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Isozyme diversity in sour, sweet, and ground cherry

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Abstract Thirty-six sour (*Prunus cerasus* L.), sweet (*P. avium* L.), and ground cherry (*P. fruticosa* Pall.) selections were evaluated for seven enzyme systems and principal coordinate analysis was used to examine isozyme divergence among these cherry species. The enzyme systems studied were phosphoglucose isomerase (PGI), isocitrate dehydrogenase (IDH), phosphoglucomutase (PGM), 6-phosphogluconate dehydrogenase (6-PGD), leucine aminopeptidase (LAP), shikimate dehydrogenase (SKDH), and malate dehydrogenase (MDH). The first principal coordinate, which accounted for 41% of the total variation, separated the diploid sweet cherry selections from the sour, ground, and sour × ground cherry tetraploids. An additional 86 selections were evaluated for up to six of the enzyme systems to determine the polymorphisms at the enzyme loci and the level of heterozygosity between the diploid sweet cherry and the tetraploid species and interspecific hybrids. 6-PGD was the most polymorphic enzyme exhibiting 16 patterns. The tetraploid cherry species were more heterozygous than the diploid sweet cherry with an average heterozygosity of 78% compared to 19% for the diploids.

Key words Cherry · *Prunus* · Isozyme · Polyploid

Introduction

Sour cherry (*Prunus cerasus* L., 2n = 4x = 32) is an allopolyploid based on shared morphological, isozyme, and chloroplast restriction fragment length polymorphism homology with its proposed progenitor species, sweet cherry (*P. avium* L., 2n = 2x = 16) and ground cherry (*P. fruticosa* Pall., 2n = 4x = 32) (Olden and Nybom 1968; Hancock and Iezzoni 1988; Hillig and Iezzoni 1988; Iezzoni et al. 1989; Iezzoni and Hancock 1994). Additionally, segregation at four isozyme loci in sour cherry fits disomic inheritance models and rejecting tetrasomic inheritance (Beaver and Iezzoni 1993). Disomic inheritance is characteristic of allopolyploids due to non-random pairing between chromosomes from the same ancestral genome (Stebbins 1977).

Sour cherry exists in maximum diversity in Europe. Its range, extending from the Mediterranean islands off Greece and Turkey to northern Russia (Hedrick 1915), spans that of its two proposed progenitor species, sweet and ground cherry (Kolesnikova 1975). Sweet cherry, the less cold-hardy cherry species, grows wild throughout the temperate regions of mainland Europe and into southern Russia; however, the greatest concentration is between and south of the Caspian and Black Seas. Ground cherry, the more cold-hardy cherry species, exists in greatest diversity in western Russia.

The expanded ecological range of many allopolyploids compared to their diploid progenitors is usually attributed to increased heterozygosity from divergent alleles on non-pairing homologous chromosomes from their progenitor species (Stebbins 1950; Hancock and Bringhamst 1981). This heterozygosity may be expressed as multiple enzymes with distinct properties which could theoretically expand the range of environments over which normal development could take place. Allopolyploid species might then have the potential to undergo substantial differentiation in response to differing selection pressures through occasional heterogenetic associations (Stebbins 1950).

Based on morphological and cold-hardiness differences, Russian investigators have divided sour cherry cultivars into two ecotypes: western European and middle-Russian (Kolesnikova 1975; Yuskev 1975, 1977). The western European group is less winter-hardy than the middle-Russian group and morphologically re-
sembles sweet cherry, while the middle Russian group is more winter-hardy and morphologically resembles ground cherry. The morphological variation found in sour cherry is most likely due to continued gene flow between sour cherry and both sweet and ground cherry (Yushev 1975; Hillig and Iezzoni 1988). Spontaneous hybrids between sour cherry and its progenitor species do occur and cold temperature may be the most important selection force.

The Michigan State University (MSU) sour cherry germplasm collection, which includes material collected from throughout the habitat range of sour cherry, exhibits morphological diversity similar to that reported by the Russian researchers (Kolesnikova 1975; Yushev 1975, 1977). The diversity for fruit and plant type in the MSU sour cherry germplasm collection suggested that the individuals were highly variable genetically (Hillig and Iezzoni 1988; Krahl et al. 1991). Therefore an isozyme study was initiated to further characterize the variation present in the sour cherry collection along with a limited collection of sweet and ground cherry. Isozymes have commonly been used for detailed studies of the genetic structure of wild populations and landraces due to their co-dominant expression and the prevalence of polymorphisms. In sweet and sour cherry, isozyme loci have been reported for nine and eight enzyme systems, respectively (Kaurisch et al. 1988, 1991; Santi and Lemoine 1990a, b; Tobutt and Nicoll 1992; Beaver and Iezzoni 1993; Granger et al. 1993).

Our objectives were to describe the isozyme diversity within and among sweet, sour, and ground cherry cultivars and their hybrids, and to compare the level of heterozygosity between the diploid sweet cherry and the tetraploid species and interspecific hybrids.

**Materials and methods**

**Plant material**

Sixty-seven sour, 26 sweet, six ground cherry cultivars and numbered selections, and seven sour x ground cherry and five sour x sweet cherry hybrids were evaluated for up to six isozyme systems. All plant materials are from the MSU collection growing at either the Clarks-ville Horticultural Experiment Station, Clarksville, Mich., or the Horticultural Research Center, East Lansing, Mich. The plant material in the MSU germplasm collection was collected, beginning in 1993, in Serbia, Bulgaria, Hungary, Poland, Germany, Sweden, and Russia.

**Isozyme procedures**

Starch-gel electrophoresis was performed on extracts from young leaves and dormant vegetative buds. The enzyme systems studied were phosphoglucone isomerase (PGI, E.C.5.3.1.9), isocitrate dehydrogenase (IDH, E.C.1.1.1.42), phosphoglucomutase (PGM, E.C.5.4.2.2), 6-phosphogluconate dehydrogenase (6-PGD, E.C.1.1.1.44), leucine aminopeptidase (LAP, E.C.3.4.11.1), shikimate dehydrogenase (SKDH, E.C.1.1.1.25), and malate dehydrogenase (MDH, E.C.1.1.1.37). Electrophoresis and staining procedures for the first five enzymes were the same as described in Beaver and Iezzoni (1993). SKDH and MDH were resolved on morpholine citrate 6.1 gels (Clayton and Tretiak 1972) and assayed according to Arulsekar and Parfitt (1986) and Vallejos (1983), respectively.

Isozyme alleles were named based on their mobilities relative to the most anodal band which was designated as the 100 allele. However, a few cherry genotype exhibited rare isozymes anodal to 100 for PGI (110 and 105) and SKDH (120). Loci of an enzyme system were numbered progressively beginning with 1 in the most anodal position. The sour cherry cultivars, ‘Montmorency’ or ‘Meteor’, were used as controls to aid in band identification as their bands had previously been diagnosed as allelic or heteromorphic for Pgm-2, 6-Pgd-1, 6-Pgd-2, Idh-2, and Lap-1 (Beaver and Iezzoni 1993). Both cultivars possess the 100 allozyme for all enzyme systems except SKDH.

**Analytical procedures**

Thirty-six selections were analyzed for all enzyme systems (Table 1). These 36 sour, sweet, and ground cherries were compared by principal coordinate (PCO) analysis (Gower 1966; Digby and Kempton 1987). A matrix of 36 genotypes x 44 isozyme bands was constructed with a '0' or a '1' entered for each isozyme band to indicate its absence or presence for every genotype analyzed. The isozyme bands utilized are defined in Fig. 1. Data were analyzed by calculating similarity matrices using the similarity statistic of Marczewski and Steinhaus: S = w/(a + b - w), where w = number of bands of common mobility and a and b are the number of bands in individuals A and B, respectively (Angus et al. 1988). The similarity matrices were used in PCO analysis using a program written in SAS/IML (SAS Inst., Cary, N.C.). The data was then plotted in two dimensions.

**Results**

The cherry selections exhibited three and two patterns for Pgm-2 and Idh-2, respectively, resulting from two previously confirmed alleles per locus (Pgm-2$^{100}$ and Pgm-2$^{75}$; Idh-2$^{100}$ and Idh-2$^{64}$) (Beaver and Iezzoni 1993)(Fig. 1). The patterns exhibited by Pgi-2 and Lap-1 were also due to two previously identified alleles per locus (Pgi-2$^{100}$ and Pgi-2$^{82}$; Lap-1$^{100}$ and Lap-1$^{65}$) plus one newly identified putative allele/locus. The novel Pgi-2 band had a mobility of 110 with a heterodimeric band at 105. The newly identified variant for Lap-1 had a mobility of 97.

Sixteen patterns encoded by nine alleles at two loci were identified for 6-PGD. 6-Pgd-1 exhibited three previously reported alleles (6-Pgd-1$^{100}$, 6-Pgd-1$^{98}$, and 6-Pgd-1$^{76}$) and heterodimeric bands with relative mobilities of 94, 82, and 88 (Beaver and Iezzoni 1993). At 6-Pgd-2, two previously identified alleles (6-Pgd-2$^{100}$ and 6-Pgd-2$^{28}$) were found (Beaver and Iezzoni 1993) along with four newly identified putative alleles with relative mobilities of 82, 72, 38, and 28. The middle band of the five-banded patterns for both of the 6-Pgd loci resulted from co-migration between a homodimer from one allele product and an intralocus heterodimer of two allele products. This is more diversity than previously reported by Kaurisch et al. (1988, 1991).

Thirteen patterns encoded by five putative alleles were found for SKDH. It is believed that these putative alleles are at a single SKDH locus since only one SKDH locus has been found in other Prunus species (Mowrey et al. 1990). Santi and Lemoine (1990a) concluded that SKDH in sweet cherry is determined by alleles at one