Are ‘Oblonga’ and ‘Frantoio’ Olives the Same Cultivar?

Diego Barranco and Isabel Trujillo

Departamento de Agronomía, Universidad de Córdoba, Apdo. 3048, 14080 Córdoba, Spain

Pilar Rallo

Instituto de Agricultura Sostenible, Apdo. 4084, 14080 Córdoba, Spain

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Abstract. The cultivar Oblonga may have originated from a volunteer seedling at an orchard near Corning, Calif., about 1940. Its main virtue is its high degree of resistance to *Verticillium dahliae*. ‘Frantoio’ is the main variety in Italy and has been planted worldwide because of its high content of top-quality oil. In the present study, we show that both cultivars have the same fifteen morphological and eight agronomical traits and both have amplified the same patterns for 22 RAPD primers and five SSRs. This indicates that ‘Oblonga’ and ‘Frantoio’ are probably the same cultivar.

The cultivated olive is characterized by the existence of numerous cultivars that were selected hundreds of years ago in small regional areas. Only a few of these cultivars have become widely planted in other areas (Barranco, 1995). In general, confusion exists regarding the names of cultivars in all olive-producing countries because these names were chosen by the growers who selected the cultivars. As a consequence, either the same name has been given to different cultivars or the same cultivar may appear with different names in different regions or countries (synonyms). This confusion extends to the many cultivar collections around the world, because of the absence or insufficiency of the identification systems. The Dept. of Agronomy of the Univ. of Córdoba uses morphological and biochemical (isoenzymes and DNA) identification methods to resolve this problem (Barranco and Rallo, 2000).

The cultivar Oblonga, whose name refers to the shape of the fruit, might have originated from a volunteer seedling at an orchard near Corning, Calif. (Hartmann et al., 1971). Its main virtue is a high degree of resistance to *Verticillium dahliae* Kleb. When grafted on ‘Oblonga’, ‘Sevillano’ develops into a large and heavy producing tree, which is resistant to this disease (Hartmann and Whisler, 1970). Because of these characteristics, it has been placed in numerous olive-variety collections and germplasm banks worldwide (Bartolini et al., 1998). ‘Oblonga’ was first incorporated into the Córdoba Olive Germplasm Bank (Spain) in 1975 in the form of cuttings sent by Professor H.T. Hartmann.

‘Frantoio’ is the main variety in Italy and has been cultivated for hundreds of years in different parts of central Italy. Some of the

most common synonyms for this variety include ‘Frantoiano’, ‘Gentile’, and ‘Razza’ (Cimato et al., 1995). Given its high content of top-quality oil, it has been planted worldwide and is grown in many countries, including Argentina, Australia, South Africa, and the United States (Bartolini et al., 1998). It was brought to the Córdoba Olive Germplasm Bank in 1970 from Pescia (Italy).

Systematic coincidence of both cultivars in the yearly evaluations of the Córdoba Olive Germplasm Bank made us suspect that they were in fact identical. We have therefore

analyzed these cultivars, using morphological, agronomic, and molecular methods (RAPDs and SSRs) to confirm our hypothesis.

Materials and Methods

Plant material. We analyzed ‘Frantoio’ and ‘Oblonga’ samples from the Córdoba Olive Germplasm Bank, Alameda del Obispo, Córdoba, as well as from their countries of origin: U.S. Germplasm Repository, Univ. of California, Winters, for ‘Oblonga’ and the Collezione di Varietà di Olivo di la Università di Firenze (Italy) for ‘Frantoio’.

Morphological and agronomic characterization. Morphological characterization was carried out according to 15 characters (Table 1) selected for their high utility for olive cultivar identification (Barranco et al., 2000). Mean values of eight agronomic traits, systematically evaluated in the Córdoba Olive Germplasm Bank over several years, were calculated (Table 2).

DNA extraction. Total genomic DNA was extracted from young leaf tissue collected in the spring, following the method described by Murray and Thompson (1980) adapted for olive by Trujillo et al. (1999).

PCR RAPDs. Twenty-two decamer oligonucleotides (Operon Technologies, Alameda, Calif.) were screened for PCR amplification: OPA-01, OPA-02, OPA-12, OPA-19, OPB-01, OPB-08, OPB-11, OPB-14, OPB-18, OPD-05, OPD-18, OPE-14, OPE-20, OPI-11, OPI-20, OPK-16, OPR-03, OPR-15, OPX-01, OPX-03, OPX-15, and OPZ-11. These primers were

Table 1. Comparison of morphological characteristics of olive cultivars Oblonga and Frantoio.

Organ	Character	Oblonga	Frantoio
Leaf	Shape	Elliptic-lanceolate	Elliptic-lanceolate
Fruit	Shape	Ovoid	Ovoid
	Symmetry	Slightly asymmetric	Slightly asymmetric
	Position of maximum transverse diameter	Toward apex	Toward apex
	Nipple	Absent	Absent
	Location of start of color change	From the apex	From the apex
Endocarp	Shape	Elliptic	Elliptic
	Symmetry	Slightly asymmetric	Slightly asymmetric
	Position of maximum transverse diameter	Toward apex	Toward apex
	Apex	Rounded	Rounded
	Base	Rounded	Rounded
	Surface	Rugose	Rugose
	Number of grooves	Medium	Medium
	Distribution of the grooves	Regular	Regular
	Termination of the apex	With mucro	With mucro

Table 2. Comparison of several traits of ‘Oblonga’ and ‘Frantoio’ olives in the Córdoba Olive Germplasm Bank (Spain).

Trait	Oblonga	Frantoio	Reference
Date of full bloom	6 May	5 May	Barranco et al. (1994)
Date of fruit ripening	11 Nov.	9 Nov.	Barranco et al. (1998)
Fruit weight (g)	3.2	2.8	Rio and Caballero (1994)
Flesh/stone ratio by weight	5.5	5.1	Rio and Caballero (1994)
Fruit abscission force/weight ratio ^a	121.8	122.1	Jaime (1998)
Growth reduction when grown on calcareous soil (%)	33	33	Cordeiro (1997)
Oil content (% fruit dry matter)	42.1	43	Rio and Caballero (1994)
Dead plants after inoculation with <i>Verticillium dahliae</i> (V4) (%)	0	0	López-Escudero (1999)

^aFruit abscission force/weight ratio in Newtons·10⁻²/g.

selected for their polymorphism and repeatability, and because they have shown unique markers in the 'Frantoio' variety in studies by other authors (Belaj, 1998). DNA amplifications were performed in 20- μ L reaction mixtures containing 20 to 40 ng of template DNA, 0.05 U AmpliTaq DNA polymerase Stoffel fragment (PE Biosystems, Norwalk, Conn.), 0.75 mM of each dNTP (Boehringer Mannheim, Germany), 20 μ M of a single primer, 25 mM $MgCl_2$, 50 mM KCl, and 10 mM Tris-HCl, pH 8.3. The reactions were performed in a thermal cycler (Gene Amp PCR System 9600, PE 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 an incubation for 6 min at 72 °C. All the reactions were repeated three times using DNA from different extractions and different lots of the AmpliTaq DNA polymerase. The amplification products were separated on polyacrylamide gels of 18 $\times$ 16 cm containing 10% acrylamide, 0.126% piperazine diacrylamide crosslinker in 0.375 M Tris-HCl (pH 8.8) in Tris glycine (0.025 M Tris, 0.192 M glycine) at 30 mA per gel during 2.5 h. The DNA was visualized by silver staining as described by Bassam et al. (1991). Gels were photographed using a DC 120 Digital Camera (Kodak, Rochester, N.Y.).

A conservative criterion for the selection of bands was used. Only reproducible and well-defined bands were considered as polymorphic markers.

Simple-sequence repeats (SSR) or microsatellites. Identification tests were also performed with five simple-sequence repeats (SSR) from olive: IAS-oli06, IAS-oli11, IAS-oli12, IAS-oli17, and IAS-oli22. These microsatellites have been recently developed and described by Rallo et al. (in press) and their polymorphism has been confirmed in the cultivar collection.

PCR reactions were performed in a 15- μ L volume containing 10 ng of olive genomic DNA (DNA template), 15 mM Tris-HCl pH 8.0, 50 mM KCl, 2.50 mM $MgCl_2$, 0.25 mM of each dNTP, 0.20 μ M of forward and reverse primers, and 1 U of AmpliTaq-Gold polymerase (PE Biosystems). Microsatellites were amplified on thermal cycler. PCR profiles consisted of one polymerase activation cycle at 94 °C for 11 min; 35 to 40 amplification cycles at two (96 °C for 30 s and 68 °C for 1 min) for IAS-oli06 and IAS-oli11, or three temperatures (96 °C for 30 s; 60/64 °C for 1 min; 72 °C for 1 min) for IAS-oli12, IAS-oli17 and IAS-oli22; and a final elongation cycle of 5 min at 72 °C. Products were run in 3.5% Metaphor agarose gels (FMC Bioproducts, Rockland, Maine), in the presence of ethidium bromide (0.5 μ g·mL⁻¹) at 110 V for 3.5 h.

Results and Discussion

In 15 morphological characters (leaf, fruit, and endocarp), the two varieties were identical, both in samples from the Córdoba Germplasm Bank and in samples from their

respective countries of origin (Table 1). Close similarity in the behavior of both varieties was also evident in eight different agronomic evaluations of the two cultivars at the Córdoba Olive Germplasm Bank (Table 2). This, together with shared morphological characteristics, suggested that they were in fact the same cultivar. Furthermore, Trujillo et al. (1995), in a study of isoenzyme variability in 155 olive cultivars, discovered that 'Oblonga' and 'Frantoio' had the same enzymatic profile in four systems tested (ME, GPI, LAP, ADH).

'Oblonga' and 'Frantoio' showed identical amplification patterns with the 22 RAPD primers used for analysis (Fig. 1). Previous studies carried out on the main Mediterranean olive cultivars (Belaj, 1998; Navarro, 1999) showed the presence of unique banding patterns for four primers (OPD-05, OPK-16, OPX-01, OPX-03) in 'Frantoio'. According to the frequencies of these patterns (0.02), the probability of finding the same banding

patterns in another cultivar would be $\approx 1.6 \times 10^{-5}$, if the patterns for the primers were independent.

When testing SSRs, both cultivars amplified identical products as observed in Metaphor agarose gels (Fig. 2). The discrimination capacity of these five SSR markers has been previously confirmed in a set of 46 representative olive cultivars (Rallo et al., in press). Ninety-five percent of these cultivars, including 'Frantoio', showed unique genotypic profiles obtained by the combination of the five loci. Furthermore, 'Frantoio' could be identified by the presence of a unique allele combination at locus IAS-oli12, not shown by any of the other cultivars studied. This "unique" combination was also perfectly amplified by 'Oblonga'.

Morphological and molecular characterization, in addition to close similarity in numerous agronomic evaluations, thus confirmed that they are probably the same cultivar.

Fig. 1. RAPD amplification products obtained in polyacrylamide gel from 'Oblonga' (O) and 'Frantoio' (F) with the primers OPA-19 (1), OPA-02 (2), OPX-01 (3), OPK-16 (4), OPD-18 (5), OPD-05 (6), OPB-18 (7), OPB-11 (8), OPX-15 (9), and OPX-03 (10). The lane at the right end of the gel contains the molecular weight marker (MW).

Fig. 2. Amplification profiles of 'Oblonga' (O) and 'Frantoio' (F) in 3.5% metaphor (FMC products) agarose gel for five SSR: IAS-oli06 (1), IAS-oli11 (2), IAS-oli17 (3), IAS-oli12 (4), and IAS-oli22 (5).

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