Journal of Advances in Biology & Biotechnology
2(4): 208-219, 2015; Article no.JABB.2015.022
ISSN: 2394-1081

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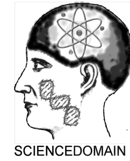

Molecular Characterization of *Prunus mahaleb* L. Genotypes using *Prunus* Simple Sequence Repeat Markers

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Authors' contributions

This work was carried out in collaboration between both authors. Authors DE and SH designed the study and author DE carried out all experiments. Author DE wrote the first draft the manuscript. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JABB/2015/15508
Editor(s):
(1) Tapan Kumar Mondal, National Research Center on DNA Fingerprinting, National Bureau of Plant Genetic Resources, India.
Reviewers:
(1) Anonymous, México.
(2) Anonymous, Turkey.
Complete Peer review History: http://www.sciencedomain.org/review-history.php?iid=877&id=39&aid=7914

Original Research Article

Received 30th November 2014
Accepted 14th January 2015
Published 28th January 2015

ABSTRACT

Turkey, located in the ecological range of mahaleb (*Prunus mahaleb* L.), has a variable genetic population. Mahaleb is the most important rootstock in terms of fruit-growing sweet cherry trees. In this study, we conducted a simple sequence repeats (SSRs) marker analysis of 60 mahaleb genotypes selected from the Anatolian gene sources for molecular characterization and investigation of the genetic relationships. A total of 33 SSR primer pairs selected from sweet cherry and peach were used for genetic identification. We found that 21 SSR markers were polymorphic, with 2 to 7 (average = 3.5) alleles per locus. The allele size varied from 70 to 550 bp. The lowest number of alleles (2) was found in the microsatellite markers EMPaS12^A, PceGA25, PS07AO2, BPPCT 034, and BPPCT 038, and the most (7) were found in EMPaSO5. The observed mean heterozygosity value for different loci was 0.74, while the expected heterozygosity was 0.56. The genotypes collected from the same region were closely related and regrouped together. In this study, the SSR markers developed from cherries and peaches were successfully used in the molecular characterization of mahaleb genotypes. The results obtained demonstrated the transferability of the SSR markers between the close relative species in *Prunus* spp. for the differentiation of the genotypes. Furthermore, the present study identified the mahaleb genotypes present in Anatolia and determined the rich genetic variability in high potentials.

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Keywords: Genetic diversity; mahaleb; molecular characterization; Prunus mahaleb L.; SSR.

1. INTRODUCTION

Mahaleb (*Prunus mahaleb* L. or *Cerasus mahaleb*) belongs to the *Prunoidae* sub familia of Rosaceae familia [1]. The origin of *P. mahaleb* L. is Western Asia and is spread over a wide area in this L., has a wide genetic variety of mahaleb spread naturally in Amasya, Ankara, Bolu, Çorum, Diyarbakır, Gümüşhane, Hakkâri, Istanbul, Kars, Mardin, Muğla, Tokat (Centrum, Erbaa, Niksar and Zile districts), Uşak and Van [2,3]. Turkey has a rich mahaleb population with a very wide range of genetic variability because it is located in the natural spreading zone of mahaleb types and cherry trees are grafted on mahaleb rootstocks.

Mahaleb rootstock is resistant to cold and arid climate and is not selective in terms of soil [4,5]. Mahaleb grows in chalky, sandy, gravelly and stony soils and its roots go deep, spreading towards its width in outcrop soils where the bedrock or ground water is close to the soil surface. Mahaleb tree adapts well to soil having irrigation problems with less water. It has been reported that cherry and sour cherries grafted on mahaleb rootstock form smaller trees, give more fruits and mature early [4,6]. So far, many varieties of *P. mahaleb* L. have been identified morphologically [7] and in studies involving RAPD markers [8], various ISSR markers [9] as well as *Prunus* rootstocks, including mahaleb rootstock, have been identified at molecular levels [10,11,12].

Turkey has different geographic and climatic conditions and therefore, different phytogeographical regions [13]. As a result of different ecogeographies, wild plant species have different regional varieties in terms of various adaptive characteristics [14]. Owing to the regional differences, Turkey's genetic variability of mahaleb population is known to be very wide.

Simple sequence repeat markers (SSRs) are genetic markers that have produced very good results in studies on the genetic variety of plants in a wide range. These markers have been used in the identification of different *Prunus* rootstocks [12,15,16].

The main objectives of the present study were to develop SSR markers, to optimize PCR conditions and to analyzes genetic diversity among *P. mahaleb* in Turkey. Natural genetic variability of mahaleb (*P. mahaleb* L.) was assessed in 60 autochtonous populations collected from different geographic regions in Turkey. As there are no mahaleb-specific SSRs, the study tested SSRs developed from cherry [17,18] and peach [19,20,21,22,23] in respect to suitability for *P. mahaleb*. Additionally, PCR conditions namely temperature were modified wherever necessary.

2. MATERIALS AND METHODS

2.1 Plant Material

In general, the fruits of mahaleb are red and yellow; fruits carrying yellow tones are known as yellow mahaleb, while the ones carrying red and black colored tones are known as black mahaleb (Fig. 1). Mahlep fruits are used in the preparation of jelly, candy and pestil, a traditional food made of dried fruit pulp. Besides, seeds are utilized in pharmaceutical and cosmetic industry.

A total of 60 genotypes of *P. mahaleb* were used for SSRs analysis. These genotypes were previously selected from wild cherry populations from Afyon, Kemalpaşa, Tokat, Turgutlu and Manisa provinces (Table 1). All the genotypes are maintained in a germplasm collection at the Ege University Horticultural Department, İzmir, Turkey. Leaf samples of this 60 analyzed genotypes, frozen in liquid nitrogen and subsequently stored at –20°C until processed.

The Afyon (Af), Kemalpaşa (Kp), Tokat (T), Turgutlu (Tur), and Manisa (Man) mahaleb genotypes used in the present study are given in Table 1 according to their fruit skin color and collection site.

2.2 DNA extraction

The DNA was isolated from 200 mg of fresh leaf tissue for each selection. The tissue was macerated in 1.5 ml Eppendorf tubes using liquid N_2 and 500 µL of extraction buffer 2X CTAB (100 mM Tris-HCl, pH 8.0; 20 mM EDTA, pH 8.0; 1.4 M NaCl; 1 % PVP, Mr. 40.000; and 2% CTAB) and 7 µL of proteinase K (Promega, Madison, WI, USA) were added to the tubes. The samples were incubated at 65°C for 30 minute, extracted thrice with 500 µL of chloroform-isoamylalcohol (24:1) and centrifuged for 10 minute at 13.000

rpm. The supernatant was transferred to a fresh tube and 1 µg of RNAse was added at 37°C for 30 minute and precipitated overnight with 1/10th volume of 3 M NaAc (pH 5.2) and 2.5 volumes of 95% ethanol. The DNA fragment was pelleted by centrifugation at 13.000 rpm for 10 minute. The pellet was washed twice with 70% ethanol (1 ml), dried at 37°C for 5 minute and redissolved in 150 µL of TE (10 mM Tris-HCl, pH 8.0 and 1 mM EDTA, pH 8.0).

2.3 SSRs analysis

A total of 33 SSRs developed in cherry and peach were screened for polymorphism for the mahaleb genotypes (Table 2). The PCR conditions for these primers for mahaleb are given in Tables 3 and 4.

The SSRs were amplified in a total volume of 25 µL, containing 30 ng of DNA, 1X PCR buffer (Applied Biosystems), 3 mM $MgCl_2$, 0.08 mM dNTP, 20 pm each primer and 0.5 U Taq DNA polymerase (Amplified Taq Gold), at different melting temperatures (Tm) and "touchdown" (TD) temperatures of the SSR primers (Tables 3 and 4). The annealing temperature was decreased by 1ºC per cycle for "touch-down" conditions (Table 3). All the SSRs primer conditions developed in this study were tested for mahaleb genotypes.

The amplified PCR products were separated by electrophoresis using 3% metaphor® agarose (Biowittaker, Maine, USA) gel (1X TBE buffer) at 5 V/cm, stained using ethidium bromide (0.5 µg/ml) and visualized under UV light. A 1-kb Plus DNA Ladder (Fermantas) was used as the molecular size standard.

2.4 Data analysis

The genetic analysis program 'IDENTITY' 1.0 [24] was used in order to calculate the number of alleles, expected and observed heterozygosity (He and Ho, respectively), estimated frequency of null alleles, and the probability of genetic identity per locus (PI) [25]. The genetic relationships among the 60 genotypes included in this study were investigated using the unweighted pair-group method with arithmetic mean (UPGMA) [26] cluster analysis of the similarity data and depicted in a dendrogram. All the analyses were computed with the NTSYS 2.0 program [27].

3. RESULTS AND DISCUSSION

A total of 60 mahaleb genotypes from different geographical regions (Afyon, Kemalpaşa, Tokat, Turgutlu, and Manisa) of Anatolia were screened. Genetic analysis of the 60 mahaleb genotypes revealed 74 alleles and the allele sizes are presented in Table 5. Amplification was successful for 26 of the 33 SSRs assayed in mahaleb, while 7 primer pairs ($EMPaSO2^B$, EMPaSO4, EMPaSO7, $EMPaS11^A$, Pchgms14, BPPCT 039, and BPPCT 040) could not be amplified in this study. Besides, PMS3, PSO8EO8, pchgms15, pchgms20 and pchgms25 had monomorphic alleles, while the rest of the primers had polymorphic alleles (Table 5).

A large size range of the amplified bands between 70 and 550 was observed in the mahaleb genotypes. The number of presumed alleles (polymorphic bands) revealed by SSR analysis ranged from 2 to 7. In general, most of the primers presented more than two alleles, although four SSR markers (PMS3, PSO8EO8, pchgms 15, pchgms 20, and pchgms 25) were monomorphic. The lowest number of alleles (two alleles) was found in the microsatellite markers EMPaS12A, PceGA25, PS07AO2, BPPCT 034, BPPCT 038 and the highest number (seven alleles) were found in EMPaSO5.

Fig. 1. Yellow and black mahaleb genotypes analyzed for genetic variability

Table 1. Mahaleb genotypes used in this study

Genotypes	Skin colour	Collection site (city)	Genotypes	Skin colour	Collection site (city)	Genotypes	Collection site (city)	Skin colour
Af 3	Black	Afyon	Kp 1(4)	Black	Kemalpaşa	T 9	Tokat	Black
Af 4	Black	Afyon	Kp 3(1)	Black	Kemalpaşa	T 10(1)	Tokat	Yellow
Af 5(2)	Black	Afyon	Kp 3(2)	Black	Kemalpaşa	T 10(2)	Tokat	Yellow
Af 6(2)	Black	Afyon	Kp 4	Black	Kemalpaşa	T 14	Tokat	Black
Af 6(3)	Black	Afyon	Kp 6	Black	Kemalpaşa	T 22(1)	Tokat	Yellow
Af 10(1)	Black	Afyon	Kp 9	Black	Kemalpaşa	T 22(2)	Tokat	Yellow
Af 10(4)	Black	Afyon	Kp 18(1)	Black	Kemalpaşa	T 28	Tokat	Yellow
Af 10(5)	Black	Afyon	Kp 18(3)	Black	Kemalpaşa	T 29	Tokat	Yellow
Af 11(2)	Black	Afyon	Kp 20(1)	Black	Kemalpaşa	T 30	Tokat	Black
Af 11(4)	Black	Afyon	K 20(2)	Black	Kemalpaşa	T 31	Tokat	Black
Af 18(1)	Black	Afyon	Kp 29(1)	Yellow	Kemalpaşa	T 32(2)	Tokat	Yellow
Af 18(2)	Black	Afyon	Kp 29(2)	Yellow	Kemalpaşa	T 33(1)	Tokat	Yellow
Af 66(1)	Black	Afyon	Kp 27(3)	Black	Kemalpaşa	T 33(3)	Tokat	Yellow
Af 66(2)	Black	Afyon	Man 2(2)	Black	Manisa	T 34(3)	Tokat	Yellow
Af 111(4)	Black	Afyon	Man 2(3)	Black	Manisa	T 36(2)	Tokat	Yellow
Af 111(5)	Black	Afyon	Man 5(1)	Black	Manisa	T 37(3)	Tokat	Yellow
Af 111(6)	Black	Afyon	Man 5(2)	Black	Manisa	T 43	Tokat	Yellow
Kp 1(1)	Black	Kemalpaşa	Man 5(3)	Black	Manisa	T 47	Tokat	Yellow
Kp 1(2)	Black	Kemalpaşa	Man 9(1)	Black	Manisa	Tur 3	Turgutlu	Black
Kp 1(3)	Black	Kemalpaşa	Man 9(3)	Black	Manisa	Tur 7	Turgutlu	Black

Table 2. SSR primer pairs used in this study to characterize *P. mahaleb* genotypes

SSR locus	Sequence (5'-3')	Species origin	References
PS12AO2	F: GCCACCAATGGTTCTTCC R: AGCACCAGATGCACCTGA	cherry	[17]
EMPaSO1[A]	F: CAAAATCAACAAAATCTAAACC R: CAAGAATCTTCTAGCTCAAACC	cherry	[18]
EMPaSO2[B]	F: CTACTTCCATGATTGCCTCAC R: AACATCCAGAACATCAACACAC	cherry	[18]
EMPaSO4	F: CATTGCTTAACCCTCTTGTTTC R: CATTACTCAAAAACGCCTCC	cherry	[18]
EMPaSO5	F: CATGTGCTTTCTCTGCCC R: TCTTCTCAAGCAATTCCCC	cherry	[18]
EMPaSO6B	F: AAGCGGAAAGCACAGGTAG R: TTGCTAGCATAGAAAAGAATTGTAG	cherry	[18]
EMPaSO7	F: ACCACATGATATCCCGAACC R: CGTGGAAAAGTAAAATAAAACCC	cherry	[18]
EMPaS10[A]	F: GCTAATATCAAATCCCAGCTCTC R: TGAAGAAGTATGGCTTCTGTGG	cherry	[18]
EMPaS11[A]	F: ACCACTTTGAGGAACTTGGG R: CTGCCTGGAAGAGCAATAAC	cherry	[18]
EMPaS12[A]	F: TGTGCTAATGCCAAAAATACC R: ACATGCATTTCAACCCACTC	cherry	[18]
EMPaS13	F: GAATTGAAGCAACCAAGCAC R: TGGCACACTCTACCTAACATTC	cherry	[18]
EMPaS14[A]	F: TCCGCCATATCACAATCAAC R: TTCCACACAAAAACCAATCC	cherry	[18]
EMPaS18	F: GGTGATTCATGAGGAATTTGG R: CCATCAACTGTCTTCTGTGTTG	cherry	[18]
UDP96-001	F: AGTTTGATTTTCTGATGCATCC R: TGCCATAAGGACCGGTATGT	peach	[19]
UDP96-005	F: GTAACGCTCGCTACCACAAA R: CCTGCATATCACCACCCAG	peach	[19]
UDP97-403	F: CTGGCTTACAACTCGCAAGC R: CGTCGACCAACTGAGACTCA	peach	[19]
PceGA25	F: GCAATTCGAGCTGTATTTCAGATG R: CAGTTGGCGGCTATCATGCTTAC	peach	[20]
PMS3	F: TGGACTTCACTCATTTCAGAGA R: ACTGCAGAGAATTTCACAACCA	peach	[20]
PMS49	F: TCACGAGCAAAAGTGTCTCTG R: CACTAACATCTCTCCCCTCCC	peach	[20]
PSO8EO8	F: CCCAATGAACAACTGCAT R: CATATCAATCACTGGGATG	peach	[20]
PS07A02	F: CAGGGAAATAGATAAGATG R: TCTAATGGTGGTGTTCATT	cherry	[21]
Pchpgms3	F: ACGCTATGTCCGTACACTCTCCATG R: CAACCTGTGATTGCTCCTATTAAAC	peach	[21]
BPPCT 034	F: CTACCTGAAATAAGCAGAGCCAT R: CAATGGAGAATGGGGTGC	peach	[22]
BPPCT 038	F: TATATTGTTGGCTTCTTGCATG R: TGAAAGTGAAACAATGGAAGC	peach	[22]
BPPCT 039	F: ATTACGTACCCTAAAGCTTCTGC R: GATGTCATGAAGATTGGAGAGG	peach	[22]
BPPCT 040	F: ATGAGGACGTGTCTGAATGG R: AGCCAAACCCCTCTTATACG	peach	[22]
pchgms12	F: CGACACTTAGCTAGAAGTTGCCTTA R: TCAAGCTCAAGGTACCAGCA	peach	[23]
pchgms14	F: GCAAAGAGTACAACAATATCTACCG	peach	[23]

SSR locus	Sequence (5'-3')	Species origin	References
	R: GGATGGTGAAGACGATGAGG		
pchgms15	F: TGTCCCCTAGCCATGCTAAT R: CGGACAGTTATTCAGGCAAT	peach	[23]
pchgms17	F: ATGCACTCAAGTGGCAAGC R: GGTTTTTGAGCAAAGATGCAC	peach	[23]
pchgms20	F1: AATTGCATCACAGCAAGAGC R1: GGGGGTTTGGTTAAGATCG F2: CCCTTACCCCCTTACCACTT	peach	[23]
pchgms24	F: CAACGAGCTCCCATGACTTT R: ACCACCACAACCAAACCATT	peach	[23]
pchgms25	F: GCCAGGAGGCTTTAACCTGT R: TCAGACCCCCTTTCATCATC	peach	[23]

Table 3. Different Touch Down (TD) temperatures for SSR primers used in this study

SSR primer	Initial denaturation	Elongation (TD) temperatures		Final extension
EMPaSO1[A] EMPaSO5 EMPaSO6B EMPaS10[A] EMPaS12[A] EMPaS13 EMPaS14[A] EMPaS15 EMPaS18	94ºC 15 min	94ºC 30 s 66-48ºC 90 s 72ºC 60 s	94ºC 30 s 48ºC 90 s 72ºC 60 s (35 cycle)	60ºC 30 min
PceGA25 PMS49 pchpgms3 PMS3 PSO8EO8	94ºC 4 min	94ºC 60 s 60-50ºC 60 s 72ºC 60 s	94ºC 60 s 50ºC 60 s 72ºC 60 s(30 cycle)	72ºC 5 min
PCHGM12 PCHGM15	94ºC 4 min	94ºC 60 s 65-58ºC 60 s 72ºC 2 min	94ºC 60 s 58ºC 60 s 72ºC 2 min (35 cycle)	72ºC 4 min
PCHGM17 PCHGM24 PCHGM25	94ºC 4 min	94ºC 60 s 65-60ºC 60 s 72ºC 2 min	94ºC 60 s 60ºC 60 s 72ºC 2 min (35 cycle)	72ºC 4 min
PCHGM20	94ºC 4 min	94ºC 60 s 65-59ºC 60 s 72ºC 2 min	94ºC 60 s 59ºC 60 s 72ºC 2 min (35 cycle)	72ºC 4 min
PS07AO2 PS12AO2	94ºC 4 min	94ºC 45 s 60ºC -50ºC 30 s 72ºC 30 s	94ºC 45 s 60ºC -50ºC 30 s 72ºC 30 s	72ºC 5 min

Table 4. Different melting temperatures (Tm) for SSR primers used in this study

SSR primer	Initial denaturation	Elongation tm temperatures	Final extension
UDP96-001 UDP96-005 UDP97-403	94ºC 4 min	94ºC 45 s 50ºC 45 s 72ºC 30 s (35 cycle)	72ºC 5 min
BPPCT 034 BPPCT 038	94ºC 60 s	94ºC 45s 57ºC 45 s 72ºC 2 min (35 cycle)	72ºC 4 min

Table 5. List of genetic parameters obtained with SSRs used in this study

Locus	N	Allelic size range (bp)	Ho	He	PI
EMPaSO1[A]	3	150, 200, 350	0.30	0.27	0.56
EMPaSO2[B]	na	na	na	na	na
EMPaSO4	na	na	na	na	na
EMPaSO5	7	70, 100, 150, 200, 250, 350, 400	0.80	0.66	0.31
EMPaSO6B	3	100, 150, 200	0.83	0.68	0.29
EMPaSO7	na	na	na	na	na
EMPaS10[A]	3	100, 150, 200	0.73	0.57	0.38
EMPaS11[A]	na	na	na	na	na
EMPaS12[A]	2	100, 150	0.92	0.74	0.21
EMPaS13	4	100, 120, 200, 250	1.0	0.53	0.55
EMPaS14[A]	5	100, 120, 150, 200, 210	0.31	0.33	0.47
EMPaS18	5	100, 120, 150, 200, 250	1.0	0.78	0.14
UDP96-001	6	100, 250, 300, 400, 500, 550	1.0	0.65	0.29
UDP96-005	3	100, 120, 150	0.52	0.41	0.52
UDP97-403	5	100, 120, 150, 250, 300	1.0	0.80	0.11
PceGA25	2	150, 200	1.0	0.63	0.33
Pchpgms3	3	100, 160, 180	1.0	0.57	0.46
PMS3	nc	500	nc	nc	nc
PMS49	3	70, 90,200	1.0	0.69	0.22
PSO8EO8	nc	200	nc	nc	nc
pchgms12	3	200, 400, 450	1.0	0.51	0.58
pchgms14	na-	na	na	na	na
pchgms15	nc	300	nc	nc	nc
pchgms17	5	150, 200, 250, 350, 400	0.52	0.43	0.46
pchgms20	nc	250	nc	nc	nc
pchgms24	3	200, 300, 400	0.15	0.28	0.57
pchgms25	nc -	300	nc	nc	nc
PS07A02	2	120, 200	0.90	0.57	0.46
PS12AO2	3	150, 200, 250	1.0	0.61	0.38
BPPCT 034	2	250, 300	0.56	0.43	0.61
BPPCT 038	2	150, 200	0.05	0.65	0.35
BPPCT 039	na	na	na	na	na
BPPCT 040	na	na	na	na	na
Total	74				
Average	3.5		0.74	0.56	

**N, number of alleles; Ho, observed heterozygosity; He, expected heterozygosity; Pı, probability of identity; nc, not calculated; na, no amplification*

The observed heterozygosity (Ho) value ranged from 0.05 (BPPCT 038) to 1.0 (EMPaS13, EMPaS18, UDP96-001, UDP97-403, PceGA25, Pchpgms3, PMS49, pchgms12, and PS12AO2), while the expected heterozygosity (He) value ranged from 0.27 (EMPaSO1[A]) to 0.80 (UDP97-403). The observed mean Ho value for different loci for all the mahalebs was 0.74, while the He value was 0.56. In general, the Ho value was higher than expected for all the microsatellites, except BPPCT 038, because of the presence of null alleles, which are alleles that failed to amplify during PCR**.** The marker BPPCT 034 had the highest probability of identity (PI) value (0.61), while the marker UDP97-403 had the lowest PI value (0.11).

A molecular analysis of 60 mahaleb genotypes was done by using 26 SSR primers and it was identified that there were two to seven alleles. Besides, observed mean Ho value for different loci for all of the mahalebs was 0.74, while the He was 0.56 in the present study (Table 5). Previous studies, total of 15 SSR markers developed from peach were screened in *Prunus* spp. (*P. domestica, P. salicina, P. armeniaca, P. dulcis, P. persica* var. *vulgaris, P. persica* var. *laevis, P. avium, P. cerasus,* and *P. malus*) and found to be polymorphic, showing 2 to 4 alleles each. The mean heterozygosity of all loci was 0.32 [19]. In a previous study, 26 microsatellites isolated and sequenced from peach and evaluated microsatellite polymorphism in 50 peach and nectarine cultivars. All the microsatellites were polymorphic, showing 2 to 8

alleles per locus, and the heterozygosity ranged from 0.04 to 0.74 (mean 0.47) and discrimination power (*PD*) ranged from 0.04 to 0.84 (mean 0.60) 26 microsatellites [28]. In another study, 31 sweet cherry cultivars were assessed with 14 SSR primers, and it was reported that there were 2 to 11 alleles (average = 5.3). The Ho value among the Lithuanian cultivars varied from 0.35 to 0.95 (mean 0.68), and the He value varied from 0.38 to 0.86 (mean 0.66) [29]. Furthermore, 10 SSR primers were used to discriminate 184 sweet cherry rootstocks, and it was demonstrated that the number of alleles ranged from 10 to 20 (average = 13.3) per locus, the average Ho value was 0.57, the average He value was 0.81, and the PI ranged from 0.06 to 0.72 [12].In a study, 10 previously described PCR primer pairs were tested on 51 sweet and sour cherry accessions to determine if the primers could differentiate the accessions. The loci were highly polymorphic, with 14 to 40 different alleles found at each locus. The heterozygosity values ranged from 0.52 to 1.00, gene diversity (PIC) values ranged from 0.905 to 0.966, and the *PD* of each locus varied from 0.896 to 0.965 for the sour cherry accessions, from 0.48 to 0.967 for the sweet cherry genotypes, and from 0.803 to 0.921 for the cherry rootstocks [30]. A total of 74 alleles (average of 3.5 putative alleles per locus) were obtained in the genetic analysis of mahaleb genotypes in this study. Our identification indicated that SSR primers were informative in agreement to values reported in the literature.

UDP96-001, UDP96-005, and UDP97-403 SSR markers were developed from peach and used in the studies of peach, nectarine, almond, and sweet cherry rootstocks [12,19,28,31]. In the present study, the size of these primers ranged from 100 to 550 bp for mahalebs (Table 5), while in the other studies, the size ranged from 120 to 170 bp for peaches and nectarines [19,28].

The peach primer pchpgms 3 obtained by Abbott [21] had been used previously in the studies of cherry, sour cherry [20,30] and cherry rootstocks [32]. In the present study, this primer presented three allele sizes between 100 and 180 bp, while it showed polymorphism in 5 alleles in cherry between 174 and 189 bp [20], 19 alleles in black cherry between 170 and 230 bp [17] and 3 alleles in sweet cherry rootstocks between 174 and 187 bp [32].

Furthermore, it was found that PSO8EO8 and PMS3 primers were monomorphic, while PMS49 and PceGA25 showed polymorphism in 3 alleles (70, 90, and 200) and 2 alleles (150 and 200), respectively (Table 5).

In a study conducted to identify the cherry accessions, PSO8EO8 primer showed polymorphism in 4 alleles between 174 and 189 bp, PSO8EO8 primer presented polymorphism in 16 alleles between 152 and 200 bp, PMS49 exhibited polymorphism in 15 alleles between 79 and 185 bp, and PceGA25 showed polymorphism in 14 alleles between 145 and 198 bp [20]. In another study conducted to identify sour cherry and sweet cherry accessions, pchpgms3, PSO8EO8, PMS3, PMS49, and PceGA25 showed polymorphisms [30].

In this work, it was found that primer pchgms14 did not amplify, while the rest of the primers (pchgms12, pchgms15, pchgms 17, pchgms 20, pchgms 24, and pchgms 25) amplified with different touchdown conditions (Table 3). Furthermore, the range size of pchgms 12, pchgms 15, pchgms 17, pchgms20, pchgms24, and pchgms 25 primers was more than 200 bp for mahalebs (Table 5), as well as peach [23].

Furthermore, in the present study, the EMPaSO2^{B}, EMPaSO4, EMPaSO7, EMPaS11^{A} primers used in mahaleb genotypes were not amplified and EMPaSO1^{A} EMPaSO5, EMPaSO6B, EMPaS10^{A}, EMPaS12^{A}, EMPaS13, EMPaS14^{A}, and EMPaS18 primers showed polymorphism between 2 and 7 alleles between 70 and 400 bp (Table 5). In previous studies, 16 wild cherry accessions were characterized with these primers, and EMPaSO4 and EMPaS18 primers were found to be monomorphic, while the rest of the primers showed polymorphism in 2 and 6 alleles between 73 and 248 bp [18]. The PS07A02 primer developed in cherry showed polymorphism in 2 alleles in mahaleb (120 and 200), while the PS12A02 primer showed polymorphism in 3 alleles (150, 200, and 250) (Table 5). In previous studies, PS07A02 and PS12A02 primers, which were used in the characterization of peach genotypes [21], were also used in the characterization of cherry genotypes [17].

In the present study, no amplification occurred in primers BPPCT 039 and BPPCT 039, while BPPCT 034 and BPPCT 038 showed polymorphism in 2 alleles between 150 and 300 bp. These primers were previously used in the identification of genetic diversity in peach and cherry [22].

Plant material that contains high levels of polysaccharides, polyphenolics, and other PCR-inhibiting compounds, just as in plant material from *Prunus* species, yields low amounts of DNA of sufficient purity and longevity [33,34]. Due to the density of the phenolic compounds and polysaccharides in mahaleb leaves, in the present study, the number of repetitions of washing steps with chloroform-isoamyl alcohol was increased in the pure DNA acquisition phase, and it was determined that treatment with proteinase K and NaAc is necessary.

Previous studies have reported that the genetic structure is highly conserved in *Prunus* species [35,36,37]. Therefore, it was concluded that the SSR markers developed in peach, cherry, and apricot were transferable in other *Prunus* species and were used by researchers to determine the genetic distinction [12,15,16,19,22,38,39]. In this study, it was determined that SSR markers developed in peach and sweet cherry are useful for discriminating among 60 mahaleb genotypes. We found the transferability of these SSR markers from cultivated species to mahalebs.

The genetic similarity measured within the mahalebs ranged between 0.69 and 0.97. The dendrogram generated from the UPGMA cluster analysis based on the Jaccard coefficient of genetic similarity was used to classify all the mahalebs genotypes. The dendrogram, which was formed with the SSR primers (Fig. 2), were divided into two main groups at 0.78 similarity levels, with the first group containing 56 genotypes and the second group containing 4 genotypes. The highest genetic similarity (97%) was observed between the yellow mahaleb genotypes T10(1) and T10(2), indicating that they were closely related. The lowest genetic similarity coefficient of 0.69 was found between T22(1) and Kp1(3) and Af10(5) and Kp1(3).

The first main group of the dendogram was further divided into two subgroups and subgroup 1.1 was divided into two groups, namely, subgroup 1.1.1 that mostly contained yellow mahaleb, including all mahaleb genotypes from Tokat region, 9 genotypes from Afyon region, and one genotype from Turgutlu region. It was determined that the Tokat and Afyon genotypes in subgroup 1.1.1 formed relatively close groups. The Afyon genotypes were included in subgroup 1.1.2 and the similarity between these two subgroups (subgroups 1.1.1 and 1.1.2) were 0.83. Furthermore, it can be observed in the dendogram that subgroup 1.2 contained 13 genotypes from Kemalpaşa region, 5 from Afyon region, 7 from Manisa region, and 1 from Turgutlu region. In addition, all the Manisa genotypes found in the population was included in this group. However, the second group in the dendrogram contained only 4 genotypes, including 3 genotypes from the Kemalpaşa region and 1 genotype from the Afyon region.

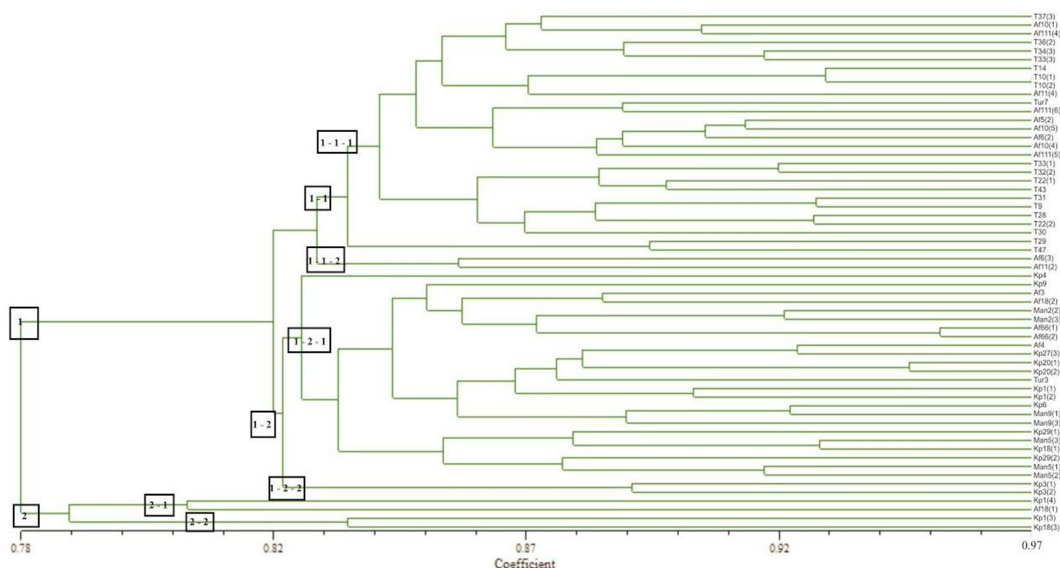

Fig. 2. Dendrogram of mahaleb rootstocks based on UPGMA analysis with SSRs primers

Using SSRs, a total of 60 mahaleb genotypes from different sites of Anatolia, including Afyon, Kemalpaşa, Tokat, Turgutlu, and Manisa were characterized in the present study. It was found that many mahaleb genotypes from the same sites were relatively close and could be grouped into the same location in the dendrogram. Furthermore, some genotypes from the same sites, although not very often, were found to be very far from each other in the dendrogram (Fig. 2). In a previous study, twenty nine *P. mahaleb* genotypes were examined with ISSR markers, and determined that the genetic variability among these 29 *P. mahaleb* genotypes was relatively high [9].

4. CONCLUSION

In the present study, primers that were previously used in cherry and peach were employed, because there were no mahaleb-specific primers. PCR conditions modifying for mahalebs and all mahaleb genotypes are characterized by these primers. Thus, the transferability of SSR markers was proven.

Moreover, the results showed that microsatellite markers for fingerprinting purposes were efficient and useful for genetic characterization, and it was found that the markers must have transferability to closely related species. In addition, the present study indicated the transfer characteristics of cherry and peach SSRs, and these markers were used to identify the level of genetic variability in mahalebs. Moreover, the identification of the Anatolian mahaleb gene sources indicated that this region has a high potential for rich genetic diversity and it was found that the gene pool of mahaleb genotypes has significant amounts of genetic variations in Anatolia.

ACKNOWLEDGEMENTS

This research was funded by the Scientific and Technological Research Council of Turkey (Contract No:105O058) and Ege University Scientific Research Fund (Contract No: 2010 ZRF 044).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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