CDFA PD/GWSS Progress Report
March 2010

I. Project Title: Map-based identification and positional cloning of *Xylella fastidiosa* resistance genes from known sources of Pierce's disease resistance in grapes.

Reporting period: March 2009 to March 2010

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III. List of objectives and description of activities

Objective 1. Completely characterize and refine the *Xf* resistance locus on chromosome 14 by genetic mapping in four populations 04190 (*V. vinifera* F2-7 x F8909-08), 9621 (D8909-15 x F8909-17), 04191 (F2-7 x F8909-17), and 04373 (*V. vinifera* F2-35 x *V. arizonica* b43-17).

Objective 2. Genetic mapping of PD resistance from resistant accessions b42-26 and b40-14.

Objective 3. Develop a BAC library for the homozygous resistant genotype b43-17 (parent of F8909-08, and F8909-17) and screen the library with closely linked markers.

Objective 4. Complete the physical mapping of *PdR1a* and *PdR1b* and initiate the sequencing of BAC clones that carry *PdR1* candidate gene.

IV. Summary of Research Accomplishments

Objective 1. We have refined the genetic position of the *PdR1a* resistance locus between marker VVCh14-56 and VVCh14-77 in the 9621 map. Both markers flank simple sequence repeat region, have distinct allelic pattern and carry unique allele size for resistant parents that is not found in susceptible *vinifera* parents. This characteristic made these markers very valuable for marker-assisted selection (MAS) in our ongoing wine grape breeding program (see companion project report). These markers also allowed us to narrow down the physical sequence distance from 300Kb to 200 Kb for the location of *PdR1*. We used these markers to test a composite set of recombinant plants in 9621 population. There are three key recombinants from the tested set of more than 900 plants. For two plants, the recombination event happened between VVCh14-78 and *PdR1a*, and other plant had a recombination event between VVCh14-81 and *PdR1a*. With the addition of new markers, the *PdR1a* locus is within a 1cM window and it completely correlates to the physical distance between the markers that were developed from Pinot noir genome sequence.

F8909-08 possesses the *PdR1b* resistance locus, which is being mapped in the 04190 population. Previously we reported that *PdR1b* maps between VvCh14-02 and VVCh14-70. Additional markers (VvCh14-28/VVCh14-29/VVCh14-30) were added to the entire set of 397 plants in the 04190 population. The greenhouse screen was repeated for key recombinants, which also helped to refine the data. In addition, marker analysis identified 14 recombinants from 15 different crosses (1,000 plants) based on resistance from F8909-08. We completed the greenhouse screen on 35 recombinants (including seedlings from *PdR1b* background crosses). The screen identified four key recombinants: in two plants the recombination event occurred between *PdR1b* and VVCh14-02; and in one plant the recombination event occurred between *PdR1b* and VVCh14-70. The greenhouse screen is being repeated for four other recombinants that had inconclusive first test results. In the most updated map, we have placed the *PdR1b* locus

between markers VVCh14-81 and VVCh14-77 (**Table 1**). Both of these markers are less than 200Kb apart based on the Pinot noir genome sequence.

The 04191 population (*V. vinifera* F2-35 x F8909-17) has 153 progeny plants and has resistance from F8909-17 (*PdR1a*), which can be examined without possible confounding effects from D8909-15, the other parent of the 9621 population, since D8909-15 has a multigenic resistance from b42-26. The resistance locus *PdR1a* is mapped in the 9621 (D8909-15 x F8909-17) population, and the 04190 population mentioned above, and refined mapping focused only on chromosome 14. The 04191 population is critical for the identification of any minor genes that might contribute to PD resistance. Therefore, we expanded the framework genetic map to all 19 chromosomes. A total of 143 SSR markers representing all 19 chromosomes were added to the set of 153 plants. Sixty of these markers were also polymorphic for the susceptible *vinifera* parent F2-7. Greenhouse-based ELISA screening used 10 green cuttings taken from each genotype to establish 5 to 6 plants that were inoculated with *Xf* in October. These plants are in the process of ELISA screening to determine their *Xf* titers. ELISA results will be available in Spring 2010, and that data will be used to conduct QTL analysis on framework map.

Objective 2. Thus far we have used three resistance sources (b43-17, b40-14 and b42-26 – Table 2). Resistance from b43-17 is inherited as a single gene, it is well placed on our genetic map and significant progress has been made in developing the physical map of that region. The preliminary data from resistance source b42-26 indicate that resistance is quantitative. We initiated genetic mapping in the F1 population from the b42-26 background (05347 –Table 2). Greenhouse screening of a subset found 48 genotypes were resistant and 13 were susceptible. A total of 337 markers were tested on small a parental data set. Results found a high level of homozygosity for b42-26 (only 113 markers were polymorphic); 184 markers were homozygous for the male parent b42-26, and 40 markers did not amplify. We completed 70 markers on a set of 64 genotypes, however 64 genotypes is not an adequate population size to map a quantitative trait. Therefore this cross was repeated in Spring 2008 to produce additional plants for the core population. DNA was extracted from an additional 100 plants to increase the core set to 164 seedlings. The seedlings were planted in the field in Spring 2009. In Summer 2010, all additional plants will greenhouse screened. The framework map for the 05347 population will be initiated in Summer 2010 to conduct QTL analysis in Winter after ELISA results are available.
Previous preliminary mapping and greenhouse screen data from the 0023 population (D8909-15 x *V. vinifera*) with resistance from *V. arizonica/girdiana* b42-26 found that PD resistance is quantitative. We are in the process of re-evaluating the 0023 population. Comprehensive results from this background will be presented in Fall 2010.

Vitis arizonica b40-14 is a third promising resistance source with resistance that seems to be homozygous and controlled by a single dominant gene. Previously, we reported that all F1 progeny from a cross of *V. rupestris* x b40-14 (the R8918 population) were resistant except three genotypes with intermediate results. Two resistant siblings were used to develop two populations: 07388 (R8918-02 x *V. vinifera*) and 07744 (R8918-05 x *V. vinifera*). We extracted DNA from 122 seedlings of the 07744 and 105 seedlings from the 07386. A total of 277 markers were polymorphic for one or the other parent in preliminary marker screening. One hundred fifty two polymorphic markers were completed on the entire set of 122 plants in the 07744 population. Mapping analysis was carried out on each parent separately. The framework map of R8918-05 was produced with 152 markers on 121 genotypes with JOINMAP (3.0). Only three markers were unlinked and the remaining 149 markers were grouped into the expected 19

chromosomes. QTL analysis was performed with MAP QTL (4.0) and the Kruskal-Wallis approach was used to complete the preliminary analysis. No association with PD resistance was found on any other chromosome except 14 – the same chromosome where *PdR1a* and *PdR1b* map to. PD resistance from b40-14 (which we have named *PdR1c*) also maps in the same general region between flanking markers VVCh14-77 and VVIN64 and within 1.5 cM. The LOD threshold for the presence of this QTL was 33 and phenotypic variation explained was 82 % (**Table 3, Fig. 1 and 2**). Considering 5% environmental variation, *PdR1c* explains 87% phenotypic variability. In 2009, crosses were made and 500 seedlings will be evaluated in 2010 with MAS to move this resistance source in breeding program.

Objectives 3 and 4. Two BAC libraries, each from different restriction enzymes, were created from the homozygous resistant b43-17. In the first phase of the project, library screening was carried out with markers VVCh14-10 and VVCh14-56, both tightly linked to *PdR1*. This process identified 24 positive clones – four of the clones were positive with both markers: H23-P13, H34-B5 and H64-M16 and H45-J22. New markers (both SSR and non-repetitive) were developed from the 695Kb region from the Pinot noir genome sequence covered by markers VVCh14-56 and VVCh14-70/77/78 (see previous reports). This region overlaps two different scaffolds from the Pinot noir genome sequence (9 and 21). Currently, *PdR1* is placed between Ch14-81 and Ch14-78 at a physical distance of ~200Kb. Based on the genetic map from the 9621 population, the physical and genetic distance correlates because 1cM is equivalent to about 216Kb. The second round of BAC library screening was carried out with the Ch14-58 marker. A total of 17 clones were positive, five of them were also positive with the VVCh14-56 marker (see details in the previous report). Clone H69J14 was selected for 454 sequencing. A total of 42,000 sequences were generated. Two different programs were used to assemble the sequence. Preliminary data analysis has enabled us to identify multiple tandem repeats of serine threonine protein kinase with a leucine-rich repeat domain gene family in the resistance region. In the susceptible Pinot noir, a total of 13 tandem repeats of the serine-threonine protein kinase gene family are present that vary from exon sequence of 744bp to 2,985 bp with no intron to 6 introns. It is essential to compare the susceptible Pinot noir region with resistant BAC clone so that all sequencing gaps are filled, but this is a challenging and time consuming process. The comparison of the PD resistance region indicates that it is quite homologous to susceptible Pinot noir outside the resistance gene region (almost 98% identical for most of the contigs). However, the b43-17 resistance region sequences that overlap with scaffold 9 of Pinot noir matched to multiple sites and the level of similarity was less. This result suggests that either the b43-17 genomic region with the PD resistance gene(s) is divergent from Pinot noir, or the available 8X assembly of Pinot noir's scaffold 9 has gaps and errors. The currently unavailable 12X coverage of Pinot noir genome would be more helpful to conduct meaningful sequence comparison. We are in the process of filling the sequence gaps for thorough analysis.

V. Publications or Reports from this Project

Riaz, S., A.C. Tenscher, R. Graziani, A.F. Krivanek and M.A. Walker. 2009. Using marker-assisted selection to breed Pierce's disease resistant grapes. Amer J Enol Vitic 60:199-207.

Cheng, D.W., H. Lin, M.A. Walker, D.C. Stenger, and E.L. Civerolo. 2009. Effects of grape xylem sap and cell wall constituents on in vitro growth, biofilm formation, and cellular aggregation of *Xylella fastidiosa*. European J Plant Path. 125:213-222.

VI. Presentations on PD Research

Impact of invasive species: breeding for resistance to PD. CSREES Review, UC Davis, Jan. 13, 2009.

Current issues in grapevine pests and diseases. UCD Wine Executive Short Course, Mar. 10, 2009.

Crape breeding with an emphasis on flavor. Recent Advances in Viticulture and Enology, Mar. 19, 2009.

PD resistant winegrapes coming soon. Temecula Grape Day, Temecula CA, April 2, 2009.

Grape breeding at UCD. International Grape Research Coordination Network for Grape Functional Genomics, Granlibaken, Lake Tahoe, CA, May 16, 2009.

Twenty years of grape breeding at UC Davis. Honorary Research Lecture, ASEV 60th Annual Meeting, Napa, CA June 24, 2009.

Breeding PD resistant winegrapes. National Grape Breeders Conference, Tallahassee, FL, Aug. 6, 2009.

Breeding grapes with resistance to Pierce's disease. Current Issues in Plant Health, FPS/UCD Extension, Davis, CA, Nov. 19, 2009.

Will you be ready for PD resistant wine grapes? Dept. Viticulture and Enology Seminar, UC Davis, CA, Nov. 20, 2009.

Breeding PD resistant grapevines. CDFA PD/GWSS Meeting, Sacramento, CA, Dec. 10, 2009.

Breeding PD resistant winegrapes. Texas Pierce's Disease Symposium, Marble Falls, TX, Mar. 2, 2010.

Abstracts

Cheng, D.W., H. Lin, M.A. Walker, E.L. Civerolo and D. Stenger. 2009. Transcriptional regulation of the grape cytochrome P450 monooxygenase gene, CYP736B expression in response to *Xylella fastidiosa* infection. Phytopathology 98:537.

Riaz, S., A.C. Tenscher, R. Graziani and M.A. Walker. 2009. Breeding winegrapes with resistance to Pierce's disease. 60th Annual Meeting, American Society for Enology and Viticulture, Napa, CA, Technical Abstracts.

VII. Research Relevance Statement

This research is studying the genetic basis of resistance to *Xf* from several sources. It is developing fine-scale genetic maps of PD resistance loci, and physically locating these loci on chromosomes so that the genes that control resistance can be studied and utilized. This research project also provides the molecular genetic support and marker-assisted selection for breeding efforts to produce PD resistant winegrapes and collaborative efforts to produce PD resistant table and raisin grapes.

VIII. Lay Summary

Genetic mapping efforts have identified a PD resistance region on chromosome 14 termed *PdR1*, which originated from *Vitis arizonica/candicans* b43-17. This resistance acts as a single dominant gene and we have mapped the two forms from the homozygous parent – *PdR1a* from F8909-17 and *PdR1b* from F8909-08. We have also mapped another form of *PdR1* from *V. arizonica* b40-14, and are examining how the multi-gene PD resistance from *V. arizonica/girdiana* b42-26 maps and relates to *PdR1*. In the future these multiple resistance forms will be combined in our PD breeding program to ensure the strongest and broadest resistance possible. The combination of these forms of PD resistance can only be done with the

tightly linked genetic markers discovered in these mapping efforts so that the combination of the various forms of resistance can be confirmed and tracked in the interbred progeny. These mapping efforts are also essential to physically locating and characterizing PD resistance genes. At present, the chromosome region that *PdR1* exists on has been sequenced and these pieces of sequence are being arranged and compared to the Pinot noir genome sequence and that of other plants to characterize their function and determine which are likely to be involved in PD resistance.

IX. Status of Funds

These funds will be spent over the course of the project.

X. Summary and Status of Intellectual Property Produced

This project may identify resistance genes that will be patentable under UC regulations. The DNA markers are also valuable breeding tools, but are closely associated with specific breeding sources.

Table 1. Key recombinants from the 9621 (*PdR1a*) and 04190 (*PdR1b*) populations. The genotypes in bold red font are key recombinants with a recombination event between the marker and the resistance locus. "0" indicates a susceptible allele and "1" indicates a resistant allele.

Genotypes with *PdR1a* background	A010	VVCh 14-56	VVCh1 4-81	*PdR1a*	VVCh 14-78	VVCh 14-77	VVCh 14-70	VVCh14 -29	VMCNg2 b7.2
-416	0	0	0	0	0	0	0	1	1
-426	0	0	0	0	0	0	0	1	1
-470	0	0	0	0	0	0	0	1	1
-554	0	0	0	0	0	0	1	1	1
-1064	1	1	1	1	1	1	0	0	0
-8	0	0	0	**0**	**1**	1	1	1	1
-194	0	0	0	**0**	**1**	1	1	1	1
-38	0	0	**0**	**1**	1	1	1	1	1
-15	1	1	1	1	1	1	1	0	0
-23	1	1	1	1	1	1	1	0	0
-901	1	1	1	1	1	1	1	0	0
-915	1	1	1	1	1	1	1	0	0
-919	0	0	0	0	0	0	0	1	1
06314-24	0	0	0	0	0	0	0	1	1
06328-05	0	0	0	0	0	0	0	1	1
04190-026	0	0	0	0	0	0	0	1	1
06317-50	1	1	1	1	1	1	1	0	0
04190-383	1	1	1	1	1	1	1	0	0
06317-50	1	1	1	1	1	1	1	0	0
04190-320	1	1	1	?	1	1	1	0	0
04190-065	1	1	1	?	1	1	1	0	0
04190-109	1	1	1	1	1	**1**	**0**	0	0
04190-381	1	1	1	**1**	**0**	0	0	0	0

Table 1. cont'd

06711A-60	0	0	0	?	1	1	1	1	1
04190-236	1	1	1	?	0	0	0	0	0
06315-49	1	0	0	0	0	0	0	0	0
06326-23	1	0	0	0	0	0	0	-	-

Table 2. Parentage and species information for populations and genotypes being used to map PD resistance.

Population / Genotype	Species / Parentage
b42-26	*V. arizonica/girdiana*
b43-17	*V. arizonica/candicans*
b40-14	*V. arizonica*
D8909-15	*V. rupestris* A. de Serres x b42-26
F8909-08 and F8909-17	*V. rupestris* A. de Serres x b43-17
F2-7 and F2-35 (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
05347	F2-35 x b42-26
07744	R8918-05 x *V. vinifera* Airen
07386	R8917-02 x *V. vinifera* Airen

Table 3. The Kruskal-Wallis analysis LOD values for the *PdR1c* locus in the 07744 population based on resistance from *V. arizonica* b40-14.

Genetic map	**Map locus**	**K* (df)**
0	VVIN70	5.392 (1) **
3.5	VVIn94	9.323 (1) ****
9.5	ctg1025882	16.293 (1) *******
10.4	VVIP26	12.764 (1) ******
10.7	VVIS70	17.315 (1) *******
11.6	UDV025	16.160 (1) *******
15.0	VVIN64	21.081 (1) *******
16.7	VVCh14-78	22.692 (1) *******
16.7	VVCh14-77	22.946 (1) *******
17.7	VVCh14-70	19.350 (1) *******
20.3	VMCNg2b7.2	17.282 (1) *******
21.5	VVMD24	20.496 (1) *******
22.0	VMC5b3	20.631 (1) *******
22.5	VMC2a5	22.915 (1) *******
22.5	VVIV69	21.978 (1) *******
23.2	UDV033	22.857 (1) *******
28.9	VMC6c10	15.577 (1) *******
36.2	VMC2c3	8.872 (1) ****
36.5	VMC2b11	8.057 (1) ****
36.9	VChr14a	7.229 (1) ***
39.0	ctg1008359	8.772 (1) ****
39.8	VMC9f4	9.360 (1) ****
41.1	VMC2h12	8.967 (1) ****

Table 3. Cont'd

Genetic map	Map locus	K* (df)
49.8	VMC1e12	3.507 (1) *
59.2	VVIP05	1.714 (1) -
61.1	VChr14b	0.398 (1) -
65.4	VVC62	0.386 (1) -

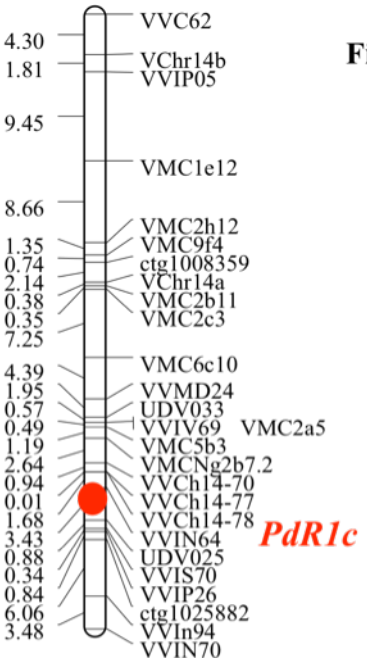

Fig. 1. SSR based genetic map of BC1 population 07744 (R8918-05 × Airen) to position the *PdR1c* locus on chromosome 14. The flanking markers are VVCh14-78 and VVIn64. It is the same genomic region of chromosome 14 where *PdR1a* and *PdR1b* has been mapped in previous studies. No other marker on other chromosomes showed linkage to PD evaluation data. A total of 89 genotypes were used for analysis. A total of 10 genotypes had missing data and 22 genotypes were not used due to ambiguous greenhouse screen results. These genotypes are currently in greenhouse screening to re-evaluate their resistance

Fig. 2. Interval mapping indicated peak at LOD 34 with a 95% confidence interval. The X-axis indicates the position of markers, LOD values are plotted on the Y-axis.