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ORIGINAL PAPER

Construction of a reference linkage map of *Vitis amurensis* and genetic mapping of *Rpv8*, a locus conferring resistance to grapevine downy mildew

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Abstract Downy mildew, caused by the oomycete *Plasmopara viticola*, is one of the major threats to grapevine. All traditional cultivars of grapevine (*Vitis vinifera*) are susceptible to downy mildew, the control of which requires regular application of fungicides. In contrast, many sources of resistance to *P. viticola* have been described in the *Vitis* wild species, among which is *V. amurensis* Rupr. (*Vitaceae*), a species originating from East Asia. A genetic linkage map of *V. amurensis*, based on 122 simple sequence repeat and 6 resistance gene analogue markers, was established using S1 progeny. This map covers 975 cM on 19 linkage groups, which represent 82% of the physical coverage of the *V. vinifera* reference genetic map. To measure the general level of resistance, the sporulation of *P. viticola* and the necrosis produced in response to infection, five quantitative and semi-quantitative parameters were scored 6 days post-inoculation on the S1 progeny. A quantitative trait locus (QTL) analysis allowed us to identify on linkage group 14 a major QTL controlling the resistance to downy mildew found in *V. amurensis*, which explained up to 86.3% of the total phenotypic variance. This QTL was named 'Resistance to *Plasmopara viticola 8*' (*Rpv8*).

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Introduction

Downy mildew, which is caused by the oomycete *Plasmopara viticola* (Berk. & Curt.) Berl.& de Toni (Dick 2002), is one of the major threats to grapevine. *P. viticola* was introduced in France from North America during the nineteenth century together with accessions of American wild *Vitis* species and rapidly spread across Europe (Galet 1977). Today, it is distributed worldwide, particularly in all of the temperate or warm regions where grapevine is cultivated. *P. viticola* is an obligate biotroph and can infect all green tissues of the grapevine, particularly leaves, reducing both the functional green leaf area and the assimilation rate of the remaining green leaf area (Moriondo et al. 2005). Inflorescences and young bunches can also be infected by downy mildew, leading to significant losses of productivity and quality (Lafon and Clerjeau 1988).

Control of downy mildew on traditional grapevine varieties requires regular application of fungicides. Nevertheless, the intensive use of chemicals becomes more and more restrictive because of their cost, their risk on human health and their negative environmental impact. Some chemical residues are detected in wines or in soils, where they can accumulate (Brun et al. 2003; Cus et al. 2010). Furthermore, some fungicide-resistant strains of *P. viticola* are now observed in the vineyard, decreasing the efficiency of these sprays (Gisi 2002; Gisi et al. 2007). The resulting

strict environmental regulations on the application of pesticides make plant breeding for disease resistance the most attractive way to control grapevine downy mildew effectively and in an environmentally friendly way.

All of the traditional cultivars of *Vitis vinifera*, which is the most widely cultivated grapevine species, are susceptible to downy mildew, although variations of susceptibility are observed among cultivars (Boubals 1959; Dubos 2002). In contrast, many sources of resistance to downy mildew have been described in species related to *V. vinifera* (Boubals 1959; Staudt and Kassemeyer 1995; Dai et al. 1995; Brown et al. 1999; Kortekamp and Zyprian 2003; Unger et al. 2007; Cadle-Davidson 2008), some of which have already been successfully introduced in grapevine to create resistant cultivars (Csizmazia and Bereznai 1968; Alleweldt and Possingham 1988; Eibach and Töpfer 2003; Cadle-Davidson 2008; Merdinoglu et al. 2009). The American and Asian *Vitis* species belonging to the *Euvitis* sub-genus or *Muscadinia* subgenus show varying levels of resistance to *P. viticola,* ranging from moderate resistance, for example in *V. rupestris*, to high resistance, for example in *V. rubra*, *V. candicans*, *V. amurensis*, *V. riparia*, *V. cinerea* or *Muscadinia rotundifolia* (Boubals 1959; Olmo 1971; Staudt and Kassemeyer 1995; Cadle-Davidson 2008; Diez-Navajas et al. 2008).

Vitis amurensis Rupr. (*Vitaceae*) is a species originating from East Asia that is mainly distributed from the northeast regions of China to northern Korea. Some accessions of *V. amurensis* display a high level of resistance to *P. viticola* (Staudt and Kassemeyer 1995; Cadle-Davidson 2008; unpublished data). Apart from showing resistance to downy mildew, *V. amurensis* exhibits numerous favorable traits, such as cold tolerance (Ma et al. 2010); resistance to powdery mildew (Wan et al. 2007), anthracnose and white rot (Li et al. 2008); and the presence of compounds of medicinal interest (Wang et al. 2000; Huang et al. 2001; Ha et al. 2009; Yim et al. 2010). These traits have prompted grapevine breeders to include this species in their breeding programs. Despite all of the favorable characteristics found in *V. amurensis*, genetic studies concerning this species are limited. The lack of knowledge about the genetic determinism of the favorable traits of *V. amurensis* is aggravated by the non-existence of a genetic map from this species. This absence of data not only precludes increasing the efficiency of breeding programs by means of marker assisted selection but also limits the exploitation of the resource provided by the genome sequence of *V. vinifera* (Jaillon et al. 2007) to understand the genetic and physiological bases of the traits of interest carried by *V. amurensis.*

The main objectives of this investigation were (1) to develop an initial genetic linkage map of *V. amurensis* to obtain preliminary data on its degree of synteny with *V. vinifera* and (2) to decipher the genetic determinism of resistance to downy mildew in *V. amurensis.* Here, we report the construction of a *V. amurensis* genetic map mainly using simple sequence repeat (SSR) and resistance gene analogue (RGA) markers and the identification of a major quantitative trait locus (QTL), named *Rpv8*, located on chromosome 14, that confers resistance to grapevine downy mildew. Comparing the genetic maps of *V. vinifera* and *V. amurensis* showed a high colinearity between the genomes of both species. The results reported here will allow us to exploit the resource of the grapevine genome sequence as well as discovering markers linked to downy mildew resistance loci for use in a marker-assisted breeding program, thus accelerating the selection of high quality wine and table grape cultivars that exhibit effective and durable resistance.

Materials and methods

Plant material

The mapping population consisted of 232 progeny from a selfing (S1) of *V. amurensis* 'Ruprecht', which is a hermaphrodite accession maintained in collection at the Geisenheim Research Center in Germany (Becker 1981). Briefly, after seed germination, young plants were transferred to 0.6 l pots until they reached the 6-leaf stage, and then they were transferred to 4 l pots and grown in the greenhouse stalked on 3 m nylon wires, on a substrate composed of 1/3 perlite and 2/3 sand, and watered daily with a complete nutritive solution (4.8% Norsk Hydro Hydrokani CPO, YARA). Biological replicates between years were produced by pruning the plants to the basal two buds in winter and allowing them to re-grow in spring to ensure uniform shoot development.

Four genotypes whose resistance phenotypes are well known and represent a range of resistance levels from susceptible to highly resistant (*V. vinifera* Cabernet Sauvignon clone 338, *V. rupestris* 'du Lot', *V. riparia* 'Gloire de Montpellier' and *M. rotundifolia* cv Regale) were grown from green cuttings under the same conditions as the S1 population plants and were used as controls in each experiment.

Evaluation of resistance to downy mildew

A strain of *P. viticola* collected from *V. vinifera* cv. Chardonnay in an experimental vineyard at INRA-Colmar (France) in 2006 was maintained on 6-week old seedlings of *V. vinifera* cv. Muscat Ottonel placed in an opened cardboard box covered with a plastic bag. After 5 days of incubation in a growth chamber (21°C, 100% relative humidity, 50 µmol/m^2/s light intensity), sporangia were recovered from infected leaves by immersion in water and gentle

Table 1 Variables scored to assess the resistance level to downy mildew

Variable name	Description	Scoring
OIV452	Symptom-based semi-quantitative scoring of downy mildew resistance adapted from the criteria of the Office International de la Vigne et du Vin (OIV; Anonymous 2009; http://news.reseau-concept.net/images/oiv/client/Code_descripteurs_2ed_FR.pdf)	From 1 (very susceptible) to 9 (totally resistant) (Fig. 1): 1 = abundant sporulation densely covering the whole disc area, absence of plant necrosis 3 = abundant sporulation present in large patches, absence of plant necrosis 5 = limited sporulation present in intercostal patches, necrotic flecks or speckles 7 = sparse sporulation, necrotic spots 9 = no sporulation, absence of necrosis or necrotic points
SDSC	Percentage of sporulating leaf discs	From 0 to 100%
SPNB	Number of sporangia per cm^2 of leaf disc measured with a Z2 Coulter Counter (Beckman Coulter)	Quantitative. Number of sporangia per ml of suspension converted to number of sporangia per cm^2 of leaf disc
NSURF	Visual semi-quantitative scoring of necrotized surface	From1 (very large) to 9 (very small) (Fig. 1)
NDSC	Percentage of necrotized discs	From 0 to 100%

shaking. The concentration of the *P. viticola* suspension was measured using a cell-counting chamber.

Sixteen leaf discs (1 cm diameter) were sampled from the fourth and the fifth fully expanded leaves from the apex of grape shoots at the 10-leaf stage. Discs were placed on wet filter paper in Petri dishes abaxial side up and then artificially inoculated by spraying with a *P. viticola* suspension at 10^5 sporangia/ml. Three replicates were performed for each individual of the S1 population and nine replicates for each control. Petri dishes were then sealed and incubated in a growth chamber at 21°C and a photoperiod of 18 h light/6 h darkness. Six days post-inoculation (dpi), inoculated leaf discs were scored for the five semi-quantitative and quantitative parameters described in Table 1 to measure the general level of resistance (OIV452), the effect of resistance on the sporulation of *P. viticola* (SDCS, SPNB) and the necrotic symptoms produced in response to infection (NSURF, NDSC).

SSR and RGA marker analysis

Genomic DNA was extracted from 80 mg of young expanding leaves using the Qiagen DNeasy® Plant Mini Kit (Qiagen S.A., Courtaboeuf, France) as described by the supplier. All microsatellite loci were amplified in an 8 µl reaction mixture containing 2.5 mM $MgCl_2$, 150 µM dNTPs, 0.25 µM of the fluorescently labeled primer (FAM, HEX or NED), 0.5 µM of the unlabeled primer, 0.025 U/µl of AmpliTaq Gold DNA polymerase, 1 ng/µl of grapevine DNA and 1× Gold Buffer. Amplifications were performed on a Perkin Elmer 9700 thermocycler programmed as follows: 10 min at 94°C, followed by 35 cycles of 45 s at 92°C, 60 s at 57°C, and 90 s at 72°C and a final step of 5 min at 72°C. Up to three different primer pairs were mixed in the same PCR reaction, taking into account the size of the amplified fragments and/or the labeling of the primers (Merdinoglu et al. 2005). The mix of four PCR products, according to their size and labeling, allowed us to analyze up to 12 markers in one injection. After a 1/5 dilution in water, 1 µl of the PCR products was added to a 19 µl mixture of formamide and HD400-ROX as the internal size standard. The mix was then denatured for 3 min at 92°C. All products for amplification and electrophoresis were obtained from Applied Biosystems, Foster City, CA. Microsatellite fragments were resolved on an automated ABI Prism 310 Genetic Analyzer (Applied Biosystems, Foster City, CA) using a 36 cm capillary filled with the POP-4 polymer. Electrophoregrams were analyzed using Genescan™ 3.1 (Applied Biosystems, Foster City, CA). Alleles were identified using Genotyper™ 2.5.2 (Applied Biosystems, Foster City, CA) and their size was determined using the HD400-ROX internal size standard.

Seven primer pairs were used to amplify RGAs (stkVa011, stkVa036, stkVa043, stkV104, rgVamu035, rgVamu092) (Di Gaspero and Cipriani 2003) and a mitogen-activated protein kinase/extracellular signal-regulated kinase kinase (MEKK) gene (forward primer: AAGCCA GGGCAAGCAAAGTCTT; reverse primer: CAAACTGT TGCTGCCAAACAAT) as sequence-tagged-sites (STSs). STSs were amplified in 8 µl reactions containing each dNTP at 150 µM, each primer at 0.5 µM, 0.025 U of *Taq* polymerase, 2.5 mM $MgCl_2$ and 10 ng of template DNA. Thermal cycling was carried out for 36 cycles of melting at 92°C for 45 s, annealing at the appropriate temperature for 60 s, and extension at 72°C for 2 min. Five of the amplification products were then digested with restriction enzymes (Di Gaspero and Cipriani 2003). StkVa043 and MEKK, which each showed a SNP, were genotyped by sequencing. The enzymatic digestion was carried out in a 20 µl reaction mixture containing 8 µl of PCR product, 0.1 mg/mL of

Table 2 Newly developed microsatellite markers

Marker name	ProbeDB PUID	Primer name	Primer sequence	Map location
Chr14V015	10595642	Chr14V015F	TGATACTGTTTGCTTGGCATAA	LG14
		Chr14V015R	GGGAGCTCATATTTCACCAA	
Chr7V001	10595643	Chr7V001F	GCATGAACCATCTTAATTTGC	LG7
		Chr7V001R	CATAATTTGGAGATGGTTTTCAA	
Chr7V003	10595644	Chr7V003F	AAGGATGACGGCTACTCCAC	LG7
		Chr7V003R	CCCATTTCAGTCTTTCCCTGT	
Chr7V004	10595645	Chr7V004F	TACCAAACCTTTTGGCCTTG	LG7
		Chr7V004R	GCTGCTCACCGTTAATGAAA	

The primer sequences are deposited in the NCBI Probe database under Probe Unique Identifiers (PUIDs) 10595642–10595645

bovine serum albumin (BSA), 3.75 U of restriction enzyme, and 1× enzyme buffer. The mixture was incubated at 37°C overnight. Digestion products were resolved on 2% agarose gels and stained with ethidium bromide.

Genetic mapping

We initially used 232 primer pairs flanking microsatellite loci from marker sets VVS (Thomas and Scott 1993), VVMD (Bowers et al. 1996, 1999), VrZAG (Sefc et al. 1999), VMC (Vitis Microsatellite Consortium, coordinated by Agrogene, Moissy Cramayel, France), UDV (Di Gaspero et al. 2005), and VVI (Merdinoglu et al. 2005) and markers newly developed in this study from the grapevine genome sequence (Table 2), mainly to improve the construction of linkage groups 7 and 14. All of them were screened for informative segregation on the parent *V. amurensis* 'Ruprecht' and six randomly chosen individuals of the S1 population. A subset of 122 polymorphic SSR markers was used to analyze the entire mapping population. For mapping purposes, the same segregation pattern was assigned to all markers (<hkxhk>: locus heterozygous in both parents, two alleles), and genotypes were encoded (hh, hk, kk) for co-dominant loci and (h-, kk) for dominant loci, following JoinMap 3.0 data entry notation (Van Ooijen and Voorrips 2001).

Linkage analysis was performed with JoinMap 3.0 (Van Ooijen and Voorrips 2001), enabling the analysis of self-pollinated populations derived from a heterozygous parent and the construction of consensus linkage maps. Recombination fractions were converted into centimorgans (cM) using the Kosambi function (Kosambi 1944). The threshold value of the logarithm of odds (LOD) score was set at 4.0 to claim linkage between markers with a maximum fraction of recombination at 0.45. The goodness-of-fit between observed and expected Mendelian ratios was analyzed for each marker locus using a χ^2 test. Markers showing segregation distortion were included in the final map if their presence did not alter surrounding marker order on the linkage group. Linkage groups were numbered according to internationally acknowledged grapevine reference genetic maps (Doligez et al. 2006; Di Gaspero et al. 2007).

QTL analysis

The genetic variance (σ_g^2) and experimental error variance (σ_e^2) required for heritability calculations were estimated using the statistical software R version 2.10.1 (The R Foundation for Statistical Computing) in the lmer function of the package lme4 by treating genotype as a random factor. Broad-sense heritability estimates were calculated on a genotype mean basis via the equation $H^2 = \sigma_g^2 /(\sigma_g^2 + (\sigma_e^2 /r))$ (Gallais 1990), where the term r refers to the number of replicates for each genotype.

Quantitative trait locus analysis was carried out by both non-parametric Kruskal–Wallis analysis and interval mapping using MapQTL 4.0 software (Van Ooijen et al. 2002). The significant LOD threshold for QTL detection at $P = 0.05$ for each linkage group was determined by 1,000 permutations of the phenotypic data. Maximum LOD values were used to estimate QTL peak positions, and the confidence intervals of QTL peaks were determined as the peak flanking regions in which LOD scores declined by two LODs.

Results

Analysis of resistance traits

The reliability of the downy mildew resistance test was assessed by scoring the five resistance parameters in the control plants *V. vinifera* Cabernet Sauvignon (susceptible), *V. rupestris*, *V. riparia* (both partially resistant) *M. rotundifolia* and *V. amurensis* 'Ruprecht' (both totally resistant, the second being the parent of the S1 mapping population). As shown in Table 3, all control plants as well as the parent of the S1 population behaved as expected for the different

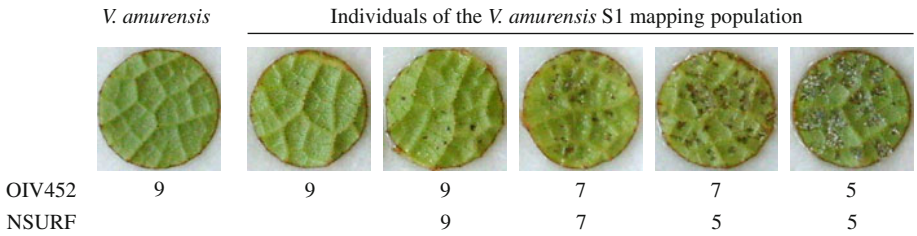

Fig. 1 Range of the segregating phenotypes more commonly observed 6 dpi on 1 cm leaf discs in the *V. amurensis* S1 mapping population. OIV452 is a symptom-based semi-quantitative scoring system of downy mildew resistance, and NSURF a visual semi-quantitative scoring system of the necrotized surface

Table 3 Control and parent mean values and confidence intervals for OIV452, SDSC, and SPNB

	OIV452	SDSC	SPNB
Cabernet Sauvignon 338	3.2 ± 0.4	99.5 ± 0.7	78,327 ± 15,153
V. rupestris 'du Lot'	4.8 ± 0.3	100.0 ± 0.0	47,520 ± 11,470
V. riparia 'Gloire de Montpellier'	7.1 ± 0.2	83.4 ± 13.2	1,291 ± 389
M. rotundifolia cv Regale	9.0 ± 0.0	0.0 ± 0.0	0 ± 0
V. amurensis 'Ruprecht'	9.0 ± 0.0	0.0 ± 0.0	0 ± 0

Table 4 Descriptive statistical parameters for OIV452, SDSC, SPNB, NSURF, and NDSC in the S1 population

	OIV452	SDSC	SPNB	NSURF	NDSC
Average	8.009	25.268	2,474	6.236	31.365
Minimum	3.333	0	0	1	0
Maximum	9	100	43,325	9	100

resistance parameters evaluated in this study. Differences in accordance with the measure of sporulation were observed by global scoring of resistance (OIV452), for which Cabernet Sauvignon, *V. rupestris* 'du Lot', *V. riparia* 'Gloire de Montpellier', *M. rotundifolia* cv. Regale and *V. amurensis* 'Ruprecht' were scored 3.2 ± 0.4, 4.8 ± 0.3, 7.1 ± 0.2, 9.0 ± 0.0, and 9.0 ± 0.0, respectively.

Resistance to downy mildew displayed a continuous variation in the S1 population and segregated as a quantitative trait, regardless of the parameter used to measure it. The distribution of OIV452 and SPNB in the S1 population ranged from 3.3 to 9 and from 0 to 43,325, respectively (Table 4), which means that the most susceptible individuals displayed partial resistance similar to the *V. rupestris* control level, whereas the most resistant were totally resistant, similarly to the *V. amurensis* 'Ruprecht' parent. The traits describing the necrotic symptoms produced in response to infection were linked to the resistance level parameters: the higher the resistance, the weaker the necrotic response to infection (Fig. 1). The highest coefficients of correlation were observed between SDSC and NSURF (0.63), between SDSC and NDSC (0.63), between OIV452 and NSURF (0.60), and between OIV452 and NDSC (0.63). OIV452, SPNB, and SDSC displayed segregation patterns where roughly sixty percent of the individuals were totally resistant, whereas the remaining part of the population showed various levels of partial resistance (Fig. 2). This segregation suggests that the differences in resistance to downy mildew observed in the S1 population from *V. amurensis* 'Ruprecht' are controlled by a dominant major locus together with minor quantitative loci, with the latter modulating the resistance level for the individuals that do not carry the major factor.

In the S1 population, the genotype factor had a highly significant ($P < 0.001$) effect on OIV452, SDCS, SPNB and NDSC scores and a very significant ($P < 0.01$) effect on NSURF. Broad-sense heritability, a direct measure of environmental effects on phenotypic variance, was calculated for downy mildew resistance using 232 genotypes and estimated for each of five scored parameters. On a genotype mean basis, the estimates were medium to high, ranging from 0.33 to 0.86, with the SDSC producing the highest heritability (Table 5).

Vitis amurensis genetic map

Of the 232 SSR primer pairs tested on the S1 population, 23 did not amplify or produced an unclear banding pattern, and 87 lacked polymorphism and were thus discarded. A total of 129 primer pairs, including 122 SSRs and 7 RGAs, allowed us to detect 134 useful loci scored on the progeny, 115 being fully informative (hh, hk, kk) and 19 displaying a dominant pattern (h-, kk). Chi-square analysis indicated segregation distortion for 14 markers (11.3%).

One hundred and thirty-two markers were mapped into 19 linkage groups, and 2 markers were linked but unmapped due to weak linkages to other markers within the group. The linkage groups were numbered LG1 to LG19 according to Adam-Blondon et al. (2004) and Doligez et al.

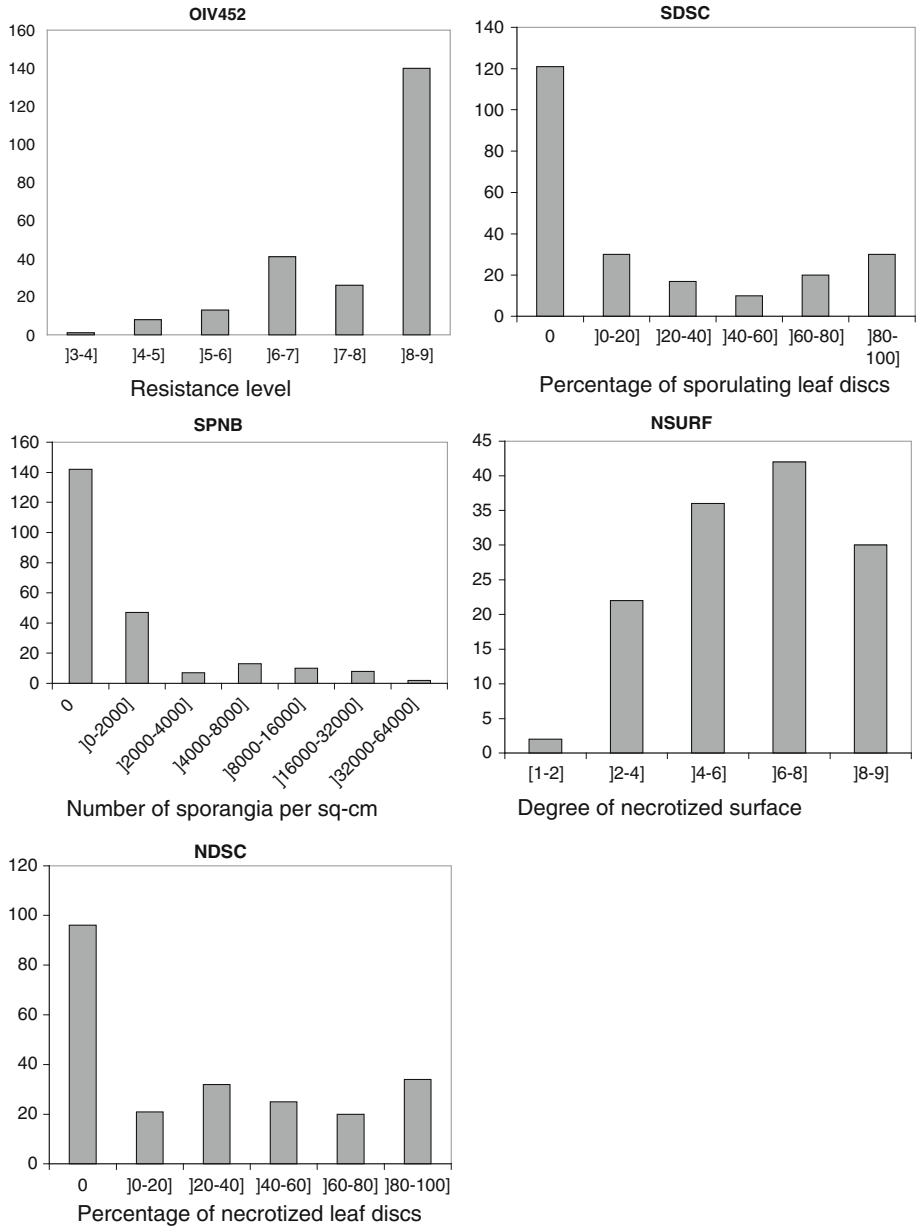

Fig. 2 Distribution of the variables scored in the *V. amurensis* S1 mapping population

(2006). The total length of the map was 975 cM, with an average distance of 7.3 cM between markers (Fig. 3). The largest group in terms of distance, LG19, consisted of nine mapped markers covering 76 cM, and the smallest, LG17, consisted of three mapped markers covering 32 cM. Only eight gaps were larger than 20 cM. The largest gap was on LG17, where the distance between marker VVIs63 and VMC9g4 was 30 cM. Locus order on the map was consistent with the *V. vinifera* reference maps (Adam-Blondon et al. 2004; Doligez et al. 2006), except in five cases where marker order was inverted in comparison to the reference map order [(LG5: VVMD14 and the group 'VMC2e9d-VVIn40-VMC2e9c'); (LG9: VVIo52 and VMC3g8-2); (LG10: UDV059 and VMC8d3); (LG12: VMC8g9 and VVIv05); (LG14: VVMD24 and VMC5b3)] and in another case where the marker VMC4h9 was expected on LG12 but was actually located on LG3. The marker order was also consistent with the order determined from the *V. vinifera* genome sequence of the French-Italian collaborative project (Jaillon et al. 2007; http://www.genoscope.cns.fr/spip/Vitis-vinifera-sequencage.html).

In comparison to the reference map of Doligez et al. (2006), the *V. amurensis* map covers up to 59% in average in genetic distance. Nevertheless, when using the common distal markers to align both maps, the coverage ratio rose to 82% due to a recombination rate that was, on average, much lower in the *V. amurensis* map than in the reference map (Table 5).

Table 5 Comparison of the genetic distances and genome coverage between the *V. vinifera* reference linkage map and *V. amurensis* genetic linkage map

	Markers common between maps		Distance between common markers			Maximum distance			
Linkage group	Start marker	End marker	Genetic distance on reference map	Genetic distance on *V. amurensis* map	Genetic distance ratio	Genetic distance on reference map	Genetic distance on *V. amurensis* map	Genetic distance ratio	Physical distance ratio
1	VMC8a7	VVIf52	69.3	54.0	0.78	87.5	72.9	0.83	1.07
2	VVIb01	VMC7g3	67.3	47.0	0.70	79.7	47.0	0.59	0.84
3	VMC8f10	VMC2e9b	53.4	39.5	0.74	70.3	71.0	1.01	1.36
4	VVIr46	VVIp37	65	46.2	0.71	90.9	46.2	0.51	0.72
5	VrZAG79	VVMD14	52.8	52.2	0.99	83.4	52.2	0.63	0.63
6	VVIp72	VMCNG4b9	75.9	41.0	0.54	82.5	41.0	0.50	0.92
7	UDV011	VVIn56	87.9	63.6	0.72	102.7	63.6	0.62	0.86
8	VMC1f10	VMC3c9	77.8	44.1	0.57	112.7	44.1	0.39	0.69
9	VVIo52	VMC3h5	48.6	38.9	0.80	104.1	38.9	0.37	0.47
10	VrZAG64	VVIv37	53.2	33.0	0.62	83.7	33.0	0.39	0.64
11	VVMD25	VVMD8	57.6	57.6	1.00	75.1	57.6	0.77	0.77
12	TT251F02	VMC8g9	72.9	56.4	0.77	81.9	59.5	0.73	0.94
13	UDV088a	VMC3b12	71.8	39.5	0.55	101.1	39.5	0.39	0.71
14	VVC62	VVIn70	94.0	64.0	0.68	94.8	64.0	0.67	0.99
15	UDV047	VMC8g3-2	35.4	33.4	0.94	37.9	33.4	0.88	0.93
16	UDV052	VMC4b7-2	69.4	41.4	0.60	92.4	41.4	0.45	0.75
17	VVIs63	VVIb09	40.3	31.9	0.79	58.0	31.9	0.55	0.69
18	VMC2a3	VVMD17	83.9	61.6	0.73	131.5	61.6	0.47	0.64
19	VVIp17a	UDV127	63.9	52.4	0.82	76.6	76.3	1.00	1.21
Total			1240.4	897.7	0.724	1646.8	975.1	0.592	0.818

QTL detection

Significant QTLs were obtained by interval mapping analysis for downy mildew resistance and necrotic symptoms produced in response to infection (Table 6). A major QTL controlling resistance to downy mildew was detected on LG14 for OIV452, DSCS and SPNB. Analysis based on DSCS gave the highest LOD score (65.45) and explained 86.3% of the total phenotypic variance and thus 100% of the genetic variance, considering the broad sense heritability estimated at 86.2%. This locus accounted for 66.5 and 36.0% of the phenotypic variation (LOD scores of 41.07 and 17.81, respectively) for OIV452 and SPNB, respectively. It was located at widely overlapping regions covering a confidence interval 7–13 cM when using any of the three disease evaluation methods, and in each case it was placed close to the SSR marker Chr14V015 (Table 6). Therefore, the locus at this position was denoted 'Resistance to *Plasmopara viticola 8*' (*Rpv8*). Analyses based on necrotic response to infection also allowed us to detect in a region overlapping *Rpv8* a major QTL that displayed a weaker effect, giving the highest LOD score (17.78) for NDSC (Table 6). The genome interval between the markers VVIp05 and VVIp22 that flanks the *Rpv8* confidence interval covered a physical distance of 15.11 Mb, which encompasses 502 genes, according to the 12× grape genome sequence (http://www.genoscope.cns.fr/externe/GenomeBrowser/Vitis/).

A minor QTL was detected for OIV452 and located on LG15 very close to VMC8g3-2. A second minor factor was detected on LG17 for NSURF next to VVIs63. Chr14V015 was chosen as a cofactor for further analysis with composite interval mapping, which gave no clear indication of there being any other QTL. Kruskal–Wallis non-parametric tests confirmed all QTLs detected with interval mapping (data not shown).

Discussion

To date, the grapevine genetic maps that have been published have been based either on *V. vinifera* intra-specific crosses (Doligez et al. 2002; Adam-Blondon et al. 2004; Riaz et al. 2004; Doligez et al. 2006; Troggio et al. 2007) or on hybrids produced from inter-specific crosses (Lodhi et al. 1995; Dalbó et al. 2000; Grando et al. 2003; Doucleff

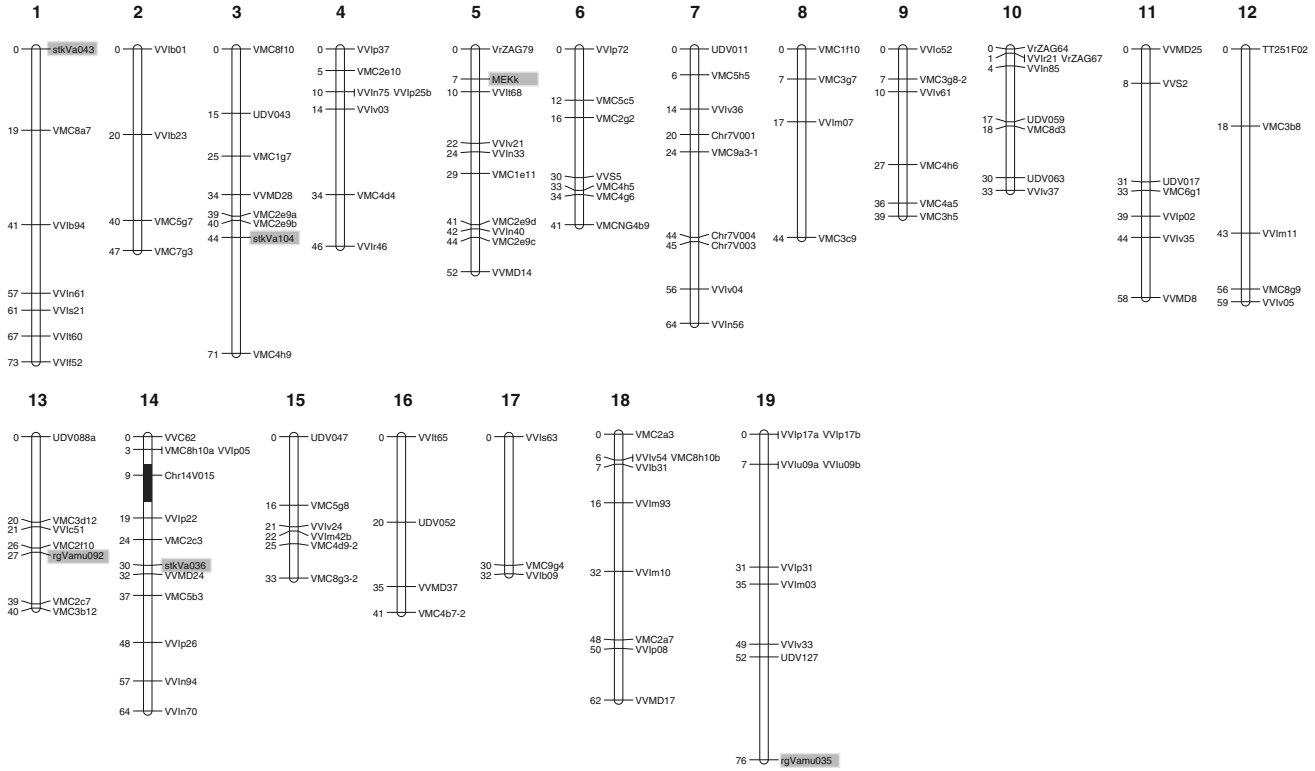

Fig. 3 *Vitis amurensis* genetic linkage map. The *black bar* on LG14 indicates the *Rpv8* position. The *grey boxes* indicate the RGA position

Table 6 QTLs detected in the S1 population for resistance to *P. viticola* and necrotic reactions observed post-infection

Trait	Linkage group	Nearest marker	Peak position	2-LOD interval	LOD score	% Phenotypic variance explained	Trait heritability	% Genotypic variance explained
SDSC	14	Chr14V015	10.9	7.9–14.9	65.45	86.3	0.862	100.1
SDSC	15	VMC8g3-2	33.4	8–33.4	2.32	4.7	0.862	5.5
SPNB	14	Chr14V015	10.9	4.9–17.9	17.81	36.0	0.413	87.3
OIV452	14	Chr14V015	11.9	6.9–15.9	41.07	66.5	0.830	80.1
OIV452	15	VMC8g3-2	33.4	10–33.4	2.74	5.6	0.830	6.7
NSURF	14	Chr14V015	12.9	0–20.5	8.18	33.0	0.333	99.0
NSURF	17	VVIs63	0	0–31.9	2.21	7.7	0.333	23.1
NDSC	14	Chr14V015	10.9	5.9–16.9	17.78	40.7	0.617	65.9

et al. 2004; Fischer et al. 2004; Lowe andWalker 2006; Welter et al. 2007; Di Gaspero et al. 2007; Salmaso et al. 2008; Bellin et al. 2009; Marguerit et al. 2009; Moreira et al. 2010). This paper reports the first grapevine genetic linkage map based on an intra-specific progeny of a *Vitis* species other than *V. vinifera*.

The high level of reproducibility and polymorphism of *V. vinifera* based SSR markers in non-*vinifera* species emphasizes the efficiency of the SSR marker system as a valuable genomic tool. The proportion of SSR markers with biased segregation observed in this study (11.3%) was lower than that reported by Grando et al. (2003) (22.4%), Lowe and Walker (2006) (16%) and Troggio et al. (2007) (20.3%) and slightly higher than that of Doligez et al. (2006) (9.2%). One marker segregation distortion clustering was found on LG7. The development of the new SSR markers Chr7V001, Chr7V003, and Chr7V004 from the 12× grapevine genome sequence permitted the improvement of the construction of LG7, which was split in two parts in the initial version of the *V. amurensis* map. The development of Chr14V015 in place of VVIq32, which was discarded because of an unclear banding pattern, enabled a more accurate detection and location of *Rpv8*. Moreover, two SSR markers, VVIv03 and VVIv54, whose positions

were unknown according to any previously published genetic linkage map and the 12× grapevine genome sequence (http://www.genoscope.cns.fr/externe/GenomeBrowser/Vitis/), were accurately located on LG4 and LG18 of our *V. amurensis* map, respectively. Finally, 132 markers were mapped into 19 linkage groups. The total length of the map was 975 cM. Considering marker order, linkage group sizes and map length, the *V. amurensis* map built in this work is consistent with other published maps (Adam-Blondon et al. 2004; Doligez et al. 2006) and with the genome sequence (Jaillon et al. 2007; http://www.genoscope.cns.fr/externe/English/Projets/Projet_ML/index.html) except for small discrepancies: four inversions in marker order between the *V. amurensis* map and the reference maps and one case where a marker was expected on LG12 and was actually located on LG3. The colinearity between the *V. amurensis* map and the previously published *V. vinifera* maps reveals a high level of macro-synteny between both genomes. This synteny facilitates the exploitation of the resource provided by the whole genome sequence of *V. vinifera* (Jaillon et al. 2007) to understand the genetic and physiological bases of the traits of interest carried by *V. amurensis.*

The segregation of the resistance to downy mildew observed in the *V. amurensis* S1 population can be explained by the effect of a dominant major locus whose presence guarantees total resistance together with minor quantitative loci that would modulate the resistance level in individuals lacking the major factor. This hypothesis was confirmed by QTL analysis, which demonstrated that downy mildew resistance derived from *V. amurensis* was mainly determined by one major QTL located on LG14 that explained between 75 and 99% of the genetic variance, depending on the method used to measure the resistance level. We have named this major locus *Rpv8,* for Resistance to *Plasmopara viticola 8*. The strong effect of *Rpv8* together with the high level of genome coverage of the linkage map leads us to assume that no significant genetic factor involved in downy mildew resistance other than those detected in this study segregates in the S1 progeny. Except *Rpv2* from *M. rotundifolia* (Wiedemann-Merdinoglu et al. 2006), the QTLs previously identified for resistance to grapevine downy mildew only confer partial resistance (Merdinoglu et al. 2003; Fischer et al. 2004; Welter et al. 2007; Bellin et al. 2009; Marguerit et al. 2009; Moreira et al. 2010), and they are all assumed to directly or indirectly originate from American *Vitis* species. *Rpv8* is thus the first QTL conferring resistance to *P. viticola* that is derived from an Asian *Vitis* species. Moreover, it clearly confers total resistance to downy mildew in the *V. amurensis* background from which it originates. Although *Rpv8* was detected in a *V. amurensis* selfed progeny, it is probably not present in all accessions of this species. According to Wan et al. (2007), *V. amurensis* is considered susceptible to downy mildew. Indeed, in that study, in natural conditions of infection in China, only one wild accession of nine was scored as partially resistant, whereas the other were scored as susceptible. These results clearly show strong variations of downy mildew resistance among *V. amurensis* accessions, as has been observed in other *Vitis* species (Boubals 1959; Wan et al. 2007; Cadle-Davidson 2008).

Resistance to downy mildew displayed a continuous variation in the *V. amurensis* S1 population, where the most susceptible individuals displayed partial resistance similar to that of the *V. rupestris* control. This suggests the existence of one or more homozygous non-segregating factors in the genetic background of *V. amurensis* 'Ruprecht' that maintains residual resistance. The traits describing the necrotic symptoms produced in response to infection are linked to the resistance level parameters; the higher the resistance, the weaker the necrotic response to infection. Moreover, a clear overlap between the regions involved in the variation of downy mildew resistance parameters and necrosis parameters was observed at the *Rpv8* locus, which confirms that the absence of necrotic response is a component of the total downy mildew resistance found in *V. amurensis* 'Ruprecht'. Therefore, more than half of the progeny presented necrotic symptoms that were observed one day post-infection. Such a necrotic response has been observed post-infection in partially resistant genotypes of *V. riparia* (Wielgoss and Kortekamp 2006), *V. rupestris* (Unger et al. 2007) and 'Bianca' cultivar (Bellin et al. 2009). We assume that this necrotic response is linked to the residual resistance that makes the most susceptible individuals of the S1 progeny partially resistant.

Rpv8 is located on the upper side of LG14, flanked by SSR markers VVIp05 and VVIp22, on a genomic region never previously described to control downy mildew resistance in grapevine or in related *Vitis* species. Based on the latest version of the grapevine genome sequence (12×, http://www.genoscope.cns.fr/externe/GenomeBrowser/Vitis/), the region delimitated by markers VVIp05 and VVIp22 is 15 Mb in size and contains 502 predicted genes, which means that it is a bit premature to speculate on putative candidates for *Rpv8* function. Nevertheless, while performing a physical map of *V. vinifera* Cabernet Sauvignon, Moroldo et al. (2008) identified 5 candidate genes for disease resistance (3 NBS-LRR genes; a receptor-like kinase; and enhanced disease resistance 1 (*EDR1*), a regulator of defense responses (Frye et al. 2001)) mapping to the region between markers VVIp05 and VVIp22. Inspection of this region in the 12× version of the grapevine genome sequence revealed that the putative receptor-like kinase was outside the interval and confirmed the presence of *V. vinifera EDR1*. Interestingly, there are at least 7 predicted NBS-LRR genes inside the interval, together with other truncated

forms that could be the result of incorrect annotation. Thus, it seems quite likely that *Rpv8* is a member of the NBS-LRR class of disease resistance genes, although we cannot discard *EDR1* as a putative *Rpv8* candidate gene.

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