

Fatty acid composition of advanced olive selections obtained by crossbreeding

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Abstract

BACKGROUND: An olive-breeding programme aimed at obtaining new cultivars for olive oil production was initiated in Spain in 1991, with oil quality being considered one of the most important objectives. In this study the oil fatty acid composition of 15 advanced olive selections coming from crosses between ‘Arbequina’, ‘Frantoio’ and ‘Picual’ cultivars was evaluated.

RESULTS: A strong genetic effect and significant differences between genotypes were obtained for all fatty acids and ratios evaluated. The results allowed the classification of genotypes into four groups according to their fatty acid composition, with the percentages of C18:1, C18:2 and saturated fatty acids being the main contributors to the total variation. The relationship between the results of the initial seedling population and those of the advanced selections indicated that an efficient selection for fatty acid composition could be carried out by considering only a single year of evaluation at the seedling stage.

CONCLUSION: A quite different fatty acid composition in the oil of 15 advanced selections and their three genitors was obtained. These results suggest that new olive cultivars with fatty acid composition fulfilling consumer and market demands could be obtained through crossbreeding in the future.

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Keywords: early selection; oil quality; *Olea europaea* L.; olive oil; variability

INTRODUCTION

In the last two decades, world olive oil production has almost doubled from 1.406×10^6 t in 1990–1991 to 2.779×10^6 t in 2006–2007, with further increases being expected in future years as new lands come into bearing.¹ Scientific demonstration that olive oil is effective in protecting human health has played a paramount role in the recent expansion of olive growing around the world.

Besides the presence of several minor components, the biological and nutritional importance of vegetable oils is linked to the nature of the fatty acids they contain.² Olive oil is characterised by a high proportion of monounsaturated oleic acid, in contrast to other vegetable oils with higher proportions of saturated fatty acids (coconut and palm) or polyunsaturated fatty acids (soybean, sunflower, safflower, flax and oils from nuts such as chestnut or walnut). Several studies have shown that a diet rich in monounsaturated fatty acids may produce a wide range of health benefits beyond improvement in cholesterol levels, suggesting that this type of diet has great potential in preventing cardiovascular disorders.^{2,3} On the other hand, fatty acid composition is also important in the commercial properties of oils, as it has been shown to

influence the stability of oils through the contribution of polyunsaturated fatty acids to oil rancidity.⁴

Research developments in recent years have promoted the initiation of olive-breeding programmes in the main olive-producing countries. Most of these programmes are focused on crossbreeding among the most outstanding cultivars and selection within the progenies. Fatty acid composition is one of the characteristics evaluated in several of these works, as oil quality is considered to be one of the most important breeding objectives in olive.^{5,6}

In Spain, an olive-breeding programme aimed at obtaining new cultivars for olive oil production was initiated in 1991 in Córdoba by performing crosses between the cultivars ‘Arbequina’, ‘Frantoio’ and ‘Picual’.⁷ Besides agronomic characteristics such as earliness of bearing and oil content, other characteristics, including fatty acid composition, are also taken into account in the evaluation process.⁸

The aim of this work was to evaluate the fatty acid composition of 15 advanced selections and the three genitors established in a comparative field trial in 2001. The relationship between the results of the initial seedling population and those of the advanced selections was also investigated.

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EXPERIMENTAL

Plant material

The selections evaluated in this work come from the reciprocal crosses between 'Arbequina', 'Frantoio' and 'Picual' olive cultivars carried out in 1991 and 1992. Four of them come from 'Frantoio' × 'Picual', five from 'Arbequina' × 'Picual' and six from 'Picual' × 'Arbequina' (Table 1). These genitors 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).⁷

Seedlings obtained were subjected to a forced growth protocol from seed germination to greenhouse and field growth, as described by Santos-Antunes *et al.*⁹ From an initial population of 748 seedlings, 15 genotypes were selected after three consecutive harvest seasons, mainly on the basis of their early bearing (short juvenile period) and high oil content. Fatty acid composition was also evaluated at the seedling stage, including only a single plant and year per genotype, in 13 of these 15 genotypes.⁸ Vegetative propagation of the 15 selected genotypes and the three genitors, used as a control, was carried out with semi-hardwood cuttings in spring 2000, and propagated trees were planted in the open field in July 2001 at 6 m × 5 m spacing. This comparative field trial was set up in a randomised block design with 16 replications and one tree per elementary plot. Several losses due to rodent damage occurred after the second year in the open field, although a minimum number of 13 trees per selection remained available (Table 1).

Table 1. Origin of selections and number of trees evaluated in comparative field trial and initial seedling population

No.	Genotype	Origin	Number of trees evaluated	
			Comparative field trial	Initial seedling population
1	Arbequina (A)	–	14	–
2	Frantoio (F)	–	11	–
3	Picual (P)	–	13	–
4	UC-I 1–19	F × P	11	1
5	UC-I 2–68	P × A	13	1
6	UC-I 4–62	F × P	11	1
7	UC-I 5–44	P × A	14	1
8	UC-I 6–9	A × P	9	–
9	UC-I 7–8	A × P	15	–
10	UC-I 7–34	P × A	13	1
11	UC-I 7–60	F × P	12	1
12	UC-I 8–7	P × A	12	1
13	UC-I 8–20	P × A	12	1
14	UC-I 9–67	A × P	12	1
15	UC-I 10–30	F × P	8	1
16	UC-I 10–54	A × P	11	1
17	UC-I 11–10	A × P	15	1
18	UC-I 11–16	P × A	12	1
	Total	–	218	13

Fatty acid composition analysis

In the comparative field trial, plants were harvested at a similar ripening index to avoid any influence of the ripening stage on the fatty acid composition. Olive fruit samples corresponding to category 4 according to Frías *et al.*,¹⁰ i.e. with black skin and white flesh, were randomly collected and kept frozen until fatty acid evaluation. Fatty acid methyl esters (FAMES) were prepared according to the procedure of Garcés and Mancha.¹¹ 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 FAME preparation. Five fruits per tree were analysed in 2005 and 2006. This methodology was also applied for fatty acid composition analysis in the initial seedling population.

FAMES prepared directly from fresh fruits were separated using a gas chromatograph equipped with a flame ionisation detector. Ten fatty acids, namely palmitic acid (C16:0), palmitoleic acid (C16:1), margaric acid (C17:0), margaroleic acid (C17:1), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), linolenic acid (C18:3), arachidic acid (C20:0) and gadoleic acid (C20:1), expressed as a percentage of FAMES, were monitored in this study. From these determinations the total unsaturated/saturated fatty acid (U/S) and oleic/linoleic acid (O/L) ratios were also calculated.

Data analysis

Average data recorded in the comparative field trial in the 2005 and 2006 harvest seasons were used for statistical analysis. These data were subjected to analysis of variance (ANOVA) to test the effect of cultivars, and separation of the means was obtained at $P \leq 0.05$ by Duncan's test using SAS software (SAS Institute Inc., Cary, NC, USA). Average data by genotype were used to perform principal component analysis (PCA) with the statistical package The Unscrambler (CAMO A/S, Trondheim, Norway). Correlations between data obtained in the initial seedling population and the comparative field trial were also calculated.

RESULTS

Genotypic variance was the main contributor to total variance for all fatty acids and ratios evaluated in the comparative field trial (Table 2). The genotype effect accounted for 50–90% of the total sum of squares in the ANOVA, with values higher than 80% for C16:1, C18:1, C18:2 and O/L. C18:1 was the fatty acid clearly predominant in all samples analysed (72.08% on average), followed by C16:0 and C18:2 (14.48 and 6.64% on average respectively). Therefore these three fatty acids accounted for 93.20% of the total fatty acid composition. Average values of C16:1, C18:0 and C18:3 were 2.32, 2.20 and 0.72% respectively. The rest of the fatty acids represent roughly the remaining 1% of the total fatty acid composition.

Table 2. Percentage of total sum of squares and significance in ANOVA for fatty acid composition (%) and calculated ratios in comparative field trial

	DF	C16:0	C16:1	C17:0	C17:1	C18:0	C18:1
Genotype	17	71.36***	80.76***	62.83***	61.36***	79.56***	84.08***
Block	15	4.25**	2.50*	4.46	3.35	1.81	1.49
Error	185	24.38	16.74	32.71	35.29	18.64	14.44
CV (%)		7.54	15.50	45.79	46.63	15.86	3.46
Mean		14.48	2.32	0.10	0.23	2.20	72.08
	DF	C18:2	C18:3	C20:0	C20:1	U/S ^a	O/L ^a
Genotype	17	89.19***	56.51***	79.94***	50.35***	70.39***	90.36***
Block	15	0.72	4.71	2.67*	4.44	3.22	0.80
Error	185	10.09	38.78	17.40	45.21	26.39	8.84
CV (%)		22.57	18.76	10.22	10.35	8.28	20.49
Mean		6.64	0.72	0.41	0.30	4.88	17.09

DF, degrees of freedom; CV, coefficient of variation. Significance: * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.

^a Total unsaturated/saturated fatty acid (U/S) and oleic/linoleic acid (O/L) ratios.

Differences between genotypes were highly significant in all cases. A high degree of variability was obtained for the main fatty acids, with ranges of variation of 12.07–18.38, 61.15–78.26 and 2.00–15.27% for C16:0, C18:1 and C18:2 respectively (Table 3). Duncan's multiple comparison procedure provided 5–12 subsets for the different fatty acids and ratios evaluated. Selections obtained by crossbreeding provided a wider range of variability than the genitors in almost all cases, the exceptions being 'Frantoio' with the lowest value for C17:0 and 'Picual' with the lowest value for C20:1.

PCA was performed on average data by cultivar. The first two principal components (PC1 and PC2) accounted for 41 and 24% of the total variance respectively (Fig. 1). PC1 was correlated positively with C18:1 and O/L and negatively with C16:0 and C18:2. PC2 was mainly associated positively with C17:0, C17:1, C18:0 and C20:0 and negatively with U/S. C16:1, C18:3 and C20:1 had less effect on PCA loadings.

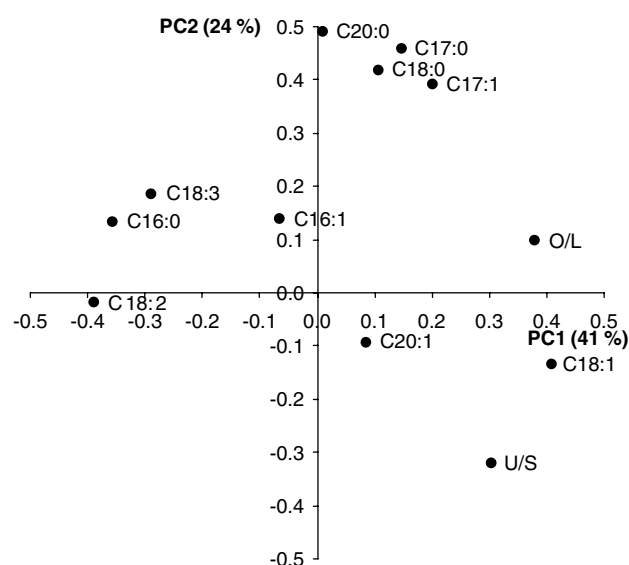


Figure 1. Plot of vector loadings on PCs 1 and 2 for fatty acids and calculated ratios.

The score biplot of PC1 and PC2 showed four groups according to the fatty acid composition of cultivars (Fig. 2). Group I, with only one cultivar ('UC-I 7-8'), had the lowest C18:1 content and a high content of C18:2, C18:3 and saturated fatty acids. Group II ('Arbequina', 'Frantoio', 'UC-I 1-19', 'UC-I 6-9', 'UC-I 8-7' and 'UC-I 10-30') was characterised by a low O/L ratio and a high C16:0 content. Group III ('UC-I 5-44', 'UC-I 7-34', 'UC-I 9-67', 'UC-I 11-10' and 'UC-I 11-16') had a medium–high C18:1 content and a high content of all saturated fatty acids except C16:0. Group IV ('Picual', 'UC-I 2-68', 'UC-I 4-62', 'UC-I 7-60', 'UC-I 8-20' and 'UC-I 10-54') showed a high C18:1 content (highest in 'UC-I 10-54') and a high U/S ratio.

The results obtained in the comparative field trial with adult vegetatively propagated plants reported here were compared with those previously obtained for these genotypes at seedling stage. Only data for the main fatty acids (C16:0, C16:1, C18:0, C18:1 and C18:2) were available at the seedling stage. A

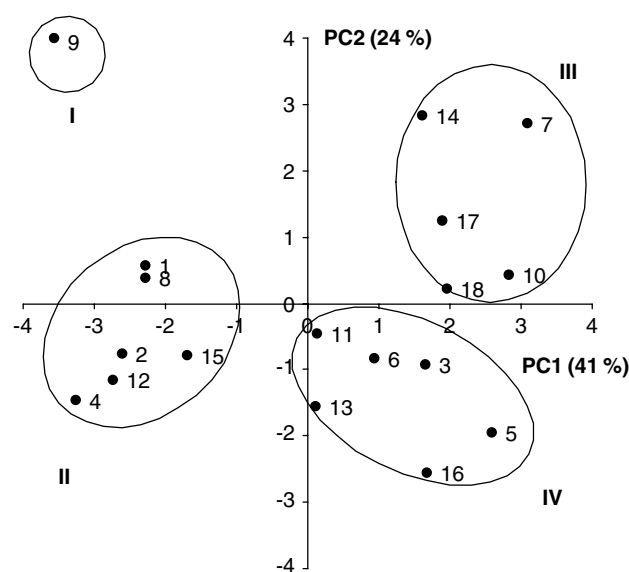


Figure 2. Plot of scores on PCs 1 and 2. Ellipses indicate the four principal groups (I, II, III and IV) described in the text. See Table 1 for correspondence of genotypes.

Table 3. Mean values by genotype for fatty acid composition (%) and calculated ratios

Genotype	C16:0	C16:1	C17:0	C17:1	C18:0	C18:1
Arbequina	16.54bc (3)	2.76c (3)	0.13c (7)	0.27cd (7)	1.65gh (16)	64.53fg (16)
Frantoio	15.08defg (7)	1.84efg (12)	0.03e (18)	0.11e (11)	1.91efg (10)	67.09e (14)
Picual	13.41ijk (12)	1.94ef (11)	0.05de (11)	0.21d (9)	2.26cd (6)	77.23abc (3)
UC-I 1–19	15.75cde (5)	1.63fgh (15)	0.05de (13)	0.07e (18)	1.68fgh (15)	63.32g (17)
UC-I 2–68	12.51kl (16)	2.34d (9)	0.08d (8)	0.23d (8)	1.51h (17)	78.17ab (2)
UC-I 4–62	13.02jkl (14)	1.55gh (16)	0.04de (15)	0.10e (13)	2.84b (4)	75.69cd (7)
UC-I 5–44	13.30jk (13)	2.63cd (5)	0.15bc (5)	0.36bc (3)	3.52a (2)	75.96bcd (6)
UC-I 6–9	18.38a (1)	4.87a (1)	0.06de (10)	0.11e (10)	1.70fgh (14)	67.76e (13)
UC-I 7–8	17.20b (2)	2.60cd (6)	0.17abc (3)	0.36bc (4)	3.05b (3)	61.15h (18)
UC-I 7–34	13.67hij (11)	3.15b (2)	0.15bc (4)	0.45a (1)	1.72fgh (12)	76.61abcd (4)
UC-I 7–60	14.58fgh (9)	2.04e (10)	0.05de (12)	0.10e (14)	2.32c (5)	74.77d (9)
UC-I 8–7	16.04cd (4)	2.48cd (8)	0.04de (16)	0.11e (12)	1.46h (18)	66.50ef (15)
UC-I 8–20	14.28ghi (10)	2.55cd (7)	0.06de (9)	0.09e (17)	1.77fgh (11)	74.64d (10)
UC-I 9–67	12.79jkl (15)	1.41h (18)	0.19a (1)	0.32bc (6)	3.66a (1)	74.60d (11)
UC-I 10–30	15.41def (6)	1.81efg (13)	0.04de (14)	0.09e (16)	2.19cde (8)	68.52e (12)
UC-I 10–54	12.07l (18)	1.43h (17)	0.04de (17)	0.10e (15)	1.72fgh (13)	78.26a (1)
UC-I 11–10	15.00efg (8)	2.75c (4)	0.14bc (6)	0.35bc (5)	2.21cde (7)	75.22cd (8)
UC-I 11–16	12.13l (17)	1.81efg (14)	0.17ab (2)	0.41ab (2)	1.98def (9)	76.10abcd (5)
Genotype	C18:2	C18:3	C20:0	C20:1	U/S ^a	O/L ^a
Arbequina	12.08b (3)	0.65ef (12)	0.42de (7)	0.32bcd (7)	4.36f (16)	5.56f (16)
Frantoio	11.85b (4)	0.99a (2)	0.40de (9)	0.33b (3)	4.74de (10)	5.84f (15)
Picual	3.21fgh (15)	0.65ef (14)	0.34hij (15)	0.26h (18)	5.25b (5)	24.76c (4)
UC-I 1–19	15.27a (1)	0.78bcd (5)	0.31j (18)	0.28fgh (15)	4.62ef (13)	4.65f (18)
UC-I 2–68	3.48efg (14)	0.46g (18)	0.32ij (16)	0.29cdefg (10)	5.94a (2)	23.68c (5)
UC-I 4–62	5.17d (8)	0.57fg (16)	0.43d (4)	0.31bcdef (9)	5.12bc (6)	15.29de (10)
UC-I 5–44	2.00h (18)	0.66def (9)	0.51c (3)	0.29defg (11)	4.71ef (11)	38.61a (1)
UC-I 6–9	5.17d (9)	0.80bc (4)	0.38efg (11)	0.31bcde (8)	3.87g (17)	13.47e (12)
UC-I 7–8	12.67b (2)	1.09a (1)	0.57b (2)	0.27gh (16)	3.75g (18)	5.09f (17)
UC-I 7–34	2.46gh (17)	0.63ef (15)	0.34hij (14)	0.32b (4)	5.28b (4)	31.94b (3)
UC-I 7–60	4.36def (13)	0.72cde (6)	0.42d (6)	0.29efgh (12)	4.75de (9)	17.53d (7)
UC-I 8–7	11.38b (5)	0.87b (3)	0.35ghi (13)	0.32b (5)	4.59ef (14)	6.02f (14)
UC-I 8–20	5.02d (10)	0.66def (10)	0.32ij (17)	0.28fgh (14)	5.09bcd (7)	15.11de (11)
UC-I 9–67	4.88d (11)	0.65ef (13)	0.60a (1)	0.32bc (6)	4.79cde (8)	16.38de (8)
UC-I 10–30	10.03c (6)	0.56fg (17)	0.39def (10)	0.26h (17)	4.53ef (15)	7.30f (13)
UC-I 10–54	4.56de (12)	0.71cde (8)	0.36fgh (12)	0.36a (2)	6.06a (1)	18.08d (6)
UC-I 11–10	2.53gh (16)	0.66def (11)	0.41de (8)	0.28fgh (13)	4.65ef (12)	31.95b (2)
UC-I 11–16	5.33d (7)	0.71cde (7)	0.43d (5)	0.37a (1)	5.77a (3)	15.33de (9)

Different letters within a column indicate significant differences at $P \leq 0.05$; ranking order in parentheses.

^a Total unsaturated/saturated fatty acid (U/S) and oleic/linoleic acid (O/L) ratios.

highly significant correlation was obtained for C16:1 ($r = 0.83$, $P < 0.001$), C18:0 ($r = 0.95$, $P < 0.001$), C18:1 ($r = 0.74$, $P < 0.01$) and C18:2 ($r = 0.90$, $P < 0.001$). A non-significant correlation was found only for C16:0 ($r = 0.49$, $P = 0.09$).

DISCUSSION

Many works indicate that the fatty acid composition of olive oil depends primarily on genetic factors, with most cultivars displaying a particular fatty acid composition which, together with other compounds, can be used to differentiate monovarietal olive oils.^{12,13} A wide variability of fatty acid composition has been reported in olive cultivar collections.^{14–16} However, for some important olive cultivars an unfavourable fatty acid composition has been reported as the main disadvantage, even imposing potential qualitative restrictions for market according to International Olive

Oil Council (IOOC) regulations.¹⁷ In such cases, crossbreeding has been considered the best strategy to provide new cultivars with improved fatty acid composition. Owing to the high level of heterozygosity of the species, any cross combination provides a wide range of variation for any characteristic, including fatty acid composition, as large as or even slightly larger than that previously observed in olive cultivar collections.^{8,18,19} As a consequence, some new cultivars showing improved oil composition have recently been released.^{17,20,21} Selections from wild populations have also been used to explore the genetic variability of the species.²²

In the present study the fatty acid composition was evaluated in 15 advanced selections obtained by crossbreeding in Córdoba, Spain. A strong genetic effect and significant differences between genotypes were obtained for all fatty acids and ratios evaluated (Table 2). Regarding the three cultivars used as

a control, 'Arbequina' and 'Frantoio' oils were characterised by a high C18:2 content and a low C18:1 content, whereas 'Picual' oil showed the opposite. These results are similar to those previously reported for the three cultivars in comparative field trials and cultivar collection evaluations performed in southern Spain.^{15,23}

The 15 selections evaluated extended the range of variation for all fatty acids, in some cases exceeding the range expected for olive oil by the IOOC,²⁴ such as C16:1 >3.5% for 'UC-I 6-9' and C18:3 >1.0% for 'UC-I 7-8'. In the initial population of 748 seedlings from which the 15 genotypes were selected, progenies showed a wide range of variation for fatty acid composition, with C16:0, C18:1 and C18:2 contents of 7.9–21.6, 43.5–84.7 and 1.6–29.2% respectively.⁸ In this work a C18:1 content of up to 78.26% and a C18:2 content as low as 2.00% were found in some of the advanced selections evaluated, providing an O/L range from 4.65 for 'UC-I 1-19' to 38.61 for 'UC-I 5-44' (Table 3). A high O/L ratio is associated with high stability and low rancidity of olive oil.⁴ It also seems to affect, in combination with other minor components, the flavour and health properties of olive oil.²⁵

PCA performed with average data by cultivar allowed the separation of four groups in the score biplot of PC1 and PC2 according to the fatty acid composition of cultivars (Fig. 2). These components reflected the same relationships among fatty acids as reported previously in olive.^{4,26} The percentages of C18:1, C18:2 and saturated fatty acids were the main contributors of variation in PC1 and PC2 and therefore in the distribution of cultivars in different groups. Similar results have been reported in the evaluation of fatty acid composition of olive oils from cultivar collections. Tous *et al.*¹⁴ and Uceda *et al.*¹⁵ also separated four groups from the evaluation of 28 and 78 monovarietal olive oils respectively by their fatty acid composition. In both cases the main criteria for classification into different groups were similar to those found in the present study. No special grouping in the PCA was observed according to the paternity of selections. All of them were distributed randomly, indicating that a high variability was obtained in all cross combinations used.

The results obtained previously in the initial seedling population and those obtained in this advanced selection trial showed a highly significant correlation for C16:1, C18:0, C18:1 and C18:2, indicating that selection for these characteristics could be performed efficiently at the seedling stage. Similar results have been obtained for other characteristics such as length of unproductive period, fruit weight, oil content and fruit removal force/fruit weight ratio.^{27,28} Previous results obtained at the seedling stage showed that variance due to yearly differences was negligibly small and that genotype variance was the main contributor to total variance for all fatty acids.²⁹ Therefore an efficient selection for fatty acid composition could

be carried out by considering only a single year of evaluation at the seedling stage.

In conclusion, the results reported in this paper show a quite different fatty acid composition of 15 advanced selections from an olive-breeding programme, allowing the classification of genotypes into four groups according to their fatty acid composition. Further multisite selection trials will help to confirm the reported fatty acid composition differences of the selections. Additionally, it has been demonstrated that early selection for fatty acid composition at the seedling stage could be performed efficiently in olive-breeding programmes.

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