Marker-assisted Selection for Powdery Mildew Resistance in Grapes

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ABSTRACT. The efficiency of marker-assisted selection for powdery mildew (*Uncinula necator* (Schw.) Burr) resistance in grapes (*Vitis* L. sp.) was studied using molecular markers associated with a major QTL (quantitative trait loci) for this trait. Initially, genetic maps were constructed from a segregating population of the cross 'Horizon' x Illinois 547-1 (a hybrid between *V. rupestris* Scheele and *V. cinerea* Engelm.). A major QTL from Ill. 547-1, the resistant parent, explained 41% of the variation. One RAPD (randomly amplified polymorphic DNA) marker and one AFLP (amplified fragment length polymorphism) marker, obtained by bulked segregant analysis, showed the highest association with powdery mildew resistance in the mapping population. Segregation of the QTL was followed in different crosses by CAPS (cleaved amplified polymorphic sequence) markers developed from these two markers. An allele-specific amplified polymorphism that segregates as present/absent was also developed from the CS25b locus. Powdery mildew resistance was evaluated visually on a 1 to 5 scale in four different seedling populations. Two populations originated from crosses using Ill. 547-1 as the resistant parent. Two other populations were from crosses with NY88.0514.03, a resistant seedling from the original 'Horizon' x Ill. 547-1 mapping population. Segregation ratio distortions were observed in some crosses. In these cases, the allele associated with the QTL for powdery mildew resistance was less frequent than the alternate allele. In all crosses, the markers were closely associated with resistance. If selection were based on markers, the percentage of susceptible individuals (classes 4 and 5) would decrease from 24% to 52% to 2% to 18%. Selection efficiency was greatest in crosses where segregation distortion was most intense.

Most commercial grape cultivars belong to the species *Vitis vinifera* L., which has high fruit quality but is highly susceptible to many fungal diseases. Related species are sources of disease resistance genes but their fruit quality is typically poor. Breeding to develop disease resistant grape hybrids with high quality is difficult due to the large amount of resources necessary to evaluate vines under field conditions. The cost of these resources limits the size of progenies evaluated. Consequently, a fast and reliable method to eliminate susceptible seedlings before planting would be advantageous.

Seedlings can be screened for disease resistance but this process is usually time-consuming, and does not always correlate with reactions of mature vines. Evaluation of young seedlings for powdery mildew resistance under greenhouse conditions was not well correlated with field ratings obtained from adult plants (Aldwinckle et al., 1975; Pool et al., 1981).

Molecular markers linked to resistance genes can be employed as an alternative selection technique. Selection of seedlings based on the presence of markers, or marker-assisted selection (MAS), is relatively fast and not influenced by environmental factors (Arus and Moreno Gonzalez, 1993). Markers linked closely to genes of interest may be obtained by bulked segregant analyses (Michelmore et al., 1991).

Powdery mildew (*Uncinula necator*) is a disease of great concern worldwide (Reisch and Pratt, 1996) and breeding programs, therefore, are developing resistant cultivars. We studied the applicability of MAS for powdery mildew resistance in grapes based on previous genomic mapping work on grapes (Dalbó et al., 2000). Resistance to powdery mildew is thought to involve more than one gene (Boubals, 1961, Eibach et al., 1989). One limitation of a MAS approach is that many genes may be necessary to reach an adequate level of resistance. However, a genomic region with a fairly strong influence on powdery mildew resistance has been identified (Dalbó, 1998), suggesting that a major gene or gene cluster is involved. Therefore, the objective of this study was to develop and evaluate markers linked to this powdery mildew resistance locus for their utility in selecting resistant genotypes in other segregating seedling populations where the same source of resistance was used.

Materials and Methods

MARKER DEVELOPMENT. A strong QTL (quantitative trait locus) for powdery mildew resistance was identified on linkage group X of Ill. 547-1 (Dalbó, 1998). Bulked segregant analysis (BSA) (Michelmore et al., 1991) was used to obtain additional markers in the region of this QTL as follows. Two DNA bulks were constructed, one with the five most resistant individuals in the progeny, and the other with the five most susceptible individuals. DNA amplifications from each bulk were compared and markers amplified in only one bulk were examined further. Two hundred thirty three randomly amplified polymorphic DNA (RAPD) primers and 55 amplified fragment length polymorphism (AFLP) primer combinations were used to polymerase chain reaction (PCR) amplify each bulk sample. At the end of this process, one RAPD marker (CS25b, primer sequence GTGTAATCGC), amplified from Ill. 547-1 and the resistant bulk, had the highest association to powdery mildew resistance

Table 1. Primer sequences of sequence tagged sites (STS) markers, 5' to 3'.

Marker	Forward	Reverse
$CS25_{997}$	TGAAAGCATTAGATTTGGGGGGGA	CCCGAAACTCAGTAATTATGCATG
$CS25_{333}$	GCATATGATTAGACACTCC	CAACACTAGTCCGGGTAC
STS-AA6	GGTCAGCTTAGAAACAAGGGATG	GACATAGACCCATCCCCAATG

and was selected to be used in this study. The DNA fragment that generated this marker was cloned (TA Cloning Kit, Invitrogen, San Diego, Calif.) from Illinois 547-1 and sequenced using an automated DNA sequencer (model ABI 377; PE Biosystems, Foster City, Calif.), at Cornell Center For Advanced Technology, Ithaca, N.Y. One pair of primers was designed from this sequence to generate a marker ($CS25_{997}$) that amplifies a DNA segment of 997 bp. Restriction enzymes were tested for detection of polymorphism to generate CAPS (cleaved amplified polymorphic sequence) markers (Akopyanz et al., 1992).

The same steps were followed to generate a CAPS marker from the AFLP marker AfAA6, linked closely to CS25b. Marker AfAA6 was found to be linked tightly to marker CS25b in the course of routine AFLP mapping. In this case, the band was excised from the polyacrylamide gel after silver staining, transferred to a tube containing 20 μL of distilled water, and boiled for 10 s. The marker was reamplified by adding 3 μL of the resulting solution to a PCR reaction with the same primers and reaction conditions used to generate the original marker. Cloning of this fragment proceeded as described for the RAPD marker.

DNA extraction. For the mapping population ('Horizon' x Ill. 547-1), DNA was extracted using a modified CTAB procedure, as described by Lodhi et al. (1995). For the other populations, DNA was extracted using a slightly modified version of the miniprep method described by Haymes (1996). In this method, leaf samples were collected by closing the lid of a 1.5-mL microcentrifuge tube on a leaf. After addition of 5 to 10 mg of polyvinylpolypyrrolidone (PVPP) and 150 μL of cetyltrimethyl ammonium bromate (CTAB) extraction buffer, samples were ground inside the tube with a plastic pestle attached to a drill. The samples were then incubated for 30 min at 65 °C, followed by a chloroform extraction and ethanol precipitation of DNA. After centrifugation for 5 min. at 8,000 g_n, the DNA pellets were washed twice with 70% ethanol and dissolved in 500 μL of distilled water.

PCR protocols. RAPD amplification was performed in a reaction volume of 25 μL containing 10 mM Tris-HCl (pH 8.0), 50 mM KCl, 2.3 mM MgCl₂, 0.1% Triton X-100, 120 μM of each dNTP, 0.4 μM primer, 40 to 50 ng genomic DNA (quantified by spectrophotometry), and 0.5 unit of *Taq* DNA polymerase (Promega, Madison, Wis.). Amplification was performed on a thermocycler (PTC-100; MJ Research Inc., Waltham, Mass.) for 39 cycles of 50 s at 94 °C, 50 s at 35 °C and 2 min at 72 °C, followed by an 8 min extension at 72 °C.

To produce AFLP markers the PCR protocol consisted of 35 cycles of 30 s at 94 °C, 30 s for annealing , and 2 min at 72 °C. The annealing temperature started at 65 °C and decreased 1 °C per cycle until it reached 56 °C. Both fluorescent primers and silver staining were used to visualize AFLP products. DNA fragments visualized by fluorescence were separated in 5% polyacrylamide gels and analyzed in a DNA automatic sequencer, model ABI 377 (Applied Biosystems Inc., Perkin-Elmer Corp.). The methodology is described in the AFLP kit from Applied Biosystems Inc. Silver stained AFLP samples were prepared according to Vos et al. (1995), separated in a 6% polyacrylamide gel and stained as described in the Promega Silver Staining Kit (Promega).

Cleaved amplified polymorphic sequence (CAPS) markers were produced by cloning and sequencing the DNA fragments corresponding to the original RAPD and AFLP markers. Longer primers (Table 1) were designed to amplify specific markers (sequence tagged sites; STSs) to identify the same loci in different genotypes. Since the resultant markers ($CS25_{997}$ and STS-AA6) appeared monomorphic, we searched for differences in restriction sites within the sequences. Ten different enzymes cleaving at four base inverted repeats were tested. Useful polymorphism was achieved with the enzymes *Rsa I* and *Taq I* at the CS25b locus, and with *Hae III*, at the AfAA6 locus.

To produce CAPS markers, the total reaction volume used was only 20 μL, the MgCl₂ concentration was 1.5 mM, and the annealing temperature was increased to 57 °C. After amplification, each tube received 0.2 units of a restriction enzyme dissolved in 5 μL of 1× Multicore buffer (Promega). The samples were incubated for 50 min at 65 °C for *Taq I* and 37 °C for other enzymes. Amplification products were separated by electrophoresis in 2% agarose gels (1% agarose/1% NuSieve GTG agarose, FMC Corp., Rockland, Maine) and visualized by staining with ethidium bromide.

An allele specific amplified polymorphism (ASAP) (Gu et al., 1995) marker, $CS25_{333}$, was also developed from the sequence of the CS25 RAPD marker. The objective was to develop a specific marker for the allele associated with powdery mildew resistance in Ill. 547-1 based on rare restriction sites of the enzymes *Rsa I* and *Taq I* present in that marker. To design specific primers, the DNA fragments of the $CS25_{997}$ marker from Illinois 547-1 and NY73.0136.17 were cloned and sequenced. Two point mutations were identified as responsible for the changes in *Rsa I* and *Taq I* restriction sites. We tried to design primers with the point mutation at the 3' end. We succeeded for the *Taq I* site but not for the *Rsa I* site, where the suggested primer would form an internal loop. As a result, the primer $CS25_{333}$-reverse, (Table 1) was extended two bases beyond the mutation point to prevent self annealing during PCR reactions. For amplification, PCR conditions were modified from those used for AFLP reactions. The changes included the starting annealing temperature at 65 °C, then decreased 1 °C per cycle until reaching 60 °C, followed by 40 cycles at 60 °C and 4 cycles at 59 °C. Before separation in agarose, the samples were transferred to an ELISA plate and 1 μL of ethidium bromide (50 μg·mL⁻¹) was added to each PCR reaction. The samples were then evaluated visually to detect amplification products both by fluorescence under ultra violet (UV) light in agarose gel and by fluorometer measurements (Cytofluor II Fluorescence Multi Well Plate Reader, Perseptive Biosystems, Framingham, Mass.).

Testing markers in other populations. Powdery mildew resistance was evaluated in the following segregating seedling populations: 'Horizon' x Ill. 547-1 (additional seedlings from original population); NY88.0514.03 ('Horizon' x Ill. 547-1) x 'Traminette' (Joannes-Seyve 23-416 x 'Gewürztraminer'); NY88.0514.03 ('Horizon' x Ill. 547-1) x NY73.0136.17 (NY33277 x 'Chancellor'); and J.S. 23-416 x Ill. 547-1.

Fig. 1. The RAPD marker CS25b, obtained by bulked segregant analysis. Lane 1, Illinois 547-1; lane 2, 'Horizon'; lane 3, Bulk 1 (five most resistant genotypes); lane 4, Bulk 2, (five most susceptible genotypes); lanes 5 to 9, resistant individuals of Bulk 1; lanes 10 to 14, susceptible individuals of Bulk 2; lane 15, *Vitis cinerea* B9; lane 16, *V. rupestris* B38. The last two individuals are parents of Illinois 547-1.

map of Ill. 547-1, constructed from a segregating seedling population of the cross 'Horizon' x Ill. 547-1 (Dalbó et al., 2000). BSA was used to find other markers in that region. A single RAPD marker (CS25b) obtained by BSA showed close linkage to the QTL and was highly associated with resistance [logarithm of odds (LOD) = 6.56; R^2 = 0.41]. This marker was present in Ill. 547-1 and one of its parents (*V. cinerea* B9), the likely source of the resistance gene(s) responsible for this QTL (Fig. 1). An AFLP marker (AfAA6), originally mapped on the 'Horizon' map, was located 1.8 cM apart from the CS25b locus. The association with powdery 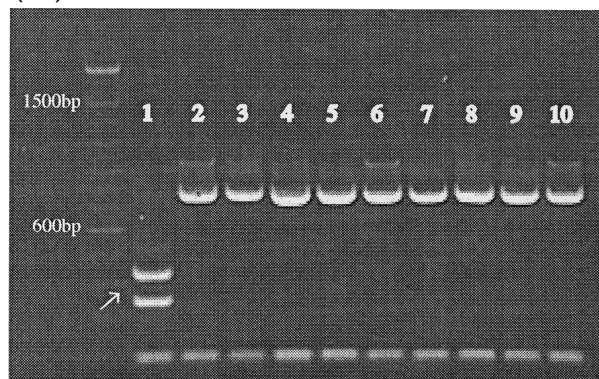mildew resistance (LOD = 6.53, R^2 = 0.38) was slightly lower, so it was used as an alternative marker to CS25b. For genetic analysis purposes, we will refer to these markers as the CS25b and AfAA6 loci.

CAPS MARKER DEVELOPMENT. The presence of relatively rare restriction sites within the sequences of original RAPD and AFLP markers allowed development of cleaved amplified polymorphic sequences (CAPS) to follow the QTL for powdery mildew resistance in different crosses. Two restriction enzymes, *Rsa I* and *Taq I*, were particularly useful for the CS25b locus, and the enzyme *Hae III*, for the AfAA6 locus.

The marker $CS25_{997}$ from Ill. 547-1 was cleaved by *Rsa I* (Fig. 2). This restriction site is absent in many varieties, including *V. vinifera* cultivars. On the other hand, a restriction site for *Taq I*, absent in Ill. 547-1, is present in most genotypes tested, including 'Horizon', 'Traminette', NY 73.0136.17, and the vinifera cultivars tested (Fig. 2). These differences were used to identify the homologous alleles in crosses with NY 88.0514.03. However, it was not possible to differentiate the two alleles of Ill. 547-1 at this locus by differences in restriction sites. Consequently, when Ill. 547-1 was used as a parent, alternative markers were used: STS-AA6/*Hae III* (Fig. 3) in the cross J.S. 23-416 x Ill 547-1 and the RAPD marker CS25b in the cross 'Horizon' x Ill. 547-1.

The original RAPD marker CS25b was monomorphic except in the cross 'Horizon' x lll. 547-1. Even in this case, it was very sensitive to PCR conditions, especially Mg concentration, in the PCR reaction mixture. Polymorphism was best observed with 2.0 to 2.3 mM of Mg. The band tended to be monomorphic above 2.5 mM and was inconsistently present below 1.8 mM.

These crosses are part of the grape breeding program at Cornell University, Geneva, N.Y. One parent in each cross was highly powdery mildew resistant, either Ill. 547-1 or its progeny NY88.0514.03. The seedlings of J.S. 23-416 x Ill. 547-1 were 6-year-old vines and were evaluated under field conditions without fungicides. The other crosses were made in 1996. Seedlings were then planted in a nursery in Apr. 1997, spaced 0.9 × 1.5 m between plants, and evaluated for powdery mildew resistance under field conditions without application of inoculum or fungicides.

Evaluation of powdery mildew resistance was made by field inspection in mid summer (August 1997), when differences among genotypes were clear. Scores were based on visual estimates of percent leaf area covered by mycelia, and scored from 1 to 5 using the following scale: 1 is up to 3% leaf area with mycelial growth, resistant; 2 is >3% to 12%, partially resistant; 3 is >12% to 25%, tolerant; 4 is >25% to 50%, susceptible; and 5 is >50%, highly susceptible.

Results and Discussion

MARKER DEVELOPMENT. This study began after discovery of a very strong QTL for powdery mildew resistance in the genetic
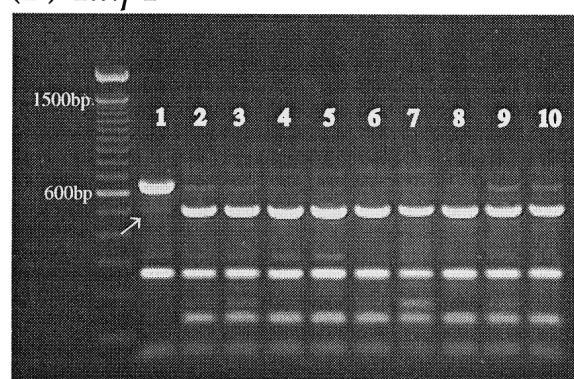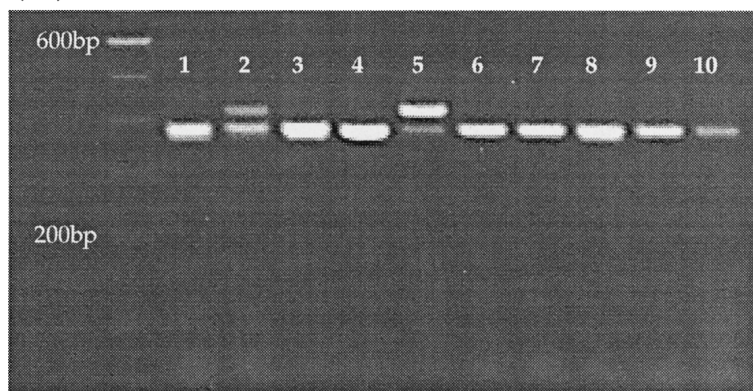
(A) *Rsa* I

(B) *Taq* I

Fig. 2. Marker CS25b997 amplified from ten genotypes (*Vitis vinifera* and interspecific hybrids) followed by digestion with either *Rsa* I or *Taq* I. The cultivars are as follows: lane 1, Illinois 547-1; lane 2, 'Horizon'; lane 3, J.S. 23-416; lane 4, 'Traminette'; lane 5, NY73.0136.17; lane 6, 'Riesling'; lane 7, 'Chardonnay'; lane 8, 'Gewürztraminer'; lane 9, 'Chenin blanc'; lane 10, 'Pinot noir'. Differences in band patterns represent differences in restriction sites and are indicated by arrows. The original band size was 997 bp.

(A) STS-AA6

(B) STS-AA6/*Hae* III

Fig. 3. (**A**) Marker STS-AA6 amplified from 10 grape genotypes (*Vitis vinifera* and interspecific hybrids). The cultivars are numbered as follows: lane 1, Illinois 547-1; lane 2, 'Horizon'; lane 3, J.S. 23-416; lane 4, 'Traminette'; lane 5, NY73.0136.17; lane 6, 'Riesling'; lane 7, 'Chardonnay'; lane 8, 'Gewürztraminer'; lane 9, 'Chenin blanc'; lane 10, 'Pinot blanc'. (**B**) Same as in A but cut with the restriction enzyme *Hae* III. The restriction fragment used to identify the allele containing a QTL for powdery mildew resistance is indicated by an arrow.

ASAP MARKER. The presence of two rare point mutations affecting the restriction sites of *Taq I* and *Rsa I* in the DNA sequence of the $CS25_{997}$ fragment (Fig. 2) allowed us to develop an ASAP marker ($CS25_{333}$) for this locus. We tried to design primers with the point mutation at the 3' end. However, one primer had to be extended two bases to prevent self annealing during PCR amplifications (see Materials and Methods).

The objective was to obtain a PCR-based marker which would amplify only when the allele associated with the QTL for powdery mildew resistance was present. Even with the limitation of having just one primer with the differential base at the 3' end, it was

possible to get a presence/absence type of segregation with the marker $CS25_{333}$ (Fig. 4). However, polymorphism was only apparent under precisely controlled reaction conditions. Small changes (1 to 2 °C) in the annealing temperature resulted in loss of polymorphism. Consequently, this marker may be subject to misscoring due to uncontrolled variations in PCR conditions.

ASAP markers are advantageous to use since gel separation is not required. Differences between presence and absence of the marker in the PCR reaction can be observed visually under UV light (Fig. 4). Using a fluorometer equipped with a plate reader, differences in fluorescence can be measured directly. Our experience indicates that by setting excitation at 360 nm and emission at 620 nm, the differences between presence and absence of a PCR product are detected more clearly, although the readings are usually low (Fig. 4). The readings varied from 4.0 to 12.0 units between samples with readings above 8.0 units for samples with the marker and below 5.0 units for the samples without it.

Use of the marker $CS25_{333}$ can be more useful when large numbers of samples must be scored, but it is more error-prone compared to CAPS markers. False negatives could occur because failures of PCR ampli-
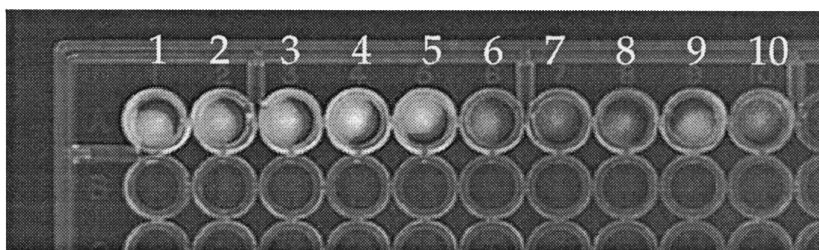
(A)

(B)

Fig. 4. ASAP marker $CS25_{333}$ amplified from 10 different individuals of the progeny NY88.0514.03 x NY73.0136.17 and visualized by two different methods. (**A**) Direct observation on an ELISA plate after addition of 50 ng of ethidium bromide to each PCR reaction. (**B**) The same samples separated in a 2% agarose gel.

Table 2. Segregation ratios for the locus CS25b in different seedling populations.

Population	CS25b+	CS25b–	Observed ratio	Expected ratio	χ^2	P
'Horizon' x Ill. 547-1	33	25	1.32 : 1	1 : 1	1.103	0.294
'Horizon' x Ill. 547-1(new cross)	25	48	0.52 : 1	1 : 1	7.247	0.007
J.S. 23-416 x Ill. 547-1[z]	31	18	1.72 : 1	1 : 1	3.449	0.060
NY88.0514.03 x 'Traminette'	17	55	0.31 : 1	1 : 1	20.056	0.000
NY88.0514.03 x NY73.0136.17	39	63	0.62 : 1	1 : 1	5.647	0.017

[z]Results based on marker STS-AA6/*Hae III*. In the other crosses, markers $CS25_{997}$/*Rsa* I or CS25b were used.

fication would be confounded with absence of the marker. Consequently, we used CAPS markers ($CS25_{997}$/*Rsa I* and STS-AA6/*Hae III*) for the genetic segregation analysis.

Distortion of segregation ratios. Distorted segregation ratios between the alleles of CS25b locus were observed in some populations. In these cases, the allele associated with the QTL for powdery mildew resistance was less frequent than the alternate allele (Table 2). The exception was the cross J.S. 23-416 x Ill.

547-1, where some selection for powdery mildew resistant genotypes had been applied previously to that population.

Segregation distortion was observed in 'Horizon' x Ill. 547-1 when the cross was remade in 1996 but not in the first cross, made in 1988. The reasons for variations in segregation ratios observed herein are unknown but similar distortions have been observed in other species (Davis and Yu, 1997). Distortions, as observed in the present study, can lead to f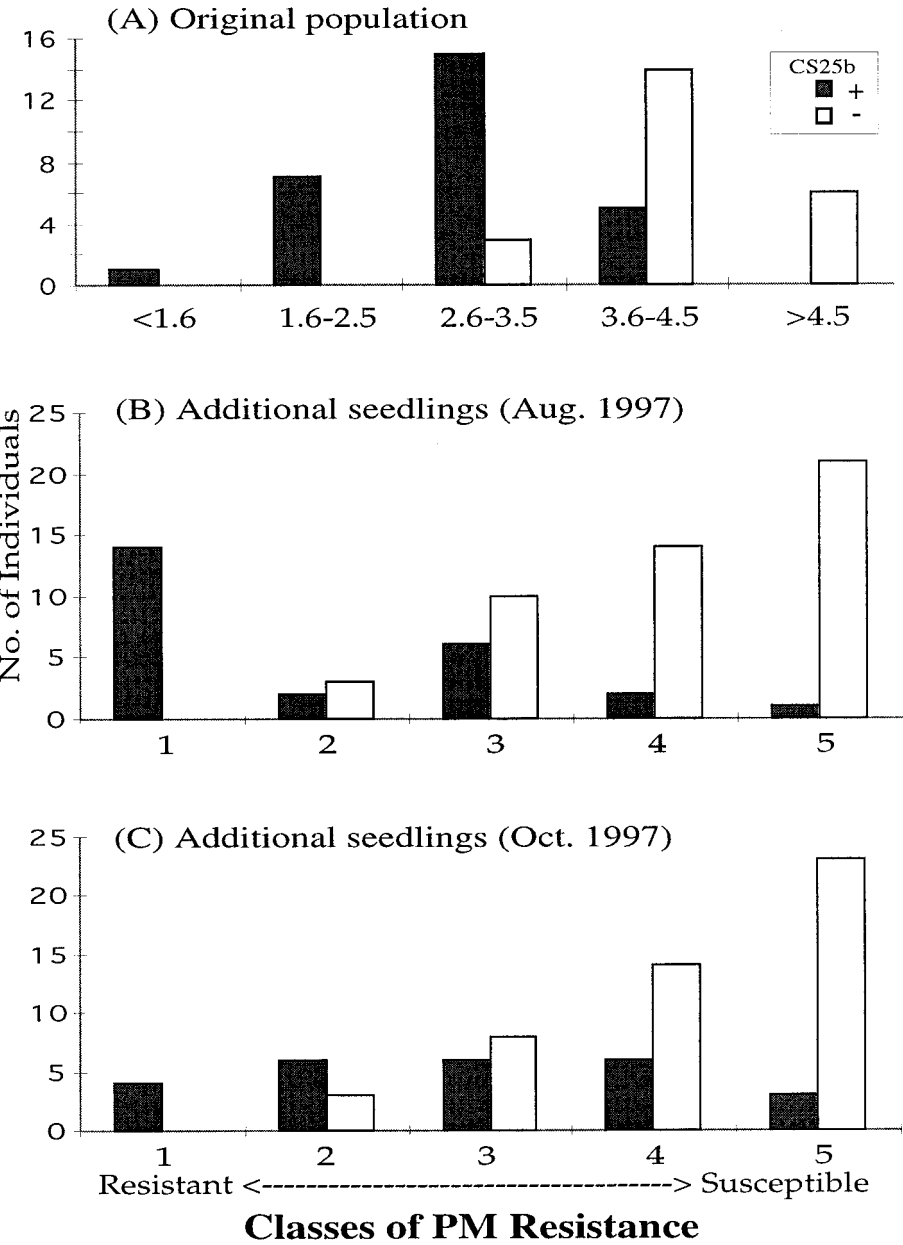aulty conclusions when genetic analyses are made based only on phenotypes. For example, the low frequency of resistant individuals in the progeny NY88.0514.03 x 'Traminette' might be interpreted as interactions of genes instead of segregation distortions at a major locus.
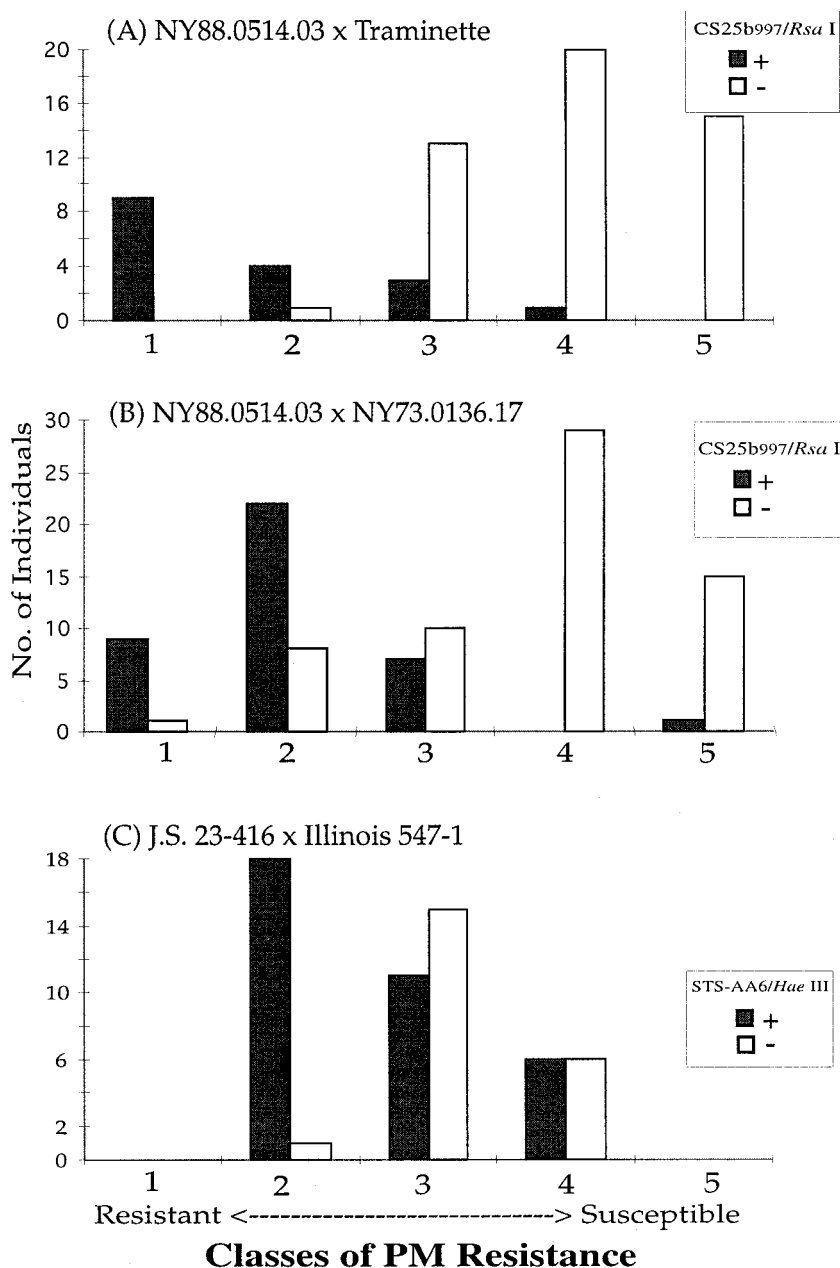
Powdery mildew resistance. The efficiency of marker CS25b in predicting powdery mildew resistance was confirmed when the cross 'Horizon' x Ill. 547-1 was remade and new seedlings were tested under field conditions (Fig. 5). The frequencies of resistance classes varied, probably due to different conditions for evaluation. In the original population (based on 5 years field data), there is a tendency to have more individuals in the intermediate classes. In the new cross (based on a single year of evaluation), the presence of individuals in the extreme classes was more frequent.

Powdery mildew resistance was normally evaluated in midsummer (20 to 30 Aug.). At this time, differences among plants were clearly visible. Later in the season, many plants classified previously as resistant were increasingly infected. Usually, this late infection was superficial, with little damage to the leaves. Some plants also showed mechanisms of tolerance, developing fewer necrotic symptoms even when visual symptoms of mycelial colonization appeared early on the leaves. These variations were not classified in our analysis.

For the cross 'Horizon' x Ill. 547-1 (new population), the comparison of the midsum-

(A) Original population

(B) Additional seedlings (Aug. 1997)

(C) Additional seedlings (Oct. 1997)

No. of Individuals

Resistant <------------------------------------> Susceptible

Classes of PM Resistance

Fig. 5. Frequency distribution of individuals by class of powdery mildew resistance and presence of the marker CS25b (from Ill. 547-1) in three segregating populations originating from the crosses: (**A**) 'Horizon' x Ill. 547-1, mapping population (average of 5 years data). (**B**) 'Horizon' x Ill. 547-1, one year-old seedling population, evaluated in midsummer (August, 97). (**C**) Same as in B but with disease severity evaluated later in the season (October, 97).

(A) NY88.0514.03 x Traminette

(B) NY88.0514.03 x NY73.0136.17

(C) J.S. 23-416 x Illinois 547-1

Resistant <----------------------------> Susceptible

Classes of PM Resistance

Fig. 6. Frequency distribution of individuals by class of powdery mildew resistance and presence of the CAPS markers $CS25b_{997}$/*Rsa* I or STS-AA6/*Hae* III in three segregating seedling populations.

mer (26 Aug. 1997) and late (10 Oct. 1997) evaluations is presented in Fig. 5. The tendency to have more individuals in the susceptible classes were more accentuated for plants carrying the marker. Apparently, the gene(s) at this locus may act by delaying fungal colonization but other genes may be necessary to prevent late season mildew growth.

The next step was to observe segregation of this QTL in different crosses. We used seedling populations that are part of the grape breeding program at Cornell University, Geneva, N.Y. In all cases, the crosses analyzed descended from the same source of powdery mildew resistance used in this study, Ill. 547-1.

For the cross J.S. 23-416 x Ill. 547-1, the population consisted of 6-year-old vines grown continuously under fungicide-free conditions. None were rated 5 (highly susceptible) in this population. This was probably due to prior selection for field-based

disease resistance that took place in years 1 and 2, and to the contribution of the female parent (J.S. 23-416), a moderately resistant cultivar. Even in these circumstances, there was a clear trend for seedlings containing the marker to belong to the more resistant classes (Fig. 6).

Two other populations descending from a resistant seedling (NY 88.0514.03) of the original mapping population were also studied. Segregation of the QTL for powdery mildew resistance was followed using a CAPS marker ($CS25_{997}$/*Rsa* I). In both crosses there was a clear association between presence of the marker and powdery mildew resistance (Fig. 6). A simulated situation, where only plants carrying the marker were selected, is presented in Table 3. The percentage of susceptible individuals (classes 4 and 5) without selection ranged from 24.5% to 52.1%. If only the plants carrying a marker (CS25b or AfAA6) linked to the QTL were saved, the percentage would be only 2.6% to 18.2% (see Table 3). Selection efficiency was greatest in crosses where segregation distortion was most intense, such as NY88.0514.03 x 'Traminette'.

Another use for marker-assisted selection is to facilitate introgression into *V. vinifera* of only a small portion of the genome of a wild species containing genes of interest with a backcrossing scheme. One major problem for conventional breeding is that disease resistant species, such as *V. cinerea* (the likely source of powdery mildew resistance in this case), contribute many genes for low fruit quality. QTL analysis can identify specific genomic regions containing genes for resistance that can be tracked in the following generations with linked markers. With selection based on markers, methods to speed up generation cycles in grapevines can be used to accelerate the process (Srinivasan and Mullins (1981). Although some undesirable genes may be linked to selective markers, most will be transmitted at smaller proportions compared to unselected crosses. The same principles are used in the advanced backcross selection technique (Tanksley and Nelson, 1996) that has been used to incorporate genes from wild species into varieties of self-pollinated crops.

Results herein also help to understand the nature of powdery mildew resistance in grapes, which is reportedly polygenic (Boubals, 1961; Eibach, 1994). Our results indicate that resistance may be due to the action of more than one gene, but at least one region of the grape genome contains genes or a gene cluster with a major effect on expression of resistance. The source of resistance studied provided similar results in crosses with cultivars of varying genetic backgrounds. Results remain to be tested in crosses of materials from the *V. cinerea* background with vinifera cultivars.

A potential concern is that our results were derived from a single location. However, previous works showed that natural

Table 3. Percentage of resistant (R) (classes 1, 2 and 3) and susceptible (S) (classes 4 and 5) individuals in the progeny of different crosses in either the whole population or in a population following simulated selection based on CS25b or AfAA6 loci.

| | No. | Vines | Before selection | | After selection | |
| | of | with | R | S | R | S |
Population	vines	marker (no.)	(%)	(%)	(%)	(%)
'Horizon' x Ill. 547.1	58	33	53.4	46.6	81.8	18.2
'Horizon' x Ill. 547-1 (new)	73	25	47.9	52.1	88.0	12.0
J.S. 23-416 x Ill. 547-1 [z]	49	31	75.5	24.5	87.1	12.9
NY88.0514.03 x 'Traminette'	72	17	51.4	48.6	94.1	5.9
NY88.0514.03 x NY73.136.17	102	39	56.9	43.1	97.4	2.6

[z]Results based on marker STS-AA6/*Hae III*.

populations of the fungus *Uncinula necator* are highly variable (Gadoury and Pearson, 1991). In the region of Geneva, New York, there are multiple pathotypes and the fungus undergoes a sexual stage prior to initiating a new season's disease cycle (Pearson and Gadoury, 1987). Because these evaluations were conducted under natural conditions for infection, it is likely that a large range of *U. necator* pathotypes were present and that resistance is stable.

We worked with just one QTL from a single source of resistance. It provided just partial resistance and could be complemented by other QTLs from other sources of resistance. Similar methodology can be used to find other QTLs in other resistant species of *Vitis*. This would offer the possibility to pyramid genes in order to achieve high levels of resistance using a small portion of the genome of wild species. The same rationale is valid for other grape diseases, traditionally considered to be under quantitative genetic control (Reisch and Pratt, 1996).

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