Allozyme Inheritance in Tetraploid Sour Cherry (Prunus cerasus L.)

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Abstract. Inheritance for seven enzyme loci was determined in seeds produced from crosses and self-pollinations involving four sour cherry parents and one open-pollinated ground cherry (P. fruticosa Pall.) parent. Segregation data were used to identify allozymes and determine whether sour cherry is a naturally occurring allo- or autotetraploid. Three allozymes were identified at the 6-Pgd-1 locus, and two were identified at each of the following loci: Pgi-2, Lap-1, Adh-1, Idh-2, Pgm-2, 6-Pgd-2. Segregating allozyme patterns for the diagnostic loci Idh-2, Pgm-2, 6-Pgd-1, and 6-Pgd-2 fit disomic inheritance models and thus confirmed the allotetraploid hypothesis for sour cherry. Chi-square tests of independence between loci indicated that Pgi-2, Adh-1, Idh-2, 6-Pgd-1, and 6-Pgd-2 were not linked.

Allozymes have not been used previously for genetic analysis or to determine polyploid type in sour cherry. Isozyme studies in sour cherry to date have been limited to zymograms, patterns, or descriptions of putative alleles from different cultivars. Fernqvist and Huntrieser (1988) presented 6-PGD zymograms for wild and cultivated sweet cherry and three sour cherry cultivars. Kaurisch et al. (1988, 1991) found polymorphisms at nine putative enzyme loci, which uniquely identified members of some cherry species. Santi and Lemoine (1990) compared isozyme polymorphisms in 33 sour cherry cultivars to those in sweet cherry. None of these studies used progeny testing in sour cherry to diagnose true alleles. Once genetic models are established, phenotypic segregation of allozyme patterns can be used to determine the mode of inheritance in tetraploid sour cherry. The objectives of this research were to identify alleles of enzyme loci and to use segregation patterns at these loci in diagnostic crosses to determine polyploid type in sour cherry.

Materials and Methods

Seeds for this study were obtained from crosses involving four sour cherry parents and one open-pollinated ground cherry parent (Table 1). Standard self- and cross-pollination techniques for cherry were used to produce the seeds (Fogle, 1975). Starch gel electrophoresis was performed on extracts from young leaves and dormant vegetative buds of parent trees. Isozyme data on progeny were obtained from individual seeds before germination. Leaves, buds, and seeds were stored at ≈ 2°C with moist paper towels in sealed plastic bags to prevent desiccation until they were used. Seeds were removed from their exocarps and mesocarps and treated with a fungicide suspension before storage. Endocarps were removed just before extraction. All material was macerated on ice the day of electrophoresis using the procedures of Krebs and Hancock (1989) with slight modifications. The extraction buffer was maintained at pH 7.5 rather than adjusted to

Table 1. Origin of cultivars and selections used in the inheritance study of sour cherry.

| Cultivar/selection | Origin                     |
|--------------------|----------------------------|
| Montmorency        | Local selection from France|
| Meteor             | Montmorency × Vladimir     |
| I 24(41)           | P. fruticosa open-pollinated|
| II 13(36)          | Cigany Meggy open-pollinated|
| II 15(4)           | Montmorency × M63 (Pandy × Nagy Gobet) |
pH 8.0, and nylon screens were not used during extraction.

Phosphoglucomutase (PGI, E.C.5.3.1.9), alcohol dehydrogenase (ADH, E.C. 1.1.1.1), isocitrate dehydrogenase (IDH, E.C. 1.1.1.42), phosphoglucose isomerase (PGM, E.C.5.4.2.2), and 6-phosphogluconate dehydrogenase (6-PGD, E.C.1.1.1.44) were resolved by 6 h of electrical current on morpholine-citrate pH 6.1 gels (Clayton and Tretiak, 1972). Leucine aminopeptidase (LAP, E.C.3.4.11.1) was resolved by 5 h of current on tris-citrate/lithium-borate pH 8.3 gels (Scandalios, 1969). All gels consisted of 12% hydrolyzed potato starch. Each gel slice was stained with 50 ml of stain solution. IDH was assayed as described by Soltis et al. (1983). All other enzymes were assayed with slight modification as reported by Arulsekar and Parfitt (1986). The LAP substrate, leucine-naphthyl amide HCl, was dissolved in 2.5 ml of N,N-dimethyl formamide per gel slice before it was added to the stain solution. Tris-HCl buffer pH 8.5 rather than pH 8.0 was used for 6-PGD.

An allele was designated by its allozyme’s mobility relative to the most anodal, frequently occurring allozyme. This most anodal allozyme represents the 100 allele of each enzyme system. Loci of the same enzyme system were numbered progressively, beginning with locus 1 at the most anodal position. Letters representing alleles in Table 2 are used for convenience only and are not the assigned allelic names.

The chi-square goodness-of-fit test compared observed progeny phenotypes to expected classes for each inheritance mode (Table 2). Phenotype, homozygous or heterozygous, rather than genotype, was scored since it does not require a subjective determination of gene dosages based on visual assessment of differential band staining intensity among heterozygotes. For those loci that did not fit the expected 3:1 ratio, a 2:1 ratio was tested. Linkage was studied using the chi-square test of independence.

Results and Discussion

Seven loci resolved well and exhibited good activity using starch gel electrophoresis: Pgi-2, Lap-1, Adh-1, Idh-2, Pgm-2, 6-Pgd-1, and 6-Pgd-2 (Fig. 1). Activity was observed slightly anodal
to \textit{Idh-2} and \textit{Pgm-2}. Kaurisch et al. (1988) had designated these bands \textit{Idh-1} and \textit{Pgm-1}. We did not score these anodal bands because of inconsistent resolution. Kaurisch et al. (1991) reported activity at two putative \textit{PGI} loci. Our results correspond with their \textit{Pgi-2}; however, we did not detect activity corresponding to their \textit{Pgi-1}.

Segregation data for crosses where the expected phenotypic ratios for disomic and tetrasomic inheritance are the same confirmed two alleles at each of the following loci: \textit{Pgi-2}, \textit{Lap-1}, \textit{Adh-1}, \textit{Idh-2}, and \textit{6-Pgd-2} (Table 3, Fig. 1). One of the crosses for \textit{Pgi-2} segregated in a 1:1 phenotypic ratio, and ‘Meteor’ selfed fit a 3:1 ratio. Segregation for two alleles at each of the \textit{Lap-1} and \textit{Adh-1} loci fit expected 3:1 ratios for ‘Montmorency’ selfed and ‘Meteor’. Three of the \textit{Idh-2} crosses fit the proposed 3:1 or 1:1 models. However, for \textit{Idh-2}, ‘Meteor’ selfed fit a 2:1 alternate ratio. For \textit{6-Pgd-2} and \textit{Pgi-2}, ‘Montmorency’ selfed data fit a 2:1 instead of the expected 3:1 ratio.

The skewed 2:1 ratios for \textit{Pgi-2}, \textit{Idh-2}, and \textit{6-Pgd-2} may have resulted from gametophytic selection. These ratios occurred only when the progeny were produced by selfing ‘Montmorency’ and ‘Meteor’. ‘Montmorency’ and ‘Meteor’ have been shown to be partially self-incompatible (Lansari and Iezzoni, 1990), which is defined as a majority of the pollen grains stopping tube growth prematurely in the style, resembling gametophytic incompatibility. This partial self-incompatibility may have caused the progeny class frequencies to deviate from the expected models.

Polymorphisms and putative alleles presented by Kaurisch et al. (1991) for \textit{6-Pgd-2}, \textit{Idh-2}, \textit{Adh-1}, and \textit{Pgm-2} are similar to our results. However, in our analysis, ‘Meteor’ has five bands for \textit{6-Pgd-1} representing three alleles and their three intralocus heterodimers. Kaurisch et al. (1991) presented only three bands that most likely correspond to our \textit{6-Pgd-1} and \textit{Pgm-1} alleles, and their heterodimer at \textit{194}. Fernqvist and Huntrieser (1988) only presented two bands for ‘Meteor’ at the putative \textit{6-Pgd-1} locus. These discrepancies, along with the difference for \textit{Pgi-2} described previously, could be caused either by variation due to different buffer systems or differences between the clones used. However, unlike the previous studies, we used progeny segregation to diagnose true alleles and reliably determine the allele dosage of the parents at all the loci presented. Segregation data for ‘Meteor’ at the \textit{Pgi-2} and \textit{6-Pgd-1} loci were consistent with two and three segregating alleles, respectively.

Segregation data confirmed two alleles at the \textit{Pgm-2}, \textit{6-Pgd-2}, \textit{Idh-2}, and \textit{Adh-1} loci. For eight crosses involving these four loci, the disomic inheritance model tested predicted only heterozygous progeny due to homozygosity of the ancestral genomes in one or both parents of each cross. In Table 2, these parental genotypes are represented as \textit{aabb} and \textit{ccdd}. \textit{Pgm-2} crosses fit the proposed two-allele disomic inheritance model with fixed heterozygosity. None of the crosses tested segregated for \textit{Pgm-2}. However, all of the 26 sweet cherry cultivars studied had only the \textit{Pgm-2} allele, and three of four ground cherry clones studied had only the \textit{Pgm-2} allele (data not presented). Therefore, the two bands of \textit{Pgm-2} in ‘Montmorency’ and ‘Meteor’ were considered allelic with both cultivars exhibiting fixed heterozygosity. Polymorphisms and putative alleles presented by Kaurisch et al. (1991) for \textit{Pgm-2} are similar to our results.

The \textit{6-Pgd-2} locus in ‘Meteor’ selfed and in I 24(41) × II 13(36) exhibited fixed heterozygosity because of disomic inheritance. The final two crosses involving ‘Meteor’ were again potentially diagnostic of disomic inheritance if the low number of homozy-
gotes are considered to be the result of pairing between chromosomes of different ancestral genomes. Tetrasomic inheritance was rejected for all 6-Pgd-2 crosses. Tetrasomic inheritance was rejected at Idh-2 for the cross I 24(41) × II 13(36). Goodness-of-fit could not be tested for disomic inheritance because of the observation of two unexpected homozygotes resulting in an undefined chi-square equation. The two homozygotes may be the result of pollen contamination or possibly pairing between chromosomes of different ancestral genomes in II 13(36). Without these two homozygotes, the data fit the proposed model for fixed heterozygosity in II 13(36).

For Adh-1, insufficient progeny were available to reject either mode of inheritance for the crosses I 24(41) × II 13(36) and ‘Meteor’ × 124(41). However, progeny from the cross 124(41) × II 13(36) were all heterozygous at Adh-1, because II 13(36) exhibited fixed heterozygosity at this locus.

Linkage between loci was tested using five segregating loci in selfed ‘Montmorency’ progeny. Lap-1 and Pgm-2 were not tested for linkage, because they were assayed from different seeds than the other loci and because Pgm-2 was fixed heterozygous in ‘Montmorency.’ None of the loci examined were linked, indicating that they may be located on different chromosomes or chromosome arms in the sour cherry genome.

A maximum of four chromosomes have been demonstrated to undergo disomic inheritance, indicating that sour cherry is an allotetraploid. However, sour cherry still may be a segmental allotetraploid where pairing relationships do not fit those predicted with strict allopolyploidy. For example, occasional pairing between chromosomes of different ancestral genomes could account for the low level of homozygous offspring for Idh-2 from the cross I 24(41) × II 13(36) and for 6-Pgd-2 from the crosses Meteor × I 24(41) and Meteor × II 15(4).

Fixed heterozygosity is a characteristic of allotetraploids when the two ancestral genomes are homozygous. When either one or both of the ancestral genomes are heterozygous (i.e., aabc or abab), the offspring segregate, with homozygous progeny produced in higher frequencies than in autopolyploids. In most of the parental selections, one or both of the ancestral genomes were heterozygous for all the enzymes investigated except Pgm-2. Presumably, sour cherry could have resulted from sexual polyploidization via an unreduced gamete from sweet cherry. Sweet cherry is an obligate outcrosser with a gametophytic self-incompatibility system; therefore, it is likely that sweet cherry is a heterozygous species and would contribute this heterozygosity to sour cherry.

### Literature Cited

Arulsekar, S. and D.E. Parfitt. 1986. Isozyme analysis procedures for stone fruits, almond, grape, walnut, pistachio, and fig. HortScience 21:928-933.

Clayton, J.W. and D.N. Tretiak. 1972. Amine-citrate buffers for pH control in starch gel electrophoresis. J. Fisheries Res. Board Can. 29:1169-1172.

Fernqvist, I. and I. Huntrisier. 1988. Use of isozyme analyses for the identification of fruit cultivars and genotypes, p. 75-82. In: Verksamhetsberattelse 1986-1987 report. Balsgard, Alnarp, Kristianstad, Sweden.

Fogle, H.W. 1975. Cherries, p. 348-366. In: J. Janick and J.N. Moore (eds.). Advances in fruit breeding. Purdue Univ. Press, West Lafayette, Ind.

Galletta, G.J. 1959. Comparative breeding, morphological and cytological studies of polyploid sour cherries (Prunus avium L.). PhD Diss., Univ. of California, Davis.

Hedrick, U.P. 1915. The cherries of New York. J.B. Lyon, Albany, N.Y.

Hruby, K. 1939. The cytology of the Duke cherries and their derivatives. J. Genet. 38:125-131.

Kaurisch, P., W. Gruppe, and W. Kohler. 1988. Enzympolymorphismen bei Kirschen (Prunus spp.) und Arbybriden (P. × spp.). Angewandte...
Botanik 62:41-52.
Kaurisch, P., M. Throii, W. Gruppe, and W. Kohler. 1991. Enzympolymorphismen bei su B-(Prunus avium) und Sauerkirschen (P. cerasus): Kultursortendiffenzierung. Angewandte Botanik 65:35-43.
Krebs, S.L. and J.F. Hancock. 1989. Tetrasomic inheritance of isoenzyme markers in the highbush blueberry, Vaccinium corymbosum L. Heredity 63:11-18.
Lansari, A. and A. Iezzoni. 1990. A preliminary analysis of self-incompatibility in sour cherry. HortScience 25:1636-1638.
Olden, E.J. and N. Nybom. 1968. On the origin of Prunus cerasus L. Hereditas 59:327-345.
Raptopoulus, T. 1941. Chromosomes and fertility of cherries and their hybrids. J. Genet. 42:91-113.
Santi, F. and M. Lemoine. 1990. Genetic markers for Prunus avium L. 2. Clonal identifications and discrimination from P. cerasus and P. cerasus × P. avium. Ann. Sci. For. 47:219-227.
Scandalios, J.G. 1969. Genetic control of multiple molecular forms of enzymes in plants: A review. Biochem. Genet. 3:37-79.
Soltis, D.E., C.H. Haufler, D.C. Darrow, and G.J. Gastony. 1983. Starch gel electrophoresis of ferns: A compilation of grinding buffers, gel and electrode buffers, and staining schedules. Amer. Fern J. 73:9-27.
Soltis, D.E. and L.H. Rieseberg. 1986. Autopolyploidy in Tolmiea menziesii (Saxifragaceae): Genetic insights from enzyme electrophoresis. Amer. J. Bot. 73:310-318.
Stebbins, G.L. 1947. Types of polyploids: Their classification and significance. Adv. Genet. 1:403-429.