Characterization and Identification of Spanish Olive Germplasm by Means of RAPD Markers

A. Belaj¹, I. Trujillo, D. Barranco, and L. Rallo

Departamento de Agronomía. ETSIAM. Universidad de Córdoba. Avenida Méndez Pidal s/n, Apdo 3048, 14080 Córdoba, Spain

Additional index words. cultivar identification, *Olea europaea* L., DNA fingerprinting

Abstract. Thirteen randomly amplified polymorphic DNA (RAPD) primers were assayed in 82 Spanish olive cultivars of economical interest. A total of 82 bands were scored giving an average of 6.3 bands per primer. A total of 4 (OPA-01) to 10 bands (OPA-19) was amplified, while the number of polymorphic fragments ranged from 2 (OPK-07) to 9 (OPA-19) with a mean of 5.7 polymorphic bands per primer. A total of 89% of the amplification products (73 bands) were polymorphic. The 13 primers yielded 184 banding patterns (14.9 per primer). The number of banding patterns per primer ranged from 4 (OPK-07) to 39 (OPA-19). Fifty-three unique banding patterns were found, the majority of them resulted from different combinations of polymorphic bands. The combination of only five primers OPA-19, OPF-06, OPX-01, OPX-03, and OPI-12, allowed identification of all the cultivars. Seventy-four cultivars (90%) were identified only by the combination of the first four primers. The addition of the fifth primer (OPI-12) was necessary for the identification of the eight remaining cultivars (10%). The ordination of the primers according to their practical discriminating capacity in this study was: OPA-19 > OPF-06 > OPX-01 > OPX-03 > OPI-12. Hence RAPD markers are recommended for olive fingerprinting in order to generate a database for olive cultivar identification.

The olive is a characteristic plant of the Mediterranean basin. Its outstanding economical importance is mainly due to the use of the olive oil as the principal source of edible oil for the people of this area for millennia. Spain is the world's leading olive producing country. As occurs in other olive producing countries, the distinctive feature of olive plant material in Spain is the abundance of very old cultivars restricted to specific areas where they originally grew (Barranco, 1997).

In an exploration survey of olive cultivars in Spain, carried out between 1972 and 1992, 262 different cultivars were identified (Barranco and Rallo, 2000). These have been conserved in the World Olive Germplasm Bank of the CIFA "Alameda del Obispo", Cordoba, Spain. The homonyms and synonyms have usually been troublesome in cultivar identification and management of olive genetic resources as names traditionally reflect common morphological traits (particularly of the fruit), place of origin or practical utility of the cultivars (Barranco and Rallo, 1984).

The high diversity of cultivars and the

confusion in naming require precise methods of discrimination for cultivar identification. Precise and rapid cultivar identification methods are also required in the management of cultivar collections as well as in olive breeding programs. Furthermore, cultivar identification represents a very important aspect for plant certification schedules in nursery industry.

Morphological data are the principal descriptors that have been used to detail the variability found in Spanish olive germplasm (Barranco, 1997; Barranco and Rallo, 1984; Barranco et al., 2000). Although these markers provide a very useful tool for identification, they are generally influenced by environmental factors and require a lengthy and expensive evaluation during the whole vegetative growth period. Enzyme markers have provided an alternative means of olive cultivar identification (Trujillo et al., 1995), but the use of pollen samples limit collection to mature trees at the time of flowering.

The introduction of DNA based markers, provides an opportunity for genetic characterization that allows direct comparison of different genetic material independent of environmental influences. Among the different markers, randomly amplified polymorphic DNA (RAPDs) are well suited for fingerprinting genotypes because of their technical simplicity, speed and low cost (Williams et al., 1990). In olive these markers have been widely used as a means of cultivar identification and genetic variability studies (Belaj et al., 2001; Besnard et al., 2001a; 2001b; Fabbri et al., 1995; Sanz-Cortés et al., 2001; Wiesman et al., 1998).

The present study reports use of RAPD markers on a set of 82 Spanish olive cultivars.

Among them, outstanding productive cultivars such as 'Picual', 'Arbequina', 'Manzanilla de Sevilla', 'Empeltre', 'Blanqueta', 'Hojiblanca', 'Morisca', and 'Villalonga' (Barranco and Rallo, 2000) were included. Furthermore, the cultivars 'Picual' and 'Arbequina' have been used in an intraspecific breeding program carried out by the Dept. of Agronomy at Cordoba Univ. Our objective was to describe the RAPD diversity among the Spanish olive cultivars, and to determine if RAPD markers can be used to accurately discriminate among the cultivars.

Material and Methods

Plant material and DNA extraction.

Eighty-two Spanish olive cultivars from the World Germplasm Bank of the CIFA "Alameda del Obispo" in Cordoba, Spain, were studied (Table 1). These cultivars, chosen on the basis of their economical importance and geographical distribution (Barranco, 1997; Barranco and Rallo, 2000), were previously described by means of pomological characterization (Barranco and Trujillo, unpublished data). Genomic DNA was extracted from young leaf tissue collected in Spring 1999, following the method described by Belaj et al. (2001).

Polymerase chain reaction. Thirteen decamer oligonucleotides from kits A, F, I, J, K, P, Q, and X from Operon Technologies (Alameda, Calif.) were used for PCR amplifications. DNA was amplified in 20 µL reaction mixtures containing 20–40 ng of template DNA, 0.05 U polymerase *AmpliTag* DNA Stoffel fragment (Applied Biosystems, Foster City, Calif.), 0.75 mM each of dNTP (Roche, Basel, Switzerland), 20 µM of the primer, 25 mM MgCl₂, 50 mM KCl, and 10 mM Tris-HCl (pH 8.3). The reactions were performed in a thermal cycler (GeneAmp PCR System 9600, Applied Biosystems) programmed for: 1 cycle of 1 min at 94 °C followed by 40 cycles: of 20 s at 94 °C, 20 s at 35 °C, and 2 min at 72 °C, for denaturing, primer annealing, and extension, respectively. The last cycle was followed by incubation for 6 min at 72 °C. All the reactions were conducted three times using DNA of different extractions and different lots of the *AmpliTag* DNA polymerase.

Separation and visualisation of the amplification products. Amplification products were separated on polyacrylamide gels as they provide a higher resolution of the amplification fragments in comparison to agarose gels (Belaj et al., 2001). Gels of 18 × 16 cm containing 10% acrylamide, 0.126% piperazine diacrylamide crosslinker in 0.375 M Tris-HCl, pH 8.8, were separated in Tris glycine (0.025 M Tris, and 0.192 M glycine) at 30 mA per gel for 3.5 h. The DNA was visualized by silver staining as described by Bassam et al. (1991). Gels were photographed using a digital camera (Kodak DC 120, Eastman Kodak Co., Rochester, N. Y.). Molecular sizes of the amplification products were estimated using a 123-base pair (bp) DNA ladder from Sigma (St. Louis).

Data analysis. RAPD bands were scored as 1 (present) or 0 (absent) in a binary matrix

Received for publication 29 July 2002. Accepted for publication 17 Mar. 2003. Contribution from the Dept. of Agronomy, Univ. of Cordoba, Cordoba, Spain. Thanks are due to Carmen del Río and the World Olive Germplasm Bank of the CIFA "Alameda del Obispo", Cordoba, for the facilities created. This research was funded by the INIA (Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria del MAPA), Project CAO 98-001-C3-1 and by the European Union project (FAIR-CT95-0689). A.B. received a PhD grant from the Agencia Española de Cooperación Internacional (AECI), Spain.

¹To whom reprint requests should be sent. E-mail: ag2belaj@uco.es

Table 1. Cultivars analysed with their register number (RN) in the collection as well as the banding patterns obtained by the primers OPA-19, OPF-06, OPI-12, OPX-01, and OPX-03.

Cultivar	RN	OPA-19	OPF-06	OPI-12	OPX-01	OPX-03	Cultivar	RN	OPA-19	OPF-06	OPI-12	OPX-01	OPX-03
Alameño de Cabra	285	A ^a	A	A	A	A	Lechín de Sevilla ^a	5	P	V	K	G	K
Alameño de Montilla	39	B	B	B	B	B	Limoncillo	35	AB	---	I	A	A
Alfajara ^a	605	C	---	C	A	A	Llumeta	226	AG	U	L	v	P
Aloreña ^a	981	---	C	D	A	C	Loaime	414	Q	---	D	E	G
Amargoso	367	C	F	D	---	-	Lucio	763	R	J	D	A	H
Arbequina ^a	231	D	D	C	C	D	Manzanilla Cacereña ^a	430	A	---	E	A	H
Argudel	1068	E	-	E	D	E	Manzanilla del Centro	499	F	---	-	G	H
Asnal	437	AJ	G	L	B	A	Manzanilla de Hellín	801	B	K	B	E	H
Azul	343	F	B	F	E	B	Manzanilla de Jaén	56	F	Y	B	G	H
Bical	387	G	E	D	A	A	Manzanilla de Montefrío	401	AD	O	A	E	G
Blanquetav	11	AF	F	---	B	F	Manzanilla de Sevilla ^a	21	S	E	A	B	A
Bolvino	46	AH	Y	---	B	F	Manzanilla del Piquito	269	T	---	M	H	G
Buidiego	---	---	---	C	K	B	Manzanilla Prieta	360	M	B	L	E	G
Caballo	333	---	Z	D	J	B	Menya	1071	U	L	N	C	---
Callosina	40	O	G	C	A	G	Mollar de Cieza	612	C	K	D	A	A
Canetera	653	Z	H	F	F	C	Morisca ^a	17	V	G	B	C	A
Cañivano Blanco	52	AL	I	A	G	B	Morona	270	N	M	F	E	G
Cañivano Negro	259	-	M	---	E	B	Morrut ^a	224	Y	N	F	E	-
Carrasqueño Alcaudete	332	F	A	G	E	G	Negrillo de Estepa	301	A	Ñ	A	E	-
Castellana ^a	576	H	I	---	E	G	Nevado Basto	305	G	I	-	A	-
Corbella	645	-	O	---	A	H	Ocal	282	S	E	A	E	G
Cornezuelo de Jaén	20	I	B	D	B	H	Ojo de liebre	413	AI	B	C	A	A
Cornicabra ^a	10	I	M	H	G	B	Palomar	661	X	---	D	C	L
Curivell	1072	B	I	I	A	A	Pequeña de Casas Ibañez	562	---	---	A	I	E
Changlot Real ^a	15	G	B	J	E	G	Pico Limón	273	AD	I	G	A	A
Chesna	374	---	O	J	B	-	Picual ^a	9	AB	P	G	E	A
Chorrúo	290	---	G	D	A	H	Picual de Almería	798	T	B	---	G	A
Empeltre ^a	13	J	C	D	E	B	Picudo ^a	3	AM	---	B	E	A
Enaguas de Arenas	586	C	A	J	A	G	Picudo de Fruto Rojo	316	AH	B	B	B	-
Farga ^a	12	---	C	F	E	I	Rapasayo	277	E	Q	D	E	M
Gatuno	380	---	---	A	C	-	Royal de Calatayud	964	C	R	C	E	I
Genovesa	970	K	A	J	E	E	Royal de Cazorla	390	AC	AM	L	E	H
Gordal de Archidona	755	---	---	---	B	G	Sevillena ^a	227	C	E	D	E	G
Gordal de Granada	761	L	V	K	G	H	Vallesa	613	AK	S	E	E	N
Gordal de Hellín	804	---	A	J	A	B	Vera	660	---	---	K	A	---
Gordal Sevillana ^a	234	AM	---	C	G	E	Verdalón	585	C	B	D	A	A
Gordalejo	396	F	---	---	G	D	Verdial de Badajoz ^a	988	C	A	I	A	G
Hojiblanca ^a	2	B	B	K	E	B	Verdial de Huevar ^a	6	AE	I	---	G	G
Imperial	297	M	G	L	E	J	Verdial Vélez Málaga ^a	883	B	A	G	E	Ñ
Jabaluna	392	N	E	C	E	H	Verdiell	677	AN	S	M	E	Ñ
Lechín de Granada ^a	54	Ñ	A	G	E	A	Villalonga ^a	364	AD	T	---	E	O

^aMajor cultivars in Spain.

^bThe capital letters represent the banding patterns for each primer.

^cMissing data.

for each primer. A conservative criterion for the selection of bands was used. Only reproducible and well-defined bands in each of the three replications were considered as potential polymorphic markers. Banding patterns, i.e., the different combinations of bands obtained for each primer, were designated by the name of the respective primer followed by a capital letter (Table 1 and Fig. 1). The identification of olive cultivars was carried out by the combination of different primers.

Results

Genetic variability. A total of 82 bands were scored giving an average of 6.3 bands per primer. Four (OPA-01) to 10 bands (OPA-19) were amplified when the 13 primers were used with the DNA of 82 Spanish cultivars (Table 2). The total number of monomorphic bands was 9 (11%) with an average of 0.69 monomorphic bands per primer. The number of polymorphic fragments ranged from 2 (OPK-07) to 9 (OPA-19) with a mean of 5.7 polymorphic bands per primer. A total of 89% of the amplification products were polymor-

phic (73 bands).

High variability in the frequency of polymorphic bands in the cultivars studied was observed. This frequency varied from 0.012 (bands present in only one cultivar of the 82 studied) to 0.988 (bands absent in only one cultivar of the 82 studied). In general, the average frequency of the above-mentioned bands was high (0.50).

The 13 primers yielded 184 banding patterns (14.9 per primer). The number of banding patterns per primer ranged from 4 (OPK-07) to 39 (OPA-19). Fifty-three unique banding patterns were found (Table 3), the majority of them resulted from different combinations of polymorphic bands found and a very few were obtained from the unique RAPD bands.

Cultivar identification with RAPD markers. The variability found with the 13 primers used made possible the identification of all the cultivars included in the study. The combination of five primers OPA-19, OPF-06, OPX-01, OPX-03, and OPI-12 allowed identification of all the cultivars (Table 1). Other combinations of primers could provide additional possibilities for identification of the cultivars.

The minimum number of necessary primers for the identification of each one of the varieties (considering only the five above-mentioned primers) is presented in Table 3.

Thirty-eight cultivars (>45% of the total) were discriminated with a single primer as a result of the presence of 53 unique patterns for these cultivars (Table 3). Twelve out of the 24 major Spanish olive cultivars (Table 1) are included in this group. Furthermore, the cultivars 'Menya', 'Pequeña de Casas Ibañez', 'Canetera', and 'Manzanilla del Piquito' were identified by unique patterns obtained by presence or absence of unique bands.

For the identification of the rest of the varieties the combination of banding patterns obtained by two or three primers was needed (Table 3). For instance, 40 cultivars (48.7% of the genotypes) were discriminated by the combination of two primers. And the combination of patterns obtained by three primers was needed only for the identification of the four remaining cultivars (6.3%).

Twenty-three cultivars with the same generic name were also included in this study such as 'Alameño' (2), 'Cañivano' (2), 'Gordal'

Fig. 1. PCR-RAPD products and schematic representation of some of the banding patterns obtained with the primer OPA-19 (a, b). the selected amplification products are indicated with arrows. The letters at the bottom of the photographs correspond to the banding patterns (b). The letters MP indicate the molecular marker, the molecular weight of the selected bands is indicated on the Y-axis (a).

(4), 'Lechín' (2) 'Manzanilla' (8), 'Picual' (2) and 'Verdial' (3) (Table 1). These homonyms were clearly discriminated by means of RAPD markers.

The number of the cultivars discriminated by the cumulative power of the five primers was also calculated. In that way, twenty-three cultivars (28% of the cultivars studied) were discriminated using only the unique banding patterns obtained by the primer OPA-19 (Fig. 2). The percentage of the cultivars identi-

fied increased to 63.4% (a total of 52 cultivars) when the primer OPF-06 was added. And the number of the cultivars identified increased to 63 (76.8%) and to 74 (90%) respectively when a third (OPX-01) and a fourth (OPX-03) primer were further summed to the first two primers for the cultivar distinction. The addition of the fifth primer (OPI-12) was necessary for the identification of the 8 remaining varieties (10%). The ordination of the primers according to their practical discriminating capacity in

this study was: OPA-19 > OPF-06 > OPX-01 > OPX-03 > OPI-12.

Discussion

The olive tree (*Olea europaea* L.) is considered a highly heterozygous species, therefore the polymorphism found in this study is expected. The autochthonous origin and very low pressure of selection throughout history of olive cultivation have influenced to the high diversity of olive cultivars in Spain (Barranco and Rallo, 1984; 2000). In terms of identified polymorphisms, our results agree with previous studies obtained by means of RAPD markers in olive (Belaj et al., 2001; Besnard et al., 2001b; Sanz-Cortés et al., 2001). To our knowledge, this study is the most complete study of Spanish olive cultivars in terms of plant material and it also represents the first attempt to create a fingerprinting database by means of RAPD markers for these cultivars.

The identification of cultivars is a very important application of molecular markers in olive. Therefore the potential of RAPD markers to yield different banding patterns for as many cultivars as possible is of great interest. And selection of the most discriminating primers reduces the cost of analysis (i.e. the number of primers and thus the number of amplifications) for reliable cultivar distinction (Tessier et al., 1999).

The combination of only four primers (OPA-19, OPF-06, OPX-01, and OPX-03) made possible the distinction of 90% of the cultivars included in the study. The discrimination power of each primer depends not only on the number of banding patterns generated but also on their frequency. The last primer (OPI-12) was kept for its ability to discriminate the few nondistinguishable pairs of cultivars and not for its efficiency. Identification of the majority of the cultivars (93.7%) by means of unique banding patterns or different combinations of the banding patterns provided by two primers is clear evidence of the high discrimination capacity of RAPD markers. However, discrimination of some of the most important

Table 2. Primers used, the total number of bands (T), polymorphic bands (P) and the number of banding patterns (BP) obtained for each primer.

Primer ²	T	P	BP
OPA-01	4	4	6
OPA-03	6	4	11
OPA-19	10	9	39
OPF-06	7	7	25
OPI-12	6	5	13
OPI-14	6	6	12
OPJ-18	5	5	10
OPK-07	5	2	4
OPK-17	7	6	12
OPP-19	6	6	13
OPQ-15	6	6	14
OPX-01	8	7	11
OPX-03	6	6	17
Total: 13	82	73	184

²Information about the fingerprinting data obtained by each primer can be obtained from the authors upon request.

Fig. 2. Percentage of the cultivars discriminated by a certain number of primers combination. 1 = OPA-19; 1+2 = OPA-19+OPF-06; 1+2+3 = OPA-19+OPF-06+OPX-01; 1+2+3+4 = OPA-19+OPF-06+OPX-01+OPX-03; 1+2+3+4+5 = OPA-19+OPF-06+OPX-01+OPX-03+OPI-12.

Spanish olive cultivars by cultivar-specific RAPD banding patterns provides the nursery industry with a powerful tool to certify their plant material. Previous studies carried out in olive by means of RAPD markers have reported the utility of these markers for reliable identification of a relatively high number of cultivars using a small number of primers (Belaj et al., 2001; Besnard et al., 2001b; Sanz-Cortés et al., 2001, Belaj, unpublished data). However, RAPDs present limitations when interpreting the results. The lack of reproduc-

ibility of the technique requires the following of a well-established protocol for routine use in the laboratory as well as a conservative criteria at the selection of the amplification products (Belaj et al., 2001).

As in previous investigations carried out by means of morphological (Barranco, 1997; Barranco and Rallo, 1984) and DNA markers (Belaj et al., 2001; Mekuria et al., 1999; Wiesman et al., 1998), our results confirm the hypothesis that generic names such as 'Gordal', 'Lechín', 'Manzanilla', 'Verdial',

etc., include different cultivars. No differences were found among the amplification profiles obtained from individuals trees of the same cultivar (data not shown). These results suggest the usefulness of different markers for an effective conservation and use of olive genetic resources in germplasm banks. Despite their great power of discrimination, molecular techniques should not be considered as substitutes of morphological characterization, but as complementary tools for the study of genetic resources (Karp et al., 1997).

Table 3. Identification of cultivars by a minimum number of primers.

Cultivar name	Primer	Cultivar name	Primer
<i>Cultivars identified by unique banding patterns (one primer)</i>			
Arbequina	OPA-19 (D); OPF-06(D)	Cornezuelo de Jaen	OPA-19 (I) +OPF-06 (B); OPA-19 (I) + OPX-01 (B); OPA-19 (I) + OPX-03 (H); OPX-01 (B) + OPX-03 (H)
Argudel	OPX-01(D)	Curivell	OPA-19 (B) +OPF-06 (I); OPA-19 (B) + OPX-01 (A); OPA-19 (B) + OPX-03 (A); OPF-06 (I) + OPX-01 (A)
Asnal	OPA-19 (AJ)	Enaguas de Arenas	OPA-19 (C) +OPI-12 (J); OPF-06 (A) + OPI-12 (J); OPI-12 (J) + OPX-01 (A); OPI-12 (J) + OPX-03 (G)
Blanqueta	OPA-19 (AF); OPF-06 (F); OPX-03 (F)	Farga	OPF-06 (C) + OPX-03 (I); OPF-06 (C) + OPI-12 (F)
Bolvino	OPF-06(Y)	Gordal de Archidona	OPX-01 (B) + OPX-03 (G)
Buidiego	OPX-01 (K)	Gordal de Hellín	OPF-06 (A) + OPX-03 (B)
Caballo	OPF-06 (F)	Gordal Sevillana	OPA-19 (AM) + OPX-01 (G); OPA-19 (AM) + OPX-03 (E); OPX-01 (G) + OPX-03 (E)
Callosina	OPA-19 (O)	Gordalejo	OPX-01 (G) + OPX-03 (D)
Canetera	OPA-19 (Z); OPX-01 (F); OPF-06 (H)	Hojiblanca	OPA-19 (B) +OPI-12 (K); OPI-12 (K) + OPX-01 (E); OPI-12 (K) + OPX-03 (B)
Cañivano Blanco	OPA-19 (AL)	Jabaluna	OPA-19 (N) +OPF-06 (E); OPA-19 (N) + OPX-03 (H);
Castellana	OPA-19 (H)	Limoncillo	OPA-19 (AB) + OPX-01 (A); OPA-19 (AB) + OPI-12 (I)
Cornicabra	OPI-12 (H)	Manzanilla Cacereña	OPA-19 (A) + OPX-03 (H); OPA-19 (A) + OPI-12 (E)
Empeltre	OPA-19 (J)	Manzanilla de Hellín	OPA-19 (B) +OPF-06 (K); OPA-19 (B) + OPX-03 (H);
Genovesa	OPA-19 (K)	Manzanilla de Sevilla	OPA-19 (S) + OPX-01 (B); OPA-19 (S) + OPX-03 (A); OPX-01 (B) + OPX-03 (A)
Gordal de Granada	OPA-19 (L)	Manzanilla Prieta	OPA-19 (M) +OPF-06 (B); OPA-19 (M) + OPX-03 (G);
Imperial	OPX-03 (J)	Mollar de Cieza	OPA-19 (C) +OPF-06 (K)
Lechín de Granada	OPA-19 (Ñ)	Morona	OPA-19 (N) + OPF-06 (M)
Lechín de Sevilla	OPA-19 (P); OPX-03 (K)	Ocal	OPA-19 (S) + OPX-01 (E); OPA-19 (S) + OPX-03 (G)
Llumeta	OPA-19 (AG); OPF-06 (U); OPX-03 (P)	Pico Limón	OPA-19 (AD) +OPF-06 (I); OPA-19 (AD) + OPX-03 (A)
Loaime	OPA-19 (Q)	Picual de Almería	OPA-19 (T) +OPF-06 (B); OPA-19 (T) + OPX-01 (G); OPA-19 (T) + OPX-03 (A);
Lucio	OPA-19 (R); OPF-06 (J)	Picudo	OPA-19 (AM) + OPX-01 (E); OPA-19 (AM) + OPX-03 (A)
Manzanilla de Jaen	OPF-06 (Y)	Picudo de Fruto Rojo	OPA-19 (AH) + OPF-06 (B)
Manzanilla del Piquito	OPX-01(H)	Sevillencia	OPA-19 (C) + OPF-06 (E)
Menya	OPA-19 (U); OPF-06 (L); OPI-12 (N)	Vera	OPI-12 (K) + OPX-01 (A)
Morisca	OPA-19 (V)	Verdalón	OPA-19 (C) + OPF-06 (B); OPF-06 (B) + OPX-01 (A)
Morrut	OPF-06 (N)	Verdial de Badajoz	OPA-19 (C) +OPI-12 (I); OPF-06 (A) + OPI-12 (I); OPI-12 (I) + OPX-01 (A); OPI-12 (I) + OPX-03 (G)
Negrillo de Estepa	OPF-06 (Ñ)	Verdial Velez Malaga	OPA-19 (B) +OPF-06 (A); OPA-19 (B) + OPX-03 (Ñ); OPF-06 (A) + OPX-03 (Ñ)
Ojo de Liebre	OPA-19 (AI)	Total	40 cultivars
Palomar	OPA-19 (X); OPX-03 (L)	<i>Cultivars identified by the banding patterns obtained by three primer</i>	
Pequeña de Casas Ibañez	OPX-01 (I)	Alfajara	OPA-19 (A) + OPI-12 (I) + OPX-01 (A); OPA-19 (A) + OPX-03 (A) + OPX-01 (A)
Picual	OPF-06 (P)	Amargoso	OPA-19 (K) + OPF-06 (F) + OPI-12 (D)
Rapasayo	OPF-06 (Q); OPX-03 (M)	Cañivano Negro	OPF-06 (M) + OPX-01 (E) + OPX-03 (B)
Royal de Calatayud	OPF-06 (R)	Manzanilla del Centro	OPA-19 (F) + OPX-01 (H) + OPX-03 (G)
Royal de Cazorla	OPA-19 (AC)	Total	4 cultivars
Vallesa	OPA-19 (AK); OPX-03 (N)		
Verdial de Huelva	OPA-19 (AE)		
Verdiell	OPA-19 (AN)		
Villalonga	OPF-06 (T); OPX-03 (O)		
Total	38 cultivars		
<i>Cultivars identified by the banding patterns obtained by two primers</i>			
Alameño de Cabra	OPA-19 (A) +OPF-06 (A); OPA-19 (A) + OPX-03 (A)		
Alameño de Montilla	OPA-19 (B) + OPX-01 (B); OPX-01 (B) + OPX-03 (B)		
Aloreña	OPX-01 (A) + OPX-03 (C)		
Azul	OPA-19 (F) +OPF-06 (B); OPA-19 (F) + OPX-03 (B)		
Bical	OPA-19 (G) +OPF-06 (E); OPA-19 (G) + OPX-03 (B); OPF-06 (E) + OPX-01 (A)		
Carrasqueño Alcaudete	OPA-19 (F) +OPF-06 (A); OPA-19 (F) + OPX-03 (G)		
Changlot Real	OPA-19 (G) +OPF-06 (B); OPA-19 (G) + OPX-01 (E); OPA-19 (G) + OPX-03 (G)		
Chesna	OPF-06 (O) + OPX-01 (B)		
Chorro	OPF-06 (G) + OPX-03 (H)		
Corbella	OPF-06 (O) + OPX-01 (A); OPF-06 (E) + OPX-03 (H)		

The development in this research of RAPD markers/fingerprints of the main and industrially important olive cultivars from Spain may help for generating a molecular database to reliably catalogue the cultivars. The utilization of this information in a systematic way will facilitate the management of cultivar collections and control the trade of plant material. In order to complete and refine the RAPD data obtained in this study, further studies with larger olive samples and other types of molecular markers, which can permit the interchange of data between different scientific groups and laboratories, SSRs, (de la Rosa et al., 2002; Rallo et al., 2000) are needed. SSRs are currently being used for the characterization and identification of the Germplasm Bank of Cordoba.

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