CSIRO PUBLISHING
www.publish.csiro.au/journals/ajar Australian Journal of Agricultural Research, 2008, **59**, 46–51

Ripening time and fruit characteristics of advanced olive selections for oil production

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Abstract. In the olive breeding program of Córdoba, Spain, the first 15 obtained selections have recently been tested in a comparative field trial, including their genitors, 'Arbequina', 'Frantoio' and 'Picual', as a control. In this work, we report the evaluation of the fruit characteristics and ripening time of those selections. Average data recorded in the comparative field trial orchard in two consecutive harvest seasons were used for statistical analysis. A high degree of variability and significant differences between genotypes were obtained for all the fruit characteristics analysed, and for ripening time and extractability index. For most of those parameters, selections showing better values than the genitors have been found. In particular, many of the selections had higher oil content than the genitors. Data for seedling plants evaluation was significantly correlated with data of the reported field trial for fruit weight, fruit removal force/fruit weight ratio and oil content. This indicates that selection in the seedling stage for these characters can be efficiently performed, even when only the first year of agronomic evaluation of the seedling is considered. On the contrary, seedling selection for fruit moisture, fruit removal force and ripening date seems to be difficult to perform. In summary, the results of the agronomic evaluation suggest that some of the selections could be released as new olive cultivars in the near future, for first time in Spain.

Additional keywords: breeding, mechanical harvesting, oil content, *Olea europaea* L., selection criteria.

Introduction

Changes in olive cultivation in recent years have motivated the spreading of some of the most outstanding cultivars outside its original area of cultivation. However, in many cases, introduction of new cultivars have not been preceded by systematic evaluation of their adaptation to the new agronomic conditions. Several studies show that the agronomic and quality characters of an olive cultivar can change dramatically depending on the area of cultivation (Tous and Romero 1994; Tous *et al*. 1997). In this sense, comparative field trials are the most efficient way to determine the best suited cultivars in any specific area of cultivation. In Spain, however, comparative field trials have not been extensively used until recent years and published results are scarce.

Testing of advanced selections is an important step in fruit breeding as information from testing trials informs breeders if new cultivars are likely to fulfill their intended use (Harding 1983). In olive, few breeding programs by crossing and selection in the progenies have been initiated in the past decades (Lavee 1990; Bellini 1992). This has intensified in recent years due to the development of procedures to shorten the juvenile period (Lavee *et al*. 1996; Santos-Antunes *et al*. 2005). As a consequence, several new cultivars have been released such as 'Barnea' (Lavee *et al*. 1986), 'Fs-17' (Fontanazza *et al*. 1998), 'Maalot' (Lavee *et al*. 1999), 'Arno', 'Tevere' and 'Basento' (Bellini *et al*. 2002) and 'Askal' (Lavee *et al*. 2003). Comparative field trials of advanced selections from breeding programs are currently carried out in several olive producing countries. Fruit characters such as fruit weight, oil content and pigmentation index are usually included in these evaluations (Lavee *et al*. 2003; Pannelli *et al*. 2006). Others, such as pulp/pit ratio, shape, pulp firmness and content of reducing sugars are also included when obtaining new cultivars for table or dual purposes is the main objective of the breeding program (Bartolini *et al*. 2006; Ripa *et al*. 2006).

In Spain, an olive breeding program aimed at obtaining new cultivars for olive oil production was initiated in 1991 in Córdoba (Rallo 1995). Fifteen genotypes were selected in an initial population of 748 seedlings on the basis mainly of their early crop (short juvenile period) and high oil content during three consecutive harvest seasons. Other characters such as fruit weight, fruit removal force and ripening time were also evaluated in the initial seedling population (León *et al*. 2004, 2005). The aim of this work was to evaluate the initial fruit characteristics in a comparative field trial with these 15 advanced selections obtained in the olive cross-breeding program of Córdoba and the three genitors as controls, and to test the efficiency of the criteria applied for selection in the seedling stage.

Materials and methods

Plant material

Selections evaluated in this work come from the first set of crosses among 'Arbequina', 'Frantoio' and 'Picual' cultivars,

 10.1071/AR07142 0004-9409/08/010046

carried out in the olive cross-breeding program of Córdoba in 1992 and 1993. Previous results of their agronomic evaluation, at the seedling stage, have been already reported (León *et al.* 2004, 2005).

These 15 selected genotypes, together with the three parents used as controls, were established in open field in July 2001 after vegetative propagation of adult semi-hardwood stem cuttings in spring 2000. Trial orchard was located in Córdoba, Spain, in a soil classified as Typic Xerofluvent of sandy-loam texture with climatic conditions typical of the Mediterranean area (Fig. 1). A randomised block design with 16 replications and one tree per elementary plot was used at 5 m distance between trees in a row and 6 m between rows. Several losses due to rodent damage occurred after the second year in open field although a minimum number of 13 trees per selection remained available. Fruit characters were systematically evaluated in two consecutive harvest seasons (2004 and 2005) in the comparative field trial orchard.

Traits evaluated

In both the initial seedling population and the comparative field trial orchard, ripening time of fruit was recorded according to the ripening index described by Frías *et al*. (1991). This method is based on colour changes of peel and pulp classified into eight groups or categories: green intense (0); yellow or yellowish green (1); green with reddish spots (2); reddish or light violet (3); black with white pulp (4); black with $<$50% purple flesh (5); black with $\geq$50% purple flesh (6); and black with 100% purple flesh (7). Ripening observations were carried out around the canopy at bi-weekly intervals from September and characterised by three numbers representing the most delayed, abundant and advanced categories observed, respectively. From these determinations, ripening was characterised by three dates expressed as days after 1 September (Barranco and Rallo 2005), initial ripening (first time in which category 2 is the most advanced category observed), middle ripening (average date in which the most abundant category observed change from 2 to 3), and end of ripening (first time in which category 4 is the most abundant or category 3 is the most delayed).

Samples of 50 fruits were collected at the end of the ripening period with a hand dynamometer Correx 2000 (Haag-Streit, Switzerland) to determine fruit removal force. Afterwards, three samples per plant were prepared to provide data on oil content components. Average fruit fresh weight was measured and then samples were dried in a forced-air oven at 105°C for 42 h to ensure dehydration. Dried samples were weighed to determine moisture content and the oil content was determined using an NMR fat analyser and expressed as a percentage on both fresh and dry weight basis. In the second harvest season, samples were also collected to determine oil extractability using an Abencor laboratory oil mill (Martínez-Suárez *et al*. 1975). This equipment consists of three basic elements: a hammer crusher, a thermo beater and a paste centrifuge. Four samples of 700 g of olive paste per tree and 3–4 trees per genotype were analysed. Extractability index (EI) was calculated using the formula: $EI = 100 \times V \times d/W \times F$, where V (mL) is the olive oil volume extracted; d is the mean olive oil density (0.915 g/mL); W (g) is the olive paste weight; and F (%) is the fruit oil content (fresh weight) measured by the NMR fat analyser.

Data analysis

Average data recorded in the comparative field trial orchard in two consecutive harvest seasons were used for statistical analysis. Data recorded were subjected to analysis of variance to test the effect of cultivars and separation of the means was obtained at $P \leq 0.05$. Correlations between measurements obtained in the initial seedling population (taking into account 1-, 2-, or 3-year data) and the comparative field trial orchard were also evaluated.

Results and discussion

Ripening time

Genotypic variance was the main contributor to total variance. Genotype effect accounts for 59–68% of total sums of squares in the analysis of ripening dates and for 46% for the length of the ripening period. The differences among genotypes were highly significant in all cases (Table 1). Average values for

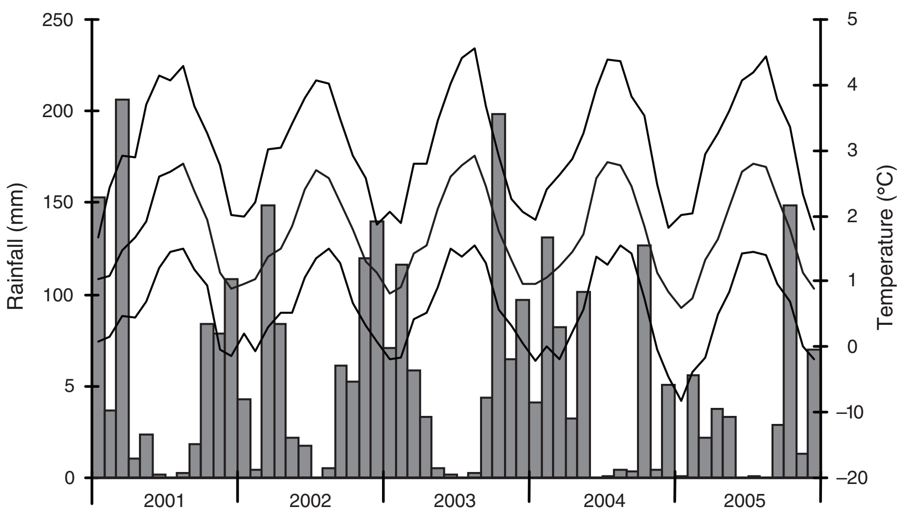

Fig. 1. Monthly average of rainfall (bars) and minimum, average and maximum temperature during the years of the comparative trial.

Table 1. Percentage of total sum of squares and significance in the ANOVA for ripening dates for olive (days after 1 September)
*$P < 0.05$, ***$P < 0.001$. n.s., not significant

	Initial	Middle	End	Length
Genotype	68.06***	63.30***	59.03***	46.35***
Block	2.28n.s.	4.45*	6.89***	7.71***
Error	29.65	32.25	34.08	45.94
CV (%)	26.10	21.09	16.98	24.85
Mean	33	71	91	60

initial, middle and end of ripening date were 33, 71, and 91 days after 1 September (4 October, 11 November, and 30 November, respectively) with an average length of 60 days.

A high degree of variability was also obtained for ripening dates (Table 2). For instance, the range of variation for middle ripening date varied from 37 days ('UC-I 1-19') to 112 days ('UC-I 9-67') after the 1 September (75 days interval), similar to the range reported for 130 cultivars in the World Olive Germplasm bank of Córdoba (Barranco and Rallo 2005). It should be noted that ripening date was not taken into account in the selection of genotypes in the seedling stage as a wide range of variation for this character in the selections was considered interesting.

Figure 2 summarises the ripening dates and length of the ripening period obtained by genotype. A general trend of shorter ripening period length in early ripening genotypes can be observed. In fact, these two characters (end of ripening date and length of the ripening period) were highly correlated ($r = 0.76$, $P < 0.001$).

Fruit characteristics

Genotypic variance was the main contributor to total variance for all the fruit characteristics analysed, being the differences among genotypes highly significant in all cases (Table 3). Genotype effect accounted for 30–84% of total sum of squares in the analysis of variance with the highest values for fruit fresh weight and oil content in fruit dry weight basis. Similar results have been obtained for fruit fresh weight and oil content in fruit dry weight basis in the evaluation of olive cultivars collections, although only 2–3 trees per cultivar were available (Del Río *et al*. 2005; Tous and Romero 2005).

A high degree of variability and significant differences between genotypes were obtained for all the fruit characteristics analysed (Table 4). The results obtained for the three genitors ('Arbequina', 'Frantoio' and 'Picual') agree in general terms with the previously reported for them in comparative field trials and cultivar collection evaluations performed in South Spain (Caballero *et al*. 2005; Del Río *et al*. 2005).

Average fruit weight and fruit removal force ranged from 1.6 to 5.5 g and 170 to 350 g in 'Arbequina' and 'UC-I 7-60', respectively. A high correlation between these two characters was reported in cultivar evaluations (Lavee *et al*. 1982; Tous and Romero 1993). Fruit removal force/fruit weight ratio, which is proportional to the acceleration to be imposed for the fruit detachment, has been used for the classification of cultivars according to the ease of detachment, and therefore for their potential mechanical harvesting aptitude by trunk shakers. In fact, a good correlation between fruit removal force/fruit weight ratio and harvest efficiency by trunk shakers has been reported (Antognozzi *et al*. 1990). In this work, 'UC-I 11-10', 'Picual', 'UC-I 2-68', and 'UC-I 4-62' showed the lowest values for this ratio, and therefore the best theoretical suitability for mechanical harvesting. On the contrary, 'Arbequina' and 'Frantoio' showed the highest values and therefore poor potential adaptation to mechanical harvesting. In any case, the values obtained here (from 51 to 113 between selections) were lower than the previously reported in the evaluation of cultivar collections: ranges from 77 to 275 in 17 cultivars from Catalonia and from 110 to 600 in 39 Italian cultivars have been reported (Preziosi and Tini 1990; Tous and Romero 1993).

The average oil content also varied widely between genotypes, with the selections 'UC-I 4-62' and 'UC-I 10-30'

Table 2. Mean values by cultivar for ripening dates for olive (days after 1 September)
Within columns, values followed by the same letter are not significantly different at $P > 0.05$. Ranking order in brackets

Genotype	Cross[A]	Initial	Middle	End	Length
'Arbequina'		33.3de (10)	82.3bcd (7)	104.1bcd (7)	70.9b (4)
'Frantoio'		14.3hi (17)	37.2i (17)	61.2ij (17)	46.9f (17)
'Picual'		43.1bc (4)	86.9bc (4)	107.6bc (4)	64.6bcde (7)
'UC-I 1-19'	F × P	9.8i (18)	37.0i (18)	53.5j (18)	66.8bcd (6)
'UC-I 2-68'	P × A	48.0ab (3)	86.0bc (5)	99.6bcde (8)	51.5f (13)
'UC-I 4-62'	F × P	34.6de (8)	83.0bcd (6)	105.8bcd (5)	71.3b (3)
'UC-I 5-44'	P × A	29.9ef (11)	57.3gh (15)	77.1gh (13)	47.3f (16)
'UC-I 6-9'	A × P	20.9g (15)	71.7def (9)	105.3bcd (6)	84.4a (2)
'UC-I 7-8'	A × P	26.3fg (12)	57.7gh (14)	73.8h (15)	47.5f (15)
'UC-I 7-34'	P × A	38.8cd (7)	59.6fg (13)	74.1h (14)	35.2g (18)
'UC-I 7-60'	F × P	38.9cd (6)	67.8efg (10)	88.6efg (11)	49.6f (14)
'UC-I 8-7'	P × A	41.7bc (5)	78.4cde (8)	94.8cdef (9)	53.1ef (11)
'UC-I 8-20'	P × A	22.7g (14)	66.8efg (11)	93.2def (10)	70.6bc (5)
'UC-I 9-67'	A × P	34.2de (9)	112.2a (1)	126.5a (1)	92.3a (1)
'UC-I 10-30'	F × P	19.8gh (16)	47.4hi (16)	71.9hi (16)	52.1ef (12)
'UC-I 10-54'	A × P	52.1a (2)	94.1b (2)	111.9b (2)	59.8bcdef (8)
'UC-I 11-10'	A × P	26.1fg (13)	65.7fg (12)	84.4fgh (12)	58.4cdef (9)
'UC-I 11-16'	P × A	52.5a (1)	90.4bc (3)	110.1b (3)	57.6def (10)

[A]Genitors: A = 'Arbequina'; F = 'Frantoio'; and P = 'Picual'.

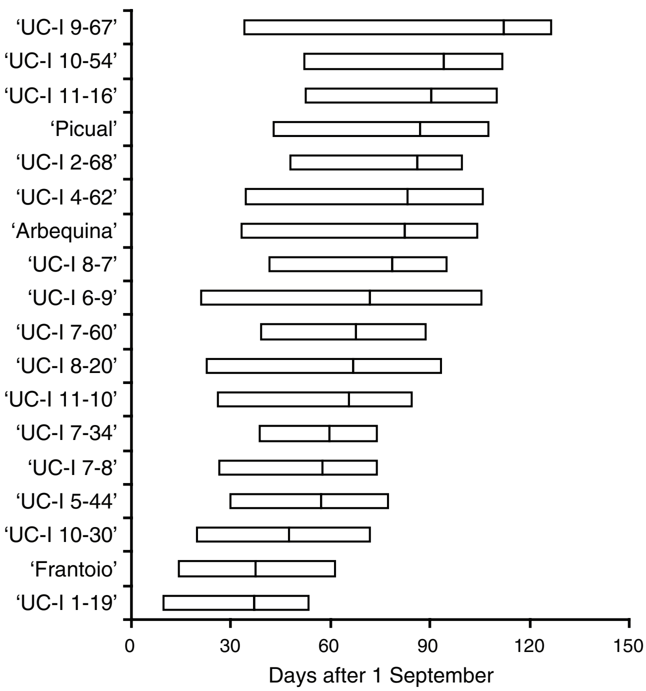

Fig. 2. Ripening dates (initial, middle and end) and length of the ripening period obtained by olive genotype.

showing the highest oil content, with an average value around 25% and 53% on fruit fresh and dry weight basis, respectively (Fig. 3). 'UC-I 7-8' and 'Frantoio' showed the lowest values for oil content in fruit fresh and dry weight basis, respectively. Oil content in fruit dry weight basis previously reported in the evaluation of cultivar collections ranged from 21 to 54% in 104 cultivars of the World Olive Germplasm bank of Córdoba (Del Río and Caballero 1994) from 30 to 53% in 35 cultivars from Catalonia (Tous and Romero 1993) and from 21 to 51% in 39 Italian cultivars (Preziosi and Tini 1990). In those cases, however, only 22, 28 and 18%, respectively, of the cultivars evaluated in these studies showed values of oil content in fruit dry weight basis higher than 46%, which contrast with 10 out of the 15 selected genotypes evaluated in this work (66%), as oil content, together with short juvenile period, was the main criteria used for selection of these genotypes in the seedling stage (León *et al*. 2004).

Mean extractability index was 0.59, ranging from 0.53 ('UC-I 7-8') to 0.76 ('UC-I 4-62'). A high correlation between extractability index and the fruit oil content (fresh weight) measured by the NMR fat analyser was found ($r = 0.74$, $P < 0.001$), i.e. a higher ease of oil extraction would be expected in genotypes with high oil content. However, a higher extractability index than expected according to their oil content measured by the NMR fat analyser was found in several genotypes such as 'UC-I 7-60', 'Picual' and 'UC-I 9-67' (Fig. 3). On the contrary, genotypes such as 'UC-I 7-34', 'UC-I 8-20' and 'UC-I 10-30' showed lower extractability index than expected, and therefore are more likely to produce emulsion and the so-called 'difficult pastes'. In a sample of 24 olive cultivars from the Olive Germplasm bank Collection of Mengíbar (Spain), mean values of extractability index ranged from 0.31 to 0.73 with the largest number of cultivars grouped in the intervals 0.4–0.6 (41.7%) and 0.6–0.8 (45.8%) (Beltrán *et al*. 2003). These authors, however, found no significant correlation between olive fruit parameters, including oil content measured by the NMR fat analyser, and extractability index. In both cases, the Abencor system has been used for oil extraction.

Efficiency of the selection criteria

Results obtained in the comparative field trial of adult vegetative-propagated plants were compared with the previously obtained for these genotypes in the seedling stage. For those seedlings, data recorded in three consecutive harvest seasons were available. Correlation was studied between the field trial data, and (1) seedling data of only the first harvest season data; (2) the average of the two first harvest season; and (3) the average values obtained in the three harvest season (Table 5).

A high significant correlation was obtained for fruit weight, fruit removal force/fruit weight ratio and oil content (particularly in fruit dry weight basis), which indicate that selection in the seedling stage for these characters can be efficiently performed. A similar correlation was found between the juvenile period of seedlings and the unproductive period of the subsequent vegetative propagated adult shoots (Santos-Antunes *et al*. 2005; León *et al*. 2007). Correlations were significant independently of the number of harvest seasons considered for seedling data. Therefore, if those results were confirmed in further experiments, only one year of evaluation in the seedling stage would be enough for an efficient selection on the basis of these characters. On the contrary, no correlation between seedling and comparative field trial data was found for fruit moisture, fruit removal force and end of ripening date. Therefore, selection for these characters at the seedling stage might be more difficult. This was also consistent independently of the number of years of seedling evaluation. These results confirm previous findings

Table 3. Percentage of total sum of squares and significance in the ANOVA for olive fruit characters
$^*P < 0.05$, $^{***}P < 0.001$

	Fruit weight (g)	Fruit removal force (g)	Fruit removal force/fruit weight	Oil content (fruit fresh weight) (%)	Moisture (%)	Oil content (fruit dry weight) (%)
Genotype	84.38***	30.61***	46.30***	48.41***	39.78***	62.20***
Block	1.60*	23.71***	20.85***	12.96***	13.12***	6.53***
Error	14.02	45.67	32.84	38.63	47.09	31.27
CV (%)	13.24	21.06	21.45	12.34	6.80	5.91
Mean	3.24	242.57	83.44	19.89	58	47

Table 4. Mean values by cultivar for olive fruit characters
Within columns, values followed by the same letter are not significantly different at $P > 0.05$. Ranking order in parentheses

Genotype	Cross[A]	Fruit weight (g)	Fruit removal force (g)	Fruit removal force/fruit weight	Oil content (fruit fresh weight) (%)	Moisture (%)	Oil content (fruit dry weight) (%)
'Arbequina'		1.61i (18)	170.8g (18)	111.1a (2)	19.97de (8)	55.39efgh (14)	44.29g (15)
'Frantoio'		2.53fgh (15)	271.6bc (4)	113.6a (1)	16.45h (17)	57.31bcde (11)	38.06i (18)
'Picual'		4.86b (2)	251.7bcde (8)	55.9gh (17)	18.18efgh (14)	60.63b (2)	45.92efg (12)
'UC-I 1-19'	F × P	2.53fgh (14)	267.8bc (5)	106.3ab (4)	20.37cd (6)	59.45bc (6)	49.83b (3)
'UC-I 2-68'	P × A	3.25cd (7)	190.5fg (17)	61.7fgh (16)	19.72def (10)	58.88bcd (7)	47.77bcde (7)
'UC-I 4-62'	F × P	4.58b (4)	272.6bc (3)	63.4efgh (15)	24.77a (2)	53.54fghi (15)	53.32a (1)
'UC-I 5-44'	P × A	3.01de (10)	281.6b (2)	93.8bc (6)	20.28cd (7)	56.65cdef (12)	46.58def (10)
'UC-I 6-9'	A × P	2.77ef (11)	226.3cdef (13)	80.8cd (8)	19.43def (11)	58.87bcd (8)	47.25cde (8)
'UC-I 7-8'	A × P	3.31cd (6)	243.4bcde (11)	76.5def (12)	14.36i (18)	66.24a (1)	41.92h (16)
'UC-I 7-34'	P × A	2.41gh (16)	249.6bcde (9)	107.3ab (3)	17.34gh (16)	58.74bcd (9)	41.67h (17)
'UC-I 7-60'	F × P	5.45a (1)	350.6a (1)	69.5defg (14)	17.73fgh (15)	60.17b (4)	44.55fg (13)
'UC-I 8-7'	P × A	2.70efg (12)	210.3defg (15)	80.3cde (9)	19.42def (12)	60.22b (3)	48.48bcd (6)
'UC-I 8-20'	P × A	3.16cd (9)	249.5bcde (10)	80.0cde (10)	20.63cd (5)	58.45bcde (10)	49.48bc (4)
'UC-I 9-67'	A × P	3.17cd (8)	253.8bcde (7)	79.9cde (11)	22.78b (3)	53.41ghi (16)	48.68bcd (5)
'UC-I 10-30'	F × P	3.37c (5)	242.9bcde (12)	75.5def (13)	24.94a (1)	52.74hi (17)	52.75a (2)
'UC-I 10-54'	A × P	2.58fgh (13)	258.6bcd (6)	104.8ab (5)	22.11bc (4)	52.03i (18)	45.96efg (11)
'UC-I 11-10'	A × P	4.58b (3)	224.7cdef (14)	51.3h (18)	19.24defg (13)	59.49bc (5)	47.10de (9)
'UC-I 11-16'	P × A	2.28h (17)	208.2efg (16)	93.8bc (7)	19.76def (9)	56.08defg (13)	44.44fg (14)

[A]Genitors: A = 'Arbequina'; F = 'Frantoio'; and P = 'Picual'.

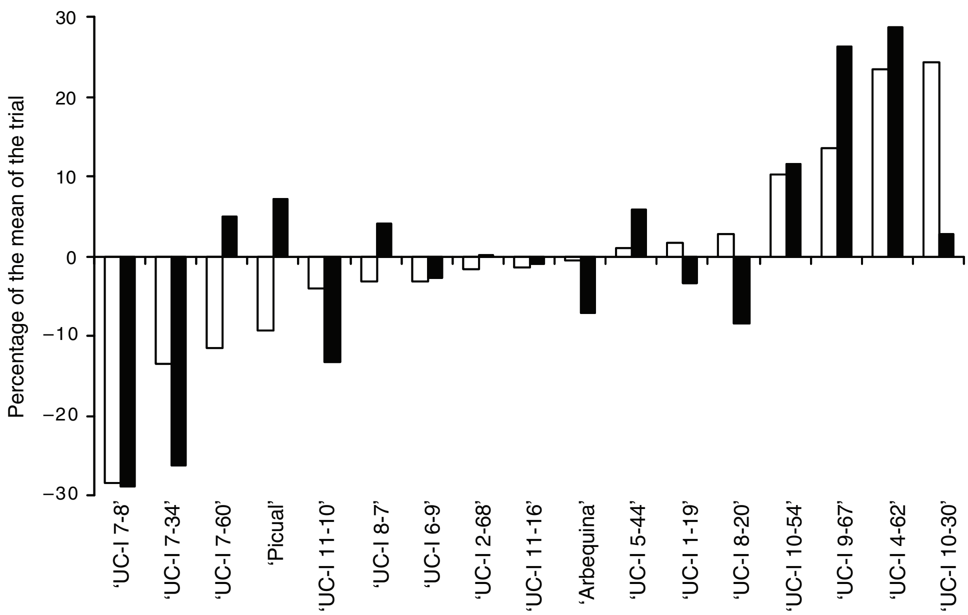

Fig. 3. Oil content on fresh weight basis measured by the NMR fat analyser (white) and extractability index (black) by genotype of olive. Values are expressed as percentage of the mean of the trial 2.

of our breeding program, in which the number of years needed for an accurate evaluation of seedlings were estimated on the basis on genetic and environmental variances, repeatability and consistency of data across years (León *et al.* 2004, 2005).

In summary, the evaluation of the initial fruit characteristics of 15 advanced selections obtained in the olive cross-breeding program of Córdoba and their three genitors has revealed a high degree of variability and significant differences between genotypes for all the fruit characteristics analysed, as was previously reported for productivity and vigour traits (León *et al.* 2007). For characters such as fruit weight, fruit removal force/fruit weight ratio and oil content, our data are indicative of the efficiency of the selection criteria used in our breeding project for these traits. Moreover, some of the selections tested showed better values than the genitors used as controls for these characters. Further evaluation would be required in the following years to obtain a proper commercial evaluation of cultivars once the trees reach their full bearing stage. Confirmation of these results will provide new olive

Table 5. Correlation coefficients between the fruit character data obtained in the seedling stage and the comparative trial, according to the number of crops evaluated at the seedling stage for olive
$^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. n.s., not significant

Fruit character	Number of crop data averaged at the seedling stage		
	1st	1st and 2nd	1st, 2nd and 3rd
Fruit weight (g)	0.886***	0.876***	0.902***
Fruit removal force (g)	0.389n.s.	0.104n.s.	0.093n.s.
Fruit weight/fruit removal force	0.714***	0.913***	0.932***
Oil content (fruit fresh weight) (%)	0.664*	0.757**	0.727**
Moisture (%)	0.308n.s.	0.591*	0.493n.s.
Oil content (fruit dry weight) (%)	0.885***	0.864***	0.907***
End of ripening (days after 1 Sep.)	0.081n.s.	0.219n.s.	0.427n.s.

cultivars, obtained for the first time in Spain from cross-breeding programs.

Acknowledgments

This work has been partly supported by project RTA2005-00031-C02-01, National Institute of Agricultural Research (INIA), Ministry of Education and Culture, Spain and by project CAO00-018-C7-2, Junta de Andalucía, Spain.

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Manuscript received 4 April 2007, accepted 3 September 2007