ASSESSING THE IDENTITY OF GRAPEVINE PLANTS FROM VINEYARDS FROM CRETE AND SAMOS BY MICROSATELLITE PROFILING

ÉVALUATION DE L'IDENTITÉ DE PLANTS DE VIGNES ISSUS DES VIGNOBLES DE CRÈTE ET DE SAMOS PAR PROFILAGE À DES LOCI MICROSATELLITES

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Abstract : Four nuclear microsatellite markers were used for testing 45 plants visually selected from 18 locations in controlled origin wine areas of Crete and Samos, and representing 7 cultivars. Discrepancies between the obtained profile and a profile from a plant of reference of a given cultivar, were found for 26 plants. This suggested that microsatellite profiling at a small number of loci was an efficient procedure in order to detect and remove inappropriate material at an early stage of selection.
In a second case, three plants were sampled from a vineyard, located in the controlled origin wine area of Dafnes in Crete, for testing their identity in comparison to cultivars of reference at 15 nuclear microsatellite loci. Only one matched its identity profile of reference. One of them was found to be a continental cultivar, named Fegi and not usually grown in Crete. This showed the resolution of microsatellite profiling for problems where misnaming at an early stage in nursery or trade could have important consequences for growers in controlled origin wine areas.

Résumé : Quatre marqueurs microsatellites nucléaires ont été utilisés pour tester l'identité de 45 plantes, sélectionnées visuellement pour leurs différences phénotypiques avec les plantes environnantes de 7 cultivars, dans 18 vignes différentes de Crète et de Samos. Des différences entre profils génétiques obtenus et profils génétiques de références pour ces cultivars ont été relevées pour 26 plantes. Cela suggère que le profilage génétique à un nombre réduit de loci microsatellite est une procédure efficace pour détecter et donc éliminer un matériel végétal impropre à une phase précoce d'un processus de sélection à partir de vignes déjà établies.
Dans un second cas, l'identité génétique de trois plantes, échantillonnées dans un vignoble privé de l'appellation contrôlée Dafnes en Crète, a été testée avec 15 marqueurs microsatellites nucléaires et les profils génétiques obtenus ont été comparés avec ceux de cultivars de référence. Une des trois plantes (dite Liatiko Div. Agr.) était identique à son profil de référence. Des deux autres, une (dite Kotsifali Div. Agr.) pouvait être un clone ou un parent de la première.
Après comparaison de son profil d'identité génétique avec 298 profils génétiques à 9 loci microsatellites nucléaire de cultivars grecs, la troisième plante (dite Kotsifaloliatiko Div.Agr.) correspondait à un cultivar habituellement non cultivé en Crète mais en Grèce continentale et nommé Fegi. Ces résultats illustrent la résolution du profilage génétique par microsatellite appliqués à des problèmes pratiques d'identification de plantes provenant de vignes, où des erreurs de nom à un stade précoce (pépinière, distribution commerciale) peuvent avoir des conséquences importantes pour le viticulteur, dans les régions d'appelation contrôlée.

Key-words: grapevine cultivars, microsatellite profiling, nSSR, *Vitis vinifera*.

Mots-clés : cultivars de vigne, profilage génétique, microsatellite, *Vitis vinifera*.

INTRODUCTION

Among the different classes of molecular markers available for molecular profiling of genotypes, microsatellites (or single sequence repeated, SSR) tend to be the tool of choice, because they are specific, highly polymorphic and co-dominant, while they also allow technically a high reproducibility between users of distant laboratories, thus enabling an easy standardization of the sizing of the alleles. Since the characterization of the first microsatellite markers in grapevine by THOMAS *et al.* (1994), more markers have been made available in the past years (BOWERS *et al.*, 1996; DI GASPERO *et al.*, 2000; LEFORT *et al.*, 2002a; SEFC

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et al., 1999), which have been used in a variety of purposes (for a review, see SEFC *et al.,* 2001). They allowed in particular for the first time the comparison of molecular genetic profiles of distant gene pools, (SEFC *et al.*, 2000; SEFC *et al.*, 2002) and the molecular markers-assisted management of genetic resources (MALETIC *et al.*, 1999 ; LOPES *et al.*, 1999). In some cases they were also useful for discriminating clonal lines (VIGNANI *et al.*, 1996). Greek genetic resources have been evaluated with previously characterized microsatellite markers and genetic profiles were made available in combination with the last advances in information technology (LEFORT and ROUBELAKIS-ANGELAKIS, 2000; LEFORT and ROUBELAKIS-ANGELAKIS, 2001; LEFORT, 2002; LEFORT *et al.*, 2002b). Markers of the same type but targeting the chloroplast genome instead of the nuclear genome can also complete the genetic information provided by nuclear microsatellites and their potential had also been assessed in Greek cultivars of grapevine (ARROYO *et al.*, 2002; LEFORT *et al.*, 2000)

Following this initial step of characterization of genetic resources of reference conserved in ampelographic collections in Greece, genetic profiling with microsatellite markers can now be used for checking the identity of plants selected from the vineyard in selection programs, or more generally in the future for controlling the conformity of cultivars and rootstocks provided by private nurseries, in order to avoid mistakes which could have harsh consequences for the wine grower.

In order to illustrate the power of resolution of molecular profiling for agricultural purposes, we report here such a kind of a procedure applied to plants of different cultivars, selected from different vineyards in controlled origin wine areas of Crete and Samos, as well as a case of identification of plant material from a private vineyard from Crete.

MATERIAL AND METHODS

I - PLANT MATERIAL

In the frame of a project of clone selection for a few cultivars from Crete and Samos, 45 grapevine plants were sampled from 18 private vineyards located in controlled origin wine areas of Crete and Samos (table I), on the basis of phenotypic differences comparatively to surrounding plants. Cuttings were cultivated in pots in the green house of the Laboratory of Plant Physiology of the University of Crete. In the second case described, three plants were sampled in a private vineyard in Agia Barbara, a location of the controlled origin wine area of Dafnes in Crete. Sampling was carried out by technicians of the Division for Agriculture of the Region of Crete and these plants are given with the label Div. Agr. along with their supposed name in table II.

II - DNA EXTRACTION

DNA was extracted from young leaves with a micro-method of DNA isolation (LEFORT and DOUGLAS, 1999) modified for grapevine (LEFORT and ROUBELAKIS-ANGELAKIS, 2001).

III - MICROSATELLITE PCR AND MICROSATELLITE PROFILE ANALYSIS

Amplification primer sequences for nuclear microsatellite loci (nSSRs) from *Vitis riparia* ssrVrZAG21, ssrVrZAG47, ssrVrZAG 62, ssrVrZAG64, ssrVrZAG67, ssrVrZAG79, ssrVrZAG83, ssrVrZAG112 (SEFC *et al.*, 1999) and from *Vitis vinifera*, VVS1, VVS2, VVS3, VVS4, VVS5 (THOMAS *et al.*, 1994), ssrVvUCH11, ssrVvUCH29 (LEFORT *et al.*, 2002a) were used for DNA amplification. Four nSSR loci were used for screening plants selected from vineyards in Crete and Samos and 15 loci were used to check identities of 3 plants from a private vineyard in Crete.

PCR amplifications were carried out in 96-well propylene plates in 20 µl final volume reaction mixtures in a PTC-100 thermal cycler (M.J. Research Inc., Watertown, MA). PCR reactions were as follows: 1 µM of each primer, 100 µM of each dNTPs (Biofinex, Praroman, Switzerland), 1.5 mM $MgCl_2$ in the buffer 75 mm Tris-HCl (pH 9.0), 50 mM KCl, 20 mM $(NH_4)2\ SO_4$, 0.5 units Taq polymerase (Biotools, Madrid, Spain) and 50 ng DNA template. The forward primer in each case was labeled with the Licor IR800 fluorochrome. The following thermal cycling protocol was applied for all loci: 95 °C for 5 min, 10 cycles of 15 s at 50 °C, 15 s at 94 °C, followed by 23 cycles of 15 s at 50 °C, 15 s at 89 °C and terminated immediately at 4 °C; except for ssrVrZAG64 which had an optimal annealing temperature of 58 °C. The amplification success was checked by electrophoresis in 2 % agarose gel in 1xTBE buffer (0.09 M Tris-borate, pH 8.3, 2 mM EDTA, pH 8.0) and ethidium bromide staining. PCR products analysis was carried out on 8 % polyacrylamide, 7M urea, 1xTBE sequencing gels in a Licor 2400 DNA Sequencer (Licor, Lincoln, NE), and alleles were sized with the software Gene Profiler v3.54 (Scanalytics, Fairfax, VA). PCR products for plants described in table I were run on the same gel. So was it in the second case, where PCR products of the 3 investigated plants were run on the same gel along with PCR products from reference plants (table II).

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Table I - Microsatellite profiles of 47 grapevine plants from Crete and Samos at 4 microsatellite loci. Expected allele sizes are those of plants of reference for a given cultivar and are according the Greek *Vitis* database (http://www.biology.uoc.gr/gvd). Allele sizes are in base pairs (bp). Discrepancies between expected and obtained allele sizes are given in bold.

Profilage de 45 plantes de vigne de Crète et de Samos à 4 loci microsatellites. Les tailles attendues des allèles sont ceux de plantes de référence pour un cultivar donné, d'après la « Greek *Vitis* Database » (http://www.biology.uoc.gr/gvd). Les tailles des allèles sont en paires de base (pb). Les différences entre tailles obtenues et attendues sont données en gras.

			Exp. Sizing		Obs. Sizing		Exp. sizing		Obs. sizing		Exp. sizing		Obs. sizing		Exp.sizing		Obs. sizing	
Name	Clone ID	Origin	VVS1	VVS1	VVS1	VVS1	VVS2	VVS2	VVS2	VVS2	ZAG21	ZAG21	ZAG21	ZAG21	ZAG62	ZAG62	ZAG62	ZAG62
Kotsifali	K 242	Boutaris Skalani, Crete	180	180	180	180	144	142	144	142	214	190	214	190	193	187	193	187
Kotsifali	K240	Orfanoudakis Kounavoi, Crete	180	180	180	180	144	142	144	142	214	190	214	190	193	187	193	187
Kotsifali	K241	Orfanoudakis Kounavoi, Crete	180	180	180	180	144	142	144	142	214	190	214	190	193	187	193	187
Kotsifali	K240b	Orfanoudakis Kounavoi, Crete	180	180	180	180	144	142	144	142	214	190	214	190	193	187	193	187
Kotsifali	K240a	Orfanoudakis Kounavoi, Crete	180	180	180	180	144	142	144	142	214	190	214	190	193	187	193	187
Kotsifaloliatiko	K27	Kalamitisi Chania, Crete	180	180	180	180	142	134	**144**	142	206	204	206	**190**	199	187	199	187
Kotsifaloliatiko	K28	Kalamitisi Chania, Crete	180	180	180	180	142	134	**144**	142	206	204	206	**190**	199	187	199	187
Kotsifaloliatiko	K26	Kalamitisi Chania, Crete	180	180	180	180	142	134	**144**	142	206	204	206	**190**	199	187	199	187
Liatiko	K162	Patsos Spiliou, Crete	187	180	187	180	142	134	**144**	134	206	206	206	206	203	199	203	199
Liatiko	K164	Patsos Spiliou, Crete	187	180	187	180	142	134	**144**	134	206	206	206	206	203	199	203	199
Liatiko	K386	Dafnes Irakleiou, Crete	187	180	187	180	142	134	**144**	134	206	206	206	206	203	199	203	199
Liatiko	K384	Dafnes Irakleiou, Crete	187	180	187	180	142	134	**144**	134	206	206	206	206	203	199	203	199
Liatiko	K161	Patsos Spiliou, Crete	187	180	187	180	142	134	**144**	134	206	206	206	206	203	199	203	199
Liatiko	K163	Patsos Spiliou, Crete	187	180	**180**	180	142	134	**132**	**132**	206	206	206	206	203	199	**195**	**185**
Liatiko	K173	Patsos Spiliou, Crete	187	180	187	180	142	134	**144**	134	206	206	206	206	203	199	203	199
Liatiko	K170	Patsos Spiliou, Crete	187	180	187	180	142	134	**144**	134	206	206	206	206	203	199	203	199
Liatiko	K172	Patsos Spiliou, Crete	187	180	187	180	142	134	**144**	134	206	206	206	206	203	199	203	199
Liatiko	K388	Dafnes Irakleiou, Crete	187	180	187	180	142	134	**144**	134	206	206	206	206	203	199	203	199
Liatiko	K174	Patsos Spiliou, Crete	187	180	187	180	142	134	**144**	134	206	206	206	206	203	199	203	199
Liatiko	K387	Dafnes Irakleiou, Crete	187	180	187	180	142	134	**144**	134	206	206	206	206	203	199	203	199
Mantilari	K309	Choudetsi Irakleiou, Crete	180	180	180	180	144	144	144	144	204	190	204	190	201	195	201	195
Mantilari	K306	Choudetsi Irakleiou, Crete	180	180	180	180	144	144	144	144	204	190	204	190	201	195	201	195
Mantilari	K308	Choudetsi Irakleiou, Crete	180	180	180	180	144	144	144	144	204	190	204	190	201	195	201	195
Mantilari	K285	Kounavoi Irakleiou, Crete	180	180	180	180	144	144	144	144	204	190	204	190	201	195	201	195
Mantilari	K310	Choudetsi Irakleiou, Crete	180	180	180	180	144	144	144	144	204	190	204	190	201	195	201	195
Mantilari	K311	Choudetsi Irakleiou, Crete	180	180	180	180	144	144	144	144	204	190	204	190	201	195	201	195
Moschato aspro	K13	Karagiannis, Samos	180	180	180	180	134	132	**132**	132	206	206	206	206	201	185	**195**	185
Moschato aspro	K9	Anastasiou, Samos	180	180	180	180	134	132	**132**	132	206	206	206	206	201	185	**195**	185
Moschato aspro	K8	Zafeiropoulou, Samos	180	180	180	180	134	132	**132**	132	206	206	206	206	201	185	**195**	185
Moschato aspro	K4	Papadam, Samos	180	180	180	180	134	132	**132**	132	206	206	206	206	201	185	**195**	185
Moschato aspro	K10	Anastasiou, Samos	180	180	180	180	134	132	**132**	132	206	206	206	206	201	185	**195**	185
Moschato aspro	K16	Papageorgiou, Samos	180	180	180	180	134	132	**132**	132	206	206	206	206	201	185	**195**	185
Moschato aspro	K15	Mattheou, Samos	180	180	180	180	134	132	**132**	132	206	206	206	206	201	185	**195**	185
Moschato aspro	K5	Papadam, Samos	180	180	180	180	134	132	**144**	**144**	206	206	206	206	201	185	**199**	187
Moschato aspro	K14	Mattheou, Samos	180	180	180	180	134	132	**132**	132	206	206	206	**202**	201	185	**195**	185
Moschato Spinas	K4	Maza Chanion, Crete	180	180	180	180	132	132	132	132	206	206	206	206	195	185	195	185
Moschato Spinas	K6	Maza Chanion, Crete	180	180	180	180	132	132	132	132	206	206	206	206	195	185	195	185
Moschato Spinas	K2	Maza Chanion, Crete	180	180	180	180	132	132	132	132	206	206	206	206	195	185	195	185
Moschato Spinas	K1	Maza Chanion, Crete	180	180	180	180	132	132	**142**	**134**	206	206	206	206	195	185	195	185
Moschato Spinas	K5	Maza Chanion, Crete	180	180	**187**	180	132	132	**144**	**144**	206	206	206	206	195	185	**203**	187
Vidiano	K185	Mikra Anogeia Rethymnis, Crete	180	180	180	180	144	142	144	142	206	202	206	202	199	187	199	187
Vidiano	K184	Mikra Anogeia Rethymnis, Crete	180	180	180	180	144	142	144	142	206	202	206	202	199	187	199	187
Vidiano	K186	Mikra Anogeia Rethymnis, Crete	180	180	180	180	144	142	144	142	206	202	206	202	199	187	199	187
Vidiano	K187	Mikra Anogeia Rethymnis, Crete	180	180	180	180	144	142	144	142	206	202	206	202	199	187	199	187
Vidiano	K188	Mikra Anogeia Rethymnis, Crete	180	180	180	180	144	142	144	142	206	202	206	202	199	187	199	187

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RESULTS AND DISCUSSION

Fourty-five plants had been visually selected from different vineyards in control origin wine areas of Crete and Samos. Selection was done on the basis of phenotypic differences compared to the cultivars constituting these vineyards. Genetic profiles at four microsatellite loci of these 45 plants were compared to genetic profiles of reference plants from the ampelographic collections of the Wine and Vine Institute (NAGREF, Lykovrissi, Athens), Institute of Viticulture, Floriculture and Horticulture (NAGREF, Katsambas, Heraklion) and the Laboratory of Plant Physiology and Biotechnology, (University of Crete, Heraklion) compiled in the Greek *Vitis* Database (LEFORT and ROUBELAKIS-ANGELAKIS, 2000; LEFORT *et al.*, 2002b; LEFORT, 2002). Four polymorphic loci were considered to be efficient enough for screening differences and thus sort potentially different material out of the plants matching expected identities. Genetic profiles are given in base pairs in table 1 where expected allele sizes and observed allele sizes can be compared.

The five selected Kotsifali plants from 2 different locations were conformed to Kotsifali as profiles matched the expected profiles.

On the other hand the three selected Kotsifaloliatiko did not match the reference profile of the Kotsifaloliatiko obtained from the collection of Lykovrisi (Athens). They differed in one allele at the locus VVS2 (144 bp instead of 142 bp) and one allele at locus ssrVrZAG21 (204bp instead of 190 bp). After searching the Nuclear Microsatellite Database of the Greek *Vitis* Database, it appeared that they might be either Zakynthino or Sefka, or a progeny of Kotsifali, which could be confirmed by further profiling at more microsatellite loci.

Concerning the 12 plants of Liatiko sampled from 2 vineyards in Crete, eleven of them did not match the profiles of the Liatiko family for one allele (144 bp instead of 142 bp) at one locus (VVS2) but did not match other profiles in the Greek *Vitis* Database. They could thus be a clone of the Liatiko family since these plants were very similar to Liatiko from an ampelographic point of view. The fact that these 11 plants came from 2 different vineyards located in the region of cultivation of Liatiko suggested that there could be a clone of Liatiko co-cultivated with Liatiko. In this case further profiling would be needed for confirmation of the clone hypothesis. The twelfth plant, clone K163 from Patsos Spiliou, differed in one allele (180 bp) at locus VVS1, 2 alleles (132 bp) at locus VVS2 and 2 alleles (195 bp and 185 bp) at locus ssrVrZAG62. It could not be a Liatiko, but rather be a Moschato Spinas (or Moschato Mazas, or Moschato Kerkyras), since it had the specific combination (195 bp, 185 bp) at the locus ssrVrZAG62, which had only been found in these 3 cultivars and also matched Moschato Spinas profile at the 3 other tested loci. This could be possible since both cultivars Liatiko and Moschato Spinas are cultivated in not very distant regions of Crete.

All the six plants of Mantilari were matching the reference profile of the Mantilari cultivar.

The nine plants of Moschato aspro from 6 different vineyards in Samos did not match the reference profile of the Moschato aspro cultivar from Lykovrisi (Athens). Seven of these Moschato plants differed from Moschato in one of the VVS2 alleles (132 bp instead of 134 bp) and one of the ssrVrZAG62 (195 bp instead of 201 bp) alleles and could be Moschato Spinas (genetic synonym Moschato Kerkyras) since they harbored the typical combination (195 bp, 185 bp) at locus ssrVrZAG62 and matched Moschato Spinas profile at the other 3 loci. Clone K5 Papadam Samos differed in 2 alleles at locus VVS2 (144 bp instead of 132 bp) and 2 alleles (199 bp and 187 bp instead of 201 bp and 185 bp) at locus ssrVrZAG62, and did not find any match in the Greek *Vitis* Database and could be an offspring of a cross or an unknown cultivar. Clone K14 Mattheou Samos could be a clone of Moschato Spinas since it differed only at one allele (202 bp instead of 206bp at locus ssrVrZAG21).

Three out of the five Moschato plants from the same location, Mazas Chanion, matched the profile of Moschato Spinas. Clone K1 Mazas Chanion had two different alleles (142 bp and 134 bp) at locus VVS2 and did not match any profile in the Greek *Vitis* Database. Clone K5 Mazas Chanion differed in 5 alleles at 3 loci making it very different from a Muscat and did not match any profile in the Greek *Vitis* Database.

Finally the five plants from one location of the cultivar Vidiano matched the reference profile for Vidiano.

The use of microsatellite markers was suitable for screening plant material selected from vineyards in purpose of selection. The results showed well the efficiency of microsatellite profiling for a quick and resolutive management of such material. The fact to be able to remove, from the selection process, all material which showed to be very different at a reduced number of microsatellite loci, will optimize the work of selection and avoid to keep unrelated cultivars.

The second case was a case of identification of plant material sampled in a private vineyard of Agia Barbara, in the controlled origin wine area of Dafnes in Crete. The controlled origin wine produced in this area is uniquely based on Liatiko, according to the rules esta-

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Table II - Microsatellite profiles at 15 microsatellite loci of cultivars of the Liatiko family, Kotsifali, Kotsifaloliatiko, Strofyliatiko, Fegi and of 3 plants sampled in a vineyard in Agia Barbara in Crete. Profiles of Fegi and Kotsifaloliatiko Div. Agr. are given at 9 and 13 loci respectively. Allele sizes are in base pairs (bp). Discrepancies between the profiles obtained for the 3 plants from the vineyard and profiles of plants of reference, according to the « Greek Vitis Database » (http://www.biology.uoc.gr/gvd), are given in bold.

Profils microsatellites à 15 loci microsatellites des cultivars Liatiko , Kotsifali, Kotsifaloliatiko, Strofyliatiko, Fegi et de 3 plantes échantillonnées dans un vignoble d'Agia Barbara (Crète).
Les profils de Fegi et Kotsifaloliatiko Div. Agr. sont donnés à 9 et 13 loci respectivement.
Les tailles des allèles sont en paires de base (pb). Les différences entre tailles obtenues chez les trois plantes issues du vignoble et observées chez des plantes de référence, d'après la «Greek Vitis Database» (http://www.biology.uoc.gr/gvd), sont données en gras.

Cultivar name	Kotsifali	Kotsifali Div. Agr.	Kotsifalolia tiko	Kotsifaloliatiko Div. Agr.	Fegi	Liatiko Div. Agr.	Liatiko Div. Agr.	Liatiko Ag. Varvara	Liatiko Alithinis	Liatiko Kounavon	Liatiko Leivadioti	Strofyliatiko
VVS2	144	142	142	**144**	144	142	142	142	142	142	142	154
VVS2	142	**134**	134	**136**	136	134	134	134	134	134	134	142
ZAG21	214	**206**	206	**204**	204	206	206	206	206	206	206	206
ZAG21	190	**204**	204	**202**	202	206	206	206	206	206	206	190
ZAG47	167	**163**	163	**172**	172	163	163	163	163	163	163	172
ZAG47	163	163	157	**172**	172	163	163	163	163	163	163	159
ZAG62	193	**203**	199	**187**	187	203	203	203	203	203	203	201
ZAG62	187	**199**	187	187	187	199	199	199	199	199	199	191
ZAG64	143	143	159	**143**	143	143	143	143	143	143	143	143
ZAG64	137	**139**	143	**137**	137	139	139	139	139	139	139	141
ZAG79	250	**252**	250	**256**	256	250	250	250	250	250	250	258
ZAG79	242	**246**	248	**256**	256	246	246	246	246	246	246	256
ZAG83	194	194	194	**190**	190	194	194	194	194	194	194	190
ZAG83	190	**188**	188	188	188	188	188	188	188	188	188	190
UCH11	244	**246**	242	**262**	262	246	246	246	246	246	246	244
UCH11	242	242	242	**248**	248	242	242	242	242	242	242	236
UCH29	211	**207**	289	289	289	289	289	207	207	289	207	211
UCH29	209	**207**	211	**215**	215	207	207	207	207	207	207	211
VVS1	180	**187**	180	180		187	187	187	187	187	187	179
VVS1	180	180	180	180		180	180	180	180	180	180	188
VVS3	212	**218**	218	**212**		218	218	218	218	218	218	218
VVS3	212	212	218	**212**		212	212	212	212	212	212	218
VVS4	174	174	174	**172**		174	174	174	174	174	174	174
VVS4	168	**174**	167	**166**		174	174	174	174	174	174	167
VVS5	90	**116**	116			116	116	116	116	116	116	116
VVS5	90	**116**	116			116	116	116	116	116	116	116
ZAG67	152	**154**	154	**149**		154	154	154	154	154	154	151
ZAG67	152	**151**	139	**132**		151	151	151	151	151	151	126
ZAG112	234	**238**	238			238	238	238	238	238	238	240
ZAG112	234	234	234			234	234	234	234	234	234	234

blished by the Ministry for Agriculture of the Hellenic Republic for controlled origin wines in Greece. Three different individual plants, representing the three forms easily distinguishable in a private vineyard of Agia Barbara and supposed to be a Liatiko, a Kotsifali and a Kotsifaloliatiko, were sampled and submitted to microsatellite profiling at 15 nuclear microsatellite loci. The obtained identity profiles were then compared to those of cultivars of reference. Microsatellite profiles of cultivars of reference and of these three plants are given in table II.

The Kotsifali sampled from the vineyard was not Kotsifali but was found very close to Liatiko. It could be in fact a subclone of Liatiko, since it differed at one allele at ssrVrZAG21 (204 bp instead of 206 bp) and one allele at ssrVrZAG79 (252 bp instead of 250 bp) and was also homozygous for the allele 207 bp at locus ssrVvUCH29, which was a characteristic of the clone Liatiko Alithinis/ Liatiko Agia Barbaras/Liatiko Leivadioti. The Liatiko sampled from the vineyard was similar to profiles of Liatiko and Liatiko Kounavon, another Cretan synonym for Liatiko. The

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Kotsifaloliatiko sampled from the vineyard was not Kotsifaloliatiko, and was not related to the Liatiko family or to Kotsifali. It appeared after searching the nuclear Microsatellite Database of the Greek Vitis Database and comparing the obtained profile with a total of 304 genetic profiles that this plant was a representative of a cultivar named Fegi, which is a cultivar originated from Magnisia, in Continental Greece and from the available data, not officially cultivated in Crete.

This work also enabled to assess a putative relationship between Kotsifaloliatiko, Kotsifali and Liatiko, since Kotsifaloliatiko was supposed to be an offspring of Kotsifali and Liatiko. Liatiko could be potentially be a direct parent of Kotsifaloliatiko, since they shared at least one allele at each of the 15 tested loci. Genetic similarity between these 2 cultivars was 63 %. The second parent remaining unknown after analyzing the profiles of the Nuclear Microsatellite Database with the programme Identity. Kotsifali could not be a direct parent but shared a common allele with Kotsifaloliatiko at 11 out of 15 tested loci and 43 % genetic similarity, which would mean that these 2 cultivars could be related. Besides that observation. no relationship was found between the cultivar Strofyliatiko and Liatiko, which had only 27 % genetic similarity. This is another example where a similar name is not enough to suggest for any genetic relationship.

These two examples of molecular profiling of plants from the vineyard may suggest on one hand that the vineyards screened in this study, which were vineyards of controlled origin wine areas, could contain a low level of contaminating cultivars. These contaminations could possibly originate from mistakes in nurseries or trade in the pastime, or simply because growers had replaced dead plants with plants of uncertified origin. The rational organisation of Greek viticulture (certification, pre-nurseries and nursery) at national level, should have reduced nowadays the extent of such a problem. Microsatellite profiling at a small number of loci showed to be a high resolution tool for helping to discard plants unrelated to the selection material, sampled in the vineyard. The identification of a low level of contaminating cultivars, in controlled origin wine areas, involves that molecular profiling should also be applied to nursery material for propagation in order to avoid propagating wrongly allocated material (cultivars or rootstocks). In this purpose, using a small number of highly polymorphic markers, multiplexed in one genotyping reaction, could already detect discrepancies between what is expected and what is obtained. Alternatively a new molecular profiling method, recently made available (PELSY and MERDINOGLU, 2002) and based on the detection of grapevine retrotransposon sequences could be applied. On the basis of the presented results we may recommend the use of a small number of markers in an initial step of general assessment of pools of plants and the completion of the genotyping only if a precise question of identity is to be solved.

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