Advances in Molecular Biology (1-2): 47-56, 2009
İstanbul Kültür University, Printed in Turkey
www.advmolbiol.org

Genetic differentiation of Turkish *Althaea* L. and *Alcea* L. species

Fatma Öztürk[1], Selcen Babaoğlu[2], M. Erkan Uzunhisarcıklı[2], Leyla Açık[2]*, Mecit Vural[2], I. Sefa Gürcan[3]

[1] Erciyes University, Faculty of Science And Art, Department of Biology, 38039, Kayseri

[2] Gazi University, Faculty of Science and Art, Department of Biology, 06500, Ankara

[3] Ankara University, Faculty of Veterinary, Department of Biostatistics, 06110, Ankara

Abstract

Random amplified polymorphic DNA (RAPD) analysis and SDS-PAGE analysis of total seed proteins were used to estimate genetic differences between/within four *Althaea (Malvaceae)* and eighteen *Alcea* species. The 4 *Althaea* and 18 *Alcea* species were found to have species-specific protein profiles. The total of 223 different RAPD bands for *Althaea* species were generated by the 11 primers and total of 116 different RAPD bands by the 8 random primers for *Alcea* species. The results of the POPGENE cluster analysis were in broad agreement with previous morphological classifications of these four species. Statistical significance within *Alcea* and *Althaea* population was tested using of molecular variance (AMOVA) approach used in Arlequin. According to the results, there is a large genetic differentiation between species *A. hirsuta* with *A. armeniaca*, *A. officinalis* with *A. hirsuta*, F_{ST}0.24 and F_{ST}0.21, respectively. There is also wide differentiation between *Alcea* species. F_{ST} statistics range from 0,126 (*A. guestii* between *A. striata* ssp. *striata*) to 0,685 (*A. biennis* between *A. acaulis*). The results suggest that RAPD and SDS-PAGE markers can be used to reveal genetic differentiation between species and genus.

Key Words: *Althaea*, *Alcea,* RAPD-PCR, SDS-PAGE

Introduction

Malvaceae family is represented by 80 genera and species over 1000 distributed worldwide. The main spreading centre of the family members is South America and majority of them are widespread (Hutchinson, 1973; Heywood, 1978). As we compare the number of species that belong to *Malvaceae* family in Turkey with the other countries, an obvious similarity is recognized but we should mention about the significant increase in Iran and Russia. While the taxa number in Turkey is 45, it is 108 in Iran and 85 in Russia (Davis *et al*., 1967; Riedl, 1969; Iljin, 1949). *Althaea (Malvaceae)* is represented by four taxa in Turkey. 18 *Alcea* taxa are recorded at Turkish flora. *Alcea striata* is represented by 2 subgenus and total taxa number is 19 (Davis *et al*., 1967).

Althaea has annual and perennial species; and characterized by 6-9 epicalyx segments connate at the base; petals up to 16 mm; anthers brownish purple; fruit a schizocarp, splitting into numerous 1-seeded indehiscent mericarps which are in 1-series around the style; carpels unilocular. *Alcea* species are characterized by epicalyx segments usually 6, rarely 7-9, connate at base; petals 30 mm or longer; anthers

* Correspondence author
Gazi University, Faculty of Science and Art, Department of Biology, 06500, Ankara
E-mail: leylaacik@gmail.com

Received: May, 13, 2009, Accepted: June, 11, 2009.

Table 1. Comparison of the morphological characters of *Althaea* species

	Duration	Leaves	Lobes of epicalyx	Sepals	Petals	Mericarps
A. cannabina	Perennial	Palmatifid-palmatisect	7-9-lobed 2.5-5 x 0.1-0.5 mm	5-7 x 3-4 mm 10-23 x 5-12 mm	Lilac-purblish	2.5-4 x 2-3.5 mm reniform glabrous
A. armeniaca	Perennial	Palmatifid-palmatipartite	6-10-lobed 2-6 x 0.5-2 mm	8-10 x 2-5 mm	Lilac-purblish 9-11 x 4-6 mm	2-3 x 1.5-3 mm orbicular stellate-pubescent hairy
A. officinalis	Perennial	Entire-palmatilobate	8-12-lobed 2-6 x 1-2 mm	6-12 x 2-5 mm	White-pinkish 7.5-15 x 6-13 mm	1.5-2.5 x 2-3 mm orbicular, reniform stellate hairy
A. hirsuta	Annual	Palmatilobate-palmatisect	7-8-lobed 4-13 x 1-4 mm	5-15 x 1-3 mm	Pinkish-lilac 6-16 x 5-10 mm	1-1.5 x 2-2.5 mm reniform glabrous

usually yellowish: fruit a schizocarp, splitting into numerous 1-seeded mericarps; carpels sub-bilocular (Table 1-2) (Davis *et al*., 1967).

The relationship between *Althaea* and *Alcea* L. had been a controversy. Linne (1753), in his Species Plantarum, assess to distinguish *Althaea* from *Alcea*. Later Willdenow (1800), De Candolle (1824), Baker (1890) fused the two genera into *Althaea*. However, Alefeld (1862), Boissier (1872) in Flora Orientalis, Iljin in Flora URSS (1949) and others insisted on keeping the two genera apart and this was also adopted by the Index Generum Phanerogamorum. Zohary (1963a; 1963b) also evaluated the two genera apart from each other after overall assessment of the studies. Fusing the genera *Althaea* and *Alcea* particularly striking in regard to the staminal tube and the carpels. The discrimination key of *Althaea* and *Alcea* genera from each other in Turkish flora rely on the difference below (Davis *et al*, 1967);

• Petals 9-16 mm; carpels unilocular; anthers purple or brownish purple ……..……. *Althaea*

• Petals 30 mm or more; carpels subbilocular, divided by an internal process of the pericarp; anthers yellowish……..……. *Alcea*

Althaea and *Alcea* (e.g. *Alcea biennis*) species are known as medicinal plants because of their usage in treating asthma and coughing. Also their flower and leaf extracts are used to treat some derm itches among public and *A. officinalis* L. roots are shown to have an antimicrobial activity (Lauk *et al*; 2003; Yücel, 2000).While there isn't any endemic taxon among these taxa, *A. armeniaca* Ten. takes most attention with its narrow distribution but in this study it has been collected from some localities and its systematic position is clearly explained. There are some difficulties in the identification of the *Althaea* and *Alcea* species because the description of this species is not explanative enough. Although species are discriminated according to the fruit characteristics this feature is not clarified enough to identify the species, and the characters used in the identification are the ones that may show variations such as petal length, petal color, indumentum, leaf division. Nevertheless *Alcea* and *Althaea* species especially *A. acaulis, A. pisidica, A. guestii, A. flavovirens, A. fasciculiflora*, *Alth. armeniaca* which is known from a few localities would be in danger of extinctions in Turkey because they all grow on roadsides. Therefore many seed samples have been collected to send the germplasm banks and to grow in special gardens in the aim of maintaining the survivability of the species.

Table 2. Comparison of the morphological characters of *Alcea* species

	Duration	Leaves	Lobes of epicalyx	Sepals	Petals	Mericarps
A. acaulis	Perennial	Palmatilobate	5-7-lobed 2-5 x 1-4 mm less than ½ as long as the calyx	10-20 x 2-5 mm	White 1.4-4 x 0.4-1.2 cm	4-5 x 4-5 mm reniform-orbicular not winged
A. striata	Perennial	Entire-palmatilobate	6-7-lobed 2-6 x 1.5-5 mm less than ½ as long as the calyx	10-20 x 4-8 mm	White, pink or yellow 1.4-4 x 0.4-1.2 cm	3-4 x 3-5 mm orbicular not winged
A. remotiflora	Perennial	Palmatilobate-palmatipartite	6-8-lobed 1-7 x 1-5 mm less than ½ as long as the calyx	10-15 x 3-6 mm	White, yellowish at base 1.5-3 x 1-2 cm	2-4 x 2-4 mm orbicular not winged
A. digitata	Perennial	Palmatipartite-Palmatisect	6-8-lobed 3-10 x 1-8 mm ½ or more than as long as the calyx	10-18 x 4-9 mm	Pink-purblish, rarely white, whitish-yellowish at base 1.5-3 x 1-2 cm	3-5 x 3-5.5 mm orbicular not winged
A. setosa	Perennial	Entire-Palmatipartite	6-8-lobed 7-16 x 3-7 mm more than ½ as long as the calyx	10-18 x 4-8 mm	Pink, rarely white, yellowish at base 2-5 x 1-2 cm	3-7 x 2.5-6 mm orbicular not winged
A. apterocarpa	Perennial	Palmatilobate-Palmatipartite	6-8-lobed 5-15 x 2-6 mm more than ½ as long as the calyx	8-22 x 3-7 mm	White, yellow or pink-purblish 3-6.5 x 1-2.5 cm	3-6 x 3.5-5.5 mm orbicular not winged
A. kurdica	Perennial	Palmatilobate-Palmatipartite	6-9-lobed 1-4 x 1-3 mm less than ½ as long as the calyx	10-15 x 4-6 mm	White, pink or yellow 2-5 x 0.7-1.2 cm	4-5 x 4-5 mm orbicular winged
A. heldreichii	Perennial	Entire-Palmatilobate	6-7-lobed 1.5-5 x 1-4 mm less than ½ as long as the calyx	6-20 x 3-10 mm	White, pinkish, yellowish at base 1-4 x 0.8-1.5 cm	2-4 x 2-5 mm orbicular winged
A. calvertii	Perennial	Entire-Palmatilobate	4-8-lobed 1-7 x 1-5 mm less than ½ as long as the calyx	10-18 x 4-8 mm	White, greenish at base base 2-4.2 x 0.5-2.5 cm	3-5 x 3-5 mm orbicular winged
A. hohenackeri	Perennial	Palmatilobate-Palmatifid	5-8-lobed 5-10 x 1.5-5 mm ½ or more than as long as the calyx	8-18 x 4-8 mm	Yellow 3.5-5.5 x 1-5.5 cm	4-6 x 2.5-5 mm orbicular winged
A. pisidica	Perennial	Entire-Palmatilobate	6-7-lobed 5-10 x 1-7 mm ½ or more than as long as the calyx	10-20 x 4-8 mm	Yellow 2.5-5.5 x 1.2-3 cm	5-6 x 5-7 mm reniform-orbicular winged

Continued in the next page

A. guestii	Perennial	Palmatilobate	7-8-lobed 4-10 x 1.5-4 mm more than ½ as long as the calyx	12-20 x 3-8 mm	yellow, white 2.5-4.5 x 1-2.5 cm	4.5-6 x 5-7 mm orbicular winged
A. biennis	Perennial	Entire-palmatilobate	6-8-lobed 5-15 x 2.5-10 mm ½ or more than as long as the calyx	9-17 x 4-8 mm	pink-purblish, white, yellowish at base 2-5 x 1-4 cm	2-4 x 2-5 mm orbicular winged
A. dissecta	Perennial	Palmatipartite-palmatisect	6-9-lobed 1-10 x 1-7 mm less than ½ as long as the calyx	10-20 x 4-7 mm	dark pink, white, yellowish at base 2.5-5.5 x 1-3.5 cm	3.5-7 x 4-8 mm reniform-orbicular winged
A. excubita	Perennial	Palmatipartite-palmatisect	5-6-lobed 1-5 x 1-4 mm less than ½ as long as the calyx	10-15 x 4-5 mm	white-bright yellow 2-5.5 x 0.8-3 cm	4-5 x 3-5 mm orbicular winged
A. flavovirens	Perennial	Palmatilobate-palmatifid	5-8-lobed 2-6 x 1-4 mm less than ½ as long as the calyx	10-20 x 4-6 mm	yellow, greenish at base 3-5 x 1.5-3 cm	4-5 x 4-5 mm orbicular winged
A. fasciculiflora	Perennial	Palmatifid-palmatisect	6-8-lobed 5-10 x 2-5 mm less than ½ as long as the calyx	15-25 x 5-10 mm	pink 2.5-5.5 x 1-3.5 cm	4-5.5 x 4-6.5 mm reniform winged

RAPD profiling distinguishes among both population level and species-level genetic variation economically and rapidly (Chapparo *et al*., 1994; Stewart and Porter, 1995). The main advantages of seed protein electrophoresis with respect to the conventional morphological approach are independence of the growing season, no need for plant cultivation, availability of material the year around, relative speed of the examination, ease of storing material, and the small sample size needed (Dinelli and Lucchese, 1999). Measures of molecular and morphological genetic variation are often used to set conservation priorities and design management strategies for plant taxa. We used a combination of both molecular (RAPD analysis) and biochemical (seed proteins) markers to better understand the genetic diversity among the species of *Althaea* and *Alcea* growing in Turkey.

Materials and methods

Plant material and DNA extraction

Plant material used in this study was 4 taxa *Althaea* and 18 taxa *Alcea* species. Total genomic DNA extractions were carried out according to method modified by Steward and Porter (1995) with the addition of 2 % PVP to 2 % CTAB. A phenol: chloroform-isoamylalcohol (25:24:1) step was also added to remove polysaccharide contaminants and DNA dissolved in TE was mixed with 2M sodium chloride before the precipitation in 100% ethanol because, most of the polysaccharides were removed effectively in a single high-salt precipitation (Fang *et al*, 1992). Isolated DNA was used in RAPD-PCR reactions.

RAPD analysis

PCR amplifications were performed as described by Williams *et al*. (1990). A set of eleven-mer oligonucleotides were used. The reaction mix contained 10X PCR buffer (Sigma), 10 mM dNTP mix, 0.2 μM random primers, 25 mM $MgCl_2$, 15 ng genomic DNA and 1U of Taq polymerase (Sigma). Amplifications were carried out in a thermocycler (Biometra, Germany) under the following temperature program; 96°C for 30 s, 30 °C for 30 s, 72 °C for 30 s, for 45 cycles. 15 μl of reaction products were separated alongside molecular weight markers (100

bp DNA ladder) by electrophoresis in 1.5 % agarose gels run at 70 V using 1 x TBE buffer. DNA fragments were visualized by ethidium bromide staining and photographed under UV light then amplification patterns were examined.

Total seed protein analysis

Protein extraction was performed according to Saraswati *et al*. (1993). Electrophoresis was carried out following the Laemmli method (1970) using 10% polyacrylamide gels at a current of 20 mA. Each run included known molecular weight marker proteins (Fermentas, UK). Proteins on the gel were fixed and stained with Comassie Brilliant Blue G-250 as described by Demiralp *et al*. (2000).

Data analysis

In the study of overall genetic variation, the fragments either in RAPD analyze or in SDS-PAGE that were readable and reproducible were used. Bands were scored as either present (1) or absent (0) for all species studied. Common band analysis was conducted using the computer programme POPGENE based on the Nei's genetic distance to determine the genetic distance values between species (Yeh and Yang 1999, Page 1996). F_{ST} is a measure of population differentiation based on genetic polymorphism data and, compares the genetic variability within and between populations. F_{ST} is always positive; it ranges between 0 (no genetic divergence within the population) and 1 (complete isolation). F_{ST} values up to 0.05 indicate negligible genetic differentiation whereas higher than 0.25 means very great genetic differentiation within the population analyzed. Statistical significance within *Althaea and Alcea* population was tested using of molecular variance (ANOVA) approach used in Arlequin (Excoffier and Schneider, 2005).

Results and discussion

RAPD and SDS-PAGE techniques were used to characterize bulk populations of 4 different *Althaea* and 18 different *Alcea* species, and establish the relationships among them. Of the 40 oligonucleotide primers tested in RAPD reactions, only 10 primers for *Althaea* species and 8 primers for *Alcea* species gave specific and suitable results (Figure 1-2). Total seed proteins' band profiles were clearly separated species tested (Figure 3). The presence or absence of high intensity and high reproducible polymorphic RAPD and seed storage protein bands were used as the character to elucidate variations of the species.

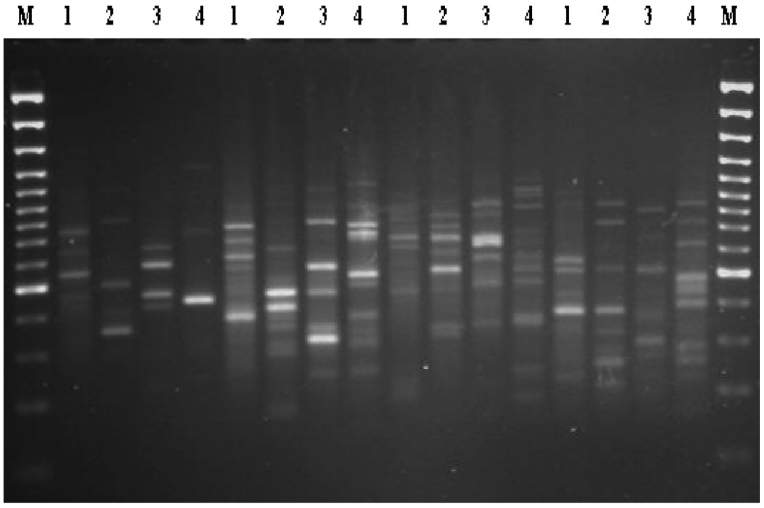

Figure 1. RAPD fragment patterns of *Althaea* species generated by the primer OPB05, LA12, B4, A2 respectively. M- DNA Ladder (100 bp) 1- *A. armeniaca*, 2- *A. officinalis,* 3- *A. hirsuta*, 4- *A. cannabina*

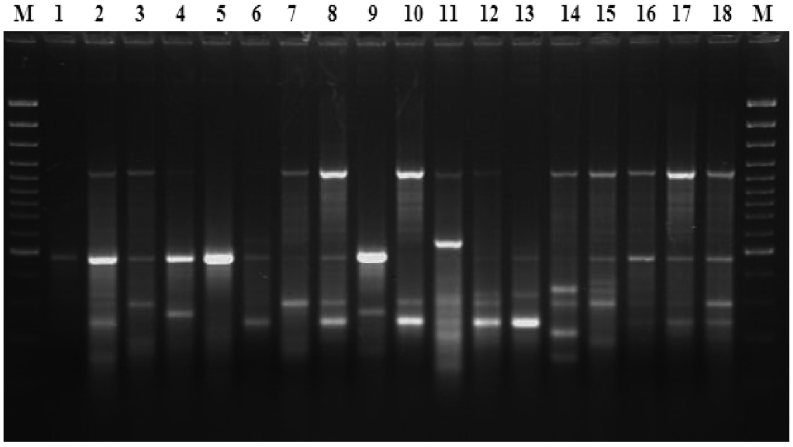

Figure 2. RAPD fragment patterns of *Alcea* species generated by the primer OPI18 M- DNA Ladder (100 bp), 1- *A. acaulis*, 2- *A. striata ssp. rufescens*, 3- *A. striata ssp. striata*, 4- *A. remotiflora*, 5- *A. digitata*, 6- *A. setosa*, 7- *A. apterocarpa*, 8- *A. kurdica*, 9- *A. heldreichii*, 10- *A. calvertii*, 11- *A. hohenackeri*, 12- *A. pisidica*, 13- *A. guestii*, 14- *A. biennis*, 15- *A. dissecta*, 16- *A. excubita*, 17- *A. flavovirens*, 18- *A. fasciculiflora*

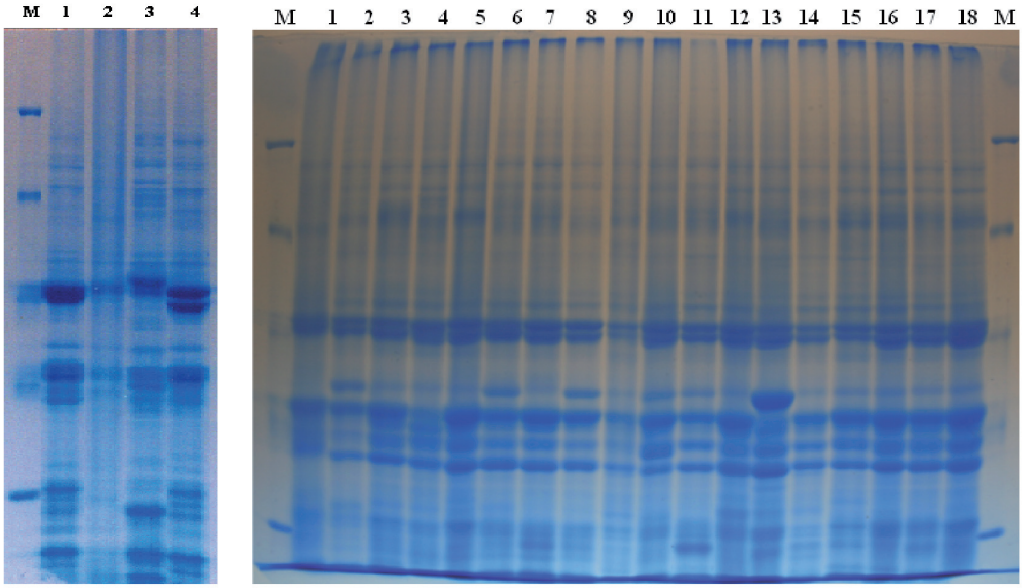

Figure 3. Protein profiles generated with SDS-PAGE of Althaea and Alcea. a) M- Protein Marker, figure, 1- *Althaea armeniaca*, 2- *A. cannabina*, 3- *A. hirsuta*, 4- *A. officinalis*. b) M- Protein Marker, 1- *Alcea acaulis*, 2- *A. striata ssp. rufescens*, 3- *A. striata ssp. striata*, 4- *A. remotiflora*, 5- *A. digitata*, 6- *A. setosa*, 7- *A. apterocarpa*, 8- *A. kurdica*, 9- *A. heldreichii*, 10- *A. calvertii*, 11- *A. hohenackeri*, 12- *A. pisidica*, 13- *A. guestii*, 14- *A. biennis*, 15- *A. dissecta*, 16- *A. excubita*, 17- *A. flavovirens*, 18- *A. fasciculiflora*

RAPD patterns displayed a considerable variability among species. For *Althaea* species, overall 223 bands ranging from 100 to 2000 bp in molecular size were analyzed and scored for polymorphism, allowing the construction of dendrogram, 171 of them were polymorphic. M13, although having produced amplification fragments it didn't show polymorphism for *Althaea* species (Table 3). For *Althaea* species based on RAPD and SDS-PAGE results the indices of differences were calculated (Table 3). The genetic distance values between *A. armeniaca, A. officinalis, A. hirsuta,* and *A. cannabina* were changed from 59% and 81%. Based on UPGMA dendrogramme (Figure 4), *A. armeniaca, A. officinalis,* and *A. cannabina* are clustered together distinct from the other taxa *A. hirsuta*. Our dendrogram shows the annual species *A. hirsuta* with distinctive hirsute hairs, outlines from the other three species. So it may be easily discriminated from the others morphologically. The other three species are perennial and hirsute hairs are never observed. *A. cannabina* and *A. armeniaca* take place in the dendrogram with the genetic distance 59%. Some morphological differences are also observed between these two genera. Although *A. cannabina* has glabrous mericarps and frequently palmate leaves, *A. armeniaca* has dense stellate hairy mericarps and 3-5 lobed palmatilobed-palmatipartite leaves. As a result it may be said that the results of the molecular studies are parallel with the studies based on the morphological characteristics. *A. officinalis* may also be distinguished from *A. cannabina* and *A. armeniaca* with having 3 lobed upper stem leaves and velutinous hairs. The genetic distance between *A. officinalis* and *A. cannabina* is 62%, the genetic distance between *A. officinalis* and *A. armeniaca* is 59% (Table 4). Statistical significance within *Althaea species* was

Table 3. Characteristics of random amplified polymorphic DNA (RAPD) primers exhibiting a polymorphic banding pattern in the set of *Althaea* and *Alcea* species.

		Althaea sp.			*Alcea sp.*		
Primer code	Primer sequences 5'… 3'	Amplified	Polymorphic	Percent	Amplified	Polymorphic	Percent
A2	TGCCGAGCTG	28	28	100	*	*	*
A7	GAAACGGGTG	19	11	57,8	*	*	*
B4	GGACTGGAGT	29	21	72,4	*	*	*
LA12	ACGACCCACG	21	21	100	*	*	*
OPO04	AAGTCCGCT	34	32	94,1	*	*	*
B18	CCACAGCAGT	10	6	60	21	21	100
M13	GAGGGTGGCGGTTCT	4	-	0	6	5	83,3
OPO06	CCACGGGAAG	19	19	100	16	15	93,7
OPB05	TGCGCCCTT	11	11	100	10	10	100
OPB10	CTGCTGGGAC	38	22	57,8	21	20	95,2
OPW06	AGGCCCGATG	10	10	100	17	17	100
OPI18	TGCCCAGCCT	*	*	*	12	11	91,6
OPU16	CTGCGCTGGA	*	*	*	13	12	92,3
TOTAL	223	181		116	111		

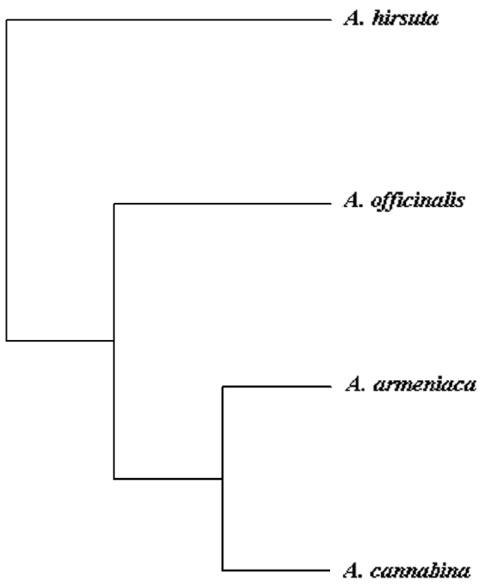

Figure 4. Dendrogram obtained by analysis combined data of RAPD and SDS-PAGE marker data from four *Althaea* species.

Table 4. Genetic distance data between *Althaea* species

Pop ID	*A. armeniaca*	*A. officinalis*	*A. hirsuta*	*A. cannabina*
A. armeniaca	**			
A. officinalis	59	**		
A. hirsuta	81	79	**	
A. cannabina	59	62	78	**

Table 5. Population pairwise F_{ST} comparisons between *Althaea* species

Pop ID	*A. armeniaca*	*A. officinalis*	*A. hirsuta*	*A. cannabina*
A. armeniaca	0.0000			
A. officinalis	0.1718	0.0000		
A. hirsuta	0.2464	0.2148	0.0000	
A. cannabina	0.1962	0.1957	0.2038	0.0000

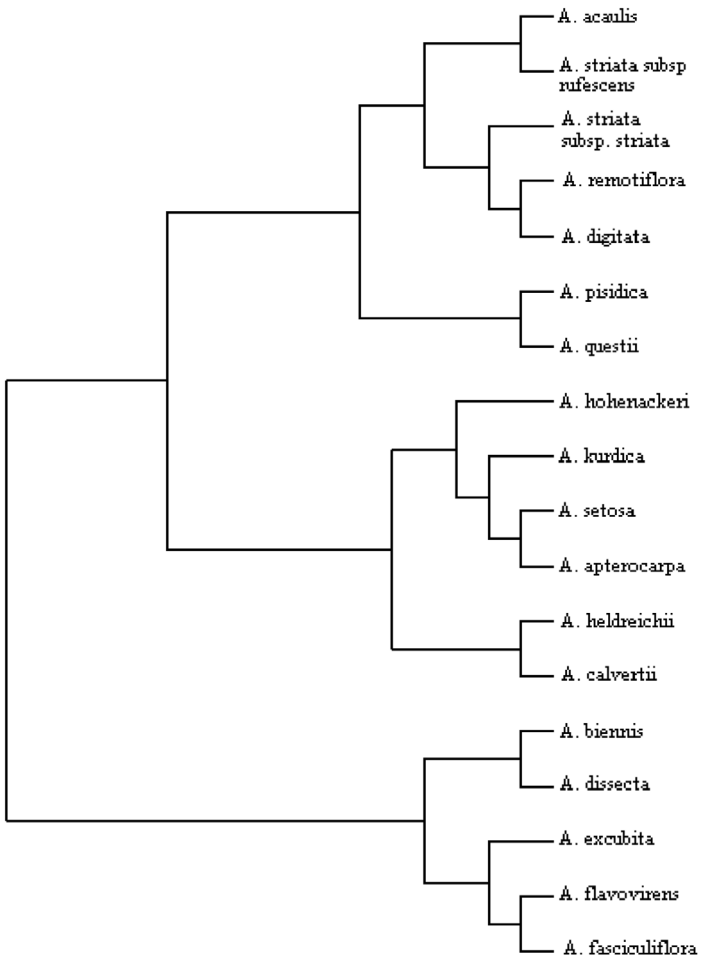

Figure 5. Dendrogram obtained by analysis combined data of RAPD and SDS-PAGE marker data from eighteen *Alcea* species.

tested using of molecular variance (ANOVA) approach used in Arlequin. The covariance components are used to compute fixation index (Table 5). According to the results, there is a large genetic differentiation between species *A. hirsuta* with *A. armeniaca*, *A. officinalis* with *A. hirsuta*, F_{ST} 0.24 and F_{ST} 0.21, respectively.

For *Alcea* species, overall 116 bands ranging from 100 to 2000 bp in molecular size were analyzed and scored for polymorphism, allowing the construction of dendrogram 101 of them were polymorphic (Figure 5). According to the results performed by the RAPD and SDS-PAGE analyses on the taxa of *Alcea* genus; the relevance value among all taxa is between 67-88% (Table 6). These values prove that these taxa are not so similar with each other but never the same. When the relevance values among the taxa are examined the highest value is between *A. remotiflora* and *A. digitata* (88%); the lowest are between, *A. fasciculiflora-A. striata* subsp. *striata* (67%) and *A. fasciculiflora-A. digitata* (67%) taxa. F_{ST} values of *Alcea* species ranges from 0.126 to 0.685. According to the results, there is the highest similarity between *A. acaulis, A. striata* ssp. *striata, A. striata* ssp. *rufescens, A. remotiflora, A. digitata, A. pisidica*

Table 6. The genetic distance data between Alcea species based on SDS- PAGE and RAPD results

pop ID	*A. acaulis*	*A.striata ssp .rufescens*	*A. striata ssp. striata*	*A. remotiflora*	*A. digitata*	*A. setosa*	*A. apterocarpa*	*A. kurdica*	*A. heldreichii*	*A. calvertii*	*A. hohenackeri*	*A. pisidica*	*A. guestii*	*A. biennis*	*A. dissecta*	*A. excubita*	*A. flavovirens*	*A. fasciculiflora*
A. acaulis	**																	
A. striata ssp. rufescens	21	**																
A. striata ssp. striata	23	19	**															
A. remotiflora	23	21	16	**														
A. digitata	20	24	21	12	**													
A. setosa	20	19	24	22	18	**												
A. apterocarpa	24	24	23	26	23	15	**											
A. kurdica	25	28	32	31	27	18	19	**										
A. heldreichii	23	24	28	26	22	20	21	22	**									
A. calvertii	25	26	27	27	23	21	21	22	19	**								
A. hohenackeri	29	26	28	27	26	17	21	22	21	22	**							
A. pisidica	24	28	25	23	20	23	26	29	26	26	28	**						
A. guestii	26	28	27	23	23	23	29	32	27	29	31	20	**					
A. biennis	29	23	26	24	25	25	27	31	27	29	26	30	26	**				
A. dissecta	29	25	26	27	26	25	27	27	28	28	26	27	27	16	**			
A. excubita	31	25	28	27	28	25	27	32	31	31	28	31	28	19	23	**		
A. flavovirens	27	25	29	27	30	25	29	30	27	28	28	28	28	25	23	16	**	
A. fasciculiflora	32	30	33	31	33	28	31	30	24	28	29	30	27	29	27	23	15	**

(0.126-0.291); on the contrary there is a large genetic differentiation between other species (Table 7). As a result it may be said that the results of the molecular studies are parallel with the studies based on the morphological characteristics.

RAPD and SDS-PAGE data evaluated by UPGMA cluster analysis three groups are constructed. First group is consists of *A. acaulis*, *A. striata* subsp. *rufescens*, *A. striata* subsp. *striata*, *A. remotiflora*, *A. digitata*, *A. pisidica* and *A. guestii* taxa; Second group involves *A. hohenackeri*, *A. kurdica*, *A. setosa*, *A. apterocarpa*, *A. heldreichii* and *A. calvertii* taxa; Third group is consisting of *A. biennis*, *A. dissecta*, *A. excubita*, *A. flavovirens* and *A. fasciculiflora* taxa. If the three group are evaluated separately; *A. acaulis-A. striata* subsp. *rufescens* (79 %), *A. remotiflora-A. digitata* (88 %) and *A. pisidica-A. guestii* (80 %) existing in the first group are very similar to each other. *A. striata* subsp. *striata* join to the *A. remotiflora-A. digitata* group. *A. acaulis* is the most significant genus in this group with its morphological differences and its relevance value to the other taxa is between 77-80 %. In the second group *A. setosa-A. apterocarpa* (85 %) and *A. calvertii-A. heldreichii* (81 %) resemble to each other. *A. kurdica* genus join to *A. setosa-A. apterocarpa* group, and *A. hohenackeri* to *A. kurdica* genus. In the third group *A. biennis-A. dissecta* (84 %)

Table 7. Population pairwise *Fst* comparisons between Alcea species

	A. acaulis	*A. striata ssp. rufescens*	*A. striata ssp. striata*	*A. remotiflora*	*A. digitata*	*A. setosa*	*A. apterocarpa*	*A. kurdica*	*A. heldreichii*	*A. calvertii*	*A. hohenackeri*	*A. pisidica*	*A. guestii*	*A. biennis*	*A. dissecta*	*A. excubita*	*A. flavovirens*	*A. fasciculiflora*
A. acaulis	0.000																	
A. striata ssp. rufescens	0,224	0.000																
A. striata ssp. striata	0.285	0.268	0.000															
A. remotiflora	0,245	0,229	0,155	0.000														
A. digitata	0,268	0,287	0,165	0,159	0.000													
A. setosa	0,527	0,543	0,633	0,665	0,490	0.000												
A. apterocarpa	0,549	0,590	0,623	0,585	0,524	0,137	0.000											
A. kurdica	0,623	0,645	0,590	0,579	0,574	0,156	0,161	0.000										
A. heldreichii	0,594	0,623	0,611	0,569	0,489	0,188	0,180	0,225	0.000									
A. calvertii	0,566	0,603	0,590	0,669	0,400	0,192	0,211	0,246	0,176	0.000								
A. hohenackeri	0,559	0,600	0,589	0,599	0,554	0,195	0,218	0,173	0,255	0,275	0.000							
A. pisidica	0,145	0,136	0,277	0,291	0,242	0,366	0,416	0,389	0,366	0,300	0,423	0.000						
A. guestii	0,135	0,126	0,286	0,271	0,265	0,389	0,401	0,445	0,379	0,327	0,456	0,244	0.000					
A. biennis	0,685	0,666	0,588	0,633	0,459	0,582	0,606	0,369	0,457	0,449	0,489	0,600	0,587	0.000				
A. dissecta	0,600	0,488	0,570	0,600	0,489	0,663	0,589	0,459	0,622	0,426	0,523	0,588	0,623	0,158	0.000			
A. excubita	0,654	0,522	0,489	0,598	0,523	0,496	0,623	0,563	0,586	0,401	0,557	0,660	0,600	0,185	0,195	0.000		
A. flavovirens	0,469	0,482	0,466	0,655	0,564	0,563	0,634	0,459	0,395	0,489	0,588	0,625	0,644	0,206	0,211	0,177	0.000	
A. fasciculiflora	0,569	0,592	0,534	0,588	0,527	0,623	0,555	0,626	0,456	0,458	0,500	0,588	0,616	0,199	0,202	0,186	0,164	0.000

and *A. flavovirens-A. fasciculiflora* (85%) are very similar to each other and *A. excubita* joins to *A. flavovirens-A. fasciculiflora* group. As morphologically *A. biennis* genus is significantly different from the other 4 taxa. Because of that its existance in this group and the relevance value between *A. dissecta* is the most extraordinary situation in all dendrogramme but its relevance value is between 73-84% and this is enough to show its difference also.

The objective of this study was to determine the genetic relationships among *Althaea* species and *Alcea* species growing in Turkey and to relate the morphological info with molecular data in order to obtain a better taxonomic classification of this species. The present results show that RAPDs and seed storage proteins are convenient and effective marker systems for *Althaea* and *Alcea* species. Data obtained from this molecular study show the variation among the *Alcea* and *Althaea* species support the morphological studies about these taxa. The genetic differentiation was observed within *Althaea* and *Alcea* species. Therefore substantial genetic differentiation was observed between the *Althaea* and *Alcea* species (results not shown), contrary to populations within species.

References

Alefeld F. Ueber die Malveen *Oest Bot Z.* 12: 246-261, 1862.

Baker EG. Synopsis of genera and species of *Malveae*. *J of Bot.* 28: 140-145, 207-209, 1890.

Boissier E. *Flora Orientalis*, Genevae et Basileae 2: 824-835, 1872.

Chapparo JX, Werner DJ, O' Malley D, Sederoff RR. Targeted mapping and linkage analysis of morphological isozyme and RAPD markers in peach. *Theor Appl Genet.* 87: 805-815, 1994.

Davis PH. *Flora of Turkey and the East Aegean Islands*. Edinb Univ Press, Edinburgh. 2: 411-420, 1967.

De Candolle A P. *Prodromus Systematis Naturalis Regni Vegetabilis* Paris. 1: 1-748, 1824.

Demiralp H, Çelik S, Köksel H. Effects of oxidizing agents and defatting on the electrophoretic patterns of flour proteins during dough mixing. *Eur Food Res Technol.* 211: 322-325, 2000.

Dinelli G, Lucchese C. Comparison between capillary electrophoresis and polyacrylamide gel electrophoresis for identification of *Lolium* species and cultivars. *Electrophoresis*, 20: 2524-2532, 1999.

Excoffier L, Laval G, Schneider S. Arlequin Version 3.0: An Integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online*. 1: 47-50, 2005.

Fang G, Hammar S, Grumet R. A quick and inexpensive methods for removing polysaccharides from plant genomic DNA. *Biotechniques*, 13: 52-56, 1992.

Hutchinson J. *The Families of Flowering Plants (Angiospermae) Dicotyledones*. Oxford Univ Press Oxford. 1: 311-313, 1973.

Heywood V H. *Flowering Plants of the World*. Oxford Univ Press London. 50-85, 1978.

Lauk L. Lo Bue A M, Milazzo I, Rapisarda A, Blandino G. Antibacterial activity of medicinal plant extracts against periodontopathic bacteria. *Phytother Res.* 17:599-604, 2003.

Iljin M M. *Flora URSS*. *Alcea* L. and *Althaea* L.,- In Komarov V.L. (ed.), Mosqua-Leningrad, 15: 64-106, 1949.

Laemmli UK. Cleavage of structural proteins during the assembly of the head of the bacteriophage T4. *Nature.* 277: 680-685, 1970.

Linne C. Species Plantarum. *Holmiae*. 2: 683-698, 1753.

Page RD. *Treeview*; An application to display phylogenetic trees on personal computers. *Computer Applications in the Biosciences*. 12: 357-358, 1996.

Riedl I. *Alcea L. and Althaea L.*(Eds) Rechinger KH Flora Iranica. Akademische Druck-u Verlagsantalt, Graz. 120: 37-86, 1967.

Saraswati R, Matoh T. Identification of *Sesbonia* species from electrophoretic patterns of seed proteins. *Trop Agric (Trinidad)*. 70: 282-285, 1993.

Stewart CN, Porter DM. RAPD profiling in biological conservation an application to estimating clonal variation in rare and endangered Iliamna in Virginia. *Biol Conserv.* 74: 135-142, 1995.

Willdenow CL. *Species Plantarum* Berolini. 694-695, 1800.

Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey S.V. DNA polimorphisms amplified by arbitrary primers are useful as genetic markers. *Nucl Acids Res.* 18: 6531-6535, 1990.

Yeh FC, Yang R. *POPGEN Version 1.31*.- University of Alberta- Alberta, Canada, 1999.

Yücel E, Tülükoğlu A. Gediz (Kütahya) Çevresinde Halk İlacı Olarak Kullanılan Bitkiler. *Ekoloji Çevre Dergisi.* 9:12-14, 2000.

Zohary M. Taxonomical studies in *Alcea* of South-Western Asia Part I. *Israel J Bot.* 11: 210-229, 1963.

Zohary M. Taxonomical studies in *Alcea* of South-Western Asia Part II. *Israel J Bot.* 12: 1-26, 1963.