

MORPHOLOGICAL, AGRONOMIC, AND QUALITY PROPERTIES OF THE TWO DEVELOPED POPULATIONS OF ALFALFA (*Medicago sativa* L.) UNDER NON-IRRIGATED CONDITIONS OF SEMIARID REGIONS

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ABSTRACT

Alfalfa is the most important forage crop in Turkey, but no alfalfa cultivar has been developed for non-irrigated conditions in the semiarid regions of Turkey. Hence, new cultivars are needed for using in rangeland improvement, artificial pasture establishment, and hay production. The two populations (L-533, and L-1739) were compared with the three control cultivars of alfalfa (Bilensoy-80, Savas and Kayseri) in two various regions of Turkey during two years. The general results showed that there were significant differences in the plant height, but no differences in stem diameter, and stem numbers among the studied genotypes. According to combined analysis results, the L-533 and Kayseri had the highest green and hay yields, but the Savas cultivar had the lowest. Moreover, as compared to the Bilensoy-80 cultivar, the L-533 and L-1739 populations produced higher green forage yields of 22.27 and 11.57%, respectively. Also, the same increase in hay yield was 18.30% and 10.13%, respectively. Excluding crude protein contents, there were statistical differences between genotypes for the acid detergent fiber, neutral detergent fiber, and digestible dry matter yield. Moreover, higher crude protein content and digestible dry matter yield were obtained from the L-533 and L-1739, but the L-533 had also the lowest neutral detergent fiber content relative to the other population, and cultivars. The results of the current study have demonstrated that the L-533, and L-1739 had high adaptation capability, yields, and quality performance under dryland conditions of a semi-arid region and could be used as commercial cultivars.

Keywords: Advanced population, alfalfa, crude protein, dry forage, green forage

INTRODUCTION

Alfalfa (*Medicago sativa* L.) with 32 million hectares growing area worldwide, is one of the most important forage legume plants for use in the hay, pasture, and silage by the livestock sector (Michaud et al., 1988; Acikgoz, 2021a). It improves soil fertility due to its ability to fixation atmospheric N (Latrach et al., 2014), protects soils that are sensitive to wind and water erosion (Abdelguerfi and Abdelguerfi-Laouar, 2002). Moreover, it is a high yielding, quality perennial legume (Xie et al., 2015; Thivierge et al., 2016; Acikgoz, 2021a). Alfalfa forage yield is highly dependent on some climatic factors, especially precipitation and temperature (Thivierge et al., 2016). Besides, the forage yield of alfalfa can change with soil type and precipitation (Hatfield et al., 2011; Lee et al., 2013), and the age of stands (Jefferson and Cutforth, 1997).

The genetic improvement of alfalfa with traditional plant breeding focuses on features such as forage yield,

abiotic and biotic stress resistances and feed nutritional value (Shi et al., 2017). Alfalfa cultivars registered in Turkey have been developed for irrigated conditions. Unfortunately, nothing has been improved for semi-arid conditions. In order to meet the increasing demand for human food, many artificial pastures have been built to increase pasture productivity, thus enabling higher amounts of forage production while simultaneously producing abundant, high-quality animal products (Huang et al., 2018). Alfalfa is widely cultivated worldwide, especially in water-constrained regions, due to its deep root system and capacity to absorb water from deeper soil layers (Sim et al., 2017). There is a broad range in hay yields in alfalfa that varies from 0.79 to 6.42 t ha⁻¹ (Chedjerat et al., 2016; Boe et al., 2020). Crude protein content, which is an important quality factor (Acikgoz, 2021c), varied between 11.9% and 26.1% (Karsli et al., 2002; Engin and Mut, 2017). These differences can be explained by genetic and environmental conditions such as different genotypes, growing seasons,

climate conditions, regions and years (Sabancı et al., 2013; Yılmaz and Albayrak, 2016; Gokalp et al., 2017; Liu et al., 2018).

Most of the rangeland areas in semi-arid regions in Turkey have degraded due to their overuse, consequently leaving them with inadequate quality and low quantity values (Gokkus, 2020). Hence, rangeland rehabilitation and management projects should be implemented urgently (Unal et al., 2012a). Establishing artificial pastures and increasing forage production areas will both decrease the pressure on rangelands and provide improvement opportunities (Tan and Yolcu, 2021). For this reason, cultivar breeding projects, having a rich and wide genetic basis, should be conducted, and many new registered cultivars should be developed.

The mass selection method has been commonly applied for cross-pollinated plants (Demir and Turgut, 1999; Acikgoz, 2021b). The aim of this method is to increase the frequencies of strongly wished traits in a population. The desired plants are selected among many genotypes, followed by these selected genotypes being harvested together and their seeds being mixed. However, this process takes a considerable amount of time (Robins and Jensen, 2020; Acikgoz, 2021b). The success of mass selection depends on the heritability of the trait in question, its visibility before pollination, and the mode of its inheritance (Demir and Turgut, 1999). The objectives of this study were (1) to identify the morphological and quality traits of the two advanced populations under non-irrigated condition, (2) to reveal the effects of mass selection by comparing the two developed populations with the control cultivars, (3) to search the interactions of year, and location over the studied genotypes.

MATERIALS AND METHODS

In the study, mass selection (Demir and Turgut, 1999) was implemented and two populations were formed between the years of 2000 and 2014, one being L-533, and the other being L-1739. The field experiments were carried out in two locations, Ankara-Golbasi, the locations of the Central Research Institute for the Field Crops, and Konya Bahri Dagdas Agricultural Research Institute in the years of 2015, 2016 and 2017 under non-irrigated conditions.

The soil properties of the Ankara Golbasi location was clay-loam, pH slightly alkaline (7.91), very poor (0.92%) in organic matter, medium (62.8 kg ha⁻¹) in phosphorus content, high (1789.8 kg ha⁻¹) in potassium content, very high (25.36%) in lime content (Anonymous, 2015a). The soil characteristics of the Konya location were clay with pH slightly alkaline (7.90), good (3.70%) in organic matter, very high (250.20 kg ha⁻¹) in phosphorus content, high (1590.1 kg ha⁻¹) in potassium content, very high (42.63%) in lime content (Anonymous, 2015b).

Total precipitation, average temperatures, and average relative humidity of the trial years of 2015, 2016, 2017, and long term averages were 537.2, 363.0, 229.8, and 399.4 mm; 10.6, 10.6, 9.9, and 12.5 °C; 64.0, 61.61, 59.9, and 58.88% at Golbasi, Ankara, respectively (Anonymous,

2018a). Total precipitation, average temperatures, and average relative humidity of the trial years of 2015, 2016, and 2017, were 284.2, 159.2, 363.6, and 324.3 mm; 11.4, 13.8, 10.6, and 11.5 °C; 64.5, 55.4, 64.8, and 59.7% at Konya, respectively (Anonymous, 2018b). When climatic values of the two regions were compared, total precipitation in Ankara had higher than that in 2015, and 2016 in Konya. The average temperature data in Konya were higher than those in Ankara. The averages of relative humidity were similar in 2015, while Ankara had higher than the values recorded in Konya in 2016 but lower in 2017.

Field experiments with two advanced alfalfa populations (L-533, and L-1739), and control cultivars (Bilensoy-80, Savas and Kayseri) were sown in a randomized complete block design (RCBD) with 4 replications between March 30 and April 2, 2015, in the provinces of Ankara and Konya. Seeds were sown by hand. The parcel size was 1.6 m x 5.0 m = 8.0 m² and consists of 8 rows with 20 cm intervals. The harvested parcel size was 4.8 m² (Anonymous, 2001). Before sowing, 30.0 kg ha⁻¹ nitrogen, and 100.0 kg ha⁻¹ phosphorus were applied soil surface and mixed into the soil.

As the generally accepted first date for plant development in the Central Anatolia Region is February 15, this date was considered the first plant development date in 2016 and 2017. Cuts were performed when 10% of the plants in the plot were at bloom. In 2016, alfalfa cutting dates were 25–31 May in Ankara and 30 April in Konya, and in the same provinces, they were determined to be 30 May–07 June and 21 May, respectively, in 2017.

10 plants were randomly chosen from each parcel for the measurement of the plant traits at the first cutting time in a growing season. Values for different morphological traits (plant height, stem number, stem diameter) and agronomic traits (green forage and hay yields) were recorded. Then, 4.8 m² of green forage from each plot was cut and the examples (500 g each) were dried at 70 °C for 48 hours. Yield values per hectare were obtained from converting plot data.

Quality traits were determined on first cutting materials of studied materials in 2016 in two regions. The samples were ground through a 1 mm screen in a Wiley mill and then used for the evaluation of quality traits. Dry matter (DM) and crude protein (CP) analyses were carried out in adherence to AOAC (1998). The contents of the neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined according to Van Soest et al. (1991) using an Ankom 2000 Fiber Analyzer (Ankom Technology Corporation, Fairport, NY, USA). Digestible dry matter yield (DDMY, kg ha⁻¹) was calculated by multiplying the dry matter yields per hectare of genotypes with the digestible dry matter ratios. Digestible dry matter ratio (DDMR) was also calculated through the use of the equation formulated as $DDMR = 88.9 - (0.779 \times ADF)$ using ADF ratios (Sheaffer et al., 1995).

Analysis of variance (ANOVA) was implemented for whole measured values with populations and cultivars as the principal factor for alfalfa genotypes in the Excel

software program of Microsoft Office 2010 (Avci, 2020). The significance of the major effects was determined using the F test. Differences among averages of studied materials were classified by the Least Significant Difference (LSD) test at a 5% level of probability (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Morphological Traits

According to combined variance analysis, significant differences were recorded for plant height among populations and cultivars, locations, years, and three binary interactions (Table 1). Combined analysis results showed that Bilensoy-80, Kayseri population, and L-533 population had similar plant heights and placed in the same first LSD group. Moreover, they were statistically higher in plant height than Savas, and L-1739 population. Since then, trials were conducted in Ankara and Konya for the following two years. Breeding studies were carried out in arid and semi-arid conditions in Ankara and two populations were developed. Therefore, Ankara had significantly higher plant heights than Konya based on two-year average data. During the second year at the two locations, plant height had become significantly higher than first year due to higher rainfall. The significant year x location interaction occurred, while plant height decreased in Ankara due to less rainfall in the second year, and the converse occurred in Konya. In the first year of the trial, the Bilensoy-80 variety was ranked first, and the Savas

variety was ranked the last. In the second year, the Kayseri population ranked first due to expected average rainfall, while the L-1739 population ranked last. The different responses of populations and cultivars to precipitation changes with years caused the genotype x year interaction to be significant. In the Ankara location, Bilensoy-80 came first and the L-533 population placed fourth. In the Konya location, Kayseri came first and Bilensoy-80 placed fourth. Although the Konya location receives less rainfall, the soil is richer in organic matter and phosphorus compared to Ankara. Genotypes have responded variously to the differences between provinces. This has caused the genotype x location interaction to be important. It has been reported that this wide range of variation in the plant heights of alfalfa depends on the genetic structure of the cultivars and the environmental conditions in which the plant grows (Demiroglu et al., 2008; Unal et al., 2012b; Dumlu et al., 2017; Gokalp et al., 2017). These findings can be enriched with sample studies as follows. Chamble and Warren (1990) and Prosperi et al. (1996) obtained similar plant height values, supporting the findings of this study. Comparisons with various previously conducted studies reveal that this experiment's results were lower than the data of Rosellini et al. (1991), Sengul and Sagsoz (2004), Unal et al. (2012b), Avci et al. (2013), Tucak et al. (2014), Turan et al. (2017), Gokalp et al. (2017). It is thought that this contradiction could potentially be explained by different genetic structures and environmental conditions.

Table 1. Results of statistical analysis of plant height (cm) for studied populations and cultivars of alfalfa at Ankara and Konya, Turkey, in 2016 and 2017

| Populations and cultivars (Genotypes) | Ankara | | | Konya | | | Two-location | | |
|--|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|--------------|
| | 2016 | 2017 | Ave. | 2016 | 2017 | Ave. | 2016 | 2017 | General Ave. |
| L-533 | 63.1 | 44.1 | 53.6 | 20.9 | 60.2 ab | 40.6 ab | 42.0 | 52.1 a | 47.1 a |
| L-1739 | 62.9 | 42.0 | 52.5 | 20.5 | 48.3 c | 34.4 c | 41.7 | 45.2 b | 43.4 b |
| Bilensoy-80 | 67.2 | 49.1 | 58.2 | 17.0 | 55.7 b | 36.4 c | 42.1 | 52.4 a | 47.3 a |
| Savas | 64.0 | 45.2 | 54.6 | 18.1 | 56.7 b | 37.4 bc | 41.0 | 50.9 a | 46.0 ab |
| Kayseri | 63.0 | 44.7 | 53.9 | 20.3 | 64.3 a | 42.3 a | 41.6 | 54.5 a | 48.1 a |
| <i>Mean</i> | <i>64.0 a</i> | <i>45.0 b</i> | <i>54.5 a</i> | <i>19.4 b</i> | <i>57.0 a</i> | <i>38.2 b</i> | <i>41.7 b</i> | <i>51.0 a</i> | <i>46.4</i> |
| F _(genotype) (0.05) | 2,6 | 0,7 | 1,7 | 2,8 | 6,9** | 6,7** | 0,3 | 3,3* | 3,1* |
| LSD (0.05) | - | - | - | - | 6,9 | 3,6 | - | 5,6 | 2,9 |
| F _(year) (0.05) | | | 164,8** | | | 1162,5** | | | 101,9** |
| F _(location) | | | | | | | 4639,2** | 48,6** | 312,5** |
| F _(year*location) (0.05) | | | | | | | | | 941,2** |
| F _(genotype*year) (0.05) | | | - | | | 5,8** | | | 2,8* |
| F _(genotype*location) (0.05) | | | | | | | 5,3** | - | 3,9** |
| F _(genotype*location*year) (0.05) | | | | | | | | | - |
| CV (%) | 3,4 | 13,8 | 8,6 | 10,4 | 7,8 | 9,2 | 8,6 | 10,6 | 8,9 |

*, **Significant at 5 and 1 % probability levels, respectively.

There were no significant differences for stem diameter among populations and cultivars in the general averages (Table 2). Conversely, a significant difference was observed among years, year x location ($p < 0.01$) and genotype x location ($p < 0.05$). In the year 2017, the stem diameter was higher than in 2016 due to higher rates of rainfall and higher biomass production. In the Ankara location, stem diameter was affected by the decrease in rainfall in the second year and became thinner. In the second year in the Konya location, stem diameter values

increased significantly due to higher rainfall and plant growth rates. This circumstance gave rise to the year x location interaction. Many researchers explained that this trait in alfalfa plants may vary due to environmental conditions (Simith et al., 1991; Demiroglu et al., 2008). In the Ankara location, which is barren and has less rainfall, the Savas variety and the L-1739 population had the thickest stem diameters, while the Kayseri population had the thinnest. The opposite occurred in the Konya location, which has bottom land and higher rainfall, the Kayseri

population was the highest, while the L-1739 population was the thinnest. This difference makes the genotype x location interaction significant. It has been previously established that this feature in the alfalfa plant may vary depending on genetic structure, soil properties, and climatic conditions (Sengul and Sagsoz, 2004; Demiroglu et al., 2008). As a result, the results of this study were lower than some previously conducted trials (Sengul and Sagsoz, 2004, Unal and Firincioglu, 2007, Unal et al., 2012b; Avci et al., 2013; Gokalp et al., 2017; Dumlu et al., 2017) and while also being higher than a piece of previous study data (Tucak et al., 2014). The leaf/stem ratio is one of the most

important factors influencing plant forage quality, and a high ratio is preferred. When the amount of stem, or crude cellulose, increases at this rate, it has a negative impact on and reduces the quality of the forage (Ozyigit and Bilgen, 2006; Budak and Budak, 2014; Acikgoz, 2021). Although a thin stem diameter is a desirable trait for palatability and animal preference (Acikgoz, 2021), it is not always preferred because it increases the risk of lying down (Kavut et al., 2009). This feature makes harvesting difficult and causes rot in plant parts that come into contact with the ground in humid conditions.

Table 2. Results of statistical analysis of stem diameter (mm) for studied populations and cultivars of alfalfa at Ankara and Konya, Turkey, in 2016 and 2017

| Populations and cultivars (Genotypes) | Ankara | | | Konya | | | Two-location | | |
|--|--------------|--------------|------------|--------------|--------------|------------|--------------|--------------|--------------|
| | 2016 | 2017 | Ave. | 2016 | 2017 | Ave. | 2016 | 2017 | General Ave. |
| L-533 | 2.2 | 2.3 | 2,3 | 1.9 | 2.7 bc | 2.3 a | 2.1 | 2.5 | 2.3 |
| L-1739 | 2.6 | 2.1 | 2,4 | 1.8 | 2.5 c | 2.1 b | 2.2 | 2.3 | 2.2 |
| Bilensoy-80 | 2.3 | 2.0 | 2,2 | 1.7 | 3.0 ab | 2.4 a | 2.0 | 2.5 | 2.3 |
| Savas | 2.7 | 2.1 | 2,4 | 1.8 | 2.9 ab | 2.4 a | 2.3 | 2.5 | 2.4 |
| Kayseri | 1.6 | 2.2 | 1.9 | 1.9 | 3.1 a | 2.5 a | 1.7 | 2.6 | 2.2 |
| <i>Mean</i> | <i>2.3 a</i> | <i>2.1 b</i> | <i>2,2</i> | <i>1.8 b</i> | <i>2.8 a</i> | <i>2.3</i> | <i>2.1 b</i> | <i>2.5 a</i> | <i>2.3</i> |
| F _(genotype) (0.05) | 2.0 | 0.6 | 1.5 | 1.0 | 6.5** | 4.1* | 1.5 | 1.7 | 0.7 |
| LSD (0.05) | - | - | - | - | 0.2 | 0.2 | - | - | - |
| F _(year) (0.05) | | | | | | | | | 26.8** |
| F _(location) | | | | | | | 9.4** | 74.9** | - |
| F _(year*location) (0.05) | | | | | | | | | 47.6** |
| F _(genotype*year) (0.05) | | | | | | 3.4* | | | - |
| F _(genotype*location) (0.05) | | | | | | | - | - | 2.8* |
| F _(genotype*location*year) (0.05) | | | | | | | | | - |
| CV (%) | 27.1 | 14.6 | 22.2 | 9.5 | 6.1 | 7.4 | 22.1 | 10.2 | 16.2 |

*, **Significant at 5 and 1 % probability levels, respectively.

The combined analysis results indicated that no significant difference for stem numbers were observed among populations and cultivars, but there were significant differences among years, locations, genotype x year interactions, and genotype x location x year interactions (Table 3). The combined analysis results showed that the 2017 year had a significantly higher stem number than the 2016 due to higher rainfall and plant production. Additionally, the same features caused the Konya location to have a statistically higher number of stems than Ankara. During the first year of the trial, when there was less rainfall, the L-1739 population was ranked first, and the Savas variety was ranked last. In the second year, the L-533 population placed first, while the L-1739 variety placed last. Due to the fact that different responses of materials to precipitation changes over time, the genotype x year interaction was significant. As the varieties and populations respond differently in different years and locations, their rankings have shifted. Subsequently, the genotype x location x year interaction became significant. Some previous trial results support these trial findings (Sengul and Sagsoz, 2004; Yilmaz, 2011; Gokalp et al., 2017). However, this trial value was lower than the data obtained by Turan et al. (2017). This difference was caused by

differences in genetic structures and growing conditions (Demiroglu et al., 2008).

Agronomic traits

Combined variance analysis results indicated that the significant differences had occurred in green forage yield among the populations and cultivars, locations and years, the interactions of year x location, genotype x year and genotype x location x year (Table 4). The general analysis results demonstrated that Kayseri and the L-533 produced the highest green forage yields due to Kayseri's good adaptability and L-533 being developed for semi-arid conditions (Table 5). However, the Savas cultivar had the lowest green forage yield. The Konya region, which received sufficient rainfall and hosts a soil structure that is rich in organic matters and phosphorus, had a higher green forage yield compared to Ankara. The yield of green forage was higher in 2017 than in 2016. This can be attributed to two reasons. Firstly, there was more rainfall in 2017. Secondly, the increased rainfall significantly improved the productivity of the fertile base land.

Depending on the amount of annual precipitation, green forage yield varied in Ankara and Konya during the trial years (Anonymous, 2018a) and (Anonymous, 2018b). In

the first year of the experiment, the Ankara location received normal annual precipitation, while the Konya location received below-average annual precipitation. This caused the green forage yield to be higher in the Ankara location during the first year. In the second year, while Konya received average rainfall, rainfall in Ankara remained below average. For this reason, the Konya location produced higher green forage yields. As a result, in both locations, the location with less than average annual rainfall produced lower yields than the location with average rainfall. Additionally, the Ankara location trial area is barren whereas the Konya location trial area is established in soil with rich organic matter and phosphorus. In this case, the high soil depth and high groundwater in the base area also gave the ability to easily meet the water needs of plants with deep taproots, such as alfalfa (Anonymous, 2015b). Therefore, the year x location interaction was significantly different. Due to the rich soil structure of the base area, the Kayseri population gave the highest yield in semi-arid conditions. Under the same conditions, the L-533 population, developed for forage purposes in semi-arid areas, ranked second in terms of green forage yield, and the L-1739 population ranked last. Population L-1739 produced the highest green forage yield

in uneven fields under semi-arid conditions. As can be observed, the presence of numerous varieties in different rows in different years has revealed the importance of the genotype x year interaction. The difference between genotypes, years (2049.0 and 4484.0 kg ha⁻¹ in 2016 and 2017, respectively), and locations (2647.0 and 3886.0 kg ha⁻¹ in Ankara and Konya, respectively) was found to be statistically significant in all four development periods in this study, and as a result, the triple interaction (genotype x location x year) was also found to be significant. When compared to Bilensoy-80, L-533, and L-1739 had 22.27 and 11.57% higher green forage yield in general averages, respectively. The yield of alfalfa, which has adapted to many ecologies around the world, can vary due to its wide range of genetic variations and ecological differences (Lowe et al., 1972; Jun et al., 2014). Green forage yield decreases when alfalfa is grown without irrigation in semi-arid conditions (Acikgoz et al., 1984; Inal, 2015). As a natural result of this circumstance, the current research value was found to be lower than those of Sengul and Tahtacioglu (1996), Cocu and Sancak (2007), Unal et al. (2012b), Avci et al. (2013), Tucak et al. (2014), Turan et al. (2017), Atis et al. (2019), and Cacan et al. (2020).

Table 3. Results of statistical analysis of stem number (number/plant) for studied populations and cultivars of alfalfa at Ankara and Konya, Turkey, in 2016 and 2017

| Populations and cultivars (Genotypes) | Ankara | | | Konya | | | Two-location | | General Ave. |
|--|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|--------------|
| | 2016 | 2017 | Ave. | 2016 | 2017 | Ave. | 2016 | 2017 | |
| L-533 | 11.8 a | 11.9 | 11.9 | 13.9 b | 19.6 | 16.7 | 12.8 b | 15.7 | 14.3 |
| L-1739 | 11.1 ab | 13.6 | 12.4 | 19.5 a | 15.1 | 17.3 | 15.3 a | 14.4 | 14.8 |
| Bilensoy-80 | 8.8 c | 11.3 | 10.1 | 15.1 b | 17.9 | 16.5 | 12.0 b | 14.6 | 13.3 |
| Savas | 9.5 bc | 12.6 | 11.1 | 14.3 b | 18.6 | 16.5 | 11.9 b | 15.6 | 13.8 |
| Kayseri | 8.8 c | 11.9 | 10.4 | 15.8 b | 19.3 | 17.5 | 12.3 b | 15.6 | 13.9 |
| <i>Mean</i> | <i>10.0 b</i> | <i>12.3 a</i> | <i>11.2 b</i> | <i>15.7 b</i> | <i>18.1 a</i> | <i>16.9 a</i> | <i>12.9 b</i> | <i>15.2 a</i> | <i>14.0</i> |
| F _(genotype) (0.05) | 4.3 * | 2.1 | 4.6 | 3.9* | 2.0 | 0.3 | 4.8 ** | 0.85 | 1.49 |
| LSD _(0.05) | 2.0 | - | - | - | - | - | - | - | - |
| F _(year) (0.05) | | | 31.9** | | | 10.2** | | | 30.2** |
| F _(location) | | | | | | | 98.8 ** | 88.5** | 186.5** |
| F _(year*location) (0.05) | | | | | | | | | - |
| F _(genotype*year) (0.05) | | | - | | | 5.4** | | | 3.8** |
| F _(genotype*location) (0.05) | | | | | | | 3.3 * | 3.2* | - |
| F _(genotype*location*year) (0.05) | | | | | | | | | 5.4** |
| CV (%) | 13.1 | 9.9 | 11.3 | 14.1 | 13.7 | 13.9 | 14.1 | 12.9 | 13.4 |

*, **Significant at 5 and 1 % probability levels, respectively.

A significant difference was observed in hay yield values among the studied populations and cultivars, years, locations, year x location interaction, and genotype x year interaction. However, no significant difference seemed on the interactions of genotype x location, and genotype x location x year (Table 5). According to general averages, the L-533 and Kayseri produced the highest hay yields (Table 5). Savas cultivar had the lowest hay yield in general averages. Both materials produced high hay yields due to Kayseri's adaptability and L-533 being developed for semi-arid conditions. The yield of hay in 2017 was greater than that in 2016. There are two reasons for this: the first is that there was more rainfall in 2017, and the second is that the

rainfall significantly increased the productivity of the fertile base land. When sufficient rainfall is received and the soil structure is rich in organic matter and phosphorus, hay yield naturally increases. Thus, the yield of hay in Konya was higher than that in Ankara. Hay yield was higher in Ankara in 2016, but higher in Konya in 2017. This dichotomy caused a significant year x location interaction. While the L-1739 population had the highest hay yield in 2016, the Bilensoy-80 cultivar had the lowest. However, in 2017, the Kayseri population produced the highest hay yield, while the Savas cultivar also had the lowest. These different performances of the studied populations and cultivars seen in different years have caused the genotype

x year interaction to be significant. Relative to Bilensoy-80, the L-533 and the L-1739 had 18.30 and 10.13% higher hay yield in general averages, respectively. It has been reported by many researchers that the differences in hay yield are caused by the genetic structures of the populations and varieties, as well as the climate and environmental conditions (Hill and Baylor, 1983; Simith et al., 1991; Jia

et al., 2006). As can be understood from these explanations, a difference between the experiments conducted under irrigated and non-irrigated conditions is expected. Therefore, the results of this study were lower than those of Sengul and Tahtacioglu (1996), Unal et al. (2012b), Yilmaz and Albayrak (2016), Dumlu et al. (2017), Turan et al. (2017), Atis et al. (2019), and Cacan et al. (2020).

Table 4. Results of statistical analysis of the green forage yields (kg ha⁻¹) for studied populations and cultivars of alfalfa at Ankara and Konya, Turkey, in 2016 and 2017

| Populations and cultivars (Genotypes) | Ankara | | | Konya | | | Two-location | | General Ave. |
|--|-----------------|-----------------|-----------------|-----------------|------------------|------------------|-----------------|------------------|---------------|
| | 2016 | 2017 | Ave. | 2016 | 2017 | Ave. | 2016 | 2017 | |
| L-533 | 7706.0 ab | 8031.0 b | 7869.0 b | 4324.0 a | 24319.0 ab | 14322.0 | 6014.9 a | 16175.0 ab | 11095.0 a |
| L-1739 | 8469.0 a | 9966.0 a | 9218.0 a | 4217.0 b | 19207.0 c | 11712.0 | 5659.0 a | 14586.0 bc | 10123.0 ab |
| Bilensoy-80 | 5250.0 cd | 6763.0 bc | 6007.0 c | 3372.0 ab | 20889.0 bc | 12131.0 | 5407.85 a | 13826.0 bc | 9069.0 bc |
| Savas | 4263.0 d | 5102.0 c | 4683.0 d | 2871.0 b | 19258.0 c | 11065.0 | 4311.1b | 12180.0 c | 7873.0 c |
| Kayseri | 6599.0 bc | 6193.0c | 6396.0 c | 2849.0 b | 28437.0 a | 15643.0 | 3566.8b | 17315.0 a | 11361.0 a |
| <i>Mean</i> | <i>6457.0 b</i> | <i>7211.0 a</i> | <i>6834.0 b</i> | <i>3527.0 b</i> | <i>22422.0 a</i> | <i>12974.0 a</i> | <i>6834.0 b</i> | <i>14816.0 a</i> | <i>9904.0</i> |
| F _(genotype) (0.05) | 8.75 ** | 10.64** | 18.35** | 4.86 ** | 6.38** | 8.30** | 9.36 ** | 5.79** | 10.45** |
| LSD _(0.05) | 1790.0 | 1770.0 | 1192.5 | 990.0 | 4820.0 | 2330.0 | 972.3 | 2430.0 | 1280.0 |
| F _(year) (0.05) | | | 4.2* | | | 699.4** | | | 234.1** |
| F _(location) | | | | | | | 96.7 ** | 416.7** | 599.5** |
| F _(year*location) (0.05) | | | | | | | | | 511.1** |
| F _(genotype*year) (0.05) | | | - | | | 4.2** | | | 10.3** |
| F _(genotype*location) (0.05) | | | | | | | 6.31 ** | 7.98** | - |
| F _(genotype*location*year) (0.05) | | | | | | | | | 5.1** |
| CV (%) | 18.1 | 15.8 | 16.9 | 18.2 | 13.9 | 17.4 | 18.8 | 15.9 | 18.1 |

*, **Significant at 5 and 1 % probability levels, respectively.

Table 5. Results of statistical analysis of the hay yields (kg ha⁻¹) for studied populations and cultivars of alfalfa at Ankara and Konya, Turkey, in 2016 and 2017

| Populations and cultivars (Genotypes) | Ankara | | | Konya | | | Two-location | | General Ave. |
|--|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|---------------|
| | 2016 | 2017 | Ave. | 2016 | 2017 | Ave. | 2016 | 2017 | |
| L-533 | 2906.0 a | 2890.0 ab | 2898.0 b | 1936.0 | 6734.0 ab | 4335.0 ab | 2421.5 a | 4812.0 ab | 3617.0 a |
| L-1739 | 3261.0 a | 3519.0 a | 3390.0 a | 1258.0 | 5420.0 b | 3339.0 c | 2259.5 a | 4470.0 abc | 3365.0 ab |
| Bilensoy-80 | 2120.0 b | 2756.0 b | 2438.0 c | 1567.0 | 5789.0 b | 3678.0 bc | 1843.1 b | 4273.0 bc | 3058.0 b |
| Savas | 1632.0 b | 1978.0 c | 1805.0 d | 1314.0 | 5639.0 b | 3477.0 c | 1473.0 c | 3809.0 c | 2641.0 c |
| Kayseri | 2752.0 a | 2652.0 b | 2702.0 bc | 1738.0 | 7462.0 a | 4600.0 a | 2245.1 a | 5057.0 a | 3651.0 a |
| <i>Mean</i> | <i>2534.0 a</i> | <i>2759.0 b</i> | <i>2647.0 b</i> | <i>1563.0 b</i> | <i>6209.0 a</i> | <i>3886.0 a</i> | <i>2049.0 b</i> | <i>4484.0 a</i> | <i>3266.0</i> |
| F _(genotype) (0.05) | 12.6 ** | 6.6** | 17.1** | 2.7 | 3.4* | 4.9** | 9.4 ** | 3.5* | 8.7** |
| LSD _(0.05) | 570.0 | 660.0 | 410.0 | - | 1430.0 | 724.0 | 366.8 | 740.0 | 400.0 |
| F _(year) (0.05) | | | - | | | 438.4** | | | 94.2** |
| F _(location) | | | | | | | 74.6 ** | 226.5** | 364.0** |
| F _(year*location) (0.05) | | | | | | | | | 299.8** |
| F _(genotype*year) (0.05) | | | - | | | - | | | 7.1** |
| F _(genotype*location) (0.05) | | | | | | | 6.6 ** | 4.3** | - |
| F _(genotype*location*year) (0.05) | | | | | | | | | - |
| CV (%) | 14.5 | 15.6 | 15.1 | 21.9 | 14.9 | 18.0 | 17.3 | 16.1 | 17.4 |

*, **Significant at 5 and 1 % probability levels, respectively.

Quality properties

No significant difference was observed for crude protein content among the populations and cultivars. In contrast, a significant difference was observed between locations (Table 6). In 2016, the Ankara location received normal rainfall, whereas the Konya location received less rainfall than the average, causing a build-up of stress in the plants. As a result of this circumstance, the Konya location had relatively lower amounts of crude protein content (Liu et al., 2018). The studied materials demonstrated nearly

similar crude protein content. The average value of this study (14.44%) was lower than data of Avci et al. (2010), Tongel and Ayan (2010), Cacan et al. (2012), Avci et al. (2013), Yilmaz and Albayrak (2016), Engin and Mut (2017), Gokalp et al. (2017), Turan et al. (2017), Atis et al. (2019), and Cacan et al. (2020). The differences in the literature regarding crude protein ratios can be derived from the cultivars used in the experiments, the ecological conditions of the studies, the delay in harvest time, and the differences in total precipitation and temperature during vegetation (Engin and Mut, 2017). Less rainfall leads to

reduced plant biomass production while simultaneously causing the leaf/stem ratio to shrink. A higher amount of stem within the total biomass amount reduces the leaf ratio (Table 3). In this case, the protein ratio also decreases

(Monirifar, 2011) (Table 6). Additionally, environmental factors (Yilmaz and Albayrak, 2016), especially drought stress, greatly impact crude protein, reducing its content (Liu et al., 2018).

Table 6. Results of statistical analysis of crude protein contents (%) and acid detergent fiber (%) for studied populations and cultivars of alfalfa at Ankara and Konya, Turkey, in 2016

| Populations and cultivars (Genotypes) | Crude protein | | | Acid detergent fiber | | |
|---|---------------|---------------|-------------|----------------------|---------------|--------------|
| | Ankara | Konya | Ave. | Ankara | Konya | General Ave. |
| L-533 | 16.7 | 13.1 | 14.9 | 26.3 a | 35.8 | 31.1 a |
| L-1739 | 16.5 | 12.6 | 14.5 | 27.9 a | 34.1 | 31.0 a |
| Bilensoy-80 | 16.1 | 12.6 | 14.3 | 27.2 a | 32.4 | 29.8 a |
| Savas | 16.4 | 11.9 | 14.2 | 22.9 b | 32.8 | 27.8 b |
| Kayseri | 16.3 | 12.1 | 14.2 | 26.5 a | 33.5 | 30.0 a |
| <i>Mean</i> | <i>16.4 a</i> | <i>12.4 b</i> | <i>14.4</i> | <i>26.2 b</i> | <i>33.7 a</i> | <i>29.9</i> |
| F _(genotype) (0.05) | 0.4 | 1.1 | 0.9 | 3.5* | 2.4 | 3.8* |
| LSD (0.05) | - | - | - | 3.2 | - | 1.9 |
| F _(location) | | | 181.7** | | | 166.7** |
| F _(genotype*location) (0.05) | | | - | | | - |
| CV (%) | 4.1 | 7.7 | 6.4 | 7.8 | 4.9 | 6.1 |

*, **Significant at 5 and 1 % probability levels, respectively.

Significant differences were determined in acid detergent fiber content among populations and cultivars, and locations (Table 6). All materials possessed similar acid detergent fiber content, excluding the Savas variety. In the Konya location, where stress conditions prevail, the decrease in total biomass and the increase in the stem (cellulose, hemicellulose and lignin) ratio in total biomass increased the acid detergent fiber content (Monirifar, 2011). This circumstance resulted the Konya location

having higher acid detergent fiber content than Ankara. The average data of this study (29.97%) was similar to Engin and Mut (2017)'s, while being larger than Atis et al. (2019)'s, and Cacan et al. (2020)'s, but lower than Avcı et al. (2010)'s, and Yılmaz and Albayrak (2016)'s. Consequently, it can be observed that this trait varies according to materials (populations and cultivars), growing conditions, and environmental conditions.

Table 7. Results of statistical analysis of neutral detergent fiber (%) and digestible dry matter yield (kg ha⁻¹) for studied populations and cultivars of alfalfa at Ankara and Konya, Turkey, in 2016

| Populations and cultivars (Genotypes) | Neutral detergent fiber | | | Digestible Dry Matter Yield | | |
|---|-------------------------|---------------|-------------|-----------------------------|----------------|---------------|
| | Ankara | Konya | Ave. | Ankara | Konya | General Ave. |
| L-533 | 36.3 b | 45.3 | 40.7 b | 1636.0 ab | 984.0 | 1310.0 a |
| L-1739 | 41.4 ab | 46.0 | 43.7 ab | 1777.0 a | 672.0 | 1225.0 a |
| Bilensoy-80 | 45.9 a | 45.2 | 45.5 a | 1071.0 cd | 819.0 | 945.0 bc |
| Savas | 37.1 b | 45.0 | 41.0 b | 928.0 d | 695.0 | 811.0 c |
| Kayseri | 44.9 a | 44.1 | 44.5 a | 1338.0 bc | 925.0 | 1132.0 ab |
| <i>Mean</i> | <i>41.1 b</i> | <i>45.1 a</i> | <i>43.1</i> | <i>1350.0 a</i> | <i>819.0 b</i> | <i>1085.0</i> |
| F _(genotype) (0.05) | 4.8* | 0.9 | 4.1* | 11.3** | 2.36 | 4.77** |
| LSD (0.05) | 6.2 | - | 3.1 | 330.0 | - | 270.0 |
| F _(location) | | | 17.7** | | | 40.3** |
| F _(genotype*location) (0.05) | | | 4.9** | | | 3.7* |
| CV (%) | 9.7 | 3.1 | 6.9 | 15.8 | 21.8 | 24.4 |

*, **Significant at 5 and 1 % probability levels, respectively.

General variance analysis results indicated that significant differences were demonstrated in neutral detergent fiber among populations and cultivars, locations, and genotype x location interaction (Table 7). While Bilensoy-80 and Kayseri had the highest neutral detergent fiber content, the Savas cultivar had the lowest value. This difference is due to the genetic constitution of a plant and the different responses they have to environmental factors (Julier et al., 2000). The aforementioned conditions for acid detergent fiber also apply to neutral detergent fiber content. Therefore, Konya had more neutral detergent fiber content. Since Bilensoy-80 and Kayseri had the highest neutral detergent fiber in Ankara, L-533 had the lowest. Kayseri had the lowest neutral detergent fiber in Konya, whereas L-1739 had the highest. This variation in genotypes' responses in regions resulted in a significant genotype x location interaction. This finding of the study (43.13%) was similar to Yilmaz and Albayrak (2016)'s; Engin and Mut (2017)'s; Atis et al. (2019)'s and Cacan et al. (2020)'s, but lower than Avci et al. (2010)'s.

A significant difference in digestible dry matter yield was observed among populations and cultivars, locations, and genotype x location interaction (Table 7). When observing general averages, due to different genetic compositions and different responses to environmental factors (Monirifar, 2011), the L-533 and L-1739 populations had the highest digestible dry matter yield among all genotypes, while the Savas cultivar had the lowest. In the Ankara location, a significant difference in digestible dry matter yield was determined among genotypes, but not in Konya. Additionally, Ankara had higher digestible dry matter yield than Konya in 2016 due to higher plant production and leaf/stem ratio (Monirifar, 2011; Annicchiarico, 2015). While L-1739 had the highest digestible dry matter yield in Ankara, Savas had the lowest. L-533 had the highest digestible dry matter yield in Konya, whereas L-1739 had the lowest. These differences in regions are rooted in a significant genotype x location interaction.

CONCLUSION

In this breeding study, two populations (L-533 and L-1739) as commercial cultivars were developed through the use of the mass selection method for use in the improvement of rangelands, the establishment of artificial pasture, and the production of quality hay in non-irrigated conditions. General analysis results demonstrated that the L-533 and Kayseri produced the highest green forage and hay yields among all genotypes. According to the general averages, L-533 and L-1739 populations had 22.27 and 11.57% higher green forage yields, respectively, compared to the Bilensoy-80 variety. Additionally, the same increase in hay yield was 18.30 and 10.13%, respectively. All studied populations and cultivars had nearly similar crude protein contents in general averages. The Savas cultivar was the lowest among all genotypes in terms of acid detergent fiber content. The L-533 population and Savas cultivar contained less neutral detergent fiber than the others. In addition, the L-533 and L-1739 populations had the highest digestible dry matter yield among all genotypes.

As a result, the two advanced populations had high adaptation capabilities, yields, and high quality performance under non-irrigated conditions in semiarid regions so these populations could be used as commercial cultivars. The findings of this study are very valuable for non-irrigated conditions, and could be used as a basis for future studies.

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