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
Development of an SSR genetic linkage map based on the 9621 family is continuing. The family segregates for PD resistance and is based on the cross of PD resistant D8909-15 x PD resistant F8909-17. We expanded the mapping population size from 116 to 188 genotypes. The current genetic linkage map consists of 217 non-AFLP markers (SSR, EST-SSR and ESTP) in 19 linkage groups. The PD resistance locus *PdR1* maps to linkage group 14 of the male parent (F8909-17), which now consists of 30 markers, 9 of which are localized within 10 cM of *PdR1*. To avoid confounding affects from resistance inherited from D8909-15 additional families derived from a susceptible by resistant cross are currently being evaluated for map based cloning of the *PdR1* locus. A family from the cross of F2-7 (a cross of two *V. vinifera* wine grapes, Cabernet Sauvignon x Carignane) x F8909-08 (a PD resistant sibling of F8909-17) has been made and is currently being screened for PD resistance via our standard greenhouse testing procedure. To saturate a narrow region around the resistance locus with molecular markers, bulk segregant analysis (BSA) in concert with the AFLP marker system has been initiated in cooperation with our report titled "Optimizing marker-assisted selection (MAS) for resistance to *Xylella fastidiosa* to accelerate breeding of PD resistant grapes."

INTRODUCTION
This project expands upon and continues a genetic mapping effort initiated with 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. The project has been mapping resistance to *Xiphinema index*, the dagger nematode, and *Xylella fastidiosa* (*Xf*) in an "F2" population designated as the 9621 family (D8909-15 x F8909-17). A genetic map of 116 individuals from the 9621 population was created primarily with AFLP markers (Doucleff et al. 2004). Our efforts were expanded to informative markers, such as microsatellites or simple sequence repeats (SSR) for two main reasons. First, a genetic map based on SSR markers provides a reliable and repeatable framework for initial 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 and ease of use. 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 linked to *Xf* resistance and optimize marker-assisted selection strategies applied to breeding programs. For fine scale mapping a narrow region around the primary resistance locus, we include procedures here. The proposal will expand to include construction and utilization of a genomic library of a resistant parental genotype for eventual cloning of the PD resistance gene.

OBJECTIVES
1. Increase the base population from 116 to 188 genotypes within the 9621 family and expand to a family based on a susceptible by resistant cross of 2,000 to 4,000 genotypes.
2. Increase the number of SSR and EST markers on the core genetic linkage map from 100 to 300 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 core framework map with an average distance of 2 to 5 cM between markers and utilize Bulk Segregant Analysis (BSA) with the AFLP marker system to saturate a 1 cM region around the *PdR1* resistance locus.

RESULTS AND CONCLUSIONS
Objective 1
The original starting material for this project was a molecular marker linkage map of the 9621 population based on 116 individuals (Doucleff et al. 2004). We expanded the core set of individuals from the 9621 to 188 genotypes to take advantage of 96-well plate based techniques and to increase resolution on the map to improve marker association with PD resistance. A second family derived from a susceptible by resistant cross of F2-7 (a *V. vinifera* wine grape, Cabernet Sauvignon x Carignane) x F8909-08 (a PD resistant sibling of F8909-17) has been made, and 40 individuals are currently being screened for PD resistance via our standard greenhouse testing procedure. An expansion of the family was made in the

Spring 2004 and a total of 4,500 seeds have been collected and placed into cold stratification. Should the initial subset of the family segregate in a 1:1 resistant to susceptible ratio as expected the expanded family of approximately 2,000 to 3,000 genotypes will be an excellent choice for fine resolution placement of the *PdR1* resistance gene. This would be the first step toward placement of resistance markers (flanking the *PdR1* locus) onto a bacterial artificial chromosome (BAC) within a genomic library in a procedure termed "chromosome landing" (Tanksley et al. 1995). Plans for construction of the library are underway.

Objective 2

The original genetic linkage map was based primarily on AFLP markers with 375 placed on the map, with an additional 32 ISSR, 25 RAPD and 9 SSR markers (Doucleff et al. 2004). Our efforts expanded to more reliable SSR markers in order to construct a repeatable framework map useful for more precise placement of primary resistance genes, QTL analysis and marker-assisted selection. Among the marker classes added to the map 310 SSR markers have been tested, 155 were polymorphic in the parents and all have been added to the map; 90 EST derived SSR markers have been tested, 60 of them were polymorphic and 46 have been added to the map; 20 EST markers (provided by Doug Adams) have been tested and 16 were added to the map (Table 1). A total of 217 markers (SSR, EST-SSR and ESTP) tested on 188 genotypes have now been utilized for map construction.

The 217 SSR markers included some that have been previously published and many that were developed by Vitis Microsatellite Consortium and are as yet unpublished. 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). We have tested and used all available informative genomic microsatellite markers for the 9621 population. Meanwhile, we also initiated collaboration efforts with the research group at INRA (Montpellier, France) to obtain primer sequences of SSR markers developed at their facility.

To develop ESTP (expressed sequence tagged polymorphism) 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 polymorphisms 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*in*f1, M*sp* I, *EcoR*V, R*sa* I) were tested to find restriction site based polymorphism among parents D89090-15 and F8909-17.

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 known function as determined by comparisons with homologs from other EST databases. We plan on selecting EST-SSR markers that show homology to genes which control disease resistance along with those that control other important morphological, physiological and agronomic traits. So far we have tested 90 EST-SSR markers from three different sources (Table 1) and 45 of informative markers were added to the entire core set of 9621 population. Our goal is to screen an additional 100-150 EST-SSR markers with putative known function and we are adding to the map as they are completed.

Objective 4

In order to develop the core framework map based on SSR markers, 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 detail. 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 217 markers (155 SSR markers, 45 EST-SSR, 16 ESTP markers and the Pierce's disease resistance locus). A total of 214 markers fall in 19 linkage groups and only 3 markers were unlinked. Total map length is 1300 cM with average distance between markers of 5.9 cM. All markers were evenly distributed. The current map is depicted in Figure 1. The largest linkage group was comprised of 30 markers and smallest group consisted of 4 markers (Table 2). The locus for Pierce's disease resistance mapped to linkage group 14 with flanking markers on each side (Figure 1). Many additional markers have been added but have not been included on the map.

To saturate a narrow region around the *PdR1* locus resistance locus with molecular markers, the strategy of bulk segregant analysis (BSA) (Michelmore et al. 1991) in concert with the AFLP marker system has been initiated in cooperation with our report titled "Optimizing marker-assisted selection (MAS) for resistance to *Xylella fastidiosa* to accelerate breeding of PD resistant grapes." Work has begun within two segregating families from susceptible by resistant crosses. One family, C8909-07 by F8909-08, segregates 1:1 resistant to susceptible and a good correlation between resistance and resistance marker alleles has been established. A bulk of the DNA from the 12 most susceptible and a bulk of the DNA 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.

Table 1. Data on number of markers mapped for the 9621 (D8909-15 x F8909-17) mapping population.

Molecular Markers		
Genomic SSR	VMC published/unpublished	134
	VVMD	10
	VVS	2
	INRA	9
EST derived SSR	Southern Cross University, Australia	4
	INRA, France	7
	Genome Facility (U.C. Davis)	35
ESTP markers	Doug Adams/NCBI data base	16
Grand Total		217

Table 2. Details of the 9621 genetic linkage map.

Linkage groups	19
Linked markers	214
Total map length	1300 cM
Average distance between markers	5.98 cM
Largest group (PD linkage group)	30 markers 80cM (group14)
Smallest group	4 markers 18cM (group 15)

Figure 1a. Riaz & Walker2004 SSR based genetic linkage map of 9621 (8909-15 X8909-17)

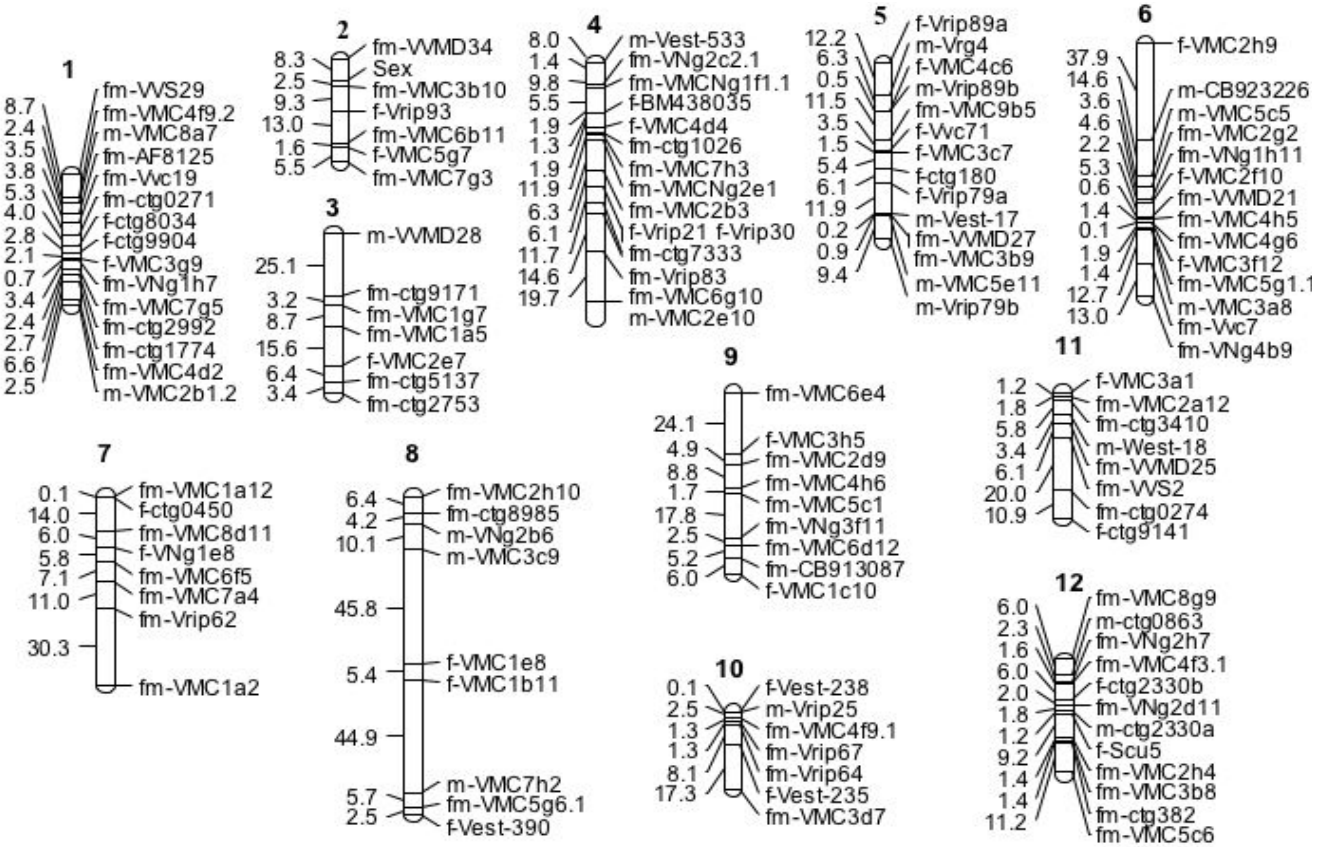
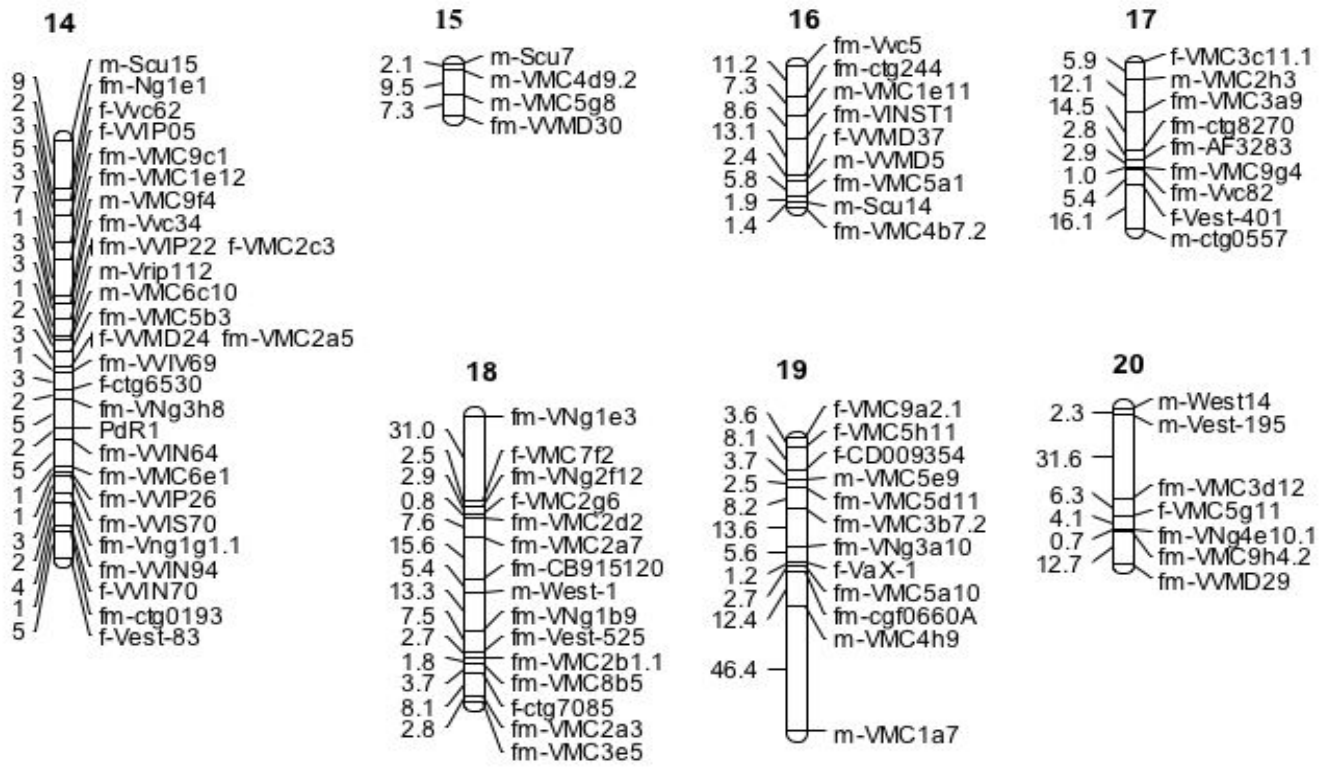


Figure 1b. Riaz & Walker2004 SSR based genetic linkage map of 9621 (8909-15 X8909-17)



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