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Genetic variability of Tunisian olive cultivars by ISSR and SSR markers

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Abstract - The present work aims to study the genetic diversity of 9 Tunisian varieties from different geographical origins; 'Chemlali', 'Chétoui', 'Jemri', 'Zarrazi', 'Zalmati', 'Oueslati', 'Chemlali Jerba', 'Neb Djmal' and 'Fakhari' based on Inter Simple Sequence Repeat (ISSR) and Simple Sequence Repeat (SSR) markers. An authorized variation of different primers is staked for the detection of polymorphism in varieties. The polymorphism percentage varies from 62.5 % to 100%. The three polymorphic primers also showed variations in the average looking informative bands (AvIb) and resolving power (RP). The primer A3 has the value of the lowest AvIb (0.28), while the primer A2 has the highest value (0.49). For RP, the lowest value (2.31) is distinguished from the primer A3 and the highest (3.96) at the primer A2. In addition, statistical analysis showed a large variation in genetic distance between varieties; the lowest (0.071) is observed in both accessions 'Chétoui' and 'Fakhari', and the greater (0,562) was distinguished between the two cultivars 'Chemlali Jerba' and 'Neb Djmal'. The analysis of the dendrogram "UPGMA" gave us 2 different groups. The geographical origin of varieties has no influence on the obtained clusters: The tested cultivars made genetic affinity groups regardless of their geographic origin. The microsatellites used revealed the presence of 10 bands in total, the primer DCA 9 as GAPU47 show a polymorphism rate of 100 %, while the other two OUB2 and OEX primers showed varying intensity bands between new cultivars studied. The analysis of the dendrogram "UPGMA" gave us 5 different groups; this revealed a significant genetic diversity.

Keywords: Olive (*Olea europaea* L.), genetic diversity, microsatellite (SSR), ISSR marker, genetic distance.

1. Introduction

Olea europaea L. represents one of the most important trees in the Mediterranean basin and the oldest cultivated plant. In fact, the genetic patrimony of the Mediterranean basin olive trees is very rich and is characterised by an abundance of varieties. Based on estimates by the FAO (2010) Plant Production and Protection Division Olive Germplasm, the world's olive germplasm contains more than 2.629 different varieties, with many local varieties and ecotypes contributing to this richness. It is likely that the number of cultivars is underestimated because of inadequate information about minor local cultivars that are widespread in different olive-growing areas. Current scientific knowledge offers the possibility of introducing new assessment systems, based not only on the varietal character phenology, usually adopted, but also on genetic traits. Various researches have been carried in the identification and assessment of the most important varieties. The first work of classification and identification of olive varieties date back to the nineteenth century. However, Ruby (1917) was the first to use the various organs of the olive tree (leaf, fruit and endocarp) to characterize and classify the varieties of this species. Since then, various studies about identification was developed from the combination of morphological, agronomic and phenological characters (Rallo et Cidraes 1975; Cimato et al. 1997; Cantini et al. 1999). The need to overcome difficulties in the morphological characterization has led some researchers to undertake new varietal identification studies based on genetic markers (enzymatic

and molecular markers). The enzymatic markers were the first developed genetic markers to identify olive varieties. However, these markers have shown their limits for identification of closely related varieties (Ouazzani et al. 1995). Recent research has focused on using morphological markers associated with molecular ones to characterize and identify olive varieties (Ercisli et al. 2009; Muzzalupo et al. 2009). The identification of varieties by using molecular markers is a crucial aim of modern horticulture, because such a technique would greatly facilitate breeding programmes and germplasm collection management. Molecular markers, mainly RAPD (random amplified polymorphic DNA), have been extensively studied in recent years for use in the identification of olive varieties. The dominant character and the problem of reproducibility of results characterizing the RAPD justify the recent resort of researchers on the use of ISSR (inter simple sequence repeats) (Pasqualone and Caponio. 2001; Vargas and Kadereit. 2001; Essadki and Ouazzani. 2003) SSR (simple sequence repeats) (Rallo et al. 2000; Carriero et al. 2002; Cipriani et al. 2002; Sefc et al. 2000) for the identification of olive varieties. In this context, this study has set the objective to study the discriminatory power of molecular markers (ISSR and SSR) for the characterization and classification of olive varieties from different regions in Tunisia.

2. Material and methods

2.1. Plant material

This work focused on 9 varieties of olives ('Chemlali Sfax', 'Chétoui', 'Chemlali Djerba', 'Fakhari', 'Neb Djmal', 'Oueslati', 'Zarrazi', Zalmati and 'Jemri'). The collections have involved five different regions from South to North of Tunisia; Djerba (33°48'27" N 10°50'42" E), Tataouine (32°51'26" N 10°17'19" E), Zarzis (33°34'32" N 11°1'53" E) , Ben Guerdene (33°7'25" N 11°13'55" E), Kairaoun (35°41'44" N 9°43'23" E), Sfax (34°56'7" N 10°36'53" E) and Beni Khalled (36°39' N 10°36' E 36.65, 10.60).

2.2. DNA extraction

Total DNA was extracted from fruits of 9 *Olea europaea* L. cultivars. The DNA extraction protocol was described by Aras et al. (2003) was modified as follow : 1 g plant material (olive leaf) were ground in 1ml CTAB 2% buffer (100 mM Tris HCl,) preheat at 60°C for 1 hour. The whole was incubated at 60°C for 1 hour and gently mixed every 10 min. After centrifugation, the supernatant was extracted for twice with chloroform/isoamyl alcohol (24:1) (600 µl). After this, 1 µl of RNase was added to every tube (700 µl of supernatant) and incubated for 30 min at 37°C. Finally, we add 600 µl of cold isopropanol to each tube and we let it precipitate all the night at -20°C.
The next day, following centrifugation (15 min at 14000g), the pellet was washed in 500 µl of 70% ethanol and dissolved in 150 µl of 1 X TE (1 mM Tris, 0.1 mM EDTA, pH 8).

2.3. PCR reaction

2.3.1. Primers used

The study of the molecular variability for these 9 varieties is carried out using two types of molecular markers ISSR (inter simple sequence repeat) and SSR (simple sequence repeat).
A set of three ISSR markers (A1, A2 and A3) as four microsatellite markers (ssrOeDCA9, GAPU47, OUB2, and OIL) were selected (Carriero et al. 2002; Cipriani et al. 2002; Sefc et al. 2000). The selected markers have been described to be ones from the very efficient for olive cultivar identification and population genetic studies (Baldoni et al. 2009).

2.3.2. PCR amplification reaction

Each PCR reaction is performed in a total volume of 25µl, containing 2µl of buffer (5x Colorless GoTaq Flexi Buffer) PCR, 2µl (100 ng) of genomic DNA, 25 mM MgCl2, and 500U of Taq polymerase. We varied the concentration of 2 mM to 4 mM NTP and the amount of primer 2 to 4µl and for a stock solution of 10 µM. The PCR reaction is performed in a thermocycler (DNA-Amp Mastercycler gradient eppendorf). The initial denaturation was performed for 5 min at 94 ° C, followed by 45 cycles (1 min at 94 ° C, 45 sec at Tm (45°C) specific primer and 1 min 72°C) and followed by a final extension of 7 min at 72 ° C. For the SSR primer, the program: 95 ° C for 5 min for denaturation, followed by 40 cycles (30 sec at 94 ° C, 45 sec at 60 ° C and 1 min at 72 ° C) and followed by a final extension of 10 min at 72 ° C (Rekik 2009).

2.4. Data Analysis

All analyzes were carried out using the program MVSP version 3 (Multi Variate Statistical Package). Polymorphic bands were recorded into a data matrix of presence (1) and absence (0) of bands. This data matrix was converted into a similarity matrix (S) using Jaccard coefficient (Jaccard 1908).
Several parameters were considered in this study, among them:
- The polymorphic bands percentage (PBP)
- The resolving power (RP) was calculated for each SSR locus according to Backus et Gilbert (1968):

$$RP = \sum Ib \qquad Ib = 1-(2|0.5-p|)$$

(Where p: is the proportion of species containing I bands)

Genetic relationships among cultivars are represented by a dendrogram based on the distances calculated from the Jaccard index. The dendrogram was constructed using the Unweight Pair Group Method using an Arithmetic average (UPGMA).

3. Results and discussion

3.1. Control of DNA quantity and quality

The quality and purity of DNA are among the most critical factors for PCR analysis. To obtain highly purified nucleic acids, adequate extraction methods should be applied. The protocol described by Aras et al. (2003) with slight modifications allows obtaining a pure and amplifiable DNA. The quality of the extracted DNA was checked by measuring the optical density (Table 1) and by electrophoresis on agarose gel (Figure 1).

Table 1. Optical density (OD) of the samples studied

Code	Variety Name	A 260/280
1	Chemlali Djerba	124
2	Fakhari	164
3	Neb Djmal	236
4	Oueslati	342
5	Zarrazi	331
6	Chemlali Sfax	240
7	Chetoui	189
8	Jemri	181
9	Zalmati	154

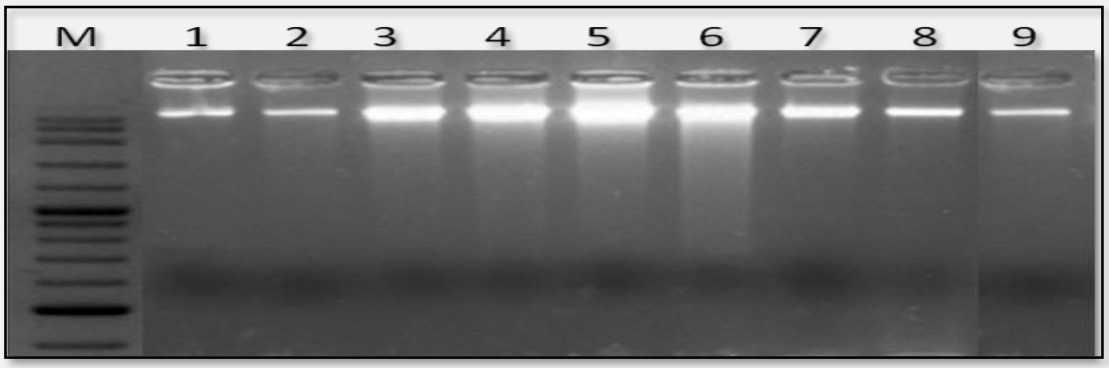

Figure 1. Electrophoretic profiles of DNA extracts of different olive cultivars

3.2. Evaluation of ISSR markers to estimate the diversity among olive varieties

Agarose gels showing the different amplification primers are shown in figure 2.

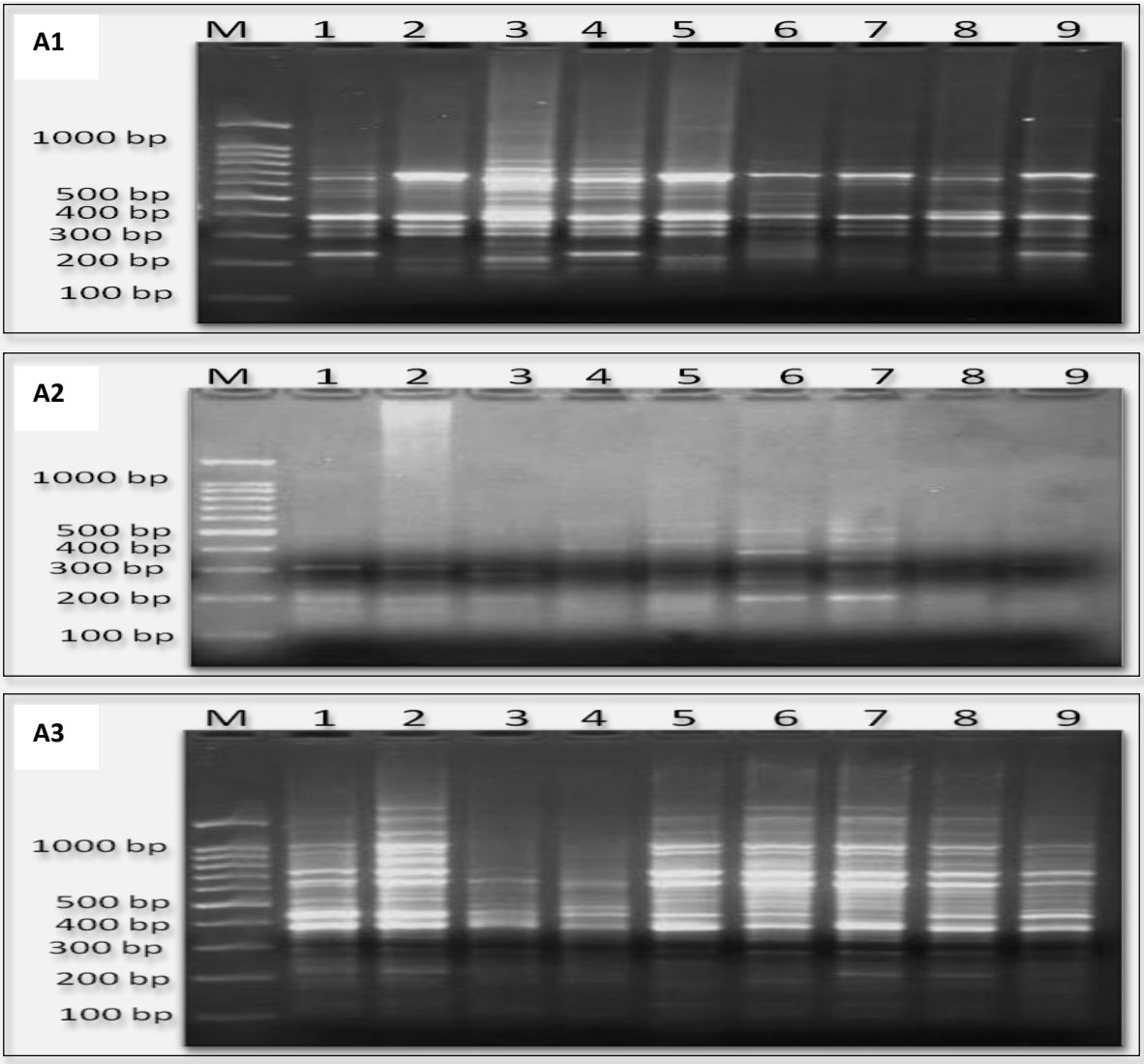

Figure 2. ISSR profiles of 9 olive varieties obtained with the primers A1 , A2, A3 ; M (molecular marker), 1: Chemlali Djerba , 2: Fakhari , 3: NebDjmal , 4: Oueslati , 5: Zarrazi , 6: Chemlali Sfax , 7: Chetoui , 8: Jemri , 9: Zalmati

The analysis of the amplification profiles obtained for each primer and comparison are summarized in the following table. The three primers have generated a total of 24 bands, the bands size varies from 200 bp and 1000 bp.

Table 2. Percentage of polymorphic bands and resolving power (RP) for ISSR primers used

	ISSR bands			Resolving Power (RP)	Average of Informativbands (AvIb)	Size of bands in bps
	Total	Polymorphe	% of polymorphism			
A1	8	6	75%	3.52	0.44	210-750
A2	8	8	100%	3.96	0.49	200-450
A3	8	5	62.5%	2.31	0.28	210-1000
Total	24	19	79.16%	3.26	0.40	200-1000

Among these 24 bands obtained, there are 19 polymorphic loci and 5 monomorphic loci. The percentage of polymorphism ranged from 62.5% to 100%. The primer A3 revealed the lowest percentage (62, 5%) while the primer A2 detected the highest percentage (100%). The three polymorphic primers also showed variations in the average of informative bands (AvIb) and the resolving power (RP). The average of AvIb ranged from 0.28 to 0.49. The primer A2 showed the highest value of AVIb (0.49) and also the highest value of Rp (3.96) so that the primer A3 has the lowest value of Rp (2.31) and the lowest value of AvIb (0.28).

3.3. Patterns of genetic diversity based on ISSR markers

Each polymorphic band was recorded into a data matrix of presence (1) and absence (0) of band. This data matrix was converted into a Similarity matrix (S) (Table 3), using Jaccard coefficient (Jaccard, 1908), and the establishment of dendrogram (Figure 3) by unweighted pair group method using an arithmetic average cluster analysis. The similarity degree between the 9 olive tree cultivars based on ISSR markers (Table 3) range from 0 to 1.

Table 3. Diagonal matrix similarity (S) of 9 olive cultivars estimated according Jaccard formula and based on ISSR marker

	C1	C2	C3	C4	C5	C6	C7	C8	C9
C1	1,000								
C2	0,786	1,000							
C3	0,467	0,533	1,000						
C4	0,385	0,357	0,600	1,000					
C5	0,714	0,923	0,467	0,385	1,000				
C6	0,846	0,923	0,467	0,385	0,846	1,000			
C7	0,733	0,929	0,500	0,333	0,857	0,857	1,000		
C8	0,643	0,714	0,615	0,417	0,769	0,643	0,667	1,000	
C9	0,769	0,714	0,400	0,417	0,769	0,769	0,667	0,692	1,000

Furthermore, the lowest genetic distances were observed between the following variety combinations: C2-C7 (0.071); C2-C5-C6 (0.077); C7-C5 (0.143); C6-C7 (0.143); C6-C5-C1 (0.154). In against part, the most distant cultivars are C1-C3 (0.615); C4-C7 (0.667); C2-C4 (0.643). All other cultivars show intermediate genetic distances can be grouped with each other with varying degrees of approximation. It should be noted that the cultivar C4 has the largest genetic distances with relatively all accessions. The (S) matrix was used to obtain a cluster diagram of cultivars based on ISSR markers (Figure 3).

The dendrogram in Figure 3 depicts the pattern of relationships between the studied cultivars. There is no clear clustering of cultivars in relation with their growing area. Nevertheless, the nine clusters can be defined by cutting the dendrogram at a genetic distance (Dg) value of 0.59. The first major group includes seven varieties 'Chetoui' from Beni Khalled; 'Chemlali Djerba' from Djerba; 'Chemlali Sfax'; 'Zarazi' from Zarzis; 'Jemri' from Ben Guerdene, 'Zalmati' from Zarzis also and 'Fakhari' from Tataouine. The second group contains two varieties 'Oueslati' and 'Neb Djmal' which are derived from kairaoun region and tataouine these cultivars have a genetic distance of 0.25.
If one looks globally at the dendrogram, there seems to be no correlation between ISSR polymorphism and growing area.

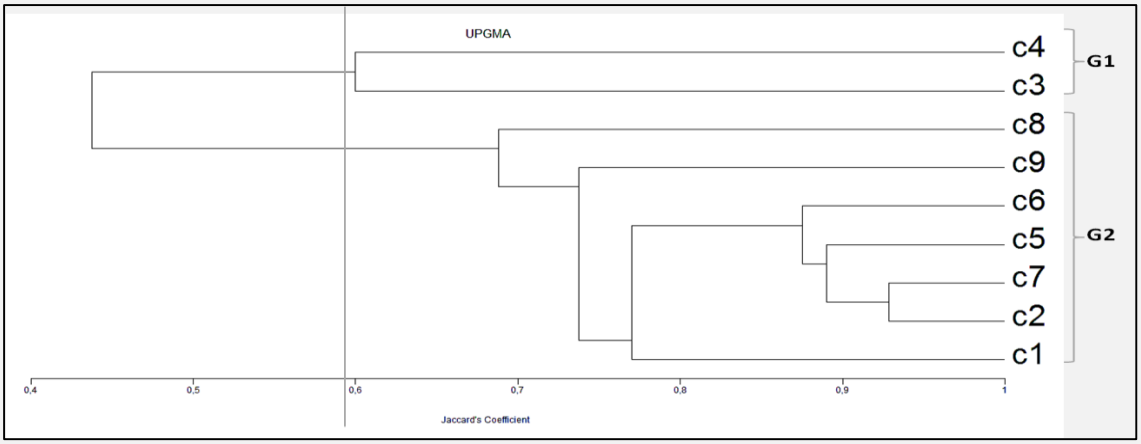

Figure 3. Dendrogram of 9 olive cultivars genrated by unweighted pair group method using an arithmetic average cluster analysis using Jaccard similarity coefficients from inter simple sequence repeat markers

3.4. Evaluation of SSR markers to estimate the diversity among olive varieties

DNA amplification profiles of different cultivars by SSR primers are present in the figure below (Figure 4).

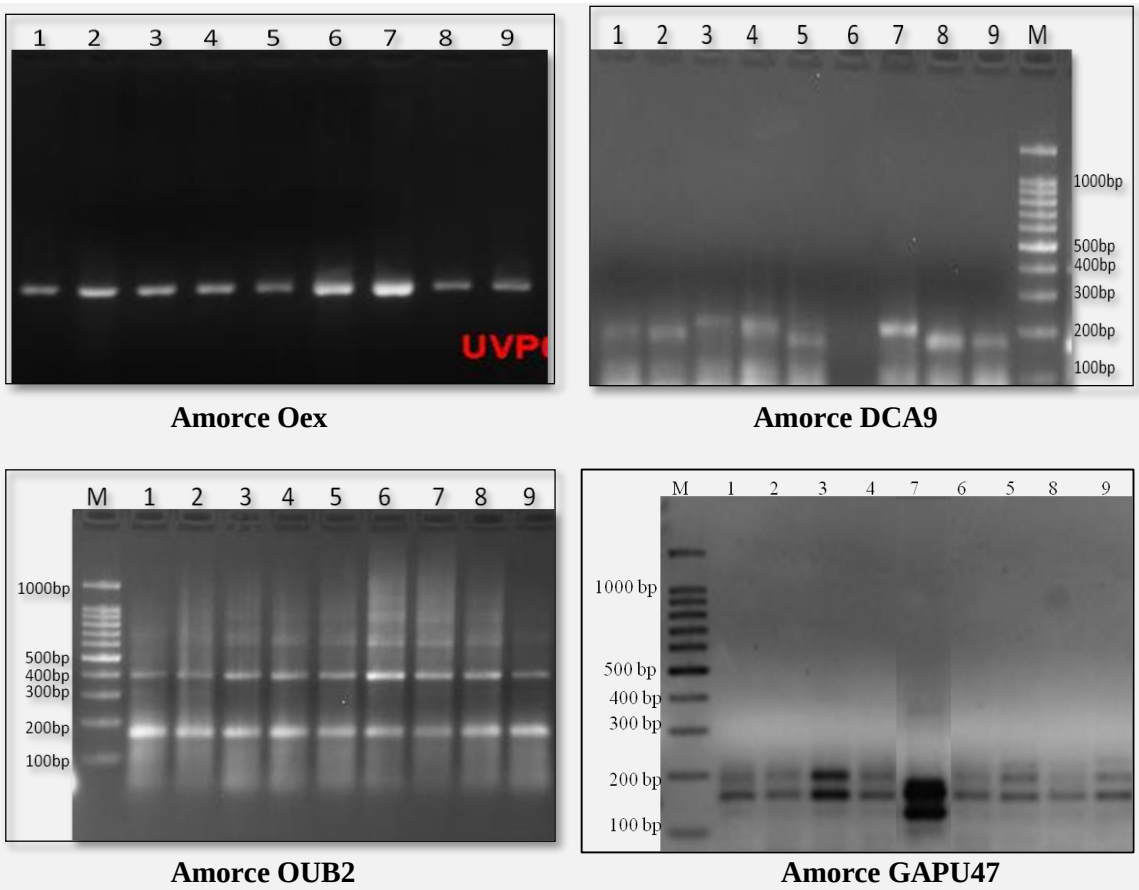

Figure 4. SSR profiles of 9 olive varieties obtained with four primers; M (molecular marker), 1: Chemlali Djerba , 2: Fakhari , 3: NebDjmal , 4: Oueslati , 5: Zarrazi , 6: Chemlali Sfax , 7: Chetoui , 8: Jemri , 9: Zalmati

The analysis of the amplification profiles obtained for each primer and comparison are summarized in the following table. The four primers have generated a total of 10 bands, the bands size varies from 140 bp and 400 bp.

Table 4. Percentage of polymorphic bands and resolving power (RP) for ISSR primers used

	SSR bands			Resolving Power (RP)	Average of Informativbands (AvIb)	Size of bands in bps
	Total	Polymorphe	% of polymorphism			
DCA 9	3	3	100%	1.8	0.45	180-210
OEX	1	0	0%	--	--	256
OUB 2	2	0	0%	--	--	180-400
GAPU47	4	4	100%	3.2	0.8	140-210
Total	10	7	70%	1.25	0.31	180-400

The analysis of OEX electrophoretic profile, we can observe a single monomorphic band (256bp). The difference between the nine cultivars is noted by the intensity of bands, which is remarkable especially in cultivars 6 and 7. Rekik (2009) was able to distinguish the difference in band intensity between Chemlali Sfax and Chétoui. Rekik (2009) succeeded in detecting and analyzing SNPs in after amplification with the primer OEX. The electrophoretic profile of the primer OUB2, revealed two bands in all cultivars; the first band (180 bp) and the second band (400 bp). DCA9 shows a total of three bands which are polymorphic, ranging from 100 to 250 bp. In this case the polymorphism rate is about 100%. Hence diversity is revealed in the studied cultivars. GAPU47 gives a total of four bands which are polymorphic, its size range from 140 bp to 210 bp.

3.5. Patterns of genetic diversity based on SSR markers

Each polymorphic band was recorded into a data matrix of presence (1) and absence (0) of band. This data matrix was converted into a Similarity matrix (S1) (Table 5), using Jaccard coefficient (Jaccard 1908), and the establishment of dendrogram (Figure 5) by unweighted pair group method using an arithmetic average cluster analysis. The similarity degree between the 9 olive tree cultivars based on ISSR markers (Table 5) range from 0 to 1.

Table 5. Diagonal matrix similarity (S1) of 9 olive cultivars estimated according Jaccard formula and based on SSR markers

	C1	C2	C3	C4	C5	C6	C7	C8	C9
C1	1,000								
C2	0,500	1,000							
C3	0,429	0,429	1,000						
C4	0,500	0,500	0,625	1,000					
C5	0,750	0,400	0,571	0,667	1,000				
C6	0,429	0,250	0,556	0,444	0,375	1,000			
C7	0,429	0,250	0,400	0,625	0,571	0,556	1,000		
C8	0,250	0,429	0,750	0,444	0,375	0,556	0,400	1,000	
C9	0,286	0,500	0,857	0,500	0,429	0,444	0,300	0,857	1,000

The smallest genetic distance value of 0.143 was observed between 'Zalmati' - Neb Djmal'/'Jemri', cultivars that differ greatly for their agromorphological and chemical characteristics. The maximum genetic distance (Dg=0.75) was found between the 'Fakhari'- 'Chemlali sfax'/'chetoui', 'Chemlali Djerba'- 'Jemri'.

This result allows us to conclude that there is a high genetic diversity with a high level of polymorphism (> 50%) between the studied cultivars. This result confirms the results reported by Kammoun (2007) based on AFLP markers.

The cluster diagram of cultivars based on SSR markers is obtained using the matrix (S1) (Figure 5).

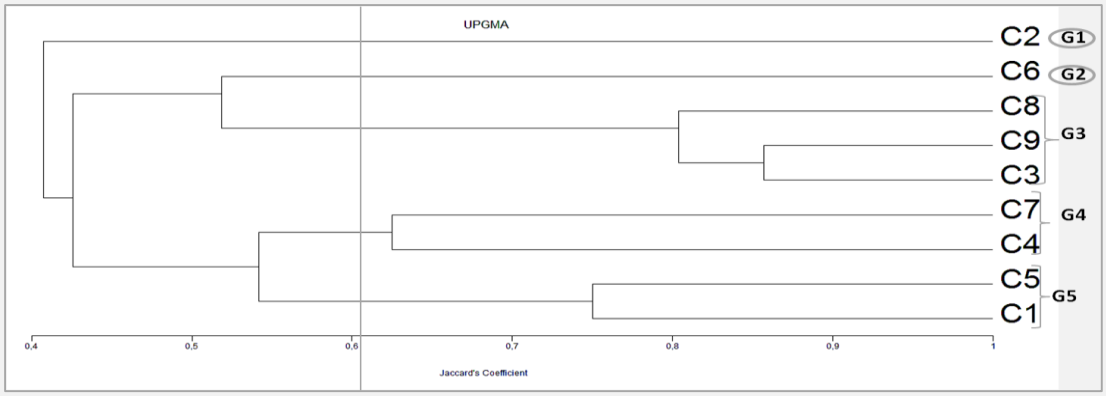

Figure 5. Dendrogram of 9 olive cultivars genrated by unweighted pair group method using an arithmetic average cluster analysis using Jaccard similarity coefficients from simple sequence repeat markers

The dendrogram in Figure 5 depicts the pattern of relationships between the studied cultivars. There is no clear clustering of cultivars in relation with their growing area. Nevertheless, the nine clusters can be defined by cutting the dendrogram at a genetic distance (Dg) value of 0.61. The two first groups G1 and G2 includes each one one variety: G1'Fakhari' from Tataouine and G2 'Chemlali Sfax'. The major group G3 includes three varieties; 'Neb Djmal', 'Zalmati' from Zarzis and 'Jemri' from Ben Guerdene. G4 contains two varieties 'Oueslati' and 'Chetoui' from Beni Khalled. And the last group G5 includes also two varieties 'Chemlali Djerba' from Djerba; 'Zarazi' from Zarzis.
If one looks globally at the dendrogram, there seems to be no correlation between SSR polymorphism and growing area.

3.6. Discussion

In the present work, we tried to study the diversity between different olive cultivars from different regions in Tunisia by molecular approach using ISSR and SSR markers. In olive, ISSR markers were successfully used to distinguish 10 Italian varieties by studying genomic DNA extracted from olive fruit (Pasqualone et al. 2001). These markers were also applied in phylogenetic analysis in olive cultivar identification (Vargas and Kadereit 2001) and within the O. europaea species (Hess and others 2000). ISSRs have been used for intracultivar variability of 201 accessions belonging to 11 Portuguese cultivars (Gemas et al. 2004). Such a high average (polymorphism rate is about 79.16%) reveals the elevated level of variability when compared with similar studies (Pasqualone et al. 2001). Similar results to our results were mentioned by Ben Abdallah (2014) when studying Tunisian and foreign varieties cultivated in Tunisia.
The use of these markers has proven very effective for the study of olive genetic diversity (Belaj et al. 2004). These markers alone or in combination with other markers have been widely used to analyze the clonal variation and the genetic variability of olive cultivars (Gemas et al. 2004; Terzopoulos et al. 2005). Previous studies have concluded that ISSR markers are effective in the phylogenetic assessment of relations in the complex Olea. europaea L. (Gemas et al. 2004) and in olive fruits and leaves identification (Pasqualone et al. 2001).
The microsatellite markers used in this study were polymorphic and demonstrated their utility in discriminating between Tunisian olive accessions. Our present study is largely in accordance and comparable with previous studies (Baldoni et al., 2009; Bracci et al., 2009; Muzzalupo et al., 2010) using SSRs as genetic analysis method to assess the genetic variability of Italian olive cultivars, including some minor exceptions.
Sarri et al. (2006) confirmed the power of SSR markers in the identification of 118 cultivars from different Mediterranean areas. In addition, several other papers used SSR technologies to study the genetic diversities of olive cultivars. In fact, Tamalli et al. (2006) investigated the diversity of 25 Tunisian olive cultivars using 10 SSR markers, and Tamalli and others (2007) studied the diversity of the 2 major Tunisian cultivars (Chemlali and Chetoui) using 11 SSR markers. Another group (Rekik et al. 2008) used 10 SSR markers to differentiate 20 Tunisian olive cultivars. A recent report by Muzzalupo et al. (2009) characterized 211 Italian olive cultivars by using 11 loci microsatellites in

order to study and to establish relationships of geographically related olive tree cultivars. Microsatellites are also very useful markers for paternity analysis (Rallo et al. 2000; Rekik et al. 2008).

Microsatellite markers are high discriminative power, which are particularly interesting to analyze the structure of genetic diversity both at the intraspecific level between species. According to several authors as Rallo et al. (2000), these SSR markers were confirmed as a powerful tool for cultivar identification. Their confirmed codominant character makes them an ideal system for paternity analysis and linkage mapping. They offer the advantage of being specific locus (Rallo et al. 2000) with informative content per locus significantly higher than RAPD (Fabbri et al. 1995) and AFLP (Kammoun 2007).

4. Conclusion

Indeed, for ISSR we got a polymorphism rate in order of 79.16%, which confirms the effectiveness of this technique for the study of genetic variability. The same for microsatellites, the resultant we obtained were interesting and clearly show genetic polymorphism of the studied cultivars.

Those results are preliminary and provide too much informations to enlarge other studies about genetic relationship. It would be interesting to extend the study of genetic variability to greater number of varieties from different regions and compare genetic diversity among cultivated genotypes and wild type of the species.

With the exception of OUB2 and Oex, oher microsatellites used confirmed to be reliable for olive cultivar classification in a germplasm collection. Therefore, the set used here can be recommended for DNA fingerprinting in other Tunisian collections and thus contribute to the establishment of a universal molecular database of olive genetic resources based on standard SSR markers as proposed by Baldoni et al. (2009).

5. References

Aras RA, Fisher W, Perez-Perez GI, Crosatti M, Ando T, Haas R, and Blaster MJ (2003) Plasticity of repetitive DNA sequences within a bacterial (Type IV) secretion system component . J. Exp. Med. 198, 1349-1360.

Backus GE, Gilbert JF (1968) The resolving power of gross earth data. Geophys J Roy Astron Soc 16: 169-205.

Baldoni L, Nicolò GC, Mariotti R, Ricciolini C, Arcioni S, Vendramin G, Buonamici A, Porceddu A, Sarri V, Ojeda MA, Trujillo I, Rallo L, Belaj A, Perri E, Salimonti A, Muzzalupo I, Casagrande A, Lain O, Messina R, and Testolin R (2009) A consensus list of microsatellite markers for olive genotyping. Molecular Breeding, Vol.24, pp. 213–231.

Belaj A, Rallo L, Trujillo I, Baldoni L (2004) Using RAPD and AFLP markers to distinguish individuals obtained by clonal selection of Arbequina and Manzanilla de Sevilla olive. HortScience, 39, 1566-1570.

Ben Abdallah K (2014) Etude de la variabilité de quelques populations d'olive par marqueurs biochimiques et moléculaires (ISSR, RAPD). Institut des Régions Arides-Medenine, Tunisie.

Cantini C, Cimato A, Graziano S (1999) Morphological evaluation of olive germplasm present in Tuscany region. Euphytica 109:173–181.

Carriero F, Fontanazza G, Cellini F, Giorio G (2002) Identification of Simple Sequence Repeats (SSRs) in olive (*Olea europaea*L.). Theoretical and Applied Genetics 104:301–307.

Cimato A, Cantini C, Sani G, Marranci M (1997) Il germopalsma dell'olivo i Toscana Regione Toscana-CNR-A.R.S.I.A.

Cipriani G, Marrazzo MT, Marconi R, Cimato A (2002) Microsatellite markers isolated in Olive (*Olea europaea* L.) are suitable for individual fingerprinting and reveal polymorphism within ancient cultivars. Theoritical and Applied Genetics 104:223–228.

Ercisli S, Barut E, Ipek A (2009) Molecular characterization of olive cultivars using amplified fragment length polymorphism markers. Genetics and Molecular Research, Vol.8, pp. 414-419.

Essadki M, Ouazzani N (2003) R ésultats préliminaires de l'identification des variétés d'olivier à l'aide des marqueurs génétiques ISSR. Olivae 97:42–45.

Fabbri A, Hormaza JI, Polito VS (1995) Random amplified polymorphic DNA analysis of olive (*Olea europaea* L.) cultivars. J Am Soc Hort Sci 120: 538–542.

FAO (2010) The Second Report on the State of the World's Plant Genetic Resources for Food and Agriculture. Rome, Italy, ISBN 978-92-5-106534-1.

Gemas V, Almadanim MC, Tenreiro R, Martins A and Fevereiro P (2004) Genetic diversity in the Olive tree (*Olea europaea* L. subsp. *europaea*) cultivated in Portugal revealed by RAPD and ISSR markers.

Hess J, Kadereit W, Vargas P (2000) The colonization history of Olea europaea L. in Macronesia based on internal transcribed spacer 1 (ITS-1) sequences, randomly amplified polymorphic DNAs (RAPD) and intersimple sequence repeats (ISSR). Mol Ecol 9:857–68.

Jaccard P (1908) Nouvelles recherches sur la distribution florale. Bull. Soc. Vaud. Sci. Nat. 44:223-227.

Kammoun N (2007) Etude de la diversité génétique de l'olivier en Tunisie : Approche pomologique, chimique et moléculaire. Thèse doctorale en sciences biologiques. 307p.

Muzzalupo I, Stefanizzi F, Perri E (2009) Evaluation of olives cultivated in southern Italy by simple sequence repeat markers. HortScience, Vol.44, pp. 582-588.

Ouazzani N, Lumaret R, Villemur P (1995) Apport du polymorphisme alloenzymatique à l'identification variétale de l'Olivier (*Olea europaea* L.). Agronomie 15:31–37.

Pasqualone A, Caponio F (2001) Inter-simple Sequence Repeat DNA markers for identification of drupes from different Olea europaea L. cultivars. European Food Research and Technology. 213:240–243.

Rallo L, Cidraes F (1975) Amélioration végétale de l'Olivier. II ème Séminaire oléicole international, Octobre 1975, Cordoba, Espagne, pp. 24–40.

Rallo P, Dorado G, Martin A (2000) Development of Simple Sequence Repeats (SSRs) in Olive tree (*Olea europaea* L.). Theoritical and Applied Genetics 101:984–989.

Rekik I, Salimonti A, Grati Kamoun N, Muzzalupo I, Lepais O, Gerber Perri E, Rebai A (2008) Characterization and identification of Tunisian olive tree varieties by microsatellite markers. HortScience 43(5): 1371–6.

Rekik I (2009) Etude de la diversité génétique de l'olivier en Tunisie en utilisant les marqueurs SSR et SNP (thèse de doctorat). Faculté des sciences de Sfax.

Ruby J (1917) Recherches morphologiques et biologiques sur l'Olivier et ses variétés cultivées en France [Thèse]. France, Faculté des sciences, Paris.

Sarri V, Baldoni L, Porceddu A, Cultrera NGM, Contento A, Frediani M, Belaj A, Trujillo I, Cionini PG (2006) Microsatellite markers are powerful tools for discriminating among olive cultivars and assigning them to geographically defined populations. Genome 49:1606–15.

Sefc KM, Lopes MS, Mendonça D, Rodrigues Dos Santos M, Laimer Da Câmara Machado M, Da Câmara Machado A (2000) Identification of microsatellites loci in olive (*Olea europaea* L.) and their characterisation in Italian and Iberian olive trees. Molecular Ecology 9:1171–1193.

Taamalli W, Geuna F, Banfi R, Bassi D, Daoud D, Zarrouk M (2006) Agronomic and molecular analyses for the characterisation of accessions in Tunisian olive germplasm collections. J Biotechnol 9(5):467–81.

Taamalli W, Geuna F, Banfi R, Bassi D, Daoud D, Zarrouk M (2007) Using microsatellite markers to characterise the main Tunisian olive cultivars Chemlali and Chetoui. J Hort Sci Biotechnol 82:25–8.

Terzopoulos PJ, Kolano B, Bebeli PJ, Kaltsikes PJ, Metzidakis I (2005) Identification of *Olea europaea L.* cultivars using inter-simple sequence repeat markers.Scientia Horticulturae, 105 (1), 45-51.

Vargas P, Kadereit JW (2001) Molecular fingerprinting evidence (ISSR, Inter-simple Sequence Repeats) for a wild status of *Olea europaea* L. (Oleaceae) in the Eurosiberian North of the Iberian Peninsula . Flora 196:142–152.