CDFA PD/GWSS Progress Report
August 2008

A. Project Title: Map-Based Identification and Positional Cloning of *Xylella Fastidiosa* Resistance Genes From Known Sources of Pierce's Disease Resistance in Grape**.**

B. CDFA Contract No:

C. Reporting period: April to July 2008

D. Principal Investigators and Cooperators:
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E. List of objectives and description of activities

1. Develop genetic linkage maps for chromosome 14 around the Xf resistance locus, *PdR1*, 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). Research activities included screening additional plants for recombinants, maintenance of plants in field, greenhouse evaluation of plants for PD resistance (both part of our PD breeding program), molecular marker development and testing on these populations, addition of markers to the entire set of mapping populations, analysis of both genetic and greenhouse screening data, and generation of genetic maps utilizing two mapping software programs.
2. Study inheritance of PD resistance from other genetic sources (b42-26 and b40-14). Research activities included generation of segregating populations, germination of seeds and maintenance of plants in the field, DNA extractions, marker testing, addition of useful markers to entire populations, greenhouse ELISA testing, and data analysis and comparisons between greenhouse phenotypes and marker data.
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. Research activities included developing and screening the BAC library.
4. Complete the physical mapping of *PdR1a* and *PdR1b* and initiate the sequencing of BAC clones that carry *PdR1a* gene candidates. Research activities included alignment of clones to scaffold 21 of the grape genome sequence, development of markers to align and determine order and orientation of the clones, and analyze and verify BAC end sequences.

F. Research accomplishments

Objective 1. As mentioned in the previous report, the resistant genotypes F8909-17 and F8909-08 inherited different sister chromatids (haplotypes) from the homozygous resistant parent b43-17. It was noted that F8909-08 has a 50 cM region in which marker segregation is distorted and the same markers are distorted in b43-17 indicating that this is a region of segregation distortion. However, the same markers on the F8909-17 map were not distorted in this region. Results of this work are presented in "Riaz S, Tenscher AC, Rubin J, Graziani R, Pao SS and Walker MA. 2008. Fine-scale genetic mapping of Pierce's disease resistance loci (*PdR1a* and *PdR1b*) and identification of major segregation distortion region along Chromosome 14 in grape. Theoretical and Applied Genetics. (In press)".

In the previous report, we mentioned that the maps from all populations mentioned above were refined. The mapping population sizes were increased and marker analysis was completed. Genetic maps were constructed by using the Join Map and TMAP mapping programs, and marker

order was consistent. The previously reported map of the 9621 population consisted of 425 progeny and *PdR1a* mapped between markers VvCh14-56/VvCh14-02 and UDV095 within a 0.6 cM genetic distance. In last three months, we have screened a total of 458 additional plants from the 9621 population with linked markers (VVIP26 and VMC2a5) and a subset of 50 recombinant plants were selected to further screen in the greenhouse. Among these plants, 189 were resistant and 188 were susceptible. Only recombinant plants were planted to the field. The 50 recombinant plants are in the process of greenhouse screening and the results will be included in the next report. The resistant source F8909-17 was also used in breeding PD resistant grapes. We selected 24 recombinant plants from five different crosses (180 plants). All additional recombinant plants are currently being greenhouse screened. It should be noted that the results from these recombinant plants might shift the location of the PD resistance locus slightly compared to that previously published and reported. It is not going to hamper our abilities to use MAS for breeding, however it will impact the positional cloning of the resistance gene. In an effort to design more clean and close markers, we utilized the Pinot noir (ENTAV 115) genome sequence available on the NCBI database. Three additional markers were polymorphic and gave clean amplification. These markers will be added to the entire set of recombinant plants as well as to the base set of 9621 population.

In the previous report, the 04190 population consisted of 361 progeny and *PdR1b* mapped between markers VvCh14-02 and UDV095/VvCh14-10 within a 0.4 cM distance. We have completed the greenhouse screening of 36 additional plants from this population. The addition of marker data was completed on this set and a map was constructed. The position of the *PdR1* resistant locus was moved from between VvCh14-02 and UDV095/VvCh14-10 to VvCh14-02 and VvCh14-28/VVCh14-29/VVCh14-30. These new markers were developed from the Pinot noir genomic region that corresponded to VMCNg3h8 clone sequence. With the clone sequence of VMCNg3h8, we obtained a contig of 99Kb size and new markers were developed. In the previous published map, VMCNg3h8 was not polymorphic for the 04190 population and that genomic region was not represented. The new markers were added to the base population of 397 plants and map was updated. It is important to note that we repeated the greenhouse screen for key recombinants and for some plants the data was further refined. The new updated map of 04190 population is presented in Fig. 1. To further refine and search for additional recombinants, we narrowed down 23 recombinants from 15 different crosses (1000 plants), in which F8909-08 was in the background. These recombinant plants are in the process of being greenhouse screened. We are continuously repeating the greenhouse ELISA screen to verify the previous screen results and to rule out any potential mistake that could happen at different stages of plant propagation, DNA extractions and data evaluation.

The majority of the markers were homozygous for the parental genotype b43-17. A total of 282 progeny from the 04373 population were used to create the 04373 map. The map of chromosome 14 for the b43-17 genotype spans 86 cM with a gap of 44 cM between two groups of markers (Fig. 1). Sixty-four plants from the 04373 population were selected for greenhouse PD screening. All these genotypes were resistant proving that b43-17 is homozygous resistant (Fig 2).

A cross of *V. vinifera* F2-35 x F8909-17 generated a fourth population, 04191. This population provides genotypes with a 50% *vinifera* background for breeding wine and table grapes as well as more recombinant plants for genetic mapping. It provides a population where resistance from F8909-17 can be examined without possible confounding effects from D8909-15. Currently, there are 212 genotypes in this population. We completed the addition of markers that are tightly linked to *PdR1* on this set, categorized resistant, recombinant and susceptible genotypes based on marker information, and selected recombinant genotypes based on flanking markers. The plants were propagated and inoculated with Xf. All marker work is complete and mapping analysis will be carried out as soon as greenhouse screen results are available.

In a previous report, we summarized the process of developing new markers from the region associated with *PdR1*. For this purpose, we utilized the Pinot noir genome sequence available on the NCBI website. A search of this sequence information allowed us select the sequences of 16 SSR markers tightly linked to *PdR1* and identify 16 contigs that provide coverage of 55.0 Kb (more detail in the June 2007 report). We developed 48 new primers and tested them on a small set of parental and progeny DNA from the three populations above. A total of 41 markers amplified cleanly and 16 of them were polymorphic in the 04190 and 9621 populations (data not shown). We added VVCh14-10 to entire set of 361 genotypes from 04190 and it co-segregated with UDV095 (Fig. 1). Two additional markers (VVCh14-56 and VVCH14-10) were added to the entire set of 425 progeny plants of 9621 population. We are in process of adding 4 additional markers to the entire set of 9621 plants to refine the map. These markers are also being used in marker-assisted selection in our PD resistance breeding program.

Objective 2. Thus far we have used three resistance sources (b43-17, b40-14 and b42-26). The populations and genotypes examined are noted in Table 1, and their segregation patterns are reported in previous reports. It is easier to manipulate single locus resistance traits in breeding and when attempting to identify genes using map-based positional cloning. Resistance from b43-17 is inherited as a single gene while resistance from b42-26 and its offspring D8909-15 is quantitatively inherited and appears to involve multiple genes that might be present on multiple chromosomes. We initiated genetic mapping in the F1 population from the b42-26 background (05347 –Table 1). The greenhouse screening data indicate that 48 genotypes are resistant and 13 are susceptible from a tested subset. 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, 40 markers did not amplify. We have completed 70 markers on a set of 64 genotypes and the remaining polymorphic markers are in process. Once all the polymorphic markers are added to the 64 progeny, we will develop the map to carry out quantitative analysis. It is important to note that the set of 64 genotypes is not an adequate number for this mapping project, and this cross was repeated in Spring 2008. Our goal is to have a set of 188 plants in the core population for developing a framework map and to carry out quantitative analysis.

In the previous report, we mentioned that b40-14 is a promising homozygous resistant genotype. We screened 45 genotypes from an F1 cross of *V. rupestris* x b40-14 and all were resistant except three genotypes with intermediate results. In Spring 2007, we made crosses with these resistant F1 genotypes to other susceptible and resistant genotypes to verify the single dominant gene mode of inheritance (07744 and 07386 – Table 1). We completed DNA extractions from 122 seedlings from 07744 and 105 seedlings for 07386. Marker testing is in the process to find a framework set of markers that span 19 chromosomes. Polymorphic markers will be added to the 122 genotypes of 07744 population. Meanwhile, all these plants will be greenhouse screened later in the season by taking cuttings from field grown plants.

For both resistance sources (b42-26 and b40-14), framework linkage maps that cover all 19 chromosomes are required to localize the resistance locus. Initially the genetic maps in F1 and BC1 populations will be developed by utilizing 96 to 188 genotypes. Once the resistance locus and QTLs are localized markers will be added to saturate appropriate linkage groups (chromosomes).

Objective 3. Genetic analyses determined that b43-17's Xf resistance segregates as a major single locus and that the full sibling progeny, F8909-08 and F8909-17, inherited different sister chromatids of chromosome 14. *PdR1* has been mapped in the F8909-17 genome, and it is possible that the PD resistance gene from F8909-08 is a different allele of the same gene or it may be a different gene. Thus a physical map of the *PdR1* region is essential. We developed two BAC libraries (each with a different restriction enzymes) from the homozygous resistant b43-17. Young leaves were used to

isolate high molecular weight DNA. Two restriction enzymes, *HindIII* and *MboI* were used to digest the DNA. The development of two libraries was done to reduce the bias in the distribution of restriction sites in the grapevine genome. In the previous report, we provided details of *Hind III* and *Mbo I* libraries and as well as the screening details. In brief, screening was carried out twice with two markers (VVCh14-10 and VVCh14-56), which are tightly linked to *PdR1* as per the previous location of the locus. There were a total of 10 positive BAC clones with marker VVCh14-10. The *HindIII* library was also screened with the other flanking marker VVCh14-56 using the same procedures. A total of 14 positive clones were identified – four of the positive clones that were selected based on the VVCh14-10 screening were also positive for the VVCh14-56 marker. These four clones are H23-P13, H34-B5 and H64-M16 and H45-J22. However, with new screening and marker data, the new position of the *PdR1* locus is between markers VVCh14-56/VVCh14-02 and VVCh14-28/29/30 (new markers, see Fig.1). The shifting in the position of the *PdR1* locus increased the distance and we will need to screen the BAC library again to find the clones that represent the new genomic region on the end of VVCh14-28/29/30 markers. We are in the process of developing markers that are polymorphic and can be used for mapping to reduce the distance and screen the library.

Objective 4. The 14 positive BAC clones that were selected with flanking marker were amplified with marker VVCh14-56, which is polymorphic (with two alleles) for b43-17 and can be used to distinguish and group clones. In an attempt to develop more markers, we utilized the 695Kb region from the Franco-Italian Pinot noir genome sequencing project that covers the marker VVCh14-56 and VVCh14-27/VMCNg2b7.2. It is important to note that this region is from two different scaffolds (9 and 21) and that it possesses stretches of unknown sequence. However, this sequence can be utilized to develop more markers to close the distance as well as to screen the BAC library. A total of 10 primers were developed that spread across 60-80Kb of physical sequence. Nine of these markers amplified successfully. We are in process of checking their polymorphism status and then they will be added to the recombinant sets from 04190 and 9621/04191 populations. Based on the previous reported position of the *PdR1* locus, we initiated shotgun sequencing of the H23P13 and H64M16 clones. These clones represent two haplotypes of b43-17. A total of 173 Kb region of H23P13 clone was assembled after primer walking. However, given the new position of *PdR1* we will be able to utilize the 35 Kb region that spans the region from marker VVCh14-56 and beyond. In the next phase of experiments the BAC library will be screened with new markers.

G. Publications or Reports from this Project

Lin, H., H. Doddapanneni, Y. Takahashi and A. Walker. 2007. Comparative analysis of ESTs involved in grape responses to *Xylella fastidiosa* infection. BMC Plant Biol. 7:8, doi:10.1186/1471-2229-7-8.

Fritschi, F.B., H. Lin and M.A. Walker. 2007. *Xylella fastidiosa* population dynamics in grapevine genotypes differing in susceptibility to Pierce's disease. American Journal of Enology and Viticulture 58:326-332.

Riaz, S., S. Vezzulli, E.S. Harbertson, and M.A. Walker. 2007. Use of molecular markers to correct grape breeding errors and determine the identity of novel sources of resistance to *Xiphinema index* and Pierce's disease. American Journal of Enology and Viticulture 58:494-498.

Fritschi, F.B., H. Lin and M.A. Walker. 2008. Scanning electron microscopy reveals different plant-pathogen interaction pattern in four *Vitis* genotypes infected with *Xylella fastidiosa*. Plant Disease 92:276-286.

Riaz, S, A.C. Tenscher, B.P. Smith, D.A. Ng and M.A. Walker. 2008. Use of SSR markers to assess identity, pedigree, and diversity of cultivated muscadine grapes. Journal of the American Society for Horticultural Science 133(4): In Press

Doddapaneni, H., H. Lin, M.A. Walker, J. Yao and E.L. Civerolo. 2008. VitisExpDB: a database resource for grape functional genomics. BMC Plant Biology 8:23 (online http://www.biomedcentral.com/1471-2229/8/23)

Riaz, S., A.C. Tenscher, J. Rubin, R. Graziani, S.S. Pao and M.A, Walker. 2008. Fine-scale genetic mapping of two Pierce's disease resistance loci and a major segregation distortion region on chromosome 14 of grape. Theoretical and Applied Genetics (In Press).

Lowe, K.M., S. Riaz and M.A. Walker. 2008. Variation in recombination rates across *Vitis* species. Tree Genomics and Genetics (In Press)

Stover, E., S. Riaz and M.A. Walker. 200X. PCR screening for *Xylella fastidiosa* in grape genebank accessions collected in the south eastern United States. American Journal of Enology and Viticulture (in final revision).

Riaz, S., A.C. Tenscher, R. Graziani, A.F. Krivanek and M.A. Walker. 200X. Using marker-assisted selection to breed Pierce's disease resistant grapes. American Journal of Enology and Viticulture (submitted)

Presentations on PD Research

A. Walker. Marker-assisted selection for Pierce's disease resistance. Applied Grape Genomics Meeting, UC Davis, July 16, 2007

A. Walker. Will there be GMOs in California vineyards – what are the issues and what can we expect. Lodi Woodbridge Grape Growers Meeting, Lodi, CA, July 17, 2007.

S. Riaz, A. Tenscher, and M. A. Walker. Molecular breeding: marker-assisted selection for Pierce's disease and powdery mildew resistance in grapevine. National Viticulture Research Conference, UC Davis, July 20, 2007

A. Walker. Grape breeding at UC Davis. North American Grape Breeder's Meeting, UC Davis, August 22, 2007

A. Walker. Breeding as an essential part of sustainable viticulture. Master's of Wine Course, Oakville, CA, October 22, 2007.

A. Walker. GMOs in viticulture – pollen movement and implications. Napa Farm Bureau, Napa, CA, November 19, 2007.

A. Walker. Classical and molecular breeding to combat PD. CDFA PD/GWSS Annual Meeting, San Diego, CA, December 14, 2007.

A. Walker. Progress breeding PD resistant wine, table and raisin grapes. San Joaquin Valley Consolidated Pest Control District Meeting, Delano, CA April 3, 2008.

A. Walker. Walker breeding program. American Vineyard Foundation Board Meeting, Modesto, CA, April 25, 2008.

A. Walker. Using marker-assisted selection to breed for Pierce's disease resistance in grape. American Society for Viticulture and Enology Annual Meeting, Portland, OR, June 18, 2008.

A. Walker. The hunt for resistance genes to combat Pierce's disease. National Viticultural Research Conference, Davis, CA, July 11, 2008.

J. Rubin and A. Walker. Genetic and phenotypic resistance to Pierce's disease in *Vitis arizonica/candicans* selections from Monterrey, Mexico. National Viticultural Research Conference, Davis, CA, July 11, 2008.

A. Walker. Pierce's disease: incidence, spread and control. Australian Society for Viticulture and Oenology Disease Workshop, Mildura, Australia, July 24, 2008.

H. Research Relevance Statement

This research project provides molecular support to our classical breeding project to create PD resistant wine and table grapes. It also aims to characterize PD resistance genes from *V. arizonica* so that their resistance to PD can be understood. This knowledge will lead to the identification of

additional resistance sources with alternative genetic control to strengthen resistance breeding. It will also lay the foundation for using PD resistant grape genes to genetically engineer wine and table grapes.

I. Lay Summary

Results from this project have allowed us to: 1) understand the segregation of PD resistance in two different backgrounds; 2) develop a framework genetic map for Xf resistance; 3) select markers for effective marker-assisted selection (MAS) in grape breeding; 4) begin development of a physical map of genomic fragments that carry the *PdR1* locus (the genetic region that contains Xf resistance), leading to map-based positional cloning of PD resistance genes. MAS has allowed the generation of PD resistant BC3 progeny with 94% of their parentage from elite *V. vinifera* wine grapes in a dramatically shortened time period. We have also constructed a bacterial artificial chromosome (BAC) library for b43-17 and located 24 BAC clones (genetic sequences), four of which carry both flanking markers on each side of PD resistance locus. Sequence analysis of one BAC clone that contains *PdR1* is in progress. This sequencing data will enable us to identify *PdR1a* resistant gene candidates.

In order to expand the range of PD resistances by exploiting other resistant accessions, we are studying the inheritance of PD resistance in two other backgrounds. Resistance in *V. arizonica* b40-14 is inherited as a single gene. We are using quantitative trait loci (QTL) analysis in the 0023 and 05347 populations to study PD resistance from *V. arizonica* b42-26 whose resistance is controlled by several genes. The genetic mapping, placement of a variety of resistance genes/traits will identify molecular markers that can be used in MAS to broaden resistance and make new varieties more durably resistant. Map-based efforts will also enable us to characterize and clone different variants of PD resistance genes, and ultimately allow the genetic transformation of susceptible grape varieties with grape resistance genes.

J. 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. 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 Airen
07386	R8917-02 x Airen

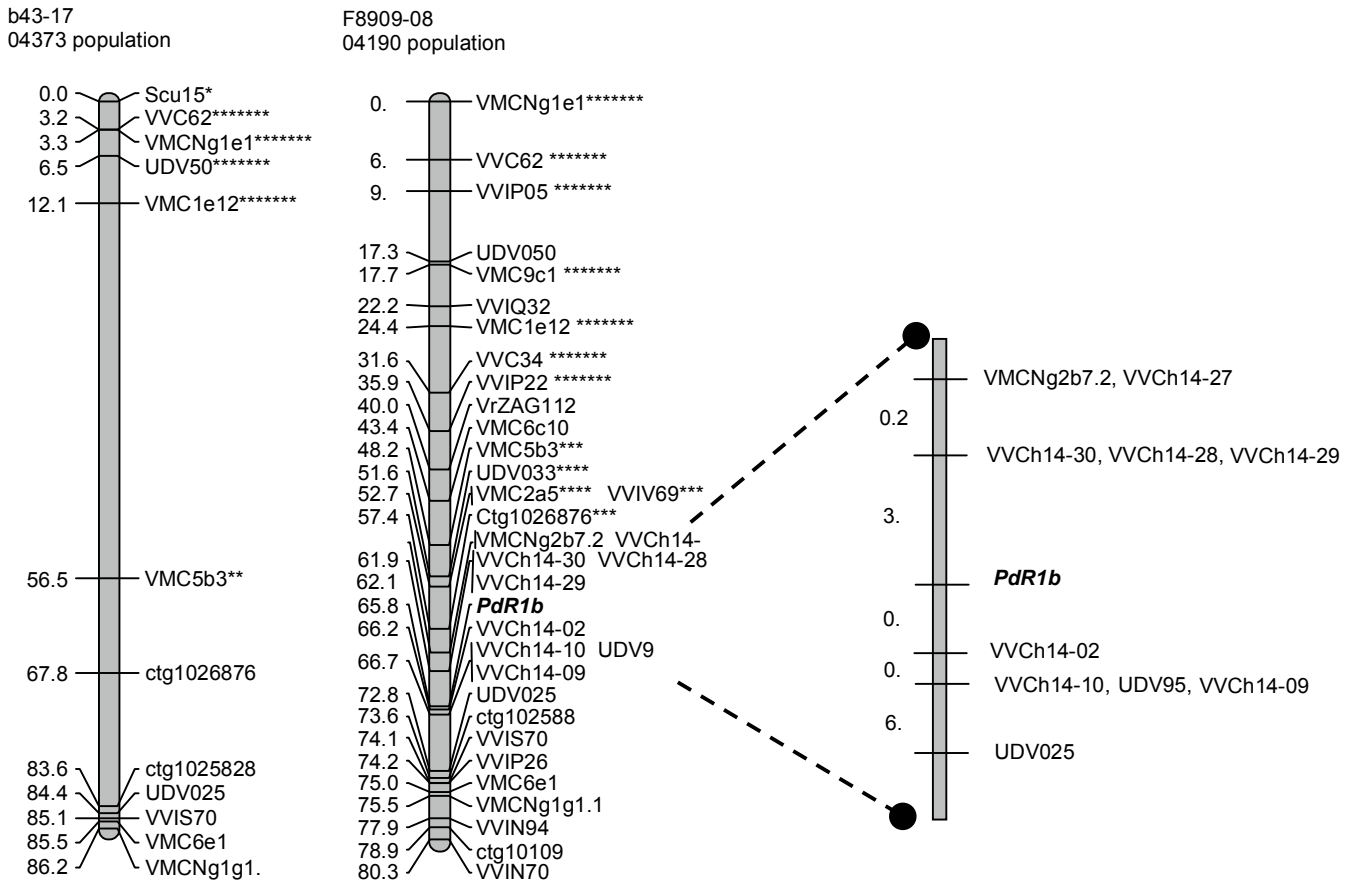

Fig. 1. SSR-based genetic map of chromosome 14 from the parental genotype 'b43-17' and progeny F8909-08 (04373, and 04190 populations, right to left).

Fig 2. Frequency distributions of Xf levels (natural log transformed cells per ml) of stem tissue extract after greenhouse testing of two populations for PD resistance. Values were derived from standardized ELISA readings. (A) 04373 n=60. (B) 04190 n=360 (see Table 1 for parentage).