Inheritance and Isozyme Diversity for GPI and PGM among Grape Cultivars

Dan E. Parfitt and S. Arulsekar
Department of Pomology, University of California, Davis, CA 95616

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Abstract. Leaf isozymes of 145 cultivars of grapes (Vitis vinifera L. and Vitis spp.) were separated via starch gel electrophoresis. They could be separated into 52 groups using glucosephosphate isomerase (GPI) and phosphoglucomutase (PGM) isozymes. Twenty-four cultivars had unique combinations of isozyme patterns. Two loci, Gpi-2 and Pgm-2, were identified and shown to be simply inherited with multiple alleles at each locus. The mean heterozygosity for the two loci (Gpi-2 and Pgm-2) among all the cultivars was 57.5%, indicating a high level of genetic variability among these cultivars. Indirect evidence for expressed linked lethal alleles was demonstrated by the missing classes in Gpi-2 and Pgm-2 segregation ratios (1:2:0 ratios) in several selfed progeny families. Chemical name used: methyl[1-(methylene)-1H-benzimidazol-2-yl]-carbamate (benomyl).

Amelographers have been concerned with the correct identification of grape (Vitis vinifera L.) cultivars for more than 50 years. Traditionally, identification techniques have involved the examination of vine and berry morphology (7). These plant characters are often not distinct and are relatively inconsistent in expression. This is especially true for leaf characters for which the greater share of morphological diversity is present. Disease, or developmental and environmental factors, can affect the apparent values of leaf characters. Amelographers have tried to overcome these problems through the use of multivariate statistical analysis of a variety of quantified leaf characters (Allleweldt and Calo, personal communication). Scanning electron microscopy of pollen has also been used for cultivar identification (1).

Alternative approaches have been developed in recent years (10), including biochemical analysis of fruit compounds—such as phenolic compounds, flavor components, berry isozymes (24), and pollen isozymes (15)—as well as serological analysis of pollen proteins (21). Isozyme techniques have been the most useful and have been highly exploited in various crop species (2, 4-6, 11, 13, 16, 18-20, 22, 23).

Starch gel electrophoresis was only partially useful for grape berry isozyme analysis (24). This may have been due to the type of tissue used or the enzyme systems studied, including indophenol oxidase, esterase, and peroxidase, all being enzymes that are partially under environmental or developmental control (9). Loukas et al. (15) studied the inheritance of nine enzymes in grape pollen. Although \( \chi^2 \) analysis supported hypotheses for simple Mendelian inheritance for eight of the enzymes, the population sizes for the progeny (8 to 24, mean = 13) were too small to provide significant support for Mendelian inheritance.

The present study was undertaken with the following objectives: a) determine whether leaf isozymes could be used to identify grape cultivars; b) test the genetic basis for the expression of these isozymes; and c) examine the extent of diversity in a selection of important grape cultivars.

Genetic analysis is reported for two enzyme systems, GPI and PGM. The genetic control of isozymes was inferred from segregating progenies of self-pollinated parent cultivars that showed multiple banded phenotypes.

Materials and Methods

Plant materials. The names of all 145 cultivars used in this study and their species identities are given in Table 1. These cultivars are maintained in the Univ. of California Foundation Plant Materials Service vineyard at Davis. Young expanding leaves, usually leaves from the third, fourth, or fifth node, were used for isozyme analysis. The methods of enzyme extraction, starch gel electrophoresis, and enzyme staining have been described (3). Samples (0.5g) of actively growing leaves were homogenized in 0.05M Tris-citrate pH 8 buffer and the crude extract was electrophoresed in a pH 8.3 Tris-citrate/lithium borate, 12% starch gel at 250 VDC for 3 to 4 hr at 4C. Four enzyme systems, glucosephosphate isomerase (GPI, EC 5.3.1.9), phosphoglucomutase (PGM, EC 2.7.5.1), leucine amino-peptidase (LAP, EC 3.4.11.1), and aspartate aminotransferase (AAT, EC 2.6.1.1) were studied. Isozyme analysis was done on leaves collected from April to mid-September during the morning hours from all of the cultivars for two consecutive years (three consecutive years for some cultivars) to check for year-to-year consistency in the results.

Progeny tests. Inheritance for GPI, PGM, and LAP enzymes were tested. AAT patterns were not sufficiently distinct or consistent for genetic analysis. Seven cultivars were bagged in 1986 to produce selfed progenies for genetic analysis. Between 200 and 800 seeds per parent were extracted from the berries and stored. Inviable seeds (floaters) were discarded. The seeds were treated with 0.2% Tersan (a benomyl preparation) fungicide for 20 min and stored at 4C until stratified. The seeds were stratified for 3 months at 4C in a mixture of 50% peat and 50% vermiculite. Stratified seeds were planted in 51 x 36 x 10-cm-deep flats in a pasteurized 50% perlite/50% standard potting mix soil. Plants were grown in the lathhouse under standard horticultural practices. Actively growing leaves were collected and analyzed for GPI, PGM, and LAP as described previously (3). Chi-square tests for allelism and linkage were performed on the GPI and PGM results for the families. Mean heterozygosity was calculated as described by Hedrick (12).

Results

Well-resolved and consistent isozyme patterns were obtained with the methods described previously for GPI and PGM. Rep-

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<tr>
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<td>8</td>
<td>5</td>
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<tr>
<td>Tannai</td>
<td>8</td>
<td>5</td>
<td><em>V. vinifera</em></td>
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</tbody>
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487
The bands were usually well-resolved. However, some cultivars occasionally had double-band patterns. For LAP, there were at least seven different banding patterns for GPI-2; four single-banded and eight triple-banded (Fig. 1). These patterns were consistent for all of the years and all of the cultivars tested.

The cultivars having GPI banding patterns 1, 2, 4, 6, 9, and 10 were selfed and the progenies were analyzed for isozyme segregation. No segregation was observed for the isozyme at the GPI-1 region. At the GPI-2 region, segregation was observed for all progenies of cultivars with three-banded patterns (Fig. 1). No segregation was observed in progenies from cultivars with pattern 1, which is single-banded at GPI-2. ‘Cabernet Sauvignon’, ‘Grey Riesling’, and ‘Symphony’ progeny families provided strong evidence for simple Mendelian inheritance at the GPI-2 locus (Table 2). Segregation analysis for single locus, Mendelian ratios at the Gpi-2 locus revealed that the isozymes with mobilities of 2.5, 1.5, 1.0, and 0.5 cm from the origin (banding patterns 1, 3, 5, and 7 in Fig. 2) were all alleles at one locus. The alleles were designated a, b, c, and d, respectively. Thus, the genotypes for patterns 1, 2, 4, 6, 9, and 10, respectively, may be written as Gpi-2aa, Gpi-2ab, Gpi-2bc, Gpi-2cd, Gpi-2ac, and Gpi-2ad. Patterns 11 and 12 show evidence of what are probably two additional alleles, but inheritance tests are needed to confirm this. The two probable alleles are located between Gpi-2a and Gpi-2b, and after Gpi-2d (near the origin).

The selfed progenies of cultivars having PGM banding patterns 1, 4, and 6 (the same cultivars and progenies used for GPI

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Table 1. Continued

PGM. Two sets of bands were observed in the gel, as was the situation for GPI. The most anodal set of isozymes was labeled PGM-1, while the less-anodal region was denoted PGM-2. Variability was observed for both regions, but the extent of variation was much greater at the PGM-2 region than for PGM-1. Although a majority of the cultivars exhibited a single band at PGM-1 (4.5 cm from the origin), some cultivars possessed a double-banded phenotype (4.5 and 4.7 cm) and others had a single band at 4.7 cm from the origin. Four different single-banded patterns and seven different double-banded patterns were present at PGM-2 (Fig. 2). Among the cultivars analyzed, 13 isozyme banding phenotypes were observed for PGM.

The selfed progenies of cultivars having PGM banding patterns 1, 4, and 6 (the same cultivars and progenies used for GPI

![Fig. 1. GPI isozyme patterns observed in grape cultivars. The alleles for which segregation was studied at the Gpi-2 locus are designated a, b, c, and d (right margin) with their corresponding mobilities (left margin).](image-url)
analysis) were analyzed for PGM segregation. No segregation was observed in the progenies of ‘Grey Riesling’, which had PGM banding pattern 1 (Fig. 2). ‘Zinfandel’ and ‘Muscat St.Vallier’, which had banding pattern 4 (Fig. 2), segregated with a single Mendelian ratio 1:2:1 (Table 2) for PGM-2. This locus is referred to as Pgm-2. The single band at the PGM-1 region did not segregate and is probably under separate genetic control. This locus is denoted as Pgm-1.

‘Pinot noir’ and ‘Cabernet Sauvignon’, which had banding pattern 6 (Fig. 2), also gave progenies that segregated for a single locus 1:2:1 ratio (Table 2) for PGM-2. Therefore, the different isozymes in PGM banding patterns 4 and 6 are multiple alleles at the same locus, Pgm-2, and the alleles are designated a, b, and c. Thus, the genotypes for banding patterns 1, 4, and 6 can be written as Pgm-2aa, Pgm-2ac, and Pgm-2ab, respectively.

‘Helena’ and ‘Symphony’, which also had the banding pattern 6 (Fig. 3), gave progenies that could fit only a 1:2:0 ratio (Table 2), probably due to linked lethal genes. Patterns 7 and 8 are putative evidence for two additional alleles, one between Pgm-2a and Pgm-2b and the other after Pgm-2c.

Linkage. The χ² linkage tests for ‘Cabernet Sauvignon’ progeny demonstrated that Gpi-2 and Pgm-2 are not linked (Table 3). Although the simple 1:2:1 χ² test for simple inheritance of Gpi-2 was negative, the linkage test for the ‘Pinot noir’ progeny also supported independent assortment under the assumption of normal 1:2:1 inheritance at both Gpi-2 and Pgm-2 loci. Alleles at Gpi-2 and Pgm-2 assort independently. The results from the present inheritance test support the conclusion of Loukas et al. (15) concerning the inheritance of Gpi and PGM.

Genetic diversity. The cultivars that were tested in this experiment represented the major cultivars and cultivar groups in V. vinifera. These cultivars were quite diverse, being separable into 52 groups on the basis of GPI and PGM analysis. Twenty-four cultivars could be uniquely identified from all of the others with these enzyme systems. The mean heterozygosity at Gpi-2 and Pgm-2 loci was 57.5%, a high level of heterozygosity.

Discussion

Cultivar diversity. Unique isozyme identities could not be assigned to many of the cultivars using only GPI and PGM enzyme systems. Development of additional enzyme staining systems or identification of heritable variation in other characters, such as DNA restriction fragments, will be needed to provide unique identities for each cultivar. At present, if unknown cultivars have the same isozyme pattern they cannot be assumed to be the same. If the cultivars have different GPI or PGM patterns, they can be shown to be genetically different cultivars.

The high level of allelic diversity at the Gpi-2 and Pgm-2 loci demonstrates that grape cultivars are highly polymorphic compared with other fruit and nut crops (4, 5, 11). This could reflect either a possible hybrid origin for V. vinifera in the distant past, as suggested by Olmo (13), or extensive introgression of foreign genes from American species in the 1860s to create the French and American hybrids as a result of attempts by growers to combat several infestations of powdery mildew (Uncinula necator), to breed for cold tolerance, and, later, for resistance to Phylloxera vitifoliae Fitch that had been imported with the American grapes (7).

Inheritance. Inheritance tests could not be used to show that the GPI-1 band was due to a separate locus, since it was monomorphic in all of the parents and all of the other cultivars examined in the identification part of the experiment. GPI-1 is known to be present in chloroplasts and is coded by a nuclear gene in other species of plants (9).

Significantly distorted inheritance ratios were observed for Gpi-2 progenies in ‘Helena’, ‘Muscat St. Vallier’, ‘Pinot noir’, and ‘Zinfandel’, as well as for Pgm-2 in progenies of ‘Helena’ and ‘Symphony’. In all progenies, except for ‘Pinot noir’, it appeared that strong selection had occurred against seedlings from one of the recessive genotype classes. Chi-square analysis for 1:2:0 ratios supported the hypothesis that one of the recessive classes had been lost from the progenies of the aforementioned parents.

The probable explanation for these results is that lethal recessive alleles were associated (linked) with one of the alleles for Gpi-2 or Pgm-2. The fact that the missing class was variously associated with Gpi-2a (‘Helena’, ‘Zinfandel’), Gpi-2b (‘Muscat St. Vallier’), or Pgm-2b (“Symphony”, ‘Helena’) suggests that the linkage is weak or that different lethal alleles have become associated with different isozyme alleles during the history of cultivar development.

The presence of deleterious recessive alleles has long been demonstrated by the severe inbreeding depression that occurs when V. vinifera is self-pollinated (17). The presence of lethal alleles is also supported by the occurrence of seedlessness (embryo abortion) in grape, a character of great economic importance (14). Embryo abortion was clearly present in the selfed seed that we collected. The frequency of “floaters” in seed lots from ‘Helena’, ‘Muscat St. Vallier’, ‘Early Muscat’, and ‘Cardinal’ was 0.3, 0.5, 0.6, and 0.8, respectively. Seed from ‘Cardinal’ and ‘Early Muscat’ had such poor germination (<5%) that the progeny could not be analyzed for this study. Seed germination for the other five cultivars was >50%. However, supporting evidence for preferential loss of the alleles mentioned previously is provided from the linkage tests in Table 3. Chi-

Fig. 2. PGM isozyme patterns observed in grape cultivars. The alleles for which segregation was studied at the Gpi-2 locus are designated a, b, and c (right margin) with their corresponding mobilities (left margin).
Table 2. Number of progeny/class, \(x^2\), and probability values for 1:2:1 and 1:2:0 single locus inheritance hypotheses for progeny from seven grape cultivars.

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<th>Genotype</th>
<th>Genotypes</th>
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<th>(P_{1:1:1})</th>
<th>(x^2);1:2:0</th>
<th>(P_{1:2:0})</th>
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</table>

<sup>a</sup> Evidence for lethal alleles at associated loci.

... Evidence for lethal alleles at associated loci.

The results also suggest that lethal recessive alleles may be associated with particular isozyme alleles in some cultivars. This hypothesis requires additional study, including the isozyme analysis of the progeny from specific additional controlled crosses. Intercrosses between the cultivars with possible lethal alleles associated with different isozyme loci have been made for future analysis as well as crosses to genotypes with no isozyme-associated lethality. Progeny from these crosses would be heterozygous at lethal loci and normal segregation ratios for the isozyme loci are expected rather than the distorted ratios found in selfed progeny families. The relationship of particular isozyme alleles to seedlessness in grapes is also being investigated.

Table 3. Chi-square tests of independence and probability values for Gpi-2 and Pgm-2 full models (1:2:4:2:1:2:1) and reduced (red.) models with missing classes (1:2:1:2:4:2:1:2:1).

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<th>No.</th>
<th>$\chi^2$</th>
<th>$P_{stat}$</th>
<th>$P_{red}$</th>
<th>$P_{stat}$</th>
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<td>Muscat St.</td>
<td>aa</td>
<td>aa</td>
<td>15</td>
<td>64.3</td>
<td>&lt;0.01</td>
<td>3.72</td>
<td>0.60</td>
</tr>
<tr>
<td>Vallier</td>
<td>aa</td>
<td>ac</td>
<td>36</td>
<td>141.2</td>
<td>&lt;0.001</td>
<td>1.65</td>
<td>0.20</td>
</tr>
<tr>
<td>Zinfandel</td>
<td>aa</td>
<td>aa</td>
<td>2</td>
<td>14.35</td>
<td>0.08</td>
<td>2.78</td>
<td>0.73</td>
</tr>
</tbody>
</table>

* Tested under reduced models when evidence from Table 1 suggested 1:2:0 ratios.

Isozyme loci are also valuable for the study of pollination biology (16), population genetics (19), and screening seedling progenies for interspecific hybrids (6, 18).

Literature Cited