Relationships among myrtle accessions from Turkey as revealed by fruit characteristics and RAPD

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Abstract

Myrtle, Myrtus communis L., is one of the most important and widespread shrubs (maquis) throughout the Mediterranean region. Its fruits ripen during the period of mid-fall to early winter and are generally dark-colored although white-colored wild forms rarely exist. In Turkey, both forms are present in addition to a white and large-fruited type, cultivated in small orchards and marketed locally. We studied two sets of myrtle accessions from Hatay, Turkey. Set 1 consisted of two wild accessions, dark- and white-colored forms, and six cultivated selections, while Set 2 contained three open-pollinated individuals from each accession of Set 1. We evaluated fruit characteristics of including fruit weight, width, length, soluble solids, pH and acidity. The accessions considerably differed in all the traits tested except for soluble solids. We also examined genotypic variation of Sets 1 and 2 using 26 RAPD primers and analyzed the data by cluster and principle components analyses. The results revealed that although some of the half-sib families (e.g., 8 a, b and c) were grouped based on their maternal parents, no overall pattern was apparent. These results suggest that myrtle pollination appeared to be not restricted to the self-pollination and the cultivated types have a narrow genetic base.

Keywords: cultivated; form; fruit color; *Myrtus communis*; wild

Introduction

Myrtle, *Myrtus communis* L., is a diploid (2n = 2x = 22) species native to the southern Europe and North Africa. Being widespread throughout the Mediterranean region, the species is one of the most important evergreen shrubs in the Mediterranean maquis and may grow up to 5 m tall. Their leaves are 3-5 cm long and contain tannins, flavonoids and volatile oils [1]. Owing to its pleasantly fragrant essential oil content, myrtle has been used for medical, food and spice purposes since ancient times. Other uses of the leaves include cattle feed, production of liqueur, cut foliage, and pot plants [2]. The flowers have five petals and sepals with a large number of stamens. They are pollinated by insects, and birds are the most common agent of dispersal although some mammals have been reported to consume and disperse their seeds, as well [3]. The seeds are snail-shaped and have a thick coat.

There are two major fruit morphologies based on the fruit color of dark and white. On the dark form, the fruits turn pale yellow and eventually dark blue on maturation during mid-fall to early winter. However, the fruit may either remain white or turn pale yellow, but never turns blue in the white-fruited form. The dark-colored form is far more common than the white-colored one. This color polymorphism has been reported from many regions such as Mallorca, Italy, and Iberian Peninsula [4].

Myrtle is widespread in the Mediterranean and Aegean Regions of Turkey [5]. Their leaves as well as fruits have been used as an antiseptic medicine in villages, while essential oil

from their leaves is utilized in perfumery [1]. Lately, the myrtle has received a significant amount of scientific attention and nutritional and their physical properties such as weight, thickness, sphericity, bulk density, and porosity were studied [6]. The essential oil and fatty acid composition of the myrtle fruits were also determined [7]. In Turkey, both color morphs are present, with the white morph being rare just as in other countries. In Turkey, there are also cultivated types with white fruits much larger than the wild forms. In Turkish, the wild form is called “mersin” or “murt”, while the cultivated form is called “hambeles”. There are small orchards in Hatay where this crop is marketed locally as table fruits. The orchards are established by seedling-grown plants since myrtle cuttings are highly subject to rot when they are prepared during the winter [8]. Furthermore, the growers consider segregations in the open-pollinated populations of the cultivated types to be negligible. This may suggest either self pollination or a narrow genetic base of the cultivated types.

The objectives of the present study were to (1) assess fruit and molecular variation among cultivated myrtle accessions from Turkey represented by six accessions; (2) compare diversity in fruit characteristics and RAPD bands between the cultivated and wild forms; and (3) determine the level of genetic similarity among the half-sib families of myrtle accessions.

Materials and methods

Two sets of plants were studied in the experiments. Set 1 consisted of eight accessions, six of which were large, white-fruited, cultivated types (31-01, 31-02, 31-03, 31-06, 31-07, 31-08) and two of which were from the wild ones (Fig. 1). The two wild accessions represented white (31-04) and dark-colored (31-05) forms of the wild myrtle populations. In the second set, Set 2, open-pollinated fruits were harvested from each of these accessions, and three seedlings were randomly selected and labeled as a, b and c. Therefore, the three seedlings from each accession were half-sib families. Information regarding the sampling locations of the accessions studied in the experiments was presented in Table 1.

Table 1. Myrtle accessions and their sampling locations in Turkey that were studied for their fruit characteristics and patterns of molecular variation.

Genotype	Longitude	Latitude	Elevation (m)
Set 1			
31-01	36º 19' 03"	36° 18' 49"	403
31-02	36º 18' 84"	36° 18' 66"	401
31-03	36° 18' 26"	36° 18' 86"	400
31-04	36° 18' 26"	36° 18' 86"	400
31-05	36° 18' 26"	36° 18' 86"	400
31-06	36° 19' 78"	36° 19' 14"	292
31-07	36° 13' 24"	36° 11' 52"	59
31-08	36° 12' 62"	36° 11' 25"	42
Set 2			
1	Open pollinated seedlings of 31-01		
2	Open pollinated seedlings of 31-02		
3	Open pollinated seedlings of 31-03		
4	Open pollinated seedlings of 31-04		
4	Open pollinated seedlings of 31-05		
5	Open pollinated seedlings of 31-06		
6	Open pollinated seedlings of 31-07		
8	Open pollinated seedlings of 31-08		

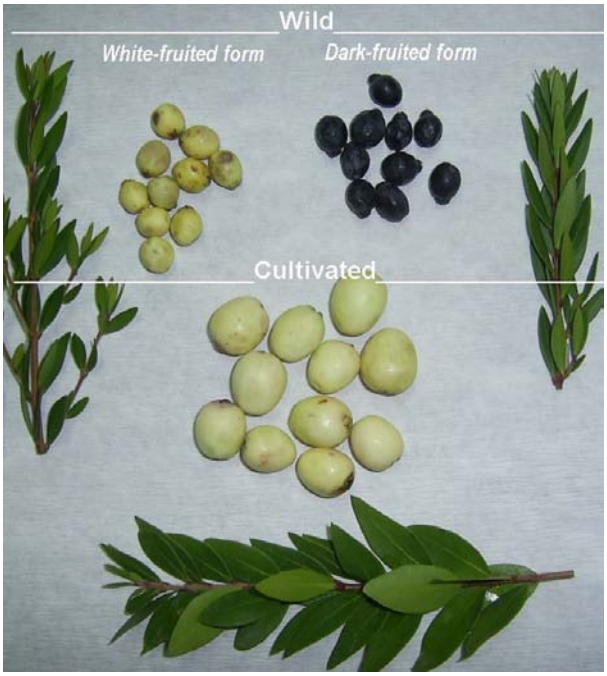

Figure 1. Fruit and leaves of wild and cultivated types. The wild types include white- and dark-fruited forms with smaller fruits than the cultivated types. The dark-fruited form is prevalent in wild populations.

The analyses were carried out for the accessions of Set 1 in 2005 and 2006. 31-04 and 31-05 were grown wild, while the other accessions were sampled from growers' orchards. The horticultural practices in these orchards were limited to pruning and training, weed management, and soil cultivation. The accessions were sampled upon their maturation at three different times (October, November, and December) as fruits ripe sequentially. At each harvesting date, four replicates of 100 fruits were randomly selected. Fruits were weighed by a scale sensitive to $\pm$ 0.01 g. Fruit width and length were measured using a digital caliper. Total soluble solids content (TSS) and titratable acidity (TA) were assessed in juice obtained from 100 fruits per subsample. TSS content was determined with a refractometer (Atago, Model ATC-1E) and TA by titration of 5 ml of fruit juice with 0.1 N NaOH to pH 8.1 and expressed as g malic acid per 100 mL juice. Juice pH was measured using a pH meter (InoLab, Model WTW).

Young leaves were collected from a single tree/seedling for each accession, immediately frozen in liquid nitrogen and stored at –80 °C. High molecular weight genomic DNA was extracted from the leaf samples following the protocol for minipreps by using CTAB [9]. DNA concentration was measured using a NanoDrop, ND 100 spectrophotometer (NanoDrop Technologies, Inc.) and gel electrophoresis. DNA was diluted in water to a final concentration of 50ng/µl and stored at –20 °C.

A hundred RAPD primers (from sets OPAC, OPAF, OPAK, OPE and OPU Operon Technologies, Almeda, CA, USA) were screened initially on a sample of the accessions. Primers that produced reproducible, polymorphic bands were used to amplify the rest of the accessions. Twenty-six 10-mer primers which were found to be polymorphic were used to generate the RAPD markers. Amplification reactions were done in 10 µl volumes containing 2x PCR Mastermix (Fermantas K0171), 1 unit of Taq DNA polymerase (Fermentas EP0402), $MgCl_2$, 30 ng of the primer and 20 ng of myrtle DNA. The mixtures were assembled at 0 °C, and then, transferred to thermal cycle, precooled at 4°C. The amplification was carried out in a model Master Gradient thermal cycler (Eppendorf) using a program consisting of an initial denaturation step of 2 min at 94°C, and then, 55 cycles of 2 min at 94°C, 1 min at 37°C, 2 min 72°C, followed by a 10 min elongation step at 72°C. PCR products were stored at 4°C before analysis.

The amplification products were separated by electrophoresis in 2 % agarose gels and 0.5 μg/ml ethidium bromide in 1x TAE buffer (40 mM Tris-Acetate, 1 mM EDTA, pH:8.0) for 3 h at 70 volts. The fragment patterns were photographed under UV light for further analysis. A 1 kb DNA ladder was used as the molecular standard in order to confirm the appropriate RAPD markers.

Fruit characteristic data from 2005 and 2006 were analyzed using SAS procedures [10]. GLM procedure was used to construct analysis of variance (ANOVA) tables, and means were calculated using TABULATE. All the factors, year, genotype, and their interactions were assumed as random factors. In construction of the ANOVAs, the subsamples were averaged. The main effect of genotypes was separated by Tukey at $P < 5\%$.

RAPD data were recorded as 1 for the presence of a band and 0 for its absence to generate a binary matrix. Only reproducible bands were scored for all the accessions tested. Sets 1 and 2 were analyzed separately by Principle Coordinate (PCoA) and Cluster analyses using NTSYS program [11]. First, a similarity matrix was generated using Jaccard coefficients. This matrix was then used for PCO. For cluster analyses, the UPGMA (Unweighted Pair Group Method using Arithmetic Average) method was used to construct dendrograms. The bootstrap values for the clusters were calculated by 1000 replicates using PAUP program [12]. The representativeness of dendrograms was evaluated by estimating cophenetic correlation for the dendrogram and comparing it with the similarity matrix, using Mantel's matrix correspondence test [13]. The result of this test is a cophenetic correlation coefficient, *r*, indicating how well dendrogram represents similarity data.

Results

Results of fruit characteristics indicated that the differences among the three sampling times (October, November and December) were similar. Therefore, they were merged to calculate averages for the experimental years. The year averages were only considerable for pH, while all the other characteristics tabulated similar means between years (Table 2). The accessions, however, had considerably different means for all the traits tested except for percentage soluble solids. The two wild accessions, 31-04 and 31-05, had the lightest fruits. Their average fruit weight was about 60% less than the cultivated accessions (31-01 – 31-03 and 31-06 – 31-08). Similarly, their fruit width and length were 29% and 27% less than the averages of the cultivated forms. The wild accessions had a higher acidity average when compared to the cultivated ones.

When the cultivated accessions were compared among themselves, it was concluded that they had a similar fruit weight, width and length. However, when soluble solids and acidity were considered, two groups of the cultivated accessions can be differentiated: (1) the group with higher soluble solids and acidity (31-01 – 31-03); and (2) the group with lower soluble solids and acidity (31-06 – 31-08).

Results of the RAPD analyses for Sets 1 and 2 are presented in Table 3. For Set 1, the 26 RAPD primers yielded 159 clear and reproducible bands, 46 of which were polymorphic. Therefore, the average polymorphic was 28.6%. The highest numbers of polymorphic bands were recovered from OPAH116, OPAJ 13 and OPAJ 19 primers. The same primers generated 119 clear and reproducible bands for Set 2, 87 of which were polymorphic, thus making up 72.9% of polymorphism. The primers OPAA13, OPAH19 and OPAH20 generated five or more polymorphic bands.

The UPGMA dendrograms for both populations are presented in Fig. 2. The dendrogram for Set 1, in Panel A, indicates that the wild accessions are separated from the cultivated ones. Indeed, there were three nodes statistically supported by bootstrap values making four groups of accessions: (1) 31-05; (2) 31-04; (3) 31-06, 31-07, 31-08; and (4) 31-

01, 31-02, 31-03. The validity of the dendrogram in reflecting the genetic relationships among the accessions was indicated by a high cophetic correlation coefficient (*r*) of 0.99. The clustering of Set 2 accessions are presented in Panel B of Fig. 2. As can be seen from the dendrogram, while some of the half-sib families were grouped based on their maternal parents, there was no overall relationship among the families. Indeed, only one of the nodes in the dendrogram was supported by bootstrap values. The cophetic correlation coefficient was lower in this group than in the dendrogram of the Set 2 accessions (*r* = 0.89).

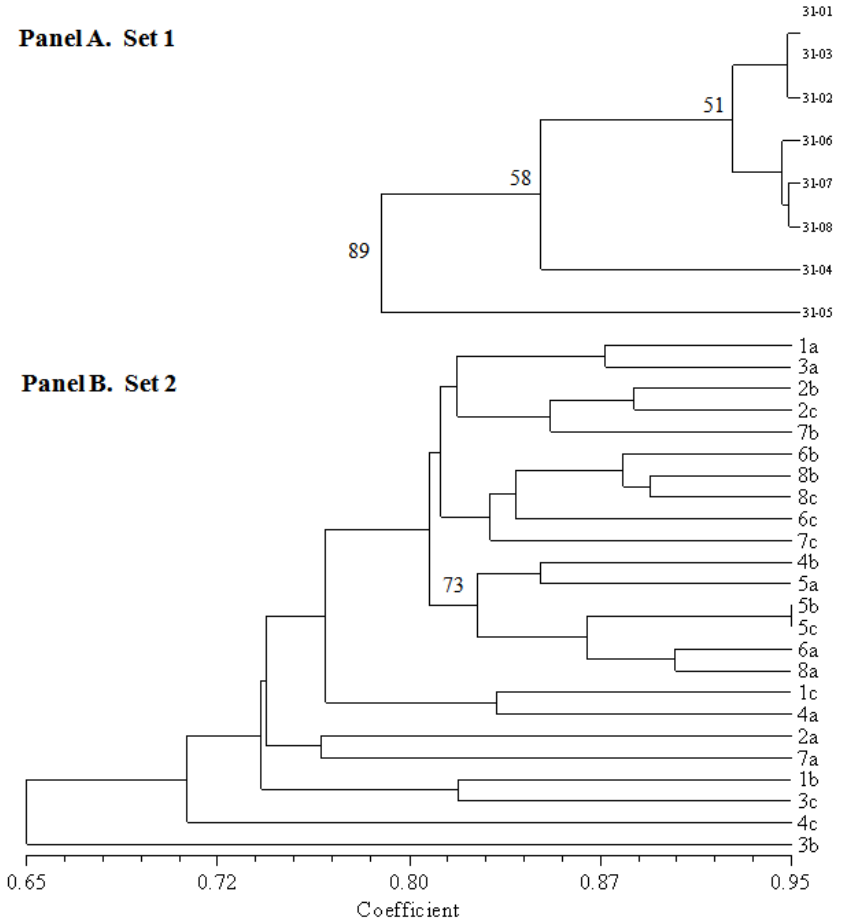

Figure 2. UPGMA dendrograms of RAPD bands generated by 26 arbitrary oligonucleotide primers for myrtle genotypes sampled from Hatay, Turkey. Numbers in nodes represent bootstrap values in percentage and are presented only when they are higher than 50%.

Results of the cluster analyses were confirmed by PCoA (Fig. 3). For Set 1, the first three PCs explained 53, 30 and 13% of the total variation, respectively making a total of 96% of the total variation. The four groups were clearly formed in the two-dimensional graph and the accessions of 31-04 and 31-05 were quite distinct from the other two groups. For Set 2, the accumulation of the first three PCs explained 35% of the total variation, and their individual values were 14, 11, and 10%, respectively. Results of PCoA and cluster were similar for Set 2, as well. Some of the families were closely located on two-dimensional graph (*e.g.*, 5a, 5b and 5c). However, the overall distribution of the accessions was poorly correlated with the maternal parent. In fact, similarity matrix presented in Table 4 indicated that the individuals of the half-sib families were not similar to each other. For example, the genetic similarity between 6a and 6b was 0.80, while accession 6a had higher similarity values to

member of the other half-sib family (4b; 0.84). Similar examples can be found in all the families tested.

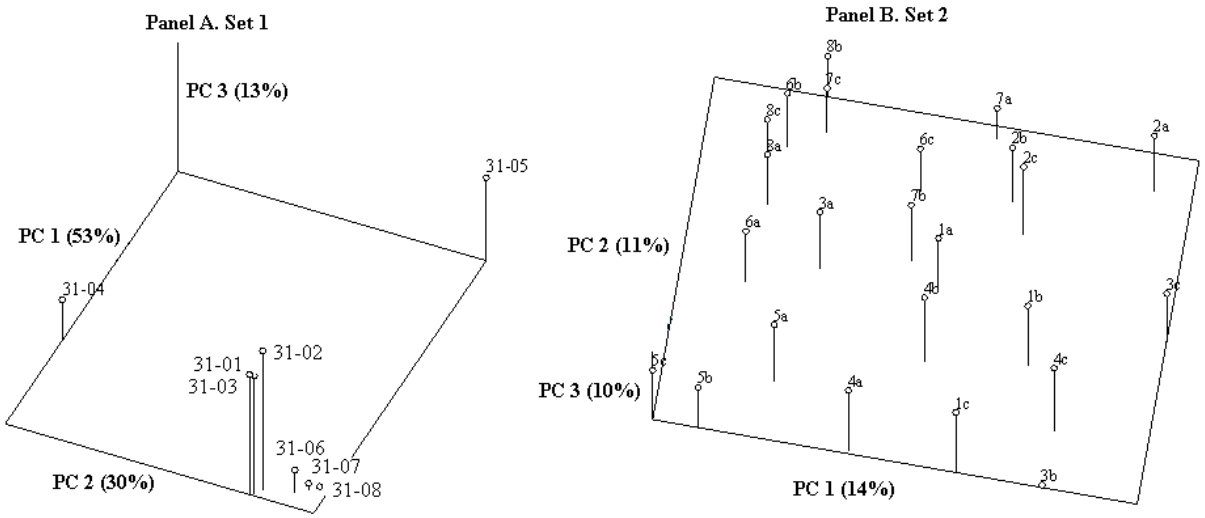

Figure 3. Principle coordinate analyses of RAPD bands generated by 26 primers for myrtle genotypes sampled from Hatay, Turkey. Set 1 includes 8 selections, while Set 2 consists of three seedlings germinated from open-pollination of each selection.

Table 2. Means and standard deviations of fruit characteristics for eight selected myrtle accessions sampled from Hatay, Turkey in 2005 and 2006.

Source	Fruit weight (g)	Fruit width (mm)	Fruit length (mm)	Soluble solids (%)	pH	Acidity
Year						
2005	9.4 ± 4.4	10.7 ± 2.4	12.9 ± 2.6	17.7 ± 2.6	5.4 ± 0.3	3.5 ± 1.4
2006	8.7 ± 4.1	10.6 ± 2.2	13.3 ± 2.2	16.6 ± 2.2	5.8 ± 0.2	3.2 ± 1.7
Genotype						
31-01	12.8 ± 1.4	12.4 ± 0.6	15.2 ± 0.5	18.6 ± 1.7	5.5 ± 0.3	3.5 ± 1.3
31-02	12.0 ± 1.0	12.1 ± 0.5	15.0 ± 0.7	18.0 ± 2.4	5.5 ± 0.4	3.1 ± 1.3
31-03	10.0 ± 1.0	11.5 ± 0.5	13.9 ± 0.5	19.4 ± 3.5	5.6 ± 0.4	3.3 ± 1.5
31-04	2.5 ± 0.6	7.1 ± 0.8	8.7 ± 1.0	19.5 ± 10.3	5.5 ± 0.3	5.4 ± 1.3
31-05	2.8 ± 0.6	7.0 ± 0.8	10.0 ± 0.9	17.9 ± 3.4	5.6 ± 0.3	4.6 ± 1.2
31-06	11.3 ± 2.3	12.1 ± 1.0	14.3 ± 0.7	15.7 ± 0.9	5.7 ± 0.2	2.5 ± 0.7
31-07	10.3 ± 3.9	11.2 ± 1.7	13.6 ± 1.7	13.7 ± 1.4	5.6 ± 0.3	2.1 ± 0.8
31-08	10.5 ± 1.6	11.8 ± 0.8	14.1 ± 0.9	14.4 ± 3.0	5.8 ± 0.2	2.4 ± 0.7
Mean	9.0 ± 4.2	10.7 ± 2.3	13.1 ± 2.4	17.2 ± 4.5	5.6 ± 0.3	3.4 ± 1.5

Table 3. Arbitrary oligonucleotide primers, the sizes of the amplified fragments, numbers of mono-and polymorphic bands and polymorphism studied to reveled molecular relationship among myrtle accessions sampled from Hatay, Turkey.

		Set 1				Set 2			
Primer code	Sequence (5’ to 3’)	Size (bp) (Min-Max)	Mono-morphic bands	Poly-morphic bands	Polymorphism (%)	Size (bp) (Min-Max)	Mono-morphic bands	Poly-morphic bands	Polymorphism (%)
OPAA1	AGACGGCTCC	350-1700	6	1	14.3	500-1900	1	4	80.0
OPAA2	GAGACCAGAC	500-1600	4	1	20.0	450-1500	2	2	50.0
OPAA3	TTAGCGCCCC	500-2000	4	3	42.9	500-2000	3	3	50.0
OPAA4	AGGACTGCTC	350-800	3	2	40.0	350-1500	1	4	80.0

OPAA6	GTGGGTGCCA	400-2000	5	0	0.0	400-1900	2	3	60.0
OPAA9	AGATGGGCAG	400-1400	6	1	14.3	450-1400	1	4	80.0
OPAA11	ACCCGACCTG	350-1500	6	2	25.0	500-1100	0	4	100.0
OPAA12	GGACCTCTTG	400-1450	5	2	28.6	400-600	1	2	66.7
OPAA13	GAGCGTCGCT	350-1500	4	2	33.3	700-1100	0	5	100.0
OPAA14	AACGGGCCAA	500-1100	4	1	20.0	500-1500	2	3	60.0
OPAA15	ACGGAAGCCC	550-900	4	0	0.0	400-1500	1	4	80.0
OPAA16	GGAACCCACA	350-1000	5	0	0.0	--	--	--	--
OPAA17	GAGCCCGACT	500-1600	6	1	14.3	400-1000	2	3	60.0
OPAA18	TGGTCCAGCC	250-1200	5	1	16.7	250-1400	2	5	71.4
OPAA19	TGAGGCGTGT	300-1100	6	1	14.3	300-1050	3	2	40.0
OPAH3	GGTTACTGCC	550-2000	4	2	33.3	550-1500	1	3	75.0
OPAH13	TGAGTCCGCA	550-1400	3	3	50.0	500-1100	1	3	75.0
OPAH16	CAAGGTGGGT	550-1500	3	4	57.1	750-1500	2	2	50.0
OPAH19	GGCAGTTCTC	350-2000	4	2	33.3	450-1900	0	6	100.0
OPAH20	GGAAGGTGAG	350-1400	6	1	14.3	350-2000	1	5	83.3
OPAJ9	ACGGCACGCA	550-1550	4	2	33.3	550-1250	1	4	80.0
OPAJ11	GAACGCTGCC	300-1500	6	1	14.3	500-1000	1	4	80.0
OPAJ13	CAGCCGTTCC	750-1700	2	4	66.7	600-1350	1	3	75.0
OPAJ15	GAATCCGGCA	700-1900	3	2	40.0	700-1250	1	3	75.0
OPAJ19	ACAGTGGCCT	500-1500	2	4	66.7	500-1000	1	3	75.0
OPAJ20	ACACGTGGTC	350-1700	3	3	50.0	400-1250	1	3	75.0

Table 4. Similarity matrixes calculated by RAPD bands generated by 26 primers for myrtle genotypes sampled from Hatay, Turkey.

Panel B. Set 2

	1a	1b	1c	2a	2b	2c	3a	3b	3c	4a	4b	4c	5a	5b	5c	6a	6b	6c	7a	7b	7c	8a	8b
1b	0.83																						
1c	0.74	0.72																					
2a	0.77	0.74	0.71																				
2b	0.78	0.73	0.79	0.85																			
2c	0.82	0.82	0.79	0.82	0.89																		
3a	0.88	0.80	0.75	0.74	0.78	0.82																	
3b	0.67	0.65	0.67	0.62	0.67	0.63	0.66																
3c	0.73	0.82	0.67	0.75	0.77	0.76	0.71	0.65															
4a	0.73	0.72	0.83	0.70	0.78	0.79	0.80	0.63	0.68														
4b	0.79	0.77	0.76	0.76	0.82	0.85	0.83	0.67	0.77	0.83													
4c	0.70	0.72	0.73	0.72	0.72	0.73	0.71	0.59	0.72	0.74	0.75												
5a	0.78	0.79	0.75	0.74	0.79	0.80	0.82	0.65	0.72	0.81	0.85	0.67											
5b	0.81	0.73	0.76	0.66	0.77	0.73	0.81	0.67	0.70	0.79	0.76	0.71	0.85										
5c	0.76	0.75	0.71	0.68	0.75	0.74	0.81	0.64	0.68	0.80	0.78	0.70	0.88	0.95									
6a	0.78	0.79	0.74	0.74	0.81	0.82	0.82	0.64	0.73	0.82	0.84	0.69	0.86	0.88	0.89								
6b	0.78	0.76	0.73	0.70	0.80	0.80	0.87	0.62	0.67	0.75	0.79	0.73	0.82	0.77	0.81	0.80							
6c	0.81	0.76	0.77	0.78	0.81	0.78	0.82	0.66	0.74	0.77	0.79	0.72	0.78	0.79	0.77	0.82	0.83						
7a	0.72	0.70	0.63	0.76	0.77	0.76	0.72	0.64	0.74	0.74	0.68	0.67	0.70	0.75	0.71	0.77	0.77	0.80					
7b	0.86	0.84	0.79	0.75	0.85	0.86	0.84	0.67	0.75	0.77	0.82	0.72	0.81	0.81	0.79	0.86	0.83	0.80	0.76				
7c	0.78	0.73	0.73	0.72	0.81	0.80	0.82	0.66	0.67	0.74	0.77	0.66	0.77	0.77	0.76	0.84	0.83	0.81	0.73	0.85			
8a	0.80	0.78	0.75	0.74	0.84	0.82	0.85	0.65	0.70	0.80	0.82	0.72	0.82	0.84	0.87	0.90	0.86	0.82	0.79	0.84	0.87		
8b	0.76	0.71	0.72	0.76	0.83	0.79	0.83	0.66	0.74	0.75	0.79	0.68	0.80	0.78	0.79	0.85	0.88	0.86	0.78	0.80	0.85	0.88	
8c	0.81	0.76	0.73	0.75	0.80	0.79	0.86	0.63	0.70	0.77	0.80	0.72	0.82	0.82	0.82	0.88	0.89	0.83	0.75	0.83	0.84	0.87	0.89

Panel B. Set 1

	31-01	31-02	31-03	31-04	31-05	31-06	31-07
31-02	0.99						
31-03	1.00	0.99					
31-04	0.87	0.87	0.87				
31-05	0.77	0.78	0.77	0.76			
31-06	0.97	0.97	0.97	0.86	0.79		
31-07	0.96	0.95	0.96	0.85	0.80	0.99	
31-08	0.97	0.96	0.97	0.86	0.79	0.99	0.99

Discussion

In our first experiments, we determined the various fruit characteristics of the cultivated and wild types of the myrtle accessions. We found that the wild and cultivated types considerably differed in fruit size parameters and acidity, but not in soluble solid and pH. Some of the variations in these traits may be attributed to the horticultural practices as the experimental accessions (Set 1) consisted of both wild and cultivated accessions. However, considering that the horticultural practices were limited and the great differences were observed, the variations were most likely to be the result of the genotypic factors. When the cultivated accessions were compared, they had similar averages for all the variables tested except for percentage soluble solid and acidity. This suggests there is a slight pomological variation among the cultivated accessions of myrtle ("habmeles" in Turkish), while they are unambiguously different from the wild accessions. The wild accessions had the highest acidity averages regardless of their fruit color. This indicates that the acidity and the fruit color are not associated. Another finding supporting this conclusion is that considerable differences recovered between the two groups of the white-fruited cultivated forms.

Our molecular analysis clearly separated the cultivated types from the wild accessions (Panel A of Fig. 2). The evolutionary relationships between the wild and cultivated types are not well-known. It is likely that the large-fruited, cultivated type was domesticated from the white-colored, wild types. The fact that all the cultivated myrtle accessions are white-fruited and the dark-fruited from is prevalent in nature indicates that the white color was preferred during the domestication process. Evolution of the Chilean strawberry, *Fragaria chiloensis*, is similar to this occurrence. There are two forms belonging to *F. chiloensis* subs. *chiloensis*, with white (f. *chiloensis*) and red fruits (f. *patagonica*), and the white-fruited form has narrower genetic base than the red-fruited forms [14]. During domestication, these separations may have been constructed by color preferences *per se*. However, it is also possible that the color forms may differ in other characteristics. The differences among the white and dark forms of the wild myrtle form have been studied for the morphological traits and nutrient composition which led to no significant differences between the two forms [4]. There are also reports from Turkey on several properties of myrtle accessions; however, they do not shed light on the comparison since the dark-fruited wild form was exclusively studied [6, 7]. Further investigations of differences in nutritional and physical properties or essential oil and fatty acid composition between these forms need to be carried out with larger sample sizes so as to represent more diverse areas in the Mediterranean Basin.

The growers have observed little morphological differences among their seedling-grown plants, thus indicating there is an insignificant genetic segregation for most horticultural traits. This may point to either presumably homozygous genome caused by self-pollination or to a narrow genetic base for the cultivated form of myrtle. Our molecular analysis for Set 2 demonstrated that the progenies of the myrtle accessions were quite variable only after one generation of open-pollination. For our data set, a lack of correlation between molecular and morphologic diversity is unlikely since even the node separating the cultivated accessions is statistically supported, although these two groups only differ in percentage soluble solids and acidity for the horticultural traits tested. We also observed much higher polymorphism in the open-pollinated individual when compared to the parental plants (28.6% vs. 72.9%) when they were studied under the same RAPD primers. Therefore, it is possible that myrtle is not allogamous as indicated [15]. Indeed, Mulas & Fadda [16] studied the floral biology of selected myrtle accessions form Italy and recovered that all accessions produced high percentages of berries both under open and self pollination.

Genetic diversity among the several myrtle populations from various regions was studied. For example, Agrimonti et al. [17] studied 113 individuals of 14 population sampled from Sardinia and Calabria using fAFLPs. Their analysis revealed that genetic variation was greater within populations (51.86%) than among populations (16.99%). In this study, polymorphism of fragment varied between 80% and 89%. Messaoud et al. [16] studied 17 populations of Tunisian wild myrtle sampled from subhumid, humid and semi-arid regions using nine isozymes and found that out of 18 loci detected, 12 were polymorphic (75%). The polymorphism detected by the RAPD primers on open-pollinated population in this study is comparable to that of Agrimonti et al. (2007) and Messaoud et al. [16]. However, we recovered much less polymorphism on our Set 1. Part of this low polymorphism may be attributed to the few accessions utilized in the study. However, the fact that most of the RAPD bands were shared among the cultivated accessions indicates that the cultivated types have a narrow genetic base.

Conclusion

The present report is the first one from Turkey describing molecular characterization of the myrtle accessions. We determined that the wild and cultivated types of the myrtle accessions can be differentiated by the RAPD molecular markers. Bruna et al. [2] investigated the genetic relationship among the germplasm collected from various regions of Italy as well as Croatia, France, Greece, Israel, Portugal and Spain and divided the genotypes into Western and Eastern Mediterranean groups using AFLP. Joint molecular analysis of our accessions with those genotypes would determine where Turkish myrtle individuals stand in the representatives of Mediterranean genotypes. The present study indicated that myrtle pollination appeared to be not restricted to the self-pollination and the cultivated types have a narrow genetic base.

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