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 a framework SSR genetic linkage map based on the 9621 family (D8909-15 (*V. rupestris* x *V. arizonica*) x (*V. rupestris* x *V. arizonica/candicans*)) is complete. The mapping population segregates for Pierce's disease (PD) resistance and was expanded from 116 to 188 genotypes. The current genetic linkage map consists of 236 non-AFLP markers (SSR, EST-SSR and ESTP-RFLP) 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 a new population has been chosen and is being prepared for mapping. This population (04190) is a cross of *V. vinifera* F2-7 x F8909-08 (sibling of F8909-17). We have confirmed a heterozygous *Xylella fastidiosa* (*Xf*) resistance inheritance (the same as F8909-17) on a subset and produced 4,500 seeds in this population for use in our mapping and positional cloning efforts. A set of 220 plants were selected for DNA extraction (to add PD group markers) and cuttings were collected from 160 plants for screening for *Xf* resistance; resistance segregates 1:1 in this population. In order to understand the stability and segregation of resistance to PD from different sources, 6 different mapping populations are under study. We are also continuing mapping efforts in the 0023 population a cross of D8909-15 x *V. vinifera* B90-116, so that we can compare these resistance sources. Extensive data for cluster and berry traits, and *Xf* resistance exists for about 200 plants in the 0023 population. A preliminary map, locates QTLs on a number of the linkage groups.

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. We have been mapping resistance to *Xiphinema index*, the dagger nematode, and *Xf* in the 9621 and 0023 populations mentioned above. The preliminary AFLP-based 9621 genetic map has been published (Doucleff et al. 2004). We then focused on adding more informative markers, such as microsatellites or simple sequence repeats (SSR) because these markers provide a more reliable and repeatable framework for initial mapping of candidate genes and quantitative trait loci (QTLs). In addition, tightly linked SSR markers are ideal for marker-assisted selection (MAS) due to their applicability across different genetic backgrounds and ease of use. This year, mapping efforts within the 9621 have concentrated on linkage group 14 which contains the PdR1 resistance locus (Krivanek et al. submitted). The addition of SSR markers to this linkage group was greatly aided by the existence of other SSR-based genetic maps of grape that have been developed within *V. vinifera* populations and by the availability of expressed sequence tag polymorphism (ESTP) markers developed by other grape researchers and available on various genetic databases. We are now applying fine-scale mapping techniques to saturate a narrow region around the primary *PdR1* resistance locus, which will lead to efforts to genetically engineer susceptible *V. vinifera* grapes with the *PdR1* gene.

OBJECTIVES
1. Complete a framework genetic linkage map of 9621 mapping population. Add SSR and ESTP markers from the PD linkage group (Chromosome 14) to additional genotypes of the 9621 population (more recombinants reduce the distance between markers).
2. Screen an additional 100-150 EST derived SSR markers for which functions are known after their comparison to homologues in available EST databases.
3. Study marker segregation linked to *PdR1* in different genetic backgrounds. Initiate genetic mapping of 04190 population (*V. vinifera* F2-7 x (*V. rupestris* x *V. arizonica/candicans* F8909-08)) with markers on linkage group 14. Apply this information in the development of a MAS system for PD resistance to assist ongoing wine grape breeding efforts.

RESULTS
Objective 1
This project began with an AFLP-based genetic map developed from 116 individuals from the 9621 population (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 paper on the portion of the AFLP-based map with *PdR1* (Krivanek, Riaz and Walker. Identification and molecular mapping of *PdR1*, a primary resistance gene to Pierce's Disease in *Vitis*. Theor. Appl. Genet.) has been submitted. Efforts have moved ahead with the use of SSR markers linked to PdR1 in our breeding program. The framework map of 9621 population is now complete with 236

primarily SSR markers (210 mapped and 26 linked). The consensus map spans 1154 cM in 19 linkage groups. Linkage group 14 is the largest group with 30 markers. The average distance between markers is 5.5 cM (a manuscript is in preparation for publication in Genome). Table 1 provides the main features of the completed SSR-based 9621 genetic linkage map. It contains 60 new EST-SSR and EST-RFLP markers that have not been mapped on any other published grape map.

Table 1. 9621 Consensus map details of the 19 chromosomes

Chromo.	Linked Markers	Mapped	Unmapped		Distance (cM)	New Markers
1	18	16	m-VMC8a7, fm-AF378125	2	91.2	8
2	11	10	VMC5g7	1	50.97	0
3	8	8		0	65.87	4
4	15	14	VMC2e10	1	79.95	4
5	17	11	f-VrZag89a, fm-VMC16d4, m-VrZag89b, f-VrZag79a, West-9, VMC4c6	6	46.77	4
6	16	10	f-VMC3f12, m-VMC3a8, fm-VVC7, fm-CF205720, f-VMC2h9	6	75.8	3
7	9	8	fm-VMC16f3	1	71.38	1
8	9	7	f-VMC1b11, f-VMC1e8	2	56.34	2
9	10	10		0	71.05	2
10	9	7	fm-ctg9946, f-vest235	2	30.87	3
11	8	8		0	48.86	4
12	13	12	fm-VMC5c6	1	33.16	4
13	9	9		0	57.29	3
14	30	28	m-VVIQ32, fm-ctg1008359	2	76.83	5
15	4	4		0	17.8	0
16	9	9		0	51.5	2
17	9	9		0	61.13	4
18	15	15		0	105.66	4
19	17	15	fm-VVIM03, m-VMC1a7	2	61.25	3
TOTAL	**236**	**210**		**26**	**1153.68**	**60**

We have extracted DNA from 300 additional genotypes from the 9621 population and will be analyzing the DNA from these plants for the markers that are contained within 15 cM of the PdR1 on linkage group 14. This increased number of individuals should yield more recombination around the PdR1 locus, finer scale positioning of markers, and get us closer to physically locating PdR1. Fine scale placement of markers in relation to a resistance locus is the first step toward screening of BAC library clones that contain the resistance gene and allows integration of a genetic linkage map to a physical map capable of locating the *PdR1* gene. This approach to clone resistance genes is termed "map-based positional cloning of genes" and it has been effectively used in other organisms to clone genes of interest. Bulk-segregant analysis (BSA) efforts are also underway with a subset of the 9621 population and 12 highly resistant and 12 highly susceptible siblings.

Objective 2

We continue to select EST-SSR markers, with known function based on comparisons of homologs from other EST databases, and to test their polymorphism for parents of two main mapping populations (9621 and 04190). This process is coupled with our efforts to increase the number of individuals on the map detailed below. In summer of 2005, we screened an additional 150 EST-SSR markers developed in Dr. Doug Cook's lab. The majority of these markers amplified successfully and 41 of them were polymorphic and useful for mapping in the 9621 and 04190 mapping populations (Table 2).

Table 2. EST-SSR markers applied to the 9621 consensus map and the linkage group they are located on

Accession No.	Putative Function	Map Location
CTG1009904	Similar to olfactory receptor MOR111-4	1
CTG1010271	AF349963_1 endoxyloglucan transferase	
CTG1011774	Nodulin-like protein [Arabidopsis thaliana]	
CTG1012992	Putative heat-shock protein [*Arabidopsis thaliana*]	
CTG1008034	Putative myosin heavy chain protein	
AF378125	AF378125_1 GAI-like protein 1 [*Vitis vinifera*]	
CTG1026392	Nuclear transport factor 2 -related	
CTG1026282	AP2 domain transcription factor, putative	
CTG1009171	RNA binding protein	3
CTG1012753	AC098693_13 Putative ubiquitin protein	
CTG1015137	S42868 serine/threonine protein kinase	
CF206266	Unknown	
CTG1007333	Probable peptidylprolyl isomerase	4
BM438035	Dehydration-induced protein RD22-like protein	
CTG1009180	Unknown	5
CTG1026305	Plastid-lipid associated protein PAP/fibrillin family	
CB923226	Protein disulphide isomerase	6
CF205720	Unknown	
CTG1026316	Amygdalin hydrolase isoform AH I precursor	
CTG1010450	ADP-RIBOSYLATION FACTOR -like protein	7
CTG1008985	Putaive arabidopsis protein	8
CB918037	Glycosyl hydrolase family 5/cellulase	9
CTG1029984	Auxin-responsive protein (Indoleacetic acid-induced protein)	
CTG1009946	Cell-cell signaling protein csgA - like	10
CTG1009141	Putaive arabidopsis protein	11
CTG1009274	Putative protein arabidopsis	
CTG1013410	Histone H1-like protein	
CTG1009382	Putative ring protein	12
CTG1010863	3-isopropylmalate dehydrogenase	
CTG1013230	Expressed protein	12
CTG1026135	S17P_SPIOL Sedoheptulose-1,7-bisphosphatase, chloroplast precursor	13
CTG1008359	Unknown	14
CTG1010193	AF448467_1 alpha-expansin	
CTG1025882	AF406809_1 glutaredoxin	
CTG1026876	Chalcone synthase	
CTG1009244	Putative protein arabidopsis	16
CTG1010557	Leaf development protein Argonaute	17
CTG1008270	Glycosyl hydrolase family 17	
AF143283	Glucose-inhibited division protein B-like protein	
CTG1007085	Putative translation initiation factor eIF-1A	18
CB915120	Eukaryotic peptide chain release factor subunit 1 (ERF1)	
CD009354	Polyadenylate-binding protein (PABP), putative	19

Objective 3

Because both parents of the 9621 population are *Xf* resistant and because the D8909-15 parent contains different *Xf* resistance loci (which derive from *V. arizonica* b42-26), we began mapping in the 04190 population to avoid confounding effects on our ability to positionally clone the *PdR1* locus. In summer 2005, we extracted DNA from 220 plants in the 04190 population before they were planted in our breeding blocks. A set of 37 SSR and EST-SSR markers were tested on small subset of eight samples (including both parents) to verify polymorphisms. Thirty-five of these markers were known to be linked to linkage group 14 based on comparisons with other published grape maps. Although all of these 35 markers were polymorphic for the 9621 population, only 29 markers were polymorphic for the 04190 and these were added to the 220 genotypes from the 04190 population. Marker order for linkage group 14 is consistent between F8909-17 (parental map) and 04190 (consensus map). A total of 111 plants inherited resistant alleles from 3 markers covering the 11 cM around the *PdR1* locus derived from F8909-08. From this observation, we conclude that resistance is segregating 1:1 in this population. Based on the presence of these resistance markers, we are now testing all resistant and 30 susceptible plants from the 04190 to verify these results with whole plant screening. These plants will be screened as part of the PD winegrape breeding effort and results are expected before the end of 2005

We are studying the expression, penetration, segregation and stability of resistance to PD from different genetic sources so that we can better predict its durability in crosses and how this locus interacts within the chromosomes. So far we have used two resistance sources (b42-26 and b43-17). The populations and genotypes we are examining are noted below.

Table 3. Parentage and species information for populations and genotypes being used to map PD resistance

Population / Genotype	Species / Parentage
b42-26	*V. arizonica*
b43-17	*V. arizonica/candicans*
D8909-15	*V. rupestris* A. de Serres x *V. arizonia* b42-26
F8909-08 and F8909-17	*V. rupestris* A. de Serres x *V. arizonica/candicans* b43-17
F2-7 and F2-35 (both females)	*V. vinifera* (Carignane x Cabernet Sauvignon)
9621	D8909-15 x F8909-17
0023	F8909-15 x *V. vinifera* B90-116
03300/5	101-14Mgt (*V. riparia* x *V. rupestris*) x F8909-08
04190	F2-7 x F8909-08
04191	F2-7 x F8909-17
04373	F2-35 x b43-17

Expected or Known Segregation Patterns:

1. 9621 Population: *PdR1* single locus for F8909-17 and multiple QTLs for D8909-15.
2. 0023 Population: multiple QTLs.
3. 03-300/5 population: *PdR1* resistance segregates 1:1 (single gene model) *Xf* greenhouse screening for entire population is in process.
4. 04-190 population: results based on resistant alleles from 6 markers, *PdR*1 segregates as 1:1 (single gene model), *Xf* greenhouse screening for entire population is in process.
5. 04-191 population: *PdR1* resistance should segregate 1:1; plant DNA extraction and addition of PD group markers are in process.
6. 04-373 population: *PdR1* resistance should segregate 1:1; plant DNA extraction and addition of PD group markers are in process.
7. 045554 population: progeny should be 93.75% *V. vinifera* and an excellent test of PdR1 in 4 backcross generations

The stability of resistance is key issue for breeding new winegrape cultivars; only genotypes that carry the resistant alleles as well as other important horticultural traits need to be selected. Therefore, it is essential to understand how resistance from different sources segregates in population. Testing of the six populations in Table 3 (9621, 0023, 03300, 04190, 04191, and 04373) that derive *Xf* resistance from both backgrounds (b42-26 and b43-17) for the presence of DNA markers and screening them for resistance to *Xf* will provide us with an understanding of resistance in different background as well as provide confidence with the stability of these resistance sources in our ongoing breeding project.

We continue to map in the 0023 population and the map results were reported last year. Since then we have determined that 75 more SSR markers are mapable. These markers are in the process of being mapped. If their addition results in a better definition of QTL location and effect we will saturate the appropriate linkage groups with markers known to exist on those groups.

We continue to study the Olmo Mexican Collection to verify its identity and the extent to which *Xf* resistance and the PdR1 locus exist in the population. We have not resolved all the confusion between the original and the USDA National Clonal Repository collections, but the work will soon be finished. We have tested all of the 51 genotypes in this collection for the presence of six SSR markers linked to the PdR1 locus. The results are being analyzed and will provide important information allowing us to correlate *Xf* screening results with the resistant alleles, distinguish new resistant alleles for breeding purposes, and determine the distribution of known resistant alleles in the entire set.

CONCLUSION

This project has enabled us to develop a framework genetic map for *Xf* resistance and now we can make progress towards physical mapping of resistance trait. Other maps are also in development in different genetic backgrounds and they will focus only on Linkage Group 14 on which *PdR1* resides, except in the case QTL analysis in the 0023. These genetic linkage maps will enable us to characterize and clone genes conferring resistance to PD, ultimately leading to genetic transformation of susceptible grape varieties with grape-based resistance genes. PD resistance makers generated in this study are used in our breeding program to optimize selection and allow the screening of larger populations and thus greater progress in the production of resistant winegrapes.

REFERENCES

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