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Variability of fatty acid composition in olive *(Olea europaea* L.) progenies

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Abstract

Seedlings from crosses between the olive cultivars 'Arbequina', 'Frantoio' and 'Picual' were evaluated for fatty acid composition over two consecutive years. Gas chromatography was used for analyzing the main fatty acids of olive oil: palmitic (C16:0), palmitoleic (C16:1), stearic (C18:0), oleic (C18:1) and linoleic (C18:2). Wide ranges of variation were obtained for all the fatty acids; as large or even larger than the ranges reported from the evaluation of olive cultivar collections. Significant differences were found between crosses for all the fatty acids analysed except for palmitic acid and between years for all of them except stearic acid, being the interaction crosses x year non significant for any of them. The wide variability observed for all the fatty acids represents a very promising base to obtain new olive cultivars with high oil quality, as vegetative propagation allows the conservation of the most interesting plants.

Key words: cross-breeding, oleic acid, olive breeding, olive oil quality, saturated, polyunsaturated.

Resumen

Variabilidad en la composición de ácidos grasos en progenies de olivo *(Olea europaea* L.)

Durante dos años consecutivos se evaluó la composición en ácidos grasos en plantas de semilla procedentes de cruzamientos entre las variedades de olivo 'Arbequina', 'Frantoio' y 'Picual'. Mediante cromatografía de gases se analizaron los principales ácidos grasos del aceite de oliva: palmítico (C16:0), palmitoleico (C16:1), esteárico (C18:0), oleico (C18:1) y linoleico (C18:2). Se obtuvieron amplios intervalos de variación para todos los ácidos grasos, tan grandes o incluso mayores que los presentados en la evaluación de colecciones de variedades de olivo. Se encontraron diferencias significativas entre cruzamientos para todos los ácidos grasos analizados excepto palmítico y entre años para todos ellos excepto esteárico. La interacción cruzamiento x año no fue significativa para ninguno de ellos. La amplia variabilidad observada para todos los ácidos grasos representa una base prometedora para obtener nuevas variedades de olivo con aceite de calidad, ya que la propagación vegetativa permite la conservación de las plantas más interesantes.

Palabras clave: mejora por cruzamiento, ácido oleico, mejora de olivo, calidad aceite de oliva, saturado, poliinsaturado.

Introduction

Fatty acid composition varies widely in vegetable oils. Standard cultivated plants are characterized by a high proportion of saturated fatty acids (coconut, palm), monounsaturated fatty acids (olive, almond, peanut) or polyunsaturated fatty acids (chestnut, walnut, and seed oils such as sunflower, soybean, safflower or flax). Several studies have shown the dietary importance of fatty acid composition of lipids: a diet rich in monounsaturated fatty acids may reduce low-density lipoprotein cholesterol and total cholesterol without altering beneficial high-density lipoprotein cholesterol levels (Matson and Grundy, 1985). Fatty acid composition has been shown to influence the stability of oils, and polyunsaturated fatty acids have been found to contribute to the rancidification of several oils (Hammond and Fehr, 1984; Tous and Romero, 1993). Finally, fatty acid composition has been found to be responsible for

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the odors and flavors associated with oil quality (Maestro-Durán and Borja-Padilla, 1990).

The importance of fatty acid composition has produced interest in the genetic manipulation of fatty acids in oil crops. Mutagenic breeding programs aimed at reducing the concentration of polyunsaturated fatty acids have been reported in several oilseeds such as soybean, sunflower and flax. Selections from these programs have been used extensively to study the inheritance of fatty acid composition and to develop new cultivars with specific fatty acid profiles (Ntiamoah *et al*., 1995; Rahman *et al*., 1996; Pérez-Vich *et al*., 1999). Conversely, breeding programs in palm tree aimed at generating more liquid oils have led to individuals with high percentages of unsaturated fatty acids (Jones, 1984).

Attempts to develop new olive cultivars have been carried out in some olive-producing countries (Lavee, 1990; Bellini, 1992; Arsel and Cirik, 1994; Trigui, 1996; Fontanazza *et al*., 1999). Most of these programs are focused on cross breeding among the most outstanding cultivars in their respective countries. However, none of these works have provided information about fatty acid composition, in spite of oil quality being considered one of the most important breeding objectives in olive (Fontanazza and Baldoni, 1990). In Spain, a breeding program was started in 1992 to obtain new olive cultivars with some of the following traits: early bearing, high productivity and oil content, resistance to peacock eye (*Spilocaea oleagina*, Cast), suitability for mechanical harvesting and high quality of olive oil (Rallo, 1995). Fatty acid composition, particularly high oleic acid percentage, has been selected as the main criterion to evaluate oil quality.

The objective of this work was to describe the variability observed for fatty acid composition in olive progenies and to compare the results obtained with the variability reported in olive.

Material and Methods

Seedlings from crosses (9 combinations) among Arbequina, Frantoio and Picual cultivars were used in this study. The parents of the breeding program were chosen on the basis of their high productivity and oil content, and their different geographical origin (Arbequina from Catalonia, Spain; Frantoio from Tuscany, Italy; and Picual from Andalusia, Spain), earliness of bearing and fatty acid composition (Rallo, 1995). Arbequina is characterised by its high palmitic and linoleic acid content whereas Frantoio and Picual have lower proportions of these fatty acids and higher proportions of oleic acid (Tous and Romero, 1993; Fontanazza and Patumi, 1994; Uceda *et al*., 1999). Seedlings from crosses made in spring 1992, 1993 and 1994 were used in this study. Crosses were made by pollination of flowers on bagged branches, and forced growth of seedlings was carried out in a greenhouse to shorten the juvenile period (Santos-Antunes *et al*., 1999). The seedlings were transplanted into the field in 1994, 1995 and 1996 respectively at 1.50 × 3.50 m. Standard cultural practices were followed in the orchard to ensure tree growth. Fatty acid composition was recorded in 1996 and 1997 once the seedlings started to flower and produce fruits.

Seedlings were harvested at a similar ripening index, as fatty acid composition can be influenced by the ripening stage. Ripening index is a colour measurement of the fruit, of the skin and flesh, weighted according to a scale from 0 to 7 (Frías *et al*., 1991). Olive fruits samples corresponding to the category 4 (black skin and white flesh) were randomly collected in each seedling and kept frozen for fatty acids evaluation. Fatty acid methyl esters (FAMEs) were prepared according to the procedure of Garcés and Mancha (1993). This method allows the digestion of fresh tissue, transmethylation of lipids, and extraction of FAMEs in one step, avoiding the necessity of oil extraction prior to FAMEs preparation (which is time consuming and impractical for processing a high number of samples). In the laboratory, ten random replicates per genotype (50 mg sample of flesh tissue from different fruits) were boiled at 80ºC for 2h with a reagent mixture containing methanol:heptane:toluene:2,2-dimethoxipropane:H_2SO_4 (39:34:20:5:2, by volume). After cooling at room temperature, two phases were formed, the upper one containing FAMEs prepared for gas chromatography analysis. FAMEs were separated in a capillary column BPX-70 (50 m, 0.25 mm I.D., 0.22 μm film thickness) using a gas chromatograph equipped with a flame ionization detector. Five fatty acids, palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), oleic acid (C18:1) and linoleic acid (C18:2), expressed as percentage of fatty acid methyl esters, were monitored in this study.

Fatty acid composition could not be evaluated in all plants since a minimum amount of fruits was required for these evaluations. The number of seedlings evaluated for fatty acid composition by year and cross is

Table 1. Number of seedlings evaluated and range of variation for fatty acid composition (%) in olive progenies by year and cross

Cross	n	Palmitic	Palmitoleic	Stearic	Oleic	Linoleic
1996						
Arbequina × Arbequina	29	10.1-18.8	1.0-5.4	1.4-4.8	58.8-80.2	1.9-14.4
Arbequina × Frantoio	28	8.6-18.9	0.9-4.6	1.2-2.7	50.9-82.6	2.2-22.3
Arbequina × Picual	26	9.3-18.2	0.9-4.2	1.3-4.9	54.7-81.0	1.9-18.4
Frantoio × Arbequina	7	9.6-16.6	0.9-4.3	1.2-2.2	57.7-82.5	2.9-15.4
Frantoio × Frantoio	8	11.5-15.5	1.4-3.4	1.5-3.4	65.6-76.7	3.5-13.4
Frantoio × Picual	5	11.3-13.4	1.3-1.6	2.0-3.4	70.9-76.2	4.1-9.0
Picual × Arbequina	45	7.9-17.5	0.8-4.3	1.2-4.4	61.3-82.7	1.6-15.2
Picual × Frantoio	3	12.3-16.4	1.7-2.1	1.6-3.8	58.3-71.7	6.0-16.7
Picual × Picual	16	9.4-17.7	0.9-3.6	1.3-3.1	59.2-82.0	2.9-15.1
1997						
Arbequina × Arbequina	48	9.8-20.9	1.2-7.1	1.1-2.6	45.6-82.9	2.6-25.8
Arbequina × Frantoio	49	11.5-21.6	1.1-6.2	1.1-3.8	44.0-77.8	2.8-25.9
Arbequina × Picual	50	8.9-21.2	0.8-8.1	1.1-4.8	43.5-84.6	1.6-29.2
Frantoio × Arbequina	15	11.6-18.9	1.1-5.4	1.0-2.2	50.0-77.2	3.6-22.1
Frantoio × Frantoio	17	12.4-16.9	1.1-3.3	1.1-4.0	54.8-74.9	3.6-23.2
Frantoio × Picual	14	10.8-16.9	1.1-3.5	1.2-8.1	54.0-79.9	3.6-21.6
Picual × Arbequina	65	8.9-19.8	0.7-4.8	1.1-4.0	46.5-84.7	1.8-25.0
Picual × Frantoio	10	10.7-17.6	1.0-2.8	1.4-4.9	49.6-79.3	2.8-23.7
Picual × Picual	20	10.8-20.1	1.1-4.3	1.3-2.6	48.3-80.9	1.7-24.4

presented in Table 1. Frequency distributions of the different fatty acids were obtained for all progenies, and by year and cross separately. Data were subjected to analysis of variance and separation of the means was obtained using the least significant difference at $P \leq 0.05$.

Results

Progenies showed a wide range of variation for the fatty acids analysed (Table 1) with the percentages of palmitic, palmitoleic, stearic, oleic and linoleic acids ranging from 7.9 to 21.6%, from 0.7 to 8.1%, from 1.0 to 8.1%, from 43.5 to 84.7% and from 1.6 to 29.2%, respectively. Monounsaturated oleic acid is distinctly predominant, followed by saturated palmitic acid and polyunsaturated linoleic acid (67%, 14% and 10% on average, respectively). Fatty acid composition varies according to the crosses evaluated although wide ranges of variation were obtained in all the combinations tested (Fig. 1). A high variability was also obtained both years for the fatty acids analysed, with a wider range of variation in 1997 than in 1996 (Fig. 2).

Significant differences were found between crosses for all the fatty acids analysed except for palmitic acid and between years for all of them except stearic acid, being the interaction crosses x year non significant for any of them (Table 2). The average proportion of oleic acid was higher in 1996 than in 1997 and the opposite trend was observed for the proportion of palmitic, palmitoleic and linoleic acid. The highest content of oleic acid was obtained in the crosses Frantoio × Arbequina, Frantoio × Picual and Picual × Arbequina and the lowest in Picual × Frantoio. For the linoleic acid, the highest content was obtained in the cross Arbequina × Picual and the lowest in Frantoio × Frantoio, Frantoio × Picual, Picual × Arbequina and Arbequina × Arbequina.

Discussion

The variability obtained for all the progenies was as large or even slightly larger than previously observed in olive cultivar collections (Tous and Romero, 1993; Fontanazza and Patumi, 1994; Uceda *et al*., 1999), and similar to that obtained in Australia from the sampling of wild populations (Sedgley and Wirthensohn, 2000). A high variability was also observed in these progenies for other characteristics such as crop, oil content and fruit characters (León *et al*., 2004). Similar results have been reported for earliness of bearing, oil content or fruit size and shape in other olive cross-breeding programs (Lavee, 1990; Bellini, 1992; Fontanazza *et*

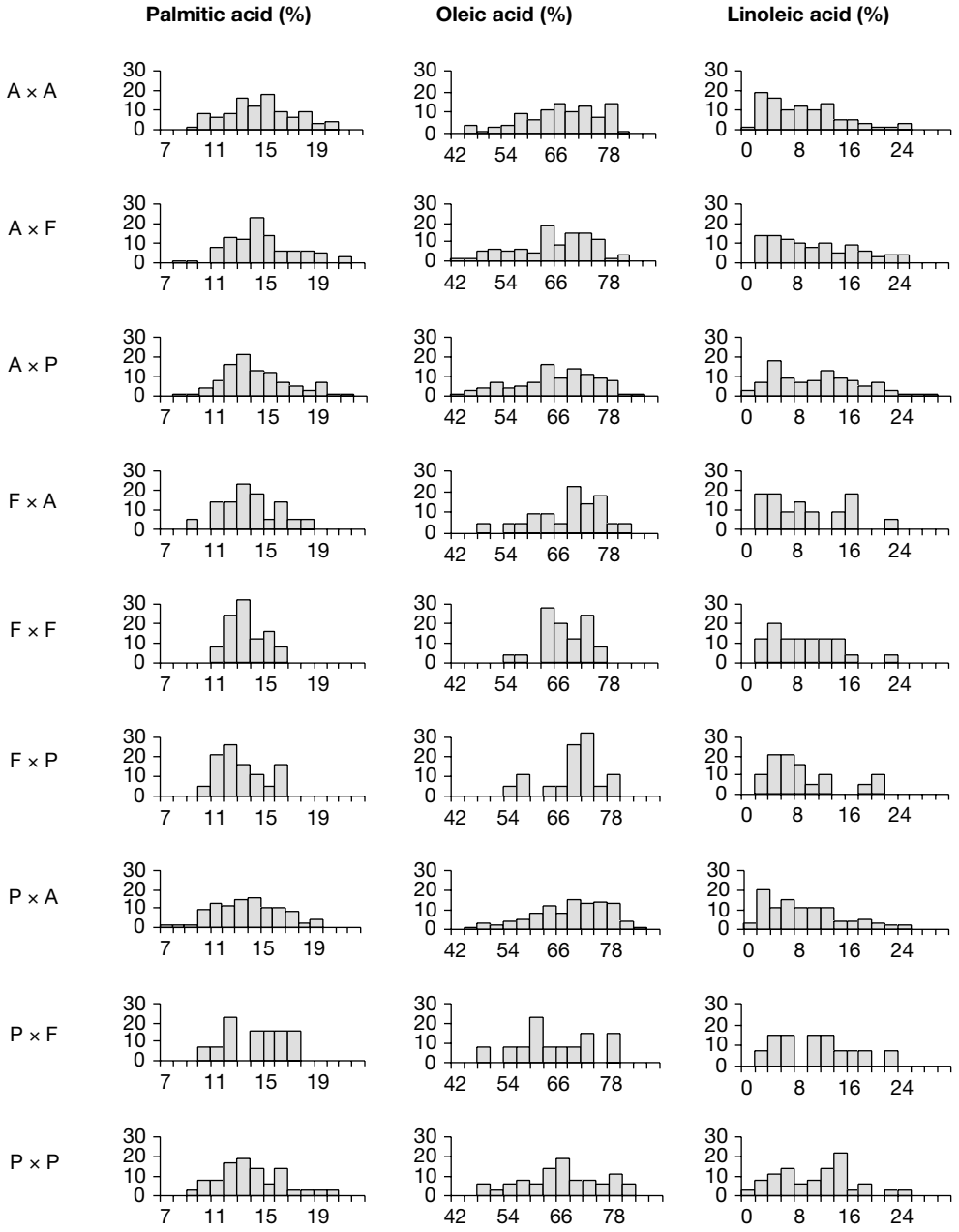

Figure 1. Frequency distributions (%) for palmitic, oleic and linoleic acid percentages in the different crosses evaluated (A: Arbequina. F: Frantoio. P: Picual).

al., 1999). Some seedlings with high oleic acid percentage (up to 85%) and others with low linoleic acid percentage (less than 2%) have been obtained. In olive, it has been demonstrated that a good quality index is assured when the oleic acid percentage is greater than 73% and the linoleic acid percentage less than 10%, producing an oleic/linoleic ratio grater than 7 (Montedoro and Garofolo, 1984; Maestro-Durán and Borja-Padilla, 1990).

The influence of genetic and environmental factors on the fatty acid composition of olive oil has been studied by several authors without clear conclusions. Some of them reported that the fatty acid composition of olive oil depends primarily on the cultivar (Uceda *et al*., 1999; Ayton *et al*., 2001), suggesting that fatty acid composition could be used for discriminating between cultivars by chemometric methods (Perri *et al*., 1999). However, a great influence of environmental

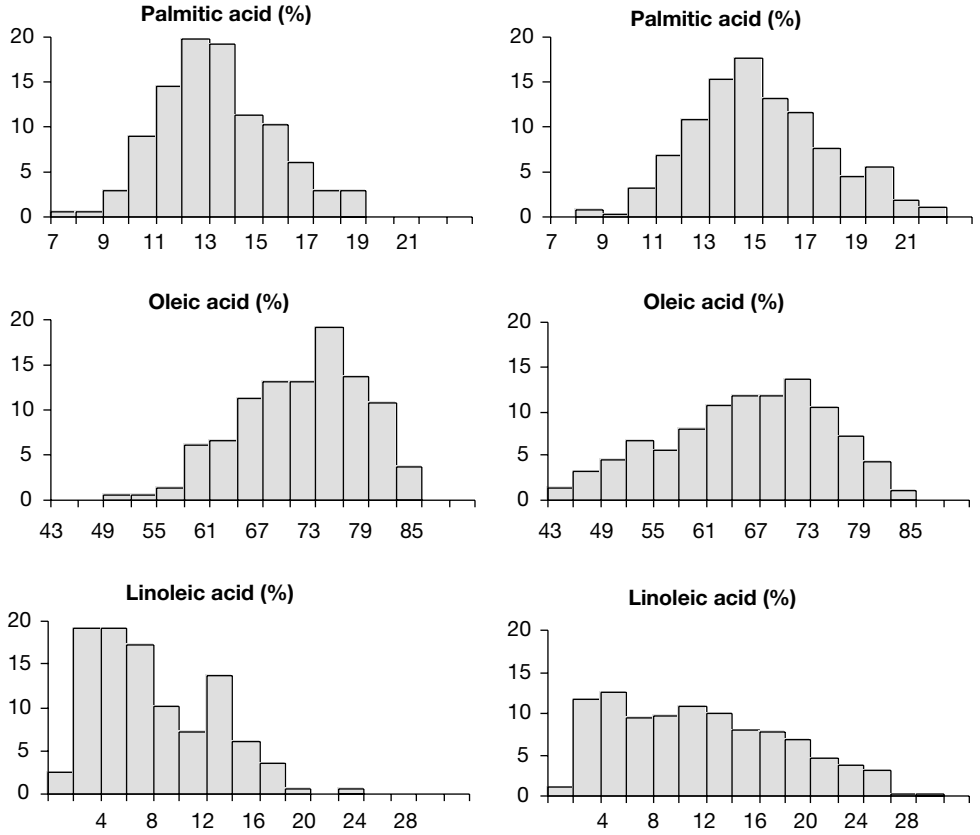

Figure 2. Frequency distributions (%) of fatty acids in olive progenies in 1996 (left) and 1997 (right).

Table 2. Analysis of variance (F values) and mean values (%) for fatty acid composition in olive progenies according to year and cross

	Palmitic	Palmitoleic	Stearic	Oleic	Linoleic
Sources of variation (df)					
Crosses (8)	3.00	6.97**	5.23*	4.62*	8.38**
Year (1)	26.06***	6.79*	4.09	52.13***	88.20***
Crosses × year (8)	0.62	0.62	0.88	0.34	0.20
Error (437)					
Crosses					
Arbequina × Arbequina	14.9	3.0 a	1.9 d	67.7 abc	9.5 c
Arbequina × Frantoio	14.9	2.8 a	1.7 d	66.1 bc	11.2 ab
Arbequina × Picual	14.4	2.6 ab	2.0 cd	66.3 bc	11.8 a
Frantoio × Arbequina	14.1	2.3 bcd	1.7 d	69.2 a	10.0 bc
Frantoio × Frantoio	13.8	2.1 cd	2.3 bc	68.7 ab	9.5 c
Frantoio × Picual	13.4	1.9 d	2.4 ab	70.1 a	9.3 c
Picual × Arbequina	14.0	2.5 abc	1.8 d	69.5 a	9.4 c
Picual × Frantoio	14.5	2.0 d	2.7 a	65.5 c	11.5 ab
Picual × Picual	14.0	2.3 bcd	1.9 d	67.4 abc	11.2 ab
Year					
1996	13.3 b	2.4 b	2.1	71.4 a	8.0 b
1997	15.0 a	2.7 a	1.8	65.7 b	11.7 a

*,**,*** Significant at 0.05, 0.01 and 0.001, respectively. For each constituent and factor, different letters indicate significant differences at $P \leq 0.05$.

factors such as year of harvest, location, season or climatic conditions (in particular the temperature and rainfall during fruit growth and ripening) on the variation of some fatty acids has also been reported (Tsimidou and Karakostas, 1993; Panelli *et al*., 1994; Tous and Romero, 1994; Tovar *et al*., 2002). Moreover, it has been previously reported in other olive breeding programs that the evaluation of olive progenies is rather complex and some characters like oil content seem to stabilize only after 2-3 years (Lavee, 1990).

The high variability observed represents a very promising base to obtain new olive cultivars with high oil quality, as asexual propagation allows the preservation of any genotype for use either as a new cultivar or as breeding stock for future generations. The high variability observed also suggests that selection in olive progenies for earliness of bearing (short juvenile period) may not suppose a risk for inferior fatty acid composition of selected plants, because a wide range of variation has been obtained from the first year, i.e. seedlings with short juvenile period, in all crosses evaluated. Further experimentation is needed to clarify the inheritance of fatty acids in olive and to determine the best breeding method to generate superior genotypes.

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References

ARSEL H., CIRIK N., 1994. General overview of olive breeding in Turkey. Olivae 52, 25-27.

AYTON J., MAILER R.J., ROBARDS K., ORCHARD B., VONARX M., 2001. Oil concentration and composition of olives during maturation in south-western New South Wales. Austral J Expt Agr 41, 815-821.

BELLINI E., 1992. Behaviour of some genetic characters in olive seedlings obtained by cross-breeding. Acta Hort 317, 197-208.

FONTANAZZA G., BALDONI L., 1990. Proposed programme for the genetic improvement of the olive. Olivae 34, 32-40.

FONTANAZZA G., PATUMI M., 1994. Influence of cultivars on the composition and quality of olive oil. Acta Hort 356, 358-361.

FONTANAZZA G., VERGARI G., PATUMI M., GIORIO G., 1999. Preliminary results of the evaluation of yield components in an F1 segregant population of olive seedlings from the cross Leccino x Kalamata. Acta Hort 474, 97-101.

FRÍAS L., GARCÍA-ORTIZ A., HERMOSO M., JIMÉNEZ A., LLAVERO DEL POZO M.P., MORALES J., RUANO T., UCEDA M., 1991. Analistas de laboratorio de almazara. Informaciones Técnicas, 6/91, Junta de Andalucía, Sevilla, Spain.

GARCÉS R., MANCHA M., 1993. One-step lipid extraction and fatty acid methyl esters preparation from fresh plant tissues. Anal Biochem 211, 139-143.

HAMMOND E.G., FEHR W.R., 1984. Improving the fatty acid composition of soybean oil. J Am Oil Chem Soc 61, 1713-1716.

JONES L.H., 1984. Novel palm oils from cloned plants. J Am Oil Chem Soc 61, 1717-1719.

LAVEE S., 1990. Aims, methods and advances in breeding of new olive (*Olea europaea*, L.) cultivars. Acta Hort 286, 23-36.

LEÓN L., RALLO L., DEL RÍO C., MARTÍN L.M., 2004. Variability and early selection on the seedling stage for agronomic traits in progenies from olive crosses. Plant Breeding 123, 73-78.

MAESTRO-DURÁN R., BORJA-PADILLA R., 1990. La calidad del aceite de oliva en relación con la composición y maduración de la aceituna. Grasas y Aceites 41, 171-178.

MATSON F.M., GRUNDY S.M., 1985. Comparison of effects of dietary saturated, monounsaturated and polyunsaturated fatty acids on plasma lipids and lipoproteins in man. J Lipid Res 26, 194-202.

MONTEDORO G., GAROFOLO L., 1984. Caratteristiche qualitative degli oli vergini di oliva. Influenza di alcune variabili: varietà, ambiente, conservazione, estrazione, condizionamento del prodotto finito. Riv Ital Sostanze Grasse 61, 157-168.

NTIAMOAH C., ROWLAND G.G., TAYLOR D.C., 1995. Inheritance of elevated palmitic acid in flax and its relationship to the low linoleic acid. Crop Sci 35, 148-152.

PANNELLI G., SERVILI M., SELVAGGINI R., BALDIOLI M., MONTEDORO G.F., 1994. Effect of agronomic and seasonal factors on olive (*Olea europaea* L.) production and on the qualitative characteristics of the oil. Acta Hort 356, 239-243.

PÉREZ-VICH B., FERNÁNDEZ J., GARCÉS R., FERNÁNDEZ-MARTÍNEZ J.M., 1999. Inheritance of high palmitic acid content in the seed oil of sunflower mutant CAS-5. Theor Appl Genet 98, 496-501.

PERRI E., PARLATI M.V., PALOPOLI A., PELLEGRINO M., RIZZUTI B., 1999. Characterization of Italian virgin olive oils using fatty acids. Acta Hort 474, 627-630.

RAHMAN S.M., TAKAGI Y., KINOSHITA T., 1996. Genetic control of high oleic acid content in the seed oil of two soybean mutants. Crop Sci 36, 1125-1128.

RALLO L., 1995. Selection and breeding of olive in Spain. Olivae 59, 46-53.

SANTOS-ANTUNES A.F., MOHEDO A., TRUJILLO I., RALLO L., 1999. Influence of the genitors on the flowering of olive seedlings under forced growth. Acta Hort 474, 103-105.

SEDGLEY M., WIRTHENSOHN M., 2000. The Australian olive improvement programme. Olivae 83, 27-30.

TOUS J., ROMERO A., 1993. Variedades de olivo. Fundación «La Caixa», Barcelona, Spain.

TOUS J., ROMERO A., 1994. Cultivar and location effects on olive oil quality in Catalonia, Spain. Acta Hort 356, 323-326.

TOVAR M.J., ROMERO M.P., ALEGRE S., GIRONA J., MOTILVA M.J., 2002. Composition and organoleptic characteristics of oil from Arbequina olive (*Olea europaea* L) trees under deficit irrigation. J Sci Food Agric 82, 1755-1763.

TRIGUI A., 1996. Improving the quantity and quality of olive production in Tunisia: unavoidable need and outlook for olive identification and breeding. Olivae 61, 34-40.

TSIMIDOU M., KARAKOSTAS K.X., 1993. Geographical classification of Greek virgin olive oil by non-parametric multivariate evaluation of fatty acid composition. J Sci Food Agric 62, 253-257.

UCEDA M., HERMOSO M., GARCÍA-ORTIZ A., JIMÉNEZ A., BELTRÁN G., 1999. Intraspecific variation of oil contents and the characteristics of oils in olive cultivars. Acta Hort 474, 659-662.