

**California Department of Food and Agriculture PD/GWSS
Progress Report — July 2016**

REPORT TITLE: Interim Progress Report for CDFA Project 14-0137-SA

PROJECT TITLE: Molecular breeding support for the development of Pierce's disease resistant winegrapes.

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INTRODUCTION

This project continues to provide molecular support to the PD resistance grape breeding project – “Breeding Pierce's disease resistant winegrapes” by acquiring and testing a wide range of resistant germplasm, associating resistance regions with markers developed by genetic mapping and then functionally characterizing the resistance genes from different backgrounds. In earlier versions of this project, genetic markers linked to *X. fastidiosa* resistance from the b43-17 background were used to perform marker-assisted selection (MAS) to accelerate our PD resistant winegrape project and the table and raisin grape breeding of David Ramming. Outcomes from the earlier two projects include genetic maps, and BAC (bacterial artificial chromosome) libraries of the highly resistant *V. arizonica* accessions, b43-17 and b40-14. A physical map of the *PdRI* locus was completed and several candidate genes were identified. Five candidate genes were cloned and constructs were developed with 35S promoter to transform tobacco, Chardonnay, Thompson Seedless and St George. These constructs are currently being prepared with grape promoters prior to renewed transformation efforts.

The current molecular breeding support project has these key objectives: to identify novel sources of PD resistance for use in broadening the genetic base of resistance; to accelerate marker discovery and the identification of new and unique resistance genes; to clone and characterize unique DNA sequences (promoters) that regulate the expression of candidate PD resistance grape genes cloned from the *PdRIb* locus; and to evaluate and compare lines transformed with native and 35S promoters. We have surveyed over 250 accessions of *Vitis* species growing in the southern US and Mexico to identify new PD resistant accessions. Analysis using population genetics methods allowed us to better understand gene flow among resistant species and their taxonomic and evolutionary relationships. We have identified PD resistant accessions that are genetically and phenotypically different, were collected from different geographic locations, and have different maternal inheritance.

The identification and characterization of resistance genes and their regulatory sequences will help determine the basis of resistance/susceptibility in grape germplasm. In addition, these genes and their promoters can be employed in production of ‘cisgenic’ plants. Cisgenesis is the transformation of a host plant with genes and promoters from that genus or species (Holmes et al. 2013). Alternatively, other well characterized *vinifera*-based promoters; either constitutive (Li et al. 2012) or activated by *X. fastidiosa* (Gilchrist et al. 2007) could be utilized. Development of *V. vinifera* plants transformed with grape genes and grape promoters might mitigate concerns about transgenic crops harboring genetic elements derived from different organisms that cannot be crossed by natural means. Proven resistance gene constructs could be transformed into a broad array of elite *V. vinifera* cultivars.

More than 250 accessions were greenhouse screened and tested with markers to determine their diversity and relationship to each other. Twenty resistant accessions were identified, and they have been used to develop breeding populations from 2012 to 2015. More than 700 seedlings from breeding populations were marker tested to ensure correct parentage and identity. We used a limited mapping strategy by utilizing markers from chromosome 14 in conjunction with greenhouse screen data of small breeding populations to determine if resistance to PD is different from the previously identified locus *PdR1*. Two new unique sources (T03-16 and b41-13) were identified as having a different resistance region than chromosome 14. More crosses were made in Spring 2016 to expand these breeding populations for map-based identification of genomic regions that contribute to resistance. Physical mapping was completed for b43-17 to clone and characterize resistance genes (*PdR1a* and *PdR1b* see earlier reports). The physical map of the *PdR1c* locus (from b40-14) is also now complete. We are continuously developing and expanding breeding populations from new promising resistant lines.

Upstream and downstream sequences as well as gene sequences of two candidate genes, ORF14 and ORF18, from *PdR1b* were verified and constructs were developed. Transformation experiments with the *PdR1* resistance gene with a native grape promoter were completed with ORF18 and transgenic lines are being developed and maintained for later resistance verification. A large scale multiple time point gene expression project was completed in the greenhouse and RNA extractions were completed for over 400 samples. We used qPCR to test the expression of candidate genes. Cultures to generate embryogenic callus of *V. vinifera* cvs. Chardonnay and Thompson Seedless and *V. rupestris* St. George are being maintained. These efforts will help us to identify candidate resistance genes by complementation and better understand how they function.

OBJECTIVES

1. Provide genetic marker testing for mapping and breeding populations produced and maintained by the PD resistance-breeding program, including characterization of novel forms of resistance.
2. Complete a physical map of the *PdR1c* region from the b40-14 background and carry out comparative sequence analysis with b43-17 (*PdR1a* and *b*).
3. Employ whole genome (WGS) sequencing (50X) of recently identified PD resistant accessions and a susceptible reference accession; use bioinformatics tools to identify resistance genes, perform comparative sequence analysis and develop SNP markers to be used for mapping.
4. Clone *PdR1* genes with native promoters.
5. Compare the PD resistance of plants transformed with native vs. heterologous promoters.

RESULTS AND DISCUSSION

Objective 1. Provide genetic marker testing for mapping and breeding populations produced and maintained by the PD resistance breeding program, including characterization of novel forms of resistance.

The development of resistant varieties requires multiple genetic sources of resistance stacked up to assure a broader and long-lasting resistance. We completed greenhouse testing of over 250 southwestern and northern Mexico *Vitis*, which included accessions collected from multiple collection trips from States bordering Mexico or that were previously collected from Mexico by Olmo. Both SSR (simple sequence repeat) and chloroplast markers were used to establish relationships with known sources of resistance currently being used in the breeding program (Riaz and Walker 2013). We found 20 highly resistant accessions and 15 accessions were selected to develop small breeding populations. In Spring 2016, we extracted DNA from the 704 individuals obtained from breeding populations that were also greenhouse screened. We carried out a limited mapping strategy by utilizing markers from chromosome 14 that are linked to the *PdR1* locus (See previous reports for details of the *PdR1* locus). This strategy allowed us to identify resistance sources whose resistance is similar to *PdR1* and those that are different among the newly identified accessions. We selected 12 SSR (Single Sequence Repeats) markers that flanked a 3.5 Mb region around *PdR1* locus (Figure 1a). The *PdR1* locus is located between markers Pd82-1b4 and ORF18-19-03. The genotypic data of all resistance accessions with 22 markers from 19 chromosomes was used to analyze how genetically distinct the resistant accessions were from each other (Figure 1b).

Based on the polymorphic markers for each breeding population, a genetic map was created to determine the relative marker order and then QTL analysis for each population was carried out. We were able to identify two accessions that were resistant to PD but none of the markers from chromosome 14 showed any association to the

resistance indicating that a distinctive resistance resides on different a chromosome and most likely is different from the *PdR1* locus.

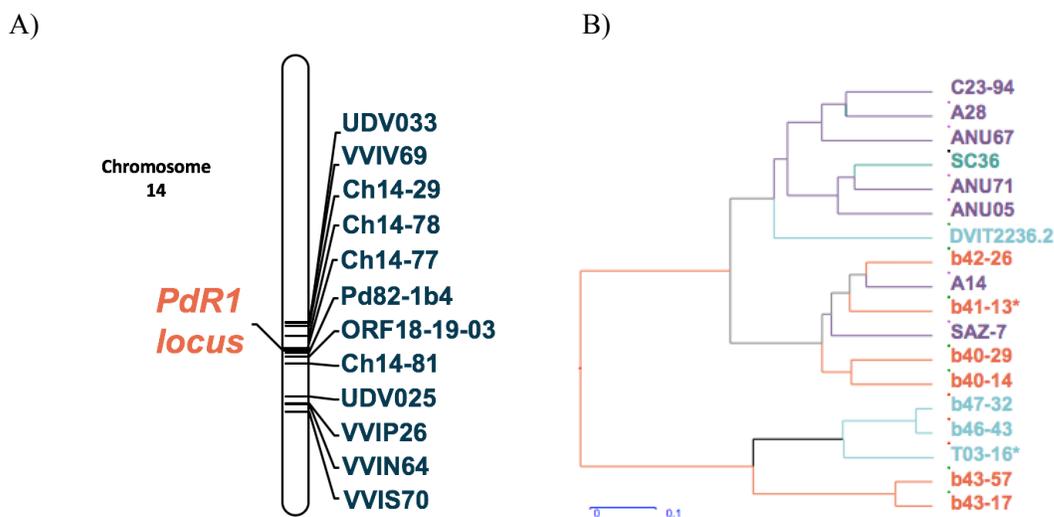


Figure 1. (A) 12 SSR markers that flank 3.5 Mb region on Chromosome 14. The *PdR1* locus resides between markers Pd82-1b4 and ORF18-19-03. (B) Dendrogram shows genetic relationship between resistant accessions; asterisks show PD resistant accessions with different source of resistance. Purple: accessions from AZ; Green: accessions from CA; Blue: accessions from Mexico; Orange: accessions from TX.

Accession T03-16 from the Big Bend region in Texas and b41-13 from Tamaulipas state in Mexico are strong candidates that do not possess *PdR1*. Figure 2 shows the location of all resistant accessions tested so far in this study. These accessions show great potential to be implemented in the PD grapevine breeding program. In order to proceed and to identify the genomic regions in these two accessions, crosses were made in Spring 2016 to expand the size of populations. In four backgrounds, we were not able to determine if resistance is different than *PdR1* due to the small population size (Table 1). We plan to expand the number of individuals in those backgrounds, greenhouse test them for PD resistance and carry out analysis next year to determine the presence or absence of *PdR1*. These results will get us one step closer to finding a new mechanism of PD resistance that we can use for our breeding program.

Table 1. Resistant accessions used for the 23 breeding populations. Resistant accessions with different source of resistance are marked as Not 14 in last column. Accessions marked as LG14 possess the *PdR1* locus. Accessions marked as ND could not be determined due to small population size and less informative markers.

Resistance source	Species description	Populations tested	Number of Screened Genotypes	Results of Limited mapping strategy
ANU5	<i>V. girdiana</i>	12-314	60	LG14
b40-29	<i>V. arizonica</i> , brushy	12-340, 12-341, 14-367, 14-368	29	LG14
b46-43	<i>V. arizonica</i> , glabrous with <i>V. monticola</i>	12-305, 14-308, 14-321, 14-322, 14-324, 14-336	159	LG14
b41-13	<i>V. arizonica-mustangensis</i> and <i>champinii</i> hybrid, red stem with hairy leaves	13-355	47	Not 14
b47-32	<i>V. arizonica</i> glabrous with <i>monticola</i> , small clusters, red stem	13-344	13	ND

SC36	<i>V. girdiana</i>	13-348	35	LG14
T03-16	<i>V. arizonica</i> glabrous	13-336	62	Not 14
A14	<i>V. arizonica</i>	14-313	25	ND
A28	<i>V. arizonica</i>	14-347, 14-364	42	LG14
ANU67	<i>V. arizonica</i> glabrous	14-362	28	ND
ANU71	<i>V. arizonica-riparia</i> hybrid	14-340	30	ND
C23-94	<i>V. arizonica</i> glabrous and brushy	14-303	44	LG14
DVIT 2236.2	<i>V. cinerea</i> like, long cordate leaves, short wide teeth, small flower cluster	14-360	30	LG14
SAZ 7	<i>V. arizonica</i>	14-363	52	LG14

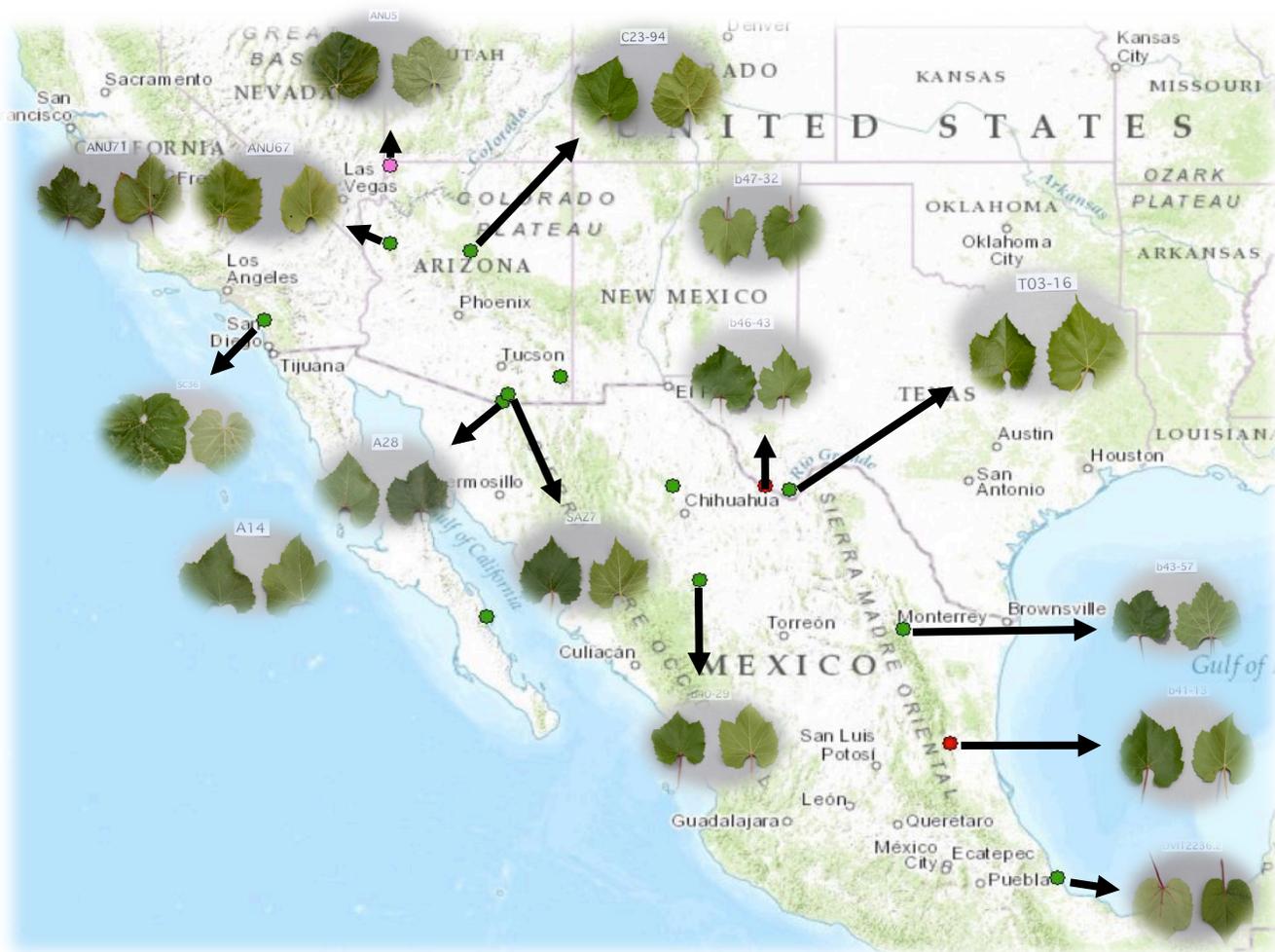


Figure 2. Geographic location of the 15 different PD resistant accessions tested in our program.

In Spring 2016, we provided molecular support to the companion PD resistance winegrape breeding project by marker testing a total of 745 seedlings from 6 crosses to identify resistant and susceptible genotypes. An additional group of 1,400 more genotypes were tested for the presence of combined resistance. The objective was to stack resistance from b42-26 and *PdR1b* as well as to develop advanced breeding lines with *PdR1c* (from the b40-14 background).

Objective 2. Complete a physical map of the *PdR1c* region from the b40-14 background and carry out comparative sequence analysis with b43-17 (*PdR1a* and *b*).

The SSR-based framework genetic map of b40-14 was completed. Greenhouse ELISA screen data was used to carry out QTL analysis and a major PD resistance locus, *PdR1c*, was identified on chromosome 14 (see previous reports for details). PD resistance from b40-14 maps between flanking markers VVCh14-77 and VVIN64 within a 1.5 cM interval. The genomic location of the *PdR1c* locus is similar to the *PdR1a* and *PdR1b*. An additional 305 seedlings were marker tested to identify unique recombinants using new SSR markers developed from the b43-17 sequence (Table 2) to narrow the genetic mapping distance. Four recombinants were identified between Ch14-81 and VVIN64, and one recombinant between the Ch14-77 and Ch14-27 markers. The new markers position the *PdR1c* locus to a 325 Kb region based on the sequence of b43-17.

A BAC library from b40-14 genomic DNA (see details in previous reports) was screened and 30 BAC clones were identified with two probes, Ch14-56 and Ch14-58. BAC clones that represent *PdR1c* were separated from the other haplotype and two BAC clones VA29E9 and VA57F4 were selected. The DNA of selected BAC clones was sequenced using PAC BIO RS II (see previous report).

A third BAC clone was sequenced to expand the region beyond the probe Ch14-58. The previous assembly consisted of two contigs with no overlap. Common probes between the *PdR1c* and *PdR1b* region were used to align the sequences in order to determine length of the gap in the assembly. A fourth BAC that overlaps with the VA30F14 and VA57F4/VA29E9 assembly was selected based on use of the new probes. Sequencing of this BAC clone was completed. New probes were designed using the sequence of *PdR1c* region to test for overlapping BACs. The assembly of four BAC clones is presented in Figure 3. A manuscript entitled “The genetic and physical map of PD resistance locus, *PdR1c*” is in preparation.

The assembly of H43-I23 from the b43-17 BAC library, which represents the *PdR1a* haplotype (F8909-17), is complete (See previous report for details).

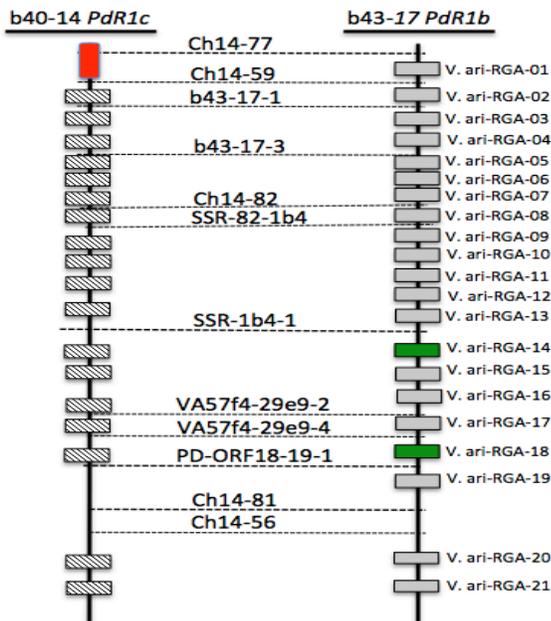


Figure 3. Sequence analysis of the *PdR1b* and *PdR1c* regions. In *PdR1c*, the assembled sequence is 426Kb. Two of the resistance genes are outside the genetic window with marker Ch14-81. The red regions represent the gap between the Ch1459 and Ch14-77 markers in the assembly.

Objective 3. Employ whole genome sequencing (WGS) (50X) of recently identified PD resistant accessions and a susceptible reference accession; use bioinformatics tools to identify resistance genes, perform comparative sequence analysis and develop SNP markers to be used for mapping.

In this proposal and as detailed in previous reports, we have proposed to use WGS to genetically map two new resistant accessions, b46-43 and T03-16, which have maintained very low bacterial levels in repeated greenhouse screens. Next generation sequencing using Illumina HiSeq and MiSeq platforms to carry out SNP discovery and identification of SNP markers linked to resistance would only be used with those resistant lines for which we have strong greenhouse screen information, information on the heritability of the PD resistance, and potential screening of the population using a limited mapping strategy.

The *V. arizonica* accession b46-43 is homozygous resistant to PD. Multiple crosses to *V. vinifera* were made to develop BC1 populations in 2014 and 2015. Breeding populations were tested with markers to verify the integrity of the crosses. Greenhouse screening of the BC1 populations with b46-43 and other resistant sources was completed (see companion project report) and results were used in conjunction with markers from the LG14 to evaluate the correlations between markers and resistance. Preliminary results indicate that there is a major PD resistance locus on ch14. However, our breeding program has already identified two other accessions that have a major PD resistance locus on ch14. In order to optimize the development of broadly resistant PD winegrapes, we would like to use PD resistance sources that map to different region so that we have the greatest chance of stacking resistance genes from multiple and diverse sources. Test results suggest that b46-43 is not a unique source of PD resistance since it maps to the same location as *PdR1*, although it does have very strong resistance to *X. fastidiosa*. In the light of these results, we will not pursue WGS to map in the b46-43 background. We will finalize the map of only ch14 for the BC1 mapping population, complete screening in the greenhouse (take down of experiment is in first week of August), with analysis in Fall 2016.

Objective 4. Cloning of *PdR1* genes with native promoters.

The physical map of *PdR1b* using four BAC clones covers 604Kb (see previous reports for details). Multiple ORFs (open reading frames) of the Leucine-Rich Repeat Receptor Kinase gene family were identified. These genes regulate a wide range of functions in plants including defense and wounding responses for both host as well as non-host specific defense. The physical distance is limited to the 82 Kb – with 5 ORFs associated with disease resistance and other plant functions described above. The main challenge is the high sequence similarity of the ORFs that are present outside the genetic window to the candidate ORFs.

We have acquired optimized binary vectors pCLB1301NH and pCLB2301NK (Feechan et al. 2013), which are capable of carrying large DNA sequences, thus allowing us to insert the candidate genes plus surrounding sequences. Two ORFs V.ari-RGA14 and V.ari-RGA18, within the resistance region boundaries, are the most likely candidates for *PdR1b*. The other 3 sequences, V.ari-RGA15, 16 and 17 are shorter and contain a large number of transposable elements (TE).

We have verified sequences upstream and downstream of V.ari-RGA14 and 18, two more likely *PdR1b* candidates. Both RGA14 and 18 (resistance gene analogs) have a very similar sequence profile with the exception that RGA-18 is 2946bp in size and lacks the first 252 bp of sequence that is part of RGA14. Functional analysis of the protein sequence of both RGA revealed that RGA-14 lacks a signal peptide in the initial part of the sequence. This result was verified using 3'RACE (rapid amplification of cDNA ends) to specifically amplify RNA from grapevines transformed with V.ari-RGA14 under the 35S promoter. The results found that mature mRNA does not contain a signal peptide, necessary for proper membrane localization, at the beginning of the sequence thus leaving RGA-18 as the strongest candidate. Sequence verification for RGA14 and RGA18 and flanking sequences was completed and fragments that contain the entire coding region plus ~3 kb upstream and ~1 kb downstream sequences were synthesized and cloned into pCLB2301NK at Genewiz, Inc. The new plasmids, called pCLB2301NK-14 and pCLB2301NK-18, were verified by restriction analysis in our lab (Figure 4). Besides the corresponding 7 kb fragment, containing RGA14 or RGA18, these plasmids contain a 35S:mGFP5-ER reporter cassette and a kanamycin-selectable marker gene with the NOS promoter.

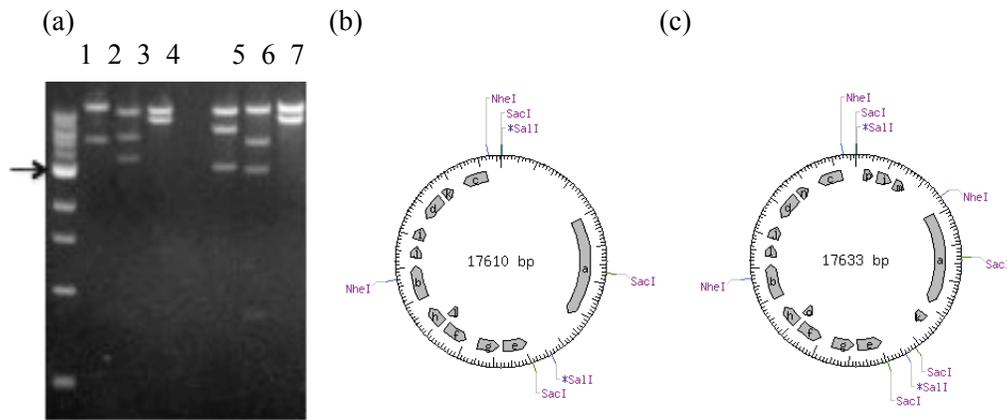


Figure 4. (a) Restriction analysis of plasmids pCLB2301NK-14 (lanes 2,3,4) and pCLB2301NK-18 (lanes 5,6,7) after digestion with NheI (lanes 2,5), SacI (lanes 3,6) and SalI (lanes 4,7). Gel image includes a 1kb ladder (lane 1) with the 3 kb fragment having increased intensity to serve as a reference band. The results on the gel match the predicted sizes inferred from the plasmid information; (b) pCLB2301NK-14 restriction map; (c) pCLB2301NK-18 restriction map.

We carried out sequence verification of genotypes U0505-22 and U0505-01, which are being used as biocontrols in our greenhouse screenings. These genotypes were originally selected for the presence of *PdR1b* markers in our breeding program. However, U0505-22 displays PD susceptibility despite being positive for the markers, which then offers the opportunity to explore the changes that could explain this behavior at the DNA level. Presently, we are focused on RGA-18 and RGA-14 sequence verification including the promoter region. Our first results have not been conclusive since direct sequencing of non-cloned PCR fragments, using primers originally designed for sequence verification of RGA14 and RGA18, produced mixed signals. Consequently, we have designed new primers to produce approx. 4.5 kb fragments that include sequences upstream and downstream of RGA-14 or RGA-18 in order to increase the specificity of the amplification and facilitate cloning by reducing the number of fragments to 2.

A large experiment with resistant and susceptible plants using multiple replicates, and time points for control (un-inoculated) and inoculated plants (see details in previous report) was completed. To date, we have completed RNA extractions from 450 samples in the above-mentioned experiment. We have also designed primers and determined primer efficiency for gene expression studies with both RGA14 and RGA18. Two different primer pairs with efficiency of greater than 90% were selected to carry out preliminary analysis with un-inoculated and inoculated samples of Chardonnay and F8909-17. Preliminary results with samples from six time points indicates that the expression level of both RGA14 and RGA18 in F8909-17 increases after day 8 in comparison to un-inoculated, peaks at day 23 and then decreases. Un-inoculated and inoculated susceptible Chardonnay did not show any expression. Gene expression analysis is underway.

Objective 5. Comparing the PD resistance of plants transformed with native vs. heterologous promoters.

We have established an *Agrobacterium* mediated transformation system followed by regeneration of plants from embryogenic callus. We have streamlined the protocol and have established cultures of pre-embryogenic callus derived from anthers of *V. vinifera* Thompson Seedless (TS), Cabernet Sauvignon, Chardonnay (CH) and the rootstock *V. rupestris* St. George (SG) (Agüero et al. 2006). In an earlier phase of this project, we transformed these varieties with five candidate genes containing the 35S cauliflower mosaic virus promoter, the nopaline synthase terminator and an *hptII*-selectable marker gene (see previous reports for details). We completed testing and found that the transgenic plants did not confer PD resistance or tolerance. These results are in accordance with the latest assembly obtained using PAC BIO SRII system and 3 additional overlapping BAC clones. They show that only one of the sequences tested, V.ari-RGA14, lays within the more refined resistance region of 82 kb defined by the two recombinants we recently obtained. The 3'RACE technique was used to amplify RNA from

V.ari-RGA14 transformed grapevines and results showed that mature mRNA does not contain the signal peptide, necessary for proper membrane localization, at the beginning of the sequence.

In addition to the embryogenic calli of Thompson Seedless (TS), Chardonnay (CH), Cabernet Sauvignon (CS) and *Vitis rupestris* St. George (SG) we have available for transformation, we developed meristematic bulks (MB) of these genotypes plus 101-14 Mgt for transformation via organogenesis (Figure 5). Slices of MB can regenerate transformed shoots in a much shorter period of time than somatic embryos. We have tested different media and selective agents and established protocols for the initiation, maintenance and genetic transformation of MB from these 5 genotypes (Xie et al. 2016). MB induction in non-*vinifera* genotypes is less efficient but still high, with about 80% of the explants producing MB after 3 subcultures in medium containing increasing concentrations of cytokinins.

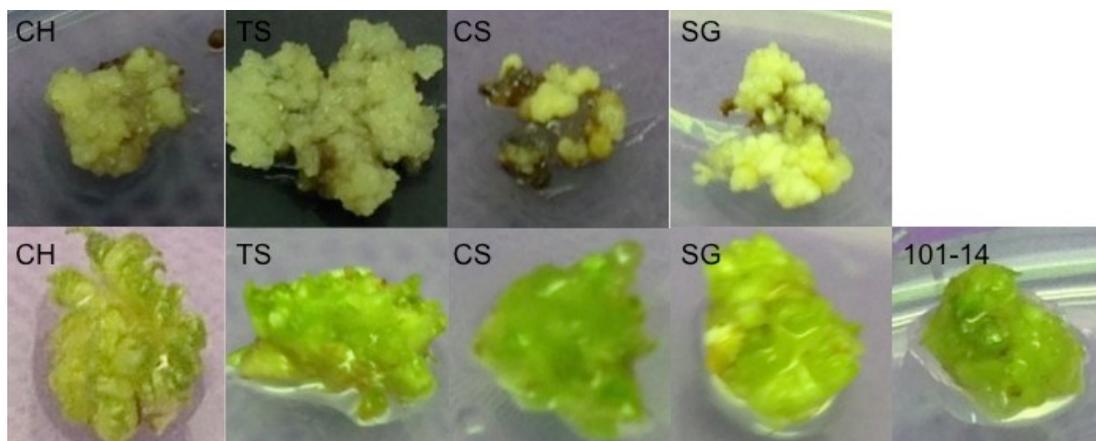


Figure 5. Embryogenic cultures (top) and meristematic bulks (bottom) of CH, TS, CS, SG and 101-14

In order to include native promoters and terminators in constructs for future genetic transformations, we have verified sequences upstream and downstream of V.ari-RGA14 and 18, the two most likely *PdR1b* candidates. Sequence verification has been completed up to 4-6 kb in the upstream region and 1 kb in the downstream region. In silico analysis of the upstream regions with PlantCare, a database of plant cis-acting regulatory elements has shown that upstream sequences contain several motifs related to drought and defense responses.

Previous transformations with *Agrobacterium tumefaciens* carrying binary plasmids that contain hygromycin (pCLB1301NH) or kanamycin (pCLB2301NK) selectable marker genes showed that both antibiotics are effective selection agents for embryogenic calli. However, MB regeneration has mainly occurred in selection with kanamycin, confirming our previous observation that MB are highly sensitive to hygromycin. Thus, pCLB2301NK was chosen to carry RGA14 and RGA18 expanded sequences and named pCLB2301NK-14 and pCLB2301NK-18 thereafter.

Agrobacterium tumefaciens strain EHA 105 pC32 was chemically transformed with pCLB2301NK-14 or pCLB2301NK-18 and subsequently used to transform embryogenic calli of *V. vinifera* cvs. Chardonnay, Thompson Seedless and rootstock *V. rupestris* St. George. Transformation experiments with pCLB2301NK-18 and pCLB2301NK-14 were initiated in March and July 2016 respectively, after synthesis and cloning was completed. In addition, *Agrobacterium* was used to transform meristematic bulks (MB) produced from the same genotypes. Table 2 shows the number of inoculated explants with *Agrobacterium* carrying pCLB2301NK-18, while Figure 6 shows the most advanced cultures growing in selection medium.

Table 2. Number of embryogenic calli (EC) and meristematic bulks (MB) inoculated with *Agrobacterium* carrying pCLB2301NK-18

Genotype	Explant	# Explants
Chardonnay	EC	994
T. Seedless	EC	603
St. George	EC	692
Chardonnay	MB	40
T. Seedless	MB	80
St. George	MB	70

We have also started the production of MB of PD susceptible genotypes selected from the 04191 population, which are 50% *vinifera*, 25% b43-17 and 25% *V. rupestris* A. de Serres (as in the original population used for *PdR1b* mapping). These genotypes can provide an additional genetic background for analysis of expression of *PdR1* candidate genes. Two of these genotypes, designated 29-42 and 47-50 exhibited great potential for the development of MB (Figure 6) and will be ready for transformation with *Agrobacterium* next month.

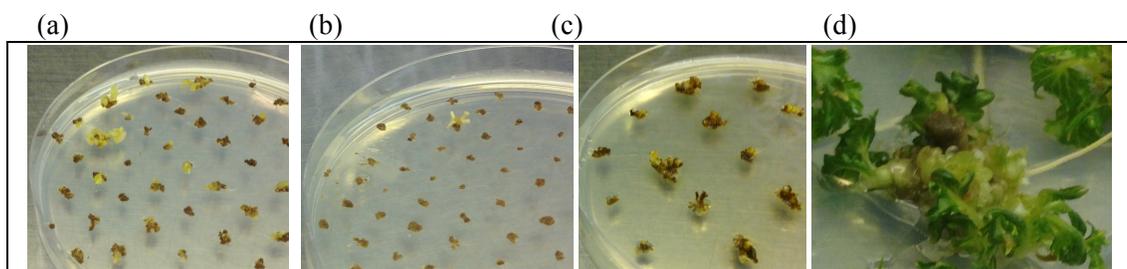


Figure 6. Embryo regeneration from embryogenic callus in (a) Thompson Seedless and (b) St. George. Shoot regeneration from meristematic bulks in (c) St. George. Meristematic bulk development in (d) genotype 47-50 from the 04191 population.

CONCLUSIONS

We completed greenhouse screening, marker testing and QTL analysis of breeding populations from 15 new resistance sources including b46-43 and T03-16. We identified T03-16 and b41-13 as possessing resistance on a different region than chromosome (ch) 14. Crosses were made to expand these breeding populations for framework map development in order to identify other genomic regions. Our primary goal is to identify new sources of resistance where resistance region is different than ch14 so we can facilitate stacking of these resistance sources with *PdR1* from b43-17, since the incorporation of multiple resistances should make resistance more durable. We have completed the genetic and physical mapping of PD resistance from b40-14. This resistance source maps within the *PdR1b* locus, but it may be an alternative gene within this complex replicated locus. Finally, we verified the sequence of two candidate genes from the *PdR1b* locus, completed transformations with ORF18 and are preparing ORF14 for complementation tests. This effort is also identifying the promoters of these genes so that we can avoid the use of constitutive non-grape promoters like CaMV 35S.

PUBLICATIONS RELATED TO WINEGRAPE BREEDING

- Xie, X., C.B. Agüero, Y. Wang and M.A. Walker. 2015. *In vitro* induction of tetraploids in *Vitis X Muscadinia* hybrids. *Plant Cell, Tissue & Organ Culture* DOI 10.1007/s11240-016-1023-4.
- Peressotti, E., C. Dolzani, L. Poles, E. Bianchi, M. Stefanini, F. Salamini, R. Velasco, S. Vezzulli, S. Riaz, M.A. Walker, B.I. Reisch, W.E. Van de Weg, M.C.A.M. Bink. 2015. A first pedigree-based analysis (PBA) approach for the dissection of disease resistance traits in grapevine hybrids. *Acta Horticulturae* 1082:113-121.

- Feechan, A., M. Kocsis, S. Riaz, W. Zhang, D.M. Godoury, M.A. Walker, I.B. Dry, B. Reisch and L. Cadle-Davidson. 2015. Strategies for *RUN1* deployment using *RUN2* and *REN2* to manage grapevine powdery mildew informed by studies of race-specificity. *Phytopathology* 105:1104-1113.
- Walker, M.A. 2015. The Genus *Vitis*, Its Species and Its Rootstocks. IN: Compendium of Grape Diseases, Disorders, and Pests, 2nd Edition, Ed. W.F. Wilcox, W.D Gubler and J.K. Uyemoto et al. APS.
- Amrine, K.C.H, B. Blanco-Ulate, S. Riaz, D. Pap, L. Jones, R. Figueroa-Baoderas M.A. Walker and Cantu, D. 2015. Comparative transcriptomics of Central Asian *Vitis vinifera* accessions reveals distinct defense strategies against powdery mildew. *Horticultural Research 2*: Article number: 15037 (2015) doi:10.1038/hortres.2015.37
- Dangl, G.S., M.L. Mendum, J. Yang, M.A. Walker and J.E. Preece. 2015. Hybridization of cultivated *Vitis vinifera* with wild *V. californica* and *V. girdiana* in California. *Ecology and Evolution* 5:5671-5684.
- Viana, A.P., M.D.V. de Resende, S. Riaz and M.A. Walker. 2016. Genome selection in fruit breeding: application to table grapes. *Scientia Agricola* 73:142-149.
- Pap, D., S. Riaz, I.B. Dry, A. Jermakow, A.C. Tenschler, D. Cantu, R. Olah and M.A. Walker. 2016. Identification of two novel powdery mildew resistance loci, *Ren6* and *Ren7*, from the wild Chinese grape species *Vitis piasezkii*. *BMC Plant Biology* (In Press)
- Xie, X., C.B. Agüero, Y. Wang and M.A. Walker. 2016. Genetic transformation of grape varieties and rootstocks via organogenesis. *Plant, Cell, Tissue and Organ Culture* (In Press)

PRESENTATIONS

Talks at Grower Meetings (Extension/Outreach) July 2015 to July 2016

- Breeding PD and PM resistant winegrapes with tasting. Daniel Roberts Client Group, Santa Rosa, CA July 10
- PD resistant wine grapes. Ventura Farm Press Interview, July 7
- Breeding PD and PM resistant winegrapes. Sonoma County Winegrape Commission, Santa Rosa, CA July 31
- PD resistant winegrapes – talk and tasting California Association of Family Farms, Valley Center, CA Aug 7
- Grape breeding at UC Davis. Chilean Table Grape Association, UC Davis, Aug 25
- Grape rootstock and scion breeding at UC Davis. North American Grape Breeders Mtg, Geneva, NY, Aug 29.
- PD Breeding Progress – report and tour. CDFA Administrators, UC Davis Oct 13
- Grape breeding at UC Davis Interview for David Pelletier for International Wine Magazine, UC Davis Oct 13
- Breeding PD resistant wine grapes – talk and tasting VEN on the Road, Santa Maria, CA Nov 5
- UCD vineyard and winery tour, and PD wine tasting with Darrel Corti. Sacramento Private School support group and auction prize, UC Davis Nov 8
- PD resistant winegrapes nearing release. FPS Annual Meeting, UC Davis Nov 10
- Breeding PD resistant winegrapes. Napa Vit Tech Meeting, Napa, CA Nov 12
- Grape breeding at UC Davis. Guest Lecturer at Chihuahua University, Chihuahua, MX Nov 25
- Breeding PD resistant winegrapes. UCD Winegrape Day, UC Davis Dec 2
- Walker grape breeding program. UC Cooperative Extension Grape Farm Advisor Meeting, UC Davis Dec 3
- PD breeding update and tasting. Oak Knoll Growers Group, Napa, CA Jan 7, 2016
- Walker grape breeding program update/tasting. Silverado Growers Management Seminar, Napa, Jan 13, 2016
- PD resistant winegrapes – update and tasting Napa/Sonoma growers meeting, Napa, CA Jan 21, 2016
- Rootstock and Scion breeding overview. Lodi Grape Day, Lodi, CA Feb. 2 2016
- PD resistant winegrape breeding and tasting, Silverado Vineyards meeting, Napa, CA April 4
- PD resistant winegrape breeding. Talk and discussion with John Dyson and Williams Salem staff, UC Davis, April 13
- PD resistant winegrape breeding and tasting for California Association of Winegrape Growers, Sacramento, CA Apr 18
- Breeding PD resistant winegrapes. Temecula Grape Day, Temecula, CA Apr 21
- Breeding PD resistant winegrapes. Alan Tenschler presenting to the AVF Board in Livermore, Apr 29
- Breeding PD resistant winegrapes. Talk and tasting for Napa winemakers and viticulturists, UC Davis, May 4
- Winegrape breeding at UC Davis. Vintage Nursery Open House, Wasco, CA May 18
- Winegrape breeding at UC Davis. International Cabernet Sauvignon Conference, Pine Ridge Winery, Napa, CA June 22

Presentations at Scientific Meetings

Xiaoqing Xie, Cecilia B. Agüero, Yuejin Wang, M. Andrew Walker. Optimizing the genetic transformation of grape fruiting and rootstock cultivars. 2016 ASEV National Meeting, Monterey, CA June 29.

Karla Huerta, Summaira Riaz, Alan Tenschler and M Andrew Walker. Characterization of Pierce's disease resistance in germplasm collected from the southwestern US and Mexico. 2016 ASEV National Meeting, Monterey, CA June 29.

Summaira Riaz, Dániel Pap, Alan Tenschler and M. Andrew Walker. Molecular strategies to stack powdery mildew resistance from multiple backgrounds in a grape breeding program. 2016 ASEV National Meeting, Monterey, CA June 29.

RESEARCH RELEVANCE

The goal of this research is to understand the genetics of PD resistance and provide genetic support to our PD resistance breeding of wine grapes. We successfully mapped the resistance genes from a form of *V. arizonica* and used the linked markers to greatly expedite our breeding program. We are now searching for additional forms of PD resistance in other species from a variety of geographic locations across the southern US and Mexico, with the goal of combining resistance from several species together to ensure durable resistance

LAYPERSON SUMMARY

We continue to identify and genetically characterize novel resistance sources from southwestern US and Mexican *Vitis* species collections; use genome sequence information to identify resistance genes; clone and characterize these resistance genes with native promoters; and develop resistance gene constructs prior to transforming them into susceptible *V. vinifera* grapes to test their function. Creating genetic maps with DNA markers allows us to identify and validate markers that could be used for marker-assisted selection and to incorporate (stack) multiple resistance genes into a single background to create more durably resistant varieties.

STATUS OF FUNDS: These funds are schedule to be spent by the end of the grant.

INTELLECTUAL PROPERTY: PD resistant varieties will be released through the Office of Technology Transfer (Patent Office) of the University of California, Davis.

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FUNDING AGENCY

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