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Genetic identification, origin and sanitary status of grapevine cultivars (*Vitis vinifera* L.) grown in Babar, Algeria

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Summary

This research focused on present grapevine biodiversity of neglected cultivars grown in 'Babar' region, Northeastern Algeria. The obtained results demonstrate the complex, rich, and even surprising inheritance of grapevine biodiversity in such a small region, with currently residual viticulture practiced only for direct consumption. Babar is one of the oldest inhabited areas in Algeria and part of the Atlas Mountains, considered very favorable for wild and cultivated vine growing since protohistoric times. Thirty-seven vines from the traditional growing area were analyzed using nuclear microsatellite (SSR) markers for cultivar identification and RT-qPCR analysis for virus detection and sanitary status evaluation. As a result, thirteen different genotypes were found, most of them showing a very good sanitary status, then constituting a valuable biological source for clonal selection. A close relatedness was evidenced with some Mediterranean varieties, resulting from previous exchanges of grapevine cultivars in the past. Furthermore, the present study highlighted the existence of three new genotypes, highly probably autochthonous of Babar region, with proposed names 'Babari', 'Babar-Algeria', and 'Amesski-Babar'. They could represent unique Algerian varieties, probably preserved over time. The conservation of these endangered genotypes is highly recommended.

K e y w o r d s : Algerian grapevine varieties; nuclear microsatellites; synonyms; virus occurrence; Pascale di Cagliari.

Introduction

Vine-growing in Algeria is done since protohistoric times by the indigenous population of North Africa, the "Numidians" or "Berbers"; through the first millennium BC, Phoenicians traded huge quantities of wine and transplanted grapevines across the Mediterranean sea; then Romans used Algeria as a granary for their empire (Meloni and Swinnen 2014); later, Muslims introduced table grapes from the Middle East in North Africa (Bouquet *et al.* 2008). The French conquest of Algeria took place between 1830 and 1847; during the French colonization, viticulture in Algeria was oriented exclusively towards European wine production (Isnard 1969, Levadoux *et al.* 1971), so that, in 1960, Algeria was the 4th largest wine producer and the world's largest exporter, representing a quarter of the volume of international transactions. This situation changed since independence, in 1962, when Algeria lost its market of wine (Meloni and Swinnen 2014). In 1996 FAO declared that destruction of forests in Africa is the main cause of genetic erosion, and in 2005 the Algerian Ministry of Environment confirmed that the loss of grapevine biodiversity was 95 % (Mediouni 1997, Vié *et al.* 2009).

This work contributes to the discovery and conservation of native Algerian grape varieties grown in unchecked places like Babar. Babar belonged to the Numidian kingdom, meeting point between the African and Greco-Eastern influence (Camps 1979). Babar is located in the North-East of Algeria (Fig. 1), stays at the foothills of the Aurès mountains, has a semi-arid climate (Drouai 2018), unique ecosystem features, and plant species unique in Algeria. Aurès has long remained a closed country, difficult to access to foreign forces and therefore relatively preserved throughout the ages. In the mountains of Babar, that are part of Khenchela province, there is no interest in viticulture. In fact, only 11 ha of vineyards exist (Directorate of Agriculture Khenchela, 2018). These vineyards have been inherited by local families over time; nowadays, remnant plants remained near to single houses and are cultivated with primitive methods; these vines are not grafted, their presumed age is between 10 and 30 years and they are used only as table grapes.

The aim of our work was to explore the grapevine varietal diversity still present in the Babar area, to discover their identity and to evaluate their sanitary status, using microsatellite (SSR) markers and virus detection through RT-qPCR analysis.

Material and Methods

P l a n t m a t e r i a l : Thirty-seven vines were sampled, the list is reported in Tab. 1 and the places of sampling in Fig. 1; only 22 of them had a variety name, highlighted in bold in the same table. Some of these names refer to the colour or dimension or shape or use of grapes. As some people inherited the vines without knowing their names, we tried to choose the most commonly used name by the indigenous people or to give the name of the area or the name of the owner to anonymous samples.

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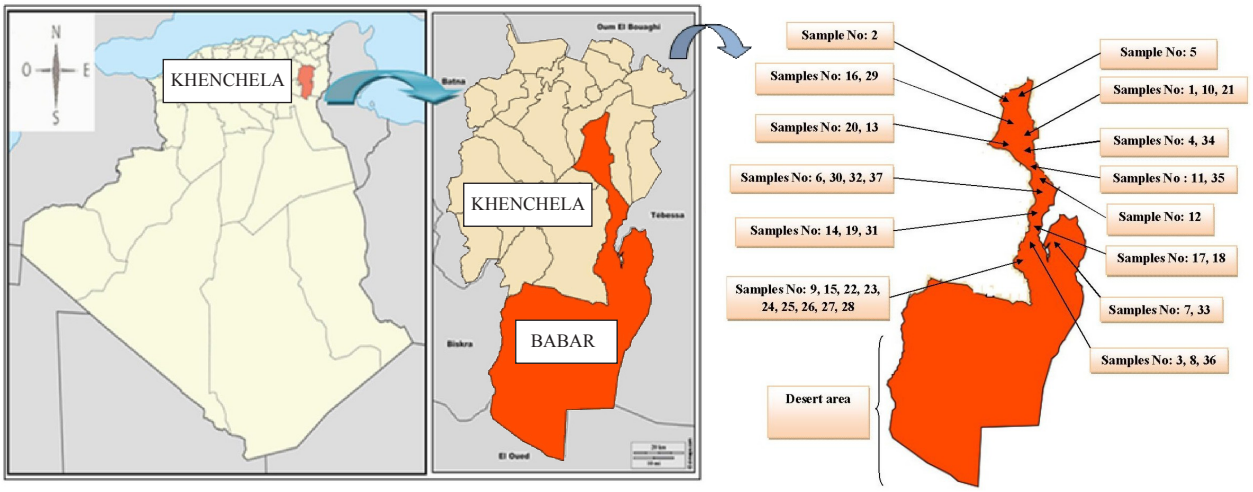

Fig. 1: Area of sampling in Babar, Algeria and samples location map.

Genotyping with SSR markers and statistics: Genomic DNA was extracted from 20 mg leaves or cambium tissue from wood (Qiagen DNeasy Plant mini-kit, Qiagen, Hilden, Germany), following the manufacturer's protocol. Twelve SSR markers were analysed, the nine proposed as common grape markers for international use within the framework of the Grapegen06 European project (VVS2, VVMD5, VVMD7, VVMD25, VVMD27, VVMD28, VVMD32, VrZAG62, VrZAG79) (MAUL *et al.* 2012), plus ISV2 (VMC6e1), ISV4 (VMC6g1) and VMCNG4b9 (MIGLIARO *et al.* 2013). The SSR analyses were performed following the protocol detailed in MIGLIARO *et al.* (2013), using fluorescent primers and an ABI3130xl genetic analyser (Applied Biosystems, Foster City, CA). SSR allele calling was performed using GeneMapper® software version 3.0, with a bin set produced with reference varieties. Identifications were performed by comparing the obtained SSR profiles with the CREA Viticulture and Enology molecular database, literature information and the *Vitis* International Variety Catalogue (*V*IVC, http://www.vivc.de).

Statistics on SSR data were computed using the following software: Cervus vs 3.0 (KALINOWSKI *et al.* 2007) and GenAlEx 6.51b2 - released June 2018 (PEAKALL and SMOUSE 2006 and 2012); GenAlEx was used also to look for possible parent-offspring relationships and to evaluate population structure using Principal Coordinates Analysis (PCoA), based on standardized covariance of genetic distances calculated for codominant markers.

Sanitary tests: Veins from leaves or cortical scrapings from canes were cut and used for the sanitary molecular analyses. Fresh samples were homogenized in liquid nitrogen, and total RNA was extracted using the RNeasy Plant Mini Kit (Qiagen) with a protocol described by MACKENZIE *et al.* (1997). One µg of RNA was treated with 1 unit of RNase-free DNase I (MBI Fermentas) for 45 min at 37 °C, and the reaction was stopped with 1 µL of 25 mM EDTA. After denaturation at 95 °C for 5 min, RNA was reverse transcribed at 42 °C for 50 min with Moloney Murine Leukemia Virus reverse transcriptase (Invitrogen) and DNA random primers (Roche Diagnostic).

Detection of the grapevine viruses *Arabis mosaic virus* (ArMV), *Grapevine fanleaf virus* (GFLV), *Grapevine leafroll-associated virus 1, 2* and *3* (GLRaV-1, 2 and 3), *Grapevine virus A* and *B* (GVA and GVB) and *Grapevine Rupestris stem pitting-associated virus* (GRSPaV) was performed by real-time PCR with primer pairs listed in Tab. 1S. All PCR assays were carried out on a Bio-Rad thermal cycler (model CFX96) in 96-well plates using the 2X Platinum SYBR Green qPCR Supermix-UDG (Invitrogen). PCR reactions were performed at least in duplicate, in a total volume of 10 µL, including 0.3 µM of each primer and 1 µL of cDNA.

Results and Discussion

Genotyping and identifications: The SSR analysis of the 37 vines with 12 SSR markers produced 13 molecular profiles; ten genotypes were identified whereas three represent novelties, being different from all those present until now in the available molecular databases and in the literature (Tab. 1 and Tab. 2).

About the identified genotypes, the most frequent refers to 'Dabouki' and encompasses eight samples; three of them have names based on berry dimension and shape (Tab. 1). According to the *V*IVC, Armenia should be the 'Dabouki's' country of origin (BASHEER-SALIMIA *et al.* 2014); GALET (2000) refers that this variety is cultivated in the Near Orient, Palestine, Lebanon, Syria, Jordan, and our study shows that it was spread also in Algeria. 'Rassegui blanc' is the second more common variety among the identified plants and was represented by five vines; it is considered a Tunisian variety (GALET 2000). 'Danugue' and 'Pascale di Cagliari' were represented by four plants each. 'Danugue' is considered a French variety, well spread in Algeria (RAIMONDI *et al.* 2015). 'Pascale di Cagliari' is an Italian cultivar, typical of Sardinia, where it is used as table grape and for wine, too. To our knowledge, and according to LOVICU (2017), this is the first report on the presence of this variety outside Italy. LAIADI *et al.* (2009) found that the presumed Algerian cultivar 'Lakhdari' corresponded to the well-known Italian 'Sangiovese', meaning that the wideness of grapevine exchanges among Algeria and other Countries included also Italy. 'Chikki', represented by two vines, showed to be synonym with 'Azanjari' and 'Agogal'; this genotype is

Table 1

Sample name, berry colour, sample name meaning, varietal correspondences, and country of origin of the 37 sampled vines. Berry colour codes: B = black, G/R = red/green, G/p = green/pink, G = green, R = red, P = purple, W = white (green/yellow)

Sample name (and number)	Berry colour	Sample name meaning	Correspondance by SSR	Country of origin
Azizao (10)	G	green, in Amazigh languages	Afus Ali	Lebanon
Arabth (14)	B	arabic, in Amazigh languages	Alphonse Lavallee	France
Nabil Athmani (8)	G	name of the owner	Chanronge (Lacombe 2013, *V*IVC 40350)	
Azanjari (13)	P	violet, in Amazigh languages	Chikki (Riahi 2010, Lacombe 2013)	Algeria
Agogalth (15)	B	black, in Amazigh languages		
Aneb Takhatelt (7)	G/p	name of a large area in Babar	Clairette	France
Datte (23)	W	palm date	Dabouki	Armenia
Azogar (24)		palm date, in Amazigh language		
Anonymous (25)				
Anonymous (26)				
Amellal (27)		white, in Amazigh language		
Afagoss (28)		watermelon, in Algerian dialect		
Lanab agahlan (30)		good taste, in Amazigh languages		
Sbaa laroussa (33)		the fingers of the bride (in Algerian dialect)		
Anabi (12)	R	related to Anabi city, eastern region of Algeria called Annaba	Danugue (Raimondi 2015, *V*IVC 3425)	France
Mokrani noir (16)	B	big black, in Amazigh languages		
Amanzo (17)	B	big, in Amazigh languages		
Anonymous (36)	G/R			
Bousada (1)	P	name of the owner	Pascale di Cagliari	Italy
Aberkan (11)		black		
Anonymous (20)				
Anonymous (21)				
Khoudja (4)	G	name of the owner	Rassegui blanc	Tunisia
Anonymous (29)				
Anonymous (32)				
Amokran (34)		great, in Amazigh languages		
Talyani (35)		Italian, in Amazigh languages		
Azbib (18)	P	used for zebib (raisins); dried grapes, in Amazigh languages	Taferielt (*V*IVC 12196)	Morocco
Anonymous (19)	B			
Rahali mohamed cherif (3)	G	name of the owner	none, proposed name: Babar-Algeria	Algeria
Amezian (5)		small, in Amazigh languages		
Aneb Babar (6)		the grapes of Babar, in Amazigh languages		
Azorith (9)		grape tree, in Amazigh languages		
Anonymous (37)				
Lanab amesski (31)	W	aromatic grape, in Amazigh languages	none, possible self of Dabouki; proposed name: Amesski-Babar	Algeria
Babari (2)	P	the grapes of Babar	none, proposed name: Babari	Algeria
Azogagh (22)	R	red, in Amazigh languages		

considered original of Algeria. Another two plants showed the SSR profile of 'Taferielt', a Moroccan variety; however, under the synonym 'Farana noir', this variety is considered as grown mostly in Algeria, and is therefore classified as an Algerian variety (Galet 2000, Zinelabidine *et al.* 2014). Finally, single vines showing the SSR profile of 'Afus Ali', 'Alphonse Lavallee', 'Chanronge', and 'Clairette' were found. Almost all these plants clearly represent a French heritage, even if the original variety name was lost. When the French landed in Algeria, in 1830, they were surprised by the amazing force of the local vines on alluvial soils such as the slopes of the Aurés and by the importance given by the natives to viticulture (Leroux 1894, Lequement 1980). Larnaude (1948) pointed out that the first vine plantations in the French colony of Algeria dated only from the end of a long period for half a century from 1830 on. 'Clairette', a renowned variety in southern France, was also the most important white variety during the French colonization of

Algeria, and it does not appear to be grown in Europe outside France (Kerridge and Gackle 2005). 'Clairette' is mentioned among the Algerian fruit trees by Laplagne-Barris (1848).

About the three novelties (Tab. 1), one genotype was represented by as many as five vines dispersed in different fields (Fig. 1); for it we propose the name 'Babar-Algeria'; our data show that it could be parent-offspring related with 'Chikki', because they share at least one allele per locus for all 12 SSR markers. We hypothesize that sample 31 could be a selfing of 'Dabouki', because its molecular profile shows segregation of 'Dabouki's' alleles and higher homozygosity than 'Dabouki'; we propose 'Amesski-Babar' as "prime name" for this vine, while being aware that it is not possible to ensure that it is a real new variety, spread by vegetative multiplication, but just a selfed progeny plant that became cultivated. For the last genotype, shared by two vines found in distant places (Fig. 1), we propose the name 'Babari'; we do not have any hypothesis on the origin of these plants, however, 'Babari' shows to be very close to 'Danugue', sharing at least one allele per locus, except for VVMD28 marker. These three novelties could represent unique Algerian varieties and highly probably grapevines specific of the Babar area.

Some genotypes corresponding to Mediterranean or international varieties showed to have been renamed, suggesting that the local names represent new synonyms.

Genetic diversity: About the genetic diversity of our genotypes, 84 alleles were found in total, with a mean of 7 alleles per locus; the mean number of effective alleles (Ne) was 4.727; mean observed and expected heterozygosis (Ho and He) were very similar being 0.788 and 0.809, respectively; the mean polymorphic information content (PIC) was 0.747 and the cumulative probability of identity (PI) $4.13 \cdot 10E^{-14}$. These data reflect the high genetic diversity of the grapevines found in Babar. PCoA showed 21.72 % diversity along axis 1, and additional 14.80 % along axis 2 (Fig. 2). Interestingly, the Armenian 'Dabouki' and its probable selfing progeny, 'Amesski-Babar', are located at the opposite of the Moroccan 'Taferielt'; 'Chanronge' is very close to 'Clairette', suggesting a French origin.

Sanitary status: The sanitary status of the plants studied was generally very good, probably since the plants are self-rooted (Tab. S2). Indeed, grafting is one of the major routes of virus spreading in grapevine. A limited survey

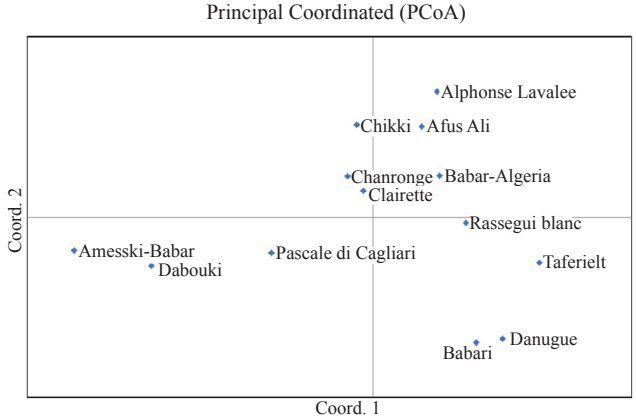

Fig. 2: PCoA graph. Coordinate 1 explains 21.72 % of variation, coordinate 2 14.80 %.

Table 2

Thirteen SSR profiles obtained with 12 microsatellite markers

Variety	VVS2		VVMD5		VVMD7		VVMD25		VVMD27		VVMD28		VVMD32		VrZAG62		VrZAG79		ISV2		ISV4		VMCng4b9	
Afus Ali	133	135	226	232	239	249	253	259	185	185	237	261	259	273	185	187	242	250	143	167	177	191	150	158
Alphonse Lavallee	133	135	226	238	249	255	243	259	185	185	247	247	253	273	185	203	238	250	141	165	187	193	158	176
Chanronge	133	139	240	240	239	239	243	243	183	189	261	261	257	273	185	187	244	246	137	165	169	187	150	164
Chikki	133	151	226	236	233	253	245	253	185	185	261	263	253	257	199	203	236	250	143	157	177	187	150	170
Clairette	137	151	226	232	239	249	245	267	179	191	231	261	257	263	185	203	250	250	141	165	177	191	158	158
Dabouki	135	151	234	236	247	249	245	249	179	183	261	261	251	273	187	203	246	246	169	175	177	187	158	160
Danugue	137	145	228	234	233	239	245	259	194	194	239	247	263	273	187	203	256	256	137	143	177	191	138	166
Pascale di Cagliari	145	151	226	234	243	249	245	267	179	181	261	261	257	273	187	203	246	260	165	165	177	177	164	166
Rassegui blanc	143	149	226	240	239	239	253	259	179	194	247	251	251	273	185	187	250	256	141	141	169	177	160	172
Amesski-Babar	135	151	236	236	247	247	249	249	179	183	261	261	251	273	203	203	246	246	175	175	177	187	158	160
Taferielt	133	135	238	240	249	249	243	245	194	194	237	263	253	273	193	203	256	256	141	165	191	191	176	176
Babari	145	151	234	240	239	249	245	253	194	194	251	261	253	263	187	201	256	256	143	169	177	191	138	158
Babar-Algeria	133	135	226	240	233	239	243	245	185	194	237	261	257	259	187	203	236	256	141	157	177	187	150	176

carried out in the past had shown a much higher prevalence of all viruses on the only 10 samples analysed (Digiaro *et al.* 1999). In the present work, twelve out of 37 plants (32.4 %) were free of all searched viruses, among them, interestingly, all vines identified as 'Rassegui blanc' (5 out of 5 plants), although collected in different areas. Indeed, some viruses, *i.e.* the *Nepoviruses* ArMv and GFLV, the *Ampelovirus* GLRaV-1 and the *Vitivirus* GVB, were not present in any of the analysed plants. The absence of *Nepoviruses* is interesting, as nematode vectors of these viruses, such as *Xiphinema* spp., are known to occur in these regions although at low densities (Louadi and Robaux 1992). GLRaV-3, associated with leafroll complex, was identified in 13 % of the collected vines, while GVA, one of the viruses associated to rugose wood complex in the form of Kober stem grooving, was detected in 24 % of total samples. Both viruses, though belonging to different genera, are known to be transmitted by many species of mealybugs. Given the reported occurrence of mealybugs in some of the investigated areas (Louadi 1992 and Robaux 1992), the coinfection of a few samples with the two viruses, and the geographic pattern of the infection, it is possible to postulate a field vector transmission for most of the infected plants. Some surveys on autochthonous varieties in nearby Tunisia showed the presence of GVA in 35 % of the analysed plants (out of 141 in total; Selmi *et al.* 2018), quite similar to the prevalence found in the present work, while GLRaV-3 was much more frequent (Mahfoudhi *et al.* 2008). The occurrence of GLRaV-2 in 16 % of the plants is intriguing. Indeed, no vector of the virus has been found so far in the world, and some evidence suggested an American origin of the virus (Angelini *et al.* 2017). Thus, it was unexpected to find it out in self-rooted grapevines, especially those of local or African origin, such as 'Taferielt', and two vines of 'Babar-Algeria' out of 5. It could be stimulating to investigate most deeply its occurrence in self-rooted autochthonous germplasm of Algeria and other nearby countries, with the aim of verifying if clustered distribution of the virus exists, which could suggest the existence of local possible vectors. Finally, the most widespread virus was GRSPaV, associated with rugose wood disease complex in the form of Rupestris Stem Pitting, recorded in 62 % of the samples. In details, the virus was randomly distributed among the different vines, and especially in 'Dabouki', where it was ascertained in all the 8 analysed samples, regardless the locality of collection; this could imply a common mother plant of all tested grapevines belonging to this variety. A prevalence of 35 % infected vines was found in autochthonous varieties in Tunisia (Selmi *et al.* 2017), while in Egypt the prevalence is much lower (16.6 %, Fattouh *et al.* 2014). However, this virus reaches infection level of 100 % in most of the countries, supposed to be mostly linked to the high grafting volume following the phylloxera outbreak in Europe and the fact that no vector of GRSPaV is known (Meng *et al.* 2006).

Conclusions

The set of twelve microsatellite markers employed in this study allowed the detection of thirteen genotypes among 37 vines from Babar region in Northeastern Algeria; ten of them revealed to be new synonyms of varieties grown around Mediterranean Countries, like 'Dabouki', 'Rassegui blanc', 'Danugue' ,'Taferielt', 'Afus Ali', 'Alphonse Lavallee', 'Chanronge', 'Clairette' and 'Chikki'. The Italian cultivar 'Pascale di Cagliari' was firstly detected outside Italy.

Furthermore, three novelties could represent unique Algerian varieties specific of Babar area: 'Babar-Algeria', which could be parent-offspring related with 'Chikki', Amesski-Babar a probable selfing of 'Dabouki', and 'Babari', looking very close to 'Danugue'. The statistical analysis reflects the high genetic diversity of Babar's grapevines.

Furthermore, the viral tests showed the very good sanitary status of Babar's grapevine in general, probably because the plants are self-rooted.

The present results contribute towards a better understanding of the genetic structure and the sanitary status of Babar's grapevines as a neglected natural resource that needs to be protected and maintained in the near future. These vines constitute a valuable biological source for clonal selection, future sustainable breeding and improvement of grapevine. Our results demonstrate the complex, rich, and even surprising inheritance of grapevine biodiversity in such a small region, with currently residual viticulture. Our findings show that Babar region was a big crossroads of exchanges between peoples, which resulted in the grapevine varietal diversity residual richness.

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