant and agricultural characters and (2) to determine the similarity and differences in respect to morphological variation of red clover accessions which naturally grow in Black Sea region.

MATERIALS AND METHODS

In this study, 48 red clover (*Trifolium pratense* L.) genotypes were investigated and 47 of them were the populations collected from 20 different locations in the Black Sea Region in 2008 (Table 1). A control cultivar "Start" was also used in the experiment.

The scarified seeds were sown in seed trays and then seedlings 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, organic matter content was 3.08%, available P content was 30.0 ppm and available K content was 116.3 ppm. The experimental area has typical Mediterranean climatic conditions (Figure 1).

Flowering date, plant height, stem number, number of node in the main stem, main stem diameter, shape of medial leaflet, and density of hairs in the main stem were determined according to UPOV criteria (UPOV, 2001). Width and length of medial leaflet measurements were done with callipers (Table 2).

Plants were harvested for hay at 50% flowering stage (Onal Asci, 2009). Forage samples were dried at 70°C for 48 h and then hay yield per plant was figured out on five different plants (Anonymous, 2010). All observations and measurements were done in the second year of the study (in the summer of 2010). The basic statistics (minimum, maximum, mean, standart error) and correlation analysis were done using SPSS 10.0 program (SPSS Inc., 1999). Hierarchical cluster analysis was carried using the Ward method in JMP 5.1 for Windows (SAS Institute Inc., 2004).

RESULTS

In terms of the first harvest time for hay, genotypes were divided into three groups (Figure 2). Harvests were done between 16 May to 17 July.

The big difference between the minimum and the maximum hay yield (30.58 and 316.01 g, respectively) indicate that large variation existed among the genotypes (Table 3). Furthermore, 29 genotypes were more productive than the cultivar Start (Figure 3).

The morphological study carried out on *T. pratense* L. revealed a high level intra-specific variability for the studied characteristics (Table 3). Plant height is a very important yield component. According to the correlations, there was a positive relationship between plant height and hay yield (0.870**). Average plant height of the red clover plants were measured as 64.48 cm, the shortest plant was found in genotype 42 (46.20 cm), while the tallest plant was determined in genotype 3 (92.20 cm) (Table 3). With reference to average plant height, 32 genotypes were taller than cultivar Start (Figure 4). The number of node ranged from 5.75 to 16.25 with an average of 11.08 (Table 3). Regarding the correlations, positive relationship existed between the plant height and node number (0.296**). Concerning the main stem diameter, it changed between 2.18 mm and 4.19 mm. Average stem number, which is the important character for hay yield, was as 87.68 (Table 3). The lowest and highest values were found as 24.00 (in genotype 46) and 154.20 (in genotype 2). In comparison, all the 28 genotypes had more stems than cultivar Start (Figure 5). According to the analysis, there was a positive correlation between stem number and hay yield (0.756**). It is also noteworthy to mention that median leaflet length ranged from 1.33 to 4.43 cm. Average median leaflet width was measured as 1.57 cm (Table 3). Shape of medial leaflet was observed as elongated and ovate (UPOV, 2001). The density of hairs observed on the 3rd internode of a fully expanded flower head ranged from very low to medium according to UPOV criteria (UPOV, 2001).

In order to determine the similarities or differences among genotypes, a cluster analysis was performed as well. The statistics of the cluster analysis, based on the ten morpho-agronomic traits, allowed the identification of eight basic groups with variation between 2 and 11 (Figure 6). According to the cluster analysis, genotypes 9 and 17 seemed to be close to each others. Cluster A contained the largest number of genotypes collected from

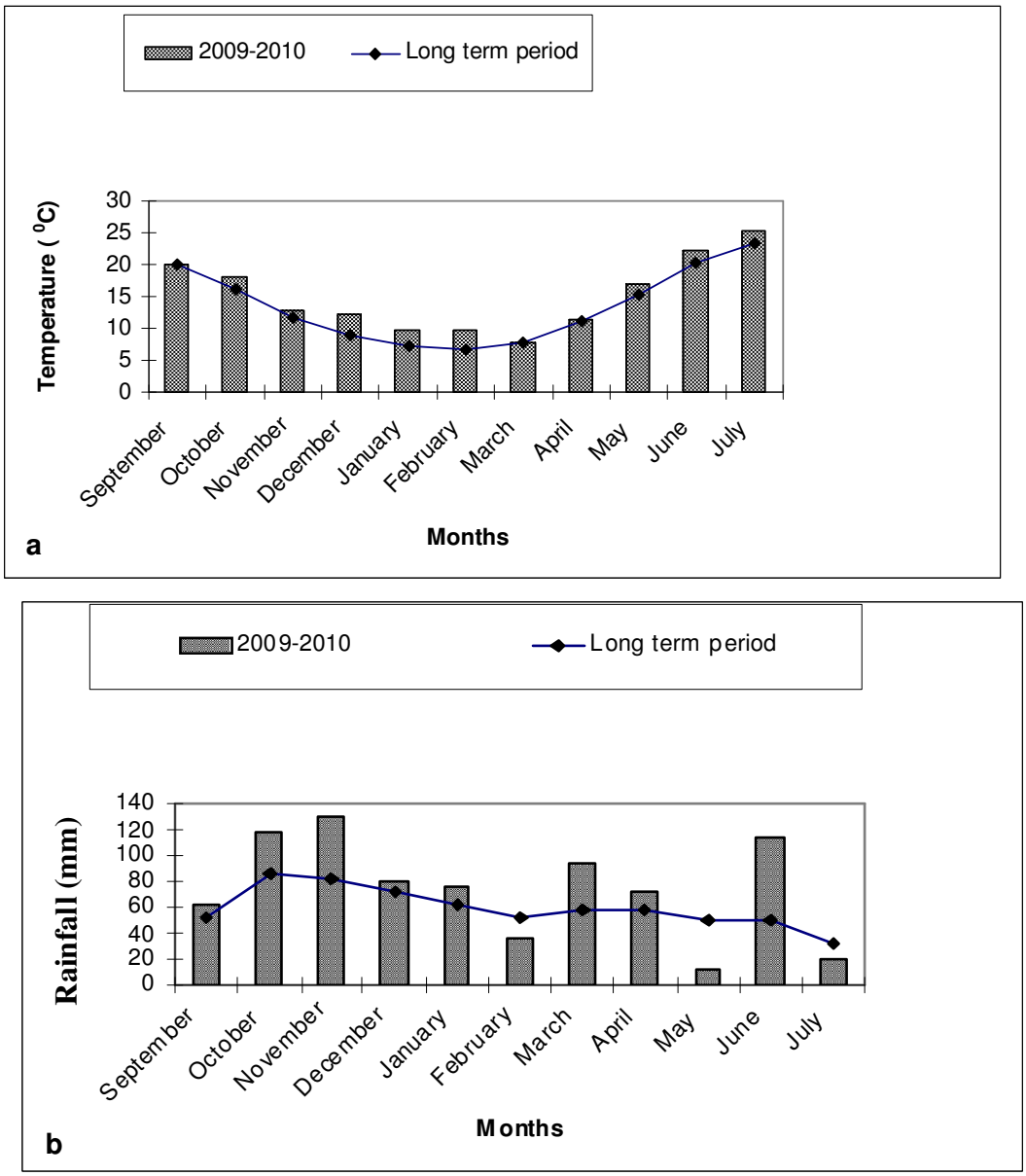

Figure 1. Climatic diagrams of Samsun. (a) Monthly means of temperature; (b) monthly means of precipitation.

nine different locations. It was noticed that there was statistically significant differences among and within the gonotypes concerning locations. In general, the genoytpes collected from near regions, were clustered in the same groups.

DISCUSSION

It is the notion among the breeders that the high level of genetic diversity in a pool contributes to variation, demonstrating the significance of selection. Results of this study reflect the existence of variation within genotypes and among the genotypes.

There were three groups with regards to the first harvest time for hay yield. Greene et al. (2004) studied 33 wild red clover population using 15 morphological traits and found that flowering time contributed to group population into three classes.

Achieved results confirm good production potential of 29 genotypes, which were comparable with cultivar Start. From the viewpoint of average hay yield, the best one was genotype 3, in addition to that, it was the tallest genotype. Also, Vasiljevic et al. (2006) suggested that selection for a longer stem may lead to increased mass yield. It was more productive than cultivars cultivated by

Table 2. Description of the characters used in the morphological study of the red clover.

Character	Description
Flowering time	Made when 3 heads per plant are flowering
Plant height (cm)	Measurements of the distance between the top point of the plant and the ground surface during harvest, using meter
Stem number	Stem number per plant
Number of node in the main stem	Counted on the longest stem including the head within 1-2 weeks after mean date of flowering
Main stem diameter	Measured 2 to 4 cm above tillering node.
Shape of medial leaflet	(1) elongated, (2) ovate, (3) rounded
Density of hairs in the main stem	Observed on the 3rd internode of a fully expanded flower head: (1) very low, (3) low, (5) medium, (7) high, (9) very high
Width of medial leaflet	(3) narrow, (5) medium, (7) broad
Length of medial leaflet	(3) short, (5) medium, (7) long

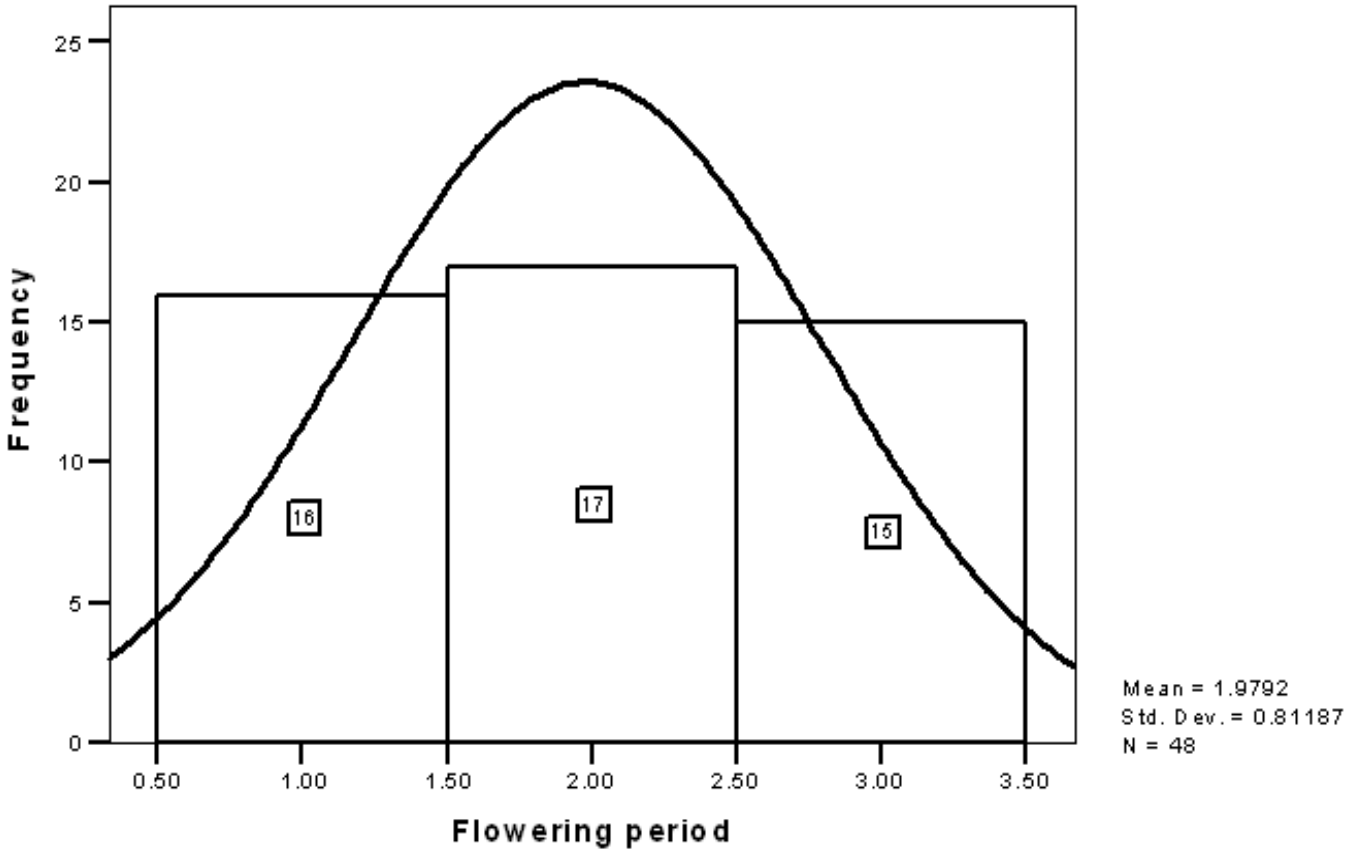

Figure 2. Flowering time of red clover.

Tucak et al. (2009).

It is known that plant height may affect the yield. Though red clover plant height ranges from 30.3 cm (Muntean, 2006) to 85 cm (Acar et al., 2001), generally it grows to 50 to 60 cm tall (Acıkgoz, 2001). In this study, plant height of genotypes 3 and 26 was higher than 85 cm, furthermore, 28 genotypes were taller than 60 cm. The stem number is a very important yield component. In this research, while some genotypes had less stem, some genotypes had more stem than the varieties which were used by Drobna (2009) who reported that stem number of red clover ranged from 66.74 to 107.10.

Table 3. Some measurements and statistical data obtained from the fisrt growth of genotypes.

Features	Number	Mean ± Standard error	Minimum	Maximum	CV (%)
Plant height (cm)	48	64.48±1.84	46.20	92.20	19.79
Main stem diameter (mm)	48	3.47±0.06	2.18	4.19	12.84
Main stem number/plant	48	87.68±4.69	24.00	154.20	37.03
Nod number/main stem	48	11.08±0.36	5.75	16.25	22.38
Hay yield/plant (g)	48	142.85±9.23	30.58	316.01	44.75
Median leaflet length (cm)	48	2.70±0.09	1.33	4.43	24.44
Median leaflet width (cm)	48	1.57±0.05	0.68	2.34	21.66

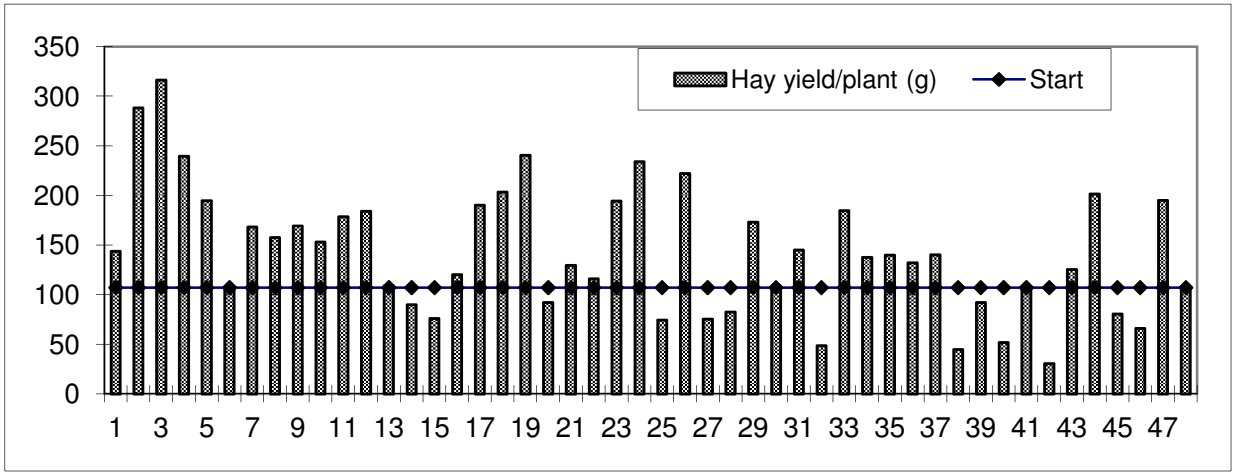

Figure 3. Hay yield/plant (g).

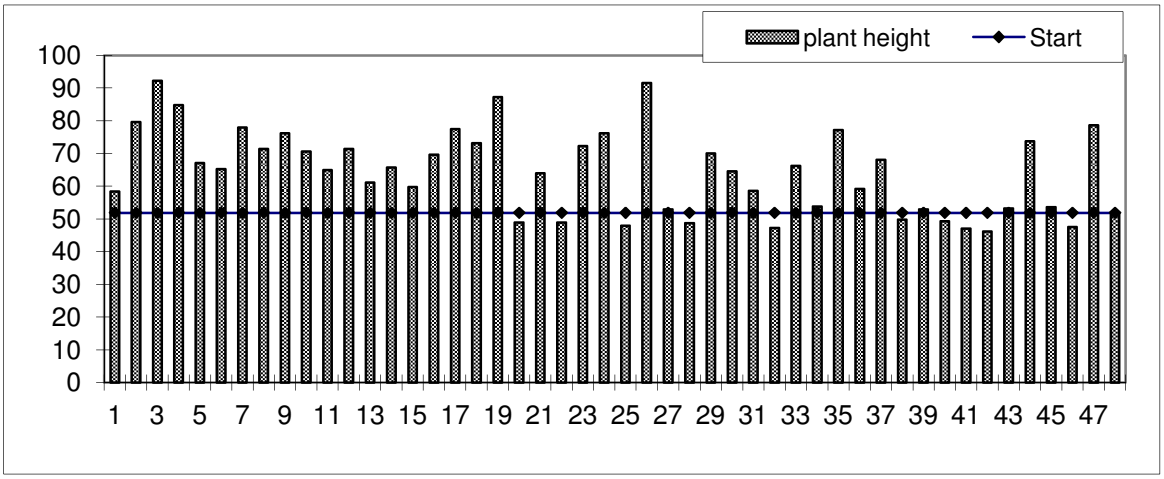

Figure 4. Plant height of populations (cm).

The node numbers of the genotypes used in this study ranged from medium to very high according to the UPOV (Anonymous, 2010).

Leaflet length for cultural varieties of red clovers was reported as 1.5 to 3.0 cm by Genckan (1983). According to IPGRI and UPOV criteria (Anonymous, 2010), except for one genotype, the others had very short leaflet.

As for leaflet width, most of the genotypes had very narrow leaflet in accordance with IPGRI and UPOV criteria (Anonymous, 2010).

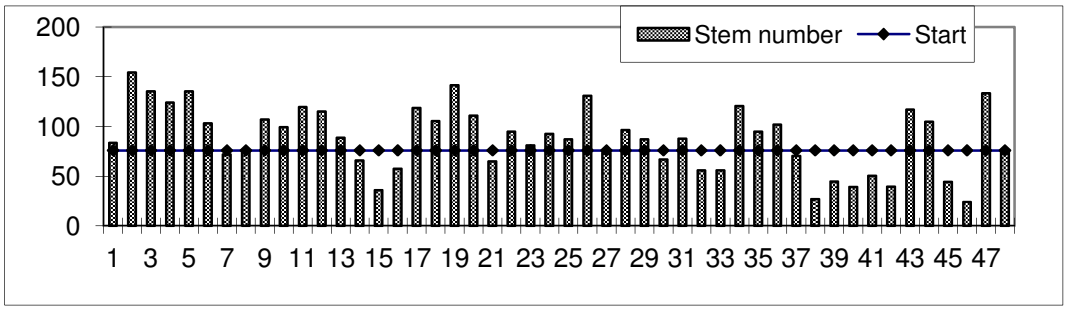

Figure 5. Stem number of populations.

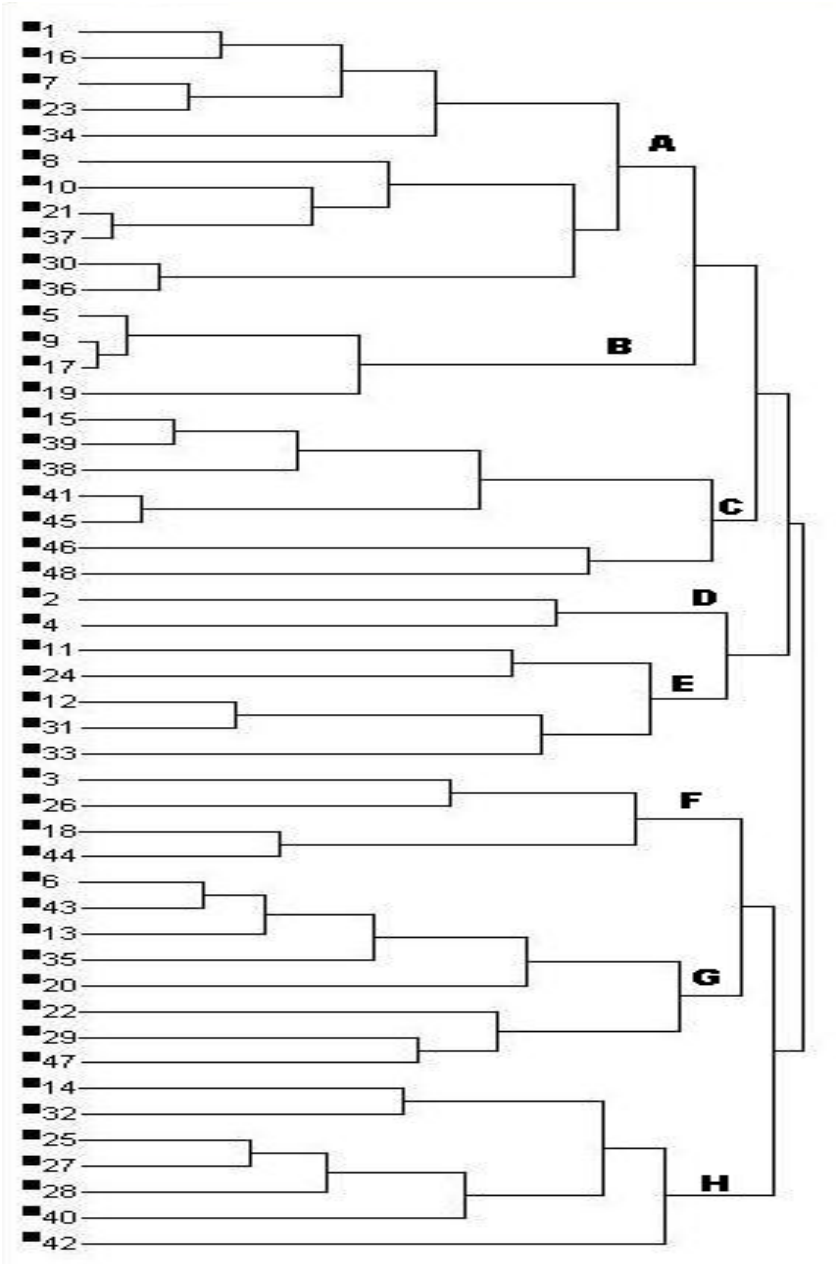

Figure 6. Dendrogram of the 48 red clover cultivar/populations based on the ten morpho- agronomic traits.

To be useful for plant breeders, genetic resources must be characterized by morphological and agronomic traits (Martins et al., 2006). For this reason, there is need to collect, characterize and evaluate remnant local genotypes before they disappear (Balkaya and Ergun, 2008). The cluster analysis has different genotypes on the basis of similarity and thus provides a hierarchical classification (Sozen and Bozoglu, 2007). The cluster analysis categorized the genotypes into eight groups. This finding reinforces some researchers' assertion that they realized different groups of populations based on the contribution of several morpho-agronomic traits (Dias et al., 2008; Drobna, 2009; Tucak et al., 2009).

These results indicate that there was a significant genetic diversity among the 48 red clover genotypes. The most diversity was found in the genotypes collected from Samsun. This will contribute to the maintenance of the diversity in red clover breeding program.

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