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Evaluation of genetic diversity among Tunisian grapevines by RAPD markers

N. Zoghlami, A. Mliki and A. Ghorbel

Institut National de Recherche Scientifique et Technique, Hammam-lif, Tunisie

Summary

Thirty-three native Tunisian grapevine varieties (*Vitis vinifera* L.), previously analysed with biochemical tools, were surveyed with RAPD markers. Eleven primers amplified 54 clear and unambiguous markers. Five groups of varieties were clearly resolved by UPGMA cluster analysis. These results allowed the discrimination of groups of varieties that could not be discriminated by biochemical analyses. In addition, the dendrogram obtained could be shown being independent from both the phenotypic characters and the origin of the accessions.

K e y w o r d s : grapevine identification, RAPD, genetic diversity, UPGMA.

Introduction

In Tunisia, grapevine cultivars have a long history of domestication; the first report of *Vitis vinifera* dating back to the Carthaginian civilisation.

Despite its economical importance, a genetical analysis of *V. vinifera* genotypes has been neglected up to date. This, however, could rapidly change due to the current availability of molecular techniques allowing clear identification of genotypes.

The present work, for the first time, investigates the genetic relationships of native Tunisian varieties and establishes a reliable taxonomic classification with the goal of unambiguously matching the genotype to the name of each variety. In fact, names given by farmers to certain varieties exhibit, in most cases, a flagrant taxonomic ignorance of the genotype.

Native varieties have been analysed previously using morphological data (Zaki *et al.* 1996) and isozymes (phosphoglucoisomerase, GPI; phosphoglucomutase, PGM; aspartate aminotransferase, AAT and peroxidase) (Ben Abdallah *et al.* 1998). Out of the 61 varieties studied, 26 have been definitively characterised. Nevertheless, the biochemical identification was not conclusive for 11 groups totalling 33 varieties (Tab. 1). As reported by Parfitt and Arulsekar (1989), accessions, having similar profiles of a particular enzymatic system, can be judged as being identical. On the other hand, those showing different profiles are considered to be inevitably genetically different. The present RAPD analysis therefore was carried out only with accessions having similar enzymatic profiles.

Material and Methods

P l a n t m a t e r i a l : The 33 grapevine varieties used in this study are part of the germplasm collection in the vineyard of the Institut National de Recherche Scientifique et Technique de Hamam-lif in Tunisia (Tab. 1). They originate from sites in almost all parts of Tunisia. Leaves were sampled from late April till the onset of May and were immediately frozen in liquid nitrogen before storage at -78 °C.

D N A e x t r a c t i o n : DNA from young, healthy leaves was extracted following the protocol of Bowers *et al.* (1993), later modified by This *et al.* (1997). This procedure was further modified in our laboratory as follows: The removal of RNA was performed by digestion for 1 h at 37 °C with 1 µl of RNAse A (10 mg·ml^{-1}) instead of 10 µl. Then, the DNA was extracted twice with an equal volume of phenol-chloroform-isoamyl alcohol (25:24:1) and centrifuged at 12,000 rpm for 10 min at 4 °C; the digestion with proteinase K was omitted.

D N A q u a n t i f i c a t i o n : The concentration of DNA was determined using three different methods:
1) Running the samples in a 0.8 % agarose gel in TAE buffer (Sambrook *et al.* 1989) and staining with ethidium bromide (0.9 µg·ml^{-1}). The DNA concentration was estimated by visual comparison of the band intensity with known quantities of lambda DNA standards (Boehringer Mannheim, Germany).
2) Measuring the absorbance of the DNA sample at 260 and 280 nm by means of a UV spectrophotometer (Pharmacia Biotech).
3) By means of a minifluorimeter TD-360 (Turner designs) according to the manufacturer's protocol.
In all cases, the final DNA concentration was adjusted to 5 ng·µl^{-1} for use in PCR analysis.

R A P D c o n d i t i o n s : The standard RAPD-PCR protocol employed was similar to that of Williams *et al.* (1990) with some modifications. Amplifications were carried out in a 20 µl reaction volume with 5.0 ng of template DNA, 2.0 µl of Taq polymerase buffer (10 mM Tris-HCl (pH 9.0), 50 mM HCl, 1.5 mM $MgCl_2$, 0.1 % Triton x 100, 0.2 mg·ml^{-1} BSA), 0.8 M dNTPs (0.2 M of each: dATP, dGTP, dTTP and dCTP) (Boehringer Mannheim, Germany), 0.7 µl of 25 mM $MgCl_2$, 5 µM of primer and 1.0 U of Taq DNA polymerase (Appligene). The reaction mixture was covered with 20 µl of mineral oil. Informative primers were selected for the study as explained in the following section.

The PCR was performed in a Biometra Trio-Thermoblock thermocycler (Göttingen, Germany), programmed for one initial step of 5 min at 94 °C, followed by 40 cycles of 40 s at

Correspondence to: Dr. A. Mliki, Institut National de Recherche Scientifique et Technique, B.P. 95, 2050 Hammam-lif, Tunisia. Fax:+216-1-430934, E-mail:ahmed.mliki@inrst.rnrt.tn

Table 1

Accessions non identified by isozymes (BEN ABDALLAH *et al.* 1998) and subjected to RAPD analysis

No.	Groups*	Varieties	Origin**
1	B_1	Blanc 1	Djebba
2		Blanc 2	Djebba
3	C_3	El Biodh	Baddar
4		Bezzoul Kelba Bidha de Sfax	Sfax
5		Razaki Rafraf	Rafraf
6		Dattier de Beyrouth	Baddar
7	D_{22}	Kahli Kerkennah	Kerkennah
8		Kahli Sfax	Sfax
9	E_1	Khdhiri 1	Djebba
10		Khdhiri 2	Djebba
11		Bahbahi	Djebba
12	E_2	Beldi	Baddar
13		Beldi Rafraf	Rafraf
14		Beldi local Rafraf	Rafraf
15	F_{11}	Chaâraoui	Rafraf
16		Bidh Hamem Rafraf	Rafraf
17	F_{12}	Hencha 2	Hencha
18		Balta 2	Balta
19		Balta 3	Balta
20		Razegui	Baddar
21		Farrani	Rafraf
22		Khalt Abiedh	Tozeur
23		Meski local Tozeur	Tozeur
24		Mahdaoui	Kerkennah
25	H_{13}	Arich Dressé	Mornag
26		Hamri Kerkennah	Kerkennah
27		Turky	Baddar
28	H_{22}	Médina	Gabès
29		Arich Jerba	Djerba
30	A	Asli Hadab	Kerkennah
31		Asli Dar Slimane	Kerkennah
32	P	Sakasly	Baddar
33		Sakasly Jerba	Djerba

* Groups biochemically discriminated by GPI, PGM, AAT and peroxidase (BEN ABDALLAH *et al.* 1998).
** Origin: area of cultivation

94 °C, 40 s at 35 °C and 1 min at 72 °C. The amplification of each genotype-primer combination was repeated twice.

Products of the PCR were separated by electrophoresis in 1.6 % agarose gels with 1xTAE buffer (40 mM Tris-acetate, 1 mM EDTA, pH 8.0) using a volt range of 3 $V \cdot cm^{-1}$ during 3 h. Lambda DNA *Eco*RI/*Hind*III digested (Boehringer Mannheim, Germany) was used as a molecular size standard.

Data analysis: Photographs were taken with a Biometra Bio-doc II™ system. In order to ensure the absence of artifacts, bands were carefully selected from replicated amplifications. Amplified bands were designated by their primer code and their size in base pairs. Data were recorded as discrete variables: 1 for the presence and 0 for the absence of a similar band. Only intense and reproducible bands appearing on the gel were scored. A similarity matrix was generated with NTSYS–pc (Numerical Taxonomy and Multivariate Analysis System; ROHLF 1989), based on the simple matching algorithm of SOKAL and SNEATH (1963). This algorithm considers RAPD bands as phenotypic rather than genetic characters and considers individuals that either possess or lack a common band as a match. Nei's genetic data index was not appropriate for this analysis, as it is based on allelic frequencies (genetic data), not detected when using RAPD markers.

The simple matching similarity coefficients were used to cluster individuals with the SAHN procedure (Sequential Agglomerative, Hierarchical and Nested cluster analysis of NTSYS), which uses the unweighed pair group method with arithmetic averages (UPGMA) to obtain genetic relationships and to cluster varieties. Results were used to construct a final dendrogram showing all different groups.

Monomorphic bands were not used for the calculation of the similarity matrices (BÜSCHER *et al.* 1993). Doubts in clustering were solved by analysis of bands with low frequency (LFBs), *i.e.* bands shared by only 25 % or less varieties (BOURQUIN *et al.* 1993).

The origin of the accessions and some phenotypic characters, such as the colour of the berry and the type of leaf hairs, were taken into consideration to examine their potential effect on the genetic similarity between accessions. In addition, data obtained by the RAPD method were compared to those of the biochemical analysis previously performed with the same varieties (BEN ABDALLAH *et al*. 1998).

Results

Selection of primers: An initial screening with 43 primers (Operon Technologies-California, British Columbia University, Canada) was carried out on 5 varieties: Mahdaoui, Bezzoul Kelba Bidha, Arbi Abiedh, Saouadi and Djebbi. Only 11 informative primers were retained, due to their ability to produce polymorphic, unambiguous and stable RAPD markers (Tab. 2).

Polymorphism analysis: The 11 selected primers were used to perform RAPD reactions with the DNA extracted from the 33 native grapevine varieties. Unambiguous DNA bands, with sizes ranging from 400 to 1980 bp, were detected (Fig. 1). Eighty-one DNA bands were obtained, which is an average of 7.3 bands per primer: 27 bands were common in all accessions and 54 were polymorphic. On average, each primer rendered 5 markers (polymorphic bands).

Overall, the polymorphic markers yielded 75 different RAPD patterns, with an average of 6.8 different patterns per primer. OPP-210 showed the highest level of polymorphism (13 informative bands) and produced 21 different RAPD patterns (Tab. 2).

Seventeen low frequency bands (LFB) were detected (Tab. 3). These variety specific markers are of a special interest for varietal identification. Two couples of varieties shared the same LFBs: Médina and Turky shared 35_{500} and 37_{1620}, Hencha 2 and Farrani shared 21_{550} and 21_{590}.

The electrophoretic patterns were translated to a 33 x 54

Table 2

Informative primers selected and degree of polymorphism obtained among the 33 varieties studied

No.	Primer code	Sequence 5' to 3'	Number of total bands	Number of polymorphic bands	LFB*	Electrophoretic patterns	Degree of electrophoretic polymorphism (%)
1	BC-379	gggCTAgggT	6	5	0	4	83
2	BC-204	TTCgggCCgT	6	5	1	4	83
3	BC-302	CggCCCACgT	10	4	1	4	40
4	K-04	gATggAACCg	2	1	0	2	50
5	OPD-08	gTgTgCCCCA	8	6	2	6	75
6	OPA-04	AATCgggCTg	11	5	4	8	45
7	OPD-12	CACCgTATCC	6	4	1	8	66
8	OPG-02	GgCACTgAgg	8	4	1	6	50
9	OPP-17	TgACCCgCCT	5	5	1	8	100
10	OPP-210	AAATgCggCA	13	12	4	21	92
11	OPP-232	CCgCTTgTTg	6	3	0	4	50
total			81	54	15	75	67

*LFB: Low Frequency Band

Table 3

Accessions possessing Low Frequency Bands

No.	Variety	Low Frequency Bands
1	Blanc 1	$6_{520}, 28_{1000}, 32_{1300}, 37_{1375}$
2	Blanc 2	$6_{520}, 28_{1000}, 32_{1300}, 37_{550}$
3	El Biodh	$6_{520}, 10_{700}, 28_{1600}, 37_{1375}$
4	Bezzoul Kelba Bidha de Sfax	6_{520}
6	Dattier de Beyrouth	10_{700}
7	Kahli Kerkennah	$10_{700}, 28_{700}$
8	Kahli Sfax	$10_{700}, 37_{550}$
9	Khdhiri 1	$28_{1000}, 31_{950}, 32_{1300}, 37_{550}, 37_{1150}$
10	Khdhiri 2	$28_{1000}, 31_{950}, 32_{1300}, 37_{550}$
11	Bahbahi	$21_{590}, 28_{1000}, 31_{950}, 37_{550}$
12	Beldi	$28_{1600}, 31_{950}, 37_{550}$
13	Beldi Rafraf	$28_{1600}, 31_{950}, 37_{550}$
14	Beldi local Rafraf	$28_{1600}, 31_{950}$
15	Chaâraoui	$10_{700}, 21_{550}, 31_{950}$
16	Bidh Hamem Rafraf	$28_{1980}, 31_{950}$
17	Hencha 2	$21_{550}, 21_{590}$
18	Balta 2	37_{550}
20	Razegui	$21_{550}, 21_{590}, 37_{1150}$
21	Farrani	$21_{550}, 21_{590}$
22	Khalt Abiedh	$21_{550}, 21_{590}, 28_{1980}, 37_{1150}$
23	Meski local Tozeur	$21_{550}, 21_{590}, 28_{1980}, 35_{500}$
24	Mahdaoui	$21_{550}, 21_{590}, 28_{1980}$
25	Arich dressé	37_{1150}
27	Turky	$35_{500}, 37_{1620}$
28	Médina Gabès	$35_{500}, 37_{1620}$
29	Arich Jerba	$6_{520}, 35_{500}, 37_{1150}$
30	Asli Hadab	$28_{700}, 35_{500}, 37_{1150}, 37_{1620}$
31	Asli Dar Slimane	37_{1620}
32	Sakasly	$10_{700}, 28_{1000}, 35_{500}, 37_{1620}$
33	Sakasly Jerba	$10_{700}, 28_{1000}, 35_{500}, 37_{1150}$

matrix form used by the NTSYS software to establish a similarity matrix. This allows the calculation of genetic distances between the accessions and the design of a dendrogram.

Genetic relationships: According to Novy *et al.* (1994), varieties having a 100 % similarity are either identical or genetically close to each other. Thus, varieties with similarity percentages >90 % were considered genetically close to each other and those with less than 40 % similarity as genetically distant. Only 8 pairs of varieties were similar, with similarity degrees (SD) >0.90 (Tab. 4).

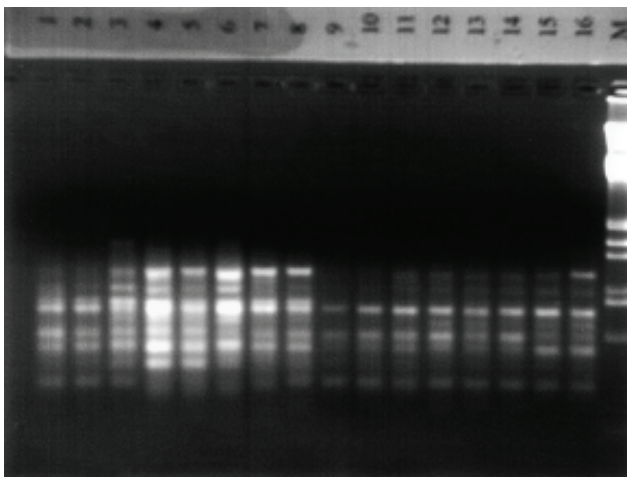

Fig. 1: Representative RAPD profiles from 16 grapevine genotypes generated by the primer OPP-210.

The simple matching similarity coefficients were used to cluster individuals using UPGMA (Fig. 2). With the 33 varieties studied, 5 groups (A, B, C, D and E) were identified.

Group A includes 8 varieties. Three pairs were genetically close with SD 0.94 (Bezzoul Kelba Bidha Sfax and Razaki Rafraf), 0.88 (Kahli Kerkennah and Kahli Sfax), and 0.94 (Blanc 1 and Blanc 2). Two more varieties, El Biodh and

Table 4

Similar accessions (>0.90 of similarity)

Varieties	Similarity, %	LFB shared	Origin	Phenotypic characters
Blanc 1 - Blanc 2	94.3	3/4	Djebba (N)*	White berries
Razaki Rafraf - BKB Sfax	94.4	0	Rafraf (N) - Sfax (S)	White berries
Beldi local Rafraf - Beldi Rafraf	92.5	2/3	Rafraf (N)	Yellowish green berries
Hencha 2 - Farrani	94.0	2/2	Hencha (S) - Rafraf (N)	White berries
Mahdaoui - Farrani	90.6	2/3	Kerkennah (S) - Rafraf (N)	White berries, different leaves (pilosity's sort)
Turky - Hamri Kerkennah	94.1	0	Baddar (N) - Kerkennah (S)	Pink berries - red berries
Médina - Turky	92.3	2/2	Gabès (S)* - Baddar (N)	Whitish berries - pink berries
Khdhiri 1 - Khdhiri 2	94.4	3/4	Djebba (N)	White berries

*(N): North of the country; *(S): South of the country; *BKB: Bezzoul Kelba Bidha.

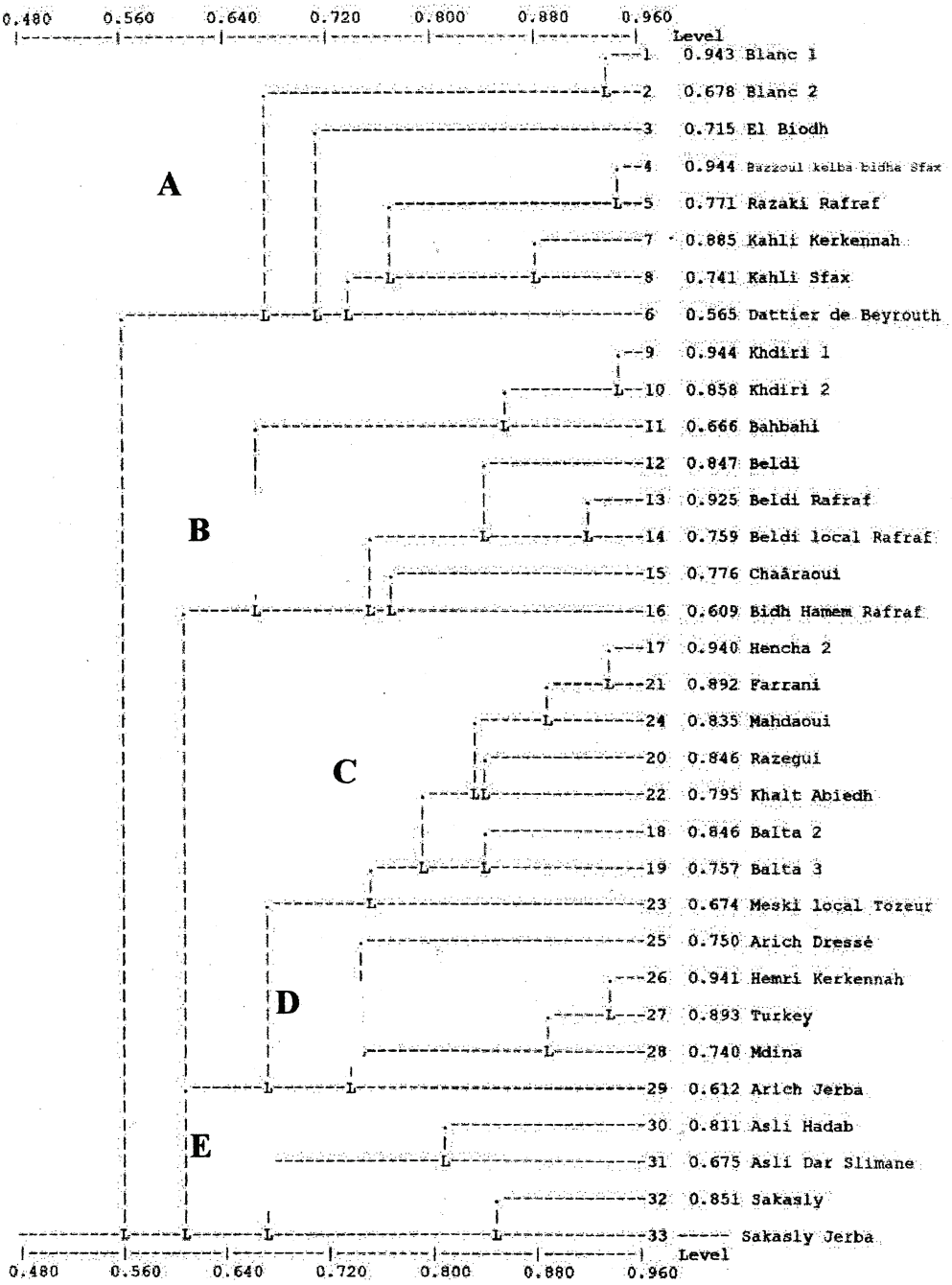

Fig. 2: UPGMA cluster analysis. A, B, C, D and E point out the clusters individualised.

Dattier de Beyrouth, were genetically distant. Group B was more heterogeneous than group A, also with 8 varieties among which two pairs were synonymous: Khdhiri 1 - Khdhiri 2 and Beldi Rafraf - Beldi local Rafraf. The other accessions were different. Group C was composed of 8 varieties. A particular sub-group was clearly defined with three homonymous varieties: Hencha 2, Farrani and Mahdaoui, with SD >0.9. Group D includes 5 varieties with a sub-group of three closely related ones (Hamri Kerkennah, Turky and Médina). Group E was too heterogeneous since the three encountered varieties were genetically distant. The variety Sakasly Jerba did not cluster with any other genotype.

Since the LFBs could contribute information to understand the dendrogram, both closely related and genetically distant varieties were compared for the shared LFBs. In addition, a few phenotypic characters were considered as well as the influence of origin of growing.

As shown on Tabs 4 and 5, similar accessions shared most of the LFBs. For instance, the varieties Hencha 2 and Farrani, Médina and Turky had exactly the same LFBs. This may lead to the hypothesis of their varietal relationship. This was also strengthened by the similarity degrees, which were 94 and 92.5 %, respectively. Nevertheless, the presence of this type of bands does not always express the affiliation between individuals. Indeed, two pairs of synonymous varieties did not share any LFB: Razaki Rafraf - Bezzoul Kelba Bidha de Sfax and Turky - Hamri Kerkennah. On the other hand, distant varieties lacked specific bands, which was in agreement with their low similarity degrees.

In relation to the geographical origin, Tabs 4 and 5 demonstrate that this factor did not influence the genetic closeness of accessions. For instance, Médina, from southern Tunisia, has been considered similar to Turky from the North. Out of the 16 genetically close varieties evaluated, only 6 had similar native sites.

By further investigation of the effect of phenotypic characters on the genetic relationship of grape varieties, it was clearly shown that they did not influence the synonymy of the varieties analysed. Two pairs out of 8 synonymous varieties were different either by the colour of their berries (*i.e.* Médina and Turky) or by their leaf hairs (Mahdaoui and Farrani) (Tab. 4). On the other hand, regarding the genetically distant accessions, they shared either the same or different phenotypic characters.

Comparison of biochemical and RAPD data : A biochemical study with two isozyme systems (GPI and PGM) in native grapes showed that the variety Razaki Rafraf was identical to the variety Dattier de Beyrouth (Ben Abedrabbou 1994). However, according to the present molecular RAPD analysis, these varieties would have a 0.77 SD, which is in disagreement with their genetic resemblance.

A second biochemical analysis was carried out in our laboratory, using 4 isozyme systems (GPI PGM, AAT and peroxidase). Out of the 61 accessions studied, 26 were discriminated and 33 still not identified. They were subjected to the RAPD analysis.

Tab. 6 presents the results of both biochemical and RAPD analysis: for each group outlined by biochemical identification, RAPD markers generated two or more sub-groups. For instance, group B_1 composed of three synonymous varieties was split into two sub-groups: B_1(a) and B_1(b); at the same time, sub-group B_1(a) included two similar varieties (Blanc 1 and Blanc 2).

Group D_{22}, including Kahli Sfax and Kahli Kerkennah, was shown to contain similar varieties with both isozymes and RAPD. Group F_{12}, with 8 varieties, was the most heterogeneous: with the RAPD markers 6 sub-groups were identified, with the special case of sub-group F_{12}(a) with three similar varieties.

In Tab. 6, Médina drew particular attention. This variety was clustered with Arich Jerba (group H_{22}) following enzymatic identification; however, after the RAPD evaluation, this variety did not belong to that group; instead Médina showed a close genetic relationship with Turky and Hamri Kerkennah, forming sub-group H_{13}(b).

As a result, the 11 groups differentiated by the isozyme analysis, including 33 varieties, were split into 25 sub-groups by the RAPD study.

Discussion

According to Devos *et al.* (1992), Penner *et al.* (1993) and This *et al.* (1997), some primers of the used set seem to be more efficient than others in producing stable and reproducible profiles. Moreno *et al.* (1995) and Ortiz *et al.* (1997) pointed out that primers yielding faint bands ought to be

Table 5

Distant accessions (<40 % of similarity)

Varieties	Similarity, %	LFB shared	Origin	Phenotypic characters
Razegui - El Biodh	36.0	0	Baddar(N) - Jerba (S)	White berries
Farrani - El Biodh	37.5	0	Rafraf (N) - Jerba (S)	White berries
Hamri kerkennah - Blanc 1	38.0	0	Kerkennah (S) - Djebba (N)	Red berries - white berries
Turky - Blanc 1	37.3	0	Baddar (N) - Djebba (S)	Pink berries - white berries
Médina - Blanc 2	39.2	0	Gabès (S) - Djebba(S)	Whitish berries - white berries

Table 6

Comparison between the data obtained by isozyme and RAPD analysis

Groups differentiated by the biochemical analysis (BEN ABDALLAH *et al.*, 1998)	Groups differentiated by the RAPD analysis
B_1: Blanc 1, Blanc 2, El Biodh	B_1(a): Blanc 1 = Blanc 2 B_1(b): El Biodh
C_3: Bezzoul Kelba Bidha de sfax = Razaki Rafraf, Dattier de Beyrouth	C_3(a): Bezzoul Kelba Bidha de sfax = Razaki Rafraf C_3(b):Dattier de Beyrouth
D_{22}: Kahli Kerkennah, Kahli Sfax	D_{22}: Kahli Kerkennah = Kahli Sfax
D_{23}: Khdhiri 1, Khdhiri 2, Bahbahi	D_{23}(a): Khdhiri 1 = Khdhiri 2 D_{23}(b): Bahbahi
E_2: Beldi Baddar, Beldi Rafraf, Beldi local Rafraf	E_2(a): Beldi local Rafraf = Beldi Rafraf E_2(b): Beldi Baddar
F_{11}: Chaâraoui, Bidh Hamem Rafraf	F_{11}(b): Chaâraoui F_{11}(b): Bidh Hamem Rafraf
F_{12}: Hencha 2, Balta 2, Balta 3, Razegui, Farrani, Khalt Abiedh, Meski local Tozeur, Mahdaoui	F_{12}(a): Hencha 2 = Farrani = Mahdaoui F_{12}(b): Balta 2, F_{12}(c): Balta 3, F_{12}(d): Razegui, F_{12}(e): Khalt Abiedh F_{12}(f): Meski local Tozeur,
H_{13}: Arich Dressé, Hamri Kerkennah, Turky	H_{13}(a): Arich Dressé *H_{13}(b): Hamri Kerkennah = Turky
H_{22}: Arich Jerba, Médina	*H_{22}(a): Médina H_{22}(b): Arich Jerba
A: Asli Hadab, Asli Dar Slimane	A_1: Asli Hadab A_2: Asli Dar Slimane
P: Sakasly Baddar, Sakasly Jerba	P_1: Sakasly Baddar P_2: Sakasly Jerba

* $H_{13}(b)=H_{22}(a)$

excluded from the analysis. In addition, CAETANO (1994) mentioned the permanent occurrence of non-functional primers. Due to this, the selection of primers is essential when dealing with discrimination analyses. The actual needed number to select primers has been questioned. For instance, VIDAL *et al.* (1999) used only two varieties. In our case, the selection of primers was performed with 5 varieties and 11 primers were retained from 43 tested.

The level of polymorphism obtained (67 %) is similar to previous RAPD studies (GRANDO *et al.* 1996). A high number of electrophoretic patterns was obtained (75), although no single primer allowed the differentiation of the 33 varieties. The most discriminating primer was OPP-210, which detected 21 different patterns. For identification purpose, LFB provided interesting information, especially the variety specific markers. Moreover, there was a positive correlation between the calculated high similarity degrees and the presence of these bands. According to the UPGMA and the LFBs analysis, the pairs Hencha 2 - Farrani and Médina - Turky, could belong to the same sortotype both by their similarity degrees (>0.90) and their identical LFB (VIDAL *et al.* 1999). Genetically distant varieties (SD <40 %) had no LFB in common. The absence of this kind of specific bands confirms their weak relationship.

Consistent with other results, our RAPD analysis seems to be appropriate for genetic relationship studies (LYNCH *et al.* 1994, TSCHAMMER *et al.* 1994, VIDAL *et al.* 1999). The minimal number of RAPD bands required to establish accu-

rate estimates of genetic similarity depends only on the number of primers used: GUIRAO *et al.* (1995) and HOREJSI *et al.* (1998) reported that a number of about 45 RAPD markers should be sufficient for the establishment of genetic relationships.

According to BOURQUIN *et al.* (1993) and BÜSCHER *et al.* (1994), DNA data enable grouping grapevine varieties in relation to their geographical origin and their phenotypic relationships. However, these factors had no clear effect on the relationship between the varieties of the present research. TSCHAMMER *et al.* (1994) reported the difficulties encountered to distinguish grapevines by phenotype. Indeed, this approach showed limitations and consequently does not seem reliable for identification purposes.

If similarity degrees are available, genetic analysis by means of RAPD may overcome some of the limitations of phenotypic analysis. IQBAL *et al.* (1997) reported on the reliability of this technique in the evaluation of genetic distances. Similarity degrees were shown to be efficient to provide information on the genetic relationships between varieties.

The comparative study carried out between data obtained in a previous biochemical identification (BEN ABDALLAH *et al.* 1998) and current RAPD showed that RAPD analysis is more efficient than the enzymatic method for discriminating between varieties. Varieties that were closely related according to the biochemical assay were better differentiated. The pairs of varieties Asli Hadab - Asli Dar Slimane, Sakasly Baddar - Sakasly Jerba and Beldi Local Rafraf - Beldi Baddar, despite their similar names and their enzymatic analogy, were shown to be different. Finally, data obtained by phenotypic characterisation and isozyme analysis were considered to be limited. In fact, both studies could not provide enough information for clustering. Nevertheless, these data are worth consideration in order to support the results of the RAPD analysis.

Conclusion

Our study enabled the definition of 5 groups of genotypes. This may help to build a definitive taxonomic sorting, which could be of great interest for breeders.

Varieties which were genetically close should be analysed with more primers, and preferably with other markers and techniques such as microsatellites providing a much more improved analysis.

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