Isozyme Locus Pgm-1 Is Tightly Linked to a Gene (V₉) for Scab Resistance in Apple

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Abstract. Pgm-1, the gene responsible for variation in the most anodal isozyme of phosphoglucomutase in apple (Malus spp.), is shown to lie ≈8 centiMorgans from the gene V₉, which confers apple-scab resistance. The proximity of the marker and the ease by which allozymic forms can be resolved suggest that Pgm-1 will be useful for following the inheritance of scab resistance conferred by V₉.

Materials and Methods

Plant material. Full-sib families from the apple breeding programs at Horticultural Research International, East Malling, England, and at the New York State Agricultural Experiment Station, Geneva, N.Y., were artificially inoculated with V. inaequalis and analyzed for allozyme variation. The two families studied at East Malling were obtained from crosses F93 (‘Jonathan’ × A849-7) and F135 (‘Idared’ × A679-12). In both crosses, the male parent was resistant to scab. At Geneva, progeny from crosses 90201 (‘Prima’ × ‘Spartan’) and 90204 (‘Liberty’ × ‘Royal Gala’) were investigated, the maternal parent being the source of resistance in each case.

Analyzing apple-scab resistance. At East Malling, tubes of scab inoculum (Strain E1) cultured on filter-paper cylinders (Kirkham, 1957) were used to prepare 250-ml suspensions. This volume was sufficient to inoculate 500 seedlings. To prevent conidia from clumping together, 2 ml of 0.01% Agral was added to each tube and the mixture was shaken vigorously. The resulting blackish liquid was filtered through muslin, diluted with distilled water to a conidial concentration of 100,000 to 120,000 conidia/ml, and immediately applied with a spray gun to leaves of 4- to 6-week-old seedlings. Family F93 seedlings were inoculated within 6 weeks of germination, whereas F135 seedlings were potted in 1985, cut back during the winter, and inoculated when they had three to four leaves. Plants were covered with polyethylene sheets spread over a wire frame to prevent the sheets from contacting the leaves. Humidity was maintained at 50% to 90% with wet cloth mats. Under these conditions, leaves remained wet for at least 18 h, after which the polythene sheets were removed. This procedure was repeated twice at 2-day intervals. Symptoms began to appear after 2 weeks, and seedlings were scored for their response to scab 1 month after the first inoculation.

At Geneva, seedlings were maintained in wooden trays and inoculated with a mixture of five races and two wild isolates of V.
in aequalis when the seedlings had at least two leaves. Frozen inoculum (270,000 conidia/ml) was thawed and sprayed on all leaf surfaces. Seedlings were maintained at 15 to 20°C for 48 h in mist rooms with >95% relative humidity and then transferred to the greenhouse. Symptoms were fully expressed after ≈2 weeks. After scoring, plants lacking scab symptoms were reinoculated as described above and reevaluated after 2 weeks.

Isozyme analysis. Nineteen enzyme systems were analyzed. Acrylamide gels, prepared according to Manganaris (1989), were used to resolve eight enzyme systems: peroxidase, esterase, endopeptidase, leucine aminopeptidase, acid phosphatase, superoxide dismutase, alcohol dehydrogenase, and aspartate aminotransferase. Horizontal starch gel electrophoresis was conducted as described by Weeden and Lamb (1985). Slices from the tris citrate–lithium borate gel were assayed for triose phosphate isomerase, glucose phosphate isomerase, glutamate dehydrogenase, and malic enzyme. Slices from the histidine gels were assayed for diaphorase, phosphoglucomutase, 6-phosphogluconate dehydrogenase, aconitase, isocitrate dehydrogenase, shikimate dehydrogenase, and malate dehydrogenase. Assay procedures followed those described by Wendel and Weeden (1990). Linkage analysis was performed using the LINKAGE-1 program (Suiter et al., 1983).

Results and Discussion

Table 1 presents segregation data for scab resistance in the four families. Seedlings designated resistant showed a typical class 1 hypersensitive reaction with pinpoint pits, while susceptible seedlings showed fungal mats with heavy sporulations. Some seedlings inoculated at East Malling displayed a class M reaction and were classified intermediate. The expression of scab reaction in the tested seedlings requires that they are actively growing and the conditions during and after inoculation are suitable. At East Malling, these requirements were not always met. The relative humidity was 50% to 80% and the temperature fluctuated between 10°C at night and 23°C during the day. Also, the seedlings were in pots and older than those in Geneva. It has been our experience that older seedlings are more difficult to infect with scab, particularly under nonideal conditions. The differences in the inoculum source and the different background of the scab-resistant parents also may partially account for the high proportion of intermediate seedlings in Table 1. In cross F135, parent A679-12 was derived from Malus ×zum, which carries a gene for resistance to the scab isolate E1 used in the East Malling experiments (unpublished data).

The near ideal conditions at Geneva allowed classification of seedlings into two categories: susceptible and resistant. Resistant plants carrying the Vf gene can be scored accurately using progenies from crosses with one highly susceptible parent (‘Spartan’, ‘Royal Gala’) and the other carrying the Vf gene but lacking polygenic resistance. Results in Table 1 agree with the hypothesis that a single dominant gene, Vf, codes for scab resistance. Resistant plants are presumed to be heterozygous (VfVf), whereas, susceptible plants are homozygous recessive.

Polymorphism was detected in 17 of the 19 enzyme systems analyzed. Segregation was recorded for 32 isozyme loci and followed expected ratios in all cases. These genes and their respective isozyme phenotypes already have been described (Chevreau et al., 1985; Manganaris, 1989; Weeden and Lamb, 1987), except for those responsible for variation in aconitase and shikimate dehydrogenase phenotypes (unpublished data). Segregation for PGM-1 is shown in Fig. 1. PGM-1 allozymes are strongly expressed in young leaf tissue and can be clearly resolved on the starch gel.

Linkage tests were conducted between Vf and the 21 isozyme loci heterozygous in the resistant parents. These tests revealed close linkage between Vf and Pgm-1 in all four families (Table 2) and independent assortment between Vf and the following loci: Aat-1, Aat-2, Aat-4, Acp-1, Acp-2, Diap-2, Enp-1, Est-1, Est-3, Est-4, Lap-1, Lap-2, Lap-4, Pgd-1, Pgm-2, Pgm-5, Prx-2, Prx-4, Prx-7, and Tpi-p. Homogeneity tests showed that the joint segregation data for Vf and Pgm-1 in the four families could be pooled, yielding a recombination value of r = 0.08 ± 0.03. As the intermediate category in the F93 and F135 families contained significant representation of all three Pgm-1 genotypes (data not shown), this category does not seem to consist solely of susceptible plants (lacking Vf) that failed to develop the high level of sporulation characteristic of susceptible genotypes. Rather, resistant genotypes also must have been present. Therefore, seedlings lacking the typical pinpoint reaction were not included in linkage calculations (Tables 1 and 2).

The close linkage between Pgm-1 and Vf (=8 centiMorgans) can be useful for screening progenies segregating for Vf. The PGM-1 phenotype is easily resolved on starch gels (Manganaris, 1989; Weeden and Lamb, 1987). The recombination frequency between Pgm-1 and Vf is low enough to make scoring for the marker as reliable as direct inoculation in many situations. In addition, the identification of the Pgm-1–Vf linkage locates Vf on linkage group 8 of the apple linkage map (Hemmat et al., 1994). This position is further confirmed by the absence of linkage between Vf and 20 other isozyme loci scattered over 11 different linkage groups.

Despite the advantages of Pgm-1 as a marker gene, there are limitations to its use. Recombination between Pgm-1 and Vf has occurred even in the few generations since the original crosses with M. floribunda 821. In A679-12, Vf is linked to the allele coding the faster migrating allozyme of Pgm-1, whereas, in A849-7, ‘Prima,’ and ‘Liberty,’ it is linked to the allele coding the slower isozyme. Similarly, certain trees such as NY75441-67, known to be heterozygous at Vf, are homozygous at Pgm-1, precluding the use of the isozyme locus as a marker in crosses involving this parent

Table 1. Segregation for resistance to Venturia inaequalis from crosses of cultivars and selections descended from Malus floribunda 821.

<table>
<thead>
<tr>
<th>Family</th>
<th>Parentage</th>
<th>Scab reaction</th>
<th>Expected</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>R</td>
<td>I</td>
</tr>
<tr>
<td>F93</td>
<td>Jonathan x A849-7</td>
<td>20</td>
<td>13</td>
</tr>
<tr>
<td>F135</td>
<td>Idared x A679-12</td>
<td>33</td>
<td>27</td>
</tr>
<tr>
<td>90201</td>
<td>Prima x Spartan</td>
<td>31</td>
<td>0</td>
</tr>
<tr>
<td>90204</td>
<td>Liberty x Royal Gala</td>
<td>17</td>
<td>0</td>
</tr>
</tbody>
</table>

*R = resistant, I = intermediate, S = susceptible.

†Chi square calculated without using I plants.

‡Indicates the scab-resistant parent (VfVf).
very difficult and time-consuming (Lamb and Hamilton, 1969).

or more genes for scab resistance using traditional methods can be
resistance is a critical goal. However, selecting those trees with two

has become particularly important now that resistance conferred
the same region of the apple linkage map as Pgm-1 (Hemmat et al., 1994), but these
were not polymorphic in families 90201 or 90204 and are not
suitable for tagging $V_f$. We are continuing our investigations of
such segregating families with the goal of identifying markers
closer to $V_f$ or those on the opposite side of $V_f$ to bracket this locus.

Identifying markers for genes conferring apple-scab resistance has become particularly important now that resistance conferred by $V_f$ seems to have been overcome in certain geographical regions (Parisi et al., 1993). Combining the independent sources of scab resistance is a critical goal. However, selecting those trees with two or more genes for scab resistance using traditional methods can be very difficult and time-consuming (Lamb and Hamilton, 1969).

Our identification of a molecular marker for $V_f$ will help pyramid scab resistance genes.

Table 2. Joint segregation analysis for the loci Pgm-1 and $V_f$.

<table>
<thead>
<tr>
<th>Family</th>
<th>Parent PGM</th>
<th>Progeny phenotype</th>
<th>Recombination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>genotype</td>
<td></td>
<td>$\chi^2$</td>
</tr>
<tr>
<td>F93</td>
<td>fs x fs'</td>
<td>ff/R  ff/S  fs/R  ss/R  ss/S</td>
<td>6.3</td>
</tr>
<tr>
<td>F135</td>
<td>fs x fs'</td>
<td>11    1  11    5    0    8</td>
<td>17.7</td>
</tr>
<tr>
<td>90201</td>
<td>fs' x fs</td>
<td>1     15   12   17   18   0</td>
<td>31.1</td>
</tr>
<tr>
<td>90204</td>
<td>fs' x ss</td>
<td>---   ---  2'   19   15   3</td>
<td>24.7</td>
</tr>
</tbody>
</table>

*Expected ratios: 1:1:2:2:1:1 when both parents heterozygous at Pgm-1 and 1:1:1:1:1 when only the resistant parent is heterozygous at Pgm-1.

*Indicates resistant parent.

*One triploid not included in the estimated $r$ value.

Literature Cited


