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Molecular identification and genetic relationships of Algerian grapevine cultivars maintained at the germplasm collection of Skikda (Algeria)

Z. Laiadi[1), 2)], M. M. Bentchikou[1)], G. Bravo[3)], F. Cabello[4)] and J. M. Martínez-Zapater[3), 5)]

[1)] Département de Biologie Végétale, Faculté des Sciences de la Nature et de la Vie, Laboratoire de Développement et Valorisation des Ressources Phytogénétiques, Université de Constantine, Algérie
[2)] Département de Biologie, Faculté des Sciences et Sciences de l'Ingénieur, Université de Biskra, Algérie
[3)] Departamento de Genética Molecular de Plantas, Centro Nacional de Biotecnología, CSIC, Madrid, Spain
[4)] IMIDRA, Alcalá de Henares, Madrid, Spain
[5)] Instituto de Ciencias de la Vid y del Vino, (CSIC, Universidad de La Rioja, Gobierno de La Rioja), CCT, Universidad de La Rioja, Logroño, Spain

Summary

We have used nuclear and chloroplast microsatellite markers to characterize a collection of 36 Algerian grapevine (*Vitis vinifera* L.) accessions maintained at the germplasm collection of Skikda (Algeria). The genetic diversity observed within the collection was comparable to what has been described for cultivated accessions of grapevine. Moreover, chlorotype C, associated to eastern accessions and highly frequent among table grape cultivars, was overrepresented in the collection. Genotype comparisons among the accessions and published cultivar genotypes identified a few synonyms within the collection as well as putative synonyms for Algerian accessions such as 'Aïn el Kelb', 'Ahmar Mechtras', 'Ahmar de Mascara' or 'Bouni' among cultivars grown in both Eastern and Western areas of the Mediterranean basin. Furthermore, the study of genetic relationships among the Algerian accessions suggests the existence of close relatedness within some groups of cultivars that could have been originated by spontaneous hybridization and seed propagation.

K e y w o r d s : Nuclear microsatellites, chlorotypes, Algerian grapevine cultivars, synonymies and homonymies, genetic relationships, *Vitis vinifera* L.

Introduction

Grapevine is an important crop in Algeria where vineyards occupied an area close to 56,500 ha in the year 2000. Among them, 32,560 ha corresponded to table grapes, 22,750 ha to wine grapes and over 1,060 ha to raisin production according to the statistics of the Algerian Agriculture Ministry. Current Algerian viticulture is related to the long and complex history of the country which results from a continuous mixture of peoples and civilizations. The Northern part of the country is within the area of distribution of the original wild species *Vitis vinifera* L. ssp. *sylvestris* from which cultivars of *Vitis vinifera* L. ssp. *sativa* would have been domesticated (This *et al.* 2006). Wild populations of *Vitis vinifera* can still be found in the coastal area of Béjaia-Jijel and in the massif of Edough of Annaba (Levadoux *et al.* 1971). In fact, before viticulture was introduced, wild grape berries were regularly consumed as a fruit by Berber populations from the Atlas Mountains (Isnard 1951). The first cultivated forms were introduced in the area by Phoenicians and Carthaginians (Isnard 1951) and those introductions and their putative derivatives resulting from spontaneous hybridizations among cultivated and wild forms could be considered the oldest cultivated vines in the region. Later on, Romans expanded viticulture until the advent of Christianity (Levadoux *et al.* 1971). The Arab culture determined a new phase in the history of Algerian viticulture more focused on the production of grapes for direct consumption, either fresh or dried as raisins (Isnard, 1951, Aldebert and Orsat 1959). The contribution of Turks during this period was not negligible, as it is attested by the presence of cultivars such as 'Chaouch', 'Sultanina', 'Corinth' or 'Rozaki' as well as several cultivars of the Middle East which were already known before the establishment of the Ottoman Empire. Much later, the French occupation increased the diversity and heterogeneity of Algerian viticulture and many wine cultivars were introduced from France and Spain as a consequence of the Phylloxera crisis in Europe (Levadoux *et al.* 1971). After independence, the viticulture sector experienced profound changes related to the new economic and social policy in the country. In fact, nearly 221,000 ha have been abandoned in connection with the re-conversion of wine vineyards. Currently, new introduced bred table grape cultivars are the most relevant at the agronomic level threatening the conservation of autochthonous germplasm.

The first studies of Algerian grapevine cultivars were performed around 1860 by Salomon in the region of Tlemcen in Western Algeria (Isnard 1951). He recognized several autochthonous cultivars in that region such as 'Courchi', 'Adari', 'Farana' and 'Aneb Lekhal' as well as cultivars from Turkey and Spain. According to Viala and Vermorel (1909), the first ampelographic characterization of a set of Algerian grapevine cultivars was performed by Leroux (1894), who analyzed autochthonous cultivars from the Blida region and Pulliat (1898) who characterized some cultivars as 'Farana' and 'Aïn el Kelb', following the names given by the author. Levadoux *et al.* 1971) provided the first general vision of Algerian ampelography

Correspondence to: Dr. J. M. Martínez-Zapater, Departamento de Genética Molecular de Plantas, CNB-CSIC, Darwin 3, 28049 Madrid, Spain. Fax: +34-915-854-506. E-mail: zapater@cnb.uam.es

with a description of the introduced wine and table grape cultivars as well as the autochthonous ones that had been analyzed or just cited in previous works. Most of those autochthonous cultivars, which did not have ampelographic similarities with any other known, can still be found in growing fields of the mountainous areas and are poorly characterized (Aldebert and Orsat 1959). Only the cultivar 'Ahmeur bou Ahmeur', well known because of its vigour and berry quality has been expanded outside Algeria and counts with a long list of synonyms (Galet 2000).

More recently, the development of molecular markers based on DNA sequence polymorphisms offer an efficient tool for genetic identification and genetic studies. Among them, microsatellites have been shown to be highly useful in grapevine given their high level of polymorphism, codominant nature and higher reproducibility than other molecular markers. Specifically, a set of six microsatellites has been shown to be sufficient for cultivar identification in this species and reference genotypes have been published (This *et al.* 2004). Furthermore, the use of a collection of chloroplast microsatellites allowed the distinction of eight *Vitis vinifera* chlorotypes and some of these chlorotypes display a marked geographic distribution in wild populations and could provide information on the Eastern or Western origin of the cultivars (Arroyo-Garcia *et al*. 2002, 2006).

Our goal in this study was to characterize part of the Algerian cultivars to establish their genetic identification and the possible genetic relationships among them and with other cultivars described in the Mediterranean region. With this purpose we have genotyped 36 autochthonous grapevine accessions from the collection of M'zej Edchiche in the eastern part of Algeria (Skikda) using twelve nuclear and four chloroplast microsatellite loci. The results provide a first genotypic characterization of these materials and open the way to develop strategies for genetic conservation and genetic improvement in this country.

Material and Methods

Plant material: Plant material consisted in 36 accessions corresponding to cultivars grown in Algeria (Tab. 1). Samples were obtained from the germplasm collection of M'zej Edchiche, Institut Téchnique d´Arboriculture Fruitière, ITAF, (Ministère de l´Agriculture) located at Skikda in the North-Eastern Algeria. This collection was created in 1990 with all accessions grafted on '1103 Paulsen'. 'Cabernet Sauvignon', 'Monastrell', 'Muscat au Petit Grains' and 'Sultanina' grown at the Spanish Germplasm Center of El Encín (Alcalá de Henares, Madrid) were used as controls to size nuclear and chloroplast microsatellite alleles as previously suggested (Arroyo-Garcia *et al.* 2006, This *et al*. 2004). Young leaf samples were collected from all the accessions, washed in sterile water, wrapped with aluminium paper, frozen in liquid nitrogen and kept at -80 °C until used.

DNA extraction and analyses: DNA was isolated from young frozen leaves using the DNeasy™ Plant Mini Kit (Qiagen, CA, USA). Extracted DNA was electrophoresed in 0.8 % agarose-gels and quantified after staining with ethidium bromide using a computational comparison with known quantities of control λ DNA. Samples were genotyped at twelve nuclear microsatellite loci and four chloroplast microsatellite loci, well characterized in previous studies. Nuclear microsatellites included 6 loci proposed by the GENRES 081 Project (European Vitis Database, www.genres.de/vitis/vitis.htm): VVS2 (Thomas and Scott 1993), VVMD5, VVMD7 (Bowers *et al.* 1996) and VVMD27 (equivalent to VrZAG47) (Bowers *et al.* 1999, Sefc *et al.* 1999), VrZAG62, and VrZAG79 (Sefc *et al.* 1999). The additional 6 microsatellite loci were selected based on the amount of genotypic information available in grapevine literature in order to facilitate genotypic comparisons. They corresponded to VVMD24, VVMD25, VVMD28, VVMD31, VVMD32 (Bowers *et al.* 1996, 1999) and VrZAG21 (Sefc *et al.* 1999). Chloroplast SSR corresponded to cpSSR3, cpSSR10, ccSSR9 and ccSSR14, previously characterized in grape (Arroyo-Garcia *et al.* 2002, 2006). Variation at these four chloroplast microsatellite loci allows the distinction of the eight chlorotypes so far described for grapevine (Arroyo-Garcia *et al.* 2002, 2006). PCR amplifications were performed in a total volume of 20 µl containing 4 ng of genomic DNA, GeneAmp (Applied Biosystems) 10X PCR buffer (10mM Tris HCl, PH 8.3, 50 mM KCl); 2 mM MgCl2; 100 µM of each dNTP; 0.1 µM of each primer (one primer from each pair was fluorescently labelled) and 0.4 U Taq DNA Polymerase (Perkin Elmer-Applied Biosystems). Amplification was performed on GenAmp® PCR System 9700 Thermocycler (PE-Applied Biosystems). After the initial denaturation at 94 °C for 5 min, the reaction followed 15 cycles of denaturation (30 s, 94 °C), annealing (30s at a temperature which varied from 49 to 62 °C depending on the locus) and extension (1min, 72 °C), followed by 20 similar cycles in which the annealing temperature was reduced 3 °C for each given locus. The final extension step was performed at 72 °C for 7 min and amplification products were stored at 4 °C. In all cases amplification was confirmed by running 5 µl of the PCR products on 2 % agarose gels and staining with ethidium bromide. The PCR products were separated in an ABI Prism 3730 DNA Sequencer (Applied Biosystems) and results were analyzed using GeneMapper Software v. 4.1 (Applied Biosystems).

Statistical analyses: Genetic diversity of the Algerian accessions was measured for nuclear microsatellites by estimating the average number of alleles per locus (*Na*), the average number of effective alleles (*Ne*) and the average gene diversity or expected heterozygosity (*He*). These genetic parameters were estimated using the software GENALEX (Peakall and Smouse 2006). Mean observed heterozygosity (*Ho*) were calculated for nuclear microsatellite loci using GENALEX. This program was also used to estimate the average probability of identity per locus (*PI*), the cumulative *PI* and the matching genotypes among Algerian accessions and published genotypes of Mediterranean grapevine cultivars. Genotypes matching all but three alleles at the twelve loci were considered to be identical. Genetic distances between individual accessions

Table 1

Accessions analyzed in this study with their corresponding genotypes at nuclear and chloroplast loci and chlorotypes

Accession name	Acc. #*	Origin	Colour**	VVS2		VVMD5		VVMD7		VVMD24		VVMD25		VVMD27		VVMD28		VVMD31		VVMD32		VrZAG21		VrZAG62		VrZAG79		cpSSR3	cpSSR10	ccSSR9	ccSSR14	Chlorotype
Aberkane	I-06	Kabylie	B	137	137	236	240	234	254	210	210	259	259	183	194	239	263	212	216	253	263	190	200	196	200	260	260	106	116	165	203	C
Adadi	I-15	unknown	W	137	137	236	240	234	254	210	210	259	259	183	194	239	263	212	216	253	263	190	200	196	200	260	260	106	116	165	203	C
Adari des Bibans	I-17	Mostaganem	W	133	137	232	238	240	248	214	218	259	275	185	189	247	261	210	216	253	253	202	214	186	204	256	258	106	116	165	203	C
Ahchichene	I-16	Kabylie	W	133	137	232	238	240	248	214	218	259	275	185	189	247	261	210	216	253	253	202	214	186	204	256	258	106	116	165	203	C
Ahmar de Mascara	I-05	Mascara	R	135	147	232	238	240	250	210	210	259	271	183	194	251	257	212	220	253	257	200	214	192	204	248	258	106	116	165	203	C
Ahmar Mechtras	I-01	unknown	P	143	149	226	232	244	254	218	218	249	249	181	194	247	261	204	212	257	275	206	206	188	188	260	260	107	115	165	202	D
Ahmed draa el Mizen	II-12	unknown	W	137	155	226	240	240	250	210	210	249	259	179	194	247	261	212	214	257	263	190	204	188	204	244	258	106	116	165	203	C
Aïn el Couma	I-20	Temcen	W	137	155	226	240	244	250	210	218	259	259	183	194	247	251	212	216	251	273	190	202	188	194	244	258	106	116	165	203	C
Aïn el Kelb	II-07	unknown	W	135	143	236	240	244	250	210	212	259	259	181	189	247	261	210	220	257	273	200	206	188	204	244	248	106	114	166	201	A
Amellal	II-18	Kabylie	W	137	155	226	240	240	250	210	210	249	259	179	194	247	261	212	214	257	263	190	204	188	204	244	258	106	116	165	203	C
Amokrane	I-04	Kabylie	W	133	137	236	240	234	254	210	210	245	259	183	185	259	261	212	212	253	253	190	204	200	204	238	260	106	116	165	203	C
Aneb el Cadi	II-09	Kabylie	W	143	145	232	234	240	254	210	210	245	245	189	194	249	263	212	220	253	263	190	214	188	200	244	258	106	114	166	201	A
Aneb Kabyle	III-11	Kabylie	W	137	155	226	240	240	250	210	210	249	259	179	194	247	261	212	214	257	263	190	204	188	204	244	258	106	116	165	203	C
Baladi	III-18	unknown	W	145	151	234	234	248	252	210	212	253	253	181	194	249	249	210	214	257	273	190	206	188	204	248	260	106	116	165	203	C
Bezzoul el Khadem	II-06	Kabylie	B	133	143	238	238	240	250	210	212	243	249	179	181	247	261	212	214	257	261	200	206	188	204	252	252	106	116	165	203	C
Boghni	II-14	Alger	W	135	137	228	232	240	250	214	218	243	259	179	179	239	247	216	216	253	273	190	200	186	194	256	262	106	114	166	201	A
Bouaber des Aures	I-10	unknown	B	133	143	232	232	234	250	210	214	243	249	191	194	247	261	214	220	251	273	206	214	204	204	248	248	106	116	165	203	C
Bouni	II-04	unknown	W	137	149	226	226	240	250	210	218	253	259	185	194	247	247	220	224	273	273	190	202	188	204	252	258	106	115	165	202	B
Cherchelli	II-19	Alger	W	149	151	238	238	240	244	212	218	245	259	179	194	251	261	204	210	251	273	206	206	186	186	252	258	106	114	166	201	A
Farana Blanc	II-13	unknown	W	143	145	228	240	240	244	210	218	245	259	179	194	251	261	204	210	251	273	206	206	186	186	258	258	106	114	166	201	A
Farana de Mascara	I-12	Mascara	W	143	145	228	240	240	244	210	218	245	259	179	194	251	261	204	210	251	273	206	206	186	186	258	258	106	114	166	201	A
Farana Noir	I-11	Medea	B	137	143	240	240	250	254	210	210	245	259	183	194	261	263	212	214	253	263	190	202	200	204	260	260	106	116	165	203	C
Ghanez	I-19	unknown	W	143	145	228	240	234	252	210	212	259	259	183	194	247	251	216	220	263	273	206	206	200	204	244	252	106	116	165	203	C
Kabyle Aldebert	I-07	Kabylie	B	133	143	232	232	234	250	210	214	243	249	191	194	247	261	214	220	251	273	206	214	204	204	248	248	106	116	165	203	C
Lekhzine	I-09	unknown	W	133	137	232	238	240	248	214	218	259	275	185	189	247	261	210	216	253	253	202	214	186	204	256	258	106	116	165	203	C
Lakhdari	II-11	unknown	W	133	133	226	236	240	264	210	216	245	245	179	185	237	247	212	212	253	257	202	204	194	196	244	260	107	115	165	202	D
Louali	II-15	unknown	W	133	137	236	240	234	254	210	210	245	259	183	185	259	261	212	212	253	253	190	204	200	204	238	260	106	116	165	203	C
Muscat de Berkain	II-16	unknown	W	133	133	228	236	234	250	210	214	245	253	179	185	249	271	212	216	241	273	190	206	186	204	252	256	107	115	165	202	D
Muscat de Fandouk 1	I-14	unknown	W	133	149	228	232	250	252	214	214	253	253	179	194	247	271	216	224	265	273	190	206	186	204	248	256	106	115	165	202	B
Muscat de Fandouk 2	I-18	unknown	W	137	143	232	238	234	250	210	210	243	249	191	194	247	261	214	220	251	273	190	206	204	204	246	258	106	116	165	203	C
Muscat el Adda	I-08	unknown	B	133	133	226	228	248	252	214	218	245	253	179	185	271	271	196	224	263	263	190	190	192	204	252	252	107	115	165	202	D
Sbaa Tolba	II-10	unknown	W	133	145	226	234	248	254	210	216	243	253	179	194	221	255	212	214	251	257	200	202	188	204	256	260	106	116	165	203	C
Sultanine Fandouk	II-02	unknown	W	145	151	234	234	240	254	210	218	243	253	181	194	221	247	210	212	251	251	190	202	188	188	248	260	106	116	165	203	C
Tadelith	I-13	unknown	B	133	143	226	226	240	254	210	210	243	259	179	183	251	261	210	216	253	257	190	204	188	200	246	250	106	114	166	201	A
Tinesrine	II-01	unknown	W	137	155	226	240	240	250	210	210	249	259	179	194	247	261	212	214	257	263	190	204	188	204	244	258	ND	ND	ND	ND	ND
Tizi Ouinine	II-08	Kabylie	W	145	151	226	228	234	240	210	218	245	253	185	194	261	261	212	220	253	253	200	202	188	188	238	252	106	116	165	203	C
Cabernet Sauvignon	Control	El Encín	B	139	151	232	240	240	240	210	218	243	253	175	189	237	239	206	210	241	241	200	206	188	194	248	248	107	115	165	202	D
Monastrell	Control	El Encín	B	133	151	226	240	250	250	210	218	245	267	179	189	247	261	206	212	241	257	200	204	188	204	252	262	106	114	166	201	A
Muscat au Petite Grains	Control	El Encín	W	133	149	228	232	250	252	214	214	253	253	179	194	247	271	216	224	265	273	190	206	186	204	248	256	106	115	165	202	B
Sultanina	Control	El Encín	W	145	151	234	234	240	254	210	218	243	253	181	194	221	247	212	212	251	251	190	202	188	188	248	260	106	116	165	203	C

* Roman numbers mean blocks and arabic numbers the plantation lines; ** berry colour: P = pink, B = black, R = red, W = white, ND = not done.

were calculated as the allele sharing distance (DAS) (Jin and Chakraborty 1994) and a dendrogram based on the distance matrix was constructed using the neighbour-joining method (Saitou and Nei 1987) using POPULATIONS v. 1.2.30 (http://bioinformatics.org, Langella, unpubl.). The dendrogram was displayed with MEGA3 (Kumar *et al.* 2004).

Results and Discussion

Genotypes at twelve nuclear and four chloroplast microsatellite loci were generated for all the analyzed accessions. Tab. 1 displays the genotypes at nuclear and chloroplast loci as well as the chlorotype assigned to each accession based on chloroplast microsatellite genotypes. The nSSR markers identified 27 different genotypes among the 36 samples analyzed. Since the cumulative Probability of Identity for the twelve loci combinations is estimated at $3.4\ 10^{-15}$, cultivar names with identical genotypes could be considered as synonyms (Tab. 2). However, the different berry colour shown by Aberkane (black) and Adadi (white) (Tab. 1) indicates that they could represent colour somatic variants of the same original genotype and therefore different cultivars. Colour somatic variation is very common in table grape (Lijavetzky *et al*. 2006) and it is a relevant trait to consider two genotypes as different cultivars.

Table 2

Synonyms found in this study

Algerian accessions	Identical genotypes
Name	**Among Algerian accessions**
Aberkane	Adadi
Lekhzine	Ahchichene, Adari des Bibans
Amellal	Ahmed draa el Mizen, Aneb Kabyle, Tinesrine
Amokrane	Louali
Kabyle Aldebert	Bouaber des Aures
Farana de Mascara	Farana Blanc
	Among other mediterranean cultivars
Aïn el Kelb	Calop blanco, Beba
Ahmar Mechtras	Mavrodaphni, Fraoula Kokkini
Ahmar de Mascara	Ahmeur Bou Ahmeur, Royal gordo, Teta de vaca
Muscat el Adda	Moscato Nero 116
Muscat de Fandouk 1	Muscat of Alexandria
Sultanine Fandouk	Sultanina
Bouni	Dominga
Lakhdari	Sangiovese
Farana de Mascara	Boal Dulce

G e n e t i c d i v e r s i t y w i t h i n A l g e r i a n a c c e s s i o n s : The remaining 27 non redundant genotypes were used to characterize the genetic diversity present in the Algerian cultivars. Genetic diversity parameters were estimated for the Algerian samples as well as for 341 cultivars for which there is public genotype information at least for 10 of the 12 microsatellite loci considered. All genotypic data were standardized for allele sizes using as control those cultivars that were in common in the different studies. The results are shown in Tab. 3. The cumulative number of alleles observed for these 12 loci is higher in the whole Mediterranean sample (158) than in the Algerian sample (95) with average number of alleles per locus (*Na*) of 13.17 ± 3.59 and 7.92 ± 1.88, respectively. This higher number of alleles is expected for larger samples as a result of the presence of a higher number of low frequency alleles. In fact, the average *Ne* per locus were not significantly different between both samples (5.32 ± 1.14 versus 5.95 ± 1.31, $P > 0.01$). Average Gene Diversity or *He* values in the two samples were also similar (0.80 ± 0.06 versus 0.81 ± 0.04, $P > 0.01$) and the same was observed for *Ho* values (0.80 ± 0.11 and 0.81 ± 0.04, $P > 0.01$) (Tab. 3). These results indicate that the Algerian samples have very similar levels of genetic diversity as those found in the whole sample of grapevine accessions from the Mediterranean region. The Probability of Identity (*PI*) per locus was also very similar in the Algeria cultivars (0.07 ± 0.04) to what was observed in the general sample (0.06 ± 0.03) resulting in very low cumulative *PI* values for the twelve loci, corresponding to $3.4\ 10^{-15}$ within the Algerian sample as compared to $7.2\ 10^{-16}$ within the total sample. These values are low enough to support the synonymies detected within the sample (Sefc *et al*. 2001). Regarding chlorotypes, most of the Algerian cultivars (56 %) carried chlorotype C. Chlorotypes A (22 %) and D (15 %) were represented within the sample at similar frequencies, while chlorotype B was found at lower frequency (7 %). Chlorotype C was previously reported to be present at higher frequencies among table grape cultivars from Near and Middle East while chlorotypes A and D were more frequently found in wine cultivars of Western (A) and Central (D) Europe. Finally, chlorotype B is detected at a low frequency (ca 8 %) in most cultivar groups analyzed (Arroyo-Garcia *et al.* 2002, 2006). These results agree with the higher relevance of table grape cultivars in Algerian viticulture and support an oriental origin for a large part of the oldest cultivars.

Table 3

Genetic diversity in Algerian and Mediterranean accessions

	Algeria*	Mediterranean accessions*
Na	7.92 ± 1.88	13.17 ± 3.59
Ne	5.32 ± 1.13	5.95 ± 1.31
He	0.80 ± 0.06	0.81 ± 0.04
Ho	0.80 ± 0.11	0.81 ± 0.04
PI (average per locus)	0.07 ± 0.04	0.06 ± 0.03
Cumulative allele number (12 loci)	95	158
Cumulative *PI* (12 loci)	$3.4\ 10^{-15}$	$7.2\ 10^{-16}$

* Mean ± SD

G e n e t i c i d e n t i t y o f A l g e r i a n a c c e s s i o n s : Most of the non redundant 27 genotypes corresponded to cultivars that are described in ampelographic literature as autochthonous Algerian cultivars. This is the case of 'Aneb el Cadi', 'Cherchelli', 'Farana Noir', 'Aberkane', 'Amellal', 'Amokrane', 'Lekhzine', and 'Tizi Ouinine' (Galet 2000). Other cultivars such as 'Bezzoul el Khadem', 'Aïn el Couma', 'Aïn el Kelb' and 'Sbaa Tolba' have been proposed to originate from different Maghreb countries, mainly Morocco, Algeria and/or Tunisia, indicating that their cultivation is spread in the area (Galet 2000). Cultivars such as 'Baladi', 'Sultanine Fandouk' and 'Muscat el Adda' have been described as having different putative origins. 'Baladi' is identified as originated either in Syria or in Spain (Vitis International Variety Catalogue, http://www.vivc.de, 'Sultanina' is a well known cultivar from Turkey (Vitis International Variety Catalogue) and 'Muscat el Adda' is known in Italy as Moscato dell'Adda (Branas and Truel 1965), which has been suggested to be a seedling of 'Muscat Hamburg' self pollination obtained by Pirovano in 1892 (Galet 2000). Finally, no references could be found for accessions with names 'Ahmar Mechtras', 'Ahmar de Mascara', 'Boghni', 'Bouni', 'Farana de Mascara', 'Ghanez', 'Kabyle Aldebert', 'Lakhdari', 'Muscat de Berkain', 'Muscat de Fandouk' and 'Tadelith'.

A comparison of Algerian genotypes with genotypes published by other authors in the Mediterranean area (Tabs 2 and 4) showed that 'Muscat de Fandouk 1', 'Muscat El Adda' and 'Muscat de Berkain' most likely correspond to 'Muscat of Alexandria', 'Moscato Nero 116' and 'Muscat Fior d'Arancio', respectively (Crespan and Milani 2001). In this way, the black berry 'Muscat El Adda' would be a synonym of one of the 'Muscat Nero' accessions analyzed previously (Crespan and Milani 2001) discarding the possibility that it could be derived from a seedling of selfed 'Muscat Hamburg', what is excluded by genotype analysis (data not shown). Furthermore, these Muscat genotypes carry chlorotypes D or B that are frequently observed within the Muscats (Arroyo-Garcia *et al*. 2002). The question remains on what is the identity of the sample known as 'Muscat de Fandouk 2' that does not seem to correspond to any of the known Muscats. Similarly, the genotype of 'Sultanine Fandouk' was identical to the genotype of the well-known Turkish cultivar 'Sultanina' (Bowers *et al.* 1996, 1999, Crespan and Milani 2001, Aradhya *et al.* 2003, This *et al.* 2004) while the genotype of 'Lakhdari' was identical to the classical Italian cultivar 'Sangiovese' (Bowers *et al*. 1996, 1999, Sefc *et al.* 2000, 2003, Crespan and Milani 2001). Furthermore, the genotype obtained for 'Ahmar de Mascara' was identical to the genotype of the classical cultivar 'Ahmeur Bou Ahmeur' as recently pointed out (Akkak *et al.* 2007). This genotype was also been found coincident with that of cultivar Tokay in the same report (Akkak *et al.* 2007). In Spain, the same genotype is cultivated under the names of Royal Gordo (Borrego *et al.* 2002, Ibanez *et al.* 2003) and Teta de Vaca, with color somatic variants white and red (Martin *et al.* 2003). Other genotypes analyzed could also be identical or closely related to genotypes characterized under different names in the Mediterranean area given their genotypic coincidence (Tab. 4). This is the case of cultivar 'Aïn El Kelb' which is described by Galet (2000) as a Tunisian cultivar but has also been described as an Algerian cultivar (Vitis International Variety Catalogue). In fact, this genotype is coincident with the genotype and chlorotype of a Tunisian accession previously analyzed under the name of 'Tebourbi' (Snoussi *et al.* 2004). Furthermore, this genotype is widely cultivated in Spain under multiple names, being 'Beba' and 'Calop blanco' or 'Calop rojo', for the red colour somatic variant, the most common ones (Martin *et al.* 2003). Its presence in the Balearic Islands as well as in the Iberian Peninsula could suggest an oriental origin likely brought by Romans. However, the relevance of this cultivar in Northern Africa suggest this region as an alternative hypothesis for its geographical origin. Algerian accession Bouni is genotypically very close at nine nuclear loci and shares the same infrequent chlorotype B with the Spanish table grape cultivar 'Dominga' (Tab. 4). 'Dominga' has been classically considered as an autochthonous cultivar of the Murcia region in Spain and it is also grown in Portugal (Galet 2000). The genotype known as 'Farana de Mascara' has a coincident genotype with 'Boal Dulce', a cultivar grown in Portugal (Aradhya *et al*. 2003, Ibanez *et al*. 2003, Martin *et al.* 2003) although in this case the chlorotype of 'Boal Dulce' is unknown. The cultivar 'Ahmar Mechtras' has a genotype which is coincident with the genotypes described as 'Mavrodaphni' (Lefort and Roubelakis-Angelakis 2000, Sefc *et al.* 2000) as well as 'Fraoula Kokkini' (Lefort and Roubelakis-Angelakis 2000, 2001). Given that 'Ahmar Mechtras' berries are pink to red as those of 'Fraoula Kokkini' (Galet 2000) and that this later cultivar has been described as present in Greece, Cyprus and in Egypt under the name of 'Roumi Ahmar' (Galet 2000), we believe it could be related to the Algerian cultivar. Further genotyping will be required to confirm this hypothesis. Finally, the genotype of the Algerian 'Baladi' analyzed in this work was not coincident with that of the Spanish synonymous cultivars (Ibanez *et al.* 2003, Martin *et al.* 2003) and the possibility that it is related with Syrian cultivars should be considered.

Genetic relationships among accessions: After genotypic comparison, 17 Algerian accessions did not correspond with any of the available published grapevine genotypes and could represent unique Algerian cultivars. With the purpose of obtaining some additional information on the genotyped accessions we analyzed their genetic relationships. The dendrogram clustered the 27 non-redundant genotypes into three major groups (Figure). The first group included accessions for which we could not find any information, such as 'Ghanez', 'Kabyle Aldebert' and 'Muscat de Fandouk 2', together with recognized Algerian cultivars, such as 'Cherchelli' and 'Amellal', and cultivars spread around the Mediterranean area such as 'Ahmar Mechtras', 'Farana de Mascara', 'Aïn El Kelb', 'Bezzoul El Khadem', 'Bouni' or 'Aïn El Couma'. This result suggests a genetic relationship between those unknown genotypes and Maghreb cultivars. A deeper analysis of each sub-cluster indicated the possible existence of close genetic relationships between some of the genotypes. In this way, 'Chercherlli' and 'Farana de Mascara' share chlorotype A which is more frequent in Western Mediterranean area and their close genetic relationship suggest they could be close relatives. Similarly, the close relation-

T a b l e 4

Genotypic identities between Algerian accessions and other grapevine cultivars

Cultivar	VVS2		VVMD5		VVMD7		VVMD24		VVMD25		VVMD27		VVMD28		VVMD31		VVMD32		VrZAG21		VrZAG62		VrZAG79		Probability*
Aïn el Kelb	35	143	236	240	244	250	210	212	259	259	181	189	247	261	210	220	257	273	200	206	188	204	244	248	
Beba, Calop blanco	135	143	236	240	244	250					181	189									188	204	244	248	$1{,}24\ 10^{-08}$
Tebourbi	135	143	236	240	244	250					181	189							200	206	188	204	244	248	$1{,}68\ 10^{-09}$
Ahmar de Mascara	135	147	232	238	240	250	210	210	259	271	183	194	251	257	212	220	253	257	200	214	192	204	248	258	
Ahmeur Bou Ahmer	135	147	232	238	240	250	210	210	259	271	183	194	251	257	212	220	253	257	200	214	192	204	248	258	$3{,}29\ 10^{-21}$
Royal gordo	**137**	147	232	238	240	250					183	194	251	257							192	204	248	258	$4{,}08\ 10^{-12}$
Teta de vaca rosa	135	147	232	238	240	250					183	194									192	204	248	258	$7{,}70\ 10^{-11}$
Ahmar Mechtras	143	149	226	232	244	254	218	218	249	249	181	194	247	261	204	212	257	275	206	206	188	188	260	260	
Mavrodaphni	143	149	226	232	244	254					181	194							206	206	188	188	260	260	$7{,}54\ 10^{-11}$
Fraoula kokkini	**133**	149	226	232	244	254					181	194							206	206	188	188	260	260	$4{,}18\ 10^{-10}$
Muscat de Fandouk1	133	149	228	232	250	252	214	214	253	253	179	194	247	271	216	224	265	273	190	206	186	204	248	256	
Moscatel de Alejandria	133	149	228	232	250	252	214	214	253	253	179	194	247	271	216	224	265	273	190	206	186	204	248	256	$1{,}94\ 10^{-19}$
Muscat el Adda	133	133	226	228	248	252	214	218	245	253	179	185	271	271	196	224	263	265	190	190	192	204	252	**252**	
Moscato nero 116	133	133	226	228	248	252	214	218	245	253	179	185	271	271	196	224	263	265	190	190	192	204	252	**256**	$3{,}78\ 10^{-21}$
Sultanine Fandouk	145	151	234	234	240	254	210	218	243	253	181	194	221	247	**210**	212	251	251	190	202	188	188	248	260	
Sultanina	145	151	234	234	240	254	210	218	243	253	181	194	221	247	**212**	212	251	251	190	202	188	188	248	260	$1{,}46\ 10^{-16}$
Bouni	137	149	226	**226**	240	250	210	218	253	259	185	194	247	247	220	224	273	273	190	202	188	204	252	258	
Dominga	137	149	226	**246**	240	250					185	194	247	247							188	204	252	258	$2{,}02\ 10^{-09}$
Lakhdari	133	133	226	236	240	264	210	216	245	245	179	185	237	247	212	212	253	257	202	204	194	196	244	260	
Sangiovese	133	133	226	236	240	264	210	216	245	245	179	185	237	247	212	212	253	257	202	204	194	196	244	260	$5{,}49\ 10^{-17}$
Muscat de Berkain	133	133	228	236	**234**	250	210	214	245	253	179	185	249	271	212	216	241	273	200	206	186	204	252	256	
Moscato fior d'arancio	133	133	228	236	**248**	250	210	214	245	253	179	185	249	271	212	216	241	273	200	206	186	204	252	256	$1{,}90\ 10^{-14}$
Farana de Mascara	143	145	228	240	240	244	210	218	245	259	179	194	251	261	204	210	251	273	206	206	186	**186**	**258**	258	
Boal Dulce	143	145	228	240	240	244					179	194	251	261	204	210	251	273			186	**188**	**252**	258	$7{,}92\ 10^{-12}$

* Probability of identity is based on allele frequencies in a set of 341 published genotypes.
Bolded alleles show disagreements among genotypes obtained in this work and published genotypes.

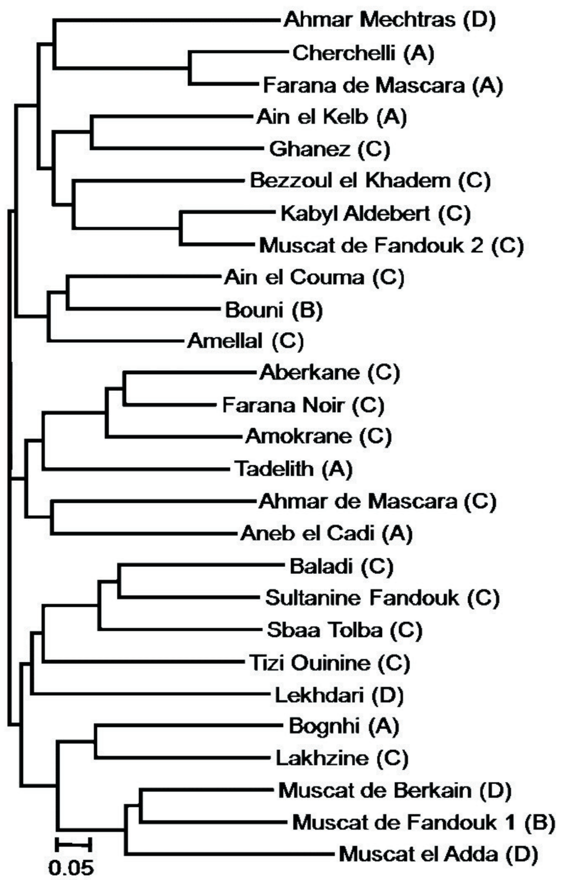

Figure: Genetic relationships among Algerian grapevine cultivars maintained at Skikda. Cultivar chlorotypes are in brackets.

ship between 'Muscat de Fandouk 2' and 'Kabyle Aldebert' and their common chlorotype C suggest that 'Muscat Fandouk 2' is not a Muscat cultivar but relates to other 'Maghreb' cultivars such as 'Bezzoul El Khadem'. Finally, the relationship among 'Bouni', 'Aïn el Couma' and 'Amellal' suggest that the origin of the Spanish 'Dominga' could be in the 'Maghreb', a hypothesis that should be confirmed with further analyses.

The second large cluster grouped classical Algerian cultivars. Some of them are very closely related and could be close relatives. This is the case of 'Aberkane', 'Farana Noir' and 'Amokrane', all sharing chlorotype C. The inclusion of cultivar 'Tadelith' within this cluster would also suggest its Algerian origin. Finally, the third large cluster grouped a heterogeneous sample of genotypes with different putative origins. Among them, 'Baladi', 'Sbaa el Tolba' and 'Sultanine Fandouk' show close genetic relationship. This could suggest a Near East origin for these cultivars related with Sultanine, a hypothesis that is supported by their common chlorotype C and against the proposed relationship between 'Baladi' and Spanish cultivars, previously suggested (Galet 2000). Another clear sub-cluster of closely related cultivars grouped the Muscats. 'Muscat de Berkain', 'Muscat Fandouk 1' and 'Muscat el Adda' all show high pair wise genetic relationships suggesting that they are close relatives, and bear typical Muscat chlorotypes such as B and D. Close genetic relationships among these three Muscat cultivars were previously reported (Crespan and Milani 2001). Finally, this cluster also included other cultivars such as 'Lakhdari' ('Sangiovese') of demonstrated Italian origin (Vouillamoz *et al.* 2007), 'Boghni' which could be related with Bogni Italian selections based on its name and two additional Algerian cultivars such as 'Tizi Ouinine' and 'Lekhzine' for which no information is available.

In conclusion, the analysis of Algerian accession genotypes allows the identification of synonyms and provides a first view of the complex origins of cultivars in this region. A characteristic feature of the analyzed accessions is the higher representation of table grape cultivars concomitantly with a higher frequency of chlorotype C. As expected from the common history of the Maghreb region, Algerian accessions are in many cases found in common with other countries in the area such as Morocco and Tunisia. Furthermore, the close genetic relationships observed among some of them suggest that there are groups of cultivars that could have been originated by spontaneous hybridization among cultivated plants followed by seed propagation. Algerian viticulture has roots both in Eastern and Western viticulture. The Eastern area could be the origin of cultivars such as 'Ahmar Mechtras', 'Sultanine Fandouk' and 'Baladi' while the Western would be represented by the presence of cultivars such as 'Farana de Mascara' or 'Cherchelli'. Algerian viticulture also includes classical cultivars, like the Mediterranean Muscats, and could have been the origin of dissemination of cultivars such as 'Ahmeur Bou Ahmeur', 'Aïn El Kelb' and perhaps 'Bouni' ('Dominga') along the Mediterranean area and specifically in Northern Africa and the Iberian Peninsula. Further analyses involving larger samples of genotypes and molecular markers are required to fully support the suggested identities and genetic relationships and to better understand and conserve this genetic diversity which study uncovers important pieces of a common Mediterranean history.

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