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Simple Sequence Repeat (SSR) Markers Differentiate Turkish Sour Cherry Germplasm

YILDIZ A. KACAR[1*], M. SELIM CETINER[2], CLAUDIO CANTINI[3], A.F. IEZZONI[4]

Abstract

Eighty-one tetraploid cherry selections, including sour cherry (*Prunus cerasus* L.) and its progenitor species *P. fruticosa* Pall., were compared using simple sequence repeat (SSR) marker analysis to determine the relationship of germplasm collected from Turkey to those collected from Russia and northern and eastern regions of Europe. SSR fragments were produced with all five primer pair – cherry selection combinations. Cherry selections exhibited high levels of polymorphism with 5 to 20 different putative alleles amplified per primer pair. The primer pair PMS 49, isolated from sweet cherry, showed a high level of polymorphism with 20 putative alleles identified. However, SSR analysis was not able to differentiate among some selections.

Overall, Turkish selections tended to group together and separated from other cherry selections. The Turkish germplasm did not exhibit many of the putative SSR alleles identified in the broader germplasm pool; however, it exhibited many novel alleles. This study demonstrated the importance of including germplasm from Turkey, an important ancestral region for sour cherry, when building a germplasm collection. It also demonstrated the utility of SSR markers in identifying genetic variation "gaps" within a germplasm collection.

Introduction

Sour cherry is believed to have originated in the Near East center which includes Asia Minor, Transcaucasia, Iran, and the highlands of Turkmenistan (19). It is in this region that geographic ranges of the progenitor species of sour cherry, ground cherry (*P. fruticosa* Pall.), and sweet cherry (*P. avium* L.) overlap. Centuries of domestication and cultivation of sour cherry in various geographic regions have resulted in the establishment of numerous landraces of sour cherry. In countries where these landraces arose, clones selected from these groups still represent a significant part of the production. Such a scenario must have occurred in Turkey, the native land of sour cherry, where it has been grown since ancient times in the northern part of Anatolia, Black Sea Region. For centuries, the local population has selected and propagated sour cherry selections from wild material. Among the most common landrace cultivars is named 'Kütahya'. This cultivar is suitable for processing as jam, marmalade, and fruit juice as well as for freezing (3).

Sour cherry landrace types in Turkey represent a wealth of morphological diversity, and the diversity in sour cherry in this material has been first reported by Oz (16). Subsequently, Burak et al. (2) studied the morphological variation among local Turkish selections and three foreign cultivars ('Rubin Weichsel', 'Montmorency', 'Early Richmond'), and found two types of 'Kütahya' sour cherry, designated as 1355 and 1310, as the most promising types for the processing industry. Onal and Gonulsen (15) selected 89 sour cherry types from the Aegean, Black Sea, Marmara, and Central Anatolia regions, and identified 26 as the most promising types of the 'Kütahya' landrace for growing in Turkish orchards. However, neither group of researchers investigated the genetic relationships among these Turkish types of sour cherry germplasm. Furthermore, there have been few reports on genetic fingerprinting of Turkish germplasm (11), and they

[1] Department of Horticulture, Faculty of Agriculture, University of Cukurova, Adana 01330, Turkey
[2] Faculty of Engineering and Natural Sciences, University of Sabanci, Istanbul, 81474, Turkey
[3] Istituto per la Valorizzazione del Legno e delle Specie Arboree, Follonica, 58022, Italy
[4] Department of Horticulture, Michigan State University, East Lansing, MI 48824, USA
[*] Corresponding author. Tel.: +90-322-3387142; Fax: +90-322-3386615 e-mail address: ykacar@cu.edu.tr

did not relate the Turkish sour cherry material to sour cherry germplasm representing a broad geographical range.

Recently the tetraploid cherry (sour and ground cherry) germplasm in the United States Department of Agriculture, Agricultural Research Service (USDA-ARS) collection was fingerprinted using 10 simple sequence repeat (SSRs or microsatellites) (12, 20) markers. These SSR primer pairs were able to amplify fragments that differentiated among all but two of 59 accessions available in the USDA/ARS collection (4). Unfortunately, the USDA/ARS collection did not include any accessions from Turkey. Because of the importance of Turkey for the geographic diversity of sour cherry, this study was undertaken to evaluate the diversity within the Turkish germplasm and compare this diversity to that previously obtained from the 59 accessions in the USDA/ARS collection using SSR marker technology. SSRs are robust markers for such an analysis because every SSR locus is defined by a unique pair of primers facilitating information exchange between laboratories (8).

Materials and Methods

Plant materials and DNA isolation. Eighty-one sour cherry cultivars, open-pollinated types and accessions, were used in this study. The plant material was obtained from Cukurova University, Adana, (Turkey), Michigan State University, and USDA-ARS tetraploid cherry collection at Geneva, N.Y., USA. The SSR data for the tetraploid cherry accessions in the USDA collection was obtained from the following web site: http://www.ars-grin.gov/gen/tartchssr.html. These data were combined with data obtained from a total of 22 sour cherry cultivars from Turkey (Table 1).

Genomic DNA was extracted from young leaves using modified protocol with extra ethanol precipitation step of that by Dellaporta et al. (6). DNA concentration was assessed using a fluorimeter (Hoefer instrument, TK 100 model).

PCR amplification of microsatellite loci. DNA was diluted in water to a final concentration of 50 ng/ µl and stored at –20 °C. PCR was performed in 25 µl reaction mixture containing the following: 50 ng DNA, 1X PCR reaction buffer, 2.5 mM $MgCl_2$, 0.02 mM dNTP mix, 2.5 µmol each of 5'and 3' end primers, 1 unit of *Taq* DNA polymerase (Boehringer Mannheim Biochemicals, Indianapolis, Ind.), and distilled water (15 µl). Five SSR primer pairs, previously isolated from peach, sweet cherry and sour cherry (Table 2) and used to fingerprint the USDA-ARS collection, were used for DNA amplification. The sour cherry primer pair (PceGA25) was obtained from a *P. cerasus* "Erdi Botermo" genomic library (A. Iezzoni, unpublished). The peach primer pair pchpgms 3 was obtained from A. Abbott (17). Each reaction mixture was overlaid by a single drop of mineral oil (70-100 µl). The amplification was carried out in a model 9600 DNA thermal cycler (Perkin-Elmer Corp., Applied Biosystems, Foster City, Calif.) using a program consisting of an initial denaturation step of 4 min at 94 °C, and then 30 cycles of 1 min at 94 °C, 1 min at 50-58 °C (annealing temperature was determined for each primer pair specifically), 1 min 72 °C, followed by a 5 min elongation step at 72 °C.

Before loading on a polyacrilamide gel, 8 µl of each reaction mix was checked on 1% agarose (Sigma A5093) gel to verify the occurrence of amplification. Amplified DNA was separated on 6% polyacrylamide gel in a Sequi-Gen® Sequencer (BIO RAD), at 80 W (~1900-2100 V) for 2.5 h, and stained with a Silver Sequence staining system (Promega, Madison, WI.). Amplified fragments were scored visually, and the fragment was estimated using a 10 base pair (bp) DNA ladder (Gibco BRL, Rockville, Md). DNA amplification and scoring of fragments were repeated in two independent experiments for all accessions to confirm repeatability of the results.

Table 1. Plant material used in the SSR analysis and geographic origin, if known, for the Turkish germplasm.

Sour cherry cultivars obtained from Turkey
Kütahya, Rubin Weichsel (RW)
Sour cherry selections from Turkey *(geographic origin)*
1319 *(Denizli, Aegean Sea)*, 1352 *(Aydin, Aegean Sea)*, 1360 *(Afyon, Aegean Sea)*, 1367 *(Aydin, Aegean Sea)*, 1391 *(Amasya, Black Sea)*, 1416 *(Nigde, central Anatolia)*, 1432 *(Konya, central Anatolia)*
Sour cherry open pollinated plants from Michigan State University collection field *(geographic origin)*
S1A1, S1A2, S2A1$_1$, S2A1$_2$, S3A1, S3A3$_1$, S3A3$_2$, 1315$_1$ *(Denizli, Aegean Sea)*, 1315$_2$ *(Denizli, Aegean Sea)*, 1355$_1$, 1355$_2$, 1873$_1$, 1873$_2$.
Tetraploid cherry germplasm accessions from USDA/ARS Plant Genetic Resources Unit (abbreviations used in Figure 1)
Altaiska o.p. IV-7-6 (**Alt**), Amarena di Pescara (**AdP**), Balaton (**Ba**), Cigany 59 (**C59**), Crisana (**Cr**), Csengodi Csokros (**CC**), Del Nord (**DN**), Dropia (**Dr**), Dwarfrich (**Dw**), Engleise Timpurii (**ET**), English Morello (**EM**), Erdi Botermo (**EB**), Erdi Jubileum (**EJ**), Erdi Nagygumolesu (**EN**), Favorit (**Fa**), Ferracida (**Fe**), Fructbare von Michurin (**FvM**), Griotte Moskovskii (**GM**), I13 [61] (**13**), I24 [63] (**63**), Ideal o.p. 25-11-50 (**Id**), III 18 [12] (**18**), Kelleriis 14 (**Ke**), Korai Pipacs Meggy (**KPM**), Lebedjankaja op 26e 2 (51) (**Le**), Lubskaya (**Lu**), Maliga Emleke (**ME**), M209 (**M2**), Mari Timpurii (**MT**), Mesabi (**Me**), Meteor (**Met**), Meteor Korai (**MK**), Mocanesti 16 (**Mo**), Montmorency (**Mont**), Nefris (**Ne**), North Star (**NS**), Oblacinska (**Ob**), BMs4 o.p. 26e-1-25 (**BMs**), BMT3 o.p. 26e-1-4 (**BMT**), BS2 o.p. 26e-1-18 (**BS**), Bt1 o.p. 26e-1-59 (**Bt**), Paza1 o.p. 26e-2-4 (**Pa**), Pandy 114 (**P114**), Pandy 279 (**P279**), Pandy 35 (**P35**), Pandy 48 (**P48**), *P. fruticosa* (**FR**), *P. fruticosa* (**FR2**), *P. fruticosa* (**FR1**), *P. fruticosa* (**FR8**), Pozog 29 (**P29**), Rexelle (**Re**), Rheinische Schattenmorelle (**RS**), Sarandi (**Sa**), Studencheskaya o.p. IV-6-15 (**Stu**), Sumadinka (**Sum**), Surefire (**Sur**), Tschernokorka (**Ts**), Ukr. Griotte (**UG**)

Data analysis. SSR fragments for each primer pair were scored as either present (1) or absent (0). Genetic similarity values (14) were calculated, and unweighted pair-group method analysis (UPGMA) cluster analysis was performed to generate dendograms using NTSYS-PC version 2.02i software program (Exeter Software, Setauket, NY).

Results and Discussion

The five SSR primer pairs used to fingerprint the sour cherry accessions produced discrete reproducible fragments for all cherry cultivars/types tested. The number of fragments amplified from an individual sour cherry selection with each primer pair ranged from one to four. Four fragments per primer pair would be the number expected if the primer pair amplified different size fragments from duplicate loci present in tetraploid sour cherry. Few fragments per primer pair from certain selections could result from low variability for that particular locus or the presence of null alleles (21).

The five SSR primer pairs used on all the cherry selections produced 63 different fragments. The mean number of putative alleles detected per primer pair in this study ranged from 5 to 20 (Table 3) with a mean value of 12.6 alleles per primer pair. This mean value is similar to that reported for *Malus ×domestica* genotypes (12.1 alleles/locus) Hokanson et al. (10) but higher than reported for other plant species (9, 13,18, 21).

Table 2. SSR locus designation, the sequences and sources of primer pairs used.

SSR	Orientation[z]	Oligo sequence (5' to 3')	Original reference	Source
PMS 49	F	TCACGAGCAAAAGTGTCTCTG	Cantini et al.	Sweet
	R	CACTAACATCTCTCCCCTCCC	(4)	cherry
PS08E08	F	CCCAATGAACAACTGCAT	Sosinski et al.	Sweet
	R	CATATCAATCACTGGGATG	(17)	cherry
pchpgms3	F	ACGCTATGTCCGTACACTCTCCATG	Sosinski et al.	Peach
	R	CAACCTGTGATTGCTCCTATTAAAC	(17)	
PceGA25	F	GCAATTCGAGCTGTATTTCAGATG	Cantini et al.	Sour
	R	CAGTTGGCGGCTATCATGCTTAC	(4)	cherry
PMS3	F	TGGACTTCACTCATTTCAGAGA	Cantini et al.	Sweet
	R	ACTGCAGAGAATTTCACAACCA	(4)	cherry

[z]F=forward, R=reverse

The primer pair PMS 49, isolated from sweet cherry, was the most informative and producing 20 different sized fragments (Table 3). Where as the primer pair pchpgms3, isolated from peach, was the least informative with only five different sized fragments identified.

In the SSR analysis of the USDA-ARS tetraploid cherry collection (Cantini et al., (4)), the primer pair PMS3 identified more fragments (16 fragment) than any of the other primer pairs used. However, in this study 17 fragments were identified, including one novel fragment in the Turkish germplasm (Table 3). The second most polymorphic SSR identified in the USDA-ARS collection was PMS 49 with 15 fragments scored. In this study, five new fragments were identified in the Turkish germplasm, the increasing the total number to 20 (Table 3). With primer pairs PS08E08 and PceGA25, the Turkish germplasm exhibited novel fragments, two and one respectively (Table 3). No novel fragments were identified in the Turkish germplasm with the SSR primer pair pchpgms3.

All selections exhibiting a 114 bp fragment with primer PMS 49 were selected from the Aegean and Central Anatolia regions, except for selection 1391 which was selected from the Black Sea region (15). All sour cherry selections exhibiting novel fragments with PMS49 were open-pollinated plants of unknown Turkish origin. Novel SSR fragments 163 and 170 bp observed with primer PS08E08 were obtained from open-pollinated plants except for selection 1391.

The Turkish germplasm exhibited all putative alleles identified in the USDA-ARS collection for SSR primer pairs PS08E08 and pchpgms3. However, the Turkish germplasm exhibited only a subset of the variation identified with SSR primer pairs PMS 3, PMS 49, and PceGA25. The Turkish germplasm only exhibited six of the 17 putative alleles for PMS 3, three of the 15 putative alleles for PMS 49, and nine of the 20 putative alleles for PceGA25. Therefore, the Turkish germplasm included some previously unidentified novel putative alleles; however, it represented only a subset of the diversity present in the USDA-ARS collection that includes material from northern and eastern Europe and Russia (4).

Table 3. Number of SSRs fragments observed in *P. cerasus* for five primer pairs[y].

SSR	Total no of fragment.	Fragment sizes (bp)
PMS 49	20	79,92,96,100,112,114,120,122,127,132,136,140,142,143,144,145, 156, 160,162,185
PS08E08	6	163,170,172,174,182,185
pchpgms3	5	174,176,178,183,189
PceGA25	15	145,161,163,165,173,175,178,181,183,185,186,188,192,195,198
PMS 3	17	147,152,155,157,167,169,172,175,177,180,185,186,188,190,195, 197, 200

[y] Novel SSR fragments identified in the Turkish germplasm are underlined.

Table 4. Turkish sour cherry selections exhibiting SSR fragments not previously identified in sour cherry germplasm.

Primer pair	Fragment size (bp)	Selection(s)
PMS 49	114	Rubin Weichsel, Kütahya, 1319, 1352, 1360, 1367, 1391, 1416, 1432, $S3A3_2$, 1355_1, 1873_1, 1873_2
	132	1315_1
	142	S1A1, S1A2, $S2A1_1$, $S2A1_2$, S3A1, $S3A3_1$, 1315_1,1315_2
	143	S1A2, 1315_1,1315_2
	145	S1A2
PS08E08	163	1391, $S2A1_1$, $S2A1_2$, $S3A3_2$, 1315_1, 1315_2, 1355_1
	170	S1A1
PceGA25	163	1319,1360,1367,1391,1416,1432
PMS 3	147	$S2A1_2$

Based on a total of 63 fragments, a similarity matrix was generated for the 81 tetraploid cherry selections using the Dice coefficient of Nei and Li (14). The dendogram constructed by UPGMA cluster analysis separated the cherry selections into two groups with a similarity value of 0.38 (Fig. 1).

The five SSR primer pairs were not able to differentiate some Turkish sour cherry selections such as 1319, 1360, 1416. These selections have slight morphological differences, and may represent sports, resulting from somatic mutations, selected during vegetative propagation from the original cultivar. Based on the five SSR markers, close genetic relationship among sports is difficult to discriminate. However, it should be noted that three of the SSR primer pairs used in this study, pchgms3, PMS 3, and PceGA25, have been placed on the *Prunus* linkage map in groups 1, 4, and 5, respectively (1).

The Turkish selections tended to group together, and were separated from all other cherry selections. This indicated that the

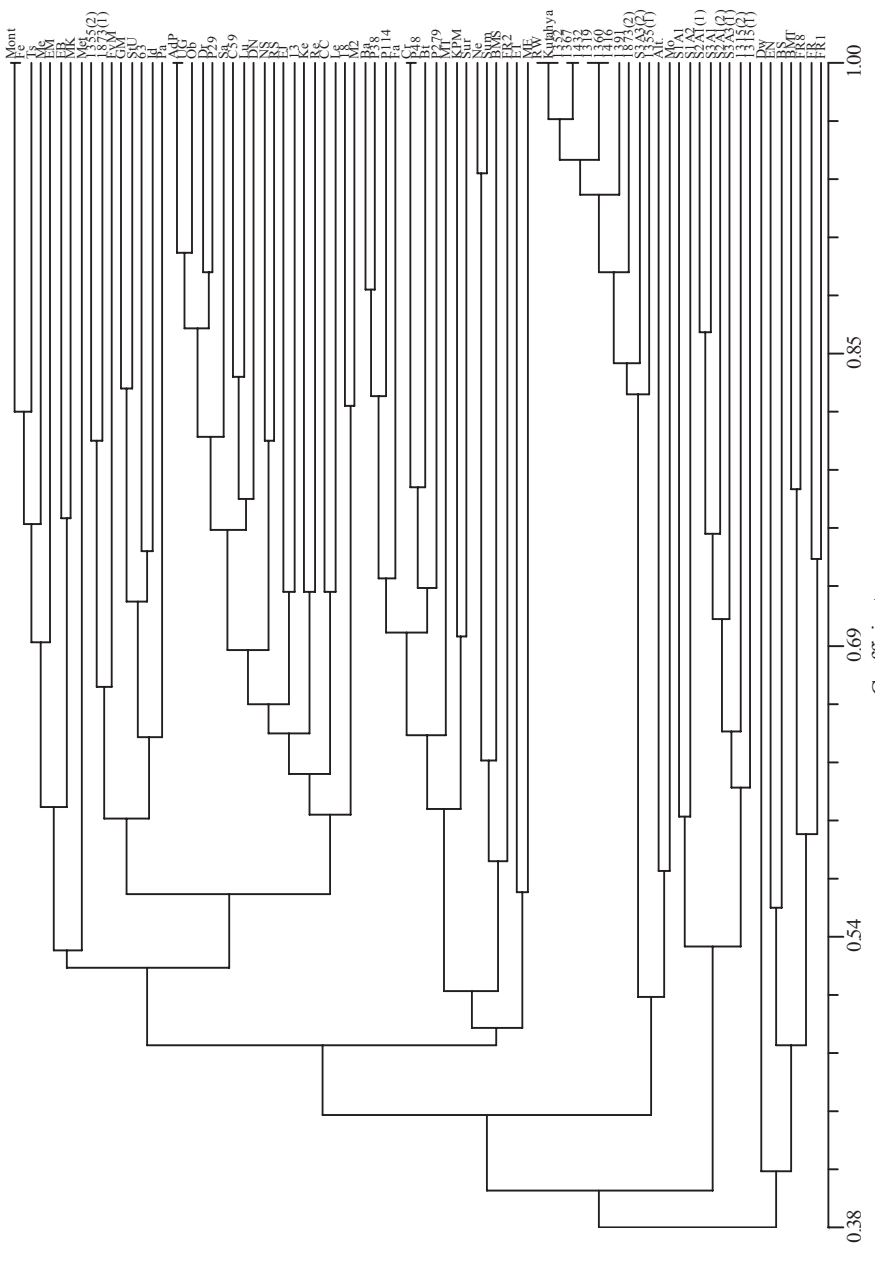

Figure 1. UPGMA dendogram of 81 sour cherry accessions from SSR data. Similarity values are shown at the bottom of the dendogram. Abbreviations are as in Table 1.

Turkish germplasm represented material that had not previously been included in the tetraploid cherry SSR analysis. The Turkish landrace selection 'Kütahya' was clearly different from the landrace selections in Germany, Russia, Serbia, and Hungary/ Romania represented by 'Rheinische Schattenmorelle', 'Lubskaya', 'Oblacinska', and 'Pandy'/ 'Crisana', respectively.

The Turkish sour cherry landrace cultivar 'Kütahya' and the German cultivar 'Rubin Weichsel' did not differ for any of the SSR markers analysed in this study. As a result, 'Rubin Weichsel' clustered with the Turkish germplasm, and was distant from all other German germplasm included in the study. Morphological studies conducted by Burak et al. (2) reported that 'Rubin Weichsel' and 'Kütahya' were morphologically different as Rubin Weichsel had a relatively horizontal growth habit, while 'Kütahya' trees were semi-vertical to vigorous. Additionally, the juice from 'Kütahya' fruit is a darker red than that of 'Rubin Weichsel'. However, the fruit yield, fruit weight, fruit length and diameter, stem length and seed weight were found to be similar for 'Kütahya' and 'Rubin Weichsel' (2). Despite these morphological differences, the genetic similarity suggests either that 'Rubin Weichsel' is of Turkish origin or that the 'Rubin Weichsel' included in this study is different from the original German clone.

In summary, some of the Turkish sour cherry selections used in this study represent novel germplasm that differs from the sour cherry germplasm most prevalent in northern and eastern Europe and Russia. The Turkish germplasm did not exhibit many of the putative SSR alleles identified in the broader germplasm. In addition, it included many novel alleles. This study demonstrates the importance of including germplasm from the entire species range when building a germplasm collection. It also demonstrates the value of SSR markers to identify "gaps" in a germplasm collection. Finally, our results demonstrate that the transportability of selected SSR markers in *Prunus*, confirming what was previously reported.

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