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Microsatellite marker-based identification and genetic relationships of millennium olive cultivars in Tunisia

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Abstract

Microsatellite markers were used to characterize the millennium olive cultivars localized in nine different archeological sites in Tunisia. Thirty genotypes were considered for genetic fingerprinting using 10 pairs of microsatellite primers. The number of alleles per locus ranged from 3 to 5, with a mean of 3.7 alleles per primer pair (a total of 37 alleles). The observed heterozygosity ranged from 0.4 to 1, while the expected heterozygosity varied between 0.37 and 0.74. The polymorphism information content values ranged also from 0.37 to 0.74. The mean polymorphism information content value of 0.61 for the SSR loci provided sufficient discriminating ability to evaluate the genetic diversity among the millennium cultivars. The UPGMA cluster analyses using Jaccard's index permitted a segregation of the thirty millennium cultivars in three main groups and revealed that most of the millennium cultivars grouped according morphological parameters of the fruit and the endocarp and no clear clustering trends were observed according to their geographic origin. As a sequel to the present work, new surveys should be made in the archeological sites localized in North and the Center of Tunisia to sample more cultivars and to draw a clearer picture of the diversity of the Tunisian millennium olive germplasm.

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Introduction

Tunisia is one of the oldest agricultural settlements in history. Evidences revealed by archeological excavations indicated that olives were cultivated before about 3000 years **(**Loussert and Brousse, 1978**)**. The ancient civilizations such as the Phoenicians and Romans have spread this culture from the North to the South of Tunisian country (Brown, 2004). Millennium olive germplasm richness has been confirmed by morphological and AFLP molecular methods, particularly in the region of 'Haouaria', 'Makthar' and 'EL Jem' localized in the center of Tunisia (Mnasri *et al.,* 2013; Mnasri *et al.*, 2014). However, there is still a need for better genetic diversity assessment and varietal identification using high throughput marker technologies such as SSR markers. Specially that, molecular methods for olive cultivar fingerprinting have been demonstrated to be effective, but microsatellite or simple sequence repeat (SSR) analysis is becoming the preferred choice for its high discriminatory power and simpler interpretation (Bracci *et al.,* 2011). In an effort to trace the provenance of olive cultivars, recent reports have provided a list of recommended SSR markers and procedures for genotyping olive (Doveri *et al.,* 2008; Baldoni *et al.,* 2009). Also, the local germplasm for limited or small cultivation areas has recently been characterized by SSR markers, suggesting high levels of genetic diversity as well as notable variability of wild and cultivated types (Rekik *et al.,* 2008 , Abdelhamid *et al.,* 2012).

Additionally, in recent years, different kinds of markers have been successfully used in olive species. Random amplified polymorphic DNAs (RAPDs) and restriction fragment length polymorphism showed good discriminatory properties (Belaj *et al.,* 2001; Besnard *et al.,* 2001; Fabbri *et al.,* 1995; Gemas *et al.,* 2000; Guerin *et al.,* 2002; Mekuria *et al.,* 1999; Sanz-Cortez *et al.,* 2001). Amplified fragment length polymorphism (AFLPs) were also used for exploring germplasm (Angiolillo *et al.,* 1999; Sensi *et al.,* 2003) and the millennium olive patrimony (Mnasri *et al.,* 2014). Recently also, single nucleotide polymorphism has been used to discriminate olive cultivars (Reale *et al.,* 2006). Although these markers have resulted in the ability to discriminate among olive cultivars, their dominant character (RAPDs and AFLPs) or poor reproducibility among different laboratories and experiments (RAPDs) are still considered major drawbacks in cultivar fingerprinting. Among the others, microsatellite markers have proved successful for germplasm fingerprinting of woody plants. These markers exhibit a high level of polymorphism. In diversity studies, because of their codominant character, they are more effective than others in estimating heterozygosity. The capacity of microsatellite primers for evaluating genetic diversity between different genotypes is the first prerequisite for genetic characterization of germplasm collections. Furthermore, microsatellites seem to be suitable for such purpose as a result of their adaptability to high-throughput studies as well as adaptability for database setup (Carriero *et al.,* 2002).

The objectives of this study were to test whether microsatellite primers developed from cultivated olive (Doveri *et al.,* 2008; Rekik *et al.,* 2008; Baldoni *et al.,* 2009; Abdelhamid *et al.,* 2012) would enable the complete fingerprinting of the Tunisian millennium olive cultivars, which has never been previously characterized by SSR molecular markers and to investigate whether the polymorphism displayed by DNA amplification with 10 different SSR primer pairs would be sufficient to distinguish among all the tested millennium accessions, particularly those presenting very close morphological features and those known as somatic clones of the same genotypes according to their phenotype (Mnasri *et al.,* 2013) and their AFLP fingerprinting (Mnasri *et al.,* 2014). The major goal is to use SSR markers to investigate the genetic relationships among the tested millennium varieties, to estimate the level of inbreeding and to detect underlying cultivar structure in Tunisian millennium olives.

Material and methods

Plant material

Samples were collected from nine archeological sites localized in the North, the Center and the South of Tunisia (fig. 1).The results of (Mnasri *et al.*, 2013) have proved the wealth and the importance of the millennium olive germplasm in these sites. The study has been carried out on a sample of 30 cultivars. Three trees were sampled at random in a representative field and analyzed for each cultivar.

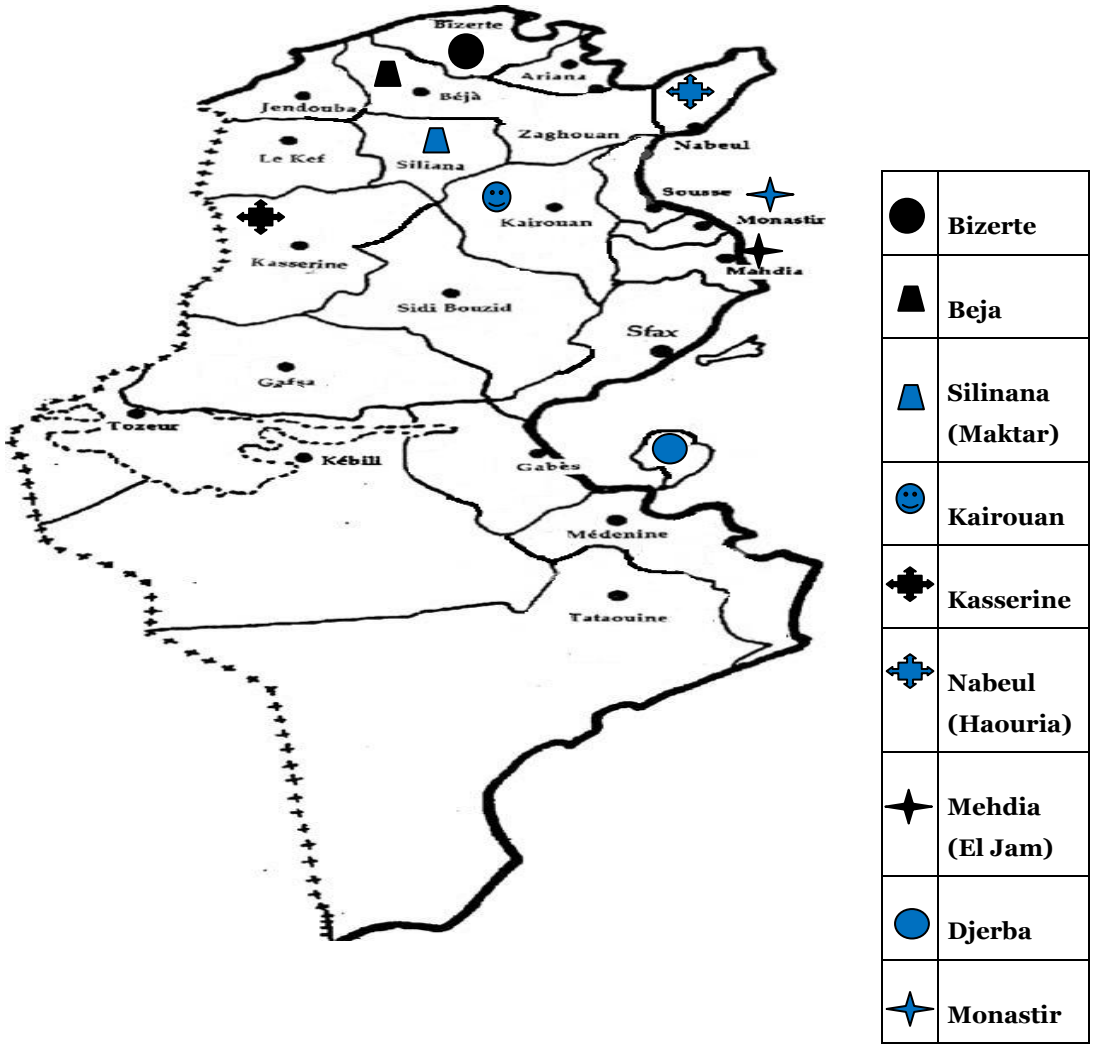

Fig. 1. Map of Tunisia indicating the position of the thirty analyzed millennium olive cultivars.

DNA extraction

Total genomic DNA was extracted from young leaf tissue following the method described by (Angiolillo *et al.,* 1999) using a CTAB buffer with a concentration measured on agarose gel by lambda ladder.

SSR markers

Ten microsatellite (SSR) markers were used in this study. Four markers (GAPU59, GAPU71A, GAPU71B, GAPU103A) from the primer set designed by Carriero *et al.* (2002), four markers (UDO03, UDO12, UDO28, UDO39) from Cipriani *et al.* (2002) and two markers (DCA9, DCA18) from Sefc *et al.* (2000) were selected for their high polymorphism among olive cultivars, their easily scored patterns and their small-scale stuttering (Table 2). The 20-µl reactions contained 50 ng template DNA, 1.5 mM MgCl2, 0.3 mM dNTP, 10 pmol of each primer, and 1.5 U Taq DNA polymerase (Gibco-BRL) in 1X PCR buffer. The cycling regime consisted of 94°C for 4 min, followed by 34 rounds of 94°C for 30 s; 50-60°C (primer pair dependent; Sefc *et al.,* 2000; Cipriani *et al.*, 2002) for 45 s and 72°C for 60 s, with a final step of 72°C for 10 min.

SSR data were analyzed using several genetic parameters such as: number of alleles per locus; observed heterozygosity (H_o, calculated as the number of heterozygotes per locus divided by the number of individuals typed); expected heterozygosity (H_e) or gene diversity (Nei, 1987), and the polymorphism information content (PIC) calculated for each locus (Botstein *et al.,* 1980). Pair wise genetic similarities were calculated using Dice similarity coefficient (Dice, 1945; Neil and Li, 1979). A dendrogram was constructed from the resultant matrix via the unweighted pair group method with the arithmetic averages algorithm (UPGMA) methods. All calculations were performed with the use of NTSYS-pc version 2.1 (Rohlf, 1998).

Results and discussion

Overall microsatellite diversity

A total of 37 alleles were observed across the ten markers, the number of alleles per locus ranging from 5 (UDO 28; UDO 39) to 3 (GAPU 59, DCA 09 and DCA 18) with a mean value of 3.7 alleles per locus (Table 2). This diversity may be associated with the variation in the loci as well as in the number of genotypes and their location. As Well, an important number of reports have indicated the high variability in the average number of alleles per locus in olive cultivars (Carriero *et al.,* 2002; De La Rosa *et al.,* 2002; Diaz *et al.,* 2006; Sarri *et al.,* 2006; Belaj *et al.,* 2010 and Abdelhamid *et al.*, 2012). Alleles sizes vary among the ten loci, differences between the longest and shortest allele ranged from 124 to 228 bp (Table 1). Genetic variability was wide as indicated by the very high values of observed heterozygosity that ranged between 1.00 at locus (GAPU 71B, UDO 12, UDO 28) and 0.4 at DCA 18, with a mean value of 0.74. The mean PIC values were high (0.61) ranging from 0.74 at UDO 39 to 0.37 at DCA 18. In accord with prior findings (Abdelhamid *et al.*, 2012), our high heterozygosity values were similar to those of several studies that used SSR markers on olive cultivars in Tunisia (Rekik, 2008 and Tamalli *et al.*,2006) as well as on the Spanish olive germoplasm (Delgado-Martinez *et al.,* 2012). Also, the mean observed heterozygosity values (Ho) of the present study was higher than previous studies using cultivars from different areas of the Mediterranean basin (Sarri *et al.,* 2006). The ten microsatellite markers were also demonstrated their utility in discriminating between the thirty millennium olive cultivars. Specific allele profiles at the locus DCA-18 (174-190pb) and GAPU-71B (126-144pb) permitted the discrimination of the accessions (Vm10, Vm16 and Vm22) from the other cultivars. Whereas, only the cultivar Vm23 present the alleles (154-154pb), (136-150pb), (166-193pb) and (154-154pb) at the locus (GAPU-71A, GAPU-103A, UDO-12 and UDO-28, respectively) and only the cultivar Vm24 present the allele (210-228 pb) at the locus GAPU-71A.

Genetic relationships among millennium olive cultivars

The SSR marker genotypes were used to evaluate the relatedness of the studied accessions by hierarchical clustering using UPGMA (Fig. 2) based on Jaccard index (1901) with NTSYS-PC (Rohlf, 1998). This analysis clearly separated the 30 millennium cultivars in three main groups, with similarity coefficients between all possible pairs of genotypes ranging from 0.2 to 1. The dendrogram showed a clear separation between the oil cultivars of the second cluster (Vm10, Vm11, Vm12, Vm1, Vm14, Vm16, Vm25 and Vm27), localized in the regions of Makthar, Haouria and El Jem and characterized by a height oil quality (Mnasri *et al.,* 2013). Whereas, the cluster 1 and cluster 3 revealed a perfect similitude between the cultivars (Vm17, Vm15, Vm18, Vm19, Vm20, Vm21, Vm26, Vm28, Vm29) and (Vm1, Vm2, Vm3, Vm4, Vm5, Vm6, Vm7, Vm8, Vm9) respectively, which are classified in the olive categories of medium to low weight fruit and they can be used with a double aptitude (Barranco *et al.*, 2000). These proved that the cultivars of the first and the third group present different clones of two principal varieties localized in the North, the Center and the South of Tunisia and confirmed our previous molecular study of the Tunisian millennium olive varieties based on 6 AFLP markers (Mnasri *et al.,* 2014). Moreover, the second cluster grouped cultivars with different DNA fingerprinting and proved the importance diversity of

the germoplasm of millennium olive varieties in the regions of Makthar, Haouria and El Jem. These results are proved by the historical story of olive in Tunisia. The ancient manuscripts revealed that the civilizations of the eastern and western Mediterranean such as the Phoenicians led to the establishment of numerous olive varieties in the North and the Center of Tunisia, and then this culture has been spread from the north to the south of Tunisia with the Roman and the Arabic civilizations (Camps –Fabrer, 1997; Loussert and Brousse, 1978).

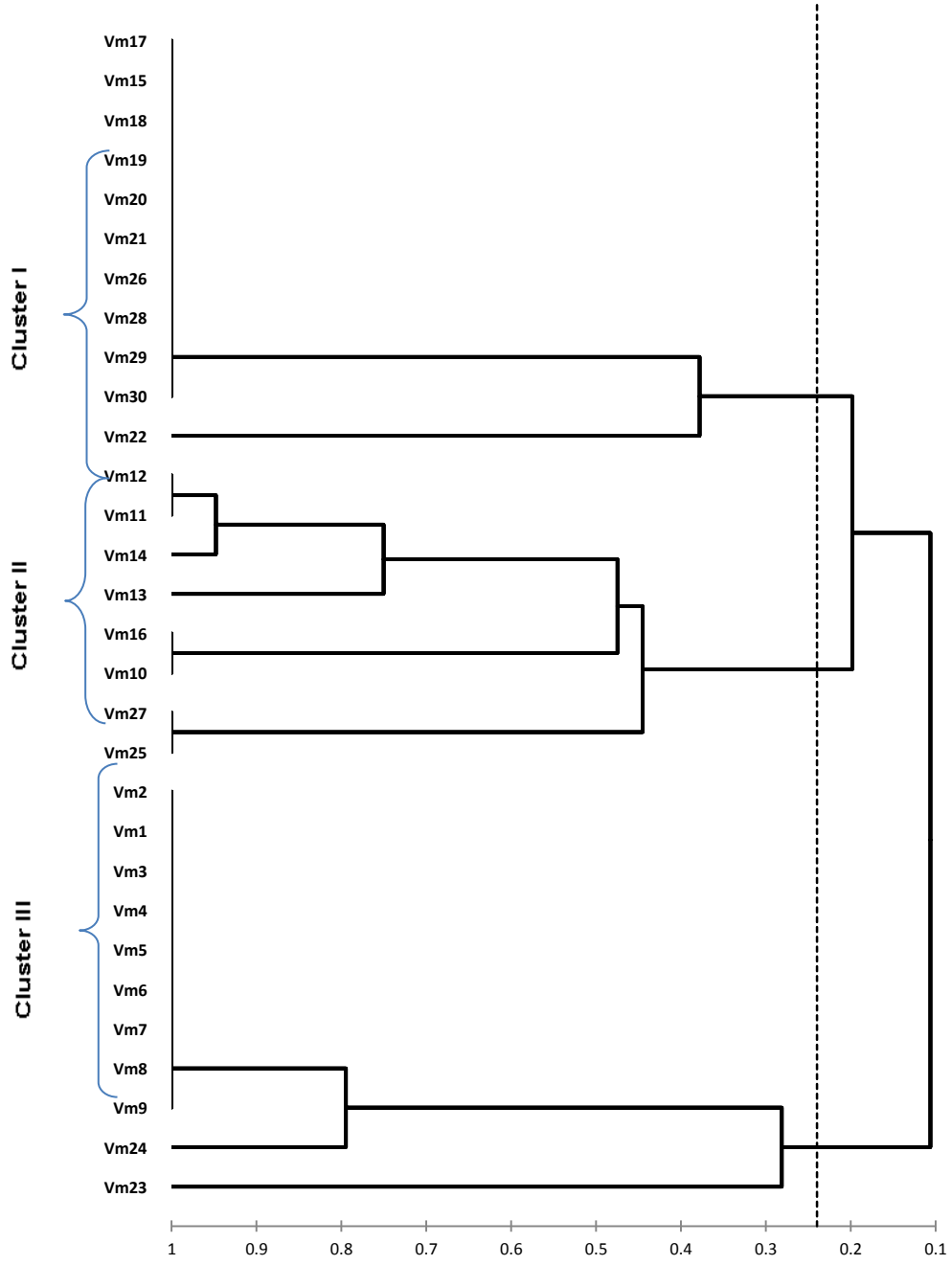

Fig. 2. Dendrogram of the thirty millennium olive cultivars based on SSR data using Jaccard's GS matrix and the UPGMA clustering method.

Moreover, Loukas and Krimbas (1983), in their isozyme study, Fabbri *et al.* (1995), in their analysis of olive cultivars by RAPD and (Kamoun *et al.,* 2006 ; Mnasri *et al.,* 2013 ; Mnasri *et al.*,2014) in their analysis of olive biodiversity in Tunisia by AFLP obtained a comparable clustering of cultivars based

on fruit and endocarp size. That these similar results emerge from analysis of different olive cultivars using different approaches would seem to indicate that fruit and endocarp size is a morphological marker that can efficiently discriminate olive germplasm. Additionally, the lack of any apparent correlation between DNA polymorphism and the origin of cultivars is consistent with the hypothesis that early after domestication, olive cultivars of horticultural value were moved widely from region to region by human migration which have favored the dispersal of olive, cultivated in the whole Mediterranean basin along many centuries (Chevalier 1948 ; Cifferi 1950 ; Fabbri *et al.,* 1995; Ouazzani *et al.,*1995; Mnasri *et al.,* 2013).

Table 1. Genotypic profiles for the ten simple sequence repeat markers used to genotyping the 30 Tunisian millennium olive cultivars.

	GAPU-59	GAPU-71A	GAPU-71B	GAPU-103A	UDO-03	UDO -12	UDO -28	UDO -39	DCA -09	DCA -18
Vm1	212-218	212-228	121-144	136-157	135-182	166-177	143-205	108-213	194-206	174-174
Vm2	212-218	212-228	121-144	136-157	135-182	166-177	143-205	108-213	194-206	174-174
Vm3	212-218	212-228	121-144	136-157	135-182	166-177	143-205	108-213	194-206	174-174
Vm4	212-218	212-228	121-144	136-157	135-182	166-177	143-205	108-213	194-206	174-174
Vm5	212-218	212-228	121-144	136-157	135-182	166-177	143-205	108-213	194-206	174-174
Vm6	212-218	212-228	121-144	136-157	135-182	166-177	143-205	108-213	194-206	174-174
Vm7	212-218	212-228	121-144	136-157	135-182	166-177	143-205	108-213	194-206	174-174
Vm8	212-218	212-228	121-144	136-157	135-182	166-177	143-205	108-213	194-206	174-174
Vm9	212-218	212-228	121-144	136-157	135-182	166-177	143-205	108-213	194-206	174-174
Vm10	212-212	212-212	**126-144**	159-159	182-202	166-182	182-210	213-232	182-194	**174-190**
Vm11	212-212	212-214	124-144	159-159	135-182	166-182	182-210	205-205	182-182	174-174
Vm12	212-212	212-214	124-144	159-159	135-182	166-182	182-210	205-205	182-182	174-174
Vm13	212-212	212-228	124-144	150-157	135-135	166-182	182-210	205-205	182-182	174-174
Vm14	212-212	212-214	124-144	159-159	135-135	166-182	182-210	205-205	182-182	174-174
Vm15	212-218	210-214	124-144	150-157	182-182	166-177	154-210	165-165	182-206	174-177
Vm16	212-212	212-212	**126-144**	159-159	182-202	166-182	182-210	213-232	182-194	**174-190**
Vm17	212-218	210-214	124-144	150-157	182-182	166-177	154-210	165-165	182-206	174-177
Vm18	212-218	210-214	124-144	150-157	182-182	166-177	154-210	165-165	182-206	174-177
Vm19	212-218	210-214	124-144	150-157	182-182	166-177	154-210	165-165	182-206	174-177
Vm20	212-218	210-214	124-144	150-157	182-182	166-177	154-210	165-165	182-206	174-177
Vm21	212-218	210-214	124-144	150-157	182-182	166-177	154-210	165-165	182-206	174-177
Vm22	212-212	212-212	**126-144**	150-157	166-182	166-177	**154-205** [a]	108-205	182-182	**174-190**
Vm23	**214-218** [a]	212-212	124-144	**136-150** [a]	166-182	**166-193** [a]	**154-154** [a]	108-213	194-206	177-177
Vm24	212-218	**210-228** [a]	121-144	136-157	135-182	166-177	143-205	108-213	194-206	174-174
Vm25	214-214	212-212	124-144	159-159	202-202	166-182	154-210	108-213	182-182	174-174
Vm26	212-218	210-214	124-144	150-157	182-182	166-177	154-210	165-165	182-206	174-177
Vm27	214-214	212-212	124-144	159-159	202-202	166-182	154-210	108-213	182-182	174-174
Vm28	212-218	210-214	124-144	150-157	182-182	166-177	154-210	165-165	182-206	174-177
Vm29	212-218	210-214	124-144	150-157	182-182	166-177	154-210	165-165	182-206	174-177
Vm30	212-218	210-214	124-144	150-157	182-182	166-177	154-210	165-165	182-206	174-177

[a] Unique alleles; unique allelic patterns are shown in bold.

Table 2. SSR locus, allelic number, Ho, He, PIC and product size range of the 10 SSR loci studied.

SSR locus	N° alleles	Observed Heterozygosity	Expected heterozygosity	PIC	Range size (pb)
GAPU59	3	0.7	0.55	0.54	212-218
GAPU71A	4	0.76	0.71	0.7	210-228
GAPU71B	4	1	0.65	0.63	121-144
APU103A	4	0.76	0.74	0.72	136-159
UDO03	4	0.56	0.58	0.57	135-202
UDO12	4	1	0.52	0.51	166-193
UDO28	5	1	0.7	0.69	143-210
UDO39	5	0.53	0.76	0.74	108-232
DCA09	3	0.73	0.65	0.63	182-206
DCA18	3	0.4	0.37	0.37	174-190
Total	**37**				
Mean	**3.7**	**0.74**	**0.62**	**0.61**	

Conclusion

In this study, we have made an attempt to characterize thirty millennium olive cultivars, localized in nine different archeological sites in Tunisia. The SSR markers showed a wide genetic diversity among the millennium cultivars, especially in the north and the center of our country, approved the AFLP molecular diversity observed among the olive accessions (Mnasri *et al.,* 2014) and suggests a high genetic potential, which could be used from the agronomic point of view to substantially improve the olive production in Tunisia. Nevertheless, the high intra-similarity between the cultivars of the first and the third cluster should be addressed in deeper detail using a larger number of SSR markers and the combination between SSR and AFLP markers to establish a fingerprint of each cultivar and to more analysis their intra-clone diversity. Specially, that the Tunisian millennium olive germplasm contains substantial diversity, which could support the national programmer's breeding objectives as well as allow participation in international programmers aiming at olive improvement and conservation.

References

Abdelhamid S, Kamoun G, Francesco M, Tiziano C. 2012. Genetic similarity among Tunisian cultivated olive estimated through SSR markers. Scientia Agricola.**70 (1),** 33-38.

Angiolillo A, Mencuccini M, Baldoni L. 1999. Olive genetic diversity assessed using amplified fragment length polymorphism. Theoretical and Applied Genetics **98,** 411–421.

Baldoni L, Cultrera NG, Mariotti R, Ricciolini C. 2009. A consensus list of microsatellite markers for olive genotyping. Molecular Breeding. **24**, 213-231.

Barranco D and Rallo L. 2000. Olive cultivars in Spain. Hortechnology **10**,107-110.

Belaj A, Trujillo I, De La Rosa R, Rallo L. 2001. Polymorphism and discriminating capacity of randomly amplified polymorphic markers in an olive germplasm bank. of the American Society for Horticultural Science. **126**, 64-71.

Belaj A, Muñoz-Diez C, Baldoni L and Satovic Z. 2010. Genetic diversity and relationships of wild and cultivated olives at regional level in Spain. Scientia Horticulturae.**124**, 323-330.

Besnard G, Baradat P, Bervillé A. 2001. Genetic relationships in the olive (*Olea europaea* L.) reflect multilocal selection of cultivars. Theoretical and Applied Genetics.**102**, 251-258.

Botstein D, White RL, Skolnick M and Davis RW.1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. American Journal in Human Genetics **32**, 314-331.

Bracci T, Busconi M, Fogher C, Sebastiani L.2011. Molecular studies in olive (*Olea europaea* L.): overview on DNA markers applications and recent advances in genome analysis. Plant Cell Reports. **30**, 449-462.

Brown JP. 2004. Wine and oil in the antique Mediterranean. Publishing «Saint- Etienne » house, France.

Camps-Fabrer H. 1997. The olive cultivation of olive in the North Africa, Evolution and history, in: International Olive Oil Council (Eds), World Encyclopaedia of Olive. Madrid, Espagne.

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.

Chevalier A. 1948. Origine of olive cultivars. Revue de botanique appliquée et d'agriculture tropicale **28**, 1–25.

Cifferi R. 1950. Primary elements for the study of the origin and the evolution of the cultivated olive. Acte du XIII congres International d'oléiculture **1**, 189–194.

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

De La Rosa R, James C, Tobutt KR. 2002. Isolation and characterization of polymorphic microsatellite in olive (*Olea europaea* L.) and their transferability to other genera in the Oleacea. Molecular Ecology.**2**, 265-267.

Delgado-Martinez FJ, Amaya I, Sánchez-Sevilla JF, Gomez-Jimenez MC. 2012. Microsatellite marker-based identification and genetic relationships of olive cultivars from the Extremadura region of Spain. Genetic Molecular. Research. **11 (2)** 918-932.

Diaz A, De La Rosa R, Martin A, Rallo P.2006. Development, characterization and inheritance of new microsatellites in olive (*Olea europaea* L.) and evaluation of their usefulness in cultivar identification and genetic relationship studies. Tree Genet. Genomes. **2**, 165-175.

Dice LR. 1945. Measures of the amount of ecologic association between species. Ecology **26,** 297-302.

Doveri S, Sabino Gil F, Diaz A, Reale S. 2008. Standardization of a set of microsatellite markers for use in cultivar identification studies in olive (*Olea europaea* L.). Scientia Horticulturae.**116**, 367-373.

Fabbri A, Hormaza JI, Polito VS. 1995. Random amplified polymorphic DNA analysis of olive (Olea europaea L.).Journal of the American Society for Horticultural Science **120,** 538–542.

Gemas VJV, Rijo-Johansen MJ, Tenreiro R, Fevereiro P. 2000. Inter-and Intra-varietal analysis of three Olea europaea L. cultivars using the RAPD techniques. Journal of Horticulture Science and Biotechnology. **75**,312–319.

Guerin RJ, Sweeney SM, Collins GG, Sedley M. 2002. The development of a genetic database to identify olive cultivars. Journal of the American Society for Horticultural Science. **127**,977–983.

Jaccard P. 1901. Comparative study of floral distribuition in the mountains of the Alps and the Jura, Bulletin Societé Vandoise des Sciences Naturelles **37**, 547-579.

Kamoun Garrati N, Lamy FM, Rebaî A, Gargouri A, Panaud O, Saar A. 2006. Genetic diversity of Tunisian olive tree (Olea europaea L.) cultivars assessed by AFLP markers. Genetic Resources and Crop Evolution **53,** 265–275.

Loukas M, Krimbas C B. 1983. History of olive cultivars based on their genetic distances. Journal of Horticulture Sciences **58**, 121–127.

Loussert L, Brousse G. 1978. Olive tree: Mediterranean Agricultural Techniques of olive production. Publishing « neuve and Larose » house, 44-111.

Mekuria GT, Collins GG, Sedgley M.1999. Genetic variability between different accession of some common commercial olive cultivars. Journal of Horticulture Science and Biotechnology.**74**,309–314.

Mnasri Rahmani S, Saddoud Dabbebi O, Ferchichi A. 2013. Preliminary characterization and morph-agronomic evaluation of millennium olive varieties in Tunisia. Journal of Biodiversity and Environmental Sciences **3 (8),** 150-155.

Mnasri Rahmani S, Saddoud Dabbebi O, Ben Saleh M, Ferchichi A. 2014. DNA fingerprinting of millennium olive varieties in Tunisia by AFLP

markers. Journal of Biodiversity and Environmental Sciences **4 (4),** 310-317.

Nei, M. 1987. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics **89**,583–590.

Nei M, Li WH. 1979. Mathematical model for studying genetic variation in terms of endonucleases. Proceedings of the National Academy Sciences of the United States of America **76,** 5269–5273.

Ouazzani N, Lumaret R, Villemur P, Di Giusto F. 1993. Leaf allozyme variation in cultivated and wild olive trees (*Olea europaea* L.). Journal of Heredity **84**, 34–42.

Reale S, Doveri S, Dıaz A, Angiolillo A, Lucentini L, Pilla F, Martin A, Donini P, Lee D. 2006. SNP-based markers for discriminating olive (Olea europaea L.) cultivars. Genome **49,** 1193-1205.

Rekik I, Salimonti A, Innocenzo M, Oliver L, Sophie G. 2008. Characterization and Identification of Tunisian Olive Tree Varieties by Microsatellite Markers. HOrtscience. **43(5)**, 1371–1376.

Rohlf, M. 1998. **NTSYS-pc. Numerical Taxonomy and Multivariate Analysis System: Version** 2.02. Exeter Software, Setauket, NY, USA.

Sanz-Cortez F, Badenes ML, Paz S, Iniguez A. 2001. Molecular characterization of olive cultivars using RAPD markers. Journal of the American Society for Horticultural Science.**126**, 7-12.

Sarri V, Baldoni L, Porceddu A, Cultrera NG. 2006. Microsatellite markers are powerful tools for discriminating among olive cultivars and assigning them to geographically defined populations. Genome. **49**, 1606-1615.

Sefc K M, Lopes M S, Mendoncxa D, Rodrigues dos Santos M, Laimer da Camara Machado M, Da Camara Machado A. 2000. Identification of microsatellite loci in olive (*Olea europea* L.) and their characterization in Italian and Iberian olive trees. Molecular Ecology. **9**,1171–1173.

Sensi E, Vignani R, Scali M, Masi E, Cresti M. 2003. DNA fingerprinting and genetic relatedness among cultivated varieties of *Olea europea* L. estimated by AFLP analysis. Scientia Horticulurae. **97**,379–388.

Taamalli W, Geuna F, Banfi R, BassiD, Daoud D, Zarrouk M. 2006. Agronomic and molecular analyses for the characterisation of accessions in Tunisian olive germplasm collections. Electronic Journal of Biotechnology **9,** 467–481.