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# Transferability of olive microsatellite loci across the genus *Olea*

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**Abstract** The transferability of microsatellite markers developed for olive cultivars (*Olea europaea* L.) has been tested and confirmed in the *Olea* complex. Thirty two genotypes, belonging to different taxa of the genus *Olea*, have been analyzed with four olive SSRs. Positive amplifications at all loci were obtained in 13 taxa (at least one accession per species). Sixty seven different alleles have been detected at the four loci analyzed. Polymorphic products have been observed at the inter- and intra-species level. Some SSR loci have shown multiple amplification products in some species. The high number of unique alleles has allowed the unambiguous discrimination of most accessions. Similarity coefficients and relationships among the *Olea* taxa have been calculated based on SSR amplification results. The reliability of SSRs as markers for intra-species variability evaluation has been confirmed while their use to explore relationships at the inter-species level is discussed, being dependent on the locus analyzed.

**Keywords** SSR · *Olea* species · *Olea europaea* · Genetic relationships

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## Introduction

Olive (*Olea europaea* L.) is the most important oil-producing crop in the Mediterranean region. It belongs to the genus *Olea*, which is divided into two sub-genera, *Olea* and *Paniculatae*; the sub-genus *Olea* is divided into two sections, *Olea* and *Ligustroides*. The section *Olea* includes the complex of *O. europaea* L., grouping the olive plants belonging to the Mediterranean olive tree (Green and Wickens 1989). The revision of the *Olea* genus taxonomy made by these authors has reconstituted most of the species in the Mediterranean area, with Africa and Asia as four subspecies within *O. europaea*:*O. europaea* subsp. *europaea* (wild and cultivated olive of the Mediterranean area), subsp. *cuspidata* (including species from Asia and South-East Africa such as *Olea cuspidata, Olea indica, Olea africana* and *Olea ferruginea*); subsp. *laperrinei*, corresponding to *Olea laperrinei* of the Sahara region; subsp. *cerasiformis*, the wild form of Macaronesian Islands. Recently, two new subspecies have been added within *O. europaea* (Medail et al. 2001; Vargas et al. 2001): subsp. *guanchica*, from Canary Islands, and subsp. *maroccana*, from Morocco.

The interest for the related species to olive is based not only on the phylogeny of the genus but involves the role played by these wild relatives as a source of variability for olive-breeding strategies in order to develop new cultivars and rootstocks, as has been demonstrated in many other cultivated species.

Microsatellites or SSRs (simple sequence repeats) have become one of the most useful molecular markers in plant breeding, widely used for cultivar fingerprinting, paternity testing and genome mapping. SSR transferability across related species and genera makes these markers very powerful for comparative genetic studies (Szewc-McFadden et al. 1996; Smulders et al. 1997). A high-cross species conservation of SSR loci has also been observed in tree species such as *Citrus* (Kijas et al. 1995), *Prunus* (Cipriani et al. 1999; Dirlewanger et al. 2002; Wünsch and Hormaza 2002), *Vitis* (Sefc et al. 1999),

*Elaieis* (Billote et al. 2001), *Picea* (Hodgetts et al. 2001) and *Pinus* (Shepherd et al. 2002).

In the past years different groups have been working for the development of microsatellites in olive (Rallo et al. 2000; Sefc et al. 2000; Carriero et al. 2002; Cipriani et al. 2002). Although most microsatellites are assumed to be transferable (Kijas et al. 1995), it has not yet been confirmed in the case of olive. The aim of the present paper is to test across different *Olea* taxa the amplification of olive SSRs developed by our group, and use them to explore the inter-relations within the *Olea* genus and between cultivated olive and the other *Olea* taxa.

## Materials and methods

### Plant material and DNA isolation

Amplification of four olive microsatellites has been tested in 32 accessions of 15 *Olea* taxa (Table 1). In order to establish the relationships between these taxa and olive, 14 *O. europaea* cultivars were included in the study (Table 1). The cultivars were chosen on the basis of previous results (Belaj et al. 2002), in order to represent the high variability within *O. europaea*, subsp. *europaea*, var. *europaea*.

DNA from the olive cultivars was isolated from young leaves following the method described by Belaj et al. (2001). DNA from the different *Olea* taxa was kindly supplied by different institutions, as specified in Table 1.

### PCR amplification

The microsatellites IAS-oli11 [$(GA)_{18}$], IAS-oli 12 [$(GA)_{17}$], IAS-oli17 [AGGAG $(AGAGGG)_7$AGGGAG] and IAS-oli22 [$(GA)_6(GT)_5$GACCT$(GA)_5$] were used for the analysis, based on

**Table 1** *Olea* taxa and olive cultivars analyzed by SSR markers

| Subgenus/Section | Species/subspecies | Taxonomy by Green and Wickens (1989) | Number of accessions and tags | Origin | Source[a] |
|---|---|---|---|---|---|
| *Olea/Olea* | *Olea africana* Mill. | *O. europaea* subsp. *cuspidata* | 3 (I1) | Kenya | KEW |
| | | | (I2) | Kenya | INRA |
| | | | (I3) | South Africa | INRA |
| | *Olea cerasiformis* Webb. & Berth. | | 2 (B1, B2) | Canary Islands | INRA |
| | *Olea chrysophylla* Lam. | *O. europaea* subsp. *cuspidata* | 2 (A2, A4) | Yemen | INRA |
| | *Olea cuspidata* Wall. | *O. europaea* subsp. *cuspidata* | 1 (G1) | China | IRO |
| | *Olea ferruginea* Royle | *O. europaea* subsp. *cuspidata* | 8 (H1) | India | IRO |
| | | | (H2,H3,H4) | Iran | INRA |
| | | | (H5,H6,H7,H8) | Pakistan | INRA |
| | *Olea indica* Klein | *O. europaea* subsp. *cuspidata* | 1 (M1) | Kenya | KEW |
| | *Olea laperrinei* Batt. & Trab. | *O. europaea* subsp. *laperrinei* | 1 (O1) | Algeria | INRA |
| | *Olea maroccana* Gren. & B. | *O. europaea* subsp. *maroccana*[b] | 2 (P1, P3) | Morocco | INRA |
| *Olea/Ligustroides* | *Olea capensis* L. | | 1 (C3) | South Africa | INRA |
| | *Olea capensis capensis* | | 1 (C4) | South Africa | INRA |
| | *Olea capensis hochstetteri* Bak. | | 2 (C1,C2) | Ivory Coast | INRA |
| | *Olea exasperata* Jacq. | | 2 (E1,E2) | South Africa | INRA |
| | *Olea lancea* Lam. | | 2 (L1) | Mauritius | KEW |
| | | | (L2) | Reunion | INRA |
| | *Olea perrieri* Chev. | | 1 (S1) | Madagascar | INRA |
| | *Olea woodiana* Knobl. | | 1 (U2) | South Africa | INRA |
| *Paniculatae* | *Olea paniculata* R. Br. | | 1 (Q1) | Australia | KEW |
| *Tetrapilus* | *Olea brachiata* (Lour.) Merr. | | 1 (F1) | Sumatra | INRA |
| | | Species | Cultivar | Origin | Source |
| | | *Olea europaea L.* | 'Arbequina' | Spain | COGB |
| | | | 'Picual' | | |
| | | | 'Ascolana Tenera' | Italy | COGB |
| | | | 'Frantoio' | | |
| | | | 'Ayvalik' | Turkey | COGB |
| | | | 'Memeçick' | | |
| | | | 'Koroneiki' | Greece | COGB |
| | | | 'Kalamon' | | |
| | | | 'Chemlali' | Algeria | COGB |
| | | | 'Chetoui' | Tunisia | COGB |
| | | | 'Meski' | | |
| | | | 'Cordovil de Serpa' | Portugal | COGB |
| | | | 'Kaissy' | Syria | COGB |
| | | | 'Picholine Maroccaine' | Morroco | COGB |

[a] COGB, Olive Germplasm Bank, Córdoba, Spain; INRA, UR Genetique et Amelioration des Plantes, Montpellier, France; IRO, Instituto di Ricerche sulla Olivicoltura, CNR, Perugia, Italy; KEW, RGB Kew Living Collection, London, UK
[b] Vargas et al. (2001)

their clear polymorphism obtained on a large group of olive cultivars (Rallo et al. 2000).

PCR reactions were performed in a 15-$\mu$l volume containing 10 ng of DNA, 15 mM of Tris-HCl pH 8.0, 50 mM of KCl, 2.5 mM of $MgCl_2$, 0.25 mM of each dNTP, 0.20 $\mu$M of forward and reverse primers and 1 U of Ampli*Taq* Gold DNA Polymerase (Applied Biosystems). Amplifications were performed in a 9600 Thermal Cycler (Applied Biosystems) following the conditions described by Rallo et al. (2000) for each locus. For fluorescent detection of fluorescence, forward primers of IAS-oli11, IAS-oli12, IAS-oli17 and IAS-oli22 were labelled with FAM, HEX, TET and TET fluorescent tags (Applied Biosystems), respectively. Reactions and runs were repeated at least twice.

### Resolving PCR products

Fluorescent amplification products were mixed with de-ionized formamide and an internal size standard (Genescan 350-TAMRA, Applied Biosystems) in a 1:12:0.5 volume ratio, denatured at 95 °C for 5 min and analyzed on the ABI PRISM 310 Genetic Analyzer (Applied Biosystems). The Genescan software, version 3.1 for Mac OS, was used for sample analysis.

### Data analysis

All alleles detected at the four SSR loci were sized (bp) with the help of Genescan software. The total number of alleles detected per locus were recorded and all samples were scored for the presence or absence of these alleles.

Genetic similarity among the different taxa was calculated with the SIMQUAL program, according to the Nei and Li (1979) coefficient: $Sij = 2a/(2a + b + c)$ where a is the number of bands present in both individuals, b is the number of bands present in *i* and absent in *j*, and c is the number of bands present in *j* and absent in *i*. Cluster analyses and construction of dendrograms were performed with the Unweighted Pair-Group Method (UPGMA) using arithmetic averages with the SAHN-clustering and TREE program of the NTSYS-pc package v 2.0 (Rohlf 1997).

## Results

### *Olea* species amplification

Amplification products at all four SSR loci analyzed have been obtained in 13 of the 15 *Olea* taxa studied (27 out of 32 accessions) (Table 2). Only two species, *Olea brachiata* and *O. cuspidata*, have failed amplification at loci IAS-oli12 and IAS-oli11, respectively, while some of the products obtained in other taxa were weak or difficult to interpret, especially when amplifying locus IAS-oli-11.

Sixty seven different amplicons were obtained in the four loci for the 32 genotypes analyzed. The number of alleles per locus ranged from 11 (IAS-oli22) to 22 (IAS-oli11). The total number of alleles per species at all four loci varied from five (*O. brachiata*) to 28 (*O. ferruginea*), these results directly related to the number of accessions per taxon. Numerous amplification products differed from each other only by one single nucleotide. These amplification products were all considered as different alleles. This kind of mutation is present either in SSRs with simple repeated motifs (IAS-oli11 and IAS-oli12) and in those with complex motives (IAS-oli17 and IAS-oli22).

Some accessions of *Olea capensis, O. exasperata, O. ferruginea, O. maroccana, O. perrieri* and *O. woodiana* have shown cases of multiple amplification (three or four amplicons per individual). In the accessions of *O. capensis* the amplification of the locus IAS-oli11 has originated multiple unspecific products, difficult to interpret, while at locus IAS-oli12 four and three clear-amplicons were detected in *O. capensis hochstetteri* and in *O. capensis capensis*, respectively. At locus IAS-oli22 there were three amplification products for the last taxon.

A high number of unique alleles has also been observed (Table 3), and about 42% of the alleles detected (28 out of 67) were present in just one accession. Eight other alleles were only present in few accessions of *O. ferruginea, O. lancea* and *O. capensis*. Locus IAS-oli11 has shown seven alleles within the 14 *O. europaea* cultivars, five of them also being present in the other taxa. For locus IAS-oli12 six alleles were obtained in the cultivars, five of them shared with the other taxa where the other 12 alleles were amplified. Loci IAS-oli17 and IAS-oli22, respectively, showed four and three alleles in the cultivars and 13 and eight in the other taxa.

Surprisingly, the most distant species to olive, such as *O. exasperata, O. lancea* and *O. paniculata*, shared with *O. europaea* one IAS-oli11 allele (144), not present in more closely related taxa. Similar results concern the IAS-oli22 allele 128 that is shared by *O. europaea, O. brachiata, O. capensis, O. exasperata, O. woodiana*, other than *O. cerasiformis, O. ferruginea, O. laperrinei* and *O. maroccana*.

### Clustering

The SSR data obtained have been used to detect their reliability to establish genetic relationships among the different *Olea* taxa. The highest similarity coefficients between species and cultivars ranged from 0.31 to 0.57, relating cultivars with the taxa *O. africana, O. cerasiformis, O. chrysophylla, O. exasperata, O. laperrinei* and *O. maroccana* (data not shown). No similarity (0) was observed between some accessions of *O. ferruginea* with most olive cultivars. Taxa have been grouped based on the UPGMA methodology according to the Nei's similarity coefficient (Fig. 1). The *O. capensis* accessions, two samples of *O. ferruginea* (H1, H4) and one of *O. exasperata* (E2), with missing values or ambiguous results in more than one SSR locus, were not included in the analysis.

Although only four SSR loci have been used, all accessions analyzed were distinguished (with the exception of the two samples of *O. lancea*). A clear grouping of samples belonging to the same species have been observed for all the species with more than one accession (*O. africana, O. cerasiformis, O. maroccana, O. chrysophylla* and *O. ferruginea*), excepting one sample of *O. maroccana*. The dendrogram topology shows a clear separation of the group of olive cultivars from the other taxa. However, the distribution of these taxa shows some

**Table 2** Cross-species amplification of olive microsatellites (*O. europaea* L.) in different *Olea* taxa

| *Olea* taxa (sample size) | SSR loci | | | | Total allele number | Mean allele number/taxon |
|---|---|---|---|---|---|---|
| | Ias-oli 11 | Ias-oli 12 | Ias-oli 17 | Ias-oli 22 | | |
| *O. africana* (3) | +/p 4 (150-158-163-171) | +/p 4 (124-126-136-138) | +/p 4 (156-169-181-186) | +/p 3 (127-130-133) | 15 | 5 |
| *O. brachiata* (1) | + 1 (142) | – | + 2 (182-188) | + 2 (115-128) | 5 | 5 |
| *O. capensis* (4) | mp** | mp/p 5 (124-126-132-134-136) | +/p 3 (171-182-230) | mp/p 4 (124-126-128-130) | 12 | 3 |
| *O. cerasiformis* (2) | +/p 3 (131-139-153) | +/p 2 (132-136) | +/m 1 (171) | +/m 2 (128-130) | 8 | 4 |
| *O. chrysophylla* (2) | +/m 1 (145) | +/p 4 (126-132-134-140) | +/p 3 (163-169-175) | +/m 1 (130) | 9 | 4.5 |
| *O. cuspidata* (1) | – | + 2 (134-136) | + 2 (156-163) | + 2 (137-141) | 6 | 6 |
| *O. exasperata* (2) | mp*/p 3 (115-127-144) | mp 3 (132-134-136) | + 1 (182) | mp/p 3 (124-128-130) | 10 | 5 |
| *O. ferruginea* (8) | mp/p 12 (128-129-133-135-136-137-139-142-150-154-159-161) | +/p 9 (120-128-130-132-134-140-145-150-154) | mp/p 5 (148-149-156-163-169) | +/p 2 (115-128) | 28 | 3.5 |
| *O. indica* (1) | mp 3 (139-144-150) | + 2 (138-147) | + 2 (156-169) | + 1 (127) | 8 | 8 |
| *O. lancea* (2) | mp* 2 (142-144) | +/m 1(116) | +/m 2 (139-232) | +/m 1(139) | 6 | 3 |
| *O. laperrinei* (1) | + 1 (142) | + 2 (128-134) | + 2 (171-177) | + 1 (128) | 6 | 6 |
| *O. maroccana* (2) | mp/p 4 (139-145-151-161) | +/ p 2 (120-122) | mp/p 5 (152-161-165-171-177) | +/p 3 (128-130-132) | 14 | 7 |
| *O. paniculata*(1) | +* 2 (142-144) | + 1 (107) | + 1 (182) | + 2 (130-132) | 6 | 6 |
| *O. perrieri* (1) | + 2 (115-142) | + 2 (132-134) | + 1 (182) | +/mp 3 (124-126-130) | 9 | 9 |
| *O. woodiana* (1) | mp* | + 2 (116-134) | mp 3 (171-177-182) | + 2 (128-130) | 7 | 7 |
| *O. europaea* (14) | +[a]/p[b] 7[c] (135-137-144-148-152-154-161)[d] | +/p 6 (120-126-128-134-138-143) | +/p 4 (169-175-181-187) | +/p 3 (128-130-132) | 22 | 1.57 |

[a] Amplification results: + (positive), – (no amplification), mp (multiple products), * (weak or ambiguous in one sample), ** (weak or ambiguous in all samples)
[b] Intraspecific polymorphism (when more than one accession per taxon): m (monomorphic), p (polymorphic)
[c] Number of alleles
[d] Allele size

**Table 3** Number of total alleles per locus for 15 *Olea* taxa, and specific alleles for species and accessions

| Locus | Allele size[a] | Total number of alleles | No. specific alleles | Accession-specific alleles | Taxon-specific alleles |
|---|---|---|---|---|---|
| IAS-oli11 | 114-127-128-129-131-133-**135**-136-**137**-139-142-**144**-145-150-151-153-**154**-158-159-**161**-163-171 | 22 | 15 | 127/E2; 128/H1; 129/H8; 131/B1; **135**/H1; 151/P3; 153/B2; **154**/H5; 158/I3; 159/H4; 163/I1; 171/I2 | 133/H4-H6; 136/H2-H3; **137**/H6-H8 |
| IAS-oli12 | 107-116-**120**-122-124-**126**-**128**-130-132-**134**-136-**138**-140-145-147-150-154 | 17 | 5 | 122/P1; 145/H6; 147/M1; 150/H3; 154/H2 | |
| IAS-oli17 | 139-148-152-156-161-163-165-**169**-171-**175**-177-**181**-182-186-188-230-232 | 17 | 12 | 149/H1; 152/P1; 161/P1; 165/P3; **175**/A2; 181/I3; 186/I3; 188/F1; 230/C2 | 139/L1-L2; 230/C1-C2; 232/L1-L2 |
| IAS-oli22 | 115-124-126-127-**128**-**130**-**132**-133-137-139-141 | 11 | 4 | 137/G1; 141/G1 | 133/I1-I2; 139/L1-L2 |

[a] Alleles shared with the *O. europaea* cultivars are indicated in bold

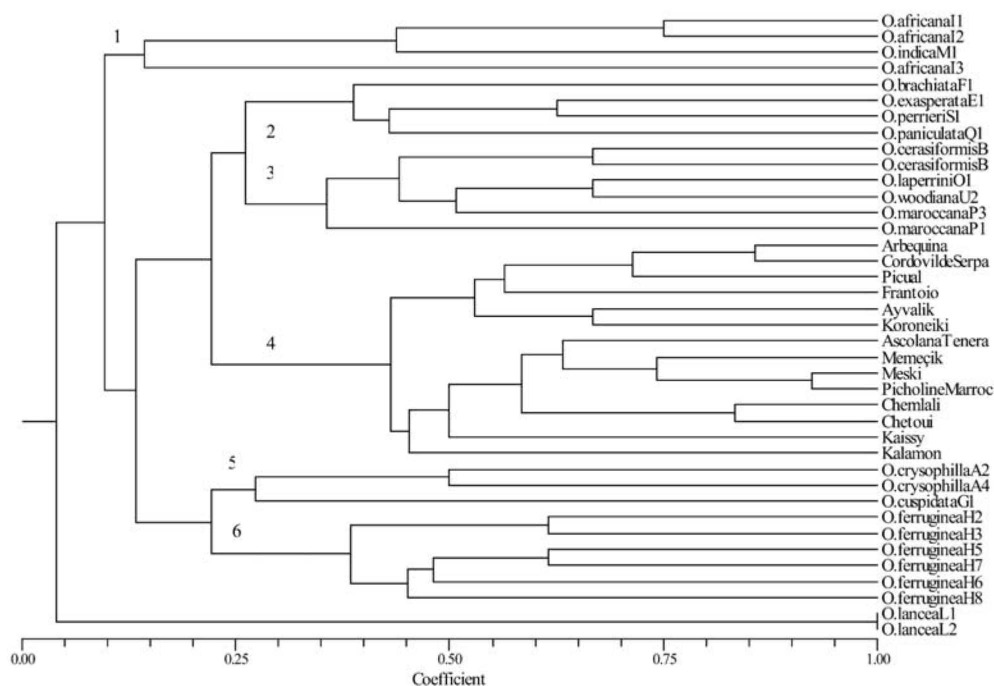


**Fig. 1** Dendrogram of *Olea* species and cultivars of *O. europaea*, based on the UPGMA methodology according to the Nei and Li's coefficient. *Numbers* in the dendrogram locate the different clusters

discrepancies with the expected relationships: *O. brachiata* and *O. paniculata*, both belonging to different subgenera, clustered together with the species *O. exasperata* and *O. perrieri*, belonging to the subgenus *Olea*, section *Ligustroides*, while the two samples of *O. lancea* showed the highest distance from the other taxa. The taxa *O. africana* and *O. indica* clustered separately from *O. chrysophylla, O. ferruginea* and *O. cuspidata*, although they all belong to subsp. *cuspidata*. *O. woodiana* (Section *Ligustroides*) grouped with *O. laperrinei*, a subspecies of *O. europaea*.

Geographic origin seems to be also influencing the clustering of the different species. A clear separation of the African species (groups 1, 2 and 3 in Fig. 1) from the taxa with an Asian origin (groups 5 and 6 in Fig. 1) can be observed, with the exception of *O. brachiata* and *O. paniculata*, included in the first group. Geography has clearly determined also the grouping of the three African accessions diffused in Kenya (I1, I2 and M1), as well as the association of species from the Canary Islands (*O. cerasiformis*) with *O. maroccana* (accessions coming from north west Morocco) and *O. laperrinei* (from Algeria).

The closest group to olive cultivars seems to be a heterogeneous cluster of species with very different origins, where some north African species (*O. laperrinei, O. maroccana* and *O. cerasiformis*) are included.

Within the group of cultivars the similarity between Chemlali and Chetoui confirms previous results obtained by the analysis with RAPDs (Belaj et al. 2002).

## Discussion

*Olea* is considered to be a rich genus including numerous species and subspecies, according to the recent revisions of the taxonomy (Green and Wickens 1989; Vargas et al. 2001). In the past years, several molecular studies, including AFLPs, RAPDs, ISSRs, repetitive DNA sequence analysis and chloroplast and mitochondrial DNA polymorphism, have also contributed to elucidate the classification of the *Olea* complex and the origin of cultivated olive (Angiolillo et al. 1999; Amane et al. 2000; Besnard and Bervillé 2000; Hess et al. 2000; Vargas and Kadereit 2001; Besnard et al. 2002b, c; Contento et al. 2002).

Microsatellite markers have a high potential for genetic studies at varietal, species and genus level, thanks to the high conservation of the flanking regions. A previous report of SSR transferability within the Oleaceae family, performed by Lefort et al. (1999), has demonstrated that SSR loci developed for *Fraxinus* were present in many taxa within the Oleaceae family. Nevertheless, only monomorphic products within *O. europaea* were obtained from the amplification of two transferable *Fraxinus* loci (unpublished data). More recently, some *Olea* species have been analyzed with two cpDNA microsatellites highly conserved in dicotyledonous angiosperms (Besnard et al. 2002a). It has also been demonstrated in other species that the frequency of insertion/deletions and base substitutions raises as the genetic distance between samples increases (Chen et al. 2002).

In our work, interspecific transferability of olive microsatellites, previously selected to discriminate *O. europaea* cultivars, has been tested within the genus *Olea*. Thirty two accessions belonging to 15 different *Olea* species have been studied. Positive amplification results at all four loci were obtained in 86% of the taxa analyzed (at least one accession per species). Nevertheless, in certain accessions of the taxa *O. capensis, O. exasperata* and *O. ferruginea* the resulting bands were weak and not clear, due in part to the appearance of multiple products.

Olive SSRs have been very polymorphic among the *Olea* taxa. Sixty seven different alleles have been observed for the four loci, most of them not shared with olive samples. In fact, over 50% of alleles unique to accessions or to species have been obtained.

Locus IAS-oli11, when used to amplify taxa quite distant from *O. europaea*, such as those belonging to different subgenera (*O. brachiata, O. paniculata*), has shown clear products with alleles common to *O. europaea*, while, when amplifying different taxa within the subgenus *Olea* (*O. capensis, O. exasperata, O. lancea, O. perrieri* and *O. woodiana*) or belonging to the same *O. europaea* species, it has produced a very high polymorphism, multiple alleles or unclear amplifications. Similar results were also detected for loci IAS-oli17 and IAS-oli22, even if they showed a lower level of polymorphism. On the contrary, locus IAS-oli12 showed amplifications more consistent with the taxonomy of the genus, with a higher polymorphism when amplifying distant taxa.

These results suggest very active mutational processes on the generation of new alleles along evolution as pointed out by Lehmann et al. (1996). Moreover, allelic size at a single locus among, and even within, species is not in accordance with a step-wise mutation model, indicating that both changes in the repeat number and in the flanking region of microsatellites are sources of variation.

Multiple amplification products were observed in the species *O. capensis, O. exasperata, O. ferruginea, O. maroccana, O. perrieri* and *O. woodiana*. The allopolyploid origin of the Oleaceae family pointed out by Stebbins (1971), could be the explanation for this phenomenon, indicating multiple priming sites along the *Olea* genome. However, while some multiple alleles were clear and unambiguous, in many cases no clear amplification products have been detected (especially at locus IAS-oli11 and when amplifying the *O. capensis* taxon) and, therefore, multiple products might be rather an effect of the unspecific priming site than a consequence of the ploidy level.

The dendrogram obtained by the analysis of these SSR data (Fig. 1), being partially consistent with the relationships within the *Olea* genus previously established by other authors, suggests that different SSR loci have evolved differently, affecting their ability to represent the real genetic differences, and suggesting that very good attention should be taken in the choice of the SSR loci for their application to establish genetic relationships at the inter-species level. In fact the electromorph size-polymorphism may be considered as an adequate measure of the genetic difference when closely related individuals are analyzed, but when the phylogenetic distances increase other variations, affecting the SSR sequence and leading to misinterpretation of the results. Future sequencing may lead to more clear conclusions on the classification and evolution of allele size (Sefc et al. 1999; Billote et al. 2001; Shepherd et al. 2002).

In summary, although the use of SSR loci for phylogenetic studies has been discussed and doubted by some authors (Russell et al. 1997), the good transferability, high polymorphism and coherent results obtained with four SSRs, indicate the potentiality of these markers. Nevertheless, results should be completed with a higher number of SSRs, currently under development for olive varieties, and coupled with information obtained with other molecular markers. Furthermore SSR reliability when exploring relationships at the inter-species level is highly dependent on the locus analyzed. The transferability of olive SSR raises the usefulness of microsatellite markers, increasing the profitability of the development procedures, which are high-cost and laborious, and to fully exploit the advantages of transferable genetic maps.

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## References

Amane M, Ouazzani N, Lumaret R, Debain C (2000) Chloroplast-DNA variation in the wild and cultivated olives (*Olea europaea* L.) of Morocco. Euphytica 116:59–64

Angiolillo A, Mencuccini M, Baldoni L (1999) Olive genetic diversity assessed using amplified fragment length polymorphism. Theor Appl Genet 98:411–421

Belaj A, Trujillo I, de la Rosa R, Rallo L, Giménez MJ (2001) Polymorphism and discriminating capacity of randomly amplified polymorphic markers in an olive germplasm bank. J Am Soc Hort Sci 126:64–71

Belaj A, Satovic Z, Rallo L, Trujillo I (2002) Genetic diversity and relationships in olive (*Olea europaea* L.) germplasm collections as determined by randomly amplified polymorphic DNA. Theor Appl Genet 105:638–644

Besnard G, Berville A (2000) Multiple origins for Mediterranean olive (*Olea europaea* L. ssp. *europaea*) based upon mitochondrial DNA plymorphisms. Life Sci 323:173–181

Besnard G, Khadari B, Baradat P, Bervillé A (2002a) *Olea europaea* (Oleaceae) phylogeography based on chloroplast DNA polymorphism. Theor Appl Genet 104:1353–1361

Besnard G, Khadari B, Baradat P, Bervillé A (2002b) 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

Besnard G, Green PS, Berville A (2002c) The genus *Olea*: molecular approaches of its structure and relationships to other Oleaceae. Acta Bot Gallica 149:49–66

Billote N, Risterucci AM, Barcelos E, Noyer JL, Amblard P, Baurens FC (2001) Development, characterization, and across-taxa utility of oil palm (*Elaeis guineensis* Jacq.) microsatellite markers. Genome 44:413–425

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

Chen X, Cho YG, McCouch SR (2002) Sequence divergence of rice microsatellites in *Oryza* and other plant species. Mol Genet Genomics 268:331–343

Cipriani G, Lot G, Huang WG, Marrazzo MT, Peterlunger E, Testolin R (1999) AC/GT and AG/CT microsatellite repeats in peach [*Prunus persica* (L) Batsch]: isolation, chacterisation and cross-species amplification in *Prunus*. Theor Appl Genet 99:65–72

Cipriani G, Marrazzo MT, Marconi R, Cimato A, Testolin R (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

Contento A, Ceccarelli M, Gelati MT, Maggini F, Baldoni L, Cionini PG (2002) Diversity of *Olea* genotypes and the origin of cultivated olives. Theor Appl Genet 104:1229–1238

Dirlewanger E, Cosson P, Tavaud M, Aranzana MJ, Poizat C, Zanetto A, Arús P, Laigret F (2002) Development of microsatellite markers in peach [*Prunus persica* (L.) Batsch] and their use in genetic diversity analysis in peach and sweet cherry (*Prunus avium* L.). Theor Appl Genet 105:127–138

Green PS, Wickens GE (1989) The *Olea europaea* complex. In: Kit T (ed) The Davis and Hedge Festschrift, Edinburgh University Press, Edinburgh, pp 287–299

Hess J, Kadereit JW, Vargas P (2000) The colonization history of *Olea europaea* L. in Macaronesia based on internal transcribed spacer-1 (Its-1) sequences, randomly amplified polymorphic DNAs (RAPD), and intersimple sequence repeats (ISSRs). Mol Ecol 9:857–868

Hodgetts RB, Aleksiuk MA, Brown A, Clarke C, Macdonald E, Nadeem S, Khasa D (2001) Development of microsatellite markers for white spruce (*Picea glauca*) and related species. Theor Appl Genet 102:1252–1258

Kijas JMH, Fowler JCS, Thomas MR (1995) An evaluation of sequence tagged microsatellite site markers for genetic analysis within Citrus and related species. Genome 38:349–355

Lefort F, Brachet S, Frascaria-Lacoste N, Edwards KJ, Douglas GC (1999) Identification and characterization of microsatellite loci in ash (*Fraxinus excelsior* L.) and their conservation in the olive family (Oleaceae). Mol Ecol 8:1075–1092

Lehmann T, Hawley WA, Collins FH (1996) An evaluation of evolutionary constraints on microsatellite loci using null alleles. Genetics 144:1155–1163

Medail F, Quezel P, Besnard G, Khadari B (2001) Systematics, ecology and phylogeographic significance of *Olea europaea* L. ssp. *maroccana* (Greuter & Burdet) P. Vargas et al. a relictual olive tree in south-west Morocco. Bot J Linnean Soc 137:249–266

Nei M, Li W (1979) Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc Natl Acad Sci USA 76:5269–5273

Rallo P, Dorado G, Martin A (2000) Development of simple sequence repeats in olive tree (*Olea europaea* L.). Theor Appl Genet 101:984–989

Rohlf FJ (1997) NTSYS-PC: numerical taxonomy and multivariate analysis system. Exeter software, New York

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

Sefc KM, Regner F, Turetschek E, Glössl J, Steinkellner H (1999) Identification of microsatellite sequences in *Vitis riparia* and their applicability for genotyping of different *Vitis* species. Genome 42:367–373

Sefc KM, Lopes MS, Mendonça D, Rodrigues Dos Santos M, Laimer da Camara Machado M, Da Camara Machado A (2000) Identification of microsatellite loci in olive (*Olea europaea*) and their characterization in Italian and Iberian olive trees. Mol Ecol 9:1171–1173

Shepherd M, Cross M, Maguire TL, Dieters MJ, Williams CG, Henry RJ (2002) Transpecific microsatellites for hard pines. Theor Appl Genet 104:819–827

Smulders MJM, Bredemeijer G, Rus-Kortekaas W, Arens P, Vosman B (1997) Use of short microsatellites to generate polymorphisms among *Lycopersicon esculentum* cultivars and accessions of other *Lycopersicon* species. Theor Appl Genet 94:264–272

Stebbins GL (1971) Chromosomal evolution in higher plants. In: Barrington EJW, Willis AJ (eds) Contemporary biology. Reading, Massachusetts

Szewc-McFadden AK, Kresovich S, Bliek SM, Mitchell SE, McFerson JR (1996) Identification of polymorphic, conserved simple sequence repeats (SSRs) in cultivated *Brassica* species. Theor Appl Genet 93:534–538

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

Vargas P, Munoz Garmendia F, Hess J, Kadereit J (2001) *Olea europaea* subsp. *guanchica* and subsp. *maroccana* (Oleaceae), two new names for olive tree relatives. Anal Jardin Bot Madrid 58:360–361

Wünsch A, Hormaza JI (2002) Molecular characterization of sweet cherry (*Prunus avium* L.) genotypes using peach [*Prunus persica* (L.) Batsch] SSR sequences. Heredity 89:56–63