ASSESSMENT OF TWO HIGHLY POLYMORPHIC MICROSATELLITE LOCI IN 103 ACCESSIONS OF *VITIS* SPECIES

ÉVALUATION DE DEUX LOCI MICROSATELLITES HAUTEMENT POLYMORPHES DANS 103 ACCESSIONS DU GENRE *VITIS*

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Abstract: We assessed the discriminative power of two nuclear microsatellite (nSSR, nuclear simple sequence repeated) loci, ssrVvUCH12 and ssrVvUCH29, which had been suspected to be highly polymorphic and to display specific alleles in the frame of a previous characterization work. As more genotyping of more plant material at a higher number of nSSR loci was needed for an extensive comparison, one hundred and three accessions of *Vitis vinifera*, other *Vitis* species, and 3 related genera were submitted to microsatellite profiling at fourteen nuclear nSSR loci, including ssrVvUCH12 and vsrVvUCH29. The loci ssrVvUCH12 and ssrVvUCH29 loci displayed each 30 alleles found in 103 accessions while the most polymorphic loci among the 12 other nSSR loci tested, were ssrVrZAG93 and VMc8a7, which displayed 31 and 23 alleles respectively. These 2 last loci had however the disadvantage to display a three banded pattern in some individuals, probably due to chimerism. Most interestingly average similarity found in this pool of 103 accessions was also lower with the combination ssrVvUCH12-ssrVvUCH29 than with the combination ssrVrZAG-VMC8a7, while observed heterozygocity was higher with the combination ssrVvUCH12-ssrVvUCH29. Comparisons were made easily visible under the form of UPGMA phenograms for each combination of loci and plant sets and the most resolutive combination was always the combination ssrVvUCH12-ssrVvUCH29.
At last, numerous specific alleles were found for rootstocks and *Vitis* species at loci ssrVvUCH12 and ssrVvUCH29, while the other combination did not show many specific alleles. These features allow to think that this 2-loci combination could be considered for managing collections of *Vitis* species in addition of previously evaluated highly polymorphic microsatellite markers. It could also be useful as a quality control tool for rootstock material in nurseries or trade.

Résumé: Nous avons évalué le pouvoir discriminant de deux loci microsatellites nucléaires (SSRn, séquences simples répétées), ssrVvUCH12 et ssrVvUCH29, que l'on suspectait être hautement polymorphe et receler des allèles spécifiques, d'après un précédent travail de caractérisation. Comme le génotypage d'un plus grand nombre de plantes à un plus grand nombre de loci était nécessaire pour en permettre une comparaison plus étendue, 103 accessions de *Vitis vinifera*, d'autres espèces de *Vitis* et de 3 genres apparentés ont été soumises au profilage par microsatellites à 14 loci microsatellites nucléaires, incluant ssrVvUCH12 et vsrVvUCH29. Au total, 30 allèles ont été caractérisés pour chacun des deux loci ssrVvUCH12 et vsrVvUCH29 dans les 103 accessions. Les deux loci les plus polymorphes parmi les 12 autres loci analysés étaient ssrVrZAG93 et VMc8a7 et avaient respectivement 21 et 23 allèles dans les 103 accessions analysées au total. Ces deux dernier loci ont par contre le désavantage d'afficher de façon reproductive un profil à trois allèles dans un certain nombre d'accessions, signe probable de chimérisme. Il est aussi particulièrement intéressant que la similarité moyenne calculée pour les 103 accessions analysées avec la combinaison des deux loci ssrVvUCH12-ssrVvUCH29 ait été trouvée inférieure à celle obtenue avec la combinaison ssrVrZAG93-VMC8a7. Par contre l'hétérozygotie obtenue avec la combinaison ssrVvUCH12-ssrVvUCH29 était supérieure à celle obtenue avec la combinaison ssrVrZAG93-VMC8a7. Les comparaisons entre les deux combinaisons de loci ont été visualisés sous la forme de phénogrammes UPGMA pour les 103 accessions et pour des sous-groupes au sein de ces 103 accessions. Dans chaque cas de comparaison, la combinaison ssrVvUCH12-ssrVvUCH29 était toujours la plus avantageuse en terme de discrimination.
De plus de nombreux allèles spécifiques ont été découverts pour des porte-greffes et des espèces de *Vitis* autre que *V. vinifera,* aux loci ssrVvUCH12 et ssrVvUCH29, alors qu'il y en avait peu pour les deux autres loci.
Ces caractéristiques permettent de penser que ces deux loci très polymorphes pourraient être considérés pour la gestion des collections ampélographiques, en combinaison avec les loci microsatellites déjà en usage. Ils pourraient également être intéressants comme outils de contrôle de qualité de certains porte-greffes dans les pépinières ou lors d'échanges commerciaux.

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INTRODUCTION

Nuclear microsatellites (simple sequences repeated, nSSRs) are polymorphic markers which display among other advantages the fact to be locus-specific, co-dominant and reproducible. Many loci have now been characterized in grapevine, where they tend more and more to become the molecular marker of choice for cultivar identification purposes as well as for pedigree reconstruction and genetic mapping. The history of their development in grapevine and their present applications have been reviewed by SEFC *et al.* (2001).

The challenge with microsatellites has always been to find highly polymorphic loci in order to reduce the number of markers to be used in routine analysis, and thus to decrease the cost of microsatellite profiling. In the frame of the evaluation of 14 nSSR loci, four of them appeared to be highly polymorphic. Two of them had been previously evaluated in rootstocks and Greek cultivars (LEFORT and ROUBELAKIS, 2001, LEFORT et al., 2002) and were compared in this work to the two most polymorphic loci out of 12 nSSR, including new loci.

MATERIALS AND METHODS

I - PLANT MATERIAL AND DNA EXTRACTION

Young, expanding leaves were collected from 103 individual accessions from the ampelographic collection of INRA Colmar (France). These accessions included 53 *Vitis vinifera* subsp. *vinifera* cultivars, 17 *Vitis vinifera* subsp. *sylvestris* individuals, 23 other *Vitis* species (including 10 rootstocks), 4 *Muscadinia rotundifolia* varieties, 5 *Ampelopsis* species and 1 *Parthenocissus* species. Total DNA was purified using the DNAeasyTM Plant Mini Kit (Qiagen, Hilden, Germany) as described by the supplier.

II - SSR ANALYSIS

Amplifications were carried out at 14 nuclear microsatellite (nSSR, nuclear simple sequence repeated) loci: ssrVvUCH12, ssrVvUCH29 (LEFORT *et al*., 2002), ssrVrZAG25, ssrVrZAG62, ssrVrZAG79, ssrVrZAG93 (SEFC et al., 1999), VVS2, VVS29 (THOMAS and SCOTT, 1994), VVMD30 (BOWERS et al., 1999), VMC1a11, VMC2a12, VMC3a9, VMC5g7 (*Vitis* Microsatellite Consortium, unpublished data), VMC8a7 (SCOTT K.D.; unpublished; forward primer: gcAgcAAcTcTcTTAcAcAccg, reverse primer gTgggAgcAcTggTTgcTTTAg). PCR amplifications were multiplexed using two different fluor labels FAM and HEX (PE Applied Biosystems, Warrington, UK). In each case the label primer was the forward primer.

Amplification reactions were performed in a 8 µl reaction mixture, with 8 ng of genomic DNA, 2.5 mM of MgCl2, 1x Buffer Gold, 150 mM of each dNTP (Boeringer Manheim, Germany), 0.2 unit of Amplitaq Gold (PE Applied Biosystems, Foster City, CA), 0.25 to 0.5 mM labeled primer and 0.5 mM of unlabelled primer. PCR was carried out in the GeneAmp®PCR System 9700 thermocycler (PE Applied Biosystems). The cycling program was as follows: 94 °C for 10 min, 35 cycles of 92 °C for 45 s, 57 °C for 1 min and 72 °C for 1 min 30, followed by 72°C for 5 min. Amplified fragments were resolved on an automated 310C ABI PRISM DNA sequencer (PE Applied Biosystems) with HD400 ROX (PE Applied Biosystems) used as an internal size standard. Such analysis was repeated twice. Band scoring was performed using GenScan (version 3.1) and Genotyper (version 2.5.2) softwares (PE Applied Biosystems).

Genetic similarity was estimated using Nei and Li coefficient method (NEI and LI, 1979). The derived phenograms were generated by UPGMA analysis on NTSYSpc (version 2.02i) software.

RESULTS AND DISCUSSION

When compared to loci ssrVvUCH12 and ssrVvUCH29, which displayed each 30 alleles, only loci ssrVrZAG93 and VMC8a7, out of the twelve other loci used in this comparison displayed a comparable polymorphism in the analysed set of 103 accessions, with respectively 31 alleles and 23 alleles. This combination was thus further kept for comparison with the ssrVVUCH12-ssrVvUCH29 combination. Microsatellite profiling results are given for the four loci ssrVvUCH12, ssrVvUCH29, ssrVrZAG93 and VMC8a7 in table 1, where allele sizes are given in base pairs. Loci ssrVvUCH12 and ssrVvUCH29 showed more alleles in these 103 accessions than reported before (Lefort et al., 2002)

It was noticeable that some accessions had a reproducible tri-allelic profile at loci ssrVrZAG93 and VMC8a7, which represent a disadvantage for software assisted-pedigree search in large data samples since most of such softwares are only able to analyse diallelic data. Such tri-allelic profiles have been already reported to occur at some loci by FRANKS et al. (2002). Eight accessions displayed a tri-allelic profile with VMC8a7: 3 *Ampelopsis* accessions and 5 *Vitis vinifera* accessions. All of the *Vitis vinifera* accessions are Muscat cultivars sharing the same third allele. Interestingly in this example, the third allele could help and identify which one of these cultivars could be the ancestor of the other Muscat cultivars with a tri-allelic pattern. At locus ssrVrZAG93, 7 accessions had a

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Table I - Microsatellite profiles at loci ssrVvUCh12, ssrVvUCh29, ssrVrZAG93 and VMC8a7. Scored allele sizes are given in base pairs (bp) (- ; not applicable).

Profiles microsatellites aux loci ssrVvUCh12, ssrVvUCh29, ssrVrZAG93 et VMC8a7. La taille des allèles est donnée en paires de bases (pb).

Accessions	VvUCH12		VvUCH29		VMC8a7			VrZAG93		
	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 3	Allele 1	Allele 2	Allele 3
A. aconitifolia	-	-	207	218	145	151	159	163	-	-
A. cordata	157	160	228	-	157	159	-	164	-	-
A. heterophylla	-	-	216	-	145	153	-	159	173	199
A. japonica	-	-	221	-	151	153	155	163	173	-
A. pedonculata	-	-	216	-	149	155	157	163	195	212
Aligoté	165	-	205	287	151	157	-	184	-	-
Aubin vert	165	-	205	287	151	157	-	184	-	-
Auxerrois	161	165	287	295	151	157	-	184	-	-
Bachet	161	165	287	295	157	159	-	184	-	-
Beaunoir	165	-	205	287	157	159	-	184	-	-
Cabernet franc	149	165	207	209	161	-	-	195	-	-
Cabernet Sauvignon	147	165	209	284	161	-	-	195	-	-
Carignan	143	165	209	-	151	155	-	184	-	-
Chardonnay	165	-	287	295	157	159	-	184	-	-
Chasselas	165	-	287	307	157	161	-	184	-	-
Chenin	159	165	209	284	157	161	-	184	-	-
Cinsaut	147	159	209	-	155	-	-	186	226	-
Clairette	147	159	307	-	155	161	-	184	203	-
Colombard	159	161	205	-	157	159	-	184	191	-
Corbeau	165	-	209	295	157	-		184	193	-
Côt	165	-	209	-	157	161	-	184	226	-
Couderc 3309 (*V. riparia* Tomenteux X *V. rupestris* Martin)	160	209	298	-	161	171	-	214	218	-
Folle blanche	147	165	205	287	157	159	-	184	-	-
Franc Noir de Haute Saône	161	165	287	295	151	157	-	184	-	-
Gamay	161	165	205	287	151	157	-	184	-	-
Gamay blanc Gloriod	161	-	205	287	157	159	-	184	-	-
Gewurztraminer	147	165	284	287	157	-	-	184	-	-
Gouais	161	165	205	295	151	159	-	184	-	-
Graisse	147	-	209	-	151	-	-	210	-	-
Grenache	147	165	213	287	157	168	-	210	-	-
Knipperlé	165	-	287	295	157	159	-	184	-	-
Kobber 5BB (V.*berlandieri* X *V .riparia*)	157	221	281	289	158	159	-	203	205	210
M. rotundifolia Carlos	147	-	298	-	145	-	-	218	236	-
M. rotundifolia Dulcet	147	-	293	-	145	-	-	197	217	-
M. rotundifolia Régale	133	147	298	-	145	150	-	236	242	-
M. rotundifolia YxC	147	-	293	-	145	-	-	197	219	-
Marsanne	159	165	307	-	155	157	-	193	195	-
Mauzac	159	165	307	-	155	157	-	195	195	-
Melon	165	-	287	295	157	159	-	184	-	-
Merlot Noir	149	165	209	295	157	161	-	184	195	-
Mission	147	165	205	287	159	165	-	210	210	-
Mourvèdre	159	165	207	307	155	157	-	184	195	-
Muscat 336	165	-	287	307	157	163	165	184	186	-
Muscat d'Alexandrie	147	165	209	295	151	161	-	184	186	-
Muscat d'Alsace	165	-	209	295	161	163	165	184	186	-
Muscat de Hambourg	147	165	209	-	151	157	-	184	186	-
Muscat Ottonel	165	209	209	307	161	163	165	184	186	-
Muscat à petit grain	165	-	209	295	161	163	165	184	186	-
Muscat Reine des Vignes	147	165	209	295	151	155	-	186	210	-
Muscat de Saumur	165	-	287	295	157	163	165	184	-	-
P. quinquefolia	123	-	254	-	150	-	-	202	-	-
Persan	147	-	284	295	157	-	-	184	-	-

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Table I (suite) - Microsatellite profiles at loci ssrVvUCh12, ssrVvUCh29, ssrVrZAG93 and VMC8a7. Scored allele sizes are given in base pairs (bp) .

Profiles microsatellites aux loci ssrVvUCh12, ssrVvUCh29, ssrVrZAG93 et VMC8a7. La taille des allèles est donnée en paires de bases (pb).

Accessions	VvUCH12		VvUCH29		VMC8a7			VrZAG93		
Peurion	165	-	287	295	157	159	-	184	-	-
Pinot Noir	165	-	287	-	157	-	-	184	-	-
Riesling	147	165	205	307	157	159	-	184	-	-
Roublot	161	165	205	287	151	157	-	184	-	-
Roussanne	147	159	307	-	155	157	-	193	195	-
Sacy	161	165	205	287	157	159	-	184	-	-
Sauvignon	147	165	284	295	157	161	-	184	-	-
Sémillon	165	-	307	-	157	-	-	184	193	-
Sylvaner	165	-	284	295	157	159	-	184	-	-
Syrah	165	-	209	287	157	-	-	184	195	-
Tannat	165	-	209	211	155	157	-	184	-	-
Ugni Blanc	161	165	209	307	157	161	-	184	195	-
V. amurensis	135	159	271	276	155	159	-	184	186	-
V. arizonica	147	204	285	-	157	-	-	195	212	-
V. berlandieri Colombard	165	173	303	307	157	-	-	184	197	203
V. berlandieri Planchon	158	183	289	291	158	162	-	191	205	207
V. berlandieri Thyers	147	159	209	211	157	159	-	184	226	-
V. candicans	144	150	284	-	163	181	-	182	208	-
V. cinerea	144	160	283	-	158	160	-	189	195	199
V. cordifolia 9 Couderc	160	162	279	283	150	172	-	219	-	-
V. doaniana	157	160	284	294	159	176	-	182	220	-
V. ishikari	183	189	274	-	155	159	-	184	201	-
V. labrusca Isabelle	161	165	284	287	157	-	-	184	202	-
V. linsecumii	165	181	284	286	159	162	-	182	189	-
V. monticola Large Bell	144	150	298	308	148	164	-	193	195	201
V. palmata	150	178	274	-	151	155	-	189	189	-
V. reticulata	187	189	274	297	159	173	-	214	222	-
V. riparia Gloire de Montpellier	221	233	281	288	159	161	-	210	-	-
V. riparia Müller	239	-	288	-	159	-	-	210	219	-
V. rubra	152	181	279	285	159	-	-	193	210	-
V. rupestris du Lot	133	158	284	294	161	181	-	189	218	-
V. silvestris 50K	147	-	209	211	157	159	-	184	224	-
V. silvestris 50L	147	159	209	211	157	159	-	184	226	-
V. silvestris 53J	147	-	209	-	157	-	-	184	226	-
V. silvestris C1S1	147	-	209	-	157	-	-	184	-	-
V. silvestris C1S2	147	-	209	-	157	-	-	184	-	-
V. silvestris C1S3	147	-	209	-	157	-	-	184	-	-
V. silvestris C1S4	147	-	209	-	157	-	-	184	226	-
V. silvestris C1S5	147	-	284	-	157	-	-	184	195	-
V. silvestris C25S1	147	-	211	-	157	-	-	184	-	-
V. silvestris C25S2B	147	-	211	-	157	-	-	184	-	-
V. silvestris C25S2H	147	-	211	-	157	-	-	184	-	-
V. silvestris C25S3	147	-	211	-	157	-	-	184	-	-
V. silvestris C25S4	147	-	211	-	157	-	-	184	-	-
V. silvestris C25S5	147	-	211	-	157	-	-	184	-	-
V. silvestris C25S6	147	165	209	-	151	157	-	184	-	-
V. silvestris C25S7	147	-	209	-	157	-	-	184	-	-
V. silvestris C25S8	147	-	209	-	157	-	-	184	226	-
V. titania	170	215	274	-	159	169	-	189	-	-
V. vulpina	150	162	279	287	161	-	-	189	210	-
Viogner	165	165	287	307	157	161	-	184	195	-

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Table II - Specific alleles for some *Vitis* species and other accessions found at loci ssrVvUCh12 and ssrVvUCh29.

Alleles were considered as specific when found in one or two species or in hybrids originated from one of these species with specific alleles. Allele sizes are given in base pairs (bp). Specific alleles are shown in bold.

Allèles spécifiques de certaines espèces de Vitis et d'autres accessions aux loci ssrVvUCh12 et ssrVvUCh29.

Un allèle est considéré comme spécifique lorsque présent dans une ou deux espèces ou chez les hybrides, ayant ces espèces pour origine. La taille des allèles est donnée en paires de bases (pb). Les allèles spécifiques sont donnés en gras.

Locus	ssrVvUCH12		ssrVvUCH29	
Name	Allele 1	Allele 2	Allele 1	Allele 2
V. arizonica	147	**204**	**285**	**285**
V. berlandieri X V. *riparia* Kobber 5BB	157	**221**	**281**	**289**
V. berlandieri Colombard	165	**173**	**303**	307
V. berlandieri Planchon	**158**	183	**289**	**291**
V. candicans	144	**150**	284	284
V. cinerea	144	**160**	**283**	**283**
V. linsecumii	165	**181**	284	286
V. riparia Gloire	**221**	**233**	**281**	**288**
V. riparia Müller	**239**	**239**	**288**	**288**
V. rubra	**152**	**181**	279	**285**
V. rupestris du Lot	**133**	**158**	284	294
V. cordifolia 9 Couderc	160	162	279	**283**
M. *rotundifolia*_Régale	**133**	147	298	298

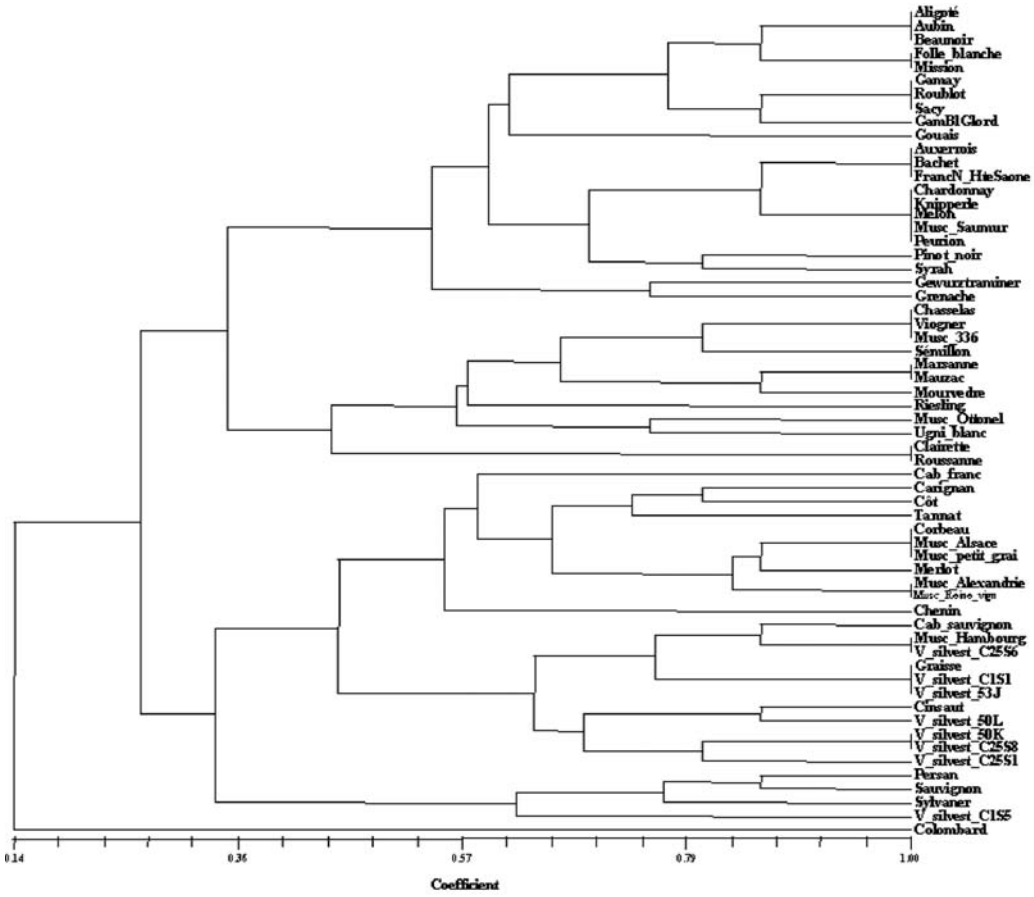


Fig. 1a - 61 accessions of *V. vinifera* profiled at 2 nSSR loci UCH12 and UCH29.

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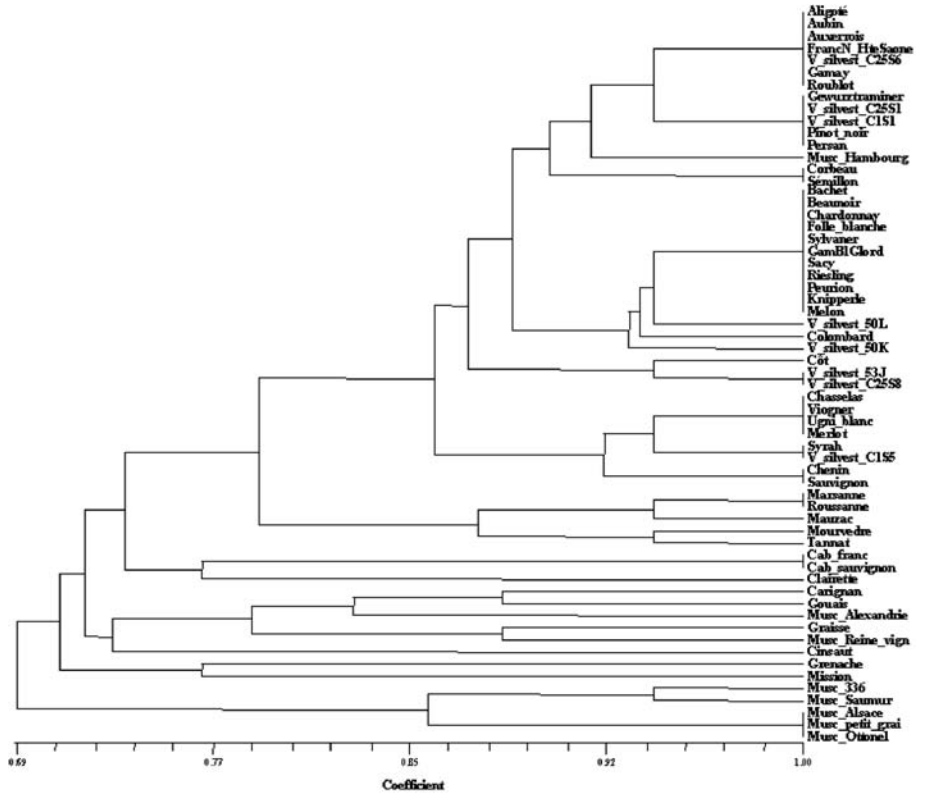


Fig. 1b - 61 accessions of *V. vinifera* profiled at 2 nSSR loci ssrVrZAG93 and VMC8a7

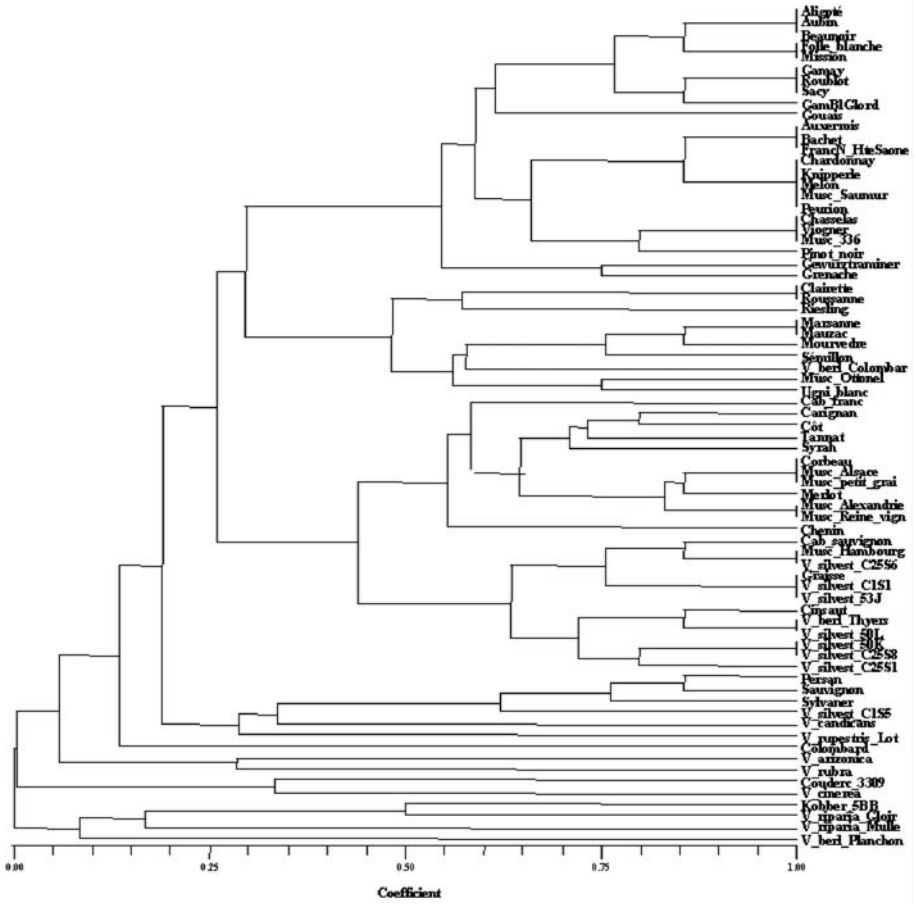


Fig. 2a - 73 accessions of *V. vinifera* and rootstocks profiled at 2 nSSR loci UCH12 and UCH29.

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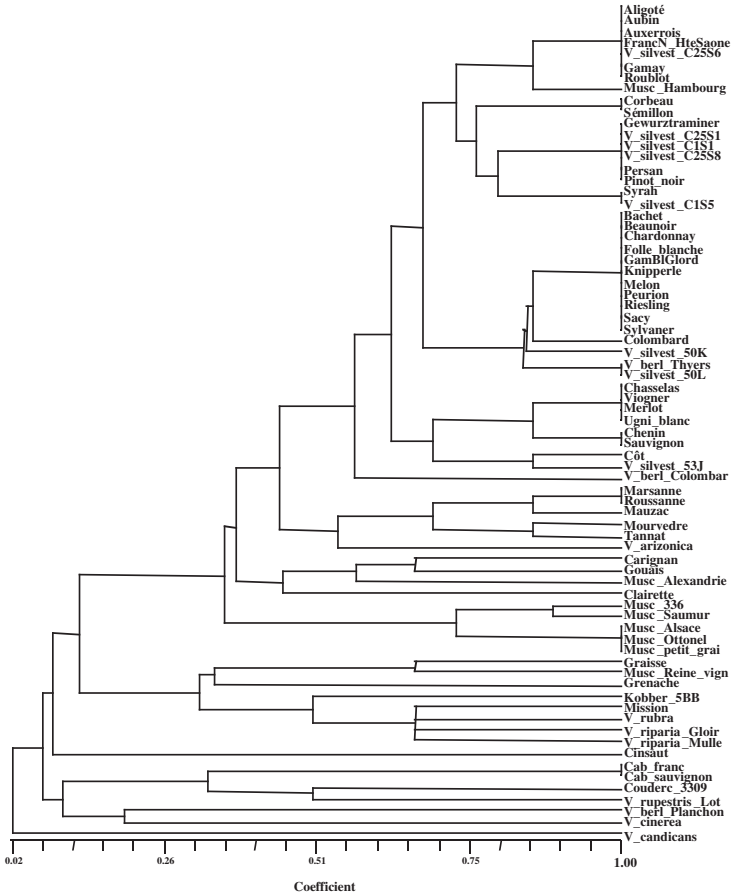


Fig. 2b - 73 accessions of *V. vinifera* and rootstocks profiled at 2 nSSR loci ssrVrZAG93 and VMC8a7.

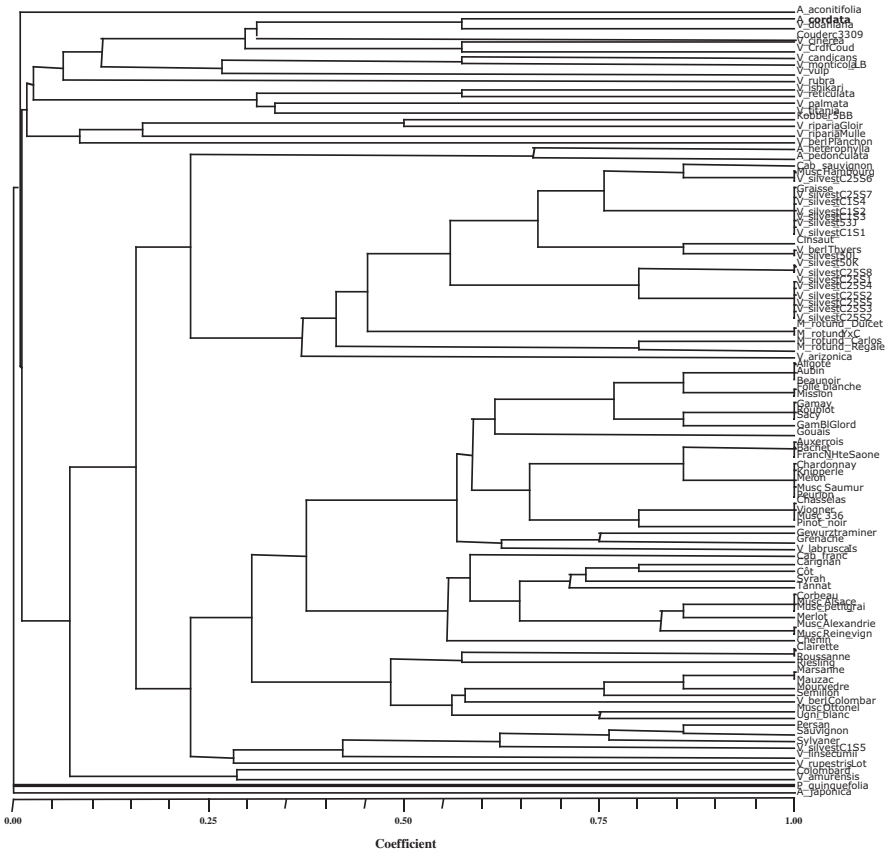


Fig. 3a - 103 accessions profiled at 2 nSSR loci UCH12 and UCH29.

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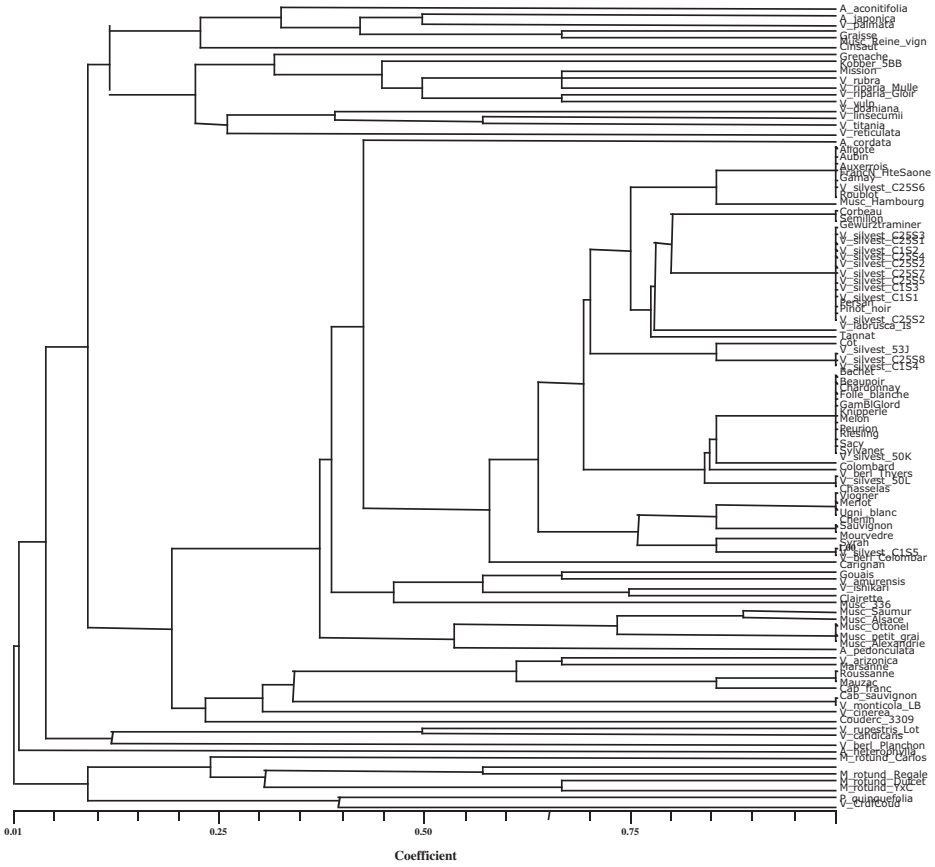


Fig. 3b - 103 accessions profiled at 2 nSSR loci ssrVrZAG93 and VMC8a7.

tri-allelic profile and none were from *Vitis vinifera* accessions. Suspicion of null alleles at ssrZAG93 was strengthened by the profiles of the Cabernet Sauvignon pedigree, which showed that the 184 bp allele of Sauvignon Blanc was not transmitted to Cabernet Sauvignon, whose 195 bp allele originated from Cabernet Franc. In this particular case the null allele was the transmitted allele.

The resolution of both combinations (ssrVVUCH12-ssrVvUCH29 and ssrVrZAG93-VMC8a7) was analysed successively in a set of 61 accessions of *Vitis vinifera* subsp. *vinifera* (53 accessions) and subsp. *sylvestris* (8 accessions) (fig. 1a and fig. 1b), in a set of 73 accessions including the previous 61 *V. vinifera* accessions and 12 other *Vitis* sp. (including common rootstocks) (fig 2a and fig. 2b) and finally in the total set of 103 accessions including 53 cultivars of *V. vinifera* subsp. *vinifera*, 17 individuals of *V. vinifera* subsp. *sylvestris*, 23 other *Vitis* sp., 5 *Ampelopsis*, 4 *Muscadinia* and 1 *Parthenocissus* species (fig. 3a and fig. 3b).

In figure 1a, it appears that the combination ssrVVUCH12-ssrVvUCH29 resolves the 61 *Vitis vinifera* accessions in 39 branches among which 25 single branches corresponding thus to a single identity profile, whereas the combination ssrVrZAG93-VMC8a7 (fig. 1b) resolves the same set of accessions in 30 branches among which 18 single branches.

Figure 2a shows that the combination ssrVVUCH12-ssrVvUCH29 resolves 73 accessions of *V. vinifera* and *Vitis* sp. in 50 branches among which 39 branches correspond to single profiles. The combination ssrVrZAG93-VMC8a7 (fig. 2b) resolved the same set of 73 accessions in 41 branches among which 30 single branches corresponded to single identity profiles.

Finally when assessed in the total set of 103 accessions, the combination ssrVvUCH12-ssrVvUCH29 (fig. 3a) produced 70 branches among which 54 single branches whereas the second combination ssrVrZAG93-VMC8a7 (fig. 3b) produced 62 branches among which 50 single branches.

Additionally to the number of alleles per locus, analysis in the total set of 103 accessions showed an observed heterozygosity of 0.849 for the combination ssrVvUCH12-ssrVvUCH29 and 0.772 for the combination ssrVrZAG93-VMC8a7. Although single locus

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heterozygosity was not high for the loci ssrVvUCH12 (Ho=0.57) and ssrVvUCH29 (Ho=0.57), the combination of these 2 loci yielded a higher hetorozygosity than the other combination. On the other hand, the average similarity, in the 103 accessions, calculated from the genetic distance matrix, was 0.19 for the combination ssrVvUCH12-ssrVvUCH29 and 0.31 for the combination ssrVrZAG93-VMC8a7. Average similarity calculations included tri-allelic profiles at loci ssrVrZAG93 and VMC8a7, thanks to the software NTSYSpc which can accommodate such data. Both combination seemed to be more resolutive in *Vitis* sp. other than *V. vinifera* but still the combination ssrVvUCH12-ssrVvUCH29 showed to be more advantageous with the presence of numerous specific alleles, which are potentially very useful for management of rootstock species with a reduced number of microsatellite markers.

The table II shows some specific alleles for a few *Vitis* species. It is especially noticeable for *V. riparia* which both species display specially long alleles (221 bp, 233 bp, 239 bp) at ssrUCH12 locus whereas this locus mainly has short alleles. The specific allele 221 bp-long of Kobber 5BB at ssrVvUCH12 comes from its parent *V. riparia* Gloire while the specific allele 289 bp-long at ssrVvUCH29 comes from the parent *V. berlandieri* Planchon and the specific allele 281 bp-long at ssrVvUCH29 comes from *V. riparia* Gloire as expected (GALET, 1956).

These specific alleles may especially be of interest for these species used as rootstocks in order to allow their management in nursery and trade with one or 2 markers.

CONCLUSION

The features of these two loci ssrVvUCH12 and ssrVvUCH29 make them valuable for a use in the management of ampelographic collections, in addition to previously evaluated highly polymorphic SSR markers. They could also find a use as quality control tools for management of rootstock material in nurseries or trade, where lowering the analytical costs through the use of a reduced number of markers is a matter of concern in order to make the sophisticated technique of genetic profiling a practicable approach.

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