

Comparative ampelographic and genetic analysis of grapevine cultivars from Algeria and Morocco

L.H. ZINELABIDINE^{1,5}, Z. LAIADI², R. BENMEHAIA³, P. GAGO⁴, S. BOSO⁴, J.L. SANTIAGO⁴,
A. HADDIOUI¹, J. IBÁÑEZ⁵, J.M. MARTÍNEZ-ZAPATER⁵ and M.C. MARTÍNEZ⁴

¹ Laboratoire de Gestion et Valorisation des Ressources Naturelles, Equipe de Génétique et Biotechnologie Végétale, Faculté des Sciences et Techniques, Université Sultan Moulay Slimane, Beni Mellal, Morocco

² 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, Route d'Aïn El Bey, 025000 Constantine, Algeria

³ Département de Biologie Végétale, Faculté des Sciences, Université de M'Sila, Ichebilila, M'Sila, Algeria

⁴ Misión Biológica de Galicia (MBG-CSIC), Consejo Superior de Investigaciones Científicas, Carballeira 8, Salcedo 36143, Pontevedra, Spain

⁵ Complejo Científico Tecnológico, Instituto de Ciencias de la Vid y del Vino (Gobierno de La Rioja, CSIC, Universidad de La Rioja, C/ Madre de Dios 51, 26006 Logroño, Spain

Corresponding author: Dr María Carmen Martínez, email carmenmartinez@mbg.csic.es

Abstract

Background and Aims: North Africa has a long history of viticulture and a wide diversity of grape cultivars. Ampelographic studies have been made of grapevine cultivars grown all over the world, but only a few describe those of Algeria and Morocco. Many Maghrebi cultivars held in germplasm banks or found growing wild in this region have recently been subjected to microsatellite profiling by different researchers, though little comparative analysis has been undertaken. The aim of the present work was to clarify the identity of the grapevine cultivars growing in the Maghreb via ampelographic and single-nucleotide polymorphism analysis.

Methods and Results: Seventy-one accessions were studied through the ampelographic construction of their mean leaves, via genotypic analysis using single-nucleotide polymorphism markers, and the comparison of these results with previously reported single sequence repeat marker profiles and ampelographic data for other grapevine material from the Maghreb.

Conclusion: New synonyms and homonyms were detected between Maghrebi cultivars. Some misinterpretations and errors of identification made during the making of the studied germplasm collections were identified.

Significance of the Study: This study helps clarify the confusion over the identity of Algerian and Moroccan grapevine cultivars and provides a general picture of grapevine diversity in the Maghreb.

Keywords: *germplasm collection, homonym, North Africa, SNP, synonym, Vitis vinifera, wild population*

Introduction

North Africa has a long history of viticulture and a great diversity of grape cultivars. In fact, the north of Algeria, Morocco and Tunisia are within the area of distribution of the original wild species *Vitis vinifera* L. ssp. *silvestris* (Gmelin) Hegi from which present day cultivated grapes (*Vitis vinifera* L. ssp. *sativa*) were domesticated (This et al. 2006). Cultivated vines were brought into the region by Phoenicians and Carthaginians (Isnard 1951, McGovern 2004) and these likely hybridised among themselves and with wild forms over centuries of viticultural history. Viticulture became consolidated in Northern Africa under Roman influence (McGovern 2004, This et al. 2006). Centuries later, Islam enhanced the expansion of table rather than winemaking cultivars (Isnard 1951, Aldebert and Orsat 1959), bringing new cultivars from Eastern regions (Fregoni 1991). More recently, European influence in the region promoted grapevine cultivation in Algeria, with colonists making their own wine (Föex 1891, Johnson 1990). The arrival of phylloxera in Europe at the end of the 19th century, however, led to a great increase in the area cultivated to the grapevine in Algeria, with French vine growers planting large vineyards. Many of the cultivars planted were those grown at that time in France (Föex 1891), while

others were brought from Spain (Isnard 1951, Levadoux et al. 1971). Today, information held by the Organisation Internationale de la Vigne et du Vin (OIV) (<http://www.oiv.int/>) Internationale de la Vigne et du Vin 2009) for the period 1986–2007 shows that Algeria and Morocco were responsible for a mean 0.42% and 0.41%, respectively, of the world's entire grape production. In Algeria, 59.41% of this production was destined for table consumption, 40.47% for winemaking and 0.10% for raisin production. In Morocco, these figures were 76.40, 23.24 and 0.36%, respectively.

Many ampelographic studies have been made of grapevine cultivars from all over the world, but only a few have described those of Algeria and Morocco (Föex 1891, Isnard 1951, Vidal 1951, Levadoux et al. 1971), the most recent being a collection of the descriptions made by Galet since 1952 under the title *Dictionnaire Encyclopédique des Cépages* (Galet 2000). In recent years, however, several molecular studies on North African cultivars have been made, identifying the accessions held in germplasm banks through the study of DNA polymorphisms. As a consequence, many of the Maghrebi cultivars have now been profiled by nuclear and chloroplast microsatellite [simple sequence repeats (SSR)] analysis (El Ouakadi et al. 2009, 2011,

Laiadi et al. 2009, Riahi et al. 2010, Zinelabidine et al. 2010). Unfortunately, these studies were published over a short period of time that made it difficult for their authors to compare their results. Thus, much confusion regarding the true number of cultivars continues to exist, the consequence of the existence of synonyms, homonyms, errors of identification and the difficulty in transcribing Arab words into the Latin alphabet.

Recently, single-nucleotide polymorphisms (SNPs – another type of molecular marker) have been used to identify grapevine cultivars, to construct genetic maps and to perform parentage analysis (Cabezas et al. 2011, Ibanez et al. 2012, Zinelabidine et al. 2012). In the genome, SNPs are more abundant than SSRs, and further, they have the advantage of being bi-allelic, co-dominant, robust and of allowing for high-throughput genotyping and automation. Importantly, their allele binning is easy (bi-allelic and based on nucleotide sequence instead of amplicon length), which makes these markers particularly suitable for comparing the data obtained by different laboratories.

The aim of the present work was to clarify the identity of the grapevine cultivars growing in the Maghreb via ampelographic and SNP analysis, and to compare the results obtained with previously published SSR marker profiles and ampelographic data. This information was used to detect synonyms, homonyms and possible errors of identification in previous reports.

Materials and methods

Plant material

The Algerian material used in this study included 34 accessions (two plants per accession) (codes A1–A34; Table 1) of the germplasm collection belonging to the M'zejj Edchiche Institut Technique d'Arboriculture Fruitière (ITAF), (Ministère de l'Agriculture), Skikda (northeastern Algeria). The Moroccan material included 37 accessions (two plants per accession) and wild plants (codes M1–M37; Table 1); these were either maintained as part of the grapevine collection of the Société du Développement Agricole (SODEA) de Meknès ($n = 19$), found growing in vineyards in the Demnate region ($n = 11$), or found growing spontaneously in non-cultivated areas in Oum-er-Rbia, Ouaoumana and El Ksiba ($n = 7$). Different leaf samples were taken from the same plants for the ampelographic and molecular analysis.

Ampelographic characterisation

After blooming, 10–11 fully expanded leaves from nodes eight to nine of green shoots were collected from each accession. Measurement of the variables required for reconstructing an average leaf for each plant was taken following the method of Martinez and Grenan (1999) (Figure 1a). Image analysis of digital photographs was performed with analySIS 3.0 software (Soft Imaging System GmbH, Münster, Germany). The number of teeth between the major veins was also recorded following the proposal of the same authors (Figure 1b).

The variables measured (lengths and angles) were used to calculate the following relationships: Rel.1:L1d/L; Rel.2:L1g/L; Rel.3:A + B + G; Rel.4:A' + B' + G'; Rel.5:a + b + g; Rel.6:a' + b' + g'; Rel.7:(S1d + S2d)/(L1d + L2d); Rel.8:(S1g + S2g)/(L1g + L2g); Rel.9:S1d/L1d; Rel.10:S1g/L1g; Rel.11:S2d/L2d; Rel.12:S2g/L2g (Martinez and Grenan 1999).

DNA analysis

DNA was isolated from young frozen leaves using the DNeasy™ Plant Mini Kit (Qiagen, Valencia, CA, USA). The genotype of most of the grapevine cultivars examined was determined with a set of 48 selected SNPs (Table 1). Genotyping was performed at CEGEN (<http://www.cegen.es>), using

the SNPlex high-throughput genotyping platform (Applied Biosystems, Foster City, CA, USA), according to Cabezas et al. (2011). The results obtained were compared with the Instituto de Ciencias de la Vid y el Vino database, which includes the genotypes of more than 6000 grapevine samples.

Data analysis

Principal component analysis (PCA) of the raw data from the above-defined leaf relationships was performed using SAS statistical software v.9.1 (SAS Institute, Cary, NC, USA). This analysis avoids problems caused by differences in leaf size because of growing conditions. SNP data were analysed using GenAlEx (Peakall and Smouse 2006, 2012).

Results

The average leaf of every cultivar studied was constructed. Figures 2 and 3 show the mean leaves constructed for the Algerian and Moroccan cultivars, respectively. The first three axes determined in PCA explained 88% of the variation between cultivars in terms of leaf morphology. Axis 1 (Prin 1) explained 49.5% of the variation, with variables related to the depth of the lateral sinuses (Rel. 7, Rel. 8, Rel. 9, Rel. 10, Rel. 11 and Rel. 12) having the greatest weight. Axis 2 (Prin 2) explained 25.28% of the variation, with variables related to the angles formed by the main veins (Rel. 3, Rel. 4, Rel. 5 and Rel. 6) showing the greatest weight. Finally, the third axis (Prin 3) explained 13.55% of the variation, with variables indicating the orbicular or cuneiform shape of the leaf (Rel. 1 and Rel. 2) having the greatest weight.

Figure 4 shows the PCA distribution plot for the studied plants with respect to the variables analysed. The accessions to the right of the graph on Prin 1 are those with shallow lateral sinuses, including accession Oum-er-Rbia 1 (M28), and the cultivars Bouchouka (M7), El Farryali (M15), Laadari (M20), Bouzouga (M11), Tagulayt (M34) and Oualgdid (M25), all from Morocco. On the left on Prin 1 lie the cultivars with deeper lateral sinuses, such as Amokrane (A12), Carignan (M12), Louali (A27), Muscat el Adda (A30), Bezzoul el Khadem (A15), Farana (M19), El Karim (M16) and Sidi Taybi (M33). Muscat de Berkaim (A28), with the most open vein angles, is located at the back of the graph on Prin 2, while El-Ksiba 2 (M18), Farana (M19) and Bezzoul el Khadem (A15), with the most closed angles, are located in the foreground. The bottom of the graph on Prin 3 is occupied by Ouaoumana 2 (M27), Farana Blanc (A20), Oum-er-Rbia 1 (M28), Bouchouka Blanc (M7) and Rarjij (M31), all plants with longer leaves, i.e. those in which the relationship between L1g or L1d with respect to L is small. The upper part of the graph is occupied by Muscat Sefrou (M24), Sultanina Fandouk (A32), El-Ksiba 1 (M17), Bouzouga (M11), Muscat de Fandouk1 (A29) and Muscat de Berkaim (A28), the leaves of which are orbicular in shape. The remaining cultivars are distributed in intermediate positions.

Given the above PCA distribution of the accessions and the mean leaf data (Figures 2 and 3), the Moroccan cultivars were found to show (at least in *grasso modo*) more variation in terms of leaf morphology than those of Algeria, which generally were characterised by deep lateral sinuses.

In the samples studied, SNP1399_81 was found to be monomorphic and SNP analysis of 58 cultivar samples (34 Algerian and 24 Moroccan) revealed a total of 39 non-redundant genotypes (Table 2). Most redundancy was found among accessions of the same country, but two matching pairs were found between accessions from different countries: Ain el Kelb (A10) with Muscat Sefrou (M24), and Muscat de Fandouk 1 (A29) with Muscat Doukkala (M23). A total of 23 different genotypes

Table 1. The Algerian and Moroccan cultivars studied in the present work.

Accession name	Code	Berry colour	Origin†	Country	SNP analysis
Aberkane	A1	B†	ITAF	Algeria	Yes
Adadi	A2	G	ITAF	Algeria	Yes
Adari des Bibans	A3	G	ITAF	Algeria	Yes
Ahchichene	A4	G	ITAF	Algeria	Yes
Ahmar de Mascara	A5	R	ITAF	Algeria	Yes
Ahmar Mechtras2	A6	P	ITAF	Algeria	Yes
Ahmar Mechtras3	A7	P	ITAF	Algeria	Yes
Ahmed dra el Mizen	A8	G	ITAF	Algeria	Yes
Ain el Couma	A9	G	ITAF	Algeria	Yes
Ain el Kelb	A10	G	ITAF	Algeria	Yes
Amellal	A11	G	ITAF	Algeria	Yes
Amokrane	A12	G	ITAF	Algeria	Yes
Aneb el Cadi	A13	G	ITAF	Algeria	Yes
Baladi	A14	G	ITAF	Algeria	Yes
Bezzoul el Khadem	A15	B	ITAF	Algeria	Yes
Boghni	A16	G	ITAF	Algeria	Yes
Bouaber des Aures	A17	B	ITAF	Algeria	Yes
Bouni	A18	G	ITAF	Algeria	Yes
Cherchelli	A19	G	ITAF	Algeria	Yes
Farana Blanc	A20	G	ITAF	Algeria	Yes
Farana de Mascara	A21	G	ITAF	Algeria	Yes
Farana Noir	A22	B	ITAF	Algeria	Yes
Ghanez	A23	G	ITAF	Algeria	Yes
Kabyl Aldebert	A24	B	ITAF	Algeria	Yes
Lakhzine	A25	G	ITAF	Algeria	Yes
Lekhdari	A26	G	ITAF	Algeria	Yes
Louali	A27	G	ITAF	Algeria	Yes
Muscat de Berkain	A28	G	ITAF	Algeria	Yes
Muscat de Fandouk1	A29	G	ITAF	Algeria	Yes
Muscat el Adda	A30	B	ITAF	Algeria	Yes
Sbaa Tolba	A31	G	ITAF	Algeria	Yes
Sultanine Fandouk	A32	G	ITAF	Algeria	Yes
Tadelith	A33	B	ITAF	Algeria	Yes
Tizi Ouinine	A34	G	ITAF	Algeria	Yes
Abbou	M1	B	SODEA	Morocco	Yes
Abbouhou	M2	B	Demnate	Morocco	Yes
Aguyar	M3	B	Demnate	Morocco	Yes
Arbia	M4	B	SODEA	Morocco	Yes
Azizi El Hor	M5	G	SODEA	Morocco	No
Bezoul el Aouda	M6	B	SODEA	Morocco	Yes
Bouchouka blanc	M7	G	Demnate	Morocco	Yes
Boujlida	M8	G	SODEA	Morocco	Yes
Boukhanzir	M9	G	SODEA	Morocco	No
Bouqseb	M10	G	SODEA	Morocco	Yes
Bouzouga	M11	B	SODEA	Morocco	Yes
Carignan	M12	B	SODEA	Morocco	Yes
Djinani	M13	G	SODEA	Morocco	Yes
El Biod	M14	G	SODEA	Morocco	Yes
El Farryali	M15	B	Demnate	Morocco	No
El Karim	M16	G	SODEA	Morocco	Yes
El-Ksiba 1	M17	Nd	Wild (El-Ksiba)	Morocco	No

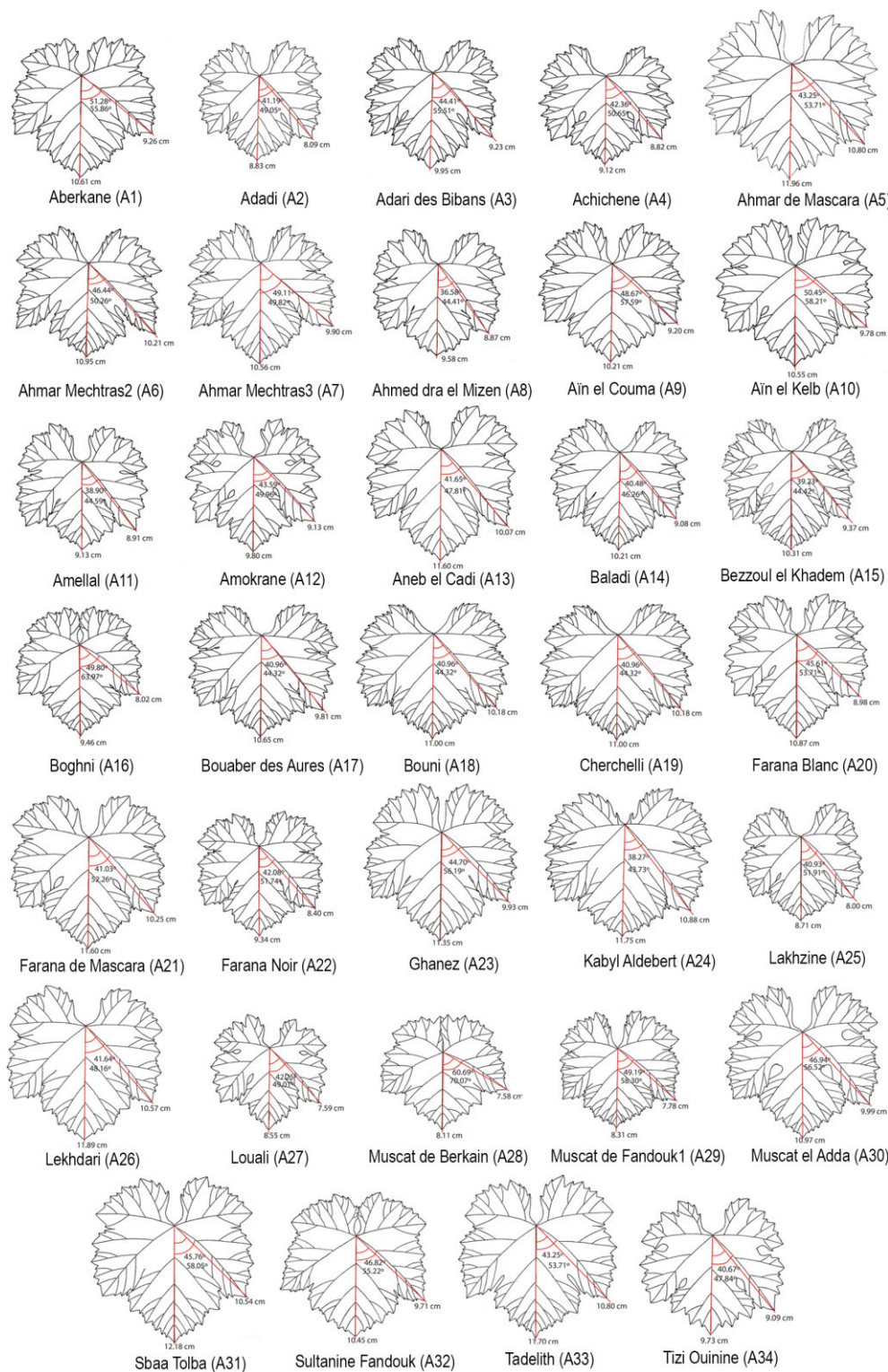


Figure 2. Mean leaves of the Algerian cultivars (A1 to A34).

Less information is available for Algeria. The only information on the extension of land under vineyards, and of the cultivars grown, is provided by the Organisation Internationale de la Vigne et du Vin (2009) (originally provided by the ITAF, by Isnard (1951), and by Levadoux et al. (1971). According to the ITAF, some 94 025 ha were given over to vineyards in 2013. The only information available on the cultivars grown (and their relative proportion) is now many years old (Isnard 1951, Levadoux et al. 1971). Although grapevine cultivation is

thought to be in recession in Algeria, the conservation of the country's grapevine genetic resources is both scientifically and economically important.

Several authors have recently published microsatellite profiles for many of the cultivars examined in the present work, insome cases using the same accessions examined here [those of the ITAF (Algeria), and the SODEA and Demnate (Morocco) collections]. The present work compiles and compares all the microsatellite and ampelographic data published for North

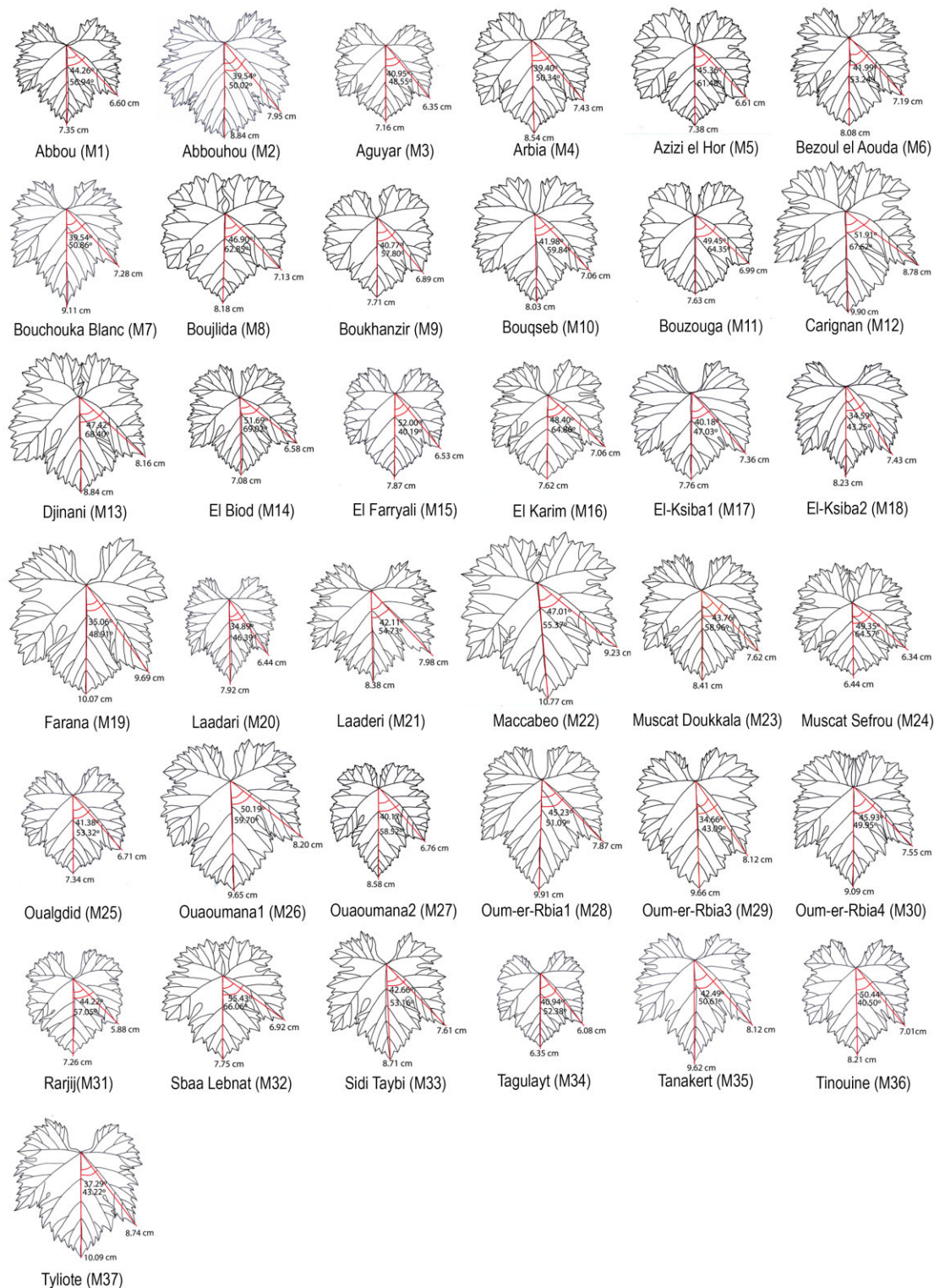


Figure 3. Mean leaves of the Moroccan cultivars and wild plants (M1 to M37).

African grape cultivars, and along with the ampelographic and SNP results obtained, provides a greater understanding of the true identity behind the names given to cultivars in different areas. Supporting Information Table S1 provides a comprehensive summary of the comparisons made and conclusions reached. Samples are grouped according to their SNP genotypes and ordered according to their codes (Table 1), except in those cases in which homonyms or similar names appear; these are grouped together for better understanding. Where synonyms,

homonyms, similar names and possible sampling and/or collection errors come together, proper identification can be complicated. For example, the cultivars Ahmed dra el Mizen (A8), Amellal (A11) and Tizi Ouinine (A34) (GEN_SNP_2313, Supporting Information Table S1), all turned out to be the same cultivar. The same happened for Amokrane (A12) and Louali (A27), (GEN_SNP_2308, Supporting Information Table S1). Another example is the complex use of 'Farana', which includes several Algerian and Moroccan cultivars (GEN_SNP_2220,

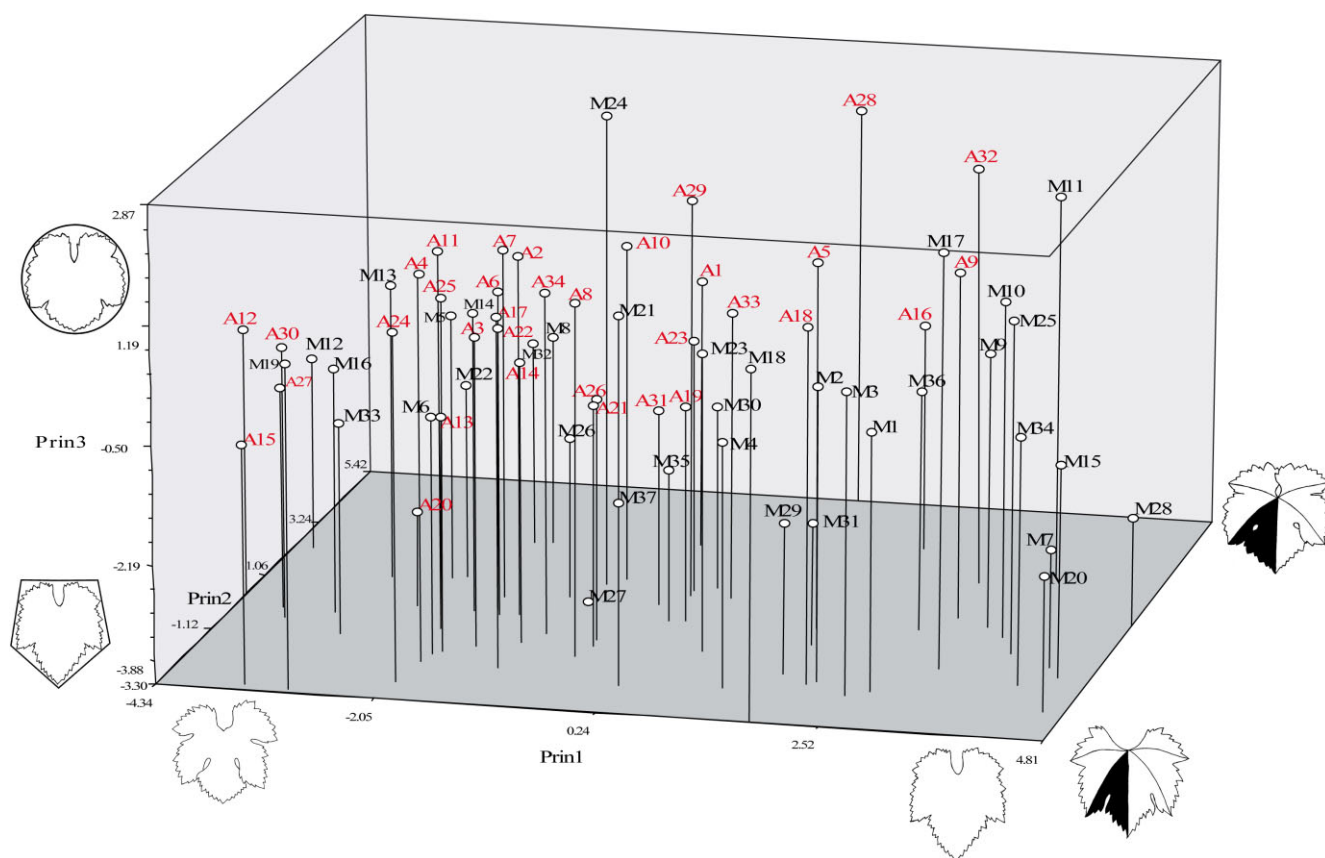


Figure 4. Principal component analysis showing the projection of the 34 Algerian (A1 to A34) and 37 Moroccan (M1 to M37) grape cultivars on the axes defined by the first three principal coordinates.

GEN_SNP_1475 and GEN_SNP_2311, Supporting Information Table S1). The present work shows that several old cultivars grown both in Spain and in the Maghreb countries, especially in Morocco (where they still grow but under different names), include Ahmeur bou Ahmeur, Airen, Cañocazo, Beba, Cayetana Blanca, Dominga, Ohanes, Planta Fina and Planta Mula (Supporting Information Table 1). This is probably the result of cultural and material exchange between North Africa and the Iberian Peninsula over many centuries. There are also cultivars of wide distribution outside the Maghreb, such as several Muscats, and Sultanina, that were also found in the Moroccan and Algerian collections. Finally, there are several cultivars (Aberkane, Achichene, Adadi, Amellal, Aneb el Cadi, Bezzoul el Khadem, Taferielt, Agueyer, Abbouhou, Bouqseb, Bouchouka and Bouzouga) for which no synonyms were found outside of the region; these cultivars are probably native to the Maghreb.

The molecular profiles for Ouaoumana 1 and Ouaoumana 2 (wild grapevine samples from Morocco) reported by Zinelabidine et al. (2010) were identical, but different to any other wild plants from different locations in Morocco. No matches were seen with the profiles of other North African materials reported by other authors (El Oualkadi et al. 2009, Laiadi et al. 2009, Riahi et al. 2010). Figure 3 shows the mean leaves for Ouaoumana 1 (M26) and Ouaoumana 2 (M27) to be similar in terms of lateral sinus depth and vein angles, even though Ouaoumana 2 (M27) has more cuneiform leaves. This great similarity in their leaves, and the above-mentioned molecular results, show that they are the same genotype.

The three wild plants found at Oum-er-Rbia (M28, M29 and M30) had clearly different leaves to one another and to plants

from the other locations. The wild plant M28 had more cuneiform leaves, M29 more closed lateral sinuses and superimposed lobules, and M30 a closed petiolar sinus and U-shaped lateral sinuses.

In terms of their vein angles, M17 and M18 from El-Ksiba also showed leaves that differed from one another, and from those of the other cultivars studied. This suggests that they represent different cultivars; however, it would appear that they are similar at the molecular level because 8 of the 11 microsatellites analysed by Zinelabidine et al. (2010) coincide.

The present results highlight the need for collaboration between countries if problems of homonyms and synonyms are to be resolved. They also show the need to combine ampelographic and molecular results. Cultivars with similar leaf or bunch morphology might be difficult to distinguish by ampelographic means, but be clearly distinguishable by molecular techniques. For example, Bouchouka Blanc (M7) and Laadari (M20), which have leaves of similar morphology, are clearly shown by molecular methods to be different cultivars. Combining these techniques can also help confirm identity. For example, Amokrane (A12) and Louali (A27) have similar leaf morphologies, but leaves may vary greatly in size (because of the properties of the soil and water availability, or even because their parent plants belong to different clones). Molecular analysis shows them to be the same cultivar.

Conversely, some cultivars show identical molecular profiles for the markers analysed, but have different ampelographic characteristics, even to the extent that the grapes of one might be red and those of the other green. This was seen for the Pinot cultivars (Pinot Noir, Pinot Blanc and Pinot Gris) and for Muscat de Frontignan Rouge and Muscat de Frontignan Blanc.

Table 2. SNP genotypes for the 34 Algerian and 24 Moroccan accessions analysed.

Code	Name	SNP Genotype number	Proposed name†	SNP1003_336	SNP1015_67	SNP1027_69	SNP1035_226	SNP1079_58	SNP1119_176	SNP1127_70	SNP1157_64	SNP1215_138	SNP1229_219	SNP1323_155	SNP1347_100	SNP1349_174	SNP1399_81	SNP1411_565	SNP1445_218	SNP1453_40	SNP1471_179	SNP1513_153	SNP191_100	SNP197_82	SNP227_191	SNP259_199
A01	Aberkane	GEN_SNP_2412	ABERKANE	AA	AG	CC	LL	AG	AC	GT	AT	CC	CC	CC	GG	AA	AA	LL	AG	AA	LL	TT	CC	CC	CC	TT
A02	Adadi	GEN_SNP_2902	—	ND	AG	CT	CT	ND	ND	ND	ND	CC	CC	ND	ND	ND	AA	ND	ND	ND	LL	TT	CC	CC	CC	TT
A03	Adari des Bihans	GEN_SNP_2310	ACHICHANE	CC	AG	CT	LL	AG	AC	GT	AT	CC	CC	CC	AG	AA	AT	AT	AG	AG	TT	TT	CC	CC	CC	TT
A04	Abchichene	GEN_SNP_2310	ACHICHANE	CC	AG	CT	LL	AG	AC	GT	AT	CC	CC	CC	AG	AA	AT	AT	AG	AG	TT	TT	CC	CC	CC	TT
A05	Ahmar de Mascara	GEN_SNP_0473	AHMEUR BOU AHMEUR	AC	AA	CT	CT	AA	AC	GG	AT	CT	CC	CC	GG	AA	TT	TT	AG	GG	CT	CT	CC	CC	CC	TT
A06	Ahmar Mechtras II	GEN_SNP_0051	FRAOULA KOKKINI	AA	AG	CC	CT	AA	CC	GG	AT	CC	CC	CC	GG	AA	TT	TT	AG	AA	TT	TT	CC	CC	CC	TT
A07	Ahmar Mechtras III	GEN_SNP_0051	FRAOULA KOKKINI	AA	AG	CC	CT	AA	CC	GG	AT	CC	CC	CC	GG	AA	TT	TT	AG	AA	TT	TT	CC	CC	CC	TT
A08	Ahmed Draa el Mizen	GEN_SNP_2313	AMELLAL	AC	AG	CT	CT	GG	AA	LL	LL	CC	CC	CC	AG	AA	TT	TT	GG	AA	LL	TT	CC	CC	CC	TT
A09	Ain el Couma	GEN_SNP_2413	AIN EL BOUMA	CC	AG	CC	CC	ND	AA	GG	LL	CC	CC	CC	AG	AA	TT	TT	GG	AA	LL	TT	CC	CC	CC	TT
A10	Ain el Kelb	GEN_SNP_2088	BEBBA	AC	AA	TT	CT	AA	AA	GG	LL	CC	CC	CC	AG	AA	TT	TT	GG	AA	LL	TT	CC	CC	CC	TT
A11	Amellal	GEN_SNP_2313	AMELLAL	AC	AG	CT	CT	GG	AA	GG	LL	CC	CC	CC	AG	AA	TT	TT	GG	AA	LL	TT	CC	CC	CC	TT
A12	Amokrane	GEN_SNP_2308	—	AA	AG	CT	CT	GG	AA	GG	LL	CC	CC	CC	AG	AA	TT	TT	GG	AA	LL	TT	CC	CC	CC	TT
A13	Aneb el Cadi	GEN_SNP_2314	—	AA	AG	CT	CT	GG	AA	GG	LL	CC	CC	CC	AG	AA	TT	TT	GG	AA	LL	TT	CC	CC	CC	TT
A14	Baladi	GEN_SNP_0021	—	AA	AG	CT	CT	GG	AA	GG	LL	CC	CC	CC	AG	AA	TT	TT	GG	AA	LL	TT	CC	CC	CC	TT
A15	Bezoulet el Khadem	GEN_SNP_2312	BEZOUL EL KHADEM D'ALGERIE	AA	AG	CC	CT	GG	CC	GG	AT	CT	CC	CC	AG	AA	TT	TT	GG	AA	LL	TT	CC	CC	CC	TT
A16	Boghni	GEN_SNP_2414	—	AC	GG	CC	CT	AA	AA	GT	TT	TT	CC	CC	AA	AA	TT	TT	GG	AA	TT	TT	CC	CC	CC	TT
A17	Bouaber des Aures	GEN_SNP_2309	KABYLE ALDEBERT	CC	AG	CT	CT	GG	AC	GG	LL	CC	CC	CC	AG	AA	TT	TT	GG	AA	TT	TT	CC	CC	CC	TT
A18	Bouni	GEN_SNP_2114	DOMINGA	CC	AG	CT	CT	AA	CC	GG	TT	CT	CC	CC	AA	AA	TT	TT	GG	AA	TT	TT	CC	CC	CC	TT
A19	Cherchelli	GEN_SNP_2220	—	CC	AG	CT	CT	AA	CC	GG	TT	CT	CC	CC	AA	AA	TT	TT	GG	AA	TT	TT	CC	CC	CC	TT
A20	Farana Blanc	GEN_SNP_2220	PLANTA FINA	CC	AG	CT	CT	AA	CC	GG	TT	CT	CC	CC	AA	AA	TT	TT	GG	AA	TT	TT	CC	CC	CC	TT
A21	Farana de Mascara	GEN_SNP_2220	PLANTA FINA	CC	AG	CT	CT	AA	CC	GG	TT	CT	CC	CC	AA	AA	TT	TT	GG	AA	TT	TT	CC	CC	CC	TT
A22	Farana Noir	GEN_SNP_2311	TAFERIEIT	CC	AG	CT	CT	ND	AA	GG	LL	CC	CC	CC	AG	AA	TT	TT	GG	AA	TT	TT	CC	CC	CC	TT
A23	Ghanez	GEN_SNP_0608	OHANES	AC	AG	CT	ND	AA	AA	GG	LL	CT	CC	CC	AG	AA	TT	TT	GG	AA	TT	TT	CC	CC	CC	TT
A24	Kabyte Aldebert	GEN_SNP_2309	KABYLE ALDEBERT	CC	AG	CT	CT	GG	AC	GG	TT	CT	CC	CC	AG	AA	TT	TT	GG	AA	TT	TT	CC	CC	CC	TT
A25	Lekzine	GEN_SNP_2310	—	CC	AG	CT	CT	GG	AC	GG	TT	CT	CC	CC	AG	AA	TT	TT	GG	AA	TT	TT	CC	CC	CC	TT
A26	Lakhdari	GEN_SNP_2315	—	CC	AG	CT	CT	GG	AA	GG	TT	CT	CC	CC	AG	AA	TT	TT	GG	AA	TT	TT	CC	CC	CC	TT
A27	Louali	GEN_SNP_2308	LOUALI	AC	GG	CC	CT	GG	AA	GG	LL	CT	CC	CC	AG	AA	TT	TT	GG	AA	TT	TT	CC	CC	CC	TT
A28	Muscet de Berkain	GEN_SNP_0799	MUSCAT FLEUR D'ORANGER	AC	GG	CC	TT	AA	AC	GT	AT	CT	CC	CC	GG	AA	AA	TT	AG	AG	CT	CT	CC	CC	CC	TT
A29	Muscet de Fandouk	GEN_SNP_2153	MUSCAT OF ALEXANDRIA	AC	GG	CC	CT	AG	AC	GT	AT	CT	CC	CC	AG	AA	AA	TT	AG	AG	CT	CT	CC	CC	CC	TT
A30	Muscet el Adda	GEN_SNP_2059	MOSCATO D'ADDA	AA	ND	TT	TT	AA	CC	GG	TT	CT	CC	CC	AG	AA	TT	TT	GG	AA	TT	TT	CC	CC	CC	TT
A31	Shaa Tolba	GEN_SNP_2220	—	ND	ND	ND	ND	AA	CC	GT	TT	CT	CC	CC	AG	AA	TT	TT	GG	AA	TT	TT	CC	CC	CC	TT
A32	Sultanine Fandouk	GEN_SNP_2126	SULTANINA	AC	AG	CT	CT	ND	CC	GG	TT	CT	CC	CC	AG	AA	AA	TT	AG	AA	TT	TT	CC	CC	CC	TT
A33	Tadelth	GEN_SNP_0608	—	ND	AG	CT	ND	ND	AA	GG	TT	ND	CC	CC	AG	AA	TT	TT	GG	AA	TT	TT	CC	CC	CC	TT
A34	Tizi Ouintine	GEN_SNP_2313	—	AC	AG	CT	CT	GG	AA	TT	CT	CC	CC	CC	AG	AA	TT	TT	GG	AA	TT	TT	CC	CC	CC	TT
M01	Abbou	GEN_SNP_2307	AGUEYER	CC	AG	CC	CT	AG	CC	GG	TT	CC	CC	CC	AG	AA	TT	TT	GG	AA	TT	TT	CC	CC	CC	TT
M02	Abouthou2	GEN_SNP_2317	ABOUHOU	CC	AG	CC	CT	AG	CC	GG	TT	CC	CC	CC	AG	AA	TT	TT	GG	AA	TT	TT	CC	CC	CC	TT
M03	Agyar1	GEN_SNP_2307	AGUEYER	CC	AG	CC	CT	AG	CC	GG	TT	CC	CC	CC	AG	AA	TT	TT	GG	AA	TT	TT	CC	CC	CC	TT
M04	Arbia	GEN_SNP_1158	PLANTA MULA	CC	AG	CC	TT	AG	AC	GT	AT	CT	CC	CC	AG	AA	AA	AA	AA	AA	AA	CT	CT	CC	CC	TT
M06	Bezoulet Anouda	GEN_SNP_0527	BEZOUL EL KHADEM	CC	AG	CC	CT	AG	AC	GG	TT	CT	CC	CC	AG	AA	TT	TT	GG	AA	TT	TT	CC	CC	CC	TT
M07	Bouchouka Blanc	GEN_SNP_2316	BOU CHOUKKA	AC	AG	CC	CC	AA	AC	GG	TT	CT	CC	CC	AG	AA	TT	TT	GG	AA	TT	TT	CC	CC	CC	TT
M08	Boujida	GEN_SNP_0966	CAÑOCAZO	AC	GG	CT	TT	AA	AC	GT	TT	CT	CC	CC	AG	AA	TT	TT	GG	AA	TT	TT	CC	CC	CC	TT
M10	Bouqseb	GEN_SNP_2306	BOU QSOB	AA	AA	CC	CT	AG	AA	GG	TT	CT	CC	CC	AG	AA	TT	TT	GG	AA	TT	TT	CC	CC	CC	TT
M11	Bouzouga	GEN_SNP_2421	BOUZOUGA	AC	AG	CC	TT	AA	AC	GG	TT	CT	CC	CC	AG	AA	TT	TT	GG	AA	TT	TT	CC	CC	CC	TT
M12	Carignan	GEN_SNP_0540	CARIGNAN	AC	AG	CC	CT	GG	CC	GG	TT	CT	CC	CC	AG	AA	TT	TT	GG	AA	TT	TT	CC	CC	CC	TT
M13	Dijnani	GEN_SNP_1089	CAVETANA BLANCA	CC	AA	CT	CT	AA	AC	GG	TT	CC	CC	CC	AG	AA	TT	TT	GG	AA	TT	TT	CC	CC	CC	TT
M14	El Biod	GEN_SNP_0885	AIREN	AC	GG	CT	CT	AA	AA	GG	TT	CC	CC	CC	AG	AA	TT	TT	GG	AA	TT	TT	CC	CC	CC	TT
M16	El Katim	GEN_SNP_0885	AIREN	AC	GG	CT	CT	AA	AA	GG	TT	CC	CC	CC	AG	AA	TT	TT	GG	AA	TT	TT	CC	CC	CC	TT
M19	Ferama	GEN_SNP_1475	—	CC	GG	CT	TT	GG	CC	GG	TT	CT	CC	CC	AG	AA	TT	TT	GG	AA	TT	TT	CC	CC	CC	TT
M21	Laaderi	GEN_SNP_2307	—	CC	GG	TT	TT	AG	CC	GG	TT	CT	CC	CC	AG	AA	TT	TT	GG	AA	TT	TT	CC	CC	CC	TT
M22	Maccabeo	GEN_SNP_1504	VILURA	CC	GG	TT	TT	AG	AC	GT	TT	CT	CC	CC	AG	AA	AA	AA	AA	AA	AA	CT	CT	CC	CC	TT
M23	Muscet Doukala	GEN_SNP_2153	MUSCAT OF ALEXANDRIA	AC	GG	CC	CT	AA	AC	GT	TT	CT	CC	CC	AG	AA	AA	AA	AA	AA	AA	CT	CT	CC	CC	TT
M24	Muscet Setrou	GEN_SNP_2088	BEBBA	AC	AA	TT	CT	AA	AA	GG	TT	CC	CC	CC	AG	AA	AA	AA	AA	AA	AA	CT	CT	CC	CC	TT
M25	Oulgid	GEN_SNP_2317	—	CC	AG	CC	CT	AG	CC	GG	TT	CC	CC	CC	AG	AA	TT	TT	GG	AA	TT	TT	CC	CC	CC	TT
M32	Shaa Bnet	GEN_SNP_0479	OLIVETTE BARTHELET	CC	AA	CT	TT	AA	AA	GG	TT	CC	CC	CC	AG	AA	TT	TT	GG	AA	TT	TT	CC	CC	CC	TT
M33	Sidi Taybi	GEN_SNP_0527	BEZOUL EL KHADEM	AC	AG	CC	CT	AA	AC	GG	TT	CT	CC	CC	AG	AA	TT	TT	GG	AA	TT	TT	CC	CC	CC	TT
M34	Tagleyate2	GEN_SNP_2316	—	AC	AG	CC	CC	AA	AC	GG	TT	CT	CC	CC	AG	AA	TT	TT	GG	AA	TT	TT	CC	CC	CC	TT
M35	Tanakarte2	GEN_SNP_2415	—	AC	AG	CC	TT	AG	CC	GG	AT	CC	CC	CC	AG	AA	TT	TT	GG	AA	TT	TT	CC	CC	CC	TT
M36	Tintimne2	GEN_SNP_2424	—	AC	GG	CC	CT	AG	AC	GG	TT	CT	CC	CC	AG	AA	AA	AA	AA	AA	AA	TT	TT	CC	CC	TT

The accessions Ghanez (A23) and Tadelith (A33) had identical SNP profiles (SNP genotype number: GEN_SNP_0608; proposed name: Ohanes), and their mean leaves were similar (Figure 2) – even clustering close to one another in PCA (Figure 4). The fact that they have different coloured grapes, however, is enough to consider them different cultivars. The reporting of contradictory results in previous studies (Supporting Information Table S1) suggests that Tadelith might be a colour sport of Ohanes, or that sampling errors were made (either in previous studies or in the present work). These results underscore the need for experts to undertake sampling.

The techniques used in the present work have certain advantages. For example, the construction of mean leaves allows the variability in morphology between leaves of the same accession to be examined. Experience tells us that when sampling is properly performed, the leaves of plants from the same cultivar are similar (with the exception of the Spanish cultivar Godello), and that when mean leaves are being constructed, the coefficients of variation for the variables measured are usually low. The technique also allows for statistical comparisons to be made. In the present study, PCA was used, which permits the simultaneous comparison of many variables. The three-dimensional display of the results obtained (i.e. for the first three axes) shows the distribution of the different accessions in terms of leaf lateral sinus, the angle formed between the main veins and the shape of the leaf (more orbicular or more cuneiform). Figure 4 shows the accessions with similar leaves in these respects to be distributed close to one another, while others with different characteristics in one or more respects to lie more distant from the corresponding axes. The construction of mean leaves also allows comparisons to be made between leaves in terms of single variables.

In grapevine molecular analyses, SNP markers are not widely used while SSRs are commonly employed. In the present work, the genotyping results of SSR analyses and SNP analyses were compared (Supporting Information Table S1). The main difficulty in SSR data comparison is the different allele binning used by different authors, which in many cases precludes large-scale comparisons. Reaching definite conclusions about the identity of some alleles is therefore impossible. This is not the case when comparing SNP data because of the bi-allelic nature of SNPs. This makes comparisons much easier.

Conclusion

In the present work, the mean leaves of the main Moroccan and Algerian grapevine cultivars were constructed. SNP analysis and comparison of published microsatellite and ampelographic data highlighted the existence of new synonyms and homonyms. The existence of some errors of identification, misinterpretations and collection or sampling errors is suggested. Together, the present results contribute towards a better understanding of grapevine diversity in the Maghreb.

Acknowledgements

Lalla Hasna Zinelabidine was supported by a fellowship from AECI and a short-term scientific mission from COST FA1003. Iván González and Elena Zubiaurre are thanked for providing technical assistance and Adrian Burton for helping with the English version of the manuscript.

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Manuscript received: 20 May 2013

Revised manuscript received: 19 September 2013

Accepted: 3 October 2013

Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site: <http://onlinelibrary.wiley.com/doi/10.1111/ajgw.12079/abstract>

Table S1. Comprehensive summary of all the microsatellite and ampelographic data published for North African grape cultivars, compared with the ampelographic and SNP results obtained in the present work.