guishing white-leaved from yellow-leaved seedlings, data for them were combined. The testcross of chlorotic *virescent* plants with F_1 plants produced normal, chlorotic, *virescent*, and chlorotic *virescent* seedlings in equal numbers.

To verify the inheritance of this mutant further and to look at the white-leaved plants, several F_2 plants were self-pollinated or crossed with yellow-leaved plants. The resulting F_3 populations segregated in ratios typical of two independent genes (Table 2). When plants having yellow leaves with green veins were crossed with white-leaved plants, there was no clear evidence of segregation.

From this study, we conclude that the expression of the 'Edisto' chlorotic muskmelon mutant is controlled by a single recessive gene that is not allelic or linked to *virescent*.

Literature Cited

1. Andrus, C.F., P.C. Grimball, and D.M. McLean. 1967. Breeding. Twenty-Eighth Annu. Rpt. of Vegetable Breeding in the Southeastern United States, Hawaii, and Puerto Rico. U.S. Vegetable Laboratory, ARS/USDA, Charleston, S.C. p. 15–16.
2. Brown, J.C., R.L. Chaney, and J.E. Ambler. 1971. A new tomato mutant inefficient in the transport of iron. Physiol. Plant. 25:48–53.
3. Cox, E.L. and K.E. Harding. 1986. Linkage relationships of the *light green* mutant in cantaloupe. HortScience 21:940 (Abstr.)
4. Dyutin, K.E. 1979. Inheritance of yellow-green coloration of the young leaves in melon (In Russian). Tsitologia i genetika 13:407–408. [Cucurbit Genetics Coop. Rpt. 5:62–65; 1982]
5. Guries, R.P. 1978. A test of the mentor pollen technique in the genus *Ipomoea*. Euphytica 27:825–830.
6. Hoagland, D.R. and D.I. Arnon. 1950. The water-culture method for growing plants without soil. Calif. Agr. Expt. Sta. Circ. 347.
7. Hoffman, J.C. and P.E. Nugent. 1973. Inheritance of a *virescent* mutant of muskmelon. J. Hered. 64:311–312.
8. Kabata-Pendias, A. and H. Pendias. 1984. Trace elements in soils and plants. CRC Press, Boca Raton, Fla.
9. McCreight, J.D. and G.W. Bohn. 1979. Descriptions, genetics, and independent assortment of red stem and pale in muskmelon (*Cucumis melo* L.). J. Amer. Soc. Hort. Sci. 104:721–723.
10. Nugent, P.E. 1980. The genetic relationship of *Virescent, Yellow-green, Glabrous*, and *Halo* mutants in muskmelon. HortScience 15:804–805.
11. Nugent, P.E. 1987. C879-J1 and J2 *virescent* mutant muskmelon breeding lines. HortScience 22:333–335.
12. Nugent, P.E. and J.C. Hoffman. 1974. Inheritance of the *halo* cotyledon mutant in muskmelon. J. Hered. 65:315–316.
13. Nugent, P.E., J.C. Hoffman, and C.F. Andrus. 1979. 'Mainstream' muskmelon. HortScience 14:192.
14. Pitrat, M., C. Ferriere, and M. Ricard. 1986. *Flava*, a chlorophyll deficient mutant in muskmelon. Cucurbit Genetics Coop. Rpt. 9:67.
15. Rufner, R. and A.V. Barker. 1984. Ultrastructure of zinc-induced iron deficiency in mesophyll chloroplasts of spinach and tomato. J. Amer. Soc. Hort. Sci. 109:164–168.
16. Sprague, H.B. 1964. Hunger signs in crops. David McKay, New York.
17. Whitaker, T.W. 1952. Genetic and chlorophyll studies of a *yellow-green* mutant in muskmelon. Plant Physiol. 27:262–268.
18. Zink, F.W. 1977. Linkage of *virescent* foliage and plant growth habit in muskmelon. J. Amer. Soc. Hort. Sci. 102:613–615.

HortScience 23(2):381–383. 1988.

Malate Dehydrogenase Isozyme Patterns in Seven *Prunus* Species

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Additional index words. electrophoresis

Abstract. **Cultivars from three cherry species, sour cherry (*Prunus cerasus* L.), sweet cherry (*P. avium* L.), and ground cherry (*P. fruticosa* Pall.), and open-pollinated progenies of *P. mahaleb* L., *P. incisa* Thunb., *P. canescens* Bois., and *P. subhirtella* Miq., and the sour cherry cultivars Cigany Meggy and Pitic de Iasi were characterized electrophoretically for malate dehydrogenase (MDH) in leaf tissue. No intraspecific variability for MDH isozymes was detected within the sour cherry, sweet cherry, or *P. fruticosa* cultivars evaluated. The codominant expression of both the sweet cherry and *P. fruticosa* bands in sour cherry supports the hypothesis that sour cherry arose by interspecific hybridization. A single isozyme band was associated with the mitochondrial subcellular leaf extract from sour cherry. Sweet and sour cherry pollen had activity for a cathodal MDH locus that was not detected in the leaf tissue. Open-pollinated populations of *P. mahaleb* and *P. canescens* each exhibited one banding pattern; however, similar progeny from *P. subhirtella, P. incisa*, and the sour cherry cultivars Pitic de Iasi and Cigany Meggy each segregated for two zymogram patterns. Sufficient polymorphism at the MDH locus has been identified to permit its use as a biochemical genetic marker in interspecific hybridizations.**

The tetraploid sour cherry (2n = 32), is considered to have originated through hybridization of sweet cherry (2n = 16), and the cold tolerant ground cherry (2n = 32), which grows wild in Russia (6). Maximum genetic diversity in sour cherry is found in Eastern Europe, where it coexists with sweet cherry and *P. fruticosa*. The major germplasm collections have been made in those countries where sour cherry diversity is highest. Other cherry species that might be useful for the genetic improvement of sour cherry include the diploid species *P. mahaleb*, of Western Asian and European origin, and *P. incisa, P. canescens*, and *P. subhirtella*, of Japanese and Chinese origins.

Isozyme gene markers have been used widely for the identification of cultivars (12), species (1), and interspecific hybrids (8) and for the measurement of genetic divergence between and within populations for ecological, systemic, and phylogenetic study (3). In a breeding program, isozyme gene markers are advantageous because they can be detected at the seedling stage, permitting early selection of desirable individuals. However, no studies on variability for isozyme loci have been reported in cherry species to the best of our knowledge.

Polymorphism for malate dehydrogenase (MDH) has been studied in numerous crop species; however, its inheritance is complicated by overlapping MDH isozymes, which are compartmentalized in the cytosol, microbodies, and mitochondria (5). In *Prunus*, polymorphism for MDH has been described in peach with the observation of three distinct banding patterns (2). The F_2 seedlings segregated in a 1:2:1 ratio for the banding patterns, which is consistent with codominant alleles at a single locus. However, because segregation was not observed for four of the bands, it was not possible to determine the number of loci involved in the control of MDH isozymes in peach. MDH is reported to be a dimer in corn (11, 13) and celery (7) and a multimer in peach (2). In peach, haploid plants derived from heterozygous parents for the MDH allozymes exhibited only homodimeric banding patterns, suggesting that, in peach, heterodimers are formed between the two enzyme products. We present additional studies of MDH in *Prunus* and

Received for publication 26 Mar. 1987. Michigan State Univ. Agricultural Experiment Station Journal Article no. 12271. We thank Bruce Parliman and Norm Foran of the U.S. Plant Introduction Station for sending plant material and James Hancock, Pat Moore, and Carol Schumann for helpful discussion and suggestions in procedure and analysis. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

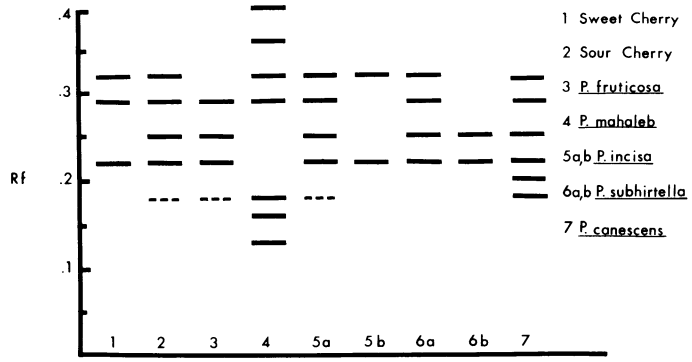

Fig. 1. Observed MDH isozyme patterns for sweet cherry, *P. fruticosa, P. mahaleb, P. incisa, P. subhirtella,* and *P. canescens*.

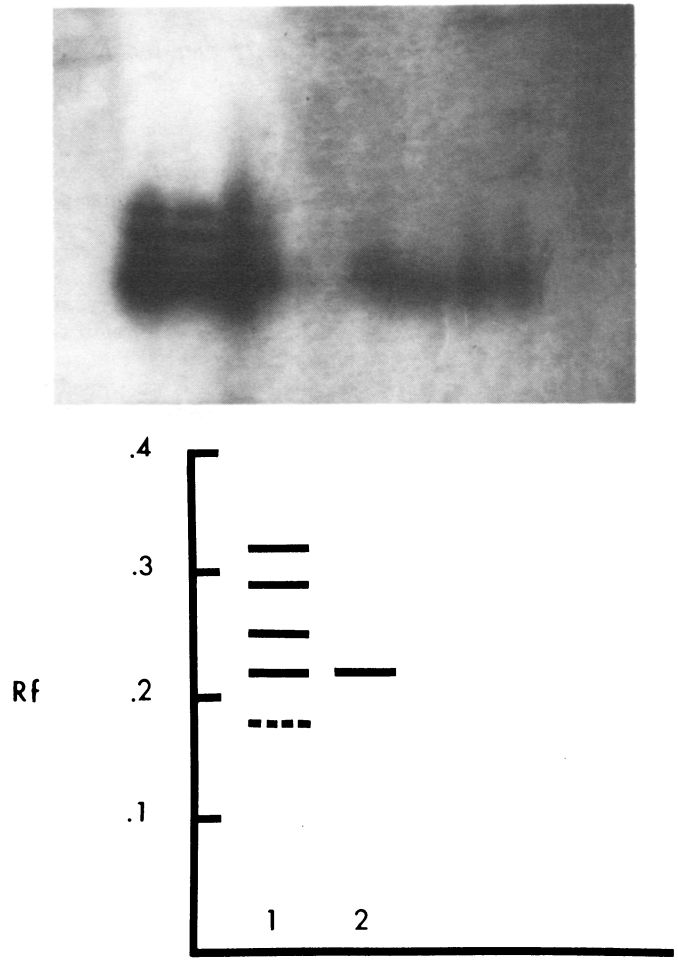

Fig. 2. MDH isozymes in total cellular (lane 1) and mitochondrial (lane 2) fractions from sour cherry.

demonstrate that this enzyme system should be useful for identifying variability in cherry.

Nineteen sour cherry cultivars, six sweet cherry cultivars, and four *P. fruticosa* clones growing at either the Clarksville Horticultural Experiment Station, Clarksville, Mich., or the Horticultural Research Center, East Lansing, Mich., were evaluated. Leaves of the sour cherry cultivars Cigany Meggy and Pitic de Iasi were kindly provided by the U.S. Plant Introduction Station, Glenn Dale, Md. Thirty-four and 22 open-pollinated progeny of 'Cigany Meggy' and 'Pitic de Iasi', respectively, were evaluated along with 20 progeny each of *P. incisa, P. subhirtella, P. canescens*, and *P. mahaleb*. Open-pollinated seeds of 'Cigany Meggy' were collected in Hungary in 1984; those of 'Pitic de Iasi', *P. incisa, P. subhirtella,* and *P. canescens* were collected in Romania in 1985. Seeds of *P. mahaleb* were purchased from Lawyer's Nursery (Plains, Mont.)

Preparation of leaf tissue for electrophoresis was done following the protocol of Weeden and Lamb (12). Pollen samples were prepared for electrophoresis by soaking 50 mg of pollen briefly in 200 µl of extraction buffer in a chilled porcelain depression plate, then grinding with a glass rod (12). Wicks were soaked directly in the homogenate; samples were not centrifuged. Mitochondrial were isolated following the method of Hanson et al. (4). The pellet enriched for mitochondria was gently resuspended in 250 µl of buffer that had been amended by adding several drops of 0.01 percent Triton X-100.

Electrophoresis was run using starch gels, with a histidine gel buffer and a Tris·citrate electrode buffer (8). All gels were run at 30 mA for ≈2½ hr, or until a bromphenol blue marker had traveled 9.5 cm or more. Wicks were removed after the first 30 min of the run. When the run was complete, gels were sliced and stained in a solution consisting of 50 ml of 0.2 M Na-malate, pH 7.0, 50 ml of 0.05 Tris·citrate, pH 8.3, 50 mg nicotinamide adenine dinucleotide (NAD), 20 mg nitroblue tetrazolium (NBT), and 5 mg of phenazine methosulfate (PMS). Gels were incubated in the dark at 32°C until distinct bands formed, then removed from the incubator and fixed in a solution of 1:1 EtOH-H_2O.

All leaf material from sour cherry cultivars, including 'Cigany Meggy' and 'Pitic de Iasi', had an identical banding pattern with the following R_f values: 0.32, 0.29, 0.25, 0.22, and 0.18 (Fig. 1). The R_f 0.18 band always stained at a lighter intensity compared to the other bands. The band at R_f 0.22 from sour cherry leaf extract was associated with the mitochondrial fraction (Fig. 2). All the sweet cherry cultivars had a banding pattern that consistently differed from the sour cherry pattern by the absence of the band at R_f 0.25 and 0.18 (Fig. 1). Also, all *P. fruticosa* selections had identical banding patterns that lacked the R_f 0.32 band found in sour cherry. The codominant expression of the sweet cherry and *P. fruticosa* bands in sour cherry supports the hypothesis that sour cherry arose from the hybridization between sweet cherry and *P. fruticosa*. Although *P. incisa* shows the same banding pattern as *P. cerasus*, taxonomic differences have resulted in its placement in a different botanical section from sweet and sour cherry and *P. fruticosa* (10).

If dimeric isozymes are expressed in sporophytic tissue and postmeiotically in pollen, a heterozygous sporophyte would exhibit two homodimeric bands and an intermediate heterodimeric band in the diploid sporophytic phase and just two homodimeric bands in the pollen grains. When pollen of two sour cherry cultivars and four sweet cherry cultivars was stained for MDH, nine and seven bands were present, respectively (Fig. 3). The five and three fast bands had R_f values identical to those present in sporophytic tissue. Either the sweet cherry cultivars are homozygous for the MDH isozyme or the isozyme is expressed premeiotically. Because sour cherry is a tetraploid with 2× pollen, the possibilities of heterozygosity for the MDH isozyme and postmeiotic expression cannot be ruled out. Four additional pollen bands, which were present below the five and three sporophytic bands in sour and sweet cherry, respectively, indicate that at least one additional MDH locus is expressed in sour and sweet cherry pollen. In *Populus nigra*, pollen showed activity for a cathodal MDH locus that was not detected in the sporophyte (9).

The open-pollinated populations of *P. mahaleb* and *P. canescens* showed only one

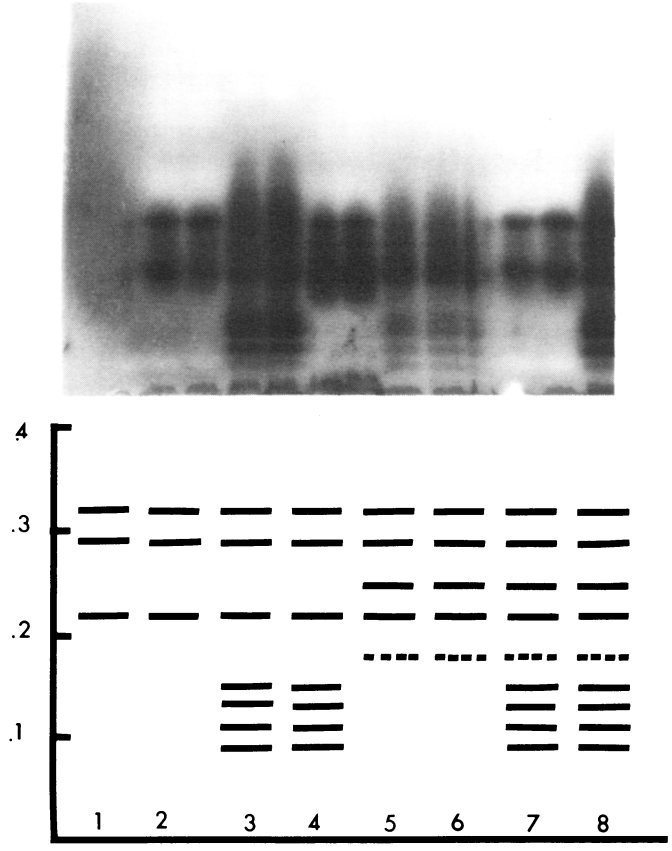

Fig. 3. MDH isozymes in sweet cherry leaves (lanes 1 and 2), sweet cherry pollen (lanes 3 and 4), sour cherry leaves (lanes 5 and 6), and sour cherry pollen (lanes 7 and 8).

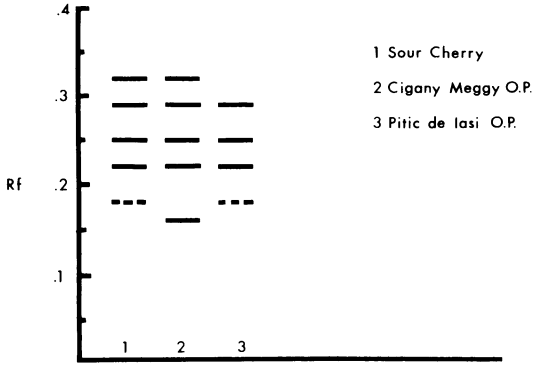

Fig. 4. Zymogram of MDH isozymes from open-pollinated progeny of the sour cherry cultivars Cigany Meggy and Pitic de Iasi.

banding pattern, with seven and six bands, respectively (Fig. 1). However, open-pollinated progeny from *P. subhirtella* and *P. incisa* each segregated for two zymogram patterns. For the 20 seedlings of *P. incisa*, five exhibited zymogram "b" and 15 exhibited zymogram "a" (Fig. 1). For the 20 seedlings of *P. subhirtella*, eight exhibited zymogram "b" and 12 exhibited zymogram "a" (Fig. 1).

Two out of 34 open-pollinated seedlings of 'Cigany Meggy' had a distinct lower band at R_f 0.16 (Fig. 4). Principal component analysis of botanical characteristics from 20 'Cigany Meggy' progeny in which one of the two individuals with the lower band was included, placed this individual at the center of the plot (K. Hillig, personal communication). Although component analysis does not rule out the possibility that this individual resulted from an outcross, it does suggest that if an outcross did occur, it was not with a different species. Therefore, the MDH isozyme variability observed in the 'Cigany Meggy' progeny represents potentially useful intraspecific polymorphism.

Four of the 22 open-pollinated seedlings of 'Pitic de Iasi' had the MDH banding pattern of *P. fruticosa*, which lacks the band at R_f 0.32 (Fig. 4, lane 3), whereas the remaining 18 of the progeny showed the banding pattern of sour cherry (Fig. 4, lane 1). Open-pollinated seeds of 'Pitic de Iasi' were collected in the Moldavia region of Romania along the Russian border, where winter temperatures frequently reached −32°C. 'Pitic de Iasi' originated here as an open-pollinated seedling from 'Plodorodnaya', a dwarf, cold-hardy, late-blooming Russian *P. fruticosa* cultivar. The paternal parent of 'Pitic de Iasi' was most likely *P. cerasus*. It is not surprising that 'Pitic de Iasi', one of the most productive cultivars in Moldavia, is an interspecific hybrid combining the superior fruit quality of sour cherry with the cold hardiness of *P. fruticosa*.

The present results do not permit conclusions concerning the inheritance of the MDH isozymes in cherry. However, these data do indicate the pertinent crosses segregating for MDH isozymes in cherry that will need to be made; tissue specificity and the subcellular location also must be determined. Unfortunately, because much of the plant material analyzed in this study was collected recently as seed, it will be several years before flowering occurs. However, sufficient polymorphism at the MDH locus has been identified to permit use as biochemical genetic markers in future intra- and interspecific hybridizations.

Literature Cited

1. Arulsekar, S., D.E. Parfitt, and G.H. McGranahan. 1985. Isozyme markers in *Juglans* ssp. Inheritance of glucose phosphate isomerase and aspartate amino transferase in *J. regia* and *J. hindsii*. J. Hered. 71:103–106.
2. Arulsekar, S., D.E. Parfitt, V. Beres, and P.E. Hansche. 1986. Genetics of malate dehydrogenase isozymes in the peach. J. Hered. 77:49–51.
3. Crawford, D.J. 1983. Phylogenetic and systemic inferences from electrophoretic studies, p. 257–288. In: S.D. Tanksley and T.J. Orton (eds.). Isozymes in plant genetics and breeding. Part A. Elsevier, Amsterdam.
4. Hanson, M.R., M.L. Baeshore, P.E. McClean, M.A. O'Connell, and H.T. Nivision. 1986. Mitochondria and mitochondrial DNA isolation. Methods Enzymol. 118:437–453.
5. Newton, K.J. 1983. Genetics of mitochondrial isozymes, p. 157–174. In: S.D. Tanksley and T.J. Orton (eds.). Isozymes in plant genetics and breeding. Part A. Elsevier, Amsterdam.
6. Olden, E.J. and N. Nybom. 1968. On the origin of *Prunus cerasus* L. Hereditas 59:327–345.
7. Orton, T.J. 1983. Celery and celeriac (*Apium graveolens* L.), p. 351–367. In: S.D. Tanksley and T.J. Orton (eds.). Isozymes in plant genetics and breeding. Part B. Elsevier, Amsterdam.
8. Parfitt, D.E., S. Arulsekar, and D.W. Ramming. 1985. Identification of plum x peach hybrids by isoenzyme analysis. HortScience 20:246–248.
9. Rajora, O.P. and L. Zsuffa. 1986. Sporophytic and gametophytic gene expression in *Populus deltoides* Marsh., *P. nigra* L., and *P. maximowiczii* Henry. Can. J. Genet. Cytol. 28:476–482.
10. Rehder, A. 1974. Manual of cultivated trees and shrubs hardy in North America. MacMillan, New York.
11. Sari Gorla, M., C. Frova, G. Binelli, and E. Ottaviano. 1986. The extent of gametophytic-sporophytic gene expression in maize. Theor. Applied Genet. 72:42–47.
12. Weeden, N.F. and R.C. Lamb. 1985. Identification of apple cultivars by isozyme phenotypes. J. Amer. Soc. Hort. Sci. 110:509–515.
13. Yang, N.S., J.C. Sorenson, and J.G. Scandalios. 1977. Genetic control of mitochondrial malate dehydrogenes: Evidence for duplicated chromosome segments. Proc. Natl. Acad. Sci USA 74:310–314.