Identification of *Vitis vinifera* L. x *Muscadinia rotundifolia* Small Hybrids by Starch Gel Electrophoresis

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*Abstract.* Four isozyme systems, glucosephosphate isomerase (PGI, EC 5.3.1.9), phosphoglucomutase (PGM, EC 2.7.5.1), isocitrate dehydrogenase (IDH, EC 1.1.1.42), and malate dehydrogenase (MDH, EC 1.1.1.37), were identified as useful in detecting intergeneric *Vitis vinifera* x *Muscadinia rotundifolia* hybrids. Polymorphism between and within the two genera was observed at the PGI-2, PGM-2, and IDH-1 loci. However, the two appeared fixed for different alleles at the MDH-3 locus. The combination of any two of the enzyme systems allowed for rapid identification of F1 hybrids at the young, pre-fruiting seedling stage.

Electrophoresis has been used by plant breeders to study polymorphisms in natural populations (6, 11), determine outcrossing rates (5), and for development of chromosome linkage maps (15). Isozymes are also excellent markers for identifying interspecific hybrids (3, 10). Early detection of such hybrids at the seedling stage makes it possible to discard non-hybrids, better using manpower and greenhouse space.

Muscadine grapes (*Muscadinia rotundifolia*) are of interest to *V. vinifera* breeders as a potential source of resistance to insects and diseases, such as powdery mildew [Uncinula necator (Schw.) Burr.] and Pierce’s disease. Most crosses have been designed to transfer those traits from *M. rotundifolia* to *V. vinifera* (9). However, there are traits within bunch grapes germlasm that would be desirable to transfer to muscadines, to develop high-quality muscadine-type table and wine cultivars (7).

Plants used in this study were juvenile greenhouse-grown seedling populations of the *V. vinifera* x *M. rotundifolia* crosses: ‘Royalty’ x ‘Tarheel’, ‘Royalty’ x ‘Noble’, ‘Cabernet Sauvignon’ x ‘Tarheel’, ‘Cabernet Sauvignon’ x ‘Noble’, B46-76 x ‘Carlos’, and P79–101 x ‘Carlos’. *Vitis vinifera* females were grown and crossed were made in Fresno, Calif. in 1986 and seedlings grown in Raleigh, N.C.

Preliminary electrophoretic analyses were performed using leaf samples obtained from parents and randomly selected offspring. Samples were prepared by blending 300 mg of leaf tissue with 56 ml 0.05 M Tris-HCl buffer and 200 mg insoluble polyvinylpolypyrrolidone (1).

Gels were prepared using 29.8 g Connaught starch, 15 g Electro starch, 15 g sucrose, and 330 ml gel buffer. Gels were run using histidine-citrate (pH 6.5) gel and electrode buffers at a constant 13 W of power for 4 hr (13). Enzyme staining systems were surveyed using standard or slightly modified techniques (1, 2, 4) and included: alcohol dehydrogenase (ADH, EC 1.1.1.1), aldolase (ALD, EC 4.1.2.13), diaphorase (DIA, EC 1.6.4.3), menadione reductase (MNR, EC 1.6.99.2), glucose-6-phosphate dehydrogenase (G6PD, EC 1.1.1.49), glutamate oxaloacetate transaminase (GOT, EC 2.6.1.1), glutamate dehydrogenase (GDH, EC 1.4.1.2), isocitrate dehydrogenase (IDH), leucine aminopeptidase (LAP, EC 3.4.11.1) malate dehydrogenase (MDH), malic enzyme (ME, EC 1.1.1.40), peroxidase (PER, EC 1.11.1.7), 6-phosphogluconate dehydrogenase (PGD, EC 1.1.1.43), glucosephosphate isomerase (PGI), phosphoglucomutase (PGM), and shikimate dehydrogenase (SDH, EC 1.1.1.25). Of these MDH, IDH, PGM, and PGI were selected since polymorphism was found between the species and resolution was good. MNR was polymorphic in both species, but could not be used in hybrid identification due to the complex banding patterns. Polymorphism also appeared to be present for LAP, GOT, and DIA, but resolution was poor. These results agree with those previously reported by Loucas et al. (8), who found polymorphism in *V. vinifera* for PGI, PGM, and IDH.

Staining results for the selected enzymes indicated two loci for IDH, PGM, and PGI, and possibly four loci for MDH (Fig. 1). Enzyme phenotypes indicated that IDH, PGI, and MDH are dimeric enzymes and PGM is monomeric, as has been reported in corn (13). In IDH there was polymorphism in the faster-migrating zone (IDH-1), with the *V. vinifera* parents ‘Cabernet Sauvignon’ and B46-76 having faster-migrating alleles than the *M. rotundifolia* parents ‘Tarheel’, ‘Noble’, and ‘Carlos’. Observed alleles were given letter designations, with A indicating the slowest allele and increasing migration indicated by succeeding letters (Table 1). ‘Tarheel’ was designated AB, ‘Noble’ and ‘Carlos’ BB, ‘Cabernet Sauvignon’ CC, and B46–76 CD. Phenotypes of the F1 progeny varied according to alleles carried by the parents and consisted of two homodimeric bands and an intralocus heterodimer band (Figs. 2 and 3). The IDH-2 zone (slow) stained inconsistently, but, when present, no apparent polymorphism was observed.

No polymorphism was observed between

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Table 1. Isocitrate dehydrogenase (IDH), phosphoglucomutase (PGM), malate dehydrogenase (MDH), and glucosephosphate isomerase (PGI) isozyme genotypes of four *V. vinifera* and three *M. rotundifolia* cultivars.

<table>
<thead>
<tr>
<th>Parents</th>
<th>IDH-1</th>
<th>PGM-2</th>
<th>MDH-3</th>
<th>PGI-2</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>V. vinifera</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Royalty</td>
<td>---</td>
<td>DD</td>
<td>BB</td>
<td>BC</td>
</tr>
<tr>
<td>Cabernet Sauvignon</td>
<td>CC</td>
<td>CD</td>
<td>BB</td>
<td>BC</td>
</tr>
<tr>
<td>B46-76</td>
<td>CD</td>
<td>AA</td>
<td>BB</td>
<td>DD</td>
</tr>
<tr>
<td>P79-101</td>
<td>---</td>
<td>AA</td>
<td>BB</td>
<td>CD</td>
</tr>
<tr>
<td><em>M. rotundifolia</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Noble</td>
<td>BB</td>
<td>BB</td>
<td>AA</td>
<td>AA</td>
</tr>
<tr>
<td>Tarheel</td>
<td>AB</td>
<td>BB</td>
<td>AA</td>
<td>AA</td>
</tr>
<tr>
<td>Carlos</td>
<td>BB</td>
<td>BB</td>
<td>AA</td>
<td>AB</td>
</tr>
</tbody>
</table>

*Observed alleles in each isozyme stain were given letter designations, with A indicating the slowest allele and increasing migration indicated by succeeding letters.*

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**Fig. 2.** IDH zymogram of progeny from 'Cabernet Sauvignon' x 'Tarheel'. Lanes C-E, H-J, K, M, O, and P-T are F₁ hybrid seedlings. Lanes A and V are 'Tarheel', lane B is 'Noble', and lanes F, G, L, and N are non-hybrid contaminants. Origin at the bottom of the figure.

**Fig. 3.** IDH, PGM, PGI, and MDH zymograms of progeny from B46-76 x 'Carlos'. Lanes A, B, and C-F represent B46-76, 'Carlos', and F₁ hybrids, respectively. Origin at the bottom of the figure.

**Fig. 4.** Zymograms from progeny from P79-101 x 'Carlos'. Lanes A is P79-101, lane B 'Carlos', and lanes C and D are F₁ hybrids. Origin at the bottom of the figure.

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**Literature Cited**

Fig. 5. PGM, PGI, and MDH zymograms of progeny from ‘Royalty’ x ‘Tarheel’. Lanes A and B are ‘Royalty’ and ‘Tarheel’, respectively. Lanes C, E, and F are F1 hybrids, and lane D a non-hybrid contaminant. Origin at the bottom of the figure.

Fig. 6. PGM, PGI, and MDH zymograms of progeny from ‘Cabernet Sauvignon’ x ‘Tarheel’ and ‘Cabernet Sauvignon’ x ‘Noble’. Lanes A, B, and G are ‘Cabernet Sauvignon’, ‘Tarheel’, and ‘Noble’, respectively. Lanes C-F are ‘Cabernet Sauvignon’ x ‘Tarheel’ F1 hybrids and lanes H-J are ‘Cabernet Sauvignon’ x ‘Noble’. Origin at the bottom of the figure.

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