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Project Title: Breeding Pierce's disease resistant winegrapes.

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INTRODUCTION

The key requirement to breed for resistance to Pierce's disease is novel germplasm, and fast and accurate phenotyping that mimics field-based assessments. The Walker lab has acquired unique and highly resistant *V. rupestris* x *V. arizonica* selections, collected a wide range of wild southwestern grape species, developed techniques for rapid screening for *Xylella fastidiosa* (*Xf)* resistance in the greenhouse (Buzkan et al. 2003, Buzkan et al. 2005, Krivanek et al. 2005a 2005b, Krivanek and Walker 2005), and developed tightly linked markers to allow marker assisted selection (MAS). These factors have allowed us to introduce extremely high levels of *Xf* resistance into commercial grapes. We have selected progeny with the PD resistance locus, *PdR1*, from the b43-17, a *V. arizonica*/*candicans* resistance source, and carried out crosses up to backcross 4 (BC4) so that we now have 97% *vinifera* level selections with high fruit quality. The progeny lines that passed the tightly linked marker test have undergone greenhouse testing at every BC generation to verify resistance multiple times. Progeny passing these tests with the highest levels of resistance are first prepared for small-scale winemaking and planted as 6 to 8 vines sets on commercial spacing and trellising in the UCD vineyard. After further evaluation grafted scions are planted in field trials in various PD hotspots. We have made wine from vines that are of the 94% *vinifera* level from the *PdR1* resistance background for 5 years and at the 97% *vinifera* level for two years. They have been very good and don't have the hybrid flaws (blue purple color and strong herbaceous aromas and taste) that were prevalent in wines from the 87% *vinifera* level. There are two forms of *PdR1*, 8909-08 and 8909-17 – sibling progeny of b43-17 and they have different alleles of *PdR1*. These selections have been introgressed into a wide range of winegrape backgrounds over multiple generations. We are also pursuing two other resistance sources b40-14, and b42-26 and breeding them into existing advanced selections in order to stack multiple resistance genes and produce more durable resistance. We have screened a wide range of southwestern United States (SWUS) species and developed breeding populations with the most promising resistant sources. However, the resistance in these later lines has yet to be characterized nor are markers developed to expedite MAS breeding.

OBJECTIVE

The overall objective of this proposal is to develop 97% *vinifera*-based PD resistant winegrapes with durable field resistance loci pyramided from diverse genetic backgrounds.

1. Identify additional unique sources of Xf resistance; develop breeding populations and phenotype them with our greenhouse screen to characterize their inheritance of resistance.
2. Develop 97% *vinifera*-based PD resistant lines of winegrapes utilizing diverse sources of resistance to Xf, conduct fruit and wine evaluations.
3. Utilize marker-assisted selection (MAS) to allow pyramiding of resistance loci, screen for resistant genotypes, and develop backcross generations by crossing resistant selections to elite *vinifera* varieties in order to produce high quality and PD resistant winegrapes.
4. Develop and maintain new and existing genetic mapping populations to assist companion mapping/genetics project; begin the mapping of fruit quality traits such as color, tannin content, flavor, and productivity in PD resistant backgrounds.

RESULTS AND DISCUSSION

Breeding PD Resistant Winegrapes

After evaluating thousands of seedlings for appearance and quality in the field and for resistance in our greenhouse test, breeding efforts involving *PdR1b* resistance as a single line of defense against PD are nearly complete. We have yet to map one or more major resistance loci in the b42-26 *V. arizonica* line however in our greenhouse test we observe select individuals with significant PD resistance. In 2013, our breeding efforts focused on stacking *PdR1b* with resistance from b42-26 to create potential parental breeding genotypes containing both resistance sources and homozygous at either *PdR1b* or the b42-26 resistance regions. This will allow a final backcrossing to elite *vinifera* cultivars to create progeny approximately 94% *vinifera* with multiple sources of PD resistance. Table 1 details the 345 individuals MAS tested for *PdR1b* and the 57 individuals planted to the field. In addition, we planted a small population from the b42-26 line created by crossing our two most resistant individuals at the 75% *vinifera* level to help better understand the inheritance of resistance in this line. PD resistant cultivars with resistance to powdery mildew would greatly enhance the desirability of new wine grape varieties. *Ren4* is a strong and unique powdery mildew resistance locus originally discovered in the Chinese species *V. romanetti*. Table 1 presents the details of MAS testing and planting of genotypes a the ≥90% *vinifera* level that contain both PD and PM resistance. Resistance to PD and PM is homozygous at one or more loci in some resistant lines and we plan to make a final cross back to pure *vinifera*. Overall, 16% of MAS tested genotypes made it to the field due to the strict selection for homozygous markers.

Table 1. 2013 PD breeding crosses MAS tested and planted to field. (# to field Notes: a. Only those R at *PdR1b* planted. b. Only those RR at *PdR1b* planted. c. Only RR at one and R at the other loci planted).

Cross PDR Type	Cross PM Type	% Vinifera	# MAS tested	# to field	# to field note
PdR1b x b42-26^2	None	86%	45	25	a
(*PdR1b* x b42-26)^2	None	86%	300	32	b
b42-26^2	None	75%	-	83	
PdR1b^2	*Ren4*^2	92%	150	33	c
PdR1b^2 x b42-26	*Ren4*	89%	220	29	b

In 2014, breeding efforts continued with the stacking of the *PdR1b* and b42-26 sources that were advanced one generation to the 92% *vinifera* level. Selfing of these will create individuals homozygous at both resistance regions for a final crossing to pure *vinifera* to produce cultivars ≥96% *vinifera* (Table 2a). Based on preliminary DNA sequence data and PD phenotypic symptom differences with *PdR1b* containing genotypes, we are renewing efforts in the b40-14 line, which is the source of *PdR1c* on LG14. Table 2b reflects crosses made in this line. Crosses to 09-367 go back to our most resistant 75% *vinifera* individuals to insure we bring along minor resistance loci as we advance to higher backcross levels. Crosses to 12-326 and 12-327 will produce progeny ≥ 94% *vinifera*. Advancing promising new SWUS resistance sources for both breeding and mapping is summarized in Table 2c. Of particular interest is the b46-43 line where all F1 individuals were resistant by both phenotype and ELISA in our greenhouse test. This population will also be used for mapping this promising new resistance source. Table 2d and 2e show crosses made to pyramid both PD and PM resistance from multiple sources into single cultivars. *Ren1* is a reliable, strain-specific PM resistance source found in a number of pure *vinifera* cultivars from Central Asia.

Table 2. PD Breeding Crosses made in 2014. A) Crosses made to pyramid *PdR1b* (F8909-08) Monterrey *V. arizonica/candicans* and b42-26 *V. arizonica* resistance lines to produce 92.2% *vinifera* progeny. B)

Chihuahua *V. arizonica* resistance source (*PdR1c*) b40-14 to produce progeny with 87.5% (09-367 crosses) or 94.75% *V. vinifera* parentage. C) Cross made to three new promising SWUS resistance lines to produce BC1 75% *V. vinifera* progeny. 08319-07 and 08326-61 are self's of Zinfandel and Cabernet Franc respectively and 100% vinifera. D) Monterrey *V. arizonica/candicans* resistance source *PdR1b* (F8909-08) to produce progeny with approximately 91% *V. vinifera* parentage with PM resistance from advanced *Ren1* and *Ren4* lines. Some crosses will produce homozygous resistant progeny at one or more loci. E) Monterrey *V. arizonica/candicans* resistance source *PdR1b* (F8909-08) pyramided with b42-26 *V. arizonica* resistance lines to produce progeny about 90% *vinifera* parentage with PM resistance from *Ren4* or both *Ren1* and *Ren4* lines. Some crosses will produce homozygous resistant progeny at one or more loci.

	Resistant Type	*Vinifera* Parent\grandparent of Resistant Type	*Vinifera* Types used in 2014 crosses	Estimated # of Seed
A	09-331	Zinfandel,Grenache\Petite Sirah,F2-35	Zinfandel\Petite Sirah; Chardonnay\Grenache	1100
B	09-367	Cabernet Sauvignon\Airen	Carignane, Nero d'Avola, Zinfandel	230
	12-326, 12-327	Carignane,Grenache\Cabernet Sauvignon	Carignane, F2-35, Nero d'Avola	515
C	ANU5, b40-29, b46-43	F2-35, Rosa Minna	08319-07, 08326-61, Alicante Bouschet	1580
D	07-365, 08-335, 11-326, 11-715	F2-35\Karadzhandal	Zinfanel\Petite Sirah	1145
E	11-394	F2-35\Karadzhandal	Zinfanel\Petite Syrah	1100

Table 3 provides a list of the PD greenhouse screens analyzed, initiated and/or completed over the last 6 months. We are making every effort in new lines to bring minor genes along with those for which we have markers. In group A we are testing 28 BC1 genotypes from the b43-17 line that were selected with markers for minor resistance genes that may have been lost during the early breeding generations with *PdR1*. In groups A as well as B we continue testing of *PdR1b* selections at the 97-98% *vinifera* level. The special focus of these trials is on white-fruited selections and those that descend from Nero d'Avola. Results from these and earlier screens have helped us to decide on the resistant selection of genotypes to advance to field trials (for example, Table 4) and to Foundation Plant Services for certification. In part of group B we are retesting 20 of the most promising F1 genotypes from the 2012 crosses from the new SWUS species selections ANU5, b40-29, and b46-43 first tested last August. BC1 crosses were made with these in Spring 2014 to expedite their incorporation into our breeding efforts (Table 2c). In groups C and D we tested genotypes from the b42-26 multigenic PD resistance line. Included in the Group C testing were 25 PD rootstock genotypes from 4 crosses made in 2011 – advanced *PdR1b* rootstocks were crossed to Ramsey, 420A or Schwarzmann. Based on phenotype scores, five of the crosses to Ramsey look particularly promising. ELISA results are pending. Fifty-three new southwestern US species were tested in groups A, C, D and F to facilitate PD resistance gene discovery work being done in our companion Pierce's disease mapping project. BC2 progeny in the b40-14 line from crosses made in 2012 are being tested in Group F.

Table 3. Greenhouse PD screens analyzed, completed and/or initiated during the reporting period.

Group	Genotypes	No. of Genotypes	Inoculation Date	ELISA Sample Date	Resistance Source(s)
A	SWUS Species, PdR1-, 09-10 PdR1b advanced	132	11/19/2013	2/18/2014	Species, b43-17
B	2010 Cross *PdR1b*, 2009 *PdR1b* cross final, 2012 SWUS Cross F1 most PDR	99	3/13/2014	6/12/14	b43-17, b40-29, b46-43, ANU05
C	*PdR1b* x b42-26 pyramided, new PD rootstocks, *PdR1a* advanced, SWUS species	165	3/20/2014	6/19/14	F8909-08, PdR1a, b42-26, Ramsey, V. species
D	b42-26 BC2 & SWUS Species	47	4/8/2014	7/8/14	b42-26, V. species
E	2013 SWUS F1 Cross Seedlings	190	4/24/2014	7/29/14	b41-13, b43-57, SC36, T03-16
F	88% b40-14, Additional 2013 SWUS Cross Seedlings	146	5/15/2015	8/14/14	b40-14, b43-57, b47-32

We have also established new field trials with cooperators in PD hot spots in both Temecula and Napa. 25 vine reps of each of 8 advanced *PdR1b* accessions were planted (Table 4). No pictures are provided since they are newly planted. Extensive details on the encouraging results from our Beringer field trial in Napa were provided in the last progress report.

Table 4. 97% *vinifera PdR1b* scions and rootstocks used for two field trials planted in 2014. 08314-15 and 08314-46 rootstocks are PD resistant stocks developed at UCD.

Genotype	Rootstock	Parentage	Scion Color
09314-102	1103 Paulsen	07370-028 x Cabernet Sauvignon	W
09330-07	08314-15	07370-039 x Zinfandel	B
09331-047	08314-46	07355-020 x Zinfandel	B
09331-133	1103 Paulsen	07355-020 x Zinfandel	B
09333-178	101-14 Mgt	07355-020 x Chardonnay	B
09333-253	1103 Paulsen	07355-020 x Chardonnay	B
09333-331	1103 Paulsen	07355-020 x Chardonnay	B
09333-370	1103 Paulsen	07355-020 x Chardonnay	B

Characterizing Xf Resistance

Table 5 shows the seedling populations produced from F1 crosses made in 2013 to fve new PD resistant *Vitis species* accessions from the southwestern US and northern Mexico, number of seedlings in testing and the number planted to the field. These populations are being considered for possible genetic mapping so that genetic markers can be developed to expedite breeding. The resistant parental

accessions were chosen based on their low ELISA values, minimal expression of PD symptoms in the greenhouse screen and their diverse geographic origins and genetic profiles. Multiple copies of each genotype were made and greenhouse testing is scheduled to becompleted in July and August to characterize the inheritance of PD resistance (Table 3 Groups E & F).

Table 5. Seedlings produced from F1 crosses made in 2013 to develop genetic maps in new accessions from the southwestern US and Mexico. F2-35 is a 100% *vinifera* female flowered progeny of Carignane and Cabernet Sauvignon. Note: numbers of selections destined to the field for these crosses is pending on the Greenhouse/ELISA results. Only the most resistant will be planted.

Resistance Source	Pure *vinifera* types Used in 2013 Crosses	Geographic Origin	Chloroplast DNA Haplotype Similar to:	LG14 *PdR1* Region Similar to:	No. of plants in the in Field	No. of genotypes in GH testing
T03-16	Palomino	Lajitas, TX	b46-43, b47-32	unique	184	62
b47-32	F2-35	Big Bend Park, TX	b46-43, T03-16	b43-17	see note	44
SC36	Grenache	Fallbrook, CA	ANU05	unique	56	35
b41-13	F2-35	Ciudad Mante, MX	b40-29, b40-14	few b43-17	85	47
b43-57	Malaga Rosada	Rinconada, MX	b43-17	b43-17	see note	50

In Spring 2014 crosses were made to seven new and one previously used SWUS PD resistant *V. species* accessions (Table 6). A subset of these will be rapidly advanced to GH testing this winter to preview the nature of their *Xf* resistance.

Table 6. F1 Crosses made in 2014 to develop genetic maps in new accessions from southwestern US and Mexico germplasm. Genotype 08326-61 is a self of Cabernet Franc, F2-35 is a cross of Cabernet Sauvignon x Carignane; both are 100% *vinifera*.

Resistant Source/ new or existing	Geographic Origin - Appearance Phenotype	Pure *Vinifera* Types used in 2013 crosses	Estimated # of Seed
A14	*V. arizonica* AZ	Pinot blanc, Nero d'Avola, Colombard	625
A28	*V. arizonica* AZ	F2-35, Rosa Minna	700
ANU67	V. arizonica Mojave	F2-35	300
ANU71	*V. arizonica/riparia* Mojave	Colombard, Grenache blanc	166
b46-43	*V. arizonica/monticola* Big Bend, TX	08326-61	210
C23-94	V. arizonica AZ	Pinot blanc, Nero d'Avola	205
2236.2	*V. cinerea* Veracruz, MX	F2-35, Malaga Rosada	450
SAZ71	*V. arizonica* San Rafael, AZ	F2-35	475

CONCLUSIONS AND LAYPERSON SUMMARY

We continue to make strong progress towards breeding PD resistant winegrapes. Aggressive vine training and selection for precocious flowering has allowed us to reduce the seed-to-seed cycle to 2 years. We are also using marker-assisted selection (MAS) for the PD resistance gene, *PdR1* (see reports from our companion project) to select resistant progeny as soon as seeds germinate. These two practices have greatly accelerated the breeding program and allowed us to produce four backcross

generations with elite *V. vinifera* wine grape cultivars in 10 years. We have screened through about 1,000 progeny from the 2009 and 2010 crosses that are 97% *vinifera* with the *PdR1b* resistance gene from *V. arizonica* b43-17. Seedlings from these crosses continue to fruit and those with high quality fruit are advanced to greenhouse testing, where only those with the highest resistance to *Xf*, after multiple greenhouse tests, are advanced to multi-vine wine testing at Davis and at PD hot spots in California. The best of these will be advanced to 25 vine commercial wine testing, the first group that was planted in 2014. We have now sent 13 scion and three rootstocks advanced selections to FPS over the last two seasons to be certified and begin the release process. PD resistance from *V. shuttleworthii* and BD5-117 is also being pursued but progress is limited due to multigenic nature of resistance. Other forms of *V. arizonica* are being studied and the resistance of some will be genetically mapped for future efforts to combine/stack multiple resistance sources and ensure durable resistance. Very small-scale wines from 94% and 97% *vinifera PdR1b* selections have been very good, and have been received well at tastings in the campus winery and at pubic tastings in Davis, Sacramento (CAWG), Healdsburg (twice), Napa, Fresno and Temecula and Santa Rosa (Sonoma Winegrape Commission).

PUBLICATIONS AND PRESENTATIONS

Talks at Grower Meetings (Extension/Outreach)

Walker grape breeding program. Napa Valley Grape Growers, UC Davis, February 8, 2013.
Grape breeding. Napa Valley Vintners. UC Davis, Feb. 27, 2013.
The vineyard of the future. Wine Executive Program, UC Davis, March 28, 2013.
Breeding PD resistant wine grapes (including new PD rootstocks). Santa Rosa Winegrape Association Meeting, Santa Rosa, CA, April 5, 2013.
Sustainable Viticulture. Haas Business School, DNV Top Tech Program, Mondavi Winery, Oakville, CA, April 20, 2013
Pest and disease threats: Decisions and the future of farming. Napa Valley 2030 – Ahead of the Curve, Napa Valley Grape Growers, Napa, May 7, 2013
Walker grape breeding program. Chilean Winegrowers Meeting, UC Davis, May 7, 2013
Grape Improvement: breeding, genetics, genomics, 'omics'. International Table Grape Symposium, Ica, Peru June 19, 2013.
Breeding resistant grapes. Diageo Central Coast Growers Meeting, Asilomar, CA, July 24.
Walker, A. 2014. PD resistant winegrapes coming soon. Current Wine and Winegrape Research Conference / Unified Grant Management, UC Davis, February 12

Papers and Presentations at Scientific Meetings

Grape Improvement: breeding, genetics, genomics, 'omics'. International Table Grape Symposium, Ica, Peru June 19, 2013.
Bistué C., Agüero C.B., Riaz S., and Walker M.A. 2013. Testing *Vitis arizonica* candidate genes for Pierce's disease resistance in *Nicotiana tobacum* /SR-1. 64th ASEV National Meeting, Monterey, CA, June 26, 2013.
Riaz, S. and Walker, M.A. 2013. Using marker-assisted selection to optimize grape breeding. Grape Research Coordination Network Meeting, UC Davis, Davis, CA, July 11, 2013.
Walker, A. 2013. Walker lab grape breeding. North American Grape Breeders Meeting, Fayetteville, AR, August 15, 2013.
Walker, M.A. 2013. Grape breeding at UC Davis, Seminar at Missouri State University, Springfield, MO, August 10, 2103.
Walker, M.A. 2013. Optimizing grape improvement with molecular tools. University of Missouri, Colombia, MO, August 11, 2013.
Grape breeding at UC Davis. Viticulture and Enology Seminar, UC Davis, Oct. 4, 2013.
Agüero, C., Bistué, C., Riaz, S. and Walker, A. 2013. Molecular characterization of the putative *Xylella fastidiosa* resistance gene(s) from b43-17 (*V. arizonica/candicans*). 2013. Pierce's Disease Research Progress Reports. Sacramento, CA, December 16-18, 2013. http://www.cdfa.ca.gov/pdcp/Research.html
Walker, A., A.C. Tenscher and S. Riaz. 2013. Breeding Pierce's disease resistant winegrapes. Pierce's Disease Research Progress Reports. Sacramento, CA, December 16-18, 2013.

Riaz, S., R Hu and M.A. Walker. 2013. Genetic mapping of *Xylella fastidiosa* resistance gene(s) in grape germplasm from the southern United States. Pierce's Disease Research Progress Reports. Sacramento, CA, December 16-18, 2013.
Walker, A. 2013. PD resistant winegrapes nearing release. Pierce's Disease Research Symposium, Sacramento, CA, December 18
Sun, Q., Y. Sun, M.A. Walker and J.M. Labavitch. 2013. Vascular occlusions in grapevines with Pierce's disease make disease symptom development worse. Plant Physiology 161:1529-1541.
Riaz, S., J-M. Boursiqout, G. Dangl, T. Lacombe, V. Laucou, A. Tenscher and M.A. Walker. 2013. Identification of mildew resistance in wild and cultivated Central Asian grape germplasm. BMC Plant Biology 13:149.
Agüero, C. B., S. Riaz, A. Tenscher, X. Xie and M. A. Walker. 2014. Functional analysis of Pierce's disease resistance genes from *Vitis arizonica*. 65th ASEV National Meeting, Austin, TX
Riaz, S., R. Hu, A. Tenscher and M. A. Walker. 2014. Comparative sequence analysis of the Pierce's disease resistance locus *PdR1.* 65th ASEV National Meeting, Austin, TX

RESEARCH RELEVANCE

The goal of this research is two-fold: to produce PD resistant wine grapes that can be used in PD hot spot in California and across the southern US; and to provide breeding, maintenance and screening support for our gene characterization and genetic mapping efforts. We now have hundreds of selections at the 97% *vinifera* level and have begun the process of determining which are most resistant and most suitable for release. Over the last two years 13 winegrape and 3 rootstock selections were sent to FPS to be certified and prepared for release.

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.