THE DISCRIMINATING CAPACITY OF SSR, RAPD AND AFLP MARKERS AND THEIR EFFECTIVENESS IN ESTABLISHING GENETIC RELATIONSHIPS IN OLIVE (*Olea europaea* L.)

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Olive (*Olea europaea* L.) is among cultivated fruit trees and the richest in genetic diversity. The longevity of this species and the low breeding pressure has contributed to the conservation of its variability, and the reduced extent of genetic erosion within its germplasm has allowed the persistence of olive diversity (Ganino *et al.*, 2007). This is why the number of known cultivars is very high and has steadily increased since ancient times, especially since the more recent progresses in taxonomy and breeding. The described olive germplasm amounts to over 1,200 cultivars, not to mention a large number of synonyms and homonyms (Bartolini *et al*., 2005). The considerable diversity in olive and the presence of cases of homonyms and synonyms stress the need for efficient and rapid discriminating methods.

Siwa oasis is a natural isolated depression in the western desert of Egypt at about 29°/06'-29°/24'N and 25°/12'-25°/12'E. The most visible evidence of the presence of olive as a cultivated fruit crop is the presence of a huge number of olive plants and stumps which were able to survive for centuries against the offences of time and environment. In these areas, mainly hilly environments, a number of olive varieties adapted and/or differentiated to constitute a rich and varied germplasm (Masini *et al.*, 2003).

Genetic variability can be evaluated in several ways (Ganino *et al.*, 2006); morphology (Taamalli *et al.*, 2006), enzymatic studies (Trujillo *et al.*, 1995), RFLP markers (Besnard; Bervillé, 2000), RAPD markers (Ozkaya *et al.*, 2006), AFLP markers (Hemeida *et al*., 2007), SSR markers (Khadari *et al.*, 2008), SNPs (Diaz Bermudez, 2005), chloroplast and mitochondrial DNA analysis (Besnard *et al.*, 2002) and NMR analysis of olive oils (Fragaki *et al.*, 2005).

Comparisons of molecular markers for measuring genetic diversity have been carried out in several plant species (Powell *et al*., 1996; Russell *et al.*, 1997; Pejic *et al.*, 1998; Garcia-Mas *et al.*, 2000) but, to our knowledge, no such studies have yet been reported in olive. A better understanding of the effectiveness of the different molecular markers is considered a priority step toward olive germplasm characterization; classification

and a prerequisite for more effective breeding programs.

The objectives of this study are: (1) to compare the discriminating capacity and informativeness of the PCR-based molecular markers RAPD, AFLP and SSR for genotype identification and genetic diversity analyses; (2) to determine the genetic similarity estimates and genetic relationships among the cultivars analyzed and (3) to compare the patterns of variability obtained with each marker.

MATERIALS AND METHODS

Plant material and microsatellite assay

Eleven olive cultivars (*Olea europaea* L.) from the Siwa oasis (Chemlali; Wetagen; Meloky; Toffahy; Hamdey; Maraky; Manzanilla; Khosha; Coronaiki; Picual and Kalamata) were included in the study.

Genomic DNA was extracted from 80 mg of leaves with the DNeasy® Plant Mini Kit (QIAGEN™, Hilden, Germany) according to the manufacturer's instructions. DNA concentration and purity were estimated by using a Gene Quant (Amessham pharmacia Biotech). The PCR conditions described by Cipriani *et al.* (2002) were used for the amplifications of the eight SSR (Table 1) primer pairs (Sefc *et al.*, 2000). Amplification reactions were performed in a final volume of 25 µl in the presence of 20 ng template DNA, 4 pmol each primer, 0.2 mM of each dNTP, 2mM $MgCl_2$, and 1U *Taq* polymerase (Sigma). Polymerase chain reaction (PCR) was carried out using a Biometra *T1* gradient thermalcycler (Biometra biomedizinische, Germany). After 5 min at 94°, 30 cycles were performed with 30s at 94°C, 30s at either 50 or 56 or 60°C and 30 s at 72°C, followed by a final extension step of 5 min at 72°C. The amplification products were resolved by electrophoresis in a 1.8% agarose gel in TAE buffers and revealed under UV illumination by ethidium bromide staining (Carriero *et al.*, 2002).

Data collection and analysis

To compare the efficiency of the three markers (RAPD, AFLP and SSR) in varietals identification, diversity and differentiation, estimated the following parameters for each assay unit (*U*, the product of PCR amplification obtained with one set of primers) used:

(1) Number of polymorphic bands (n_p);

(2) Number of monomorphic bands (n_{np});

(3) Number of polymorphic bands / assay unit (n_p/U);

(4) Number of loci (*L*): in the case of RAPD and AFLP markers the theoretical maximum number of loci is equal to total number of bands (n_p+n_{np}) obtained for each marker type;

(5) Number of loci per assay unit: $n_u = L/U$ (Taamalli *et al.*, 2006);

(6) Number of banding patterns for each molecular marker (T_p);

(7) Average number of patterns per assay unit (*I*);

(8) Confusion probability (*Cj*) of the *j*-th assay unit: *I*

$Cj = \sum_{i=1}^{I} P_i (Np_i - 1) / N\text{-}1$ where p_i is the frequency of the *i*-th pattern; N, sample size; *I*, total number of patterns generated by the *j*-th assay unit;

(9) Discriminating power (D_j) of the *j*-th assay unit as reported by Tessier *et al.* (1999): $D_j = 1 - C_j = 1 - \sum_{i=1}^{I} P_i (Np_i - 1) / N\text{-}1$;

(10) Limit of D_j as *N* tends toward infinity: $D_L = \lim (D_j) = 1 - \sum_{i=1}^{I} P_i^2$;

(11) Effective number of patterns per assay unit: $P = 1/1\text{-}D_L$;

(12) Average number of alleles per locus (n_{av}). For SSRs the average number of alleles per locus is calculated according to the formula: $n_{av} = n_p/L$: For RAPDs and AFLPs two alleles per assay are considered ($n_{av} = 2$) according to Powell *et al*. (1996);

13) Expected heterozygosity (H_{ep}) of the polymorphic loci for a genetic marker: $H_e = 1 - \sum P_i^2$ where p_i is the allele frequency for the *i*th allele and the arithmetic mean of the expected heterozygosity of the polymorphic loci: $H_{ep} = \sum H_{np}/n_p$; where *n* is the number of markers analyzed;

14) Fraction of polymorphic loci (β) according to Powell *et al*. (1996): $\beta = n_p/n_p. + n_{np}$;

15) Expected heterozygosity (H_e) as reported by Powell *et al.* (1996): $H_e = \beta \sum H_{np}/n_p$;

16) Effective number of alleles per locus (n_e) according to Morgante *et al*. (1994): $n_e = 1/\sum P_i^2$ where *p* is the frequency of the *i*th allele;

17) Total number of effective alleles (N_e) as defined by Pejic *et al*. (1998): $N_e = \sum n_e$;

18) Assay efficiency index (A_i) according to Pejic *et al.* (1998): $A_i = N_e/U$;

19) Effective multiplex ratio (*E*) according to Powell *et al*. (1996): $E = n_u \beta$;

20) Marker index (*MI*) as defined by Powell *et al*. (1996): $MI = E\, H_{ep}$.

Only reproducible and well defined bands in the replications were considered as potential polymorphic markers. For each primer, the alleles were scored as 1 (present) or 0 (absent) and a similarity matrix using the similarity coefficient of Jaccard (1908) was constructed from the whole SSR data. Pair wise distances between DNA cultivars were calculated and analyzed using the unweighted pair-group method (UPGMA). Cluster analysis was performed using NTSYS-pc version 2.11a (Rohlf, 2000).

RESULTS AND DISCUSSION

Microsatellites were successfully amplified in all eleven olive cultivars with the eight primer pairs used. Two molecular criteria were used to select the

eight SSR loci: (1) clear amplified DNA fragments and (2) based on previous results (Bandelj *et al.*, 2002) primer pairs will be referred to as loci and DNA bands as alleles. These SSR loci revealed a total of 142 alleles ranging from two at the ssrOeUA-DCA08 locus to 12 at the ssrOeUA-DCA07 and ssrOeUA-DCA17 loci (Table 2), with an average number of 7.75 alleles per locus. Overall observed heterozygosity values (per assay unit) ranged from 0.28 to 0.98 with a mean value of 0.75 (Table 2). The expected heterozygosities (0.80) showed slightly higher values than the observed heterozygosities.

In current work (Hemeida *et al*., 2007) used different markers, *i.e.*, RAPDs and AFLPs on the same eleven olive cultivars. This work has been extended in the present study to fingerprint these cultivars with SSR. The different types of markers, *i.e.*, RAPD, AFLP and SSR, revealed different levels of genetic similarity among the eleven olive cultivars. This could be due to the difference in polymorphism detection mechanisms by different types of markers. The combining data obtained from the different types of markers may reveal more informative genetic relationships.

The high level of polymorphism observed in this study for all three marker systems is consistent with results from previous studies carried out on olive cultivars by means of different molecular markers (Carriero *et al.*, 2002; Samaee *et al.*, 2003; Taamalli *et al.*, 2006), thereby confirming the great diversity within the cultivated olive germplasm (Baali-Cherif and Besnard, 2005). The higher level of polymorphism detected in olive cultivars by SSR markers than with RAPDs and AFLPs highlights the discriminating capacity of the former. This result is in accordance with previous studies where SSRs were compared to other marker systems (Besnard *et al.*, 2002; Baldoni *et al.*, 2006; Casas *et al.* 2006; Ganino *et al.*, 2007).

All three markers proved to be effective in discriminating the eleven cultivars analyzed. The results obtained are summarized in Table (3). The total number of polymorphic bands ranged from 59 for SSRs to 130 for RAPDs. For instance, the total number of bands scored for RAPDs and AFLPs was relatively high, 143 and 123, respectively, with 81% and 75% of them being polymorphic. In contrast, the lowest number of total bands was obtained for SSR markers, with an intermediate value of the percentages of polymorphism (77%).

The variability observed at SSR loci was expected because of the unique mechanism by which this variation is generated: replication slippage is thought to occur more frequently than single nucleotide mutations and insertion/deletion events, which generate the polymorphisms detectable by AFLP and RAPD analyses (Taamalli *et al.*, 2006). The codominant nature of the markers permits the detection of a high number of alleles per locus and

contributes to higher levels of expected heterozygosity being reached than would be possible with RAPDs and AFLPs. However, this result also depends on the species under study. In barley (Russell *et al.*, 1997) and in tetraploid potato (McGregor *et al.*, 2000), for example, AFLPs scored a higher level of expected heterozygosity (also called diversity index) than SSRs and RAPDs.

The similar levels of polymorphism and expected heterozygosity observed in olive with AFLP and RAPD analyses are consistent with results obtained in other plant species (Powell *et al.*, 1996; Garcia-Mas *et al.*, 2000) and are probably due to how variation is sampled. However, in rice, Fuentes *et al.* (1999) found that AFLPs detected higher levels of polymorphism than RAPDs, while in barley (Russell *et al.*, 1997), AFLPs scored the lowest polymorphism when compared to other markers.

The total number of bands patterns per assay unit for each marker type ranged from 142 for SSR markers to 774 for AFLPs, with an intermediate value of 760 for RAPDs, the number of banding patterns per assay unit for RAPDs (47.50), was somewhere between the values found for SSRs (17.75) and AFLPs (258).

Small values of average confusion probability were obtained for the three markers, especially for the AFLPs (0.07) and SSRs (0.12). The discriminating capacity (*D*), negatively correlated to the confusion probability, showed the highest value for AFLPs (0.93), an intermediate value for SSRs (0.88), while RAPDs showed the lowest value (0.77). D_L values, estimated for the three markers, were close to the actual discriminating power of each of them (0.89, 0.83 and 0.74, respectively, Table 3).

For the SSR markers, the effective number of alleles per locus (n_e) was 1.98, while for RAPDs and AFLPs these values were slightly lower, 1.79 and 1.63, respectively. This was reflected in lower values of the expected heterozygosity for both RAPD and AFLP markers. The very low value of the effective number of alleles per locus for SSR markers in comparison to the average number of alleles per locus (N_e) may suggest the presence of many unique or less frequent alleles. The highest assay efficiency (*Ai*) and marker index (*MI*) values were observed for AFLPs (67.92 and 10.11, respectively) and the lowest for SSRs (1.99 and 0.39, respectively). The values for RAPD markers were intermediate between those of AFLPs and SSRs. The high value of the marker index for AFLPs is the result of a very high multiplex ratio component (E = 34.85). The very high values of assay efficiency and marker index for AFLPs highlights the distinctive nature of these markers. This is due to the simultaneous detection of several polymorphic markers per single reaction.

The fact that all three marker systems showed very low levels of confusing probability support their utility in identification studies. The values of

average discriminating power followed the order AFLP > SSR > RAPD, as a direct consequence of their confusion probability values. Similarly, in grape Tessier *et al.* (1999) obtained higher values of D_L for SSRs than for RAPDs. Therefore, AFLPs and SSRs should probably be preferred than RAPDs for olive variety identification and plant certification (Cipriani *et al.*, 2002). A high frequency of microsatellites amplifying multiple loci has also been reported in olive by Carriero *et al.* (2002). This phenomenon is relatively common in species with an allopolyploid origin, although this has not been clearly demonstrated in olive and may be due to genome fusion and chromosome duplication events during evolution (Minelli *et al.*, 2000).

The relatively high values of the effective number of patterns per assay units (*P*) for all the markers used give evidence of their discrimination capacity. This is very important for the management of germplasm banks where numerous cultivars need to be accurately characterized and identified (Baldoni *et al.*, 2006). AFLPs showed the highest value of *P*, probably due to the high number of loci (or bands) simultaneously analyzed. The very conservative criteria that were applied for the selection of polymorphism may have reduced, to some extent, the values of *P* obtained for RAPDs.

The utility of a given marker is a balance between the level of polymorphism it can detect and its capacity to identify multiple polymorphisms (Besnard *et al.*, 2002). The *MI* for AFLP data is related to the effective multiplex ratio (*E*) value. In other words, it depends more on the high number of alleles (polymorphic bands) obtained in each profile than on the allelic heterozygosity found among cultivars. Both RAPDs and AFLPs have higher multiplex ratios than SSRs in the cultivar set studied. In soybean, however, SSRs scored higher values of *MI* than did RAPDs (Powell *et al.*, 1996). These results reinforce the need for specific studies of marker comparisons for each plant species. Similarly to the *MI* values, the information measured as the assay efficiency index (*Ai*), which correlates with the number of effective alleles per assay, was greater for AFLPs than for the other markers (AFLPs > RAPDs > SSRs). Very high values of *Ai* detected by AFLPs, against RAPDs, SSRs and RFLPs were reported in maize inbred lines by Sanz-Cortes *et al*. (2001).

The study has demonstrated that the three marker systems may have different applications in olive, according to their characteristics: SSRs had the highest polymorphism, H_e and *D* and *P*; AFLPs were characterized by the highest *MI*, *D* and *P* values but the lowest H_e; finally, RAPDs had an intermediate value of *MI* but the lowest values of H_e, *D* and *P*. Such properties, together with other considerations of practical and economical nature, must be taken inconsideration when choosing a marker system for specific applications.

Using eight primers, the SSR analysis was performed on the bulked DNA samples representing the eleven olive cultivars (*Olea europaea* L.). Figure (2) illustrates the different SSR profiles. Moreover, all three markers showed a degree of similarity in dendrogram topologies (Fig. 2), though with some differences in the positioning of some cultivars at the main groups. In the AFLP tree (Fig. 2A), two main clusters were observed: Cluster I, including three cultivars and Cluster II, eight cultivars. The Chemlali and Wetagen cultivars clustered together in the Group (Ia). The Picual and Kalamata cultivars as well as the Hamdey, Khosha and Maraky cultivars also grouped at Cluster II in different groups.

The dendrogram obtained with RAPD markers (Fig. 2B) showed a similar topology with some exceptions. For instance, cultivar Hamdey grouped in Cluster II instead of Cluster I as it did with AFLPs, while some cultivars, such as cultivars Kalamata / Picual / grouped together in Cluster I instead of the Cluster II of AFLPs. At the subgroup level, some associations were maintained in both the AFLP and RAPD dendrograms. This was the case of cultivars Chemlali/Wetagen and Picual/Kalamata.

The dendrogram obtained with SSR markers (Fig. 2C) was to some extent less similar (at the subgroup level) to that obtained with AFLP markers than the dendrogram resulting from RAPDs. The following differences were observed: cultivars Picual and Kalamata grouped together at Cluster I instead of Cluster II, Manzanilla cultivar from Cluster II in the AFLP dendrogram grouped at Cluster I with SSRs. Furthermore, cultivars Meloky /Khosha and Toffahy/Hamdey clustered together, as they did not with AFLP markers, but were part of Cluster II. Some interesting common associations of cultivars were observed in all three markers. For instance, cultivars Chemlali/ Wetagen and Picual/Kalamata grouped together at the same subgroup for all markers, and cultivar Manzanilla clustered with almost the same Cluster in RAPDs and SSRs markers.

The general dendrogram (Fig. 2D), constructed using the combined data of the three sets of molecular markers, was similar to those obtained separately with each marker. However, there were some differences, which led to a better representation of the relationships for most of the cultivars. The dendrogram revealing the relationships at the cultivars level, comprised two main clusters, one containing Chemlali and Wetagen cultivars were together in the first Cluster, while the other cluster revealed two groups. The first one consisted of four cultivars, *i.e.*, Meloky, Manzanilla and Picual / Kalamata, while the Toffahy and Maraky/Khosha cultivars as well as Hamdey clustered together in the second Group (IIb). Moreover, the Chemlali and Picual cultivars were clustered together with cultivars Wetagen and Kalamata, respectively, similarly to what was observed in the separated dendrograms

for each marker. Furthermore, the three techniques have discriminated most genotypes effectively, but SSR markers were able to discriminate cultivars Chemlali/ Wetagen and Picual/Kalamata.

All three techniques may provide useful information on the level of polymorphism and diversity in olive, showing their utility in the characterization of germplasm accessions. For RAPD analysis, the problems of reliability and transferability among laboratories should be considered (Jones *et al.*, 1997). We have found that reliable RAPD data can be generated following a standard protocol, replication of amplification reactions and a conservative criterion of bands selection. The higher informativeness of SSRs and AFLPs, together with the abovementioned problems for RAPDs, will limit its use in DNA fingerprinting (Grati-Kamoun *et al.*, 2006). However, they will remain useful where financial investment is limited.

Both, RAPDs and AFLPs, were efficient in detecting genetic similarities in olive, while the codominant nature of SSRs will make it the marker of choice for segregation studies and genome mapping in olive. A better understanding of the effectiveness of the different molecular markers is considered a priority step toward olive germplasm characterisation and classification, and a prerequisite for more effective breeding programs (Khadari *et al.*, 2008).

SUMMARY

RAPDs, AFLPs and SSRs were compared in terms of their informativeness and efficiency in a study of genetic diversity and relationships among eleven olive cultivars (*Olea europaea* L.) cultivated in Siwa oasis. SSRs presented a higher level of polymorphism and greater information content, as assessed by the expected heterozygosity, than AFLPs and RAPDs. The lowest values of expected heterozygosity were obtained for AFLPs, which nevertheless were the most efficient marker system due to their capacity to reveal the highest number of bands per reaction and because of the high values achieved for a considerable number of indexes. All three techniques discriminated the genotypes effectively, but SSRs were able to discriminate the cultivars Chemlali, Wetagen, Picual and Kalamata. For all markers, a similarity in dendrogram topologies was obtained although some differences were observed. Both, RAPDs and AFLPs, were efficient in detecting genetic similarities in olive, while the codominant nature of SSRs will make it the marker of choice for segregation studies and genome mapping in olive. A better understanding of the effectiveness of the different molecular markers is considered a priority step toward olive germplasm characterisation and classification, and a prerequisite for more effective breeding programs.

REFERENCES

Baali-Cherif, D. and G. Besnard (2005). High genetic diversity and clonal

growth in relict populations of *Olea europaea* subsp. *laperrinei* (Oleaceae) from Hoggar, Algeria. Ann. Bot., 96: 823-830.

Baldoni, L., N. Tosti, C. Ricciolini, A. Belaj, S. Arcioni and G. Pannelli (2006). Genetic structure of wild and cultivated olives in the Central Mediterranean Basin. Ann. Bot., 98: 935-942.

Bandelj, D., J. Jakse and B. Javornik (2002). DNA Fingerprinting of Olive Varieties, Food Technol. Biotechnol., 40: 185-190.

Bartolini, G., G. Prevost, C. Messeri and G. Carignani (2005). Olive germplasm: cultivars and word-wide collections. Web site FAO: http://www.apps3.fao.org/wiews/olive/oliv.jsp.

Besnard, G. and A. Berville´ (2000). Multiple origins for Mediterranean olive (*Olea europaea* L. ssp. europaea) based upon mitochondria DNA polymorphisms. Life Science, 323: 173-181.

Besnard, G., B. Khadari, P. Baradat and A. Berville (2002). Combination of chloroplast and mitochondrial DNA polymorphisms to study cytoplasm genetic differentiation in the olive complex (*Olea europaea* L.). Theor. Appl. Genet., 105: 139-144.

Carriero, F., G. Fontanazza, F. Cellini and G. Giorio (2002). Identification of sample sequence repeats (SSRs) in olive (*Olea europaea* L.). Theor. Appl. Genet., 104: 301-307.

Casas, R., G. Besnard, P. Scho, L. Balaguer and P. Vargas (2006). Extensive gene flow blurs phylogeographic but not phylogenetic signal in *Olea europaea* L. Theor. Appl. Genet., 113: 575–583.

Cipriani, G., M. Marrazzo, R. Marconi, A. Cimato and R. Testolin (2002). Microsatellite markers isolated in olive are suitable for individual fingerprinting and reveal polymorphism within ancient cultivars (*Olea europaea* L.). Theor. Appl. Genet., 104: 223-228.

Diaz Bermudez, A. (2005). Desarrollo y caracterizacio´n de nuevos microsate´ lites y SNPs y aplicacio´n en la mejora gene´ tica del olivo (*Olea europaea* L.), PhD. tesis, Departamento de Gene´ tica, Universidad de Co´rdoba ETSIAM, Spain, pp 180.

Fragaki, G., A. Spyros, G. Siragakis, E. Salivaras, P. Dais (2005) Detection of extra vergin olive oil adulteration with lampante olive oil and refined olive oil using nuclear magnetic resonance spectroscopy and multivariate statistical analysis. J. Agric. Food Chem., 53: 2810-2816.

Fuentes, J., F. Escobar, A. Alvarez, G. Gallego, M. Duque, M. Ferrer, J. Deus and M. Tohme (1999). Analyses of genetic diversity in Cuban rice varieties using isozyme, RAPD and AFLP markers. Euphytica, 109: 107-115.

Ganino, T., A. Bartolini and A. Fabbri (2006). A The classification of olive germplasm- a review. J Hortic Sci Biotech., 81: 319-334.

Ganino, T., D. Beghe, S. Valenti and A. Fabbri (2007). RAPD and SSR markers for characterization and identification of ancient cultivars of *Olea europaea* L. in the Emilia region, Northern Italy. Genet Resour Crop Evol., 54: 1531-1540.

Garcia-Mas, J., M. Oliver, H. Gomez-Paniagua and M. de Vicente (2000). Comparing AFLP, RAPD and RFLP markers for measuring genetic diversity in melon. Theor. Appl. Genet., 101: 860-864.

Grati-Kamoun, N., F. Mahmoud, A. Rebai, A. Gargouri, O. Panaud and A. Saar (2006). Genetic diversity of Tunisian olive tree (*Olea europaea* L.) cultivars assessed by AFLP markers. Genetic Resources and Crop Evolution, 53: 265-275.

Hemeida, A., A. Sanaa, M. Taha and M. Nassr (2007). Characterization of some Siwan olive cultivars (*Olea europaea* L.) using agronomic and molecular markers. Egypt. J. Genet. Cytol., 36: 267-284.

Jaccard, P. (1908) Nouvelle recherches sur la distribution florale. Bulletin de la Société Vaudoise des Sciences Naturelles, 44: 223-270.

Jones, C., K. Edwards, S. Castaglione, M. Winfield, F. Sala, C. Wiel, G. Bredemeijer, B. Vosman, M. Matthes, A. Daly, R. Brettschneider, P. Bettini, M. Buiatti, E. Maestri, A. Malcevschi, N. Marmiroli, R. Aert, G. Volckaert, J. Rueda, R. Linacero, A. Vazquez and A. Karp (1997). Reproducibility testing of RAPD, AFLP and SSR markers in plants by a network of European laboratories. Mol. Breed., 3: 381-390.

Khadari, B., J. Charafi, A. Moukhli and M. Ater (2008). Substantial genetic diversity in cultivated Moroccan olive despite a single major cultivar: a paradoxical situation evidenced by the use of SSR loci. Tree Genetics & Genomes, 4: 213-221.

Masini, A., H. F. El-wakil and M. Th. Hassan (2003). Study of flora composition and distribution in Siwa Oasis. J. Adv. Agric. Res., 8: 241-245.

McGregor, C., C. Lambert, M. Greyling, J. Louw and L. Warnich (2000). A comparative assessment of DNA

fingerprinting techniques (RAPD, ISSR, AFLP and SSR) in tetraploid potato (*Solanum tuberosum* L.) germplasm. Euphytica, 113: 135-144.

Minelli, S., F. Maggini, M. Gelati, A. Angiolillo and P. Cionini (2000). The chromosome complement of *Olea europaea* L.: characterization by differential staining of the chromatin and in situ hybridization of highly repeated DNA sequences. Chromosome Res., 8: 615-619.

Morgante, M., A. Rafalski, P. Biddle, S. Tingey and A. Olivieri (1994). Genetic mapping and variability of seven soybean sample sequence repeat loci. Genome, 37: 763-769.

Ozkaya, M., E. Cakir, Z. Gokbayrak, H. Ercan and N. Taskin (2006). Morphological and molecular characterization of Derik Halhali olive (*Olea europaea* L.) accession grown in Derik-Mardin province of Turkey. Sci. Hortic., 108: 205-209.

Pejic, I., P. Ajmone-Marsan, M. Morgante, V. Kozumplik, P. Castiglioni, G. Taramino and M. Motto (1998). Comparative analysis of genetic similarity among maize inbred lines detected by RFLPs, RAPDs, SSRs and AFLPs. Theor. Appl. Genet., 97: 1248-1255.

Powell, W., M. Morgante, C. Andre, M. Hanafey, J. Vogel, S. Tingey and A. Rafalski (1996). The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. Mol. Breed., 2: 225-238.

Rohlf, F. J. (2000). On the use of shape spaces to compare morphometric method. Hystrix Italian J. Mammology (n.s.), 11: 8-24.

Russell, J., J. Fuller, M. Macaulay, B. Hatz, A. Jahoor, W. Powell and R. Waugh (1997). Direct comparison of levels of genetic variation among barley accessions detected by RFLPs, AFLPs, SSRs and RAPDs. Theor. Appl. Genet., 95: 714-722.

Samaee, Z., S. Shobbar, H. Ashrafi and M. Hosseini-Mazinani (2003). Molecular characterization of olive germplasm in Iran by use of Random Amplified Polymorphic DNA (RAPD): Correlation with phenotypic studies. Acta Hort., 623: 169-175.

Sanz-Cortes, F., M. Badenes, S. Paz, A. Iniguez and G. Llacer (2001). Molecular characterization of olive cultivars using RAPD markers. J. Am. Soc. Hort. Sci., 126: 7-12.

Sefc, K. M., M. Lopes, D. Mendonca, M. Rodrigues Dos Santos, M. Laimer Da Camara Machado and A. Da Camara Machado (2000). Identi-

fication of microsatellite loci in olive (*Olea europaea*) and their characterization in Italian and Iberian olive trees. Mol. Ecol., 9: 1171-1173.

Taamalli, W., F. Geuna, R. Banfi, D. Bassi, D. Daoud and M. Zarrouk (2006). Agronomic and molecular analyses for the characterization of accessions in Tunisian olive germplasm collections. Electronic J. Biotechnology, 9: 467-481.

Tessier, C., J. David, P. This, J. Boursiquot and A. Charrier (1999). Optimization of the choice of molecular markers for varietal identification in *Vitis vinifera* L. Theor. Appl. Genet., 98: 171-177.

Trujillo, I., L. Rallo and P. Arus (1995). Identifying olive cultivars by isozyme analysis. J. Am. Soc. Hort. Sci., 120: 318-324.

Table (1): Repeat motifs, primer sequences and annealing temperatures (T_a) and for eight microsatellite loci analyzed in eleven olive cultivars (*Olea europaea* L.).

Locus	Repeat motif	Primer sequence (5'–3')	T_a
ssrOeUA-DCA04	$(GA)_{16}$	CTT AAC TTT GTG CTT CTC CAT ATC C	55°C
AJ279855		AGT GAC AAA AGC AAA AGA CTA AAG C	
ssrOeUA-DCA05	$(GA)_{15}$	AAC AAA TCC CAT ACG AAC TGC C	50°C
AJ279856		CGT GTT GCT GTG AAG AAA ATC G	
ssrOeUA-DCA07	$(AG)_{19}$	GGA CAT AAA ACA TAG AGT GCT GGG G	60°C
AJ279857		AGGGTAGTCCAACTGCTAATAGACG	
ssrOeUA-DCA08	$(GA)_{18}$	ACAATTCAACCTCACCCCCATACCC	55°C
AJ279858		TCACGTCAACTGTGCCACTGAACTG	
ssrOeUA-DCA10	$(TA)_{14}$ $(GA)_{17}$	CGT GAC CAC CTA AAT CCG CCC C	50°C
AJ279860		CTG TCC AGA GCT AAA GGT TTC G	
ssrOeUA-DCA13	$(GA)_{15}$	GAT CAG ATT AAT GAA GAT TTG G	55°C
AJ279862		AAC TGA ACC TGT GTA TCT TGC ATC C	
ssrOeUA-DCA16	$(GT)_{13}(GA)_{29}$	TTAGGTGGGATTCTGTAGATGGTTG	50°C
AJ279865		TTTTAGGTGAGTTCATAGAATTAGC	
ssrOeUA-DCA17	$(GT)_9(AT)_7$AGAT A$(GA)_{38}$	GATCAAATTCTACCAAAAATATA	50°C
AJ279866		TAATTTTTGGCACGTAGTATTGG	

Table (2): Allele sizes (bp) detected in analysis of eleven olive varieties, number of amplified alleles per locus (n) and observed heterozygosity (Ho).

Allele*	Locus							
	DCA04	DCA05	DCA07	DCA08	DCA10	DCA13	DCA16	DCA17
A	165	229	145	145	216	161	180	196
B	158	223	130	135	206	143	170	189
C	150	217	125		200	128	143	185
D	148	195	121		190	113	139	166
E	135	180	111		168	109	134	147
F	132		107		163		130	144
G	130		103		155		125	127
H	129		96		143		121	124
I	121		94		130			119
J			90					110
K			88					108
L			84					100
n	***9***	***5***	***12***	***2***	***9***	***5***	***8***	***12***
Ho	***0.94***	***0.73***	***0.58***	***0.28***	***0.89***	***0.89***	***0.81***	***0.68***

*The letters indicate alleles at each locus. Note: Loci ssrOeUA-DCAno are designated DCAno.

Table (3): Levels of polymorphism and comparison of informativeness obtained with RAPD, AFLP and SSR markers in eleven olive cultivars.

Indexes with their abbreviations		Marker system		
		RAPD*	AFLP*	SSR
Polymorphic fragments		617*	587*	109
Percentages of polymorphism	%	81*	76*	77
Number of assay units	U	16	3	8
Number of polymorphic bands	n_p	130	105	59
Number of monomorphic bands	n_{np}	13	18	3
Average number of polymorphic bands/assay unit	n_p/U	8.13	35.00	7.38
Number of loci	L	143	123	8
Number of loci/assay unit	n_u	8.94	41	1.00
Number of banding patterns	T_p	760*	774*	142
Average number of patterns/assay unit	I	47.50	258	17.75
Average confusion probability	C	0.23	0.07	0.12
Average discriminating power	D	0.77	0.93	0.88
Average limit of discriminating power	D_L	0.74	0.89	0.83
Effective number of patterns/assay unit	P	4.85	9.10	5.88
Average number of alleles per locus	n_{av}	2.00	2.00	7.38
Expected heterozygosity of the polymorphic loci	H_{ep}	0.64	0.49	0.79
Fraction of polymorphic loci	β	0.91	0.85	0.99
Expected heterozygosity	H_e	0.31	0.25	0.80
Effective number of alleles per locus	n_e	1.79	1.63	1.98
Total number of effective alleles	N_e	257.76	203.75	15.91
Assay efficiency index	A_i	16.11	67.92	1.99
Effective multiplex ratio	E	8.14	34.85	0.99
Marker index	MI	2.77	10.11	0.39

*The amplified product assessed in a previous work (Hemeida *et al*., 2007).

*From Hemeida *et al*. (2007), results here for RAPD and AFLP analysis.

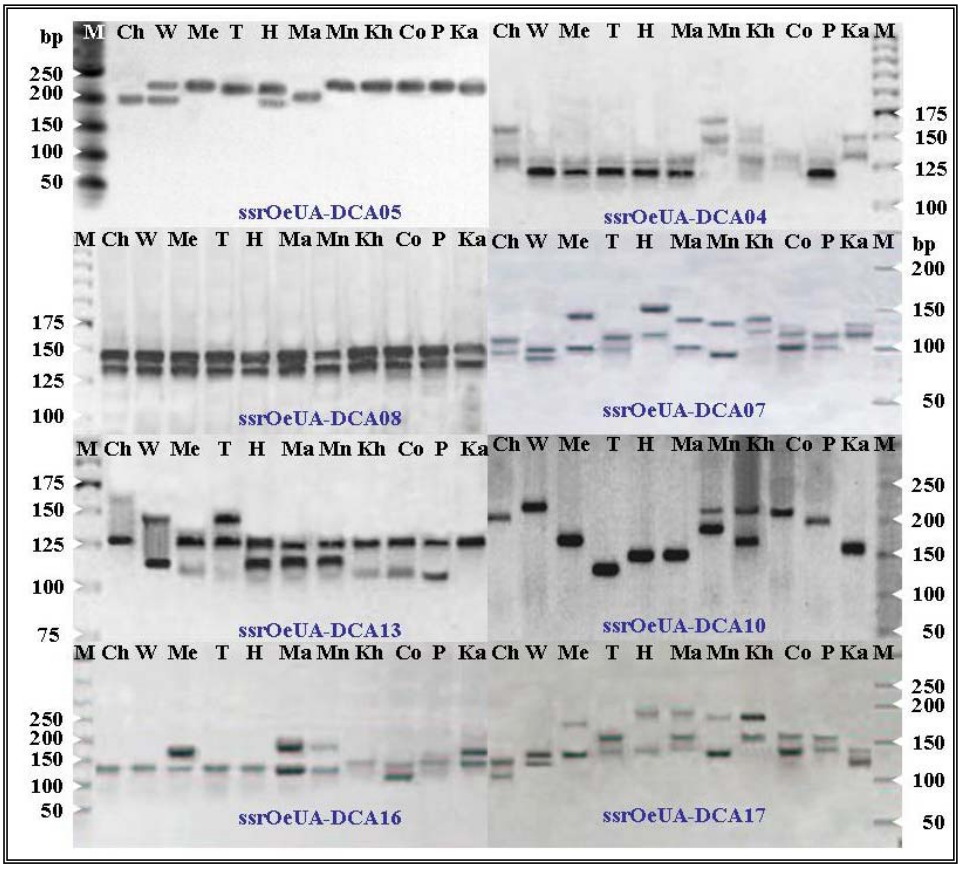

Fig. (1): Photographs showing SSR patters from the eleven Siwan olive cultivars analyzed using eight primer pairs. Chemlali: Ch; Wetagen: W; Meloky: Me; Toffahy: T; Hamdey: H; Maraky: Ma; Manzanilla: Mn; Khosha: Kh; Coronaiki: Co; Picual: P; Kalamata: K. and M: DNA marker.

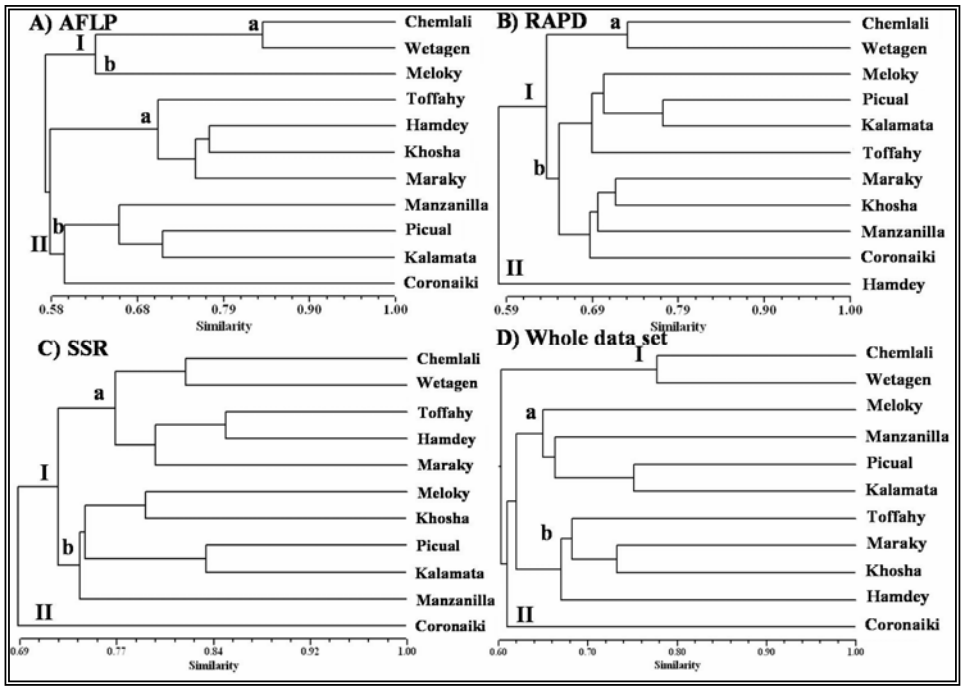

Fig. (2): Dendrograms of eleven olive cultivars obtained using AFLP, RAPD and SSR markers separately (A-C) and the whole data set of the three markers (D).