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 L.), 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.

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 alleles. 

Table 1. Tetraploid cherry germplasm accessions assayed for SSR polymorphisms.\(^3\)

| 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     |
| Ujfeherti 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     |
| Droppia                         | 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     | B1 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 Nagygymoelus              | 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       |
| Kellneris 14                    | PI 592877     | Rheinische Schattenmorelle       | PI 592846     |
| Korai Pipacs Meggy              | PI 592875     | Sarandi                          | PI 592882     |
| Lebedjanskaja op 26e 2 (51)     | GPRU 77       | Studencheskaja o.p. IV-6-15      | PI 592872     |
| Lubskaya                        | PI 592881     | Sumadinka                        | PI 592871     |
| Maliga Emleke                   | PI 592862     | Surefire                         | PI 592840     |
| M209                            | GPRU 70       | Tschemorkorka                    | PI 592869     |
| Mari Timpurii                   | GPRU 78       | Ukr. Griotte                     | PI 592865     |

\(^3\)PI and GPRU numbers are those of the USDA/ARS Plant Genetic Resources Unit, Cornell University, Geneva, N.Y.
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 amplified 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 propagules 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).
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 result from homozygous genomes A100 A 100 B100 B104, 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

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

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

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

| Locus            | Heterozygosity | PIC\(^a\) | Probability of matching fingerprints | Discrimination power\(^b\) |
|------------------|----------------|-----------|-------------------------------------|--------------------------|
| 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                    |

\(^a\)All values were rounded to three significant digits after computation. 
\(^b\)Gene diversity or polymorphic information content.

\(^c\)Discrimination power is one minus the probability of a matching fingerprint.

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