Vitis **55**, 79–81 (2016) DOI: 10.5073/vitis.2016.55.79-81

Rpv14, a new genetic source for *Plasmopara viticola* resistance conferred by *Vitis cinerea*

I. Ochssner, L. Hausmann and R. Töpfer

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

Summary

A biparental population segregating for downy mildew resistance was studied to identify resistance linked molecular markers. The progeny of 202 individuals from a cross of V3125 (susceptible breeding line) with 'Börner' (resistant rootstock) was phenotyped in the field in four seasons and by evaluating artificially infected leaf discs. QTL mapping revealed a major resistance locus on chromosome 5 that explained up to 17.4 % of the phenotypic variance. This new resistance locus was named *Rpv14*. It was transmitted from the male grandparent *V. cinerea* Arnold to 'Börner' and is associated with the marker GF05-13.

Key words: downy mildew, *Plasmopara viticola*, MAS, resistance, *Rpv14*.

Introduction

Plasmopara viticola, the causal agent of downy mildew, is considered one of the most threatening grapevine pathogens. The introgression of resistance traits therefore is a major task in modern grapevine breeding programs. Breeding efforts aim at stacking different resistance loci from American and Asian *Vitis* species by the use of closely-linked molecular markers for the early selection of seedlings. Recent scientific work has thus focused on the identification and characterization of resistance loci from various grapevine resources to use this information for an introgression of the respective traits by marker-assisted selection (MAS). For downy mildew, resistance-related QTLs have already been described originating from *Muscadinia rotundifolia* (Merdinoglu *et al.* 2003), *Vitis riparia* (Marguerit *et al.* 2009, Moreira *et al.* 2011), *Vitis rupestris* (Di Gaspero *et al.* 2012) and *Vitis amurensis* (Blasi *et al.* 2011, Schwander *et al.* 2012, Venuti *et al.* 2013). Here, we describe a new locus conferring resistance against *P. viticola* (*Rpv14*) that is derived from *Vitis cinerea* and located on chromosome 5.

Material and Methods

A progeny of 202 F_1 individuals derived from V3125 ('Schiava Grossa' x 'Riesling') and 'Börner' (*V. riparia* Gm183 x *V. cinerea* Arnold) was used for evaluation of *P. viticola* leaf resistance. V3125 is a highly pathogen-susceptible, high-quality *V. vinifera* breeding line, while the interspecific hybrid and rootstock cultivar 'Börner' shows different degrees of tolerance to *Erysiphe necator* (powdery mildew), downy mildew, *Guignardia bidwellii* (black rot) and *Daktulosphaira viticola* (phylloxera). All vines, the mapping population and both parents, were planted with two plants of each genotype in a vineyard at Geilweilerhof (49° 13' 5" N, 8° 2' 45" E) in 1999 and 2002.

Downy mildew infections of leaves were screened in the field three times from July to September in four growing seasons (2009-2012). Minimal fungicide protection management was applied once before flowering mid of May in each year as reported by Rex *et al.* (2014). Dates of evaluation were chosen when the conditions for downy mildew were favorable and indicator vines (*V. vinifera* 'Müller Thurgau') showed symptoms. Evaluation was carried out according to OIV descriptor 452 (OIV 2009).

In addition to the field evaluation, leaf disc infection assays were performed in 2010/2011 using the method described by Schwander *et al.* (2012). The highly susceptible 'Müller Thurgau' was used as a positive control. In two leaf disc assays the grandparents of the population were also evaluated regarding the infection status.

For QTL analysis three types of phenotypic data sets were used: median values of field data, leaf disc assays and a combined data set (leaf discs + field). Field data derived from the year 2011 were excluded from the calculations due to the low infection status of the plants. QTL analysis was performed using an improved genetic map of the mapping population (Fechter *et al.* 2014). Both consensus and paternal maps were used for calculation with the program MapQTL 5.0 by interval mapping (Van Ooijen 2004). Linkage group-specific and significance thresholds were determined by permutation tests (1000 permutations) and the thresholds set to a value of $p < 0.05$. The QTL graph was drawn with MapChart 2.2 (Voorrips 2002).

Results and Discussion

Phenotpying in the field revealed different levels of resistance in the progeny ranging from complete resistance (= 1) to high susceptibility (= 9), with the female parent V3125 showing a medium to high degree of *P. viticola* infection level (7) and 'Börner' almost complete resist-

Correspondence to: Dr. L. Hausmann, Julius Kuehn-Institute (JKI), Federal Research Centre for Cultivated Plants, Institute for Grapevine Breeding Geilweilerhof, 76833 Siebeldingen, Germany. E-mail: ludger.hausmann@julius-kuehn.de

ance (2), respectively. This result was also confirmed in all leaf disc assays. In addition, high resistance (1) was observed for *V. cinerea* Arnold – the male parent of 'Börner' – and only a slight infection status (2) for *V. riparia* Gm183. Both parents of V3125, however, showed medium (5; 'Riesling') respective high (8; 'Schiava Grossa') susceptibility towards *P. viticola* infection in the leaf disc assays.

Interval mapping revealed a major QTL region for downy mildew resistance on chromosome 5 that explained up to 17.4 % of the total phenotypic variance. A significant association with marker VMC9b5 was identified in every year in all data sets from both field evaluation and leaf disc assays (Table). A second linkage to the two adjacent markers GF05-04 and VMC5e11 was detected only with the leaf disc assay data. Further QTL analysis with the parental maps revealed that the resistance is clearly associated with the three markers VMC9b5, GF05-13 and UDV_111 of the male parent 'Börner' (*V. riparia* Gm183 x *V. cinerea* Arnold) (Figure), with allele sizes observed as follows (*V. riparia* Gm183/*V. cinerea* Arnold): 240 bp/228 bp (VMC9b5); 0 bp/294 bp (GF05-13) and 94 bp/114 bp (UDV_111). From the two paternal grandparents *V. cinerea* Arnold appeared to be the only source with significant impact on *P. viticola* resistance within the mapping population. Using the average of all field evaluations, 95 % of the highly resistant genotypes carried the *V. cinerea* allele in the QTL region (data not shown). Additional tests with further genotypes using the nearest markers to the QTL peak revealed that plants carrying the *V. riparia* allele are generally more susceptible to *P. viticola* infection than the ones carrying the *V. cinerea* allele. On chromosome 5, QTLs for resistance to *P. viticola* have already been described for different grapevine sources such as 'Regent' (Fischer *et al.* 2004), 'Chardonnay' (Bellin *et al.* 2009) and 'Solaris' (Schwander et al. 2012). However, the QTL presented here mapped into a different genomic region of chromosome 5 and is therefore considered as a new resistance locus and designated *Rpv14*. According to the current table of *P. viticola* resistance loci it is the first one from *V. cinerea* (www.vivc.de, → Database search, → Data on breeding and genetics).

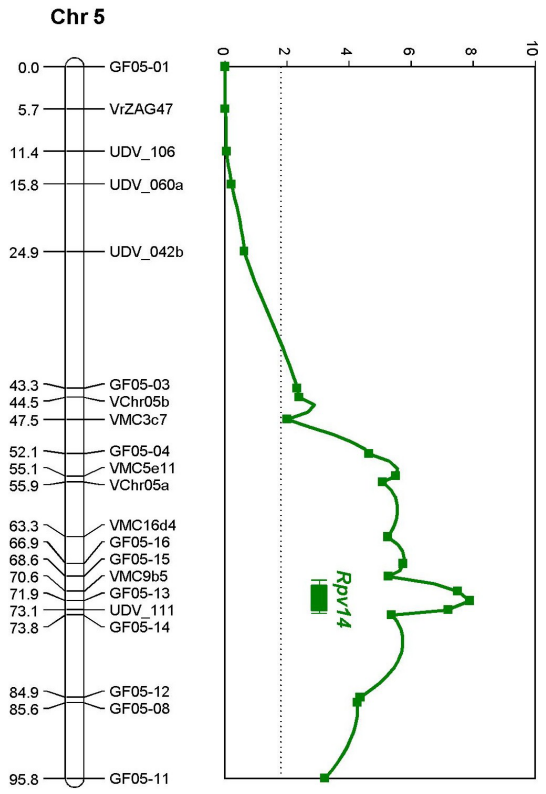

Figure: Left, paternal ('Börner') genetic map of chromosome 5 with marker positions in cM. Right, LOD profile for downy mildew resistance based on the combined data set of both field trials and leaf disc assays (solid line with squares). The 1-LOD (thick bar) and 2-LOD (thin line) support intervals for the *Rpv14* QTL encompass the region of markers VMC9B5, GF05-13 and UDV_111. The dotted line shows the linkage group-specific LOD threshold.

Table

Results of the QTL analysis for resistance to *Plasmopara viticola* in the mapping population V3125 x 'Börner' on chromosome 5 using the consensus map or the paternal map and different phenotypic data sets. cM = centiMorgan; LOD = logarithm of the odds

Data set	QTL position (cM)	Support interval 1-LOD (cM)	LOD peak	LOD threshold α = 0.05	Nearest marker	Variance explained (%)
Consensus map						
Field data	65.5	63.5-68.3	6.8	2.8	VMC9b5	16.1
	73.6	68.3-79.8	6.5	2.8	UDV_111	16.4
Leaf disc assays	49.9	30.5-54.3	4.6	2.7	GF05-04	10.5
	65.5	60.2-70.6	5.3	2.7	VMC9b5	12.2
Leaf discs + field	52.9	49.9-59.2	7.1	2.7	VMC5e11	16.6
	65.5	63.5-68.3	8.1	2.7	VMC9b5	17.4
	73.6	68.3-79.8	7.4	2.7	UDV_111	16.4
Paternal map						
Field data	70.6	68.6-73.8	5.3	3.0	VMC9b5	12.0
	79.8	73.8-86.6	4.4	3.0	-	11.6
Leaf disc assays	38.9	27.9-46.5	3.7	2.8	-	9.6
	70.6	63.3-79.8	4.7	2.8	VMC9b5	10.5
Leaf discs + field	71.9	69.6-73.8	7.9	1.8	GF05-13	17.0

An additional smaller QTL effect explaining up to 8.6 % of the total variance was observed on chromosome 1 with the corresponding marker GF01-09, but only when field data were used for calculation. Allele sizes for *V. riparia* Gm183 and *V. cinerea* Arnold were 100 bp and 97 bp, respectively. This smaller effect was not detected with data from leaf disc assays. Significant loci conferred by *V. riparia* Gm183 could not be observed although this genotype showed virtually no susceptibility towards *P. viticola* in either field evaluations or leaf disc assays. After extensive MQM mapping, very small effects could also be observed on linkage group 7 within the V3125 x 'Börner' population, but the allele distribution at this locus remains unclear due to insufficient marker density in this region. Resistance to *P. viticola* infections in *V. riparia* might consist of several minor loci which are then transferred to the progeny in different combinations, leading to the observed effects. Two or more resistance loci have been described also for other *V. riparia* accessions (Marguerit *et al.* 2009, Moreira *et al.* 2011).

Modern breeding intends to combine (stack) several resistance loci in one plant, thus making it more difficult for the pathogen to overcome the resistance of the plant (Eibach *et al.* 2007). This, however, is most likely possible by combining different resistance mechanisms. Since up to now the different mechanisms of resistance have not yet been resolved, the combination of loci from different sources is the current strategy of choice in grapevine breeding. The *V. cinerea* resistance locus *Rpv14* on chromosome 5 presents such a new source which is being introgressed into a *V. vinifera* genetic background. The respective molecular markers are available for MAS based breeding efforts.

Acknowledgements

We gratefully thank the German Federal Ministry of Education and Research (BMBF) for funding (grant no. 0315460B). Our further thanks go to A. Preiss and M. Daum for technical assistance, F. Schwander for helpful discussions and R. Eibach and his team for grapevine breeding.

References

Bellin, D.; Peressotti, E.; Merdinoglu, D.; Wiedemann-Merdinoglu, S.; Adam-Blondon, A. F.; 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 localized necrosis at the infection site. Theor. Appl. Genet. **120**, 163-176.

Blasi, P.; Blanc, S.; Wiedemann-Merdinoglu, S.; Prado, E.; Rühl, E. H.; Mestre, P.; Merdinoglu, D.; 2011: Construction of a reference linkage map of *Vitis amurensis* and genetic mapping of *Rpv8*, a locus conferring resistance to grapevine downy mildew. Theor. Appl. Genet. **123**, 43-53.

Di Gaspero, G.; Copetti, D.; Coleman, C.; Castellarin, S. D.; Eibach, R.; Kozma, P.; Lacombe T.; Gambetta, G.; Zvyagin, A.; Cindric, P.; Kovács, 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.

Eibach, R.; Zyprian, E.; Welter, L.; Töpfer, R.; 2007: The use of molecular markers for pyramiding resistance genes in grapevine breeding. Vitis **46**, 120-124.

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

Fischer, B. M.; Salakhutdinov, I.; Akkurt, M.; Eibach, R.; Edwards, K. J.; Töpfer, R.; Zyprian, E. M.; 2004: Quantitative trait locus analysis of fungal disease resistance factors on a molecular map of grapevine. Theor. Appl. Genet. **108**, 501-515.

Marguerit, E.; Boury, C.; Manicki, A.; Donnart, M.; Butterlin, G.; Nemorin, A.; Wiedemann-Merdinoglu, S.; Merdinoglu, D.; Ollat, N.; Decroocq, S.; 2009: Genetic dissection of sex determinism, inflorescence morphology and downy mildew resistance in grapevine. Theor. Appl. Genet. **118**, 1261-1278.

Merdinoglu, D.; Wiedemann-Merdinoglu, S.; Coste, P.; Dumas, V.; Haetty, S.; Butterlin, G.; Greif, C.; 2003: Genetic analysis of downy mildew resistance derived from *Muscadinia rotundifolia*. Acta Hortic. **603**, 451-456.

Moreira, F. M.; Madini, A.; Marino, R.; Zulini, L.; Marco Stefanini, M.; Velasco, R.; Kozma, P.; Grando, M. S.; 2011: Genetic linkage maps of two interspecific grape crosses (*Vitis* spp.) used to localize quantitative trait loci for downy mildew resistance. Tree Genet. Genomes **7**, 153-167.

OIV; 2009: Descriptor list for grape varieties and *Vitis* species, 2nd ed. O. I. V. (Off. Int. Vigne Vin), Paris, http://www.oiv.org

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

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.

Van Ooijen, J. W.; 2004: MapQTL® 5. Software for the Mapping of Quantitative Trait Loci in Experimental Populations. Kyazma B.V., Wageningen.

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**, e61228.

Voorrips, R. E.; 2002: Software for the graphical presentation of linkage maps and QTLs. J. Heredity **93,** 77-78.

Received December 12, 2015