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AFLP marker-based identification and genetic relationships of olive cultivars in the region of Hbebsa "North West of Tunisia"

Mnasri Rahmani Sameh[1*], Saddoud Debbabi Olfa[1], Ferchichi Ali[2]

[1]*National Gene Bank of Tunisia, Street Yesser Arafet, 1080, Tunis, Tunisia*

[2]*National Institute of Agronomy of Tunisia, 43 Street Charles Nicolle 1082-Tunis Mahrajène Tunisia*

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Abstract

In the region of Hbebsa, little is known about the olive germplasm, and even though there is an important olive biodiversity, studies about characterization and evaluation are scarce. The aim of this work was to make a molecular characterization by the use of amplified fragment length polymorphism (AFLP) markers. A total of 13 olive varieties were genotyped using different EcoRI–MseI AFLP primer combinations. Auto radiographs revealed 92 polymorphic markers in a total of 237 detected fragments. A set of redundant marker patterns was identified and deleted from the binary data matrix; data analysis demonstrated a high degree of polymorphism with an average of 35%. The analysis of AFLP profiles found in our set of olive cultivars showed a wide genetic diversity among olive germplasm. The UPGMA cluster analyses using Jaccard's index and the Principal coordinate analysis (PCO) revealed that the genetic diversity was predominantly structured according to fruit size. The data obtained can be used for the varietal survey and construction of a database of olive varieties grown in the region of Hbebsa and providing also additional information that could form the basis for the national design of breeding programs.

***Corresponding Author:** Mnasri Rahmani Sameh ✉ mnasrisameh@yahoo.fr

Introduction

The olive (Olea europaea L. subsp. europaea, 2n = 2x = 46) is considered one of the most important fruit crops of the Mediterranean basin (De la Rosa et al., 2003; Gemas et al., 2004) and is characterized by a large number of varieties, mainly used for oil or seasoned fruit production (Mataix and Barbancho 2006). In Tunisia, there are different olive cultivars known, but culture depends on two prevailing cultivars, 'Chetoui' in the northern and 'Chemlali' in central and southern parts of the country. Conversely, several minor varieties are maintained in restricted areas. The number is probably underestimated because of the scarce information on minor local varieties widespread in the different olive growing areas, but this genetic diversity is threatened by modernization of production practices and by changes in agricultural and commercial policies. Thus, there is an urgent need to study and to inventory these traditional varieties before their lost (Abaza et al., 2005; Baccouri *et al.,* 2007).

The identification of olive tree cultivars has been traditionally carried out by morphological, agronomic and chemical traits (Mehri et al., 1997; Koutsaftakis et al., 2000; Kamoun et al., 1999; Visioli et al., 2002, Mehri et al., 2007 and Mnasri et al., 2013). However, these morphological and phenological markers have the disadvantage of the small number of polymorphism detected and of being environmentally dependent (Trigui and Msallem, 2002). Besides that, some of the phonological characteristics are only accessible for a limited period or when the olive tree achieves a mature stage, which may delay the correct identification.

DNA-based markers are particularly useful for the correct identification of varieties, due to their independence of environmental conditions and several of them have been successfully applied for olive for example, random amplified polymorphic DNA (RAPDs) (Bogani et al.,1994), amplified fragment length polymorphism (AFLPs) (Angiolillo et al., 1999), sequence characterized amplified regions (SCARs) (Busconi et al., 2006), inter simple sequence repeats (ISSRs) (Hess et al., 2000), single nucleotide polymorphism (SNPs) (Reale et al., 2006) and simple sequence repeats (SSRs) (Poljuha et al., 2008). AFLP marker technology was confirmed to be a powerful tool not only for studying variation between populations of the genus Olea as shown by Angiolillo et al. (1999), but also for characterising intraspecific variation among cultivated accessions of Olea europaea L. subsp. europaea. In Tunisia Kammoun et al. (2006) assessed genetic diversity among 29 different olive varieties using nine AFLP primer combinations and Taamalli et al. (2006) revealed the deference between 25 Tunisian olive cultivars by the use of five AFLP primer combinations and ten SSR loci .

In the region of "Hbebsa" localized in the North West of Tunisia, little is known about the olive germplasm, and even though there is an important olive biodiversity (Mnasri et al., 2013) studies about characterization and evaluation are scarce. The aim of this work was to make a molecular characterization by the use of six AFLP primer combinations. This study will explore for the first time in Tunisia the genetic diversity of 13 minor olive varieties in the region of "Hbebsa". The use of molecular markers AFLP will be essential to verify the denomination of each cultivar and increase the knowledge about the diversity of this species as well as to allow participation in international programmers aiming at olive improvement and conservation.

Material and methods

Samples were collected from the region of Hbebsa localized in the North West of Tunisia. The results of Mnasri et al. (2013) have proved the wealth and the importance of the olive germplasm although the desertification of the studied site. The study has been carried out on a sample of 13 cultivars. The common name, end-use of all cultivars studied is given in Table 1. Three trees were sampled at random in a representative field and analyzed for each cultivar.

AFLP analysis

AFLP analysis was performed as previously described for olive (Angiolillo et al., 1999). Four EcoRI primers (E-AAC, E-ACC, E-ACA and E-AAG) and six MseI primers (M-CTC, M-ACG, M-ATT, M-AGG, M-GCT and M-CAA) with three selective nucleotides were used. A total of six highly polymorphic primer combinations were screened (Table 2) among those previously tested on the Tunisian olive varieties by Kammoun et al., (2006).

Data analysis

AFLP results were scored for presence (1) and absence (0) of amplified fragments. Pair wise genetic similarities were calculated using Dice similarity coefficient (Dice, 1945; Neil and Li, 1979). Principal coordinate analysis has been used to highlight the pair wise relationship between cultivars. 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

AFLP markers (Vos et al., 1995) allow multi-locus screening of a genome in absence of preliminary sequence knowledge and represent the best choice for studies into genetic relationship or for accurate evaluation of intra-cultivar variability (Belaj et al., 2002; Powell et al., 1996).

In this present study, a total of 237 AFLP markers were analyzed, of which 92 were polymorphic (35%). The number of amplified fragments varied from 9 (P-AAG/M-ATT) to 75 (P-AAC/M-CTC) with an average of 28 fragments per primer combination. The average percentage of polymorphism ranged from 22% for P-AAG/M-ATT to 44% for P-ACA/M-GCT primer combination (Table 2). This result is consistent with earlier findings indicating the wide genetic basis of olive germplasm in Tunisia (Taamaali et al., 2006 and Kamoun et al., 2006).

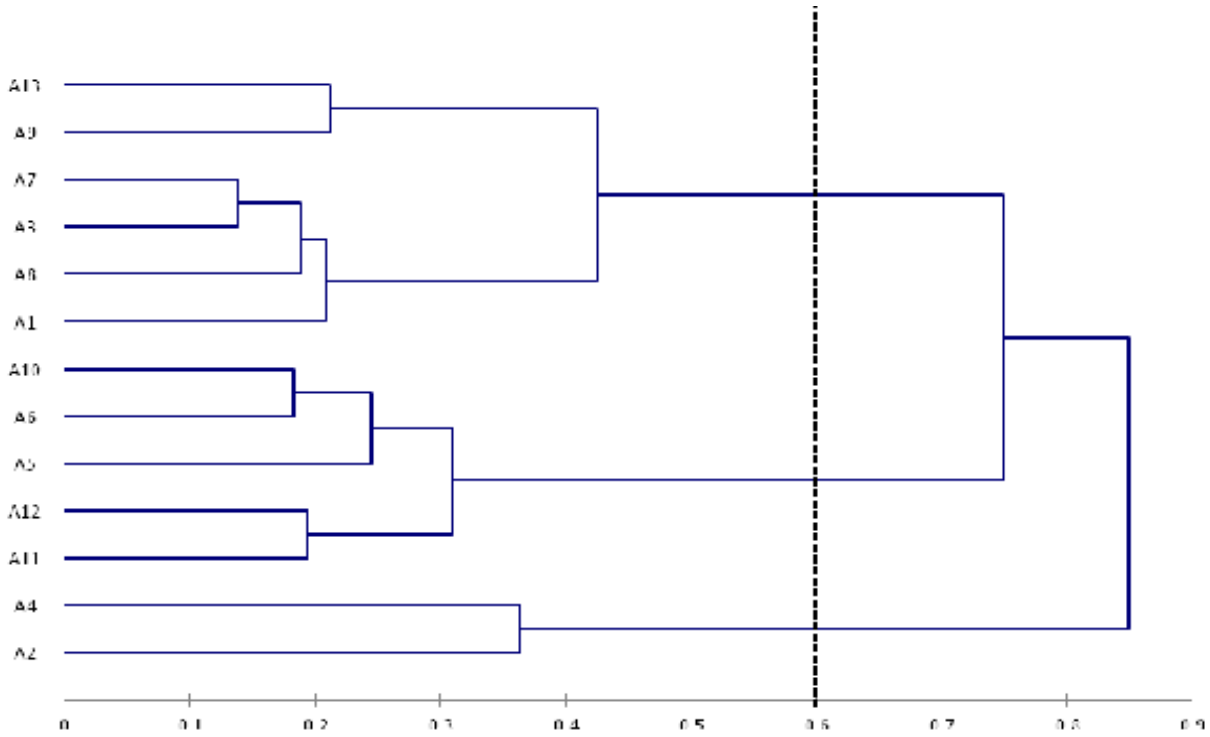

Fig. 1. UPGMA dendrogram based on dice similarity matrix between Samples in the region of Hbebsa.

The diversity of the studied sample was approached by calculating a dendrogram of genetic similarity (fig 1) based on Jaccard index (1901) with NTSYS-PC (Rohlf, 1998). Three main groups were revealed by cutting the dendrogram at a GS value of 0.6. Cluster 1 consisted of the accessions A2 and A4, locally named "Meski" and "Besbessi". These cultivars are among the most important Tunisian table variety (fruit weight > 5g), essentially used for canning. The second cluster is sub-devised on two main subgroups. The group 1 showed cultivars with medium-sized fruits (2-4g) A11 and A12, locally named "Neb Jmel" and "Gerboui" and characterized by their height oil quality (Taamalli et al., 2006 and Mnasri et al., 2013) . The second sub-group included three medium-fruited accessions A5, A6 and A10, locally named "Tounsi" , "Toffahi" and "Oueslati" and typically used for oil and canning. Cluster 3 consisted of six oil varieties which have a small average fruit weight of about 1.5 g, A1, A8, A3, A7, A9 and A13, locally named ("Kbiret Louzir", "Unknown", "Jemri", "Unknown", "Chemlali" and "Sahli"). Kamoun et al., (2006), Taamaalli et al., (2006) and Abdelhamid et al., (2013), in their analysis of Tunisian olive cultivars by AFLP and SSR obtained a comparable clustering of cultivars based on fruit size. These similar results emerge from analysis of different olive cultivars using different approaches would seem to indicate the efficiently of the morphological marker (fruit size) to discriminate olive germplasm.

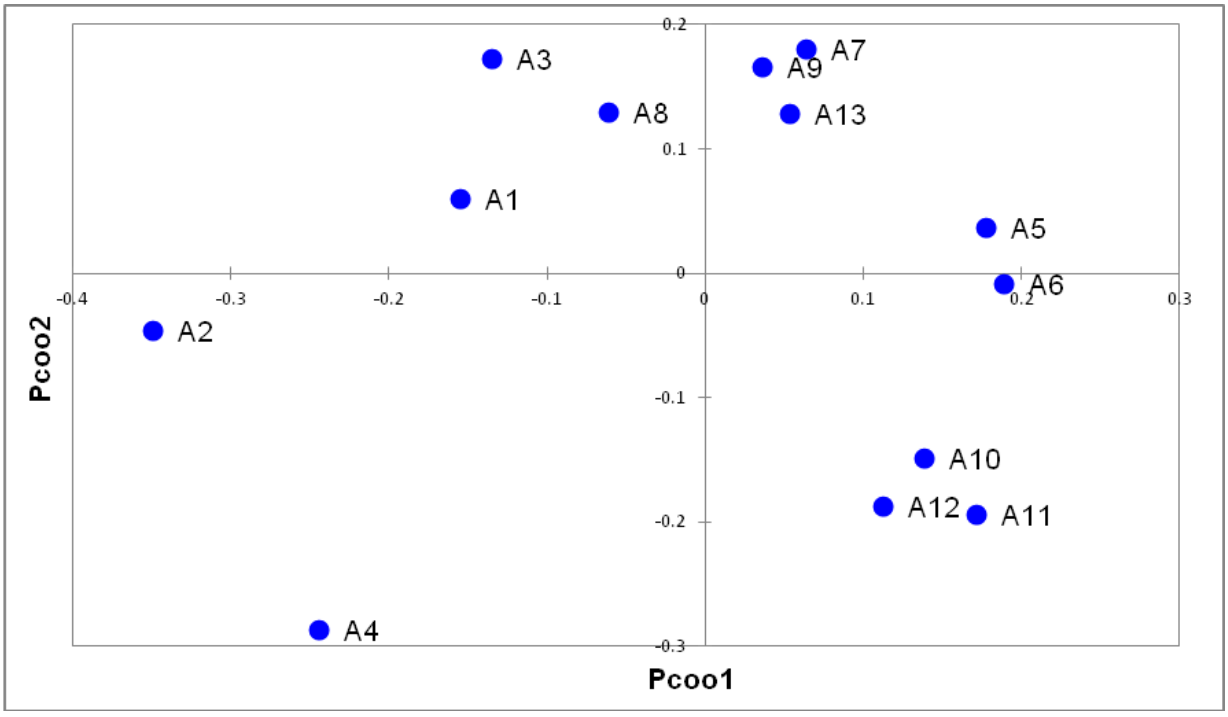

Fig. 2. Principal coordinate plot of olive genotypes for the first and second principal coordinates estimated with 92 AFLP markers using the GS matrix.

The taxonomic structure was further investigated by Principal coordinate analysis (PCO), based on the same matrix of pair wise distances. PCO consists on a representation of the dissimilarity among several cultivars in a reduced multidimensional Q space. On the first three principal coordinates, preservation of the original pair wise distances is very good (cophenetic correlation of 0.88). The 13 tested cultivars were separated along the two-principal dimensional PCO plot (fig. 2) into three main clusters. The first two principal components accounted for 39.82% of the variance, seems to support the results obtained by cluster analysis. The pattern was comparable to the clustering observed in the UPGMA dendrogram (fig 1). The PCO separated the oil producing cultivars characterized by small seize fruits from the table varieties essentially used for canning

and the medium fruit seize cultivars typically utilized for oil and canning.

The AFLP distance matrix showed a rather high variability among the accessions examined and that most of olive accessions in the region of Hbebsa clustered according to their fruit size. For instance small-fruited accessions clustered in Group 3. Accessions that have medium to large sized-fruits clustered in Group 2 and 1. Genetic differentiation based on fruit size has been observed in previous studies. Kamoun et al. (2006), in their AFLP study of 29 Tunisian olive varieties, obtained a clustering of olive cultivars into two main groups according to fruit size. Using the AFLP and SSR techniques, Taamalli et al. (2006) reported that most of Tunisian cultivars clustered according to their fruit size or commercial use. Moreover, Fabbri et al. (1995), in their study based on RAPD markers clustered seventeen olive cultivars into two main groups according to fruit size and oil content.

Table 1. List of the studied accessions.

Accession	Common name	Use
A1	Kbiret Louzir	Oil
A2	Meski	Table
A3	Jemri	Oil
A4	Besbessi	Table
A5	Tounsi	Oil and Table
A6	Toffahi	Oil and Table
A7	Unknown	Oil
A8	Unknown	Oil
A9	Chemlali	Oil
A10	Oueslati	Oil and Table
A11	Neb Jmel	Oil and Table
A12	Gerboui	Oil and Table
A13	Sahli	Oil

Table 2. Polymorphism rates of the six primer combinations.

Primer combination	Total number of bands*	NPB*	PR*(%)
E-AAC/MCTC	75	30	40
EACC/MACG	47	19	40
EAAG/MATT	9	2	22
EACA/MAGG	25	7	28
EACC/MCAA	34	13	38
EACA/MGCT	47	21	44
Total	237	92	
Mean	28	15	35

NPB: Number of Polymorphic Bands, PR: Polymorphism Rate in Percent unit

Conclusion

The results obtained in this work, aimed at testing the reliability of the AFLP markers for cultivar discrimination and clarifying the local cultivars" identity" in the region of Hbebsa. The AFLP markers showed a wide genetic diversity among the cultivars and approved the phenotypic and biochemical diversity observed among the olive accessions (Mnasri et al., 2013) and suggests a high genetic potential, which could be used from the agronomic point of view to substantially improve the production of Hbebsa. These findings are of great relevance and open large horizons. This is particularly useful for the widely cultivated (two-third of total olive area) cultivars Chemlali and Chetoui which are vigorous and well adapted to arid regions but give oil, (Kamoun et al., 2002) having undesirable acid composition (low levels of oleic acid and high level of palmitic and linoleic acid).

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