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Research Note

Marker assisted selection (MAS) for powdery mildew resistance in a grapevine hybrid family

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K e y w o r d s : *Vitis vinifera* L., *Muscadinia rotundifolia* Michx., powdery mildew resistance, *Run*1 locus, molecular markers, MAS.

Introduction: Fungal disease resistance became a cardinal point of the grape breeding in the 19th century, when pathogens such as powdery mildew (*Uncinula or Erysiphe necator* Schwein) and downy mildew (*Plasmopara viticola* Berk. et Curtis ex. de Bary Berl. et de Toni) were carried to Europe from North America. European viticulture faced a new challenge and a significant environmental risk factor because of the necessity of repeated fungicide applications during the vegetation period, since traditional cultivars of *Vitis vinifera* origin do not carry any resistance to the mildew fungi. All sources providing high or partial resistance to these pathogens are low-quality wild *Vitis* species, therefore introgression of the resistance genes from them requires many back-crosses with *Vitis vinifera* in order to produce high quality cultivars (Fischer *et al*. 2004). *Muscadinia rotundifolia* was described as totally resistant to powdery mildew (PM) species due to a single locus called *Run*1 (for **R**esistance to ***U**ncinula **N**ecator* 1). This dominant gene was introduced into the *V. vinifera* genome using a pseudo-backcross strategy (Bouquet 1986). Molecular markers closely linked to *Run*1 locus have been identified (Pauquet *et al*. 2001; Donald *et al.* 2002), allowing MAS to be used in a breeding program.

Our research aimed at the application and validation of molecular markers linked to *Run*1 PM resistance locus in BC_5 individuals based on the results of Donald *et al*. (2002) and Barker *et al*. (2005) with PCR-RFLP (GLP1-12P1-P3), and SSR (simple sequence repeat) markers (VMC4f3.1, VMC8g9), respectively. The objective of the involvement of the SSR markers was to find a one-step way for identification of the PM resistant lines beside the reliable two-step PCR-RFLP.

Material and Methods: P l a n t m a t e r i a l : Individuals of BC_5 hybrid family (named 02-2) deriving from the {(*M. rotundifolia* x *V. vinifera*) BC_4} x Cardinal (Table) were applied (Kozma 2002).

E v a l u a t i o n o f p o w d e r y m i l d e w s y m p t o m s : The seedlings (4-6 leaf stage) were gravitationally infected with the conidia of the pathogen (in addition to the continuous abundance of the fungus) in the greenhouse in Pécs. Based on the symptoms after 3 weeks the plants were grouped as symptomless and PM-susceptible.

I s o l a t i o n o f D N A : The DNA was isolated with DNeasy® Plant Mini Kit (Qiagen, Biomarker Ltd., Gödöllő, Hungary) as described by the manufacturer.

P C R - R F L P a n d S S R a n a l y s e s : For PCR-RFLP the GLP1-12P1-P3 primers and *Eco*RI restriction analysis were applied (Donald *et al.* 2002). In SSR analyses VMC4f3.1 and VMC8g9 (Di Gaspero *et al*. 2000; pers. comm.) were used. PCRs and SSR allele size deter-

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Comparison of the results of phenotyping for powdery mildew symptoms and genotyping with molecular markers (shaded numbers indicate the „resistant allele" sizes)

Variety/ population	Phenotype		Molecular markers					
	Symptomless/ resistant	Susceptible/ sensitive	GLP1-12P1-P3 Endonucleolytic cleavage of PCR fragment with *Eco*RI enzyme		VMC4f3.1 alleles (bp)		VMC8g9 alleles (bp)	
	R	S	R yes	S no	R 186	S 184	R 160	S 167
Cardinal	-	+	-	+		164:164		179:179
VRH 3082-1-42 BC_4	+	-	+	-	184:186		160:167	
02-2 hybrid family BC_5	67	62	66	63	164:186 61	164:184 68	160:179 66	167:179 63
Ratio of recombinants			1/129 = 0.007		13/129 = 0.100		5/129 = 0.038	

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minations were carried out as it was described by Halász *et al.* (2005). The VMC8g9 PCR products were separated on a 3.5 % Metaphor agarose gel (Cambrex Bio Science, Rockland, ME, USA) in 1xTBE buffer.

Results and Discussion: As a first step 20 resistant and 20 susceptible BC_5 plants of the 02-2 hybrid family were selected according to PM symptoms on leaves and were tested with PCR-RFLP markers. One 870 bp DNA fragment was amplified both in healthy and susceptible plants (Figure, A). Symptomless and susceptible individuals could only be discriminated by restriction analysis of the PCR product. *Eco*RI cleaved the DNA amplicon of the symptomless leaves into two pieces (670 and 200 bp), while it did not split the PCR product of the susceptible samples (Figure, B).

Based on these results, altogether 142 seedlings from the BC_5 family were screened. SSR primers provided a way to monitor outcrosses, too. As a result, lines carrying „alien" alleles could be excluded from further analyses. The Table summarizes the data concerning the two parents (VRH 3082-1-42 (BC_4) and Cardinal) and the 129 progeny.

In the case of all three markers recombinants were obtained (Table): PM resistant individuals, whose GLP1-12P1-P3 (BC_4) PCR amplicons remained uncut after *Eco*RI cleavage or the SSR alleles coupled with the resistance were missing from them. In the case of VMC4f3.1 SSR a 186 bp, while in case of VMC8g9 a 160 bp allele proved to be a PM resistance linked marker (Table). In spite of the fact that the linkage of the applied markers proved to be lower than 100 %, they can be successfully applied in MAS since 90-99 % of the plants selected in this way will carry the *Run*1 PM resistance gene. With regard to economy the VMC8g9 is the most favourable of the three markers because the discriminative 160-167 bp fragments can be separated on an agarose gel of high resolution (Figure, C) following a simple PCR allowing of the routine analyses of many samples at the same time.

Based on physical and molecular mapping of *Run*1 locus (Barker *et al*. 2005) it can be expected that more tightly linked SSR or gene-specific markers will be available making MAS even more efficient and reliable. MAS benefits the breeders, providing a possibility for early screening of PM resistant seedlings and accelerating the selection in the case of pyramiding of resistance genes of different origin (e.g. *Run*1 and *Ren*1; Kozma *et al.* 2007).

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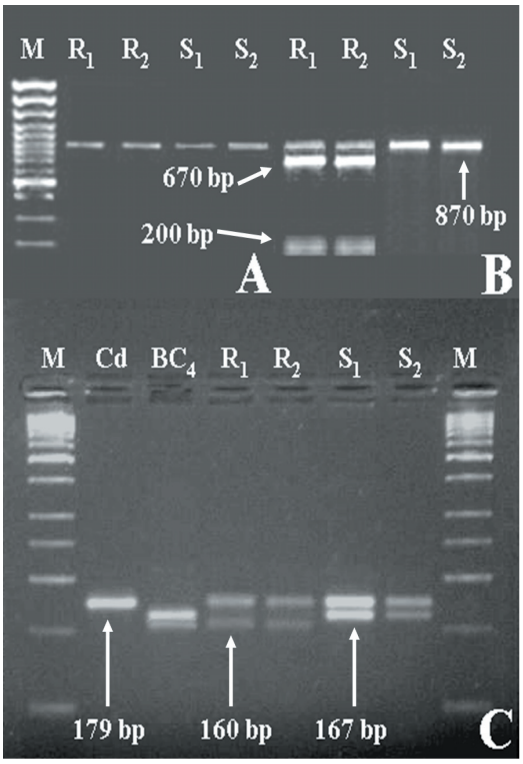

Figure: Distinction of resistant/symptomless and sensitive/susceptible genotypes by PCR-RFLP (GLP1-12P1-P3) and VMC8g9 SSR primers. (**A**): R_1, R_2: resistant and S_1, S_2 sensitive lines produced PCR fragments of the same size (870 bp); M: DNA molecular weight marker (Fermentas GeneRuler™ 100 bp ladder plus). (**B**): *Eco*RI restriction pattern of resistant (R_1, R_2) and sensitive (S_1, S_2) lines. Amplicons of the sensitive lines remained uncut. M: DNA molecular weight marker (Fermentas GeneRuler™ 100 bp ladder plus). (**C**): SSR profile of Cardinal (Cd), BC_4 (VRH 3082-1-42), R_1, R_2 resistant and S_1, S_2 sensitive genotypes. M: DNA molecular weight marker (Fermentas GeneRuler™ 50 bp DNA ladder).

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