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Molecular Characterization and Study of Genetic Relationships among local Cultivars of the Moroccan fig (Ficus carica L.) using Microsatellite and ISSR Markers

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Abstract*— *Molecular characterization of Moroccan local fig (Ficus carica L.) germplasm was performed on the cultivars present in a collection of the National School of Agriculture of Meknes. A total of 22 fig samples were analysed using 7 ISSR primers and 9 loci S.S.R. A total of 54 I.S.S.R. polymorphic bands with an average of 8 per primers and 42 S.S.R. alleles with means 5 alleles per locus were revealed by these analyses. The ISSR markers allowed distinguishing 22 molecular profiles and S.S.R. loci differentiated between 21 different profiles. Pairwise Comparing, 87% of cultivars pairs were differentiated by 7 to 24 alleles and 89% by 9 to 29 ISSR bands. The statistical analysis and genetic distances have shown a wide molecular diversity in the collection, where the average observed heterozygosity was 0.42. The average similarity between cultivars is 70% using SSR markers and 71.6 for ISSR markers.

The same SSR profile was obtained for Nabout1 and Nabout2 with 0 allele difference. Small differences of 1 to 6 alleles were obtained among cultivars which have the same names, which presumably corresponds to somaclonal variations obtained through intense vegetative propagation over long periods, while the differences over 7 alleles suggests the problems of homonyms.

Keywords*— *Ficus carica L., genetic diversity, ISSR markers, molecular characterization, SSR markers, varietal identification.

I. INTRODUCTION

The common fig (*Ficus carica* L. Moraceae) is one of the oldest fruits grown in the Mediterranean [1]. In Morocco, fig cultivars are very diverse and offer a wide range of cultivars particularly in the north [2]. However, because of numerous cases of synonymy (several names for the same cultivar) and homonymy (several cultivars under the same name), pomological characterization of the fig is insufficient to select authentic cultivars needed for genetic improvement programs. To avoid confusion varietal problems, the use of molecular markers is essential to correctly identify the fig cultivars and establish genetic identity for each cultivar.

Currently there are several molecular marker techniques, isozymes were the first genetic markers used in genetic characterizations and have been applied in several fruits species including figs [3, 4, 5, 6]. However, their utility was limited because small number of isozyme systems available, the low polymorphism level obtained and the influence of environmental factors.

The emergence of several techniques based on the Polymerase Chain Reaction (PCR) has developed several types of molecular markers. Random amplified polymorphic DNA (RAPD), the amplified fragment-length polymorphism (AFLP) and simple sequence repeat (SSR), has given the opportunity for genetic resources characterization. Their advantages are made they are highly polymorphic and are not easily influenced by environmental factors [7, 8, 9, 10]. RAPD. markers have previously been used for the characterization of fig cultivars [11, 4, 12], but because of the use of short arbitrary sequences and hybridization relatively low temperatures, these markers cannot be exchanged between laboratories according to standard protocols [13]. To avoid this limitation, a comparison of previous data obtained by RAPD, ISSR and SSR markers on the analysis of 30 fig cultivars showed that SSR and ISSR

markers are complementary tools for reliable characterization of this species [14].

In this study, ISSR and SSR markers were used to characterize the local fig accessions, preserved in the collection of Meknes National School of Agriculture in Morocco, and furnish a molecular database for the breeding fig projects.

II. MATERIALS AND METHODS

2.1. Plant Material

The fig collection located at the experimental station of the National School of Agriculture (ENA, Meknes, Morocco) was the subject of the study. The collection encloses 22 local cultivars collected in northern and central Morocco (Table 1). In some cases, several cultivars were classified under the same name (see, for example, "Ghoudane"). Molecular characterization was performed on a tree by cultivar.

Table.1: Name, code, use and origin of the studied accessions

Cultivars	Code	use	Origin	Cultivars	Code	Use	Origin
Ounq Hmam	OQH	Fresh	My Driss Zerhoun	Ghoudane2	GHD2	Fresh	Taounate
Hzzat	HZZ	Fresh	My Driss Zerhoun	Hamriya	HAM	Fresh	Taounate
Chaâri	CHA	Fresh	My Driss Zerhoun	Sebti	SEB	Fresh	Taounate
Ournakssi	OUR	Fresh and Drying	My Driss Zerhoun	Nabout 2	NAB2	Drying	Taounate
El Fassi	FAS	Fresh	Taounate	Nabout 1	NAB1	Drying	Taounate
Lemtel2	LEM2	Fresh	Taounate	Ghoudane4	GHD4	Fresh	Taounate
Lemtel1	LEM1	Fresh	Taounate	Ghoudane1	GHD1	Fresh	Taounate
Arguel	ARG	Drying	Taounate	Génotype19	GEN19	Fresh	Taounate
El Beida	BEI	Fresh and Drying	Taounate	Géenotype20	GEN20	Drying	Taounate
El Khoubzi	KHO	Fresh and Drying	Taounate	Oulmessia Hamra	OLMH	Fresh	Oulmes
Ghoudane3	GHD3	Fresh	Taounate	Oulmessia Beida	OLMB	Fresh	Oulmes

2.2. Molecular analysis

a. DNA extraction

The DNA was extracted from 30 mg of freeze-dried leaves using the technique CTAB of Saghai Maroof et *al.* [15] with modifications. Molecular characterization was made in the laboratory of the Research Unit "Plant Breeding and Phyto-Genetic Resources Conservation" of the Regional Centre for Agricultural Research in Meknes based on recent studies of Achtak et *al.* [16].

b. Molecular analysis using ISSR markers

Analysis the ISSR markers was performed using primers selected from seven thirty available at said laboratory: F1, F8, F11, IMA834-Z, IMA 5-3 IMA5-Z, UBC-841 (Table 2). The choice was made, after testing polymorphism, amplification of DNA and the reproducibility of the results on five fig cultivars randomly chosen. PCR Amplification was performed in a final volume of 25 µl containing: 2.5 mM $MgCl_2$, 1X PCR buffer, 0.2 mM of each dNTP, 0.5 mM of each primer, 1 unit Taq DNA polymerase and 20 ng of template DNA. Polymerase chain reaction (PCR) was carried out using a Mastercycler Eppendorf using the following conditions: an initial denaturation at 94 °C for 4 min, followed by 35 successive cycles. Each cycle comprises a succession of three steps: denaturation at 94 ° C for 1 min, a hybridization phase at the optimum temperature according to the primer for 1 min (Table 2) and an elongation at 72 ° C for 1 min. After the end of the cycles, the program is completed by a final elongation step at 72 ° C for 10 min. Amplified products were electrophoresed on 2% of agarose followed by staining with ethidium bromide and visualized using an UV trans-illuminator related to an imaging system.

Table 2 : Sequences and specific hybridization temperature of ISSR primers

Locus	Primer sequences (5'----------3')	Hybridization temperature
F1	AGAGAGAGAGAGAGAGTA	41 °C
F8	AGAGAGAGAGAGAGAGCC	46 °C
F11	CACACACACACACACAAC	41 °C
IMA-5-3	CACACACACACACACATG	45 °C
IMA-5-Z	CACACACACACACACAGT	45 °C
IMA 834-Z	AGAGAGAGAGAGAGAGYT	50 °C
UBC-841	GAGAGAGAGAGAGAGYC R3'	46 °C

c. Molecular analysis using specific SSR loci

We selected 9 SSR locus of 17 primers which have been used by Achtak et *al.* [16]. These loci belonging to the three sets of primers: LMFC [17], MFC [18, 19] ; Ahmed et *al.* 2007), FSYC [19]. Analyses were performed according to the PCR conditions developed by Achtak et *al.* [19]. Amplification reactions were performed in a final volume of 25 µl in the presence of 20 ng template DNA, 4 pmol reverse primer and 0.5 mM of each primer, 0.2 mM of each deoxynucleotide, 2.5 mM MgCl2, 1 U Taq polymerase (Qiagene) and 1x of taq buffer. Polymerase chain reaction (PCR) was carried out using a Mastercycler Eppendorf. After 5 min at 94°, 35 cycles were performed with 45s at 94°C, 45 s at either 55 or 60°C (Table 3) and 1 min at 72°C, followed by a final extension step of 10 min at 72°C.

Electrophoresis was performed on 6% polyacrylamide gel which was prepared from a 40% solution of acrylamide, 7.5 M urea and 1X TBE buffer. The revelation was made according to the steps indicated by Benbouza et *al.* [19].

Table 3: Sequences and specific hybridization temperatures of SSR loci

Locus	Primer Sequences (5'----------3')	Hybridization temperature
LMFC30 -F	TCTTTTTAGGCAGATGTTAG	55 °C
LMFC30 -R	TTGTCCGTTTCTTATACAAT	
MFC2 –F	GCTTCCGATGCTGCTCTTA	55 °C
MFC2 -R	TCGGAGACTTTTGTTCAAT	
MFC3 -F	GATATTTTCATGTTTAGTTTG	55 °C
MFC3 -R	GAGGATAGACCAACAACAAC	
FSYC01 -F	CAAATGAAAAACACAAATTTGCCAAC	55 °C
FSYC01 -R	TGCAAGTACTAATTCCTCTGCCGTG	
MFC9 -F	GGAGGCAAAACGACAAACGACAT	60 °C
MFC9 -R	CAAGGAACCAAGCGGGAGGG	
MFC11 -F	CAAAAGAGAAGACCAGCATC	60°C
MFC11 -R	GACGAGGGAAGGAGAGACAC	
LMFC19 -F	AATGAATGGAAATGATCTTG	55°C
LMFC19 -R	CTTATGAAAACTCGGTAGAAG	
LMFC34 -F	GTATTGGATCTTGATTATGTTT	55 °C
LMFC34 -R	GTTACAAAGTACAGGTAAGCA	
MFC4 -F	CCAAACTTTTAGACAACTT	55 °C
MFC4 -R	TTTCTCAACATATTAACAGG	

2.3. Data analysis

The sizes of the bands produced by ISSR were calculated using the Mesurim pro software and that generated by the SSR loci were manually measured with respect to the marker's size.

Genetic relationships among fig cultivars were studied Genetic relationships between olive genotypes were studied on the basis of a similarity matrix [21]. Genetic distances were calculated using Clustering Calculator program developed by Brzustowski [22] through Simple Matching Coefficient. Thus, based on the comparison two by two of genotypes, a histogram of according to the number of alleles which distinguish them has been established.

Two phenograms were drawn based on the unweighted pair group method with arithmetic mean algorithm using the NTSYS-pc program ver. 2.11g [23].

The index PIC (Polymorphism information content) related to the genetic diversity of each primer used was calculated using the formula of Botsein et *al.* [23]

PIC j = n $(1- \sum P^2ij) / (n -1)$, with j: primer concerned, n: The size of the i band and Pij: frequency of marker i revealed by the primer j through the band sum.

For each SSR locus, observed heterozygosity's values were calculated using the GENETIX 4.0 software [25]. The importance of heterozygosity deficiency was assessed using exact tests of Genepop3 software. Furthermore, a factorial correspondence analysis was performed using the Genetics software 4.0 [26] on the SSR markers matrix.

III. RESULTS AND DISCUSSION

3.1. Molecular characterization of cultivars by ISSR markers

Seven I.S.S.R. primers selected, following their polymorphism and the bands clarity, revealed a total of 54 markers varying between 4 to the primer IMA-834-Z and 11 for the primer IMA-5-Z, with an average value of 8 markers primer (Table 4). This number reflects the high level of polymorphism among cultivars revealed by the selected primers. The result obtained is equal to twice the

mean of markers observed by [27], using four ISSR markers in a comparison study of the efficiency of RAPD, SSR and ISSR techniques on the Mediterranean fig cultivars. This difference could be explained by the number and type of ISSR primers used and the plant material studied. In addition, a study conducted on Asian and European cultivars by Ikegami et *al.* [28] showed that the primer UBC-812, the most commonly used to evaluate the genetic diversity was generated nine band. The highest percentage of primers polymorphism the was obtained for the primer IMA-5-Z (92%) and the lowest percentage was observed for F1 primer (63%) (Table 4).

Tableau 4: List of seven I.S.S.R. primers used, their sequence, repeat type (R.T.), hybridization temperature (HT), bands sizes, number of polymorphic markers (P), monomorphic markers (M) and index of polymorphism information content (PIC)

Name	sequence (5'-3')	H.T. (°C)	bands sizes (pb)	Polymorphism %	Number of bandes		
					P	M	PIC
IMA-5-3	CACACACACACACACATG	45	233 - 1235	88	7	1	0,98
F1	AGAGAGAGAGAGAGAGTA	41	910 - 2566	63	5	3	0,96
F11	CACACACACACACACAAC	41	338 - 2561	90	9	1	0,99
IMA-834-Z	AGAGAGAGAGAGAGAGYT	50	834 - 819	80	4	1	0,91
IMA-5-Z	CACACACACACACACAGT	45	488 - 2824	92	11	1	0,99
UBC-841	GAGAGAGAGAGAGAGYC R3'	46	166 - 2692	82	9	2	0.98
F8	AGAGAGAGAGAGAGAGCC	46	288 - 3764	64	9	5	0,98
				80	54	14	0,97

di : dinucleotide

Generally, the number of I.S.S.R. markers generated is correlated positively with the number of primers used. However, this number can be greatly influenced by the analysed plant species and the nature of the migration gel used [29, 30].

Indeed, in comparison with other species, the percentage of polymorphic bands revealed by ISSR primers was very high in *Asparagus acutifolius* L. (100%) [31], *Lupinus spp.* (99%) [32] and in *Oryza sativa* (80.9%) [29], but lower in *Bombyx mori* (64%) [33].

All primers used generated polymorphic profiles with varying and significant index of genetic diversity (Table 4). Indeed, the index analysed according to the procedure of Botsein et *al.* [24] confirmed the genetic variability of seven primers used. The diversity index, which tends towards one, reveals a significant degree of polymorphism. The more its value tends towards one, the primer is polymorphic and vice versa. Thus, the diversity index is 0.91 for the primer IMA-834-Z which has one monomorphic marker on a total of 4 and maximum of 0.99 in the F11 and IMA-5-Z primers which have given almost 100% of polymorphic bands. However, the average value of the diversity index (0.97 ± 0.205) obtained for all the primers consolidates and justifies the choice of seven ISSR primers to analyse our twenty-two fig accessions. In order to confirm the genetic diversity of the collection, pairwise comparison shows that 89% of cultivars pairs are differentiated by 9 to 29 ISSR bands. Thus, the average similarity obtained by the ISSR is 71.6%.

Getting a high number of ISSR polymorphic primers is an important utility. It increases the reliability of the interpretations of the results; especially in our case where profiles generated are reproducible. The number of the polymorphic primers used to analyze various plant species differs according to the authors. Pradeep et *al.* [33] used seven primers, twelve by Wiesner et *al.* [30], 23 by Sica et *al.* [31], a number of 30 per Talhinhas et *al.* [33] and 41 Young et *al.* [34].

Furthermore, the primers that we used in this study are type di-nucleotide repeat. A similar study by Konate [35] in carob showed that they are more efficient than the primers tri-nucleotide repeat.

3.2. Molecular characterization of cultivars by SSR markers

Forty-two alleles were obtained using nine microsatellite loci. The number of alleles per locus varies from three alleles (FSYC01, LMFC19, LMFC34 and MFC4) to 12 alleles (MFC2) per locus with an average of five alleles. Allele size ranges from 100 bps at the FSYC01 loci to 305 bp in the LMFC19 locus (Table 5).

The observed heterozygosity ranged from 0.045 to 0.772 for FSYC01 and MFC9 loci, and 0.42 as the average for all loci. Compared to the value of expected heterozygosity, a significant excess of heterozygosity was observed for the locus MFC9, probably because a selection effect that would take place within a limited gene pool (Table 5). For the fixation index (Fis), it ranges from 0.078(MFC11) to 0.906 (FSYC01) locus, indicating that the genotypes studied have a heterozygosity

deficiency for the loci, except for MFC9 locus, the Fis is negative (-0.406) with an excess significant heterozygote probability. This situation may be explained by the fact that this collection comes from a prospection directed to cultivars with agronomic performance characters in a limited gene pool [27] have discovered a heterozygosity deficiency for four SSR loci (MFC1, MFC2, MFC3, MFC7) in the molecular characterization study (SSR and ISSR) of Moroccan fig germplasm. However, for a larger study of 277 cultivars prospected in Morocco, 15 of 17 loci showed excess heterozygosity [16]. Allelic frequencies ranged from 0.019 for seven alleles belonging to the locus MFC2, MFC9 and FSYC01, to 0.87 for LMFC19-302 allele, with an average of 0.18 and 0.22 as estimated standard deviation.

Table 5: Number and size of alleles and diversity parameters for each of the nine SSR loci

Locus	Range of size (pb)	diversity parameters					
		N	H_0	H_e	P	F_{is}	PI
LMFC30	250-261	4	0,2273	0,7035	0,0000	0,689	0,143
MFC 2	158-190	12	0,5909	0,7955	0,0000	0,279	0,061
MFC 3	108-114	4	0,5455	0,6746	0,0000	0,214	0,163
MFC 9	190-220	3	0,7727	0,5425*	0,0000	-0,406	0,297
MFC 11	182-206	7	0,7273	0,7696	0,0002	0,078	0,077
FSYC01	100-106	3	0,0455	0,4638	0,0000	0,906	0,376
LMFC19	300-305	3	0,1818	0,2800	0,0000	0,371	0,537
LMFC34	214-219	3	0,3636	0,5010	0,0091	0,296	0,306
MFC 4	240-244	3	0,3636	0,4298	0,0035	0,176	0,369
9 locus	100-305	5	0,42	0,58	0,0000	0,29	0,26

N: number of alleles; **Ho:** observed heterozygosity; **He:** expected heterozygosity; **P:** Hardy-Weinberg Test probability; **Fis:** Fixation Index; PI: Probability of identity; * significant excess heterozygosity.

Compared with other collections, it appears that the ENAM collection has diversity parameters such as 5 alleles per locus, and observed heterozygosity of 0.42 are similar to European and Asian cultivars studied by SSR markers where the number of alleles per locus was 5.2 and an observed heterozygosity 0.44 respectively [28]. Diversity levels close to our results have been seen in other collections in Morocco and elsewhere but with heterozygosity higher values. Indeed, the fig collections No. 1 and No. 2 of the Ain Taoujdate Experimental Field of CRRA-Meknes, have seven and six respectively as the average number of alleles per locus and 0.79 and 0.63 as the average observed heterozygosity. A similar level of diversity was shown in the Porquerolles Mediterranean collection (France), with an average of six alleles per locus and 0.54 observed heterozygosity [16]. In contrast, a low polymorphism (3.9 alleles per locus, and 0.38 observed heterozygosity) was detected in Extremadura Spanish collections [17].

The total number of 42 alleles allowed to distinguish 21 SSR profiles whose two Nabout genotypes the same profile. Among the two-hundred-thirty-two cultivars pairwise comparison, only 19 SSR profile pairs were differentiated by less than six alleles. Other pairs were distinguished by 7 to 24 alleles (Fig. 1).

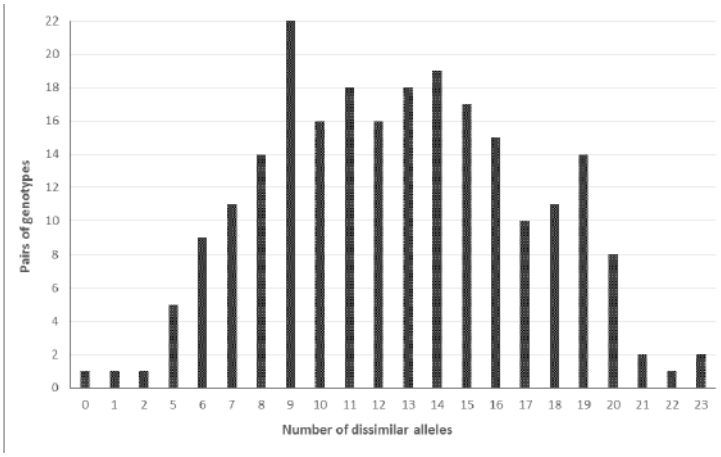

Fig. 1. Frequency distribution of genetic dissimilarity for all pairwise combinations among 22 fig genotypes of ENA Meknes collection

Similar results were reported in a large study of the Moroccan fig [16]. Relatives cultivars (low allelic distinction) presumably correspond to soma-clonal variations via intense vegetative propagation over long periods, while distinct genotypes (over 6 alleles) are issued from seed (sexual reproduction). Getting close cultivars probability, which differ only by 1 to 3 SSR alleles via sexual reproduction, is very low [36]. Thus, descendants analysis of two olive crossbreeding, "Picholine marocaine (Parent female)" x Picholine de Languedoc (Male Parent) " [37; 38] and "Olivière"

(female parent) x "Arbequina" (Parent male) [39] using 36 and 47 SSR loci shows that the descendants are different by at least 16 and 18 alleles respectively. These results indicate that cultivars from the ENAM collection really correspond to prospecting trees belonging diverse populations.

3.3. Cultivars hierarchical classification and factorial correspondence analysis

a. SSR Markers

The SSR data exploitation allowed the all cultivars genetic characterization. Indeed, the dendrogram generated by statistical analysis based on the UPGMA method and similarity distances calculation shows a wide intra-collection molecular diversity (Fig. 2).

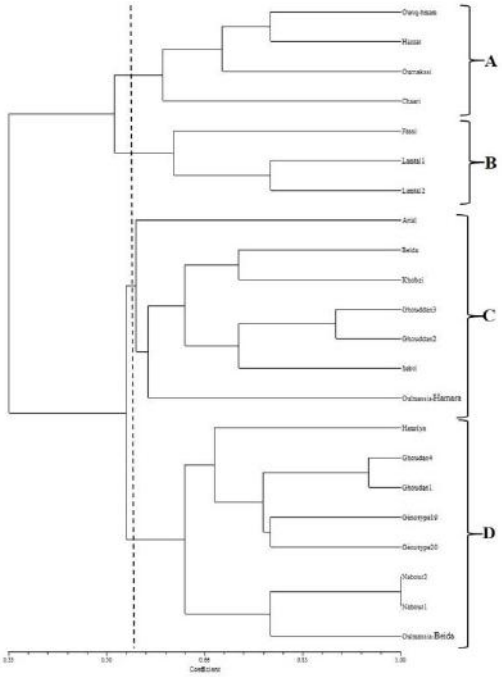

Fig. 2. Genetic relationships among fig cultivars. The dendrogram was based on a similarity matrix UPGMA algorithm of SSR markers.

Approximately 56% of similarity, the resulting dendrogram allowed distinguishing four clusters (A, B, C and D) which each includes 4, 3, 7 and 8 cultivars respectively. Cluster A contains Chaari, Ournaksi, Ounq Hammam and Hzzat cultivars, latter two cultivars belong the same sub-cluster. Similarly, Lemtel1 and Lemtel2 constitute a sub-cluster and belong to the cluster B with the Fassi cultivar. However, Beida and Oulmessia Hamra, Khoubzi and Sebti, Ghoudane2 and Ghoudane3 constitute three sub-clusters and create cluster C with Arial and Hamriya cultivars. Finally, the cluster D contains the rest of cultivars with Oulmessia Beida as single and three sub-clusters constituted by Genotypes19 and Gentypes20 cultivars, Ghoudane1 and Ghoudane4, Nabout1 and Nabout2. The peculiarity of pairs cultivars Lemtel1-Lemtel2, Ghoudane2-Ghoudane3 and Ghoudane1-Ghoudane4 that they are very close and have the same name (Pomological resemblance fruit), suggesting the problems of homonyms and somaclonal variations. The analysis also revealed four cases of homonymy among cultivars (Ghoudan1-Ghoudan, Ghoudan1-Ghoudan3, Ghoudan2-Ghoudan4 and Ghoudan3-Ghoudan4) since they are pairwise genetically and pomology different. Low allelic variations and synonyms and homonyms problems were reported in the fig by Achtak et *al.* [16] and in several other fruit trees [40; 41; 27; 42].

The plot of factorial correspondence for the first, second and third axes, which explained 23.6%, 12.29% and 10.43% of the variance, respectively, giving a cumulative variability of 46.32% (Fig. 3). This plot shows the existence of four similar groups to the four groups revealed by the dendrogram including the same homogeneous cultivars. According to the figure, a clear genetic structure of the studied population (Dendrogram and FCA) is clearly reveals in the analysis. Thus, we find, after this distinction into separate groups, they are from different gene pools and are selected and planted based on their agronomic performance.

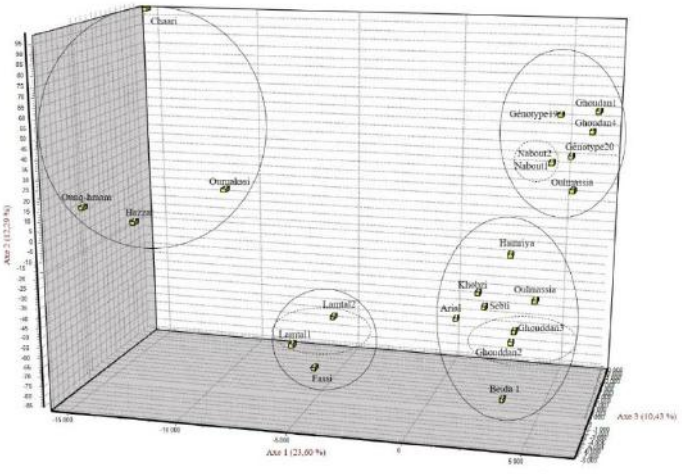

Fig. 3. Factorial correspondence analysis of 22 genotypes as defined in Table 1. the analysis allowed distinguishing for four separate groups.

b. ISSR Markers

The different gels and 54 revealed bands have resulted in the phenogram (Figure 4), which illustrates the genetic relationships existing between 22 fig cultivars. At 77% dissimilarity, the dendrogram below shows four different groups: G1, G2, G3 and G4 respectively containing nine, four, five, and four genotypes (cultivars) that are homogeneous within each group.

Cultivars having the same names lamtal1 and lamtal2, Ghodane2 and Ghoudane3 are very close in pairs, confirming the results obtained using the SSR markers. This confirmation of the molecular similarities shows that the variations between these genotypes are due to the

soma-clonal variations between cultivars with genetic and phenotypic profiles very close.

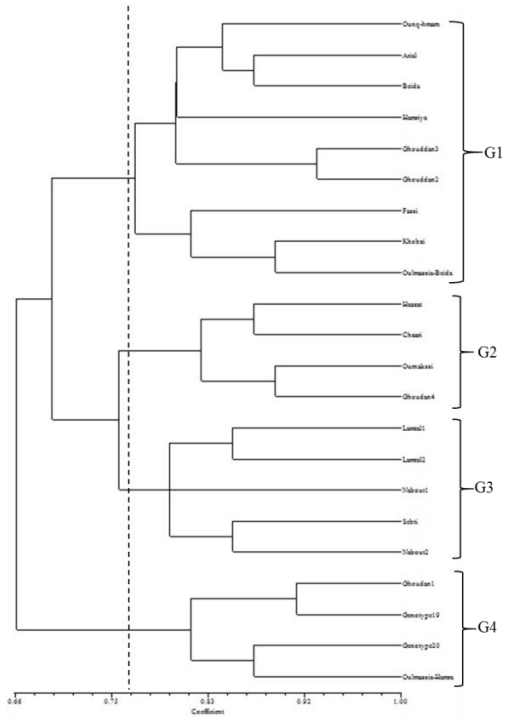

Fig.4. Dendrogram the fig cultivars generated by ISSR based on the UPGMA algorithm and similarity matrix

The dendrogram confirms the high genetic diversity between collection genotypes, however, does not reflect the same homogeneous groups generated by the SSR markers dendrogram (Fig. 4). Our results are in agreement with Ikegami el *al.* [28] studies, on Asian and European cultivars using RAPD, ISSR and SSR markers, which found significant differences in the hierarchical classification generated by the three methods. This can be explained by a low correlation coefficient between the three markers types and different adjustment degrees. Moreover, according to several authors, ISSR give better profiles emanating especially the length of primer sequence, involving a high annealing temperature, compared with the RAPD and RFLP methods, gives thick and reproducible bands [43; 44; 45].

c. Detailed study of cultivars genetic profiles

Pomological characterization, based on 25 qualitative and quantitative traits related to fruit, for the same cultivars realized by Ait Haddou et *al.* [46] has revealed the similarities and differences traits between cultivars having the same denomination (Table 6).

Cultivars are similar for most morphological characters except for Oulmessia Hamra and Oulmessia Beida (Table 6). The molecular marker numbers that does not exceed six alleles per SSR locus for all cultivars except for Oulmessia Hamra and Oulmessia Beida that differ by eight alleles genetically confirms this similarity.

Table 6: Pomological characters most discriminating cultivars bearing the same names in Moroccan collection of ENA Meknes [46].

Accessions	FT	MP	FW (g)	Cal	PL (mm)	FD (mm)	FS1	FS2	SAV	BCE	IPC	PTX	ICF	QS
Lemtel2	B	Midseason	42,37	24	3,97	44,03	Globular	Ovoid	Yes	Light green	Red	Coarse	Small	Medium
Lemtel1	B	Midseason	44,96	23	4,21	44,38	Globular	Ovoid	Yes	Green	Red	Coarse	Without	Medium
Ghoudane3	B	precocious	53,17	19	4,95	47,96	Globular	Ovoid	Yes	Purple	Dark red	Medium	Without	Medium
Ghouane2	B	precocious	55,05	18	4,75	48,20	Globular	Ovoid	Yes	Purple	Dark red	Medium	Medium	Medium
Nabout2	U	Tardive	30,95	34	4,07	39,14	Globular	Ovoid	Yes	Green	Dark red	Coarse	Without	Medium
Nabout1	U	Tardive	31,08	34	3,84	36,28	Globular	Ovoid	Yes	Green	Red	Coarse	Without	Medium
Ghoudane4	B	Midseason	45,73	22	5,03	44,86	Globular	Ovoid	Yes	Black	Dark red	Small	Without	Large
Ghoudane1	B	Midseason	44,56	23	4,92	44,73	Globular	Ovoid	Yes	Black	Dark red	Medium	Without	Large
Genotype19	B	Midseason	42,49	24	5,39	44,30	Globular	Ovoid	Yes	Black	Red	Coarse	Without	Medium
Genotype20	B	precocious	42,39	24	5,53	44,64 44,52	Globular	Ovoid	Yes	Black	Red	Medium	Without	Large
Oulmessia Hamra	U	Midseason	43,52	23	10,9	44,93	Globular	piriformis	No	Violet	Amber	Coarse	Without	Large
Oulmessia Beida	U	Tardive	45,65	22	7,2		Globular	Ovoid	Yes	Yellowish	Dark red	Medium	Small	Medium

FT: fruit type; **PM**: maturity period; **FW**: fruit weight; **Cal**: caliber; **PL**: peduncle length; **FD**: fruit diameter; **FS1**: fruit shape as (width / length); **FS2**: fruit shape depending on where of the maximum width; **SAV**: symmetry along the vertical axis; **BCE**: background color of epidermis; **IPC**: internal pulp color; **PTX**: pulp texture; **ICF**: internal cavity of the fruit; **QS**: quantity of seeds.

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Pairwise molecular profiles comparison of the accessions, having the same denominations, has also identified the differences between cultivars and compared relative to pomological results.

The Lemtel cultivar is represented by two different denominations that have the same characteristics except for pomological fruit weight, the epidermis background color and the fruit internal cavity (Table 6). Indeed, this similarity is confirmed by the molecular profiles that differ only by five SSR markers. These accessions are therefore considered genetically closer. The Nabout cultivar is represented by two similar accessions at all Pomological characters (Table 6). Both accessions are all characterized by globular ovoid figs, a green epidermis and as weight 31g. Furthermore, they have a difference in fruit diameter. This Pomological similarity between the two denominations is validated by the same SSR profile. This result is different from the study of Charafi [47] which shows allelic differences between cultivars of Naboute of Ouazzane Regione cooperative (Janane Rif) and those of the experimental field of Ain Taoujdate (INRA-Meknes).

The cultivar Ghoudane includes four accessories which Ghoudane1 and Ghoudane4 have the same pomological traits and therefore constitute same genotype. However, Ghoudane2 and Ghoudane2 accessions are close together and different than Ghoudane1 and Ghoudane4 by the period of maturity, size, epidermis background color and the seed quantity (Table 6). Given the significant differences between pomological Ghoudane1 and Ghoudane4, firstly, and secondly Ghoudane2 and Ghoudane3, each pair would thus consist of a same cultivar. This distinction is confirmed by molecular results also revealed the similarity among Ghoudane1 and Ghoudane4 with only one SSR allele difference and 2 SSR alleles between Ghoudane2 and Ghoudane3. Ghoudane Polytonality was already demonstrated by Achtak et *al.* [16]. Varietal confusion, synonyms and homonyms problems, low allelic variations related to somaclonal variations, are all very common problems and traits in the fig [16].

Genotype19 and genotype20 are close genetically (5 different alleles), they are also similar for the majority of pomological traits (Table 6), except for the maturity period, texture and the quantity of seeds. Since these characters are not very discriminating, we can consider the genotype19 and genotype20 are derived from the same genetic origin.

Olmessia Hamra and Oulmessia Beida cultivars will differ by the majority pomological characters (Table 6) and 8 SSR markers. Therefore, they can be considered as two separate cultivars and Oulmessia denomination comes from their origin in addition to the genotype color.

IV. CONCLUSION

Molecular characterization allowed characterizing the genetic profiles of 22 fig cultivars from the ENA-Meknes collection. The two cultivars Nabout 1 and 2 have the same SSR profile and pair's accessions Ghoudan1 and Ghoudan4, Ghoudan2 and Ghoudan3, Genotype19 and Genotype20, Lamtel1 and Lamtael2 are genetically very close. This similarity was also confirmed by the pomological characterisation. The remaining accessions are different and therefore can be regarded as separate cultivars. The complementarity of the two approaches is therefore confirmed and the establishment of a genetic identity for each cultivar should consider the polyclonality of some varieties.

The selection of authentic reference cultivars within polyclonal cultivar is not easy when we consider the genetic diversity of plant materi*al.* In addition to the homonymies and synonymies problems and soma-clonal variations which are widespread in this species. For agronomic considerations, we suggest to consider the reference cultivar denomination having the better pomological characteristics. Choosing the most important cultivar is justified by the aboriginal always opted for the best genotypes for this culture. In the same direction, it is essential to make intra-varietal characterizations by combining pomological and molecular characterization using SSR markers to select the best clones of each variety. The collections should contain the selected clones that are references for any breeding program. Thus, follow up these collections will quantify phenotypic variation for this species particularly sensitive to climate variations.

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