Theor Appl Genet (2008) 117:671–681
DOI 10.1007/s00122-008-0802-7

ORIGINAL PAPER

Fine-scale genetic mapping of two Pierce's disease resistance loci and a major segregation distortion region on chromosome 14 of grape

S. Riaz · A. C. Tenscher · J. Rubin · R. Graziani · S. S. Pao · M. A. Walker

Received: 22 January 2008 / Accepted: 9 May 2008 / Published online: 31 May 2008

Abstract A refined genetic map of chromosome 14, which contains the Pierce's disease (PD) resistance locus, was created from three grape mapping populations. The source of PD resistance in these populations was b43-17, a male form of *Vitis arizonica* Engelm. that is homozygous resistant. The resistance locus segregated as a single dominant gene and mapped as *PdR1a* in the F1 selection F8909-17 (9621 population) and as *PdR1b* in a sibling F1 selection F8909-08 (04190 population). These two full sibs inherited either allele of the Pierce's disease resistance locus from the b43-17 parent, which is homozygous at that locus. The 9621 population consisted of 425 progeny and *PdR1a* mapped between markers VvCh14-56/VvCh14-02 and UDV095 within a 0.6 cM genetic distance. The 04190 population consisted of 361 progeny and *PdR1b* mapped between markers VvCh14-02 and UDV095/VvCh14-10 within a 0.4 cM distance. Many of the markers present on chromosome 14 were distorted with an excess of female alleles in the 04190 and 04373 population (developed from a cross of *V. vinifera* L. F2-35 × b43-17) indicating that potential gametophytic factors are present in this region. Common markers from this region within the 9621 population were not distorted except Scu15. When these markers were compared to *V. vinifera*-based maps of chromosome 14 they were also distorted suggesting the involvement of gametophytic factors, and prompting the identification of this region as *Vitis*-segregation distortion region 1 (*V-SDR1*). The refined genetic maps developed from this study can be used to identify and clone genes that confer resistance to Pierce's disease.

Communicated by H. Nybom.

Electronic supplementary material The online version of this article (doi:10.1007/s00122-008-0802-7) contains supplementary material, which is available to authorized users.

S. Riaz · A. C. Tenscher · J. Rubin · R. Graziani · S. S. Pao · M. A. Walker (✉)
Department of Viticulture and Enology,
University of California, Davis, CA 95616, USA
e-mail: awalker@ucdavis.edu

Introduction

Pierce's disease (PD) is a serious impediment to viticulture in the southern areas of North America, Central America, and some parts of South America (Hopkins and Purcell 2002). The disease is caused by the xylem-limited bacterium *Xylella fastidiosa* (Wells et al.) Symptoms are expressed as xylem vessels become blocked by bacterial aggregation and the formation of gums and tyloses, leading to desiccation and plant death within a few years. The vast majority of the world's wine, table and raisin grape vineyards are planted to cultivars of the European bunch grape (*Vitis vinifera* L.), which is highly susceptible to PD. Grape species native to the southern United States and Mexico resist PD, but the genetic control of their resistance is poorly understood. Despite this lack of knowledge, breeders have created PD resistant grape cultivars by crossing highly resistant species and selections with *V. vinifera* cultivars. However, acceptance of these PD resistant hybrids has been limited because their fruit quality is inferior to that of *V. vinifera* cultivars.

In recent years, forms of *V. arizonica* Engelm. from northern Mexico have been proven to be resistant to *X. fastidiosa* (Krivanek and Walker 2005; Riaz et al. 2007). Olmo collected b43-17 a form of *V. arizonica* near Monterrey, Mexico that appears to be introgressed with *V. candicans* Engelm. ex A. Gray) (Riaz et al. 2007). Thirteen F1 progeny from a cross of the susceptible *V. rupestris* Scheele cv. A. de Serres and b43-17 were resistant to *X. fastidiosa* suggesting that b43-17's PD resistance is homozygous (unpublished data). One

of the F1 resistant progeny, F8909-17 was crossed to D8909-15 a resistant half-sib (same *V. rupestris* parent crossed with b42-26, a form of *V. arizonica* that appears to be introgressed with *V. girdiana* Munson) to develop the 9621 population, which was used to develop a genetic map (Doucleff et al. 2004). This population was also part of a study on the inheritance of *X. fastidiosa* resistance, in which Krivanek et al. (2005a) used a 4 × 4 factorial mating design to evaluate *X. fastidiosa* resistance from *V. arizonica* hybrid selections under greenhouse conditions. They used complex segregation analysis to determine that a major gene with a dominant allele was responsible for the *V. arizonica*-based resistance. Krivanek et al. (2006) later used the 9621 population to genetically map a primary locus for PD resistance, *PdR1*, derived from the male F8909-17 parent. Both parents of the 9621 population are PD resistant, but Krivanek et al. (2006) were only able to map resistance from F8909-17 as a single dominant locus. Riaz et al. (2006) increased the 9621 population size and added more markers to the above genetic map to create a refined map using 210 simple sequence repeat (SSR) and EST-derived SSR markers. This map located *PdR1* on chromosome 14 between two SSR markers spanning a genetic distance of 7 cM (Riaz et al. 2006).

F8909-08, a PD resistant full sibling of F8909-17 has been extensively used in efforts to breed PD resistant wine, table and raisin grapes. Proof that the PD resistance locus from F8909-08 maps to the same region as its sibling F8909-17 is needed so that markers linked to *PdR1* can be effectively used to assist resistance breeding. Thus, this study presents the genetic mapping of the *PdR1* locus in a population generated from a cross of the pistillate *V. vinifera* F2-7 × F8909-08. A genetic map of b43-17, the parental *V. arizonica* selection, is also presented. The main objectives of this study were: (1) to verify that resistance from b43-17 is controlled by a single dominant homozygous locus; (2) to verify inheritance of *PdR1* as a single dominant gene; (3) to determine whether F8909-08 and F8909-17 inherited the same allele of *PdR1*; (4) to develop a fine-scale map of *PdR1* in F8909-08 and F8909-17 based populations; and (5) to examine the nature of segregation distortion on a major segment of chromosome 14 by developing a genetic map in a population derived from b43-17. These genetic maps also provide the framework for physical mapping of the PD resistance gene(s) from the homozygous resistant b43-17.

Materials and methods

Plant materials

Three mapping populations were created or expanded. **04190**: this population was based on b43-17, a staminate *V. arizonica/candicans* type collected near Monterrey, Nuevo Leon, Mexico by Olmo in 1961 (Riaz et al. 2007). b43-17 was crossed to the PD susceptible *V. rupestris* cv. A. de Serres to produce 13 progeny (Fig. 1b). One of the staminate progeny, F8909-08, was crossed to the pistillate *V. vinifera* F2-7 to produce the 04190 population from which 361 progeny were used to create a genetic map of *PdR1* originating from F8909-08 (Fig. 1E). **9621:** this mapping population (D8909-15 × F8909-17) was used to position *X. fastidiosa* resistance in past mapping studies (Krivanek et al. 2006; Riaz et al. 2006) (Fig. 1a, b, d). It was expanded from 181 to 425 individuals to enable fine-scale mapping of *PdR1* originating from F8909-17. **04373**: this population of 282 individuals was created from a cross of the PD susceptible *V. vinifera* F2-35 × b43-17 (Fig. 1c). It was created to confirm b43-17's homozygous dominant single gene resistance, and to develop a map of chromosome 14 so that segregation distortion could be studied. These populations and parents are maintained in the Department of Viticulture and Enology vineyards at the University of California, Davis.

Young leaf tissue was taken from both field- and greenhouse-grown plants of progeny from the 04190, 04373 and the expanded 9621 populations (Fig. 1c, d, e). DNA was

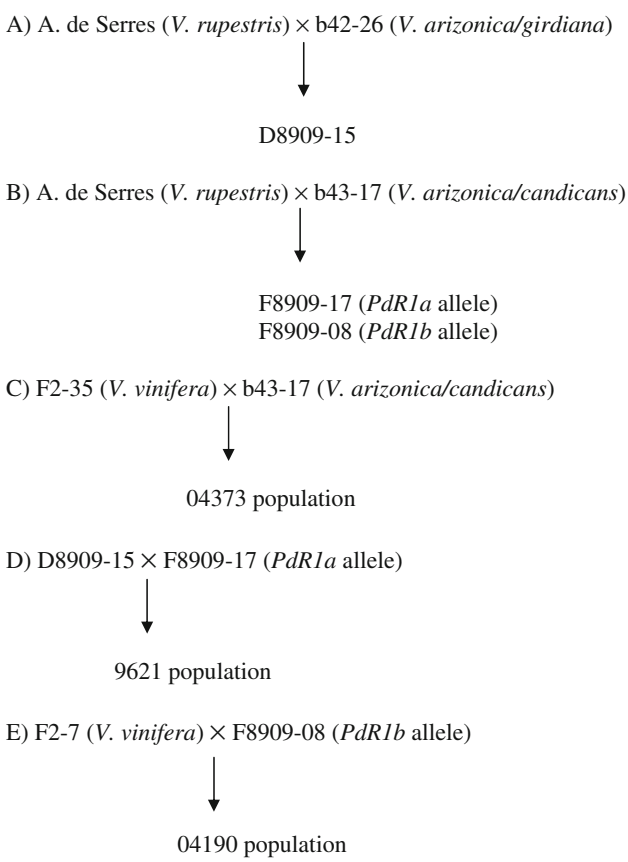

Fig. 1 Description of the crosses and the relationships among the different parents that were used to develop three mapping populations for the study of *PdR1* allelic differences

extracted using a modified CTAB procedure as described by Lodhi et al. (1994).

Disease evaluation

All the individuals in the 04190 and 9621 population, and 60 progeny from the 04373 population, were evaluated for *X. fastidiosa* resistance using the greenhouse-based technique described by Krivanek et al. (2005b). There were at least four replicate test plants for each genotype, and they were inoculated with an isolate of *X. fastidiosa* obtained from the Stag's Leap area of Napa, CA, which was maintained in susceptible greenhouse-grown *V. vinifera* cv. Chardonnay plants. Bacteria were isolated and maintained following procedures outlined in Krivanek et al. (2005b). In preparation for inoculations, actively growing bacteria were washed from Petri plates with ddH_2O, and the cell suspension was standardized to a 0.25 absorbance at 600 nm (approximately 6×10^8 CFU/ml as determined by culture plating). Plants were needle inoculated (Hopkins 1980) above the nearest node 20–30 cm above the base of each shoot with 10 µl of bacterial suspension. Plants were re-inoculated 3 days later below that node to ensure successful inoculation.

Plants were sampled 12 weeks post inoculation by taking a 0.5 g piece of stem tissue from 10 cm above the point of inoculation. Samples were placed into grinding bags (Agdia, Elkhart, IN, USA) with a phosphate-buffered saline (PBS), 0.05% Tween, and 2% soluble polyvinylpyrrolidone (PVP-40) buffer (Nome et al. 1981). Samples were lightly crushed with a hammer and further processed using a Homes 6 mechanical homogenizer (Bioreba, Longmont, CO, USA), and the resulting extract was stored at −20°C until analyzed with ELISA.

A modified double antibody sandwich ELISA procedure as described in Krivanek and Walker (2005) was used to quantify *X. fastidiosa* levels in plant samples. To obtain homogeneous variances and normally distributed residuals in the ELISA data set, average values of the cells/ml concentrations were natural log transformed and plants were separated into resistant and susceptible categories. For reference, the susceptible female parent and inoculated susceptible positive control (*V. vinifera* cv. Chardonnay) were included. In this study, the susceptible parent and positive control had values greater than or equal to natural log 15 cells/ml, while the resistant parent had a mean natural log value of 8.5 (cells/ml).

Fingerprinting, segregation analysis and mapping

An allelic profile of A. de Serres, b43-17, F8909-08, and F8909-17 was generated for 33 simple sequence repeat (SSR) markers (details in Table 1 and Table 1 Supplemental) taken from chromosome 14 based on information published in the previous genetic map of the 9621 population (Riaz et al. 2006). Additional marker information was obtained from the integrated genetic map based on five different *V. vinifera* populations (Doligez et al. 2006), and from the Pinot noir genome sequence information available on the NCBI database (http://www.ncbi.nlm.nih.gov/). The amplified products were run on polyacrylamide gels with a size standard sequencing reaction (Promega, Madison, WI, USA). Markers that were polymorphic within each population were amplified on the entire progeny and then separated on denaturing 5% polyacrylamide sequencing gels as described by Riaz et al. (2006). Gels were visualized with a silver staining kit (Promega, Madison, WI, USA), all markers were scored and the gels were scanned to archive a digital image. The "locus genotype frequency" function in JoinMap 3.0 was used to calculate χ^2 values for each marker in order to test for the expected 1:1 segregation frequency distribution. Data for markers on chromosome 14 that were common to a Cabernet Sauvignon × Riesling map (C × R) (S. Vezzulli, personal communication) and to the international *V. vinifera* reference map Riesling × Cabernet Sauvignon (R × C) (Riaz et al. 2004) were also used to determine the expected 1:1 segregation frequency distribution. Data were available for 87 genotypes and six markers (VMCNg1e1, VMC1e12, VMC6c10, VVMD24, VMC2a5 and VMC6e1) from the C × R population and for eight markers and 153 genotypes from the R × C population (Table 3). The linkage analysis for the 04373, 9621 and 04190 populations was performed with

Table 1 Source, code and reference for markers used to develop chromosome 14 genetic maps from populations derived from b43-17, F8909-17 and F8909-08

Marker symbol	Source	Reference
VrZAG	University of Agriculture, Vienna, Austria	Sefc et al. (1999)
VVC	INRA, France	Decroocq et al. (2003)
VMC	University of Madrid, Spain	Arroyo-Garcia and Martinez-Zapater (2004)
VMC, VMCNg	Vitis Microsatellite Consortium	Agrogene SA (Moissy Cramayel, France)
VVI	NCBI uni-STS	Merdinoglu et al. (2005)
ctg	University of California, Davis	http://cgf.ucdavis.edu/
UDV	University of Udine, Italy	Di Gaspero et al. (2005)
Scu	Southern Cross University, Australia	Scott et al. (2000)
A010	NCBI uni-STS	Doligez et al. (2006)
VVCh14	Viticulture and Enology, Davis	Unpublished

JoinMap 3.0 (Van Ooijen and Voorrips 2001) as well as with TMAP (Cartwright et al. 2007). Map units in centimorgans (cM) were derived from the Kosambi (K) mapping function (Kosambi 1944). Maps of chromosome 14 were drawn with MapChart 2.1 software (Voorrips 2002).

Results

Analysis of PD resistance data

A total of 60 progeny from the 04373 population were screened for resistance to *X. fastidiosa* to verify that b43-17 was homozygous resistant to PD. All 60 progeny plants had natural log transformed values ranging from 8.5 to 13.5 (cells/ml) and were considered to be resistant (Fig. 2a). The susceptible parent F2-35 and other inoculated susceptible biological control had natural log transformed values greater than 16.5 (cells/ml). These results indicated that the genotype b43-17 is indeed homozygous resistant to PD and that all F1 progeny are heterozygous. Two full sibs F8909-08 and F8909-17 (Fig. 1b) were used to generate the 04190 and 9621 populations, respectively (Fig. 1d, e). A total of 360 progeny from the 04190 population were screened for *X. fastidiosa* resistance: 171 plants were resistant and 189 were susceptible (Fig. 2b, and see Krivanek et al. 2006). The natural log transformed values in cells/ml of the susceptible female parent, progeny and positive controls were $\geq$15.5. The resistant progeny had natural log transformed values ranging from 8.5 to 12.5 cells/ml. Four hundred and twenty-five progeny were screened in the 9621 population; 218 were resistant and 207 were susceptible (data not shown). Resistance to PD segregated 1:1 in both the 04190 and 9621 populations indicating that the resistant male parents (F8909-08 and F8909-17) were heterozygous for resistance controlled by a single dominant locus or gene.

Fingerprint profiles of b43-17, F8909-08 and F8909-17

The SSR fingerprint profiles of the parents A. de Serres and b43-17, and progeny F8909-08 and F8909-17 are provided in Table 1 Supplemental. b43-17 was homozygous for 15 of the 33 markers from chromosome 14, and was polymorphic, with two alleles, for 18 of the tested markers. In each case F8909-08 and F8909-17 inherited different alleles of the 18 polymorphic markers. Figure 3 is a gel image of nine of these 18 markers showing that for each marker F8909-17 and F8909-08 inherited a different allele from b43-17. The allelic profile of b43-17 (*V. arizonica/candicans*) was very different from that of A. de Serres (*V. rupestris*). These parental genotypes only had alleles of similar size at eight of the 33 markers, confirming that these parents were very different from each other (supplemental Table 1).

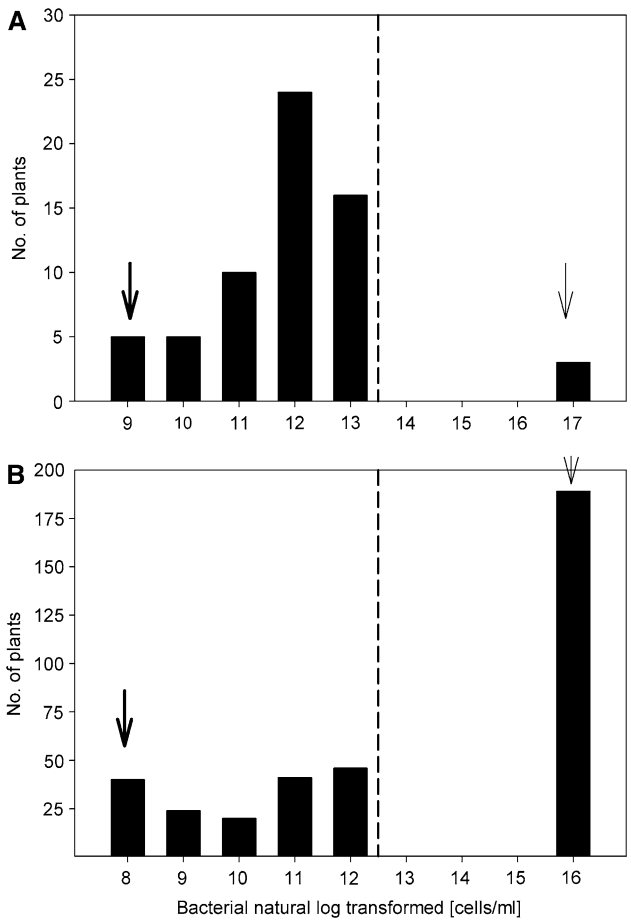

Fig. 2 Frequency distributions of *Xylella fastidiosa* levels (natural log transformed cells per ml) extracted from stem tissue after greenhouse testing of two populations for resistance to Pierce's disease. Values were derived from standardized ELISA readings. **a** 04373 (*V. vinifera* F2-35 × b43-17) $n = 60$. **b** 04190 (*V. vinifera* F2-7 × F8909-08) $n = 360$. The *dotted line* indicates the selected threshold level for resistant genotypes. The *thick black arrow* indicates the mean scores for the resistant male parent and *thin arrow* indicates the mean score for the susceptible parent, positive control and susceptible progeny

b43-17 genetic map

The b43-17 chromosome 14 genetic map was developed with 12 polymorphic markers that were easy to amplify and score on set of 282 progeny plants from the 04373 population. The majority of markers that were polymorphic and mapped in F8909-08 and F8909-17 were not polymorphic for b43-17. The total map length was 86.2 cM with a large gap of 44.4 cM between markers VMC1e12 and VMC5b3 (Fig. 4). The marker order was relatively consistent among b43-17 and siblings F8909-08 and F8909-17, except in the region of the chromosome where a set of markers (UDV025, VVIS70, VVIP26, ctg1025882, VMC6e1, and VMCNg1g1.1) were less than 1 cM from each other. Local inversions were observed for these markers in the F8909-08

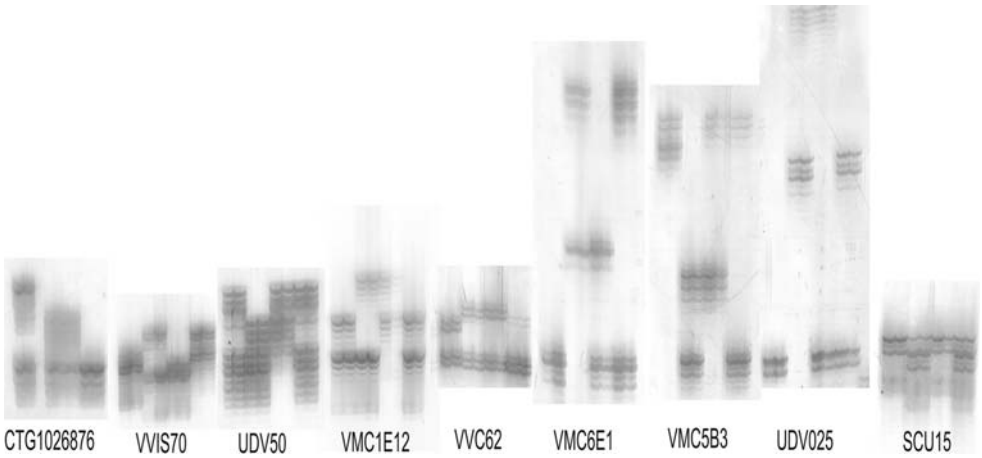

Fig. 3 Gel image of nine polymorphic SSR markers for the A. de Serres female parent, b43-17 male parent, and their progeny F8909-08 and F8909-17 indicating that the progeny inherited a different sister chromatid from b43-17, the resistant male parent. There are eight samples for each marker (two per genotype). Samples *1*, *2* are A. de Serres, *3*, *4* are b43-17, *5*, *6* are F8909-08 and *7*, *8* are F8909-17

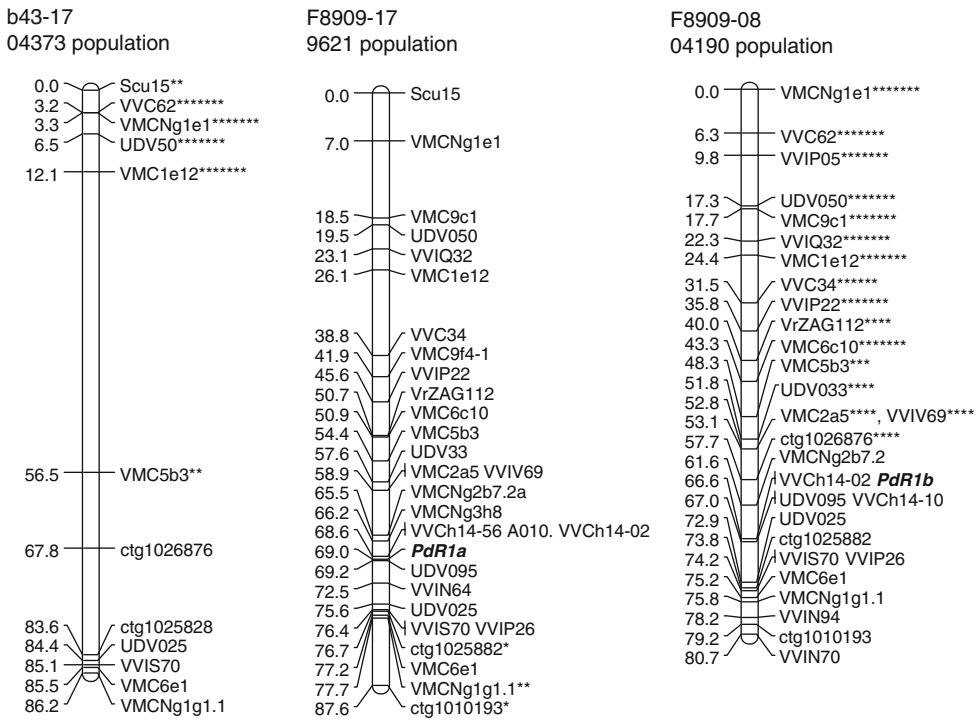

Fig. 4 Chromosome 14 genetic maps of the homozygous resistant parent b43-17 and its two full sib progeny F8909-17 (*PdR1a*) and F8909-08 (*PdR1b*)

and F8909-17 progeny and in the b43-17 parental genotype. More recombinant genotypes are required to determine the exact order of these markers. Marker order was relatively consistent in comparison to the integrated map of grapevine developed from five *V. vinifera*-based populations (Doligez et al. 2006).

The greenhouse screen data for *X. fastidiosa* resistance in the F1 04373 population indicated that b43-17's resistance is controlled by a single dominant and homozygous locus, and all F1 progeny are heterozygous resistant. The fingerprint profile with SSR markers points out that the F1 genotypes, F8909-17 and F8909-08, each possess a different sister chromatid of chromosome 14 from b43-17. These sister chromatids may carry the same resistance gene, two allelic forms of same resistance gene, or may have different resistance genes depending upon the genetic diversity present in the parents of b43-17. Because of this potential difference, resistance from F8909-17 was designated *PdR1a* and resistance from F8909-08 was designated *PdR1b*.

F8909-17 genetic map

A total of 29 molecular markers and the phenotypic disease resistance locus *PdR1a* were mapped onto chromosome 14 (Fig. 4). The total map length was 87.6 cM with an average distance of 2.9 cM between markers. The largest gap, 12.7 cM, was between marker VMC1e12 and VVC34. The *PdR1a* locus mapped between markers VvCh14-56/VvCh14-02/A010 and UDV095 at a distance of 0.4 and

0.2 cM, respectively. These results were in agreement with Riaz et al. (2006) where the flanking markers were VMCNg3h8 and VVIN64. Four new markers flanking the *PdR1a* locus mapped in between VMCNg3h8 and VVIN64 (Fig. 4) and the genetic distance between *PdR1a* and the nearest flanking marker (VMCNg3h8 and VVIN64) was reduced from 4.3 and 2.7 to 0.4 and 0.2 cM, respectively. The addition of genotypes and markers reduced genetic distances around *PdR1a* and positioned it more precisely compared to previously published maps of the 9621 population (Krivanek et al. 2006; Riaz et al. 2006).

F8909-08 genetic map

Resistance to *X. fastidiosa* segregated 1:1 in the 04190 population (Fig. 2b; Table 2). A total of 28 molecular markers and the *PdR1b* resistance locus mapped to chromosome 14 (Fig. 4). Twenty-three of the mapped markers were in common with the 9621 chromosome 14 map. The total map length was 80.7 cM with an average distance of 2.8 cM between markers (Fig. 4). The resistance locus, *PdR1b*, co-segregated with the marker VvCh14-02 and it was 0.4 cM from UDV095/VvCh14-10. The resistance locus mapped in between the same markers on the chromosome 14 of F8909-08 (04190 population) and F8909-17 (9621 population), suggesting that the position of the resistance locus is the same in both genotypes. It will now be necessary to compare the *PdR1* containing genomic sequences of these two siblings to resolve whether they possess different alleles of the same gene or different resistance genes.

Segregation distortion

The genotypic frequency distributions for each marker on the three maps of chromosome 14, the number of homozygous and heterozygous genotypes, and χ^2 values and their significance levels are provided in Table 2. The F8909-08 and b43-17 maps had a large number of markers with distorted segregation ratios on one end of chromosome 14, suggesting the presence of a locus or loci causing segregation disorder (Table 2; Fig. 4). However, with the exception of Scu15, markers on the F8909-17 chromosome 14 map were not distorted. This observation implies that genomic differences exist between the sister chromatids of F8909-08 and F8909-17. The distorted markers spanned a genetic distance of 56.5 and 57.7 cM in b43-17 and F8909-08, respectively (Fig. 4). In order to study the transmission of male gametes, alleles for each marker were always labeled as "a" for female and "b" for male to distinguish patterns of homozygotes (aa) and heterozygotes (ab) for all crosses across chromosome 14. The distortion was due to a greater than expected number of homozygotes, implicating the involvement of male gametophytic selection factors in this region of the chromosome (Table 2). Segregation distortion was also observed for common markers on chromosome 14 in the *V. vinifera*-based maps of Riesling × Cabernet Sauvignon (Riaz et al. 2004) and its reciprocal cross of Cabernet Sauvignon × Riesling (S. Vezzulli, personal communication). The markers VMCNg1e1, VMC6c10, VVMD24, VMC2a5 and VMC5b3 were distorted at significance levels of 0.05–0.01 when Cabernet Sauvignon was used as the male parent (Table 3). When Cabernet Sauvignon was used as the female parent, only VMC6c10 and VVMD24 were distorted (S. Vezzulli, personal communication). However, the Cabernet Sauvignon × Riesling mapping population consisted of only 87 individuals. In both cases, the distorted markers had a higher than expected number of homozygotes, which indicates that factors responsible for preferential male gamete selection are also present on chromosome 14 in *V. vinifera*-based genotypes.

Discussion

PdR1, an allelic form of PD resistance originating from F8909-17 was previously mapped in the 9621 population (Krivanek et al. 2006; Riaz et al. 2006). The study presented here pursued the fine-scale mapping of allelic forms of PD resistance introgressed from the homozygous resistant genotype b43-17. The first stage of the study proved that b43-17 was homozygous resistant by greenhouse screening and ELISA evaluation of 60 plants from the 04373 (*V. vinifera* F2-35 × b43-17) population. All 60 progeny had low levels of *X. fastidiosa* in their stems with values for bacterial cells/ml (natural log transformed) ranging from 8.5 to 13.5, while the susceptible female parent and positive controls had values equal to 17 (Fig. 2a). *PdR1* segregated 1:1 in the 04190 and 9621 populations, whose resistance derived from F8909-08 and F8909-17, respectively. The mapping results presented here found that these full siblings inherited different chromatids from b43-17, thus they represent either two alleles of same gene or different genes for PD resistance.

Fine-scale mapping of *PdR1a* and *PdR1b* locus

Two programs (JoinMap 3.0 and TMAP) were used to determine the correct marker order on the chromosome 14 genetic maps from the three populations. JoinMap 3.0 uses the goodness-of-fit statistic to evaluate the quality of marker order and the contributions of individual loci (Stam 1993; Van Ooijen and Voorrips 2001). TMAP uses a likelihood model for genetic mapping that includes the possibility of genotyping errors (Cartwright et al. 2007). This program also provides the percentage of error for each marker. There were no differences in marker order between

Table 2 Genotype frequencies for markers on chromosome 14 in populations derived from (a) b43-17,(b) F8909-17 and (c) F8909-08

	Marker name	Homozygotes	Heterozygotes	Missing data	χ^2	Significance level
a) b43-17 (04373 pop)	VMCNg1e1	144	112	26	4	**
	SCU15	170	91	21	23.9	*******
	VVC62	169	97	16	19.5	*******
	UDV50	170	94	18	21.9	*******
	VMC9c1	159	96	27	15.6	*******
	VMC1e12	165	84	33	26.4	*******
	VMC5b3	157	120	5	4.9	**
	ctg1026876	148	127	7	1.6	–
	UDV095	140	110	32	3.6	*
	VMCNg1g1.1	130	139	13	0.3	–
	VMC6e1	137	135	10	0	–
	VVIS70	135	144	3	0.3	–
	UDV025	133	128	21	0.1	–
	ctg1025828	142	129	11	0.6	–
b) F8909-17 (9621 pop)	Scu15	222	180	23	4.4	**
	VMCNg1e1	210	204	11	0.1	–
	UDV050	163	165	97	0	–
	VMC9c1	200	206	19	0.1	–
	VVIQ32	201	214	10	0.4	–
	VMC1e12	214	197	14	0.7	–
	VVC34	213	186	26	1.8	–
	VMC9f4-1	203	210	12	0.1	–
	VVIP22	198	213	14	0.6	–
	Vrip112	212	209	4	0	–
	VMC6c10	211	209	5	0	–
	VMC5b3	216	196	13	1	–
	UDV33	207	205	13	0	–
	VMC2a5	207	217	1	0.2	–
	VVIV69	207	217	1	0.2	–
	VMCNg3h8	171	195	59	1.6	–
	VMCNg2b7.2a	188	199	38	0.3	–
	A010	203	173	47	1.5	–
	VvCh14-56	200	177	48	1.4	–
	PdR1a	207	218	0	0.3	–
	UDV095	216	196	13	1	–
	VVIN64	194	226	5	2.4	–
	VVIS70	226	194	5	2.4	–
	VVIP26	225	198	2	1.7	–
	ctg1025882	218	184	23	2.9	*
	VMC6e1	227	195	3	2.4	–
	VMCNg1g1.1	232	180	13	4.4	**
	UDV025	218	193	14	1.5	–
	ctg1010193	219	184	22	3	*
c) F8909-08 (04190 pop)	VMCNg1e1	243	114	4	46.6	*******
	VVC62	244	111	6	49.8	*******
	VVIP05	230	111	20	41.5	*******
	UDV050	228	129	4	27.4	*******

Table 2 continued

	Marker name	Homozygotes	Heterozygotes	Missing data	χ^2	Significance level
	VMC9c1	229	126	6	29.9	*******
	VVIQ32	225	131	5	24.8	*******
	VMC1e12	221	132	8	22.4	*******
	VVC34	222	132	7	22.9	*******
	VVIP22	218	132	11	21.1	*******
	Vrip112	191	138	32	8.5	****
	VMC6c10	219	138	4	18.4	*******
	VMC5b3	198	147	16	7.5	***
	UDV033	197	142	22	8.9	****
	VVIV69	211	149	1	10.7	****
	VMC2a5	209	151	1	9.3	****
	ctg1026876	205	151	5	8.2	****
	VMCNg2b7.2	192	157	12	3.5	*
	VvCh14-2	183	162	16	1.3	–
	PdR1b	189	171	1	0.9	–
	VvCh14-10	188	169	4	1.0	–
	UDV095	188	169	4	1.0	–
	UDV025	178	177	6	0.0	–
	ctg1025882	177	172	12	0.1	–
	VVIS70	180	181	0	0.0	–
	VVIP26	177	180	4	0.0	–
	VMC6e1	172	180	9	0.2	–
	VMCNg1g1.1	172	186	3	0.6	–
	VVIN94	182	175	4	0.1	–
	ctg1010193	181	172	8	0.2	–
	VVIN70	168	172	21	0.1	–

Significance levels at alpha 0.05 = **, 0.01 = ***, 0.005****, 0.001 = *****, 0.0005 = ******, 0.0001 = *******

Table 3 Genotype frequencies for markers in common with the Riesling × Cabernet Sauvignon genetic map

Marker name	Homozygotes	Heterozygotes	Missing data	$\chi 2$	Significance level
VMCNg1e1	85	60	8	4.3	**
VMC1e12	67	80	6	1.1	–
VrZAG112	80	62	11	2.3	–
VMC6c10	86	59	8	5	**
VVMD24	82	58	13	4.1	**
VMC5b3	92	60	1	6.7	***
VMC2a5	86	61	6	4.3	**
VMC6e1	75	73	5	0	–

Significance levels at alpha 0.05 = **, 0.01 = ***

the two programs. Marker order on the parental and full sibling progeny maps was consistent, with the exception of a few local inversions in lower end of the chromosome (Fig. 4). The correct order and distance among tightly linked markers is greatly affected by the number of recombinants, differences in recombination rates between parents, and loci with segregation disorder (Tanksley et al. 1992). Where local inversions in marker order existed, the markers were linked within a 1 cM distance. Statistical power is limited when determining the correct order of closely linked markers. Overall, the marker order on all three maps was in agreement with SSR markers common to the integrated framework map constructed from five *V. vinifera* populations (Doligez et al. 2006).

The Pierce's disease resistance loci, *PdR1a and PdR1b*, mapped between markers VvCh14-56/A010/VvCh14-02 and UDV095/VvCh14-10, on the F8909-17 and F8909-08 maps, respectively. The flanking markers for *PdR1a* from F8909-17 were within a 0.6 cM distance. The flanking markers for *PdR1b* from F8909-08 were within a 0.4 cM distance. Previously, Riaz et al. (2006) found that flanking markers bordering *PdR1* spanned a 7.1 cM distance on the F8909-17 map. The addition of more markers and recombinants has markedly reduced the distance between markers linked to the *PdR*1 locus.

Potential segregation distortion factors

Molecular markers provide valuable data for the identification of regions associated with segregation distortion. This study involved only chromosome 14 on which PD resistance resides. Riaz et al. (2006) previously reported that the percentage of distorted markers in the 9621 consensus map was higher than reported on other published maps of *Vitis* where 17% of the markers were distorted, while 7% of the markers were distorted in the F8909-17 male parent. The Riaz et al. (2006) study also observed distorted markers on chromosome 14 and speculated that segregation distortion regions existed and that the skewed distortion was due to male gametophytic selection (Ottaviano et al. 1982). Segregation distortion regions have been reported in other crop species including tomato (Paterson et al. (1988), rice (Xu et al. 1997), alfalfa (Jenczewski et al. 1997), coffee (Ky et al. (2000), maize (Lu et al. (2002), and wheat (Kumar et al. 2007). However, very few is known about the role of gametophytic factors, including genetic incompatibility, environmental effects and the genetic control of pollen viability and morphology in grape. One of the limitations to understanding segregation disorder is the need for reciprocal crosses (Kumar et al. 2007). In this study all three populations were crosses of pistillate by staminate individuals because the wild grape species are dioecious, which prevents reciprocal crosses.

The difference in the rate of segregation distortion between the full sibs F8909-17 and F8909-08 was also indicative of b43-17's diverse genetic background. More than 50% the markers were distorted in the F8909-08 and b43-17 maps. In addition, the ratio of homozygotes to heterozygotes was higher across the markers indicating that there was preferential selection for male gametes carrying certain allelic combinations. Few of these markers were in common with the maps from the reciprocal crosses of *V. vinifera*-based Riesling × Cabernet Sauvignon and Cabernet Sauvignon × Riesling (Table 3). Five chromosome 14 markers in common with the Riesling × Cabernet Sauvignon population were distorted and the ratio was skewed toward homozygotes indicating that male gametophytic factors also play a role in the same general region of chromosome 14 in a *V. vinifera* background. When Cabernet Sauvignon was used as the female parent, two of these markers (VMC6c10 and VVMD24) were distorted (S. Vezzulli, personal communication) indicating that both Riesling and Cabernet Sauvignon have preferential selection of pollen. Virtually all *V. vinifera* cultivars are hermaphroditic, very fruitful and potentially carry favorable gametophytic allelic combinations, perhaps diluting the effect of deleterious alleles, which may be more pronounced in populations developed from wider crosses of *Vitis* species. Reciprocal crosses are rarely created with hermaphroditic grape cultivars because of the time and expense of maintaining populations of this perennial, long generation time plant. Therefore, it is difficult to study regions with segregation distortion in the available *V. vinifera*-based maps. Wide crosses among different species provide an opportunity to investigate differences in regions where segregation disorder is detected. This study reports the first genomic region in a *V. arizonica* background harboring potential male gametophytic factors and proposes the name *Vitis*-segregation disorder region 1 (*V-SDR1*) to identify it. This region is also found in the *V. vinifera*-based genetic maps described above and in a *V. riparia* × *V. champinii* map (Lowe and Walker 2006).

Utilization of the Pinot noir genome sequence

The availability of the grape genome sequence provides an opportunity to explore and compare the sequences of genomic regions of interest (Jaillon et al. 2007; Velasco et al. 2007). When the sequences of markers closely linked to PD resistance were blasted against the *V. vinifera* cv. Pinot noir grape genome sequence, a 109 Kb region on scaffold 21 on chromosome 14 was identified. There were 13 putative genes in that region, five were annotated with molecular function. Future comparisons of sequences of regions containing *PdR1a* and *PdR1b* to the Pinot noir genome sequence will provide important information about the differences among *Vitis* species at the sequence level. Plants that are resistant to PD restrict *X. fastidiosa* levels in their xylem and restrict bacterial movement both vertically and across xylem vessels (Krivanek and Walker 2005, Krivanek et al. 2005b). Susceptible plants have much higher *X. fastidiosa* levels in the xylem and allow vertical and cross vessel movement. It is likely that resistance is due to structural changes in vessel anatomy or to differences in the biochemical composition of cell walls. However, further research on the nature of this resistance and its genetic basis will depend on the physical mapping and sequencing of the *PdR1* region.

Future directions

This study provided an opportunity to investigate and map PD resistance from two separate lines obtained from the homozygous resistant *V. arizonica/candicans* selection b43-17. The 9621 (F8909-17-based) and 04190 (F8909-08-based) populations segregate for PD resistance and allowed mapping of the *PdR1a* and *PdR1b* resistance loci to within a 0.6 cM region. These results set the stage for map-based cloning of genes that confer resistance to PD. The next step is to generate a bacterial artificial chromosome (BAC) library to develop a physical map of the *PdR1* region. The tightly linked markers from the genetic map will greatly facilitate physical mapping of this region. In addition, the *V-SDR1* region will be exploited in other backgrounds to improve knowledge of the nature of gametophytic factors in grape. The Pinot noir genome sequence will also be exploited to compare the nature of the genes associated with disease resistance and segregation disorder.

Acknowledgments Research funding from the California Department of Food and Agriculture's Pierce's Disease Board, the California Grape Rootstock Improvement Commission and the Louis P. Martini Endowed Chair funds is gratefully acknowledged. The authors also wish to thank Rong Hu, Rita Zhu, Kurt Kabica, Geoffrey Dervishian and Daniel Ng for assisting with the laboratory, greenhouse and fieldwork needed for this study.

References

Arroyo-Garcia R, Martinez-Zapater JM (2004) Development and characterization of new microsatellite markers for grape. Vitis 4:175–178

Cartwright DA, Troggio M, Velasco R, Gutin A (2007) Genetic mapping in the presence of genotyping errors. Genetics 176:2521–2527

Decroocq V, Favé MG, Hagen L, Bordenave L, Decroocq S (2003) Development and transferability of apricot and grape EST microsatellite markers across taxa. Theor Appl Genet 106:912–922

Di Gaspero G, Cipriani G, Marrazzo MT, Andreetta D, Prado Castro MJ, Peterlunger E, Testolin R (2005) Isolation of (AC) n-microsatellites in *Vitis vinifera* L. and analysis of genetic background in grapevines under marker assisted selection. Molec Breed 15:11–20

Doligez A, Adam-Blondon A-F, Cipriani G, Di Gaspero G, Laucou V, Merdinoglu D, Meredith CP, Riaz S, Roux C, This P (2006) An integrated SSR map of grapevine based on five different populations. Theor Appl Genet 113:369–382

Doucleff M, Jin Y, Gao F, Riaz S, Krivanek AF, Walker MA (2004) A genetic linkage map of grape utilizing *Vitis rupestris* and *Vitis arizonica*. Theor Appl Genet 109:1178–1187

Hopkins DL (1980) Use of pin-prick inoculation technique demonstrates variability in virulence of Pierce's disease bacterium, pp177–180. In: Proceedings of VIIth international conference viruses grapevines (ICGV), Niagara Falls, Canada

Hopkins DL, Purcell AH (2002) *Xylella fastidiosa*: cause of Pierce's disease of grapevine and other emergent diseases. Plant Dis 86:1056–1066

Jaillon O, Aury JM, Noel B, Policriti A, Clepet C, Casagrande A, Choisne N, Aubourg S, Vitulo N, Jubin C, Vezzi A, Legeai F, Hugueney P, Dasilva C, Horner D, Mica E, Jublot D, Poulain J, Bruyere C, Billault A, Segurens B, Gouyvenoux M, Ugarte E, Cattonaro F, Anthouard V, Vico V, Del Fabbro C, Alaux M, Di Gaspero G, Dumas V, Felice N, Paillard S, Juman I, Moroldo M, Scalabrin S, Canaguier A, Le Clainche I, Malacrida G, Durand E, Pesole G, Laucou V, Chatelet P, Merdinoglu D, Delledonne M, Pezzotti M, Lecharny A, Scarpelli C, Artiguenave F, Pe ME, Valle G, Morgante M, Caboche M, Adam-Blondon AF, Weissenbach J, Quetier F, Wincker P (2007) The grapevine genome sequence suggests ancestral hexaploidization in major angiosperm phyla. Nature 449:463–467

Jenczewski E, Gherardi M, Bonnin I, Prosperi JM, Olivieri I, Huguet T (1997) Insight on segregation distortions in two intraspecific crosses between annual species of *Medicago* (Leguminosae). Theor Appl Genet 94:682–691

Kosambi DD (1944) The estimation of map distances from recombination values. Ann Eugen 12:172–175

Krivanek AF, Famula TR, Tenscher A, Walker MA (2005a) Inheritance of resistance to *Xylella fastidiosa* within a *Vitis rupestris* × *Vitis arizonica* hybrid population. Theor Appl Genet 111:110–119

Krivanek AF, Stevenson JF, Walker MA (2005b) Development and comparison of symptom indices for quantifying grapevine resistance to Pierce's disease. Phytopathology 95:36–43

Krivanek AF, Walker MA (2005) *Vitis* resistance to Pierce's disease is characterized by differential *Xylella fastidiosa* populations in stems and leaves. Phytopathology 95:44–52

Krivanek AF, Riaz S, Walker MA (2006) Identification and molecular mapping of *PdR1*, a primary resistance gene to Pierce's disease in *Vitis*. Theor Appl Genet 112:1125–1131

Kumar S, Gill BK, Faris JD (2007) Identification and characterization of segregation distortion loci along chromosome 5B in tetraploid wheat. Theor Appl Genet 278:187–196

Ky C-L, Barre P, Lorieux M, Thouslot P, Akaffou S, Louarn J, Charrier A, Hamon S, Noirot M (2000) Interspecific genetic linkage map, segregation distortion and genetic conversion in coffee (*Coffea* sp.). Theor Appl Genet 101:669–676

Lodhi MA, Reisch BI, Weeden NF (1994) A simple and efficient method for DNA extraction from grapevine cultivars and *Vitis* species. Plant Mol Biol Rep 12:6–13

Lowe KM, Walker MA (2006) Genetic map of the interspecific grape rootstock cross Ramsey (*Vitis champinii*) × Riparia Gloire (*Vitis riparia*). Theor Appl Genet 112:1582–1592

Lu H, Romero-Severson J, Bernardo R (2002) Chromosomal regions associated with segregation distortion in maize. Theor Appl Genet 105:622–628

Merdinoglu D, Butterlin G, Bevilacqua L, Chiquet V, Adam-Blondon AF, Decroocq S (2005) Development and characterization of a large set of microsatellite markers in grapevine (*Vitis vinifera* L.) suitable for multiplex PCR. Mol Breed 15:349–366

Nome SF, Docampo D, Goheen AC, Raju BC, Nayland G (1981) An enzyme-linked immunosorbent assay (ELISA) for detection of Pierce's disease bacteria in plant tissues. (Abstr) Phytopathology 71:107

Ottaviano E, Sari-Gorla M, Pe E (1982) Male gametophytic selection in maize. Theor Appl Genet 63:249–254

Paterson A, Lander E, Hewitt JD, Peterson S, Lincoln SE, Tanksley SD (1988) Resolution of quantitative traits into Mendelian factors by using the complete linkage map of restriction fragment length polymorphisms. Nature 335:721–726

Riaz S, Dangl GS, Edwards KJ, Meredith CP (2004) A microsatellite marker based framework linkage map of *Vitis vinifera* L. Theor Appl Genet 108:864–872

Riaz S, Krivanek AF, Xu K, Walker MA (2006) Refined mapping of the Pierce's disease resistance locus, *PdR1*, and *Sex* on an extended genetic map of *Vitis rupestris* × *V. arizonica*. Theor Appl Genet 113:1317–1329

Riaz S, Vezzulli S, Harbertson ES, Walker MA (2007) Use of molecular markers to correct grape breeding errors and determine the identity of novel sources of resistance to *Xiphinema index* and Pierce's disease. Am J Enol Vitic 58:494–498

Scott KD, Eggler P, Seaton G, Rossetto M, Ablett EM, Lee LS, Henry RJ (2000) Analysis of SSRs derived from grape ESTs. Theor Appl Genet 100:723–726

Sefc KM, Regner F, Turetschek E, Glossl J, Steinkellner H (1999) Identification of microsatellite sequences in *Vitis riparia* and their applicability for genotyping of different *Vitis* species. Genome 42:367–373

Stam P (1993) Construction of integrated genetic linkage maps by means of a new computer package: JoinMap. Plant J 3:739–744

Tanksley SD, Ganal MW, Prince JP, de Vicente MC, Bonierbale MW, Broun P, Fulton TM, Giovannoni S, Grandillo S, Martin GB, Messeguer R, Miller JC, Miller L, Paterson AH, Pineda O, Roder MS, Wing RA, Wu W, Young ND (1992) High density molecular linkage maps of tomato and potato genomes. Genetics 132:1141–1160

Van Ooijen JW, Voorrips RE (2001) JoinMap version 3.0, software for the calculation of genetic linkage maps. Plant Research International, Wageningen

Velasco R, Zharkikh A, Troggio M, Cartwright DA, Cestaro A, Pruss D, Pindo M, FitzGerald LM, Vezzulli S, Reid J, Malacarne G, Iliev D, Coppola G, Wardell B, Micheletti D, Macalma TM, Facci M, Mitchell JT, Perazzolli M, Eldredge G, Gatto P, Oyzerski R, Moretto M, Gutin N, Stefanini M, Chen Y, Segala C, Davenport C, Demattč L, Mraz A, Battilana J, Stormo K, Costa F, Tao Q, Si-Ammour A, Harkins T, Lackey A, Perbost C, Taillon B, Stella A, Solovyev V, Fawcett JA, Sterck L, Vandepoele K, Grando MS, Toppo S, Moser C, Lanchbury J, Bogden R, Skolnick M, Sgaramella V, Bhatnagar SK, Fontana P, Gutin A, Van de Peer Y, Salamini F, Viola R (2007) High quality draft consensus sequence of the genome of a heterozygous grapevine variety. PLoS ONE 2:e1326

Voorrips RE (2002) MapChart: software for the graphical presentation of linkage maps and QTLs. J Hered 93:77–78

Xu Y, Zhu L, Xiao J, Huang N, McCouch SR (1997) Chromosomal regions associated with segregation distortion of molecular markers in F2, backcross, doubled haploid, and recombinant inbred populations in rice (*Oryza sativa* L.). Mol Gen Genet 253:535–545