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Search for molecular markers associated with resistance against powdery mildew of *Vitis vinifera L.*

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Evaluation of resistance to powdery mildew is of high value for breeding grapevines. RAPD and SSR technique were applied to develop genetic markers associated with resistance to Uncinula necator *(powdery mildew). Two populations with about 40 individuals were screened with 195 RAPD oligomeres and more than 40 SSR loci for polymorphism linked to powdery mildew. Among the RAPD markers more than 80% of the primers gave distinct band patterns by genotyping offspring from 'Grüner Veltliner' x 'Seyval blanc' (Kl.1979) and only 65% with 'Welschriesling' x 'Sirius' (Kl.1977). Seven RAPD markers show significant correlation with absence of powdery mildew infection. SSR markers even produced several polymorphic fragments but with less correlation to the resistance trait. In the population Kl.1979 the level of Oidium infection was low compared to the population Kl.1977. Even most loci of the population Kl.1977 show only alleles typical for* Vitis vinifera*. As several resistance related alleles are necessary to find field resistance we could confirm that resistance to powdery mildew is a multilocus phenomenon. RAPD markers of one population could not be applied to the other population due to their different origin of the resistance sources. SSR markers seem to have a more common nature but with less correlation to the trait.*
Keywords: Grapevine, *Vitis vinifera*, SSR, RAPD, hybridization, population

Entwicklung von molekularen Markern gegen Echten Mehltau der Rebe (*Vitis vinifera L.*). *Für die Rebenzüchtung scheint die Bewertung und Erfassung einer Resistenzquelle unerlässlich. Um einen Zugang zu der genetischen Information der Oidiumresistenz zu erlangen, wurden RAPD- und SSR-Marker eingesetzt. Dazu wurden zwei Populationen mit jeweils über 40 Einzelindividuen mit 195 RAPD- und mehr als 40 SSR-Markern analysiert. Ziel war das Auffinden von Genen, die in Verbindung mit der Oidiumresistenz stehen. Beim Einsatz der RAPD-Marker konnten mehr als 80 % der Primer-Unterschiede im Bandenmuster in der Nachkommenschaft von 'Grüner Veltliner' x 'Seyval blanc' (Kl.1979), aber nur 65 % bei der Nachkommenschaft 'Welschriesling' x 'Sirius' (Kl.1977) gefunden werden. Sieben RAPD-Marker zeigten signifikante Korrelation mit der Abwesenheit von Echtem Mehltau. Auch SSR-Marker zeigten erhebliche Unterschiede in den Allellängen, aber mit nur geringer Verbindung zu den Resistenzeigenschaften. In der Population Kl.1979 war außerdem der Befall mit Oidium viel geringer als in der Population Kl.1977. Ursache für diese Unterschiede könnten die in Kl.1977 überwiegenden* V. vinifera*-Allele sein, die keine Resistenz tragen. Um Feldresistenz gegen Oidium zu erreichen, benötigt man eine komplexe multigenetische Resistenzquelle. Die gewonnenen RAPD-Marker einer Population konnten nicht auf die andere übertragen werden. Möglicherweise ist die unterschiedliche Herkunft der Resistenzquellen dafür verantwortlich. SSR-Marker zeigen zwar eine geringere Kopplung zur Resistenzeigenschaft, sind aber eher auch zwischen Populationen vergleichbar.*
Schlagwörter: Rebe, *Vitis vinifera*, SSR, RAPD, Kreuzungszüchtung, Neuzüchtungen

Le développement de marqueurs moléculaires contre l'oïdium de la vigne (*Vitis vinifera L.*). *L'évaluation et l'enregistrement d'une source de résistance semble indispensable pour la sélection des vignes. Des marqueurs RAPD et SSR ont été utilisés pour avoir accès à l'information génétique de la résistance à l'oïdium. À cette fin, deux populations avec plus de 40 individus chacune ont été analysées à l'aide de 195 marqueurs RAPD et plus de 40 marqueurs SSR. Le but a été de trouver des gènes liés à la résistance à l'oïdium. Avec les marqueurs RAPD,*

plus de 80 % des différences de primers dans le dessin de la bande ont pu être trouvées dans la descendance de 'Grüner Veltliner' x 'Seyval blanc' (Kl.1979), mais 65% seulement dans la descendance de 'Welschriesling' x 'Sirius' (Kl.1977). Sept marqueurs RAPD présentaient une forte corrélation avec l'absence d'oïdium. Les marqueurs SSR présentaient de grandes différences dans les longueurs des allèles, mais n'étaient que faiblement liés aux caractéristiques de résistance. En outre, l'attaque d'oïdium a été beaucoup plus faible dans la population Kl.1979 que dans la population Kl.1977. La cause de ces différences pourrait résider dans les allèles de V. vinifera *qui prévalent dans Kl.1977 et qui ne portent pas de résistance. On a besoin d'une source de résistance multigénétique complexe afin d'obtenir une résistance sur le champ à l'oïdium. Les marqueurs RAPD obtenus d'une population ne pouvaient pas être transférés à l'autre. Cela est probablement dû à l'origine différente des sources de résistance. Il est vrai que les marqueurs SSR présentent une liaison plus faible avec la caractéristique de résistance, mais on peut mieux les comparer également entre les populations.*

Mots clés : vigne, *vitis vinifera,* SSR, RAPD, hybridation, population

In grape breeding one of the major goals is to introduce resistance against powdery mildew (EIBACH, 1994) to the European grapevine cultivars. As in *Vitis vinifera* sufficient resistance genes against mildew are absent, other *Vitis* species like *V. riparia*, *V. labrusca*, *V. rupestris* and *V. cinerea* were used as donor plants in crossbreeding (RÜHL et al., 1997). The seedlings of these interspecific crosses could not convince by their wine quality but still show high resistance against powdery mildew. The aim of breeding cultivars with high wine quality comparable to other *V. vinifera* cultivars but with resistance to this fungal pathogen could not be reached with a single step (DIEHL, 1987). Several back crossings are needed for acceptable wine quality. The information about resistance to powdery mildew gained by breeding programs is not easily transferable (DALBO et al., 2001) and is mainly based on specific genotypes. It is supposed that the inheritance of the resistance is complex and only very few progenies carry that trait with comparable resistance than the donor plant. Nevertheless we do know some mechanisms, which are responsible for the resistance phenomenon (BLAICH et al., 1989) but it can be supposed that in different species there exist different mechanisms. Nevertheless field resistance is based on several defence mechanisms and their interaction (BLAICH et al., 1989). The resistance is not based on one gene but on several alleles and therefore is considered as a multilocus phenomenon. The extent of resistance differs on grapes, leaves and canes, but is not inherited independently (ROY et RAMMING, 1990). As we had to perform the analyses on young seedlings we evaluated leaf resistance to *Uncinula necator* (powdery mildew) according to the O.I.V. descriptor (O.I.V., 1983).

The use of RAPD technique for tracing polymorphism related to specific traits has already been demonstrated for seedlessness (STRIEM et al., 1996; LAHOGUE et al., 1998; ADAM-BLONDON et al., 2001), while other markers like SSR and AFLP are proved mainly for the identification and characterization approach (BOWERS et al., 1996; REGNER et al., 2000). As we could link Muscat flavour with a specific allele of VRZAG 79 the proceeding in marker assisted selection encouraged for further activities (REGNER et al., 2004). RAPD markers are a useful tool for preselecting sequences from each locus at the genome. The low costs and the simple performance as well as the possibility for easily sequencing these markers favour their application in genotyping segregating populations. Nevertheless it would be necessary to transform them into SCAR markers for further studies. Microsatellite markers have been used for cultivar identification with grapevine for some years (THOMAS et al., 1993; BOWERS et al., 1996; REGNER et al., 2000). The six most polymorphic loci (VVS2, VVMD5, VVMD7, VVMD27, VRZAG62, VRZAG79) are appropriate for differentiating all grapevine cultivars analysed so far (REGNER et al., 2001). Since more than 40 SSR markers have been developed and are published by different laboratories (THOMAS et al., 1993; BOWERS et al., 1996; SEFC et al., 1999) sufficient microsatellite loci are now available for investigations into the correlation with resistance traits of the progeny. Accordingly to that procedure the marker VVS3 could be linked to the sex determination of grape flowers (DALBO et al., 2000).

Two populations were available for the search of markers associated with resistance to powdery mildew. The population of 'Grüner Veltliner' x 'Seyval blanc' showed a high percentage of resistant seedlings while the cross 'Welschriesling' x 'Sirius' resulted only in a few resistant descendants. Both populations were used for segregation analyses and genotyping. The seedlings were divided into resistant and susceptible offspring

and not less than 20 cultivars were screened by several RAPD markers.
The objective of the presented work was to develop markers associated with resistance against powdery mildew and verify their suitability for marker assisted selection.

Materials and methods

Plant material

The plant material is kept at the department for grapevine breeding at the Höhere Bundeslehranstalt und Bundesamt für Wein- und Obstbau Klosterneuburg. The populations used for the analysis are Kl.1977 and Kl.1979. Kl.1977 contains the larger amount of seedlings and consisted of more than 200 vines of the cross 'Welschriesling' x 'Sirius'. Kl.1979 was derived from the cross 'Grüner Veltliner' x 'Seyval blanc' and consisted of about 120 seedlings. All the vines are still kept in the nursery field planted under hydroponic culture conditions on their own roots and in a dense planting (20 x 20 cm) formation. Therefore only few grapevines could develop flowers for blooming and producing grapes. Hence resistance screening could not sufficiently be performed under field conditions and was additionally done with an in vitro infection study assay (Stein et al., 1985).

DNA-analysis

DNA was extracted from young leaves by following the protocol described by Thomas et al. (1993) and modified by Regner et al. (1998).
The VVS markers were developed by Thomas and Scott (1993) and the VVMD markers by Bowers et al. (1996) as well as by Bowers and Meredith (1999). The VRZAG markers (Sefc et al., 1999) and VRG (Regner et al., paper submitted 2004) were obtained from investigations into simple sequence repeats of *Vitis riparia*. Additionally several VMC (Vitis Microsatellite Consortium) markers were applied, however these markers show no significant correlation and are not free for the public domain.

RAPD-analysis

RAPD analyses were carried out step by step. At first only a small number of progenies together with the parents were screened to find polymorphism. The second step was the development of half of the samples and only if the marker seemed still promising for the association in resistance, all selected seedlings were analysed.
Decamer oligonucleotides were obtained from Operon Technologies (Alameda, USA, kit B, C, D, E) and Metabion GmbH (Martinsried, Germany, kit G, M, N) as well as MWG GesmbH, Ebersberg, Germany (kit A, F, H, V). Amplification was performed in 20 µl of the buffer solution, which consisted of 16 mM $(NH_4)_2SO_4$, 67 mM Tris-HCl pH = 8.8, 1.5 mM $MgCl_2$, 0.01 % Tween 20, 0.1 mM each dNTP (GenXpress, Maria Wörth, Austria), 0.2 µM primer, 1 Unit Biotherm Taq DNA polymerase (GenXpress, Maria Wörth, Austria), and 20 ng genomic DNA of grapevine.
An Omnigene (Hybaid, London) thermocycler processed 40 cycles of 30 sec. at 92°C, 90 sec., at 38°C and 60 sec. at 72°C. The arbitrarily amplified fragments were separated on a 2% agarose gel and detected by staining with ethidium bromide. Documentation was done by taking Polaroid photographs.

PCR-protocol

The amplification of the SSR loci was performed by following our general protocol but applying specific annealing conditions. The general PCR protocol applied for these studies was 2 min. denaturation at 94°C and 35 cycles with annealing phase for 30 sec. (temperature between 45°C and 55°C) and denaturation for 15 sec. at 92°C. The annealing temperature for each locus was set according to the original protocol. A final extension of the fragments was performed at 72°C for 5 min. Due to the different size range of the involved loci multiplex PCR was feasible. At least the alleles of three loci were separated on a sequencing gel.

DNA-electrophoresis

Yield of DNA fragments was estimated by running an aliquot of the sample on a 2 % agarose gel stained with ethidium bromide. The samples were denaturated by heating up with formamide and loaded together with a size standard (Genescan 350 Tamra, Appl. Biosystems, Vienna) to a 6 % polyacrylamid gel. Detection of the SSR fragments labelled with 6-FAM, HEX and TET was carried out by an automated sequencer (ABI 373, Perkin-Elmer, Vienna). Labelling with different fluorescent colouring agents facilitated the application of multiplex PCR.

Sequencing

The isolation of fragments was done from 2 % agarose gel by removing slices containing the DNA and processing them with a PCR elution system called NucleoSpin Extract (Machery-Nagel, Düren, Germany). The purified fragments were ligated into a pGEM-T vector by using the pGEM-T ligation kit (Promega, Mannheim, Germany). The vectors were transferred into E. coli JM 109 by $CaCl_2$ transformation and selected due to their lac- operon system by X-gal screening. Colonies with PCR inserts were incubated in LB medium over night and plasmid DNA was gained by following the procedure recommended for the purification with NucleoSpin Plasmid kit (Machery-Nagel, Düren, Germany). The M13 universal primers could be used for sequencing the fragments. The sequencing reactions were performed according to the protocols of the RR Dye-Deoxy Terminator Cycle Sequencing Kit (Appl. Biosystems, Vienna). Sequence comparison was done by the database programme "blast search" and preliminary calculations were performed by using statistical software SSPS.

Results and Discussion

The populations Kl.1977 and Kl.1979 were evaluated with respect to their behaviour to Oidium infection during two seasons. The level of resistance was defined for leaves according to the OIV descriptor Nr.455 (Table 1). The medium value of resistance in 1977 is 4.8 and all above can be regarded as more resistant plants (Table 2). All loci listed in this table are contributing to the resistance trait. The most important is VVMD 36 while VRZAG 79 only improves the resistance phenomenon with a small non-significant rate. Plants carrying at the locus VVMD 36 the allele 238 show significant higher resistance (6.2) than without this allele (4.2).The evaluation of in vitro infection studies provided us with additional information. Both the in situ and the in vitro value of resistance are considered for the definition of the resistance degree. In Kl.1979 the level of resistance was remarkably higher and reached the mean value of 6.08, whereas the mean degree of resistance was 4.8 in Kl.1977. On the other side seedlings of Kl.1979 suffer especially from phylloxera on the formation of leaf galls and from erinose. In both populations several plants were observed with symptoms of Mg-deficiency. By genotyping the populations Kl.1979 and Kl.1977 with RAPD markers many polymorphic fragments could be observed. Among 195 oligomeres more than 80 % of the primers gave distinct band patterns when genotyping 'Grüner Veltliner' x 'Seyval blanc' and only 65 % were polymorphic with 'Welschriesling' x 'Sirius'. The following oligomeres were selected as the most promising ones: **Kl.1979**: U17, GtO5, B3; AP12, V8, V17, Dec4, and for **Kl.1977**: C9, BC302, N15, H10, A14, N12, M18 (Fig. 1).

Table 1:
Evaluation of oidium related resistance (O.I.V. 455) of the population Kl.1979 derived from a cross 'Grüner Veltliner' x 'Seyval blanc'.
(Resistance average: 6,08)

Geno-type	Oidium resistance	Geno-type	Oidium resistance
Seyval blanc	9	935	6
Gr. Veltliner	1	936	7
853	7	941	9
854	4	942	5
855	3	943	9
857	5	944	9
861	5	945	5
864	5	946	9
865	5	947	8
867	3	948	5
868	3	949	9
871	7	950	9
872	3	1047	9
873	3	1048	6
874	7	1049	7
875	7	1053	3
884	3	1054	3
885	3	1055	3
886	7	1056	8
888	3	1057	9
889	5	1058	9
915	6	1059	8
916	6	1060	6
926	9	1061	8
934	9	1062	7

These RAPD markers allowed the amplification of DNA fragments linked to the observed trait. The correlation of the markers to the trait was calculated and verified by the significance testing, while the effect of a marker is shown (Table 3 + 4). Some of them were selected as linked to the resistant genotypes. Only a few (U17, B3, C9) of them could be ligated into a pGEM-T vector. The sequence of these fragments was gained by using M13 universal primers and a cycle sequencing protocol (sequence not shown). By comparing the data with database (Blast Search) no exact alignment or homology could be detected.

The effect of a marker depends on the amount of plants screened and was calculated as the difference of the re-

Table 2:
Evaluation of the genotypes (number of DNA) Kl.1977 for differentiation to sensitive and resistant vines

Genotype	Oidium Resistance	VVMD36		VVMD21		VVMD8		VRZAG7		VVMD7		VVS1		VRZAG79		VRZAG15	
Sirius	7	1		1		1		1		1		1		1		1	
Welschriesling	1		0				0		0		0		0		0		0
1182	8		0	1		1		1		1		1		1		1	
1183	8	1		1		1		1			0	1		1		1	
1184	8		0	1			0	1			0	1			0	1	
1185	3		0		0	1		1			0	1			0		0
1186	1		0		0		0		0		0		0	1			0
1187	3	1			0		0	1			0		0	1			0
1188	3		0		0	1		1			0		0		0	1	
1189	3		0		0	1		1			0		0		0		0
1190	3	1		1			0		0		0		0		0	1	
1191	6	1			0		0		0		0	1		1			0
1192	5		0		0	1		1			0	1			0		0
1193	3		0		0		0	1			0	1		1		1	
1194	3	1		1			0		0		0	1			0	1	
1195	3		0		0		0		0		0		0		0	1	
1196	5	1		1			0	1		1		1			0		0
1197	5		0	1			0		0	1			0		0		0
1198	5		0		0		0	1			0	1			0		0
1199	3		0		0		0	1		1			0	1			0
1200	1		0	1			0		0		0		0	1		1	
1201	3		0	1			0	1		1		1			0	1	
1202	3		0		0		0		0	1		1		1			0
1203	3		0		0		0		0	1		1		1			0
1204	3		0		0		0	1		1		1		1		1	
1205	3		0		0		0		0	1			0		0	1	
1206	3		0		0	1			0		0	1		1		1	
1207	3		0		0	1			0		0	1			0	1	
1208	3		0		0		0		0	1		1		1			0
1209	3		0		0	1			0		0	1		1		1	
1211	6	1		1		1			0	1		1			0		0
1212	8	1			0		0		0		0	1		1		1	
1213	8	1			0	1			0	1		1		1		1	
1214	3		0		0	1			0	1			0	1		1	
1215	7		0		0	1		1			0		0	1			0
1216	8	1			0		0	1			0		0	1			0
1217	8	1		1			0		0		0		0		0	1	
1218	6		0		0		0		0		0		0	1		1	
1219	8		0		0	1			0		0		0	1			0
1220	6	1		1			0		0		0		0	1			0
1221	8		0		0		0	1			0		0		0	1	
1230	5	1			0		0	1		1			0	1		1	
1241	3		0	1			0	1			0	1		1			0
1291	7		0		0		0		0	1			0		0		0
1292	6	1			0		0		0	1		1			0		0
1293	8		0		0		0	1		1		1		1			0
433	9	1		1		1		1		1		1		1		1	
Resistance average	4.8	**6.2**	4.2	**5.5**	4.5	**5.4**	4.5	**5.4**	4.4	**5.2**	4.6	**5.1**	4.5	**5**	4.6	**5**	4.7

sistance degree. The mean value of resistance of vines carrying the marker was compared to that of vines lacking the fragment. The marker effect is the slope of the regression line.

Due to the large number of markers and their small contribution to the resistance it can be confirmed that this kind of resistance is a multi-allelic one. Therefore to reach a similar degree of resistance in the offspring as in the donor plant it is necessary that most resistance associated alleles are inherited. From crosses of suscep-

Table 3:
RAPD markers which show correlation to the resistance trait of the population Kl.1979

RAPD marker	U 17	V 17	B 3	V 8	Dec 4	GTO 5	Ap 12
Length	350 bp	1200 bp	900 bp	300 bp	2500 bp	800 bp	450 bp
Correlation	0,78	0,71	0,67	0,68	0,47	0,57	0,37
Significance	0,01	0,01	0,01	0,01	0,05	0,01	0,05
Marker effect	4,2	3,9	3,6	3,6	2,7	2,0	1,5

Table 4:
RAPD markers correlating to the oidium resistance trait of the population Kl.1977

RAPD marker	N 12	M 18	N 16	BC 302	B 3	A 14	C 9
Length	1100 bp	600 bp	800 pp	500	400	350 bp	450
Correlation	0,79	0,87	0,82	0,44	0,52	0,49	0,43
Significance	0,01	0,01	0,01	0,01	0,05	0,05	0,05
Marker effect	3,8	3,9	4,0	1,4	2,3	2,4	1,4

tible x resistant genotypes only a few plants out of a large population reach the same or a similar level of resistance as the donor plant. The number of these alleles is estimated to be very high. Each backcross represents a kind of dilution of corresponding alleles. Only by chance genotypes carrying all relevant parental genes will be found. Therefore we guess that for powdery mildew resistance marker assisted selection is not a proper tool in screening high numbers of seedlings.
The kind of inheritance of resistance related alleles could be seen much better by genotyping the populations with microsatellites. Due to the high number of analyses we have already done with genuine *Vitis vinifera* cultivars we easily could define the alleles derived from the incrossing of other *Vitis* species (Table 5 + 6). In 'Seyval blanc' the percentage of foreign *V. vinifera* alleles is higher than in 'Sirius'. Besides at some loci 'Seyval' is homozygous for foreign alleles whereas 'Sirius' is heterozygous. For the calculation of the correlation and observations of the segregation it was an advantage to have two populations available. As the most important resistance alleles are in Kl.1979 homozygous we considered that segregation in Kl.1977 is more helpful to gain information.
As there was no single marker which could be obviously identified as closely linked to the resistance trait it can be supposed that several markers are necessary to reach a level of resistance sufficient for field observations. The idea how to get access to the right marker is to follow the alleles of the parents. First, the donor plant 'Seyval blanc' shows high resistance (O.I.V. value for Oidium resistance = 8 to 9), 'Sirius' (= 7) was esti-

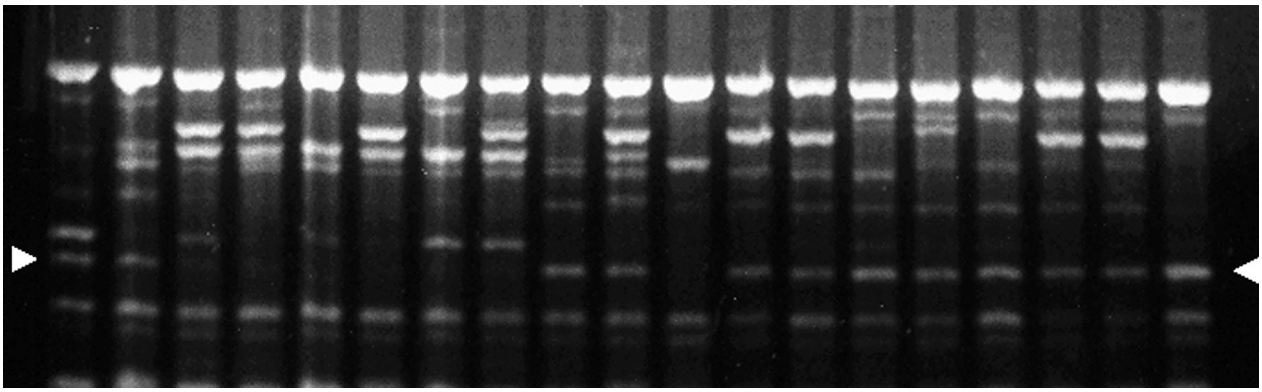

Figure1: Genotyping with RAPD M18 marker resulted in fragments associated with Oidium resistance. Lane 1 to 9 represent oidium susceptible seedlings, lane 10 is the resistant parent 'Sirius', lane 11 is 'Welschriesling' and the other lanes up to 20 correspond to resistant seedlings

Table 5:
SSR alleles of the cultivars 'Grüner Veltliner' x 'Seyval blanc' (Kl.1979). Alleles in bold letters derived from other Vitis species

Locus	Seyval blanc		Gr. Veltliner	
VVS 1	181	***193***	162	181
VVS 2	133	133	133	151
VVS 3	214	216	214	
VVS 4	169	177	167	175
VVS 29	***174***	179	171	
VVMD 5	226	***252***	232	232
VVMD 6	189	207	189	199
VVMD 7	***237***	243	247	257
VVMD 8	***171***	***189***	143	147
VVMD 14	228	245	228	228
VVMD17	220		220	222
VVMD 21	***235***	243	243	249
VVMD 24	***206***	212	212	217
VVMD 25	***241***	***241***	245	253
VVMD 26	251	271	251	251
VVMD 27	189	189	189	194
VVMD 28	239	***255***	237	251
VVMD 31	210	***220***	210	216
VVMD 34	236	238	236	
VVMD 32	251	271	240	256
VVMD 36	***238***	***238***	254	264
VRZAG 7	157	***163***	157	159
VRZAG 12	***156***	164	153	164
VRZAG 14	163	185	163	
VRZAG 15	165	***197***	167	167
VRZAG 21	204	208	202	206
VRZAG 25	238	247	238	247
VRZAG 26	130	***204***	130	
VRZAG 29	120	***124***	116	118
VRZAG 30	149	149	149	149
VRZAG 62	179	187	193	203
VRZAG 64	142	160	140	144
VRZAG 67	141	***169***	128	161
VRZAG 79	260	***262***	246	250
VRZAG 82	254	264	268	
VRZAG 83	188	200	194	200
VRZAG 93	199	209	191	199
VRZAG 112	231	238	236	244
VRG 1	240		194	234
VRG 2	164	170	158	164
VRG 3	212	230	194	210
VRG 4	188	198	188	190
VRG 5	***228***		164	178

Table 6:
SSR alleles of the cultivars 'Welschriesling' x 'Sirius' (Kl.1977). The alleles derived from other Vitis species are bold marked

Locus	Sirius		Welschriesling	
VVS 1	190	***193***	183	190
VVS 2	133	151	135	151
VVS 3	212	212	212	218
VVS 4	168	168	168	168
VVS 5	102	124	110	
VVS 29	179	181	179	179
VVMD 5	228	232	226	238
VVMD 6	207	***214***	199	207
VVMD 7	***237***	247	247	257
VVMD 8	147	***185***	143	146
VVMD 14	228	244	220	244
VVMD17	212	220	220	222
VVMD 21	***233***	249	249	249
VVMD 24	***204***	212	212	217
VVMD 25	***239***	253	253	253
VVMD26	251	251	249	255
VVMD 27	189	192	185	189
VVMD 28	237	***245***	249	261
VVMD 31	204	214	212	216
VVMD 32	251	273	242	273
VVMD 34	238	238	238	240
VVMD 36	***238***	264	264	264
VRZAG 7	157	***191***	157	159
VRZAG 12	157	157	150	153
VRZAG 14	164		224	
VRZAG 15	167	***195***	167	177
VRZAG 21	204	***224***	192	208
VRZAG 25	227	236	227	240
VRZAG 26	126		130	
VRZAG 29	114	114	114	118
VRZAG 30	151	151	151	151
VRZAG 62	179	193	193	195
VRZAG 64	140	144	158	160
VRZAG 67	141	154	141	141
VRZAG 79	246	***262***	252	252
VRZAG 82	254	274	254	267
VRZAG 83	190	190	188	194
VRZAG 93	188	208	188	198
VRZAG 112	227	227	231	236
VRG 1	112	137	214	226
VRG 2	131	156	156	164
VRG 3	185	241	137	
VRG 4	135	150	150	225
VRG 5	282	***327***	192	282

mated slightly weaker. On the other side the cultivars 'Grüner Veltliner' and 'Welschriesling' are susceptible to infection by *Uncinula necator*. Therefore the resistance is based in both populations on the alleles of the paternal genotype. As 'Seyval blanc' and 'Sirius' are derived from a very complex hybridisation procedure, the original source of resistance could not be defined. 'Grüner Veltliner' and 'Welschriesling' lack any essential resistance to powdery mildew. However, it could be concluded that alleles derived from the donor plants correlating to the resistance phenomenon are true associated ones (Table 7 +8). At some loci especially in Kl.1979 va-

Table 7:
SSR markers and correlation to the resistance trait and marker effect of the population Kl.1979

Locus	VVMD31	VVS1	VVMD24	VVMD7	VRZAG15	VRZAG7	VRZAG79
Correlation	0,55	0,35	0,3	0,29	0,25	0,15	0,15
Significance	0,01	0,05	0,05	0,05	0,05	no	no
Marker effect	2,0	1,4	1,0	1,0	1,0	0,6	0,6

Table 8:
SSR markers and their correlation as well as the effect to powdery mildew resistance of the population

Locus	VVMD36	VVMD21	VVMD8	VRZAG7	VVMD7	VVS1	VRZAG79	VRZAG15
Correlation	0,42	0,21	0,18	0,18	0,11	0,1	0,09	0,07
Significance	0,01	0,05	no	no	no	no	no	no
Marker effect	2,0	1,0	0,9	0,8	0,6	0,6	0,4	0,3

Table 9:
Incidence of new alleles at some SSR loci in population Kl.1979

Locus	VRG 1	VRZAG 93	VRZAG 29	VRZAG 7
Parental	199:230	191:199	114:116	155:157
alleles	243:-	199:209	118:122	155:161
	12x 205	11x 168	8x 120	3x 163
New alleles	2x 255	4x 188	1x 128	16x 167
		3x 215		10x 115

ried alleles (Table 9) could be observed as being very frequent.

The possibilities how a locus will be influenced by the alleles derived from other *Vitis* species are numerous. If the locus is homozygous all progenies should carry that allele as we could see for Kl.1979 at the loci VVMD 21 and VVMD36. The presence of these alleles in all seedlings does not allow to study segregation. Nevertheless as the trait of oidium-resistance is a complex and multi-allelic phenomenon all genotypes could be positively influenced by this allele. On the other hand we used the second population Kl.1977 to follow the segregation of these alleles. Calculation of the medium resistance in allele carrying genotypes compared to seedlings lacking the allele was shown to be the effect of the marker. The most efficient contribution of an SSR marker in Kl.1977 was found at the locus VVMD 36. Even all other loci like VVMD 21, VRZAG 7, VVMD 8, VVMD 7, VVS1, VRZAG 79, VRZAG 15 can be associated to the resistance trait. All these loci only could be verified by their influence as they were heterozygous and segregating in a Mendelian manner. Several SSR loci are not inherited in a Mendelian way and even some loci showed a tendency for mutations within a population. These deviations represent obstacles in marker search.

Nevertheless in a relatively high number of progenies no amplification of parental alleles could take place. As the parents show at this locus no null allele the reason for the disappearance of the alleles could be recombination errors during hybridisation. But what is more confusing is the fact that it is not clear if the allele is present and can be activated despite small changes in the sequence homology or if it is truely absent. On the other hand it is not guaranteed that a present allele is active if mutations have changed the allele sequence. Even a deviation of two base pairs could mean that one repeat of the sequence is missing and has inactivated the whole region. Especially in Kl.1979 we found a lot of loci with new incidences of null alleles. An explanation for this higher frequency could be the recombination problems of very different genotypes. At one locus (VVMD 26) not any seedling of 'Seyval blanc' has carried the parental allele with 271bp length. All descendants showed the second allele of 'Seyval blanc', a new recombined allele length or a null allele. Therefore this allele is supposed to be a barrier for hybridisation with 'Grüner Veltliner'. In general, mutations of alleles are not a rare event but happened very frequently in combinations of very heterozygous genotypes as 'Seyval blanc' and 'Grüner Veltliner'. At the loci VRG 1, VRZAG 29, VRZAG 93 and VRZAG 7 some of the deviations of the heritage are shown (Table 8). Furthermore it would be interesting to observe the stability of newly created alleles during further propagation and even new hybridisation.

Selection of a grapevine cultivar for the release to the

public needs a very careful evaluation procedure. Usually the expenditure of time for the breeding process is estimated as being too long. But considering that about fifty characters have to be proved, the long periods are justified. Helpful for the acceleration of the breeding work would be only genetic markers which enable to minimize time frame. As we could not find only few markers dividing the population into resistant or susceptible vines the usefulness for marker assisted selection of powdery mildew is questionable.

These findings are in contrast to the resistance found in Illinois 547-1 (4) derived by crossing *V. rupestris* x *V. cinerea*. In a population descendant from Illinois 547-1 a major QTL could be verified for marker assisted selection. Due to the direct influence of American *Vitis* species it is questionable if the selected material is useful for viticulture. However, the genetic base of resistance against Oidium may differ in various *Vitis* species. Therefore the source of resistance is responsible for the kind of the genetics.

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