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Sweet Cherry Cultivar Identification by Using SSR Markers

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Abstract: In this study, sweet cherry varieties and types grown in Turkey were described using SSR markers. This study was undertaken to develop DNA marker profiles that could be used to distinguish among the sweet cherry cultivars used in production in Turkey. For microsatellite analysis, 13 SSR primers isolated from sweet cherry (*P. avium* L.) sour cherry (*P. cerasus* L.) and peach (*P. persica* L. Batsch) were used on sweet cherry cultivars and types. Two primer pairs did not give amplification with genotypes analyzed. Two-primer pairs amplified monomorphic fragment for the sweet cherry varieties therefore they were uninformative for the sweet cherry genetic analysis. Genetic similarities were calculated and a dendrogram has been established. All of the 8 SSR primers used and cherries have produced amplified bands. For each primer the alleles obtained has been between 1 and 6, in total 38 alleles have found. Through these analyses the similarities between these varieties have been converted into numerical values. This happens to be the first study kind towards the molecular identification of sweet cherry genetic resources in Turkey.

Key words: DNA fingerprinting, sweet cherry (*P. avium* L.), variety identification, SSR, microsatellite

INTRODUCTION

Sweet cherries are believed to have originated in a region that includes Turkey and other countries bordering the Caspian and Black Seas. Even today wild cherry trees can be found in the Eastern Black Sea region and most of the nursery owners obtain their wild sweet cherry seed (syn. mazzard) that is used as a rootstock for budding from that region. Centuries of domestication and wild cultivation of sweet cherries have resulted in numerous landraces. In Turkey where the landraces arose, clones selected from these groups still present and significant part of production. There are several varieties or their types grown in different regions of Turkey[1].

Suitable climatic conditions in Turkey and excellent export market demand has resulted in the rapid increase of production. However, much confusion still exists as to the cultivars used in production and their trueness to type. One common method used to distinguish among plant cultivars is the development of DNA marker profiles that distinguish among the different types. Simple Sequence Repeat (SSR) markers that were developed in *Prunus* species were readily available for this purpose. SSR markers have several advantages over other molecular markers. They are in fact abundant in most genomes and co-dominant, therefore, their information content is very high; SSRs are PCR based, thus requiring little DNA for the amplification; every SSR locus is defined by a unique pair of primers, so that information exchange between laboratories is easy[2]. It is widely held that SSRs isolated from a source genome can be transferred to different individuals of the same species or the same genus.

For this purpose microsatellite markers that are isolated from peach, sweet and sour cherry for sweet cherry varieties to assess the genetic relationship between varieties, to verify how useful are peach, sweet and sour cherry microsatellites when applied to sweet cherries and to detect the level of polymorphism of each microsatellite in sweet cherries.

MATERIALS AND METHODS

Plant materials and DNA isolation: Ten sweet cherry varieties were genotyped with the SSR markers. They include seven cultivars that are widely planted in Turkey (0900 Ziraat, Malatya Dalbasti, Akşehir Napoleon, Omerli, Uluborlu, Allahdiyen and Mustafa Kemal Pasa) and three selections with foreign origin: Starks Gold, Noir de Guben, Bing. The plant material used in this study was obtained from Cukurova University, Pozanti Agricultural Experimental Center, Turkey.

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Table 1: SSR locus designation, the primer sequences and plant species source

Locus	Orientation[a]	Oligo sequence (5' → 3')	Isolated plant species
PMS 49	F	TCACGAGCAAAAGTGTCTCTG	Sweet cherry
	R	CACTAACATCTCTCCCCTCCC	
pceGA77	F	CCCAATGAACAACTGCAT	Sweet cherry
	R	CATATCAATCACTGGGATG	
PS08E08	F	ACGC[b]TATGTCCGTACACTCTCCATG	Peach
	R	CAACCTGTGATTGCTCCTATTAAAC	
pchpgms3	F	CCTTACCACTGGCATCATCA	Sour cherry
	R	CAGCTGAGCAGGCAACAAAA	
pceGA34	F	GAACATGTGGTGTGCTGGTT	Sour cherry
	R	TCCACTAGGAGGTGCAAATG	
PS12A02	F	GCCACCAATGGTTCTTCC	Sweet cherry
	R	AGCACCAGATGCACCTGA	
pceGA25	F	GCAATTCGAGCTGTATTTCAGATG	Sour cherry
	R	CAGTTGGCGGCTATCATGCTTAC	
PMS2	F	CACTGTCTCCCAGGTTAAACTC	Sweet cherry
	R	CCTGAGCTTTTGACACATGC	
PMS3	F	TGGACTTCACTCATTTCAGAGA	Sweet cherry
	R	ACTGCAGAGAATTTCACAACCA	
PMS30	F	CTGTCGAAAGTTTGCCTATGC	Sweet cherry
	R	ATGAATGCTGTGTACATGAGG	
PMS67	F	AGTCGCTCACAGTCAGTTTCTC	Sweet cherry
	R	TTAACTTAACCCCTCTCCCTCC	
B4G3	F	CATTGTTCATGGGAGGAATT	Peach
	R	AGAACATTCCTAAAGGAGCA	
GA65	F	GAGAAGCATCCAATGGCAAAGTT	Sour cherry
	R	CGGCGGCATCAATATACCTCAA	

[a]F=Forward, R= Reverse; [b]= pchpgms3 in original primer sequence was G[5]

Young leaves were collected from a single tree for each genotype, immediately frozen in liquid nitrogen and stored -80°C. High molecular weight genomic DNA was extracted from the leaf samples following the method of Dellaporta *et al.*[3]. DNA concentration has been assessed using a spectrophotometer and gel electrophoresis.

PCR amplification of microsatellite loci: DNA was diluted in water to a final concentration of 50 ng μL^{-1} and stored -20°C. PCR was performed in 25 μL reaction volumes. The reaction mixtures contained: 50 ng DNA, 1X PCR reaction buffer, 2.5 mM $MgCl_2$, 0.02 mM dNTP mix, 2.5 pM each of 5 and 3 end primers, 1 unit of Taq DNA polymerase (Boehringer Mannheim Biochemicals, Indianapolis, Ind.) and distilled water. Thirteen previously described SSR primer pairs (PMS 49, PS08E08, pchpgms3, pceGA77, pceGA34, PS12A02, pceGA25, PMS2, PMS3, PMS30, PMS67, B4G3 and GA65) were used to genotype the ten sweet cherry cultivars (Table 1).

Each reaction mixture was overlaid by one drop of mineral oil. The amplification was carried out in a thermocyler (Perkin Elmer Cetus DNA Termal Cycler 480) using a program consisting of an initial denaturation step 4 min 94°C and then 30 cycles of 94°C, 1 min, 50-58°C 1 min (annealing temperature was determined for each primer pair specifically), 72°C 1 min, followed by a 5 min elongation step at 72°C.

Before loading on a polyacrilamide gel, 8 μL of each reaction mix were checked on agarose gels (0.9%) to verify quality. Amplified DNA was separated high resolution in 6% polyacrilamide gel in a Sequi-Gen® Sequencer (BIO RAD), run at 80 W for 2.5 h and stained with Silver Sequence staining system (Promega, Madison, Wis.). The bands of amplified DNA were scored visually and size was estimated using 10 bp (base pair) ladder (Gibco BRL, Rockville, Md).

Data analysis: SSR fragments for each primer pair were scored as present (1) or absent (0). Genetic similarity values[4] were calculated and UPGMA (Unweighted Pair-group Method Analysis) cluster analysis was performed to generate dendrograms with the programme NTSYS-PC version 2.02i (Exeter Software, Stauket, NewYork, NY, USA).

RESULTS AND DISCUSSION

Of the thirteen primer pairs tested, two primer pairs (B4G3 and GA65 isolated from peach and sour cherry, respectively) did not result in any amplification products for the genotypes analyzed. Two of the remaining eleven primer pairs each amplified a monomorphic fragment for the sweet cherry cultivars tested (PMS 49: 142 bp, pchpgms3: 182 bp). Therefore, these two primer pairs were uninformative for distinguishing among the sweet cherry selections (Table 2). Previous studies also found that the SSR pchpgms3, that was isolated from peach sequence, Sosinski *et al.*[5] is monomorphic in cherry. Downey and

Table 2: Expected and obtained fragment size for each locus

Locus	Expected size range	Reference	Obtained allele size
PMS 49	79-185	[14]	142
S08E08	180	[5]	
	172-185	[14]	170, 172, 182, 185
pchpgms3	170-230	[6]	
	179	[5]	
	174-189	[14]	182
pceGA77	166	Iezzoni (unpublished)	165, 170, 178, 180
pceGA34	140-174	[6]	145, 160, 167, 209, 212, 225
PS12A02	200	[5]	
	150-178	[6]	170, 172, 173, 185
pceGA25	145-198	[14]	158, 160, 192, 195, 207
PMS2	132-152	[14]	140, 145, 148
PMS3	152-200	[14]	185,186, 200, 210
PMS30	119-175	[14]	131, 140, 155, 170
PMS67	144-191	[14]	150, 162, 167

Iezzoni[6] reported that pchpgms3 is monomorphic in *Prunus serotina*. Additionally, Wünsch and Hormaza[7] reported that pchpgms3 identified one common fragment in a survey of 76 sweet cherry genotypes.

The nine remaining SSR primer pairs produced 37 fragments with an average of 4.1 putative alleles per locus. The number of putative alleles per locus ranged from three (PMS2 and PMS67) to six (pceGA34). Sizes ranged from 131 bp (PMS30) to 225 bp (pceBA34) (Table 2).

Primer pair PceGA34 isolated from sour cherry was most informative for sweet cherry as six putative alleles were identified. The amplification of microsatellite loci in sweet cherry was possible sour cherry microsatellites but SSR primers that are isolated from peach were uninformative for sweet cherry cultivars. This can be due to the different position of peach and sour cherry in relation to sweet cherry in *Prunus* phylogeny. The main cultivated *Prunus* species belong to three of them: *Amygdalus* (peach and almond), *Prunophora* (plum and apricot) and *Cerasus* (sour and sweet cherries). Subgenus *Cerasus* species diverged earlier from the ancestral *Prunus* species than the other two subgenera[7].

The range of putative alleles per polymorphic SSR (three to six) identified in this study is similar to that identified in other surveys of sweet cherry. Clarke and Tobutt[8] genotyped 14 sweet cherry varieties with nineteen SSR primer pairs and identified two to seven putative alleles per polymorphic SSR locus. Vaughan and Russell[9] tested 16 wild cherry accessions with 10 SSR primer pairs and identified between two and six putative alleles per polymorphic SSR.

A low of level of polymorphism in sweet cherry has also been detected by Stockinger *et al.*[10] and Gerlach and Stosser[11] using RAPD markers, by Beaver *et al.*[12] and Granger[13] with isozyme markers, probably reflecting a narrow genetic base in sweet cherry germplasm[7].

A similarity matrix was generated for the 37 fragments using Dice coefficient of Nei and Li[4]. The dendrogram

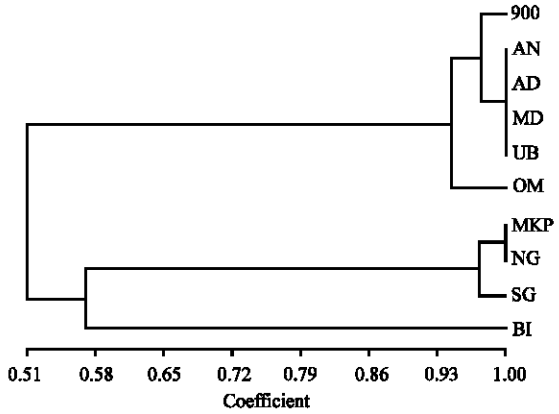

Fig. 1: UPGMA dendrogram of 10 sweet cherry genotypes from SSR data. Similarity values are shown at the bottom of the dendrogram. (0900:0900 Ziraat, AN:Akşehir Napoleon, AD:Allahdiyen, MD: Malatya Dalbastı, UB: Uluborlu, Omerli, MKP: Mustafa Kemal Pasa, NG: Noir de Guben, SG: Starks Gold, BI: Bing)

constructed by UPGMA clusture analysis is illustrated in Fig. 1. Based on this dendrogram, the genotypes can be separated two major groups with a similarity value of 0.51. The Group I consists of local cherry varieties 0900 Ziraat, Akşehir Napoleon, Allahdiyen, Malatya Dalbasti, Uluborlu and Omerli. The second group includes Mustafa Kemal Pasa Napoleon, Noir de Guben and Starks Gold sweet cherry varieties and Bing sweet cherry variety which completely different origin. For the Turkish sweet cherry cultivars Malatya Dalbasti, Akşehir Napoleon, Uluborlu, Allahdiyen showed identical SSR patterns and distinct from 0900 Ziraat and Omerli, but all of them shared at least one of the two alleles for each locus. Thus, showing a certain degree of genetic relatedness. Noir de Guben and Mustafa Kemal Pasa sweet cherry varieties gave the same profiles.

Molecular characterization of fruit tree scions and rootstock is essential for the interest of fruit tree breeders, germplasm collections and commercial sector such as nurseries. Since most temperate fruit species are vegetatively propagated, the identification of genotypes allow standardisable reference for identification of any cultivar and control of its propagation, independently of any factors that limit or influence phenotypic characterization, such as environment the time of year or the age of the tree. Furthermore, the possibility of studying the genetic diversity among different cultivars and populations will benefit cherry breeding programmes by helping to take decisions on parental genotypes for crosses and germplasm management to maximized to conserved diversity. This is becoming increasingly important to conserve the existing variability in the wild stands of this species scattered through most European and some Asian countries, especially due to the progressive narrowing of the genetic[7].

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