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Molecular Clarifications of Grapevine Identities in Algerian Germplasm Collections using Microsatellite Markers

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Abstract

A total of twenty-three accessions of autochthonous grapevine cultivars from the collection of 'Tegnennif' Mascara in Northwestern Algeria were genotyped using nine nuclear microsatellite loci (SSR) to characterize their genetic diversity, and compare them with previous reports on cultivars at the germplasm collection of Skikda in Northeastern Algeria. The total number of alleles was sixty-three, the mean number of alleles per locus was 7.9, while the expected and observed heterozygosity values were 0.790 and 0.861 respectively. The most informative loci was VVMD5 with an effective number of alleles (Ne=6.62), while the low cumulative PI value was estimated to be 4.52×10^{-10} reflecting the high discriminative power of the chosen markers for the investigated set of grapevines. This study identifies three cases of synonyms within the collection, ten cases of real duplication, and four cases of homonymous grape cultivars. These involved nine different genotypes, six cases of pairs with the same name in both collections, while the remaining two cases are slightly different. In any case, the genetic relationship, based on the shared alleles' distance between the cultivars in both collections, showed very high levels of similarity between homonymous accessions in both collections which indicates a significant number of shared alleles in the studied loci. The results reported here are significant towards a better characterization of grapevine accessions and can help in future germplasm management and the breeding efforts in Algeria.

Keywords: Germplasm collection, SSR, Characterization, Genetic diversity, Homonyms, Synonyms.

1. Introduction

Among the processes necessary for the conservation of genetic resources is the establishment of genetic collections to build the first barrier against genetic erosion. Therefore, there is a big need to identity and clarify the real number, evaluate genetic diversity, and study relationships among accessions located in the smallest possible area. Germplasm collections of grapevines were created throughout the world with the aim to conserve the existing autochthonous diversity (El Oualkadi *et al*., 2009). The native genotypes are very well-adapted to local environmental conditions, and probably contain genes of different interests that could be considered as important resources to plant breeders and geneticists (Santana *et al*., 2008). One of the important troubles in grapevine cultivar identifications is the abundance of synonyms and homonyms at international, national, and regional levels, complicated by errors in the material propagation, altogether leading to a high number of mistakes and repetitions in germplasm banks (Sefc *et al*., 2000; Diaz-losada *et al*., 2013). During the last few years, accessions from germplasm collection of Skikda have already been identified and characterized using molecular analyses with nuclear and chloroplastic microsatellites (Laiadi *et al*., 2009) with ampelometric and SNP analyses (Zinelabidine *et al*., 2014). On the contrary, the accessions from the germplasm collection of Mascara have only been characterized through phyllometric measurements (Laiadi *et al*., 2013). However, the common morphological identification has several limitations, and does not necessarily provide enough evidence for the correct identification of the accessions (Nebish *et al*., 2017). Molecular markers such as SSRs (simple sequence repeats) or microsatellites have been demonstrated to be a powerful tool for cultivar identification, and are extensively used for the characterization of grapevine collections (Ibañez *et al*., 2003; Martin *et al*., 2003; This *et al*., 2004; Costantini *et al*., 2005; Santana *et al.*, 2008; Laiadi *et al.* 2009; Zinelabidine *et al*., 2014; Maletić *et al*., 2015), and for the verification of synonymies or homonymies (Sefc *et al*., 2000; Crespan and Milani, 2001; Schneider *et al*., 2001; Regner *et al*. 2006; Ferreira *et al*., 2015; Goryslavets *et al*., 2015; Nebish *et al*., 2017).

This study is conducted to evaluate the genetic diversity within the Mascara germplasm collection, and to identify duplicates, synonyms, homonyms, and genetic relationships of accessions after comparing them with previous reports of genetic profiles from the germplasm collection of Skikda that was considered a duplicate of the first one. This study aims at enhancing the understanding of genetic diversity among different genotypes in the germeplam bank of grapevines at the ITAFV 'Institut

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2. Material and Methods

2.1. Plant Material and DNA Extraction

The plant materials consisted of twenty-three samples that were obtained from the grapevine collection located at the Experimental Station of Teghennif (Mascara) in Northwestern Algeria. These accessions belong to varieties that are classified as critically-endangered according to the 'Institut Technique de l'Arboriculture Fruitière et de la Vigne (ITAFV)' in Algeria.

Genomic DNA was isolated from young frozen leaves using the DNeasy^{TM} Plant Mini Kit (Qiagen, CA, USA). The DNA quality was determined on agarose gels (1 %) and the concentration was measured by NanoDrop ND-1000 spectrophotometer (Peqlab, Erlangen, Germany). Based on several criteria, the following nine microsatellites were selected and amplified in a multiplex PCR (Ibáñez *et al.,* 2009b): VVS2 (Thomas and Scott, 1993), VVMD5, VVMD27, VVMD28 (Bowers *et al.*, 1999), ssrVrZAG29, ssrVrZAG62, ssrVrZAG67, ssrVrZAG83 and ssrVrZAG112 (Sefc *et al*., 1999). All forward primers were labeled with either 6-FAM, VIC and NED. Labels and multiplex PCR amplifications were done according to Ibañez *et al.* (2009a) in a total volume of 25 µL containing 5 ng of genomic DNA.

2.2. Data Analysis

To detect identical genotypes, « Identity 1.0 » software (Wagner and Sefc, 1999) was used. The obtained profiles were compared with those published genotypes in the Skikda collection in Algeria (Laiadi *et al*., 2009). Several genetic diversity parameters were calculated using GenAlEx 6.41 (Peakall and Smouse, 2006): the number of alleles per locus (Na), the number of effective alleles (Ne), Shannon's Information Index, gene diversity or expected heterozygosity (He), probability of identity per locus (PI), and cumulative PI. The genetic distances between individual accessions 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) by Populations v. 1.2.30 (http://bioinformatics.org, Langella, unpubl.), while Mega5.2 (Tamura *et al.,* 2011) was used to display it.

3. Results

3.1. Statistical Analyses.

A total of nineteen different SSR profiles were obtained for the twenty-three accessions studied with the nine markers (Table 1). Marker ssrVrZAG29 (ZAG29) showed a very low polymorphism in the present study: only two alleles and two genotypes in the whole population, and thus the polymorphism analysis was not used for the diversity study (Table 2). A total of sixty-three alleles were detected at the remaining eight SSR loci, ranging from 4 (ZAG83) to10 (VVS2) and 11 (VVMD28) and with an average of 7.87 per locus.

The most informative locus was VVMD5 with 6.62 effective alleles and the lowest probability of identity (PI= 0.041), followed by VVS2 (Ne=6.17; PI=0.045), while the least informative (apart from ZAG29) was ZAG83 with four alleles (Ne=3.02) and the highest probability of identical genotypes (PI=0.17). These findings are in line with results found by Boz *et al.* (2011) on fifty-five grape cultivars from Southeast Anatolia (Turkey) using fourteen SSR markers.

The observed heterozygosities (Ho) were very high, with a mean value of 0.861. The highest level (0.94) was detected at VVMD27 and ZAG 67, while the lowest (0.68) was at ZAG83.

Values of Probability of Identity (PI) ranged between 0.041 and 0.168, with four out of eight markers close to the value of 0.050 at which a grapevine microsatellite is considered hyper- polymorphic (Sefc *et al.,* 2001).The cumulative PI or probability to obtain individuals with identical profile at all eight loci was estimated as 4.52 X 10^{-10}, similar to those found by other authors for the same number of markers in grapevine, such as 6.93 X 10^{-12} (Ibáñez *et al.,* 2003) or 1.2 X 10^{-8} (Hvarleva *et al.,* 2004).

Table 1. Genetic profiles of 23 Algerian *V. vinifera* L. cultivars analyzed at 9 microsatellite loci. Allele sizes are given in base pairs (bp)

N°	Geno-type	Cultivar	VVS2		VVMD5		VVMD27		VVMD28		ZAG29		ZAG62		ZAG67		ZAG83		ZAG112	
1	**1**	Aberkane	137	137	236	240	183	194	239	263	109	109	196	200	137	153	190	192	236	236
2	**2**	Aneb el Cadi	143	145	232	236	189	194	249	263	109	109	188	200	137	137	195	195	232	238
3	**3**	Adadi des Bibans	133	137	232	240	183	189	247	261	109	109	186	204	123	137	190	195	227	232
4	**3**	Aïn el Couma	133	137	232	240	183	189	247	261	109	109	186	204	123	137	190	195	227	232
5	**4**	Bouaber des Aures	133	143	232	232	191	194	247	261	109	109	204	204	123	129	190	190	236	240
6	**3**	Ahchichene	133	137	232	240	183	189	247	261	109	109	186	204	123	137	190	195	227	232
7	**5**	Aïn el Kelb	135	143	236	240	181	189	247	261	109	109	188	204	129	137	195	195	227	238
8	**6**	Ahmar Mechtras	135	147	232	238	183	194	251	257	109	109	192	204	137	153	195	201	232	238
9	**7**	Valenci Noir	133	135	240	242	194	194	237	263	109	109	194	204	129	137	192	195	227	229
10	**1**	Muscat Noir	137	137	236	240	183	194	239	263	109	109	196	200	137	153	190	192	236	236
11	**8**	Torki	133	135	240	242	//	//	235	261	109	109	194	204	123	137	195	195	227	229
12	**9**	Tizi Ounine1	133	143	226	240	183	185	247	261	109	111	188	200	129	137	192	195	227	232
13	**10**	Ghanez	133	137	236	238	183	194	247	251	109	109	200	204	129	137	190	201	236	238
14	**11**	Elwali	137	143	240	242	183	194	261	261	109	109	200	204	123	137	190	195	227	236
15	**12**	Farrana	143	145	228	240	179	194	251	261	109	109	186	186	147	149	195	195	227	227
16	**13**	Bezzoul el Khadem	133	143	238	238	179	181	247	261	109	111	188	188	129	158	190	190	229	229
17	**14**	Tizi Ounine 2	145	151	226	228	181	185	261	263	109	111	188	204	137	158	192	195	232	236
18	**15**	SbaaTolba	151	153	238	242	183	189	260	263	109	109	188	188	//	//	195	201	227	234
19	**7**	Tadelith	133	135	240	242	194	194	237	263	109	109	194	204	129	137	192	195	227	229
20	**16**	Sidi Ahmed draa el Mizen	137	155	226	242	179	194	247	261	109	109	188	204	123	137	190	192	227	240
21	**17**	Raisin de Bouni	137	149	226	248	185	194	237	247	109	109	188	204	123	137	190	192	232	245
22	**18**	El mokrani	133	149	232	240	179	194	237	247	109	111	192	204	137	153	190	195	227	245
23	**19**	Muscat d'Adda	133	133	226	228	179	185	271	271	109	109	192	204	123	147	190	195	232	259

Table 2. Genetic parameters obtained in eight SSR markers for 19 distinct genotypes: Statistical results for 8 microsatellite markers used in the present study, namely Observed number of alleles (Na), effective number of alleles (Ne), Shannon's Information index (I), observed heterozygosity (Ho), expected heterozygosity (He), and Probability of identity.

Locus	Na	Ne	I	Ho	He	PI
VVS2	10	6.171	2.009	0.895	0.838	0.045
VVMD5	8	6.624	1.966	0.895	0.849	0.041
VVMD27	7	4.985	1.754	0.944	0.799	0.066
VVMD28	11	5.685	2.001	0.895	0.824	0.051
ZAG62	7	4.198	1.638	0.789	0.762	0.090
ZAG67	7	3.951	1.610	0.944	0.747	0.096
ZAG83	4	3.021	1.216	0.684	0.669	0.168
ZAG112	9	5.823	1.937	0.842	0.828	0.050
Sum	63	40.457				
Mean	7.9	5.057		0.861	0.790	
Cumulative						4.52×10^{-10}

3.2. Identification of Synonyms

nSSR markers identified nineteen out of the twenty-three genotypes analyzed, (Table 1). Given the low value found for cumulative PI, cultivar names with identical genotypes could be considered as synonyms, if no critical morphological differences are found between them such as berry color.

Ahchichene had been previously shown to be a synonym for Adari des Bibans in the collection of Skikda (Laiadi *et al.,* 2009; Zinelabidine *et al.,* 2014). In this work, this synonym has been confirmed, and Aïn el Couma also showed the same DNA profile, while it had been reported by the same authors as a different cultivar in Skikda collection. Muscat Noir matched Aberkane, which means 'black' in the Berber dialect in Northern Algeria; it refers to the berry colour. Tadelith and Valenci Noir, both with a black berry color, also had the same DNA profiles.

3.3. Cluster Analysis

Considering the twenty-three accessions analyzed here as well as the twenty-seven unique accessions from Skikda collection (Laiadi *et al*., 2009), thirty-six non-redundant cultivars were found from both collections, with five common SSR markers in both studies. A dendrogram based on a genetic distance measure from those markers was constructed using the Weighted Neighbour-Joining method for a preliminary evaluation of genetic relatedness between the investigated cultivars (Figure 1). Accessions could be easily distinguished in the dendrogram despite being homonymous. When five similar pairs were shared, there is a significant number of alleles for the five loci 'Bouni'or 'raisin de Bouni'(≠ 2 /10 alleles) , 'Tizi Ouinine (S) with Tizi Ouinine2 (M) (≠ 4 /10 alleles) and 'Ghanez' (≠ 4 /10 alleles). The last pair is considered as the unique pair in a separate group that did not appear close to other accessions. 'Tadelith', 'SbaaTolba', 'Elmokrani' or 'Amokrane' in the Skikda collection are three cases of homonymous pairs. They were organized in different sub clusters which in fact did not show a clear relationship between them for the five loci used in this comparison.

As for 'Elmokrani' and 'Elwali' from Mascara , at first they were thought to be replicated from 'Amokrane' or its synonyms 'Louali' from Skikda, but eventually it seems to be completely different between them and among other groups, as well as between those cultivated in the Skikda collection. The same cases were recorded with 'Raisin de Bouni' in Mascara and 'Bouni' in Skikda collection, where it was thought that the name used for the same cultivar can be changed in different growing areas, but it turned out that they were different at least in two alleles with both loci VVMD5 and VVMD28. Finally 'Farana de Mascara' or 'Farrana' was different from that called 'Farrana Noir' regarding the color of the berry which was black in the latter and white in the former although they were located in the same branch in the cluster.

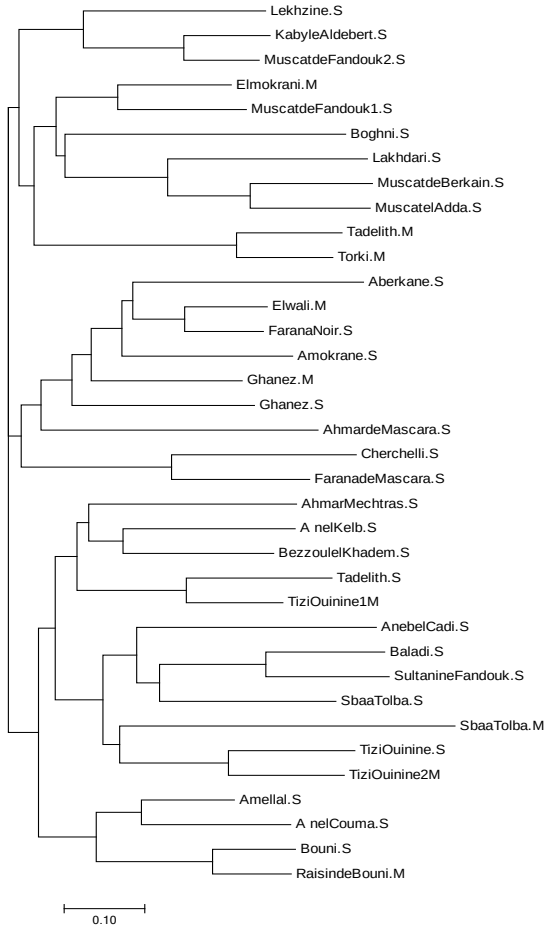

Figure 1. Dendrogram of 36 Algerian grapevine cultivars genotyped at five nSSR loci. The dendrogram was built using the Weighted Neighbour-Joining method with Populations software 1.2.32. (M. Mascara, S. Skikda).

4. Discussion

4.1. SSR Genetic Diversity

The results obtained from statistical analyses agree well with those of previous works on Bulgarian (Hvarleva, 2004) and Turkish genotypes (Boz *et al.,* 2011), in particular in the variation range of alleles per locus; VVMD28 was also found among the most discriminating loci in a group of Muscats by Crespan and Milani (2001).

The values of observed heterozygosity are similar to those found by some previous studies on grapevines, such as 84.3 % in Castilian cultivars obtained with the GENRES 081 set of six nuclear loci (Santana *et al.,* 2008) and higher than 81.0 % for ninety-six genotypes (Ibáñez *et al.* 2003), 80.6 % for 163 genotypes (Martín *et al.,* 2003), and 78.4 % for seventy-three genotypes (Fernández-González *et al.,* 2007). These high values of heterozygosity may indicate that most grapevine cultivars were originally obtained through hybridization, and fixed later by clonal propagation. The gene diversity or expected heterozygosity (He) values were lower than the Ho values for every marker, which may indicate either the absence of null alleles or to their very low frequency (Ibáñez *et al.,* 2003). Gene diversity varied between 0.67 for ZAG83 and about 0.85 for VVS2, VVMD5, while the average gene diversity value was 0.79, similar to that found by other authors for He (Díaz-Losada *et al.,* 2013; Goryslavets *et al.,* 2015)

4.2. Management of Germplasm Collections

The comparison of the nineteen non-redundant genotypes of Mascara accessions with the twenty-seven previously published for the Skikda collection (Laiadi *et al.,* 2009) showed some matches. Many accessions conserved in Mascara are considered as duplicated in the Skikda collection. Several cases were detected in Table 3: Aberkane (1 in Table 1), Aneb el Cadi (2), Aïn el Kelb (5), Bezzoul el Khadem (16), and Muscat d'Adda (23), show the same names and genotypes in both collections; Ahmar Mechtras from Mascara and Ahmar de Mascara from Skikda are two cultivars with the same first name 'Ahmar' meaning red in Arabic; this cultivar from Mascara is different in the second name only. Ahchichene (6) and its synonym Adadi des Bibans (3) from the Mascara collection also matched in names and genotypes in both collections with an additional synonym, Lekhzine, in the Skikda collection as reported by Laiadi *et al.* (2009). The synonym of Sidi Ahmed Draa el Mizen (20) in the Mascara collection occurred as a result of changes in the grammatical gender of the denomination (Díaz-Losada *et al.,* 2013) with Ahmed Draa el Mizen presented by Amellal in Skikda collection. Each of these pairs shares the same berry color. 'Farrana' (15) or 'Farhana' meaning 'happy' in Arabic according to Levadoux *et al.* (1971) shows the same genotype with Farana de Mascara.

Table 3. Genotypic identities of Mascara and Skikda germoplasm collections.

Accessions name of Skikda	Identical genotypes from Mascara	Berry colour
Lekhzine.S	Adadi des Bibans.M	White
Aberkane S	Aberkane.M	Black
Amellal.S	Sidi Ahmed Draa el Mizen.M	White
Aneb el Cadi.S	Aneb el Cadi.M	White
Muscat d'Adda.S	Muscat el Adda.M	Black
Aïn el Kelb.S	Aïn el Kelb.M	White
Bezzoul el Khadem.S	Bezzoul el Khadem.M	Black
Ahmar de Mascara.S	Ahmar Mechtras.M	Pink to Red
Farrana de Mascara.S	Farrana.M	White
Kabyle Aldebert.S	Bouaber des Aures.M	Black

Four groups of homonyms were detected between the two collections involving nine different genotypes (Table

4): cases of pairs among them taking the same names in both collections are 'Ghanez' 'SbaaTolba' 'Tadelith' and 'Tizi Ouinine 1, 2'.

Form the comparison with previous results by the same authors regarding the Skikda germplasm collection published in 2009 and 2014, nine synonyms affecting eighteen genotypes were detected. False duplicated or homonyms were found for four cultivars, involving ten genotypes. Nine genotypes were identified in the collection of Mascara that have not been described previously using SSRs.

Thus, the total current number of accessions to represent local grapevines maintained at the both collections is thirty-six.

Table 4. Homonymous grapevine cultivars analyzed at 5 microsatellite loci (allele sizes are given as base pairs). S. accession from Skikda collection (Laiadi *et al*. 2009). M. accession from Mascara collection.

N°	Homonymous Cultivar name	VVS2		VVMD5		VVMD27		VVMD28		VrZAG62	
1	Ghanez (S)*	143	145	228	240	183	194	247	251	200	204
	Ghanez (M)	133	137	236	238	183	194	247	251	200	204
2	SbaaTolba (S)*	133	145	226	234	179	194	221	255	188	204
	SbaaTolba(M)	151	153	238	242	183	189	260	263	188	188
3	Tadelith (S)*	133	143	226	226	179	183	251	261	188	200
	Tadelith (M)	133	135	240	242	194	194	237	263	194	204
4	TiziOuinine (S)*	145	151	226	228	185	194	261	261	188	188
	TiziOuinine 2 (M)	145	151	226	228	181	185	261	263	188	204
	TiziOuinine 1 (M)	133	143	226	240	183	185	247	261	188	200

5. Conclusion

The present study provides the first molecular database for Algerian grapevine germplasm management. The germplasm collection of 'Teghennif' thus contains nineteen out of the twenty-three analyzed accessions of autochthonous original cultivars with three cases of recorded duplications involving seven genotypes. This study shows the important genetic diversity in germplasm collections for *Vitis* in Algeria. Unfortunately, only five SSRs were utilized in the comparison of profiles; however it becomes clear now that during the transfer of samples there was a clear confusion in the proper labeling among the cutting materials that were transferred from the mother germplasm collection. In practice, stickers on cutting materials may be easily dropped completely, leading to a possible mislabeling especially if the person who transferred the cutting is not specialized in the field. The right thing to do in such cases is to remove all questionable material from the GenBank and replace it with true to type cutting material, thus, reducing the cost of effort, time, and money to verify its identity.

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Author contributions

All authors have participated in the research and article preparation. Rahali M. and Achour H. have participated in the analysis and interpretation of data and manuscript revision.

Conflict of interest disclosure

The authors declare no conflict of interest.

References

Bowers JE, Dangl GS and Meredith CP. 1999. Development and characterization of additional microsatellite DNA markers for grape. *Am J Enol Vitic*, **50**: 243-246.

Boz Y, Bakir M, Çelikkol BP, Kazan K, Yilmaz F, Çakir B. Aslantaş Ş. Söylemezoğlu G, Yaşasin AS, Özer C, Çelik H and Ergül A. 2011. Genetic characterization of grape .*Vitis vinifera* L.. germplasm from Southeast Anatolia by SSR markers. *Vitis*, **50**: 99–106.

Costantini L, Monaco A, Vouillamoz JF, Forlani M and Grando MS. 2005. Genetic relationship among local *Vitis vinifera* cultivars from Campania (Italy). *Vitis*, **44**: 25-34.

Crespan M and Milani N. 2001. The Muscats A molecular analysis of synonyms, homonyms and genetic relationship within a large family of grapevine cultivars. *Vitis*, **40**: 23-30.

Díaz-Losada E, Tato Salgado A, Orriols-Fernández I, Ramos-Cabrer AM and Pereira-Lorenzo S. 2013. New Synonyms and Homonyms for Cultivars from Northwestern Spain. *Am J Enol Vitic*, **64**: 156-162.

El Oualkadi A, Ater M, Messaoudi Z, Laucou V, Boursiquot JM, Lacombe T and This P. 2009. Molecular characterization of Moroccan grapevine germplasm using SSR markers for the establishment of a reference collection. *J Int Sci Vigne Vin*, **43**: 135-148.

Fernández-González M, Mena A, Izquierdo P and Martínez J. 2007. Genetic characterization of grapevine (*Vitis vinifera* L.) cultivars from Castilla La Mancha .Spain. using microsatellite markers. *Vitis*, **46**: 126-130.

Ferreira V, Pinto-Carnide O, Mota T, Martín JP, Ortiz JM and Castro I. 2015. Identification of minority grapevine cultivars from Vinhos Verdes Portuguese DOC Region . *Vitis*, **54** (Special Issue) 53–58.

Goryslavets S, Bacilieri R, Risovannaya V, Memetova E and Laucou V. 2015. Genetic diversity of ancient grape cultivars of the Crimea region. *Vitis*, **54** (Special Issue): 37-41.

Hvarleva T, Rusanov K, Lefort F, Tsvetkov I, Atanassov A and Atanassov I .2004. Genotyping of Bulgarian *Vitis vinifera* L. Cultivars by microsatellite analysis. *Vitis*, **43**: 27-34.

Ibáñez J, De Andres M T, Molino A and Borrego J. 2003. Genetic study of key spanish grapevine varieties using microsatellite analysis. *Am J Enol Vitic*, **54**: 22-30.

Ibáñez J, Vargas AM, Palancar M, Borrego J and De Andrés MT. 2009a. Genetic relationships among table-grape varieties. *Am J Enol Vitic*, **60**: 35-42.

Ibañez J, Velez MD, De Andres MT and Borrego J. 2009b. Molecular markers for establishing distinctness in vegetatively propagated crops a case study in grapevine. *Theor Appl Genet*, **119**:1213-1222.

Laiadi Z, Bentchikou MM, Bravo G, Cabello F and Martínez-Zapater J. 2009. Molecular identification and genetic relationships of Algerian grapevine cultivars maintained at the germplasm collection of Skikda (Algeria). *Vitis*, **48**: 25-32.

Laiadi Z, Bencharif S, Lakhrif Z, Bentchikou M M and Mohand-Larbi R. 2013. First ampelometric study of autochthonous grapevines in Algeria Germplasm collection of Mascara. *Vitis*, **52**: 21–27.

Levadoux L, Benabderrabou A and Douaouri B. 1971. Ampélographie Algérienne : Cépages de Cuve et de Table Cultivés en Algérie. SNED.

Maletić E, Pejić I, Karoglan Kontić J, Zdunić, Preiner D, Šimon S, Andabaka Ž, Žuljmihaljević M, Bubola M, Marković Z, Stupić D and Mucalo A. 2015. Ampelographic and genetic characterization of Croatian grapevine varieties. *Vitis*, **54** (Special Issue): 93–98.

Martin JP, Borrego J, Cabello F and Ortiz JM. 2003. Characterization of Spanish grapevine cultivar diversity using sequence-tagged microsatellite site markers. *Genome*, **46**: 10-18.

Nebish A, Ochssner I, Maul E, Topfer R, Hausmann L, Hovhannisyan A, Devejyan H, Melyan G and Aroutiounian R. 2017. Genetic identification and characterization of Armenian grapevine cultivars. 40th World Congress of Vine and Wine, BIO Web of Conferences 9, 01020 **.** *Sofia, Bulgaria.*

Peakall R and Smouse PE. 2006. GENALEX 6 genetic analysis in excel population genetic software for teaching and research. *Mol Ecol Notes*, **6**: 288-295.

Regner F, Hack R and Santiago JL. 2006. Highly variable Vitis microsatellite loci for the identification of Pinot Noir clones. *Vitis*, **45**: 85-91.

Saitou N and Nei M .1987. The neighbor-joining method - a new method for reconstructing phylogenetic trees. *Mol Biol Evol*, **4**: 406-425.

Santana JC, Hidalgo E, De Lucas AI, Recio P, Ortiz JM, Yuste J, Arranz C and Rubio JA . 2008. Identification and relationships of accessions grown in the grapevine (*Vitis vinifera* L.) germplasm bank of Castilla y Léon .Spain. and the varieties authorized in the VQPRD areas of the region by SSR-marker analysis. *Genet Resour Crop Evol*, 55: 573-583.

Schneider A, Carra A, Akkak A, This P, Laucou V and Botta R .2001. Verifying synonyms between grape cultivars from France and Northwestern Italy using molecular markers. *Vitis*, **40**: 197-203.

Sefc KM, Regner F, Turetschek E, Glossl J and Steinkellner H. 1999. Identification of microsatellite sequences in *Vitis riparia* and their applicability for genotyping of different *Vitis* species. *Genome*, **42**: 367-373.

Sefc KM, Lopes MS, Lefort F, Botta R, Roubelakis-Angelakis KA, Ibáñez J, Pejic I, Wagner HW, Glossl J and Steinkellner H. 2000. Microsatellite variability in grapevine cultivars from different European regions and evaluation of assignment testing to assess the geographic origin of cultivars. *Theor Appl Genet*, **100**: 498-505.

Sefc KM, Lefort F, Grando MS, Scott KD, Steinkellner H and Thomas, MR. 2001. Microsatellite markers for grapevine a state of the art. *In:* **Molecular Biology & Biotechnology of Grapevine,** Roubelakis-Angelakis KA.(Ed.). Kluwer Academic Publishers, The Netherlands .pp 30.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M and Kumar S .2011. MEGA5 Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol*, **28**: 2731-2739.

This P, Jung A, Boccacci P, Borrego J, Botta R, Costantini L, Crespan M, Dangl GS, Eisenheld C, Ferreira-Monteiro F, Grando MS, Ibáñez J, Lacombe T, Laucou V, Magalhães M, Meredith CP, Milani N, Peterlunger E, Regner F, Zulini L and Maul E. 2004. Development of a standard set of microsatellite reference alleles for identification of grape cultivars. *Theor Appl Genet*, **109**: 1448-1458.

Wagner HW, Sefc KM .1999. Identity 1, Centre for Applied Genetics, University of Agricultural Science, Vienna.

Zinelabidine LH, Laiadi Z, Benmehaia R, Gago P, Boso S, Santiago JL, Haddioui A, Ibáñez J, Martínez-Zapater JM and Martínez MC. 2014. Comparative ampelographic and genetic analysis of grapevine cultivars from Algeria and Morocco. *Aust J Grape Wine R*, **20**: 324–333.