

Full Length Research Paper

Development of new alfalfa cultivar suitable for the dry continental conditions of the central Anatolia, Turkey

Derya Guloglu

Süleyman Demirel Üniversitesi, Isparta, Bitkisel ve Hayvansal Üretim Bölümü, Aksu Mehmet Süreyya Demiraslan Meslek Yüksekokulu, Turkey. E-mail: deryaguloglu@sdu.edu.tr. Tel: 00905069542080.

Accepted 26 June, 2011

Cultivated alfalfa (*Medicago sativa* L.) is an important forage legume, grown for hay in Turkey. There is need to increase the area of forage under cultivation in Turkey and to exploit all available germplasm for increased animal production. Alfalfa can be harvested for 4 to 5 years before the stand deteriorates. The study reports a recurrent selection from 160 plants collected from seven provinces of the Central Anatolia, with aim to breed superior cultivar resistant to dry conditions. Evaluation of yield and yield components showed that newly developed cultivar averaged 11% more yield; which was significantly higher ($p < 0.05$) compared to the best cultivar currently used in central Anatolia.

Key words: *Medicago sativa* L., Kayseri alfalfa, stem number per plant, floret number per raceme, pod set, spiral number per pod, thousand seed weight.

INTRODUCTION

Cultivated alfalfa (*Medicago sativa* L., $2n = 4x = 32$) is a tetraploid perennial, open pollinated legume with polysomic inheritance (Barnes et al., 1988). It is the most important forage legume grown for hay, dehydrated forage, pellets, silage and grazing. Alfalfa residues increase soil organic matter. Its root system mobilize nutrients deep within the soil profile, improves soil structure, permeability to water and water retention capacity. Furthermore, alfalfa cultivation is cheap compared to other forage legumes (Sevimay et al., 2005a,b) and requires low inputs of herbicides and pesticides and no N fertilizers due to N-fixation by the symbiont *Sinorhizobium meliloti*, and it can be harvested for up to 4 to 5 years before the stand deteriorates. Anatolian farmers are poor and face problems in feeding their animals due to scarce availability of forage during dry summers. This makes it necessary to develop drought hardy cultivars that are generally based on selection of open pollinated plants within field nurseries of genotypes surviving severe droughts.

The selection experiments must be replicated to ensure that spatial and temporal variability of environmental conditions allow adequate screening of plants meeting the objectives. Development of a variety with superior adaptation to drought and high temperatures would significantly assist the farmers during harsh summers to feed their animals. Turkey is very rich in *Medicago* germplasm: out of 60 alfalfa species, 30 are found in

Turkey. There is need to increase forage area under cultivation in Turkey and exploit all available germplasm for increased animal products. This study reports a recurrent selection protocol using genetically diverse material under field conditions to develop improved alfalfa populations for their tolerance to dry conditions of central Anatolia using 7 elite genotypes selected from 160 clones collected from seven provinces of the central Anatolian region; with aim to breed superior field clones of alfalfa with subsequent effects on biomass production that could mark the beginning of a significant shift in the agricultural systems of this region.

MATERIALS AND METHODS

The material used in this research includes 7 clones collected during 2002, from seven provinces of Central Anatolia region. These were selected for fast growth, rooting and large leaves from among 160 clones. A Polycross was conducted among above mentioned seven clones during 2003 by open pollination through insects, and wind, etc. The seed from each plant was collected and sown in 8 lines each of 5 m length, with seed rate of 20 kg/ha. Out of these plants, 50 clones per plant were established in wooden boxes (containing sand in greenhouse) for rooting, after removing leaves. The temperature of glasshouses was maintained to 18 to 20°C and after care was taken by irrigating them routinely as and when required. These clones rooted easily in 2 to 3 weeks and were transferred to plastic tubes containing sand clay and peat (1:1:1) for further growth and development. These

were transferred to plots on 23 April 2006. Each 2 × 5 m plot contained 10 plants in randomised complete block design with three replications (10 clones × 3 replications = 30 clones for each line) and were allowed random pollinate. The following parameters were recorded on each of the 7 genotypes:

Plant height (cm)

The height of each clone in each plot was measured from the soil surface to the tip of the tallest shoot of each plant at the time of seed harvest on August 18, 2006 and August 20, 2007.

Number of shoots per clone

Number of shoots per clone were counted by counting all shoots that developed above root crown (Gulcan, 1974).

Number of flowers per raceme

Number of flowers per cluster in each were measured by counting the number of flowers on main stalk, which were marked using a thread (Sevimay, 1992).

Number of fruits per raceme

Frequency of fruiting and number of fruiting and non fruiting flowers were counted on the marked clusters following Tamkoç (1985) and Sevimay (1992).

Number of wrinkles per seed

Number of wrinkles per seed was also determined (Tamkoç, 1985). (Sevimay, 1992).

Number of seeds per fruit

Number of seeds per fruit was also determined (Sevimay, 1992).

Number of fruits per plant

Seed yield per plant was also determined by weighing them on sensitive weight balance (Sevimay, 1992).

1000 seed weight

1000 seed weight of seeds from each clone in each plot and replication was also determined.

RESULTS AND DISCUSSION

Plant length

Data concerning plant height is presented in Table 2 statistical analysis of the data revealed insignificant variation for plant height between the years. However, taller plants were produced during the first year (2006) of the experiment compared to second year (2007). Data pertaining to number of shoots per plant is presented in

Table 3. Statistical analysis of the data revealed insignificant variation for number of leaves per plant between the years. However, in general the clones had more shoots per plant during the second year (2007) compared to the first year (2006).

Number of flowers per plant

Data pertaining to number of flowers per plant is presented in Table 4. Statistical analysis of the data revealed insignificant variation for number of flowers per plant over the years. However, in general the clones showed more number of flowers per plant during first year (2006) compared to second year (2007).

Number of fruits per plant

Data pertaining to number of fruits per plant is presented in Table 5. Statistical analysis of the data revealed insignificant variation for number of fruits per plant over the years. However, in general the clones showed more number of fruits per plant during first year (2006) compared to second year (2007).

Percentage of pod set

Data pertaining to percentage of pod set per plant is presented in Table 6. Statistical analysis of the data revealed insignificant variation for percentage of pod set over the years. However, in general the clones showed a higher percentage of pod set during first year (2006) compared to second year (2007).

Number of wrinkles per seed

Data pertaining to number of wrinkles per seed is presented in Table 7. Statistical analysis of the data revealed significant variation for number of wrinkles per seed over the years (Table 1). Number of wrinkles per seed over years ranged 2.5 to 2.87 during 2006 and 2.54 to 3.12 during 2007.

Number of seeds per pod

Data pertaining to number of seeds per pod is presented in Table 8. Statistical analysis of the data revealed insignificant variation for number of seeds per pod during first year with range of 3.46 to 4.23 seeds per fruit during first year. Significant variations ($p < 0.01$) were recorded among number of seeds per pod during 2nd year. The results showed maximum number of 4.37 seeds per pod in clone 125 and minimum number of 3.57 seeds per pod in clone number 123.

Table 1. Locations and clone numbers of the clones selected for their performance and used in polycross.

Clone no	Origin
114	Kirşehir- Malya TIGEM alfalfa plots
120	Cicekdagi- Kirsehir 1180 m height
123	Cicekdagi- TIGEM alfalfa plots
125	Farmers field at a distance of 10 km from Yozgat
139	Sivas- Ulas TIGEM alfalfa plots
159	Konya- Kulu (Makas) surroundings
170	Ankara – Golbasi surroundings

Table 2. Plant height (cm) of seven clones during 2006 on August 15, 2006 and August 20, 2007.

Clone no	Mean plant height during 2006	Clone no	Mean plant height during 2007
114	97.15	114	96.64
125	96.35	125	95.33
123	88.82	123	88.38
170	86.35	139	85.67
139	86.18	170	85.32
159	83.75	159	83.28
120	78.60	120	77.92
Means of years	88.17		87.50

Means with the same letter are not significantly different using duncans multiple range test at $P<0.05$.

Table 3. Number of shoots per plant of seven clones allowed to pollinate under natural conditions of growth.

Clone no	Mean number of shoots per plant during 2006	Clone no	Mean number of shoots per plant during 2007
114	40.23	114	40.50
139	37.70	139	37.43
125	36.87	125	36.93
123	33.27	123	33.87
120	25.97	120	28.67
159	25.40	159	25.90
170	23.47	170	23.33
Mean	31.84		32.38

Means with the same letter are not significantly different using duncans multiple range test at $P<0.01$.

Table 4. Number of flowers per plant of seven clones allowed to pollinate under natural conditions of growth.

Clone no	Mean number of flowers per plant during 2006	Clone no	Mean number of flowers per plant during 2007
125	43.40	125	44.33
170	42.47	123	42.23
123	41.63	170	42.17
120	40.93	120	41.70
114	39.97	114	41.63
139	37.63	139	37.27
159	34.10	159	34.00
Mean	40.01		40.47

Means with the same letter are not significantly different using duncans multiple range test at $P<0.01$.

Table 5. Number of fruits per plant of seven clones allowed to pollinate under natural conditions of growth.

Clone no	Mean number of fruits per plant during 2006	Clone no	Mean number of fruits per plant during 2007
114	24.37	114	24.33
120	22.07	120	22.40
170	20.83	170	21.00
123	20.63	123	20.73
125	19.97	125	20.20
139	19.37	139	18.57
159	17.80	159	17.60
Mean	20.72		20.69

Means with the same small letter are not significantly different letter using duncans multiple range test at $p<0.01$.

Table 6. Percentage of pod set of seven clones allowed to pollinate under natural conditions of growth.

Clone no	Percentage of pod set during 2006	Clone no	Percentage of pod set 2007
114	61.18	114	58.46
120	53.92	120	53.71
159	52.18	159	51.76
139	51.45	170	49.83
123	49.60	139	49.80
170	49.07	123	49.12
125	46.02	125	45.60
Ort	51.92		51.18

Means with the same small letter are not significantly different letter using duncans multiple range test at $p<0.01$.

Table 7. Number of wrinkles per seed of seven clones allowed to pollinate under natural conditions of growth.

Clone no	Number of wrinkles per seed during 2006	Clone no	Number of wrinkles per seed during 2007
114	2.87	123	3.12
120	2.79	120	3.04
125	2.76	139	2.90
159	2.75	170	2.87
123	2.69	125	2.86
139	2.75	114	2.69
170	2.56	159	2.54
Ort	2.71		2.86

Means with the same small letter are not significantly different letter using duncans multiple range test at $p<0.05$.

Table 8. Number of seeds per pod of seven clones allowed to pollinate under natural conditions of growth.

Clone no	Number of seeds per fruit during 2006	Clone no	Number of seeds per fruit during 2007
125	4.23 a	125	4.37a
170	3.83 b	114	4.10 ab
114	3.83 b	159	4.00 abc
159	3.80 b	170	3.97 abc
139	3.50 c	123	3.80 bc
120	3.47 c	120	3.70 bc
123	3.46 c	139	3.53 c

Means with the same small letter are not significantly different letter using duncans multiple range test at $p<0.01$.

Table 9. Seed yield per plant of seven clones allowed to pollinate under natural conditions of growth.

Clone no	Seed yield per plant during 2006		Clone no	Seed yield per plant during 2007	
114	25.96	a	114	27.32	a
125	24.52	ab	125	25.31	ab
120	22.75	ab	120	23.67	b
139	21.33	ab	139	22.53	b
170	18.47	ab	159	19.34	c
159	18.43	ab	123	19.27	c
123	18.02	b	170	17.95	c
Ort	21.35			22.20	

Means with the same small letter are not significantly different letter using duncans multiple range test at $p < 0.01$.

Table 10. Thousand (1000) seed weight of seven clones allowed to pollinate under natural conditions of growth.

Clone no	1000 seed weight during 2006		Clone no	1000 seed weight during 2007	
123	2.96		114	3.10	
139	2.90		125	2.92	
170	2.83		120	2.90	
120	2.75		123	2.87	
114	2.72		170	2.84	
159	2.64		139	2.71	
125	2.51		159	2.68	
Mean	2.76			2.86	

Seed yield per plant

Data pertaining to seed yield per plant is presented in Table 9. Statistical analysis of the data revealed significant variation for number of seeds per plant during first year with range of 18.02 to 25.96 seeds per plant during first year and 17.95 to 27.32 seeds per plant during second year. Mean of the two years showed mean maximum number of 26.64 seeds per plant in clone number 114 and mean minimum number of 18.21 seeds per plant in clone No. 123.

Thousand (1000) seed weight

Data pertaining to thousand (1000) seed weight is presented in Table 10. Statistical analysis of the data revealed insignificant variation for thousand seed weight during both years with range of 2.51 to 2.96 gram during first year and 2.68 to 3.10 grams during second year. As a result of polycross among these clones, a single high yielding clone named Cafer Kislik was selected by bulking all seeds together. The agronomic production of Cafer Kislik was compared with five standard cultivars commonly used in the area for six harvests over two years at experimental fields of the department of field crops, Ankara University, Ankara. Before experimentation

six soil samples were taken at the depth of 0.3 m and were analysed. Soil analysis results showed that it had clayed texture with pH of 8.20 EC dS/m of 0.19, lime of 5.92%, N of 0.19 mg/l, P of 12.3 mg/l, K of 4.30 mg/l and organic matter of 3.8%. All cultivars were tested in randomised block system in four replications. The borders of plots were surrounded by border plants, so that the border influence was eliminated. The examined trial had 100 plants per cultivar per plot in 5 m long rows with plant to plant distance of 20 cm and row to row distance of 0.90 cm. The climatic conditions were representative of central Anatolia. Analysis of harvests 1, 2 and 4 during the first year showed insignificant differences among the five standard cultivars and proposed cultivar Cafer Kislik (Table 11). However, sharp difference among green yield was observed during harvests 3, 5 and 6. General total of yield also showed sharp differences among yield of all cultivars.

In general, the proposed cultivar Cafer Kislik was the highest yielding cultivar among all. It was followed by Cv. Daisy, P. Bologna, Bilensoy and Gea. The lowest yield was recorded from Cv. Savas. Green yield of these cultivars was again tested during 2nd year under the same environmental conditions of Ankara. Analysis of first harvest during 2nd year showed insignificant differences among yield of all cultivars (Table 11). However, sharp difference among green yield was

Table 11. A comparison of yield in kg/ha of proposed cv. Cafer Kislik with five standard alfa cultivars used in the Central Anatolia during first year.

Cultivars	First harvest	2nd harvest	3rd harvest	4th harvest	5th harvest	6th harvest	Total
Proposed Cafer Kışlık	2991	2563	1547 a	1918	1461 a	1282 a	11760 a
Daisy	1950	2422	1481 ab	1638	1289 b	928 d	10707 b
P.Bologna	2820	2500	1414 bc	1710	1391 ab	1150 b	10985 b
Savaş	2861	2477	1348 c	1520	1293 b	598 e	10097 c
Bilensoy	2739	2458	1410 bc	1760	1342 b	1042 c	10751 b
Gea	2717	2488	1383 bc	1692	1337 b	1028 cd	10644 b
Source of variation (%)	6.34	2.59	5.48	14.83	4.99	7.02	3.28

Means with the same letter are not significantly different using duncans multiple range test at $P<0.01$.

Table 12. A comparison of yield of proposed cv. Cafer Kislik with 5 standard alfa cultivars used in the Central Anatolia during 2nd year.

Cultivars	First harvest	2nd harvest	3rd harvest	4th harvest	5th harvest	6th harvest
Proposed Cafer Kışlık	2622	1732 a	1751 a	1506 a	991 a	8602 a
Daisy	2510	1597 bc	1552 cd	1141 c	876 b	7674 c
P.Bologna	2478	1640 ab	1714 ab	1496 a	1007 a	8335 b
Savaş	2550	1525 c	1488 d	1197 b	866 b	7625 c
Bilensoy	2450	1692 ab	1704 ab	1500 a	977 a	8322 b
Gea	2509	1652 ab	1629 bc	1478 a	967 a	8234 b
VK (%)	6.59	3.98	4.56	1.60	4.77	2.06

Means with the same letter are not significantly different using duncans multiple range test at $P<0.01$.

observed during remaining harvests. General total of green yield during 2nd year again showed Cafer Kislik was the best cultivar measured (Table 12). It was followed by cv. P. Bologna, Bilensoy and Gea. Yield of cv. Daisy fluctuated and reduced. Again, Savaş had the lowest yield. Average precipitation had range of 0 to 50 cm and 0-65.6 cm during 2008 and 2009, respectively. Average temperature had range of -5.7 24.3°C and -0.3-21.1°C during 2008 and 2009, respectively. April to August 2008 received average of 15.6 cm with total of 78 cm rainfall. April to August 2009 received average of 33 cm with total of 165 cm rainfall. Whereas, April to August 2008 and 2009 received average temperature of 18°C and 16.46°C. Two years of analysis clearly show that Daisy is phenotypically unstable and behaved variably during two years with total yield of 10707 kg/ha during 2008 and total yield of 7674 kg/ha during 2009. different environmental conditions.

In this case however, all other cultivars are genetically stable and behaved similarly under variable environmental conditions. The yield of proposed cv. Cafer Kislik remained stable and superior to all other cultivars popularly used in the area. One feature of this experiment was that Cafer Kislik yielded consistently better during two years. Forage crop researchers know that it is very difficult to find an alfalfa variety that will yield consistently. Based on morphological and phenological characteristics, the proposed synthetic variety Cafer Kislik developed in

this study, is ideally adapted to the conditions of central Anatolia. In fact, proposed cv. Cafer Kislik begin blooming a few days earlier than normally used cultivars and exhibit longer duration of flowering and greater amount of vegetative growth than conventional cultivars, that made it superior to other cultivars during experimental trials. To date, suitable seed production environments have not been identified for proposed cultivar Cafer Kislik and management practices for seed production have not been developed.

DISCUSSION

Alfalfa is a dicotyledonous plant. Most winter hardy alfalfa varieties have several nodes originating below the soil surface (2.5 to 7.6 cm) in the seeding year. Contractile growth greatly aids winter survival of alfalfa by providing soil insulation for the perennial over-wintering crown structures. Also uninjured by frost and hard winter these buds gave rise to branch rhizomes in the next spring compared to other standard cultivars in agreement with Moser and Jennings (2003), with very similar observations about alfalfa. This result would encourage the conclusion by Busbice (1970), based on theoretical considerations, that selection of parents should be based on both their inbred and outcross yield, thereby capitalizing on additive and non-additive gene action.

However, an incomplete cross-fertilization and a certain proportion of selfing in the polycross, determined by the behaviour of pollinators, cannot be excluded (Strickler and Vinson, 2000), which may have biased upwards the correlations between progeny. Moreover, consistent ranking at the top during two years indicates that selected proposed cultivar has improved yield potential compared to other cultivars used in this study. Future advances in alfalfa hybrid production may offer more higher yield potential for this crop through polycross breeding.

REFERENCES

- Barnes DK, Goplen RP, Baylor JE (1988). Highlights in US and Canada. In: A.A. Hanson (ed.), Alfalfa and alfalfa improvement. Agronomy: n. 29. ASA, CSSA, SSSA Publishers, Madison, Wisconsin, USA, pp. 1-23.
- Busbice TH (1970). Predicting yield of synthetic varieties. *Crop Sci.*, 10: 265-269.
- Moser LE, Jennings JA (2003). Grass legume structure and morphology. In : Barnes RF, Nelson CJ, Moore KJ, Collins M eds. Forages, the science of grassland agriculture, Volume II 6th edition. Blackwell Publishing, pp. 15-36.
- Sevimay CS, Guloglu D, Khawar KM (2005a). Karyotype Analysis of Eight Turkish Vetch (*Vicia sativa* L.) cultivars. *Pak. J. Bot.*, 37(2): 313-317.
- Sevimay CS, Khawar KM, Çöçü S, Özcan S (2005b). Somatic embryogenesis in White Clover (*Trifolium repens* L.). *Periodicum Biologorum*, 107(1): 101-105.
- Strickler K, Vinson JW (2000). Simulation of the effect of pollinator movement on alfalfa seed set. *Environ. Entomol.*, 29: 907-918.