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Isozyme Locus *Pgm-1* Is Tightly Linked to a Gene (V_f) 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 V_f, 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 V_f.**

Apple scab, caused by *Venturia inaequalis* (Cke.) Wint., is one of the most serious diseases of apple. In areas with favorable environmental conditions for disease development, no fewer than eight to ten fungicide sprays per year are applied to avoid losses in apple orchards. Surveys of *Malus* germplasm have identified several clones with a high level of apple-scab resistance. When assayed under greenhouse conditions, distinct reaction classes can be identified: no visible sign of infection (class 0), pinpoint pits with no sporulation (class 1), irregular chlorotic or necrotic spots with no sporulation (class 2), few restricted sporulating lesions (class 3), a mixture of class 2 and class 3 lesions (class M), or extensive and abundantly sporulating lesions (class 4) (Shay and Hough, 1952; Williams and Kuc, 1969).

Although several sources of apple-scab resistance have been identified, most breeding programs have relied on the V_f gene derived from *M. floribunda* 821. The resistant reaction in later generations of *M. floribunda* 821 backcrosses with susceptible commercial cultivars is usually a chlorotic lesion with a variable level of sporulation, probably depending on the presence of minor genes contributing to resistance (Rousselle et al., 1974). The resistance reaction is conditioned by the dominant V_f gene (Hough, 1944; Williams et al., 1966) and may be expressed as a class 1, 2, 3, or M reaction. Resistance classes are distinguishable from the fully susceptible class 4 response, in which sporulation occurs earlier, more abundantly, and uniformly on the susceptible inoculated leaves. Thirty-four apple varieties have been released carrying mainly this source of scab resistance (Crosby et al., 1992). Yet, none of these varieties has been widely accepted commercially, although their quality has been comparable if not superior to susceptible varieties (Durner et al., 1992).

Tagging genes coding for agronomic characteristics with molecular markers has great potential for plant breeders. A high level of allozyme polymorphism has been revealed in apples, and genetic studies have identified >20 polymorphic isozyme loci (Chevreau et al., 1985; Manganaris, 1989; Weeden and Lamb, 1987). The present study investigates the potential for using one of these loci, *Pgm-1*, as a marker for V_f.

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' x A849-7) and F135 ('Idared' x A679-12). In both crosses, the male parent was resistant to scab. At Geneva, progeny from crosses 90201 ('Prima' x 'Spartan') and 90204 ('Liberty' x '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.*

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inaequalis 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 20C 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, glucosephosphate 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 10C at night and 23C 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* ×*zumi*, 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 V_f gene can be scored accurately using progenies from crosses with one highly susceptible parent ('Spartan', 'Royal Gala') and the other carrying the V_f gene but lacking polygenic resistance. Results in Table 1 agree with the hypothesis that a single dominant gene, V_f, codes for scab resistance. Resistant plants are presumed to be heterozygous (V_fv_f), 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 allozyme 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 V_f and the 21 isozyme loci heterozygous in the resistant parents. These tests revealed close linkage between V_f and *Pgm-1* in all four families (Table 2) and independent assortment between V_f 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 V_f and *Pgm-1* in the four families could be pooled, yielding a recombination value of $r = 0.08 \pm 0.03$. 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 V_f) 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 V_f (≈8 centiMorgans) can be useful for screening progenies segregating for V_f. The PGM-1 phenotype is easily resolved on starch gels (Manganaris, 1989; Weeden and Lamb, 1987). The recombination frequency between *Pgm-1* and V_f 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*–V_f linkage locates V_f on linkage group 8 of the apple linkage map (Hemmat et al., 1994). This position is further confirmed by the absence of linkage between V_f 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 V_f has occurred even in the few generations since the original crosses with *M. floribunda* 821. In A679-12, V_f 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 V_f, 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.

		Scab reaction[z]			Expected		
Family	Parentage	R	I	S	ratio	χ^{2y}	*P*
F93	Jonathan x A849-7[x]	20	13	17	1:1	0.24	0.62
F135	Idared x A679-12[x]	33	27	25	1:1	1.10	0.29
90201	Prima[x] x Spartan	31	0	32	1:1	0.02	0.90
90204	Liberty[x] x Royal Gala	17	0	22	1:1	0.64	0.42

[z]R = resistant, I = intermediate, S = susceptible.
[y]Chi square calculated without using I plants.
[x]Indicates the scab-resistant parent (V_fv_f).

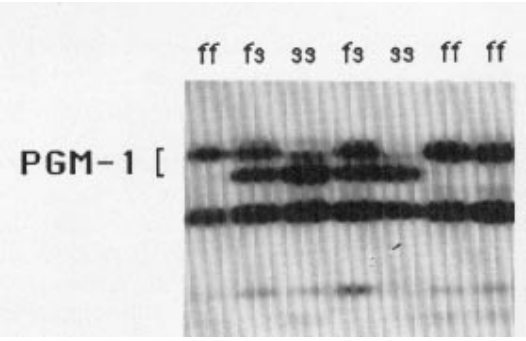

Fig. 1. Phosphoglucomutase phenotypes from apple (family 90201). The most anodal zone of activity, designated PGM-1, is segregating, as is the most cathodal zone. Genotypes for PGM-1 are given at the top of the figure. The intense band across the center of the figure is PGM-3 and is an excellent control for the PGM assay. Not only is it the most active PGM isozyme in apple, but it is also monomorphic throughout nearly all the domesticated apple germplasm and can be used to quickly identify the region of the gel containing the PGM-1 activity.

(unpublished data). Obviously, a marker more closely linked to V_f is preferable; however, such a marker almost certainly will be a DNA polymorphism, for nearly all known protein polymorphisms identified in apple assort independently of V_f (Hemmat et al., 1994). Certain DNA markers have been mapped to 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.

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

Family	Parent PGM genotype	Progeny phenotype ff/R	ff/S	fs/R	fs/S	ss/R	ss/S	χ^{2z}	*P*	Recombination frequency ± SE
F93	fs x fs^y	1	3	7	6	6	0	6.3	0.043	10 ± 10
F135	fs x fs^y	11	1	11	5	0	8	17.7	<0.001	5 ± 5
90201	fs^y x fs	1	15	12	17	18	0	31.1	<0.001	3 ± 3
90204	fs^y x ss	---	---	2^x	19	15	3	24.7	<0.001	10 ± 5
									Pooled estimate	8 ± 3

[z]Expected ratios: 1:1:2:2:1:1 when both parents heterozygous at *Pgm-1* and 1:1:1:1 when only the resistant parent is heterozygous at *Pgm-1*.
[y]Indicates resistant parent.
[x]One triploid not included in the estimated *r* value.