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ORIGINAL PAPER

A framework map from grapevine V3125 (*Vitis vinifera* 'Schiava grossa' × 'Riesling') × rootstock cultivar 'Börner' (*Vitis riparia* × *Vitis cinerea*) to localize genetic determinants of phylloxera root resistance

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Abstract Grapevine rootstock cultivar 'Börner' is a hybrid of *Vitis riparia* and *Vitis cinerea* Arnold that shows high resistance to phylloxera (*Daktulosphaira vitifoliae* Fitch). To localize the determinants of phylloxera root resistance, the susceptible grapevine V3125 (*Vitis vinifera* 'Schiava grossa' × 'Riesling') was crossed to 'Börner'. Genetic framework maps were built from the progeny. 235 microsatellite markers were placed on the integrated parental map. They cover 1,155.98 cM on 19 linkage groups with an average marker distance of 4.8 cM. Phylloxera resistance was scored by counting nodosities after inoculation of the root system. Progeny plants were triplicated and experimentally infected in 2 years. A scan of the genetic maps indicated a quantitative trait locus on linkage group 13. This region was targeted by six microsatellite-type markers newly developed from the *V. vinifera* model genome sequence. Two of these appear closely linked to the trait, and can be useful for marker-assisted breeding.

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Introduction

Grapevine is one of the most important horticultural crops in the world. Its production reached more than 66 million tons, and the area harvested achieved over 7.5 million hectares in 2007 (FAOSTAT, http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567). The worlds viticulture is based on domesticated *Vitis vinifera* ssp. *vinifera* L. cultivars that are resistant to phylloxera (*Daktulosphaira vitifoliae* Fitch) at their leaves, but highly susceptible at their roots. This aphid-like sucking insect (Hemiptera, Sternorrhycha, Aphidoidea and Aphididae) causes galls on tips of primary roots by swellings of the root cortex called "nodosities" and hyperplasic so-called "tuberosities" in older wooden root parts. Nodosities act as nutrient sinks. They contain elevated starch levels and an altered amino acid composition (Kellow et al. 2004) to feed the inhabitant larvae developing into an egg-producing louse. Phylloxera root infection causes vine decline and finally plant death, also due to secondary infections. The parasite was accidentally introduced from America to Europe in the middle of the nineteenth century. Since this time resistance breeding is used to solve the problem. Rootstocks derived from hybridizing American wild *Vitis* species with root resistance (predominantly *V. riparia*) have been successfully used to safe European viticulture. High quality scions of wine-producing *V. vinifera* ssp. *vinifera* are grafted in commercial viticulture. Mostly *V. berlandieri* × *V. riparia* hybrids make up the acknowledged rootstock cultivars currently in use. However, there is continuous effort to

improve phylloxera resistance levels, as the available rootstocks are only partially resistant (Schäller 1965). They develop tuberosities that they isolate by cork layers from the inner tissue of the roots (Niklowitz 1954), thereby reducing further damage. In contrast, the rather new rootstock cultivar 'Börner' (a *V. riparia* × *V. cinerea* hybrid) exhibits a typical hypersensitive response to phylloxera attack with local necrosis (El-Nady 2001; Van Heeswijck et al. 2003) leading to high level or absolute resistance. It is assumed that this trait was transmitted from its *V. cinerea* Arnold parent, and is expected to operate differently from the resistance determinants inherited from *V. riparia*.

Grapevine breeding takes decades to release an advanced cultivar. Molecular markers linked to absolute phylloxera resistance of the roots could greatly accelerate this process by selecting individuals at early stages of the evaluation. In a first step toward this aim, we constructed genetic framework maps analyzing a population of segregating plants from the cross of V3125 × 'Börner'.

Several genetic maps of grapevine have been published since 1995. The first maps were based on dominant RAPD and AFLP markers (Lodhi et al. 1995; Dalbó et al. 2000; Doligez et al. 2002; Grando et al. 2003; Fischer et al. 2004; Doucleff et al. 2004). A large number of highly length-polymorphic, often co-dominant SSR (simple sequence repeat) markers were then developed through international joint activity in the Vitis Microsatellite Consortium (VMC, managed through AGROGENE, Moissy Cramayel, France) and additional efforts (Merdinoglu et al. 2005; Di Gaspero et al. 2005). Recently established maps are based on SSR loci (Riaz et al. 2004; Doligez et al. 2006; Mandl et al. 2006; Lowe and Walker 2006; Di Gaspero et al. 2007; Welter et al. 2007; Troggio et al. 2007). They may be supplemented with single nucleotide polymorphisms (SNP) (Lamoureux et al. 2006; Riaz et al. 2006) or markers derived from EST analysis (e.g. Ablett et al. 2000; Moser et al. 2005; Peng et al. 2007) and genomic sequencing (Salmaso et al. 2008).

However, the published genetic maps are targeted to localize genetic determinants of traits like flower sex, fungal disease resistances, morphological traits, phenological characteristics or berry quality (e.g. Cabezas et al. 2006; Grando et al. 2004). Genetic maps developed from an interspecific rootstock cross of 'Ramsey' × 'Riparia Gloire' (Lowe and Walker 2006) and from *V. rupestris* × *V. arizonica/candicans* crosses (Riaz et al. 2006, 2008) aimed to localize other characteristics of rootstock cultivars such as *Xyllela fastidiosa* or *Xiphinema index* resistance (Xu et al. 2008). No study did yet address the mapping of grapevine phylloxera resistance. In addition, very few genetic data are available for *Vitis cinerea*, resistance donor and one of the predecessors of 'Börner'. This investigation presents the genetic maps derived from the wide interspecific cross of V3125 × 'Börner' based on 241 SSR markers to construct the female, male and integrated framework maps to localize genetic determinants of phylloxera resistance. This effort is the first step toward the development of marker-assisted breeding for high-level phylloxera resistance. It also promotes further investigation of the corresponding genomic region and the molecular mechanisms of the defense response encountered in 'Börner'.

Materials and methods

Mapping population and parental plants

The mapping population consisted of 188 progeny individuals obtained by the cross of V3125 (*V. vinifera* ssp. *vinifera* 'Schiava grossa' × 'Riesling') × 'Börner' (*V. riparia* Gm183 × *V. cinerea* Arnold) as pollen donor in 1998 and 2001. Absolute phylloxera resistance is thought to have been transmitted from *V. cinerea* Arnold as this genotype is known to possess the high-level resistance. The seedlings were planted in the fields of the Institute in 1999 and 2002. V3125 is a breeding line of the Institute for Grapevine Breeding Geilweilerhof that shows promising characteristics for wine quality, but is entirely susceptible to root-phylloxera and other pathogens. 'Börner' is a rootstock cultivar with complete root resistance to phylloxera released by the Institute of Viticulture, Grapevine Breeding and Wine Technology of the Geisenheim Research Centre in 1991.

Samples of *V. riparia* Gm183 and *V. cinerea* Arnold were obtained from the Geisenheim Research Centre. Leaf samples of breeding line V3125, 'Börner', 'Schiava grossa' and 'Riesling' were from the grapevine germplasm collection of the Institute for Grapevine Breeding Geilweilerhof.

DNA extraction

Young leaves (second and third insertion from the apices) of the test plants were collected in the beginning of the vegetation period and stored after shock-freezing with liquid nitrogen at −70°C. The material was grinded in the frozen state for DNA extraction. DNA was isolated using the Qiagen DNeasy plant mini prep kit (Qiagen GmbH, Hilden, Germany).

Genotyping

The primer pairs flanking microsatellite loci from marker sets VVS (Thomas and Scott 1993), VVMD (Bowers et al. 1996, 1999), VrZAG (Sefc et al. 1999), SCUvv (Scott et al. 2000), VMC (Vitis Microsatellite Consortium, managed through AGROGENE, Moissy Cramayel, France), UDV (Di Gaspero et al. 2005) and VVI (Merdinoglu et al. 2005)

were screened for informative segregation. All forward primers were 5′-end labeled with fluorescent dyes (NED, HEX or FAM). Additional sequence-tagged microsatellite site (STMS) markers were developed for the "lower" part of linkage group 13, where the QTL region for phylloxera root resistance was located. From the grapevine genome sequence of PN40024 available at the Grape Genome Browser (http://www.genoscope.cns.fr/externe/GenomeBrowser/Vitis/) the sequences of scaffolds 45 (CU459262), 149 (CU459366), 152 (CU459369), 181 (CU459398) and 210 (CU459427) were screened for dinucleotide repeat motifs. For suitable loci flanking primer pairs were designed to produce marker sizes in the range of 100–500 bp (Table S1). The markers were tested on the parental genotypes and 13 randomly picked progeny plants of the population. Polymorphic markers were run over the entire mapping population. Selected markers were also analyzed in the predecessors of V3125 and 'Börner'.

Polymerase chain reactions (PCR) were performed in an ABI 9700 thermal cycler in 10-µl reaction mixtures containing 5 ng template DNA, 5 pmol of each primer, 0.2 mM of each dNTP, 1 µl 10× buffer (Invitek, Berlin, Germany), 0.3 U *Taq* DNA polymerase (Invitek, Berlin, Germany) and 1.5 or 2 mM $MgCl_2$. Amplification conditions were optimized for each primer pair using various annealing temperatures (50, 56 or 60°C) or gradient PCR. The amplification was achieved using 3-min initial denaturation at 94°C, 40 cycles of 1 min at 94°C, 1 min annealing at optimal temperature and 2 min synthesis at 72°C, followed by a final 7-min incubation at 72°C. PCR products were analyzed by capillary electrophoresis (ABI 3130 Genetic Analyzer; Applied Biosystems, Foster City, California, USA). Peaks were identified by size and height with GeneMapper 4.0 software. Any ambiguous genotype was re-run, re-amplified or left as unknown.

Genetic mapping

The double pseudo-testcross strategy (Grattapaglia and Sederoff 1994) was applied to construct the maps. Marker segregation was analyzed with regard to goodness-of-fit to the expected Mendelian ratio using the Chi-square test. Markers showing distorted segregation were included in the mapping computation as described in other mapping studies (e.g. Riaz et al. 2006; Welter et al. 2007; Salmaso et al. 2008). The genotypic data were subjected to linkage and recombination analysis with JoinMap®4.0 software (van Ooijen 2006) applying the Kosambi function to estimate genetic map distances (Kosambi 1944). LOD (logarithm of the odds) score thresholds equal or greater than five were used to determine linkage groups (LGs) with the exception of LG 16 of the consensus that was created at LOD 3 to fuse two groups split in the 'Börner' map. The maximal recombination fraction permitted was 0.4. Fully informative markers with four or three segregating alleles (ab × cd, ef × eg), double heterozygous markers (hk × hk) and markers segregating only from the maternal genotype (lm × ll) were used to construct the female map; fully informative markers, double heterozygous markers and markers segregating only from the parental genotype (nn × np) were used to build the male map. All marker sets together yielded the integrated map. The resulting linkage groups were numbered according to acknowledged references (Doligez et al. 2006; Di Gaspero et al. 2007).

Estimation of genomic length and map coverage

The genome length estimation was determined by a method of moments estimator (Hulbert et al. 1988). The observed genome length was based on the sum length of all linkage groups for each linkage map. Observed genome map coverage rate was calculated as described by Lowe and Walker (2006).

Phenotypic evaluation of phylloxera resistance

The mapping population was scored for phylloxera resistance in the years 2006 and 2007. The individual plants were vegetatively propagated. Wooden cuttings were rooted in triplicate and propagated in fine Fruhstorfer soil (FE Pikiererde, Flormaris, Wangerland, Germany) in 12-cm pots in the greenhouse. At the beginning of the vegetation period (middle of may), phylloxera leaf galls containing mature, yellowish eggs raised on leaf-susceptible grapevine cultivars (e.g. rootstock cultivars SO4 or 125 AA) in the greenhouse were used to inoculate the test plants by depositing 8–12 large galls (~3 mm in diameter) 3 cm deep in the soil of the potted test plants with the gall openings directed toward the root system. This inoculation was repeated after 3–4 weeks. Three to four weeks after the second experimental inoculation the plants were taken out from their pots, the roots carefully cleaned from attaching soil and the nodosities counted per root system. In 2006, 98 individual plants were triplicated and tested, while 163 individuals could be analyzed this way in 2007.

QTL analysis

The maximum numbers of nodosities observed on the root systems of each of the triplicated test plants grown and infected in 2006 and in 2007 were used directly as the quantitative score of susceptibility resp. resistance. The data were computed separately for both years based on the integrated map, the 'Börner' and V3125 maps and the individual genotypic profiles with MapQTL®5 (Van Oijen 2004). In addition, log-transformed values of the maximal

nodosity numbers were used to scan the integrated map for segregation of QTLs. To explore the biostatistical variation, a scan was also done omitting the samples with very low nodosity numbers (less than ten nodosities maximally observed or log < 1).

First, Kruskal–Wallis analysis and interval mapping (IM) over the linkage groups in 1-cM intervals was performed to identify trait-linked markers and QTL areas. Markers appearing in linkage or closely flanking the trait were then selected as cofactors. Several rounds with alternative cofactor selections were run. Cofactors were verified with the "Automatic cofactor selection" tool of MapQTL®5. Restricted MQM and full MQM QTL mapping computations were then performed. The statistic threshold of the LOD score significance at $P = 0.05$ (5%) for each linkage group was determined by at least 1,000 permutations.

Results

Analysis of SSR and STMS markers

Four-hundred microsatellite (SSR) and ten sequence-tagged microsatellite site (STMS) flanking primer pairs were at first tested in a small subset of 13 randomly chosen progeny plants and the two parental types of the population. Out of these, 167 SSR markers were found informative and analyzed over the complete segregating population. The others were discarded due to production of monomorphic bands, too complex multiple banding patterns or failing amplification. A preliminary genetic mapping and QTL analysis (see below) hinted at the location of quantitative factors for phylloxera root resistance located on LG 13. This LG was therefore specifically targeted by six more STMS markers developed from the grapevine genome sequence. Altogether, 104 primer pairs yielded full co-dominant information exhibiting four alleles (ab × cd), and ten more segregated with three alleles (ef × eg). Eight primer pairs produced bands segregating from both parents with the same size scored as double heterozygous markers following the pattern hk × hk. In some cases SSR amplification yielded one to a few products segregating only from one parent, either because the marker was homozygous (resulting in a monomorphic product) or missing in the alternative parent. 54 markers segregated only from the maternal genotype (lm × ll), and 69 markers segregated exclusively from the paternal genotype (nn × np). In these cases, allelism cannot be deduced from the marker data. The double heterozygous, only maternally and solely paternally segregating markers were therefore recorded as individual bands like dominant markers, increasing the total number of markers to more than the number of microsatellite loci. Altogether, this analysis yielded 245 informative markers. Double heterozygous dominant markers and fully informative codominant markers segregated in a 3:1 resp. 1:1:1:1 ratio, and were placed both on the male and the female parental linkage maps.

The map of V3125

115 fully informative, eight double heterozygous and 58 maternal markers (181 markers in total) were used to construct the *V. vinifera* map. Linkage and recombination analysis assembled 174 markers into 19 groups. These 19 LGs cover a total of 1,116.11 cM. Their size varies from 14.9 to 95.3 cM with an average length of 58.74 cM. The mean distance between markers is 6.41 cM. The largest gap of 29 cM is found on LG 19 (Table 1; Fig. 1). The estimated genome length is 1,108.42 cM and the observed genome coverage accounts for 100%.

Comparing the V3125 map with the reference linkage map (Doligez et al. 2006), allowed to assign all linkage groups of V3125 by shared markers. In general, the markers kept the same order except for some inversions between closely linked markers on LGs 3 (VMC3f3 and VVMD36), 8 (VMC2f12 and VMC1f10)), 10 (VMC8d3 and UDV59a and VrZAG67 and VMCNGRegVR1) and 12 (VMC1g3.2 and UDV24). UDV88 produced amplificates that mapped to three different linkage groups (LGs 3, 13 and 15), while it was localized only to LG 13 in the reference map. No other potential locus translocation between linkage groups was evident.

The 'Börner' map

The 115 fully informative, eight double heterozygous and 76 paternally segregating markers (199 in total) were used to construct the male map. Linkage and recombination analysis placed 190 markers into 20 linkage groups and a doublet. These 21 linkage groups cover a total of 1,070.18 cM. The average distance between markers is 5.63 cM. The largest gap spans 36.31 cM on linkage group 15 (Table 1; Fig. 1). The estimated genome length was 1,397.56 cM and the genome coverage reached 76.5%.

'Börner' is a hybrid of *V. riparia* and *V. cinerea*. In comparison to the maps published for *V. riparia* (Lowe and Walker 2006; Grando et al. 2003) the map elaborated here appears better covered with SSR markers. Possible locus shifts are hard to detect due to the rather low number of markers in common in both maps. In comparison to the reference linkage map of *V. vinifera* (Doligez et al. 2006) most of the SSR markers are found in the same order except for small inversions already identified in the female map. Furthermore, six new STMS markers targeted to LG 13 (where a QTL had been preliminarily mapped in 'Börner',

Table 1 Markers numbers and lengths of the linkage groups of V3125, 'Börner' and the resulting consensus map giving average marker distances and estimated genome coverage

LGs[a]	Consensus map			Map of female parent V3125			Map of male parent 'Börner'		
	Covered length (cM)	No. of markers	Average distance (cM)	Covered length (cM)	No. of markers	Average distance (cM)	Covered length (cM)	No. of markers	Average distance (cM)
1	63.87	12	5.32	65.46	8	8.18	65.80	12	5.48
2	32.91	7	4.70	14.94	3	4.98	32.43	7	4.63
3	54.24	15	3.62	54.52	14	3.89	46.32	11	4.21
4	67.85	11	6.17	71.85	9	7.98	72.45	10	7.25
5	51.81	13	3.99	45.41	11	4.13	49.83	9	5.54
6	38.54	5	7.71	40.67	5	8.13	26.03	3	8.68
7	68.25	17	4.01	73.68	12	6.14	65.83	12	5.49
8	92.51	15	6.17	93.95	11	8.54	89.70	10	8.97
8 associated	4.33	2	2.17	–	–		4.21	2	2.11
9	57.49	7	8.21	58.38	7	8.34	38.89	5	7.78
10	33.47	7	4.78	33.23	7	4.75	33.09	7	4.73
11	42.90	9	4.77	41.88	6	6.98	40.70	7	5.81
12	52.33	8	6.54	52.24	8	6.53	52.80	7	7.54
13	77.83	37	2.10	75.95	19	4.00	63.84	27	2.36
14	78.37	17	4.61	78.66	12	6.56	66.33	13	5.10
15	38.77	5	7.75	40.55	5	8.11	61.80	5	12.36
16	74.39	9	8.27	57.51	4	14.38	42.47	4	10.62
							18.59	4	4.65
17	33.25	11	3.02	32.04	6	5.34	33.10	11	3.01
18	90.75	19	4.78	89.92	13	6.92	100.44	15	6.70
19	102.11	15	6.81	95.27	14	6.81	65.56	9	7.28
$O(G)$[b]	1,155.97	241	4.80	1,116.11	174	6.41	1,070.18	190	5.63
$E(G)$[c]	1,209.36			1,108.42			1,397.56		
C_o^d (%)	95.59			100.69			76.57		

$E(G) = M(M-1)X/K$ where M is the number of markers; X is the maximum observed map distance among the locus pairs above a threshold LOD Z; K is the number of locus pairs having LOD values at or above Z. The value used for Z in this table was 6

[a] Linkage group numbering according to the reference map (Doligez et al. 2006)

[b] $O(G)$, observed genome length, based on the sum length of all linkage groups for each linkage map

[c] $E(G)$, estimated genome length in cM, calculated by the moment estimator (Hulbert et al. 1988)

[d] C_o, observed genome map coverage, determined as the percentage of $O(G)/E(G)$ (Lowe and Walker 2006)

see below) are found to be co-linear to the physical map of *V. vinifera* genotype PN40024 from which they were derived. This result indicates overall synteny between the genome of *V. riparia* and *V. cinerea* wild species genotypes and *V. vinifera* at the level of genetic maps and encouraged the construction of an integrated map.

The integrated map

Using all the marker sets together, a total of 241 markers were aligned into 19 linkage groups and a doublet derived from 'Börner', covering 1,155.97 cM with an average distance between markers of 4.80 cM. LG 16 was constructed at LOD 3 fusing two corresponding linkage groups of the 'Börner' map. All other linkage groups were defined at a minimum linkage of LOD 5. The largest LG contains 37 markers and covers 77.83 cM, with 2.10 cM average distance between them. The smallest group is a doublet with two markers in 4.33 cM distance.

The 19 linkage groups were easily aligned to the IGGP (International Grapevine Genome Program; http://www.vitaceae.org) reference map (Doligez et al. 2006) for numbering and marker order comparison. The small marker doublet contains accessory markers of linkage group 8 (Table 1; Fig. 1). The largest gap of 28.9 cM is found on linkage group 19. The estimated genome length is 1,209.36 cM, and the observed genome map coverage reached 95.6%.

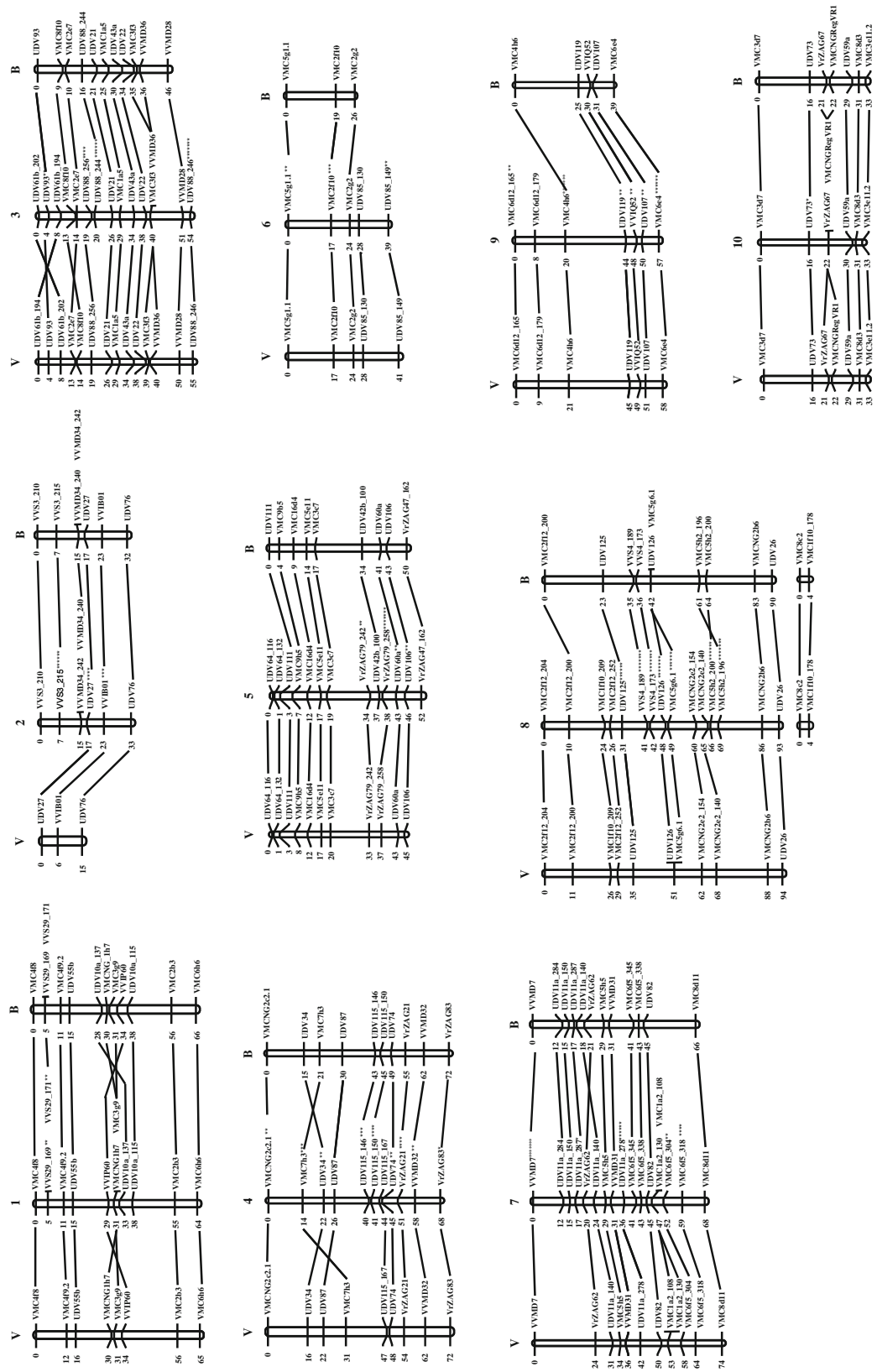

Fig. 1 Linkage maps of V3215 ('Schiava grossa' × 'Riesling') × 'Börner' (*V. riparia* 183 GM × *V. cinerea* Arnold). For each linkage group, the V3125 map is shown on the *left*, the Börner map is on the *right* and the consensus map is presented in the *middle*. Markers with skewed segregation are indicated by *asterisks* according to their level of distortion with (*$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.005$, ****$P \leq 0.001$)

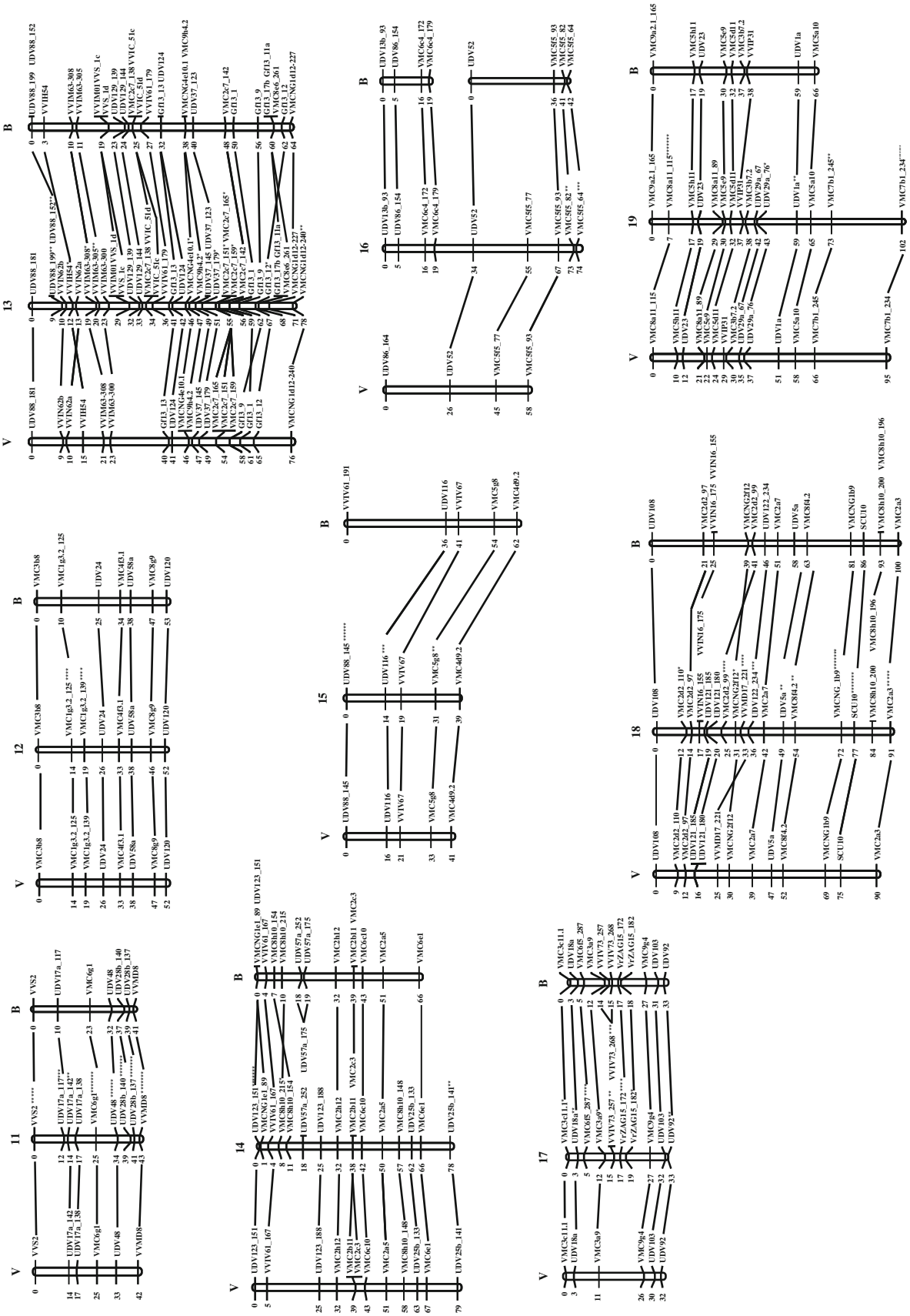

Fig. 1 continued

Distorted segregation

Distortion of marker segregation is a common phenomenon in genetic mapping, and may be caused by disturbed meiotic recombination and gametic or zygotic selection, especially in crosses involving a hybrid parent carrying homologous chromosomes from two different species such as 'Börner'. A Chi-square test indicated unbalanced segregation for several markers. Out of the mapped 241 markers, 81 (33.6%) showed significant segregation distortion at $\alpha \geq 0.05$. 38 biased markers (codominant or double heterozygous dominant) segregated from both parental types, 19 markers from the female parent and 24 markers from the male parent. Distorted markers were included in the map if they did not disturb flanking marker order (as checked in control map calculations omitting the distorted markers). They were found spread around the genome in 18 out of 19 linkage groups. In many cases, there was unbalanced repartition predominantly between the two alleles segregating from 'Börner' (LGs 1, 2, 4, 6, 8, 11, 15, 16 and 17). In contrast, LGs 5, 7, 12 and 19 exhibited biased segregation of the female alleles. The distorted markers on LGs 3, 9, 13, 14 and 18 showed no preferential allelic misbalance from either parental type. Markers with segregation distortion are indicated in the integrated map in Fig. 1.

Phenotypic scoring of phylloxera root resistance

The phenotypic scores of phylloxera root resistance were obtained by experimental inoculations of triplicated test plants with phylloxera leaf galls containing mature eggs. The resulting nodosities were counted per rootstock. These nodosity numbers varied considerably within the three replicates of each individual plant tested in both years of experimentation. As this experimental system used insect egg inoculation, physiological variations in larval development, their successful finding and attachment to the rootlets and establishment of the galls can hardly be avoided. Low nodosity numbers therefore may just result from less successful inoculations and not really represent the level of resistance of the host plant. To minimize this kind of effects the maximal number of nodosities scored per individual plant was considered as the most reliable indicator of the quantitative resistance phenotype. Data were obtained in the year 2006 on a subset of 98 F1 plants (due to different levels of wood maturity not all individuals could yet be vegetatively propagated in 2006) and on almost the complete population of 163 individuals in 2007. The segregation of the phenotype of maximal nodosity scores is shown in Fig. 2.

QTL analysis

Preliminary QTL analysis based on the 'Börner' map and the integrated framework map had indicated one QTL on

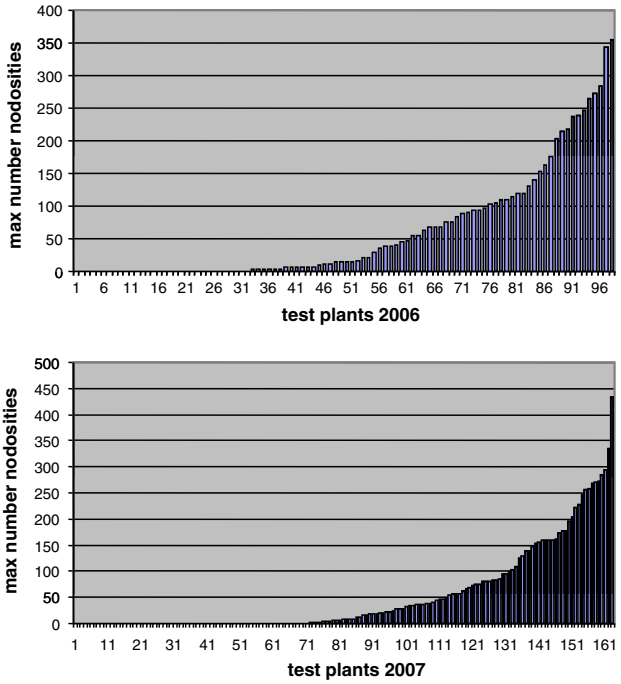

Fig. 2 Segregation of the scores obtained for the maximal number of nodosities on triplicated test plants in 2006 (*upper graph*) and 2007 (*lower graph*)

LG 13. This region of interest was therefore targeted with new microsatellite-type STMS markers deduced from the *Vitis* genome sequence and indicated by the prefix "Gf" in the map. The computation of QTL analysis based on the supplemented map confirmed a single significant QTL on LG 13 of the 'Börner' map. Based on the phenotypic data of 98 test plants from the year 2006 this QTL reached a LOD score of 10.68 (LG 13 specific threshold was LOD 3.1, $P \leq 0.05$) at position 52.3 cM of 'Börner' LG 13, and explained 42.9% of the phenotypic variance observed that year. In the integrated map the same QTL had a maximum of LOD 12.04 (significance threshold LOD 4.5) at position 59 cM and explained 43.8% of variance. When log-transformed values of the maximal nodosity numbers were used to scan the integrated map, again only one QTL was found almost in the same position of 60.1 cM on LG 13. It reached a maximal LOD value of 24.98 (clearly surpassing the LG-specific significance threshold of LOD = 8.7), and explained 72% of the experimental variance computed this way. The resistance phenotype was linked to alleles d of the flanking markers Gf13_1 and Gf13_9 originating from *V. cinerea*. Omitting the samples with small nodosity counts (<10) from the analysis did not considerably change this result.

The results using 163 test plants in 2007 showed the QTL in the same region with a LOD score of 10.8 (threshold 2.7) at its maximum at position 54.3 cM of LG 13 in the 'Börner' map. It explains 29% of the variance observed in

Table 2 Summary of QTL characteristics identified on LG 13

Year of phenotypic data	LG 13 map	LOD_{max} after MQM analysis	Significance threshold ($\alpha = 0.05$)	LOD_{max} position (cM)	Variance explained (%)	Cofactor	Confidence interval ($LOD_{max} - 2$) (cM)
2006	'Börner'	10.68	3.1	52.3	42.9	Gf13_9	50.2–59.7
2006	Consensus	12.04	4.5	59	43.8	Gf13_1	57–62.2
2006 (log trans.)	Consensus	24.98	8.7	60.1	72.5	Gf13_1	59–62.2
2007	'Börner'	10.8	2.7	54.3	29	Gf13_9	50.3–59.7
2007	Consensus	11.34	4.3	61.15	29.5	Gf13_1	57–67
2007 (log trans.)	Consensus	22.6	16.6	60.1	50.9	Gf13_1	56–62.2

2007. In the integrated map, the maximal LOD score of 11.34 was reached at position 61.15 cM of LG 13 (significance threshold LOD 4.3) explaining 29.5% of variance. Analysis of the integrated map based on log-transformed values of maximal nodosity counts showed the very same and single QTL on LG13 with a LOD maximum of 22.6 (significance threshold 16.6) at position 60.1 cM, explaining 51% of the observed variance computed in log transformation. Again, omitting very small numbers of nodosities ($n < 10$ or $\log < 1$) from the analysis did not significantly change this result. In the Börner LG 13 map, the best cofactor was Gf13_9 in both years, while the QTL appeared best represented by cofactor Gf13_1 in the integrated map. These two markers are direct neighbors in both maps, separated by 3 cM in the integrated resp., 6 cM in the male map. Further details of the QTL mapping results are summarized in Table 2. One example is graphically represented in Fig. 3. In any case, the phenotypic trait of very low nodosity numbers was clearly associated with one of the 'Börner' alleles (labeled "d") of markers Gf13_1 (allele size 211 bp) or Gf13_9 (allele size 336 bp) originating from *V. cinerea*. No other significant QTL were found in the 'Börner', the V3125—or the integrated parental map.

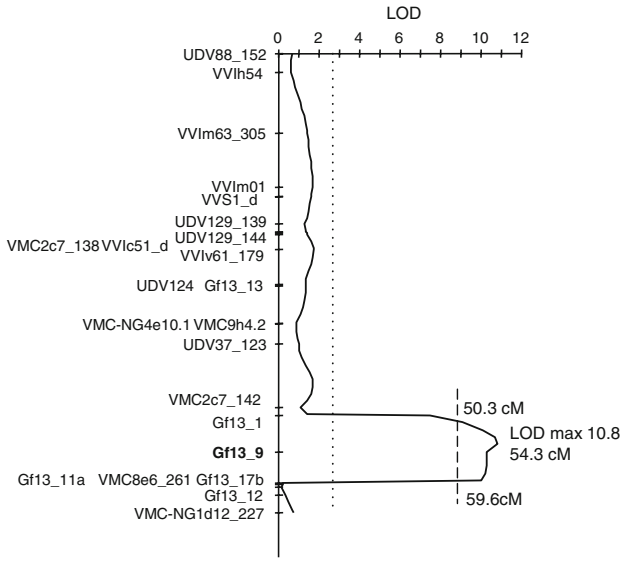

Fig. 3 QTL for phylloxera root resistance on LG 13 of the 'Börner' map obtained with phenotypic data from the year 2007 on 163 individuals. The peak reached a LOD maximum of 10.8 and explained 29% of the variance observed. The QTL region as defined by MQM mapping stretches over a confidence interval of $LOD_{max} - 2$ from 50.3 to 59.6 cM (*dashed line*). The *dotted line* represents the linkage group-specific statistical threshold value (see text). The cofactor confirmed by MapQTL®5 and used in MQM analysis was Gf13_9, indicated in *bold*

Grapevine genome sequence information at the QTL

In the end of the year 2007 two grapevine genome sequences became public in their first high quality drafts. These are derived from a largely homozygous 'Pinot noir' derivative called PN40024 analyzed in the frame of a French-Italian consortium (Jaillon et al. 2007) and a heterozygous 'Pinot noir' clone ENTAV 115 investigated in Italy (Velasco et al. 2007). Alignments of the marker sequences flanking the phylloxera root resistance QTL on LG 13 of 'Börner' were performed using the BlastN algorithm (Altschul et al. 1997) at NCBI (http://blast.ncbi.nlm.nih.gov) or the French genome browser (http://www.genoscope.cns.fr). These alignments confirmed the positioning of the markers to the "lower" region of LG 13 (Fig. 1). The sequence of VMC_NG1d12 matched to scaffold 149 of PN40024 resp. contig VV78X021204.3 of 'Pinot noir' with the better score of 593 bits and an *E* value of $3e^{-168}$ for the similarity to VV78X021204.3. However, alternative alignments of partial stretches (up to five matches with *E* values less than e^{-10}) could be identified on the same genomic contig. This indicates a repetitive structure in the QTL region that also explains the genetic pattern of multiple bands obtained from this marker sequence. Similar results were obtained by aligning locus VMC2c7 that showed high similarity to contig VV78X224390.3 from 'Pinot noir' (414 bits, $E = 7e^{-113}$) over 239 bp and also two possible matches of shorter stretches to contig VV78X192244.3. In contrast, the marker sequence of VMC8e6 (producing a marker segregating only from 'Börner' in this study) matched in one

clear alignment of 559 bits and an *E* value of e^{-157} over 282 identical base pairs to scaffold 45 of chromosome 13 in the PN40024 assembly.

The grapevine genome sequences were exploited to develop markers specifically targeted to the QTL region. The sequence of the newly developed marker Gf13_1 corresponds to a single site in scaffold 152 of PN40024 (microsatellite at position 890320) and contig VV78X108101.17 of 'Pinot noir'. The unique locus Gf13_9 is located on scaffold 45 (compound repeat at position 1473370) of PN40024 and contig VV78X117761.12 from 'Pinot noir'. The physical distance between the QTL flanking markers Gf13_1 and Gf13_9 adds up to about 1.5 Mb according to the PN40024 assembly.

It is known that the lower part of chromosome 13 of 'Pinot noir' contains 54 disease resistance gene analogs mainly of the NBS-LRR type (Velasco et al. 2007), making it likely that also 'Börner' resp. *V. cinerea* contains such resistance genes in this genomic location. One or several closely linked candidates may be functional in phylloxera root resistance.

Discussion

Genetic map synteny

The framework maps established here for QTL analysis are based on microsatellite-derived markers mapped in other grapevine crosses. Comparison of the marker order of the interspecific 'Börner' and its integrated map with *V. vinifera* V3125 to the reference map (from five different *Vitis vinifera* crosses; Doligez et al. 2006) or to maps from two crosses involving resistance donors (Di Gaspero et al. 2007) shows overall the same marker order. This finding indicates a high level of synteny maintained across *Vitis* species. Consequently, new STMS markers were developed after a first search for QTL indicating the location of resistance factors on chromosome 13. These targeted STMS markers were based on the *V. vinifera* genome sequences (Jaillon et al. 2007; Velasco et al. 2007). In testing them on the cross population six out of ten markers were informative. Five mapped in co-linear order in the hybrid 'Börner'. The sixth marker, Gf13_13, derived from "random" (not yet arranged in order within LG 13) scaffold 181 mapped nearly in the center of linkage group 13. Thus, scaffold 181 can now be positioned between scaffolds 74 (VVIc51) and 120 (VMC_NG4e10.1) in the physical map of PN40024 (http://www.genoscope.cns.fr/externe/GenomeBrowser/Vitis/).

The two STMS markers Gf13_1 and Gf13_9 are flanking the QTL for phylloxera root resistance. They delimit a region of 6 cM in the 'Börner' map, resp. 3 cM in the integrated map or 1.5 Mb in the PN40024 model genome sequence. This results in a resolution of 0.25–0.5 Mb per cM observed in this genomic region.

The development of targeted markers strongly reduced the interesting interval size of 12 cM between VMC2c7 and VMC8e6 in the preliminary map. This reduction facilitates further physical mapping of the region of interest, and allows to start molecular analyses of this resistance QTL. Development of even more closely linked markers labeling phylloxera root resistance and the understanding of the responsible genes at this QTL through detailed molecular analysis should be accomplishable.

Segregation distortion

The genotyping in the V3125 × 'Börner' population indicated a high number of markers significantly deviating from the expected segregation ratios. The level of this segregation distortion considering the integrated map is 33.6% at $\alpha \geq 0.05$, a value higher than previously observed in *V. vinifera* and *Vitis* hybrid crosses (19–20%, Salmaso et al. 2008; 11.6%, Troggio et al. 2007, 3–13%, Di Gaspero et al. 2007; 7–11%, Doligez et al. 2006) or in a wide cross of *V. rupestris* × *V. arizonica/candicans* (17%, Riaz et al. 2006, 2008). The predominant part of distorted markers was transmitted from 'Börner', less distorted markers were segregating from V3125. These effects may be caused by difficulties in the meiotic synapsis and recombination between the homologous chromosomes of 'Börner' derived from the two wild species *V. riparia* and *V. cinerea* in comparison to the *V. vinifera* line V3125. Even if there is overall synteny in the genetic maps, the homology may be locally disturbed across some chromosomal regions inhibiting inter- and intrachromosomal recombination leading to gametic selection. Effects of zygotic selection may be operating as well as found in other crosses engaging a cultivar with a complex pedigree (Welter et al. 2007).

The highly distorted markers of 'Börner' and V3125 were found dispersed around the genome on 18 out of 19 linkage groups, with accumulation in LGs 4, 6, 9, 11, 15, 17 and 18. Riaz et al. (2006) described the presence of pronounced segregation bias regions on LGs 6, 7 and 14 in their wide cross. Major distortion in LGs 7 and 14 was not evident here. Most other studies observe rather even spreading of the distorted markers around the genome. However, clustering of skewed markers was observed in LGs 9 and 18 in a 'Syrah' × 'Pinot noir' mapping study (Troggio et al. 2007), two groups also identified in the V3125 × 'Börner' cross to harbor clusters of distorted loci. It is yet unclear which mechanisms of marker distortion are operating in these cases. The future annotation of the whole grapevine genome sequences and the phenotypic effects of the genes in those regions may help to understand these phenomena in the near future.

Marker UDV88 (GenBank Accession BV097062) produced several amplificates, some of which exhibited strongly distorted segregation patterns and mapped to LGs 3 and 15, locations not yet reported for this marker locus. In contrast, the genetic position found for its less-distorted products on LG 13 is in agreement with the reference map and confirmed in the genome sequence.

The distal positions of UDV88 amplificates found in LGs 3 and 15 therefore may be false. However, a search of the grapevine genome sequence of PN40024 indicated the presence of sequence stretches complementary to the primers flanking this locus on several scaffolds, including one assigned to chromosome 15 (scaffold 37, score 34 bits, *E* value 0.074 for primer A). Clear amplification of this locus may therefore be hampered by partial redundance of the flanking sequences in the genome.

The intention of this work was to construct framework maps suited for first QTL analysis. The interesting QTL region was then targeted by increased marker coverage for detailed QTL studies. This approach is efficient, time- and cost-saving and can exploit available genome sequence data.

The rootstock 'Börner' as genetic resource

This paper presents the first map for 'Börner' and the first genetic mapping of a QTL for phylloxera resistance. A recent study on biometric traits related to phylloxera susceptibility from *V. vinifera* × *V. rupestris* hybrid AXR1 suggested two genes involved in nodosity formation, but the picture for the genetic determinants of tuberosities is not yet clear (Roush et al. 2007). This cultivar failed as phylloxera resistant rootstock. In contrast, the 'Börner' rootstock is an interspecific hybrid from the partially phylloxera root resistant *Vitis riparia* and the completely phylloxera root resistant *Vitis cinerea* Arnold. It has demonstrated high-level root resistance against all strains of phylloxera available for testing (Schmid and Rühl 2003; Schmid et al. 2003). In addition, it possesses good grafting affinity and adaptation to a wide range of soil types. Hence, 'Börner' is a highly attractive genetic resource for further rootstock breeding. Labeling its resistance determinants by molecular markers was the main objective of this investigation. This was achieved for a major QTL.

The localization of a single reproducible QTL identified markers that should be useful tools for rootstock resistance breeding. It was found on LG 13 clearly linked to alleles of markers Gf13_1 and Gf13_9 originating from *V. cinerea* Arnold. This QTL explained around 43% of the variance of nodosity counts as such and 72% of the variance after log transformation of the data in 2006 based on 98 F1 plants. In 2007, more F1 plants were available for phenotyping. Using 163 plants, the QTL on LG 13 explained around 29% of the variance of nodosity numbers and 51% of the variance after log transformation of the data. Variability of the strength of QTL effects over several seasons is not unusual and observed also for other traits (e.g. Cabezas et al. 2006). In the case of the phylloxera resistance phenotyping it may have been influenced also by a variability of the vigor of the insect population raised for the experimental inoculation. Such variation could have been caused by environmental influences affecting the physiology of the host/pathogen interaction during the propagation of phylloxera even if the galls employed for the experimental inoculations had been raised on susceptible host plants under standard conditions in the greenhouse.

This study is the first to provide markers for marker-assisted selection of complete phylloxera root resistance. However, there may be additional QTLs not segregating in this cross, and hence going undetected in this study. Alternative crosses with 'Börner' are necessary to conclude about this point. Also, the newly identified trait-linked markers need to be rigorously tested in a range of further 'Börner' crosses. Furthermore, the map now available can be used to detect genetic factors governing other traits segregating in this cross. Such analyses are under way.

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References

Ablett E, Seaton G, Scott K, Shelton D, Graham MW, Baverstock P, Lee SL, Henry R (2000) Analysis of grape ESTs: global gene expression patterns in leaf and berry. Plant Sci 159:87–95

Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 25:3389–3402

Bowers JE, Dangl GS, Vignani R, Meredith C (1996) Isolation and characterization of new polymorphic simple sequence repeat loci in grape (*Vitis vinifera* L.). Genome 39:628–633

Bowers JE, Dangl GS, Meredith C (1999) Development and characterization of additional microsatellite DNA markers for grape. Am J Enol Vitic 50:243–246

Cabezas JA, Cervera MT, Ruiz-Garcia L, Carreno J, Martinez-Zapater JM (2006) A genetic analysis of seed and berry weight in grapevine. Genome 49:1572–1585

Dalbó MA, Ye GN, Weeden NF, Steinkellner H, Sefc KM, Reisch BI (2000) A gene controlling sex in grapevine placed on a molecular marker-based genetic map. Genome 43:333–340

Di Gaspero G, Cipriani G, Marrazzo MT, Andretta 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. Mol Breed 15:11–20

Di Gaspero G, Cipriani G, Adam-Blondon A-F, Testolin R (2007) Linkage maps of grapevine displaying the chromosomal locations

of 420 microsatellite markers and 82 markers for R-gene candidates. Theor Appl Genet 114:1249–1263

Doligez A, Bouquet A, Danglot Y, Lahogue F, Riaz S, Meredith CP, Edwards KJ, This P (2002) Genetic mapping of grapevine (*Vitis vinifera* L.) applied to the detection of QTLs for seedlessness and berry weight. Theor Appl Genet 105:780–795

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 mapping 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

El-Nady MF (2001) Untersuchungen zum Mechanismus der Reblausresistenz der Unterlagsrebsorte 'Börner'. Dissertation, Johannes Gutenberg-Universität Mainz

Fischer B, Salakhutdinov I, Akkurt M, Eibach R, Edwards KJ, Toepfer R, Zyprian EM (2004) Quantitative trait locus analysis of fungal disease resistance factor on a molecular map of grapevine. Theor Appl Genet 108:505–515

Grando MS, Bellin D, Edwards KJ, Pozzi C, Stefanini M, Velasco R (2003) Molecular linkage maps of *Vitis vinifera* L. and *Vitis riparia* Mchx. Theor Appl Genet 106:1213–1224

Grando MS, Sevini F, Moser S, Marino R, Dalla Serra A, Versini G (2004) Genetic mapping of aroma compounds in grape. Bull de l'OIV 2004:881–882

Grattapaglia D, Sederoff R (1994) Genetic linkage maps of *Eucalyptus grandis* and *Eucalytus urophylla* using a pseudo-testcross mapping strategy and RAPD markers. Genetics 137:1121–1137

Hulbert SH, Ilott TW, Legg EJ, Lincoln SE, Lander ES, Michelmore RW (1988) Genetic analysis of the fungus *Bremia lactucae*, using restriction fragment length polymorphisms. Genetics 120:947–958

Jaillon O, Aury J-M, 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, DelFabbro C, Alaux M, DiGaspero G, Dumas V, Felice N, Paillard S, Juman I, Moroldo M, Scalabrin S, Canaguier A, LeClainche I, Malacrida G, Durand E, Pesole G, Laucou V, Chatelet P, Merdinoglu D, Delledonne M, Pezotti M, Lecharny A, Scarpelli C, Artiguenave F, Pe ME, Valle G, Morgante M, Caboche M, Adam-Blondon A-F, Weissenbach J, Quetier F, Wincker O (2007) The grapevine genome sequence suggests ancestral hexaploidization in major angiosperm phyla. Nature 449:463–468

Kellow AV, Sedgley M, Van Heeswijck R (2004) Interaction between *Vitis vinifera* and grape phylloxera: changes in root tissue during nodosity formation. Ann Bot 93:581–590

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

Lamoureux D, Bernole A, LeClainche I, Tual S, Thareau V, Paillard S, Legeai F, Dossat C, Wincker P, Oswald M, Merdinoglu D, Vignault C, Delrot S, Caboche M, Chalhoub B, Adam-Blondon A-F (2006) Anchoring of a large set of markers onto a BAC library for the development of a draft physical map of the grapevine genome. Theor Appl Genet 113:344–356

Lodhi MA, Daly MJ, Ye GN, Weeden NF, Reisch BI (1995) A molecular marker based linkage map of *Vitis*. Genome 38:786–794

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

Mandl K, Santiago J-L, Hack R, Fardossi A, Regner F (2006) A genetic map of Welschriesling × Sirius for the identification of magnesium-deficiency by QTL analysis. Euphytica 149:133–144

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

Moser C, Segala C, Fontana P, Salakhutdinov I, Gatto P, Pindo M, Zyprian E, Toepfer R, Grando MS, Velasco R (2005) Comparative analysis of expressed sequence tags from different organs of *Vitis vinifera* L. Funct Integr Genomics 5:208–217

Niklowitz X (1954) Histologische Studien an Reblausgallen und Reblausabwehrnekrosen (*Viteus vulpinae* CB. auf *Vitis vinifera* und *Vitis riparia*). Phytopathologische Z 24:299–340

Peng FY, Reid K, Liao N, Schlosser J, Lijavetzky D, Holt R, Martinez Zapater JM, Jones S, Marra M, Bohlmann J, Lund ST (2007) Generation of ESTs in *Vitis vinifera* wine grape (Cabernet Sauvignon) and table grape (Muscat Hamburg) and discovery of new candidate genes with potential roles in berry development. Gene 402:40–50

Riaz S, Dangl GS, Edwards KJ, Meredith CP (2004) A Microsatellite 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 the extended genetic map of *Vitis rupestris* × *V.* arizonica. Theor Appl Genet 113:1317–1329

Riaz S, Tenscher AC, Rubin J, Graziani R, Pao SS, Walker MA (2008) Fine-scale genetic mapping of two Pierce's disease resistance loci and a major segregation distortion region on chromosome 14 of grape. Theor Appl Genet 117:671–681

Roush TL, Granett J, Walker MA (2007) Inheritance of gall formation relative to phylloxera resistance levels in hybrid grapevines. Am J Enol Vitic 58:234–241

Salmaso M, Malacarne G, Troggio M, Faes G, Stefanini M, Grando MS, Velasco R (2008) A grapevine (*Vitis vinifera* L.) genetic map integrating the position of 139 expressed genes. Theor Appl Genet 116:1129–1143

Schäller G (1965) Ergebnisse der Rebenunterlagenzüchtung mit *Vitis cinerea* Arnold. Der Züchter 35:250–255

Schmid J, Rühl EH (2003) Performance of *Vitis cinerea* hybrids in motherblock and nursery—preliminary results. Acta Hort 617, ISHS 2003:141–145

Schmid J, Manty F, Rühl EH (2003) Experience with phylloxera tolerant and resistant rootstocks at different vineyard sites. Acta Hort 617, ISHS 2003:85–93

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, Glössl J, Steinkellner H (1999) Identification of microsatellite sequences in *Vitis riparia* and their applicability for genotyping of different *Vitis* species. Genome 42:367–373

Thomas MR, Scott N (1993) Microsatellite repeats in grapevine reveal DNA polymorphisms when analyzed as sequence-tagged sites (STSs). Theor Appl Genet 6:985–990

Troggio M, Malacarne G, Coppola G, Segala C, Cartwright DA, Pindo M, Stefanini M, Mank R, Moroldo M, Morgante M, Grando MS, Velasco R (2007) A dense single-nucleotide polymorphism based genetic linkage map of grapevine (*Vitis vinifera* L.) anchoring Pinot noir bacterial artificial chromosome contigs. Genetics 176:2637–2650

Van Heeswijck R, Bondar A, Croser L, Franks T, Kellow A, Powell K (2003) Molecular and cellular events during the interaction of phylloxera with grapevine roots. In: Rühl EH and Schmid J (eds) Proceedings of phylloxera infested vineyards. Acta Hort 617, ISHS 2003, pp 13–15

Van Oijen JW (2004) MapQTL®5, software for the mapping of quantitative trait loci in experimental populations. Kyazma B.V, Wageningen

Van Ooijen JW (2006) JoinMap®4.0, software for the calculation of genetic linkage maps in experimental populations. Kyazma B.V, Wageningen

Velasco R, Zharkikh A, Troggio M, Cartwright DA, Cestaro A, Pruss A, Pruss D, Pindo M, FitzGerald LM, Vezulli S, Reid J, Malacarne G, Iliev D, Coppola G, Wardell B, Micheletti D, Macalma T, Facci M, Mitchell JT, Perrazzolli M, Eldredge G, Gatto P, Oyzerski R, Moretto M, Gutin N, Stefanini M, Chen Y, Segala C, Davenport C, Dematte 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 SM, 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) A high quality draft consensus sequence of the genome of a heterozygous grapevine variety. PLoS One 2(12):e1326. doi:10.1371/journal.pone.0001326

Welter LJ, Göktürk-Baydar N, Akkurt M, Maul E, Eibach R, Töpfer R, Zyprian EM (2007) Genetic mapping and localization of quantitative trait loci affecting fungal disease resistance and leaf morphology in grapevine (*Vitis vinifera* L.). Mol Breed 20:359–374

Xu K, Riaz S, Roncoroni NC, Jin Y, Hu R, Zhou R, Walker MA (2008) Genetic and QTL analysis of resistance to Xiphinema index in a grapevine cross. Theor Appl Genet 116:305–311