Segregation of Scab Resistance in Three Apple Populations: Molecular Marker and Phenotypic Analyses

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Abstract. The codominant PCR marker AL07-SCAR closely linked to the V_f gene for scab resistance was used to genotype seedlings in three apple populations in which each parent ('GoldRush', 'Enterprise', 'Pristine', and CQR10T17) was resistant to apple scab. The marker was used to predict the genotype at the V_f locus. Each parent was heterozygous. In two populations (CQR10T17 x 'GoldRush' and 'Pristine' x 'GoldRush') seedlings segregated 1:2:1 for fragments associated with V_fV_f , V_fv_f , and v_fv_f as predicted by Mendelian segregation. However, in another population ('GoldRush' x 'Enterprise') the ratio was 1.5:1:1.5, suggesting some type of selection against heterozygotes. Fruiting seedlings were rated for the presence of fruit scab. No scab was observed on seedlings homozygous for the PCR marker linked to V_f , a small amount of scab was observed on one heterozygous seedling out of 35, and 22 of 26 seedlings that were homozygous recessive, had fruit scab.

The apple scab disease, caused by the fungus *Venturia inaequalis*, is one of the most serious diseases affecting apples worldwide. This pathogen infects leaves thereby reducing photosynthetic capacity and also attacks fruit causing cosmetic blemishes. A number of apple cultivars have been bred that are resistant to scab, most of them owing their resistance to the presence of the V_f gene, originally found in the crab apple *Malus floribunda* 821 (Janick et al., 1996).

Breeding fruit trees that have superior fruit quality and horticultural characteristics, in addition to disease resistance traits, is an enduring challenge. The use of genetic markers, however, can speed up the process of selecting for a specific trait, provided that suitable markers tightly linked to genes controlling that trait have been identified.

Many genetic markers have been identified that are linked to the V_f gene. These include isozymes (Manganis et al., 1994), RAPDs (Gianfranceschi et al., 1996, Tartarini, 1996) and AFLPs (Xu and Korban, 2000). High resolution mapping has ordered these markers relative to the V_f locus and several are very tightly linked (Xu and Korban, 2000). Some of the RAPD and AFLP markers have been converted into more robust PCR markers (Tartarini et al., 1999, Xu et al., 2001). One of these, AL07-SCAR, lies about 0.2 cM from the V_f locus (Xu and Korban, 2000). This marker has the advantage of being codominant, i.e. it can distinguish between individuals that are heterozygous or homozygous for either allele at this locus. We have used the

AL07-SCAR marker to predict the inheritance of the V_f gene in three apple populations derived from scab resistant parents and that share the scab resistant cultivar 'Goldrush' as a common parent. Molecular genetic analysis was compared with field evaluation of scab resistance in these populations.

The objectives of this study were to determine the inheritance of V_f and whether the molecular marker genotype was a good predictor of scab infection in field conditions. Furthermore, the identification of homozygous V_fV_f genotypes would be valuable for use as parents to produce seedling populations in which all individuals were scab resistant.

Materials and Methods

Three populations were generated by controlled hybridizations in Spring 1996, all made with scab resistant parents. These populations were A) 'GoldRush' x 'Enterprise', B) CQR10T17 x 'GoldRush', and C) 'Pristine' x 'GoldRush' (Table 1). These parents were all selections from the PRI (Purdue-Rutgers-Illinois) apple breeding program (Crosby et al., 1992). Based on parentage (resistant x susceptible), 'GoldRush' and 'Pristine' were assumed to be heterozygous for V_f (Crosby et al., 1994b, Janick et al., 1995), whereas the genotypes of 'Enterprise' and CQR10T17 were unknown since both parents were heterozygous for scab resistance and so they could be either homozygous or heterozygous for V_f (Crosby, 1994a).

Seeds from these hybridizations were germi-

nated and grown initially in a greenhouse before they were transferred to the field. Populations A, B, and C consisted of 410, 337, and 578 seedlings, respectively. Approximately the first 100 trees from each population were used in this study. Within each population, trees were planted randomly down the row, so the 100 trees selected should have provided a reasonable representation of each population without any obvious bias in sampling. When trees were in their fifth growing season, leaf samples were collected from each plant as well as from the four parents. Samples were also collected from *M. floribunda* 821 (scab resistant) and 'Gala' (scab susceptible).

During Fall 2001 five leaf discs were collected from each tree using a 7 mm diameter hole punch and placed into 1.5 ml microfuge tubes. Fresh leaf tissues were placed in liquid nitrogen and then stored in a -20°C freezer until DNA extractions were performed. Genomic DNA was extracted from frozen leaf discs as described by Doyle and Doyle (1990).

PCR amplifications were performed on the extracted DNA as described by Tartarini et al., (1999), except that 10-μL reaction volumes were used containing 25 ng of genomic DNA, 0.1% Triton X-100, 2 mM MgCl₂, 250 nM of each primer, 200 μM dNTPs, de-ionized water, and 1 U Taq polymerase (Promega, Madison, Wis.). The primers used to amplify the AL07-SCAR marker were: forward primer 5'-TGGAAGAGAGATCCAGAAAGTG-3' and reverse primer 5'-CATCCCTCCACAAATGCC-3' (Tartarini et al., 1999). The primers were purchased from MWG Biotech (High Point, N.C.). The amplification conditions for the ALO7 marker were as follows: 3 min at 95 °C, followed by 40 cycles of 30 sec at 95 °C, 1 min at 60 °C, 2 min at 72 °C, followed by 10 min at 72 °C. The samples were stored at 4 °C. Five microliters of each PCR was analyzed by agarose gel electrophoresis. The PCR products were separated in 1% agarose gels at 130 v for 45 min using a Tris-acetate buffer. The DNA fragments were stained with ethidium bromide, visualized under UV light and photographed. A 100-bp ladder of DNA fragments (BioRad, Hercules, Calif.) was used as a size standard.

The presence of scab was rated on the same trees used for the PCR study during the summer of 2002. Trees received no fungicide applications. Scab infections were more obvious on fruit than leaves, therefore fruit scab was assessed and rated as 0 (no visible scab), 1 (≤2 lesions/tree), 2 (3 to 5 lesions/tree), or 3 (>5 lesions/tree). Only those trees bearing fruit could be assessed for fruit scab and scored for resistance or susceptibility. The proportion of trees bearing fruit was 32%, 21%, and 30% for populations A, B, and C, respectively.

Table 1. Segregation of the AL07-SCAR marker in seedlings of three apple populations. All parents were heterozygous (466/724). The 466-bp fragment is linked to V_f whereas the 724-bp fragment is linked to v_f .

Population	466/466	466/724	724/724	Chi square
A) 'GoldRush' x 'Enterprise'	37	23	36	26.07**
B) CQR10T17 x 'GoldRush'	28	51	27	0.17
C) 'Pristine' x 'GoldRush'	23	52	24	0.27

**Significantly different from 1:2:1 ratio at $p = 0.01$.

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Fig. 1. PCR amplification pattern of AL07-SCAR marker to apple DNA on an agarose gel stained with ethidium bromide. Lanes 1 = CQR10T17 (466/724); 2 = 'Gala' (724/724); 3 = *M. floribunda* 821 (466/466); 4 = 466/466 seedling; 5 = 724/724 seedling; 6 = 466/724 seedling. The 466-bp fragment is linked to V_f whereas the 724-bp fragment is linked to v_{fj} .

Results and Discussion

The PCR primers for AL07-SCAR amplified a fragment of 466 bp using template DNA from the scab resistant *M. floribunda* 821 (Fig. 1, lane 3). DNA from the susceptible cultivar 'Gala' produced a fragment of 724 bp (Fig. 1, lane 2). These results are in agreement with those presented by Tartarini et al., (1999) who showed that the 466 bp PCR product was linked to the V_f gene whereas the 724-bp fragment was associated with the allele for susceptibility to scab. Because this marker is co-dominant, PCR amplification of DNA from trees that are heterozygous at this locus should produce both fragments. Two examples of this are shown in Fig. 1. DNA from both CQR10T17 and a tree from one of the segregating populations analyzed in this study (lanes 5 and 6, respectively) resulted in amplification of both the 724- and 466-bp fragments, indicating these plants are heterozygous.

In addition to these two fragments a third PCR product, between 724 bp and 466 bp, was observed in samples from heterozygous individuals. This intermediate PCR product amplified from heterozygous plants has not been reported previously in apple and was very reproducible under the conditions used in these experiments. This fragment may have been a heteroduplex between the 724- and 466-bp fragments, as has been found in cranberry (Novy and Vorsa, 1996). The nature of this fragment has not been investigated further.

All four cultivars used as parents of the populations examined in this study ('GoldRush', 'Pristine', 'Enterprise' and CQR10T17) produced the three-band pattern, indicating each was heterozygous at the AL07-SCAR marker, close to the V_f locus. Since the parents for all three populations appeared to be heterozygous (Table 1), the progeny would be expected to segregate at the V_f locus with a ratio of 1:2:1 V_fV_f : V_fv_{fj} : $v_{fj}v_{fj}$, resulting in a phenotypic ratio of 3 resistant : 1 susceptible. Approximately 100 trees from each of the three populations were genotyped by PCR with marker AL07-SCAR. Populations B and C segregated with the expected ratios, with 74.5% and 75.8% of the seedlings, respectively, containing at least one copy of the marker allele linked to the V_f gene and expected to be resistant to apple scab (Table 1). However, Population A ('GoldRush' x 'Enterprise') did not give the expected segregation ratio. The observed ratio was close to 1.5:1:1.5, indicating a pronounced under-representation

of the heterozygous class. The most likely explanation for the observed segregation ratio is that there has been some inadvertent selection against heterozygous plants. No segregation for dwarfs or lethals was observed in this population. Additional genotypic analysis with other molecular markers may be able to provide an answer to this question. Interestingly, the reciprocal cross ('Enterprise' x 'GoldRush') did not set fruit and suggests either some type of incompatibility (unlikely in a gametophytic system) or an adverse response to emasculation (Janick, unpubl.).

The frequency of recombination between AL07-SCAR and V_f has been estimated to be between 0.2% and 2.0% in various studies (Tartarini et al., 1999, Xu and Korban, 2000).

Because the AL07-SCAR marker is tightly linked to the V_f gene, it should be possible to use this marker to predict the scab resistance phenotype of an individual tree with some confidence. Any plant carrying at least one copy of the AL07-SCAR allele that gives the 466 bp product would be expected to be resistant to scab. There is some evidence to suggest that trees homozygous for V_f are more resistant than heterozygous trees (Sansavini et al., 2002). Trees that had been genotyped from the three populations were assayed for fruit scab. Over all three populations, none of the seedlings homozygous for the 466-bp PCR product (linked to V_fV_f) exhibited fruit scab while a single heterozygous (466/724) tree out of 35 showed a small amount of scab (Table 2). Susceptibility to scab in this one tree may have resulted from a recombination between the V_f locus and the marker. Of the trees that were homozygous for the 724-bp fragment, scab was evident on 22 of the seedlings. Four trees were scab-free and these were likely to be escapes, i.e., susceptible but not showing symptoms of disease, since we observed the effects of natural field infection and did not use controlled inoculations of the pathogen. It is interesting to note that even in Population A, where we observed non-Mendelian segregation ratios for the PCR marker, the marker genotype was still an excellent predictor of scab resistance. Additional data will be required to determine if trees that are homozygous for the 466-bp product demonstrate a higher degree of scab resistance than those that are heterozygous. Overall, these results indicate that the AL07-SCAR marker genotype of trees can be used as a useful and

reliable tool to predict scab resistance in these and other apple populations.

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Table 2. Ratings of severity of fruit scab on fruiting seedlings in three apple populations. The 466-bp fragment is linked to V_f whereas the 724-bp fragment is linked to v_{fj} .

Population or genotype	Fruit scab rating ^c				Total
	0	1	2	3	
A) 'GoldRush' x 'Enterprise'					
466/466	12	0	0	0	12
466/724	8	1	0	0	9
724/724	0	1	6	3	10
B) CQR10T17 x 'GoldRush'					
466/466	4	0	0	0	4
466/724	12	0	0	0	12
724/724	1	1	2	2	6
C) 'Pristine' x 'GoldRush'					
466/466	6	0	0	0	6
466/724	14	0	0	0	14
724/724	3	1	5	1	10

^aRatings were 0 (no visible scab), 1 = ≤2 lesions/tree, 2 = 3 to 5 lesions/tree, or 3 = ≥5 lesions/tree.