MOLECULAR CHARACTERIZATION OF MOROCCAN GRAPEVINE GERMPLASM USING SSR MARKERS FOR THE ESTABLISHMENT OF A REFERENCE COLLECTION

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Abstract

Aims: This study aims to characterize the SODEA ampelographic collection in Meknès, the only germplasm repository in Morocco. To assess the usefulness of this germplasm, a study was conducted to verify the trueness to type of the genotypes and to provide the first database for a reference collection in Morocco. A core collection was then established to define a small sample easier to handel further characterization.

Methods and results: Ninety-four grapevine samples from the collection were analyzed using 20 nuclear SSR markers. From these samples, we identified 67 grapevine genotypes. The nuclear SSRs revealed a high diversity within the SODEA collection: 202 alleles were detected with a mean of 10.1 alleles per locus. Analysis of molecular data with the software DARwin was used to classify the genotypes into six groups according to their origin or their genetic relatedness. The 18 autochthonous cultivars were differentiated according to geographical origin (North vs. South). We established a core collection among this germplasm using MSTRAT: the complete diversity present in the collection was captured with only 34 individuals. Nevertheless, an optimal core collection representing 89% of the diversity was constituted by only 17 cultivars. Among these 17 individuals, 5 are autochthonous.

Conclusion: The collection of SODEA represents a unique resources of grapevines for Morocco. It contains several important autochthonous cultivars in terms of diversity and agronomic utilisation.

Significance and impact of the study: The study showed potential and interest of the cultivars present in the collection of SODEA, suggesting that their utilisation may be important for the farmers.

Keywords: *Vitis vinifera* L, core collection, SSR markers, local germplasm

Résumé

Objectifs : Cette étude a pour objectif de caractériser la collection ampélographique de la SODEA à Meknès, l'unique conservatoire de ressources génétiques vignes existant au Maroc. Pour évaluer l'intérêt de ce conservatoire, une étude a été conduite afin de vérifier l'identité exacte des génotypes et d'établir la première base de données relative à cette collection de référence. Une *core* collection a ensuite été établie afin de définir un sous-échantillon restreint, plus facile à gérer pour de futures analyses.

Méthodes et résultats : Quatre-vingt quatorze échantillons de vigne prélevés dans la collection ont été analysés en utilisant 20 marqueurs microsatellites. À partir de ces échantillons, nous avons identifié 67 génotypes distincts. L'analyse de ces marqueurs a révélé une grande diversité au sein de la collection de la SODEA : 202 allèles ont été détectés avec une moyenne de 10,1 allèles par locus. Les données moléculaires ont été analysées avec le programme DARwin. Les génotypes ont pu être répartis en 6 groupes, en relation avec leur origine ou leur relation génétique. Les 18 cultivars autochtones ont été différenciés en fonction de leur origine géographique (Nord vs. Sud). Nous avons établi une *core* collection à partir de ces ressources génétiques en utilisant le logiciel MSTRAT : la diversité totale présente dans ce conservatoire peut être représentée par 34 individus. Toutefois, une *core* collection optimisée et constituée seulement de 17 cultivars permet de représenter 89 % de la diversité. Parmi ces 17 individus, 5 sont autochtones.

Conclusion : La collection de la SODEA représente l'unique ressource en variétés de vignes au Maroc. Elle renferme plusieurs cépages autochtones importants en termes de diversité et d'intérêt agronomique.

Signification et impact de l'étude : L'étude a permis de montrer le potentiel et l'intérêt des cultivars présents dans la collection de la SODEA et suggère que leur utilisation pourraît être importante pour les agriculteurs.

Mots-clés : *Vitis vinifera* L., *core* collection, marqueurs microsatellites, ressources génétiques locales

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INTRODUCTION

The evolution of cultivar uses (Bouquet and Boursiquot, 1999) shows, in the last six decades, a drastic reduction of diversity, with the overrepresentation of internationally renewed cultivars such as Cabernet-Sauvignon, Chardonnay and others. This evolution is thus performed to the detriment of the traditional autochthonous cultivars. This will lead to a substantial loss of genetic diversity, called genetic erosion.

In order to limit this genetic erosion, germplasm collections were created throughout the world with the aim to conserve existence of autochthonous diversity.

Contrary to the plants with sexual multiplication for which one seeks to preserve allelic diversity, perennial heterozygous plants with vegetative multiplication such as the grapevine require the conservation of genotypes (Brown, 1995). The size of collection thus increases proportionally to the number of samples. The perenniality of grape allows the installation of collections in field. Alleweldt and Dettweiler (1994) designate 141 collections of grapevines on a world level, located in 35 countries. Most of these collections take place in Europe (France, Italy, Romania...). However, the management of such collections require space, time and intensive labor. This management can also be rendered more difficult because of synonymies, homonymous or simply errors of identification.

In grape, synonymies are in fact very common (Sefc *et al.*, 2001; Riaz *et al.*, 2004; Montaner *et al.*, 2004; Ortiz *et al.*, 2004; Walker and Lowe 2006; This *et al.*, 2006a) and convergences of names for distinct cultivars (homonyms) also occur because of some peculiar features of the cultivars or because they grow in different regions (This *et al.*, 2006a). That is a major problem in the management of grapevine germplasm collections (Lopes *et al.*, 1999; Martin *et al.*, 2003).

In the early 1990, nuclear microsatellite markers were shown to be useful in determining cultivar identity and identifying mistakes (Thomas *et al.*, 1993; Thomas *et al.*, 1994). Germplasm collections have already been characterised using microsatellite markers (Aradhya *et al.*, 2003; Lopes *et al.*, 1999; Martin *et al.*, 2003; Sefc *et al.*, 2000) thus demonstrating the usefulness of these markers to solve problems of homonymies or synonymies (Sefc *et al.*, 2000; This *et al.*, 2006b). The markers have also been very useful for analyses of genetic diversity (Aradhya *et al.*, 2003; Lopes *et al.*, 1999; Martin *et al.*, 2003; Sefc *et al.*, 2003).

Genetic resources stored in gene banks are sampled with a final purpose of evaluating and utilising them efficiently, as well as studying phenotypic and genotypic diversity. Phenotypic analysis can be quite tedious and may require definition of core subsets. The aim in defining core subset is to preserve in the sample as much diversity present in the original collection as possible. The concept of core collections (or core subsets) was introduced to increase the efficiency of characterising and utilising the collections stored in gene banks while preserving the genetic diversity of the collection (Frankel and Brown, 1984; Brown, 1989).

In Morocco, the only grapevine repository belongs to the SODEA (an agricultural development company located in Meknès). The collection was created by J.P. Vidal in 1952 at the former École Nationale d'Agriculture (now INA) in Meknès. Vidal collected autochthonous vines from different regions, and especially from the Rif region in northern Morocco. The collection was enriched thanks to further prospecting in Morocco, and with foreign cultivars of table and wine grapes to reach 94 accessions.

The transfer of the collection from Meknès to the SODEA took place in 1985. Unfortunately, this transfer was made by workmen of low expertise without scientific supervision. Errors may thus have arisen at the time of transfer: mix up in the wood harvesting, in grafting, installation, use of marcots for further plant replacement, etc.

Accurate identification of the 94 accessions of this collection is thus a preliminary step. Members of the user community typically request germplasm based on traits known to be present in specific species, cultivars, accessions or genotypes (Kresovich and McFerson, 1992; McFerson *et al.*, 1996; Peeters and Galwey, 1988). In order to identify the exact content of the germplasm collection and the extent of its diversity, we analyse the 94 accessions with 20 nuclear SSR microsatellite markers (Di Vecchi Staraz *et al.*, 2007; Lacombe *et al.*, 2007).

After correct identification of the cultivars, a core subset of this collection was developed based, on the 20 SSR markers. In order to improve the potential usefulness of this collection, we also analysed some cultural characteristics of the cultivars, on the duplicate present in INRA Vassal grapevine collection, Marseillan, France (http:/www.montpellier.inra.fr/vassal).

MATERIAL AND METHODS

1. Plant material

The Moroccan grapevine collection located at the SODEA (Meknès, Morocco) was used for this study. The collection contained 94 accessions (mainly *Vitis vinifera*) represented by 22 plants per accession collected essentially from Northern of Morocco (Table 1). We defined an accession as one or several grapevine plants under the

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same denomination and registration number. Molecular characterization was performed on one plant per accession sampled in the field.

2. Molecular analyses

Young leaves were collected in spring and freeze dried (for 24 hours at 0.370 mbar and -55 °C after harvesting). Extraction was performed with a Qiagen DNeasy Plant Mini Kit with minor modifications: addition of 1% w/v of PVP-40 to the AP1 solution, addition of 180 µL AP2 and centrifugation for 10 min at 6,000 rpm.

Microsatellite analyses were performed on 20 microsatellite markers (SSRs) well distributed across the 19 grape chromosomes (Doligez *et al.*, 2006) as previously described (Di Vecchi Staraz *et al.*, 2007; Lacombe *et al.*, 2007): 2 of the VMC series (VMC1b11, VMC4f3; Vitis Microsatellite Consortium, Adam-Blondon *et al.*, 2004), 9 of the VVI series (VVIv67, VVIn16, VVIp60, VVIp31, VVIv37, VVIq52, VVIb01, VVIn54, VVIn73, Merdinoglu *et al.*, 2005), 8 of the VVMD series (VVMD28, VVMD27, VVMD21, VVMD25, VVMD32, VVMD7, VVMD24, VVMD5; Bowers *et al.*, 1996, 1999), and VVS2 (Thomas and Scott, 1993; Thomas *et al.*, 1994).

Amplifications were performed using a PTC100 (MJ Research) apparatus. Reactions were performed on a mixture (20 µL final volume) containing genomic diluted DNA (10 ng/µl), 2 µL of buffer 10X (Qiagen), 200 µM of deoxynucleoside triphosphate, 2.5 mM/$MgCl_2$, 0.08 µL Taq polymerase (Qiagen), 0.32 pM of the unlabelled primer and a variable quantity of the labelled primer depending on the marker.

Amplification conditions included an initial denaturation of 4 min at 95 °C followed by 35 cycles of 1 min at 94 °C, 1 min at 56 °C for all loci (except for locus VMC1b11 and VMC4f3 at 60 °C), 2 min at 72 °C, with a final extension for 6 min at 72 °C. Multiplex were planned with a maximum of three colours, taking into account the size of the amplified fragments and up to seven markers per sequencing run were mixed as previously described by Di Vecchi Staraz *et al.* (2007) and Lacombe *et al.* (2007).

3. Data analysis

Standard measures of genetic variation, including number of alleles per locus, allele frequencies, observed heterozygosity (Ho), and their standard deviation were calculated using the macro MS® Excel Microsatellite Toolkit elaborated by Park, (2001). Expected heterozygosity values were computed under the Hardy-Weinberg hypothesis according to the no-bias model (Nei, 1987) using Genetix 4.05 software (Belkhir *et al.*, 2004).

We estimated the frequency of null alleles for each locus using the program Micro-Checker (Van Oosterhout *et al.*, 2004). The Discrimination power D was calculated as follows: $Dj= \sum pi\ (Npi-1)/(N-1)$ where pi was the frequency of the i-th molecular pattern revealed by primer or locus j, and N was the number of genotypes (Tessier *et al.*, 1999).

A clustering was built using DARwin v.5.0.156 software (Perrier *et al.*, 2003) and applying the weighted neighbour-joining method to the dissimilarity matrix.

To know the cultivars which represent more diversity and could be used later in the other studies, a core collection starting from the 67 single individuals was established using the MStrat software (Gouesnard *et al.*, 2001).

4. Agronomic traits

Eighteen agronomic characters used for descriptions of the cultivars were taken from the data available in the data base of Vassal (http://bioweb.supagro.inra.fr/collections_vigne/main.php).

RESULTS

1. Identification of genotypes based on SSR

Because a safety duplicate of the autochthonous cultivars was send to INRA Vassal collection by J.P. Vidal in 1954, molecular data from the SODEA collection was compared to the data available with the same markers on the Vassal collection (Laucou *et al.*, in preparation). As a first step, the profiles of the 94 accessions were compared to each others: based on a complete concordance of the SSR data, we could differentiate 67 different genotypes among the 94 accessions (Tables 1 and 2).

Forty-nine of the genotypes were assigned to only one accession (for example Merlot rouge, genotype 1) whereas 18 genotypes were common to 2 to 6 accessions, for example Sultanine rosée and Delight had the same genotype (8). The most repeated genotype was genotype 24 which corresponds, under different names, to cv. Muscat d'Alexandrie.

The unique profiles were then compared to the 2,853 genotypes of the Vassal collection. Except for five genotypes, the 62 others correspond to genotypes present in the Vassal collection and therefore to identified cultivars (Table 1). The 94 accessions can be thus divided into five groups:

a. Five accessions (referred as U in table 1) showed an original profile not present in the Vassal collection. The 69 MAR accession (genotype 47) named Aramon

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Table 1 - List of grapevines analysed in this study sorted according to their SSR genotype.

Accession number	Accession name	Genotype	Prime name in Vassal collection	Country of origin	Use [1]	Identification [2]
01MAR	Merlot rouge	1	Merlot	France	C	S
02MAR	Cot chernagua	2	Cot de Chéragas	Algeria	C	T
03MAR	Mireille	3	Mireille	France (cross)	T	I
04MAR	El bioud	4	Airen	Spain	CT	T
05MAR	El karim	4	Airen	Spain	CT	L
06MAR	Zerhoune	4	Airen	Spain	CT	L
07MAR	El gouz	5	El Gouz	South of Morocco	T	I
08MAR	IP 75	6	Sultana moscata = Pirovano 75	Italy (cross)	T	S
09MAR	Ceprissize	7	Rosa menna di vacca	Romania ou Hungary	T	PM
10MAR	Sultanine rosée	8	Sultanine	Turkey	T	T
11MAR	Delight	8	Sultanine	Turkey	T	PM
12MAR	Sultanine	8	Sultanine	Turkey	T	I
13MAR	Bezoult al aouda	9	Bezoul el Khadem	South of Morocco	T	L
14MAR	Sidi taybi	9	Bezoul el Khadem	South of Morocco	T	L
15MAR	38K	10	Dattier de Beyrouth	Middle East	T	P
16MAR	Rosaqui	10	Dattier de Beyrouth	Middle East	T	PM
17MAR	Olivette blanche	10	Dattier de Beyrouth	Middle East	T	PM
18MAR	Dattier de beyrouth	10	Dattier de Beyrouth	Middle East	T	I
19MAR	Sbaa Labnat	11	Olivette Barthelet	North of Morocco	T	S
20MAR	Blanc de ghafsai	12	Blanc de Rhafsaï	North of Morocco	T	T
21MAR	Djinani	13	Jaen	Spain	C	X
22MAR	Bouzouga	14	Bouzouga	South of Morocco	T	I
23MAR	Boujlida	15	Cañocazo	Spain	C	X
24MAR	Tiniouine	16	Tiniouine	South of Morocco	T	I
25MAR	Tyni	17	Tini	North of Morocco	T	T
26MAR	IP46	18	Delizia di Vaprio = Pirovano 46 a	Italy (cross)	T	T
27MAR	Calmere	19	Calmeria	USA (cross)	T	T
28MAR	Bougseb	20	Dedo de dama	North of Morocco	T	L
29MAR	Boukhenzir	21	Bou Khanzir blanc	North of Morocco	T	T
30MAR	Azizi el hour	21	Bou Khanzir blanc	North of Morocco	T	L
31MAR	Musca teracina	22	Muscat de Terracina	Italy	T	T
32MAR	Musca setubal	23	Diagalves	North of Morocco	CT	PM
33MAR	Muscat hbique	24	Muscat d'Alexandrie	Unknown	CT	L
34MAR	Muscat Sali	24	Muscat d'Alexandrie	Unknown	CT	L
35MAR	Muscat zerhoun	24	Muscat d'Alexandrie	Unknown	CT	L
36MAR	Muscat	24	Muscat d'Alexandrie	Unknown	CT	L
37MAR	Muscat doukhala	24	Muscat d'Alexandrie	Unknown	CT	L
38MAR	Muscat d'alexandrie	24	Muscat d'Alexandrie	Middle East	CT	I
39MAR	Muscat pearson	25	-	U.K. (interspecific cross)	Unk	U
40MAR	Muscat madersfield	26	Muscat Madresfield Court	U.K. (cross)	T	T
41MAR	Muscat sefrou	27	Jerez fina	Spain	C	PM
42MAR	Valency	27	Jerez fina	Spain	T	X
43MAR	Valency royale	27	Jerez fina	Spain	T	X
44MAR	Asserghine 1	28	-	South of Morocco	Unk	U
45MAR	Valency noir	29	Danugue	Spain	T	S
46MAR	Azizi el jeia	30	Azizi el Jaïa	North of Morocco	T	T
47MAR	Aguyer 1	31	Abouhou	South of Morocco	T	X
48MAR	Aguyer 2	31	Abouhou	South of Morocco	T	X
49MAR	Asserghine n°2	32	-	South of Morocco	Unk	U
50MAR	Muscat sefrou n°2	33	Muscat d'Istamboul	Middle East	C	L
51MAR	Muscat romain	33	Muscat d'Istamboul	Middle East	C	L
52MAR	Bouchouka	34	Bouchouka	South of Morocco	T	I
53MAR	Carignan	35	Carignan	Spain	C	I
54MAR	Grenache noir	36	Grenache	Spain	C	I
55MAR	Grenache	36	Grenache	Spain	C	I
56MAR	X1	37	Alicante Henri Bouschet	France (cross)	C	P
57MAR	Tempranillo	38	Syrah	France	C	PM
58MAR	Syrah	38	Syrah	France	C	I
59MAR	Mourvedre	39	Mourvèdre	Spain	C	I
60MAR	Morastel	39	Mourvèdre	Spain	C	PM
61MAR	Cinsault	40	Aspiran noir	France	C	PM
62MAR	Ugni blanc	41	Ugni blanc	Italy	C	I
63MAR	Maccabeo	42	Macabeu	Spain	C	T
64MAR	Taferielt	43	Taferielt	North of Morocco	CT	I
65MAR	Gros vert	44	Gros vert	France	T	I
66MAR	Sijani	44	Gros vert	France	T	X
67MAR	Muscat italia	45	Italia = Pirovano 65	Italie (cross)	T	L
68MAR	Cognac	46	Ahmeur bou Ahmeur	North of Morocco	T	S
69MAR	Aramon SODEA	47	-	Morocco	C	U
70MAR	Plant Y	48	Negra mole	Spain	C	X
71MAR	Plant X	49	Nave	North Africa	C	S
72MAR	Clairette	50	Clairette	France	C	I
73MAR	Ferrana	51	Bourboulenc	France	C	PM
74MAR	Chasselas	52	Ruby seedless	USA (cross)	T	PM
75MAR	Kings ruby	52	Ruby seedless	USA (cross)	T	S
76MAR	Muscat hambourg	53	Muscat de Hambourg	Unknown	T	I
77MAR	Ohanez (Omenez)	54	Aledo	Spain	T	PM
78MAR	Hybride noir	55	Colobel = Seibel 8357	France (interspecific cross)	C	L
79MAR	Hybride blanc	56	-	Morocco	Unk	U
80MAR	Aabou	57	Aguyar	North of Morocco	T	X
81MAR	Laaderi	57	Aguyar	North of Morocco	T	X
82MAR	Olivette noir	58	Dattier noir	Middle East	T	PM
83MAR	X2	59	Alphonse Lavallée	France or U.K. (cross)	T	P
84MAR	Alphonse Lavallee	59	Alphonse Lavallée	France or U.K. (cross)	T	I
85MAR	Servent	60	Servant	France	T	T
86MAR	Aarbia	61	Planta mula	Spain	CT	L
87MAR	Cabernet blanc	62	Semillon	France	C	PM
88MAR	Cabernet Sauvignon	63	Cabernet-Sauvignon	France	C	I
89MAR	Perlette	64	Perlette	USA (cross)	T	I
90MAR	Cardinal	65	Cardinal	USA (cross)	T	I
91MAR	Raisin tomate	66	Maticha	North of Morocco	T	T
92MAR	X3	66	Maticha	North of Morocco	T	P
93MAR	Jacomar	67	Seibel 1020	France(interspecific cross)	C	X
94MAR	X4	67	Seibel 1020	France (interspecific cross)	C	P

1 - Use for wine (C), Table (T) or both (CT), unknow (Unk)

2 - Identification refers to codes described in the text

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Table 2 - Allele sizes (bp) at 20 SSRs for the 67 cultivars involved in the diversity analysis (first part).

Cultivar name	VMC1b11		VMC4f3		VVIb01		VVIh54		VVIn16		VVIn73		VVIp31		VVIp60		VVIq52		VVIv37		VVIv67		VVMD21		VVMD24		VVMD25		VVMD27		VVMD28		VVMD32		VVMD5		VVMD7		VVS2	
Merlot	171	184	171	202	290	294	165	165	149	149	263	267	182	188	311	328	77	79	159	169	368	368	241	246	206	210	238	248	184	188	227	233	239	239	223	234	239	247	137	149
Cot de Chéragas	171	184	177	185	290	290	165	167	151	151	263	263	178	188	319	324	83	83	157	167	353	368	241	247	206	208	240	254	178	186	243	257	239	255	223	238	239	249	131	141
Mireille	171	171	205	207	290	294	163	165	155	155	263	263	178	192	315	319	83	83	159	173	368	368	253	263	206	210	238	240	176	178	233	267	271	271	229	234	243	247	131	131
Airen	184	184	171	185	290	290	165	165	149	151	263	263	188	188	319	319	79	83	157	159	348	362	241	263	206	210	254	254	178	191	233	243	251	271	223	232	243	255	141	143
El Gouz	165	165	187	202	290	294	165	166	149	151	261	263	182	190	315	317	79	83	157	177	353	386	241	253	206	210	238	262	176	180	247	257	249	255	232	238	239	249	149	149
Sultana moscata = Pirovano 75	165	184	179	189	290	294	165	165	149	155	261	263	178	186	315	315	83	83	155	171	370	372	247	253	210	215	248	248	191	191	216	267	249	263	225	232	239	249	147	149
Rosa menna di vacca	184	188	165	171	290	294	165	177	147	149	263	263	178	182	324	326	79	79	159	167	358	361	241	253	206	210	240	248	178	182	235	257	251	271	229	244	239	247	137	141
Sultanine	165	184	187	189	290	298	163	165	151	155	261	263	178	182	315	317	79	83	155	177	353	353	247	253	206	215	238	248	178	191	216	243	249	249	232	232	239	253	143	149
Bezoul el Khadem	165	169	202	202	290	290	165	175	147	155	263	263	182	190	315	319	79	83	167	177	371	371	247	253	204	210	238	244	176	191	243	247	249	255	232	236	233	247	131	153
Olivette Barthelet	184	188	182	184	290	294	165	165	149	151	263	263	188	190	319	319	79	79	157	157	353	362	247	247	206	206	254	254	186	191	243	243	255	271	234	236	249	249	133	135
Blanc de Rhafsaï	165	188	171	202	290	306	165	167	151	151	263	263	174	184	319	328	83	83	159	159	353	372	253	253	206	206	238	240	178	191	233	259	255	271	234	236	243	249	133	143
Jaen	167	188	185	202	290	306	165	167	149	151	263	263	174	178	319	319	79	83	159	173	362	372	247	253	206	208	240	254	178	178	233	247	251	255	232	234	243	249	135	143
Bouzouga	165	184	184	202	290	290	165	167	147	151	263	263	178	190	315	315	83	83	157	157	353	386	241	247	204	206	254	254	176	182	243	259	251	261	236	238	249	253	149	149
Cañocazo	184	188	185	206	290	306	165	165	151	151	263	263	186	188	315	324	79	83	159	173	353	368	247	253	206	206	240	254	182	191	233	247	255	271	232	234	243	249	141	143
Tiniouine	165	169	187	202	290	290	165	166	147	155	263	263	182	190	317	319	79	83	145	177	355	371	241	253	210	210	248	248	176	191	247	257	249	255	232	232	233	249	149	149
Tini	165	178	184	185	290	306	165	166	149	151	261	263	182	190	311	319	79	83	155	157	355	361	253	253	206	206	254	254	182	191	235	247	255	255	223	229	249	253	133	135
Delizia di Vaprio = Pirovano 46 a	165	184	171	206	294	306	165	165	149	149	261	263	190	192	319	319	77	79	159	171	361	386	246	253	206	210	238	248	191	191	233	243	263	271	223	229	249	251	131	131
Calmeria	165	184	184	189	294	298	163	165	147	151	263	263	178	178	315	315	79	83	149	177	353	358	247	253	206	206	248	254	180	191	243	243	249	261	232	232	233	239	131	149
Dedo de dama	169	184	185	202	290	294	165	167	147	151	263	263	174	178	315	317	79	83	149	157	353	372	247	247	206	210	254	270	182	191	233	259	251	251	223	236	249	253	137	149
Bou Khanzir blanc	171	184	171	185	290	290	166	167	149	151	261	263	178	186	313	317	79	83	157	159	361	371	247	247	206	206	254	254	178	191	233	257	251	251	223	223	249	253	141	149
Muscat de Terracina	182	184	165	182	294	294	163	165	147	151	263	263	182	186	315	324	77	83	157	161	359	361	241	263	210	215	238	240	176	191	247	257	257	271	223	225	249	249	131	133
Diagalves	184	188	185	185	290	290	165	165	151	151	263	263	174	178	317	324	83	83	157	157	353	368	247	247	206	206	254	254	178	182	233	259	251	271	232	236	243	253	141	149
Muscat pearson SODEA	171	184	179	206	288	294	165	165	147	151	263	263	178	186	303	319	77	79	167	171	353	386	247	253	206	210	238	248	176	182	259	267	251	263	225	234	249	251	131	131
Muscat Madresfield Court	165	173	171	180	290	294	165	165	147	149	263	263	178	186	303	315	77	83	159	167	357	371	253	263	206	210	248	254	176	182	259	267	271	271	225	225	239	249	147	149
Asserghine 1 SODEA	169	184	187	202	290	290	165	167	147	149	263	263	174	190	315	317	79	83	155	177	353	353	241	247	206	210	238	254	176	182	243	247	249	255	223	238	239	249	131	135
Danugue	188	188	182	206	290	290	165	165	149	149	256	263	174	182	319	319	79	83	149	167	353	362	241	253	206	206	240	254	191	191	235	243	261	271	225	232	233	239	135	143
Azizi el Jaïa	171	184	184	185	290	306	165	165	151	151	261	263	182	190	311	319	79	83	167	173	353	360	247	253	206	206	254	256	182	191	235	259	255	255	225	229	249	253	133	135
Abouhou	165	169	184	202	290	290	165	165	151	155	263	263	182	190	315	317	79	83	157	177	355	386	241	253	204	210	248	254	176	182	243	247	249	261	234	238	249	249	149	149

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Table 2 - Allele sizes (bp) at 20 SSRs for the 67 cultivars involved in the diversity analysis (second part).

Cultivar name	VMC1b11		VMC4f3		VVIb01		VVIh54		VVIn16		VVIn73		VVIp31		VVIp60		VVIq52		VVIv37		VVIv67		VVMD21		VVMD24		VVMD25		VVMD27		VVMD28		VVMD32		VVMD5		VVMD7		VVS2	
Asserghine 2 SODEA	169	171	184	187	290	290	165	165	151	157	263	263	178	188	315	317	79	83	163	163	353	371	248	248	206	210	254	254	182	182	243	247	251	261	223	225	233	249	133	149
Muscat d'Istamboul	184	188	179	184	290	294	165	165	147	151	256	263	186	190	315	319	77	77	157	171	361	386	253	263	208	210	248	254	176	186	243	257	255	271	225	234	249	251	131	141
Bouchouka	165	169	185	187	290	290	165	165	151	151	263	263	178	184	315	317	79	79	149	149	371	371	247	253	206	210	248	254	176	182	243	259	249	251	223	238	249	253	131	149
Carignan	173	184	177	185	290	294	165	165	149	151	263	263	174	176	315	324	77	79	159	167	358	371	247	251	206	212	240	254	178	182	247	257	249	251	223	225	239	239	141	143
Alicante Henri Bouschet	182	188	171	206	290	294	163	167	149	157	256	263	174	182	319	319	77	83	157	167	353	361	241	247	206	208	240	240	178	191	243	259	249	271	223	236	239	243	131	143
Syrah	165	188	171	206	290	294	163	165	149	151	261	263	180	188	315	315	83	83	159	161	357	377	246	263	206	212	240	240	186	188	216	227	239	271	223	229	239	239	131	131
Mourvèdre	171	188	177	177	290	290	165	165	151	157	263	263	178	190	315	319	83	83	161	167	353	360	241	247	206	215	240	262	176	186	243	257	239	255	223	238	249	249	131	149
Grenache	188	194	185	205	288	290	163	167	151	157	256	263	174	182	315	319	77	83	157	167	353	360	241	247	208	214	240	254	191	191	243	243	239	249	223	238	239	243	135	143
Aspiran noir	165	184	177	187	290	294	163	167	149	151	263	263	178	186	315	319	83	83	157	159	358	360	247	253	206	210	240	248	176	178	227	257	239	261	223	223	247	249	131	131
Ugni blanc	184	184	171	185	294	294	163	167	149	149	256	263	182	188	324	330	77	83	159	167	361	372	241	247	206	206	240	254	176	180	243	247	249	271	223	229	249	253	131	141
Macabeu	184	184	177	185	290	294	165	165	151	151	263	263	174	194	315	324	79	83	155	157	368	371	241	251	206	208	238	240	186	191	235	257	249	255	232	234	239	239	131	143
Taferielt	165	184	171	173	290	306	165	167	151	151	263	263	184	188	319	328	79	83	149	159	353	361	241	253	206	206	238	240	191	191	233	259	251	271	236	238	249	249	131	133
Gros vert	169	184	171	184	294	294	165	167	149	149	263	263	180	188	319	319	79	83	149	167	353	368	247	253	206	210	238	254	176	191	233	247	239	271	223	236	243	249	131	135
Italia = Pirovano 65	171	184	171	206	290	294	163	165	155	157	263	263	178	186	319	319	81	83	167	173	368	371	247	253	206	210	238	248	176	191	233	243	251	271	229	236	243	247	131	147
Ahmeur bou Ahmeur	184	184	187	187	290	294	165	167	149	151	263	263	182	190	319	319	77	83	155	157	353	361	247	253	206	206	254	266	180	191	247	253	251	255	229	236	239	249	133	145
Jerez fina	184	188	184	185	290	294	163	165	149	151	256	263	188	190	315	319	77	79	157	159	361	368	247	253	206	208	254	254	178	186	243	257	255	271	234	238	243	249	133	141
Aramon SODEA	182	184	177	177	288	294	163	163	151	157	263	263	182	188	315	319	79	83	159	167	361	361	241	247	206	210	240	248	176	182	227	257	239	255	223	223	239	243	131	149
Negra mole	184	188	165	202	290	290	166	167	149	151	263	263	174	190	319	319	79	83	159	159	347	361	241	263	204	208	240	254	178	178	243	257	251	271	219	238	239	239	141	143
Nave	184	188	185	206	290	294	165	165	149	151	256	263	174	190	315	315	77	77	159	159	360	368	247	247	206	208	240	254	178	186	235	243	239	271	225	238	239	243	131	141
Clairette	182	184	171	187	288	294	165	165	149	157	263	263	178	182	315	319	83	83	159	173	357	360	247	247	206	210	240	262	176	188	227	257	255	261	223	229	239	249	135	149
Dattier de Beyrouth	184	184	165	187	290	294	165	177	149	149	263	263	172	182	315	315	77	79	147	159	353	357	253	253	206	206	248	254	182	182	233	257	257	271	223	229	239	249	131	133
Bourboulenc	182	182	171	187	288	290	163	165	149	149	263	263	182	188	317	319	79	83	159	167	353	360	247	247	206	210	240	248	182	186	233	257	255	255	223	223	239	239	131	131
Ruby seedless	165	184	179	184	290	294	165	167	149	151	256	263	186	188	315	315	77	83	159	171	368	368	247	253	206	215	238	248	191	191	257	267	263	271	225	234	239	249	131	149
Muscat de Hambourg	165	171	171	206	294	294	165	165	149	155	263	263	178	186	315	319	77	81	159	167	368	386	247	253	210	210	248	254	176	182	235	243	271	271	229	236	247	249	133	147
Aledo	165	188	185	187	294	294	165	167	149	151	263	263	188	190	319	319	79	83	157	159	353	368	253	253	206	208	238	254	178	186	243	247	251	271	229	238	239	243	131	141
Colobel = Seibel 8357	167	171	163	206	290	298	161	161	145	151	254	254	174	176	319	319	83	83	159	159	329	333	226	247	204	214	240	248	182	188	235	259	249	271	223	223	239	251	131	131
Hybride blanc SODEA	171	184	173	180	290	294	167	167	149	149	263	263	174	188	315	321	79	83	147	167	353	368	247	247	206	210	254	254	178	178	233	257	271	271	223	229	237	239	133	143
Aguyar	169	184	187	187	290	290	165	165	147	155	263	263	182	190	315	317	79	83	157	177	355	371	241	253	206	210	248	254	176	191	247	257	249	255	223	232	233	249	135	149
Dattier noir	184	188	163	202	290	290	139	159	149	151	263	263	172	188	315	316	77	83	151	157	353	355	247	263	206	215	244	248	178	191	243	243	249	273	225	229	239	253	141	147
Alphonse Lavallée	165	173	165	206	294	294	163	165	149	149	256	263	186	186	319	324	77	79	155	159	353	368	247	247	206	210	238	254	182	182	243	243	251	271	223	236	249	255	131	133
Servant	184	188	177	187	290	294	163	165	151	157	263	263	182	188	317	319	83	83	157	167	353	368	247	253	206	215	238	254	180	182	243	257	239	249	223	232	243	249	131	131
Planta mula	165	188	171	173	290	306	165	165	149	157	263	263	186	190	315	319	79	83	167	173	353	361	241	253	206	210	240	240	178	180	243	253	255	271	234	238	239	249	131	131
Semillon	171	188	165	171	290	294	163	163	149	151	263	263	190	192	315	315	79	83	167	167	360	371	247	247	214	215	240	248	172	182	233	243	239	271	234	236	239	257	131	131
Cabernet-Sauvignon	184	184	171	177	290	290	165	179	151	151	263	267	188	188	303	311	77	83	159	159	360	368	247	255	206	215	238	248	172	186	233	235	239	239	229	238	239	239	137	149
Perlette	165	188	165	187	290	290	165	165	149	155	261	263	172	178	315	317	79	83	147	177	368	368	247	253	206	206	238	240	176	178	233	243	249	271	232	234	247	253	131	143
Cardinal	173	184	165	165	290	294	165	165	149	155	256	263	178	186	315	319	79	83	155	159	353	368	247	263	206	206	254	254	176	182	243	267	251	271	223	234	249	249	133	133
Muscat d'Alexandrie	165	184	179	205	290	294	165	165	147	149	263	263	186	190	315	319	77	77	159	171	371	386	253	263	210	210	248	248	176	191	243	267	263	271	225	229	249	251	131	147
Maticha	171	184	165	202	290	306	165	167	147	151	263	263	174	184	319	319	77	79	157	159	361	371	241	247	206	206	238	270	186	191	233	259	251	257	225	238	249	253	133	137
Seibel 1020	165	180	168	206	284	290	167	167	145	151	254	263	176	182	315	320	87	87	159	159	333	377	226	247	204	210	236	248	188	198	235	247	251	251	229	250	239	251	131	149

was certainly mislabelled since it does not correspond to the true to type cv. Aramon. We consequently renamed it as cv. Aramon faux SODEA;

b. Twenty-two accessions (I) were identical in the two collections (same genotypes, same names);

c. Five accessions (for example X1) whose names were probably lost (P), and whose genotypes were identical to a cultivar in the Vassal collection;

d. Forty-eight accessions from each collection share SSR genotypes but have different names in the SODEA and Vassal collection, they can be subdivided into:

- Fourteen accessions whose names are not completely identical (T): Cot chernagua, Sultanine rosée, Blanc de ghafsai, Tyni, Clamere, Boukhenzir, Valency and Maccabeo. They are probably either spelling errors (especially the autochthonous cultivars), or French translations of Arabic names (e.g. Raisin tomate vs. Maticha, genotype 66).

- Seven cases of known synonyms (S) reported by Galet (2000), i.e. the same cultivars but different names, such as Cognac, (genotype 46, and known synonym of cv. Ahmeur Bou Ahmeur) or Valency noir (genotype 29, known synonym of Danugue) for example.

- Sixteen possible cases of new synonyms which correspond to names of a person or a locality (L).

- Eleven accessions (X) which shared genotypes with an accession in the Vassal collection but had a unique name. Due to the absence of reference, we cannot draw conclusions, but they may represent new synonyms.

e. Fourteen obvious errors (PM), because they shared their genotype with true to type cultivars belonging to the Vassal collection, but bore the name of another cultivar.

The following work will be conducted on the 67 unique genotypes.

2. Nuclear SSR polymorphism and structure of diversity

Table 3 - Genetic variability present in the sample analysed in this study.

Locus	Number of alleles	Number of Genotypes	Expected heterozygosity	Observed heterozygosity	Discriminating power (D)	null allele present
VMC1b11	11	23	0.79	0.84	0.79	No
VMC4f3	17	41	0.90	0.90	0.91	No
VVIb01	6	10	0.59	0.63	0.59	No
VVIh54	10	13	0.58	0.53	0.59	No
VVIn16	6	13	0.71	0.72	0.72	No
VVIn73	5	6	0.31	0.32	0.31	No
VVIp31	12	29	0.86	0.93	0.87	No
VVIp60	13	20	0.73	0.68	0.73	No
VVIq52	5	9	0.64	0.72	0.65	No
VVIv37	14	32	0.84	0.82	0.84	No
VVIv67	18	39	0.87	0.87	0.88	No
VVMD21	9	16	0.73	0.78	0.74	No
VVMD24	7	14	0.65	0.72	0.65	No
VVMD25	10	18	0.77	0.75	0.77	No
VVMD27	10	24	0.82	0.82	0.83	No
VVMD28	10	25	0.84	0.93	0.85	No
VVMD32	9	25	0.82	0.81	0.83	No
VVMD5	10	25	0.85	0.87	0.85	No
VVMD7	10	21	0.78	0.82	0.79	No
VVS2	10	26	0.82	0.78	0.83	No
Total	202	429	-	-	-	-
Mean	10.1	21.45	0.75	0.76	0.75	-

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A total of 202 alleles were obtained using twenty SSR loci on the 67 unique cultivars (Table 3). The number of alleles per locus varied from five (locus VVIN73 and VVIq52) to 18 (locus VVIv67) with an average of 10.1 alleles per locus. A total of 429 genotypes were detected, VVIv67 presents the bigger number of genotypes (39) and VVIn73 the lowest number. Observed heterozygosity ranged from 0.32 (VVIn73) to 0.93 (VVIp31) with an average over all loci of 0.76 (Table 3).

Compared to the expected heterozygosity value, a significant heterozygote deficiency was observed for VVIh54, VVIp60, VVIv37, VVMD25, VVMD32 and VVS2 loci, probably due to a selection effect that would have occurred within a limited gene pool.

The discriminating power of each SSR locus used in this study varied from 0.31 to 0.91 (Table 3). The discriminating power variation among loci was due to the number of alleles per locus, and also to alleles frequencies. Locus VVMD5, VVMD28, VVIv37, VVMD27, VVMD32 and VVS2 were more discriminating. The test of null allele carried out on the whole set of genotypes studied did not revealed the presence of any null allele in this population (Table 3).

A dendrogram was drawn using SSR data for the 67 unique genotypes present in the SODEA collection (figure 1). The clustering distinguished seven well-differentiated groups (A, B, C, D, E, F and G). We have observed good differentiation of the cultivars according to their origin or their genetic relatedness: Group A corresponds to the genotypes of Muscat or classical table grape cultivars of diverse origins (except cv. Ugni blanc, a wine cultivar); Group B corresponds to wine cultivars of French origin, including the two interspecific hybrids; Seibel 8357 (*V. vinifera* x *V. rupestris* x *V. aestivalis* x *V. cinerea* x *V. berlandieri*) and Seibel 1020 (*V. rupestris* x *V. lincecumii* x *V. vinifera* cv. Aramon); Group C

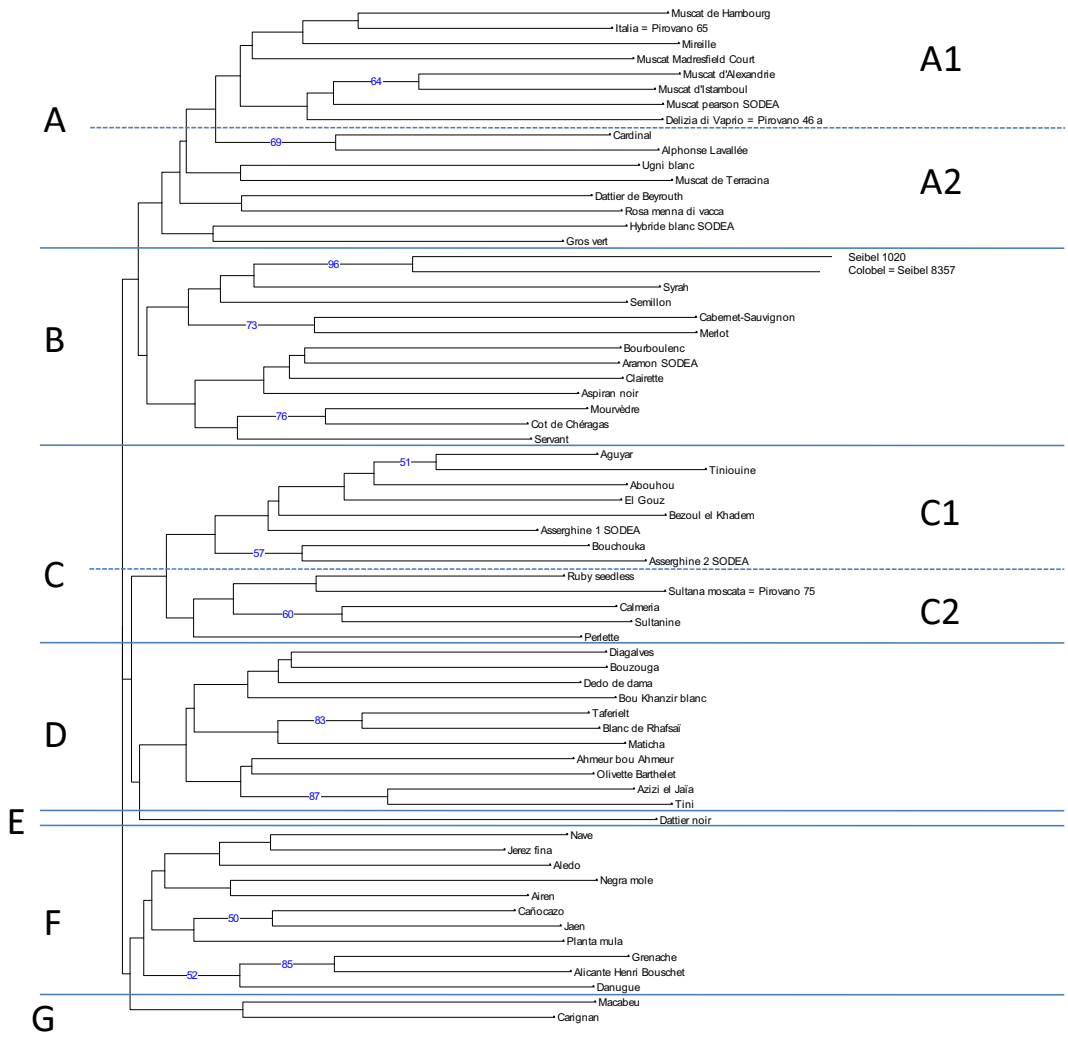


Figure 1 - Dendrogram illustrating the genetic relationships established between 67 cultivars belonging to the SODEA collection using the 20 microsatellite markers based on weighed Neighbor-Joining method.

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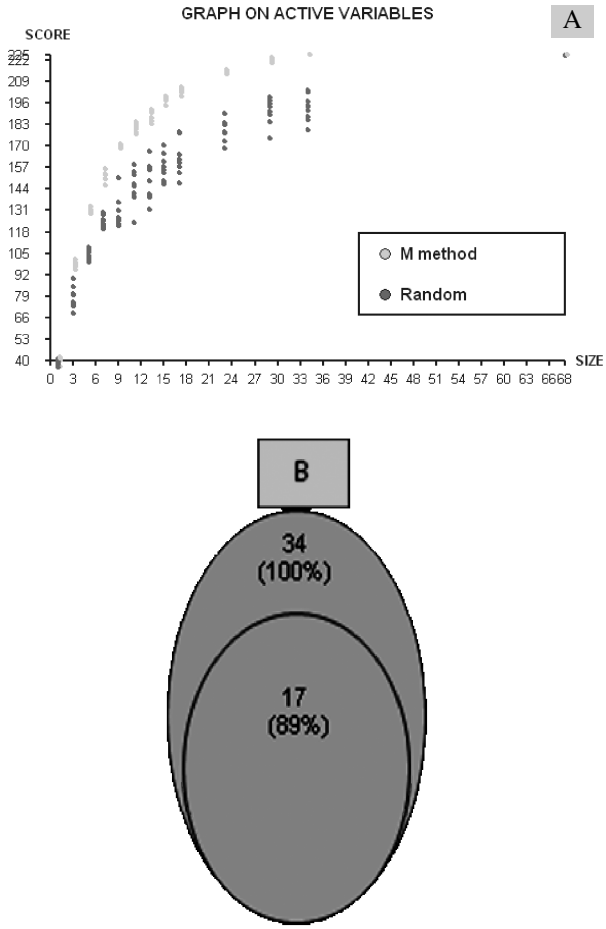


Figure 2 - Construction of the core collections. Curve of redundancy on the basis of 20 SSR markers both with random method (dark dots) or M method (light dots) (A) and size of the nested core collections (B). Amount of diversity captured, represented as % of the alleles, is indicated in brackets.

corresponds to the seedless cultivars related to cv. Sultanina and to the autochthonous cultivars; Group D corresponds to the autochthonous cultivars; Group F corresponds to a cultivar from Spain. Finally, the cv. Dattier noir in group E was well separated from all the other groups, and the cv. Macabeu and cv. Carignan were bulked into the G group. Based on ampelographic descriptions, groups A and C can be differentiated into two sub groups each (A1, A2) and (C1, C2).

3. Establishment of the core collection

The core subset was defined by a two-step procedure:

- First redundancy of the collection was analyzed using MSTRAT setting with 10 repeats and 1,000 iterations. The redundancy curve (figure 2 A) allowed to define the optimal size of the core collection. The optimal size would be 17 cultivars representing 89% of the diversity while 100% of diversity could be obtained with 34 cultivars (figure 2 B).

- The definition of the core collection itself was then performed using 17 or 34 as core size and setting 10 repeats and 1,000 iterations. The best core subsets based on Shannon index were then selected. It leads to two nested core collections of 17 and 34 individuals respectively. The content of both cores is presented in table 4. The smallest core includes five autochthonous cultivars, while the largest core collection, nine (Table 4).

4. Agronomics traits

The complete data for the 16 cultivars present in Vassal are given in table 5 (data not available for the two unique cultivars). The sixteen cultivars can be differentiated into three groups:

- Nine cultivars present medium berries (weight between 3.4 g and 4.9 g) and medium bunches (weight between 141 g and 1,015 g).

- Six cultivars present bigger berries (weight between 5 g and 7 g) and bunches (weight between 298 g and 1,205 g).

- Three cultivars with double use (table and wine).

For the shoot attitude, the cultivars are divided in four groups: semi-erect to erect (1 cultivar), semi-erect (9 cultivars), semi-erect to horizontal (5 cultivars), horizontal (1 cultivar).

DISCUSSION

This work analyses the content of the SODEA collection, the only grapevine germplasm collection in Morocco and assesses the usefulness of the collection in terms of genetic resources for future uses such as selection or reinstallation in local vineyards.

I - Identification of the cultivars

The first step was to identify the cultivars present in the collection. To correctly identify genotypes, we compared their profiles with the profiles of cultivars belonging to the Vassal collection, an internationally recognized collection for trueness to type and because most of the autochthonous cultivars were send to Vassal as safety duplicates. Among the 94 accessions analysed, we identified 67 different genotypes with the 20 SSR which correspond without any doubt to different cultivars.

As often observed in collections, some samples were mislabelled or simply unidentified. Our analyses have enabled identification of all the samples except for five cultivars: cv. Hybrid blanc, cv. Aserghine 1, cv. Aserghine 2, cv. Aramon faux SODEA and cv. Muscat

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Table 4 - Nested genetic core collections of 17 and 34 cultivars.

Autochthonous cultivars are represented in bold.

Size	Variety name	Nbr of alleles
17	Merlot	202
17	Rosa Menna di Vacca	202
17	**Bezoul el Khadem**	202
17	**Dedo de Dama**	202
17	Muscat de Terracina	202
17	**Azizi el Jaïa**	202
17	**Asserghine n°2**	202
17	Syrah	202
17	Grenache	202
17	**Ahmeur bou Ahmeur**	202
17	Negra Mole	202
17	Colobel = Seibel 8357	202
17	Hybride blanc	202
17	Dattier noir	202
17	Cabernet-Sauvignon	202
17	Muscat d'Alexandrie	202
17	Seibel 1020.	202
34	Mireille	225
34	Sultana moscata = Pirovano 75	225
34	**Blanc de Rhafsaï**	225
34	**Tiniouine**	225
34	**Tini**	225
34	**Bou Khanzir blanc**	225
34	Muscat Pearson	225
34	Danugue	225
34	Carignan	225
34	Mourvèdre	225
34	Ugni blanc	225
34	Macabeu	225
34	Gros vert	225
34	Clairette	225
34	Muscat de Hambourg	225
34	Alphonse Lavallée	225
34	Semillon	225

Pearson SODEA which were unique and did not correspond to any of the 2,853 true to type cultivars in the Vassal collection.

Among the 62 other genotypes, we identified several cases of spelling and translation errors and thus the accession name can be modified using the prime names. Many synonyms were also confirmed by molecular analysis as well as new synonyms.

Few real errors were encountered (14). These were probably due to errors made at the time of the installation of this collection (mislabelling of cuttings, errors in plantation, loss of identification plates, etc.) or during maintenance (errors in replanting, cuttings of wrong accessions, etc.) due to the absence of scientific supervision.

Based on the SSR profiles and the geographical origin of the accessions, we identified 18 cultivars of autochthonous origin in the SODEA collection. The diversity of these 18 autochthonous cultivars was compared to that of Vassal collection (El Oualkadi *et al.*, submitted).

2. Level of diversity in the collections

The study of the diversity of SODEA was important to understand which diversity is present in this collection and should be conserved for later reference collection. The number of alleles detected (202 alleles with a mean equal to 10.1/locus) was higher compared to the 6.3 alleles on average in random samples of 13 individuals from European regions (Sefc *et al.*, 2000). Aradhya *et al.* (2003) did not detect more than an average of 6.9 alleles in groups of up to 20 accessions. Similar results were obtained by Bowers *et al.* (1999) on 380 cultivars (11alleles/locus) and by Sefc *et al.* (2000) on 164 cultivars (9.8 alleles/locus).

Expected heterozygosity (gene diversity) levels of the studied loci ranged from 0.31 (locus VVIn73) to 0.90 (locus VMC4f3). The lowest observed heterozygosity was detected at VVIn73 locus with 0.32 and the highest one at VVMD28 and VVIp31 with 0.93. We found that the observed proportions of heterozygous individuals (observed heterozygosities) were significantly ($p<0.05$) lower than the expected ones at 9 out of 20 microsatellites loci.

The dendogram based on SSR markers has enabled the definition of six groups which were furthers subdivided into nine clusters when adding ampelographic data. This structuration reflects either the geographical origin of the cultivars (group B and group E) or the genetic proximity to either Sultanina group C2 or to Muscat cultivars (group A1), or table cultivars (group A2).

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Table 5 - Agronomic data for 16 autochthonous cultivars from SODEA collection and present actually in Vassal collection.

Accession number	Name	202 [1]	204 [2]	223 [3]	225 [4]	6 [5]	502 [6]	503 [7]	Use	Sexe	Colour Pel	236 [8]	301 [9]	302 [10]	303 [11]	304 [12]	Degre of alcool	Must acidity	Wine acidity
07MAR	El Gouz	very short (< 10 cm) to short (10 à 15 cm)	very loose to loose	ellipsoid to ovoid	green yellow	semi-erect	141	3.9	T	H	White	Simple	-3		0.7	1	0	0	0
13MAR	Bezoul el Khadem	very short (< 10 cm) to medium (15 to 20 cm)	very loose to medium	ellipsoid	blue black	horizontal	311	6.3	T	H	NR	Simple	8			5	0	0	0
20MAR	Blanc de Rhafsaï	medium (15 to 20 cm)	dense to very dense	ellipsoid	green yellow	erect to semi-erect	980	5.7	T	H	White	Simple	3		2.3	5	0	0	0
22MAR	Bouzouga	medium (15 to 20 cm) to very long (> 30 cm)	medium	ellipsoid	red - dark red violet - blue black	semi-erect to horizontal	340	4.7	T	H	black	Simple	5		2	3.8	0	0	0
24MAR	Tiniouine				blue black	semi-erect			T	H	black	Simple	7						
25MAR	Tini	medium (15 to 20 cm)	medium	ellipsoid	red- dark red violet - blue black	semi-erect	851	4.4	T	H	Red	Simple	4		2.7	4.3	0	0	0
28MAR	Dedo de dama	medium (15 to 20 cm) to very long (>30cm)	medium to very dense	ellipsoid	green yellow	semi-erect to horizontal	1205	5	T	H	White	Simple	12		3	4.6	0	0	0
29MAR	Bou Khanzir blanc	medium (15 to 20 cm)	medium	ellipsoid	green yellow	semi-erect	671	4.8	T	H	White	Simple	11		1.7	3.9	0	0	0
32MAR	Diagalves	very short (< 10 cm) to very long (> 30 cm)	very loose to very dense	ellipsoid to cylindric	dark red violet- blue black - green yellow		504	4.2	CT	H	White	Simple	10		2.1	4	10.4	4.2	4.2
46MAR	Azizi el Jaïa	medium (15 to 20 cm)	dense to very dense	ellipsoid	green yellow	semi-erect	236	3.4	T	H	White	Simple	3		2.7	3.7	0	0	0
47MAR	Abouhou	very short (< 10 cm) to short (10 à 15 cm)	very loose to medium	ellipsoid to ovoid	blue black	semi-erect to horizontal	347	7	T	F	black	Simple	8		2.9	5.1	0	0	0
52MAR	Bouchouka	medium (15 to 20 cm) to very long (> 30 cm)	medium	ellipsoid	green yellow	semi-erect to horizontal	1015	4.6	T	H	White	Simple	2		3.7	5	0	0	0
64MAR	Taferielt	medium (15 to 20 cm) to very long (> 30 cm)	dense to very dense	ellipsoid to obovoid	blue black	semi-erect	469	4.9	CT	H	black	Simple	9		4.3	5	8.4	4.5	2.8
68MAR	Ahmeur bou Ahmeur	very short (< 10 cm) to very long (> 30 cm)	medium to very dense	ellipsoid to obtuse ovoid	rose- red- dark red violet	semi-erect	595	6.5	T	H	Red	Simple	8	5	2.7	4.6	10.6	4.2	3.5
80MAR	Aguyar	medium (15 to 20 cm) to very long (> 30 cm)	dense to very dense	ellipsoid		semi-erect to horizontal	298	5.8	T	H	black	Simple	10		3	5.5	0	0	0
91MAR	Maticha	very short (< 10 cm) to medium (15 to 20 cm)	medium to very dense	obloid	green yellow	semi-erect	536	4.9	CT	H	White	Simple	10		3.3	4.8	8.7	7.2	5.4

[1]: OIV code for Bunch: length (peduncle excluded)
[2]: OIV code for Bunch: density
[3]: OIV code for Berry : form
[4]: OIV code for Berry: color of skin
[5]: OIV code for Shoot: attitude (before tying)
[6]: OIV code for Bunch: medium bunch weight
[7]: OIV code for Berry: medium berry weight
[8]: OIV code for Particular flavor
[9]: OIV code for Mean time of bud burst in days (/ 19 march)
[10]: OIV code for Mean time of full bloom (/ 27 may)
[11]: OIV code for Mean time of beginning of berry ripening (/ 20 july)
[12]: OIV code for Mean time of full physiological maturity of the berry (/14 august)

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The autochthonous cultivars were separated into two distinct groups, C1 and D. The C1 group containing mainly cultivars from southern Morocco (Atlas region) and the group D comprising germplasm from northern Morocco (Rif region).

As revealed by their agronomical features, the cultivars present in the collection represent either table grape or wine grape with quite diverse features, especially a very variable precocity and berry or bunch size which could enable the Moroccan growers to propose quite diverse production.

3. Construction of the core collections

Two core collections were build (the optimal one and the one representing 100% of the diversity) following Le Cunff *et al.* (2008), in order to define sample easier to manage, and in order to define a working subset on which to start more defined analyses, such as stress resistance or precise agronomical or technological traits. A small number of 17 cultivars enabled the capture of 89% of the diversity and 34 cultivars was enough to capture 100% of the diversity. There were five or nine autochthonous cultivars in the 17 and 34 member core samples, respectively, in accordance with the high level of diversity among autochthonous cultivars previously observed (El Oualkadi *et al.*, submitted).

The comparison with other models is not easy, as they have different biological characteristics. The original collection did not reach the same global diversity of the species, and the analyses are seldom performed in the same way. Nevertheless, the core collections developed for *A. thaliana* (McKhann *et al.*, 2004) or *M. truncatula* (Elwood *et al.*, 2006) using the same method have required higher percentages of individuals. The same capture was obtained using bigger samples, since Vassal collection contains more cultivars (Le Cunff *et al.*, 2008).

Our result shows that the characterisation of germplasm collections by means of microsatellites is very useful in the management and the identification of cultivars. Now this collection can be suggested as the unique reference of grapevines in Morocco.

In spite of the diversity already revealed in the SODEA collection, it may represent only a part of the grape diversity present in Morocco. Additional prospection could enrich the collection to build a representative collection in Morocco (El Oualkadi *et al.*, in preparation).

CONCLUSION

The present study provides the first molecular database for Moroccan grapevine germplasm management. The SODEA collection thus contains 67 cultivars (23 wine cultivars, 35 table cultivars and 5 with double use), 18 of autochthonous origin, and 47 of foreign origin. The germplasm have displayed diverse plant materials and appears to represent a major component of the Moroccan grapevine genetic resources and the unique reference collection for grapevines in Morocco. Future collection and introductions must include molecular analysis in order to optimise the genetic diversity of the germplasm and to avoid duplications observed in the present collection. The diversity revealed in our study has confirmed the interest of the collection and should consequently ensure its durability and its use by the producers.

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