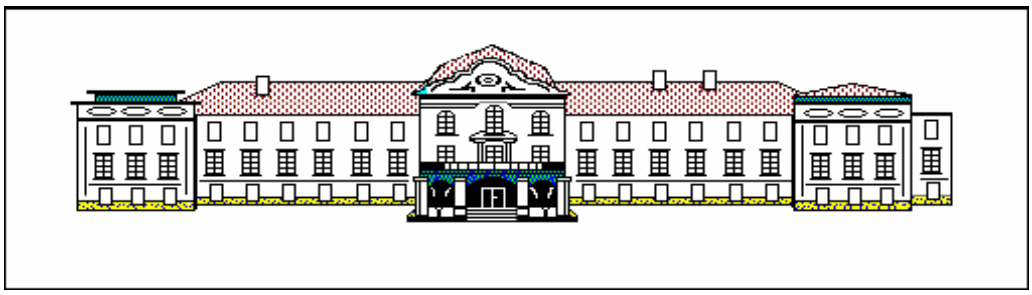

Szent István University
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PHD THESIS

Marker assisted selection of powdery and downy mildew resistance grape genotypes

STELLA MOLNÁR

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PhD School

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BACKGROUND AND OBJECTIVES

Grapevine plantations around the world are jeopardized by many pathogens and pests. It was back in the mid 19th century when the fungus species appeared in Europe that have still been causing major damage in the sensitive *Vitis vinifera* plantations ever since. Downy mildew (*Plasmopara viticola* Berk. et Curtis) and powdery mildew (*Erysiphe necator* Schwein.) are two of the major fungal diseases of grapevine (Bényei *et al.* 1999). Each of them (downy mildew and powdery mildew) is able to cause a yield loss of up to 100% in susceptible varieties unless chemical pesticides are used.

The European practice of breeding grapevine varieties with resistance to powdery mildew and downy mildew infections started in the 19th century with the active involvement of Hungarian grapevine breeders. As the varieties of *V. vinifera* cultivated for centuries in Europe were defenseless against pests (*Erysiphe necator* Schwein. and *Plasmopara viticola* Berk. et Curtis), resistance genes from North-American and Asian *Vitis* species were used for the launch of the breeding programs.

The species originating from North-America and Asia have adapted to versatile environments and to indigenous grapevine pests. Thus several of them can be used as resistance gene sources (powdery mildew, downy mildew, phylloxera) in the breeding programs. *V. riparia*, *V. rupestris* and *V. berlandieri* played an outstanding role in the stop of phylloxera infection on the European continent. Researchers in powdery mildew and downy mildew resistance breeding crossed *Muscadinia* or *Vitis rotundifolia, V. riparia, V. rupestris, V. berlandieri, V. labrusca, V. cinerea, V. lincecumii,* and *V. aestivalis* between each other or with *V. vinifera* varieties (Galet, 1988).

Crossing these gene sources with valuable wine and table grape varieties results in resistant varieties of good quality. Keeping such good quality requires careful selection and backcrossing practiced through generations. A major tool for this is the use of molecular markers for breeding in the form of marker-assisted selection (MAS).

In most plants the genes resistant to various pathogens show a highly conservative structural pattern. In particular, they contain nucleotide binding sites (NBS), leucine-rich repeats (LRR), leucine zipper (LZ) regions and receptor-like kinases (RLK) (Meyers *et al.* 1999).

While permitting both the selection of progeny with resistance gene(s) and the reduction of next generation's size, marker-assisted selection (MAS) ensures the identification of resistant

and susceptible genotypes by the detection of DNA sequence differences closely linked to resistance genes. Identification at DNA level was practiced first with the RFLP method and later, when the PCR technique was already available, through the use of random, gene-specific and microsatellite primers. The specific primers designed along the conservative sequences of resistance genes proved to be suitable markers for various plant species including grapevine (Donald *et al.* 2002; DiGaspero and Cipriani, 2003). The construction of integrated maps also facilitated the application of microsatellite or SSR (Simple Sequence Repeats) and BAC-derived markers widely used for genotyping (Adam-Blondon *et al.* 2004, Riaz *et al.* 2004; Barker *et al.* 2005, Doligez *et al.* 2006, DiGaspero *et al.* 2007). Physical and microsatellite maps enabled researchers to identify DNA markers linked to the *RUN1* and *RPV1* genes (Merdinoglu *et al.* 2003; Barker *et al.* 2005; DiGaspero *et al.* 2007).

M. rotundifolia grown in the subtropical regions of the United States has immunity-level resistance to powdery mildew, downy mildew and phylloxera (Small, 1913, Bouquet, 1980). This fact earned *M. rotundifolia* a great attention already in the early days of grapevine resistance breeding. However, its crossing with *V. vinifera* was first hindered by the difference in the chromosome numbers of the two species (*M. rotundifolia*: 2n=40; *V. vinifera*: 2n=38). Despite such difference, in 1968 Jelenkovic and Olmon managed to produce partly fertile progeny which, once backcrossed with *V. vinifera*, enabled researchers to perform an introgression of the resistance genes of *M. rotundifolia* into the *V. vinifera* genome (Jelenkovic and Olmo, 1968; Bouquet, 1986; Pauquet *et al.* 2001). Classic genetic analysis has shown that powdery mildew resistance is caused by *RUN1* (**R**esistance to ***U**ncinula **n**ecator*), a dominant gene from *M. rotundifolia*, while downy mildew resistance is the result of *RPV1* (**R**esistance to ***P**lasmopara **v**iticola*).

Through the use of the *M. rotundifolia* x *V. vinifera* hybrid created by Bouquet such hybrid families were produced between 1999 and 2002 in Pécs where *M. rotundifolia* x *V. vinifera* BC_4 hybrids were crossed with quality varieties of *V. vinifera*. (Kozma and Dula, 2003). The leaves of the individuals of hybrid populations were tested and evaluated on the basis of artificial and natural infections. This thesis discusses the molecular selection of the generation produced from the crossing of BC_4 x Cardinal and BC_4 x Kishmish moldavskij.

Based on the above results, we started the molecular analysis of (*M rotundifolia* x *V. vinifera*) BC_4 x *V. vinifera* cv. Cardinal BC_5, created at PTE Research Institute for Viticulture and Enology in Pécs, with DNA markers linked to *Run1* and *Rpv1* loci on individuals showing severe symptoms of powdery mildew infection on their leaves and on symptomless individuals.

We used the results of the BC_4 × Cardinal hybrid family for genotyping the BC_4 x Kishmish moldavskij progeny.

Objectives

1. To select *RUN1/RPV1* genotypes in the progeny of two hybrid families created at PTE Research Institute for Viticulture and Enology in Pécs – BC_4 x Cardinal (02-02) and BC_4 x Kishmish moldavskij (02-01) – with 3 microsatellite markers, 2 BAC-derived markers and 1 RGA (resistance gene analogue) marker.

2. To specify the exact size of microsatellite marker alleles linked to the *RUN1* and *RPV1* resistance genes.

3. To establish – based on the co-segregation of markers involved in the study – the reliability of each marker for the selection of resistant genotypes among the individuals of a given hybrid family.

4. To develop a simple and quick routine test method based on agarose gel electrophoresis for the selection of genotypes carrying *RUN1/RPV1* resistance genes.

MATERIALS AND METHODS

Plant materials

150 individuals of the (*M. rotundifolia x V. vinifera*) BC_4 x *Cardinal* 02-2 hybrid family selected for PM symptoms and 50 unselected seedlings of the (*M. rotudifolia x V. vinifera)* BC_4 x Kishmish moldavskij 02-01 hybrid family, created at PTE Research Institute for Viticulture and Enology in Pécs, as well as the parents.

Method

DNA isolation

DNA was isolated from young grape leaves with Qiagen's DNeasy Plant Mini Kit according to the manufacturer's protocol.

Molecular markers used for the study

The study of the BC_4 × Cardinal (02-02) hybrid family was conducted with CAPS technique, also known as PCR-RFLP markers, using the GLP1-12P1-GLP1-12P3 primers (Donald *et al.* 2002).

The fluorescent-labeled primers used for the microsatellite analyses of the BC_5 (02-02) progeny were as follows: VMC8g9 and VMC4f3.1 (Barker *et al.* 2005).

The same hybrid family was used to test the applicability of CB69.70 and CB137.138 i.e. BAC-derived dominant markers (unpublished personal communication).

For genotyping the BC_4 × Kishmish moldavskij family we used two SSR loci (VMC8g9 and VMC1g3.2 (also fluorescently labeled); Merdinoglu *et al.* 2003) and two BAC-derived loci (CB69.70 and CB137.138).

PCR, PCR-RFLP (CAPS) and SSR evaluation

PCRs were carried out as described by Halász *et al.* (2005 a and b). Bio-Rad iCycler was used for the reaction, the conditions of which were as follows: 2 minutes at 95˚C, followed by 35 cycles of 30 seconds at 95˚C, 30 seconds at 57-58˚C, 90 seconds at 72˚C, followed by a final incubation of 2 minutes at 72˚C. For the GLP1-12P1 - GLP1-12P3 (Donald *et al.* 2002.) primers we applied PCR-RFLP also known as CAPS (Cleaved Amplified Polymorphic

Sequence) technique (Konieczny and Ausubel, 1994.). For such purpose the amplicons had to undergo a restriction digestion after PCR. 10 µl of the PCR product was cleaved with *EcoR*I restriction enzyme by mixing 0.5 µl *EcoR*I (10 u/µl; Fermentas) enzyme, 3 µl water and 1.5 µl buffer (Fermentas, Tango ^{TM}) and then by keeping the mixture at 37 ^{o}C for 1.5-2 hours.

The forward primers used for microsatellite analyses were labeled with CY-5 fluorescent dye (Metabion, Merck Kft., Budapest).

ALF – Automatic Laser Fluorescent analyses

The PCR products were separated on 8% denaturing polyacrylamide gel (Reprogel, GE Healthcare Bio Sciences, AP Hungary Kft., Budapest). The allele sizes were determined with ALF Express II DNA analyzer (Amersham Biosciences, AP Hungary Kft., Budapest) using ALFexpress^{TM} sizer as a molecular weight standard.

RESULTS

Application of molecular markers linked to *RUN1* powdery mildew resistance gene in the BC_4 x Cardinal (02-02) hybrid family

As a first step 20 symptomless and 20 infected plants of the 02-02 hybrid family were examined with the GLP1-12P1 - GLP1-12P3 primers described by Donald *et al.* (2002).

When using the primers, all 40 plants showed an 870 bp fragment (as it was expected). Although it verified the successful operation of the GLP1-12P1 - GLP1-12P3 primer pair (Figure 1), the resulting monomorph pattern was not suitable for the separation of PM infected and symptomless individuals.

After the digestion of the PCR product with *Eco*RI enzyme (PCR-RFLP) the genotypes could be clearly distinguished from each other: in addition to the 870 bp fragment, 670 bp and 200 bp pieces appeared on the agarose gel for the symptomless leaves, while the enzyme did not split the DNA amplicon of the susceptible samples (Figure 1). This proved not only the applicability of the GLP1-12P1 - GLP1-12P3 primers as *RUN1* markers but also that the resistant individuals are heterozygous in the *Run1* locus.

Then the remaining 150 individuals of the (*M. rotundifolia x V. vinifera*) BC_4 x *Cardinal* 02-02 hybrid family were tested with the same primers. 66 symptomless individuals showed the R1; R2 genotype pattern (Figure 1) after the *Eco*RI digestion of the 870 bp PCR fragment and 63 individuals showed S1; S2 genotype. Except for one sample, it was the same as the phenotyping results.

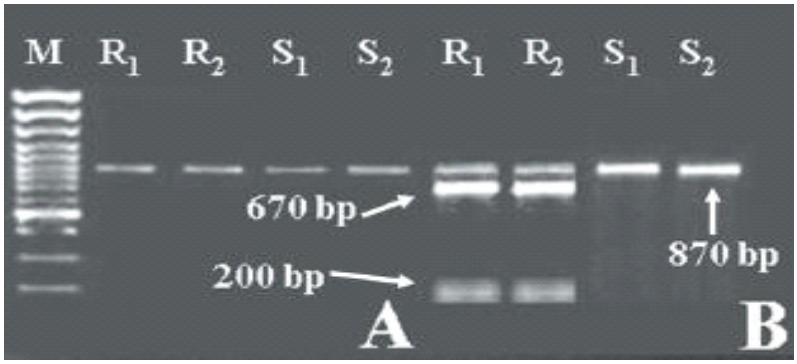


Figure 1: Testing the individuals of the 02-02 hybrid family with GLP1-12P1 - GLP1-12P3 primer pair

M: Molecular weight marker (Fermentas 100 bp ladder plus)

R1; R2 (A): free from powdery mildew symptoms

S1; S2 (A): PCR fragments of infected lines with GLP1-12 – GLP1-12P3 primer pair

R1; R2 (B): symptomless (resistant) individuals after the restriction digestion of the PCR product (*Eco*RI)

S1; S2 (B): infected (susceptible) individuals after the restriction digestion of the PCR product (*Eco*RI)

In addition to GLP1-12P1 - GLP1-12P3, two SSR primers (VMC4f3.1 and VMC8g9) were also involved in the study. Literature data only referred to the co-segregation of VMC4f3.1 and VMC8g9 with PM resistance but did not supply any information about the allele size of markers linked to resistance genes. Our objective was to determine the exact allele size of the marker linked to the *RUN1* gene. Figure 2 shows a part of the ALF analysis made with VMC4f3.1. The genotype of the resistant BC_4 parent is 184:186, while that of sensitive Cardinal is 164:164 bp. The genotype of resistant individuals in the segregating BC_5 progeny is 164:186 bp with VMC4f3.1 marker, while that of susceptible individuals is 164:184 bp. It means that the allele size of the resistant marker is 186 bp.

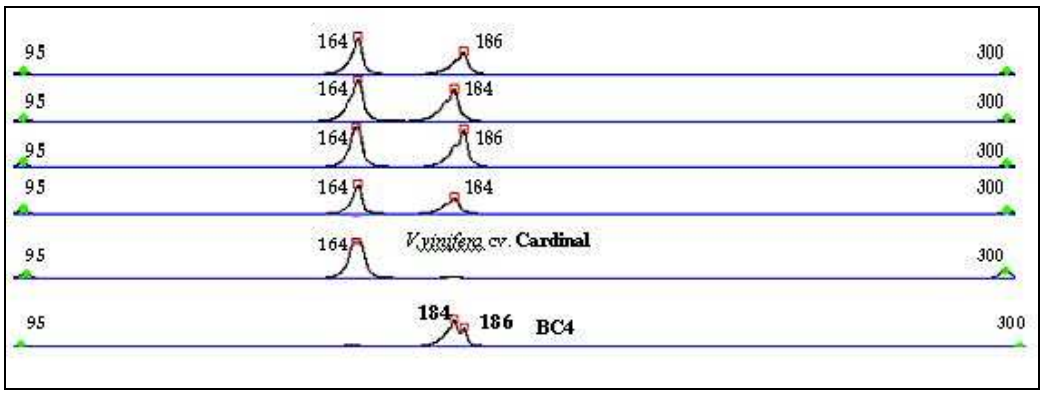
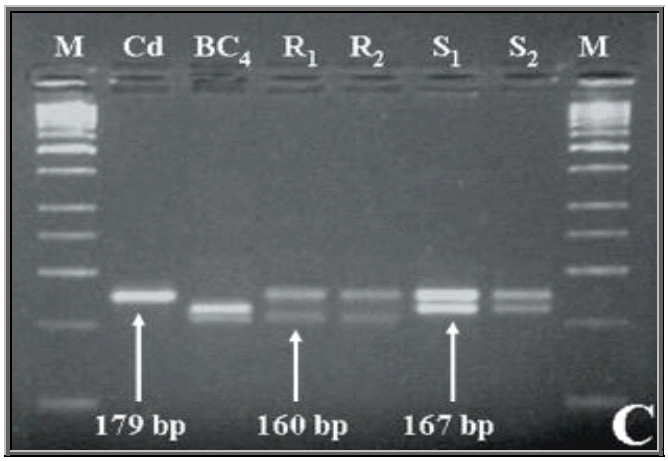


Figure 2: Electrophoretogram obtaiend with ALF Express II of some genotypes obtained with VMC4f3.1 microsatellite primer

The genotype of resistant BC_4 is 160:167 bp with the VMC8g9 primer, while that of susceptible Cardinal is 179:179 bp. The genotype of resistant individuals in the segregating BC_5 progeny is 160:179 bp with the VMC8g9 marker, while that of susceptible individuals is 167:179 bp meaning that the 160 bp fragment is linked to the *RUN1* resistance gene. As there is a 7 bp difference in length between the marker allele sizes of susceptible and resistant individuals, the fragments were separated also on 3.5% Metaphor agarose gel. This way we have developed a simple agarose gel-based method, requiring no fluorescent labeling of primers and no use of DNA fragment analyzer, for the selection of *RUN1* genotypes (Molnár *et al.* 2007). The results are shown in Figure 3.



Cd: Cardinal (sensitive)
BC_4: resistant parent (VRH 3082-1-42I

R_1; R_2 (C): resistant samples
S_1; S_2 (C): susceptible samples

M: Molecular weight marker (Fermentas GeneRuler™ 50 bp DNA Ladder)

Figure 3: Testing the individuals of the 02-02 hybrid family with VMC8g9 primer – separation of fragments on Metaphor gel (3.5%)

The microsatellite analysis also enabled us to exclude the individuals, based on allele sizes from foreign pollination, that do not correspond to parental combinations. Thus evaluation was performed for 129 individuals. In the case of those 129 plants we were able to clearly separate allele sizes and, based on the presence or lack of fragments, to distinguish between infected and symptomless individuals (Table 1). The use of all three markers ensures reliable screening. Evaluated with χ^2 test, the ratio of 1:1 between observed phenotypes and marker genotypes can also be statistically verified.

Table 1: Comparison of powdery mildew symptoms with molecular marker results (shaded numbers indicate resistance allele sizes)

Variety	Phenotype		Molecular markers					
	Symptomless/ resistant	Infected/ susceptible	**GLP1-12P1P3**		**VMC4f3.1** alleles (bp)		**VMC8g9** alleles (bp)	
	R	**S**	**R** (PCR fragment digested with *Eco*RI)	**S** (PCR fragment not digested with *Eco*RI)	**R** 186	**S** 184	**R** 160	**S** 167
Cardinal	-	+	-	+		164: 164		179: 179
BC_4	+	-	+	-	184: 186		160: 167	
BC_5 plants	67	62	66	63	164: 186	164: 184	160: 179	167: 179
					61	68	66	63
χ^2 test	0,193		0,068		0,378		0,068	
χ^2 test with Yates' correction	0,124		0,031		0,193		0,031	
χ^2 value (P=0.5%)	3,84							
Ratio of "recombinant" genotypes			1/129=0,007		13/129=0,100		5/129=0,038	

In addition to the 3 markers described above, another two BAC-derived dominant markers (CB69.70 and CB137.138) were involved in the study of the 02-02 BC_5 hybrid family. Our objective was to verify the applicability of such BAC-derived dominant markers in 02-02 BC_5 progeny, which makes screening an even simpler process. An agarose gel electrophoresis, following the PCR reaction, proved the applicability of the two dominant markers in the separation of resistant and susceptible individuals. CB69.70 amplified a 157 bp fragment for resistant individuals but generated no PCR products for susceptible samples. Similarly to the case of CB69.70, the reaction made with CB137.138 ended in the appearance or lack of the DNA fragment. A 277 bp fragment indicated the resistant individuals.

Application of molecular markers linked to *RUN1* and *RPV1* downy mildew resistance gene in the BC_4 x Kishmish moldavskij hybrid family

Based on the results obtained with the BC_4 x Cardinal hybrid family, our study was extended to 50 unselected seedlings of the 02-01 hybrid family originating from *(M. rotundifolia* x *V. vinifera)* BC_4 x Kishmish moldavskij progeny. No artificial infection data were available for this hybrid family. Only one on-field survey was made of the symptoms of a natural downy mildew infection.

As in the BC_4 x Cardinal family the VMC8g9 marker showed a reliable co-segregation with powdery mildew resistance (*RUN1* genotype) – meaning also an *RPV1* genotype according to literature data – and satisfactory correspondence with the PCR-RFLP results, we started the study of the BC_4 x Kishmish moldavskij family with this VMC8g9 marker. Based on the results, CB69.70 and CB137.138 as well as SSR marker VMC1g3.2 were also involved in the study. VMC1g3.2 was used instead of VMC4f3.1 as during the study of the BC_4 × Cardinal progeny there was only a difference of 2 bp between susceptibility and resistance marker alleles, requiring several repetitions with ALF Express II. Table 2 shows the possible VMC8g9 and VMC1g3.2 genotypes of the parents and the 02-01 BC_5 progeny.

Table 2: Parent genotypes in the 02-01 BC_5 hybrid family

	VMC8g9	VMC1g3.2.
BC4	160 : 167	122 : 140
Kishmish moldavskij	160 : 174	128 : 142
RUN1+/RPV1+	160 : 160	122 : 128
genotypes	160 : 174	122 : 142
run1-/rpv1-	160 : 167	128 : 140
genotypes	167 : 174	140 : 142

As in the VMC8g9 locus the susceptible Kishmish moldavskij parent also contains a 160 bp marker allele – indicating resistance in the BC_4 x Cardinal family –, selection in the progeny is based not on the presence of the 160 bp marker allele but on genotype (Table 2).

The PCR carried out with BAC-derived dominant markers (CB69.70 and CB137.138) produced – just like in the case of the 02-02 hybrid family – a 157 bp fragment in some

individuals tested with the CB69.70 primer and a 277 bp fragment in the BC_4 x Kishmish moldavskij progeny when using the CB137.138 primer. No fragments were produced in the remaining individuals.

Table 3 shows the comparison of downy mildew symptoms with marker results, the exact allele sizes and the presence or lack of fragments.

Table 3: Comparison of downy mildew symptoms with marker results

Sample No.	Result of outdoor downy mildew symptoms	CB69.70	CB137.138	VMC8g9	VMC1g3.2
BC4	Ø	+	+	160 : 167	122 : 140
Kishmish moldavskij	+	Ø	Ø	160 : 174	128 : 142
Cardinal	+	Ø	Ø	179 : 179	136 : 140
"Resistant" genotypes				160 : 174 160 : 160	122 : 128 122 : 142
Susceptible genotypes				160 : 167 167 : 174	128 : 140 140 : 142
5	+	Ø	Ø	167 : 174	140 : 142
7	+	Ø	Ø	160 : 167	128 : 140
8	Ø	+	+	160 : 174	122 : 142
9	Ø	Ø	+	160 : 174	122 : 142
10	+	Ø	Ø	160 : 167	128 : 140
12	+	Ø	Ø	160 : 167	128 : 140
13	Ø	+	+	160 : 174	122 : 142
16	Ø	+	+	160 : 174	122 : 142
17	+	Ø	Ø	160 : 167	128 : 140
19	+	Ø	Ø	167 : 174	140 : 142
23	Ø	+	+	160 : 160	122 : 128
25	+	Ø	Ø	**160 : 160**	128 : 140
26	Ø	+	+	160 : 160	**128 : 140**
30	+	Ø	Ø	160 : 167	128 : 140
32	Ø	Ø	Ø	160 : 174	122 : 128
37	+	Ø	Ø	167 : 174	140 : 142
41	Ø	+	Ø	160 : 160	122 : 128
44	Ø	+	+	160 : 160	122 : 128
46	+	Ø	Ø	160 : 167	**122 : 128**
49	+	Ø	Ø	167 : 174	140 : 142
53	Ø	+	+	160 : 160	122 : 128
54	Ø	+	+	160 : 174	122 : 142
55	Ø	Ø	Ø	160 : 160	122 : 128

Sample No.	Result of outdoor downy mildew symptoms	CB69.70	CB137.138	VMC8g9	VMC1g3.2
64	Ø	+	+	160 : 160	122 : 128
68	Ø	+	+	160 : 160	122 : 128
69	Ø	+	+	160 : 174	122 : 128
70	Ø	+	+	160 : 160	122 : 128
73	+	Ø	Ø	160 : 167	128 : 140
76	Ø	+	+	160 : 174	122 : 142
81	Ø	+	+	160 : 174	122 : 142
85	Ø	+	+	160 : 160	122 : 128
87	Ø	+	+	160 : 174	122 : 142
90	Ø	+	+	160 : 174	122 : 142
95	Ø	+	+	160 : 160	122 : 128
100	+	Ø	Ø	160 : 167	128 : 140
101	+	Ø	Ø	160 : 167	128 : 140
104	Ø	+	+	160 : 174	122 : 142
108	+	**Ø**	**Ø**	**160 : 160**	**122 : 142**
113	+	Ø	Ø	167 : 174	128 : 140
115	+	Ø	Ø	167 : 174	140 : 142
119	Ø	+	+	160 : 160	122 : 128
122	Ø	+	+	160 : 160	122 : 128
128	+	Ø	Ø	167 : 174	140 : 142
133	+	Ø	Ø	160 : 167	128 : 140
140	Ø	**Ø**	+	160 : 174	122 : 142
148	Ø	+	+	160 : 174	122 : 142
152	Ø	**Ø**	+	160 : 174	122 : 142
153	+	Ø	Ø	167 : 174	140 : 142
163	Ø	**Ø**	+	160 : 174	122 : 142
167	Ø	+	+	160 : 174	122 : 142

* Discrepancies marked with blue.

The tests performed with the four markers (CB69.70, CB137.138, VMC8g9 and VMC1g3.2) indicated discrepancies for 11 samples either in terms of on-field downy mildew symptoms or marker results.

The segregation ratios obtained with the CB69.70, CB137.138, VMC8g9 and VMC1g3.2 markers are shown in Table 4.

Table 4: Summary of data of downy mildew symptoms and marker genotypes in the BC_4 x Kishmish moldavskij family

Phenotype: Downy mildew symptomless	**Phenotype: Downy mildew infected**	**VMC8g9 resistant genotype: *RUN1+/RPV1+* genotype**	**VMC8g9 sensitive genotype**	**VMC1g3.2 resistant genotype: *RUN1+/RPV1+* genotype**	**VMC1g3.2 sensitive genotype**	**CB69.70 positive genotype: *RUN1+/RPV1+* genotype**	**CB69.70 sensitive genotype**	**CB137.138 positive genotype: *RUN1+/RPV1+* genotype**	**CB137.138 sensitive genotype**
30	20	32	18	31	19	24	26	27	23

Despite the assumed “recombinants” both VMC8g9 and VMC1g3.2 as well as CB137.138 markers can be efficiently applied for quick screening.

NEW SCIENTIFIC RESULTS

- We have been the first to use molecular markers linked to the *RUN1/RPV1* powdery mildew / downy mildew resistance genes for determining the genotype composition of the BC_4 x Cardinal (02-02) and BC_4 x Kishmish moldavskij hybrid families (02-01).
- We have proved the applicability of the GLP1-12P1 - GLP1-12P3 primers used in CAPS (PCR-RFLP) for selection purposes.
- We have determined the exact allele size of markers linked to resistance gene in VMC4f3.1 and VMC8g9 microsatellite loci.
- We have verified the applicability of CB69.70 and CB137.138 in the BC_4 x Cardinal hybrid family.
- We have developed a simplified genotyping method based on agarose gel electrophoresis for VMC8g9.
- We have proved that VMC8g9 is suitable for MAS in the BC_4 x Kishmish moldavskij family despite the fact that the susceptible parent also carries a 160 bp marker allele. However, selection can be reliably performed on the basis of genotype.
- We have determined the exact allele size of VMC1g3.2, which is linked to resistance gene (*RUN1*/*RPV1)*.
- With the involvement of the VMC1g3.2 primer we have confirmed the tight *RUN1*/*RPV1* linkage.
- We have verified the applicability of the CB69.70 and CB137.138 dominant markers in the BC_4 x Kishmish moldavskij hybrid family.

DISCUSSION AND RECOMMENDATIONS

Our results have proved that the individuals of various phenotypes belonging to the BC_4 x Cardinal (02-02) and BC_4 x Kishmish moldavskij (02-01) hybrid families can be separated from each other in the early stage of leaf development by the use of molecular markers linked to powdery mildew and downy mildew resistance genes. We have worked out a routine selection method that is suitable for the cost-effective sorting of valuable progeny from cleaved hybrid populations. It also helps to reduce significantly the costs of maintenance, phenotypic selection and assessment.

Through the study of 150 seedlings of the (VRH3082-1-42) BC_4 x Cardinal hybrid family we have proved that the RGA-derived GLP1-12P1 - GLP1-12P3 primers (Donald *et al*. 2002) are perfectly suitable – following a successful PCR and then a digestion with *Eco*RI enzyme – for the reliable separation of genotypes. This proves not only the applicability of the GLP1-12P1 - GLP1-12P3 primers for use as *RUN1* markers but also that the resistant individuals are heterozygous in the *Run1* locus. Additional two markers (VMC8g9 and VMC4f3.1) were involved in the SSR analysis (Barker *et al*. 2005). For both primers we have determined the size of the marker allele linked to the *RUN1* gene. VMC4f.3.1 produced the highest (10%), while GLP1-12P1P3 gave the lowest (0.7%) recombination rate. For VMC8g9 the presumably recombinant individuals showed a rate of 3.8%. In fact, allele sizes can be precisely determined with the use of an ALF Express II DNS fragment analyzer and the fluorescent labeling of primers. That is why for VMC8g9 we have developed a simple agarose-gel based method for the electrophoretic separation of resistant and susceptible samples, the allele sizes of which show a length difference of 7 bp only.

Dominant BAC-derived markers (CB69.70 and B137.138) were also involved in the study in order to identify a quick and simple selection method. We have proved the applicability of the CB69.70 and CB137.138 markers in the individuals of the 02-02 hybrid family.

Based on the results obtained with the 02-02 hybrid family, our study was extended to 50 unselected outdoor seedlings of the (VRH3082-1-42) BC_4 x Kishmish moldvskij (02-01) hybrid family. As several authors have already confirmed the tight *RUN1/RPV1* link, we completed the VMC8g9, CB69.70 and CB137.138 markers with the VMC1g3.2 SSR locus as this marker had already been used by some researchers

for the selection of *RPV1+* genotypes. All four markers are suitable for the separation of individuals carrying PM and DM resistance genes (*RUN1+/RPV1+*) from the individuals not carrying such genes (*run1-/rpv1-*).

Based on our results, we suggest that the markers used during our work (GLP1-12P1 – GLP1-12P3, VMC8g9, VMC4f.3.1, VMC1g3.2, CB69.70 and CB137.138) should be involved in further selection studies. They may be suitable for the examination of hybrid families where one of the crossing partners (resistant parent) belongs to VRH2082-1-42 BC_4 or any other backcross generation (e.g. VRH3082-1-49, VRH3084-2-56, VRH3099-10-57) where the source of PM/DM resistance gene is *Muscadinia rotundifolia*. We have developed a reliable marker-based system that can be safely used for the selection of *RUN1/RPV1* genotypes following a PCR and polyacrylamide or agarose electrophoresis. The applicability of VMC8g9, VMC1g3.2, CB69.70 and CB137.138 markers was successfully proved (Katula-Debreczeni *et al*. 2010) also in other hybrid families of cumulative resistance genes (Kozma *et al.* 2006), which further confirms the results of this thesis.

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2. Lencsés Andrea Kitti, Katuláné Debreceni Diána, Galbács Zsuzsanna, **Molnár Stella**, Halász Gábor, Hoffmann Sarolta, Veres Anikó, Szőke Antal, Heszky László, Kozma Pál, Kiss Erzsébet (2008): Molekuláris módszerek alkalmazása a kárpát-medencei szőlő génforrások megőrzésére. „Fiatal Kutatók az élhető Földért" – A Tudomány Ünnepe. FVM Budapest, 2008.11.24.

Posters

2. Galbács Zsuzsanna, **Molnár Stella**, Halász Gábor, Hoffmann Sarolta, Veres Anikó, Galli Zsolt, Szőke Antal, Tóth Zsófia, Pilinszky Katalin, Wichmann Barnabás, Kiss Erzsébet, Kozma Pál, Heszky László (2006): Mikroszatellit ujjlenyomat alkalmazása"hungaricum" szőlőfajták pedigré elemzésére. Új típusú gazdasági kihívások és válaszok a bolognai folyamatban. **Debreceni Egyetem Mezőgazdaságtiudományi Kar, Szent István Egyetem Mezőgazdságtudományi Kar közös tudományos ülése**. Debrecen, 2006. december 7.