Morphological Limitations in Floral Development among Olive Tree Cultivars

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Abstract

Although the olive tree flowers abundantly, only a very small proportion of those flowers eventually set fruit. It appears that high flower production is a strategy of this species in order to overcome limitations to pollination and fruit set and assure reproductive success. Those limitations include developmental problems or anomalies in floral structure at different levels of organization, specifically 1) the formation of staminate flowers due to pistil abortion and 2) incomplete ovule development. We determined the incidence of pistil abortion and incomplete ovule development in 6 Spanish olive cultivars in three different years, in order to assess the relative influence of genetic and environmental factors and to evaluate the possible interactions between the two different levels of morphological organization. Inflorescences were obtained from adult trees in the World Olive Germplasm Bank of the Junta de Andalucía IFAPA station in Cordoba, Spain, and were processed using standard histological procedures. The results support a strong influence of both genetic and environmental factors, yet independence in pistil and ovule developmental controls.

INTRODUCTION

The olive tree, *Olea europaea* L., flowers abundantly, but only a very small proportion of those flowers eventually set fruit. The final fruit set in commercial olive trees is frequently 1-2%, and even though this is considered to produce a good level of yield (Lavee, 1996), it is quite low compared to many tree fruit crops. Abundant flowering appears to form part of an evolutionary strategy to overcome limitations of fruit set, such as those related to flower quality, and to guarantee offspring quality (Stephenson, 1981).

The olive tree is monodioecious, producing a mixture of perfect or hermaphroditic flowers and imperfect or staminate flowers on paniculate inflorescences. The perfect flowers contain a fully formed pistil with its corresponding ovary, and thus the ability to form a fruit. However the imperfect flowers are staminate, caused by varying degrees of pistil abortion; and only produce pollen. Traditionally, in evaluating olive tree floral quality with respect to yield, researchers have considered the flower number per inflorescence and the percentage of perfect flowers, and have noted the influence of both genetic and environmental factors (Rallo and Fernández-Escobar, 1985; Lavee, 1996). Reale et al. (2006) observed cultivar tendencies for both inflorescence flower number and percentage of imperfect flowers in five Italian cultivars grown in the Umbria region. Uriu (1960) determined an effect of both moisture availability and nutritional status and Perica et al. (2001) found that foliar application of boron increased the percentage of perfect flowers. Reale et al. (2009) observed higher starch content in hermaphrodite (perfect) than staminate (imperfect) pistils, evidence of the role of carbohydrate nutrition. Also the percentage of imperfect flowers may be higher when there are more flowers per inflorescence (Reale et al., 2006).

A second level of floral developmental restriction or anomaly frequently occurs within the ovary, in the ovules. While the basic requirement for fruit production is a perfect flower with a complete, ovary-containing pistil, olive ovaries may also vary in their capacity to form a fruit. The transformation of an olive ovary into a fruit requires the fertilization and development of a seed from at least one of the four ovules present in the ovary. That capacity may be limited by the occurrence of incompletely developed ovules which lack an embryo sac, the entity in which the final steps of fertilization are carried out and the embryo is initiated. This phenomenon was first described by Rallo et al. (1981) in the non-fruiting cultivar Swan Hill, but also occurs in fruiting cultivars (Martins et al., 2006; Rapoport and Martins, 2006; Fernandez-Escobar et al., 2008).

In this study we studied different aspects of floral development of six Spanish olive cultivars. We evaluated the relative influence of genetic and environmental factors and the possible interactions between the two different levels of morphological organization. We sampled the same single trees per cultivar during two different years (2003, 2004), and two different trees per cultivar for one year (2008).

MATERIALS AND METHODS

Plant Material

Six Spanish olive cultivars (‘Arbequina’, ‘Empeltre’, ‘Hojiblanca’, ‘Lechín de Sevilla’, ‘Manzanilla de Sevilla’ and ‘Morisca’) were evaluated, using adult trees in the World Olive Germplasm Bank of the Junta de Andalucía IFAPA station in Cordoba, Spain. All olive trees showed a high flowering level and a good nutritional status. One tree of each cultivar was evaluated during the years 2003 and 2004, and two trees per cultivar in the year 2008.

Morphological and Histological Evaluation

Thirty inflorescences per olive tree were collected from central positions of flowering shoots located around the tree canopy. The inflorescences contained a mixture of closed and open flowers in order to facilitate observations and assure that the time of sampling was close to anthesis. They were immediately fixed in a mixture of formalin, acetic acid, 95% ethanol and distilled water (10:5:50:35 v/v/v/v) under vacuum for 1 h, for conservation. Total flower number per inflorescence and perfect flower number were counted. The percentage of perfect flower per inflorescence was calculated from those values.

For the histological evaluation, pistils were selected from the inflorescences and dehydrated through a tertiary butyl alcohol series, then infiltrated and embedded in Histosec© paraffin at melting point 56-58°C (Merck, Darmstadt, Germany). A maximum of three pistils was obtained per inflorescence. Transverse sections were cut at 12 µm thickness with a rotary microtome, mounted on glass slides and stained with 0.05% toluidine blue for 20 min prior to paraffin removal (Sakai, 1973).

Ovary quality was evaluated according with the number of fully developed ovules present in the ovary. The ovules were observed and characterized according to normal and anomalous embryo sac development. As the olive ovary contains four ovules, the ovaries were rated according to the number of normal, fully developed ovules of the four, that is N/4, with N presenting values from 1 (one fully developed ovule) to 4 (four fully developed ovules). Furthermore, an ovary rating was calculated by averaging the number of fully developed ovules, providing an ovary rating score between 1 and 4.

Statistical Analysis

Variability was evaluated among cultivars, trees and years. Recorded data were compared among both years and trees by analysis of variance, using the SPSS v.15. Moreover, the relationships among several parameters were determined by the coefficients of correlation.

RESULTS AND DISCUSSION

Inflorescence Characteristics: Flower Number and Perfect Flowers

There were significant differences among cultivars for flower number per inflorescence, perfect flower number and perfect flower percent (Table 1). These results are similar to those obtained by Reales et al. (2006) for five Italian olive cultivars, and indicate a genetic influence on these parameters. The number of flowers per inflorescence was shown as a variable parameter among cultivars, ranking from 12.6 to 17.3 (Table 1). However, perfect flowers per inflorescence was a much more highly variable, with values between 5.62 and 15.47.

The percentage of perfect flowers was not significantly different among trees within each cultivar for 'Arbequina' and 'Empeltre'. The greatest differences were observed for 'Lechín de Sevilla' and 'Morisca', while for the remaining cultivars ('Hojiblanca' and 'Manzanilla de Sevilla'), the differences were intermediate (data not shown). The variability among trees and years for individual cultivars is likely due to environmental factors, as has been shown in previous studies (Uriu et al., 1960; Rallo and Fernández-Escobar, 1985; Lavee, 1996; Perica et al., 2001). Still, 'Arbequina' and 'Empeltre' were little affected by either year or tree differences.

Ovule Development

Ovule development categories for all trees are shown in Figure 1 and summarized by cultivar average ovary rating in Table 1. Cultivar tendencies may be observed in the distribution of ovaries in different categories (Fig. 1) and by ovary rating the cultivars into three statistically separate groups (Table 1). These initial results suggest the potential use of these characteristics for olive breeding or varietal choice. The variability among trees and years suggests that there is also an environmental effect, but further observations are required to obtain sufficient data to test the influence of this factor. In a study of olive trees with a range of nitrogen status, a range of ovule development categories was also observed, but there was no significant effect due to nitrogen level.

Relationship between Perfect Flowers and Ovule Development

A correlation between perfect flower development and ovule development showed a positive tendency but was not statistically significant ($R^2 = 0.328$). The cultivar values for perfect flower development and ovule development (expressed as ovary rating), however, showed contrasting tendencies (Table 1). For example both parameters were high for 'Arbequina', intermediate for 'Hojiblanca' and 'Manzanilla de Sevilla', and low for 'Lechin de Sevilla'. In contrast, perfect flower development was high but ovary rating low for 'Empeltre', and perfect flower development low but ovary rating high for 'Morisca'.

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Tables

Table 1. Flower quality parameters for each one of the six olive cultivars evaluated. The values represent the means of 25 inflorescences/tree for four trees in three years (one tree/cultivar in 2003 and 2004, and two in 2008).

Cultivar	Flowers per inflorescence	Perfect flower per inflorescence	Perfect flower (%)	Ovary rating
Arbequina	16.4 b[x]	15.47 a	94.0 a	3.85 a
Empeltre	12.6 e	11.32 b	90.2 a	3.16 b
Hojiblanca	13.9 d	9.43 c	67.6 c	3.18 b
Lechín de Sevilla	17.3 a	7.63 d	44.9 d	2.02 c
Manzanilla de Sevilla	14.8 c	10.59 b	72.9 b	2.98 b
Morisca	13.7 d	5.62 e	41.0 d	3.62 a

[x]Means with different letters within each column indicate significant differences (Duncan's test, $p \leq 0.05$).

Figures

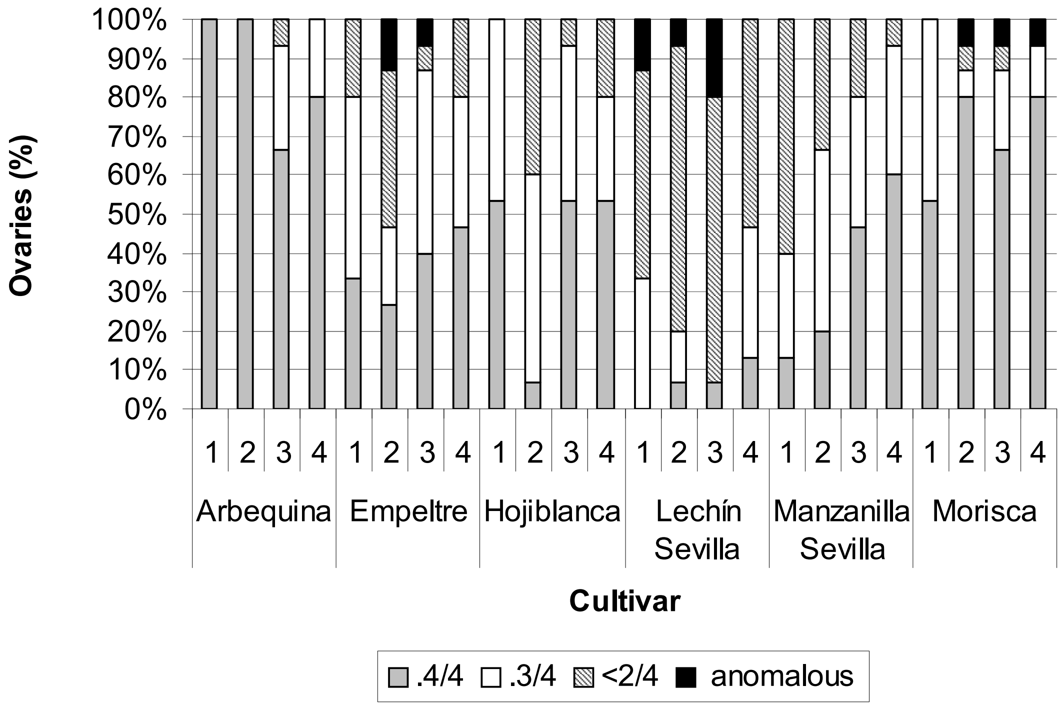

Fig. 1. Ovary distribution of each cultivar according to categories based on the number of normal, fully developed ovules of the four, that is N/4, with N presenting values from 1 (one fully developed ovule) to 4 (four fully developed ovules). An additional category "anomalous" was considered for other anomalies such as the presence of only 3 ovules. Each column represents 15 ovaries from 1 tree/year (Column 1, 2003; column 2, 2004; columns 3 and 4 two different trees in 2008).