MAP BASED IDENTIFICATION AND POSITIONAL CLONING OF *XYLELLA FASTIDIOSA* RESISTANCE GENES FROM KNOWN SOURCES OF PIERCE'S DISEASE RESISTANCE IN GRAPE

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ABSTRACT
Genetic mapping efforts continue in the 9621 population (D8909-15 x F8909-17). *Xylella fastidiosa* (*Xf*) resistance is segregating in this population both the *Vitis arizonica* and a *V. arizonica/V. champinii* are resistant. We have increased the mapping population size from 116 to 188 individuals, thus increasing recombination frequencies and taking advantage of 96-well plate based techniques. We are adding the original 375 AFLP markers to the additional 72 individuals. Three hundred and ten SSR markers have been tested of which 192 amplified and 165 were polymorphic in the population – these have been added to the map. Fourteen of 20 EST markers from Doug Adams' lab were also added. We have also added 16 EST derived SSR markers from Doug Cook's database of ESTs with the goal of adding 100 to 150 more by June. *Xf* resistance maps to linkage group 14. There are two flanking SSR markers on one side and an SSR and AFLP marker on the other side of the *Xf* resistance locus derived from F8909-17.

INTRODUCTION
This project expands upon and continues a genetic mapping effort that has also received funding from the California Grape Rootstock Improvement Commission, the Fruit tree, Nut tree and Grapevine Improvement Advisory Board, the California Table Grape Commission and the American Vineyard Foundation. Last year's CDFA GWSS/PD Research Board proposal was entitled "An Expanded Genetic Map of *Vitis rupestris* x *Muscadinia rotundifolia* for Fine Scale Mapping and Characterization of Pierce's Disease Resistance". The name was changed to reflect current knowledge of the mapping population's true parentage. The project has been mapping resistance to *Xiphinema index*, the dagger nematode, and *Xylella fastidiosa* (*Xf*) in an "F2" population made from "siblings" selected from an F1 *V. rupestris* x *M. rotundifolia* population generated by Dr. Olmo in 1988. We have recently discovered that these crosses were largely contaminated by pollen other than the applied *M. rotundifolia* (more details are included in "The genetics of and breeding for Pierce's disease resistant grapes" project summary). The 9621 mapping population is a cross of D8909-15 (*V. rupestris* x *V. arizonica*) x F8909-17 (*V. rupestris* x a *V. arizonica/V. champinii* type). Because of this population's segregation for, and presence of high resistance to, *X. index* and *Xf* it is an extremely valuable asset for map based positional cloning of these resistance genes.

A genetic map of 116 individuals from the 9621 population was created primarily with AFLP markers. The AFLP marker system is very robust, however, dominant inheritance of markers can cause limitations with highly heterozygous crops like grape. We began using more informative markers, such as microsatellites or simple sequence repeats (SSR) for two main reasons. First, a framework genetic map with SSR markers provides essential infrastructure for targeted physical mapping of candidate genes and quantitative trait loci (QTLs). Secondly, SSR markers tightly linked to resistance and phenotypic traits of interest are ideal for marker-assisted selection due to their applicability across different genetic backgrounds. The grape genetic research community formed the International Grape Genome Program (IGGP) to increase coordination and cooperation and to enhance knowledge of the grape genome. Use of the SSR marker system is common among the different research groups so that our mapping efforts can be linked to others. Integrating the 9621 genetic linkage map to other mapping populations will facilitate targeting genomic regions that harbor quantitative trait loci. Comparison to other maps will allow us to identify more markers that are tightly linked to *Xf* resistance and optimize marker-assisted selection strategies in the breeding program (see the "Optimizing marker-aided selection (MAS) for *Xylella fastidiosa* resistance to accelerate the breeding of PD resistant grapes" progress report). It will also more fully support efforts to locate and identify the gene(s) responsible for *Xf* resistance.

OBJECTIVES
1. Increase the core mapping population size from 116 to 188 individuals (more recombinants reduce the distance between markers).
2. Use genomic SSR and ESTP (expressed sequence tag polymorphism) markers as the core marker system and increase the number of SSR markers on the genetic linkage map to 200 (initial efforts were only 100 SSR markers).
3. Screen an additional 100-150 EST derived SSR markers for which functions are known after their comparison to homologues in available EST databases.
4. Develop the core framework map with an average distance of 2-5 cM between markers.

RESULTS AND CONCLUSIONS

Objective 1. The starting material for this project was a complete AFLP marker based genetic map of the 9621 population. This map was initiated several years ago and was based on 116 individuals with 375 AFLP, 32 ISSR, 25 RAPD and 9 SSR markers. We expanded the core set of individuals from the 9621 to 188 individuals and have extracted the complete set of DNA.

Objective 2. The SSR markers used included some that have been previously published and many that were developed by Vitis Microsatellite Consortium. All markers were tested on a small set of 8 DNA samples including both parents and run on 6 % polyacrylamide gels. DNA on the gels was visualized by silver staining with a commercial kit (Promega). Only informative markers were used on the entire set of 188 genotypes. We have tested and used all available informative genomic microsatellite markers for 9621 population. (Table 1).

To develop ESTP markers, sequences of grape cDNA were obtained from Dr. Doug Adams (Department of Viticulture and Enology, UC Davis). Potential PCR primers were designed using the computer program PRIMER 0.5. Primers were selected to have similar properties to facilitate standard conditions for PCR reactions. Primers are 20 to 23 nucleotides long with GC contents of 50-60% and melting temperature ranging from 59-64°C. Amplification and polymorphism for each EST was tested on 2% agarose gels. If length base polymorphism were not revealed, then a set of 10 different restriction enzymes (*Hind*III, *EcoR*I, *Ava* II, *Bst*NI, *Dra*I, H*ae* III, H*inf*1, M*sp* I, *EcoR*V, R*sa* I) were tested to find restriction site based polymorphism among parents D89090-15 and F8909-17. Polymorphic ESTP markers were added on entire progeny of 188 genotypes.

Table 1. Data on number of markers tested and useful for the D8909-15 x F8909-17 mapping population.

Markers	Tested	Amplified	Useful for Map
SSR	310	192	165
EST (D. Adams)	20		14
Total	330		179

We are in the process of developing a collaboration with researchers at INRA (Montpellier, France) to gain access to more SSR markers based on the comparisons with their genetic linkage map of Syrah x Grenache.

Objective 3. There are now a large number of EST derived SSR markers available, in addition to the genomic SSR markers from the Vitis Microsatellite Consortium. The EST derived SSR markers are more valuable if the cDNA sequence from which the EST was derived has a know function as determined by comparisons with homologs from other EST databases. We plan on selecting EST-SSR that show homology to resistance genes to different pathogens and genes that control other important morphological, physiological and agronomic traits. Dr. Doug Cook developed a database with a large number of EST derived SSR markers. Our goal is to screen 100-150 EST-SSR markers with putative known function by June 2004. Thus far, we obtained sequences for 50 markers from the http://cfg.ucdavis.edu/ web site. Twenty-five markers have been screened and 16 were polymorphic in the 9621 population. We are in the process adding them to the genetic linkage map.

Objective 4. Preliminary linkage analysis for each parent was carried out with MAPMAKER 2.0. Each segregating locus was paired with a "dummy" locus, resulting in a doubled data set. Linkage groups obtained from the doubled data set were then divided into two symmetrical sets of groups and one set was chosen for further detailed. The "first order" and "compare" commands were used to determine the probable order of all markers in each linkage group. The integrated linkage analysis to obtain the sex-average map was performed with JOINMAP 2.0. (LOD 5.0 and recombination frequency 0.45). Using the fixed sequence command the order of markers was determined relative to the established order obtained from the initial MAPMAKER analysis. Map units in centimorgans (cM) were derived from the Kosambi (K) mapping function. The integrated consensus map analysis was carried out with JOINMAP 3.0. The consensus linkage map was developed with 156 markers (141 SSR markers, 14 ESTP markers and the Pierce's disease resistance locus). A total of 153 markers fall in 20 linkage groups and only 3 markers were unlinked. Total map length is 935 cM with average distance between markers of 6.19 cM. All markers were evenly distributed. The largest linkage group was comprised of 13 markers (105cM) and smallest group consisted of 4 markers (17cM). The locus for Pierce's disease resistance mapped to linkage group 14 with two flanking markers on each side. We continue to add markers - an additional 24 were added to the entire population, but not included in the above analysis. Our goal is to reduce the distance between markers from 6 cM to 2-5 cM; the required distance to initiate the map based positional cloning of genes.

CONCLUSIONS

We are continuing to optimize our genetic linkage map and shorten the distances between markers linked to *Xf* resistance. We are preparing to begin map-based positional cloning. More individuals need to be put on the map and tested for *Xf* resistance to increase the recombination frequency and shorten map distances. We have about 2,000 9621 seedlings in the field to allow these efforts. This project is greatly benefiting our marker-assisted selection project ("Optimizing marker-aided selection (MAS) for *Xylella fastidiosa* resistance to accelerate the breeding of PD resistant grapes") by fine-tuning makers.

FUNDING AGENCIES
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