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Research Article

Genetic Characterization of Pierce's Disease Resistance in a *Vitis arizonica/monticola* Wild Grapevine

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Abstract: Pierce's Disease (PD) is a bacterial disease that threatens vineyards across the US and Mexico. Genetic resistance against this disease has been achieved using the resistance locus *PdR1*, which was found in a wild grapevine from Mexico. In order to broaden the genetic base, we aimed to identify additional unique sources of resistance. The objective of this study was to characterize PD resistance in accession b46-43, a PD resistant wild grapevine from Texas. The manifestation of PD resistance in b46-43 is different from b43-17, with even cane lignification, minor leaf scorch and minimum bacterial growth. It was hypothesized that b46-43 possibly carry resistance that differ from the *PdR1* locus in b43-17. A total of 318 seedlings from population 14399 were evaluated for PD resistance and genotyped. The framework genetic map was developed and a quantitative trait locus (QTL) analysis was carried out. The QTL analysis identified a major locus that explained 55.5% of the phenotypic variation on chromosome

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14, at the genomic position *PdR1* was mapped in earlier studies. No minor loci were identified on other chromosomes. The rarity of additional loci indicates that PD resistance may have a common origin and it is widespread as a result of gene flow.

Key words: genetic resistance, grapevine breeding, Pierce's disease, QTL, *Xylella fastidiosa*, wild grapevines

Introduction

Pierce's Disease (PD), an important disease of grapes, is caused by the xylem-limited bacterium, *Xylella fastidiosa*. PD has been reported in the southern regions of North America and central region of South America (Hopkins and Purcell 2002, Walker et al. 2019). *Xylella fastidiosa* has a broad host range that includes landscape, horticultural and agricultural crops within 300 different plant species and is vectored by several sap-feeding insects like sharpshooters and spittle bugs (Rapicavoli et al. 2015). As bacteria colonize the water-conducting xylem vessels, it clogs them by inducing the formation of biofilms. As a defense response, grapevines produce an outgrowth of the parenchyma cells in the xylem vessels, called tyloses, which severely limits water uptake, resulting in desiccation and eventual plant death (Sun et al. 2013).

Pierce's Disease has limited grape production in California, the Gulf Coastal states, and Mexico (Webster et al. 2014). All commercially grown wine, table and raisin grapes belong to the species *Vitis vinifera* that are susceptible to *X. fastidiosa* and succumb to the disease. Different strategies to control its vectors are in use, including insecticides and removal of riparian vegetation that house both vectors and bacterium (Alston et al. 2013). Nonetheless, these methods are expensive, and harmful to human health, the environment, and riparian ecosystems. As of today, no cure is available once the plants are infected (Kyrkou et al. 2018). Hence, breeding of grape varieties with resistance to the pathogen is the only environmentally friendly approach.

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The Grape Breeding Program at University of California, Davis has identified PD resistance in many North American wild grape accessions collected from Mexico and the Southwestern US (Riaz et al. 2018). The genomic location of *PdR1* locus was identified on chromosome 14 in accession b43-17 (Krivanek et al. 2006), and marker-assisted selection was used to develop PD resistant winegrape selections with superior fruit quality. In 2019, five of these selections were released (Walker et al. 2019). The accession b46-43, collected from the Big Bend area in southwestern Texas was among other tested accessions with strong resistance to PD after multiple greenhouse screening trials. Accession b46-43, a putative hybrid of *V. arizonica* and *V. monticola*, had markedly better cane lignification and less leaf abscission and desiccation in comparison to accession b43-17 in greenhouse-based screenings. It also harbored very low bacterial populations when inoculated with *X. fastidiosa*. The accession b46-43 also had a different allelic profile with the nuclear and the chloroplast markers and population diversity metrics placed it in a different genetic clade from b43-17 (Riaz et al. 2018). These results suggested that b46-43 may possess a different form of PD resistance from accession b43-17.

The aim of a successful breeding program is to broaden the genetic base of resistance and stack resistance genes that potentially have different modes of action. In order to achieve this goal, detailed genetic characterization of accession b46-43's PD resistance was required to evaluate its potential use in the breeding program. In this study, we characterized the PD resistance of accession b46-43. A F1 and a pseudo backcross 1 (pBC1) populations were developed and phenotype PD resistance under greenhouse conditions. A framework genetic map was developed using simple sequence repeat (SSR) markers and finally a multiple Quantitative Trait Loci (QTL) analyses were carried out to identify major and potential minor loci that contribute to PD resistance.

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Materials and Methods

Plant material. The PD resistant accession b46-43, a natural hybrid between *V. arizonica* and *V. monticola,* was collected from Big Bend, TX (Fig. 1A) by Harold P. Olmo in 1961. The maternal PD susceptible *V. vinifera* 08326-61 (a selfed progeny from Cabernet Franc) was crossed with the male accession b46-43 to produce F1 population 12305 (Fig. 1B). It was discovered that PD resistance was controlled by a homozygous dominant allele as 100% of the progeny were PD resistant. One male F1 resistant seedling plant, 12305-55, was crossed with the susceptible female F2-35 to develop a pseudo backcross (BC1) population, used for the genetic mapping. Its PD resistance segregated in a 1:1 ratio for the pBC1 population, supporting b46-43's homozygous dominant nature. All F1 and pBC1 breeding population individuals were maintained at the Department of Viticulture and Enology, University of California, Davis, CA.

Disease evaluation. Three trials to evaluate PD resistance were carried out under greenhouse conditions following the protocols described by Krivanek and Walker (2005) in three trials during 2015 (TD151217), 2016 (TD160809) and 2017 (TD171031). A total of 318 seedling plants were tested at least once with four biological replicates each across the three trials. A set of grape accessions with known strong and intermediate PD resistance, as well as the susceptible *V. vinifera* cultivar Chardonnay (un-inoculated and inoculated), were used as references in every screen. The use of reference plants allowed comparison among screen results across different trials and years.

Disease onset was evaluated using three different metrics after 12-14 weeks post inoculation, when the susceptible reference plants were showing visible disease symptoms. The plants were evaluated for their degree of cane maturation and necrosis development designated as Cane Maturation Index (CMI) as described in an earlier study (Krivanek and Walker 2005), the mean percentage area of scorch on four leaves above and nearest to the point of inoculation (POI) and leaf loss was recorded as Leaf Scorch-Leaf Loss (LS-LL) (Krivanek et al. 2006). Finally, the enzyme-linked immunosorbent assay (ELISA) was

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used to quantify the *X. fastidiosa* titer in ±0.5 g of stem tissue as described in (Krivanek and Walker 2005).

The bacterial titer data was normalized for titers lower than 10,000 and higher than 6,500,000 cfu/ml and transformed to the natural log (*X. fastidiosa* cfu/ml (ln)) to obtain homogeneous variances. The statistical analysis was performed using the lme4 package (Bates et al. 2015) in R (R Core Team 2019), and JMP Pro14 (Copyright 2018, SAS Institute Inc.).

Genotyping, framework genetic map and QTL Analysis. DNA was extracted from young leaf tissue using the modified CTAB method (Riaz et al. 2011) which excluded the RNAase step and precipitated DNA with one cycle of chloroform-isoamyl alcohol. Then, DNA dilutions were made. A total of 497 SSR markers from previously published marker series (Thomas et al. 1993, Bowers et al. 1999, Scott et al. 2000, Decrooq et al. 2003, Arroyo-Garcia and Martinez-Zapater 2004, Riaz et al. 2004, Di Gaspero et al. 2005, Merdinoglu et al. 2005, Doligez et al. 2006, Cipriani et al. 2008, the Vitis Microsatellite Consortium managed by AGROGENE) were tested on a subset of eight samples including parents, grandparents and progeny. The SSR marker sequences are available at NCBI database (http://ncbi.nlm.nih.gov). Polymorphic markers were selected to genotype the pBC1 population. Polymerase Chain Reactions (PCR) were based on a previously described protocol (Riaz et al. 2008). PCR products were separated using an ABI 3500 capillary electrophoresis analyzer with GeneScan 500-Liz Size Standard (Life Technologies, Carlsbad, CA, USA) and analyzed with GeneMapper 4.1 software (Applied Biosystem Co., Ltd., USA). The parents and grandparents of the population were used as internal references to verify and standardize the allele calls between plates.

In the first step, a genetic map was completed for only chromosome 14 using the 318 seedlings and 21 SSR markers using JoinMap4 (Van Ooijen 2006). The phenotypic data from all three trials was used to complete a Quantitative Trait Locus (QTL) analysis with MapQTL6 (Van Ooijen 2009). Both

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Interval Mapping (IM) and the Kruskal-Wallis (KW) non-parametric tests were performed and the K* significance (p-values) were obtained for every marker for all three phenotypic scores.

Later, a framework genetic map was developed to cover all nineteen chromosomes using a set of 92 seedling plants. Parental and a consensus genetic maps were constructed using the phenotypic data with the software previously described. The Kosambi mapping function was used to calculate the centiMorgan (cM) distance using linkage with a recombination frequency smaller than 0.4 and LOD larger than 1. A permutation test (PT) was carried out to determine the genome-wide and chromosome-wide LOD thresholds for significant QTLs based at a 5% significance level and with 1000 permutations. The automatic selection cofactor analysis was carried out with a p-value of 0.02 and 200 iterations with the consensus map. Using the selected cofactors, the multiple-QTL model approach (MQM mapping) was also performed to increase the power of identifying multiple QTLs and QTL-environment interactions.

Results

Disease evaluation and segregation. Statistical analysis of the three trials using the reference plants found that there was a significant effect of the trial date on the bacterial titer at the $p < 0.05$ level (F-statistics: 58.15 on 15 and 303 DF, p-value: < 2.2e-16). The post-hoc analysis found no statistical differences between the 2015 and 2016 trials. However, the 2017 trial was significantly different with a lower overall mean (11.569) (Supplemental Table 1). The values of bacterial titer of the reference plants common in the three trials were similar to those we have observed in previous greenhouse screenings for PD resistance (Riaz et al. 2018), confirming consistent results across different years (Table 1). The threshold of natural log of above 13 for the bacterial titer values was considered to be susceptible based on the intermediate reference genotypes. Figure 2 shows the phenotypic distribution of the seedling plants. According to the Chi-square test, the resistance segregated in a 1:1 Mendelian ratio (143:175, $X^2=3.22$, 1 d.f., $p=0.073$).

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Genotyping, framework genetic map and QTL Analysis. *QTL analysis for chromosome 14*. Two markers (VMC6c10 and PD82-1b4) were removed due to missing data; hence, the genetic map for the resistant parent was developed with the allelic data of 19 markers for chromosome 14. The total map length was 98.6 cM with an average distance of 5.18 cM between markers (Fig. 3A). The KW analysis indicated all markers of chromosome 14 had highly significant association to the three metrics of disease evaluation. Fifteen out of nineteen SSR markers were significantly associated to the phenotypic data. Similarly, the IM analysis identified marker ch14-78 with the highest percentage of phenotypic variation for the bacterial titer, the CMI and the LS-LL explaining 44.2%, 22.5% and 11.5%, respectively (Supplemental Table 2, Fig. 3B).

Results from the IM analysis suggested the presence of a QTL on chromosome 14 in the similar genomic region where the *PdR1* locus was identified in accession b43-17 (Krivanek et al. 2006) (Fig. 3B). Given the high percentage of phenotypic variation explained for the *PdR1* locus in b43-17 (72%) compared to the results in this breeding population, it was inferred that other major or minor loci found on another chromosome in b46-43 were potentially contributing to PD resistance.

*Framework genetic map***.** A total of 195 markers were fully informative for both parents and used to generate the parental and consensus genetic maps (Table 2). One hundred and seventy-seven markers were used for the consensus map, which had 23 linkage groups (LG) representing all 19 chromosomes (Supplemental Figure 1). Chromosomes 5, 7, 12 and 19 were fragmented due to a lack of recombination. The consensus map covered 988.03 cM, with an average of 7.69 markers per chromosome, and a 5.58 cM distance between markers. For the 12305-55 and F2-35 parental maps, 20 LG and 21 LG were obtained, respectively. The F2-35 parental map covered 830 cM with 126 markers. There was an average of 6 markers per chromosome and an average distance of 6.58 cM between markers (Table 2). For the PD resistant line 12305-55, the genetic map covered 924.9 CM with 185 markers. There were 9.25 markers per chromosome with an average distance of 4.99 cM between markers. Marker order between the three

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maps was consistent (female and male parental genetic maps not shown), except for chromosomes 14, 16 and 18 where marker order was slightly different in the 12305-55 map compared to the consensus map. Seven markers with high segregation distortion affected the order of other markers on chromosome 14 and they were excluded from the consensus map.

The KW results found that markers on chromosome 14 were highly significant for all three phenotypic scores for the consensus map (Supplemental Table 3). For the bacterial titers on the consensus map, 11 markers on chromosome 14 and three markers outside of chromosome 14 were significant (p-value < 0.01) on the consensus map (UDV21 on chromosome 3, VMCNG3F11 and VMCNG1e.2.2 on chromosome 9). For the CMI score, VVMD31 on chromosome 7, SC02-24 and VMCNG1e2.2 on chromosome 9, UDV101 and ctg5592 on chromosome 10, 4 markers on chromosome 12, UDV78 on chromosome 15, and VMC3c11.1 on chromosome 17 were significant. For the LS-LL, 9 markers on chromosome 14 were highly significant. Interestingly, six markers on chromosome 1 were also significant for LS-LL, as well as VMC3b10 on chromosome 2, VVIp77 and CTG0624 on chromosome 4, VVIn33 and VVC71 on chromosome 5, VMC5c5 on chromosome 6, VMC2f6 on chromosome 13, VVIq61and UDV78 on chromosome 15, and Vchr19a on chromosome 19.

The IM analysis identified a major QTL only on chromosome 14 between markers chr14-29 and UDV25 using the bacterial concentration and CMI phenotypic data (Fig. 4A), corroborating previous results. The phenotypic variation percentage explained was 55.1%, 27.8% and 13.2% for the bacterial titer, the CMI and the LS-LL scores, respectively. The genome wide threshold for a significant QTL was 4.2, 4.3, and 4.2 for the bacterial titer, CMI, and LS-LL, respectively. Unfortunately, no significant QTL were identified on any other chromosome (Fig. 4A).

The automatic cofactor selection analysis selected five SSR markers on chromosome 14: ctg6140, CF2513, VChr14a, VMC2b11, and UDV25. The Multiple QTL Model (MQM) mapping confirmed the QTL found on chromosome 14 with a 33% phenotypic variation explained at marker UDV25 (Fig. 4B).

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Marker UDV25 on chromosome 14 was the most closely linked marker across all three phenotypic parameters – bacterial titer, CMI and LS-LL scores. The MQM analysis did not identify any other additional QTLs apart from chromosome 14.

Discussion

Breeding for disease resistance in crops has proven to be a useful approach that prevents infection, disease spread, and reduces the use of pesticides (Stuthman et al. 2007). The PD resistance breeding program at the University of California, Davis hosts an extensive collection of wild *Vitis* spp. that is a valuable and unique gene pool. The PD resistant accession b43-17, has served as the resistance source in the development of multiple red and white winegrape selections that are up to 97% *V. vinifera* with high fruit and wine quality and strong resistance to the disease (Walker et al. 2019). Nonetheless, to ensure long lasting and broader resistance, plant breeders like to stack different forms of resistance in the same background. The merging of different resistance loci is of great value for combining different mechanisms of resistance that might stop the establishment of a pathogen at different stages of infection.

This study genetically characterized PD resistance in the accession b46-43 collected from Texas, in an effort to identify new sources of PD resistance. In the first phase, the 318 seedlings from the breeding population 14399, were evaluated for PD resistance in greenhouse-based screens and genotyped using SSR markers on chromosome 14. Then, a framework map representing 19 chromosomes was developed using 92 seedlings. The main goal was to identify unique major and minor loci that contribute to PD resistance and develop markers that could be employed in the breeding program.

Interestingly, based on the phylogenetic analysis with nuclear SSR markers, b46-43, a *V. arizonica/monticola* hybrid, was unique and placed in a different clade from the *PdR1*-bearing accession b43-17 (Riaz et al. 2018). Additionally, b46-43 possesses desirable horticultural traits. For instance, b46-43 did not show leaf scorch and had excellent cane maturation during the greenhouse evaluations, and erect habit on the field in comparison to b43-17 (collected from Monterrey, Mexico), which had

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moderate amount of leaf scorch and leaf loss, a shrub-like growth habit, and occasional irregular cane maturation, while possessing very low bacterial titers.

Based on these observations, we expected to potentially identify a different chromosome bearing PD resistance in b46-43. However, the results of this study identified a major locus on chromosome 14 in the similar genomic region where the *PdR1* locus was previously mapped in b43-17 (Krivanek et al. 2006, Riaz et al. 2008). The values of phenotypic variations were lower, indicating the possibility of other major and/or minor QTLs on the other genomic regions (Supplemental Table 2). The QTL analysis with the expanded genetic map of 92 seedlings also confirmed the results of a major locus on chromosome 14 that explained up to 55% of the phenotypic variation (Fig. 4A). No minor QTLs on other chromosomes were identified. These results are in line with another study by Riaz *et al*. (2018) that reported on nine accessions with PD resistance in the similar genomic region on chromosome 14 with phenotypic variations explained from 17.3% to 86.4 %. It is still unknown why this broad phenotypic variation exists within the same locus. One plausible explanation is the allelic diversity of the resistance genes, and the impact of their hybrid nature. A recent study by Riaz *et al*. (2020) revealed that resistance to PD is widespread in Southwestern US wild grapevines, particularly within the grape species *V. arizonica*. The accession b46-43 described in this study is a *V. arizonica/monticola* hybrid based on the morphological description, consistent with the description of all other accessions that harbor PD resistance on the chromosome 14 (Riaz et al. 2018).

It might be possible that these accessions have differences in the underlying genes within the genetic boundaries of the *PdR1* locus. Other studies have identified similar differences also (Cochetel et al. 2021). Cochetel compared sequences of grape powdery mildew resistance locus *Run1* haplotype A and B from cultivar Trayshed. The number of resistance genes were different between haplotypes representing the same locus. In the same study, they identified a similar situation in the *Run2* locus on chromosome 18. Furthermore, the genetic window of *PdR1* locus in b43-17 is less than 1 cM (Riaz et al. 2008). In

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comparison, the genetic window of *PdR1* locus in b46-43 is around 10 cM. High resolution genetic mapping of this region is necessary to bring the genetic window of *PdR1* locus in b46-43 to around 1 cM to deduce if it is the same region.

In general, the presence of major QTLs makes it more challenging to map and validate minor loci (Yang et al. 2013). The inability to find minor effect loci in this population could be the result of several factors. For example, it is well-known that population sample size has a large influence on the accuracy of mapping minor effect QTLs. In this study, a total of 92 seedlings were used to construct the framework genetic map. Previous studies have shown that populations with 60 individuals are sufficient to identify major loci (Riaz et al. 2018), but may not be capable of finding minor loci. An additional study with a larger population size to develop a framework map would allow to carry out the QTL analysis in the absence of a major locus (Pap et al. 2016).

The search for minor loci may also have been impeded by poor marker coverage. It is possible we could not detect these minor QTLs due to the limited number of SSR markers used for the framework genetic map. The consensus map was constructed from 177 markers with an average of 7.69 markers per linkage group (Table 2). Ideally, more markers should have been used to ensure there were no gaps on any chromosome. The KW analysis determines the association of phenotype to each SSR marker. The KW results found few SSR markers were significant outside of chromosome 14 (Supplemental Table 3). Nonetheless, the Interval Mapping analysis we did not identify any minor QTL in those regions. To consider a major or minor QTL to be significant, multiple markers in a region of a chromosome should be significant. In contrast, multiple markers on chromosome 1 were significant for the LS-LL score. Ideally, more markers should be added to chromosome 1 to validate this association. As mentioned previously, infected plants of b46-43 do not lose as many leaves as b43-17. It would be interesting to see if this trait could be linked to this chromosome, but further analysis is needed. If after the addition of

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more markers, the LS-LL score is still significant, it may be hypothesized a minor locus may influence leaf loss.

Lastly, the results from this study also validated the measurement of *X. fastidiosa* titers as the most reliable parameter to identify resistance loci. These results are in line with previous studies that also found that CMI and LS-LL scores were not as strongly correlated to the *PdR1* resistance locus as compared to bacteria titers (Riaz et al. 2006, S. Riaz et al. 2008). However, the visible disease symptoms are helpful to select superior potential parental selections that have minimum leaf scorch, leaf loss, and better cane lignification.

Conclusion

The genetic mapping and QTL analysis identified a major locus on chromosome 14 in accession b46-43. The genomic region was similar where *PdR1* locus had been previously identified in accessions collected from the southwestern US and Mexico. No minor QTL was identified on other chromosomes. The accession b46-43 can be used in the breeding program as it possesses desirable horticultural traits in addition to PD resistance. Only further comparative genomic analysis of the region between all accessions that have resistance on chromosome 14 can fully elucidate differences between b46-43 and b43-17 *PdR1* locus. This comparison could also shed more light on the evolution and mechanism of resistance, candidate genes and their molecular interactions. Finally, conservation of North American wild grapevines has great value for the viticulture industry because of their outstanding potential for breeding resistance to a wide range of biotic and abiotic problems.

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Table 1. Least square means (lsmeans) comparison of bacterial titer in biocontrols and 14399 genotypes across three trials. Genotypes connected by the same letter are statistically the same. According to the Tukey's test, genotypes are divided in three categories: resistant (R), tolerant (T) and susceptible (S). Tolerant genotypes have higher standard deviations as they perform differently depending on environmental effects. Similar trends in other PD trials are usually observed.

Genotype	lsmeans	SE	df	lower CL	upper CL	Groups	Category
b43-17	9.25	0.229	305	8.81	9.7	a	R
b46-43	9.43	0.318	305	8.8	10.1	ab	R
14399-029	9.58	0.329	305	8.93	10.2	ab	R
14399-047	10.63	0.329	305	9.99	11.3	bd	T
14399-055	10.26	0.34	305	9.59	10.9	abc	T
12305-55	10.25	0.318	305	9.62	10.9	abc	T
U0505-01	10.99	0.225	305	10.54	11.4	cd	T
14399-066	10.91	0.329	305	10.27	11.6	bd	T
Blanc de Bois	11.94	0.232	305	11.48	12.4	de	T
U0505-35	12.77	0.245	305	12.28	13.2	e	T
Roucaneuf	12.95	0.232	305	12.49	13.4	e	T
14399-009	14.66	0.329	305	14.01	15.3	f	S
U0505-22	14.9	0.236	305	14.44	15.4	f	S
Chardonnay	14.17	0.218	305	14.74	15.6	f	S

p-value <0.05

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Table 2. Summary of consensus and parental maps from population 14399. All three maps had different numbers of linkage groups that represented all 19 chromosomes.

Consensus Map				12305-55 Map (Male Resistant Parent)				F2-35 (Female Susceptible Parent)			
Chr	**Linkage Group**	**No. of markers used**	**cM covered**	**Chr**	**Linkage Group**	**No. of markers used**	**cM covered**	**Chr**	**Linkage Group**	**No. of markers used**	**cM covered**
1	1	9	36.476	1	1	9	35.8	1	1	6	25.9
2	2	10	57.708	2	2	10	58.2	2	2	3	43.1
3	3	7	64.26	3	3	7	56.9	3	3	3	44
4	4	10	63.789	4	4	10	51.3	4	4	10	37.8
5	5	9	36.307	5	5	9	43.8	5	5	7	28.9
5	6	2	1.124	5	6	3	10.5	6	6	6	49.4
6	7	9	52.031	6	7	8	37.9	7	7	6	43.9
7	8	8	23.618	7	8	8	21.9	8	8	7	46.2
7	9	2	6.185	8	9	11	79.3	9	9	4	3.8
8	10	10	61.21	9	10	11	43	9	10	5	25.7
9	11	11	46.625	10	11	7	40	10	11	7	53.8
10	12	7	46.8	11	12	8	57.6	11	12	7	37.4
11	13	7	36.319	12	13	10	51.4	12	13	2	1.3
12	14	4	16.763	13	14	10	37.6	12	14	10	53.8
12	15	6	16.136	14	15	24	64.1	13	15	9	58.5
13	16	10	45.01	15	16	7	34.1	14	16	7	46.7
14	17	16	57.395	16	17	8	30.8	15	17	8	71.8
15	18	7	39.803	17	18	7	34.5	16	18	2	7.5
16	19	8	47.372	18	19	10	47.1	17	19	4	4.5
17	20	8	54.572	19	20	8	88.9	18	20	8	85.4
18	21	10	96.667					19	21	5	60.6
19	22	3	25.106								
19	23	4	56.76								
Total		177	988.03	Total		185	924.7	Total		126	830
Average		7.69	42.958	Average		9.25	46.235	Average		6	39.523

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Figure 1. A) Geographical location of accession b46-43 found in Big Bend National Park, TX. B) Diagram of crosses for 14399 population. PD resistant accession was crossed to the susceptible *V. vinifera* 08326-61, a highly homozygous selfed Cabernet franc. All individuals in population 12305 were PD resistant, showing b46-43 was homozygous resistant. Individual 12305-55 from 12305 population (F1) was selected as the paternal parent for the development of the pseudo-backcross (pBC1) 14399. A susceptible *V. vinifera* F2-35 was selected as the female parent.

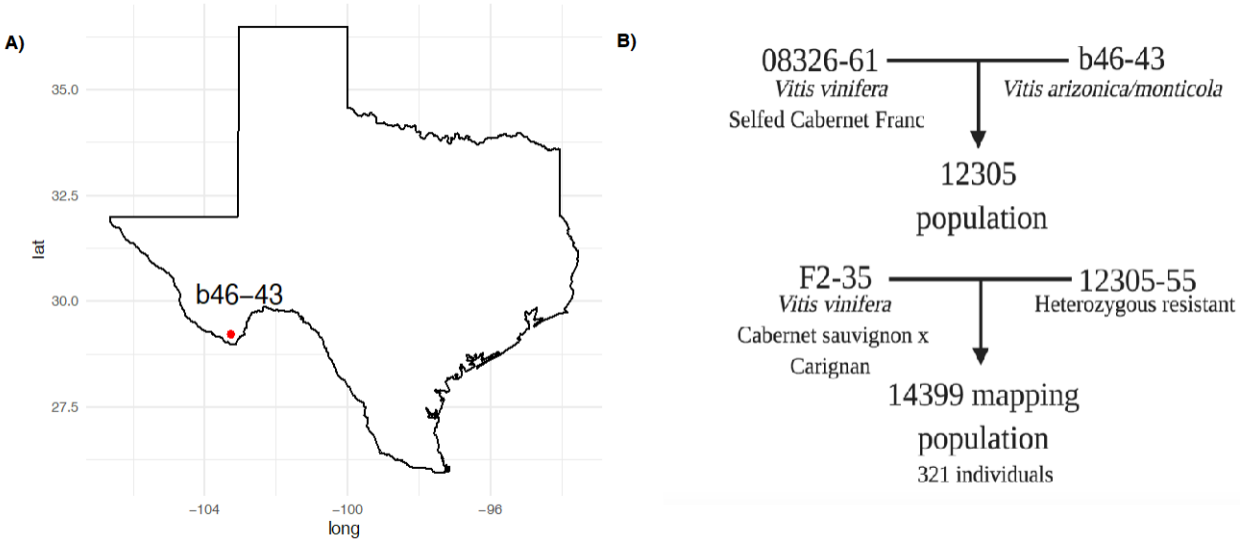

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Figure 2. Phenotypic distribution of population 14399 across three trials. Bar plot shows the natural logarithm of the bacterial titer (*X. fastidiosa* cfu/ml) ranges from 9 to 16. Number over bars shows the number of 14399 population genotypes in the bacterial titer range. (n=318). Dashed line indicates the selected threshold separation between resistant and susceptible genotypes (1:1 ratio).

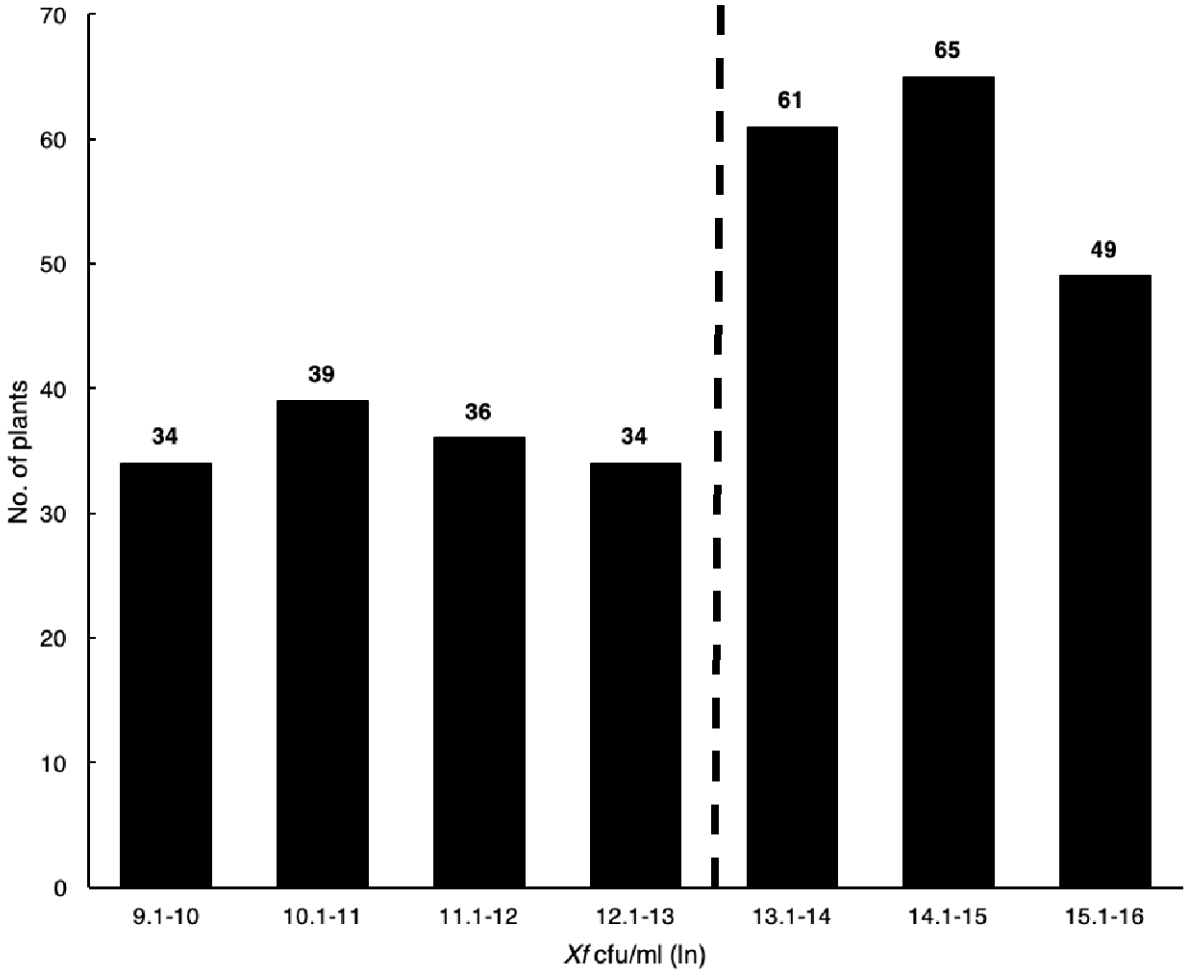

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Figure 3. A) Chromosome 14 linkage genetic map refined mapping. B) Identification of QTL by limited genetic mapping. Interval mapping results identified a major locus on chromosome 14 in the same location as the previously discovered *PdR1* locus. Straight thin horizontal line represents LOD value (1.5) from the Permutation Test for QTL detection. 1.5 was the lowest LOD calculated for the bacterial titer (*X. fastidiosa* cfu/ml (ln)), followed by 1.6 for CMI index and 1.7 for LS-LL index.

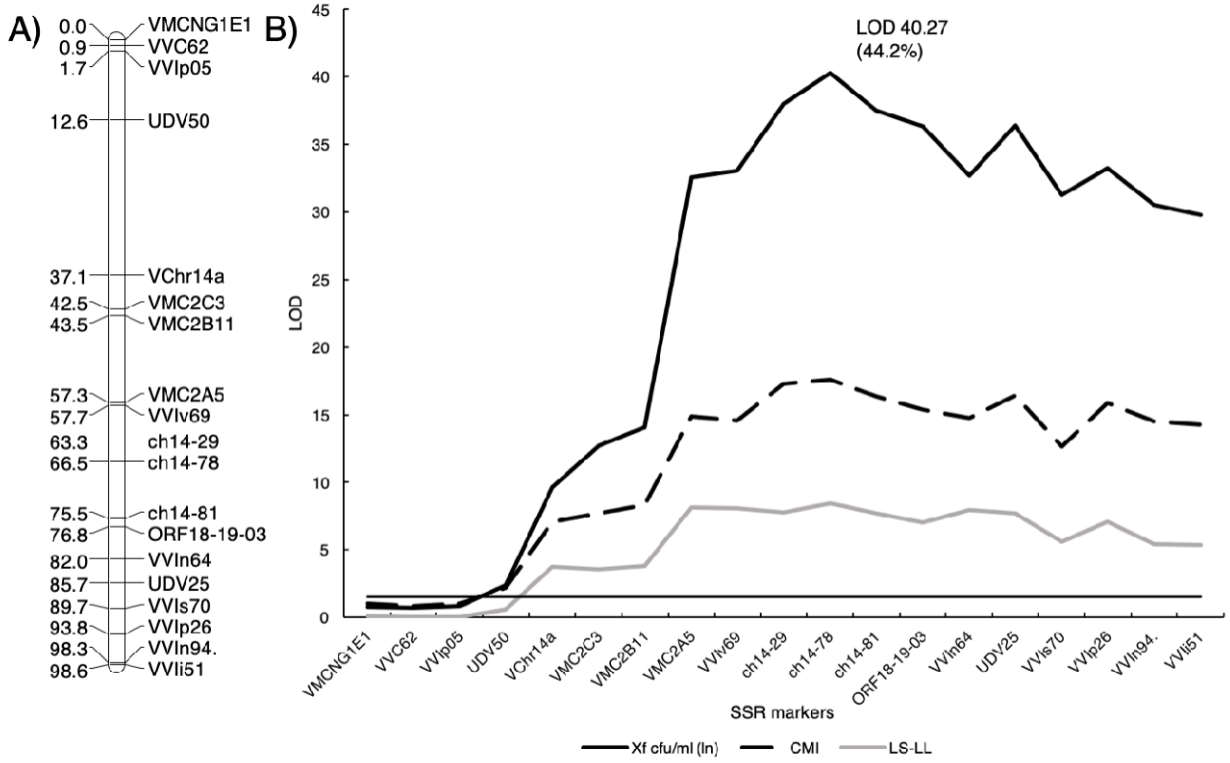

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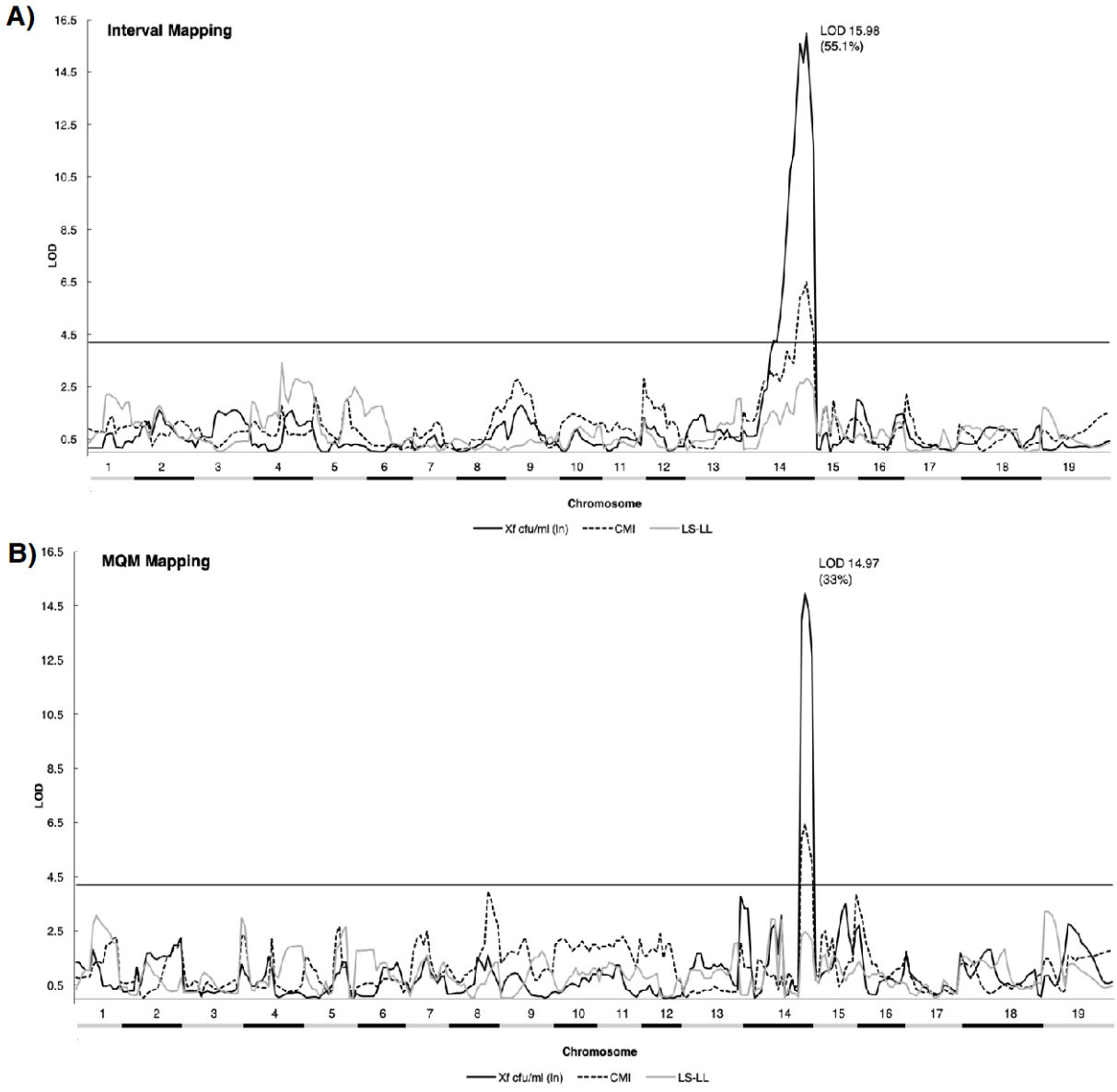
Figure 4. Identification of QTL on chromosome 14 with interval and MQM mapping. A) results of interval mapping for all 19 chromosomes for the *Xf* bacterial titer (*X. fastidiosa* cfu/ml (ln)), CMI and LS-LL scores using consensus map. B) Results of MQM mapping on all 19 chromosomes using the consensus map. Markers ctg6140, CF2513, VChr14a, VMC2b11 and UDV25 were used as cofactors. Horizontal black line shows the Genome-Wide PT value (4.2 for bacterial titer, 4.3 for CM and 4.2 for LS-LL).



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Supplemental Table 1. Tukey test results on effect of trial date on the bacterial titer. Biocontrols and five 14399 population genotypes tested in all three trial dates were included in the analysis. Trial in 2015 (TD151217) and 2016 (TD160809) were statically the same and trial in 2017 was different from both.

Takedown Date	mean	std	r	Min	Max	Q25	Q50	Q75	groups
TD151217	12.32605	2.4434	119	9.2	15.7	9.6	12.2	14.75	a
TD160809	12.125	2.2321	76	9.2	15.7	10.075	12	14.025	a
TD171031	11.56935	2.4052	124	9.2	15.7	9.2	10.95	13.725	b

p-value <0.05

Supplemental Table 2. QTL results for chromosome 14 in population 14399 from 2015, 2016 and 2017 trials. A total of 318 individuals were used for the analysis. LOD values in bold are statistically significant according to the LOD threshold calculated with the Permutation test. The permutation test values were 1.5, 1.6 and 1.7 for bacterial titer, CMI and LS-LL, respectively.

Locus	Position	*Xf* cfu/ml (ln)			CMI			LS-LL		
		Signif.	LOD	% Expl.	Signif.	LOD	% Expl.	Signif.	LOD	% Expl.
VMCNG1E1	0	*	0.79	1.1	*	1.05	1.5	-	0.12	0.2
VVC62	0.87	**	0.71	1	**	0.86	1.2	-	0.02	0
VVIp05	1.718	*	0.81	1.2	*	1.01	1.5	-	0.03	0
UDV50	12.575	****	**2.37**	3.4	***	**2.18**	3.1	-	0.6	0.9
VChr14a	37.09	*******	**9.6**	13	*******	**7.07**	9.7	*******	**3.77**	5.3
VMC2C3	42.527	*******	**12.69**	16.8	*******	**7.71**	10.6	*******	**3.58**	5
VMC2B11	43.505	*******	**14.06**	18.4	*******	**8.32**	11.4	*******	**3.79**	5.3
VMC2A5	57.347	*******	**32.57**	37.6	*******	**14.86**	19.4	*******	**8.11**	11.1
VVIv69	57.71	*******	**33.08**	38.1	*******	**14.54**	19	*******	**8.06**	11
ch14-29	63.308	*******	**38.03**	42.3	*******	**17.27**	22.1	*******	**7.78**	10.7
ch14-78	66.532	*******	**40.27**	*44.2*	*******	**17.62**	22.5	*******	**8.43**	11.5
ch14-81	75.49	*******	**37.51**	41.9	*******	**16.35**	21.1	*******	**7.7**	10.6
ORF18-19-03	76.8	*******	**36.34**	40.9	*******	**15.39**	20	*******	**7**	9.6
VVIn64	81.996	*******	**32.67**	37.7	*******	**14.77**	19.3	*******	**7.96**	10.9
UDV25	85.67	*******	**36.41**	41	*******	**16.45**	21.2	*******	**7.72**	10.6
VVIs70	89.708	*******	**31.29**	36.4	*******	**12.63**	16.7	*******	**5.61**	7.8
VVIp26	93.786	*******	**33.27**	38.2	*******	**15.89**	20.6	*******	**7.11**	9.8
VVIn94.	98.25	*******	**30.51**	35.7	*******	**14.51**	18.9	*******	**5.44**	7.6
VVIi51	98.588	*******	**29.77**	35	*******	**14.27**	18.7	*******	**5.34**	7.4

*: 0.1; **: 0.01; ***: 0.001;****: 0.0001; *****: 0.00001; ******: 0.000001; *******: 0.0000001.

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424 **Supplemental Table 3.** One-way ANOVA Kruskal-Wallis results test from the QTL analysis with the
425 consensus map showing the K- test for all chromosomes. Markers on chromosome 14 were highly
426 significant for all three phenotypic parameters. LS-LL showed multiple markers outside chromosome 14
427 to be significant. Markers not shown did not show high significance (p-value < 0.01) for any of the
428 phenotypic parameters.

Chromosome	Locus	*Xf* cfu/ml (ln)		CMI		LS-LL	
		K*	Signif.	K*	Signif.	K*	Signif.
1	VVIo02	0.106	-	0.013	-	4.913	**
1	VVIs21	3.84	-	2.589	-	12.65	***
1	VMC2b3	2.716	-	3.082	-	10.2	**
1	VMC9F2	0.925	-	1.466	-	11.49	***
1	VVIf52	0.129	-	0.557	-	5.433	**
1	VMC9d3	2.421	-	2.113	-	10.49	**
2	VMC3b10	2.902	*	3.129	*	5.694	**
3	UDV21	8.128	**	1.953	-	0.329	-
4	VVIp77	4.311	-	3.787	-	11.17	**
4	CTG0624	3.903	-	3.493	-	10.62	****
5	VVIn33	1.365	-	5.613	-	7.952	**
5	VVC71	0.639	-	3.283	-	8.693	**
6	VMC5c5	0.011	-	0.322	-	4.346	**
7	VVMD31	0.955	-	4.092	**	0.139	-
9	SC02-24	3.364	-	9.407	**	1.114	-
9	VMCNG3F11	5.491	**	6.176	**	0.936	-
9	VMC2d9	8.556	**	2.243	-	0.498	-
10	UDV101	3.343	-	8.665	**	3.424	-
10	CTG5592	2.382	-	8.236	**	2.685	-
12	vmc4a9	0.351	-	4.788	**	0.79	-
-12	VMC2h4	3.429	-	8.047	**	2.746	-
-12	VMC3b8	0.041	-	5.239	**	1.222	-
13	VMC2f6	1.412	-	1.63	-	10.9	**
14	UDV50	9.86	****	9.224	****	3.889	**
14	VChr14a	16.615	*******	12.2	******	6.355	**
14	VMC2C3	16.858	*******	12	*****	6.446	**
14	VMC2B11	24.534	*******	13.16	******	5.518	**
14	VMC6C10	30.794	*******	15.99	****	8.589	**
14	VMC2A5	35.837	*******	18.15	******	8.006	**
14	VVIv69	38.478	*******	18.08	******	6.362	*
14	ch14-29	39.427	*******	23.29	*******	9.37	****
14	VVIp26	33.869	*******	20.61	******	5.801	-14
UDV25	47.781	*******	33.78	*******	12.57	***	14
VVIi51	33.77	*******	20.84	*******	9.324	****	15
VVIq61	2.898	-	5.002	-	8.508	**	15
UDV78	2.449	-	10.53	**	8.084	**	17
VMC3c11.1	6.276	*	9.968	**	0.993	-19	
	Vchr19a	0.762	-	3.638	-	9.241	**

K*: Kruskal-Wallis test statistic
*: 0.1; **: 0.01; ***: 0.001; ****: 0.0001; *****: 0.00001; ******: 0.000001; *******: 0.0000001.

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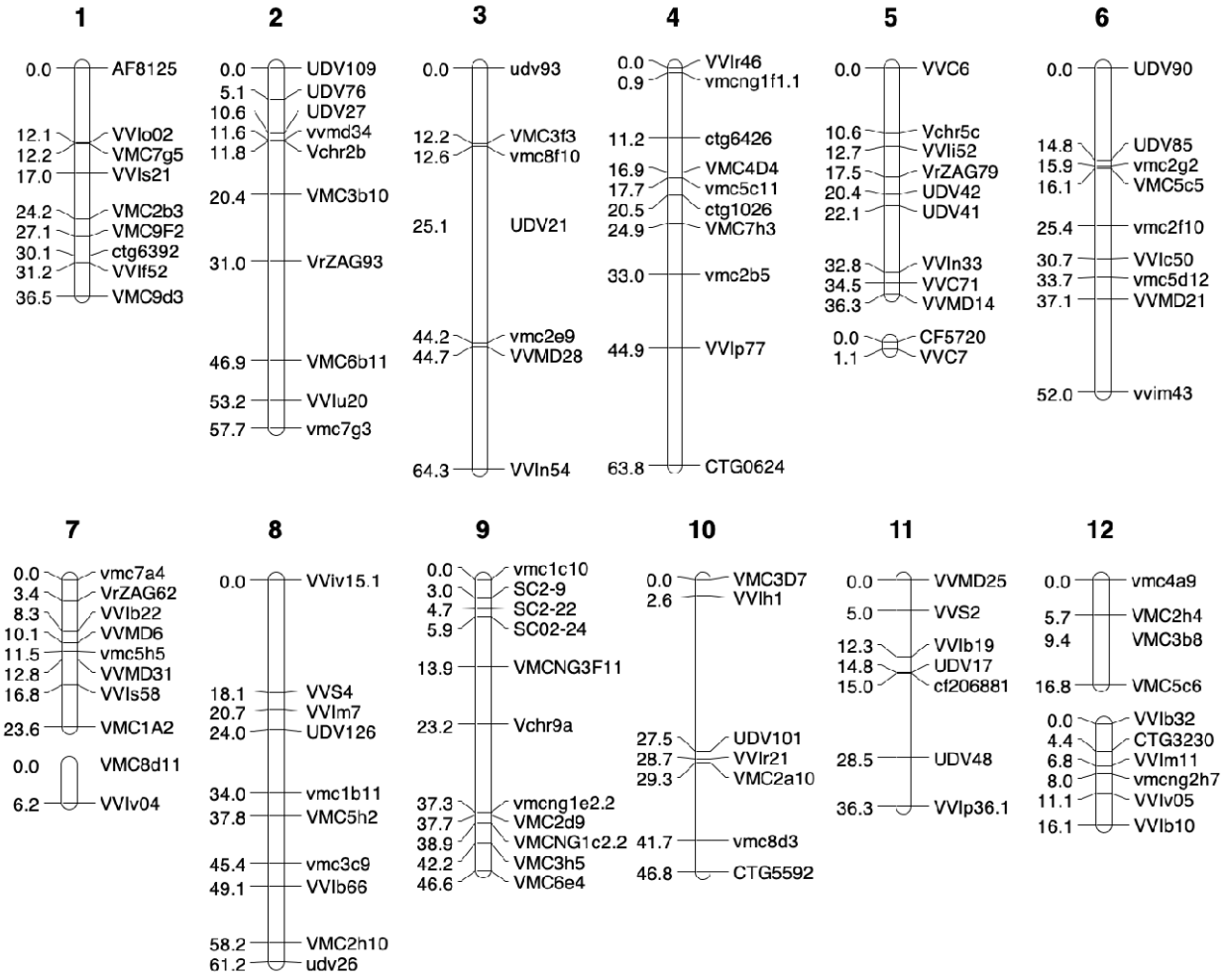
Supplemental Figures

Supplemental Figure 1. Consensus linkage genetic map of the pBC1 population 14399 (*V. vinifera* and *V. arizonica/monticola* hybrids) generated with 177 polymorphic SSRs using JoinMap4. Markers on chromosomes 5, 7, 12 and 19 did not have sufficient linkage and were linked to two different groups.



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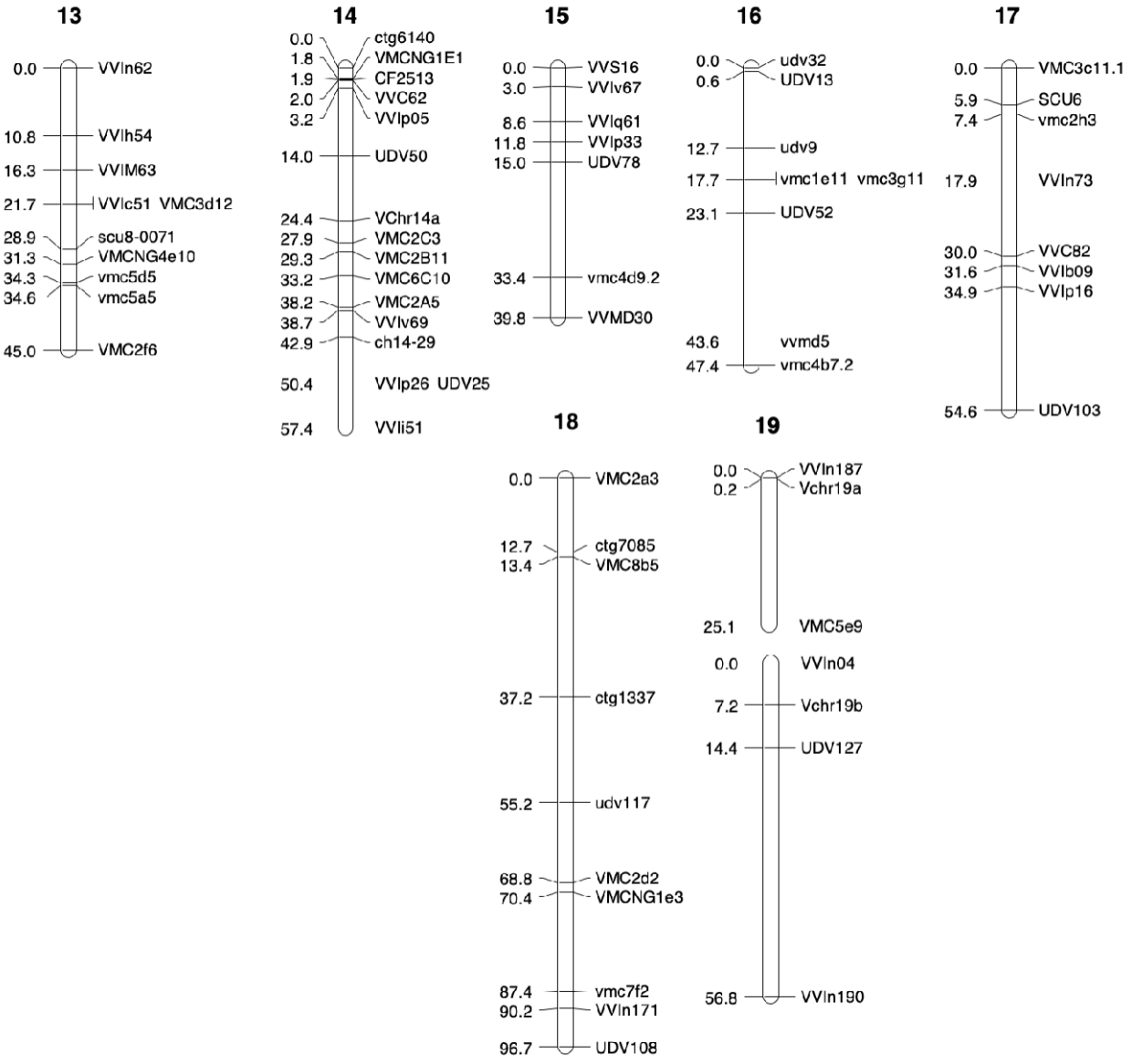

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