OPTIMIZING MARKER-ASSISTED SELECTION FOR RESISTANCE TO *XYLELLA FASTIDIOSA* TO ACCELERATE BREEDING OF PIERCE'S DISEASE RESISTANT GRAPES OF HIGH FRUIT QUALITY

Project Leaders:

Andrew Walker
Dept. of Viticulture and Enology
University of California
Davis, CA 95616
awalker@ucdavis.edu

Alan Krivanek
Dept. of Viticulture and Enology
University of California
Davis, CA 95616

Summaira Riaz
Dept. of Viticulture and Enology
University of California
Davis, CA 95616

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ABSTRACT

Efforts at identifying molecular markers linked to *Xylella fastidiosa* (*Xf*) resistance are continuing. Our primary focus is on resistance derived from b43-17, a *Vitis arizonica/candicans* type collected near Monterrey, Nuevo Leon, Mexico. The '9621' *V. rupestris* x *V. arizonica* hybrid mapping family (PD resistant D8909-15 x PD resistant F8909-17) was used to localize *PdR1*, a primary PD resistance locus within the linkage map of the male parent F8909-17 (progeny of b43-17) and identify candidate linked resistance markers. In more recent research, a comparative mapping strategy between the '9621' linkage map and other SSR maps within *Vitis* was used to identify 9 SSR markers within 10 cM of the resistance locus. Resistance from the female parent D8909-15 has not yet been localized to a genetic map. The strategy of bulk segregant analysis (BSA) in concert with the AFLP marker system has been initiated to saturate the region around the resistance locus and is expected to yield an additional 20 to 50 markers linked to the resistance trait. All candidate resistant markers have been and will continue to be applied to breeding populations derived from '8909' x *V. vinifera* and ('8909' x *V. vinifera*) x *V. vinifera* back-cross generations in order to confirm resistance marker effectiveness in *V. vinifera* backgrounds and continue with marker assisted selection for development of high quality PD resistant grapes.

INTRODUCTION

Several American *Vitis* species are native to the regions where PD is endemic, and resistance from these sources has been introgressed into many different cultivars grown in the south-eastern United States. The acceptance of the new hybrid cultivars has been limited due in part to some undesirable non-vinifera fruit quality traits. The development of high quality PD resistant cultivars will be facilitated by the use of molecular markers to achieve a more precise introgression of the resistance genes into domesticated backgrounds and avoid introgression of undesirable traits (Figure 1). Backcross introgression via molecular markers has been accomplished successfully in other crops (Young and Tanksley 1989). This type of introgression is generally termed Marker Assisted Selection (MAS), whereby indirect selection on a trait of interest (such as disease resistance) is made by screening for the presence of a DNA marker allele tightly linked to the trait. MAS for disease resistance can also be used to eliminate susceptible genotypes in a breeding population early in the selection process, which allows for evaluation of much larger effective populations. Larger effective population sizes increase the opportunity to identify genotypes with high disease resistance and good horticultural qualities (such as good flavor traits, color, berry and cluster size. etc.). Other key aspects of the MAS process include avoiding confounding environmental effects on the trait phenotype and accelerating breeding progress while saving space and time, allowing for more efficient use of resources (Paterson et al. 1991, Kelly 1995). Rapid screening time is particularly valuable when applied to perennial crops such as grape with relatively long generation times (Alleweldt 1988, Striem et al. 1994). To effectively use linked markers in MAS only requires that the markers be highly reproducible, linked in coupling phase i.e. on the same homologous chromosome, and within 5 centimorgan (cM) mapping units of the resistance locus (Kelly 1995).

Within grapevines, markers linked to powdery mildew resistance (Dalbo et al. 2001, Pauquet et al. 2001), downy mildew resistance (Luo et al. 2001) and seedlessness (Lahogue 1998) have been published. In the case of powdery mildew resistance, MAS has already been successfully utilized for screening a grape breeding population. We are successfully developing a MAS system for screening PD resistant genotypes that will greatly benefit our breeding of PD resistant wine grapes.

OBJECTIVES

Our overall objective is to identify DNA markers that are tightly linked to the primary locus or loci required for complete resistance to PD within *Vitis*. Research will focus on PD resistance as inherited from *V. arizonica* and will utilize an established *V. rupestris* x *V. arizonica* genetic map. These markers will be utilized for MAS to eliminate susceptible seedling progeny our continuing PD resistance breeding program.

Sub-objectives

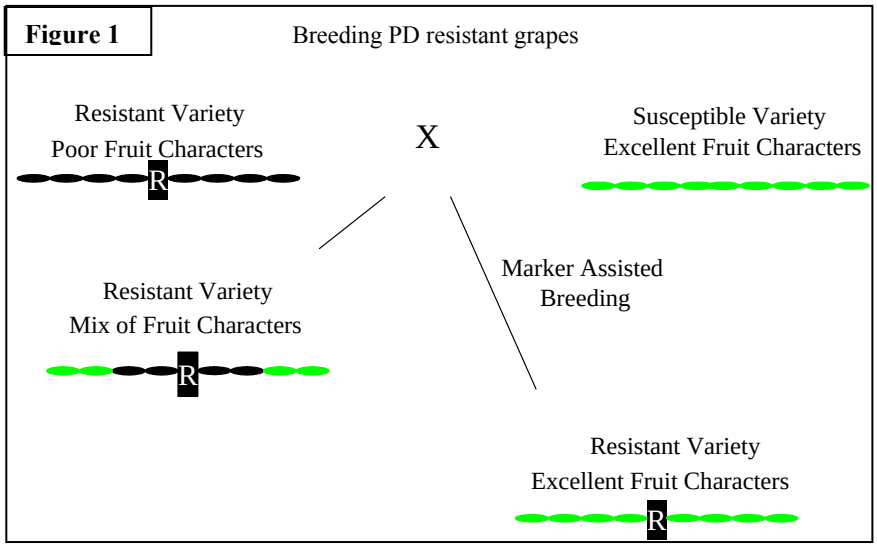
1. Continue with a comparative mapping strategy between the *V. rupestris* x *V. arizonica* 9621 (D8909-15 x F8909-17) linkage map and other SSR maps within *Vitis* in order to identify additional SSR markers linked to resistance.

2. Utilize Bulk Segregant Analysis (BSA) with the AFLP marker system to saturate with markers the region around the previously mapped *Xf* resistance locus and eventually convert confirmed candidate markers to stable SCAR primers.
3. Confirm candidate marker linkage to resistance within families derived from resistant by susceptible crosses such as the '8909' x *V. vinifera* and ('8909' x *V. vinifera*) x *V. vinifera* back-cross generations.

RESULTS AND CONCLUSIONS

Sub-objective 1.

Initial mapping of the PD resistance locus *PdR1* in the male parent F8909-17 of the 9621 family localized it to chromosome 14, and identified 6-8 SSR markers on the same linkage group. Marker placement on published SSR linkage maps of *Vitis* were used to preferentially target chromosome 14, bringing the total number of SSR markers on the linkage group up to 30. Approximately 9 SSR markers are localized within a 10 cM distance of the resistance gene. These SSR markers are reliable and are the easiest of the molecular markers to incorporate within a MAS breeding program. Correlation tests of these candidate markers to PD resistance when functioning within a *V. vinifera* genetic background are underway and described in sub-objective 3. The SSR marker analysis has allowed us to confirm that marker alleles linked in coupling to PD resistance alleles of the *PdR1* locus in another PD resistant progeny of b43-17 (F8909-08) are different than the alleles linked in coupling the resistance alleles in F8909-17. It is apparent from these results that b43-17 is homozygous resistant for the PdR1 locus, and that F8909-17 inherited its resistance allele from one chromosome 14 and F8909-08 inherited its resistance allele from the homologous chromosome 14. In either case the markers linked to resistance will function for MAS, however, different alleles linked in coupling to the resistance alleles will have to be followed through the downstream MAS process. Placement of SSR markers to chromosome 14 via the comparative mapping strategy continue as the markers become available, however, the number of SSR markers that can be targeted to a specific chromosomal region via comparative mapping is limited.



Sub-objective 2.

For high density marker saturation within a narrow window around the *PdR1* locus, a bulk segregant analysis (BSA) strategy (Michelmore et al. 1991) in concert with the AFLP marker system was chosen as the method of choice. Initial BSA was attempted within the 9621 family, however, confounding effects of the resistance loci within the D8909-15 parent made the attempt more difficult than expected. To avoid confounding affects from resistance inherited from other genetic backgrounds and focus the BSA procedure only on the *PdR1* locus, work has begun within two segregating families from susceptible by resistant crosses. The first family, 99217 (C8909-07 x F8909-08) consists of 33 genotypes, has been screened for PD resistance (Krivanek et al. submitted) and segregates 1:1 resistant to susceptible (Table 1). DNA has been extracted from these genotypes, flanking SSR markers were run and a good correlation between resistance and resistance marker alleles has been established (Table 1). A bulk of the DNA from the 12 most susceptible and a bulk of the DNA from the 12 most resistant genotypes are in process and will be tested for AFLP polymorphisms utilizing florescent primers and visualized on a PE 3100 sequencer. The second family derived from a susceptible by resistant cross is a *V. vinifera* x F8909-08 family; it consists of 40 genotypes and has been designated as 0062. Testing of this family for PD resistance is currently underway via our standard greenhouse testing procedure (Krivanek et al. in press; Krivanek and Walker in press). It is expected that the progeny in this family will segregate in a 1:1 manner, and if so, DNA extraction and BSA procedures will be undertaken as with the 99217 family. Candidate AFLP markers will be converted to stable and more reliable SCAR primers before incorporation into the MAS program.

Sub-objective 3.

Work is progressing with two distinct breeding populations for testing of candidate resistance markers and initial application of those markers to MAS. One family is a cross of the PD resistant F8909-08 to a female *V. vinifera* wine grape F2-7 (Cabernet Sauvignon x Carignane) and designated as the 0062 family. A second breeding population consists of a cross of F8909-08 to several elite *V. vinifera* table grape genotypes (the 500 series). A subset of the 500 series has been screened for PD resistance and screened for markers flanking the *PdR1* locus. Five confirmed resistant genotypes have been utilized in the development of the first backcross generations BC1 (backcrossed to additional elite *V. vinifera* genotypes). The BC1 population (25000 series) consists of approximately 200 individuals and was planted in the field in 2003. Marker analysis for flanking markers to the *PdR1* locus has been completed for the 25000 series and the marker information was utilized in selection of genotypes for the spring of 2004 crosses for the development of the BC2 generations. Subsets of candidate

resistant and susceptible genotypes within the 25000 series have shown improved fruit quality (Figure 2) and are currently being screened to confirm the correlation between the resistance markers and the PD resistance trait. We are also utilizing these populations to confirm the effectiveness and economics of the MAS relative to our greenhouse screening procedure.

Table 1. Resistance classification and marker genotypes for the individuals of the full-sib family derived from the susceptible by resistant cross of C8909-07 x F8909-08. * = Genotypes selected for Bulk Segregant Analysis procedure.

Genotype	Overall resistance level to PD	Mean natural log (cells/ml)	Mean CMI score	Mean % leaf scorch	Alleles of SSR markers flanking the PdR1 resistance
99217-21 *	Resistant	9.51	1.00	58.3	Rr / Rr
99217-40 *	Resistant	9.70	1.33	75.0	rr / Rr
99217-18 *	Resistant	9.77	2.75	95.0	Rr / Rr
99217-41 *	Resistant	10.19	4.25	76.3	Rr / Rr
99217-35 *	Resistant	10.55	1.33	100.0	rr / Rr
99217-19 *	Resistant	11.08	2.50	76.7	rr / Rr
99217-01 *	Resistant	11.52	2.25	90.0	rr / Rr
99217-23 *	Resistant	11.57	3.00	87.5	Rr / Rr
99217-34 *	Resistant	11.83	3.75	65.0	Rr / Rr
99217-46	Resistant	11.87	5.75	100.0	Rr / Rr
99217-27 *	Resistant	12.20	4.25	100.0	Rr / rr
99217-22 *	Resistant	12.29	4.00	100.0	Rr / Rr
99217-12 *	Resistant	12.50	4.00	95.0	Rr / Rr
99217-38	?	12.69	5.00	100.0	Rr / Rr
99217-36	?	13.09	5.00	100.0	rr / rr
99217-50	?	13.52	4.25	83.8	Rr / Rr
99217-14	Susceptible	14.06	5.50	88.8	rr / Rr
99217-07	Susceptible	14.87	5.50	100.0	rr / rr
99217-04 *	Susceptible	15.42	6.00	100.0	rr / rr
99217-33 *	Susceptible	15.59	5.75	100.0	rr / rr
99217-06 *	Susceptible	15.80	5.25	68.3	rr / rr
99217-09 *	Susceptible	15.81	5.75	100.0	rr / rr
99217-10	Susceptible	15.82	4.75	100.0	rr / rr
99217-13 *	Susceptible	15.84	5.50	100.0	rr / rr
99217-42	Susceptible	15.85	4.25	75.0	rr / Rr
99217-15 *	Susceptible	15.87	5.25	100.0	rr / rr
99217-32 *	Susceptible	15.87	5.50	100.0	rr / rr
99217-28 *	Susceptible	15.91	5.75	100.0	rr / rr
99217-05 *	Susceptible	15.91	5.75	100.0	rr / rr
99217-37 *	Susceptible	15.92	5.25	100.0	rr / rr
99217-26 *	Susceptible	15.95	5.50	100.0	rr / rr
99217-24 *	Susceptible	16.04	6.00	100.0	rr / rr

Figure 2.

Vitis arizonica PD Resistant poor fruit quality

Hybrid BC1-25017 with flanking PD resistance markers Improved fruit quality

Vitis vinifera PD Susceptible Excellent fruit quality

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