GENETIC MAPPING OF *XYLELLA FASTIDIOSA* RESISTANCE GENE(S) IN GRAPE GERMPLASM FROM THE SOUTHERN UNITED STATES.

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LAYPERSON SUMMARY

Genetic mapping of two different forms of *Vitis arizonica* has identified a region on chromosome 14 that is responsible for Pierce's disease (PD) resistance, we named it *PdR1*. We mapped two forms of *PdR1* from *V. arizonica/candicans* b43-17, identified a minor gene on chromosome 19 (*PdR2*), and have mapped a third form, *PdR1c*, which originated from *V. arizonica* b40-14. These forms are both single dominant genes for PD resistance. The resistance of *V. arizonica/girdiana* b42-26 is controlled by multiple genes. This resistance is being studied to determine if fine-scale mapping will allow markers to be placed closely enough to these multiple resistance genes to be useful for marker-assisted selection (MAS) in our breeding program. We plan to combine these varying resistance sources in our breeding program to ensure broad and durable resistance to PD. These mapping efforts are also essential to physically locating and characterizing PD resistance genes, so that we can study how they work and predict how well or how long they will function. We expanded our search for plant material that possesses resistance to PD by selecting and greenhouse screening 52 wild grape species accessions that were collected from different parts of the southern US and Mexico. Greenhouse screening identified 10 accessions with good resistance and that can be used to developing breeding and mapping populations. These populations will be used to determine the inheritance of PD resistance, develop framework genetic maps, and identify resistance regions for the development of markers to facilitate the breeding program.

This research project provides the genetic markers critical to the successful classical breeding of PD resistant wine, table and raisin grapes. Identification of markers for the PD resistance gene, *PdR1*, has allowed us to reduce the seed-to-seed cycle to two years and attain four backcross generations to produce resistant vines with 97% *vinifera* in 10 years. The development of these markers also led to the identification of six genetic sequences that house the resistance gene. These sequences are in the process of testing to verify their function. These efforts will help us better understand how, and which of, these genes function, and could lead to the identification of PD resistance genes from grape that would be available to genetically engineer PD resistance in *V. vinifera* cultivars

INTRODUCTION

We are mapping Pierce's disease (PD) resistance in different forms of *Vitis arizonica*. The breeding program produces and screens the seedling populations upon which the genetic mapping efforts depend. The tightly linked genetic markers generated in these mapping efforts are used to optimize and greatly accelerate the PD breeding program. These markers are essential to the successful introgression of resistance from multiple sources, because although the resistance genes may vary, the expression of resistance (the phenotype) is the same. Only the markers can verify that different resistance sources are being successfully combined. We are also identifying resistance in other southern grape species in an effort to discover new resistance genes. Once the species are identified we will genetically map the resistance, identify genetic markers that are tightly linked to the resistance and use them to pyramid resistance from different backgrounds into a single line. We are pursuing two other resistant *V. arizonica* forms: b42-26 *V. arizonica/girdiana* from Loreto, Baja California; and b40-14 *V. arizonica* from Chihuahua, Mexico. Although they are morphologically distinct from b43-17, they both posses strong resistance to PD and greatly suppress *Xylella fastidiosa (Xf)* levels in stem tissue after greenhouse screening. We have also widened the search for additional resistance sources by screening species collections from different parts of southern US and Mexico. Initial greenhouse screen results indicate that we have 10 other accessions that possess strong PD resistance.

OBJECTIVES

1. Complete the genetic mapping of additional QTLs in the 04191 (*V. vinifera* F2-7 x F8909-17 (*V. rupestris* x *V. arizonica/candicans* b43-17) population.
2. Greenhouse screen and genetically map PD resistance from other forms of *V. arizonica*: b42-26 (*V. arizonica/girdiana*) and b40-14 (*V. arizonica*).
3. Evaluate *Vitis* germplasm collected (250 accessions) from across the southwestern US to identify accessions with unique forms of PD resistance for grape breeding. Determine the inheritance of PD resistance from *Muscadinia rotundifolia*, develop new and exploit existing breeding populations to genetically map this resistance.
4. Complete the physical mapping of *PdR1a* and *PdR1b* and initiate the sequencing of BAC clones that carry *PdR1a* gene candidates.

RESULTS AND DISCUSSION

Objective 1. The genetic mapping of the 04191 (*V. vinifera* F2-7 x F8909-17 (*V. rupestris* x *V. arizonica/candicans* b43-17)) population was carried out to identify additional minor QTL(s) other than the major locus on chromosome 14 (*PdR1a*). A total of 139 SSR markers representing all 19 chromosomes were added to the set of 153 seedlings, of which 141 were greenhouse screened for resistance. A genetic map was constructed with a LOD score of 5.0 and a recombination frequency of 0.40. A total of 136 markers were grouped on 19 chromosomes. We confirmed the major locus, *PdR1a*, on chromosome 14 that explained 78% of the variation with a 95% confidence level (**Figure 1**). This work also identified a minor QTL (*PdR2*) with a LOD 2.3 that explains 7% of the phenotypic variation on chromosome 19, and which peaks at marker CB918037 (**Figure 2**). This QTL is within a 10 cM interval – a very large genetic distance for map-based positional cloning purposes. In order to narrow this region, we developed seven SSR markers based on the Pinot noir genome sequence (PN 40024). These markers should allow us to reduce the gap from 10 cM. In order to test whether the QTL on chromosome 19 has an additive effect in conjunction with the *PdR1a* locus, we analyzed the resistant and susceptible genotypes with a least square means test for chromosomes 14 and 19 (**Table 1**). It was clear that both loci work independently of each other and they do not have an additive impact. The mean ELISA values of resistant and susceptible plants with the *PdR1a* locus were very different, however, the mean values of resistant and susceptible plants for the *PdR2* locus were higher for the resistant plants.

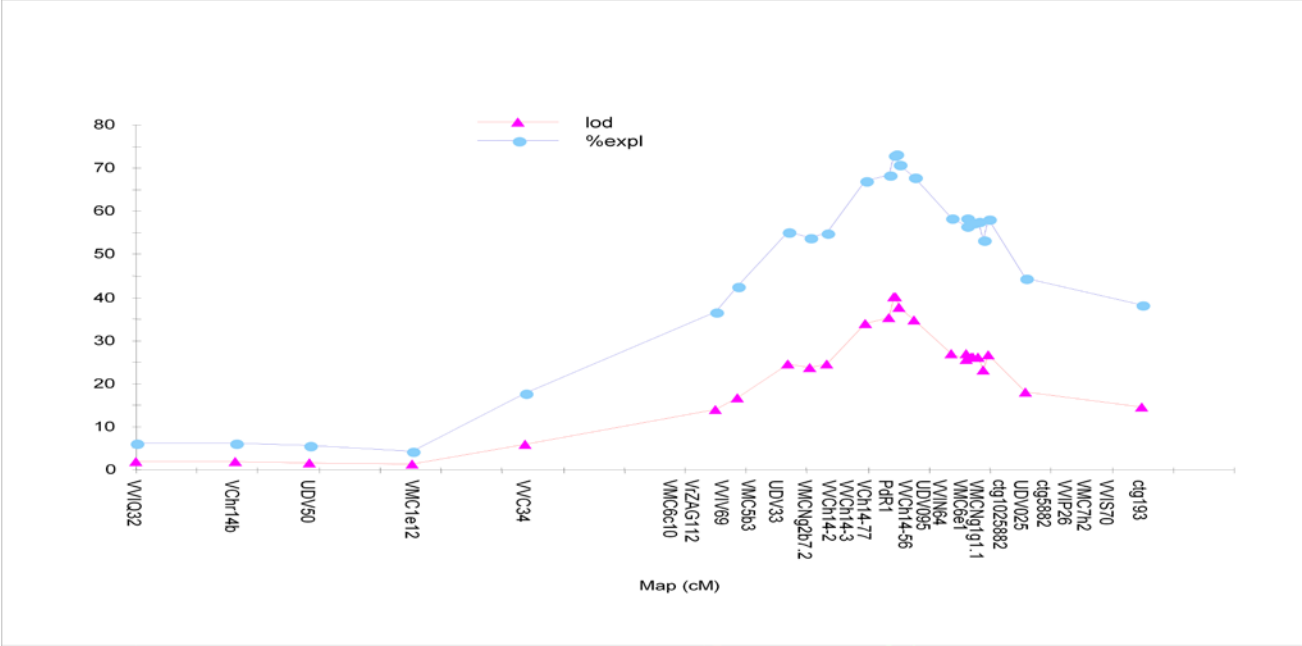


Figure 1. Updated interval mapping analysis of the *PdR1a* locus on LG 14.

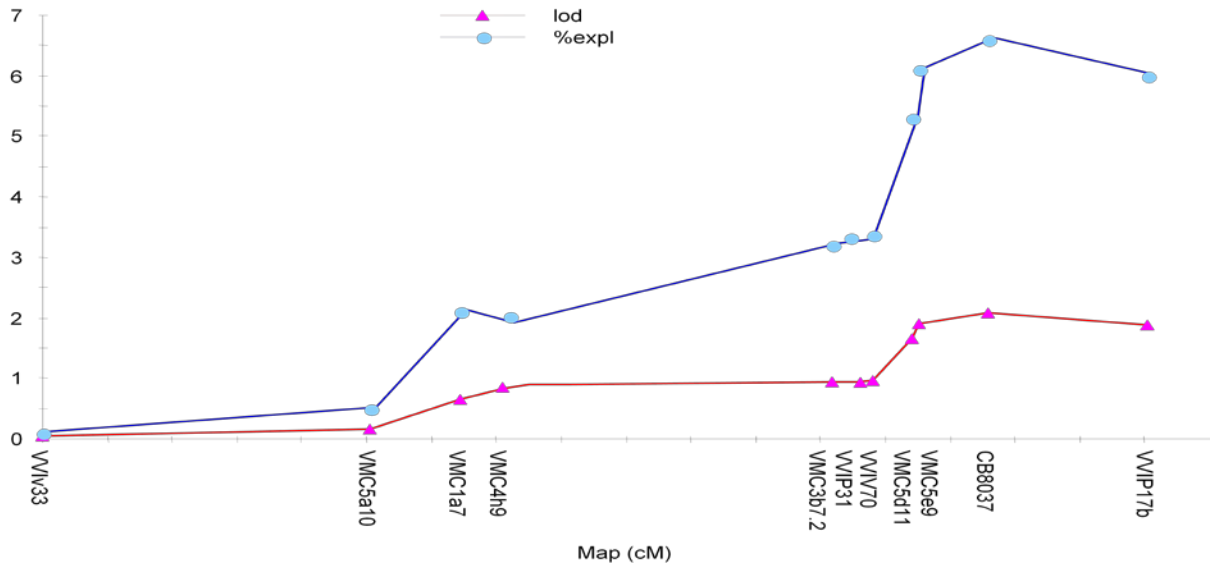
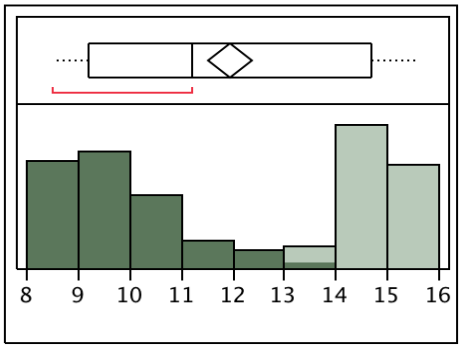


Figure 2. Interval mapping analysis of the *PdR2* locus on LG 19.

Table 1. The distribution of natural log transformed ELISA values from a greenhouse testing of the 04191 population.

Mean (ln cfu/ml)



Quantiles		
100.0%	maximum	15.6
99.5%		15.6
97.5%		15.6
90.0%		15.28
75.0%	quartile	14.7
50.0%	median	11.2
25.0%	quartile	9.2
10.0%		8.8
2.5%		8.5
0.5%		8.5
0.0%	minimum	8.5

Moments	
Mean	11.941844
Std Dev	2.6467273
Std Err Mean	0.2228946
Upper 95% Mean	12.382519
Lower 95% Mean	11.501169
N	141

Objective 2. Resistance to PD in *V. arizonica/girdiana* b42-26 is strong but is controlled by multiple genes. We completed preliminary QTL analysis with 64 greenhouse screened genotypes (**Figure 3**) from the 05347 population (*V. vinifera* F2-35 x b42-26) using data from 71 SSR markers. They were analyzed with the Kruskal-Wallis test, which allows association of each marker to the phenotypic trait. Because we know the chromosomes the markers reside on, we can get a rough map from this analysis. The results indicated that markers from chromosome 10 and 14 (and to a lesser extent 2 and 11) are associated with PD resistance. This allows us to focus mapping efforts on markers known to exist on these chromosomes, which will greatly accelerate the identification of genomic regions responsible for b42-26's resistance.

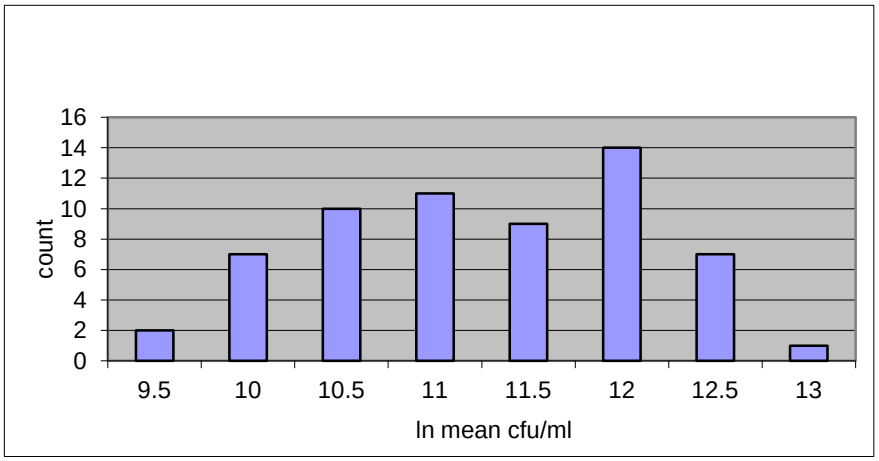


Figure 3. Distribution of ELISA values (ln means in cfu/ml) of greenhouse tested progeny from the 05347 population.

The genome of b42-26 is very homozygous, which is unusual for *Vitis* species and suggests that this collection is from a very localized population which has been inbred over time. This is likely given its collection location from a coastal valley along Baja California's east desert coast. This also means that many of the homozygous SSR markers are not useful for mapping. Thus, we developed 71 new SSR markers from clone sequences generated by the Vitis Microsatellite Consortium (the original source of SSR markers for grape). We acquired primer sequences of an additional 200 markers that have not been used to test b42-26. Marker testing on small set progeny and the parents is underway. We are also adding markers to develop a framework map for the entire population. We are now mapping with the complete set of 239 seedlings with 30 to 50 markers known to exist on chromosomes 2, 10, 11 and 14.

A single dominant gene controls PD resistance in *V. arizonica* b40-14. Two resistant siblings from the R8918population (*V. rupestris* x *V. arizonica* b40-14) were used to develop the 07388 (R8918-02 x *V. vinifera*) and 07744 (R8918-05 x *V. vinifera*) populations. Two hundred and twenty-seven markers were polymorphic for one of the parents; 152 were analyzed on the entire set of 122 plants; a framework map of R8918-05 was produced with MAP QTL (4.0) and the Kruskal-Wallis approach was used to complete the preliminary analysis. PD resistance mapped only on chromosome 14 – the same chromosome where *PdR1a* and *PdR1b* mapped. PD resistance from b40-14 (which we have named *PdR1c*) maps in the same general region between flanking markers VVCh14-77 and VVIN64 and within 1.5 cM. The LOD threshold for the presence of this QTL was 33 and 82% of the phenotypic variation was explained (**Figure 4**). In 2009, crosses were made with F1 resistant selections from 07744 population.

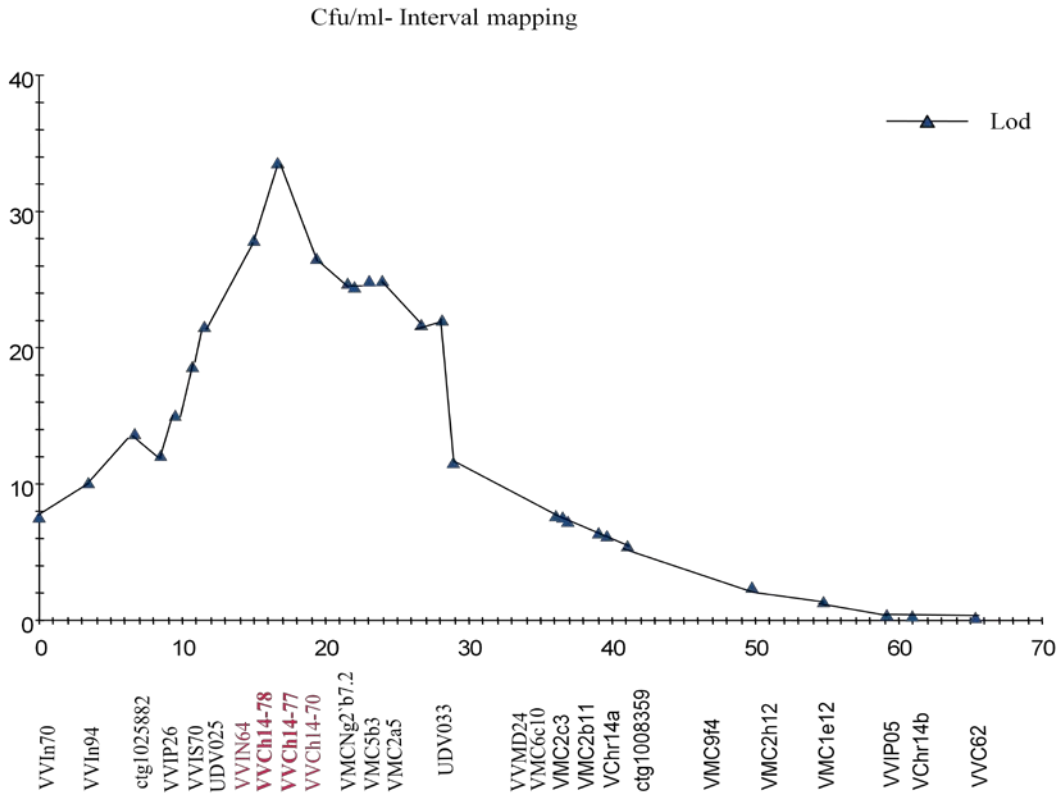


Figure 4. Interval mapping of *PdR1c* from the 07744 population indicating a peak at LOD 34.0 with a 95% confidence interval. The X-axis indicates the position of the markers; LOD values are plotted on the Y-axis.

Objective 3. *Vitis* species growing in the southern US have co-evolved with *Xf* and resist PD. To date we have focused on accessions of *Vitis* species that Olmo collected in northern Mexico in 1960. In addition to these accessions, we have more than 250 accessions collected from PD hot spots in Texas, New Mexico, Arizona, Nevada and California. Fifty-two of these from across this geographic range (including the 15 accessions from Mexico) are being evaluated with our greenhouse screen. Based on early evaluations of cane maturation and leaf scorch and leaf loss, we have identified 10 accessions that had very good scores for these two parameters. Three of these accessions were collected collected from Texas and Arizona (**Table 2**). The ANU5 accession was collected near Utah along the Virgin River and is a very easterly selection of *V. girdiana*. A recent collection trip to southwest Utah found this population is expanding and may prove very valuable. This accession also has excellent salt tolerance and is being utilized in our rootstock breeding program. ELISA results will be ready in November or early December 2011. **Figure 5** shows where the *Vitis* accessions current under test were collected. Many of the collection sites have multiple accessions associated with them, but they are not seen in this depiction. The green dots indicate accessions with promising resistance.

Working with this germplasm will expand the pool of resistance genes available for breeding, identify potentially unique sources of resistance, and identify geographic regions with high levels of resistance so that areas can be identified for future collections. Studying the inheritance of resistance in these accessions will be the next area for investigation followed by characterizing the nature of resistance so that multiple forms can be combined to broaden PD resistance.

Objective 4. We have employed three categories of sequencing (shotgun reads, fosmid reads and 454 sequencing) to localize the BAC clone H69J14 that carries the *PdR1* gene(s). Now that this sequence is assembled, we have been able to identify six genes ranging in size from 2Kb to 3.1Kb in the resistance region. Copies 1 through 4 are 97 to 99% similar and may be tandem repeats of one gene. They are also up to 78% similar to four copies of genes on the Pinot noir PN40024 sequence (**Figure 6**). We utilized CENSOR software to screen query sequences against a reference collection of repeats to generate a report classifying all of the detected repeats. All four PN40024 genes carry DNA transposons as well as LTR retrotransposons confirming that the region is very complex.

Table 2. List of promising accessions from Mexico and US that performed well in the greenhouse screen. CMSSI is the cane maturation index, LS-LL is an index of leaf scorch and leaf loss, both values are recorded before samples are run to detect *Xf* in stems with ELISA.

Genotype	Mean (CMSSI)	Mean (LS-LL Index)	Sex	Source
ANU5	0.0	1.5	F	Littlefield, AZ - near I15 bridge crossing Virgin River
B40-14	3.8	2.1	M	80km n Chihuahua
B40-29	0.0	0.8	M	80km n Chihuahua
B41-13	1.0	0.8	F	near Ciudad Mante - Ciudad de Maiz
B42-26	0.8	1.4	M	Loreto, B.C - 200 km N La Paz
B43-17	2.5	2.3	M	Guadalupe, near Monterrey
B46-43	0.0	1.0	M	Big Bend Park, 250km W San Antonio
B46-48	0.0	0.8	F	Big Bend Park, 250km W San Antonio
B47-28	0.0	1.9	F	Big Bend Park, 250km W San Antonio
B47-32	0.0	0.9	M	Big Bend Park, 250km W San Antonio
B47-5	0.0	1.7	F	Big Bend Park, 250km W San Antonio
T 03-16	0.0	1.4		Hwy 170 W of Lajitas, TX
TX9714	2.7	1.0	M	N. Hondo, Medina Co., TX

Figure 5. Source of *Vitis* species collections currently under greenhouse evaluation for resistance to PD. The green dots indicate accession with promising resistance based on foliar symptoms. ELISA results are due in mid to late November 2011.

We utilized different tools from www.expasy.org/tool/ to conduct pattern and profile searches of the PD resistance genes. There is very strong evidence of a leucine rich repeat (LRR) region in five of the candidate genes. **Figure 7** displays differences in the LRR regions in the *PdR1b-1* gene. There is no signal sequence in the protein sequence, which suggests that the resistance gene product is not secreted. There is also no indication of a coiled-coil, which suggests that the PD resistance gene is not a member of the CC-NB-LRR class of resistance proteins. The protein sequences do carry trans-membrane domains, however they lack the kinase domain. Interestingly, the PdR1b-6 gene candidate that was very different from the other candidates with protein kinase domains.

Currently we have cloned and verified the sequence of copy 1 and copy 6 candidate genes and are developing constructs for transformation experiments to determine which of these gene candidates confers resistance to PD. (See companion report "Molecular characterization of the putative *Xylella fastidiosa* resistance gene(s) from b43-17 (*V. arizonica*)).

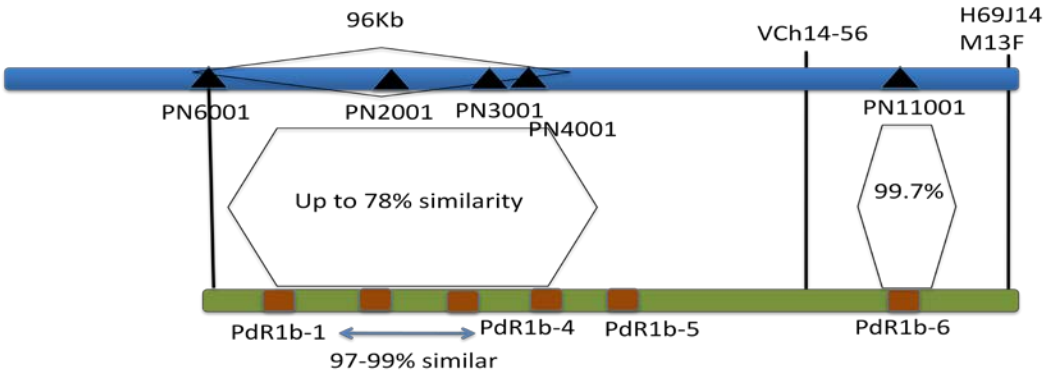


Figure 6. A direct comparison of the H69J14 clone sequence to the Pinot noir PN40024 sequence is not possible due to major re-arrangement of repetitive elements between the two genomes.

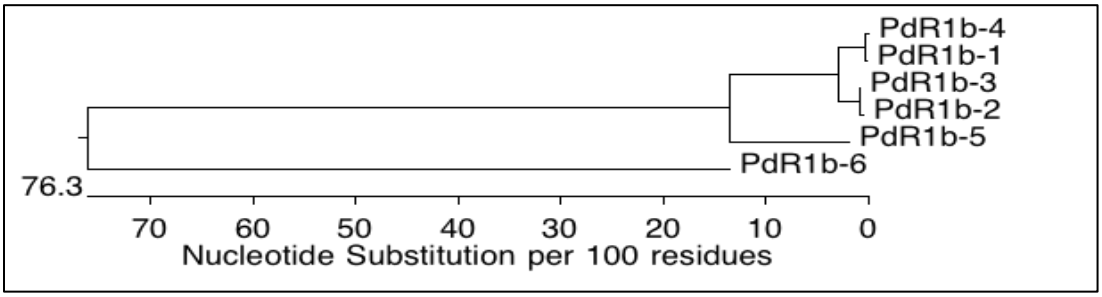


Figure 7. An alignment tree of the six candidate PD genes displaying their similarity.

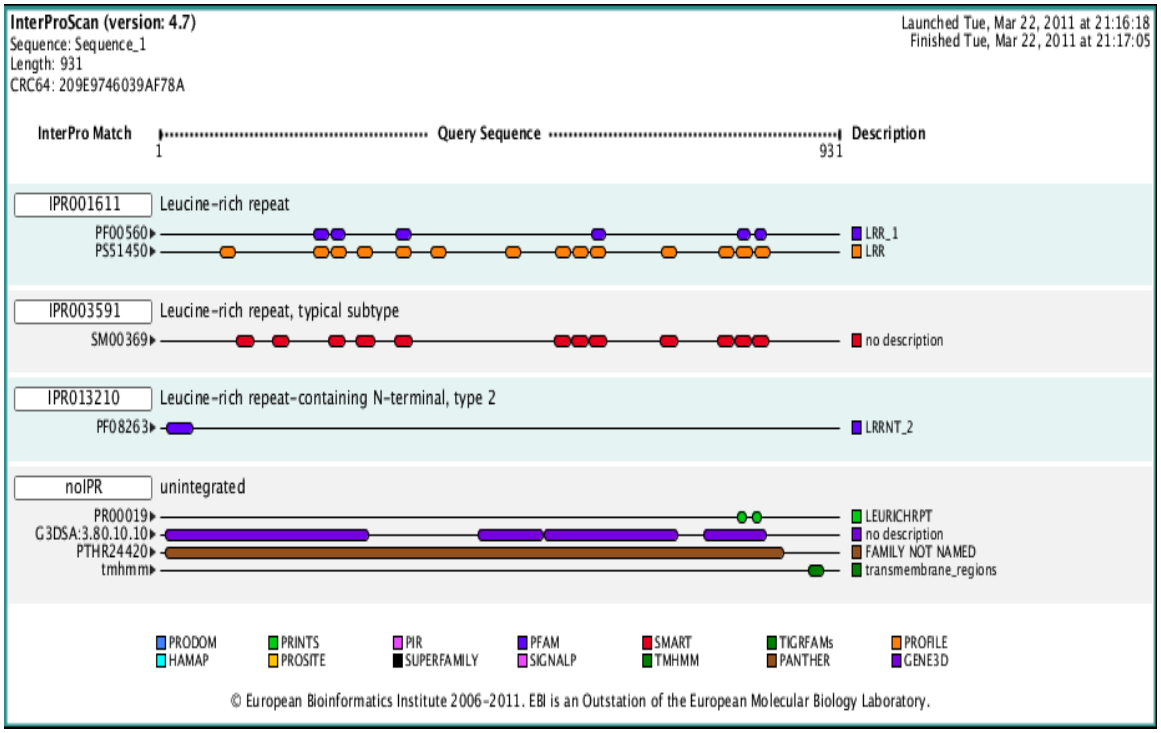


Figure 7. Interpro scan results of the *PdR1b-1* gene.

FUNDING AGENCIES

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