Research Note

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

S. MOLNÁR1), Z. GÁLÁCS1), G. HALÁSZ2), S. HOFFMANN3), E. KISS1), P. KOZMA3), A. VIERES1), Z. GALLI1), A. SZŐKE1) and L. HESZKY1)

1) Szent István University, Institute of Genetics and Biotechnology, Gödöllő, Hungary
2) Höhere Bundeslehranstalt und Bundesamt für Wein- und Obstbau, Klosterneuburg, Austria
3) Research Institute of Viticulture and Enology, Pécs, Hungary

Key words: Vitis vinifera L., Muscadinia rotundifolia Michx., powdery mildew resistance, Run1 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 due 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.

Isolation of DNA: The DNA was isolated with DNeasy® Plant Mini Kit (Qiagen, Biomarker Ltd., Gödöllő, Hungary) as described by the manufacturer.

PCR-RFLP and SSR analyses: For PCR-RFLP the GLP1-12P1-P3 primers and EcoRI 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-

Table

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

<table>
<thead>
<tr>
<th>Variety/population</th>
<th>Phenotype</th>
<th>GLP1-12P1-P3 Endonucleolytic cleavage of PCR fragment with EcoRI enzyme</th>
<th>Molecular markers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>R S yes R S no</td>
<td>R S alleles (bp)</td>
</tr>
<tr>
<td>Cardinal</td>
<td></td>
<td>+ + - + 164:164</td>
<td>179:179</td>
</tr>
<tr>
<td>VRH 3082-1-42</td>
<td></td>
<td>+ - + - 184:186</td>
<td>160:167</td>
</tr>
<tr>
<td>BC4</td>
<td></td>
<td>+ - + - 184:186</td>
<td>160:167</td>
</tr>
<tr>
<td>family BC5</td>
<td></td>
<td>+ + + + 184:186</td>
<td>160:167</td>
</tr>
<tr>
<td>Ratio of recombinants</td>
<td>1/129 = 0.007</td>
<td>13/129 = 0.100</td>
<td>5/129 = 0.038</td>
</tr>
</tbody>
</table>

Correspondence to: Dr. E. Kiss, Institute of Genetics and Biotechnology, Páter Károly str 1, Gödöllő, 2100 Hungary, Fax: +36-28-522069.
E-mail: Kiss.Erzsbe@iskk.szie.hu
Results and Discussion: As a first step 20 resistant and 20 susceptible BC₁ 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. EcoRI 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 BC₁ 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. "alien" alleles could only be discriminated by restriction analysis of the PCR product. EcoRI 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).

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In the case of all three markers recombinants were obtained (Table): PM resistant individuals, whose GLP1-12P1-P3 (BC₁) PCR amplicons remained uncut after EcoRI 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 Run1 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 Run1 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. Run1 and Ren1; KOZMA et al. 2007).

The research is supported by the grants from the Hungarian Scientific Research Fund (OTKA K 62535, M 36630 and M 45633) and GrapeGen06 EU project.


Figure: Distinction of resistant/symptomless and sensitive/susceptible genotypes by PCR-RFLP (GLP1-12P1-P3) and VMC8g9 SSR primers. (A): R₁, R₂; resistant and S₁, S₂ sensitive lines produced PCR fragments of the same size (870 bp); M: DNA molecular weight marker (Fermentas GeneRuler™ 100 bp ladder plus). (B): EcoRI restriction pattern of resistant (R₁, R₂) and sensitive (S₁, S₂) lines. Lineages of the sensitive lines remained uncut. M: DNA molecular weight marker (Fermentas GeneRuler™ 100 bp ladder plus). (C): SSR profile of Cardinal (Cd), BC₁ (VMC8g9 VRH 3082-1-42), R₁, R₂ resistant and S₁, S₂ sensitive genotypes. M: DNA molecular weight marker (Fermentas GeneRuler™ 50 bp DNA ladder).


Received February 19, 2007