California Department of Food and Agriculture PD/GWSS
Interim Progress Report — February 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

A successful resistance-breeding program depends on germplasm to provide a wide genetic base for resistance. Identification, characterization and manipulation of novel sources of resistance are prerequisite for breeding. This evolved 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, tag resistance regions with markers 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 b43-17 background were used to perform marker-assisted selection (MAS) to accelerate our PD resistant winegrape 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 *PdR1* 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.

The new merged 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 *PdR1b* 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. Breeding populations from new promising resistant lines were developed. These populations will be tested to study the inheritance of resistance and genetic maps will be developed to identify genomic regions associated with resistance, and genetic markers will be used for the stacking of multiple resistance genes to breed winegrapes with durable PD resistance.

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 its own genes and promoters (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.

Over the last year, 250 accessions were greenhouse screened and tested with markers to determine genetic mapping distances. Twenty resistant accessions were identified, and they have been used to develop breeding populations from 2012 to 2015. Breeding populations were marker tested to ensure correct identity. Resistance loci were identified on genetic maps, markers were developed for breeding, and 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. A large scale multiple time point gene expression project was completed in the greenhouse and RNA extractions were completed for over 400 samples. qPCR experiments were used 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 for use in transgenic experiments. Experiments to utilize the *PdR1* resistance gene with a native grape promoter are underway. 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.

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). Crosses were made with five PD resistant *V. arizonica* accessions from the southwestern US and Mexico to develop small breeding populations. A subset of seeds from these crosses was germinated and greenhouse screened to characterize the inheritance of their PD resistance.

Greenhouse testing of the F1 and BC1 (backcross 1) breeding populations using two of the resistant accessions, b46-43 and T03-16, commenced. Genomic DNA was isolated from 177 seedlings in the BC1 population from the b46-43 background and marker testing on a small set of seedlings and parental DNA was completed.

In Spring 2015, we provided molecular support to the companion PD resistance wine grape breeding project by marker testing a total of 1,237 seedlings from 17 crosses to determine PD resistant and susceptible genotypes. Most of these crosses were designed to stack resistance from b42-26 and *PdR1b* as well as to develop advanced breeding lines with the *PdR1c* (the b40-14 background).

Table 1 presents the breeding populations that were developed with new resistance sources (for details, see previous reports). In Spring 2015, we completed propagation of 4-5 replicates for the subset of crosses mentioned

in Table 1. These plants were inoculated with *X. fastidiosa* at the end of August and the results of this assay are presented and discussed in the companion PD breeding project report.

Table 1. Crosses that are under greenhouse testing to determine the mode of inheritance of their resistance to Pierce's disease

Cross ID	Female Name	Male Name	Seedlings tested
14-360	F2-35	DVIT 2236.2 (*V. nesbittiana*)	90
14-367	F2-35	12340-13	50
14-321	Rosa Minna	12305-55	28
14-308	Rosa Minna	12305-55	19
14-364	Rosa Minna	A28	19
14-347	Rosa Minna	A28	23
14-322	Rosa Minna	12305-56	15
14-313	A14	Colombard	53
14-324	F2-35	12305-56	47
14-340	ANU71	Grenache blanc	38
14-303	C23-94	Nero d'Avola	64
14-362	F2-35	ANU67	31
14-363	F2-35	SAZ 7	52
14-368	F2-35	12340-14	35
14-336	F2-35	12305-83	14
Total			578

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 325 Kb based on the sequence of b43-17.

Table 2. Positioning of genetic markers in relationship to PD resistance in *V. arizonica* b40-14

Genotype	14-29	14-27	VVCh14-77	SSR82-1b4	ELISA Results	ORF18 to 19-3	14-81	VVIn64	UDV025	VVIp26
09367-35	+	+	+	+	R	+	+	+	+	+
09367-37	+	+	+	+	R	+	+	+	+	+
09367-38	+	+	+	+	R	+	+	+	+	-
09367-41	-	-	-	-	S	-	-	-	+	
12325-78	+	+	+	+	R	+	+	+	-	-
12326-18	+	+	+	+	R	+	+	-	-	-
12327-54	-	-	-	-	S	-	-	+	+	+
09367-26	-	-	-	-	S	-	-	+	+	
09367-30	-	-	-	-	S	-	-	+	+	+
09367-07	-	-	+	+	R	+	+	+	+	+
09367-12	+	-	-	-	S	-	-	-	-	-
09367-40	+	+	+	+	R	+	+	+	+	+
09367-43	+	+	+	+	R	+	+	+	+	+

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 (Figure 1). The assembly of four BAC clones is presented in Figure 2. Currently we are annotating and doing comparative sequence analysis of this region with the susceptible corresponding sequence of PN40024 and 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. There was complete homology between the overlapping BAC clone sequences of the two different haplotypes. The BAC clone H43I23 has ORF16 to ORF20 and all five ORFs have sequences that are identical to the *PdR1b* haplotype. Based on these results, we conclude that there is complete sequence homology between haplotype a, and b of the *PdR1* locus; therefore cloning and functional characterization of genes from either haplotype will be sufficient for future work. Complete sequence homology indicates that the parents of b43-17 must be closely related and may have a first-degree relationship and may have acquired resistance from same parent.

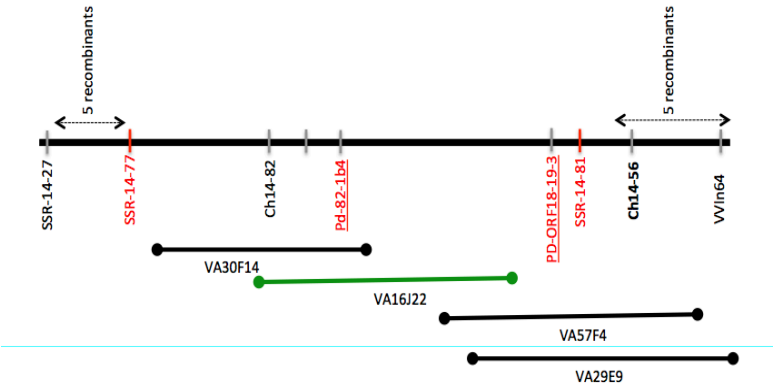

Figure 1. The alignment of four BAC clones. Markers in bold were used as probes to screen library, marker in red are SSR markers. Two underlined markers were developed from b43-17 sequence; SSR14-27 was designed from the PN40024 seq.

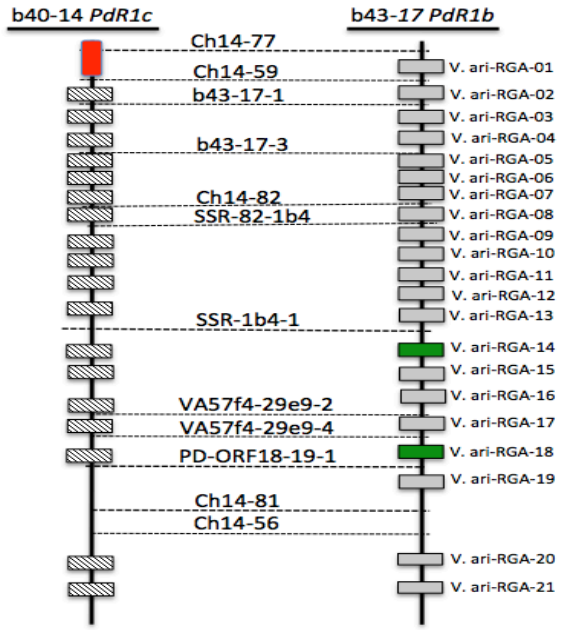

Figure 2. Sequence analysis of the *PdR1b* and *PdR1c* regions. In *PdR1c*, the assembled sequence is 426Kb. Two of the resistance genes are outside the genetic window with marker Ch14-81. The red regions represent the gap between Ch1459 and Ch14-77 marker in the assembly.

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 funded proposal and previous reports, we have proposed to use WGS to genetically map two new resistant accessions, b46-43 and T03-16, which have shown very low bacterial levels in repeated greenhouse screens. Next generation sequencing using IIlumina 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 access whether there is any correlation. 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 wine grapes, we would like to use PD resistance sources that map to different region so that we can stack resistance genes from multiple 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 (plants are being propagated), with analysis in Spring and Summer 2016. Based on the results from the b46-43 background, we expanded the analysis of ch14 markers that are linked to the resistance to all other resistant accessions that we have used to develop breeding populations (Table 3). We selected 12 markers that span the *PdR1* locus and used them to genotype all of the breeding populations described in Table 3. DNA was extracted from 704 seedling plants maintained in the field. Genotyping with 12 SSR markers is complete and currently we are analyzing data from each population in conjunction with the greenhouse screen data. The results will allow us to infer and identify genotypes with resistance on chromosomes other than chromosome 14; only those unique genotypes will be considered for the WGS work.

Table 3. List of resistant sources that have been employed to develop breeding populations and currently in the process of analysis to determine if resistance is other than chromosome 14.

Year of Cross	Cross ID	Resistance source	Number of genotypes screened in the greenhouse
2012	12314	ANU5	27
2012	12340, 12341, 14367, 14368	b40-29	29
2012	12305, 14308, 14321, 14322, 14324, 14336	b46-43	60
2013	13355	b41-13	47
2013	13360	b43-57	20 & 44
2013	13344	b47-32	20 & 31
2013	13348	SC36	35
2013	13336	T03-16	62
2014	14313	A14	29
2014	14347, 14364	A28	42
2014	14362	ANU67	30
2014	14340	ANU71	30
2014	14303	C23-94	44
2014	14360	DVIT2236.2	30
2014	14363	SAZ 7	52

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 further 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 RGA-18 has been completed and a 7 kb fragment, which contains the entire RGA-18 coding region plus ~3 kb upstream and ~1 kb downstream sequences is being synthesized and cloned into pCLB2301NK at Genewiz, Inc.

We are also carrying 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, but 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.

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 an 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 will be carried out on complete data sets in Spring 2016.

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) and 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 technique of 3'RACE 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 3). 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. submitted). 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. For this reason, we have also started the production of MB of PD susceptible genotypes selected from 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.

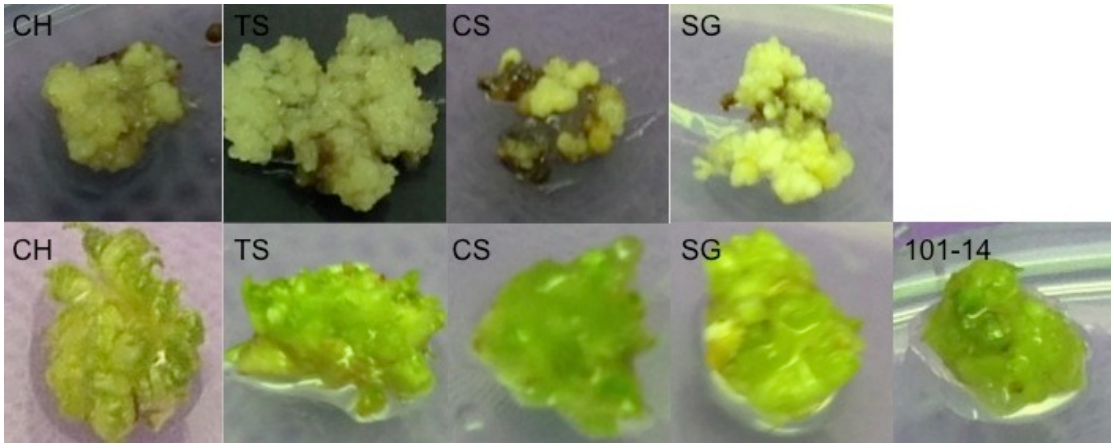

Figure 3. Embryogenic cultures (top) and meristematic bulks (bottom) of CH, TS, CS, SG and 101-14

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 strongest *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. Transformations with V.ari-RGA18 coding region plus ~3 kb upstream and ~1 kb downstream will begin in March, 2016.

Transformations with *Agrobacterium tumefaciens* carrying binary plasmids pCLB1301NH and pCLB2301NK, will be used to clone *PdR1b* candidate genes and their regulatory regions. These plasmids have been designed to overcome stability problems associated with the presence of large genomic fragments (Feechman et al. 2013). They carry the hygromycin (pCLB1301NH) and kanamycin (pCLB2301NK) selectable marker genes, respectively. Both plasmids also carry mGFP5-ER as a reporter gene. We have transformed embryogenic calli and MB of TS, CH and SG with both plasmids to test the use of the hygromycin and kanamycin genes under the control of the NOS promoter in contrast with our previous results using the same genes under the control of the 35S promoter. Although inoculated explants are still growing in selection medium, they are clearly showing that hygromicin and kanamycin are effective selection agents for embryogenic calli. However MB regeneration has only occurred in selection with kanamycin, confirming our previous observation that MB are highly sensitive to hygromicin.

CONCLUSIONS

We completed greenhouse screening of breeding populations from 15 new resistance sources including b46-43 and T03-16. We are using markers linked to *PdR1b* locus to decipher if resistance whether is contributed from ch14. 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 to prepare them for complementation tests. This effort is also identifying their promoters so that we can avoid the use of constitutive non-grape promoters like CaMV 35S.

PUBLICATIONS AND PRESENTATIONS

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/s11240-015-0801-8.

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 (submitted)

Talks at Grower Meetings (Extension/Outreach)

Breeding for PD and PM resistance Napa Valley Grape Growers, Napa, CA March 4 2015

Vineyard of the Future Wine Executive Program lecture, UCD. Mar. 27
California viticulture. UC Berkeley DNV Business program Napa, CA. Apr. 18
Breeding for PD and PM resistance Diageo Winemakers, UC Davis, April 23, 2015
Breeding new winegrape varieties – PD, PM and beyond. Napa Marriot, May 6
A look to the future – what's in store for CA vineyards Anderson Valley Tech Conference, Philo, CA. May 15
ASEV Portland
Breeding PD and PM resistant winegrapes – talk and 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 Association Meeting, 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
Walker grape breeding program update and tasting. Silverado SIMCO Growers Management Seminar, Napa, CA Jan 13
PD resistant winegrapes – update and tasting Napa/Sonoma growers meeting, Napa, CA Jan 21

Presentations at Scientific Meetings

Walker, A. , A. Tenscher and S. Riaz. 2015. Breeding Pierce's disease resistant winegrapes. Proceedings of the 2015 Pierce's Disease Research Symposium.
Walker, A., S. Riaz, C. Agüero and D. Cantu. 2015. Molecular breeding support for the development of Pierce's disease resistant winegrapes. Proceedings of the 2015 Pierce's Disease Research Symposium.

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 scheduled 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.

LITERATURE CITED

Agüero CB, Meredith CP, Dandekar AM (2006) Genetic transformation of *Vitis vinifera* L. cvs. Thompson Seedless and Chardonnay with the pear PGIP and GFP encoding genes. *Vitis* 45:1-8

Feechan, A., Anderson, C., Torregrosa, L., Jermakow, A., Mestre, P., Wiedemann-Merdinoglu, S., Merdinoglu, D., Walker, A. R., Cadle-Davidson, L., Reisch, B., Aubourg, S., Bentahar, N., Shrestha, B., Bouquet, A., Adam-Blondon, A.-F., Thomas, M. R. and Dry, I. B. (2013), Genetic dissection of a TIR-NB-LRR locus from the wild North American grapevine species Muscadinia rotundifolia identifies paralogous genes conferring resistance to major fungal and oomycete pathogens in cultivated grapevine. The Plant Journal, 76: 661–674. doi: 10.1111/tpj.12327

Gilchrist D., and Lincoln J. (2008) Functional testing and characterization of Pierce's disease-induced promoters from grape. Proceedings of the Pierce's Disease Research Symposium.

Holmes IB, Wendt T, and Holm PB (2013) Intragenesis and cisgenesis as alternatives to transgenic crop development. Plant Biotech. J. 11:395–407

Li ZT, Kim KH, Jasinski JR, Creech MR, and Gray D. (2012) Large-scale characterization of promoters from grapevine (*Vitis* spp.) using quantitative anthocyanin and GUS assay systems. Plant Sci. 196:132-142

Riaz, S and M.A. Walker. 2013 Phylogeographic analysis of resistance to Pierce's Disease in North American and Mexican species. 64th Annual Meeting of the American Society for Enology and Viticulture, Monterey, CA.

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