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Olive genetic diversity assessed using amplified fragment length polymorphisms

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Abstract Amplified fragment length polymorphism (AFLP) analysis was used to study the genetic variation within and among populations of genus *Olea*. A group of genotypes, all of them cultivated varieties of a single species, *Olea europaea*, was compared with wild olives and with a group of individuals belonging to different *Olea* species. Five primer combinations were used which produced about 290 polymorphic bands. The data obtained were elaborated with the Nei's genetic similarity coefficient, applying different clustering methods and the Principal Coordinate Analysis. Cultivars, wild olives and North-West African species formed groups clustering together at a similarity level of 0.56, while the *Olea* species from East Africa and Asia grouped separately. Species from the Indian Ocean and Australia showed the highest diversity. We hypothesize that cultivars and wild plants are different forms of the same *O. europaea* species. The *Olea* from East Africa and Asia may be assigned to a different species, while the role of *O. laperrini* as well as that of *O. maroccana* as an intermediary form is confirmed.

Key words AFLPs · Diversity · Phylogeny · *Olea europaea*

Introduction

Olive (*Olea europaea* L.) is an important oil-producing crop which can be found throughout the Mediterranean basin. It is a long-living diploid (2n = 46) tree with a large number of varieties, most of which are self-incompatible. Unlike other crops, olive germplasm has not suffered any genetic erosion because turnover with new genotypes has not occurred and old plants are able to survive for a long time without cultivation. Therefore, its entire variability has been preserved until now without it having been either studied or exploited. Olive cultivation has a very long history which started from the Third Millennium B.C. (Loukas and Krimbas 1983) in the eastern region of the Mediterranean sea and spread later around the basin following land and maritime routes to Italy, Spain, North Africa and France.

According to Chevalier (1948) the cultivated olive is of Asian origin and derived from the selection of large-fruited forms of *O. chrysophylla* or from the hybridization of this species with others which have since disappeared. Ciferri (1950) confirmed the second hypothesis but considered *O. ferruginea* to be the ancestor species.

Found in the same range as the domesticated olive, wild plants are present in the maquis and in uncultivated areas and show some morphological differences with cultivars, such as a smaller fruit size and a lower oil content in the mesocarp (Terral 1996; Liphschitz et al. 1991). Two distinct wild olives have been recognized: *oleaster* and feral forms. *Oleaster* occupies primary niches in undisturbed areas (Liphschitz et al. 1991) as a constituent of evergreen plant associations. Despite its uncertain origins and genetic relationship to the olive cultivars, it has been considered to be a different form within the same *O. europaea* L., either as a subspecies (subsp. *oleaster* (Hoffm. & Link) Hegi) or a variety [var 'sylvestris' (Mill.) Lehr. = var 'oleaster' (Hoffm. & Link) DC.] (Zohary and Hopf 1994), and it should be regarded as the wild stock from which the cultivated fruit tree derived. Feral forms occur in secondary habitats such as disturbed areas or abandoned fields. They have been considered to be non-cultivated ecotypes of *O. europaea*, kept in a continuous juvenile

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stage by grazing (Rugini and Lavee 1992), derived from grafting stocks (Zohary and Spiegel-Roy 1975) or segregating seedlings of cultivated clones. Other authors (Zohary and Hopf 1994) suggest that they are products of hybridization between cultivated clones and adjacent wild *oleasters*, and that their 'wild' characteristics are a result of the segregation of highly heterozygous subjects and the absence of agronomical cares such as fertilization and irrigation, practices which have a great influence on increasing fruit size. Wild olives everywhere have the same chromosomal number as cultivars, are completely interfertile and show a good grafting affinity (Zohary and Spiegel-Roy 1975).

Besides the high variability within *O. europaea* species, all of the genus *Olea* is particularly rich and more than 100 species have been classified. Recently, a general revision of the genus taxonomy was proposed, and the number of species was drastically reduced (Mazzolani and Altamura Betti 1978, 1981; Altamura et al. 1987). A further step was taken by Green and Wickens (1989) who, on the basis of morphological, karyological, anatomical, palynological and biochemical evidence, included most of the species of the *Olea* complex within the *O. europaea* species. Nevertheless, Zohary (1994) stated that the geographical isolation of *O. africana*, *O. chrysophylla* and *O. ferruginea* and their morphological differences from *O. europaea* fully justify their classification as independent species. Loukas and Krimbas (1983) stated that *O. laperrini* represents an intermediary form between *O. europaea* and *O. chrysophylla*.

Up to now, the variability of the *Olea* germplasm has only been described in terms of morphology and agronomical behavior; biochemical and molecular analyses were recently carried out using isozymes (Ouazzani et al. 1993; Trujillo and Rallo 1995) and random amplified polymorphic DNA (RAPD) (Fabbri et al. 1995), but these were mainly aimed at cultivar identification. The relationships among cultivated olive, wild forms and related species need to be extensively explored. A better understanding of the genetic structure of wild populations and related species represents a first step towards answering numerous important questions such as the following. How many forms of olive deserve to maintain the rank of species? Which of them have contributed to the domestication of varieties? Which wild populations are truly distinct from one another and merit further study of their potential in order to improve the cultivated olive? In the study presented here AFLP (amplified fragment length polymorphism) analysis was used to establish the relationships among related species and both cultivated and wild forms of olive, and to evaluate their genetic distances.

AFLP markers, recently developed by Vos et al. (1995), have been widely employed because of their effectiveness and reliability (Lu et al. 1996; Prabhu and Gresshoff 1994). Studies on genetic relationships and evolution have already been carried out with AFLPs in numerous crop plants like soybean (Maughan et al. 1996), lettuce (Hill et al. 1996), wild bean (Tohme et al. 1996), lentil (Sharma et al. 1996), peanut (He and Prakash 1997), tea (Paul et al. 1997), einkorn wheat (Heun et al. 1997), sunflower (Hongtrakul et al. 1997) and potato (Milbourne et al. 1997).

Materials and methods

Plant material

A group of 43 olive varieties, 30 wild olives and nine *Olea* species was included in the screening (Table 1). In order to best represent the variability within the cultivated germplasm, we chose varieties from the most distant locations of the Mediterranean basin. Wild olives were collected in Sicily because of its geographic position in the center of the Mediterranean basin. Two locations were chosen on the opposite sides of the island, very close to cultivated areas, so that they could be considered as disturbed areas where only feral olive plants are supposed to be present. One sample of wild olive classified as *O. europaea* var 'sylvestris' from the Balearic Islands was also included in this group. To understand the relationships between wild and cultivated olives living in the same ecosystem we also considered a group of cultivars typical of Sicily. The *Olea* species were included in the study because of both their hypothetical relationships to the cultivated olive and their geographic origin. Three individuals for *O. maroccana*, *O. chrysophylla* and the Iranian *O. ferruginea*, two individuals for *O. africana* and one for each of the other species were sampled. The material was kindly provided by the Olive Germplasm Bank of Cordoba (Spain), the Laboratoire d'Arboriculture Fruitiere, INRA, of Montpellier (France), the Kew Living Collection of London (UK) and the collection of the Olive Research Institute, CNR, Perugia (Italy).

DNA extraction

Genomic DNA was extracted from fresh leaves according to Saghai-Maroof et al. (1984) with the following modifications. Five to six grams of ground leaves were incubated with 2 × CTAB buffer for 1 h at 65°C. Chloroform extraction was repeated twice, and RNA was removed from the aqueous solution by treatment with RNase (10 μg/μl) for 1 h. After the isopropanol/ethanol precipitations DNA was resuspended in TE buffer. About 20 μg DNA per gram of fresh tissue was obtained.

AFLP analysis

The AFLP technique was carried out as described by Vos et al. (1995). Genomic DNA (0.5 μg) was double-digested using both *Eco*RI and *Mse*I enzymes, and adaptors were ligated to the obtained fragments. Five microliters of template DNA from a 1:1 diluted ligation mixture was used for PCR preamplification with primers carrying one selective nucleotide. Twenty cycles were carried out at 94°C for 30 s, 56°C for 60 s and 72°C for 60 s in a 480 DNA Thermal Cycler (Perkin Elmer). The preamplification products, diluted 1:10, were used as template for hot selective amplification. Primers with three selective nucleotides were used: four *Mse*I primers (M-CAC, M-CAA, M-CTG, M-CTT) and three *Eco*RI primers (E-AGC, E-ACT, E-AAC) (Table 2). *Eco*RI primers were end-labeled with γ-[^{33}P]-ATP, and the following PCR conditions were used: first cycle at 94°C for 30 s; 65°C for 30 s; 72°C for 60 s. The annealing temperature was then reduced every 3 cycles by 1°C and after 11 cycles it reached the optimal annealing temperature of 56°C.

Twenty-five additional cycles were done at these temperatures (94°C for 30 s; 56°C for 60 s; 72°C for 60 s) to complete the second amplification. To determine the size of the AFLP fragments, we used an AFLP DNA ladder ranging in length from 30 to 330 bp (Gibco-BRL). The hot-amplified products were run on a 6% polyacrylamide gel. The reproducibility of the AFLP fingerprints was assessed on three DNA samples by replicating the entire procedure starting from the original DNA for all the primer combinations.

Data analysis

AFLP polymorphic bands were scored as present (1) or absent (0) on autorads. Estimates of similarity among all genotypes were calculated according to the Nei and Li (1979) definition of similarity: $Sij = 2a/(2a + b + c)$, where Sij is the similarity between two individuals i and j, 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. The matrix of similarity was analyzed by the Unweighted Pair-Group Method (UPGMA) and the dendrogram was obtained using NTSYS-PC software, Version 1.80 (Rohlf 1993). Principal Coordinate Analysis (PCA) was performed using the SAS PRINCOMP procedure, release 6.12 (SAS Institute 1994). The Neighbor-Joining method and the Dollo parsimony criterion were applied to estimate phylogeny from the distance matrix and from the raw data, respectively, thus obtaining two unrooted trees. To this purpose, the NJ and DOLLOP tree-building methods from the PHYLIP Package (Felsenstein 1993) were used.

Table 1 Olive cultivars, wild plants and related species of *Olea* analyzed using AFLP markers

Country of cultivation	Cultivar	Abbreviation	Sources[a]
Italy	Ascolana Tenera	ATE	IRO
	Cassanese	CAS	IRO
	Cellina	CEL	IRO
	Dolce Agogia	DAG	IRO
	Frantoio	FRA	IRO
	Leccino	LEC	IRO
	Pendolino	PEN	IRO
	San Felice	SFE	IRO
Italy-Sicily	Biancolilla	BIA	IRO
	Giarraffa	GIA	IRO
	Moresca	MOR	IRO
	Ogliarola Messinese	OGM	IRO
	Nocellara del Belice	NOB	IRO
	Nocellara Etnea	NOE	IRO
	Passalunara	PAS	IRO
	Santagatese	SAN	IRO
	Tonda Iblea	TIB	IRO
	Zaituna	TUN	IRO
Spain	Arbequina	ARB	COGB
	Cornicabra	CRN	COGB
	Empeltre	EMP	COGB
	Lechin de Sevilla	LDS	COGB
	Picual	PIC	COGB
	Villalonga	VIL	COGB
France	Bouteillan	BOU	COGB
	Lucques	LUC	COGB
	Olivier	OLI	COGB
	Picholine	PCH	COGB
Greece	Kalamata	KAL	IRO
	Koroneiki	KOR	COGB
	Valanolia	VAL	COGB
Turkey	Domat	DOM	COGB
	Ayvalik	AYV	COGB
Syria	Kaissy	KAI	COGB
	Zaity	ZAI	COGB
Lebanon	Souri	SOU	COGB
Israel	Merhavia	MER	COGB
Egypt	Toffahi	TOF	COGB
Morocco	Picholine Marocaine	PHM	COGB
Tunisia	Chetoui	CHE	COGB
Algeria	Sigoise	SIG	COGB
Portugal	Galega	GAL	COGB
Croatia	Oblica	OBL	COGB

Wild Olives	Origin	Sources[a]
M1-M17	Italy, Sicily, Trapani, Menfi	IRO
L1-L12	Italy, Sicily, Messina, Ali	IRO
Olea europea var 'sylvestris' Mill.	Balearic Islands	KEW

Table 1 Continued

Species	Origin	Diffusion area	Abbreviations	Sources[a]
Olea africana Mill.	Kenya	East Africa, South Arabia	afr1	KEW
Olea africana Mill.	Kenya	East Africa, South Arabia	afr2	INRA
Olea chrysophilla Lam.	Yemen	S-E Africa, Asia	chr1	INRA
Olea chrysophilla Lam.	Yemen	S-E Africa, Asia	chr2	INRA
Olea chrysophilla Lam.	Yemen	S-E Africa, Asia	chr3	INRA
Olea cuspidata Wall.	China	East Asia	cus	IRO
Olea ferruginea Royale	India	S-W Asia	fer1	IRO
Olea ferruginea Royale	Iran	S-W Asia	fer2	INRA
Olea ferruginea Royale	Iran	S-W Asia	fer3	INRA
Olea ferruginea Royale	Iran	S-W Asia	fer4	INRA
Olea indica Klein	Kenya	South Asia	ind	KEW
Olea lancea Lam	Mauritius Island	Indian Ocean	lan	KEW
Olea laperrini Batt. & Trab.	Algeria	Algeria	lap	INRA
Olea maroccana Gren. & B.	Morocco	Morocco	mar1	INRA
Olea maroccana Gren. & B.	Morocco	Morocco	mar2	INRA
Olea maroccana Gren. & B.	Morocco	Morocco	mar3	INRA
Olea paniculata R. Br.	Australia	Australia	pan	KEW

[a]IRO, Institute of Olive Research, CNR, Perugia, Italy; COGB, Olive Germplasm Bank, Cordoba, Spain; INRA, UR Genetique et Amelioration des Plantes, Montpellier, France; KEW, RGB Kew Living Collection, London, UK

Table 2 Oligonucleotide adaptors and primer combinations used for AFLP analysis

Name		Sequence
*Eco*RI adaptor		5′-CTCGTAGACTGCGTACC-3′ 3′-CTGACGCATGGTTAA-5′
*Mse*I adaptor		5′-GACGATGAGTCCTGAG-3′ 3′-TACTCAGGACTCAT-5′
Primers used in preamplification		
*Eco*RI + 1-A	E-A	5′-GACTGCGTACCAATTC + A-3′
*Mse*I + 1-C	M-C	5′-GATGAGTCCTGAGTAA + C-3′
Primer combinations used in selective AFLP amplification		
*Eco*RI + 3-AGC	E-AGC	5′-GACTGCGTACCAATTC + AGC-3′
*Mse*I + 3-CAC	M-CAC	5′-GATGAGTCCTGAGTAA + CAC-3′
*Eco*RI + 3-ACT	E-ACT	5′-GACTGCGTACCAATTC + ACT-3′
*Mse*I + 3-CAA	M-CAA	5′-GATGAGTCCTGAGTAA + CAA-3′
*Eco*RI + 3-AGC	E-AGC	5′-GACTGCGTACCAATTC + AGC-3′
*Mse*I + 3-CTG	M-CTG	5′-GATGAGTCCTGAGTAA + CTG-3′
*Eco*RI + 3-ACT	E-ACT	5′-GACTGCGTACCAATTC + ACT-3′
*Mse*I + 3-CAC	M-CAC	5′-GATGAGTCCTGAGTAA + CAC-3′
*Eco*RI + 3-AAC	E-AAC	5′-GACTGCGTACCAATTC + AAC-3′
*Mse*I + 3-CTT	M-CTT	5′-GATGAGTCCTGAGTAA + CTT-3′

Results

The AFLP fingerprinting (Fig. 1) of the 90 olive genotypes tested using five random primer combinations, EAGC/MCAC, EAGC/MCTG, EACT/MCAA, EACT/MCAC, EAAC/MCTT (Table 2), revealed a total number of 419 amplified DNA fragments ranging in length from 40 to 400 bp; 288 of them turned out to be polymorphic and were distributed across the entire lanes. The average percentage of polymorphism ranged from 51% for EACT/MCAA to 83% for EAGC/MCAC, and only 121 unambiguous bands were used for genetic analysis (Table 3). Some bands were specific to one group of genotypes: 2 bands were present in the cultivar and related species groups and absent in the wild one, while 2 others were specific to the wilds and related species; 1 band was only present in the related species, and another 1 was only present in the wilds.

Nei's genetic similarity estimated within the different groups (Table 4) showed the highest values in the cultivar group, ranging from 0.93 for the Sicilian varieties

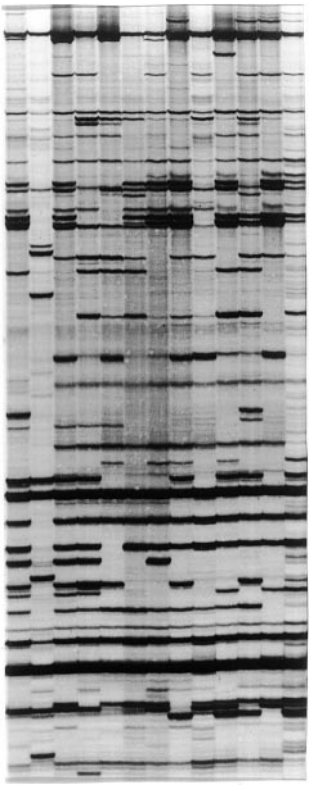


Fig. 1 Example of AFLP banding patterns in olive using the primer combination E-AGC/M-CTG

'Ogliarola Messinese' and 'Passalunara' to 0.53–0.58 between 'Sigoise' and a large group of varieties.

Similarities in the wild population ranged from 0.87 (L5-L11) to 0.51 (M13-L2). However the highest variability (0.15–0.85) was observed when the various species were compared. *O. lancea* had values of 0.15–0.21 when compared with most of the other species, and very low values were also observed among *O. paniculata* and *O. indica*, *O. africana*, *O. cuspidata*, *O. maroccana* (around 0.30). The greatest similarity was observed between *O. indica* and *O. africana* (0.85), between *O. laperrini* and *O. maroccana* (0.75), while *O. ferruginea* was about 0.70 with *O. cuspidata*, *O. chrysophylla* and *O. africana*. Very high similarity values were observed at the intra-species level in *O. maroccana* (0.93) and in the Iranian *O. ferruginea* (0.90) group.

When similarity between groups was compared (Table 4) the lowest values were obtained between wild olives and most of the species. *O. maroccana* was the most similar having 0.74 and 0.76 with M10 and M14, respectively. This species was also quite close to the cultivars' group, particularly to 'Lechin de Sevilla' (0.72) and to 'Arbequina' and 'Koroneiki' (0.69). On the contrary, *O. paniculata* and *O. lancea* values averaged 0.21–0.36 with most of the cultivars. Between wild olives and cultivars the values of the Balearic Islands' sample averaged 0.55, slightly higher values were found only with 'Koroneiki' (0.64) and 'Lechin de Sevilla' (0.63), while the wild olives from Sicily showed a wider range of values. The highest similarity was shown by M12 with most of the cultivars, ranging from 0.82 with 'Lechin de Sevilla' to 0.66 with 'Dolce Agogia'. M13 showed the lowest values (0.33–0.54). The UPGMA dendrogram, derived from the similarity matrix described above, showed three main distinct groups (Fig. 2). One of these included the species *O. cuspidata*, *O. ferruginea*, *O. chrysophylla*, *O. africana* and *O. indica*, (similarity of 0.58). The second one consisted of two subgroups: one containing the wild plants from Sicily and the *O. europaea* var 'sylvestris' (similarity 0.66) and the other, at a lower level of similarity (0.62), including the species *O. maroccana* and *O. laperrini*. The third cluster, showing a within-similarity of 0.70, included all the cultivars and only few wild genotypes (M7, M8, M12, M16). These four samples have shown a high morphological affinity with the cultivated genotypes in terms of leaf shape and size (data not shown). The species *O. paniculata* and *O. lancea* were excluded from these groups (similarity lower than 0.30). In the group of cultivars the varieties from Sicily clustered in a subgroup with a similarity higher than 0.75 which also included cvs 'Merhavia' from Israel and 'Zaity' from Syria. Cultivars 'Cellina' and 'Frantoio', sharing the same band pattern, were determined to be equal, while 'S. Felice' showed the lowest affinity with all the others.

The Principal Coordinate Analysis (Fig. 3), where the first two principal components accounted for 76.1% of the variance, was able to separate the different groups, supporting the results obtained with the cluster analysis. Wilds and cultivars remained clearly

Table 3 Polymorphim rates related to the five primer combinations

Primer combination	Total number of bands	Polymorphic bands	Polymorphism (%)	Scored bands
E-AGC/M-CAC	80	66	83	26
E-AGC/M-CTG	64	48	75	22
E-ACT/M-CAA	102	52	51	18
E-ACT/M-CAC	65	45	69	23
E-AAC/M-CTT	108	77	71	32
Total	419	Total 288	mean 68	Total 121

Table 4 Some values of genetic similarity (Nei and Li 1979) within and among the groups

Cultivars				Wilds				Related species			
Highest: 0.93		Lowest: 0.53–0.58		Highest: 0.87		Lowest: 0.51		Highest: 0.68–0.85		Lowest: 0.15–0.30	
Ogl. Messinese	Passalunara	Sigoise	Santagatese Zaity Moresca Cornicabra Merhavia Ogl. Messinese Zaituna Lucques Nocell. Etnea	L5	L11	M13	L2	*O. africana* *O. cuspidata* *O. Chrysophylla* *O. africana* *O. laperrini*	*O. indica* *O. ferruginea* *O. maroccana*	*O. paniculata* *O. lancea*	*O. indica* *O. africana* *O. cuspidata* *O. maroccana* Others
Related species-cultivars				Wilds-cultivars				Wilds-related species			
Highest: 0.60–0.72		Lowest: 0.21–0.36		Highest: 0.61–0.82		Lowest: 0.33–0.54		Highest: 0.74–0.76		Lowest: 0.21–0.23	
O. maroccana	Lechin Sev. Arbequina Koroneiki	*O. paniculata* *O. lancea*	Most of the cultivars	M12	Lechin Sev. Dolce Agogia	M13	Most of the cultivars	M10 M14	*O. maroccana*	L9 L4 M2	*O. paniculata*
O. ferruginea	Cornicabra			M8	Sigoise Ascolana						
O. chrysophilla	Zaituna Picholine Tonda Ibela Lucques				Tenera Picholine						
				O. europaea sylvestris	Koroneiky Lechin Sev.						

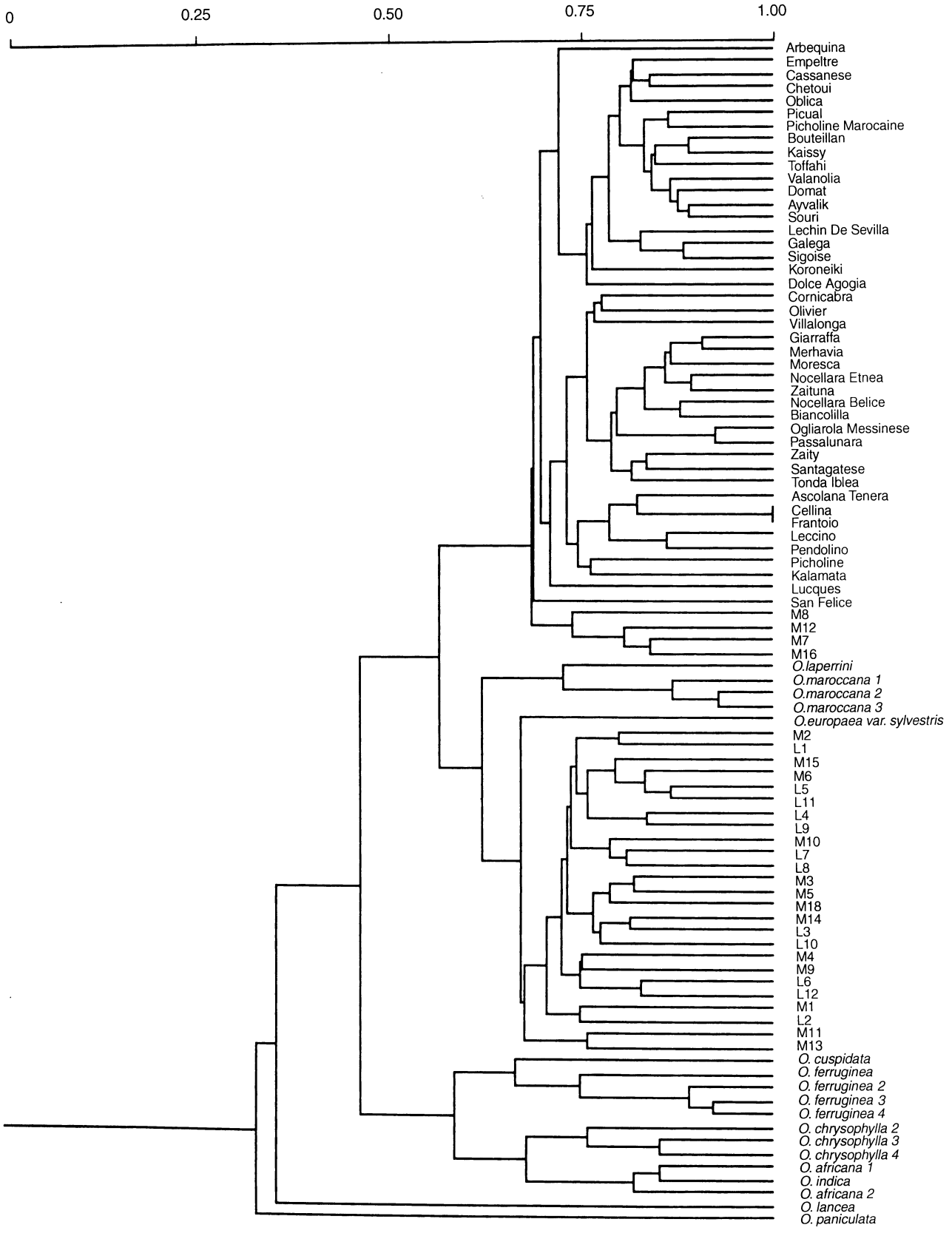


Fig. 2 Dendrogram of olive genotypes based on AFLP data using the Nei's genetic distance matrix of similarity and the UPGMA clustering method

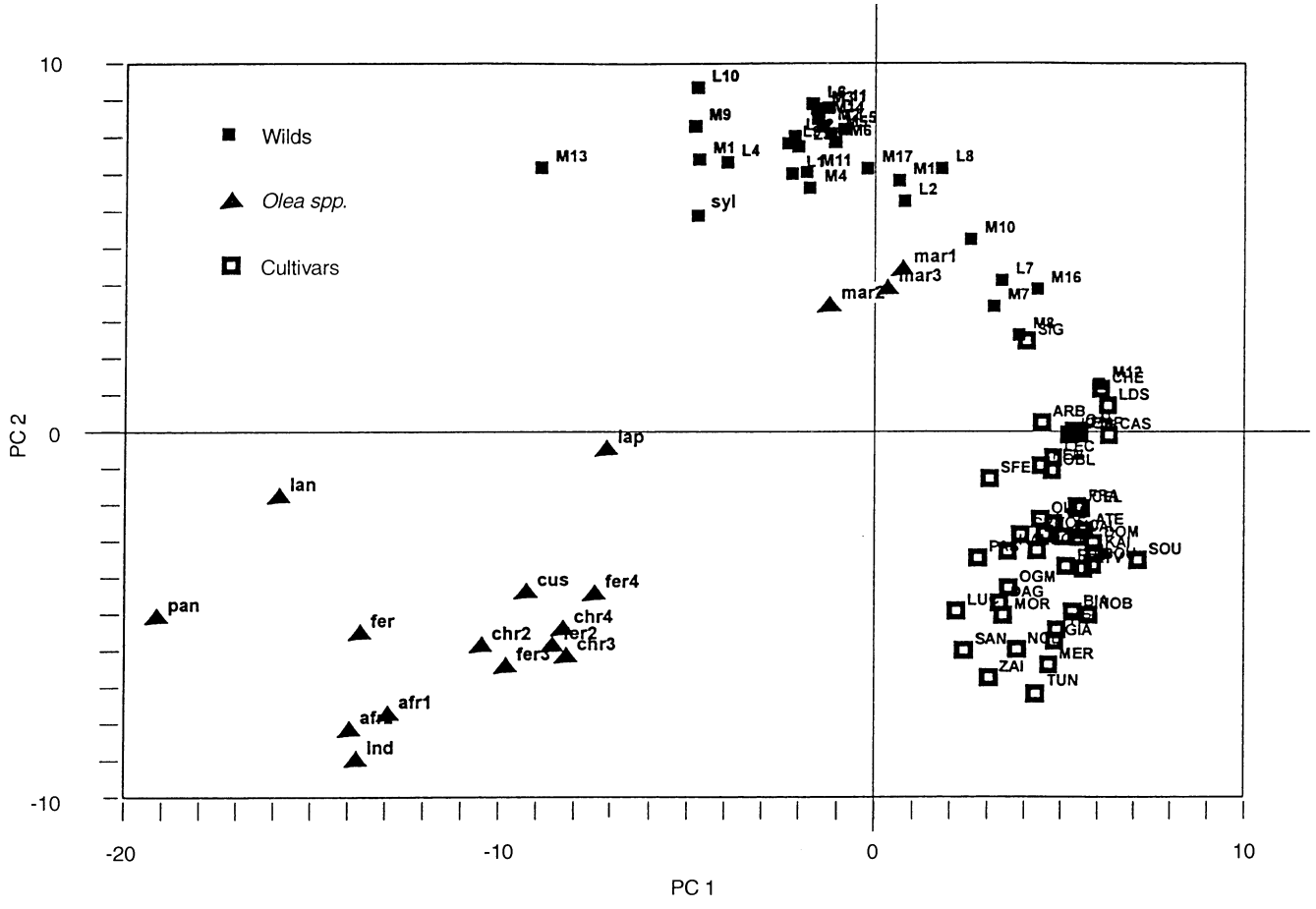


Fig. 3 Principal Coordinate plot of olive genotypes for the first and second principal coordinates estimated with 121 AFLP markers, using the genetic similarity matrix

separated and the M7, M8, M12, M16 samples were in an intermediate position together with cv 'Sigoise' and the wild L7.

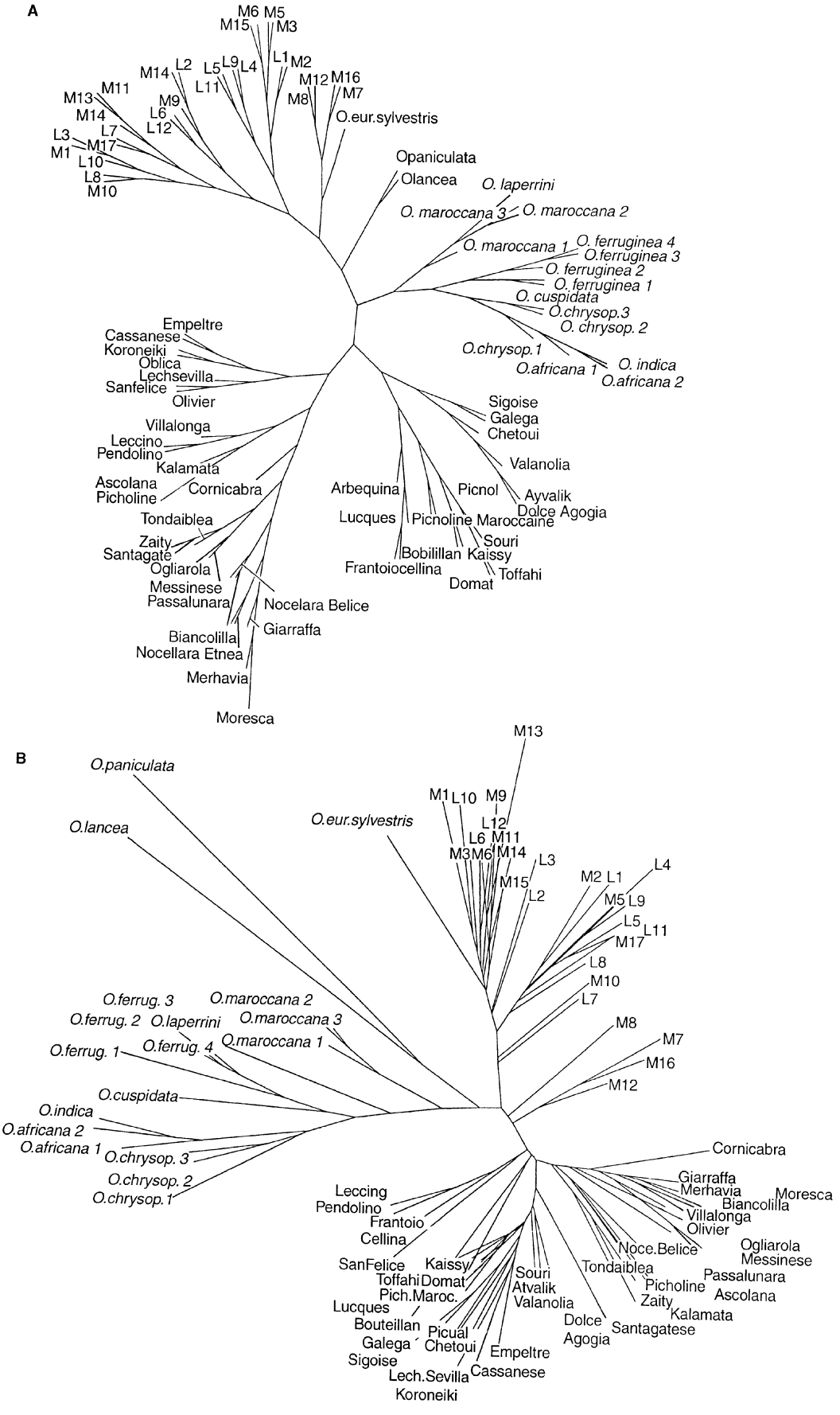
The unrooted trees produced using the Dollo and the Neighbor-Joining methods (Fig. 4) also separately grouped the cultivars, the wilds and the related species. The cluster of Sicilian cultivars was confirmed, while the other varieties assumed different positions within the group. In both trees *O. lancea* and *O. paniculata* were positioned between the wilds and the other species, contrasting with the UPGMA and PCA analyses. The Dollo program turned out to be slightly affected by the way and order of data entering, determining minor changes within each group.

Discussion

In this study a group of genotypes, all cultivated varieties of the species *O. europaea*, was compared by AFLP analysis with wild plants, whose genetic relationships with the cultivars are still highly controversial, and with a group of individuals belonging to hypothetically different species. The ability of AFLP to distinguish different levels of variability has been established in other taxa, such as lentil (Sharma et al. 1996) and lettuce (Hill et al. 1996) and has turned out to be a powerful tool for olive as well.

The AFLP analysis demonstrated that cultivated and wild olives separate into two clearly distinct groups. The level of genetic variability within the group of cultivars is similar to that observed in the one of the wilds. Among the cultivars studied, only those from Sicily showed high similarity and clustered together, while the other varieties did not show any particular affinity based on their area of cultivation. This is in agreement with the complexity of the history of olive domestication. Over a period of 5,000 years there was more likely an intense exchange of propagation material, such as cuttings, shions and ovules, all around the Mediterranean coasts than a unidirectional flux from East to West (Loukas and Krimbas 1983). As regards

Fig. 4A, B Unrooted trees generated using Dollo **A** and Neighbor-Joining **B** methods


A
M6
M5
M15
M3
L1
M2
M14
L2
L5
L9
L4
M12
M16
M11
L11
M8
M7
M13
M9
M14
L6
O.eur.sylvestris
L12
L3
L7
M17
M1
L10
L8
M10
Opaniculata
Olancea
O. laperrini
O. maroccana 3
O. maroccana 2
O. maroccana 1
O. ferruginea 4
O.ferruginea 3
O. ferruginea 2
O. ferruginea 1
O. cuspidata
O.chrysop.3
O. chrysop. 2
O.chrysop.1
O. indica
O.africana 1
O.africana 2
Empeltre
Cassanese
Koroneiki
Oblica
Lechsevilla
Sanfelice
Olivier
Sigoise
Galega
Chetoui
Villalonga
Leccino
Pendolino
Kalamata
Valanolia
Ascolana
Picholine
Cornicabra
Arbequina
Picnol
Ayvalik
Dolce Agogia
Tondaiblea
Lucques
Picnoline Maroccaine
Zaity
Souri
Santagate
Bobilillan
Kaissy
Ogliarola
Frantoiocellina
Toffahi
Messinese
Domat
Passalunara
Nocelara Belice
Giarraffa
Biancolilla
Nocellara Etnea
Merhavia
Moresca
B
M13
O.paniculata
M1
L10
M9
O.lancea
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L12
L6
M11
M3
M6
M14
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L4
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M2
L1
L2
M5
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L11
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L8
M10
L7
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O.maroccana 2
M8
O.ferrug. 2
O.laperrini
O.maroccana 3
M7
O.ferrug. 1
O.ferrug. 4
O.maroccana 1
M16
M12
O.cuspidata
O.indica
O.africana 2
O.africana 1
O.chrysop. 3
O.chrysop. 2
O.chrysop.1
Cornicabra
Giarraffa
Merhavia
Moresca
Leccing
Biancolilla
Pendolino
Villalonga
Frantoio
Olivier
Cellina
Noce.Belice
Ogliarola
Messinese
SanFelice
Kaissy
Tondaiblea
Souri
Passalunara
Toffahi
Domat
Atvalik
Picholine
Pich.Maroc.
Valanolia
Ascolana
Zaity
Lucques
Kalamata
Bouteillan
Dolce
Santagatese
Picual
Agogia
Galega
Chetoui
Empeltre
Sigoise
Cassanese
Lech.Sevilla
Koroneiki

wild olives, the supposed higher variability within this group compared with the one of the cultivars (Terral 1996) was not confirmed in our study. Despite the fact that the wild olives were collected in Sicily, they did not show a close relationship to the Sicilian cultivars, rather they were more similar to other varieties cultivated in distant locations. Moreover wild genotypes coming from the two sites on the island did not cluster separately. On the basis of these results it is possible to assign the wild olives included in this study to a form of *Olea europaea* which evolved separately from the cultivated varieties. Therefore, they should be considered as *oleasters*. Only a few feral plants (M7, M8, M12, M16) showed a greater similarity to the cultivars, comparable to that of the cultivars among themselves. It is possible to hypothesize for these plants an origin from the hybridization between oleasters and cultivars (Zohary and Hopf 1994) once diffused in the island. On the contrary, their origin as seedlings coming from the cross or the self-pollination of cultivated clones (Rugini and Lavee 1992) can be excluded because they share with the other wilds some peculiar bands totally absent in the cultivars. However, this evidence does not exclude that other plants not considered in this study but showing 'wild characters' could originate from cultivar dissemination.

With regard to the *Olea* species, a gradient of similarity was related to the geographic origin of the species: the closer to the Mediterranean area the higher was the affinity to the cultivated and wild forms of olive; the farther the origin the fewer the similarities.

The dendrogram and the PCA graph showed that the species from North-West Africa, *O. laperrini* and *O. maroccana*, have a closer affinity to the group of wild olives. This, however, was not confirmed by the Dollo and Neighbor-Joining analyses which placed them near to the other species. Our data did not support the hypothesis of Chevalier (1948) which assigned *O. laperrini* to a primitive cycle of *O. europaea*. *O. laperrini* and *O. maroccana* could instead represent different forms which evolved separately from the central Mediterranean area due to their geographical isolation in the Algerian Sahara and the Moroccan mountains, respectively.

The species diffused across East Africa and Asia (*O. ferruginea*, *O. chrysophylla*, *O. africana*, *O. cuspidata*, and *O. indica*), despite their diverse origin, had a high level of similarity comparable to that observed for the different forms of *O. europaea* (Fig. 2). This would justify the assignement of these five species to a single one, separated from *O. europaea*, as reported by Mazzolani and Altamura Betti (1978). It appears more difficult, instead, to justify the assignment of all these species to *O. europaea* subspecies *cuspidata*, as reported by Green and Wickens (1989).

Finally, our results confirm the great distance of the species *O. lancea* and *O. paniculata* from the others, which was already observed by Green and Wickens (1989) who assigned *O. lancea* to the Section *Ligustroides*.

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