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DNA Fingerprinting of Tetraploid Cherry Germplasm Using Simple Sequence Repeats

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Abstract. **The U.S. Department of Agriculture (USDA), Agricultural Research Service (ARS) tetraploid cherry (*Prunus* L. sp.) collection at Geneva, N.Y., contains ≈75 accessions of sour cherry (*P. cerasus* L.), ground cherry (*P. fruticosa* Pall.), and their hybrids. Accurate and unambiguous identification of these accessions is essential for germplasm preservation and use. Simple sequence repeats (SSRs) are currently the markers of choice for germplasm fingerprinting because they characteristically display high levels of polymorphism. Recently SSR primer pairs from sweet cherry (*P. avium* L.), sour cherry, and peach [(*P. persica* L. Batsch (Peach Group)] have been reported. Ten SSR primer pairs were tested on 59 tetraploid cherry accessions to determine if they could differentiate among the accessions. Scorable SSR fragments were produced with all primer-accession combinations. The cherry accessions exhibited high levels of polymorphism with 4 to 16 different putative alleles amplified per primer pair. Most of the putative alleles were rare with frequencies <0.05. Heterozygosity values ranged from 0.679 to 1.00, while gene diversity values ranged from 0.655 to 0.906. The primer pairs differentiated all but two of the 59 cherry accessions. Based upon the ability of the SSR data to differentiate the cherry accessions and the high level of gene diversity, we propose that all the tetraploid cherry accessions in the USDA/ARS collection be fingerprinted to provide a mechanism to verify the identity of the individual accessions. The fingerprinting data are available on the World Wide Web (http://www.ars-grin.gov/gen/cherry.html) so that other curators and scientists working with cherry can verify identities and novel types in their collections and contribute to a global database.**

About 75 accessions of sour cherry (*P. cerasus*), ground cherry (*P. fruticosa*), and their hybrids are maintained in the U.S. Department of Agriculture (USDA), Agricultural Research Service (ARS) tetraploid cherry (*Prunus* L. sp.) collection at the Plant Genetics Resources Unit (PGRU) in Geneva, N.Y. This collection consists of cultivars, landrace cultivars, and open-pollinated seedlings. With clonally propagated crops it is especially important to accurately identify germplasm accessions so that the user community can confidently use the accessions. First, it is common for the same cultivar or landrace cultivar to be identified by two or more different names. For example, the same sour cherry landrace cultivar is called 'Pándy' in Hungary, 'Crisana' in Romania, and 'Köröser Weichsel' in Germany (Iezzoni et al., 1990). In addition, some of the older landraces are best represented in the germplasm collection by a set of selections since individuals belonging to these landrace cultivars differ for horticultural traits. Within the 'Pándy' landrace, types are available that differ most significantly in bloom and ripening time (Iezzoni et al., 1990).

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Genetic fingerprinting is an efficient and unambiguous strategy used to accurately identify and catalogue accessions preserved in germplasm repositories (Hokanson et al., 1998; Lamboy and Alpha, 1998). However, to achieve this goal, the genetic fingerprints must be repeatable across laboratories and must display a sufficient amount of genetic variability among the accessions so that individual accessions have unique fingerprints. The molecular markers of choice for genetic fingerprinting are frequently simple sequence repeats (SSRs, also termed microsatellites) (Litt and Ludy, 1989) due to their hypervariability, abundance, and relatively simple diagnostic polymerase chain reaction (PCR) procedure (Powell et al., 1996). Recently SSR primer pairs from sweet cherry (*P. avium*), peach [*P. persica* (Peach group)] (Cipriani et al., 1999; Sosinski et al., 2000), and sour cherry (Downey and Iezzoni, 2000) have been developed; however, the utility of these primers for fingerprinting *P. cerasus* and *P. fruticosa* germplasm has not been determined. Therefore, our objective was to fingerprint a subset of the USDA/ARS tetraploid cherry accessions with 10 SSR primer pairs and determine if there is sufficient polymorphism to differentiate the accessions.

Materials and Methods

Genomic DNA was extracted from young leaves of 59 tetraploid cherry accessions (Table 1) using the extraction protocol of

Stockinger et al. (1996). The DNA was quantified by fluorometry, diluted to a final stock concentration of 300 ng·mL^{-1}, and a 50 ng·mL^{-1} dilution was then used as template for PCR. Ten SSR primer pairs that were isolated previously from sour cherry, sweet cherry, and peach (Table 2) were used for DNA amplification. The PCR reaction was prepared containing a final concentration of 1× PCR reaction buffer, 2.5 mM $MgCl_2$, 0.2 mM dNTP mix, 2.5 pmol of each primer, 50 ng of template DNA, 0.6 units of Taq DNA polymerase (Boehringer Mannheim Biochemicals, Indianapolis, Ind.) and distilled water to a total volume of 25 mL. The amplification was carried out in a thermocycler (model 9600; Perkin Elmer Applied Biosystems, Inc., Foster City, Calif.) using: 94 °C for 5 min, then 30 cycles of 94 °C for 1 min, 55 °C for 1 min, 72 °C for 1 min; and finally 72 °C 5 min. To detect the DNA fragments, 4 mL of the PCR product was loaded on a 6% polyacrylamide gel in a 50 cm 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 a 10 base pair (bp) ladder (Gibco BRL, Rockville, Md.). To ensure reproducibility of fragment sizing, each accession per primer sample was replicated two to four times.

Allele frequencies, number of alleles per locus, direct count heterozygosity, gene diversity (Weir, 1990) [also called polymorphic information content (PIC) (see, Röder et al., 1995)], and discrimination power (Jones, 1972; Kloosterman et al., 1993) were calculated using the computer program 'SSRS' written by W.F. Lamboy. The discrimination power is the probability that two tetraploid cherry accessions can be distinguished by their SSR profiles. It is calculated as one minus the probability that the SSR profiles will be identical (Jones, 1972). Accessions showing only one fragment at a locus were considered to be homozygous for that fragment. The mean heterozygosity reported may represent both intra- and inter-locus heterozygosity since it is possible for each primer pair to amplify the duplicate loci in a tetraploid.

Results

All 10 primer pairs amplified fragments in the sour cherry accessions tested (Table 3). The fragments (bp sizes) scored for each accession in this study can be found on the World Wide Web at URL http://www.ars-grin.gov/gen/cherry/html. Only two selections, 'Montmorency' and 'Ferracida', had identical fingerprints, and the fingerprinting data even distinguished among five selections from the same landrace: 'Crisana', 'Pándy 279', 'Pándy 114', 'Pándy 48', and 'Pándy 35'.

No more than four fragments were amplified for each accession/primer pair combination. Four fragments per primer pair are the maximum number of fragments expected assuming that the primer pair amplifies both duplicate loci. Since segregation data for the scored fragments are not available, all fragments have been given tentative allele and locus designations. Hence, all primer pairs herein are referred to as loci, and fragments generated by each primer pair in an amplification reaction are referred to as alleles.

Table 1. Tetraploid cherry germplasm accessions assayed for SSR polymorphisms.[z]

Identification	Accession no.	Identification	Accession no.
Altaiskaja o.p. IV -7-6	PI 592857	Meteor	PI 592848
Amarena di Pescara	PI 592861	Meteor Korai	PI 592864
Ujfehertoi Furtos	PI 592878	Mocanesti 16	PI 592879
Cigany 59	PI 592853	Montmorency	PI 592845
Crisana	GPRU 81	Nefris	PI 592880
Csengodi Csokros	PI 592860	North Star	PI 592841
Del Nord	PI 592844	Oblacinska	PI 592873
Dropia	PI 592859	BMs4 o.p.26e-1-25	PI 592887
Dwarfrich	PI 592842	BMT3 o.p. 26e-1-4	PI 592884
Engleise Timpurii	PI 592855	BS2 o.p. 26e-1-18	PI 592888
English Morello	PI 592847	Bt1 o.p. 26e-1-59	PI 592886
Erdi Botermo	PI 592856	Paza1 o.p. 26e-2-4	PI 592885
Erdi Jubileum	PI 592868	Pandy 114	PI 592867
Erdi Nagygyumolesu	GPRU 76	Pandy 279	GPRU 67
Favorit	PI 592876	Pandy 35	GPRU 66
Ferracida	PI 592883	Pandy 48	GPRU 68
Fructbare von Michurin	PI 592870	*P. fruticosa*	PI 592843
Griotte Moskovskii	PI 592890	*P. fruticosa* (FR2)	PI 592850
I 13 (61)	GPRU 75	*P. fruticosa* (FR1)	PI 592851
I 24 (63)	GPRU 74	*P. fruticosa* (FR8)	PI 592852
Ideal o.p. 25-11-50	PI 592858	Pozog 29	PI 592854
III 18 (12)	GPRU 80	Rexelle	GPRU 79
Kelleriis 14	PI 592877	Rheinische Schattenmorelle	PI 592846
Korai Pipacs Meggy	PI 592875	Sarandi	PI 592882
Lebedjanskaja op 26e 2 (51)	GPRU 77	Studencheskaya o.p. IV-6-15	PI 592872
Lubskaya	PI 592881	Sumadinka	PI 592871
Maliga Emleke	PI 592862	Surefire	PI 592840
M209	GPRU 70	Tschernokorka	PI 592869
Mari Timpurii	GPRU 78	Ukr. Griotte	PI 592865
Mesabi	PI 592849		

[z]PI and GPRU numbers are those of the USDA/ARS Plant Genetic Resources Unit, Cornell University, Geneva, N.Y.

Table 2. SSR primer designations, sequences, and references.

Primer Pair	Orientation[z]	Sequence (5' to 3')	Reference/lab source
pchpgms3	F	ACG C[y]TA TGT CCG TAC ACT CTC CAT G	Sosinski et al. (2000)
	R	CAA CCT GTG ATT GCT CCT ATT AAA C	
PS08E08	F	CCC AAT GAA CAA CTG CAT	Sosinski et al. (2000)
	R	CAT ATC AAT CAC TGG GAT G	
PMS2	F	CAC TGT CTC CCA GGT TAA ACT	D. Struss
	R	CCT GAG CTT TTG ACA CAT GC	
PMS30	F	CTG TCG AAA GTT TGC CTA TGC	D. Struss
	R	ATG AAT GCT GTG TAC ATG AGG C	
PMS49	F	TCA CGA GCA AAA GTG TCT CTG	D. Struss
	R	CAC TAA CAT CTC TCC CCT CCC	
PMS3	F	TGG ACT TCA CTC ATT TCA GAG A	D. Struss
	R	ACT GCA GAG AAT TTC ACA ACC A	
PceGA25	F	GCA ATT CGA GCT GTA TTT CAG ATG	A. Iezzoni
	R	CAG TTG GCG GCT ATC ATG TCT TAC	
PMS40	F	TCA CTT TCG TCC ATT TTC CC	D. Struss
	R	TCA TTT TGG TCT TTG AGC TCG	
PceGA59	F	AGA ACC AAA AGA ACG CTA AAA TC	A. Iezzoni
	R	CCT AAA ATG AAC CCC TCT ACA AAT	
PMS67	F	AGT CTC TCA CAG TCA GTT TCT	D. Struss
	R	TTA ACT TAA CCC CTC TCC CTC C	

[z]F = forward, R = reverse.
[y]This fourth nucleotide, a C in our primer, is a G in the original pchpgms3 primer (Sosinski et al., 2000).

The primer pairs amplified from 4 to 16 alleles with a mean of 10.7 alleles/locus (Table 3). The majority of the alleles were rare with frequencies of <0.05 (Tables 4 and 5). Only 14% of the alleles had frequency values >0.2. Heterozygosity levels for the loci identified by each primer pair ranged from 0.678 to 1.00 with a mean value of 0.946 (Table 6). The primer pairs that had heterozygosity values of 1.00 likely amplifed products from both homoeologous loci due to the large number of accessions that exhibited four fragments per primer pair. Genetic diversity or polymorphism information content ranged from 0.655 to 0.906 with a mean value of 0.810. Based upon discrimination power and the probability of matching a fingerprint, primer pair PMS3 was the most informative and the primer pair PS08E08 was the least informative.

Discussion

The present study demonstrated that SSR primers can be used to differentiate among all but two of the 59 tetraploid cherry accessions examined in the USDA/ARS collection. The two accessions not differentiated, 'Montmorency' and 'Ferracida', both originated in France. Additionally, these two selections are difficult to distinguish phenotypically, suggesting they may be the same cultivar. SSR fingerprinting successfully separated the five selections of the 'Pándy'/'Crisana' landrace. For example, of the 27 fragments present in 'Pándy 114', four (15%) of these fragments were absent in 'Pándy 35'. It is possible that genetic diversity may develop among clonal propagales of the same selection over time due to the accumulation of somatic mutations and preference of plant propagators for certain novel phenotypes (Cervera et al., 2000). In the case of the over 300 year old 'Pándy'/'Crisana' landrace, it is likely that early plant selectors would have propagated for their gardens, types that differed from the original type for some important characteristics. For example, a series of 'Pándy' clones with different ripening times would have been very desirable since sour cherry is a perishable fruit crop. SSR profile differences have also been found among Italian olive (*Olea europaea* L.) landrace cultivars (C. Cantini, personal communication).

The mean number of alleles per locus for the sour cherry accessions of 10.7 is considerably higher than the 3.1 of diploid tomato (*Lycopersicon* Mill sp.) species (Smulders et al., 1997)

Table 3. Number of SSR fragments observed and fragment sizes in base pairs (bp) for each of the 10 SSR primer pairs used to fingerprint 59 tetraploid cherry accessions.

Primer	No.	SSR fragments observed (size in bp)
pchpgms3	5	174, 176, 178, 183, 189
PS08E08	4	172, 174, 182, 185
PMS2	8	132, 135, 137, 138, 145, 148, 150, 152
PMS30	11	119, 124, 132, 134, 136, 142, 154, 159, 161, 171, 175
PMS49	15	79, 92, 96, 100, 112, 120, 122, 127, 136, 140, 144, 156, 160, 162, 185
PMS3	16	152, 155, 157, 167, 169, 172, 175, 177, 180, 185, 186, 188, 190, 195, 197, 200
PceGA25	14	145, 161, 165, 173, 175, 178, 181, 183, 185, 186, 188, 192, 195, 198
PMS40	11	88, 96, 98, 100, 103, 106, 108, 111, 113, 115, 118
PceGA59	10	181, 186, 189, 194, 200, 206, 217, 219, 221, 226
PMS67	13	144, 148, 150, 154, 162, 168, 170, 172, 174, 176, 178, 181, 191

Table 4. SSR fragments[z] detected in 59 tetraploid cherry accessions and their relative frequency (f).

pchpgms3		PS08E08		PMS2		PMS30		PMS49	
bp	f	bp	f	bp	f	bp	f	bp	f
174	0.194	172	0.418	132	0.190	119	0.012	79	0.051
176	0.129	174	0.052	135	0.258	124	0.301	92	0.008
178	0.076	182	0.381	137	0.098	132	0.116	96	0.059
183	0.318	185	0.149	138	0.055	134	0.052	100	0.025
189	0.282			145	0.350	136	0.110	112	0.051
				148	0.012	142	0.081	120	0.025
				150	0.031	154	0.006	122	0.127
				152	0.006	159	0.006	127	0.025
						161	0.040	136	0.280
						171	0.197	140	0.025
						175	0.081	144	0.025
								156	0.212
								160	0.017
								162	0.051
								185	0.017
PMS3		PceGA25		PMS40		PceGA59		PMS67	
bp	f	bp	f	bp	f	bp	f	bp	f
152	0.026	145	0.006	88	0.161	181	0.059	144	0.006
155	0.039	161	0.297	96	0.065	186	0.238	148	0.192
157	0.026	165	0.018	98	0.121	189	0.213	150	0.285
167	0.032	173	0.158	100	0.191	194	0.208	154	0.029
169	0.032	175	0.133	103	0.141	200	0.005	162	0.105
172	0.039	178	0.012	106	0.050	206	0.005	168	0.041
175	0.175	181	0.030	108	0.015	217	0.010	170	0.128
177	0.149	183	0.055	111	0.166	219	0.010	172	0.058
180	0.091	185	0.055	113	0.020	221	0.005	174	0.029
185	0.097	186	0.067	115	0.060	226	0.248	176	0.047
186	0.052	188	0.018	118	0.010			178	0.03
188	0.058	192	0.024					181	0.023
190	0.019	195	0.109					191	0.023
195	0.084	198	0.018						
197	0.032								
200	0.045								

[z]SSR fragments are identified by their length in base pairs (bp).

and 7.4 of hexaploid wheat (*Triticum aestivum* L.) (Prasad et al., 2000). The high value is more consistent with the mean number of alleles per locus identified in apple [*Malus sylvestris* (L.) Mill. var. *domestica* (Borkh.) Mansf.] (12.1; Hokanson et al., 1998), avocado (*Persea americana* Mill.) (9.5; Lavi et al., 1994), and 26 grape (*Vitis vinifera* L.) cultivars (8.4, Thomas and Scott, 1993), but less than the mean number of alleles per locus identified in a *Vitis* L. sp. collection (27.6; Lamboy and Alpha, 1998).

The mean number of alleles per locus in the tetraploid cherry accessions is estimated since the calculations are based upon two assumptions which would lead to an underestimation and an overestimation of the heterozygosity values, respectively. The first assumption is that any accession showing only one fragment from a primer pair is homozygous for that fragment. Hence, if the accession is heterozygous for the fragment and a null allele, the levels of heterozygosity and gene diversity among accessions would be underestimated. The second assumption is that any accession exhibiting two bands is heterozygous at a single locus. However, since sour cherry is an allopolyploid, each "locus" could represent the amplification products from two homoeologous loci. For example, a duplex state visualized as two fragments, could result from homozygous genomes $A^{100}A^{100}B^{104}B^{104}$ or heterozygous genomes $A^{100}A^{104}B^{100}B^{104}$, this being the assumed condition. Previous allozyme inheritance data demonstrated that sour cherry is composed of both genome types, i.e., homozygous or heterozygous genomes (Beaver and Iezzoni, 1993). Assigning alleles to the two putative homoeologous loci for all 59 selections is impractical since it would require segregation data from crosses involving all 59 selections. Therefore, since the heterozygosity values were calculated as a sum over the two putative homoeologous loci, the mean heterozygosity represents both intra- and inter-genome-locus heterozygosity.

Based upon the success of this study using SSR data to differentiate a set of cherry accessions, we propose that the other

Table 5. Number of alleles belonging in various allelic frequency classes for the 10 loci studied.

Allelic frequency class	Alleles within a class (no.)
<0.050	46
0.05–0.099	24
0.100–0.149	13
0.150–0.199	9
>0.200	15

Table 6. Heterozygosity, gene diversity, probability of two fingerprints matching by chance, and discrimination power at each locus.[z]

Locus	Heterozygosity	PIC[y]	Probability of matching fingerprints	Discrimination power[x]
pchpgms3	1.000	0.759	0.1327	0.8673
PS08E08	0.982	0.655	0.3867	0.6133
PMS2	1.000	0.761	0.1555	0.8445
PMS30	0.895	0.828	0.0319	0.9680
PMS49	0.678	0.846	0.0438	0.9562
PMS3	0.946	0.906	0.0075	0.9925
PceGA25	1.000	0.844	0.0324	0.9676
PMS40	1.000	0.865	0.0160	0.9840
PceGA59	1.000	0.790	0.1739	0.8261
PMS67	0.964	0.843	0.0242	0.9758
Mean	0.946	0.810	---	---
All loci	---	---	0.182×10^{-12}	≈1.00

[z]All values were rounded to three significant digits after computation.
[y]Gene diversity or polymorphic information content.
[x]Discrimination power is one minus the probability of a matching fingerprint.

tetraploid cherry accessions at the PGRU also be fingerprinted to provide a molecular profile to verify the identities of individual accessions. With the fingerprinting data made available on the World Wide Web it should be possible for curators and scientists working with cherry germplasm to verify identities and novel types in their collections, and contribute to a common global database of tetraploid cherry germplasm.

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