

Co-Localization of QTLs for Seedlessness and Downy Mildew Resistance in Grapevine

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Abstract

A genetic linkage map was constructed using a pseudo-testcross strategy based on a cross between the seedless *Vitis vinifera* ‘Crimson Seedless’ and the complex hybrid ‘Villard Blanc’, resistant to downy mildew. A total of 315 DNA markers, including 262 AFLP, 48 simple sequence repeats (SSR), 2 SCARs (sequence characterized amplified region) and 3 minisatellite markers were used to generate a map for each parent. For both parents, 19 linkage groups were obtained, covering 1,111.0 cM and 926.0 cM for ‘Villard Blanc’ and ‘Crimson Seedless’, respectively. The position of SSR loci in the obtained maps is consistent with the genomic sequence. Quantitative Trait Loci (QTLs) for seedlessness and resistance to downy mildew were investigated. Two major effect QTLs for downy mildew resistance and seedlessness were mapped on the same region of linkage group 18. These QTLs explain 25.0-55.7% and 54.0-62.4% of the total variance, respectively. The MIKCC-Type MADS box gene *VvAG3*, whose orthologue is involved in *Arabidopsis* carpel and ovule development, and located in the confidence interval of the seedlessness QTL detected on the LG 18, could be considered as a candidate gene to control seed development in grape. Co-localizations were found in the same region, between the position of the *Rpv3* locus, which is very rich in TIR-NBS-LRR genes, and the main QTL identified for downy mildew resistance. Our results demonstrate that the same region of LG 18 contains important genetic determinants for seedlessness and downy mildew resistance in grapevine. Moreover, assessing the allelic variation at these agronomically important loci provides a basis for the development of marker-assisted selection for seedlessness and downy mildew simultaneously.

INTRODUCTION

The understanding of genetic and molecular mechanisms driving seedlessness in table-grapes is justified by their economic relevance, and most breeding programs have focused on the generation of new table grape cultivars combining seedlessness with other berry quality traits, such as large size, muscat flavour, or crispiness (Loomis and Weinberger, 1979). Since *Vitis vinifera* does not carry any resistance to downy mildew fungi (*Plasmopara viticola*), fungicide applications each growing season became indispensable for production (Cadle-Davidson, 2008). Therefore, grapevine breeding combining resistance, e.g., from American *Vitis* species, with other important berry quality traits of *V. vinifera* became an important strategy to combat the fungi in grape vineyards towards a more environmentally friendly and cost-efficient viticulture (Fisher et al., 2004).

To further investigate the complex genetic control of seedlessness and downy mildew resistance simultaneously in table-grapes, we have analyzed a progeny derived from a cross between the seedless *Vitis vinifera* ‘Crimson Seedless’ and the complex hybrid ‘Villard Blanc’, resistant to downy mildew. For this purpose we constructed parental genetic maps, mainly based on amplified fragment length polymorphism (AFLP)

and microsatellite markers, and performed QTL analyses of seed dry matter (SDM) and resistance to *P. viticola* in leaves. Our results confirm the existence of two major effect QTLs affecting both seed dry matter and downy mildew resistance in the same region of the distal part of the LG18. In addition, we show the potential usefulness of an LG18 microsatellite locus, VMC7F2, as a marker for seedlessness and downy mildew simultaneously.

MATERIAL AND METHODS

An F₁ segregating population was obtained from the cross between the cultivars ‘Crimson Seedless’ and ‘Villard blanc’ (Seyve-Villard 12.375) performed in 1998 via embryo rescue (Ramming et al., 1990). Ninety-four individuals of the population were randomly chosen for genotyping. The progeny was genotyped for 48 SSRs originated from different marker sets: VVS (Thomas and Scott, 1993), VVMD (Bowers et al., 1996, 1999), VrZAG (Sefc et al., 1999), VMC (Vitis Microsatellite Consortium), Scu (Scott et al., 2000) and VVC (Decroocq et al., INRA, unpublished). PCR over SSR loci was performed on the parents and the progeny in standard reactions of 20 µL following the protocols described by the corresponding authors. AFLP markers were produced after Vos et al. (1995) and polymorphisms were tested for 63 primer combinations. The parents and the progeny were also genotyped for three minisatellites (GACA₍₄₎, GTG₍₅₎ and M13 universal core sequence) and two SCAR markers (VR2, Fisher et al. 2004 and SCC8, Lahogue et al., 1998). DNA extraction and the molecular marker detection were carried out as described by Lefort and Douglas (1999) and Creste et al. (2001) respectively.

Seeds and seed traces were classified following Bouquet and Danglot (1996) in the year 2002 for 62 individuals of the population. Five classes of seedlessness were scored according to the percentage of SDM: class 5 (normal seeds, 72-80% SDM), class 4 (64-72% SDM), class 3 (56-64% SDM), class 2 (48-56% SDM) and class 1 (seed traces with 40-48% SDM). The mapping population was scored for resistance to downy mildew in the year of 2002 simultaneously in two different places. The evaluations were performed at the Tropical Experimental Station for Viticulture (Jales, SP, Brazil), omitting any fungicide protection, under natural field infection conditions and at Embrapa Uva e Vinho (Bento Gonçalves, RS, Brazil) in greenhouses after artificial inoculation. The degree of resistance to downy mildew was evaluated on leaves following the OIV index 452 (Anonymous, 1983). A final disease resistance score was attributed considering the lower level of resistance/susceptibility to downy mildew in the different evaluations and a mean value of resistance was also calculated based on the scores of the two phenotypic evaluations.

The genotypic information was subjected to genetic mapping through linkage and recombination analysis with JOINMAP 4.0 software (Van Ooijen and Voorrips, 2001), applying the Kosambi function (Kosambi, 1944). LOD score thresholds equal or greater than 4.0 were used to determine linkage groups. The maximal recombination fraction permitted was 0.4. Putative QTLs were primarily identified on individual parent linkage maps by interval mapping (Young, 1996). Subsequently, molecular markers coinciding or closely flanking the LOD maxima of QTLs were used as co-factors in multiple QTL analysis (restricted MQM and full MQM mapping). The linkage group specific and genome wide significance thresholds of QTL LOD scores were determined by permutation tests (1,000 permutations, $P \geq 0.05$) of the quantitative trait data (Churchill and Doerge, 1994) employing MAPQTL 4.0 software (Van Ooijen et al., 2000). The non-parametric Kruskal-Wallis test was used to detect significant associations between single marker genotypes and raw phenotypic data.

RESULTS AND DISCUSSION

Following the double pseudo-testcross strategy, marker sets from either parent were processed separately and both maps. The complete maternal map consisted of 150 markers (113 AFLPs, 33 SSRs, two SCARS and two minisatellites) mapped on 19 linkage groups covering 926 cM, with an average interval length of 6.21 cM. The complete

paternal map consisted of 189 markers (149 AFLPs, 37 SSRs, one SCAR, and mini-satellites) mapped on 19 linkage groups covering 1,111 cM with an average interval length of 5.87 cM. The linkage groups were numbered according to the international agreement achieved within the IGGP (International Grape Genome Program) except when no SSR could be mapped (data not shown). Most of the mapped SSR loci were placed in similar positions as compared to other mapping experiments in grape (Cabezas et al., 2006; Bellin et al., 2009; Mejia et al., 2007; Costantini et al., 2008; Welter et al., 2007).

QTL analysis was performed separately on the parental maps for each set of phenotypical data obtained and are shown on Table 1. Two major effect QTLs for downy mildew resistance and seedlessness were mapped in the same region of the distal part of the linkage group 18. These QTLs explained between 25.0-55.7% and 54.0-62.4% of total observed variance for downy mildew resistance and seedlessness, respectively. Since these two major QTLs explained a large percentage of total phenotypic variance independently for the two distinct traits (seedlessness and downy mildew resistance) they turn out to be good candidates to test linked markers that could be useful in table grape breeding. The closely linked microsatellite loci VMC7F2 (Pellerone et al., 2001), of segregation type abxcd (double heterozygous), was initially selected as possible marker, with genotype 194:210 bp in 'Villard blanc' and 198:200 bp in 'Crimson Seedless'. An uneven distribution of seedlessness classes against the VMC7F2 alleles was clearly observed (Fig. 1A), indicating a dominant effect of a Crimson Seedless allele for seedlessness in the F₁ progeny. A very similar situation was observed for the downy mildew resistance (Fig. 1B) where a skewed distribution towards the 194 bp allele of 'Villard blanc' is observed in the more resistant individuals of the progeny. The 198 bp and the 194 bp alleles of the VMC7F2 loci in 'Crimson Seedless' and 'Villard blanc' were thus found to be significantly associated with seedlessness ($\chi^2= 39.8$, $P \leq 0.0001$) and downy mildew resistance ($\chi^2= 65.73$, $P \leq 0.0001$), respectively. Therefore the VMC7F2 locus and possibly other loci in the same region could be good candidates for marker-assisted breeding of seedlessness and downy mildew simultaneously. In fact, the usefulness of the VMC7F2 locus for marker-assisted selection of seedlessness has already been proposed by Cabezas et al. (2006). However, the results of our work demonstrate that depending on the design of the cross, the same marker can also be used to simultaneously guide the selection for both traits.

General reliability of our results was supported by similar findings in other segregating populations for seedlessness (Doligez et al., 2002; Cabezas et al., 2006; Mejia et al., 2007; Costantini et al., 2008) and downy mildew resistance (Fisher et al., 2004; Welter et al., 2007; Bellin et al., 2009), where QTLs for both traits were found in the same region of the chromosome 18 and, in some of the studies, linked to the same SSR marker (VMC7F2), as in the case of seedlessness (Cabezas et al., 2006; Costantini et al., 2008) and the recently named *Rpv3* locus (Bellin et al., 2009), indicating that these loci could be closely linked. The results presented in this paper confirmed the previous findings showing that the major QTLs for seedlessness and for 'Villard blanc'-derived downy mildew resistance (*Rpv3* locus), are closely linked and co-localize to the same chromosomal region in LG 18.

With the availability of the grape genome sequence, searching the vicinity of the microsatellite VMC7F2 allowed us to identify groups of candidate genes that can be associated to seedless and downy mildew resistance. This region of the LG 18 is very rich in TIR-NBS-LRR genes (Di Gaspero et al., 2007). The evidence that resistance to downy mildew is based on the ability to mount hypersensitive response (HR) and that the class of genes clustered at the *Rpv3* locus are NBS-LRRs leads to the expectation that these genes might be essential for the onset of HR in grapes. Finally, the microsatellite VMC7F2 is very close to the predicted gene for *V. vinifera* MADS-box protein *VvAG3* (Boss et al., 2002; Diaz-Riquelme et al., 2009) whose orthologous is involved in *Arabidopsis* carpel and ovule development. Therefore, based on the flower carpel-specific expression already observed for this gene during grapevine inflorescence and berry development (Boss et al.,

2002; Diaz-Riquelme et al., 2009), *VvAG3* may be considered as candidate gene to control seed development in grape.

CONCLUSIONS

Conservatively, we detected the co-localization of two major QTLs responsible for the variation in seedlessness and the *Rpv3* locus for downy mildew resistance in the ‘Crimson Seedless’ × ‘Villard blanc’ F₁ progeny. The VMC7F2 microsatellite, closely linked to these two major QTLs, was shown to be a useful tool towards the design of a marker-assisted program for table grape improvement. Further work will be required to assess the allelic variation of other loci at this agronomically important region to provide a better basis for the development of marker-assisted selection for seedlessness and downy mildew simultaneously.

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Tables

Table 1. Description of the QTLs detected by parametric (Interval Mapping and MQM mapping) and non-parametric (Kruskal-Wallis) analysis for seedlessness and resistance to *Plasmopara viticola* based on the ‘Crimson Seedless’ × ‘Villard blanc’ segregating population.

Trait	Place/ Phenotype	QTL position				LOD maximum ^c		LOD threshold		Variance %	Kruskal-Wallis	
		Map	LG ^a	Peak position	Nearest marker ^b	Interval mapping	MQM	LG specific	Genome wide		K value	<i>p</i> value
Resistance to <i>P. viticola</i>	Jales	SV	18	82,8	VMC7F2	20,02	20,33	5,5	6,6	55,7	58,1	0.0001
	Bento	SV	18	84,6	22M4	4,76	4,76	9,0	10,2	25,0	18,5	0.0001
	Gonçalves											
	Final score	SV	18	82,8	VMC7F2	8,09	8,09	6,8	7,7	38,8	29,4	0.0001
	Mean value	SV	18	84,6	22M4	16,16	16,16	3,8	4,9	55,9	50,0	0.0001
Seedlessness	Dry matter	CS	18	11,7	VMC7F2	13,18	13,18	5,5	13,1	62,4	36,5	0.0001
	Phenotypic class	CS	18	7,5	46M6	10,45	-	-	-	54,0	34,2	0.0001

a Linkage groups named as per the International Grape Genome Program (IGGP) nomenclature.

b Nearest molecular markers to the peaks of the QTLs. 22M4 and 46M6 represents AFLP markers closely flanking VMC7F2.

c Maximum LOD score obtained by interval mapping and full MQM mapping.

Figures

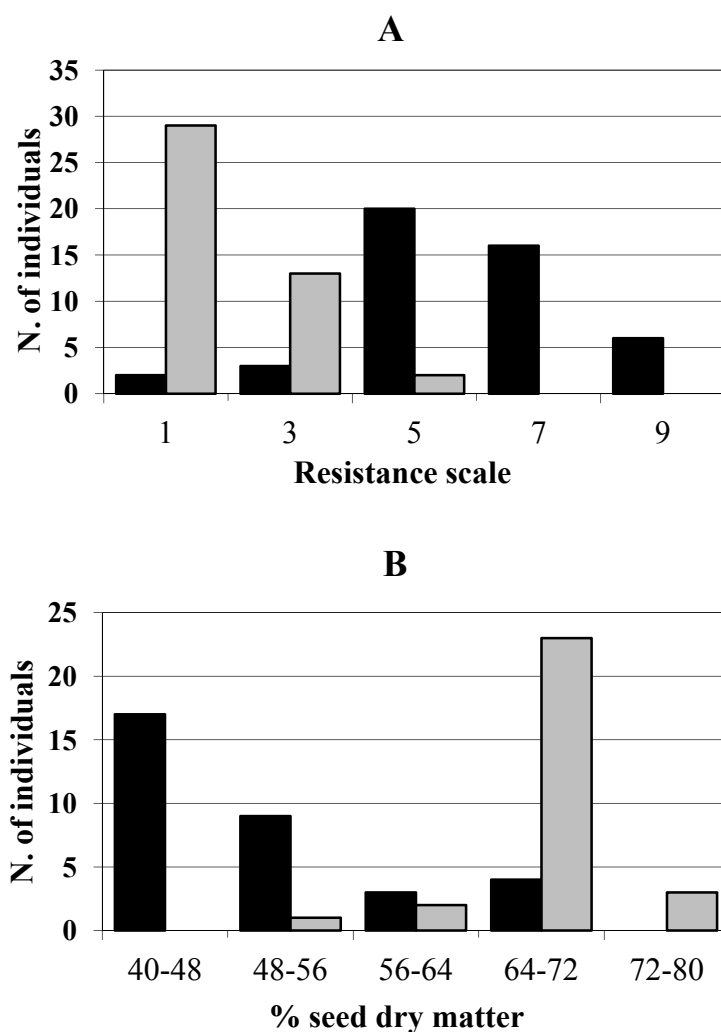


Fig. 1. Distribution of phenotypic traits of the 'Crimson Seedless' × 'Villard blanc' segregating population. A) Distribution of resistance level to *P. viticola* evaluated in Jales, 2002, for the individuals containing the 194 or 210 bp alleles of microsatellite marker VMC7F2, inherited from the resistant genitor 'Villard blanc'. Resistance scale used as described by OIV 452 descriptor. B) Distribution of percentage of seed dry matter for the individuals containing the allele 198 bp or allele 200 of microsatellite marker VMC7F2, inherited from the seedless genitor 'Crimson Seedless'. The VMC7F2 marker was used as dividing criteria to identify the two subpopulations with different alleles.

