MARKER-ASSISTED SELECTION FOR RESISTANCE TO *XYLELLA FASTIDIOSA*: ACCELERATED BREEDING OF PIERCE'S DISEASE RESISTANT GRAPES

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ABSTRACT
Efforts at identifying genetic markers tightly linked to *Xylella fastidiosa* (*Xf*) resistance are continuing. These efforts are primarily focused on the 9621 mapping population (D8909-15 x F8909-17) and are in cooperation with fine-scale mapping efforts discussed in our report "Map based identification and positional cloning of *Xylella fastidiosa* resistance genes from known sources of Pierce's disease resistance in grape." This project is adding 200 SSR markers to the 9621 map and has positioned *Xf* resistance from the male parent F8909-17 on the lower arm of linkage group 14, where *Xf* resistance is flanked by multiple markers. Resistance from the female parent D8909-15 maps as a quantitative trait locus. The addition of additional SSR marker is expected to reduce the distance between SSR markers and *Xf* resistance to about 2 cM and lead to the development and utilization of very specific sequence characterized amplified region (SCAR) primers. These markers will be applied to populations in the breeding program derived from D8909-15 and F8909-17 and tested for effectiveness in *Xf* resistance backgrounds derived from other species.

INTRODUCTION
Marker Assisted Selection (MAS) is the process 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 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 high horticultural qualities (such as good fruit size, color, texture etc.). Other key aspects of the MAS process is that confounding environmental effects on the trait phenotype can be avoided and progress in breeding programs can be accelerated 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).

Markers linked to grape resistance genes of other diseases have been published. AFLP and RAPD markers tightly linked to powdery mildew resistance (Dalbo et al. 2001, Pauquet et al. 2001) and downy mildew resistance (Luo et al. 2001) are some examples. To effectively use such 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 cM (cM = centimorgan, a mapping unit representative of the distance between two loci or genes) of the resistance locus (Kelly 1995). Conversion of AFLP and RAPD markers to SCAR primers allows for a more reproducible marker system and identifying tightly linked markers is a direct function of numbers of markers screened. In the case of powdery mildew resistance MAS has already been successfully utilized for screening a grape breeding population (Dalbo et al. 2001) and it is expected that this project will have a high chance of success for developing a functional MAS system for screening PD resistant genotypes. Markers tightly linked to PD resistance should have immediate benefits toward accelerating the breeding of PD resistant wine, table and raisin cultivars.

OBJECTIVES
1. Refine localization of primary QTL's associated with PD resistance derived from *Vitis arizonica.*
2. Saturate regions of primary QTL's with AFLP markers via Bulk Segregant Analysis (BSA).
3. Identify tightly linked flanking markers around PD resistance QTL's and convert to SCAR primers.
4. Confirm candidate marker linkage to resistance within a (8909 x *V. vinifera* table grape) family.
5. Utilize resistance markers to eliminate susceptible progeny within a (8909 x *V. vinifera*) x *V. vinifera* table grape backcross generation and future generations of the continuing UCD/USDA collaborative PD resistance breeding program.

RESULTS AND CONCLUSIONS
Objective 1. This proposal expands upon a portion of a project funded by the AVF and last year by the CDFA entitled "The Genetics of Resistance to PD". That project developed a genetic map in a *Vitis rupestris* x *V. arizonica* population (9621 = D8909-15 x F8909-17; see Walker, Tenscher, Ramming Progress Report in this proceedings for more detail on this population) segregating for *Xylella fastidiosa* (*Xf*) resistance and was based on about 500 DNA markers. The parents of this cross were half siblings sharing a common *V. rupestris* parent which is susceptible to PD. The progeny D8909-15 is a female

vine and derives its PD resistance from a *V. arizonica* collected in Baja California. The progeny F8909-17 is a male vine and derives its PD resistance from an apparent *V arizonica / V. champinii* hybrid collected in northern Mexico west of Monterrey. PD resistance from the F8909-17 male vine has been localized to the lower arm of a single linkage group when resistance is mapped as a single dominant trait. PD resistance for the D8909-15 female parent cannot be localized on the map when scoring PD resistance as a single dominant trait. When scoring PD resistance in a quantitative manner, preliminary results indicate that this resistance is localized at multiple positions (i.e. QTL's within the D8909-15 genome).

Progress has been made on expanding this map with additional individuals and developing a framework map based on highly reproducible SSR markers. The map now includes 188 individuals with SSR marker data and 140 individuals that have PD resistance data. To date, approximately 310 SSR markers have been tested and 165 of them proved to be useful within the 9621 mapping population. Our objective is place a total of 200 SSR markers on the framework map such that each linkage group will have 8-10 highly reproducible markers. Markers will be selected for even spacing across the genome at distances of 5-7cM, which will allow good coverage for refined QTL mapping.

Objective 2. Thus far we have identified one SSR marker and 2 AFLP markers linked in coupling phase within ~ 10cM of the primary *Xf* resistance locus of F8909-17. Following accurate placement of the F8909-17 locus and D8909-15 *Xf* resistance QTL's on the framework map in Objective 1, Bulk Segregant Analysis (BSA) (Michelmore et al. 1991) will be used to saturate regions of resistance loci with AFLP markers. We have arrangements to use a PE 3100 sequencer for large scale marker screening. A goal of 200 primer combinations should lead to identification of 2-4 markers flanking the *Xf* resistance gene within a 2-cM window. Utilization of SSR markers to link the 9621 map other genetic maps should also lead to selective placement of a variety of markers around primary *Xf* resistance QTL's.

Objective 3. Candidate AFLP markers linked to *Xf* resistance identified in Objective 2 will be confirmed by separately evaluating marker patterns on each individual within the DNA bulks. The framework 9621 population will be used to precisely localize all confirmed resistance markers, after which Sequence Characterized Amplified Region (SCAR) primers will be developed from the tightly flanking markers.

Objective 4. A family derived from an D8909-15 x *vinifera* table grape (0023) has been evaluated for PD symptoms based on cane maturation, and *Xf* bacteria numbers in stem tissue based on ELISA data at 16 weeks post inoculation. DNA has been extracted from these screened genotypes and each will be tested for the presence or absence of resistance markers identified in Objective 3, so as to confirm the correlation with the resistance phenotype. Correlation of the markers with resistance will be recalculated if different from the original mapping population.

Objective 5. A backcross population (D8909-15 x *vinifera* table grape) x an advanced seedless *vinifera* table grape was made in the spring of 2002 and 2003. Highly resistant genotypes: 0023-19, 0023-54, 0023-63, 0023-98 (stem bacteria numbers of less than 60,000 cells/ml) were backcrossed to several advanced table grape selections in order to establish a large advanced breeding population. From the 2002 crosses 1,693seeds were collected and 654 seedlings were planted in the field the summer of 2003. Markers shown to be linked to resistance in Objectives 1-4 will be used on this population to identify candidate resistant and susceptible genotypes to confirm the effectiveness and economics of the MAS relative to our greenhouse screening procedure.

REFERENCES

Alleweldt, G., and J.V. Possingham. 1988. Progress in grapevine breeding. Theor. Appl. Genet. 75: 669-673.

Dalbo, M.A., G.N. Ye, N.F. Weeden, W.F. Wilcox, and B.I. Reisch. 2001, Marker-assisted selection for powdery mildew resistance in grapes. J. Am. Soc. Hort. Sci. 126: 83-89.

Kelly, J.D. 1995. Use of random amplified polymorphic DNA markers in breeding for major gene resistance to plant pathogen. HortScience 30: 461-465.

Lahogue, F., P. This, and A. Bouquet. 1998. Identification of a codominant SCAR marker linker to the seedlessness character in grapevine. Theor. Appl. Genet. 97: 950-959.

Luo, S.L., P.C. He, P. Zhou, and X.G. Zheng. 2001. Identification of molecular genetic markers tightly linked to downy mildew resistant genes in grape. Acta Genet. Sinica 28: 76-82.

Michelmore R.W., I. Paran , R.V. Kesseli. 1991. Identification of markers linked to disease-resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. Proc Natl Acad Sci USA 88: 9828-9832.

Paterson, A.H., S.D. Tanksley, and M.E. Sorrells. 1991. DNA markers in plant improvement. Adv. Agr. 46: 39-90.

Pauquet, J., A. Bouquet, P. This, and A-F Adam-Blondon. 2001. Establishment of a local map of AFLP markers around the powdery mildew resistance gene Run1 in grapevine and assessment of their usefulness for marker assisted selection. Theor. Appl. Genet. 103: 1201-1210.

Striem, M.J., G. Ben-Hayyim, and P. Spiegel-Roy. 1994. Developing molecular genetic markers for grape breeding, using polymerase chain reaction procedures. Vitis 33: 53-54.

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