USE OF SSR MARKERS TO IDENTIFY GRAPEVINE CULTIVARS REGISTERED IN THE CZECH REPUBLIC

UTILISATION DE MARQUEURS SSR POUR L'IDENTIFICATION DE VARIÉTÉS DE VIGNES ENREGISTRÉES EN RÉPUBLIQUE TCHÈQUE

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Abstract: In this study we analyzed 51 grapevine cultivars registered in the Czech Republic using six highly informative SSR loci, and determined SSR profiles typical for each cultivar. All cultivars were easily distinguished on the basis of SSR allele length, except for berry color mutants (Pinot and Chasselas group). The average number of detected alleles per locus was 13.2, while observed heterozygosity is between 0.745 (VrZAG 79) and 0.922 (VVMD 7). The lengths of the alleles were compared with results from other laboratories; lengths were either the same or different by 1-3 bp. We also tested a new approach to microsatellite data comparison using a coding system based on reference alleles as a size standard (THIS et al., 2004). The obtained profiles were mostly in accordance with profiles of reference cultivars (THIS et al., 2004); we discuss the possible reasons for observed discrepancies.
A third allele in the microsatellite spectrum was observed in five samples where template DNA originated from leaves. Additional analyses with DNA isolated from phloem-enriched samples (L2 meristem layer) were performed and typical diallelic profiles obtained. The reason for the detected triallelism is assumed to be a periclinal chimerism.

Résumé : Dans cette étude, nous avons analysé 51 variétés de vignes enregistrées en République tchèque en utilisant six loci microsatellites hautement informatifs, puis nous avons déterminé les profils microsatellitess typiques pour chaque variété. Toutes les variétés ont été facilement différenciées sur la base de la longueur des allèles microsatellites à l'exception des mutants des groupes variétaux Pinot et Chasselas (mutation de la couleur des raisins). Le nombre moyen d'allèles détecté par locus était de 13,2 alors que l'hétérozigocité est comprise entre 0,745 (VrZAG 79) et 0,922 (VVMD 7). Les longueurs des allèles ont été comparées avec les résultats obtenus dans d'autres laboratoires; les longueurs étaient soit les mêmes soit différentes de 1-3 bp. Nous avons également testé une nouvelle approche de comparaison de données microsatellites en utilisant un système de codage basé sur des allèles de référence utilisés comme taille standard (THIS *et al*., 2004). Les profils obtenus étaient en général en accord avec les profils des variétés de référence (THIS *et al.*, 2004). Les éventuelles raisons des discordances observées sont discutées.
Lors de ces travaux de profilage microsatellite, un troisième allèle a été observé dans cinq échantillons dont l' ADN avait été extraits de feuilles. Des analyses supplémentaires ont été réalisées avec de l'ADN isolé du phloème (couche méristèmatique L2) et des profils typiques à deux allèles ont alors été obtenus. Il est supposé que la présence simultanée de trois allèles soit due au chimérisme périclinal.

Key words: SSR, cultivar identification, *Vitis vinifera*, genotyping

Mots clés : microsatellite, identification de variété, *Vitis vinifera*, profilage génétique

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INTRODUCTION

Grapevine has been grown in Bohemia (Czech rep.), since the second century when a Roman legion broke through to Moravia and Slovakia and brought the first plants, mostly Malvasia and Chasselas (SEDLO, 1994). Czech viticulture expanded greatly in 14th century in time of Czech King Karel IV (CEPICKA *et al.*, 2001). There are currently 18,700 ha of vineyards in the Czech Republic, with one-third of them yet to reach fruit-bearing age.

There are 58 grapevine and rootstock cultivars registered in the Czech Republic. Many of them are ancient cultivars that are widespread throughout Europe, while 18 were bred in the Czech Republic (formerly Czechoslovakia). More than 700 *Vitis vinifera* and interspecific cultivars are grown in the germplasm collections in Karlstejn, Lednice and Znojmo.

Ampelographic data are still mostly used for cultivar identification and differentiation. New identification systems based on different molecular genetic methods have been tested (SSR - TESSIER *et al.*, 1999; FATAHI *et al.*, 2003; SSR and AFLP- FOSSATI *et al.*, 2001; retrotranspozon- based markers PELSY *et al.*, 2003 etc.). Thus far, microsatellite markers are the most efficient tool for cultivar identification (SEFC *et al.*, 2001). Many SSR loci derived from the Vitis genome have already been described and collected in the NCBI database (http://www.ncbi.nlm.nih.gov/Database/index.html). It is evident from comparative analyses that allele sizes observed in different laboratories vary for a particular cultivar (GRANDO *et al.*, 1998). These discrepancies could have resulted from the unique PCR fragments separation conditions in each experiment. The GENRES 081 European project recommended a batch of SSR markers most suitable for differentiation of grapevine cultivars. This unified system has been employed for cultivar description by various research groups throughout Europe, and established the possibility of comparing obtained results. To overcome the problems described above, THIS *et al.* (2004a) proposed a strategy for data comparison using reference alleles detected in well known cultivars.

Over the last few years, a number of studies have described a third allele in grapevine microsatellite profiles (HOCQUIGNY *et al.*, 2004; FRANKS *et al.*, 2002; RIAZ *et al.*, 2002). The presence of this new allele is usually explained as a consequence of periclinal chimera, as differences occur in obtained SSR profiles when DNA is isolated from different cell layers of the meristem of an individual.

In the present study we analyzed 51 grapevine cultivars registered in the Czech Republic using the six highly informative SSR markers recommended by THIS *et al.* (2004). We used various statistical parameters to assess the suitability of individual loci in terms of their ability to differentiate cultivars. We then constructed an overall table with recorded alleles lengths and corresponding codes (developed by THIS *et al.*, 2004) for each cultivar. Three alleles were observed in the case of some loci and cultivars; the impact of this effect on utilization of SSR for cultivar identification is also discussed.

MATERIAL AND METHODS

I - PLANT MATERIAL

51 grapevine cultivars and rootstocks registered in the Czech Republic in 2003 (table 1). Cuttings were acquired from the collection of the Central Institute for Supervising and Testing in Agriculture, Department of Viticulture in Oblekovice and Research Institute for Grapevine Breeding in Polesovice (table I).

II - DNA EXTRACTION

DNA was isolated from young leaves and phloem with the DNeasy Plant Mini Kit of Qiagen (Hilden, Germany). Shoots of the cultivars with "tri-allelic" SSR profiles were cropped in February. Skin was prune away by the sharp scalpel and phloem-enriched tissue was scrape directly into mortar with liquid nitrogen and grinded. DNA was quantified on 1 % agarose gel stained with ethidium bromide by computational comparison (GeneTools, Version 3.00.22, SynGene, England) with known quantities of λDNA (Takara, Tokyo, Japan).

III - MICROSATELLITE LOCI

Ssix microsatellite loci recommended by the Genres 081 project (THIS *et al.*, 2004) were used. One oligonucleotide of each primer pair was labeled with different fluorescent agents: VVS2 - NED (THOMAS *et al.*, 1993), VVMD5 -NED, VVMD7 -FAM (BOWERS *et al.*, 1996), VVMD27 -FAM (BOWERS *et al.*, 1999), VrZAG62 - JOE, VrZAG79 -JOE (SEFC *et al.*, 1999).

IV - PCR PROTOCOL

Amplification was performed in a reaction volume of 25 µl containing 2 ng of genomic DNA, 1 x buffer (10 mM Tris HCl, pH 8.8; 1.5 mM $MgCl_2$; 150 mM KCl and 0.1 % Triton X-100), 0.2 mM of each dNTP (Promega, Madison, USA), 0.2 µM both primers and 0.5 unit of DNA Polymerase (DyNAzyme II, Espoo, Finland). Amplification was performed on a T-gradient thermocycler (Biometra, Goettingen, Germany). After initial denaturation at 94 °C for 3 min., 40 cycles followed, consisting of: denaturing (30 s at 94 °C), annealing (45 s at 45 - 55 °C, temperature varied for individual locus) and extension (45 s at 72 °C). The last cycle of the extension was prolonged to 9 min and the amplifica-

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Tableau I - Grapevine cultivars registered in the Czech Republic

Variétés de vignes enregistrées en République tchèque

Ab.	name of variety Nom de la variété	colour couleur
Ag	Agni	N
Al	Alibernet	N
An	André	N
Ar	Ariana	N
CM	Cabernet Moravia	N
CS	Petit Cabernet	N
Fr	Blaufrankish	N
Me	Merlot	N
MP	Portugais Bleu	N
Ne	Neronet	N
RM	Pinot Noir	N
Sv	St. Laurent	N
Zw	Zweigeltrebe	N

Table grapes / raison de table

Ab.	name of variety	colour
Ark	Arkadia	B
Di	Diamant	B
CHB	Chasselas Blanc	B
CHÈ	Chasselas Rose	Rg
JB	Julski biser	B
Ol	Olsava	Rg
PK	Panonia Kincse	B
Po	Pola	N
Vi	Vitra	N

Ab.	name of variety Nom de la variété
Au	Aurelius
De	Dì vín
Ch	Chardonnay
IO	Irsai Oliver Muscotaly
Ke	Kerner
Le	Lena
Mal	Malverina
MM	Muscat Moravia
MO	Muscat Ottonel
MT	Müller Thurgau
Ng	Neuburger Blanc
Pa	Pálava
RB	Pinot Blanc
RS	Pinot Gris
RR	Riesling Blanc
RV	Riesling Italico
Sg	Sauvignon Blanc
SZ	Gruner Silvaner
TÈ	Traminer rot
VÈR	Veltliner (Malvoise rose)
VZ	Gruner Veltliner
Ve	Veritas

rootstock / porte greffe

Ab.	name of variety
Am	Amos
K 5BB	Kober 5BB
SO 4	SO - 4
Cr 2	Craciunel 2
T 5C	Teleki 5C
125 AA	125 AA
K 1	LE - K/1

Ab. - abbreviations assigned by the central Institute for Supervising and Testing in Agriculture. Berry color: white and yellow (B), blue and black (N), red (Rg), rose (Rs)

Ab. - abréviations attribuées par l'Institut central de Surveillance et de Contrôle en Agriculture. Couleur des raisins : blanc et jaune (B), bleu et noir (N), rouge (Rg), rose (Rs)

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Table II - Genetic parameters of 6 microsatellite loci used for analysis of 51 cultivars.
Paramètres génétiques des 6 locus microsatellites utilisés pour l'analyse des 51 variétés.

	VVS 2	VVMD 5	VVMD 7	VVMD 27	VrZAG 62	VrZAG 79
number of alleles/ nombre d´allèles	13	12	14	14	12	14
number of allele combination/ nombre de combinaison d'allèles	25	27	22	22	18	30
number of unique allele combination/ nombre de combinaison d'allèle unique	12	16	10	10	8	20
He	0.842561	0.856017	0.865629	0.836986	0.791619	0.896194
Ho	0.843137	0.862745	0.921569	0.843137	0.745098	0.745098
probability of null alleles/ Probabilité d'allèles nuls	-0.000313	-0.003625	-0.029985	-0.003349	0.025966	0.079684
PI	0.072040	0.070550	0.059849	0.077355	0.128893	0.037274

He - expected heterozygosity, Ho - observed heterozygosity, PI - probability of identity
He - hétérozygocité attendue, Ho - hétérozygocité observée, PI - probabilité d'identité

tion products were then stored at 4 °C. Amplifications were confirmed by running 2.5 µl of the PCR product on 5.2 % non denaturating PAGE stained with ethidium bromide.

V - ALLELE SIZING

DNA fragments were analyzed by the ABI Prism 310 genetic analyzer running GeneScan Software (version 3.7, Applied Biosystems, CA, USA) through GA 310 POP-4, a pre-formulated liquid polymer matrix, heated to 60 °C. All amplified samples at six loci for each cultivar were run together. 0.5 - 2 µl of PCR samples (according to signal intensity on PAGE) were pooled together before injection and mixed with 12 µl of deionized formamide and 0.5 µl of fluorescently labeled DNA size standard (GeneScan 400 HD ROX). The mixture was denatured for 4 min at 95 °C and then immediately cooled in ice.

Data were analyzed using « Identity 1.0 » software (WAGNER and SEFC, 1999; www.boku.ac.at/zag/forsch/identity.htm). Similarity of quantitative data was calculated using Nei and Li / Dice similarity index (NEI and LI, 1979). Dendrogram was constructed by means of the unweighted pair group method using arithmetic averages (UPGMA method, SNEATH and SOKAL, 1973). The tree was generated by the NT-SYSpc software (Version 2.11T, Exeter Software, NY, USA).

RESULTS AND DISCUSSION

Fifty-one cultivars registered in the Czech Republic were genotyped at six microsatellite loci. All but five tested cultivars had a unique allelic profile. No allelic differences were detected within the following groups: Pinot blanc, gris and noir, and Chasselas blanc and rose. As with other studies (BOWERS *et al.*, 1996; SEFC *et al.*, 1998; CRESPAN and MILANI, 2001; FATAHI *et al.*, 2003), we were unable to distinguish berry color mutants using SSR markers. Explanation is the discovery of a retrotranspozon insertion into a gene of the anthocyans biosynthesis pathway (KOBAYASHI *et al.*, 2004)

I - DATA ANALYSIS

The number of alleles detected ranged from 12 to 14, with an average of 13.2 alleles per locus (table 2). The expected heterozygosity (NEI, 1973) among all cultivars was higher than in other similar studies (HVARLEVA *et al.*, 2004; FATAHI *et al.*, 2003), and ranged from 0.792 to 0.896. SEFC *et al.* (2000) observed similar values when testing microsatellite variability in grapevine cultivars from different European regions. They also observed that

heterozygosity varies between 0.745 and 0.922. Relatively high heterozygosity of Czech registered cultivars reflects the presence of rootstocks and interspecific hybrids in the collection. The positive probabilities of null alleles (PAETKAU *et al.*, 1995) were obtained from the VrZAG 62 (0.0260) and VrZAG 79 (0.0797) loci. This value for VrZAG 79 is relatively high, as previously reported by ZULINI *et al.* (2002) and HVARLEVA *et al.* (2004). The cumulative probability to obtain no relative individuals with an identical profile at all tested loci (PI) was 6.027. 10-8. For the collection of cultivars, we also calculated the number of allele combinations and unique allele combination for each locus (table II). This value describes the usefulness of the tested loci. (table II)

II - DETECTION OF PERICLINAL CHIMERISM

During SSR analysis of DNA isolated from young leaves, more than two alleles were observed in some cases. Other authors have detected tri-allelic profiles at some loci in clones of Chardonnay, Pinot noir (RIAZ *et al.*, 2002), Pinot gris and blanc (HOCQUIGNY *et al.*, 2004), Primitivo (FRANKS *et al.*, 2002) and in all 15 clones of Pinot Meunier, using the VVS 2 locus (HOCQUIGNY *et al.*, 2004, FRANKS *et al.*, 2002). Based on the analysis of two meristem layers, these studies concluded that the observed triallelism resulted from periclinal chimerism. This kind of somatic mutation is most simply explained as the result of slipped strand mispairing on the larger allele (FRESCO and ALBERTS, 1960) or polymerase slippage (SCHLOTTERER and TAUTZ, 1992). More than two alleles were detected in this study in the case of Pinot blanc, Riesling blanc, Merlot and rootstocks 125 AA and Craucinel 2 (examples: figure 1). The DNA of these samples was isolated from the phloem and reanalyzed. Typical di-allelic profiles were observed; allele sizes were the same or varied by 3 pb at most from the values described by SEFC *et al.* (1998), REGNER *et al.* (2000) and BOWERS *et al.* (1999). The only distinctive result was

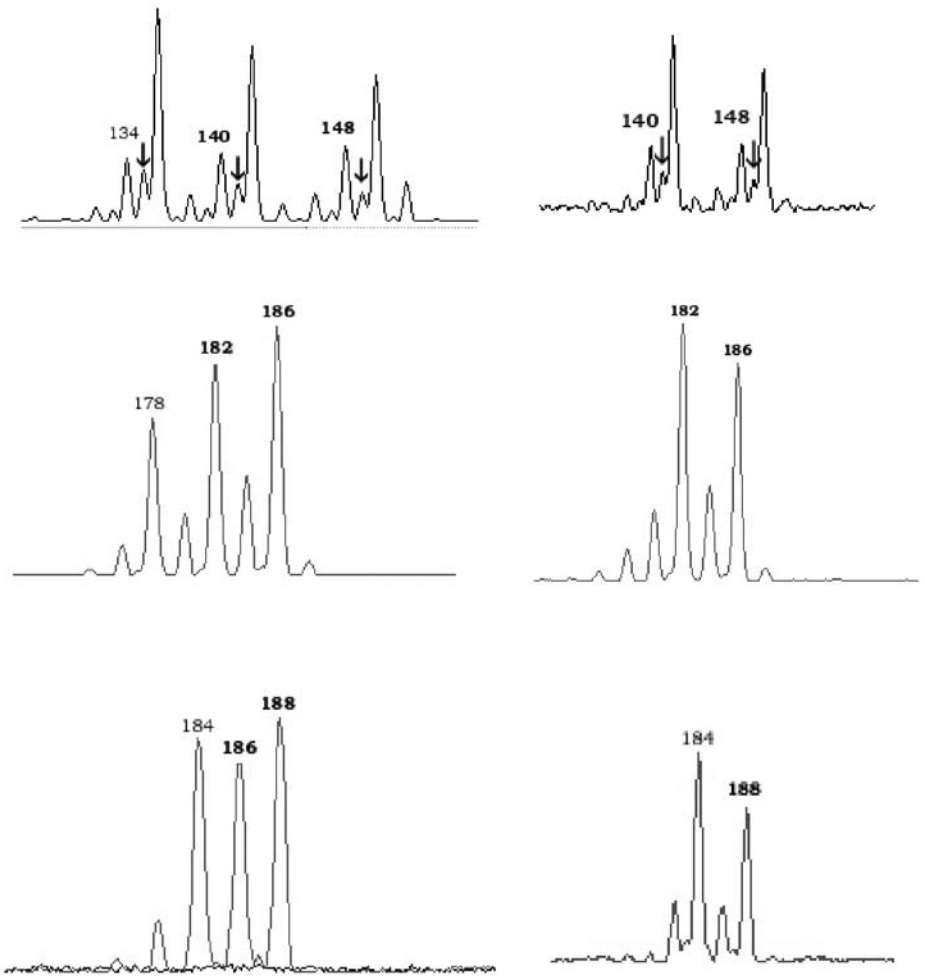


Figure 1 - Examples of detected trialelism, the bold letter mark the standard alleles.
DNA isolated from young leaves DNA isolated from phloem

Exemples de présence simultanée de trois allèles, les allèles en gras sont les allèles standard pour ces variétés
DNA isolé dans de jeunes feuilles DNA isolé dans le phloème

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found in Merlot, which is discussed below. Since the phloem consists only of the L2 meristem layer, we propose that the chimerism is the cause of detected triallelism. It is clear that a particular plant tissue for DNA isolation should be recommended as part of revised protocols that focus on cultivar identification via SSR markers. However, it appears that the L1 layer is more sensitive to mutations, as we found a mutant allele in the L2 meristem layer of a sample of Merlot. FRANKS *et al.* (2002) and RIAZ *et al.* (2002) also detected mutant alleles in both meristem layers. Thus, it is problematic to recommend this particular tissue for DNA isolation. (figure 1).

III - COMPARISON OF RESULTS

We compared the obtained allele sizes of some cultivars with the results of other laboratories. In south Moravia, the main vine-growing region of the Czech Republic, climatic conditions are similar to those in the Austrian viticulture area. Our results are compared mostly with SSR profiles detected by SEFC *et al.* (1998) and REGNER *et al.* (2000). Classic cultivars (Pinot, Sauvignon, Traminer etc.) were analyzed many times by several laboratories; we compared our results with allele sizes detected by GRANDO *et al.* (1998), BOWERS *et al.* (1999) and DETWEILER *et al.* (2000). Allele sizes determined in this study are the same or closely similar (variances were less than 3 bp) to the results of the above studies. Differences may relate to the distinct protocols used for PCR and separation of the fragments.

IV - CODING OF THE ALLELE SIZES

To facilitate the comparison of results between different laboratories, we transformed our results into the coding system (http://www.genres.de/eccdb/vitis/) generated by the group of research teams associated with the GENRES 081 project (THIS *et al.*, 2004). The reference cultivars of Chardonnay, Pinot, Merlot, Cabernet Sauvignon, Traminer, Kober 5 BB, and Teleki 5C were chosen from the collection of cultivars registered in the Czech Republic, and corresponding cultivar codes were assigned to the allele sizes (table III). Obtained allele sizes mostly correspond with those previously published. Discrepancies were detected in Kober 5BB and Teleki 5C; these rootstocks were therefore not used as reference cultivars (discussed below).

We found an allele in Merlot that varied from the standard allele of the reference cultivar. Three alleles (184 : 186 : 188) for VVMD 27 were detected during the ana-

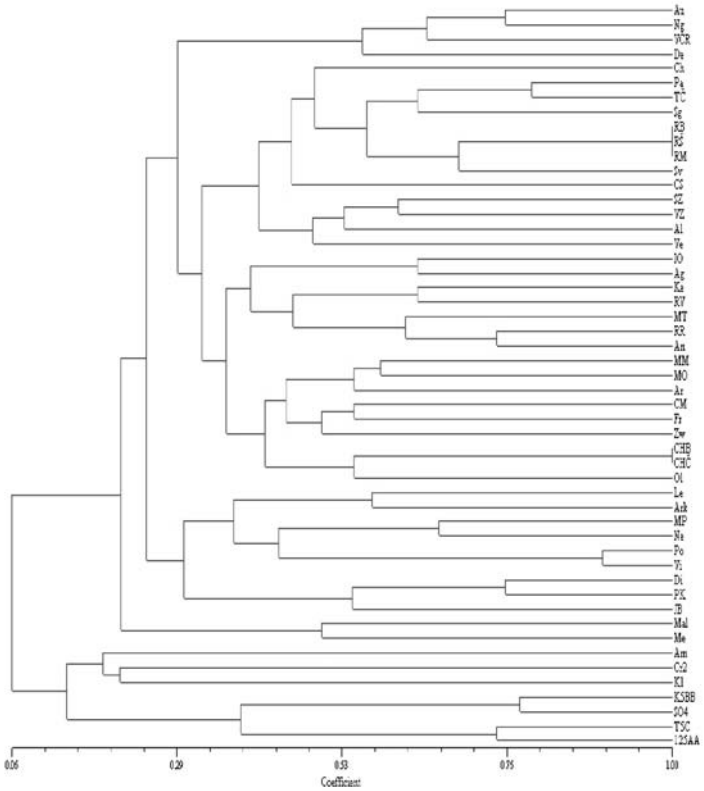


Figure 2 - The dendrogram of relationships among analyzed cultivars derived from allele frequencies of 6 microsatellite loci.

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Table III - Allele size in base pairs (bp) and coded microsatellite profiles for 51 varieties uniformly coded by the PCR fragments of selected reference cultivars.

Longueur des allèles en paire de bases (pb)et profils microsatellites codés pour 51 variétés codées uniformément par les fragments RCP de variétés choisies comme référence.

var.	VVS 2	VVMD 5	VVMD 7	VVMD 27	VrZAG 62	VrZAG 79
Au	128 : 148	224 : 238	244 : 246	186 : 186	203 : 203	241 : 247
	VE1 : SI1	CF1 : CF2	ME2 : MU2	CS2 : CS2	CF2 : CF2	CH1 : SI1
De	128 : 148	**236 : 238**	**240 : 250**	186 : 191	**187 : 191**	**239 : 247**
	VE1 : SI1	CH2 : CF2	TR1 : SU2	CS2 : MU2	CH1 : VE1	n+4 : SI1
Ch	134 : 140	**232 : 236**	236 : 240	178 : 186	187 : 195	241 : 243
	CH1 : CH2	CH1 : CH2	CF1 : TR1	CF1 : CS2	CH1 : CH2	CH1 : CH2
IO	**132 : 152**	224 : 234	244 : 246	176 : 178	203 : 203	247 : 251
	BA2 : MAR2	CF1 : MU2	ME2 : MU2	MU1 : CF1	CF2 : CF2	SI1 : VI2
Ke	**148 : 152**	224 : 234	244 : 254	182 : 186	193 : 193	**235 : 241**
	SI1 : MAR2	CF1 : MU2	ME2 : TR2	PI1 : CS2	CF1 : CF1	RO1 : CH1
Le	132 : 142	224 : 230	236 : 246	**176 : 176**	187 : 203	**235 : 245**
	BA2 : SU1	CF1 : TR1	CF1 : MU2	MU1 : MU1	CH1 : CF2	RO1 : CF1
Mal	**130 : 136**	**234 : 238**	**248 : 248**	**178 : 188**	193 : 193	**253 : 257**
	BA1 : CF1	MU2 : CF2	FE2 : FE2	CF1 : ME2	CF1 : CF1	MU2 : CF2
MM	130 : 148	224 : 226	236 : 254	176 : 178	**177 : 193**	**239 : 251**
	BA1 : SI1	CF1 : MU1	CF1 : TR2	MU1 : CF1	n+4 : CF1	PI1+2 : VI2
MO	130 : 140	224 : 226	236 : 240	176 : 186	187 : 193	251 : 255
	BA1 : CH2	CF1 : MU1	CF1 : TR1	MU1 : CS2	CH1 : CF1	VI2 : 44MA1
MT	140 : 148	224 : 226	244 : 254	**178 : 178**	193 : 193	**241 : 241**
	CH2 : SI1	CF1 : MU1	ME2 : TR2	CF1 : CF1	CF1 : CF1	CH1 : CH1
Ng	128 : 148	224 : 238	244 : 250	**186 : 190**	191 : 203	247 : 247
	VE1 : SI1	CF1 : CF2	ME2 : SU2	CS2 : 4MG1	VE1 : CF2	SI1 : SI1
Pa	148 : 148	230 : 236	240 : 254	186 : 186	187 : 193	241 : 247
	SI1 : SI1	TR1 : CH2	TR1 : TR2	CS2 : CS2	CH1 : CF1	CH1 : SI1
RB	134 : 148	**226 : 236**	236 : 240	182 : 186	187 : 193	**237 : 243**
	CH1 : SI1	MU1 : CH2	CF1 : TR1	PI1 : CS2	CH1 : CF1	PI1 : CH2
RS	134 : 148	**226 : 236**	236 : 240	182 : 186	187 : 193	**237 : 243**
	CH1 : SI1	MU1 : CH2	CF1 : TR1	PI1 : CS2	CH1 : CF1	PI1 : CH2
RR	140 : 148	**224 : 232**	246 : 254	178 : 186	193 : 203	241 : 243
	CH2 : SI1	CF1 : CH1	MU2 : TR2	CF1 : CS2	CF1 : CF2	CH1 : CH2
RV	132 : 148	**224 : 236**	244 : 254	182 : 186	**193 : 195**	247 : 247
	BA2 : SI1	CF1 : CH2	ME2 : TR2	FE1 : CS2	CF1 : CH2	SI1 : SI1
Sg	130 : 148	226 : 230	236 : 254	172 : 186	187 : 193	241 : 243
	BA1 : SI1	MU1 : TR1	CF1 : TR2	CS1 : CS2	CH1 : CF1	CH1 : CH2
SZ	**148 : 150**	226 : 230	**240 : 244**	186 : 191	187 : 203	241 : 243
	SI1 : SI2	MU1 : TR1	TR1 : ME2	CS2 : MU2	CH1 : CF2	CH1 : CH2
TÈ	148 : 148	230 : 236	240 : 254	186 : 186	187 : 193	**243 : 249**
	99R2 : SI1	TR1 : CH2	TR1 : TR2	CS2 : CS2	CH1 : CF1	CH2 : TR2
VÈR	130 : 148	230 : 238	244 : 250	186 : 191	191 : 203	247 : 247
	BA1 : SI1	TR1 : CF2	ME2 : SU2	CS2 : MU2	VE1 : CF2	SI1 : SI1
VZ	130 : 148	**230 : 230**	244 : 254	186 : 191	193 : 203	241 : 245
	BA1 : SI1	TR1 : TR1	ME2 : TR2	CS2 : MU2	CF1 : CF2	CH1 : CF1
Ve	130 : 140	222 : 224	240 : 246	186 : 191	193 : 203	241 : 245
	BA1 : CH2	n+2 : CF1	TR1 : MU2	CS2 : MU2	CF1 : CF2	CH1 : CF1
Ag	**140 : 152**	**224 : 238**	244 : 246	178 : 186	203 : 203	**243 : 247**
	CH2 : MAR2	CF1 : CF2	ME2 : MU2	CF1 : CS2	CF2 : CF2	CH2 : SI1
Al	130 : 148	**224 : 230**	236 : 240	186 : 191	187 : 187	**243 : 253**
	BA1 : SI1	CF1 : TR1	CF1 : TR1	CS2 : MU2	CH1 : CH1	CH2 : MU2
An	140 : 148	**226 : 238**	246 : 254	176 : 186	193 : 203	**233 : 243**

Les profils à allèle unique pour chaque locus sont indiqués en gras.

Unique allele profile for each locus are given in bold.

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var.	VVS 2	VVMD 5	VVMD 7	VVMD 27	VrZAG 62	VrZAG 79
	CH2 : SI1	MU1 : CF2	MU2 : TR2	MU1 : CS2	CF1 : CF2	n-2 : CH2
Ar	140 : 148	226 : 226	236 : 254	176 : 186	193 : 193	**233 : 245**
	CH2 : SI1	MU1 : MU1	CF1 : TR2	MU1 : CS2	CF1 : CF1	n-2 : CF1
CM	**140 : 144**	**224 : 224**	**236 : 260**	176 : 186	193 : 203	235 : 243
	CH2 : CF2	CF1 : CF1	CF1 : CF2	MU1 : CS2	CF1 : CF2	RO1 : CH2
CS	136 : 148	230 : 238	236 : 236	172 : 186	187 : 193	245 : 245
	CF1 : SI1	TR1 : CF2	CF1 : CF1	CS1 : CS2	CH1 : CF1	CF1 : CF1
Fr	140 : 140	224 : 238	236 : 246	176 : 191	193 : 203	**233 : 247**
	CH2 : CH2	CF1 : CF2	CF1 : MU2	MU1 : MU2	CF1 : CF2	n-2 : SI1
Me	136 : 148	224 : 234	236 : 244	**184 : 188**	193 : 193	257 : 257
	CF1 : SI1	CF1 : MU2	CF1 : ME2	VIA1 : ME2	CF1 : CF1	CF2 : CF2
MP	140 : 148	224 : 230	240 : 252	178 : 191	187 : 187	**245 : 255**
	CH2 : SI1	CF1 : TR1	TR1 : PO2	CF1 : MU2	CH1 : CH1	CF1 : 44MA1
Ne	148 : 148	226 : 230	240 : 252	178 : 191	**185 : 203**	**255 : 255**
	SI1 : SI1	MU1 : TR1	TR1 : PO2	CF1 : MU2	MU1 : CF2	44MA1 : 44MA1
RM	134 : 148	226 : 236	236 : 240	182 : 186	187 : 193	237 : 243
	CH1 : SI1	MU1 : CH2	CF1 : TR1	PI1 : CS2	CH1 : CF1	PI1 : CH2
Sv	134 : 148	226 : 226	236 : 254	182 : 186	**191 : 193**	235 : 243
	CH1 : SI1	MU1 : MU1	CF1 : TR2	PI1 : CS2	VE1 : CF2	RO1 : CH2
Zw	134 : 140	224 : 226	236 : 236	**176 : 182**	193 : 193	**235 : 237**
	CH1 : CH2	CF1 : MU1	CF1 : CF1	MU1 : PI1	CF1 : CF1	RO1 : PI1
Ark	**132 : 132**	234 : 234	240 : 246	176 : 191	185 : 187	241 : 245
	BA2 : BA2	MU2 : MU2	TR1 : MU2	MU1 : MU2	MU1 : CH1	CH1 : CF1
Di	130 : 140	230 : 234	236 : 240	182 : 182	187 : 187	247 : 247
	BA1 : CH2	TR1 : MU2	CF1 : TR1	PI1 : PI1	CH1 : CH1	SI1 : SI1
CHB	130 : 140	226 : 234	236 : 244	182 : 186	193 : 203	247 : 255
	BA1 : CH2	MU1 : MU2	CF1 : ME2	PI1 : CS2	CF1 : CF2	SI1 : 44MA1
CHÈ	130 : 140	226 : 234	236 : 244	182 : 186	193 : 203	247 : 255
	BA1 : CH2	MU1 : MU2	CF1 : ME2	PI1 : CS2	CF1 : CF2	SI1 : 44MA1
JB	**130 : 152**	230 : 234	**230 : 236**	182 : 182	187 : 195	245 : 245
	BA1 : MAR2	TR1 : MU2	MU1 : CF1	PI1 : PI1	CH1 : CH2	CF1 : CF1
Ol	140 : 140	**230 : 244**	236 : 244	178 : 186	193 : 203	257 : 257
	CH2 : CH2	TR1 : VE2	CF1 : ME2	CF1 : CS2	CF1 : CF2	CF2 : CF2
PK	130 : 140	230 : 234	240 : 246	**182 : 191**	185 : 187	**247 : 253**
	BA1 : CH2	TR1 : MU2	TR1 : MU2	PI1 : MU2	MU1 : CH1	SI1 : MU2
Po	132 : 148	230 : 234	244 : 246	178 : 191	187 : 203	257 : 257
	BA2 : SI1	TR1 : MU2	ME2 : MU2	CF1 : MU2	CH1 : CF2	CF2 : CF2
Vi	132 : 148	234 : 234	**246 : 246**	178 : 191	187 : 203	257 : 257
	BA2 : SI1	MU2 : MU2	MU2 : MU2	CF1 : MU2	CH1 : CF2	CF2 : CF2
Am	132 : 142	**238 : 248**	238 : 262	**178 : 204**	**187 : 199**	251 : 255
	BA2 : SU1	CF2 : n+28	n+20 : 5C1	CF1 : 1MG2	CH1 : 5C1	VI2 : 44MA1
K 5BB	138 : 146	234 : 264	230 : 262	200 : 204	199 : 213	247 : 251
	GO2 : 99R2	MU2 : 1MG2	MU1 : 5C1	5C1 : 1MG2	5C1 : 11R2	SI1 : VI2
SO4	**144 : 144**	234 : 264	230 : 262	200 : 208	199 : 213	247 : 251
	CF2 : CF2	MU2 : 1MG2	MU1 : 5C1	5C1 : 16C2	5C1 : 11R2	SI1 : VI2
Cr 2	**138 : 142**	**250 : 250**	**248 : 258**	**204 : 204**	**201 : 201**	**253 : 253**
	GO2 : SU1	33C1 : 33C1	FE2 : 99R2	1MG2 : 1MG2	SCH2 : SCH2	MU2 : MU2
T5C	138 : 146	234 : 264	**228 : 262**	200 : 208	191 : 209	249 : 257
	GO2 : 99R2	MU2 : 1MG2	FE1 : 5C1	5C1 : 16C2	VE1 : 5C2	TR2 : MU2
125 A/	**138 : 144**	**224 : 264**	**228 : 248**	200 : 208	191 : 209	249 : 257
	GO2 : CF2	CF1 : 1MG2	FE1 : FE2	5C1 : 16C2	VE1 : 5C2	TR2 : CF2
K 1	**134 : 134**	**226 : 250**	**252 : 262**	**183 : 210**	**179 : 199**	**253 : 261**
	CH1 : CH1	MU1 : 33C1	PO2 : 5C1	GO1 : SCH2	44MA2 : 5C1	MU2 : 99R2

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lysis of DNA isolated from leaves. DNA isolated from the phloem showed the typical diallelic profile (184 : 188). Thus allele 186 bp is probably present only in the L1 layer of the meristem in our sample. THIS *et al.* (2004) claimed that the typical profile for Merlot at locus VVMD 27 is 186 : 188, with the corresponding code CS2 : ME2. BOWERS *et al.* (1999) and NARVÁEZ *et al.* (2001) detected similar allele sizes: (189 : 191). A mutation of the smaller allele in the L2 meristem layer is probably the reason for the different profile in our sample of Merlot. A mutant allele in the L2 meristem layer was also found by RIAZ *et al.* (2002) in the case of two clones of Pinot noir.

Differences from reference cultivars were also detected in the allele sizes of Kober 5BB and Teleki 5C rootstocks. Our obtained SSR profile of VVMD 5 locus is the same as the profile described by SEFC *et al.* (1998), but the shorter allele differs from the reference cultivar by approximately 13 bp (THIS *et al.*, 2004). Likewise, the shorter allele of VrZAG 62 varies by 8 bp from the standard; both alleles of VVS 2 were also different. In Kober 5BB, the differences (2-4 bp) were found on both alleles of VVMD27 and VrZAG 62 loci. The allele sizes of other loci that were transformed to the codes correspond with the standards for both rootstocks (THIS *et al.*, 2004). On the basis of the above results, it is clear that our samples of Kobber 5BB and Teleki 5C rootstocks differ from the samples of the same cultivars used in the GENRES 081project (table III).

Transformation of allele sizes to the codes is easily achieved; this system of data adaptation enables the comparison of results acquired by different researchers using distinctive laboratory protocols.

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