# Identification of olive (*Olea europaea* L.) genotypes using SSR and RAPD markers

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## 1. INTRODUCTION

Olive (*Olea europaea* L.) is one of the most ancient cultivated fruit tree species in the Mediterranean basin. It is a predominantly allogamous species showing a high degree of outcrossing which leads to considerable levels of heterozygosity and DNA polymorphism among individuals (Angiolillo *et al.*, 1999; Rallo *et al.*, 2000). The wide genetic patrimony and the large number of synonyms and homonyms in olive require precise methods of discrimination for cultivar identification and classification. Different techniques have been used to evaluate olive diversity. Morphological, agronomical or biochemical characterisation has been adopted for variability evaluation (Barone *et al.*, 1994; Barranco *et al.*, 2000; Ouazzani *et al.*, 1993; Trujillo *et al.*, 1995). The introduction of DNA markers provided a good discriminatory system, independent of environmental conditions. The random amplified polymorphic DNA (RAPD) technique has been applied in several studies to successfully distinguish between olive cultivars (Belaj *et al.*, 2001; Fabbri *et al.*, 1995; Guerin *et al.*, 2002; Mekuria *et al.*, 1999). Nowadays simple sequence repeat (SSR) have been proven to be very suitable markers for cultivar identification and identity typing in olive as they are transferable, highly polymorphic and co-dominant markers (Carriero *et al.*, 2002; Cipriani *et al.*, 2002; Rallo *et al.*, 2000).

The objectives of this paper are: (1) to identify by SSR and RAPD markers DNA fingerprints of 100 accessions of olive trees; (2) to compare the above-mentioned markers for their ability to discriminate between genotypes; (3) to verify cultivar identities using a database of DNA fingerprints.

## 2. MATERIALS AND METHODS

### 2.1. Plant material and DNA extraction

All the samples were obtained from the National Olive Variety Assessment (NOVA) collection, located at the University of Adelaide, Roseworthy Campus (South

Australia). Ten presumed Mission olive cultivar samples were obtained from cultivated olives planted around the early Mission sites in the USA.

For the RAPD and SSR analysis, total genomic DNA was isolated from fresh leaf material following the procedure previously described by Guerin *et al.* (2002).

### 2.2. RAPD analysis

Four random 10-mer primers were used in the study: GC01, GC05, OPZ11 and GC18. RAPD amplifications and NOVA cultivar identification were performed as described by Guerin *et al.* (2002).

### 2.3. SSR analysis

Nine primer pairs for olive microsatellite loci were used in the analysis; the sequences were obtained from previously published papers: UDO-006, UDO-008, UDO-024, UDO-031 (Cipriani *et al.*, 2002), EMO-2 (De La Rosa *et al.*, 2002), SSR-3, SSR-4, SSR-14 and SSR-16 (Sefc *et al.*, 2000). The forward primers were labelled with fluorescent tags, FAM, NED and HEX. PCR reactions were performed in a total volume of 20 µl containing 40 ng genomic DNA, 1 Buffer PCR, 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.5 M of forward and reverse primers and 1.25 units of Taq Polymerase (Invitrogen), adopting the following profile: initial denaturation at 95°C for 5 min, 34 cycles of 45 s at 95°C, 45 s at the annealing temperature depending on the primer pair (from 50 to 57°C), 45 s at 72°C, followed by a final extension step of 45 min at 72°C. PCR products were first checked on 1.75% agarose gel and then analysed using an automated sequencer (96 capillary ABI Prism 3700 DNA Analyser - Applied Biosystems, USA). The results were scored by Genscan software version 3.5.1 and the presence or absence of alleles was translated to a binary matrix by allocating 1 or 0 for presence or absence of alleles, respectively. A similarity matrix among accessions was calculated using SIMQUAL (Similarity of Qualitative Data), cluster analysis was performed on the estimated similarities using the unweighted pair group method with arithmetic average (UPGMA) and SHAN algorithm, and the resulting clusters were expressed as a dendrogram using NTSYS-pc (Exeter Software v.2.02k).

### 2.4. Identification of Mission samples

The genetic database established from NOVA DNA fingerprints was used to identify 10 presumed Mission samples. Six of the 9 above-mentioned microsatellite primers were tested: UDO-006, UDO-008, UDO-031, EMO-2, SSR-3 and SSR-16. Dendrogram showing genetic similarities was constructed using UPGMA.

## 3. RESULTS

### 3.1. RAPD and SSR

Both molecular markers, RAPD and SSR, proved to be highly effective in discriminating the 100 NOVA cultivars analysed. The four 10-mer RAPD primers, as

reported by Guerin and co-workers (2002), generated 26 polymorphic bands which allowed 58 different genotypes to be distinguished. Fifteen groups, each of them containing cultivars with different names but the same DNA fingerprint (synonyms), were found in the NOVA collection. Eight of these groups have been identified as being genetically similar to known cultivars obtained from international collections, whereas seven groups remain unidentified.

All nine microsatellite markers were polymorphic, revealing a total of 64 alleles, ranging from 4 to 10 alleles per locus with a mean value of 7.1 alleles per locus.

The dendrogram obtained with SSR markers (Figure 1) was similar to that obtained with RAPDs. The following differences were observed: cultivars Pueblana, WA Mission, Paragon and Bouteillon that produced the same fingerprint as Frantoio (Italy) using RAPDs were distinguished with SSRs; however Paragon still had the same genotype as Bouteillon; cultivar Gros Reddeneau that had the same fingerprint as other four accessions using RAPD markers was discriminated with SSRs.

### 3.2. Mission identification

After comparison of the Mission samples to the database, genetically similar groups were identified and are shown in figure 2: the samples in Group 1 are all similar to each other and to Manzanillo from Cordoba, Spain; the samples in Group 2 were similar to each other and to the Mission sample from UC Davis, USA; the sample from San Louis (Group 3) was similar to Nevadillo Blanco from UC Davis; however, San Diego "AB" and San Francisco Solano 3 did not match any of the other samples.

## 4. CONCLUSION

Guerin and co-workers (2002) have demonstrated the capacity of 26 polymorphic bands from four RAPD markers to assess the identity of the accessions in the NOVA collection; this result is in accordance with previous studies (Belaj *et al.*, 2001; Mekuria *et al.*, 1999).

However, the co-dominant nature of microsatellite markers permitted the discrimination of a higher number of genotypes, as indicated in other studies (Belaj *et al.*, 2003; Powel *et al.*, 1996). Belaj *et al.* (2003) also found that SSRs discriminated between two cultivars Frantoio and Cellina that had the same fingerprint generated by RAPDs and AFLPs. The group of 11 individuals that had the same DNA fingerprint as cultivar Frantoio was confirmed, using SSRs, the largest group of synonyms in NOVA collection.

The DNA fingerprints collected from NOVA collection have been used for the construction of a genetic database that was used to identify eight out of ten presumed Mission olive cultivars.

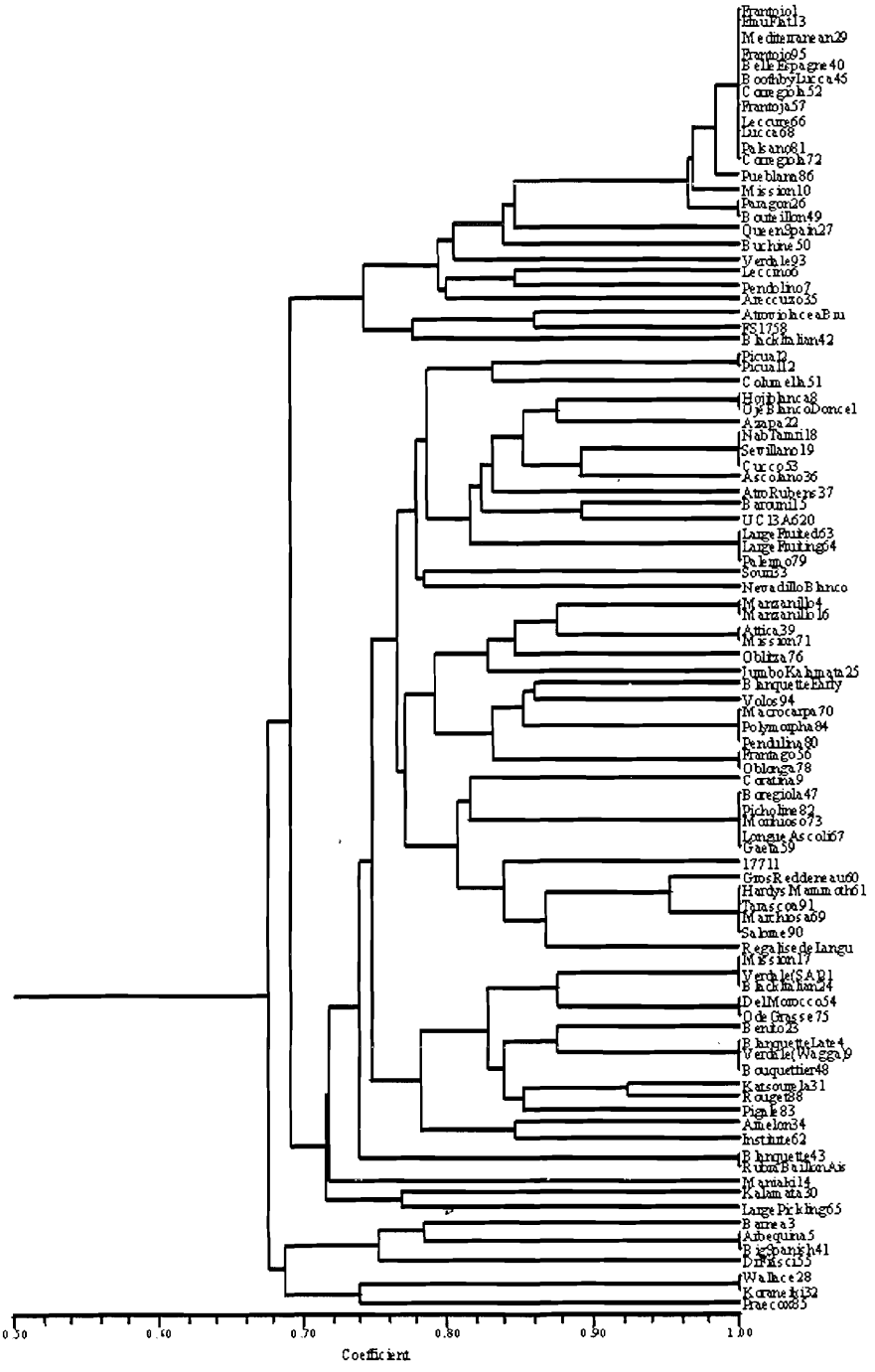


**Figure 1. Dendrogram of genetic similarity of 100 NOVA collection accessions using 9 SSRs**

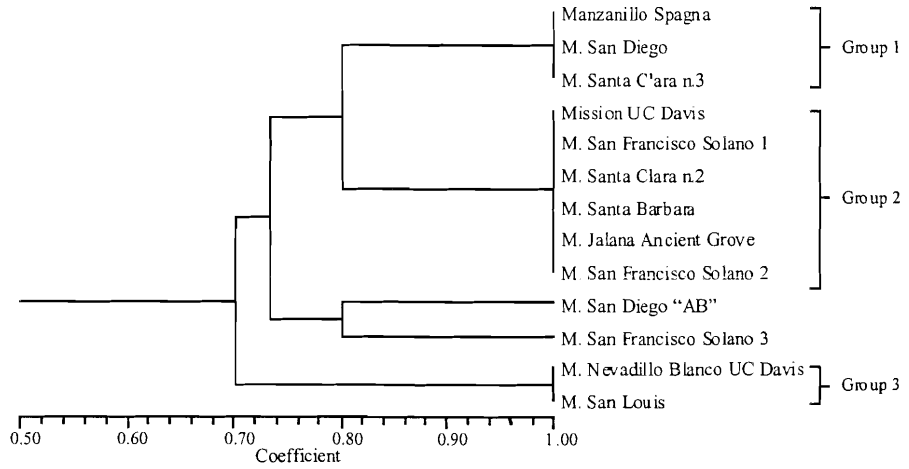


**Figure 2. Dendrogram of genetic similarity of ten Mission samples, Manzanillo (Spain), Mission (UC Davis) and Nevaldillo Blanco (UC Davis)**

## 5. ACKNOWLEDGEMENTS

We thank Susan Sweeney of PIRSA (Primary Industries and Resources South Australia) for access to the NOVA site; Bill Krueger, tree crops Farm Advisor UCCE Glenn County USA, and MOPREP (Mission Olive Preservation, Restoration and Education Project) members for providing Mission samples.

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**Identification of olive (*Olea europaea* L.) genotypes using SSR and RAPD markers**

SSR and RAPD markers were used to identify DNA fingerprints of 100 accessions of olive trees (*Olea europaea* L.) from the National Olive Variety Assessment (NOVA) collection, located at the University of Adelaide, Roseworthy Campus (South Australia). The markers were compared for their ability to discriminate between genotypes and their level of polymorphism per marker. The results were similar for both types of markers, and it was shown that several cultivars in the collection had similar genotypes. However, SSRs were more discriminatory as more genotypes were identified with these, than with RAPD markers. The DNA fingerprints collected have been used for the construction of a genetic database that was used to identify eight out of ten presumed Mission olive cultivar samples obtained from cultivated olives planted around the early Mission sites in the USA.