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ORIGINAL ARTICLE

Quantitative trait loci affecting pathogen resistance and ripening of grapevines

Eva Zyprian[1] · Iris Ochßner[1] · Florian Schwander[1] · Silvio Šimon[1] · Ludger Hausmann[1] · Martina Bonow-Rex[1] · Paula Moreno-Sanz[2] · Maria Stella Grando[2] · Sabine Wiedemann-Merdinoglu[3] · Didier Merdinoglu[3] · Rudolf Eibach[1] · Reinhard Töpfer[1]

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Abstract Grapevines (*Vitis vinifera* L.) form the basis of viticulture, and are susceptible to diseases such as downy mildew (*Plasmopara viticola*) and powdery mildew (*Erysiphe necator*). Therefore, successful viticulture programs require the use of pesticides. Breeding for resistance is the only eco-friendly solution. Marker-assisted selection is currently widely used for grapevine breeding. Consequently, traits of interest must be tagged with molecular markers linked to quantitative trait loci (QTL). We herein present our findings regarding genetic mapping and QTL analysis of resistance to downy and powdery mildew diseases in the progenies of the GF.GA-47-42 ('Bacchus' × 'Seyval') × 'Villard blanc' cross. Simple sequence repeats and single nucleotide polymorphisms of 151 individuals were analyzed. A map consisting of 543 loci was screened for QTL analyses based on phenotypic variations observed in plants grown in the field or under controlled conditions. A major QTL for downy mildew resistance was detected on chromosome 18. For powdery mildew resistance, a QTL was identified on chromosome 15. This QTL was replaced by a novel QTL on chromosome 18 in 2003 (abnormally high temperatures) and 2004. Subsequently, both QTLs functioned together. Additionally, variations in the timing of the onset of veraison, which is a crucial step during grape ripening, were studied to identify genomic regions affecting this trait. A major QTL was detected on linkage group 16, which was supplemented by a minor QTL on linkage group 18. This study provides useful information regarding novel QTL-linked markers relevant for the breeding of disease-resistant grapevines adapted to current climatic conditions.

Keywords Grapevine genetic mapping · SSR and SNP markers · QTL analysis · Downy mildew resistance · Powdery mildew resistance · Veraison

Introduction

Grapevine plants (*Vitis* spp.) provide the basis of viticulture, which is a particular branch of agriculture with a long history. Grape production is part of the European economy and culture in suitable growing regions. The production of wine and table grapes relies on the European grapevine species *Vitis vinifera* ssp. *vinifera*. All cultivars undergo vegetative propagation to maintain their characteristics, including very old cultivars like 'Riesling', which has existed for centuries. Modern vineyards in Europe consist of monocultures, which are highly susceptible to pathogens accidentally introduced from North America during the nineteenth century. Important pathogens include *Erysiphe necator* Schwein. [syn. *Uncinula necator* (Schw.) Burr.; anamorph *Oidium tuckeri* Berk.], which is an ascomycetous fungus that causes powdery mildew disease, and

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✉ Eva Zyprian
eva.zyprian@jki.bund.de

[1] Julius Kuehn Institute, Federal Research Centre for Cultivated Plants, Institute for Grapevine Breeding Geilweilerhof, 76833 Siebeldingen, Germany

[2] Research and Innovation Centre, Fondazione Edmund Mach (FEM), Via E. Mach 1, 38010 San Michele all'Adige, Italy

[3] INRA Centre de Recherche de Colmar, UMR Santé de la Vigne et Qualité du Vin, 68021 Colmar Cedex, France

Plasmopara viticola (Berk. & Curt) Berl. & de Toni, which is an oomycetous pathogen responsible for downy mildew on leaves and fruits. Protection from mildew diseases requires extensive use of fungicides, and agrochemicals are used more extensively during grape production than in the growth of other agricultural crops (http://ec.europa.eu/agriculture/markets/wine/studies/vine_en.pdf). The excessive use of pesticides raises environmental concerns and does not fit the current trends in sustainable agricultural practices. Therefore, the generation of improved grapevine cultivars for the production of high quality wine grapes that are resistant to mildew diseases is a major aim of grapevine breeding programs.

Breeding exploits diversity generated from controlled crosses of selected parental lines like a *V. vinifera* cultivar and a generally interfertile *Vitis* sp. resistance donor. Resistance traits have been introgressed predominantly from accessions of American wild species that are naturally resistant because they co-evolved with pathogens. After a cross, progenies have to be evaluated over long periods under greenhouse conditions and in experimental fields to select and propagate the most promising genotypes. Recently, this tedious evaluation process has been replaced by new genetics-based strategies. Advanced breeding methods employ marker-assisted selection using trait-linked molecular markers to indicate the inheritance of a particular genomic region. In contrast to classical phenotypic analyses, this new selection process enables the identification of individuals with multiple loci for resistance traits.

Marker-assisted selection requires trait-linked genetic tags. Traits of interest are usually of a quantitative nature that can be linked to markers by quantitative trait locus (QTL) analysis. This method involves screening segregating molecular markers in genetic maps by linkage/recombination analysis as well as segregating phenotypic traits in a mapping population. Genetic mapping in grapevines involves the segregation of heterozygous markers in the F_1 population, and mainly relies on length-polymorphic microsatellite loci (e.g., simple sequence repeats; SSRs) (Zhang et al. 2009; Schwander et al. 2012; Fechter et al. 2014; Rex et al. 2014). The development of new SSR markers targeted to specific genomic regions has been facilitated by the sequencing of a grapevine reference genome in an inbred line (Jaillon et al. 2007) and a specific clone (Velasco et al. 2007) of 'Pinot noir' ('Late Burgundy'). Furthermore, because of genome sequencing and gene diversity studies, available information regarding single nucleotide polymorphisms (SNPs) is expanding. Markers for SNPs are attractive for marker-assisted selection because of their abundance in the largely heterozygous and highly diverse genomes of grapes (Myles et al. 2010) and their ease of analysis. However, some breeders still question the utility of SNP markers. To establish SNPs as practical tools for marker-assisted selection, they need to be included in genetic maps and QTL studies. In this study, we applied a combination of SSR and SNP markers to construct a genetic map from a cross population that segregates for multiple traits relevant to breeding. Our objective was to generate a robust framework map for QTL analysis, but also to investigate the value of SNP markers in increasing marker coverage and enhancing the power of QTL detection.

We characterized the progenies of a cross between the breeding line GF.GA-47-42 (maternal genotype; 'Bachus' × 'Seyval') with 'Villard blanc' (paternal genotype; Seibel 6468 × Seibel 6905; syn. 'Subereux'), which is a French hybrid. Their pedigrees are provided in Fig. 1. The parental lines exhibit differing levels of powdery and downy mildew resistance traits derived from an American *Vitis* sp. (Akkurt et al. 2007; Di Gaspero et al. 2012; Venuti et al. 2013). 'Villard blanc' plants are highly and moderately resistant to *P. viticola* and *E. necator*, respectively. In contrast, the breeding line GF.GA-47-42 exhibits moderate and high levels of resistance to *P. viticola* and *E. necator*, respectively. The generated map was used for QTL analysis of segregating resistance traits. This approach was chosen to identify new trait-linked markers or allelic variants of known QTLs to enable further molecular characterizations.

Powdery and downy mildew resistance traits have been studied globally. Several loci for mildew resistance traits from different origins have been characterized as QTLs in genetic mapping studies (http://www.vivc.de/docs/dataonbreeding/20130521_Table%20of%20Loci%20within%20VITIS.pdf). Breeders typically aim to combine several loci to reduce the possibility that pathogens will quickly overcome plant resistance through simple mutation events or by the emergence of pathogen variants adapted to particular host resistance factors (Delmotte et al. 2014). Optimizing loci combinations requires a thorough understanding of the genes encoded at resistance loci and the functional components of defense pathways. Plant responses to pathogens are mediated by complex regulatory pathways triggered by the perception of pathogens (e.g., through pathogen-secreted effectors) (Tena et al. 2011; Jiang and Tyler 2012). Ideally, several pathogen receptors and key compounds of defense reactions should be combined to obtain sustainable disease resistance. Both *E. necator* and *P. viticola* are obligate biotrophic pathogens that infect plant cells through the formation of haustoria. They invade epidermal cells from epiphytically growing *E. necator* mycelia or mesophyll cells from intercellularly propagating *P. viticola* hyphae (Gessler et al. 2011; Gadoury et al. 2012). Improving cellular defense mechanisms depends on analyses of resistance loci from various sources and their allelic variants, which requires the identification and molecular characterization of QTLs.

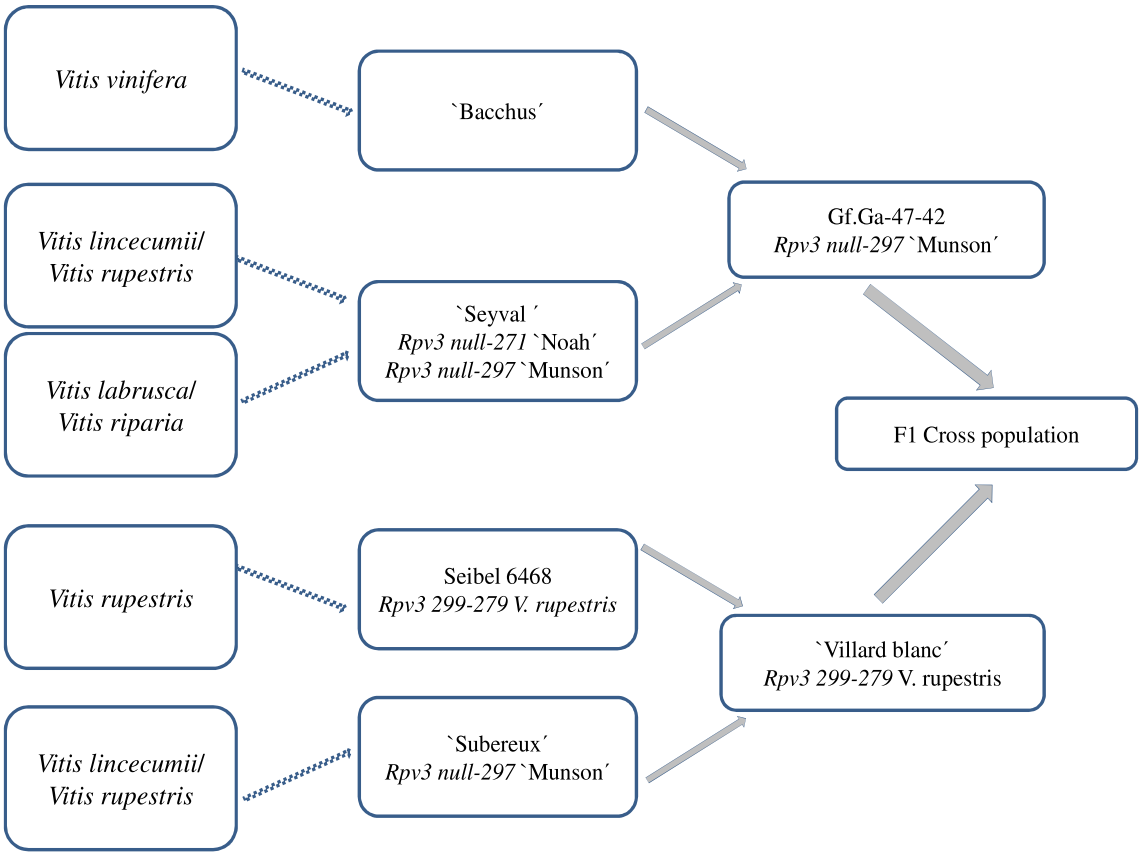


Fig. 1 Pedigree of the cross population including the lineage of *Rpv3* allelic variants according to Di Gaspero et al. (2012). Direct relationships are indicated by *solid arrows*, while more distant relationships with additional crosses intermingled are shown with *dotted arrows*

The parental lines of the mapping populations, GF.GA-47-42 and 'Villard blanc', differ considerably in their expression of certain traits, including flowering time and ripening behavior. GF.GA-47-42 is early ripening, while 'Villard blanc' is a mid- to late-ripening cultivar. Researchers have recently focused on such phenology-related characteristics because climatic changes can decrease grape quality if ripening occurs under unfavorable conditions (e.g., elevated night temperatures causing a loss of acidity). For these reasons, breeders prefer selecting grapes with a defined ripening period. In grapevines, fruit ripening is accompanied by cell wall softening, sugar accumulation, decreases in organic acid contents, accelerated growth, and initiation of anthocyanin pigmentation in red-berried cultivars (Barnavon et al. 2000). In practice, fruit softening can be assessed by manually checking the texture of individual berries. The transition point when small, hard, and green berries start to soften is called veraison. This physiological change can be used to indicate fruit maturation. We determined the time interval between the flowering stage and the onset of veraison in the progenies of the GF.GA-47-42 × 'Villard blanc' cross over several years. We also screened for QTLs affecting this trait.

Materials and methods

Plant materials

The analyzed segregating population consisted of 151 progeny plants from the GF.GA-47-42 × 'Villard blanc' cross (Fig. 1). GF.GA-47-42 ('Bacchus' × 'Seyval') ripens early and exhibits high and moderate levels of resistance to powdery and downy mildew diseases, respectively. It is a green-berried grapevine breeding line that was generated in the breeding program at the JKI Institute for Grapevine Breeding Geilweilerhof, Germany. Material from this line is abundant, so it was used as the maternal parent after manual emasculation of its hermaphroditic flowers. 'Villard blanc' served as a pollen donor (Zyprian et al. 2005). Pollen was collected from plants at the JKI Institute for Grapevine Breeding Geilweilerhof.

The GF.GA-47-42 and 'Villard blanc' lines were crossed in 1989, and the progenies were planted in an experimental vineyard in 1996. Vines were grown on their own roots at the Institute for Grapevine Breeding Geilweilerhof (N49°21.675, E8°04.433). The vineyard was cane pruned (to 10–12 buds) with 1.8 × 1.1 m (row × vine)

spacing, and a plant density of 5050 vines per hectare. Each progeny genotype was represented by one vine in one experimental plot and two more plants per genotype in a second plot planted in 2000. One of the experimental plots was not treated with fungicides to enable the assessment of natural powdery and downy mildew symptoms. Hardwood cuttings were harvested to grow plants in the greenhouse for leaf disc infection experiments involving *P. viticola*.

DNA extraction

Small sections (~1 cm^2) of young, expanding leaves from field-grown plants were cut, immediately transferred to plastic bags to prevent desiccation, cooled on ice, and flash-frozen in liquid nitrogen upon arrival in the laboratory. The samples were homogenized by shaking once or twice for 30 s at a frequency of 30 Hz in the presence of two 3-mm diameter steel beads per sample in a Retsch mill tissuelyser (Retsch, Hahn, Germany). We isolated DNA from the homogenates using the peqGold Plant DNA mini kit (Peqlab Biotechnologie GmbH, Erlangen, Germany) following the manufacturer's instructions. The quality of the purified DNA was checked using a nanophotometer (i.e., UV spectrophotometry) (Implen, Munich, Germany) and by gel electrophoresis on 0.8 % agarose gels.

Simple sequence repeat marker analysis

Sequence length polymorphisms of microsatellites (i.e., SSRs) were detected in 151 F_1 individuals and parental lines. A multiplex polymerase chain reaction (PCR) was performed using SSR-flanking primer pairs, which included fluorescent labeling at the 5′ ends of the forward primers (Schwander et al. 2012). In grapevines, many amplification products at SSR loci are transferable between different cultivars and breeding lines, yielding informative sequence length polymorphisms. The SSR marker sets from the VMC (Vitis microsatellite consortium), UDV, VVI (http://www.ncbi.nlm.nih.gov/probe), and VCHr (Cipriani et al. 2008) series could be used. Additional primer pairs for informative segregation analyses have been published (Zhang et al. 2009; Schwander et al. 2012; Fechter et al. 2014; Rex et al. 2014).

These markers were developed using the WebSat program (Martins et al. 2009) to optimize the coverage (i.e., markers for the "GF" series) of the reference genome sequence (Jaillon et al. 2007) available in the Genoscope browser (http://www.genoscope.cns.fr/externe/GenomeBrowser/Vitis/). Previously unpublished primer pairs for newly developed markers are presented in Supplemental Table 1a. All data were compiled in spreadsheets for further analysis.

Single nucleotide polymorphism analysis

Variabilities in SNPs were detected using automated systems, which slightly limited the number of analyzed individuals. A set of 137 individuals from the segregating population and the parents/grandparents were genotyped using Illumina bead array technology with 384 SNP markers (Emanuelli et al. 2013). Additionally, two sets of 48 SNP markers each were run on a Biomark system applying the FluiDigm methodology with 144 individuals. A set of 47 established SNPs (Cabezas et al. 2011) was analyzed together with a single SNP marker derived from plastid DNA (i.e., cp4527) (Hunt et al. 2010). A 47-SNP set developed at the Institute for Grapevine Breeding Geilweilerhof (Zyprian unpublished) was also used with the cpSNP marker.

The two sets of 48 primer pairs were used to amplify 47 DNA templates per run and one non-template control (48 samples). The primers and fluorescently labeled oligo probes were designed and synthesized by FluiDigm (South San Francisco, CA, USA). Allele-specific PCR products were generated after pre-amplification of the target regions by duplex quantitative real time PCR using FR48.48 dynamic arrays (FluiDigm) and a Biomark platform. Genotypes were assigned based on the relative fluorescence intensities of the two alternative dyes labeling the allele-specific probes targeting each SNP locus. Samples were processed and analyzed according to the instructions of the software supplier FluiDigm. The new SNP markers developed at the JKI Institute for Grapevine Breeding Geilweilerhof are listed in Supplemental Table 1b.

There is an overlap of the marker sets analyzed with the two different techniques. In total, 35 out of the 48 markers described by Cabezas et al. (2011) were also present in the 384-SNP set used by Emanuelli et al. (2013). Nine SNPs from gene diversity studies at the Institute for Grapevine Breeding Geilweilerhof were also included in the 384-marker set. Out of these redundant markers, 23 were associated with genetic segregation and yielded useful data for evaluating the reliability of both SNP genotyping platforms.

Genetic mapping

A genetic map was constructed using linkage/recombination analysis after coding the observed alleles according to the specifications of JoinMap 4.1 software (Kyazma B.V., Wageningen, the Netherlands). A double pseudo-testcross strategy (Grattapaglia and Sederoff 1994) was applied. The diploid and widely heterozygous grapevines exhibited co-dominant inheritance of many sequence length polymorphic microsatellite markers (e.g., Zhang et al. 2009; Schwander et al. 2012). Up to four different alleles may be

present in the F_1 population following a cross. Segregating SSR markers detected four alleles (i.e., maternal alleles a and b × paternal alleles c and d), three alleles (i.e., maternal alleles e and f × paternal alleles e and g, with e representing the same allele size in both parents), or two alleles segregating from the double heterozygous parental genotypes (i.e., h and k × h and k). Additionally, some markers produced amplification products that segregated from only the maternal genotype (i.e., lm × ll, with "lm" indicating the segregating amplificate) or the paternal genotype (i.e., nn × np, with "np" representing the segregating marker). These are indicated by the addition of allele sizes or the letters a or b to the SSR names in the genetic maps. Biallelic SNPs segregated from the heterozygous maternal genotype (i.e., lm × ll), the heterozygous paternal genotype (i.e., nn × np), or from the double heterozygous parental genotypes (i.e., hk × hk).

All data were recorded in a large matrix in spreadsheets. This matrix was analyzed by JoinMap 4.1 (Van Ooijen 2006) to calculate the segregation distortion (by the chi-square test), construct linkage groups (LGs), and determine marker linkage phases and marker order. The regression mapping algorithm was applied using the Kosambi function for calculating genetic distances in the JoinMap program. Distorted markers were kept unless they changed the order of surrounding markers. Separate maternal and paternal segregating marker sets were used to construct parental maps that were integrated afterwards. Because singularity errors occurred in these maps in downstream QTL analyses, the corresponding marker data were converted to "DH" type to construct parental maps suitable for QTL analysis, as described in the MapQTL6 instructions (Van Ooijen 2009). Additionally, the complete set of markers was used to generate an integrated map (Fig. 2).

Phenotyping

Downy and powdery mildew resistance traits were scored over several years in the pesticide-free experimental field plot under natural infection pressure (i.e., downy mildew: 1999, 2000, and 2003; powdery mildew: 1999, 2000, 2003, 2004, 2005, and 2006). Disease scoring was completed in late summer (August/September) when control (susceptible) *V. vinifera* plants clearly exhibited disease symptoms. The leaves and fruits were assessed separately. Scores were determined based on the overall impression of each plant. Assessments of powdery mildew symptoms were not influenced by leaf-loss due to severe downy mildew infestation. The degree of infection was recorded according to the following L'Organisation Internationale de la Vigne et du Vin (OIV; www.oiv.int) classification descriptors: 452 (*Plasmopara* resistance of leaves), 453 (*Plasmopara* resistance of clusters), 455 [*Oidium* (*Erysiphe*) resistance of leaves], and 456 [*Oidium* (*Erysiphe*) resistance of clusters]. Related to these classifications, an inverse notation evaluating infestations instead of resistance was used to avoid any confusion between non-infection and complete resistance (1 = no symptoms, 9 = heavy infestation). Additionally, the percentage of leaf area affected and the amount of necrosis were estimated based on the overall impressions of the test plants.

A detailed analysis of leaf discs from individual plants artificially inoculated with *P. viticola* sporangia under greenhouse conditions was completed in 2005. This methodology was established at INRA Centre de Recherche de Colmar (France) (Bellin et al. 2009; Deglene-Benbrahim et al. 2010). For each plant, the fourth and fifth leaves beneath the shoot apex were detached and rinsed with water. Eight leaf discs (1-cm-diameter) were excised with a cork borer and transferred to wet paper in a Petri dish with the abaxial side facing up. Discs were sprayed with a *P. viticola* suspension (100,000 sporangia/ml). The *P. viticola* strain was isolated from 'Chardonnay' leaves in an INRA experimental vineyard located in Colmar, France. The pathogen was propagated by applying suspensions (10,000 sporangia/ml) on susceptible *V. vinifera* seedlings. The infected seedlings were incubated in a growth chamber at 21 °C and 100 % relative humidity, with a 16-h photoperiod until the samples sporulated. Sporangia were collected by soaking leaves carrying sporangiophores (with mature sporangia) in sterile water. After inoculations, Petri dishes were incubated under controlled conditions (i.e., 21 °C and 16-h photoperiod). After a 24-h incubation, the sporangial suspensions were dried with sterile paper, and the Petri dishes were left for 5 days under the same conditions. For each Petri dish, the degree of infection was scored according to OIV descriptor 452-1. Indicators of pathogen infection related to sporulation and necrosis were recorded (Supplemental Table 2). Additional leaf disc assays were completed with three replicates in 2010 according to OIV descriptor 452-1 as previously described (Schwander et al. 2012). Average values were calculated for QTL analyses. All untransformed data were used for QTL analyses.

The timing of the onset of veraison was evaluated by daily inspection in five growing seasons (i.e., 1998, 1999, 2008, 2009, and 2010). Furthermore, the interval between flowering and the onset of veraison was recorded for 4 years (i.e., 1999, 2008, 2009, and 2010). Flower characteristics were assessed at full bloom (OIV descriptor 302), during which more than 50 % of calyptrae were released, filaments were erect with free anthers, styles were fully accessible, and a fruity-sweet scent was released from the nectaries. The onset of veraison was determined by testing ten representative berries per cluster. Veraison was defined as the stage in which 15–20 % of fruits softened (manually assessed) and turned bright green. For QTL analyses, the

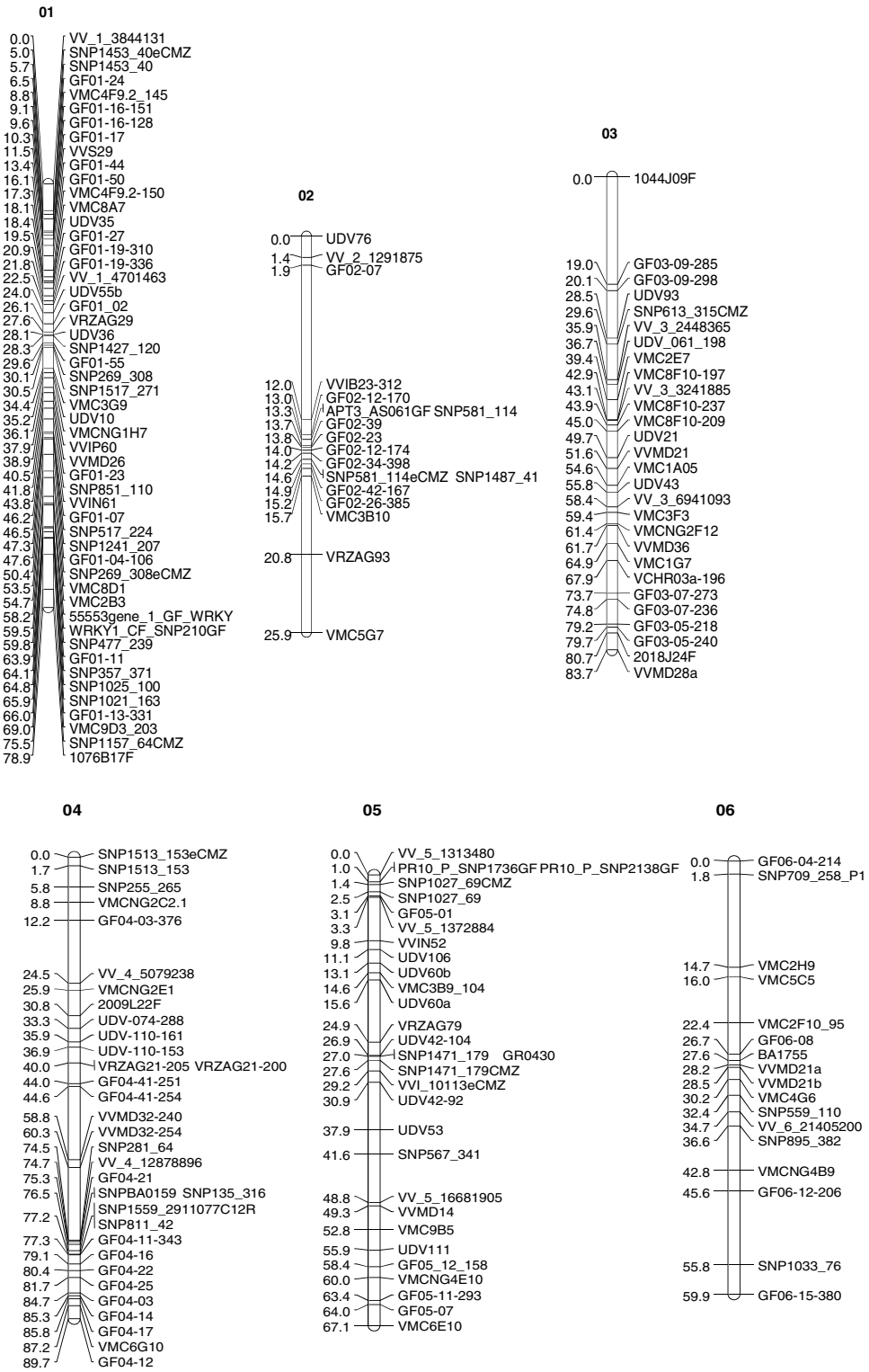
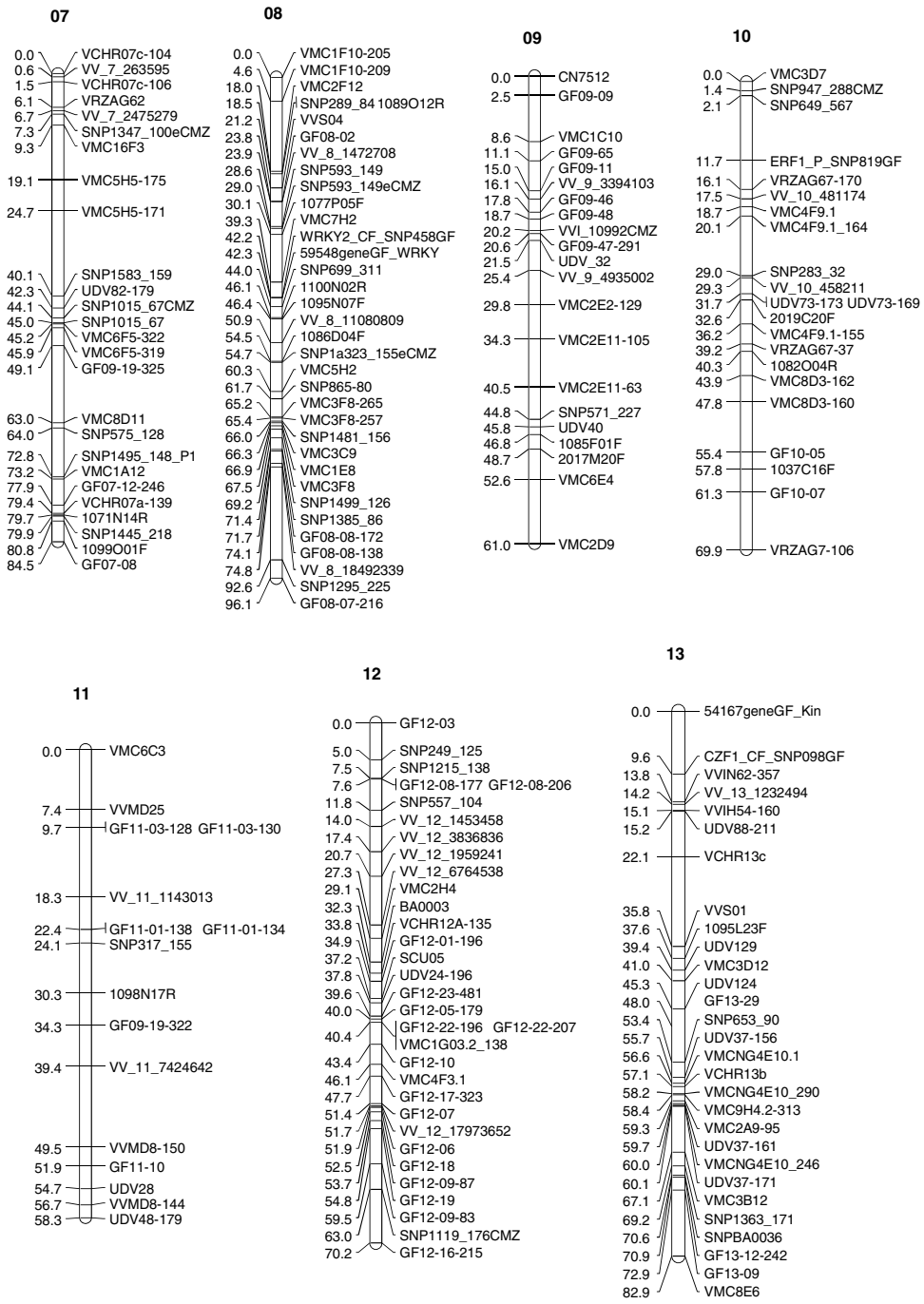


Fig. 2 Integrated genetic map of the F_1 population from the GF.GA-47-42 × 'Villard blanc' cross. Genetic distances are indicated in cM (*left side*). Marker positions are provided (*right side of the bars*) and represent the linkage groups. Linkage group numbers and orientation are based on the reference genome sequence available in the Genoscope browser (http://www.genoscope.cns.fr/externe/GenomeBrowser/Vitis/)

veraison dates were recorded relative to June 1. For QTL analyses of the interval between flowering and the start of veraison, the number of days between both stages was determined. Untransformed data were used for subsequent QTL analyses because they exhibited clear continuous variations and a roughly bimodal distribution (Supplemental Fig. 1a and b).

Statistical analysis of all resistance and phenology-related traits was completed using the R software (https://www.r-project.org/). Spearman rank

Fig. 2 continued



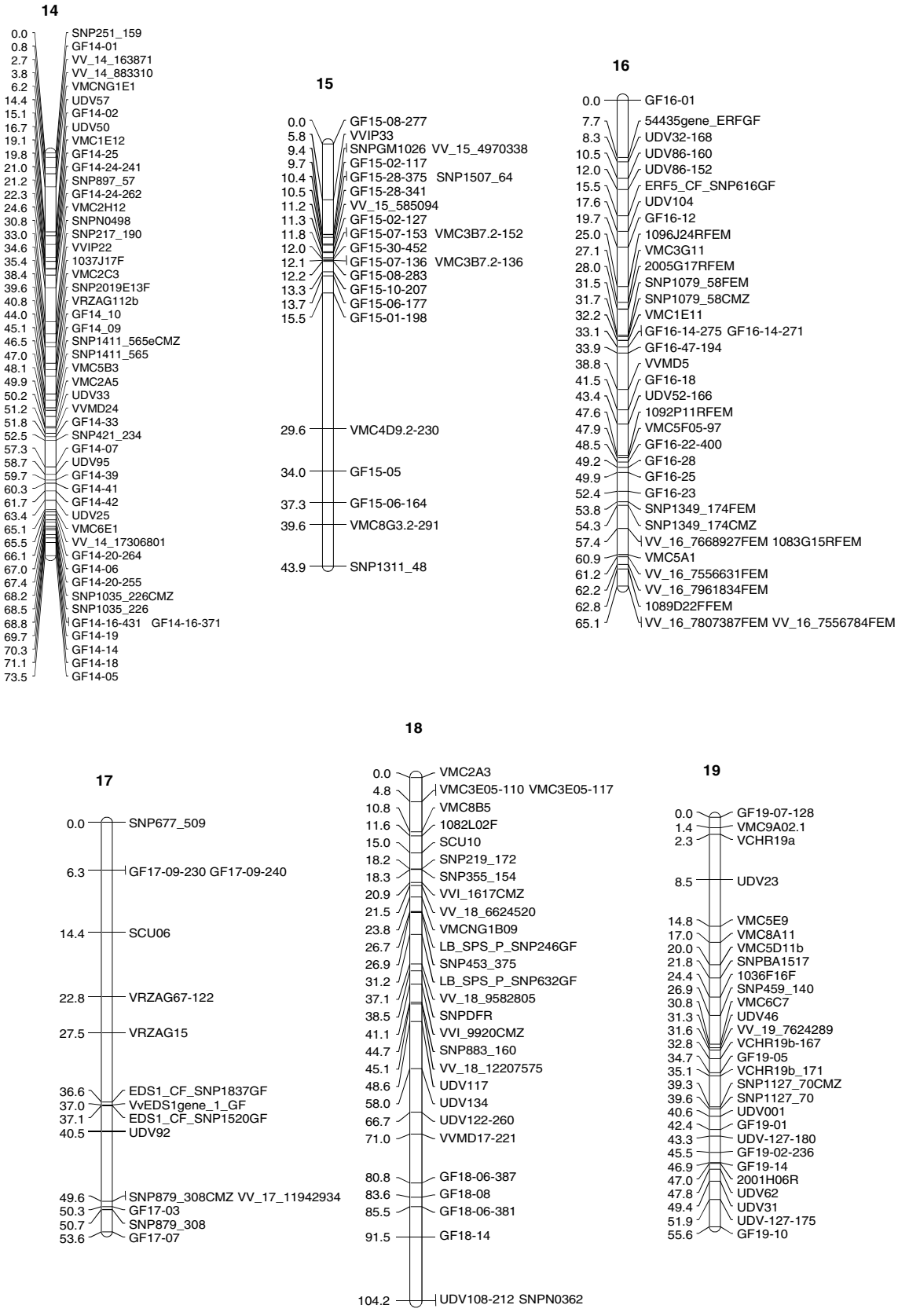
correlation coefficients and the corresponding p values were calculated.

Quantitative trait locus analysis

An integrated genetic map was screened for the segregation of QTLs using MapQTL6 software (Van Ooijen 2009). This analysis was repeated with the separate parental maps to enable comparisons. The first step involved interval mapping to detect QTLs. The QTL-flanking markers were then used as co-factors, and the analysis was extended to permit multiple QTL mapping (MQM). The significance thresholds of logarithm of odds (LOD) scores ($p = 0.05$) were determined for each LG using permutation tests with at least 1000 permutations (included in the JoinMap4.1 software).

Because climatic conditions seemed to affect QTL analysis, weather records for the Rhineland-Palatinate region were considered. They were obtained from the official German weather service (Deutscher Wetterdienst; http://www.

Fig. 2 continued



dwd.de/DE/klimaumwelt/klimaatlas/klimaatlas_node.html). The next closest weather stations were located 16 km (Bad Bergzabern) and 36 km (Weinbiet) away from the experimental plots in Siebeldingen.

Results

Marker information and reliability of single nucleotide polymorphism genotyping

The mapping of SSR loci yielded informative segregation for 301 SSR-flanking primer pairs and resulted in 349 mapped loci. From the 384 SNP assays analyzed on the Illumina chip, 21 failed to produce data and 179 were monomorphic. Out of the 184 SNP assays yielding segregating alleles, 19 had more than 10 % missing data and were excluded from linkage/recombination analysis. The remaining 165 markers (43 %) were included in the mapping matrix. The 47 markers described by Cabezas et al. (2011) were unable to genotype four loci and resulted in monomorphic patterns at 17 SNP loci. Technical problems were associated with one of the SNPs with informative segregation, resulting in more than 10 % missing data. This SNP was excluded from further analysis. The remaining 25 markers (53 %) were used for genetic mapping, and are annotated with "CMZ" in Fig. 2. Of the 47 SNPs developed at the JKI Institute for Grapevine Breeding Geilweilerhof, two were unsuitable for FluiDigm analysis and 19 were monomorphic. From this set, 26 SNP markers (55 %) were used to construct the genetic map (annotated with "GF" in Fig. 2). These contained SNPs from coding sequences and promoter regions of candidate genes differentially expressed in the defense responses of resistant and susceptible grapevine cultivars (Zyprian unpublished) or genes involved in flowering (Fechter et al. 2012).

Single nucleotide polymorphisms within one gene are expected to map to identical positions because their close genetic locations cannot be resolved by recombination in roughly 150 individuals. This redundancy was exploited to check the reliability of the SNP genotyping platforms. All of the markers were mapped to identical locations or in close proximities. For the subsequent genetic analyses, one representative marker was used to decrease the calculation load for mapping. The SNP genotypes and segregation patterns are provided in Supplemental Table 3.

We analyzed 23 SNP markers (10.6 % of SNP markers) in duplicate using Illumina hybridizations and FluiDigm genotyping technology. The duplicate samples ensured the reproducibility of genotyping with these two analytical techniques. An integrated map generated using the data of 216 SNP loci (without any SSR markers) indicated 13 markers were positioned right next to each other or in identical positions in the genetic map (data not shown). This validated the results and indicated that both techniques were suitable. Additionally, the cpSNP quality control marker exhibited the expected uniform genotype in the mapping population (i.e., maternal inheritance). All progenies contained a T at the SNP marker cp4527 locus similar to the maternal GF.GA-47-42 genotype (and its predecessors 'Bacchus' and 'Seyval'), while the paternal allele (containing a G) from 'Villard blanc' (Seibel 6468 cp4527: G × 'Subereux' cp4527: T) was not inherited.

Our analyses indicated SNP genotyping results were reproducible between Illumina bead hybridizations and FluiDigm assays on nanofluidic chips. Approximately 50 % of the markers yielded informative segregation and proved useful for genetic mapping. Finally, 194 SNP markers (out of 216) were included in the genetic map.

Genetic map

A preliminary genetic map of the population analyzed in this study was previously generated using amplified fragment length polymorphism (AFLP) markers (Zyprian et al. 2005). However, the dominant nature of this marker type and its tendency to cluster prevented its efficient use in QTL analyses. Our integrated map was based on co-dominant SNPs and SSRs, and exhibited better marker coverage. The integrated genetic map included 19 LGs, which corresponded to the number of chromosomes in the haploid *Vitis* sp. genome. The numbering and orientation of LGs complied with the internationally recognized standard (www.vitaceae.org) and the reference genome sequence (http://ensembl.gramene.org/Vitis_vinifera/Info/Index).

Our map contained 543 loci covering a genetic distance of 1324.1 cM. Marker saturation of the individual LGs varied from an average distance of 1.5 cM on LG14 to an average distance of 3.89 cM on LG11. No gaps of ≥20 cM were detected. The relevant data are summarized in Table 1, and the integrated map is provided in Fig. 2. Separate maps for male and female lines were also used for QTL analyses. These maps consisted of 20 LGs. In the male map, LG7 was split into two groups, while the female map indicated LG9 was split into two groups.

Quantitative trait locus analysis for *Plasmopara viticola* resistance

Resistance to *P. viticola* was assessed separately for leaves and berries over three seasons under field conditions with natural infection pressure. The results for 2003 were affected by unusually high temperatures. Because this pathogen requires humidity and wet leaves or moist berries for infection, its propagation may have been strongly impaired by the extended period of high temperatures and

Table 1 Marker saturation of linkage groups

Integrated map				Female map GF.GA-47-42				Male map `Villard blanc´			
LG	nloc	cM	Average marker distance (cM)	LG	nloc	cM	Average marker distance (cM)	LG	nloc	cM	Average marker distance (cM)
1	52	78.94	1.55	1	52	69.21	1.36	1	42	82.59	2.01
2	18	25.92	1.52	2	14	32.03	2.46	2	12	20.72	1.88
3	28	83.65	3.10	3	20	62.82	3.31	3	17	67.19	4.20
4	34	89.69	2.72	4	21	55.00	2.75	4	23	97.83	4.45
5	30	67.09	2.31	5	25	64.90	2.70	5	16	74.44	4.96
6	17	59.94	3.75	6	11	44.74	4.47	6	13	49.71	4.14
7	26	84.50	3.38	7	24	82.63	3.59	7a	6	25.87	5.17
8	35	96.10	2.83	8	23	63.93	2.91	7b	2	6.20	6.20
9	21	61.02	3.05	9a	8	20.97	3.00	8	24	78.96	3.43
10	22	69.86	3.33	9b	7	24.96	4.16	9	18	62.79	3.69
11	16	58.30	3.89	10	14	53.24	4.10	10	14	97.69	7.51
12	33	70.18	2.19	11	11	46.22	4.62	11	11	73.87	7.39
13	29	82.90	2.96	12	25	57.71	2.40	12	21	65.05	3.25
14	50	73.53	1.50	13	27	78.03	3.00	13	15	110.43	7.89
15	24	43.92	1.91	14	54	49.43	0.93	14	46	78.33	1.74
16	36	65.1	1.86	15	12	50.03	4.55	15	9	48.42	6.05
17	15	53.64	3.83	16	30	71.04	2.45	16	24	68.80	2.99
18	29	104.23	3.72	17	11	57.42	5.74	17	8	33.89	4.84
19	28	55.61	2.06	18	25	94.59	3.94	18	17	94.93	5.93
				19	21	48.15	2.41	19	21	64.99	3.25
	Sum = 543 loci	Sum = 1324.1 cM	2.71		Sum = 435 loci	Sum = 1127.03 cM	2.60		Sum = 359 loci	Sum = 1302.70 cM	3.64

Overview of marker saturation of linkage groups in the integrated map and the maps for GF.GA-47-42 (female) and 'Villard blanc' (male)

LG linkage group, *nloc* number of loci, *cM* centimorgan extension of an individual group

lack of moisture. In June, July, and August, 2003, the average temperature reached 19.7 °C because of several heat waves. The temperature was 2.2 °C higher than normal in southwest Germany (i.e., Rhineland-Palatinate region), and precipitation levels decreased by 25 %. Regarding leaf disease resistance, clear correlations were observed between 1999 and 2000, but not when data from 2003 were included (Supplemental Table 4a). To supplement the field data, a detailed analysis of leaf discs artificially inoculated with *P. viticola* sporangial suspensions under controlled conditions was completed in 2005. In 2010, leaf disc assays were conducted in triplicate according to OIV descriptor 452-1. The results were correlated with the phenotypic data from 1999 and 2000 as well as with the leaf disc assay results from 2005 (Supplemental Table 4a).

The QTL results associated with the phenotypic data are summarized in Table 2. The *P. viticola* leaf resistance field data indicated a strong QTL on LG18 localized to marker GF18-08. The resistance traits associated with GF18-08 and the flanking GF18-14 marker appeared to be derived from the haplotype corresponding to Seibel 6468 alleles, which are inherited from 'Villard blanc'. While GF18-08 is fully informative with four segregating alleles, GF18-06 segregates with a single 387-bp paternal allele (i.e., nn × np) and a 381-bp maternal variant (i.e., lm × ll). The paternal marker was mainly observed linked to resistance traits, whereas the maternal variant was associated with traits related to necrotic reactions.

The highest LOD score (28.15) for a *P. viticola* resistance QTL in leaves was observed in 2000 during evaluations of downy mildew resistance (i.e., percentage of leaf area affected). It explained up to 57.6 % of the observed phenotypic variance, and was delimited to a confidence interval of 80.8–85.4 cM on LG18 after MQM analysis. The QTL was detected at a similar position in 1999, but appeared less pronounced and shifted to a different region on LG18 in 2003. A prominent QTL with an overlapping confidence interval to that of the major QTL identified in 1999 and 2000 field data was reproduced in the in vitro leaf disc assays. Its highest LOD value was 21.37, which explained 51.5 % of the phenotypic variance when the data for the production of sporangia was analyzed (i.e., per ml or per cm^2 leaf surface). The QTL was reproduced in triplicate leaf disc infection assays in 2010. Some minor QTLs for leaf resistance to *P. viticola* were also detected, but may not be significant because of their limited reproducibility. These QTLs are listed in Table 2, but are not discussed further.

Analyses of berry resistance to *P. viticola* yielded similar results. There were significant correlations between the phenotypic data of leaf and berry infections in 1999 and 2000 (for correlations and *p* values cf. Supplemental Table 4a). Therefore, the leaf resistance QTL on LG18 was determined to also affect berry resistance. However, it was less efficient in berries, as indicated by its highest LOD value (7.1–7.3) in 2000. Additionally, the QTL exhibited the same positional shift in 2003 as the leaf resistance QTL. Data from OIV-based evaluations of berry resistance in 1999 and 2003 did not reach the LG-specific threshold significance level for a QTL. The QTL was detected only when berry damages (expressed as a percentage) from 1999 to 2003 were analyzed. Several other QTLs were identified, but none were reproducible at the same confidence interval for more than 1 year (Table 2).

The strength of the LG18 QTL was similar to that of the GF18-06-387 marker in the 'Villard blanc' paternal map. In contrast, no QTL was observed in the maternal map when field data were analyzed. Only analyses of data from leaf discs scored for necrosis revealed a QTL on LG18 at marker GF18-14 (highest LOD: 3.07; LG-specific significance threshold LOD: 1.8; 9.8 % of phenotypic variance explained; confidence interval: 9 cM). Thus, the major QTL on LG18 identified in this study mainly corresponds to 'Villard blanc' alleles. The specific necrotic reactions observed after artificial inoculation appear related to genetic determinants transmitted from GF.GA-47-42.

Quantitative trait locus analysis for *Erysiphe necator* resistance

Resistance to *E. necator*, the fungus responsible for powdery mildew, was evaluated under natural infection conditions in 6 years (i.e., 1999, 2000, 2003, 2004, 2005, and 2006). Leaves and berries were assessed separately. Additionally, the percentage of berries affected by infection was determined. Leaf resistance data were generally consistent over time, except in 2004 (Supplemental Table 4b). The climatic extremes in 2003 most likely decreased the natural disease pressure in subsequent years. *Erysiphe necator* is susceptible to the high temperatures, increased light intensity, and UV irradiation (Austin and Wilcox 2012; Choudhury et al. 2014) that predominated in 2003. However, leaf and berry symptoms were correlated with each other throughout the six analyzed seasons (Supplemental Table 4b).

The results of QTL analyses are summarized in Table 3. Regarding leaf resistance, a significant QTL was detected on LG15 in four seasons (i.e., 1999, 2000, 2005, and 2006). Its highest LOD score was 7.1, which explained up to 19.4 % of the phenotypic variance observed by MQM. Its confidence interval surrounded markers GF15-10, GF15-07, and GF15-28. Additionally, this was the only significant QTL for leaf resistance in 1999 and 2000, with contributions from both parents. The resistance trait was mainly

Table 2 QTL analysis of *Plasmopara* resistance traits

Evaluation	Trait	QTL IM[a]	QTL MQM[a]	MQM cofactor	LOD max	Sign. threshold LG	%expl.	Marker	Confidence interval[b]
Field data	*Plasmopara* leaf 1999	**LG18**		–	13.7	3.0	34.2	GF18-06-387	69.8–85.5 cM
			LG18	GF18-08	13.7	3.0	34.2	GF18-06-387	80.8–85.5 cM
Field data	*Plasmopara* leaf % 1999	**LG18**		–	8.49	3.1	22.8	GF18-08	70.0–87.5 cM
			LG18	GF18-08	8.49	3.1	22.8	GF18-08	70.0–87.5 cM
Field data	*Plasmopara* leaf necrosis 1999	**LG18**		–	16.72	3.0	40.0	GF18-06-387	71.8–85.5 cM
			LG18	–	16.72	3.0	40.0	GF18-06-387	80.8–85.5 cM
Field data	*Plasmopara* berry 1999	LG07		–	3.44	2.9	12.0	VMC5H5	14.2–28. 4 cM
		LG15		–	3.5	3.1	12.3	GF15-01-198	13.7–17.4 cM
			LG15	–	3.78	3.1	12.4	GF15-01-198	14.7–17.5 cM
			LG18	GF18-08	3.5	3.0	11.6	GF18-06-387	76.8–81.6 cM
Field data	*Plasmopara* berry % 1999	LG09		–	8.1	2.7	26.0	VMC2E11_105	33.7–36.2 cM
		LG11		–	5.0	2.9	17.1	VV_11_7424642	37.3–41.4 cM
		LG15		–	5.2	3.8	17.6	GF15-01-198	24.7–17.5 cM
			LG09	–	7.95	2.7	25.3	VMC2E11_105	33.7–37.3 cM
			LG11	–	5.0	2.9	16.8	VV_11_7424642	39.3–41.4 cM
			LG15	–	5.22	3.8	17.5	GF15-01-198	14.7–17.5 cM
Field data	*Plasmopara* leaf 2000	**LG18**		–	21.87	3.2	48.7	GF18-08	78.8–85.4 cM
			LG18	GF18-08	21.87	3.2	48.7	GF18-08	80.8–85.5 cM
Field data	*Plasmopara* leaf % 2000	**LG18**		–	28.15	3.0	57.6	GF18-06-387	78.8–85.4 cM
			LG18	GF18-08	28.15	3.0	57.6	GF18-06-387	80.8–85.4 cM
Field data	*Plasmopara* leaf necrosis 2000	**LG18**		–	13.4	3.1	33.5	GF18-06-387	76.8–82.6 cM
		LG09		–	3.78	2.9	10.9	GF9-09	0.0–9.6 cM
			LG18	GF18-08	12.9	3.1	32.6	GF18-06-387	80.8–83.6 cM
			LG09	–	4.15	2.9	8.6	GF09-09	0.0–7.5 cM
Field data	*Plasmopara* berry 2000	**LG18**		–	5.77	3.0	17.0	GF18-06-387	65.7–82.6 cM
		LG16		–	4.17	3.1	12.6	VV_16_7668927	52.7–59.2 cM
			LG18	GF18-8	5.17	3.0	15.3	GF18-06-387	80.8–85.5 cM
			LG16	–	3.48	3.1	9.4	VV_16_7668927	52.7–59.2 cM
Field data	*Plasmopara* berry % 2000	**LG18**		–	7.36	3.1	21.4	GF18-06-387	76.8–83.6 cM
		LG19		–	4.1	2.9	12.5	VCHR19a	0.0–4.3 cM
			LG18	GF18-08	7.15	3.1	20.8	GF18-06-387	80.8–83.6 cM
Field data	*Plasmopara* leaf 2003	LG08		–	3.72	3.1	11.8	VMC5H2	47.4–78.8 cM
			LG08	–	3.95	3.1	12.1	VMC5H2	46.6–52.9 cM

Table 2 continued

Evaluation	Trait	QTL IM[a]	QTL MQM[a]	MQM cofactor	LOD max	Sign. threshold LG	%expl.	Marker	Confidence interval[b]
Field data	*Plasmopara* leaf % 2003	**LG18**		–	6.42	3.0	19.4	UDV117	51.3–57.6 cM
		LG10		–	4.34	2.8	13.6	VMC3D7	0.0–9.1 cM
		LG01		–	3.26	3.1	10.4	VMC2B3; VMC8D1	50.6–57.3 cM
			LG18	GF18-08	4.83	3.0	13.6	UDV117	46.6–53.9 cM
			LG10	–	4.0	2.8	11.4	VMC3D7	0.0–10.1 cM
			LG01	–	3.01	3.1	8.7	VMC8D1	50.6–57.3 cM
Field data	*Plasmopara* leaf necrosis 2003	LG01		–	3.71	3.4	11.7	SNP1157_64CMZ	71.5–78.0 cM
			LG01	–	4.21	3.4	12.7	SNP1157_64CMZ	71.5–78.0 cM
Field data	*Plasmopara* berry 2003	–		–	–	–	–	–	–
Field data	*Plasmopara* berry % 2003	LG11		–	3.94	2.7	12.5	GF11-03	0.0–24.1 cM
		LG18		–	3.32	3.1	10.6	UDV117	45.6–56.9 cM
			LG11	–	4.14	2.7	12.8	VVMD25	0–24.1 cM
			LG18	GF18-08	3.21	3.1	10.1	UDV117	45.6–54.9 cM
In vitro leaf discs	Sporangia density per sporulation plot	**LG18**		–	19.54	3.0	48.2	GF18-08; GF18-06-387	79.8–85.5 cM
			LG18	GF18-08	19.54	3.0	48.2	GF18-08; GF18-06-381	80.8–85.5 cM
In vitro leaf discs	Sporangia per cm^2 leaf surface	**LG18**		–	21.37	3.1	51.5	GF18-06-387	77.8–82.6 cM
			LG18	GF18-08	20.75	3.1	50.5	GF18-08; GF18-06-381	81.6–85.5 cM
In vitro leaf discs	Sporangia per ml	**LG18**		–	21.37	3.1	51.5	GF18-06-387	77.8–82.6 cM
			LG18	–	20.75	3.1	50.5	GF18-06-381; GF18-08	81.5–86.8 cM
In vitro leaf discs	Sporangia size	**LG18**		–	13.97	3.2	37.7	GF18-08; GF18-06-387	80.2–85.5 cM
			LG18	GF18-08	13.97	3.2	37.7	GF18-08	80.2–85.5 cM
			LG15	–	4.55	2.7	9.2	GF15-01-198	14.7–18.5 cM
In vitro leaf discs	Absence/ presence of necrosis	**LG18**		–	12.78	3.1	34.9	GF18-08	81.6–86.5 cM
		LG11		–	4.12	3.0	12.9	GF11-03; VV_11_114301	4.0–19.3 cM;
			LG18	GF18-08	12.78	3.1	34.9	GF18-08	80.8–85.5 cM
			LG11	–	3.35	3.0	6.9	VMC6C3; VVMD25	0–9.7 cM
In vitro leaf discs	Necrosis frequency	**LG18**		–	6.66	3.1	20.6	GF18-06-381	79.8–90.5 cM
			LG18	GF18-08	6.88	3.1	20.6	GF18-06-381	80.8–85.5 cM
In vitro leaf discs	Necrosis size	**LG18**		–	12.78	3.1	34.9	GF18-08	81.6–86.4 cM

Table 2 continued

Evaluation	Trait	QTL IM[a]	QTL MQM[a]	MQM cofactor	LOD max	Sign. threshold LG	%expl.	Marker	Confidence interval[b]
			LG18	GF18-08	13.55	3.1	36.6	GF18-06-381; GF18-08	81.6–85.4 cM
			LG 06	–	3.6	2.7	7.3	SNP559_110	22.4–40.6 cM
In vitro leaf discs	Absence/ presence of necrotic patches	**LG18**		–	8.04	3.1	23.7	GF18-08	80.8–86.5 cM
		LG06		–	3.98	2.7	12.5	VMCNG4B9	35.7–43.8 cM
			LG18	GF18-08	8.04	3.1	23.7	GF18-08	80.8–85.5 cM
			LG17	–	4.2	2.8	10.0	EDS1 CF SNP1520	37.1–39.1 cM
			LG06	–	3.8	2.7	9.2	SNP895_383	32.1–43.8 cM
In vitro leaf discs	Absence/ presence of necrotic spots	**LG18**		–	18.46	3.0	46.2	GF18-08	80.8–86.5 cM
		LG08		–	3.56	3.1	11.3	VMC2F12	0.0–19.4 cM
			LG18	GF18-08	18.46	3.0	46.2	GF18-08	80.8–85.5 cM
			LG08	–	3.54	3.1	6.1	1089O12R	12.6–22.2 cM
			LG06	–	3.71	2.8	6.3	VMC4G6	21.9–33.cM
In vitro leaf discs	Number of necrotic leaf discs/inoculated leaf discs	**LG18**		–	12.4	3.1	34.1	GF18-08	80.8–86.5 cM
		LG11		–	3.12	2.9	10.0	VVMD25	3.0–20.3 cM
			LG18	GF18-08	12.4	3.1	34.1	GF18-08	80.8–85.5 cM
			LG11	–	5.58	2.9	11.3	VV_11_7424642	34.3–42.4 cM
			LG12	–	4.19	2.9	8.7	VV_12_6764538	15.0–29. 14 cM
In vitro leaf discs	Evaluation adapted to OIV descriptor 452-1	**LG18**		–	14.7	3.0	39.1	GF18-06-387	77.8–82.6 cM
			LG18	GF18-08	14.2	3.0	38.1	GF18-08	18.7–23.4 cM
			LG07	–	3.8	2.9	8.0	VMC6F5_319	41.6–44.5 cM
			LG09	–	3.53	3.0	7.4	VMC2E11_105	33.7–37. 3 cM
			LG11	–	3.44	2.9	7.2	VV_11_7424642	35.3–43.4 cM
In vitro leaf discs	Leaf disc assays average of triplicate OIV 452-1	**LG18**		–	7.26	3.1	21.7	GF18-06-387	73.4–89.5 cM
			LG18	GF18-08	7.22	3.1	21.6	GF18-06-387	80.8–85.4 cM
			LG14	–	5.15	3.2	12.9	VVIP22	32.0–45.5 cM

Quantitative trait locus analysis of *Plasmopara viticola* resistance traits

IM results from interval mapping, *MQM* results from multiple QTL analysis. The cofactor used for MQM computations is indicated. Significance thresholds specific for each linkage group (sign. threshold LG) were calculated using at least 1000 permutations. The percentages of variance explained by the respective QTLs (%expl.) for genetic markers and confidence intervals are listed

[a] Bold indicates the linkage group with the major QTL

[b] Confidence interval is LOD max ±1 LOD

Table 3 QTL analysis of *Erysiphe necator* resistance traits

Trait	QTL IM[a]	QTL MQM[a]	MQM cofactor	LOD max	Sign. threshold LG	%expl.	Marker	Confidence interval[b]
Erysiphe leaf 1999	**LG15**		–	8.73	2.8	23.4	GF15-10-207	11.3–13.7 cM
	LG07		–	3.91	2.9	11.2	SNP1583_159; UDV82-179	34.4–38.9 cM
		LG15	GF15-28-341	6.55	2.8	18.1	GF15-28-341	10.4–11.2 cM
Erysiphe berry 1999	**LG15**		–	7.18	3.1	20.3	GF15-08-283	11.2–13.7 cM
	LG18		–	3.42	3.0	10.2	VMC3E5-110	0.0–7.8 cM
		LG15	GF15-28-341	5.12	3.1	14.9	GF15-28-341	10.4–11.2 cM
		LG18	–	3.78	3.0	9.6	VMC3E5-110	0.0–8.8 cM
Erysiphe berry % 1999	**LG15**		–	6.3	2.8	19.9	GF15-07-153	6.8–13.7 cM
		LG15	GF15-28-375	5.7	2.8	18.1	GF15-02-117	9.3–10.4 cM
Erysiphe leaf 2000	**LG15**		–	9.05	2.7	24.1	GF15-10-207	11.2–13.7 cM
	LG12		–	3.6	2.9	10.4	GF12-16-215	60.5–70.2 cM
		LG15	GF15-28-341	7.1	2.7	19.4	GF15-28-341	10.4–11.2 cM
		LG12	–	3.96	2.9	9.2	GF12-16-215	61.5–70.2 cM
		LG08	–	4.5	3.0	10.4	VMC1F10-205	0.0–6.6 cM
Erysiphe berry 2000	**LG15**		–	3.97	2.8	12.0	GF15-01-198	12.2–27.5 cM
	LG09		–	3.44	2.9	10.5	GF09-65	9.6–13.1 cM
		LG15	GF15-28-375	3.2	2.8	10.0	GF15-28-375	9.3–10.4 cM
		LG09	–	4.39	2.9	11.9	GF09-65	9.6–14.1 cM
Erysiphe berry % 2000	**LG15**		–	3.49	2.9	10.6	SNP1507-64	0.0–11.8 cM
		LG15	GF15-28-341	3.49	2.9	10.6	SNP1507-64	10.4–11.2 cM
		LG09	–	3.58	2.9	9.7	GF09-65	9.6–14.1 cM
Erysiphe leaf 2003	**LG18**		–	3.83	3.1	12.1	UDV117	46.6–54.9 cM
	LG05		–	3.92	2.8	12.3	SNP1027_69CMZ	0.0–6.3 cM
		LG18	UDV117	3.67	3.1	10.2	UDV117	47.6–54.9 cM
		LG05	SNP1027_69CMZ	3.63	2.8	10.1	SNP1027_69CMZ	0.9–2.5 cM
Erysiphe berry 2003	LG14		–	3.75	2.9	11.8	VMC2H12	47.6–54.9 cM
	LG05		–	3.3	3.1	10.6	UDV42-92	26.9–36.9 cM
		LG14	VMC2H12	3.75	2.9	11.8	VMC2H12	22.3–31.0 cM
		LG05	–	3.37	2.9	9.5	VMC9B5	44.6–56.9 cM
Erysiphe berry % 2003	LG14		–	5.18	3.1	16.1	VVIP22	31.0–36.4 cM
		LG14	VVIP22	5.18	3.1	16.1	VVIP22	33.0–35.4 cM
Erysiphe leaf 2004	**LG18**		–	3.53	3.1	11.1	SPS_P_ SNP632GF	28.2–34.1 cM
		LG18	SPS_P_SNP632GF	3.53	3.1	11.1	SPS_P_ SNP632GF	28.2–34.1 cM
Erysiphe berry 2004	**LG18**		–	3.99	3.1	12.5	SPS_P_ SNP632GF	29.2–35.1 cM
	LG16		–	3.57	3.1	11.2	UDV104	16.6–19.6 cM
		LG18	SPS_P_SNP632GF	3.99	3.1	12.5	SPS_P_ SNP632GF	29.2–35.1 cM
Erysiphe berry % 2004	LG16		–	4.56	3.1	14.1	GF16-01	0.0–5.3 cM
		LG16	GF16-1	4.56	3.1	14.1	GF16-01	0.0–5.3 cM
Erysiphe leaf 2005	**LG15**		–	3.28	2.9	11.3	VMC3B7.2_152	2.0–25.5 cM
	LG18		–	3.56	3.1	12.2	VV_18_9582805	26.9–47.6 cM
		LG15	VV_18_9582805; GF15-01-198	4.5	2.9	13.5	GF15-01-198	13.3–26.5 cM
		LG18	–	4.6	3.1	14.0	VV_18_9582805	32.1–45.6 cM

Table 3 continued

Trait	QTL IM[a]	QTL MQM[a]	MQM cofactor	LOD max	Sign. threshold LG	%expl.	Marker	Confidence interval[b]
Erysiphe berry 2005	–		–	–	–	–	–	–
Erysiphe berry % 2005	LG13		–	3.26	2.9	11.2	VVIH54-160	0.6–22.1 cM
		LG13	–	3.26	2.9	11.2	VVIH54-160	14.2–16.1 cM
Erysiphe leaf 2006	**LG15**		–	5.14	2.9	15.9	GF15-7-153	10.5–13.7 cM
	LG18		–	3.62	3.1	11.4	VV_18_12207575	41.7–57.9 cM
		LG15	GF15-07-153; VV_18_12207575	5.14	2.9	15.9	GF15-07-153	11.2–11.8 cM
		LG18	–	4.02	3.1	10.6	VV_18_12207575	44.7–48.9 cM
Erysiphe berry 2006	**LG15**		–	3.66	2.9	11.7	GF15-05	30.6–37.3 cM
		LG15	GF15-05; CZF1_CF_SNP098GF	4.21	2.9	12.1	GF15-05	30.6–37.3 cM
		LG14	–	4.46	3.1	11.3	VMC2C3	33.6–44.0 cM
		LG13	–	3.22	2.9	9.3	CZF1_CF_SNP098GF	0.0–14.2 cM
Erysiphe berry % 2006	–		–	–	–	–	–	–

Quantitative trait locus analysis of *Erysiphe necator* resistance traits

IM results from interval mapping, *MQM* results from multiple QTL analysis. The cofactor used for MQM computations is indicated. Significance thresholds specific for each linkage group (sign. threshold LG) were calculated using at least 1000 permutations. The percentages of variance explained by the respective QTLs (%expl.) for genetic markers and confidence intervals are listed

[a] Bold indicates the linkage group with the major QTL

[b] Confidence interval is LOD max ±1 LOD

linked to the 341-bp GF-15-28 allele and the 136-bp GF-15-07 allele (Table 3). These are double heterozygous markers (hk × hk) that are present in 'Seyval', 'Subereux', and Seibel 6468 lines. Screening of the separate female and male maps confirmed both parental genotypes contributed to this locus.

A different QTL was observed during the abnormally hot 2003 and in 2004. It was present on LG18 (Table 3), and had a maximum LOD value of 4.6 (explaining 14 % of the phenotypic variance). Interestingly, a nearby QTL region on LG18 re-appeared in 2005 and 2006, functioning with the LG15 locus whose effects were lower than in 1999 and 2000. The *E. necator* resistance QTL on LG18 had weaker effects and larger confidence intervals over time than the QTL on LG15. The QTL on LG18 was linked to SNP markers, including SPS_P_SNP632GF, VV18_9582805, and VV18_12207575, as well as to the SSR marker UDV117. Because of its relatively small effects that are difficult to detect, the position of the confidence interval varied. This QTL appears to be inherited from the 'Villard blanc' cultivar.

A QTL on LG15 near marker GF15-28 also affected the susceptibility of berries to natural *E. necator* infections in 1999 and 2000. This QTL was less potent in berries than in leaves, and shifted its position to GF15-05 in 2006. A minor QTL on LG18 was observed in a similar confidence interval in 2004.

Quantitative trait locus analysis of the onset of veraison and the time interval between flowering and veraison

Veraison is a critical developmental stage in grapevine fruit ripening, during which the final ripening phase of berries is initiated. This stage is a major determinant of maturity time (Sadras and Petrie 2011). The onset of veraison was determined over 5 years (i.e., 1998, 1999, 2008, 2009, and 2010). Additionally, flowering time was recorded over 4 years (i.e., 1999, 2008, 2009, and 2010). There were clear correlations between years (Supplemental Table 4c). The QTL for veraison detected on LG16 had a maximum LOD value of 35, which explained up to 70 % of the observed phenotypic variance (Table 4). This major QTL was located within a narrow range (i.e., 43.4–46.4 cM) around markers UDV52-166 and SNP1092P11R in all years, with minimal changes in its position. This QTL likely corresponds to the VMC1E11 marker in the vicinity of UDV52 (on LG16) that influences the onset of berry ripening in the progenies of the 'Regent' × 'Lemberger' cross (Fischer et al. 2004). Therefore, we named this QTL *Ver1*. In all years, the genotypic classes "ac" and "ad" at marker UDV52-166 resulted in a later onset of veraison than the "bc" and "bd" genotypes. Thus, the early onset of veraison trait was inherited from the maternal parent (i.e., the early ripening GF.GA-47-42).

A relatively weak QTL on LG18 detected during the interval mapping of the GF.GA-47-42 × 'Villard blanc'

Table 4 QTL analysis of Véraison and the interval of flowering to Véraison

Trait	Year	LG carrying QTL[a] (IM)	LG Carrying QTL[a] (MQM)	MQM Cofactor	Significance threshold	LOD_{max}	%expl	Confidence interval[b]	Linked marker(s)
Veraison	1998	**LG16**			2.9	17.4	56.6	42.4–46.4 cM	UDV52-166; SNP1092P11R
Veraison	1998	LG18			3.0	3.39	15.0	0.0–16 cM	VMC3E5, VMC8B5
Veraison	1998		LG14	UDV52-166	3.2	5.29	10	71.1–73.5	GF14-05, GF14-18
Veraison	1998		**LG16**	UDV52-166	2.9	17.4	56.6	42.5–46.4 cM	UDV52-166; SNP1092P11R
Veraison	1999	**LG16**			3.1	25.9	56.3	42.5–46.4 cM	UDV52-166;GF16-18
Veraison	1999	LG18			3.0	5.94	17.3	28.0–38.1 cM	SPS_P_SNP632GF; VV_18_9582805
Veraison	1999		**LG16**	UDV52-166	3.1	25.9	54.9	42.4–46.4 cM	UDV52-166; GF16_18
Veraison	1999		LG18	UDV52-166	3.0	3.38	4.6	36.2–48.1 cM	SNP883_160; SNPVVI_9920
Veraison	1999		LG14	UDV52-166	2.9	4.8	6.4	62.3–73.5 cM	GF 14-05
Veraison	2008	**LG16**			3.0	35.27	70.0	43.4–46.4 cM	UDV52-166; SNP1092P11R
Veraison	2008		**LG16**	UDV52-166	3.0	35.27	70.0	43.4–46.4 cM	UDV52-166; SNP1092P11R
			LG12	UDV52-166	2.9	3.82	4.0	54.8–65.9 cM	GF12-09; SNP1119_176
			LG14	UDV52-166	2.8	4.82	4.9	47.0–61.3 cM	GF14-33; SNP421_234
Veraison	2009	**LG16**			3.2	32.8	63.5	40.8–45.4 cM	UDV52-166; SNP1092P11R
Veraison	2009	LG18			3.1	6.13	17.2	27.9–34.2 cM	SPS_P_SNP632
Veraison	2009		LG01	UDV52-166	3.2	4.73	5.1	3.3–17.8 cM	GF01-13; SNP1021_163
Veraison	2009		LG05	UDV52-166	3.0	4.41	4.8	43.5–51.28 cM	SNPVV-5_16681905; VVMD24
Veraison	2009		**LG16**	UDV52-166	3.2	32.25	62.8	40.8–46.4 cM	UDV52-166; GF16-18
Veraison	2010	**LG16**			2.9	32.5	64.9	41.4–46.4 cM	UDV52-166; GF16-18
Veraison	2010	LG18			3.0	4.54	13.6	28.9–36.2 cM	SPS_P_SNP632
Veraison	2010		LG14	UDV52-166	3.1	4.78	45.3	47.0–68.1 cM	UDV33; VMC2A5
Veraison	2010		**LG16**	UDV52-166	2.9	32.27	64.6	41.5–46.4 cM	UDV52-166; GF16-18
Flow-Ver	1999	**LG16**			3.0	6.82	18.9	29.0–37.9 cM	VVMD5; GF16-47
Flow-Ver	1999	LG17			2.9	3.64	10.6	36.9–38.1 cM	EDS1_CF_ SNP1520GF
Flow-Ver	1999		**LG16**	UDV52-166	3.0	4.97	14.2	40.8–47.4 cM	UDV52-166; GF16-18
Flow-Ver	1999		LG17	UDV52-166	2.9	3.53	8.8	36.9–38.1 cM	EDS1_CF_ SNP1520GF
Flow-Ver	2008	**LG16**			3.1	8.27	22.7	42.4–47.4 cM	UDV52-166; SNP1092P11R
Flow-Ver	2008		**LG16**	UDV52-166	3.1	8.27	22.7	42.4–47.4 cM	UDV52-166; SNP1092P11R
Flow-Ver	2009	**LG16**			3.0	33.3	63.8	41.4–46.4 cM	UDV52-166; SNP1092P11R
Flow-Ver	2009		LG05	UDV52-166	2.9	5.34	5.6	51.3–66.0 cM	UDV111; GF05-12
Flow-Ver	2009		**LG16**	UDV52-166	3.0	33.3	63.8	41.4–46.4 cM	UDV52-166; GF16-18
Flow-Ver	2010	**LG16**			3.0	13.3	33.8	41.4–47.4 cM	UDV52-166; SNP1092P11R
Flow-Ver	2010		**LG16**	UDV52-166	3.0	13.3	33.8	41.4–47.4 cM	UDV52-166; SNP1092P11R
Flow-Ver	2010		LG09	UDV52-166	2.9	4.24	8.3	33.7–39.27 cM	VMC2E11

Quantitative trait locus analysis for the timing of the onset of veraison and the interval between flowering and veraison

IM results from interval mapping, *MQM* results from multiple QTL analysis. The cofactor used for MQM computations is indicated. Significance thresholds specific for each linkage group (sign. threshold LG) were calculated using at least 1000 permutations. The percentages of variance explained by the respective QTLs (%expl.) for genetic markers and confidence intervals are listed

[a] Bold indicates the linkage group with the major QTL

[b] Confidence interval is LOD max ±1 LOD

cross affected the onset of veraison in 4 out of 5 years. This QTL was responsible for 13.6–17.3 % of the phenotypic variance, and had a confidence interval of 27–38 cM over 3 years. However, in subsequent MQM analyses, the QTL dropped below the LG-specific significance level or shifted its position. This may have been because of the effects of small inconsistencies in marker order or haplotype determination resulting from missing data (Van Ooijen pers. comm. to Iris Ochßner). Because of its repeated appearance in this study and in extended data sets from 2011 to 2012 (data not shown), we named this QTL *Ver2*. It co-segregates with marker SPS_P_SNP632GF.

An earlier investigation of this population reported the presence of QTLs affecting flowering time on LGs 1, 4, 8, 14, 17, and 19 (Fechter et al. 2014; Zyprian unpublished). To better characterize the genetic basis of developmental behaviors among individuals in this population, we searched for QTLs influencing the interval between the flowering stage and veraison in 1999, 2008, 2009, and 2010. The results are provided in Table 4. A major effect was detected for *Ver1* on LG16, with a maximum LOD value of 33.3, which explained up to 63.8 % of the observed phenotypic variance. However, we identified some minor QTLs, which were previously associated with flowering time.

Discussion

Reliability and utility of single nucleotide polymorphism markers

We investigated the reproducibility of SNP genotyping using two different technologies, Illumina bead hybridizations and FluiDigm assays. Both yielded fairly consistent results. The majority of the 384 SNPs tested by hybridization were originally detected in *Vitis vinifera* wine- and table-grape varieties as well as in two *Vitis vinifera* ssp. *sylvestris* accessions (Emanuelli et al. 2013). Our investigation revealed that ~50 % of the tested SNPs were informative and transferable in a cross between grapevines with different genetic backgrounds and complex pedigrees. Integrations with SSR markers in the parental or integrated genetic maps did not cause any problems. Additionally, QTL analyses identified SNPs associated with traits important in grapevine breeding (e.g., SPS_P_SNP632 on LG18 linked to *Ver2*; Table 4). Using SNP genotyping with FluiDigm assays enables the application of small, well-selected sets of trait-linked SNP markers in breeding programs.

Quantitative trait loci for *P. viticola* resistance

Analyses of the susceptibility of field-grown grapevines and artificially inoculated leaf material resulted in the detection of a major QTL for downy mildew resistance on LG18. The most prominent effects were derived from 'Villard blanc' alleles, but there were also contributions from GF.GA-47-42. An important downy mildew resistance QTL was identified on chromosome 18 of the grapevine cultivar 'Regent' (Fischer et al. 2004; Welter et al. 2007), and was named *Rpv3* in the 'Bianca' cultivar (Bellin et al. 2009). Both cultivars are closely related to 'Villard blanc' (Akkurt et al. 2007). In the 'Regent' genetic map, the QTL was originally localized close to a sequence characterized amplified region (SCAR)/cleaved amplified polymorphic sequence marker in the alcohol dehydrogenase gene. The QTL was close to the UDV112 marker, and was mapped to Chr18_random. Previous genetic maps were mainly based on random amplified polymorphic DNA (RAPD) and AFLP markers, and did not contain many SSR loci, which are frequently transferable between different grapevine varieties. Therefore, confidence interval comparisons between the old 'Regent' map and the new 'Villard blanc' genetic map are limited. However, the *Rpv3* locus in the 'Regent' map was confirmed in the 'Regent' × 'Red Globe' cross (van Heerden et al. 2014), and was localized to marker VMC7F2, which is the same as GF18-08. The GF18-08 marker was detected using re-designed primers (for improved amplification efficiency) specific for the sequence flanking the same SSR as VMC7F2. The VMC7F2 marker is also an *Rpv3*-linked marker in the 'Bianca' genetic map, corresponding to position 26.896.989 of chromosome 18 in the 12× reference genome sequence (Jaillon et al. 2007). Therefore, the identified QTL corresponds to *Rpv3*, which confirms the earlier results. Its confidence interval was delimited to a 4.7 cM genomic region, which is smaller than the corresponding region in 'Regent' grapevine [i.e., 17–35 cM (Welter et al. 2007) or 16.6 cM (van Heerden et al. 2014)]. This part of the genome contains a cluster of nucleotide-binding site, leucine-rich repeat resistance gene analogs (Velasco et al. 2007; Moroldo et al. 2008). It will be a challenge to identify which genes are responsible for downy mildew resistance. Because there are contributions from both parents of the mapping population, we suggest the locus should be called *Rpv3-1* in 'Villard blanc' and *Rpv3-2* in GF.GA-47-42 grapevines.

Using the *Rpv3*-flanking SSR loci UDV305 (position 24.868.404) and UDV737 [position 26.050.225 in the PN40024 (12x) sequence], Di Gaspero et al. (2012) reported that the continual use of limited sources of downy mildew resistance in breeding programs has generated seven different *Rpv3* haplotypes, which have been retained in resistant grapevine cultivars. One of these haplotypes is *Rpv3* 299_279, which is present in the cultivars 'Villard blanc', 'Regent', and its parent 'Chambourcin'. It corresponds to *Rpv3-1* in our study. This haplotype was traced back to *V. rupestris* based on an allelic survey of UDV305

Table 5 Haplotypes of *Rpv3*

Marker	Substituted marker	Position	`Regent´			`Villard blanc´		Seibel 6468	`Subereux´		GF.GA-47-42		`Seyval´	
GF18-14	UDV737	26,050,358	100	**84**	105	**84**	0	**84**	**105**	*102*	96	*102*	**74**	*102*
GF18-08	VMC7F2	26,897,795	393	**399**	383	**399**	387	**399**	**383**	*389*	392	*389*	**0**	*389*
Haplotype according to Di Gaspero et al. (2012)			**Rpv3 299–279 *V. rupestris***						*Rpv3 null-297 Munson*				Rpv3 null-271 Noah*Rpv3 null-297 Munson*	
			Rpv3-1					*Rpv3-2*						

Haplotypes of *Rpv3* determined in this study using published data (Di Gaspero et al. 2012). The origins of the alleles are indicated by differences in font (e.g., bold, italics, or regular)

(Di Gaspero et al. 2012). The breeding line GF.GA-47-42 has 'Seyval' as its resistant parent. 'Seyval' carries two *Rpv3* haplotypes, namely null-271 and null-297, which differ from each other and from the 'Villard blanc' haplotype. It was suggested that null_271 originated from the cultivar 'Noah' [(*V. labrusca* × *V. riparia*) × *V. labrusca*], while null-297 was derived from 'Munson' (*V. lincecumii* × *V. rupestris*) (Di Gaspero et al. 2012). It was unclear which of these *Rpv3* haplotypes was transmitted to GF.GA-47-42. Analysis of the alleles at markers GF18-14 and GF-18-08 at the *Rpv3* locus (Table 5) indicates GF.GA-47-42 carries the 'Munson' *Rpv3* haplotype, which is called *Rpv3-2*. Considering *Rpv3-1* was ineffective against a special *P. viticola* isolate (Delmotte et al. 2014), identifying allelic variants like *Rpv3-2* generates important information relevant for breeding disease-resistant cultivars.

Investigations of *P. viticola* fungicide resistance revealed the genetic variability within isolates from France and Italy is higher than originally expected (Genet and Jaworska 2013). If the *P. viticola* strains in our viticulture area (Rhineland-Palatinate region) are similarly genetically diverse, this may account for part of the variability observed in QTL analyses over time. It may also explain why some unstable QTLs were detected only in a few years. However, in our study, the major QTL corresponded to *Rpv3* on LG18, and field strains able to overcome the resistance mechanism encoded by *Rpv3-1* and *Rpv3-2* were not detected.

Quantitative trait loci for *Erysiphe necator* resistance

We detected a major QTL mediating *E. necator* resistance on LG15. A QTL for *E. necator* resistance was identified earlier in the 'Regent' cultivar (Welter et al. 2007) based on RAPD/AFLP markers and the SCAR marker ScORA7-760 (Akkurt et al. 2007). This locus is called *Ren3* (i.e., resistance to *Erysiphe necator*). The amplicon sequence of ScORA7-760 partially maps to PN40024 chromosome 15 at position 9.410.227, which is very close to the trait-linked marker GF15-28 (i.e., a revised VVIV67 located at position 10.897.461), and is linked to the major QTL. Thus, this locus corresponds to *Ren3*, which contains contributions from both parents. The locus was delimited to a confidence interval of 0.8 cM, which is much smaller than that reported in previous studies.

We also identified a new QTL on LG18 affecting *E. necator* resistance. It appears to mainly affect leaf resistance, and was detected following the extremely warm and dry 2003 growing season. The *E. necator* resistance loci on LG18 were previously described in the Chinese grapevine *V. romanetii* (*Ren4*) and in two cultivars of *Muscadinia rotundifolia* (*Run2.1* and *Run2.2*) (Riaz et al. 2011; Mahanil et al. 2012). These loci overlap with *Rpv3* around

marker VMC7F2 (26.8 Mb). It is possible the disease resistance QTL on LG18 may overlap with *Run2* or *Ren4*. However, this QTL is definitely from a different resistance source than that of *Run2* and *Ren4*. Therefore, we propose this QTL should be named *Ren8*. Because fewer QTLs have been reported for *E. necator* resistance than for *P. viticola* resistance (Gadoury et al. 2012), a thorough characterization of the *Ren8* locus has important implications for breeding disease-resistant cultivars. Additionally, *Run2* and *Ren4* were studied using pathogen strains from the USA (Ramming et al. 2011), while *Ren3* and *Ren8* were identified during infections with common European pathogen strains.

Interestingly, *Ren8* was identified during and after a period of high temperatures. The reasons for this are unknown. It is possible that the increased exposure to heat and sunlight in 2003 reduced and/or altered the pathogen population, and remnants of these newly adapted "best fitting strains" were still present in 2004 and beyond.

Candidate genes in the *Rpv3*, *Ren3*, and *Ren8* loci

On a molecular level, there is synteny between the reference genome sequence of PN40024, a pathogen-susceptible *V. vinifera* line, and the genomic sequence of disease-resistant grapevine breeding materials. Evidence for this is provided by the fact that several SSR markers can be transferred from the reference genome sequence into breeding lines with complex pedigrees, including non-*V. vinifera* accessions (see the "Simple sequence repeat marker analysis" subsection of "Materials and methods"). However microsynteny in the range of a few hundred or several thousands of bp may be disturbed in genomic regions introduced by introgression from wild species resistance gene donors during breeding (Dudenhöffer 2012). The introgressed regions should undergo molecular analyses, and it may also be useful to search for candidate genes in the regions associated with resistance in the model genome. Such genes may be present in non-functional alleles in susceptible grapevines. Accordingly, we screened the reference genome sequence using the current annotations of the 12× version present in the Gramene database (http://ensembl.gramene.org/Vitis_vinifera/Info/Index). A 0.4-Mb window around the genomic positions of the trait-linked markers was used.

For *Rpv3*, the positional candidates contain a leucine-rich repeat protein-encoding gene (VIT_18s0041g01790). Marker GF15-28 in the *Ren3* locus is located just a few bp upstream of a serine/threonine protein kinase gene (Vit_15s0021g00970). The *Ren8* locus identified on LG18 is near a nucleotide-binding protein gene (VIT_18s0075g00790) and a Toll/interleukin-1 receptor domain gene that encodes a protein similar to the tobacco mosaic virus resistance protein N from tobacco (VIT_18s0075g00660). All of these annotated genes encode proteins with functions that are common among the major classes of plant resistance genes (Dangl and Jones 2001). Additionally, the *Ren8* locus is close to a gene encoding a Smg-4/UPF3 family protein (VIT_18s0075g00710). This type of gene is involved in regulating defense responses in *Arabidopsis thaliana* (Jeong et al. 2011). Further profiling of expression patterns and investigations of sequence diversity in resistant grapevines will clarify the functional relevance of these candidate genes.

Quantitative trait locus analysis and identification of candidate genes associated with veraison

We identified a prominent QTL (*Ver1* on LG16) associated with the timing of the onset of veraison. *Ver1* was accompanied by a QTL (*Ver2* on LG18) that had a smaller effect. The strong effects of *Ver1* were also associated with the required interval between flowering and the onset of veraison. A QTL affecting veraison on LG16 was previously described (Fischer et al. 2004) in the genetic map of the 'Regent' cultivar using more primitive marker technology, which prevented an accurate analysis of its localization. A veraison QTL on LG16 was identified by Costantini et al. (2008) in a cross between table grape cultivars 'Italia' and 'Big Perlon'. In their study, the QTL had a confidence interval of about 5 cM around marker VMC1E11, and was flanked mostly by AFLP markers. VMC1E11 is positioned at 13.708.473 in the 12× PN40024 sequence, while UDV52 starts at position 15.756.966 on LG16. These QTL regions may be identical. However, the QTL around UDV52 inherited from GF.GA-47-42 has a smaller confidence interval (~2 cM). The position of *Ver1* determined in this study differs from that reported in a previous study (Duchene et al. 2012), in which a QTL associated with veraison on LG16 around marker VVMD37 was determined to have a relatively large confidence interval (i.e., extending over 15 cM). However, because of the lower marker coverage of LG16 in their study, and the fact they analyzed a different mapping population (i.e., 'Riesling' × 'Gewürztraminer'), the available genetic information is not completely comparable.

Analyses of the genes present surrounding *Ver1* around UDV52 revealed the presence of a group of three transcription factor genes, including *CBF4* (VIT_16s0100g00380), and two transcription factor genes involved in ethylene-activated signaling pathways (VIT_16s0100g00390 and VIT_16s0100g00400). *CBF4* is involved in responses to low temperatures as well as freezing tolerance in grapevine (Xiao et al. 2008). The two ethylene-responsive transcription factor genes may be better candidates as determinants of veraison onset. Regarding the *Ver2* locus on LG18, there are two interesting candidate genes. First, there is a gene encoding a histone deacetylase and chromatin remodeling factor

SNF2. Chromatin remodeling influences the accessibility of nucleosome-covered DNA segments and is involved in developmental processes (Gentry and Hennig 2014) such as berry ripening. Second, there is a gene encoding a flowering time control FCA-like protein (VIT_18s0075g00650) about 0.4 Mb from the QTL peak at marker SPS_P_SNP632GF. This gene may be involved in regulating developmental processes, including the timing of flowering. It may also affect the interval between flowering and the onset of veraison, as well as the downstream pathways leading to veraison.

In conclusion, this study on the segregating population resulting from a cross between GF.GA-47-42 and 'Villard blanc' further characterized previously identified resistance QTLs. The allelic forms of the *Rpv3* locus mediating resistance to downy mildew were designated as *Rpv3-1* and *Rpv3-2*. For powdery mildew, the *Ren3* locus was further delimited, and a new minor QTL, *Ren8*, was identified. A major QTL for the onset of veraison, *Ver1*, was detected and shown to be supplemented by a minor QTL, *Ver2*. New tools for marker-assisted breeding may be developed using the information generated in this study. Furthermore, all of the identified QTL regions carry promising candidate genes that should be functionally characterized. This will increase our understanding of resistance to obligate biotrophic pathogens and fruit ripening in grapevine.

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Compliance with ethical standards

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Conflict of interest All authors have no conflicts of interest to declare.

Ethical standards The authors declare that all experiments conducted in this study complied with the applicable laws in Germany and France. This article does not describe any studies with human participants or animals performed by any of the authors.

References

Akkurt M, Welter L, Maul E, Töpfer R, Zyprian E (2007) Development of SCAR markers linked to powdery mildew (*Uncinula necator*) resistance in grapevine (*Vitis vinifera* L. and *Vitis* sp.). Mol Breeding 19:103–111

Austin CN, Wilcox WF (2012) Effects of sunlight exposure on grapevine powdery mildew development. Phytopathology 102:857–866

Barnavon L, Doco T, Terrier N, Ageorges A, Romieu C, Pellerin P (2000) Analysis of cell wall neutral sugar composition, beta-galactosidase activity and a related cDNA clone throughout the development of *Vitis vinifera* grape berries. Plant Physiol Biochem 38:289–300

Bellin D, Peressotti E, Merdinoglu D, Wiedemann-Merdinoglu S, Adam-Blondon AF, Cipriani G, Morgante M, Testolin R, Di Gaspero G (2009) Resistance to *Plasmopara viticola* in grapevine 'Bianca' is controlled by a major dominant gene causing localised necrosis at the infection site. Theor Appl Genet 120:163–176

Cabezas JA, Ibanez J, Lijavetzky D, Velez D, Bravo G, Rodriguez V, Carreno I, Jermakow AM, Carreno J, Ruiz-Garcia L, Thomas MR, Martinez-Zapater JM (2011) A 48 SNP set for grapevine cultivar identification. BMC Plant Biol 11:153

Choudhury RA, McRoberts N, Gubler WD (2014) Effects of punctuated heat stress on the grapevine powdery mildew pathogen, *Erysiphe necator*. Phytopathol Mediterr 53:148–158

Cipriani G, Marrazzo M-T, Di Gaspero G, Pfeiffer A, Morgante M, Testolin R (2008) A set of microsatellite markers with long core repeat optimized for grape (*Vitis* spp.) genotyping. BMC Plant Biol 8:127

Costantini L, Battilana J, Lamaj F, Fanizza G, Grando MS (2008) Berry and phenology-related traits in grapevine (*Vitis vinifera* L.): From Quantitative Trait Loci to underlying genes. BMC Plant Biol 8:38

Dangl JL, Jones JDG (2001) Plant pathogens and integrated defence responses to infection. Nature 411:826–833

Deglene-Benbrahim L, Wiedemann-Merdinoglu S, Merdinoglu D, Walter B (2010) Evaluation of downy mildew resistance in grapevine by leaf disc bioassay with *in vitro*- and greenhouse-grown plants. Am J Enol Vitic 61:521–528

Delmotte F, Mestre P, Schneider C, Kassemeyer HH, Kozma P, Richart-Cervera S, Rouxel M, Deliere L (2014) Rapid and multiregional adaptation to host partial resistance in a plant pathogenic oomycete: evidence from European populations of *Plasmopara viticola*, the causal agent of grapevine downy mildew. Infect Genet Evol 27:500–508

Di Gaspero G, Copetti D, Coleman C, Castellarin SD, Eibach R, Kozma P, Lacombe T, Gambetta G, Zvyagin A, Cindric P, Kovacs L, Morgante M, Testolin R (2012) Selective sweep at the *Rpv3* locus during grapevine breeding for downy mildew resistance. Theor Appl Genet 124:277–286

Duchene E, Butterlin G, Dumas V, Merdinoglu D (2012) Towards the adaptation of grapevine varieties to climate change: QTLs and candidate genes for developmental stages. Theor Appl Genet 124:623–635

Dudenhöffer J (2012) Diploma thesis: Physikalische Kartierung der Resistenzregion gegen den Echten Mehltau aus der Rebsorte 'Regent'. In: Faculty of Chemistry and Life Sciences. Karlsruhe Institute of Technology (KIT), Karlsruhe

Emanuelli F, Lorenzi S, Grzeskowiak L, Catalano V, Stefanini M, Troggio M, Myles S, Martinez-Zapater JM, Zyprian E, Moreira FM, Grando MS (2013) Genetic diversity and population structure assessed by SSR and SNP markers in a large germplasm collection of grape. BMC Plant Biol 13:39

Fechter I, Hausmann L, Daum M, Rosleff Sörensen T, Viehöver P, Weisshaar B, Töpfer R (2012) Candidate genes within a 143 kb region of the flower sex locus in *Vitis*. Mol Genet Genomics 287:247–259

Fechter I, Hausmann L, Zyprian E, Daum M, Holtgräwe D, Weisshaar B, Töpfer R (2014) QTL analysis of flowering time and ripening traits suggests an impact of a genomic region on linkage group 1 in *Vitis*. Theor Appl Genet 127:1857–1872

Fischer BM, Salakhutdinov I, Akkurt M, Eibach R, Edwards KJ, Töpfer R, Zyprian EM (2004) Quantitative trait locus analysis

of fungal disease resistance factors on a molecular map of grapevine. Theor Appl Genet 108:501–515

Gadoury DM, Cadle-Davidson L, Wilcox WF, Dry IB, Seem RC, Milgroom MG (2012) Grapevine powdery mildew (*Erysiphe necator*): a fascinating system for the study of the biology, ecology and epidemiology of an obligate biotroph. Mol Plant Pathol 13:1–16

Genet JL, Jaworska G (2013) Characterization of European *Plasmopara viticola* isolates with reduced sensitivity to cymoxanil. Eur J Plant Pathol 135:383–393

Gentry M, Hennig L (2014) Remodelling chromatin to shape development of plants. Exp Cell Res 321:40–46

Gessler C, Pertot I, Perazzolli M (2011) *Plasmopara viticola*: a review of knowledge on downy mildew of grapevine and effective disease management. Phytopathol Mediterr 50:3–44

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

Hunt HV, Lawes MC, Bower MA, Haeger JW, Howe CJ (2010) A banned variety was the mother of several major wine grapes. Biol Lett 6:367–369

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, The French-Italian Public Consortium for Grapevine Genome Characterization (2007) The grapevine genome sequence suggests ancestral hexaploidization in major angiosperm phyla. Nature 449:463–465

Jeong HJ, Kim YJ, Kim SH, Kim YH, Lee IJ, Kim YK, Shin JS (2011) Nonsense-mediated mRNA Decay Factors, UPF1 and UPF3, contribute to plant defense. Plant Cell Physiol 52:2147–2156

Jiang RHY, Tyler BM (2012) Mechanisms and evolution of virulence in oomycetes. Annu Rev Phytopathol 50(50):295–318

Mahanil S, Ramming D, Cadle-Davidson M, Owens C, Garris A, Myles S, Cadle-Davidson L (2012) Development of marker sets useful in the early selection of *Ren4* powdery mildew resistance and seedlessness for table and raisin grape breeding. Theor Appl Genet 124:23–33

Martins WS, Lucas DC, Neves KF, Bertioli DJ (2009) WebSat—a web software for microsatellite marker development. Bioinformation 3:282–283

Moroldo M, Paillard S, Marconi R, Fabrice L, Canaguier A, Cruaud C, De Berardinis V, Guichard C, Brunaud V, Le Clainche I, Scalabrin S, Testolin R, Di Gaspero G, Morgante M, Adam-Blondon AF (2008) A physical map of the heterozygous grapevine 'Cabernet Sauvignon' allows mapping candidate genes for disease resistance. BMC Plant Biol 8:66

Myles S, Chia J-M, Hurwitz B, Simon C, Zhong G-Y, Buckler E, Ware D (2010) Rapid genomic characterization of the genus *Vitis*. PLoS One 5:e8219

Ramming DW, Gabler F, Smilanick J, Cadle-Davidson M, Barba P, Mahanil S, Cadle-Davidson L (2011) A single dominant locus, *Ren4*, confers rapid non-race-specific resistance to grapevine powdery mildew. Phytopathology 101:502–508

Rex F, Fechter I, Hausmann L, Topfer R (2014) QTL mapping of black rot (*Guignardia bidwellii*) resistance in the grapevine rootstock 'Börner' (*V. riparia* Gm183× *V. cinerea* Arnold). Theor Appl Genet 127:1667–1677

Riaz S, Tenscher AC, Ramming DW, Walker MA (2011) Using a limited mapping strategy to identify major QTLs for resistance to grapevine powdery mildew (*Erysiphe necator*) and their use in marker-assisted breeding. Theor Appl Genet 122:1059–1073

Sadras VO, Petrie PR (2011) Climate shifts in south-eastern Australia: early maturity of Chardonnay, Shiraz and Cabernet Sauvignon is associated with early onset rather than faster ripening. Aust J Grape Wine Res 17:199–205

Schwander F, Eibach R, Fechter I, Hausmann L, Zyprian E, Töpfer R (2012) *Rpv10*: a new locus from the Asian *Vitis* gene pool for pyramiding downy mildew resistance loci in grapevine. Theor Appl Genet 124:163–176

Tena G, Boudsocq M, Sheen J (2011) Protein kinase signaling networks in plant innate immunity. Curr Opin Plant Biol 14:519–529

van Heerden CJ, Burger P, Vermeulen A, Prins R (2014) Detection of downy and powdery mildew resistance QTL in a 'Regent' × 'Red Globe' population. Euphytica 200:281–295

Van Ooijen JW (2006) JoinMap®4, Software for the calculation of genetic linkage maps in experimental populations. Kyazma B.V., Wageningen

Van Ooijen JW (2009) MapQTL6®, Software for the mapping of quantitative trait loci in experimental populations of diploid species. Kyazma B.V., 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 T, Facci M, Mitchell JT, Perazzolli 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 QZ, 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

Venuti S, Copetti D, Foria S, Falginella L, Hoffmann S, Bellin D, Cindric P, Kozma P, Scalabrin S, Morgante M, Testolin R, Di Gaspero G (2013) Historical introgression of the downy mildew resistance gene *Rpv12* from the Asian species *Vitis amurensis* into grapevine varieties. PLoS One 8(4):e61228

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 Breeding 20:359–374

Xiao H, Tattersall EAR, Siddiqua MK, Cramer GR, Nassuth A (2008) CBF4 is a unique member of the CBF transcription factor family of *Vitis vinifera* and *Vitis riparia*. Plant, Cell Environ 31:1–10

Zhang JK, Hausmann L, Eibach R, Welter LJ, Töpfer R, Zyprian EM (2009) A framework map from grapevine V3125 (*Vitis vinifera* 'Schiava grossa' x 'Riesling') x rootstock cultivar 'Börner' (*Vitis riparia* x *Vitis cinerea*) to localize genetic determinants of phylloxera root resistance. Theor Appl Genet 119:1039–1051

Zyprian E (unpublished) QTL analysis of flowering and veraison time

Zyprian E, Eibach R, Töpfer R (2005) Eine neue genetische Karte der Weinrebe aus der Kreuzung "Gf.Ga-47-42 × "Villard blanc". Deutsches Weinbau Jahrbuch 2006 Verlag Eugen Ulmer 57:151–158