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# Variability and early selection on the seedling stage for agronomic traits in progenies from olive crosses

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## Abstract

Yield per tree, ripening date and oil content components (fruit fresh weight, flesh moisture, flesh/stone ratio both on fresh and dry weight basis, flesh and fruit oil content on dry weight basis) have been studied during 3 years in seedlings from crosses among the olive cultivars 'Arbequina', 'Frantoio' and 'Picual'. Genetic and environmental variances and year-to-year consistency of data were estimated. Most of the traits evaluated showed a range of variability as large or even larger than either the range observed in a random sample of cultivars from the World Olive Germplasm Bank of Cordoba or the range reported in the evaluation of olive cultivars collections. Between-years correlation coefficients showed that for a character such as oil content the values obtained in the first year could be reliable indicators of the values obtained in following years. Observations over 2 years may be required for characters such as fruit weight or flesh/stone ratio on a fresh weight basis and even more than 2 years may be required to estimate yield per tree.

**Key words:** *Olea europaea* — fruit characters — oil content — repeatability — variance components

The diversity, antiquity and restricted distribution outside their areas of origin characterize olive cultivars in Spain (Barranco and Rallo 2000). None of the cultivars presently grown come from cross breeding programmes, because the few that have been conducted did not give any positive result (Humanes et al. 1967). For this reason an intraspecific cross-breeding programme was initiated in 1992 in order to obtain new olive cultivars. This programme aims at obtaining cultivars that include at least some of the following traits: early bearing, high productivity, high oil content, and high oleic acid percentage. Other traits such as suitability for mechanical harvesting and resistance to peacock eye are also systematically evaluated (Rallo 1995).

The evaluation of progenies and selection of the most interesting genotypes come in the last stages of breeding programmes. Early evaluation and selection at the seedling stage decreases generation time and increases the success of the breeding effort, which is almost directly related to the space occupied by the progenies and the time used to evaluate them (Hansche 1983). Moreover, collecting data is expensive so unwanted seedlings must be eliminated as soon as possible (Allard 1960) but early elimination is only possible if the first data collected are accurate enough to predict performance in the following years.

Estimates of genetic variance, repeatability and consistency of data are interesting parameters because of their influence on the efficiency of selection and the cost of handling a large number of seedlings. Estimates of genetic and environmental components of variation for quantitative traits have been applied successfully in many fruit and nut crops (Janick and Moore 1996) but this information is still limited in olive. Some authors have studied the inheritance of several characters (Lavee 1990, Bellini 1992, Fontanazza et al. 1999) but no studies concerning the relative contributions of genetic and environmental variability to the phenotypic expression of quantitative characters in olive progenies have been reported.

The aims of this work are to describe the variability observed in olive progenies for several agronomical traits and to estimate genetic and environmental variances and year-to-year consistency for these traits in order to improve the efficiency of olive breeding programmes.

## Materials and Methods

**Plant materials:** Seedlings from crosses (nine combinations) among the 'Arbequina', 'Frantoio' and 'Picual' cultivars of olive, *Olea europaea* L., have been used in this study. Parents were chosen on the basis of high productivity and oil content, different geographical origin ('Arbequina' from Catalonia, Spain; 'Frantoio' from Tuscany, Italy; and 'Picual' from Andalusia, Spain), and differences in earliness of bearing and oleic acid content (Rallo 1995). A total of 288 seedlings from crosses made in spring 1992 and transplanted into an open field (after a period of forced growth in a greenhouse) in April 1994 were used in this study. The experimental design was in randomized blocks with nine crosses, four blocks and eight plants for a plot. In addition, a random sample of 40 cultivars (including the three parents of the breeding programme) from the World Olive Germplasm Bank, established at the experimental farm of CIFA 'Alameda del Obispo' of Cordoba, has been evaluated each year for oil content components. These cultivars are assumed to be representative of the whole variability of the Germplasm Bank for comparison with results from the progenies.

**Characters evaluated:** Fruits were harvested at a similar ripening index, as fruit traits are influenced by the ripening stage. The ripening index is a colour measurement of the fruit (skin and flesh) scored on a scale from 0 to 7 (Frías et al. 1991). In this work, samples were collected for analytical determination once enough olive fruits corresponding to the category 4 (black skin and white flesh) were observed.

Ripening date, expressed as days after September 1, and total yield per tree were recorded at the above-mentioned stage.

Three samples of 50 fruits per seedling each were prepared to provide data on oil content components. Average fruit weight was measured and, after removing and cleaning the stones, flesh and stone weight were also recorded. Afterwards, three 70 g samples of unmilled flesh were dried in a forced-air oven at 105°C for 42 h, to ensure dehydratation. After weighing the dried samples to determine the moisture content, the oil percentage was measured in an NMR OXFORD 4000 analyzer (Oxford Analytical Instruments, Abingdon, England) equipped with a 150 ml sample vase. The analysis mode of the NMR was as described by Del Río and Romero (1999). The flesh/stone ratio (both on a fresh and dry weight basis), and the fruit oil content on a dry weight basis were calculated from these measurements. Oil content components could not be evaluated on all seedlings because a minimum amount of fruits was required for these evaluations. Yield and ripening date were evaluated in 127, 162 and 241 seedlings in 1996, 1997 and 1998 respectively. Oil content components were evaluated in 82, 112 and 225 seedlings in 1996, 1997 and 1998 respectively.

**Data analysis:** Mean oil content components and the proportion of plants exceeding a certain threshold value in the progenies and the sample of cultivars from the Germplasm bank were compared by means of Student's *t*-test and $\chi^2$-tests. The average values over years for the parents were taken as threshold values. Analyses of variance were also performed with genotype and year as random effects, including only those genotypes that were evaluated in all years to avoid unbalanced data (140 genotypes for yield and ripening time data, and 69 genotypes for oil content components data). The additive linear model for the statistical analysis was: $P_{ij} = \mu + g_i + y_j + e_{ij}$, where $P_{ij}$ is the phenotypic value of the $i$th genotype in the $j$th year, $\mu$ is overall mean, $g_i$ is the genotype effect, $y_i$ is the year effect and $e_{ij}$ is the residual effect. From this model, repeatability ($r$) was estimated as $r = \sigma^2_G/(\sigma^2_G + \sigma^2_e/n_y)$, where $\sigma^2_G$ is the variance between genotypes, $\sigma^2_e$ is the residual variance and $n_y$ the number of years evaluated. Pearson's correlation coefficients between the normalized data obtained in the first and second, and first and third harvest season were also calculated to determine the consistency of year-to-year data.

## Results

The average annual yield per tree was above 4 kg, with a maximum value of almost 34 kg in some seedlings. The accumulated yield per tree increased up to almost 10 kg on average and a 42 kg maximum. The average ripening date was at the end of November, with seedlings ripening from mid September until the end of February. Mean values for fruit fresh weight, flesh moisture and flesh/stone ratio on a fresh weight basis were significantly higher in the sample of cultivars than in the progenies (Table 1). The proportion of plants exceeding the threshold value was also higher in the sample of cultivars (Table 1, Fig. 1). However, a wide range of variation was observed in the progenies for these characters. Some seedlings with a fruit fresh weight up to 7 g or a flesh/stone ratio up to 14, much higher than the best parent, have been observed. For the flesh/stone ratio on a dry weight basis there were no differences between the progenies and cultivars from the Germplasm Bank (Table 1, Fig. 1). However, the range of variation for this character was higher in the progenies than in the sample of cultivars from the Germplasm Bank (0.75–5.12 vs. 1.17–3.82). Finally, for the oil content (expressed as the percentage of flesh or fruit dry weight) a clear trend toward higher oil content in the progenies was observed, although no significant differences between the progenies and the sample of cultivars were obtained, based neither on the mean values nor by the percentage of plants surpassing the threshold values

Table 1: Mean values and percentage of plants exceeding a threshold value for the different characters evaluated in the progenies and in the sample of cultivars from the Germplasm Bank

| | Mean values | | | % Plants > threshold[1] | | |
|---|---|---|---|---|---|---|
| Traits | Progenies | Cultivars | $t$ | Progenies (n = 419) | Cultivars (n = 135) | $\chi^2$ |
| YPT | 4.3 | – | – | – | – | – |
| RD | 85.0 | – | – | – | – | – |
| FrFW | 2.9 | 4.4 | 14.09** | 56.7 | 82.1 | 32.22** |
| FM | 66.0 | 68.8 | 3.56** | 67.7 | 85.1 | 17.18** |
| F/S FW | 6.4 | 7.1 | 3.79** | 68.3 | 80.6 | 8.65** |
| F/S DW | 2.4 | 2.5 | 1.22 | 54.7 | 64.2 | 4.35* |
| OC FDW | 62.4 | 61.6 | 1.32 | 39.0 | 35.8 | 0.33 |
| OC FrDW | 43.7 | 43.3 | 0.45 | 23.1 | 20.9 | 0.53 |

YPT, yield per tree (kg); RD, ripening date (d.a. 1 Sept); FrFW, fruit fresh weight (g); FM, flesh moisture (%); F/S FW, flesh/stone ratio on fresh weight basis; F/S DW, flesh/stone ratio on dry weight basis (%); OC FDW, oil content on flesh dry weight basis; OC FrDW, oil content on fruit dry weight basis (%).
[1] Threshold value: average value of the parents for the different characteristics evaluated.
*, ** Significant at P = 0.05 and P = 0.01, respectively.

(Table 1, Fig. 1). Again it is worth noting that the wide range of variation obtained in the progenies, with some of them reaching oil contents up to 59 or 76% on a fruit or flesh dry weight basis respectively.

Analyses of variance based on genotypes and years are presented in Table 2. Variances because of yearly differences were high for yield per tree, fruit fresh weight, flesh moisture, and flesh/stone ratio on a fresh weight basis. Genotypic variance was the main contributor to total variance for the oil content, both on a flesh or fruit dry weight basis. Finally, for the flesh/stone ratio on a dry weight basis and ripening date, genotype and residual variances were equally important contributors to the total variance. Repeatability estimates from 3 years of data showed that the lowest values obtained were for yield per tree and flesh moisture (0.32–0.41), intermediate values were obtained for ripening date, fruit weight and flesh/stone ratio (0.72–0.75), and the highest values for oil content on both a flesh or fruit dry weight basis (0.78–0.82).

Yield per tree almost doubled from the first to the second year, and almost tripled from the second to the third year (Table 3). Fruit fresh weight and flesh/stone ratios were equal the first 2 years, but decreased drastically the third year. Oil content also decreased slightly over years but it seemed to stabilize. High correlation coefficients were obtained for the oil content, both on a flesh or fruit dry weight basis, between the first/second and first/third years (Table 3, Fig. 2a). Most of the genotypes evaluated were located in the first and third quadrants of the scatter plots between 2 years' normalized data, i.e. these genotypes had oil contents higher or lower than the mean respectively in both years. Low correlation coefficients were obtained between yield the first/second and first/third years (Table 3, Fig. 2b) and the same distribution of genotypes was obtained in each quadrant of the scatter plots. Correlation coefficient between second/third year data was 0.42, practically the same that obtained when the production of the first 2 years compared with the third (data not shown).

Intermediate results were obtained for the other characters evaluated. High correlation coefficients between the first/second year data and lower between the first/third year data were observed for fruit fresh weight, flesh moisture, and flesh/

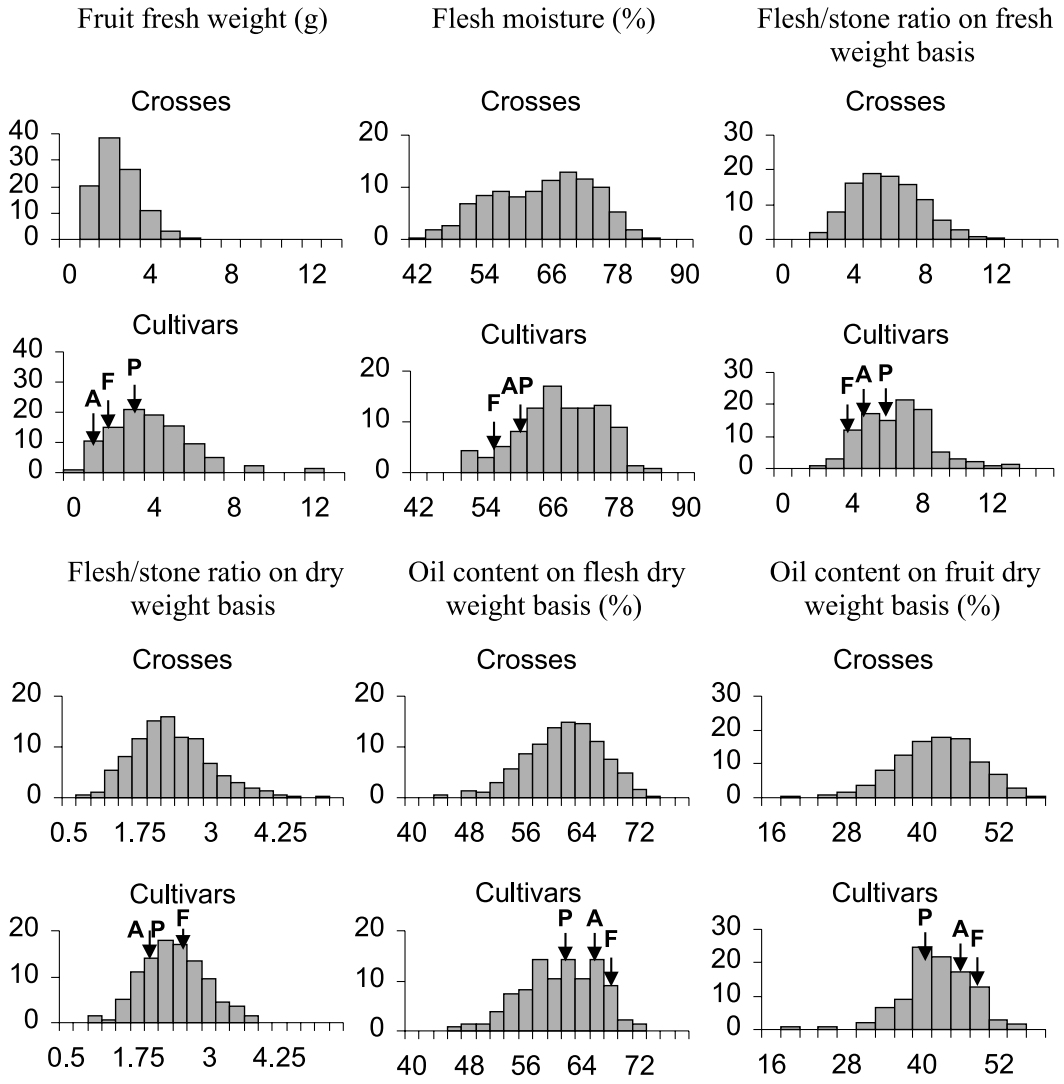


Fig. 1: Frequency distributions for oil content components in the progenies and in the sample of cultivars from the Germplasm Bank (values for parents are indicated by arrows: A, 'Arbequina'; F, 'Frantoio'; P, 'Picual')

Table 2: Mean square and repeatability for yield, ripening date and oil content components

| Source of variation | d.f.[1] | YPT | RD | FrFW | FM | F/S FW | F/S DW | OC FDW | OC FrDW |
|---|---|---|---|---|---|---|---|---|---|
| Mean square | | | | | | | | | |
| Genotype | 139/68 | 15** | 1995** | 1.1** | 49 | 3.6** | 0.60** | 55.4** | 76.5** |
| Year | 2/2 | 2890** | 24908** | 57.1** | 3185** | 204.7** | 3.60** | 857.5** | 927.5** |
| Residual | 278/136 | 8 | 510 | 0.3 | 34 | 1.0 | 0.15 | 10.2 | 16.6 |
| Repeatability[2] | | 0.41 | 0.74 | 0.74 | 0.32 | 0.72 | 0.75 | 0.82 | 0.78 |

For description of traits, see footnote in Table 1.
** Significant at P = 0.01.
[1] Degrees of freedom for YPT and RD (left), and oil content components (right).
[2] Repeatability estimated as $r = \sigma_G^2/(\sigma_G^2 + \sigma_e^2/n_y)$, where $\sigma_G^2$ is the variance between genotypes, $\sigma_e^2$ is the residual variance and $n_y$ the number of years evaluated.

stone ratio both on a fresh or dry weight basis. The opposite trend was obtained for ripening date (Table 3).

## Discussion

High yield is the main goal for any plant breeder although, because of its complicated measurement in fruit species, it is not usually included in fruit breeding programmes until the last stages. Promising results have been obtained in this work, with some seedlings cropping up to 42 kg in the first three harvest seasons (5 years after planting at most). The Germplasm Bank of Cordoba found that the accumulated yield from the different cultivars varied from 2 to 53 kg/tree 6 years after planting (Del Río and Caballero 1994) although these

Table 3: Mean values for characters evaluated in 1996, 1997 and 1998, and correlation coefficients between 1996/1997 and 1996/1998 data

| | Mean value | | | Correlation coefficient | |
|---|---|---|---|---|---|
| Traits | 1996 | 1997 | 1998 | 1996 vs.1997 | 1996 vs. 1998 |
| YPT | 1.69 | 2.78 | 10.05 | 0.043 | 0.133 |
| RD | 81.36 | 69.14 | 95.82 | 0.464** | 0.636** |
| FrFW | 3.01 | 3.71 | 1.93 | 0.709** | 0.482** |
| FM | 70.38 | 76.48 | 62.82 | 0.416** | 0.253** |
| F/S FW | 6.61 | 7.89 | 4.57 | 0.691** | 0.356** |
| F/S DW | 2.31 | 2.22 | 1.91 | 0.717** | 0.467** |
| OC FDW | 65.90 | 61.10 | 59.15 | 0.655** | 0.607** |
| OC FrDW | 45.55 | 41.76 | 38.46 | 0.679** | 0.574** |

For description of traits, see footnote in Table 1.
** Significant at P = 0.01.

cultivars were grown at 7 × 7 m spacings (seedlings evaluated in this work were grown at 3 × 1.5 m). The range of variation for ripening date in the progenies was wider than that reported in some olive cultivar collections (Tous and Romero 1993, Barranco et al. 1998). These results contrast with these obtained in other species such as plum or plumcot progenies, in which the ripening date of seedlings was within the range of variation of the parents or slanted towards the early parent (Ledbetter et al. 1994, Theiler-Hedtrich 1994). The range of variation observed for the oil content components evaluated (fruit fresh weight, flesh moisture, flesh/stone ratio both on a fresh and dry weight basis, and flesh and fruit oil content on a dry weight basis) in the sample of cultivars from the Germplasm Bank is similar to the range reported in some olive cultivar collections of Greece (Tsatsarelis et al. 1984) and worldwide (Del Río and Caballero 1994), and is even higher than the range reported in Italian collections (Preziosi and Tini 1990) and from Catalonia (Tous and Romero 1993). It seems, therefore, that the sample of cultivars evaluated is representative of the whole variability of olive cultivars. For the different oil content components evaluated in the progenies, a wide variability has been exposed. Similar results have also been reported for these and other traits in other olive cross-breeding programmes (Lavee 1990, Bellini 1992, Fontanazza et al. 1999). However, the proportion of large-fruit seedlings has been much higher than previously reported. Bellini (1992) obtained only 11% of seedlings with a fruit weight higher than 3 g, although most progenies evaluated came from crosses between table olive cultivars with greater fruit size (in this work around 26% has been obtained).

Therefore, the progenies have shown a range of variability as large or even larger than either the range observed in a random sample of cultivars from the World Olive Germplasm Bank of Cordoba or the range reported in the evaluation of olive cultivar collections. These results agree with the experience of plant breeders, which indicates that the cultivars of the asexually propagated species are very heterozygous and good segregation can be obtained by means of sexual reproduction (Allard 1960). Moreover, significant genetic diversity among the three parents has also been reported in studies of nuclear and chloroplast DNA (Besnard et al. 2002, Contento et al. 2002).

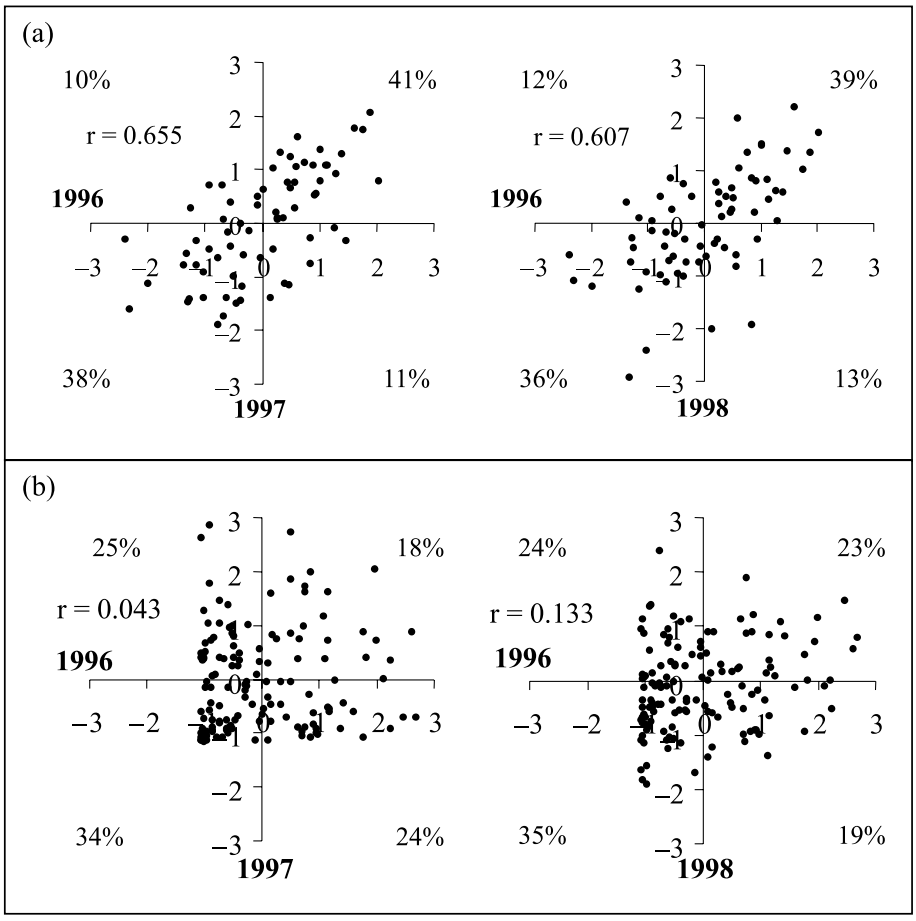


Fig. 2: Scatter plots and correlation coefficients between (a) normalized values of oil content on a flesh dry weight basis and (b) normalized values of yield obtained the first and second (left), and the first and third year (right). The percentage of genotypes in each quadrant is shown

Analysis of variance performed with genotypes and years, showed that variances because of yearly differences and residual variance (which includes genotype × year interactions and other random effects not accounted for) were high for characters such as yield per tree, fruit weight or flesh/stone ratio on a fresh weight basis. Therefore, the evaluation during two or more years would allow a better estimation of these characters in the progenies. The high repeatability obtained for characters such as oil content suggest that the values obtained in the first year could be reliable indicators of the values obtained in following years. Repeatability also gives a good indication of broad-sense heritability, which is relevant for selection among clonally-propagated plants such as olive. The only work regarding heritability estimation of agronomic traits in olive trees reported values of 0.6 for fruit weight and flesh/stone ratio from the evaluation of 23 cultivars, over 2 years, using two plants/cultivar and 50 fruits/plant (Fanizza 1982). Similar conclusions were obtained studying the correlation coefficients among the normalized data obtained in the different years. For traits considered as positive, such as yield or oil content, the most interesting genotypes are those with values higher than the mean of the different years evaluated (i.e. those located in the first quadrant of the normalized plot among 2 years data) whereas those genotypes with negative values in all years could be discarded. The genotypes located in the other quadrants would be most complicated, particularly those with negative values in the first year and positives the following ones, because they would be eliminated if decisions are taken only with 1 year of data (potentially interesting genotypes might be discarded). Most of the genotypes evaluated for oil content are located in the first and third quadrant, so that selection for this character may be easily carried out. The opposite results have been obtained for other characters such as yield, in that the same number of genotypes was observed in each quadrant. In any case, it should be noted that evaluation of olive progenies is somewhat complex because some characters seem to stabilize only after 2–3 years, as has been previously reported in other olive breeding programmes (Lavee 1990).

Reports on other fruit and nut species have indicated that field trials should be conducted over additional years rather than using more tree replications or samples per year to maximize the efficiency in breeding programmes (Hansche and Beres 1966, Yamada et al. 1993, Yao and Mehlenbacher 2000). Other authors have reported opposite results with high variances because of differences among trees of the same genotype and samples within tree (Iezzoni 1986). In olive samples from different cultivars, Del Río and Romero (1999) reported that the use of just one sample was really enough to estimate oil content and there was no increase in accuracy when using more replications. On the other hand, replicates over plants can be more expensive than replicates over years because forced growth of seedlings, which is the strategy usually followed to shorten the juvenile period (Santos-Antunes et al. 1999), requires expensive greenhouses, is time-consuming and limits the number of seedlings that can be forced (Rallo 1995).

In summary, olive progenies have shown wider ranges of variation than representative random samples of cultivars from the Germplasm Bank for some of the characteristics evaluated. This transgression of the previous generation variability limits has been an habitual strategy in fruit breeding programmes because asexual propagation allows one to preserve any genotype for use either as a new cultivar or as a breeding stock for future generations. Analysis of variance performed with genotypes and years, and correlation coefficients among the data of the different years showed that for characters such as oil content the values obtained in the first year could be reliable indicators of the values obtained in the following years, mainly in the first-stage of selection on the whole progeny populations. Once a few genotypes have been selected, measurements should be obtained from many tree replications over many years and locations to clarify the genetic properties of selected genotypes. Further experimentation is needed to clarify the inheritance of these characteristics and to determine the most interesting breeding method to generate superior genotypes.

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