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## Genetic characterization and identification of the table grape accessions preserved in the living collection of Ain Taoujdate (Morocco)

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## Summary

We have characterized the 60 table grape accessions preserved at the living collection of the Domaine Expérimental de Ain Taoujdate (Morocco) through DNA analyses. Genetic profiling based on 13 SSRs and 240 SNP markers identified up to 40 different genotypes, denoting a certain level of redundancy. This information was useful to detect many cases of misspelled accessions, some misnamed varieties, and several potential new synonymies. The comparison of these genetic profiles with international databases led to the identification of 58 accessions as 38 table grape varieties, half of them corresponding to obtentions bred in recent programs of table grape improvement. Only two accessions (named “Diamant Noir” and “Sultanine Rosée”) did not match any known genetic profile. We found that “Sultanine Rosée” does not correspond to ‘Kishmish Rozovyi’, the described pink-berried variant of ‘Sultanina’. Indeed, it turned out to be a grape variety not catalogued in international genetic databases that arose from the cross between ‘Sultanina’ and ‘Fokiano’, which we suggest to name ‘Sultanine Rose Faux’. Besides, the duo detected between the accession “Diamant Noir” and the variety ‘Moscato D’Adda’ suggests that it might correspond to the table grape variety named ‘Diamante Nero’ (‘Pirovano 57’ × ‘Moscato D’Adda’). We proved that molecular-assisted parentage analyses could be an efficient approach to suggest an identity for grapevine varieties that lack a matching genotype in international catalogues.

## Keywords

Conservation; germplasm; genetic identification; SNP; SSR; *Vitis vinifera* L.

## Introduction

Grapevine (*Vitis vinifera* L.) is one of the oldest agricultural fruit crops, mostly cultivated to produce wine, table grapes,

raisins, and juice (Reisch *et al.*, 2012). The number of different varieties all over the world is estimated between 6.000 and 10.000, although only few hundreds have actual commercial relevance (Wolkovich *et al.*, 2018). In fact, a high number of the numerous available varieties are confined in grapevine collections, commonly represented by few living plants (This *et al.*, 2006). Nowadays, these underused genetic resources are in the spotlight, as they can have useful traits to solve the limited performance of traditional cultivars to face current viticulture challenges (Wolkovich *et al.*, 2018). In addition, they can help to understand grapevine diversification processes (Dong *et al.*, 2023), as well as to identify the molecular and genetic mechanisms underlying traits of interest (Sargolzaei *et al.*, 2020). Therefore, the establishment and maintenance of living grapevine collections is essential. Some of the largest grapevine repositories in the world are those of Vassal-Montpellier (INRAe, Marseillan-Plage, France), Geilweilerhof (JKI, Siebeldingen, Germany), El Encín (IMIDRA, Alcalá de Henares, Madrid, Spain), Conegliano (CREA-VIT, Conegliano, Italy), and Rancho de la Merced (IFAPA, Jerez de la Frontera, Andalucía, Spain), which preserve thousands of grapevine accessions of *V. vinifera* L. and other related *Vitis* species (Lacombe *et al.*, 2013; Nicolas *et al.*, 2016; Cretazzo *et al.*, 2022). In addition to these major collections, there are numerous national and regional repositories that host different minor grapevine varieties, some of them of unknown identity (Maul *et al.*, 2015).

Despite their importance for basic science and applied research, living grapevine collections are difficult to establish and maintain, and they are very often threatened by funding limitations (Migicovsky *et al.*, 2019). Consequently, a careful curation to reduce the number of unnecessary duplicates and mislabeled accessions is convenient for enabling efficient and effective management practices. In grapevine, this stage is especially relevant, given the high number of existing synonyms (use of different names to refer to the same variety) and homonyms (use of the same name to refer to different varieties) (This *et al.*, 2006). The curation of grapevine collections can be done through ampelography: the exhaustive description of



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numerous morphological traits of different grapevine organs (leaves, bunches, shoots, etc.). However, it needs a high level of expertise and experience to ensure good results. Nowadays, this process is frequently approached with the help of genetic markers. Among them, the use of standard sets of microsatellites (SSRs) and/or single nucleotide polymorphisms (SNPs) have proved to be a highly efficient system for the curation of grapevine repositories of diverse origin (Maul *et al.*, 2015; Cunha *et al.*, 2016). For example, the genetic profiling of the 411 accessions of the Grapevine Germplasm Bank of Aragón (Spain) at 26 SSR markers led to the identification of 156 *V. vinifera* L. unique genotypes (Ghrissi *et al.*, 2022). Similarly, the characterization of the 288 accessions preserved at the Portuguese National Ampelographic Collection detected 263 different genotypes (Cunha *et al.*, 2016). More recently, the characterization of 37 grapevine accessions preserved at the Nabaldyan Grapevine Collection of Armenia at seven SSR and 240 SNP markers led to the identification of 27 different genetic profiles (Nebish *et al.*, 2021). In addition to the detection of duplicated genotypes, findings from these works revealed some new synonyms and homonyms among cultivars, as well as multiple mislabeled accessions, and some potential new varieties.

The first living grapevine collection of Morocco was established in 1952 by J.P. Vidal at the former École Nationale d'Agriculture in Meknès (Morocco) (El Oualkadi *et al.*, 2009). This collection hosted 94 accessions of local grapevine varieties (mostly collected in Northern Morocco) and international table and wine grape cultivars of different origins (El Oualkadi *et al.*, 2009). Some years later, this collection was transferred to the Société du Développement Agricole (SODEA), and the 94 grapevine accessions were genetically characterized at 20 SSRs *loci* to verify their trueness-to-type (El Oualkadi *et al.*, 2009). This approach led to the identification of 67 different

grapevine genotypes, including 18 putative autochthonous varieties. This collection was used to explore the genetic diversity and origin of local Moroccan genetic resources, as well as to test their relationships with grapevine cultivars from other regions (Zinelabidine *et al.*, 2010; El Oualkadi *et al.*, 2011; Zinelabidine *et al.*, 2014). Unfortunately, this collection is no longer operational. In parallel, another grapevine collection was set in the Domaine Expérimental de Ain Taoujdate (Ain Taoujdate, Morocco), which is managed by the Centre Régional de la Recherche Agronomique of Meknès. This collection was founded in 1997 after merging its own genetic resources with some transferred from the SODEA collection, and others from the Domaine Expérimental d'El Menzeh (Centre Régional de la Recherche Agronomique of Kénitra, Morocco). The 74 accessions preserved in Ain Taoujdate have never been characterized, in spite of the many errors that might have arisen during the construction of this collection, as reported in similar situations (El Oualkadi *et al.*, 2009; Alifragkis *et al.*, 2015). Consequently, the main objective of this work was to perform the genetic characterization of the 60 table grape accessions preserved at the living grapevine collection of Ain Taoujdate, in order to detect possible duplicated and mislabeled accessions. This information was useful to detect some cases of misnames and new potential cases of synonyms in the collection, and it is in use now for the adequate management of these grapevine genetic resources.

## Material and Methods

### Plant material

The 60 table grape accessions preserved at the grapevine collection of the Domaine Expérimental de Ain Taoujdate (Ain Taoujdate, Morocco) have been studied (Table 1). For each

Table 1: Genetic identification results of 60 table grape accessions of the Domaine Expérimentale de Ain Taoujdate (Morocco), after SNP and SSR profiling.

Accession name (code)	Accession origin <sup>a</sup>	Variety name	VIVC number	ICVV genotype number	Comments on identification	Use <sup>b</sup>	Breeder data <sup>b</sup>	Origin <sup>b</sup>
Oubouhou (L14X24)	DEAT	Abouhou	35	2317	Prime name, misspelling	T	No	Morocco
King's ruby (L9X3)	DEAT	Abouhou	35	2317	Misnomer (King's Ruby, VIVC N:10314)	T	No	Morocco
Muscat douille (L6X22)	SODEA	Afus Ali	122	2036	-	T/W	No	Lebanon
Dattier de bayrouth (L9X1)	DEAT	Afus Ali	122	2036	Synonym, misspelling	T/W	No	Lebanon
Ahmer bouamar (L11X10)	DEAT	Ahmeur bou Ahmeur	140	0473	Prime name, misspelling	T/W	No	Algeria
Alphonse la vallée (L11X9)	DEAT	Alphonse Lavallee	349	1032	Prime name, misspelling	T/W/R	No	France
Gros grain (L5X17)	SODEA	Alphonse Lavallee	349	1032	-	T/W/R	No	France
EMD (X1K)	DEEM	Alphonse Lavallee	349	1032	-	T/W/R	No	France
Teresa de prevane (L10X6)	DEAT	Pirovano 190	9446	4528	Misnomer/Mispelling (Teresa Pirovano VIVC N: 12367)	T	Yes	Italy

Table 1: Continued.

Accession name (code)	Accession origin <sup>a</sup>	Variety name	VIVC number	ICVV geno-type number	Comments on identification	Use <sup>b</sup>	Breeder data <sup>b</sup>	Origin <sup>b</sup>
Enselia (L13X17)	DEAT	Beba	22710	2088	New synonym	T/W	No	Spain
Doukalia (L13X2)	DEAT	Bezoul el Khadem de Tunisie	1315	0527	Berry colour somatic variant, misspelling	T	No	Tunisia
Maria prevane (L10X5)	DEAT	Cardinal	2091	0343	Misnomer/Mispelling (María Pirovano, VIVC N: 7402)	T/W	Yes	USA
Cardinal (L15X27)	DEAT	Cardinal	2091	0343	Prime name	T/W	Yes	USA
Early Iardinal (L4X13)	SODEA	Cardinal	2091	0343	Synonym/Mispelling (Cardinal Early, VIVC N: 14398)	T/W	Yes	USA
Rival (L4X16)	SODEA	Cardinal	2091	0343	New synonym	T/W	Yes	USA
Porlam (L6X24)	SODEA	Cardinal	2091	0343	New synonym	T/W	Yes	USA
- (X29DN)	DEAT	Cardinal	2091	0343	-	T/W	Yes	USA
Christmas (L4X14)	SODEA	Christmas Rose	2654	4268	Prime name	T	Yes	USA
Dabouki (L3X9)	SODEA	Dabouki	3309	0992	Prime name	T/W	No	Israel
2S Arabia lybie (L2X5)	SODEA	Dabouki Arub	24600	2558	New synonym	T/W	No	Asia Minor
Danane (L12X16)	DEAT	Danam	3418	1015	Prime name, misspelling	T	Yes	France
Danan (L6X21)	SODEA	Danam	3418	1015	Prime name, misspelling	T	Yes	France
Datal (L12X15)	DEAT	Datal	3436	0156	Prime name	T	Yes	France
Arдона (L3X12)	SODEA	Dattier de St. Vallier	3437	9078	Misnomer (Arдона, VIVC N: 17386)	T/W	Yes	France
jaamen (L2X6)	SODEA	Delhro	3504	4529	New synonym	T	Yes	France
Delcia divapine (L9X4)	DEAT	Delizia di Vaprio	3510	0197	Prime name/Mispelling	T/W	Yes	Italy
ESP nobi (L12X13)	DEAT	Dominga	4985	2114	-	T	No	Spain
Flame sedlees (L3X10)	SODEA	Flame seed-less	4141	2053	Prime name/Mispelling	T	Yes	USA
ESP.NOA (L11X12)	DEAT	Imperial Napoleon	5517	2166	-	T	No	Spain
Carrière (L5X18)	SODEA	Italia	5582	0264	Misnomer (Carriere, VIVC N: 2127)	T/W	Yes	Italy
Muskat d'italie A (L7X26)	SODEA	Italia	5582	0264	Synonym	T/W	Yes	Italy
Olivette noire (L8X30)	SODEA	Italia	5582	0264	Misnomer (Olivette Noire, VIVC N: 8759)	T/W	Yes	Italy
Dattier de bayrouth (X1Db)	DEAT	Italia	5582	0264	Misnomer/Mispelling (Dattier de Beyrouth, VIVC N: 122)	T/W	Yes	Italy
Lual (L15X25)	DEAT	Lival	6865	0354	Prime name, misspelling	T	Yes	France
- (X4K)	DEEM	Michele Palieri	7704	0928	-	T	Yes	Italy
Perlina (L13X18)	DEAT	Muscat Hamburg	8226	2047	Misnomer (Perlina d'Inverno, VIVC N: 20592; Perlina Saba, VIVC N: 9166)	T/W	No	UK
Sultanine (L16X29)	DEAT	Muscat Hamburg	8226	2047	Misnomer (Sultanina, VIVC N: 12051)	T/W	No	UK
Muskat de ham-bourg (L16X30)	DEAT	Muscat Hamburg	8226	2047	Prime name	T/W	No	UK
Muskat madine (L12X14)	DEAT	Muscat Madresfield Court	8235	0800	New synonym	T	Yes	UK
Ergilluie (L10X8)	DEAT	Muscat of Alexandria	8241	2153	New synonym	T/W/R	No	Greece

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Table 1: Continued.

Accession name (code)	Accession origin <sup>a</sup>	Variety name	VIVC number	ICVV geno-type number	Comments on identification	Use <sup>b</sup>	Breeder data <sup>b</sup>	Origin <sup>b</sup>
FAL (X5K)	DEEM	Muscat of Alexandria	8241	2153	-	T/W/R	No	Greece
Diamant noir (L14X22)	DEAT	-	-	4533	-	-	No	-
Sultanine Rosée (L15X28)	DEAT	-	-	4534	-	-	No	-
Esp. N.E (L11X11)	DEAT	Ohanes	8716	0608	-	T/W	No	Spain
Sabat kanstantini (L9X2)	DEAT	Sabalkanskoi – Ohanes Red	10432-21500	0798	Prime name/Mispelling	T	No	Portugal
Oliviette noir (L15X26)	SODEA	Olivette Noire	8759	0530	Prime name/Mispelling	T/W	No	Hungary
Chenasan (L7X25)	SODEA	Portan	9612	0964	Misnomer/Mispelling (Chenasan, VIVC N: 2521)	T/W	No	France
Chelva (L5X20)	SODEA	Red Globe	9972	2157	Misnomer (Chelva, VIVC: 22710; Chelva, VIVC N: 2520)	T	Yes	USA
FMH (X2K)	DEEM	Red Globe	9972	2157	-	T	Yes	USA
1S Early supérieur (L1X2)	SODEA	Rutilia	4432	2473	-	T	Yes	Argentina
Flem des valliers (L2X8)	SODEA	Rutilia	4432	2473	New synonym	T	Yes	Argentina
Rutra (L8X29)	SODEA	Rutilia	4432	2473	Prime name/Mispelling	T	Yes	Argentina
Pause precoce (L10X7)	DEAT	Sicilien	11775	0702	Synonym/Mispelling	T/W	No	France
2B Jaamen (L1X4)	SODEA	Sugraone	12087	0622	New synonym	T	Yes	USA
Sultanine musquée (L16X31)	DEAT	Sultana Moscata	12050	2205	Synonym	T	Yes	Italy
Thomson (L7X28)	SODEA	Sultanina	12051	2126	Synonym/Mispelling	T/W/R	No	Turkey
Rezouki (L14X21)	DEAT	Taferielt	12196	2311	New synonym	T/W/R	No	Morocco
Tapperial (L14X23)	DEAT	Taferielt	12196	2311	Prime name/Mispelling	T/W/R	No	Morocco
Danlas (X3K)	DEEM	Triomphe d'Alsace	12650	7292	Misnomer (Danlas, VIVC N: 3423)	W	Yes	France
Trijoli (L13X19)	DEAT	Tripoli	12653	2534	Prime name/Mispelling	T	Yes	Italy

<sup>a</sup> DEAT: *Domaine Expérimental de Ain Taoujdate*; DEEM: *Domaine Expérimental d'El Menzeh*; SODEA: *Société du Développement Agricole*. <sup>b</sup> Use, breeder data and origin of the variety according to VIVC database. For Use, T: Table grape; R: Raisin grape; W: Wine grape.

accession, 5-6 young leaves were collected *in situ*, preserved in ice, and lyophilized using an Alpha 2-4 equipment (Martin Christ, Germany). Then, samples were stored at room temperature until DNA extraction

### DNA extraction and genotyping

Total genomic DNA was extracted from lyophilized young leaves using the NZY Plant/Fungi gDNA Isolation Kit (NZYTech, Lisbon, Portugal), following manufacturer indications. DNA quality and quantity was evaluated using a NanoDrop Spectrophotometer (Thermo Scientific, Wilmington, USA). DNAs were genotyped for 13 SSR markers (*VVS2*, *VVMD5*, *VVMD7*, *VVMD25*, *VVMD27*, *VVMD28*, *VVMD32*, *VrZAG62*, *VrZAG79*,

*VrZAG29*, *VrZAG67*, *VrZAG83*, and *VrZAG112*) in two multiplex PCRs, as described in Nebish *et al.* (2021) and Tello *et al.* (2024). The first nine SSR markers of this set are internationally acknowledged for grapevine identification, and stored in the *Vitis* International Variety Catalogue (VIVC) online database. Later on, PCR products were separated by capillary electrophoresis, performed in an ABI 3130XL genetic analyzer (Applied Biosystems, Foster City, CA, USA) at the Centro de Investigación Biomédica de La Rioja (CIBIR). The size of the PCR fragments were rated using GeneMapper v.4.1 (Applied Biosystems, Darmstadt, Germany). Each analysis included a 'Tempranillo Tinto' DNA as positive control and a non-template as negative control. All non-redundant genetic profiles were genotyped at 240 SNP loci (Zinelabidine *et al.*, 2012),



using Fluidigm technology at the Sequencing and Genotyping Unit of the Universidad del País Vasco (UPV/EHU) (Maraš *et al.*, 2020). This set includes a subset of 48 SNPs for variety identification (Cabezas *et al.*, 2011) and 192 additional SNPs for robust parentage analyses (Lijavetzky *et al.*, 2007; Zinelabidine *et al.*, 2012). The chloroplast haplotype (chlorotype) was identified for each genotype based on three chloroplast SNPs (SNP\_NG\_C\_001, SNP\_NG\_C\_003, and SNP\_NG\_D\_003) that allow the differentiation of the four major types (A, B, C, and D) defined by Arroyo-García *et al.* (2006), as previously detailed (Maraš *et al.*, 2020).

### Genetic identification and parentage analysis

The non-redundant genetic profiles obtained for 13 SSRs and 240 SNPs were pairwise compared with the 6,354 references available in the *Vitis* International Variety Catalogue VIVC, (access 02/2024), and the 3,574 non-redundant genetic profiles stored at the Instituto de Ciencias de Vid y del Vino database (ICVV-DNA) for genetic identification. On the other hand, the non-redundant SNP genetic profiles were merged with those available at the ICVV-DNA database for a wide search of possible first-order kinship relationships, using the software Cervus v.3.0 (Kalinowski *et al.*, 2007) as described in Cunha *et al.* (2020). The robustness of each proposed relationship was evaluated using the natural logarithm of the overall likelihood ratio (LOD) score. For trios (two parents and offspring), the maximum number of mismatching SNPs was set to two. For duos (parent-offspring), only one mismatching SNP was allowed, and only those with a LOD > 25 were considered. All the new relationships found with SNPs were confirmed using SSR data. If different between the two parents, chlorotype data were used to determine the female progenitor in the proposed trios, considering that chloroplasts are inherited from the maternal parent (Arroyo-García *et al.*, 2006).

## Results

### Variety Identification

The combined SSR and SNP genotyping strategy followed in this work was useful to identify 40 different genetic profiles from the 60 table grape accessions preserved at the collection of the Domaine Expérimentale de Ain Taoujdate (Table 1). These genetic profiles are fully provided in the Supplementary Material. The pairwise comparison of these 40 profiles with those stored at the VIVC and ICVV-DNA databases led to the successful identification of 38 profiles as known grapevine varieties, all of them table grape varieties or with a double table/wine use (Table 1). Out of these 38 varieties, 19 were identified as table grape obtentions released from breeding programs of renowned breeders like Alberto Pirovano (v.g. ‘Sultana Moscata’), Paul Truel (‘Danam’), or Harold P. Olmo (‘Red Globe’). The remaining 19 identified varieties corresponded to traditional varieties grown for table grape production from different Mediterranean regions, including Spain (like ‘Beba’, or ‘Dominga’), France (‘Sicilien’), Tunisia (‘Bezoul el Khadem de Tunisie’), and Morocco (‘Abouhou’ and ‘Taferielt’). Lastly,

we did not find any matching genetic profile for two accessions, named “Diamant Noir” and “Sultanine Rosée” (Table 1).

The most abundantly found genotype corresponded to the variety ‘Cardinal’, which was found six times in the collection, followed by ‘Italia’ (four times), and ‘Alphonse Lavallee’, ‘Muscat Hamburg’, and ‘Rutilia’, detected three times (Table 1). The genetic identification of these accessions led to find up to twelve misnames in the collection, like the variety ‘Abouhou’, which was found to be wrongly preserved under the name of the variety ‘King’s ruby’ (synonym of ‘Ruby seedless’). Similarly, the variety ‘Italia’ was found to be preserved as ‘Carriere’, ‘Olivette Noire’, and ‘Dattier de Beyrouth’ (syn. ‘Afus Ali’), and the variety ‘Red Globe’, as ‘Chelva’ (Table 1). We also identified multiple spelling errors in the names given in the collection to some of the identified varieties. For example, the variety ‘Abouhou’ was preserved under the misspelled name “Oubouhou”, ‘Doukkali’ (synonym of ‘Bezoul el Khadem de Tunisie’) was preserved as “Doukalia”, ‘Lival’ as “Lual”, ‘Panse precoce’ (syn. ‘Sicilien’) as “Pause precoce”, ‘Taferielt’ as “Tap-perial”, and ‘Tripoli’ as “Trijoli”. We identified new potential local synonyms for some of the identified varieties too. This is the case of “Rival” and “Porlam”, names used in the collection to refer to the variety ‘Cardinal’, or “Muskat madine” to refer to the variety ‘Muscat Madresfield Court’. Other potential new synonyms are “Enselia” for ‘Beba’, “Aarabia lybie” for ‘Dabouki Arub’, “Ergilluie” for ‘Muscat of Alexandria’, “Flem des Valliers” for ‘Rutilia’, and “Rezouki” for ‘Taferielt’.

### Pedigree Analyses

Parentage analysis based on 240 SNP data revealed the full pedigree of 16 genotypes, including 15 bred varieties whose pedigree was already supported by SSR and/or SNP markers in previous works (Table 2). In addition, we found the full pedigree of the unidentified accession “Sultanine Rosée” (‘Fokiano’ × ‘Sultanina’). According to chlorotype data, ‘Fokiano’ (chlorotype A) acted as mother in the cross, since “Sultanine Rosée” and ‘Sultanina’ have different chlorotypes (A and C, respectively). Furthermore, stenospemocarpy of ‘Sultanina’ prevents its role as female parent in crosses. Besides, we identified three duos not reported before in the bibliography (Table 3). One of them involved the variety ‘Moscato D’Adda’ and the unidentified accession “Diamant Noir”.

## Discussion

### Genetic profiling reveals the varietal identity of most of the table grape accessions preserved in the collection of Ain Taoujdate

DNA profiling is widely recognized as a powerful tool to detect duplicates in germplasm collections, which might ultimately lead to reduce the number of accessions to maintain (Migicovsky *et al.*, 2019). Here, the analysis of the 60 grapevine accessions preserved at the Moroccan collection of the Domaine Expérimentale de Ain Taoujdate revealed 40 different genetic profiles, so we found a level of redundancy that is in the range of that found in other grapevine repositories (El

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| Table 2: Summary of the full trios detected by SNP analyses.

Offspring (Chlorotype) <sup>a</sup>	Parent 1 (Chlorotype)	Parent 2 (Chlorotype) <sup>a</sup>	SNPs compared/mismatched	LOD	Described Pedigree	References
Alphonse Lavallee (B)	Dodrelyabi (B)	Muscat Hamburg (D)	225/1	68.36	Dodrelyabi × Muscat Hamburg	Lacombe <i>et al.</i> (2013)
Cardinal (B)	Alphonse Lavallee (B)	Koenigin der Weingaerten (A)	234/1	96.34	Alphonse Lavallee × Koenigin der Weingaerten	Lacombe <i>et al.</i> (2013)
Danam (D)	Muscat Hamburg (D)	Dabouki (D)	218/2	78.43	Dabouki × Muscat Hamburg	Ghaffari <i>et al.</i> (2014); Lacombe <i>et al.</i> (2013); Vargas <i>et al.</i> (2009)
Datal (B)	Muscat of Alexandria (B)	Afus Ali (A)	164/0	62.27	Afus Ali × Muscat of Alexandria	Lacombe <i>et al.</i> (2013)
Dattier de St. Vallier (D)	Muscat Hamburg (D)	Villard blanc (C)	224/0	81.35	Muscat Hamburg × Villard blanc	Margaryan <i>et al.</i> (2021)
Delhro (B)	Alphonse Lavallee (B)	Csaba Gyoengye (C)	214/2	69.21	Alphonse Lavallee × Csaba Gyoengye	Lacombe <i>et al.</i> (2013)
Delizia di Vaprio (C)	Sicilien (C)	Muscat of Alexandria (B)	218/1	63.23	Sicilien × Muscat of Alexandria	Lacombe <i>et al.</i> (2013); Ghaffari <i>et al.</i> (2014)
Italia (C)	Bicane (C)	Muscat Hamburg (D)	229/2	65.05	Bicane × Muscat Hamburg	Lacombe <i>et al.</i> (2013); Ghaffari <i>et al.</i> (2014)
Lival (B)	Alphonse Lavallee (B)	Luglienga bianca (D)	224/1	80.78	Alphonse Lavallee × Luglienga bianca	Lacombe <i>et al.</i> (2013); Vargas <i>et al.</i> (2009)
Michele Palieri (A)	Molinera (A)	Alphonse Lavallee (B)	214/0	91.21	Molinera × Alphonse Lavallee	Lacombe <i>et al.</i> (2013)
Muscat d'Istanbul (A)	Beba (A)	Muscat of Alexandria (B)	230/1	72.91	Muscat of Alexandria × Beba	Lacombe <i>et al.</i> (2013)
Pirovano 190 (C)	Delizia di Vaprio (C)	Angelo Pirovano (D)	209/2	81.22	Delizia di Vaprio × Angelo Pirovano	Lacombe <i>et al.</i> (2013)
Sicilien (C)	Bicane (C)	Pascal blanc (-)	225/1	85.09	Bicane × Pascal blanc	Lacombe <i>et al.</i> (2013)
Sultana Moscata (B)	Muscat of Alexandria (B)	Sultanina (C)	164/0	62.68	Muscat of Alexandria × Sultanina	Lacombe <i>et al.</i> (2013)
<i>Sultanine Rose Faux</i> (A)	Fokiano (A)	Sultanina (C)	214/1	49.16	-	This work
Tripoli (-)	Angelo Pirovano (D)	Italia (C)	223/0	91.97	Angelo Pirovano × Italia	Lacombe <i>et al.</i> (2013); Ghaffari <i>et al.</i> (2014)

<sup>a</sup> Chlorotypes obtained in this work are highlighted in bold.

Oualkadi *et al.*, 2009; Nebish *et al.*, 2021; Ghrissi *et al.*, 2022). This redundancy might derive from the origin of this collection, as it was constructed after merging grapevine accessions from three different repositories (Table 1). Most of the duplicated accessions corresponded to widely propagated and cultivated table grape varieties like 'Cardinal', 'Italia', 'Alphonse Lavallee' or 'Muscat Hamburg', which are preserved in numerous grapevine collections around the world VIVC, (access 02/2024), so the concern of disappearance of these varieties is very low. Consequently, if the different accessions of these varieties do not present any remarkable phenotypic variation of interest, their presence in the collection of Ain Taoujdate could be limited to just one representative accession per genotype. This curation would ultimately render in a

more efficient use of limited human and budgetary resources, and surplus could be aimed for the preservation of a wider number of local cultivars. In fact, we only identified four table grape varieties from Maghrebian regions (including just two indigenous varieties from Morocco, 'Abouhou' and 'Taferi-elt'), even when it is known that the Maghreb is home of a high number of indigenous cultivars used for table grape production (Laiadi *et al.*, 2009; Zoghalmi *et al.*, 2009; Zinelabidine *et al.*, 2010; El Oualkadi *et al.*, 2011). The interest of preserving and exploring these underused indigenous varieties is increasing in the last decades, as grapevine genetic diversity is a powerful tool to fight current viticulture concerns (Wolkovich *et al.*, 2018). This is of special interest for traditional varieties adapted to semi-arid conditions, as they might hold

beneficial traits of adaptation to drought and warmer climate conditions (Morales-Castilla *et al.*, 2020).

Misnaming is a common issue in grapevine repositories, and it is suggested to affect up to 10% of the accessions preserved in national collections (Dettweiler-Münch, 1992). The origin of misnaming is uncertain, but it mostly derives from human errors committed during the management of plant resources, or during the transference of plant material between collections (El Oualkadi *et al.*, 2009; Nebish *et al.*, 2021). The genetic characterization of the table grape genetic resources preserved at the collection of Ain Taoujdate led to the identification of 12 misnamed accessions (20% of accessions). For example, the varieties ‘Italia’ and ‘Abouhou’ were found to be preserved as ‘Carriere’ and ‘King’s Ruby’ (syn. ‘Ruby seedless’), respectively. Another example of misnaming is the one found for the accession named “Teresa de prevane” (‘Teresa Pirovano’ misspelled), a pink-berried table grape variety bred by Alberto Pirovano in 1926 (‘Muscat of Alexandria’ × ‘Sultanina’) VIVC, (access 02/2024). Interestingly, the SSR profile obtained for this accession did not match the genetic profile of ‘Teresa Pirovano’, but it matched the genetic profiles of two varieties, ‘Barone Dell’Aterno’ (white-berried) and ‘Pirovano 190’ (pink-berried). These two varieties were generated in the same breeding cross (‘Delizia di Vaprio’ × ‘Angelo Pirovano’) (Lacombe *et al.*, 2013), and they share the same genetic profile for the seven SSRs reported in the VIVC database VIVC, (access 02/2024). Given that “Teresa de prevane” has pink berries, we definitely identified this grapevine accession as ‘Pirovano 190’.

Genetic profiling also revealed that many varieties were not preserved under their prime names, but using known synonyms. Synonymy is quite common in grapevine, as varieties are commonly renamed as soon as they are moved from one region to another (Cipriani *et al.*, 2010). For example, ‘Italia’ was preserved under its known synonym ‘Muscat d’Italie’, and ‘Afus Ali’ under the name “Dattier de bayrouth” (‘Dattier de Beyrouth’, misspelled). Interestingly, we identified the accession “Doukalia” (‘Doukkali’, misspelled) as ‘Bezoul el Khadem de Tunisie’. ‘Doukkali’ grape production dominates the Moroccan table grape market (Zinelabidine *et al.*, 2014), where it is also found as ‘Bezoul el Aouda’ and ‘Sidi Taybi’ (Zinelabidine *et al.*, 2010). The terms ‘Doukkali’ and “Doukalia” derives from Doukkala, a region in western Morocco. Today, ‘Doukkali’ and ‘Bezoul el Khadem de Tunisie’ are considered synonyms of the same variety VIVC, (access 02/2024). Nonetheless, the Moroccan variety ‘Doukkali’ has red berries (Kalili *et al.*, 2023) whilst the Tunisian variety ‘Bezoul el Khadem de Tunisie’ is registered in the VIVC database as a black-berried cultivar VIVC, (access 02/2024). Consequently, ‘Doukkali’ is probably a somatic variant of ‘Bezoul el Khadem de Tunisie’ or vice versa, showing somatic variation for berry skin colour. Besides, we found that some varieties were preserved using names that could be considered as new potential synonyms, since (to our knowledge) these terms have not been used to refer to any other variety (Table 1).

Lastly, we identified many misspelled names of the varieties preserved in the collection (“Danane” for ‘Danam’, “Lual” for ‘Lival’, or “Trijoli” for ‘Tripoli’), a common issue when transcribing Arabic names to French, as previously noted by El

Oualkadi *et al.* (2009). One case of extreme misspelling was the one found for the accession “Sabat kanstantini”, genetically identified as the variety ‘Sabalkanskoi’. Interestingly, the SSR profile of this accession matched the one of the variety ‘Ohanes Red’ stored at the VIVC database, which does not store any SSR data for ‘Sabalkanskoi’. However, the comparison of the SNP profile obtained for “Sabat kanstantini” with the 3,574 genetic profiles stored at the ICVV-DNA database revealed that it matched the one obtained after the previous analysis of two samples named “Sabalkanskoi” and two samples named “Red Ohanes” (syn. ‘Ohanes Red’). The “Sabalkanskoi” samples previously analyzed in our facilities came from Australia and Argentina, whilst the “Red Ohanes” samples came from the Spanish National Grapevine Collection of IMIDRA (Madrid, Spain), and from Almería (Southern Spain). Thus, our results based on SNP data indicated that ‘Sabalkanskoi’ and ‘Ohanes Red’ should be considered synonyms of the same variety. Given that the name ‘Ohanes Red’ might be wrongly considered as a red-berried variant of the Spanish white-berried variety ‘Ohanes’, we recommend the use of ‘Sabalkanskoi’ as the prime name of this variety. In fact, this variety is preserved in 21 international collections as ‘Sabalkanskoi’, and only in two as ‘Ohanes Red’ VIVC, (access 02/2024), suggesting that the name ‘Sabalkanskoi’ is more widely used at an international level. ‘Ohanes Red’ and ‘Sabalkanskoi’ are alleged varieties from Portugal and the former USSR, respectively VIVC, (access 02/2024). Although the genetic characterization performed in this work did not provide any additional clue to support if ‘Sabalkanskoi’ originated in Central Asia and then spread to the Iberian Peninsula (or if it happened the other way around), extended genetic structure studies cluster this variety with multiple new breeding table grape cultivars and some genotypes from the Balkans (Lau-cou *et al.*, 2018).

### Parentage analyses aid to guess the varietal identity of two unidentified accessions

DNA-based markers have proved to be a highly efficient tool to curate the breeding information of numerous grape varieties (Vargas *et al.*, 2009; Lacombe *et al.*, 2013). Here, our combined SSR and SNP genetic profiling approach confirmed the available breeding information of 15 table grape varieties, which were previously supported by means of SSR data (Table 2). Interestingly, we revealed the full pedigree of the unidentified accession named “Sultanine Rosée”. The variety ‘Sultanine Rose’ (registered in the VIVC database under the prime name ‘Kishmish Rozovyi’) is acknowledged as a pink-berried somatic variant of the white-berried seedless variety ‘Sultanina’ (Lijavetzky *et al.*, 2006), and it is confirmed by its full match for the SNP markers’ genotypes obtained in several accessions of those varieties included in the ICVV-DNA database. However, the genetic profile of the “Sultanine Rosée” accession preserved at the collection of Ain Taoujdate did not match the genetic profile of ‘Sultanina’ (Supplementary file) nor any of those preserved at the VIVC and ICVV databases, suggesting that it is a new (non-genotyped) variety. As for the true-to-type ‘Sultanine Rose’ (syn. ‘Kishmish Rozovyi’), the accession “Sultanine Rosée” is a pink-berried variety with seedless grapes, so we propose the name ‘Sultanine

| Table 3: Summary of the duos detected by SNP analyses.

Variety 1 (Chlorotype) <sup>a</sup>	Variety 2 (Chlorotype) <sup>a</sup>	SNP compared/mismatched	Pair LOD score
Dabouki (D)	Dabouki Arub (D)	217/0	44.78
<b>Diamant Noir (C)</b>	Moscato D'Adda (D)	210/1	29.64
Dominga (B)	Marsaoui (A)	223/1	29.53

<sup>a</sup> Chlorotypes obtained in this work are highlighted in bold.

Rose Faux' to refer to this variety. Interestingly, we found that 'Sultanine Rose Faux' is an offspring of 'Sultanina', obtained after its cross with the black-berried Greek variety 'Fokiano' (Table 2). In contrast to the widely spread variety 'Sultanina', 'Fokiano' is considered a rare and ancient Greek grapevine variety mostly grown in Eastern Greece, the Aegean Islands, and Crete. 'Fokiano' is also known under synonyms like 'Damaskinato', 'Erikaras', 'Giouroukiko', 'Iri-kara', and 'Rikaras' (Stavarakaki and Biniari, 2016). The limited extent of cultivation of 'Fokiano' and its role as mother in the cross suggests that 'Sultanine Rose Faux' might have originated as a spontaneous cross somewhere in Greece. However, we cannot discard that 'Sultanine Rose Faux' originated in a breeding program aimed to obtain new varieties with seedless grapes and improved features. In fact, 'Fokiano' has already been used in breeding programs, as seen for the red-fleshed variety 'Academician Trubilin' ('Fokiano' × 'Alicante Henri Bouschet') (Zamanidi *et al.*, 2011).

We also identified some duos of interest, not reported before in the bibliography (Table 3). We found a link between the varieties 'Dabouki Arub' and 'Dabouki', two alleged table grape varieties from Central Asia (Riaz *et al.*, 2013), as well as between the cultivars 'Marsaoui' and 'Dominga'. The latter could be explained as both cultivars are half-siblings that share a progenitor, 'Heptakilo' (Ghaffari *et al.*, 2014; Tello *et al.*, 2024). Interestingly, we found a duo between 'Moscato D'Adda' and the unidentified accession named "Diamant Noir". The name 'Diamant Noir' is acknowledged as a synonym of the black-berried table grape variety 'Diamante Nero', bred in 1931 by Alberto Pirovano VIVC, (access 02/2024) by crossing 'Pirovano 57' and 'Moscato D'Adda'. This breeding information agrees with the duo found in this work. In fact, the duo 'Diamante Nero' – 'Moscato D'Adda' has been previously supported by genetic analyses based on 20 SSR markers (Lacombe *et al.*, 2013). Unfortunately, we could not find genetic data of the true-to-type variety 'Diamante Nero' for a pairwise comparison with the genetic profile obtained for our accession. As for the true-to-type 'Diamante Nero', our accession "Diamant Noir" has hermaphrodite flowers and black and seeded berries with no special taste. Altogether, it is reasonable to think that the accession "Diamant Noir" preserved in Ain Taoujdate corresponds to the variety 'Diamante Nero'.

## Conclusions

The combined availability of standard sets of SSR and SNP molecular markers, and wide databases storing numerous genetic profiles and other data from varieties of diverse origin facil-

itated the proper identification of the table grape accessions preserved at the living collection of Ain Taoujdate (Morocco). We proved that molecular-assisted parentage analyses can be useful to provide valuable information on the origin and likely varietal identity of accessions lacking a matching reference genotype in wide international catalogues. Ultimately, the verification of the identity of the plant material preserved in this collection aided towards more efficient practices of management and conservation of genetic resources. Now, the varieties preserved in Ain Taoujdate can be supplied to local grape producers with variety certification.

## Supplementary Material

Supplementary data associated with this article can be found at Zinelabidine, L. H., Charafi, J., Haddioui, A., Martínez Zapater, J. M., Ibáñez, J., & Tello, J. (2024). SSR and SNP profiles obtained for 40 non-redundant grapevine genotypes found in the living collection of Ain Taoujdate (Morocco) [Data set]. Zenodo. <https://doi.org/10.5281/zenodo.11481989>.

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## Conflicts of interest

The authors declare that they do not have any conflicts of interest.

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