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Full Length Research Paper

Olive (*Olea europea)* inflorescence: A quantitative analysis of flowers differentiation among seven olive cultivars

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ABSTRACT

The formation of flowers in sufficient number and quality is a prerequisite for a successful subsequent fruit set. However, although in Mediterranean Basin, olive trees are characterized by abundant flowering; flower differentiation after pistil abortion is followed by a very relatively low fruit set. At anthesis, a comparative study on 7 olive inflorescence cultivars was carried out by selecting 30 inflorescences per cultivar, for laboratory evaluation. Using a light microscope, the number of hermaphrodites, pistillate, and staminate were identified and counted. The inflorescence indexes including flowering rate, the average number of the various types of flowers/ inflorescence as well the percentage of inflorescence based on inflorescence flower position's structure were computed and compared. Olive flowers appear in paniculate inflorescences with 10 to 19 flowers, and the rate of hermaphrodite flowers per inflorescence varied from 36.1 to 54.7% for Pikual and Yerli cultivars, respectively. The research revealed that although the number of flowers and their respective distribution on the inflorescences varies widely from each cultivar, there are clustered into three main inflorescence structures (or classes). However, those structures are not significantly correlated or determinants of the proportion of the 3 types of flowers (hermaphrodite, pistillate, staminate). The olive flower differentiation results in a variable proportion of hermaphrodite, pistillate and staminate among olive cultivars, and this variability is not related to inflorescence indexes. There is a need to make further investigations on pollination and fruit formation development abnormalities in order to understand the low fruit setting in olive trees.

Keywords: Anthesis, hermaphrodite, inflorescence, olive flowers, pistillate, staminate.

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INTRODUCTION

Originating from the Middle East, the olive crop (*Olea europea*) is a very economically important plant particularly, mostly due to its fruits with saturated and healthy fatty acids. Olive (*Olea europea*) fruits cultivation expanded to the Eastern Mediterranean basin, many centuries ago; with Spain and Italy producing more than 60% of the world's olive oil production (Terral et al., 2004; Caceres et al., 2016). Flower development in the olive tree, *Olea europaea*, is a lengthy process that takes two to three months and involves the extension and branching of the inflorescence axis as well as the production of individual flowers (Rapport et al., 2016). The formation of flowers in sufficient number and quality is a prerequisite for a successful fruit set and subsequent crop yield. The flowers of the olive tree *Olea europaea* are born on paniculate inflorescences, which form from buds in the leaf axils of the shoot development from the previous season. The axillary buds resume growth and start inflorescence differentiation after winter dormancy during which they are still undifferentiated as reproductive structures (De la Rosa et al., 2000). The olive tree is characterized by an abundant flowering, but which is followed by a very relatively low fruit set resulting in a very low yield. Generally, for a mature tree, less than 15% of the flowers can set fruits, from which only less than 5% produce mature fruits (Reale et al., 2006). Olive trees flower abundantly, but only a small proportion of the flower can set fruits and produce mature fruits. Olive flower differentiation from hermaphrodites to staminate flowers has been mentioned as the major limiting factor for fruit sets in olive trees (Moutier, 2000; Lavee, 2007). During, olive flower development, the formation of functionally staminate rather than fully functional hermaphrodite flowers is one of the major factors determining the low fruit setting; as flowers with aborted or underdeveloped pistils are incapable of producing fruit (Moutier, 2000).

On the other hand, the formation and differentiation of olive flowers have been related to genetic potential variability, environmental variables as well as nutrition competition (Erel et al., 2013). In addition, the final production of olive trees is negatively related to the previous production, and this alternate bearing is also determined by the flowering load. Thus, even though olive trees are denominated andromonoecious plant species; the formation of pistillate flowers may occur as compensation mechanisms of the flowering level, depending on cultivars (Reale et al., 2009; Liu et al., 2014). In fact, the flower differentiation and the low fruit set may vary among olive fruit varieties. In spite of its long history, limited comparative investigation studies have been carried out to understand the changes that occur in flower differentiation and fruit set (Chiappetta et al., 2015). In an attempt to explain this biological phenomenon, several hypotheses have been discussed, including but not limited to nutrition resource competition, and environmental stress that mostly may reduce the pollen quality or genetic potentials (Reale et al., 2009). The phonological time of olive flowers has been widely described by a series of development stages, reflecting the degree of flower bud as a percentage of different stages within the inflorescence, fruiting branches, and the total tree canopy (Sanz-Cortés et al., 2002). In addition, very few studies have described the features of the structural flower at anthesis (Serrano et al., 2008); they are also very limited findings describing an ovary and stamen structure as well as processes related to fertilization success in olive fruit production (Rapoport, 2012; Reale et al., 2009). Thus, this study was aimed at characterizing bio-morphological features of the flowers in olive trees by investigating the inflorescence structure and making a quantitative analysis of the proportion of perfect, pistillate and staminate flowers among olive cultivars at anthesis.

MATERIALS AND METHODS

Description of research site and olive grove

The research investigation was carried out in an olive grove of the European University of Lefke (EUL), North Cyprus. The 30 years olive grove was laid out in a completely randomized block design including seven cultivars, which are namely: Abrosona, Arbekuina, Koronaki, Toska, Pikual, Sikitita, Yerli. The climate is Mediterranean with sandy loamy soils, well-drained, with mild soil salinity (Terral et al., 2004).

Data collection and analysis

In spring 2021, 3 trees/cultivar have been selected and observed closely till anthesis (at least 60% of the selected tree flowers open). The trees on the border of the olive grove were left out to ensure that all of the diverse soil conditions and their distinct topological features were represented in the sample. Furthermore, the trees chosen are all completely productive and between the ages of 30 and 35 years. It's also worth noting that all of the trees chosen were in their 'on year' phase. At the anthesis, ten inflorescence shoots (panicle) from each of the selected olive trees were harvested randomly along the top, middle and base of the canopy. In total, thirty inflorescences from each olive cultivar have been selected. The samples were kept in ice bag conditions and transported to the laboratory for inflorescence analysis. The olive inflorescence architecture and flowering rates were evaluated and classified into 3 classes based on Seifie et al. (2008) olive inflorescence architectural evaluation criteria. The relationship between flower gender differentiation and flower architecture in olive trees was evaluated by collecting inflorescences based on their ramification structures (terminal, primary, and secondary laterals), as well as keeping track of the number of flowers that appeared on those inflorescences. After that, using a light microscope (Leica 10×10), the gender type of the flowers was confirmed, and the number of flowers per inflorescence was recorded according to the gender type of the flowers: perfect, pistillate, and staminate flowers.

Data obtained for the inflorescence indexes were recorded and processed into Genstat.14 for one-way ANOVA to create a comparative quantitative study. Duncan Multiple Range (DMR) test at P5% was used to determine the mean separation, and the Pearson correlation coefficient between the different indices was also computed.

RESULTS AND DISCUSSION

Olive trees flower abundantly, but only a small amount of the flowers can produce fruitlets. The result revealed that the percentage of aborted flowers (staminate flowers with only male organs complete and functional; flowers with an invisible ovary, or with a style or stigma atrophied), and pistillate flowers varied significantly among the considered cultivars (Table 1). The rate of hermaphrodite flowers and female flower sterility are key parameters that determine the olive tree yield (Terral et al., 2004; Chiappetta et al., 2015). The number, the gender of flowers as well as their distribution on the inflorescences vary between cultivars (Table 1). Olive flowers appear in paniculate inflorescences with 10 to 19 flowers, with an average number of flowers per inflorescence ranging from 11 to 16 flowers respectively for Sikitita and Yerli cultivars. On the other hand, the rate of hermaphrodite, staminate and pistillate flowers vary significantly across cultivars (Figure 1), with the high-rate value respectively observed for Yerli (54.7%), Pikual (49.6%), and Abrosona (15.4%). In contrast, the low rate of hermaphrodite, staminate and pistillate flowers was observed for Pikual (36.1%), Yerli (31.4%), and Arbekuna (12.7%), respectively. Hermaphrodite flowers are those whose floral parts or capillaries are normally formed (no deformity), allowing them to accept viable compatible and fecundating pollen in their receptive stigma (Serrano et al., 2008). A hermaphrodite Olive flower consists of four green sepals, four white-yellowish petals, one pistil, and two stamens; Staminate flowers in olive trees result from a failure to complete pistil development while pistillate flowers have a lack of stamens (Figure 1). Thus, for all the investigated varieties in the present study, the number of hermaphrodite flowers is relatively higher compared to the one of the stamens or pistillate. Furthermore, andromonoecy in olive trees results from a failure to complete pistil development. While stamens develop faster and big compared to associated pistils, the staminate flowers have collapsed or shrinking pistils like unfunctional pistillate flowers. As reported by Reale et al. (2009), the interruption of pistil growth might be associated with the absorption and the reallocation of the resources that were invested in the pistils. Thus, during, olive flower development (O. *europea*), the formation of functionally staminate rather than fully functional hermaphrodite flowers is one of the major factors determining the low fruit setting; as flowers with aborted or underdeveloped pistils are incapable of producing fruit (Moutier, 2000).

Table 1. Variability of flowering indexes among olive cultivars.

	Abrosona	Arbekuina	Koronaki	Pikual	Sikitita	Toska	Yerli
Average number of flowers/inflorescence	14.6^{c}	13.3^{b}	16.2^{d}	12.4^{ab}	11.7^{a}	14.9^{d}	16.3^{d}
Average number of hermaphrodite/ inflorescence	6.5^{abc}	5.6^{ab}	$6.8\ b^{c}$	4.9^{a}	7.4^{bc}	7.1^{bc}	7.8^{c}
Average number of staminate flowers/inflorescence	6.0^{bcd}	6.7^{bd}	5.1^{ab}	7.1^{d}	5.1^{abc}	6.7^{bcd}	4.4^{a}
Average number of Pistillate flowers/inflorescence	1.7^{a}	1.9^{a}	2.7^{a}	2.2^{a}	2.4^{a}	2.4^{a}	1.7^{a}

Means followed by the same letter in the same row are statistically not significant according to Duncan's multiple range test (P = 0.05).

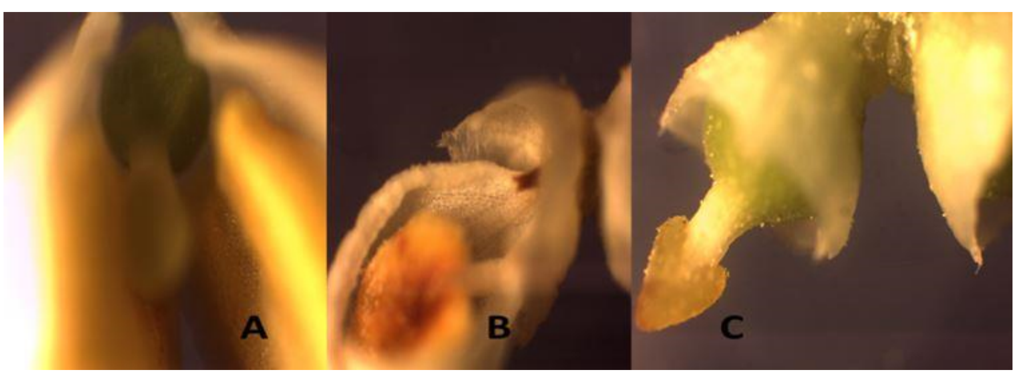

Figure 1. A view by light microscope of hermaphrodite (A), staminate (B) and pistillate (C) flower.

Although, the number of flowers and their respective distribution on the inflorescences is specific for each cultivar (Figures 2 and 3); inflorescences have been classified into three following classes: Class I (flowers directly attached to the main inflorescence stem), Class II (flowers directly attached to the main inflorescence stem,

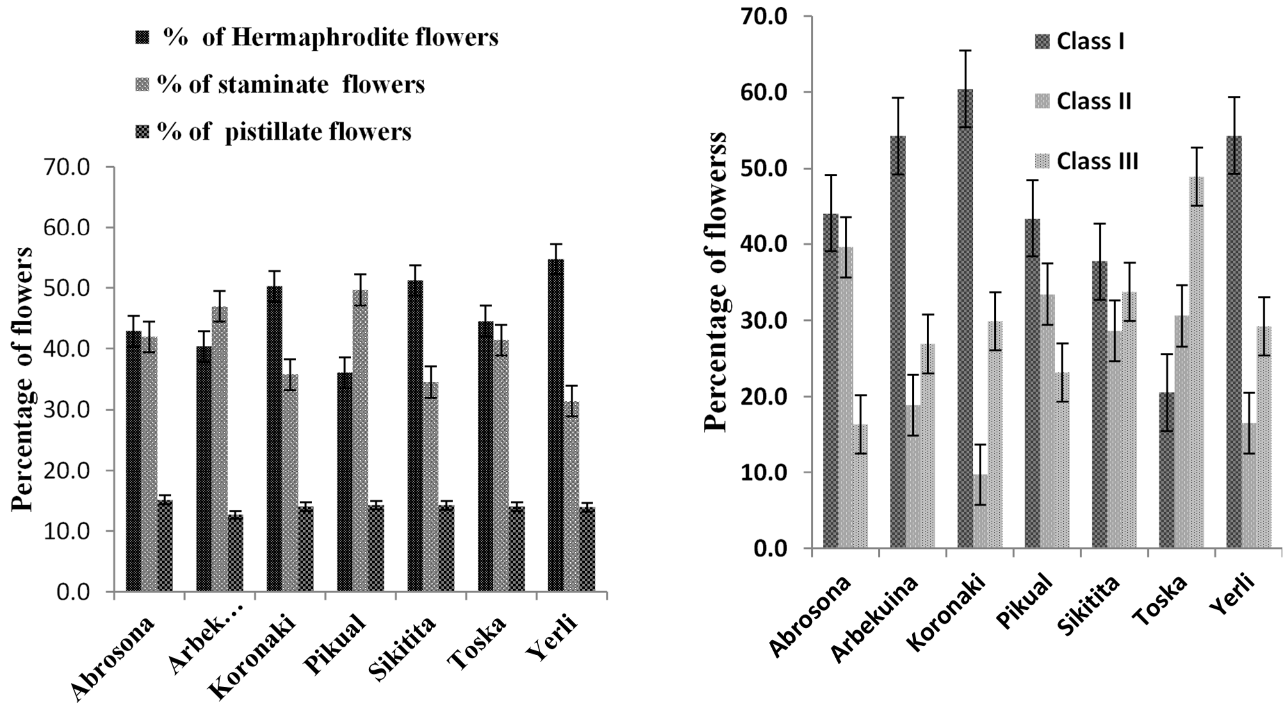

Figure 2. Gender of flowers and inflorescence architectural structure.

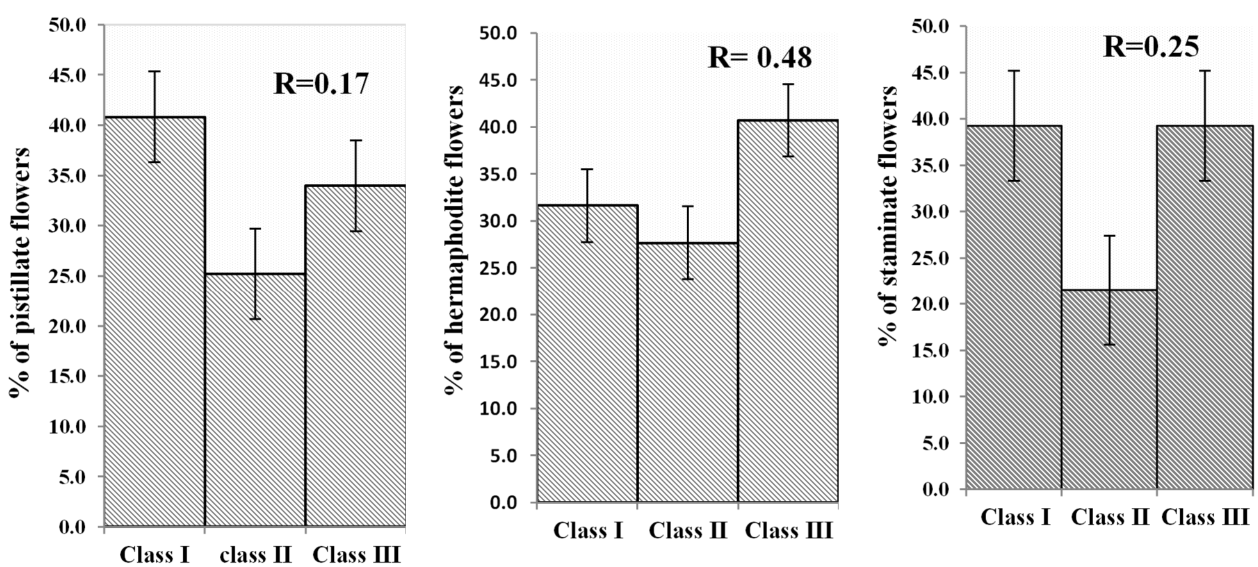

Figure 3. Gender of flowers distributed per inflorescence structure.

and with only one lateral ramification), and Class III (flowers directly attached to the main inflorescence stem, and having 1st and 2nd lateral or more ramifications).

As shown in Figure 2, there is huge variability in the proportion of hermaphrodites (bisexual) to staminate flowers among the olive cultivars. Generally, for a mature tree, only less than 15% of the flowers produced can set fruits, from which less than 5% will produce mature fruits (Reale et al., 2006). The effects of flower location on the inflorescence on opening day, gender, and petal persistence were investigated by some researchers (Krause, 2008; Seifie, 2008). Perfect flowers bloomed at the start of the flowering season, whereas staminate blooms bloomed later. In all cultivars, flower location on the inflorescence had a substantial impact on an opening day. Blooms on the principal branches and terminal flowers opened first, followed by flowers on the secondary branches. In Manzanillo and Mission, a study revealed that flower position had a substantial impact on gender (Seifie et al., 2008).

The fruit setting more than flower differentiation is the major limiting factor for the yield of olive trees. During, olive flower development (*O. europaea*), the formation of functionally staminate rather than fully functional hermaphrodites flowers has been reportedly found as one of the factors determining the low fruit setting; as flowers with aborted or underdeveloped pistils are incapable of producing fruit (Moutier, 2000). The formation and differentiation of olive flowers are determined by genetic potentials and environmental variability as well as nutrition competition (Erel et al., 2013).

Although the number of staminate flowers has no direct effect on the yield, their appearance in high numbers may suggest a competition between flowers, which may cause high pistil abortion lateral positions. The influence of the inflorescence structure, especially the position of the flowers on the inflorescence had been repeatedly discussed and their high influence on the gender of the flowers was confirmed. It is also stated that olive flowers compete within the inflorescence and may develop to be staminate, especially in poorly nurtured positions such as the laterals (Rosati et al., 2011; Erel et al., 2013). Previous findings support the idea that pistil abortion is caused by ovaries competing for resources, and they suggest that genetic differences in pistil abortion among olive cultivars might be explained by differences in pistil mass and sink strength (Ji et al., 2010; Zhu et al., 2013). Furthermore, in cultivars with a high percentage of staminate flowers, the hermaphrodite flowers are likely to be in terminal positions and the staminate in lower positions. The influence of flower position on the inflorescence opening day and petal persistence has been also demonstrated by Rosati et al. (2011) and Seifi et al. (2015). These researchers found out that terminal flowers and the flowers located on the primary branches opened earlier than the flowers located on the secondary branches, and the branch arising immediately next to the terminal flower had the latest flowers to open and the lowest percentage of hermaphrodite flowers.

CONCLUSION

The reproductive biology of olive trees goes through four main and important stages namely, flower induction and initiation, flower differentiation, pollination and fertilization, and fruit setting. The high number of hermaphrodite flowers is likely to happen in Koronaki, Sikita and Yerli olive cultivars. The number of flowers and their distribution on the inflorescence are specific for each cultivar. The present study revealed that the amount of hermaphrodite, pistillate and staminate flowers vary widely and significantly among the olive cultivars. Though, the olive flower differentiation process, the proportion of hermaphrodite or pistillate flowers is higher than one of the staminate flowers at the anthesis. The effect of the flowering rate and the inflorescence structure on the various inflorescence indexes is not significant. The staminate flowers in olives result from the pistil's growth interruption. In brief, the statistical analysis revealed a significant difference between considered varieties in the investigated inflorescences indexes except for the average number of pistillate flowers per inflorescence. More studies shall reveal the effect of the flower's position and inflorescence structure at anthesis as well as the fruit set development stage.

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