



Agromorphological, yield and quality characteristics of two populations of alfalfa developed by mass selection

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ABSTRACT: This study was designed to test the two alfalfa advanced populations (L-1737 and L-1738) with four control cultivars (Bilensoy-80, Gözli, Savaş and Plato) for agromorphological, yield and quality properties under irrigated conditions in two various locations in Ankara, Turkey in the years of 2013, 2014, and 2015. The experiments were arranged in a randomized block design with four replications. Results of the study showed that there were statistically significant differences in plant height and stem diameter as well as stem number among the study materials. Statistically significant differences were observed for green forage yield ($P < 0.05$), while not in dry matter yield among the study materials. The average green forage and dry matter yields were reported as 67.0 t ha⁻¹ and 13.0 t ha⁻¹, respectively. Green forage and dry matter yields in the advanced populations of L-1737 and L-1738 were 69.2 and 67.4 t ha⁻¹; 13.7 and 13.6 t ha⁻¹, respectively. Those yield values were 6.5 and 3.7%; 4.3 and 3.4% higher in green forage and dry matter yields of the advanced populations of L-1737 and L-1738 compared to the control cultivar Bilensoy-80, respectively. These increases in green forage and dry matter yields were due to the effect of the mass selection method. Statistically significant differences were observed for acid detergent fiber, neutral detergent fiber, and relative feeding value among the study materials, except crude protein contents. It was concluded that the two advanced populations had good adaptation ability, high yield and good quality performance under irrigated conditions and could be used as commercial cultivars.

Key words: alfalfa, advanced populations, green forage, dry matter.

Características agromorfológicas, produtivas e de qualidade de duas populações de alfafa desenvolvidas por seleção massal

RESUMO: Este estudo foi desenhado para testar duas populações avançadas de alfafa (L-1737 e L-1738) com quatro cultivares controle (Bilensoy-80, Gözli, Savaş e Plato) para propriedades agromorfológicas, produtividade e qualidade sob condições irrigadas em dois locais diferentes em Ancara, Turquia, nos anos de 2013, 2014 e 2015. Os experimentos foram dispostos em delineamento de blocos casualizados com quatro repetições. Os resultados do estudo mostraram que houveram diferenças estatisticamente significativas na altura das plantas e diâmetro do colmo, bem como no número de colmos entre os materiais avaliados. Foram observadas diferenças estatisticamente significativas para a produtividade de forragem verde ($P < 0.05$), enquanto não foram observadas diferenças na produção de matéria seca entre os materiais estudados. As produtividades médias de forragem verde e matéria seca foram de 67.0 t ha⁻¹ e 13.0 t ha⁻¹, respectivamente. Os rendimentos de forragem verde e matéria seca nas populações avançadas de L-1737 e L-1738 foram 69.2 e 67.4 t ha⁻¹; 13.7 e 13.6 t ha⁻¹, respectivamente. Esses valores de rendimento foram 6.5 e 3.7%; 4.3 e 3.4% maiores em rendimentos de forragem verde e matéria seca das populações avançadas de L-1737 e L-1738 em relação à cultivar controle Bilensoy-80, respectivamente. Esses aumentos nos rendimentos de forragem verde e matéria seca foram devidos ao efeito do método de seleção massal. Diferenças, estatisticamente significativas, foram observadas para fibra em detergente ácido, fibra em detergente neutro e valor alimentar relativo entre os materiais estudados, exceto nos teores de proteína bruta. Concluiu-se que as duas populações avançadas apresentaram boa capacidade de adaptação, alta produtividade e bom desempenho em condições irrigadas, podendo ser utilizadas como cultivares comerciais.

Palavras-chave: alfafa, populações avançadas, forragem verde, matéria seca.

INTRODUCTION

Alfalfa (*Medicago sativa* L.) is very important resource of good quality forage and known as Queen of the Forages (SHI et al., 2017). Alfalfa

ranks first in terms of sown area and production among forage legumes in Turkey (ANONYMOUS, 2021). It is perennial crop having high yield potential, as well as palatable with high quality forage. It may be sown alone or in a mixture with some other grasses

(wheatgrass, smooth brome, festuca, orchard grass etc) (AÇIKGÖZ, 2001; ALBAYRAK & EKIZ, 2005).

It's extensively produced under irrigated conditions and moreover, it's possible to be grown without irrigation under semi-arid conditions (AÇIKGÖZ, 2001) in marginal cereal areas where grain yield is low or limited.

Many alfalfa cultivars are required to increase forage production for meeting the annual forage requirement under irrigated conditions. For this reason achieving, alfalfa breeding projects or programs, covering rich genetic resources and well organized, have been conducted in Turkey. Dry matter production, silage, grazing and quality are the main sought and desired features in alfalfa breeding programs.

Alfalfa breeding programs are mainly concentrated on yield increasing, improving forage nutritive value, and raising of tolerance to abiotic/biotic stresses (TUCAK et al., 2014). A breeding program must contain rich sources of germplasm with desirable genes that control phenotypic traits (RUMBAUGH et al., 1988). Moreover, the breeder would need a wide range of genetic variations for selection of required characteristics and improvement of new cultivars with desirable traits (FRAME, 1994; BALOCH et al., 2017). In cross-pollinated plants such as alfalfa, new combinations occur in each generation due to open pollination, and the plants are largely heterozygote (DEMIR & TURGUT, 1999). This is an important potential for the breeder and presents a great opportunity to develop new cultivars. Alfalfa is the one species with high genetic complexity at individual and population level due to its autotetraploidy and allogamy that is characterized by a high level of heterozygosity and severe inbreeding depression (DEMIR & TURGUT, 1999; LABOMBARDA et al., 2000; TUCAK et al., 2010).

It is important to know and evaluate the heritability of the traits addressed in breeding programs and the results of these programs. Especially rapid genetic improvement was observed in difficult-to-improve low-heritability traits (YABE et al., 2018). Low success rate and slow progress to breed resistant cultivars are major problems in alfalfa breeding (SHI et al., 2017).

Selection is one of the oldest and most widely used conventional breeding techniques. With the effect of that, the genetic composition of the population has changed positively and the frequency of positive genes in the gene pool has also increased (DEMIR & TURGUT, 1999; TUCAK et al., 2014).

Many different forms of selection may be realized within single populations to improve array of

characteristics (RUMBAUGH et al., 1988). Based on the phenotype, open pollination commercial cultivars are obtained by mass selection (DEMIR & TURGUT, 1999). Mass selection, which is an effective, fast and minimum labour requirement, is used for cross-pollinated crops like alfalfa (AÇIKGÖZ, 2001). The method of mass selection is explained in two different ways such as a source population of individual plants is examined, and the most desirable plants, or seed from the most desirable plant, are selected to form the improved populations (MILLER & HANNA, 1995). Selection offers a unique opportunity to find new and valuable germplasm.

Recently, in most commercial alfalfa breeding programs, most used methods for the development of alfalfa cultivars are based on recurrent phenotypic selection, with or without progeny testing, with tendency to accumulate desirable alleles at high frequency into a population (LI & BRUMMER, 2012). Development of new alfalfa cultivars and/or populations is difficult and a very long-lasting process in which mainly is evaluated all the available germplasm of large number individual plants in nursery over multiple cuttings per year and multiple years (TUCAK et al., 2014).

Constantly maintenance genetic diversity and variability of alfalfa breeding pool during the selection through introductions of distances materials as a new source of genetic diversity is a prerequisite for successful breeding and developing of new cultivars/populations with high yield and good forage quality (TUCAK et al., 2011; ŽIVKOVIĆ et al., 2012). Dudley et al., (1963), Hanson et al., (1972) and Tysdal et al., (1942) stated that mass selection or phenotypic recurrent selection can be used in strain building to improve any genetically controlled characteristic that has a relatively high heritability (RUMBAUGH et al., 1988). The genetic increases in alfalfa yields have been small compared with those realized in most grain crops (HILL et al., 1988). The same authors explained the reasons for this with factors such as the perennial growth habit of alfalfa, its winter survival, and the storage of photosynthates for the production of the next crop. Also, equivalent gains per selection cycle for alfalfa and annual crop may translate into lower gains for alfalfa, as experimental strains are evaluated over several years for decision making in selection programs.

In this study, mass selection or phenotypic selection was implemented and improved two populations called the advanced populations as first population (L-1737) with 16 accessions, the second population (L-1738) with 13 accessions between 1999 and 2012. Two advanced populations were

tested with control cultivars in regional yield trials for their performance and potential from 2013 to 2015. Those trials were also organized to determine the morphological characteristics, yield, and quality properties under irrigated condition in Turkey and to investigate whether these advanced populations have better performance over standard commercial cultivars.

MATERIALS AND METHODS

In this study, mass selection (ŞEHİRALI & ÖZGEN, 1988; DEMİR & TURGUT, 1999) was performed and two populations were improved during the period of 1999 to 2012, one (L-1737) with 16 accessions, the other (L-1738) with 13 accessions. At the first period of this study, all study materials were planted and screened in nursery plot for morphological characteristics, especially plant height, stem diameter and stem number. Then the promising and desirable plants were selected and others were removed from field. After that, they all were harvested together. Phenotypic selection was realized with the three cycles in the various growing seasons. Lastly, they were seeded in isolated areas for basic seed production. During this period, two populations were called advanced population, the first population (L-1737) with 16 accessions, the second population (L-1738) with 13 accessions.

The field trials were performed in Yenimahalle and Gölbaşı locations of The Central Research Institute for the Field Crops in the years of 2013, 2014 and 2015. Yield trials with two advanced alfalfa populations (L-1737, and L-1738), and control cultivars (Bilensoy-80, Gözülü, Savaş and Plato) were sown in a randomized complete block design with 4 replications. Seeds were sown manually by hand. The plot size was $1.6\text{ m} \times 5.0\text{ m} = 8.0\text{ m}^2$, consisting of 8 rows spaced at 20 cm. The harvested plot size was 4.8 m^2 . The trials were set up in a fallow field.

After seeding, 40.0 kg ha^{-1} ammonium nitrate, and 75.0 kg ha^{-1} triple super phosphate were implemented, and was mixed into the soil. Then the top layer of soil was compressed with a roller. Weed control was made by hand hoeing when necessary.

Five cuttings were performed from 16 May-02 June to 02-10 October in 2014; from 01-10 June to 20 October in 2015 in Yenimahalle; from 29 May-02 June to 02-16 October in 2014 (except Savaş cultivar), from 15-22 June to 01-07 October (only four cuttings) in 2015 in Gölbaşı.

The growing start of plants at the spring is commonly accepted as 15th February (15.02.2014 and 15.02.2015) for the Central Anatolia Region.

The cutting times were determined as 10% of the flowering period. The 10 plants were selected from each plot for measuring the plant characters at the second cutting time in a vegetation period. Data on various morphological properties (stem numbers per plant, plant height, stem diameter) and agronomical characters (green forage and dry matter yields) were recorded.

Dormancy scores for populations were tested as 1-9 scale in the fall period in 2015 (GRAY & ANDERSON, 1995). Experiment plots were irrigated twice at each cutting and after.

After that, a 4.8 m^2 of 8.0 m^2 of each plots was harvested for green forage and samples (each 500g) were dried at $70\text{ }^\circ\text{C}$ for 48 h. Quality properties such as NDF, ADF and RFV were studied. Dry matter content was determined according to the method used by TEKKANAT & SOYLU (2005), crude protein ratio according to (NIR) (KUTLU, 2008), ADF ratio (NIR) (KUTLU, 2008), NDF ratio (NIR) (KUTLU, 2008), RFV by calculated with formula as $\text{DDM} \times (120) / \text{NDF} \%$ (STARKEY et al., 1993).

The soil characters of two locations are presented as follows. The soil of Gölbaşı location was clay-loam, pH slightly alkaline (8.04), poor (1.32%) in organic matter, medium (63.7 kg ha^{-1}) in phosphorus content, high (2074.6 kg ha^{-1}) in potassium content, very high (27.86%) in lime content (ANONYMOUS, 2016a). The soil of Yenimahalle location was clay-loam with pH slightly alkaline (7.99), poor (1.26%) in organic matter, medium (87.0 kg ha^{-1}) in phosphorus content, high (1562.1 kg ha^{-1}) in potassium content, in lime content medium (7.24%) (ANONYMOUS, 2016a).

During the experimental seasons of 2013, 2014, and 2015, total precipitation, average temperatures and average relative humidity were 267.4 mm; 383.8 mm and 385.3 mm; $10.5\text{ }^\circ\text{C}$, $11.5\text{ }^\circ\text{C}$ and $10.6\text{ }^\circ\text{C}$; 64.0 %, 72.6 %, and 71.2 % at Gölbaşı, respectively (ANONYMOUS, 2016b). Long term average precipitation, temperatures, and relative humidity are 395.5 mm and $10.1\text{ }^\circ\text{C}$, and 73.4 % at Gölbaşı, respectively. For long term on Gölbaşı location, average precipitation and relative humidity were higher than those in trial years but average temperature was lower compared with trial years.

During the experimental seasons of 2013, 2014, and 2015, total precipitation, average temperatures, and average relative humidity were 234.4 mm; 313.2 mm and 328.3 mm; $13.3\text{ }^\circ\text{C}$, $13.8\text{ }^\circ\text{C}$ and $12.7\text{ }^\circ\text{C}$; 54.0 %, 59.6 %, and 59.2 % at Yenimahalle, respectively (ANONYMOUS, 2016b). Long term average precipitation, temperatures, and relative humidity are 396.1 mm and $11.8\text{ }^\circ\text{C}$, and 61.3% at Yenimahalle,

respectively. For long term on Yenimahalle location, average precipitation and relative humidity were higher than those in trial years but average temperature was lower than those in trial years.

When we compare climatic data between two locations, total precipitation and average relative humidity in Gölbaşı became higher than those in Yenimahalle, but the average temperature in Gölbaşı was lower than that in Yenimahalle.

Analysis of variance (ANOVA) was performed for all studied traits in these experiments, with genotype as the principle factor for alfalfa cultivars. The significance of the main effects was estimated by the F test. Differences among alfalfa genotypes were compared using Duncan test at 5% level of probability.

RESULTS AND DISCUSSION

Agro-morphological characteristics

Plant height (PH, cm)

In the combined variance analysis results, PH in alfalfa was significantly affected by location, year and cultivar/population (Table 1). The location Gölbaşı had statistically significantly higher mean PH than Yenimahalle. The second year was significantly higher PH average than the first year. However, the interaction of year and location was reported to be significant, and the year effect also varied greatly from location to location. Thus, the second year had significantly a higher PH average than the first one in Gölbaşı, but in Yenimahalle, the situation was the

opposite. The cultivar Bilensoy-80 and Gözülü showed a statistically significant higher PH averages than the other cultivars and populations. PH averages over locations was significantly influenced by cultivars/populations. Thus, the cultivar Bilensoy and Gözülü showed statistically significant higher PH averages than the others. The advanced population L-1737 and Plato cultivar went in the same PH average group (B group). The other advanced population L-1738 and Savaş cultivar also became in C group of PH average. Overall average of PH in study materials was found such as 78.9 cm (68.4-87.6 cm).

Former a study for PHs were measured as ranging between 61.0 and 68.0 cm in alfalfa plant (VOLENEC et al., 1987). CHAMBLEE & WARREN (1990) reported that PH of alfalfa populations changed from 29 to 60 cm according to population and year. The plant lengths and coefficients of variation of alfalfa populations in two groups were determined such as 80.5 cm and 18.0%, and 82.5 cm and 60.0%, respectively (ROSELLINI et al., 1991). Furthermore, PHs in alfalfa were reported such as 63.0 cm - 67.0 cm in spring and 37.0- 46.0 cm in summer (PROSPERI et al., 1996). Moreover PHs in alfalfa were measured such as 63.1 cm (ÜNAL et al., 2012); 62.0 -68.2 cm (AVCI et al., 2013); 73.66 cm (66.77- 79.39 cm) (TUCAK et al., 2014); 77.9-82.3 cm (TURAN et al., 2017); 78.1-85.72 cm (GÖKALP et al., 2017). This study results occurred higher than the findings of VOLENEC et al., (1987); CHAMBLEE & WARREN (1990); PROSPERI et al., (1996); ÜNAL et al., (2012) and AVCI et al., (2013), but similar to the values of ROSELLINI et al., (1991);

Table 1 - Plant heights (cm) of alfalfa genotypes.

Cultivar/Population	-----Plant heights-----								
	-----Yenimahalle-----			-----Gölbaşı-----			-----Two-location average-----		
	2014	2015	Ave.	2014	2015	Ave.	2014	2015	Ave.
Bilensoy 80	89.5	74.9	82.2	81.1	102.5	91.8	85.3	88.7	87.0 A*
Gözülü	90.0	76.3	83.1	84.6	99.6	92.1	87.3	87.9	87.6 A
Savaş	67.2	70.0	68.6	58.7	77.7	68.2	63.0	73.8	68.4 C
Plato	78.9	73.0	76.0	76.5	91.5	84.0	77.7	82.3	80.0 B
L-1737	81.5	75.0	78.3	72.8	87.5	80.2	77.2	81.3	79.2 B
L-1738	71.6	68.8	70.2	64.0	80.3	72.2	67.8	74.5	71.2 C
Mean	79.9 B ²	73.0 C	76.4 B ⁺	72.9 C	89.8 A	81.4 A	76.4 B ¹	81.4 A	78.9

^{*)}The average values with the same letter in a column are not statistically different from each other according to the Duncan test at $P \leq 0.05$.

⁺⁾The average values of locations are not statistically different from each other at $P \leq 0.05$.

¹⁾The average values of years are not statistically different from each other at $P \leq 0.05$.

²⁾The average values of location-year combinations are not statistically different from each other according to the Duncan test at $P \leq 0.05$.

TUCAK et al., (2014); TURAN et al., (2017) and GÖKALP et al., (2017).

Stem diameter (SD, mm)

Results of the combined variance analysis showed that SD in alfalfa was a significantly affected by year and cultivar/population (Table 2). The second year was a higher SD than the first year over locations. It was not significantly seemed change between means of two locations. However, the effect of the year in SD significantly varied from location to location. In Gölbaşı location, the second year was significantly higher SD than the first year, but opposite status appeared in Yenimahalle. In this study, the overall average for SD in study materials was reported such as 3.2 mm (2.8 to 3.6 mm).

In alfalfa, SD changed from 2.46 mm to 3.26 mm, this variation occurred with the effect of the year (ÜNAL & FIRINCIOĞLU, 2007). Furthermore, SDs of alfalfa were discovered such as 2.86 mm (ÜNAL et al., 2012); 3.0-3.2 mm (AVCI et al., 2013); 2.08 mm (1.79-2.30 mm) (TUCAK et al., 2014); 3.19-3.32 mm (GÖKALP et al., 2017). Besides SD values in this study were similar to the results of ÜNAL & FIRINCIOĞLU, (2007), ÜNAL et al., (2012), AVCI et al., (2013), and GÖKALP et al., (2017). But they were higher than the values of TUCAK et al., (2014).

Stem number per plant (SNP)

The combined variance analysis results indicated that SNP in alfalfa was a significantly affected by location, year, cultivar/population and

year*location interaction (Table 3). Yenimahalle location and the second year had was a significantly higher SNP than Gölbaşı and the first year, respectively. A significantly change occurred in SNP in two locations by year effect. In the combined analysis, Savaş cultivar was highest SNP in all materials and it had similar group with advanced populations. Moreover, SNP in the study materials did significantly change over two locations. In this study, overall average of SNP was identified such as 21.7 number per plant (19.7-23.3 number per plant) (Table 3). TURAN et al., (2017) counted such as 29.9 -33.4 for SNP in alfalfa, their values were higher than the values of this study. This may be reasoned in different growing conditions and various genetic materials.

Dormancy score and status

Using of fall dormancy ratings is highly important to select proper dormancy for local climatic conditions to maximize fall growth and minimize spring frost damage or winter kill in cooler areas (POOLE et al., 2003). The study materials were measured semi-dormant, except the cultivar Savaş dormant type (Table 4). Dormant and semi dormant types are available easily to be grown in the Central Anatolia Region.

Agronomic traits

Green forage yield (GFY, t ha⁻¹)

The general variance analysis results emerged that there was a significant differences in GFY among the study materials, locations and years (Table 5). Mean value of GFY in Gölbaşı was

Table 2 - Stem diameters (mm) of alfalfa genotypes.

Cultivar/Population	Stem diameters								
	Yenimahalle			Gölbaşı			Two-location average		
	2014	2015	Ave	2014	2015	Ave	2014	2015	Ave
Bilensoy 80	3.5	3.3	3.4	3.4	4.2	3.8	3.5	3.7	3.6 A*
Gözlü	3.6	3.3	3.4	3.4	3.4	3.4	3.5	3.3	3.4 A
Savaş	2.6	2.9	2.8	2.3	3.9	3.1	2.5	3.4	3.3 BC
Plato	3.3	3.3	3.3	3.3	3.5	3.4	3.3	3.4	3.2 AB
L-1737	3.3	3.3	3.3	3.2	3.2	3.2	3.2	3.2	3.0 AB
L-1738	2.7	2.9	2.8	2.3	3.1	2.8	2.7	3.0	2.8 C
Mean	3.2 AB ²	3.1 B	3.2	2.9 B	3.5 A	3.3	3.1 B ¹	3.3 A	3.2

*The average values with the same letter in a column are not statistically different from each other according to the Duncan test at $P \leq 0.05$.

[†]The average values of locations with the same letter are not statistically different from each other at $P \leq 0.05$.

¹The average values of years with the same letter are not statistically different from each other at $P \leq 0.05$.

²The average values of location-year combinations with the same letter are not statistically different from each other according to the Duncan test at $P \leq 0.05$.

Table 3 - Stem numbers per plants of alfalfa genotypes.

-----Stem numbers per plants-----									
Cultivar/ Population	-----Yenimahalle-----			-----Gölbaşı-----			-----Two-location average-----		
	2014	2015	Ave.	2014	2015	Ave.	2014	2015	Ave.
Bilensoy 80	22.8	27.3	25.0 A*	18.6 AB	19.9	19.3 CD*	20.7	23.6	22.1 AB*
Gözlü	19.6	26.5	23.0 AB	17.3 BC	21.0	19.1 CD	18.4	23.7	21.1 BC
Savaş	16.9	30.5	23.7 AB	22.3 A	23.5	22.9 AB	19.6	27.0	23.3 A
Plato	20.8	28.1	24.4 A	13.0 C	16.8	14.9 E	16.9	22.4	19.7 C
L-1737	20.9	28.2	24.5 A	16.2 BC	20.0	18.1 D	18.6	24.1	21.3 A-C
L-1738	19.4	28.8	24.1 AB	20.2 AB	22.4	21.3 BC	19.8	25.6	22.7 AB
Mean	20.1 BC ²	28.2 A	24.1A+	17.9 C	20.6 B	19.3 B	19.0 B ¹	24.4 A	21.7

*¹The average values with the same letter in a column are not statistically different from each other according to the Duncan test at $P \leq 0.05$.

*²The average values of locations with the same letter are not statistically different from each other at $P \leq 0.05$.

¹The average values of years with the same letter are not statistically different from each other at $P \leq 0.05$.

²The average values of location-year combinations with the same letter are not statistically different from each other according to the Duncan test at $P \leq 0.05$.

statistically significant higher than that in Yenimahalle. The first year was statistically significant higher GFY than the second year. The advanced population, L-1737, gave statistically higher GFY than Plato, Savaş and Bilensoy-80. Other advanced population, L-1738, was higher GFY than Plato cultivar and so was similar GFY to Savaş and L-1737. Gölbaşı had higher GFY than Yenimahalle location. Gölbaşı location had higher total precipitation and average humidity than Yenimahalle location. At the same time average temperature in Gölbaşı became lower than Yenimahalle. Those favourable climatic conditions led to a significant increase in GFY. Overall average of GFY in this study were obtained such as 67.0 t ha^{-1} (60.3 to 73.0 t ha^{-1}).

The advanced populations had more green forage yield than the cultivars as Bilensoy-80 and Plato. Two experiments were conducted under Erzurum conditions with a continental climate and

two various results in GFY were determined such as 57.7 t ha^{-1} (ŞENGÜL, 1996) and 29.5 t ha^{-1} (ŞENGÜL & TAHTACIOĞLU, 1996). Moreover, some trials conducted in past, GFYs in alfalfa crop were detected such as 83.8 - 89.1 t ha^{-1} (AVCI et al., 2013), 83.59 t ha^{-1} (73.93 - 101.12 t ha^{-1}) (TUCAK et al., 2014), 37.9 t ha^{-1} (29.2 - 43.5 t ha^{-1}) (TURAN et al., 2017), 67.3 - 69.6 t ha^{-1} (ATIŞ et al., 2019) and 71.96 t ha^{-1} (ÇAÇAN et al., 2020). Results of this study were similar to the findings of ATIŞ et al., (2019) and ÇAÇAN et al., (2020). However they were lower than the findings of AVCI et al., (2013), and TUCAK et al., (2014), but higher than the findings of ŞENGÜL (1996), ŞENGÜL & TAHTACIOĞLU (1996), and TURAN et al., (2017).

Evaluations of influence of mass selection method

GFYs in the advanced populations of L-1737 and L-1738 were 69.2 and 67.4 t ha^{-1}

Table 4 - Dormancy score and status of tested cultivars and populations.

Cultivar/Population	Dormancy score	Dormancy status
Bilensoy 80	5.3	Semi-dormant
Gözlü	5.5	Semi-dormant
Savaş	2.2	Dormant
Plato	5.5	Semi-dormant
L-1737	4.8	Semi-dormant
L-1738	3.8	Semi-dormant

¹, respectively (Table 5). The averages of control cultivars, Bilensoy-80 (extensively grown, well known, first registered synthetic cultivar) and Plato (lowest yield value of all control cultivars) were 66.4 t ha⁻¹, 65.0 t ha⁻¹ and 60.3 t ha⁻¹ respectively. Those yield values were 4.2 and 1.5%; 6.5 and 3.7%; 14.7 and 11.7% higher in green forage yield of the advanced populations of L-1737 and L-1738 compared to the averages of control cultivars, Bilensoy-80 and Plato, respectively. Those increases in GHY could be accepted as a result of the mass selection method.

Dry matter yield (DMY, t ha⁻¹)

In the general variance analysis results, there wasn't a significant difference in DMY among the study materials, but a significant difference seemed between locations and within years (Table 6). Location*year interaction was also statistically significant. Yenimahalle and the first year had statistically significant higher DMY mean than Gölbaşı and the second year, respectively. DMY location values were the opposite of GFY values, which may be due to the moisture content in GFY. Overall average of DMY in this study materials had 13.5 t ha⁻¹ (12.9-14.3 t ha⁻¹). However, variation in DMY among the cultivars and populations was not statistically significant. The advanced populations gave as much dry matter yield as the cultivars. In the some previous studies, DMYs in alfalfa were determined such as 11.8 t ha⁻¹ (9.8-17.9 t ha⁻¹) (ŞENGÜL & TAHTACIOĞLU,1996); 11.4 t ha⁻¹ (10.4-12.2 t ha⁻¹) (ÜNAL et al., 2012); 13.1 t ha⁻¹

(7.8-16.2 t ha⁻¹) (TURAN et al., 2017); 15.1- 16.0 t ha⁻¹ (ATIŞ et al., 2019) and 24.19 t ha⁻¹ (ÇAÇAN et al., 2020). This study results were in full agreement with the findings of TURAN et al., (2017) and ŞENGÜL & TAHTACIOĞLU (1996). But they were lower than the results of ATIŞ et al. (2019) and ÇAÇAN et al., (2020). Moreover, they were also higher than the results of (ÜNAL et al., 2012).

Evaluations of influence of mass selection method

DMYs in the advanced populations of L-1737 and L-1738 were 12.2 and 12.1 t ha⁻¹, respectively (Table 6). The averages of control cultivars, Bilensoy-80 (extensively grown, well known, first registered synthetic cultivar) and Plato (the lowest yield value of all control cultivars) were 11.8 t ha⁻¹, 11.6 t ha⁻¹ and 11.4 t ha⁻¹, respectively. Those yield values were 2.4 and 1.6%; 4.3 and 3.4%; 6.5 and 5.6% higher in dry matter yield of the advanced populations of L-1737 and L-1738 compared to the averages of control cultivars, Bilensoy-80 and Plato, respectively. As a result, it can be easily said that the mass selection method causes the increases described above in DMY.

Quality properties

Crude protein content (CP, %):

No statistically significant difference seemed between study materials and years, but opposite status for locations in the general variance analysis results (Table 7). Yenimahalle was higher CP content in Gölbaşı. In Yenimahalle, the second

Table 5 - Green forage yields (t ha⁻¹) of alfalfa genotypes.

Cultivar/ Population	Green forage yields								
	Yenimahalle			Gölbaşı			Two-location average		
	2014	2015	Ave.	2014	2015	Ave.	2014	2015	Ave.
Bilensoy 80	59.0	58.7	58.9	71.6	70.5	71.0	65.3	64.6	65.0 BC*
Gözlü	66.6	60.5	63.6	90.9	73.9	82.4	78.8	67.2	73.0 A
Savaş	66.8	65.0	65.9	71.8	65.8	68.8	69.3	65.4	67.4 AB
Plato	67.8	52.8	60.3	58.6	62.2	60.4	63.2	57.5	60.3 C
L-1737	64.0	64.4	64.2	83.2	65.2	74.2	73.6	64.8	69.2 AB
L-1738	66.7	62.6	64.6	75.1	65.2	70.1	70.9	63.9	67.4 AB
Mean	65.2	60.7	62.9 B [†]	75.2	67.1	71.2 A	70.2 A [‡]	63.9 B	67.0

*The average values with the same letter in a column are not statistically different from each other according to the Duncan test at P ≤ 0.05.

†The average values of locations with the same letter are not statistically different from each other at P ≤ 0.05.

‡The average values of years with the same letter are not statistically different from each other at P ≤ 0.05.

§The average values of location-year combinations with the same letter are not statistically different from each other according to the Duncan test at P ≤ 0.05.

Table 6 - Dry matter yields (t ha⁻¹) of alfalfa genotypes.

-----Dry matter yields-----									
Cultivar/ Population	-----Yenimahalle-----			-----Gölbaşı-----			-----Two-location average-----		
	2014	2015	Ave.	2014	2015	Ave.	2014	2015	Ave.
Bilensoy 80	15.0	12.7	13.8	16.2	8.9	12.5	15.6	10.8	13.2
Gözlü	15.7	12.4	14.1	20.3	8.7	14.5	18.0	10.6	14.3
Savaş	15.9	12.1	14.0	16.6	8.6	12.6	16.2	10.4	13.3
Plato	15.7	12.4	14.0	14.2	9.4	11.8	15.0	10.9	12.9
L-1737	15.2	12.4	13.8	18.2	9.1	13.7	16.7	10.7	13.7
L-1738	16.1	12.2	14.1	16.9	9.2	13.1	16.5	10.7	13.6
Mean	15.6 B	12.4 C	14.0 A+	17.1 A	9.0 D	13.0 B	16.3 A ¹	10.7 B	13.5

³⁾The average values of locations with the same letter are not statistically different from each other at $P \leq 0.05$.

¹⁾The average values of years with the same letter are not statistically different from each other at $P \leq 0.05$.

²⁾The average values of location-year combinations with the same letter are not statistically different from each other according to the Duncan test at $P \leq 0.05$.

year had higher CP content than the first year, but no significant difference appeared between years in Gölbaşı. The general average for CP content in this study materials was 22.6%, and that ranged between 22.4% and 22.9%. As a result, the study materials were quite similar to each other in terms of CP content. Moreover, for this reason the cultivars and advanced populations were not also statistically different from each other. SARUHAN & KUŞVURAN (2011) reported that CP ratio in alfalfa changed related to the year effect (18.68% and 21.93%) and cultivar (17.4% and 22.67%). CP ratios in alfalfa were found such as 17.00% (15.95-18.21%) (ŞENGÜL et al.,

2003); 16.5% (ÇAÇAN et al., 2012); 18.5-19.4% (AVCI et al., 2013); 18.38-20.45% (İNAL, 2015); 16.60% in 2011 and 16.13% in 2012 (YILMAZ & ALBAYRAK, 2016); 24.2-26.1% (ENGİN & MUT, 2017); 18.88% (17.06-18.88%) (GÖKALP et al., 2017); 16.5-17.5% (TURAN et al., 2017); 19.6-19.9% (ATIŞ et al., 2019) and 23.0% (21.7-24.2%) (ÇAÇAN et al., 2020). When the results of this study are compared with the trial results mentioned above, they were lower than ENGİN & MUT (2017)'s, but higher than ŞENGÜL et al., (2003)'s; ÇAÇAN et al., (2012)'s; AVCI et al., (2013)'s; İNAL, (2015)'s; YILMAZ and ALBAYRAK (2016)'s; GÖKALP et

Table 7 - Crude protein contents (%) of alfalfa genotypes.

-----Crude protein contents-----									
Cultivar/ Population	-----Yenimahalle-----			-----Gölbaşı-----			-----Two-location average-----		
	2014	2015	Ave.	2014	2015	Ave.	2014	2015	Ave.
Bilensoy 80	22.4	23.4	22.9	22.3	21.6	21.9	22.3	22.5	22.4
Gözlü	22.6	23.5	23.0	21.9	21.9	21.9	22.2	22.7	22.4
Savaş	23.1	24.0	23.6	21.7	21.9	21.8	22.4	22.9	22.7
Plato	22.9	23.8	23.3	22.7	22.0	22.4	22.8	22.9	22.9
L-1737	23.3	24.5	23.8	21.9	21.7	21.8	22.6	23.0	22.8
L-1738	21.9	23.8	22.9	21.9	22.1	22.0	21.9	23.0	22.4
Mean	22.7 B ²	23.8 A	23.2 A+	22.1 C	21.8 C	22.0 B	22.4	22.8 ¹	22.6

³⁾The average values of locations with the same letter are not statistically different from each other at $P \leq 0.05$.

¹⁾The average values of years with the same letter are not statistically different from each other at $P \leq 0.05$.

²⁾The average values of location-year combinations with the same letter are not statistically different from each other according to the Duncan test at $P \leq 0.05$.

al., (2017)'s; TURAN et al., (2017)'s and ATIŞ et al. (2019)'s. In addition, The values of this study were similar to SARUHAN & KUŞVURAN (2011)'s and ÇAÇAN et al., (2020)'s.

Acid detergent fiber content (ADF, %):

In the variance analysis results over years and locations, significantly differences were found for ADF content among study materials, years, and locations. (Table 8). Moreover, only location*year interaction was statistically significant. When the average values of the two locations are considered, Gözlü gave the highest ADF content of all study materials. Gölbaşı and the first year had higher ADF content than Yenimahalle and the second year, respectively. Overall ADF content average in all study materials became 35.6% (varied from 34.7 to 36.3%). In Gölbaşı, Plato had the lowest ADF content of all study materials and the first year was significantly higher ADF content than the second year. In Yenimahalle, the first year became also higher ADF content. In the preceding studies, ADF contents in alfalfa were reported such as 33.50-36.94% (İNAL, 2015); 31.54% in 2011-34.99% in 2012 (YILMAZ & ALBAYRAK, 2016); 27.5- 29.7% (ENGIN & MUT, 2017); 26.9-27.0% (ATIŞ et al., 2019) 23.0% (20.9-24.7%) (ÇAÇAN et al., 2020). Considering references given above, this study values were the same as the values of İNAL (2015), and YILMAZ & ALBAYRAK (2016), but higher than the values of ENGIN & MUT (2017), ATIŞ et al., (2019), and ÇAÇAN et al., (2020).

Neutral detergent fiber content (NDF, %)

Considering the combined statistical variance analysis results, significant differences seemed among study materials, locations and years (Table 9). In addition to year*location interaction was statistically significant. Gölbaşı was significantly higher NDF content than Yenimahalle. The first year had also higher NDF content than the second year. Overall NDF content average in study materials had 46.5% (45.2-47.0%). All study materials had the same NDF contents, except Plato cultivar.

In Gölbaşı location, no significant difference appeared between years but opposite status occurred in Yenimahalle. In the some former studies, NDF contents in alfalfa were found as 45.73-47.46% (İNAL, 2015); 43.94% in 2011 and 43.48% in 2012 (YILMAZ & ALBAYRAK, 2016); 43.5% (41.9-45.8%) (ENGIN & MUT, 2017); 41.5% (ATIŞ et al. 2019); 38.6% (33.3-41.1%) (ÇAÇAN et al., 2020). This study values were in accordance with the values of İNAL, (2015), but higher than the values of YILMAZ & ALBAYRAK (2016), ENGIN & MUT (2017), ATIS et al., (2019), and ÇAÇAN et al., (2020).

Relative feeding value (RFV)

In overall variance analysis results, statistically significant difference seemed for RFV among study materials, locations and years (Table 10). In addition to these, only location*year interaction was statistically significant. The second year was also higher RFVs than the first year. As Gölbaşı had higher NDF and ADF contents, it took

Table 8 - Acid detergent fiber contents (%) of alfalfa genotypes.

Cultivar/ Population	-----ADF-----								
	-----Yenimahalle-----			-----Gölbaşı-----			-----Two-location average-----		
	2014	2015	Ave.	2014	2015	Ave.	2014	2015	Ave.
Bilensoy 80	36.4	34.4	35.4	36.7	36.1	36.4	36.5	35.3	35.9 AB*
Gözlü	36.3	34.7	35.5	37.0	37.1	37.1	36.6	35.9	36.3 A
Savaş	35.9	33.8	34.8	37.8	36.2	37.0	36.9	35.0	35.9 AB
Plato	35.9	33.4	34.6	34.8	34.9	34.8	35.3	34.1	34.7 C
L-1737	35.1	32.8	34.0	37.7	36.1	36.9	36.4	34.4	35.4 B
L-1738	36.1	33.6	34.8	37.3	35.3	36.3	36.7	34.4	35.6 AB
Mean	35.9 B ²	33.9 C	34.9 B ⁺	36.9 A	36.0 B	36.4 A	36.4 A ¹	34.9 B	35.6

*The average values with the same letter in a column are not statistically different from each other according to the Duncan test at $P \leq 0.05$.

⁺The average values of locations with the same letter are not statistically different from each other at $P \leq 0.05$.

¹The average values of years with the same letter are not statistically different from each other at $P \leq 0.05$.

²The average values of location-year combinations with the same letter are not statistically different from each other according to the Duncan test at $P \leq 0.05$.

Table 9 - Neutral detergent fiber contents (%) of alfalfa genotypes.

-----NDF-----									
Cultivar/ Population	-----Yenimahalle-----			-----Gölbaşı-----			-----Two-location average-----		
	2014	2015	Ave.	2014	2015	Ave.	2014	2015	Ave.
Bilensoy 80	47.2	45.3	46.3	47.1 A	47.8	47.5 A	47.2 A	46.6 A	46.9 A*
Gözlü	47.0	45.4	46.2	47.5 A	48.1	47.8 A	47.2 A	46.7 A	47.0 A
Savaş	47.0	44.7	45.8	48.4 A	47.5	48.0 A	47.7 A	46.1 AB	47.0 A
Plato	45.7	43.6	44.6	45.2 B	46.3	45.8 B	45.4 B	45.0 C	45.2 B
L-1737	45.9	43.5	44.7	48.1 A	47.3	47.7 A	47.0 A	45.4 BC	46.2 A
L-1738	47.1	44.4	45.7	47.5 A	46.4	47.0 A	47.3 A	45.4 BC	46.4 A
Mean	46.6 A ²	44.5 B	45.6 B+	47.3 A	47.2 A	47.3 A	47.0 A ¹	45.9 B	46.5

*¹The average values with the same letter in a column are not statistically different from each other according to the Duncan test at $P \leq 0.05$.

²The average values of locations with the same letter are not statistically different from each other at $P \leq 0.05$.

¹The average values of years with the same letter are not statistically different from each other at $P \leq 0.05$.

²The average values of location-year combinations with the same letter are not statistically different from each other according to the Duncan test at $P \leq 0.05$.

lower RFV than Yenimahalle location (Table 8 and 9). The Plato cultivar, having lower ADF and NDF contents, produced the highest RVF in all materials. The advanced population, L-1737, gave the second highest RFV in dry forage production among the materials. Common average for RFV had 124.4, and study materials changed from 121.8 to 129.0. RFV is well known as a product of dry matter intake ratio (DMI) and digestible dry matter ratio (DDM). The DMI and DDM are negatively correlated with NDF

content and ADF content in dry forage, respectively. Thus, higher NDF and ADF contents in dry forage reason lower DMI and DDM as well as lower RFV. The RFV value in dry forages over 151, between 150-125, 124-103, 102-87, 86-75, and less than 75 are categorized as prime, premium, good, fair, poor and rejected, respectively (KIRAZ, 2011). Considering these index values, Cultivar Plato and the advanced population, L-1737, are classified as premium, while the rest all of materials are as good. RFVs in alfalfa

Table 10 - Relative feeding values of alfalfa genotypes.

-----Relative feeding value-----									
Cultivar/ Population	-----Yenimahalle-----			-----Gölbaşı-----			-----Two-location average-----		
	2014	2015	Ave.	2014	2015	Ave.	2014	2015	Ave.
Bilensoy 80	120.8	129.1 B	124.9 BC*	120.5	119.9	120.2 D*	120.6	124.5	122.6 BC*
Gözlü	122.0	128.2 B	125.1 BC	119.3	117.8	118.6 D	120.7	123.0	121.8 C
Savaş	122.7	132.1 AB	127.4 AB	115.4	121.9	118.7 D	119.1	127.0	123.0 BC
Plato	125.1	135.7 A	130.4 A	129.3	125.6	127.5 AB	127.2	130.7	129.0 A
L-1737	125.9	137.1 A	131.5 A	116.5	121.3	118.9 D	121.2	129.2	125.2 B
L-1738	122.5	133.3 AB	127.9 AB	118.3	125.5	121.9 CD	120.4	129.4	124.9 BC
Mean	123.2 B ²	132.6 A	127.9 A+	119.9 C	122.0 BC	121.0 B	121.5	127.3	124.4

*¹The average values with the same letter in a column are not statistically different from each other according to the Duncan test at $P \leq 0.05$.

²The average values of locations with the same letter are not statistically different from each other at $P \leq 0.05$.

¹The average values of years with the same letter are not statistically different from each other at $P \leq 0.05$.

²The average values of location-year combinations with the same letter are not statistically different from each other according to the Duncan test at $P \leq 0.05$.

crop genotypes were measured in some previous alfalfa trials as 118.4-125.3 (INAL, 2015); 108-125 (AVCI et al., 2017); 154.2-154.6 (ATIŞ et al., 2019) and 173.8 (159.6- 208.1) (ÇAÇAN et al., 2020). If the reference values given above were assessed with this study values together, it seemed that this study values were similar to INAL (2015)'s and AVCI et al., (2017)'s, but were lower than ATIS et al., (2019)'s and ÇAÇAN et al., (2020)'s.

CONCLUSION

The results of the study are summarized as follows. Firstly, overall averages of PH, SD and SNP in alfalfa materials were found such as 78.9 cm; 3.2 mm and 21.7 numbers, respectively. The study materials were identified as semi-dormant, except the cultivar Savaş dormant type. Secondly, overall averages of GFY and DMY in alfalfa study genotypes were obtained such as 67.0 t ha⁻¹ and 13.5 t ha⁻¹, respectively. Thirdly, overall averages of CP, ADF and NDF contents in study genotypes was 22.6%, 35.5% and 45.6%, respectively. Moreover, general average for RFV became 124.4 in study materials. Lastly, it was also concluded that the two advanced populations, L-1737, and L-1738, had good adaptation ability, high yield and good quality performance under irrigated conditions in semiarid regions and could be used as commercial cultivars.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

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