GENETIC CHARACTERIZATION OF ALBANIAN GRAPEVINE CULTIVARS BY MICROSATELLITE MARKERS

CARACTÉRISATION GÉNÉTIQUE PAR MARQUEURS DES CÉPAGES ALBANAIS

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Résumé : L'Albanie voisine de la Grèce partage une partie de son histoire antique avec cette dernière. La vigne est cultivée depuis longtemps dans cette région. Si des cultivars français et autrichiens ont été introduits plus récemment au 20e siècle, les sélectionneurs albanais modernes ont aussi tenté de conserver les ressources génétiques natives dans des collections ampélographiques et ont créé quelques nouveaux métis entre cultivars albanais et étrangers, tels que Kallmet x Cabernet Sauvignon ou Kallmet x Alicante Bouschet. La plupart des collections ont été malheureusement détruites lors de l'effondrement du régime communiste en 1990 et la reconstruction d'une collection a été initiée ces dernières années dans le cadre de projets de coopération internationaux. Vingt-huit cultivars de *Vitis vinifera* et un individu de *Vitis sylvestris*, issus de la nouvelle collection ampélographique albanaise ont été caractérisés par profilage génétique. Vingt-huit profils d'identité génétique uniques ont été générés par amplification par la réaction en chaîne de la polymérase à 11 loci microsatellites nucléaires pour les 29 accessions examinées. Deux cultivars, Shesh i bardhë et Pucalla apparaissent être génétiquement identiques donc synonymes. Les profils génétiques des cultivars albanais ont été comparés à 8 loci microsatellite aux 29 cultivars grecs les plus communément cultivés en Grèce. Les cultivars albanais se sont révélés être proches des cultivars grecs originaires du Péloponnèse. Un cultivar grec Dempina s'est révélé être très proche de deux cultivars albanais Debina teki et Debina kala. Un autre cultivar présentait un profil tri-allélique à 5 loci sur un total de 10 loci analysés. Le nombre important de loci à profils tri-alléliques plaiderait en faveur de la triploïdie de ce cultivar bien que le chimérisme ne puisse être exclu.

Abstract : A recently restored ampelographic collection of Albanian grapevine accessions has been submitted to genetic profiling with eleven nuclear microsatellite markers, widely used in other studies. Microsatellite profiling resulted in 28 single profiles for 29 accessions. Two cultivars, Shesh I bardhë and Pucalla, were found to be synonyms. Genetic profiles of Albanian cultivars were compared at 8 microsatellite loci to 29 most commonly cultivated Greek cultivars. Albanian cultivars were found to be more closely related to Greek cultivars from Peloponnese. One Greek cultivar named Dempina was found to be genetically close to two Albanian cultivars Debina teki and Debina kala, which are homonyms Another cultivar, known as Toska or Sinambel displayed a tri-allelic profile at 5 loci over 10 analysed loci. Such a high number of tri-allelic loci found in one individual favours the hypothesis of triploidy but the chimerism hypothesis cannot be excluded without further work.

Mots clefs : Albanie, Grèce, microsatellite, ressources génétiques, vigne, *Vitis vinifera*

Key words : Albania, Greece, mplasm, grapevine, microsatellite, *Vitis vinifera*

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INTRODUCTION

Albania and Greece are neighbouring countries and share part of their ancient past including agriculture. Agriculture established in Albania around 3200 BC and among other species, grapevine has been cultivated for a long time in this region. During the past century, French and Austrian cultivars were introduced and from the mid 50's, Albanian grape breeders attempted to conserve the native genetic resources in ampelographic collections and created new crossings between native and foreign cultivars, such as Kallmet-Bukmirë x Cabernet sauvignon or Kallmet-Bukmirë x Alicante Bouschet. Most of the collections, including new crossings, were unfortunately destroyed during the collapse of the former regime in the early nineties and the present challenge is to restore these ampelographic collections. This task was undertaken in the past years by Albanian and international organisations (LERIN, 1998).

Genetic profiling with microsatellite markers, also known as simple sequence repeats (SSR) is nowadays the most popular tool for molecular marker assisted management of plant and animal genetic resources, for their high reproducibility, their co-dominance and their neutral evolution. Furthermore, microsatellite analysis has been semi-automated with the use of DNA sequencers allowing many individuals to be scored simultaneously. Considerable theoretical work has also been done on microsatellites and many analytical softwares have been developed.

In grapevine, such markers were developed about ten years ago and have been applied in a variety of utilizations (for a review see SEFC *et al*., 2001). When a population (or a cultivar in grapevine) is genetically well characterized, the genotype of an individual of unknown origin could be used to assign that individual to a population. This procedure is known as genetic assignment (AVISE, 1994). A specific use of genetic assignment is the parentage analysis where unknown pedigrees can be resolved by using co-dominant genetic markers (such as microsatellites). The co-dominant nature of microsatellite markers make them valuable tools for retrieving unknown pedigrees (BOWERS and MEREDITH, 1997, CRESPAN, 2003 ; MEREDITH *et al*., 1999 ; PILJAC *et al*., 2003; SEFC *et al*., 1998b). Other approaches aimed to assess the relationships between geographically distant gene pools (SEFC *et al*., 2000 ; 2003) or the chloroplast genome diversity and to identify chloroplast donor plants (ARROYO *et al*., 2002). The main effort in the past years carried on combining ampelographic information with genetic profiles obtained with microsatellites (SEFC *et al*., 1998a, BOWERS *et al*, 1999, LOPES *et al*. 1999 ; MALETIC *et al*., 1999, HVARLEVA *et al*., 2004 ; LEFORT and ROUBELAKIS-ANGELAKIS, 2001; ORTIZ *et al*., 2004; PINTO-CARNIDE *et al*., 2003) and arranging the resulting information into publicly available databases (Grando, unpublished ; LEFORT and ROUBELAKIS-ANGELAKIS, 2000 ; RUSSANOV *et al*., 2003).

In this work, we have attempted for the first time to ascertain the genetic identity of Albanian cultivars, coming from a recently re-established collection, with the use of microsatellite markers. We also attempted to estimate the genetic relationship of Albanian cultivars with a set of 29 cultivars from all over Greece, including some most cultivated cultivars.

MATERIALS AND METHODS

Frozen leaves from 28 *Vitis vinifera* L. cultivars and 1 *Vitis silvestris* were used for DNA extraction according to the method described by LEFORT and ROUBELAKIS-ANGELAKIS (2001). Eleven microsatellite loci were used: VVS1, VVS2, VVS3, VVS4 (THOMAS and SCOTT, 1993), ssrZAG21, ssrZAG47, ssrZAG62, ssrZAG79 (SEFC *et al*., 1999), ssrVvUCH2, ssrVvUCH19, ssrVvUCH40 (LEFORT *et al*., 2002).

PCR reactions were performed in 10 µL reaction mix consisting of 0.2 mM of each dNTP, 2mM $MgCl_2$, 8 pmol of the forward primer, 6 pmol of the reverse primer 0,3 units Taq DNA polymerase in the buffer provided by the manufacturer (Biotools, B&M Labs, Madrid, Spain) and template DNA 50-100 ng. In each case the forward primer was labelled with Licor IR800 fluorochrome. PCR amplifications were carried out in a PTC-100 thermal cycler (MJ Research, Inc., Waltham, Mass.). The amplification protocol for all loci but ssrVvUCH40 was 5 min at 95 ºC, 10 cycles of 15 sec at 50 ºC and 15 sec at 94 ºC, followed by 23 cycles of 15 sec at 50 ºC and 15 sec at 89 ºC and terminated immediately at 4 ºC. For the locus ssrVvUCH40 amplification protocol was as follows: 3 min at 94 ºC followed by 30 cycles of 1min at 94 ºC, 45 sec at 62 ºC and 45 sec at 72 ºC. The program was terminated with 5 min at 72 ºC and then at 4 ºC.

Amplified products were run in 6 % Long Ranger polyacrylamide (BMA, Rockland, Me, USA), 7mM Urea sequencing gels in a Licor 4200 DNA sequencer and alleles were sized with the software Gene Profiler v3.54 (Scanalytics, Fairfax, VA, USA). Ambiguities were corrected by visual inspection.

Genetic profiles of Greek grapevine cultivars was retrieved from the Greek *Vitis* Database (http://www.biology.uch.gr/gvd/) (LEFORT and ROUBELAKIS-ANGELAKIS, 2000). In order to confirm that newly obtained genetic profiles were identical to profiles previously obtained, four DNA samples from the DNA samples database, with the code numbers 3, 49, 487 and 489, and corresponding to the cultivars Syriki, Kotsifali, Liatiko and

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Table I - Names of Albanian and Greek cultivars analysed with microsatellite markers, given with their area of cultivation.

Data for Greek cultivars were retrieved from the Greek Vitis Database (LEFORT and ROUBELAKIS-ANGELAKIS, 2001)

Noms et secteurs de culture des cépages albanais et grecs analysés avec des marqueurs microsatellites.

Les données des cépages grecs sont extraites de la base de données grecque Vitis (LEFORT ET ROUBELAKIS-ANGELAKIS, 2001).

Albanian cultivars		Greek cultivars	
Name	Area of cultivation	Name	Are of cultivation
Bora	Korça, South Eastern Albania	Fileri Mantineias	Arcadia (Peloponnesus)
Debina kala	Skrapar, South Eastern Albania	Fokiano	Dodecanese, North and West Aegean islands, Cyclades, central and East Macedonia, Thrace, Peloponnesus, Crete, Minor Asia, Bulgaria
Debina teki	Skrapar, South Eastern Albania	Fraoula kokkini	Peloponnesus, south Ionian islands, central Greece, Euboea, Crete, Aegean islands, Cyclades, Thessalia, central and west Macedonia, Attica
Dimnor	Tirana, Central Albania	Giouroukiko	Cyclades
Durrsaku	Durres, Central Albania	Kolokythas lefkos	Macedonia, Thrace, Thessaly,
Kallmet x Alicante Bouchet	Crossing	Kontegalo	Ionian islands, Peloponnesus
Kallmet x Alicante Bouschet	Crossing	Korithi mavro	Ionian islands, Euboea, Beotia, Achaiea, Messinia (Peloponnesus), Gravias, Desfina (Fokida)
Kallmet x Alicante Bouschet	Crossing	Kotsifali	Crete, Cyclades
Kallmet Bukmirë	Rreshen, Northern Albania	Kotsifaloliatiko	Crete
Kallmet x Kabernet Sauvignon	Crossing	Kritiko mavro	Crete, Cyclades
Karaj	Tirana, Central Albania	Krystalli	Thessaly, Central and West Macedonia, Peloponnesus, Crete
Kumbullor	Tirana, Central Albania	Liatiko	Crete, Cyclades, Dodecanese, Peloponnesus, Chalcidice, Ionian islands
Pucalla 4	Berat ,Central Albania	Mavrodafni	Achaea, Ionian islands Elia, Chalcidice, Magnisia
Pules i Bylyshit 1	Tepelene, South Albania	Moschardina	Ionian islands
Pules i Bylyshit 2	Tepelene, South Eastern Albania	Moschato	Aegean islands Macedonia, Thessaly, Euboea, Ionian islands
Rrushi i arrës	Tirana, Central Albania	Moschofilero	Peloponnesus
Serina e Drenovës	Korca, Southeastern Albania	Moschopatata	Aegean islands, south Peloponnesus
Serina e zezë	Mallakaster, Central Albania	Moschoudi	Achaiea, South Aetolia- Akarnania, Ionian islands, Samos, Elia
Serina e zezë -Prishta	Skrapar, Central Albania	Mouska	Achaea
Serina Landare	Korça, South Eastern Albania	Petrachladi	Crete
Shaban	Tirana, Central Albania	Piperionos	Ionian islands, Achaiea
Shaban	Tirana, Central Albania	Razaki Moschato	Synonym of cv. Italia
Shesh i bardhë	Tirana, Central Albania	Romeiko mavro	Crete, Cyclades
Shesh i zi	Tirana, Central Albania	Rompola	Ionian islands, Epirus, minor occurrences in Arcadia (Peloponnesus)
Sinambel (Toska)	Berat, Central Albania	Strofyliatiko	Cyclades
Sumbullor	Tirana, Central Albania	Syriki	Ionian islands, south Cyclades, Peloponnesus, Crete, Euboea, South and West Greece
Sypule	Tirana, Central Albania	Tsardana	Crete
V. silvestris	Vlora, Southeastern Albania	Vidiano	Crete
Zhullaf	Berat, Central Albania	Vilana	Crete

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Table II - Genetic profiles of 27 Albanian cultivars, 1 Albanian *Vitis sylvestris* and 1 Greek grapevine cultivar (Dempina) at 11 microsatellite loci. Allele sizes are given in base pairs (bp). Dashes indicate un-scored loci. The cultivar Toska (Sinambel) has been excluded from the analysis because it reports three-banded profile.

Profils génétiques des 26 cépages albanais, d'un *Vitis sylvestris* albanais, et d'un cépage grec (Dempina) à 11 loci microsatellites. Les tailles des allèles sont données sur des paires de base (bp). Les tirets indiquent les loci non marqués. Le cépage Toska (Sinambel) a été exclu de l'analyse parce qu'il présente un profil tri-allélique.

Cultivar	VVS1		VVS2		VVS3		VVS4		ZAG21		ZAG47		ZAG62		ZAG79		UCH2		UCH19		UCH40	
Bora	188	186	144	132	218	212	174	168	210	208	167	159	195	189	242	242	180	162	192	192	270	258
Dempina	188	180	154	142	218	212	174	168	206	190	163	159	195	187	-	-	164	162	192	192	-	-
Debina Kala	-	-	144	132	212	212	174	168	210	208	167	159	195	189	242	242	180	162	192	192	270	258
Debina teki	188	186	154	134	218	212	168	168	190	190	167	163	195	189	250	242	180	162	192	192	294	258
Dimnor	180	180	138	134	218	212	174	168	208	204	167	159	189	187	258	242	164	162	-	-	260	258
Durrsaku cv	178	179	142	132	218	218	174	168	204	202	167	157	189	189	258	250	180	162	198	192	286	272
KAB1	188	180	144	142	218	218	174	172	208	202	171	157	189	189	242	236	180	154	202	192	270	268
KAB2	186	180	134	132	218	218	174	168	208	206	171	157	189	189	258	256	180	154	192	188	272	258
KAB3	188	180	144	142	218	218	174	172	208	202	171	157	189	189	242	236	180	154	202	192	268	272
Kallmet-Bukmirë	188	186	142	134	218	218	174	168	208	208	159	157	203	189	258	236	180	180	202	188	268	258
Karaj	188	180	142	142	218	218	168	168	202	190	163	157	195	189	250	250	164	162	198	192	286	258
KKS	186	180	144	142	218	218	174	168	214	208	167	157	203	189	242	236	180	162	188	186	268	268
Kumbullor	188	180	142	132	218	218	168	168	210	204	159	159	203	189	-	-	162	144	192	192	276	258
Pucalla 4	188	180	142	132	218	212	168	168	210	204	159	159	205	189	258	242	162	142	192	192	276	258
Pules i Bylyshit 1	188	180	142	134	212	212	174	168	210	190	163	155	193	189	258	250	-	-	192	192	294	268
Pules I Bylyshit 2	-	-	154	144	212	212	174	168	210	190	159	157	157	195	258	242	162	162	202	192	270	258
Rrushi I arrës	180	180	154	134	218	218	174	168	208	208	159	159	195	187	258	250	162	162	198	192	260	258
Serina e zezë	179	179	152	148	218	218	174	174	208	190	171	157	205	189	256	246	174	146	198	192	292	272
Serina e zezë-Prishta	186	179	154	150	212	212	174	174	208	190	159	157	197	189	250	250	174	162	202	192	272	270
Serina Landare	-	-	154	142	212	212	174	168	210	208	163	159	203	189	250	242	162	162	192	192	270	268
Serinae Drenoves	188	188	142	134	212	212	174	168	210	190	167	159	205	203	-	-	180	162	192	192	294	294
Shaban	188	180	142	142	212	212	174	174	204	190	163	163	203	201	250	242	162	162	198	192	294	268
Shaban	180	179	142	132	218	218	174	168	208	204	159	157	205	189	258	236	162	162	192	192	272	272
Shesh I bardhë	188	180	142	132	-	-	-	-	210	204	159	159	205	189	-	-	162	142	192	192	276	258
Sumbullor	188	180	142	132	212	212	174	168	208	190	163	159	197	189	250	242	162	162	192	192	-	-
Sypule	180	180	142	138	218	218	174	168	210	204	163	159	189	187	258	250	164	162	198	192	294	260
Vitis sylvestris	180	180	154	134	218	218	174	168	204	190	169	161	197	195	250	244	172	162	198	192	294	260
Zhullaf	188	180	142	132	218	218	174	168	208	190	163	157	193	189	250	242	162	162	192	192	294	268

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Kotsifali Agr, of the Greek *Vitis* Database, were amplified and run together with Albanian samples. This was necessary for allowing comparison between Greek and Albanian cultivars.

Observed and expected heterozygosities were calculated with FSTAT software (GOUDET, 2000). IDENTITY 1.0 software (WAGNER and SEFC, 1999) was used for calculation of probability of identity, probability of null alleles, synonyms and potential paternity relationships. Genetic distance among Albanian cultivars as well as differences between Albanian and Greek cultivars were calculated with Microsat software (MINCH *et al*., 1997) using the formula -log (proportion of shared alleles) with 500 bootstraps. KITSCH program of the PHYLIP package (FELSENSTEIN, 1995) was used for calculation of multiple phenograms and CONSENCE program of the same package was used to extract the consensus tree according to the majority rule.

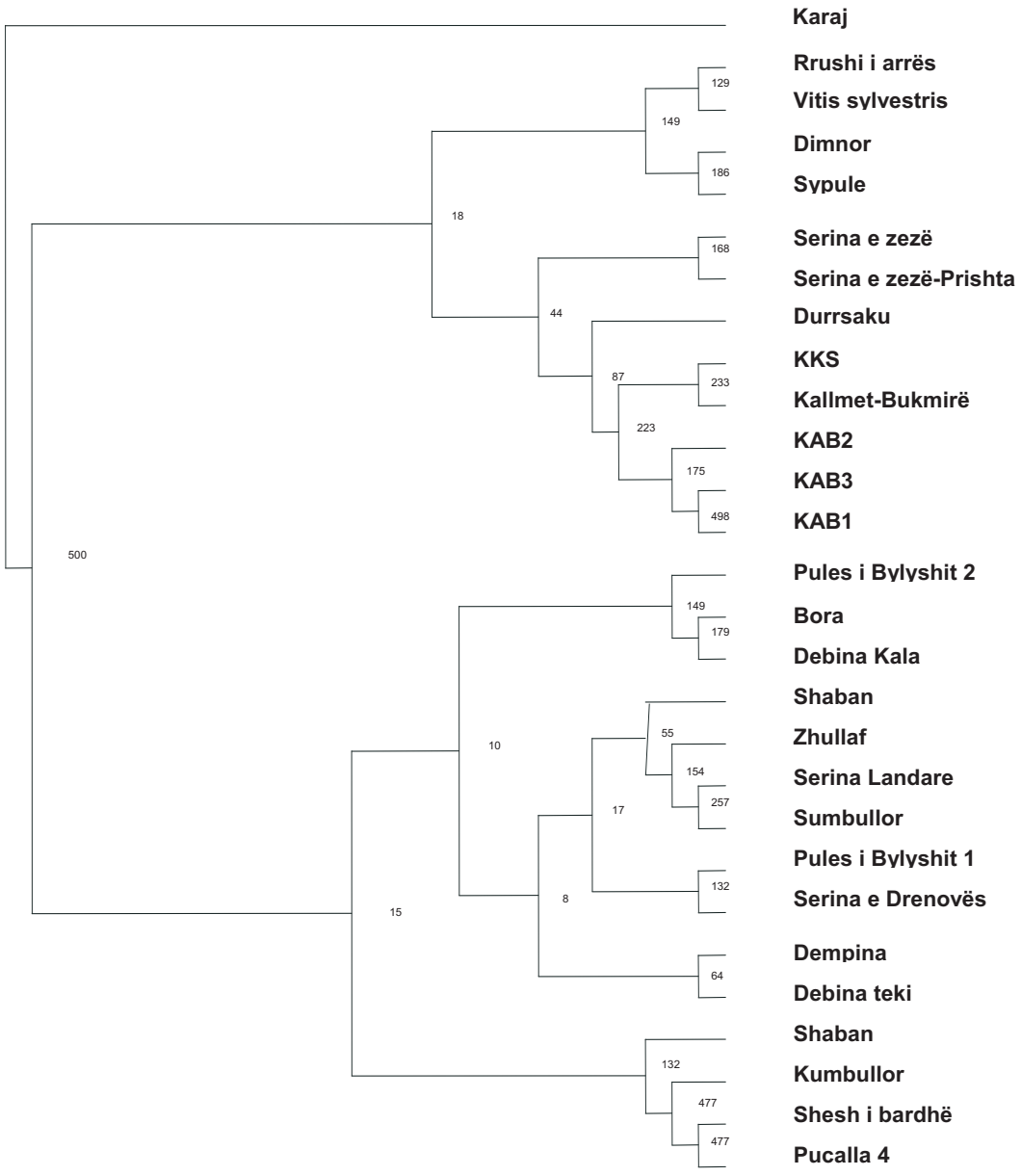


Figure 1 - Relationships among Albanian grapevine cultivars assessed with 11 SSR markers.

A Greek cultivar Dempina and an Albanian individual of *Vitis sylvestris* were included in the analysis. Numbers on the nodes represent the absolute number of 500 bootstraps that supported each node. KAB1, KAB2 and KAB3 are offspring of the crossing Kallmet x Alicante Bouschet. KKS is an offspring of a crossing Kallmet x Cabernet Sauvignon.

Rapports entre les cépages albanais évalués avec 11 marqueurs SSR.

Un cépage Dempina et un cépage individuel albanais de *Vitis sylvestris* étaient inclus dans l'analyse. Les numéros sur les nœuds représentent le nombre absolu de 500 rééchantillonages qui ont supporté chaque nœud. KAB1, KAB2 et KAB3 sont le résultat du croisement entre Kallmet et Alicante Bouschet. KKS est le résultat du croisement entre Kallmet et Cabernet Sauvignon.

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RESULTS AND DISCUSSION

I - LOCI EVALUATION

Eleven microsatellite loci were chosen for the identification of 29 grapevine cultivars from Albania and their genetic profiles were compared with published profiles of 29 Greek cultivars (Greek *Vitis* database; LEFORT and ROUBELAKIS-ANGELAKIS, 2000). Names of Albanian and Greek cultivars that were used in this study, as well as their distribution area are presented in table I. Genetic profiles of the cultivars for all loci are shown in table II. These loci have been previously published (THOMAS and SCOTT, 1993 ; SEFC *et al*. 1999, LEFORT *et al*., 2002), and five of them (namely VVS2, ZAG21, ZAG47, ZAG62,ZAG79) are the most commonly used for cultivar genetic characterization. Indeed, these loci were also highly informative in the present study. They have many alleles (range from 7-9, table III), high gene diversities (i.e. expected heterozygosities) ranging

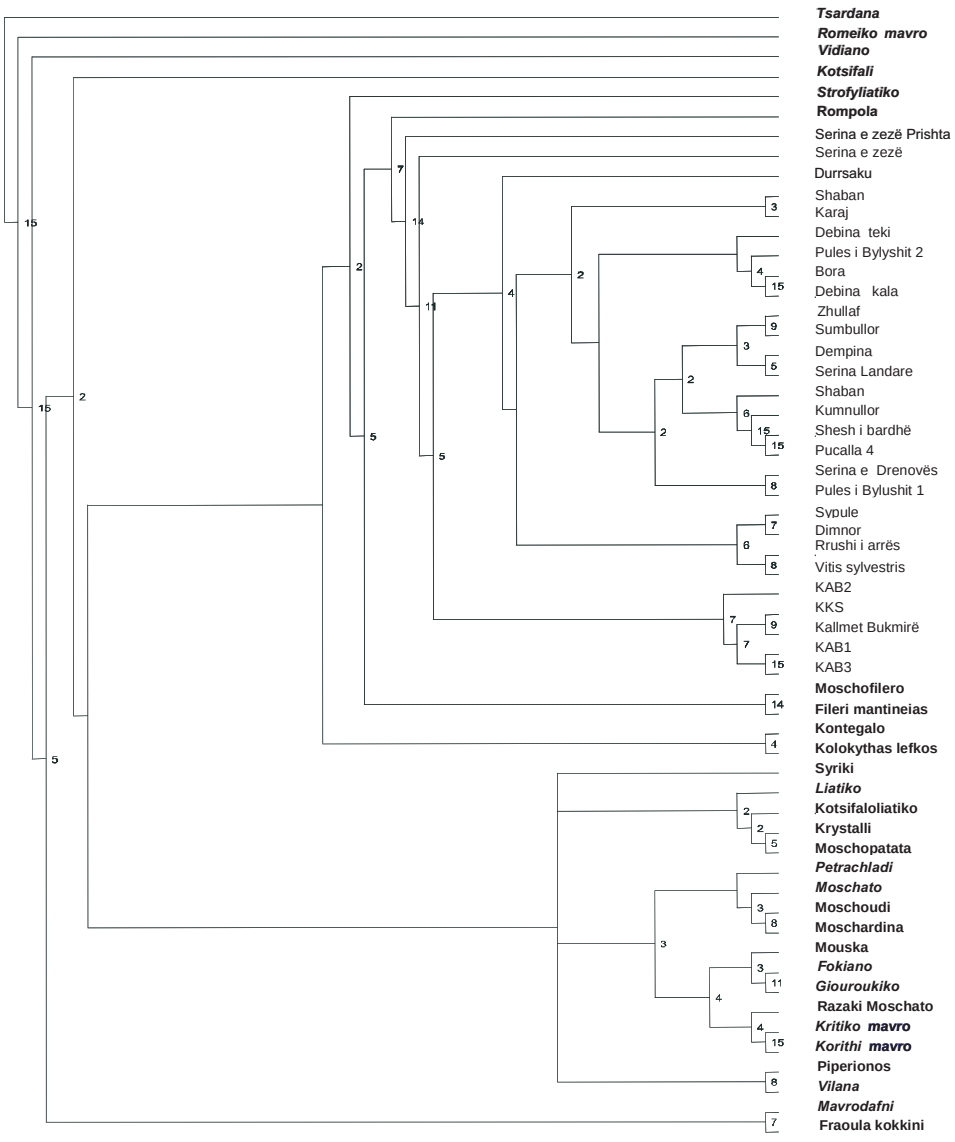


Figure 2 - Relationships between Albanian and Greek cultivars

(In bold, cultivars from Crete and the Cyclades are shown in bold italics) assessed with 8 nSSR markers (VVS1, VVS2, VVS3, VVS4, ssrZAG21, ssrZAG47, ssrZAG62, ssrZAG79). Numbers on the nodes represent the absolute number of 500 bootstraps that supported each node. KAB1, KAB2 and KAB3 are offspring of the crossing Kallmet x Alicante Bouschet. KKS is an offspring of a crossing Kallmet x Cabernet Sauvignon.

Rapports entre les cépages albanais et grecs

(En gras apparaissent les cépages en provenance de Crète, et en gras italique ceux qui proviennent des Cyclades), évalués avec 8 marqueurs nSSR (VVS1, VVS2, VVS3, VVS4, ssrZAG21, ssrZAG47, ssrZAG62, ssrZAG79). Les numéros sur les nœuds représentent le nombre absolu de 500 rééchantillonages qui ont supporté chaque nœud. KAB1, KAB2 et KAB3 sont le résultat du croisement entre Kallmet et Alicante Bouschet. KKS est le résultat du croisement entre Kallmet et Cabernet Sauvignon.

Table III - Analysis of genetic profiles : number of alleles, observed and expected heterozygosity, probability of identity (PI) and probability of null alleles at 11 nuclear microsatellite loci.

Analyse des profils génétiques : nombre d'allèles, hétérozygocité observée et attendue, probabilité d'identité (PI) et probabilité d'allèles nuls à 11 loci microsatellites nucléaires.

Locus	Number of alleles	Ho	He	Probability (PI) of Identity	Probability of Null Alleles
VVS1	5	0.760000	0.679200	0.266828	-0.048118
VVS2	9	0.928571	0.790816	0.12079	-0.076923
VVS3	2	0.185185	0.475309	0.614178	0.196653
VVS4	3	0.740741	0.534979	0.542516	-0.134048
ssrVrZAG21	7	0.892857	0.789541	0.137847	-0.057733
ssrVrZAG47	8	0.821429	0.772959	0.147195	-0.027338
ssrVrZAG62	9	0.857143	0.734694	0.140206	-0.070588
ssrvrZAG79	7	0.833333	0.760417	0.173265	-0.04142
ssrVvUCH2	9	0.703704	0.65775	0.213704	-0.02772
ssrVvUCH19	5	0.518519	0.475995	0.361565	-0.02881
ssrVvUCH40	9	0.884615	0.850592	0.074748	-0.018385
	73 alleles				
Mean	**MNA=6.63**	**0.738736**	**0.683841**	**PI for all loci 3.05728E-08**	

from 0.734 (ZAG62) to 0.790 (VVS2), low probabilities of obtaining identical profiles (PI) (range from 0.120 (VVS2) to 0.173 (ZAG79)).

Loci VVS3 and VVS4 are the less informative. They have only 2 and 3 alleles respectively (table III), low values for gene diversities (0.47 and 0.53) and high probabilities of obtaining identical genotypes between two genetically different cultivars (PI) (0.614 and 0.542). Furthermore, for locus VVS3 the probability of null alleles is positive (0.196). Probably this is the reason of the exclusion of these two loci from cultivar identification studies. Loci UCH19 and VVS1 are more informative than VVS3 and VVS4, but not as informative as the set of the most commonly used loci. They both have 5 alleles but locus VVS1 has a higher gene diversity (0.679 compared to 0.476 of locus UCH19) and a lower PI (0.266 compared to 0.361 of locus UCH19) (table III).

Locus UCH2 has 9 alleles, which is a high number but gene diversity is lower than the diversity of the five common loci (0.657). It is also lower than the diversity of locus VVS1, which has 5 alleles. Probability of identity is also higher in this locus than in the set of the common five loci.

Locus UCH40 had the highest discrimination power within the set of 11 loci. It has 9 alleles, the highest gene diversity (0.850) and the lowest PI (0.074) among all loci. This locus, to our knowledge, has not previously been used for genetic characterization of cultivars. Results from the present study, strongly suggest that this locus could be added to the core set of highly informative loci for genetic identification of grapevine cultivars.

In total, 73 alleles have been observed for all 11 loci with mean alleles per locus 6.63. When all cultivars were considered as a population, mean expected heterozygosity for all loci (gene diversity) is 0.683, which is lower than the observed heterozygosity (0.738) (table III). The observed heterozygosity of each locus separately is higher than the expected heterozygosity, with the exception of the less informative locus VVS3. Total probability of identity is 3.057x10-8, which is practically 0, which indicates that the set of 11 microsatellite loci is adequate to characterize a high number of genetically different cultivars. Moreover, all loci (but VVS3) display negative probabilities of obtaining null alleles.

II - RELATIONSHIPS AMONG ALBANIAN CULTIVARS AND BETWEEN ALBANIAN AND GREEK CULTIVARS

Relationships among the Albanian cultivars are shown in the phenogram of figure 1. The relationships among the cultivars have been interpreted as the negative logarithm of proportion of shared alleles. Two major clusters were formed with 100 % bootstrap support (500 is the absolute number of bootstraps and equals to 100 %). The first one includes 12 *Vitis vinifera* cultivars and one *Vitis silvestris*. This was congruent with present theories, which suggested modern cultivars to be locally related to native

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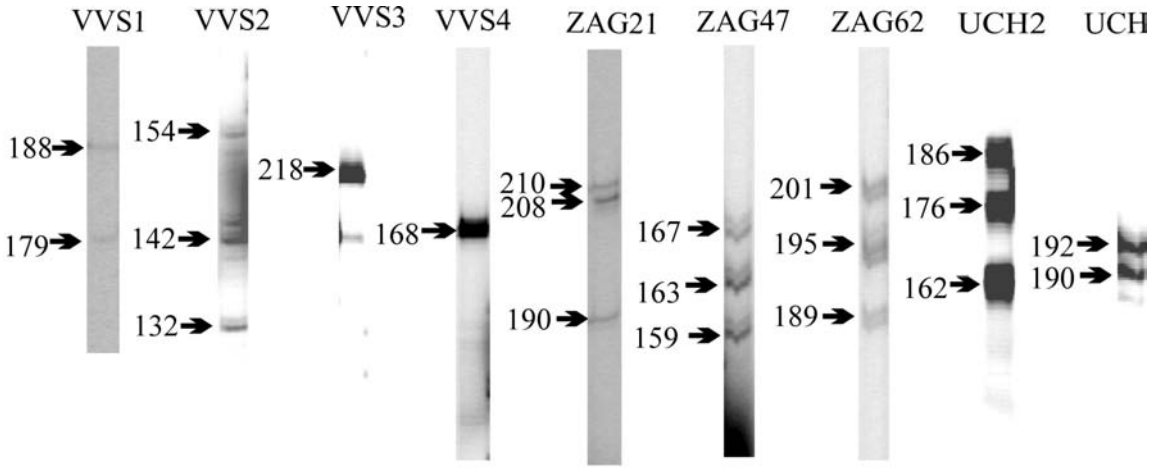


Figure 3 - Genetic profiles of the Albanian cultivar Sinambel or Toska at 10 microsatellite loci. At five loci (VVS2, ZAG21, ZAG47, ZAG62 and UCH2) this cultivar displayed a three-banded profile.

Profils génétiques du cépage albanais Sinambel ou Toska à 10 loci microsatellites. À 5 loci (VVS2, ZAG21, ZAG47, ZAG62 et UCH2), ce cépage affiche un profil tri-allélique»

wild grapes (ARROYO-GARCIA *et al*., 2003; SEFC *et al*., 2003). The second cluster included all the other cultivars except the cultivar Karaj, which appeared distinct from the other two clusters. Four cultivars are homonyms: Serina e zezë, Serina e zezë Prisha, Serina Landare and Serina e Drenovës. Microsatellite profiling proved that none of them were synomyms. Two of them sharing 45 % of their alleles segregate close to each other in the same cluster.

Previous studies have reported that similar names (homonymy) of cultivars often do not infer any genetic similarity (FATAHI *et al*., 2003) or might be the result of misnaming (MEREDITH *et al*., 1999). However, there are several cases that similar names are referred to genetically related cultivars (LEFORT and ROUBELAKIS-ANGELAKIS, 2001). In the Albanian set, two cultivars shared the name Debina (Debina kala and Debina teki). Any possible relationship with the Greek cultivar Dempina was assessed and all three cultivars segregated in the second cluster (Fig. 1), with Debina teki and the Greek Dempina being very closely related, indicating that in this case name similarity reflects genetic proximity (Fig. 1). Microsatellite profiling did not support any direct parent/offspring relationship, even if these two latter cultivars had a high proportion of shared alleles at 9 loci. The Greek Dempina is cultivated only in northwest Greece, the border region with Albania. Given the important connections between Albania and northwest Greece, it is possible that the Greek Dempina had been transmitted to Greece from Albania or vice versa.

Two cultivars with the identical name Shaban showed to be different and shared only 36 % of their alleles ruling out any direct parent/offspring relationship. Two cultivars Shesh I bardhë and Pucalla 4 were found to be synonyms with identical genetic profiles.

Concerning retrieved pedigrees, though 10 nSSR loci are not sufficient for ascertaining a pedigree with a high probability, it appeared that there is a parent/offspring relationship between the cultivars Debina kala and Bora, both being potentially parent or offspring to one another, unless the only allele of difference be due to a mutation.

The cultivars KKS, KAB1, KAB2, KAB3 coming from the crosses (Kallmet-Bukmirë x Cabernet Sauvignon) or (Kallmet-Bukmirë x Alicante Bouschet) displayed microsatellite profiles in agreement with their pedigree, where the allele from the Albanian parent can be found at each locus.

Genetic patterns, that are responsible for similarities within each cluster, or differences between clusters, should now be assessed in relation with morphological, physiological or ampelographic characteristics.

Albanian cultivars were compared with profiles of 29 Greek cultivars deposited in the Greek *Vitis* Database. The chosen cultivars are the most common cultivated in Greece and were genotyped at 8 common loci with the Albanian cultivars (VVS1, VVS2, VVS3, VVS4, ZAG21, ZAG47, ZAG62 and ZAG79). In order to avoid discrepancies in comparison of Albanian cultivars with Greek ones, the genotypes of four Greek cultivars (Syriki, Kotsifali, Liatiko and Kotsifali Agr) that were well characterized and deposited in database, were amplified again and were run together with the Albanian cultivars.

The genetic relationships between Albanian cultivars (in plain) and Greek cultivars (in bold and bold italic) are illustrated in figure 2. Cultivars from Crete and the

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Table IV - Number of alleles per locus, alleles length in base pairs (bp) and alleles frequencies (in brackets).
Nombre d'allèles par loci, longueur des allèles en paires de base et fréquence des allèles (entre parenthèses)

Locus	Nb of alleles	Alleles in bp and allele frequency in brackets
VVS1	5	178 (0.02), 179 (0.1), 180 (0.44) 186 (0.12), 188 (0.32)
VVS2	9	132 (0.18), 134 (0.14), 138 (0.036), 142 (0.36), 144 (0.11) 148 (0.018), 150 (0.018), 152 (0.018), 154 (0.122)
VVS3	2	212 (0.39), 218 (0.61)
VVS4	3	168 (0.48), 172 (0.04), 174 (0.48)
ssrVrZAG21	7	190 (0.23), 202 (0.07), 204 (0.16), 206 (0.04), 208 (0.30), 210 (0.18), 214 (0,02)
ssrVrZAG47	8	155 (0.02), 157 (0.21), 159 (0.36), 161 (0.02), 163 (0.18), 167 (0.12), 169 (0.02), 171 (0.07)
ssrVrZAG62	9	157 (0.02), 187 (0.07), 189 (0.46), 193 (0.04), 195 (0.14), 197 (0.05), 201 (0.02), 203 (0.11), 205 (0.09)
SsrVrZAG79	7	236 (0.1), 242 (0.31), 244 (0.02), 246 (0.02), 250 (0.29), 256 (0.04), 258 (0.21)
ssrVvUCH2	9	142 (0.04), 144 (0.02), 146 (0.02), 154 (0.06), 162 (0.54), 164 (0.07), 172 (0.02), 174 (0.04), 180 (0.20)
ssrVvUCH19	5	186 (0.02), 188 (0.06), 192 (0.7), 198 (0.13), 202 (0.09)
ssrVvUCH40	9	258 (0.23), 260 (0.07), 268 (0.17), 270 (0.11), 272 (0.13), 276 (0.06), 286 (0.04), 292 (0.02), 294 (0.15)

Cyclades are shown in bold italic while other Greek cultivars are shown in bold. Data for Greek cultivars were retrieved from Greek *Vitis* database (LEFORT and ROUBELAKIS-ANGELAKIS, 2000).

Although nodes are weakly supported (low bootstrap values), it is striking that Albanian cultivars are grouped in one cluster with native cultivars from neighbouring Southwest and central Greece (Moschofilero, Fileri Mantineias, Rompola, Strofyliatiko, Kontegalo, Debina, Kolokythas lefkos) with which they share a common genetic background. On the other hand they appear distantly related with cultivars from Crete and the Cyclades (with the exception of the cultivar Strofyliatiko), both regions far from Albania. This is congruent with the hypothesis of a post-glacial era recolonization after the last glacial maximum (18 000 C14 years ago) from a Greek glacial era refugium postulated by pollen evidence reviewed by BENNETT *et al.* (1991) and WILLIS (1994). Another likely hypothesis is the transmission of domesticated grape cultivars from Greece since Greek colonies (Appolonia, Epidamnus) were established on the Albania costs from the 7th century BC by Corinth (CABANES, 1994). Domestication in Albania also remains an open hypothesis. These results should anyway be interpreted with attention since the human involvement in dispersion of cultivated organisms has been well documented for many species.

III - CHIMERISM OR TRIPLOIDY ?

Microsatellites are co-dominant markers, what means that they can interpret both alleles of a heterozygous diploid organism. As a result they can also reveal chimerism or different levels of ploidy. Among the Albanian grapevine cultivars, the cultivar Sinambel (also named Toska) appeared with a three-banded profile in half of the analyzed loci (five out of 10, Fig. 3) (we could not amplify locus ZAG79). Contamination with DNA from other individuals could be excluded because PCR products from this individual were run several times and if contamination had occurred, four, but not three alleles could also have been detected. The ratio three-banded loci over all loci is greater than 0.5 because each locus has not the same probability of being three-banded. For example locus VVS3 has only two alleles, which means that never would be observed three alleles in that locus. It is obvious that the more alleles a locus has, the more probable is to observe three different alleles in a locus if the individual is triploid or chimera. Indeed, the cultivar Sinambel was homozygous for loci VVS3 (2 alleles) and VVS4 (3 alleles, He=0.534), and heterozygous for loci VVS1 (5 alleles, He=0.679), UCH19 (5 alleles, He=0.475).

It was recently shown that chimerism due to somatic mutation accumulation during the long-term clonal propagation of grape cultivars which were mainly propagated by cuttings is a reality in grapevine, with some cultivars more prone to accumulate these somatic mutations than others (FRANKS *et al*., 2002). Such a chimerism is thought to be the basis of a rich clonal diversity among old famous cultivars such as Pinot (aged more than 2000 years). HOCQUIGNY *et al*. (2004) analysed 145 accessions including « Pinot gris », « Pinot noir », « Pinot blanc », « Pinot meunier », and « Pinot mouré » cultivars with 50 microsatellite markers amplified from leaf tissue DNAs and demonstrated a prevalence of chimerism in this Pinot collection of INRA Colmar. The explanation of a tri-allelic profile at a given locus in grapevine leaf tissue would be the presence of a periclinal chimera meristem structure, in which genetically different cell layers coexist. Whether such somatic mutations participate to the phenotype is not clear. BERTSCH *et al*, (2003) showed for instance that Chardonnay clone 96 is a periclinal chimera plant composed of two distinct cell

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layers at least. Separating the two cell layers L1 and L2 through somatic embryogenesis allowed for regenerating L1 plants, which did not show any phenotypic differences compared to the parent clone in greenhouse conditions. This suggested in this case that Chardonnay 96 phenotype was not the result of any interactions between the two distinct cell layers L1 and L2.

Triploid, aneuploid or polyploid grapevines are also known to exist (NOTSUKA *et al*., 2000 ; PARK *et al*., 2002). In several cases triploids or polyploids were artificially produced for their large berries or their seedlessness trait but there are no records of naturally occurring tri- or polyploid *Vitis vinifera*.

It is difficult to decide whether the three-banded individual is a chimera or a triploid but there are arguments that support the one or the other aspect. First, it is difficult to accept triploidy because a triploid grapevine cultivar has never been observed in the wild . Secondly, chimerism has been observed in old grapevine varieties such as Pinot or Chardonnay (FRANKS *et al*., 2002; HOCQUIGNY *et al*., 2004). However, the number of loci that appeared three-banded is extremely high (five out of nine loci, given that VVS2 is not informative). HOCQUIGNY *et al*. (2004) found five three-banded loci out of 50. If we accept that most of the analyzed loci in this study are not linked (as resulted from linkage analysis-data not shown) and that chimerism results of the accumulation of somatic mutations in old grapevine cultivars, we should then accept a high mutation rate across the whole genome, or multiple genomic duplication in this examined individual. In contrast, the alternative hypothesis of triploidy sounds more reasonable. However, further work is needed to confirm chimerism or triploidy of Sinambel. This work should combine morphological data (such as size of leaves and berries, seedlessness or not) with more genetic data, such as microsatellites profiling of more individuals of the cultivar and different tissues of each individual, caryotype analysis).

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