



Original article

Improvement of Superior Genotypes from Anatolian Sage (*Salvia Fruticosa* Mill.) Populations By Clonal Selection

Fatma Uysal Bayar ^{a,*} & Kenan Turgut ^b

^a Department of Medicinal and Aromatic Plants, Bati Akdeniz Agricultural Research Institute, Antalya, Turkey

^b Department of Field Crops, Faculty of Agriculture, Akdeniz University, Antalya, Turkey

Abstract

This research was conducted with the purpose of obtaining high quality plants by selection breeding of Anatolian sage (*Salvia afruticosa* Mill.) in different locations of Antalya province. In this research, clonal individual plants belong to *Salvia fruticosa* Mill. species, were collected from 15 different populations in the flora of Antalya. The clonal selection method was used in the breeding of this species, which is propagated clonally. Dry herbage yield was between 748.34 and 1135.15 kg/da for A clones, while it was between 748.34 and 1135.15 kg/da for B clones in terms of the population mean. The highest dry leaf yield was determined 534.36 and 605.867 kg/da for A and B clones respectively. Furthermore, 1,8-cineole, camphor and caryophyllene were determined as the main components of essential oils. The proportion of 1,8-cineole was determined between 34.51-73.49%. In this research, it was observed that there was a large variation between clonal lines, and some of them were determined as important in terms of morphological characteristics, yield and quality.

Keywords: *Salvia Triloba*, Yield, Quality, Clonal Selection.

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* Corresponding author:

Fatma Uysal-Bayar is a doctor in the Department of Medicinal and Aromatic Plants at Bati Akdeniz Agricultural Research Institute, Antalya, Turkey. Her research interests include the medicinal and aromatic plants.
Email: uysal.fatma@tarimorman.gov.tr

INTRODUCTION

Salvia is the largest genus, which has 900 species and distributed all over the world (Güner et al., 2000). Some species of this genus have an economical value due to perfumery, cosmetic, medicinal and flavouring usage (Newall et al., 1996). *Salvia* species contain 1,8-cineole, which is a pharmacologically important essential oil component. Furthermore, some species are consumed as herbal tea (İpek, 2005). Especially *S. officinalis*, *S. fruticosa*, *S. tomentosa* and *S. sclarea* have an economical value. Export rate of sage of Turkey among the medicinal and aromatic plants was 3.6% in 2015 with 2.070 tons and 8 million US dollar. In 2015, 19 tons of total amount of sage was obtained from field production, 838 tons by import, and the rest of that was obtained by collection from the nature (Temel et al., 2018). *Salvia officinalis* is not found in Turkey naturally, however, researches about growing was done before, and it is produced in small amounts (Arslan, 1998). *S. fruticosa* Miller (Syn. *S. triloba*), Anatolian sage, can be found widely in nature in Southwestern Anatolia. Leaves of *S. fruticosa* Miller are consumed as herbal tea. The essential oil obtained from the leaves, is named ‘apple oil’ and it is exported substantially. It has benefits for respiratory tract infection, neurological disease, diarrhea, and it has painkiller effect (Baytop, 1999). Increase of export and domestic consumption, low production potential, rapid urbanization, industrialization, environmental pollution and especially insensible plant collection from the nature put pressure on *S. fruticosa* Mill. Species. When the potential of Turkey is evaluated, previous breeding studies for the solution of the problems were not found sufficient. In Turkey, only a variety named “Kark” was registered in 2019 by the Aegean Agricultural Research Institute with the method of mass selection. By this experiment, it was aimed that obtain new varieties that having high quality and agronomic characteristics by selection studies for *S. fruticosa* Mill. species, which were collected from different locations. Moreover, it was aimed that providing cheaper, high quality, easier and large production by obtaining superior varieties with vegetative method using clonal selection, thus, preventing destroy of *S. fruticosa* Mill. populations in the nature and saving plant genetic resources for economy of the country.

MATERIAL and METHODS

Material

In this experiment, *Salvia fruticosa* Mill. species was used as a plant material, which was collected from 15 different populations in Antalya flora. Individual plants were collected clonally from the populations. Location, date, altitude and coordinate information of the collected population are presented in Table 1.

Table 1. Location, date, altitude and coordinate information regarding the populations where the plants are collected

Population number	Collection area	Altitude (m)	Coordinates	
FK1	Kemer-Kuzdere-Sümbüllü	105	40 50 53 K	36 27 88 D
FK2	Kemer-Kuzdere	120	40 52 71 K	36 27 69 D
FK3	Kemer-Kiriş-Bayraklı tepe	31	36 34 59 K	30 34 33 D
FK4	Kemer-Teleferik	137	36 32 06 K	30 32 35 D
FK5	Kemer-Göynük kanyonu	20	36 40 96 K	30 33 43 D
FKM1	Kumluca-Gelidonya feneri	57	36 14 11 K	30 24 41 D
FKM2	Kumluca-Adrasan	5	36 18 67 K	30 27 26 D
FKM3	Kumluca-Olimpos	257	36 34 18 K	30 26 23 D
FD1	Demre-Üç ağız	7	40 12 93 K	35 76 46 D
FD2	Demre-Kekova	68	40 10 49 K	35 75 57 D
FD3	Demre-Sülüklü	57	40 13 17 K	35 76 64 D
FD4	Demre-Gökkaya	5	40 10 18 K	35 75 49 D
FKS1	Kaş-Yavu	7	40 15 88 K	35 75 56 D
FKS2	Kaş-Gökseki	95	40 10 12 K	35 73 74 D
FKS3	Kaş-Kalkan	13	40 12 01 K	35 72 06 D

Experimental Field

This research was conducted in the Aksu experiment field belongs to the Field Crops Department of Western Mediterranean Agricultural Research Institute in Antalya province of Turkey between 2011-2013.

Climate characteristics and soil samples

Antalya province, where the experiment was conducted, has a hot and dry summer, and warm and rainy winter. In April 2011, which involves that first planting date of A-clones, the average temperature was 16 °C while the total precipitation and the relative humidity were 98 mm and 70%, respectively. In March 2012, which was the harvest date of A-clones, the average temperature was 12.5 °C while the total precipitation and the relative humidity were 56 mm and 58.6% respectively. The climate data belong to this period showed similarity with the data for long years. Moreover, in March 2013 that was the harvest time of B-clones, the average temperature, the total precipitation, and the relative humidity were 13.3 °C, 19 mm, and 69.7% respectively (Table 2).

Table 2. Climate data belong to Meteorology Directorate of Antalya Province between 2010-2013

pH	8.60	Highly alkaline
Lime (%)	24.80	Very high
EC (micromhos)	197.00	Saltless
Sand (%)	15.00	
Clay (%)	43.00	Silty clay
Silt (%)	42.00	
Organic Matter (%)	1.88	Low
P (ppm)	28.00	High
K (ppm)	212.00	Sufficient
Ca (ppm)	3687.00	High
Mg (ppm)	583.00	High
Fe (ppm)	5.40	High
Mn (ppm)	6.50	Low
Zn (ppm)	0.20	Deficient
Cu (ppm)	1.90	Sufficient

Table 3. Some physical and chemical characteristics of the soil of experimental field

Months	Average temperature (oC)			Average for many years	Total Precipitation (mm)			Average for many years	Proportional Humidity (%)			Average for many years
	2011	2012	2013		2011	2012	2013		2011	2012	2013	
January	10.2	8.9	10.8	10.2	100.0	234.0	203.0	245.7	72.3	67.0	74.4	67.2
February	10.9	9.2	12.1	11.1	142.0	122.0	59.0	133.2	72.7	62.6	74.9	67.1
March	12.7	12.5	13.3	13.7	38.0	56.0	19.0	48.2	69.7	58.6	69.7	66.4
April	16.0	16.7	17.7	16.4	98.0	41.0	34.0	55.8	70.0	72.2	67.1	67.1
May	19.8	20.5	22.5	21.0	189.0	74.0	56.0	49.8	72.3	71.0	66.6	66.6
June	25.4	26.0	25.4	25.9	6.0	4.0	0.0	4.2	58.1	66.6	61.6	61.2
July	28.1	29.4	28.0	28.9	0.0	0.0	16.0	3.0	65.5	60.3	57.8	60.3
August	28.8	29.1	28.7	28.8	11.0	0.0	0.0	1.8	57.6	52.4	57.6	62.9
September	25.9	25.0	24.7	25.1	22.0	2.0	19.0	27.0	61.0	65.2	58.0	61.3
October	18.6	19.5	18.1	20.3	259.0	124.0	89.0	134.4	62.2	73.5	53.4	62.7
November	12.4	16.3	15.9	15.4	20.0	26.0	179.0	77.8	57.8	73.9	71.5	66.5
December	10.4	11.8	9.6	11.6	125.0	263.0	53.0	182.5	71.7	79.0	58.0	66.2
Total/Average	18.3	18.7	18.9	19.0	1010.0	946.0	727.0	963.4	66.6	66.9	64.3	64.6

The soil that belongs to experimental area, had a clayey and silty, saltless, very high limy and highly alkaline structure with a low organic substance, high phosphor, high calcium, very high magnesium, moderate potassium, sufficient manganese, iron and copper, and insufficient zinc. Soil of the experimental field had silty clay texture, saltless content, very high lime, highly alkaline texture, low organic matter, high phosphorus, high calcium, very high magnesium, medium level potassium, sufficient manganese, iron, copper and insufficient zinc (Table 3).

Method

In the experiment, the clonal selection method was used for vegetatively propagated plants. Populations in the natural flora, were used as a genetic variation resource. In the first year of the study, variation resource was generated with 14067 clonal plants which were collected from 284 individual plants in the nature. Location information and collection dates of population are given in Table 1. In the second year of the study, 230 individual plants were selected from variation resource as A clone. A clones were collected from each plant and planted as 10 plants for each single line. In the third year of the study, 17 B clones were selected from A clones. The study was conducted according to randomized block experimental design with three replications. Parcels were established in single line, 70 cm intrarow and 4 m length. 6 superior clones selected among B clones were registered as C clones, and breeding values were determined (Demir, 1990).

Obtainment of Data

Rooting rate; cuttings collected from each plants were rooted in peat:perlite medium. Afterwards, rooted cuttings were counted and the results were calculated as percentage. Plant height; before the harvest, the height between the soil surface and the highest point of the plant was determined as centimeter. Shoot number; before the harvest, main shoots formed in the plant were counted.

A and B clones were harvested in 29 March 2012 and 13 March 2013, respectively at the level of 10 cm height from top of the plant. After that, fresh herbage yield per decare was determined as kg/da. In 500g samples from the fresh herbage, leaf rate (%) was determined with separating leaf and stalk, and fresh leaf yield was calculated per decare using this rate. Dry herbage and dry leaf yields were calculated by collecting 500 g sample from the fresh plant, drying in 35°C and determination of loss in moisture. Essential oil rate was obtained by hydrodistillation method using Clevenger apparatus. 300 mL distilled water was added on 20 g sample, distillation occurred in 3 hours and the essential oil rate was calculated. An essential oil component analysis was made using GC-MS (Gas chromatography (Agilent 7890A), Mass spectrometer (Agilent 5975C) and Capillary column (HP Innowax Capillary; 60.0 m x 0.25 mm x 0.25 µm) according to Özek et al., (2010). The samples were diluted with 1:100 hexane for analysis. GC-MS/FID analysis was carried out at split mode of 50:1. Injection volume and temperature were adjusted as 1 µL and 250°C respectively. Helium (99.9%) was the carrier gas at a constant flow rate of 0.8 mL/min. The oven temperature was programmed as follow; 60oC for 10 minutes, increased at oC /minute to 220oC and held for 10 minutes at 220oC According to this programme, total analysis time took 60 minutes. MS spectra was monitored between 35-450 amu and the ionization mode was used electronic impact at 70 eV. The relative percentage of the components was calculated from GC-FID peak areas, and components were identified by WILEY, NIST and OIL ADAMS libraries.

Evaluation of Data

Weight Rank Method was used for selection B clones from A clones. Class score (CS) and relative score (RS) that are used in the Weight Rank Method, are given in Table 2. Each genotype was evaluated according to selection criteria, and total scores of genotypes in terms of the all characteristics (dry herbage and leaf yield, essential oil rate, rooting rate of cuttings) that are presented in Table 4 were calculated as a result of CS x RS. As a result of calculations, genotypes that had 4.5 scores or more were selected as B-clones. 17 genotypes that had 4.5 scores or more were taken to the experiment as a B-clone. As a result of calculation, genotypes which had 4.5 and higher score, were selected as B clone (uysal, 2015). Statistical differences in measurement and observation of populations in the field work, were determined with variance analysis with generalised linear model at 5% and 1% significance levels. In the case of statistically significant differences, Duncan test was applied at 5% significance level for comparison of means (Gülümser et al., 2006).

Table 4. Selection criteria, class and relative scores

Selection criteria	Classes	Class Score	Relative Score (%)
Dry herbage yield (kg da ⁻¹)	Lower than 450 kg da ⁻¹	1	30
	Between 450 and 900 kg da ⁻¹	3	
	Higher than 900 kg da ⁻¹	5	
Dry leaf yield (kg da ⁻¹)	Lower than 250 kg da ⁻¹	1	30
	Between 250 and 450 kg da ⁻¹	3	
	Higher than 450 kg da ⁻¹	5	
Essential oil rate (%)	Lower than 1,8%	1	35
	Between 1,8% and 2,5%	3	
	Higher than 2,5%	5	
Rooting rate of cuttings (%)	Lower than 60%	1	5
	Between 60% and 80%	3	
	Higher than 80%	5	

RESULTS and DISCUSSIONS

Data belong to A clones

In the first year of the experiment, rooting rate, dry herbage and dry leaf yields, the essential oil rate of A clones were determined. Rooting rate of 230 individual plants, which were selected as A clones, varied between 16% and 100%. Furthermore, 29 clonal lines had 100% rooting rate. Ayanoğlu and Özkan (2000) obtained 78.75% rooting rate for sage (*S. officinalis* L.) as the highest, Kara et al., (2011) obtained 81.00% rooting rate for the same species. These values shows similarity with several A clones in this experiment.

While dry herbage yield was between 149.24 and 2288.73 kg/da for all the populations of A clones, dry leaf yield was between 72.77 and 1062.63 kg/da (Table 5). While Bayram et al., (1999) obtained between 1028.80 and 2055.57 kg/da dry herbage yield of Anatolian sage (*S. fruticosa* Mill.) in the first year and between 2870.30 and 6558.60 kg/da in the second year in Bornova (İzmir) ecological conditions, Bayram (2001) obtained 639.00 kg/da dry herbage yield in same conditions. Baranauskiene et al., (2011) had between 50.00 and 270.00 kg/da dry leaf in Lithuanian conditions for the same species. These studies show similar and lower results with A clones in this experiment. While the essential oil rate of all populations generally was between 1.00 and 3.75%, the highest one was obtained from FK5-3. When the studies by different researchers in different locations were observed, the essential oil rate of Anatolian sage (*S. fruticosa* Mill.) was found between 0.9 and 2.8% by Başer and Kırimer (2006), 2.9% by Kocabaş et al., (2007) and 1.14-4.58% by Çiçek et al., (2011). These studies show similar results with A clones in this experiment. The difference between results in the experiments, can be explained by years, climatic conditions, different locations where plant materials were collected and different harvest times.

Table 5. Some yield and quality characteristics of A-clones

Clonal Line	Dry herbage yield (kg da ⁻¹)	Dry leaf yield (kg da ⁻¹)	Essential oil rate (%)	Rooting rate of cuttings (%)	Clonal line	Dry herbage yield (kg da ⁻¹)	Dry leaf yield (kg da ⁻¹)	Essential oil rate (%)	Rooting rate of cuttings (%)
FK2- 1	725.61	322.49	2.50	66.00	FKM1-24	1046.04	404.09	1.75	61.54
FK2- 2	632.93	291.65	2.00	62.00	FKM1-25	1712.89	823.50	2.00	34.37
FK2- 3	820.69	518.63	2.00	74.00	FKM1-26	1472.87	953.04	2.25	87.88
FK2- 4	889.09	451.18	2.00	70.00	FKM1-27	656.28	366.02	2.25	61.22
FK2- 5	598.37	358.56	1.90	84.00	FKM1-28	1132.52	677.05	1.75	43.18
FK2- 6	903.51	481.87	2.00	92.00	FKM1-29	707.36	353.68	2.00	75.86
FK2-7	1207.16	566.13	2.00	30.00	FKM1-30	269.37	149.65	2.25	17.86
FK2-8	476.82	254.11	2.15	88.57	FKM1-31	802.22	385.12	1.67	19.67
FK2- 9	352.74	224.47	2.25	58.00	FKM1-32	1212.62	358.28	2.50	43.81
FK2- 10	375.86	265.64	2.75	48.00	FKM1-35	1049.30	473.64	1.90	55.88
FK2- 11	1036.33	459.47	1.80	80.00	FKM1-37	1257.77	708.31	1.75	39.13
FK2- 12	693.15	366.08	2.50	22.00	FKM1-38	2122.09	986.72	2.00	100.00
FK2- 13	778.56	373.32	2.25	42.00	FKM1-40	1094.58	466.92	1.50	82.05
FK2- 14	985.92	492.96	1.75	80.00	FKM1-41	1035.94	544.24	2.00	72.34
FK3-1	695.07	269.83	2.00	94.34	FKM1- 47	446.40	235.68	2.25	53.66
FK3-3	609.35	416.21	2.75	82.76	FKM1-50	932.10	418.21	1.85	76.31
FK3-4	1392.45	786.83	2.25	91.67	FKM2-1	865.39	482.14	2.25	100.00
FK3-5	531.30	260.23	2.00	91.23	FKM2-2	910.15	491.48	1.83	100.00
FK3-6	858.85	445.33	1.75	96.15	FKM2-3	1169.49	517.35	2.00	97.43
FK3-7	602.50	258.93	1.50	96.67	FKM2-4	935.71	579.48	2.00	93.94
FK3-8	978.09	463.85	1.65	49.25	FKM2-5	911.03	667.50	1.75	89.47
FK3-9	626.87	280.13	2.15	35.80	FKM2-6	385.49	318.45	2.40	100.00
FK3-11	433.19	221.33	1.90	100.00	FKM2-7	218.26	105.28	2.15	95.24
FK3-12	1000.96	402.24	2.00	100.00	FKM2- 8	696.40	360.07	1.75	56.00
FK3-13	983.44	468.54	2.25	100.00	FKM2- 10	645.16	317.46	2.00	62.00

Clonal Line	Dry herbage yield (kg da⁻¹)	Dry leaf yield (kg da⁻¹)	Essential oil rate (%)	Rooting rate of cuttings (%)	Clonal line	Dry herbage yield (kg da⁻¹)	Dry leaf yield (kg da⁻¹)	Essential oil rate (%)	Rooting rate of cuttings (%)
FK3-14	1025.18	397.10	2.00	100.00	FKM2-11	842.46	516.43	1.75	100.00
FK3-15	529.68	271.63	2.25	92.19	FKM2- 14	441.80	234.10	2.00	44.00
FK3-16	1021.13	536.93	2.50	100.00	FKM2- 15	652.77	393.36	1.75	12.00
FK3-17	710.05	456.52	1.75	90.91	FKM2- 16	1062.91	471.18	1.75	26.00
FK3-19	552.01	283.50	2.50	100.00	FKM3-1	325.24	200.26	1.90	94.20
FK3-20	1035.25	557.70	2.00	80.00	FKM3-2	868.74	560.69	1.75	93.48
FK3-21	919.72	329.32	2.00	62.07	FKM3-3	1099.58	679.31	1.67	94.74
FK3-22	594.68	378.43	1.75	59.52	FKM3-4	563.38	357.56	1.50	90.10
FK3-23	594.95	246.48	1.75	46.15	FKM3-5	917.92	459.19	2.80	88.00
FK3-24	1013.45	702.66	2.00	25.00	FKM3-6	694.37	403.62	2.50	52.70
FK3- 25	501.19	230.17	2.00	36.36	FKM3-7	264.08	143.12	1.75	64.61
FK3-26	649.06	328.92	1.90	92.31	FKM3-8	226.08	151.83	2.75	85.71
FK3- 27	855.90	515.76	1.75	66.00	FKM3-9	358.70	195.28	1.50	66.25
FK3- 28	873.51	493.72	2.25	90.00	FKM3-10	520.17	218.61	2.50	46.43
FK3- 29	484.16	265.94	2.15	40.00	FKM3-11	880.20	420.09	2.75	93.48
FK3- 30	637.36	381.55	1.65	96.00	FKM3-12	536.62	264.66	2.50	72.73
FK4-1	309.44	203.35	2.25	100.00	FKM3-13	360.45	163.84	2.50	84.51
FK4-2	752.33	363.40	3.00	96.97	FKM3-14	914.45	386.33	2.00	42.50
FK4-3	578.86	385.35	2.50	100.00	FKM3-15	446.26	135.82	1.75	80.33
FK4-4	783.61	342.51	2.83	100.00	FKM3-16	685.50	260.35	1.75	78.72
FK4-5	785.59	409.18	2.50	93.10	FKM3-17	712.75	451.02	2.50	76.92
FK4-6	1397.34	789.09	2.17	76.92	FKM3-18	1024.91	486.35	2.25	76.92
FK4-7	1439.28	731.49	1.75	43.75	FKM3-19	1681.59	711.50	1.50	58.00
FK4-8	931.45	485.77	2.65	91.80	FKM3-20	573.72	289.45	1.25	86.52
FK4-9	817.92	574.78	2.75	100.00	FKM3-21	718.49	279.74	2.00	64.38
FK4-10	1055.76	674.51	2.00	91.11	FKM3- 24	378.96	191.13	2.00	75.25
FK4-11	1320.60	745.90	2.50	100.00	FKM3- 25	467.04	234.21	2.25	28.00
FK4-12	621.77	270.87	2.15	100.00	FKM3- 26	567.29	290.09	2.15	18.75
FK4-13	781.56	432.16	2.00	100.00	FKM3- 28	537.77	353.17	1.90	34.00
FK4-14	1154.21	659.15	2.75	94.91	FKM3- 29	1080.35	585.43	1.75	58.06
FK4-15	693.81	443.66	2.90	92.73	FD1- 1	1596.24	870.68	1.75	34.00
FK4-16	681.65	285.44	1.67	58.33	FD1- 2	512.38	290.22	2.90	58.00
FK4-17	902.25	614.39	1.90	70.77	FD1- 3	812.13	447.12	2.00	48.00
FK4-18	723.59	302.90	2.33	100.00	FD1- 4	786.14	443.94	1.75	34.00
FK4-19	1216.55	663.57	1.75	97.34	FD1- 5	834.77	355.68	1.75	20.00
FK4-20	518.46	274.76	2.50	97.34	FD1- 6	1119.27	664.21	1.50	18.00
FK4- 21	640.79	306.86	2.50	100.00	FD1- 10	684.29	300.12	1.50	14.00
FK4- 22	976.93	452.63	2.50	96.00	FD2-1	959.99	491.81	2.25	84.21
FK4- 23	539.05	336.43	2.00	36.36	FD2- 2	413.08	215.76	1.60	37.04
FK4- 24	835.72	413.64	2.50	46.00	FD2-3	1203.11	661.05	1.48	64.70
FK4- 25	1208.45	491.65	2.50	74.00	FD2-4	1851.54	724.59	2.25	54.05
FK4- 26	1124.56	545.24	3.00	42.00	FD2-5	1169.01	511.44	2.25	100.00
FK4- 28	1142.19	503.96	2.75	52.00	FD2-6	833.10	503.89	1.75	100.00
FK4- 29	657.83	417.95	2.50	22.00	FD2-7	481.93	243.63	2.90	81.82
FK4- 30	883.53	510.83	2.65	26.00	FD2-8	972.07	463.52	2.25	88.57
FK4- 32	775.76	427.35	2.75	70.00	FD2-9	1697.43	757.31	2.25	90.91
FK4- 33	695.46	403.01	2.75	42.00	FD2-10	1512.77	576.29	2.25	51.85

Clonal Line	Dry herbage yield (kg da⁻¹)	Dry leaf yield (kg da⁻¹)	Essential oil rate (%)	Rooting rate of cuttings (%)	Clonal line	Dry herbage yield (kg da⁻¹)	Dry leaf yield (kg da⁻¹)	Essential oil rate (%)	Rooting rate of cuttings (%)
FK4-35	666.94	409.67	2.50	100.00	FD2- 11	632.77	347.00	2.00	37.04
FK4- 36	504.31	276.47	2.75	28.00	FD2-13	1570.81	652.49	2.40	63.49
FK4-37	518.74	309.48	2.25	64.38	FD2-14	1695.94	847.97	2.25	68.18
FK4-38	953.88	553.66	2.00	80.00	FD2-15	1184.06	510.77	2.15	79.31
FK5-1	250.46	136.37	2.25	63.16	FD2-17	576.53	276.19	1.75	96.87
FK5-3	432.96	200.97	3.75	53.12	FD2-18	693.15	366.94	2.00	94.12
FK5-4	1520.27	556.56	1.50	69.77	FD2-19	1740.29	723.90	2.09	100.00
FK5-5	467.39	245.38	1.25	55.55	FD2-20	1245.14	547.11	2.25	100.00
FK5-6	782.18	489.59	1.75	55.55	FD4- 2	884.50	329.88	2.75	20.00
FK5-7	793.68	410.73	3.40	90.48	FD4-9	1539.12	837.83	2.00	12.00
FK5-8	419.76	233.64	2.00	16.00	FD4-10	796.47	391.44	2.10	16.34
FK5-9	645.12	310.61	2.00	95.31	FD4- 11	1673.19	901.40	2.25	34.00
FK5-10	2288.73	1062.63	1.90	82.86	FD4-12	1158.20	412.88	2.25	16.00
FK5-11	845.31	415.44	2.25	71.05	FD4-13	1068.14	487.22	2.50	12.00
FK5-12	1000.26	475.48	2.25	95.31	FD4- 16	820.16	379.84	2.75	18.75
FK5-13	511.15	262.13	2.50	84.00	FKS2-1	1115.54	511.08	2.50	83.33
FK5-14	793.51	414.52	1.75	100.00	FKS2-2	846.83	492.93	2.50	76.59
FK5-15	928.82	480.87	2.50	100.00	FKS2-3	610.96	225.09	2.25	65.57
FK5-16	442.49	231.15	2.65	98.57	FKS2-6	588.76	314.19	2.50	56.98
FK5- 17	1091.23	492.82	2.50	92.59	FKS2-10	851.96	440.96	1.90	34.61
FK5- 18	1225.88	576.59	1.75	74.19	FKS2-12	1093.18	678.60	2.40	56.52
FK5- 19	949.64	376.74	2.25	100.00	FKS2-13	985.09	447.77	1.75	65.62
FK5- 20	161.32	72.77	1.75	42.86	FKS2-14	905.01	465.19	2.25	28.12
FKM1-1	871.98	289.23	2.50	63.77	FKS2-15	653.61	335.00	2.25	61.36
FKM1-2	811.62	293.86	2.00	71.75	FKS3-2	620.60	372.94	2.00	90.00
FKM1-3	933.34	433.55	2.00	66.00	FKS3- 3	303.27	149.60	2.65	82.35
FKM1-4	720.19	315.33	2.15	100.00	FKS3-4	580.68	294.21	2.25	71.79
FKM1- 5	373.16	235.34	2.00	53.62	FKS3-5	979.31	522.30	2.10	86.67
FKM1- 6	149.24	82.79	1.50	54.24	FKS3-6	669.67	370.15	2.75	38.89
FKM1-7	1146.58	470.78	2.25	93.44	FKS3-7	974.99	418.04	2.90	76.92
FKM1-8	1358.57	470.28	2.50	35.09	FKS3- 8	848.59	515.22	2.50	54.54
FKM1-9	835.11	451.51	1.50	99.15	FKS3-9	1260.78	475.35	2.00	56.25
FKM1-10	544.07	268.88	2.00	95.00	FKS3-10	1303.10	639.76	2.00	62.96
FKM1-11	634.35	362.21	2.40	89.32	FKS3- 11	601.53	280.71	1.90	36.84
FKM1-12	755.43	304.16	2.15	86.44	FKS3- 12	250.19	192.06	1.90	60.00
FKM1-13	799.07	331.76	1.00	97.65	FKS3- 13	241.74	125.71	1.75	59.37
FKM1-14	775.40	319.63	1.50	89.39	FKS3- 14	836.64	509.83	2.00	43.48
FKM1-15	828.39	565.00	1.75	55.04	FKS3- 15	795.01	452.52	1.75	73.33
FKM1-16	1035.21	365.98	2.50	60.00	FKS3- 17	1013.55	625.50	2.15	78.18
FKM1- 17	915.31	498.13	1.50	70.27	FKS3- 18	161.50	106.14	2.75	25.64
FKM1-18	927.53	464.92	2.00	96.08	FKS3- 19	609.97	365.15	2.25	96.55
FKM1-19	907.24	447.23	2.25	86.49	FKS3- 20	805.00	274.65	2.25	92.00
FKM1- 21	777.44	555.31	2.25	87.80					
FKM1-23	713.88	358.41	2.00	79.63					

Some phenological and morphological characteristics of B-clones

Rooting rate, branch number and plant height of B clones, which were selected from A-clones, were determined according to the weighted rank method. Some phenological and morphological characteristics belong to B clones are shown in Table 6, and variance analysis is given in Table 7. As a result of statistical analyses, there was 1% significant difference between clonal lines in terms of rooting rate, branch number and plant height. When the groups were observed, there were not big differences between clonal lines in terms of rooting rate. Lots of clonal lines were in the same group and rooting rate varied between 31.48% and 100%. The highest rooting rate was determined on FK3-16, FK4-9, FK4-11 and FK5-7 at 100%. Clonal lines, which had the highest rooting rate, belong the population in Kemer location. Clonal lines from similar locations showed similar branch numbers that were between 18.86 and 48.61, and the highest one was obtained from FK4-22. Mossi et al., (2011) determined branch number of *S. fruticosa* Mill as 30. This values is similar with this experiment, however, it showed a lower branch number than FK4- 22 (48.61) clonal line. Karık (2013) stated that the branch numbers varied between 13 and 15. These values are considerably lower than this experiment. Using objective criterion for determination of branch numbers is very hard for *S. fruticosa* Mill. In the experiment, apical dormancy was prevented and sub branching induced by topping one month after planting. Higher branch number in this experiment can be explained by different counting method.

When the groups were observed, all clonal lines participated in different groups and plant heights varied between 46.77 cm and 117.57 cm. The highest plant height was obtained from FK4-22 (Table 3), moreover, the same clonal line had the highest branch number. Bayram (2001) studied with *S. fruticosa* Mill. in Bornova (İzmir) ecological conditions, individual plants were selected from 17 different locations and A clones were obtained. Average plant height was determined 46.4 cm, while this number shows similarity with FK5-7 clonal line (46.77 cm) in our experiment. Mossi et al., (2011) stated the plant height of *S. fruticosa* Mill. as 67.80 cm. This number shows similarity with FK4-8, FK4-14, FK4-15 and FKM3-5 clonal lines (65.17, 67.27, 61.97, 62.45 cm). Dudai et al. (1999) indicated plant height of *S. officinalis* L.x *S. fruticosa* Mill hybrid as between 29 - 84 cm in Israel. These numbers are lower than FK3-16, FK4-2, FD2-9, FKS3-7 and FKS3-8 clonal lines (91.97, 97.27, 90.47, 92.10 and 95.32 cm, respectively) in our experiment. The difference between plant height numbers in our experiment with the other studies, can be explained by agricultural practices, planting time, different ecologies, climatic and geographical conditions and measurement in different periods.

Table 6. B- Some phenological and morphological characteristics of B-clones

Clonal line	Rooting rate of cuttings (%)	Plant branch number (per plant ⁻¹)	Plant height (cm)
FK3-16	100.00 a	36.93 b	91.97 de
FK4-2	98.49 ab	27.26 cde	97.27 b
FK4-8	94.90 ab	29.40 bcde	65.17 j
FK4-9	100.00 a	29.16 bcde	59.10 l
FK4-11	100.00 a	33.78 bc	82.37 f
FK4-14	96.91 ab	27.60 cde	67.27 i
FK4-15	95.12 ab	23.79 def	61.97 k
FK4- 22	98.00 ab	48.61 a	117.57 a
FK4- 32	79.83 d	28.35 cde	73.47 g
FK5-7	88.49 bcd	28.40 cde	46.77 m
FK5-15	100.00 a	45.17 a	59.88 l
FKM1-16	78.16 d	18.86 f	80.97 f
FKM3-5	92.28 abc	21.77 ef	62.45 k
FD2-9	94.64 ab	31.58 bcd	90.47 e
FD4-13	31.48 f	35.21 bc	71.23 h
FKS3-7	82.64 cd	24.30 def	92.10 d
FKS3- 8	67.10 e	24.95 def	95.32 c

*Means followed by the same letters are not statistically significant according to Duncan's Multiple Range Test

Table 7. Variance analysis results related some phenological and morphological characteristics of B-clones

Variation resources	Degrees of Freedom	Rooting rate of cuttings	Plant branch number	Plant height
Block	2	402.75 **	92.22 *	843.68 **
Genotype	16	909.33 **	182.80 **	996.29 **
Error	32	40.94	23.28	0.83
CV (%)		7.26	15.92	1.18

*Significant at 5% level ** Significant at 1% level

Some yield values belong to B clones

Yield values belong to B clones are given in Table 8, and variance analysis is given in Table 9. As a result of statistical analyses, there was a significant difference at the 1% level in terms of fresh herbage yield, fresh leaf yield, dry herbage yield, dry leaf yield, and dry leaf rate of clonal lines. When the groups were observed, many clonal lines participated in different groups. While fresh herbage yield varied between 1115.20 and 3728.00 kg/da, fresh leaf yield was between 615.87 and 2050.40 kg/da. The lowest fresh herbage and fresh leaf yields were obtained from FK4-32. In addition, the highest fresh herbage and fresh leaf yields were obtained from FD2-9 and FK4-14 respectively. Clonal lines with high yields belong to the populations from Demre-Kekova, Kemer-Teleferik and Kemer-Kiriş. Clonal lines

participated in different groups and each clonal line with high yield is from different populations, this situation demonstrated the difference in and among the populations.

Bayram et al (1999) stated that fresh herbage yield of Anatolian sage in Bornova ecological conditions showed a change between the first (1028.80 and 2055.57 kg/da) and second years (2870.30-6558.60 kg/da). In another study, Bayram (2001) determined the fresh herbage yield of Anatolian sage as 639.00 kg/da in Bornova ecological conditions. Mossi et al., (2011) determined fresh herbage yield of Anatolian sage 1174.00 kg/da in Brazil ecological conditions. Karık (2013) determined the highest herbage yield of Anatolian sage (*S. fruticosa* Mill.) 4533.73 kg/da in the first year and 5372.85 kg/da in the second year. In the same study, the yield was 3506.67 kg/da in the first year and 5181.70 kg/da in the second year. When these studies are compared with our study, the highest fresh herbage yield (3728.00 kg/da) was higher than in the studies of Bayram (2001) and Mossi et al., (2011). Moreover, Bayram et al., (1999) and Karık (2013) obtained similar results in the first year, however, they got higher results in the second year. In the light of this information, when the clonal lines with the highest fresh herbage yields (FD2-9, FK4-14 and FK3-16) are cultivated for long years, the high yield can be obtained.

While dry herbage yield varied between 555.03 and 1357.93 kg/da, dry leaf yield was between 290.70 and 605.87 kg/da among clonal lines. The highest dry herbage and dry leaf yields were obtained from FD2-9 in direct proportion to fresh herbage yield. Bayram (2001) determined dry leaf yield of Anatolian sage 161.30 kg/da in Bornova (İzmir) ecological conditions. Baranauskiene et al., (2011) stated dry leaf yield of same species 50.00 and 270.00 kg/da in Lithuanian conditions, furthermore, Mossi et al., (2011) determined 210.00 kg/da in Brazil conditions. Bayram et al., (1999) determined dry herbage yield of Anatolian sage 475.40-871.00 kg/da in the first year and 666.67-2058.73 kg/da in the second year in Bornova (İzmir) ecological conditions. Karık (2013) determined the highest dry herbage yield 1494.86 kg/da in the first year and 2209.58 kg/da in the second year. In the same study, population means were determined 1068.20 kg/da in the first year and 1537.96 kg/da in the second year. When the highest dry herbage yield (1357.93 kg/da) in our study was compared with other studies, it was higher than the first year values, and lower than in the second year values of the other studies.

Table 8. Yield values belong to B clones

Clonal line	Fresh herbage yield (kg da ⁻¹)	Fresh leaf yield (kg da ⁻¹)	Dry herbage yield (kg da ⁻¹)	Dry leaf yield (kg da ⁻¹)
FK3-16	2474.67 b	1219.90 d	1246.46 b	429.53 d
FK4-2	1400.00 g	753.83 h	601.87 k	290.70 j
FK4-8	1587.20 f	925.00 fg	745.17 hi	388.63 fg
FK4-9	1432.00 g	882.63 g	654.33 j	459.80 c
FK4-11	2308.00 c	1307.33 c	1056.46 c	596.70 a
FK4-14	2556.83 b	1491.43 b	923.37 d	527.33 b
FK4-15	1411.20 g	777.60 h	555.03 l	362.93 h
FK4- 22	1217.60 h	543.00 j	781.53 g	362.10 h
FK4- 32	1115.20 h	615.87 i	724.57 i	365.90 h
FK5-7	1163.20 h	800.87 h	634.93 j	364.60 h
FK5-15	1171.20 h	646.77 i	743.03 hi	384.70 h
FKM1-16	2195.20 cd	807.07 h	828.20 f	372.77 gh
FKM3-5	1792.00 e	1018.73 e	734.33 i	367.37 h
FD2-9	3728.00 a	2050.40 a	1357.93 a	605.87 a
FD4-13	2248.00 cd	1320.27 c	854.50 e	389.80 f
FKS3-7	2141.37 d	967.07 ef	780.00 g	334.47 i
FKS3- 8	1155.20 h	676.60 i	761.23 gh	412.20 e

*Means followed by the same letters are not statistically significant according to Duncan's Multiple Range Test

Table 9. Variance analysis results related yield characteristics of B-clones

Variation resources	Degrees of Freedom	Fresh herbage yield (kg da ⁻¹)	Fresh leaf yield (kg da ⁻¹)	Dry herbage yield (kg da ⁻¹)	Dry leaf yield (kg da ⁻¹)
Block	2	159436.66 **	64476.89 **	42081.87 **	11300.00 **
Genotype	16	1496023.33 **	446538.02 **	141180.05 **	23044.26 **
Error	32	6579.45	1535.06	234.83	95.34
CV (%)		4.43	3.96	1.86	2.37

** Significant at %1 level

Quality characteristics belong to B clones

As a result of statistical analyses, there was a significant difference at the 1% level among clonal lines in terms of essential oil rate and rosmarinic acid amount. Quality values belong to B clones are shown in Table 10, and variance analysis is given in Table 11. When the groups were observed, while many clonal lines participated in different groups in terms of essential oil, FD2-9 and FK5-7 were in the same group. Essential oil rates varied between 1.25% and 3.80%. The highest essential oil rates were obtained from FK4-9 (3.80%), FD2-9 (3.63%) and FK5-7 (3.60%). When the studies by different researchers in different locations were observed, essential oil of Anatolian sage was determined 2.8% by Bayrak and Akgül (1987), 2.3-3.5% by Ceylan and Kaya (1988), 1.95% by Baydar et al., (1999), 3.68% by Bayram (2001), 0.9-2.8% by Başer and Kırimer (2006), 2.9% by Kocabaş et al., (2007), 1.5% by Karık and Öztürk (2009), 2.3% by Aşkun et al., (2010), 0.98% by Mossi et al., (2011) and 3.52% by Karık (2013). It can be said that the essential oil rate in our study was higher than in these studies. The

reason of this situation can be explained by different ecological conditions that materials collected from, different genotypes of plants, different ecological conditions of the experimental fields, different years, agricultural practices and different harvest times.

When the groups were observed, many clonal lines were in different groups in terms of rosmarinic acid amount. Rosmarinic acid amount varied between 2.68 and 8.89 mg/g. The highest rosmarinic acid amount was obtained from FK3-16 clonal line, and FK4-14, FKS3-8, FK4-22 followed that. Four clonal lines that had the highest rosmarinic acid amount, had lower than 3% essential oil rate. Cao et al., (1993) determined 5% rosmarinic acid in *S. fruticosa* Mill. Dinçer et al., (2012) studied with natural and cultivated varieties of *S. fruticosa* Mill that had 5.33 and 5.50 mg/g rosmarinic acid respectively. Rosmarinic acid of *S. fruticosa* Mill in this study, were in higher amounts and range than the other studies. Durling et al., (2007) stated that antioxidant characteristic of sage is related to the carnosic acid, carnosol and rosmarinic acid. In the light of values in this experiment, it can be said that Anatolian sage (*S. fruticosa* Mill.) is an important antioxidant source.

Table 10. Quality values belong to B clones

Clonal line	Essential oil rate (%)	Rosmarinic acid amount (mg g ⁻¹)
FK3-16	2.75 g	8.89 a
FK4-2	3.08 d	6.26 g
FK4-8	2.50 h	6.95 e
FK4-9	3.80 a	6.95 e
FK4-11	2.50 h	2.68 n
FK4-14	2.88 e	7.94 b
FK4-15	2.50 h	5.70 i
FK4- 22	2.86 ef	7.31 d
FK4- 32	2.50 h	6.07 h
FK5-7	3.60 b	5.25 j
FK5-15	3.33 c	4.25 m
FKM1-16	2.50 h	6.01 h
FKM3-5	1.25 i	6.57 f
FD2-9	3.63 b	6.95 e
FD4-13	2.75 g	4.77 l
FKS3-7	2.82 f	4.95 k
FKS3- 8	2.50 h	7.82 c

*Means followed by the same letters are not statistically significant according to Duncan's Multiple Range Test

Table 11. Variance analysis results related quality characteristics of B-clones

Variation resources	Degrees of Freedom	Essential oil rate	Rosmarinic acid amount
Block	2	0.34 **	0.00 N. S.
Genotype	16	1.09 **	6.93 **
Error	32	0.00	0.00
CV (%)		1.09	1.12

N.S: Not Significant * :Significant at % 1 level

Table 12. Essential oil components belong to B clones (%)

Component	FK3 - 16	FK4 - 2	FK4 - 8	FK4 - 9	FK4 - 11	FK4 - 14	FK4 - 15	FK4 - 22	FK4 - 32	FK5 - 7	FK5 - 15	FKM1 -16	FKM3 -5	FD2 - 9	FD4 - 13	FKS3 - 7	FKS3 - 8	
1	α-pinene	4.40	3.11	3.92	4.70	3.16	3.79	3.60	2.81	3.80	3.51	5.09	5.31	3.41	3.34	2.96	3.25	8.42
2	Tujen	-	-	-	-	0.56	-	-	-	-	-	-	-	-	-	-	-	-
3	Kamfen	3.23	-	2.44	5.61	-	2.41	1.80	3.28	2.28	0.83	-	0.61	-	-	-	1.99	3.38
4	β-pinene	6.20	7.23	7.16	6.52	6.75	7.26	6.31	2.92	6.11	5.08	3.47	2.63	4.22	8.38	9.51	5.87	9.59
5	Mirsen	2.72	6.60	6.27	8.09	4.51	1.77	3.09	1.68	4.17	2.72	6.58	2.55	4.73	3.11	2.75	2.35	5.33
6	α-terpinen	0.46	-	-	-	-	-	-	-	-	0.45	0.68	0.48	-	-	-	-	0.62
7	Limonen	1.23	0.78	0.83	1.03	0.72	0.83	0.78	1.03	0.84	0.83	1.20	1.05	0.87	0.87	1.15	0.84	1.72
8	1,8-sineol	47.79	60.11	56.85	34.51	67.05	60.95	53.18	55.14	42.63	59.42	60.60	61.39	67.72	68.12	73.49	60.52	45.61
9	Gamma terpinen	0.77	0.70	0.68	0.85	0.79	0.66	0.58	0.42	0.55	0.78	0.98	0.68	0.47	0.51	0.46	0.51	1.04
10	Cimen	0.75	-	-	0.62	-	-	-	0.56	-	0.42	1.13	0.69	0.66	0.45	-	0.50	1.96
11	Tiranton	0.56	0.58	0.67	0.65	0.65	0.59	0.61	0.55	0.56	0.58	0.59	0.58	0.61	0.59	0.57	0.60	0.57
12	Tujon	2.86	3.46	2.95	5.30	4.35	5.53	2.46	2.68	2.97	1.24	-	1.44	1.78	0.97	1.41	0.60	4.35
13	Sabinen hidrat	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.49	-
14	Kafur	7.49	1.11	9.15	16.91	1.45	5.66	3.29	16.88	5.23	2.71	3.07	0.96	1.71	2.81	0.72	5.21	8.29
15	Linalool	-	-	-	-	-	-	-	-	-	-	-	0.43	-	-	-	-	-
16	Bornil asetat	-	0.71	-	-	-	-	1.15	1.24	1.00	-	-	-	-	-	-	-	-
17	Terpinen-4-ol	0.74	0.75	0.77	1.01	0.79	0.82	0.64	0.86	0.55	0.61	0.74	0.62	0.52	0.59	0.80	0.44	1.09
18	Karyofillen	7.01	6.48	3.68	7.51	3.15	3.79	14.12	5.19	18.23	4.10	3.49	9.89	10.14	3.59	3.57	7.69	4.48
19	Kaleren	0.67	-	-	-	-	-	-	-	0.48	0.43	-	0.85	-	-	-	-	-
20	Aromadendren	2.72	1.34	0.56	-	1.33	0.61	1.17	-	1.83	1.86	0.95	3.49	-	-	0.89	-	-
21	α-humulen	4.98	1.04	0.71	1.31	1.78	-	1.20	0.92	1.77	7.65	7.31	1.20	0.93	-	0.87	2.55	1.34
22	α-terpineol	-	0.51	-	-	-	-	0.83	-	-	0.61	-	0.85	-	4.02	-	0.52	-
23	Borneol	2.19	-	1.09	0.98	-	1.10	1.55	2.75	1.41	-	-	-	-	-	-	1.60	1.16
24	Leden	-	0.73	-	-	-	-	-	-	-	1.19	-	1.56	-	-	-	-	-
25	Delta cadinen	-	-	-	-	-	-	0.53	-	-	-	-	-	-	-	-	0.96	-
26	Karyofillen oxit	0.56	0.75	0.61	1.59	-	1.08	1.47	1.09	1.05	0.49	-	-	1.63	0.54	-	0.76	1.05
27	Azulen	1.24	3.09	1.65	2.83	2.23	2.35	1.14	-	3.92	3.10	2.98	1.74	-	0.90	-	1.88	-
28	Jupinen	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.88	-
29	Spathulenol	-	0.93	-	-	0.76	0.82	0.51	-	0.62	0.60	-	0.43	-	-	0.85	-	-
Total		98.57	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	99.21	98.86	99.43	99.40	98.79	100.00	100.00	100.00
Undetectable		1.45	-	-	-	-	-	-	-	-	0.82	1.14	0.59	0.63	1.22	-	-	-

Totally, 29 components were identified in essential oil and these components formed approximately 100% of essential oils. The main components of essential oils were 1,8-cineole, camphor and caryophyllene. It was determined that the proportion of 1,8-cineole was between 34.51 and 73.49%. The highest 1,8-cineole rate was obtained from FD2-9 and FD4-13 clonal lines from the populations in Demre location. Camphor that was the another main component of essential oils, varied between 0.72 and 16.91%. Moreover, caryophyllene rate was determined between 3.15 and 18.23%. Besides the main components, there were α -pinene (2.81-8.42%), camphene (0.61-5.61%), β -pinene (2.63-9.59%), and thujone (0.60-5.53%) that were relatively higher than the other components (Table 12).

Karioti et al., (2003) determined 1,8-cineole as main essential oil component of *S. fruticosa* Mill. Başer and Kırimer (2006) indicated that the main components of *S. fruticosa* Mill. which was found in Turkey, were 1,8-cineole (35-51%) and camphor (7-13%). Aşkun et al., (2010) and Kocabaş et al., (2010) determined 1,8-cineole as the main component of essential oil of *S. fruticosa* Mill. with the rates of 52.8% and 50.7% respectively. Karik (2013) determined the highest 1,8-cineole and camphor rates for the same species 35.80% and 26.50 respectively. It was observed that 1,8-cineole is the main component in the essential oil in the studies about Anatolian sage (*S. fruticosa* Mill.). In addition, while Skoula et al., (2000) found 1.90-11-5% β -thujone in the essential oil of *S. fruticosa* Mill., Karik (2013) found 0.60-2.25%. Thujone in our study (0.60-5.53%) was lower than the study of Skoula et al., (2000), however, it was similar with the study of Karik (2013). Different results among the experiments can be because of the different ecology, where plant material collected from, and different genotypes. As it is seen in Table 7, the range of essential oil component rates were high. This situation can be explained by the wideness of total area and high variation.

Conclusion

In this experiment, which was conducted with the purpose development of new, high quality and agronomic varieties by clonal selection, it was determined that Anatolian sage (*S. fruticosa* Mill.) that are found in different locations in Antalya province, showed a large variation, and there were superior clonal varieties. In the light of obtained data, FK3-16, FK4-9, FK4-14, FK5-7, FD2-9 and FD4-13 clonal lines were selected as C clones. It can be said that, selected clones can be used as medicine, herbal tea, cosmetic and ornamental as a result of breeding experiments in the future. Note: As a result of the studies carried out in the continuation of this study, two variety candidates 'UYSAL' and 'TURGUT' were applied for registration.

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