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Self-incompatibility and pollination relationships for four Greek olive cultivars

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ABSTRACT

The present three-year study aimed to (1) determine the number of flowers per inflorescence, (2) determine the proportion of hermaphrodite flowers and (3) investigate the genetic and environmental components of self-incompatibility through controlled pollination trials, followed by microscopic observation of in vivo pollen tube growth for olive cultivars '*Koroneiki*', '*Kalamata*', '*Mastoidis*' and '*Amygdalolia*'. Significant differences between cultivars and years were observed for number of flowers/inflorescence and hermaphrodite flowers percentage. The highest number of flowers was observed for 'Kalamata' panicles followed by '*Koroneiki*', whereas the lower numbers were for '*Mastoidis*' and '*Amygdalolia*'. The highest percentage of hermaphrodite flowers was observed for '*Koroneiki*', followed by '*Kalamata*' while lower levels were counted for '*Mastoidis*' and '*Amygdalolia*'. '*Koroneiki*' showed a noteworthy capability (3.6–8.7% of hermaphrodite flowers) to set fruit through self pollination. Lower fruit set rates were counted for '*Mastoidis*' (1.7–2.6%) and 'Amygdalolia' (0.5–2.4%). The lowest level of fruit set was observed for '*Kalamata*' (0.4–2%). In the case of cross pollination, the highest fruit set rates were observed for 'Koroneiki', intermediate rates were counted for '*Mastoidis*', followed by '*Amygdalolia*', and the lowest levels of fruit set were counted for '*Kalamata*'. In the framework of the sporophytic SI system, we attributed *R2R4* to '*Mastoidis*' and '*Kalamata*', *R1R2* to 'Amygdalolia' and *R4R6* to '*Koroneiki*'. Based on the results of these experiments, recommendations were released on cross pollination requirements and combinations with compatible cultivars for setting up new olive orchards to ensure high fruit sets and adequate yields.

1. Introduction

The olive tree is wind pollinated and is cropped favorably under Mediterranean climate. It is considered andromonoecious species producing hermaphrodite as well as male flowers (Cuevas and Polito, 2004). Most cultivars (cv) produce a small amount of fruits through self-pollination, however, fruit set is improved by cross-pollination (Breton and Bervillé, 2012; Cuevas, 1992; Fernandez-Escobar and Gomez-Valledor, 1985). The magnitude of fruit-set increase through cross-pollination compared to open-pollination is an indicator of self-incompatibility and appropriate threshold choice is necessary for drawing accurate conclusions (Breton and Bervillé, 2012). The ratio index of self-incompatibility (ISI), fruit set by cross pollination/fruit set by open-pollination, is widely used to define whether a cross has succeeded or failed (Zapata and Arroyo, 1978).

Self-incompatibility (SI) is more likely to exist in a cv. that shows remarkable increase in fruit set following cross-pollination in comparison to self-pollination rather than in a cv. that does not exhibit this feature. This feature does not exist in other plant species such as apple, where SI is a qualitative attribute (i.e. usually no fruit is produced in absence of cross pollination) (Broothaerts and van Nerum, 2003). In those species, it is easier to characterize a cv. based on fruit set behavior and to study the inheritance of the mechanism that controls SI.

In the case of the olive, even cvs characterized as self-incompatible produce fruits, at varying degrees, following pollination by their own pollen. Consequently, indicators of SI are quantitative (Breton and Bervillé, 2012): lower (but existing) fruit set (Cuevas et al., 2001) and slower (but existing) pollen tube growth (Spinardi and Bassi, 2012) in self- compared to cross-pollination. This is a serious obstacle to accurately characterize each olive cv. in terms of SI behavior.

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It is common in the literature the same cv. to be characterized as self-incompatible in one study, partially self-incompatible in another study and self-compatible in a third one (Cuevas et al., 2001). This could occur, either due to the impact of one or several environmental factors or due to lack of adequate pollinizers under the dominant wind of the year. However, in other species harboring a SSI mechanism temperature has no such effect, and if so in the olive, one can infer that open-pollination could be sometimes a limiting factor (de Nettancourt, 2001).

In Greece, olive orchards generally consist of a single cv. Higher and more stable yields are observed in orchards where more than one cvs are present, compared to single-cultivar orchards even when the selected principal cv. has been classified as self-fertile (Androulakis and Loupassaki, 1990). The need for pollinizers in orchards with fully or partially self-sterile cvs has been widely reported (Cuevas et al., 2001; Lavee et al., 2002; Wu et al., 2002). Similar experiments carried out in Crete revealed that some cvs may be partially self-compatible (Androulakis and Loupassaki, 1990).

Recently, SI in the olive has been advocated to be directed by the sporophytic and not by the gametophytic functional mechanism (Breton and Bervillé, 2012). This means that the pollen grain carries the two determinants specified by the mother tree of the pollen. Consequently, the host pollinated tree must not carry any of them, unless dominance relationships exist between the two S-alleles of this pair in the male. If so, the recessive S-determinant is not recognized on the pollen tube progressing through the stigma tissues and thus it may fertilize the ovule. Consequently, in the trial, if the mate has been done in the other direction, the cross is expected to fail, due to dominance relationship functions in the pollen and the pollen tube only. Furthermore, the self-compatibility rates, has been suggested to be enhanced in cvs carrying an S-allele pair with dominance relationships, whereas those carrying a pair with no dominance relationships are self-incompatible (Breton et al., 2014).

Four cvs of Greek origin ('*Koroneiki*', '*Kalamata*', '*Mastoidis*' and '*Amygdalolia*') with major international importance were examined for inflorescence architecture, self-pollination, open pollination and in crosses between each other. The present three-year study aimed to: (1) determine the number of flowers per inflorescence, (2) determine the proportion of hermaphrodite flowers – also called by olive specialists as complete or perfect flowers and (3) investigate the genetic and environmental components of olive SI through controlled pollination trials, followed by microscopic observation of in vivo pollen tube growth. The genetic basis of SI in the framework of the sporophytic SI system was considered. Additionally, based on the results of this study, recommendations were released, regarding the cross pollination requirements and combinations with compatible cvs for setting up new orchards to ensure high fruit sets and adequate yields.

2. Materials and methods

2.1. Plant materials and growth conditions

Thirty to forty year-old irrigated trees belonging to '*Koroneiki*', '*Mastoidis*', '*Kalamata*' and '*Amygdalolia*' were selected based on vegetative growth and flowering uniformity. Mean air temperature in this area was 18 °C, relative humidity (RH) was 64% and annual rainfall was 600–800 mm in the Institute of Olive Trees and Subtropical Plants, N.AG.RE.F., Chania–Crete, Greece (N.AG.RE.F. meteorological station, Chania).

Field observations and experiments were carried out, over the three-year period 2005–2007 (N.AG.RE.F., Chania). Mean daily temperatures during April and May of the three experimentation years are presented in Fig. 1.

2.2. Mate pollination trials

Four to twelve olive trees per cv. were used each year depending on flowering uniformity. The number of flowers/inflorescence was determined at the beginning of flowering for each cv. and year, in a random sample of inflorescences (replicate inflorescences for the three years: 69, 40, 160 for '*Koroneiki*', 46, 80, 54 for '*Mastoidis*', 53, 80, 160 for '*Kalamata*' and 36, 20, 60 for '*Amigdalolia*') distributed around the canopy of the selected trees. The percentage hermaphrodite versus total flowers was determined at the beginning of flowering for each cv. and year, in a random sample of inflorescences (replicate inflorescences for the three years: 18, 40, 100 for '*Koroneiki*', 10, 80, 32 for '*Mastoidis*', 12, 80, 32 for '*Kalamata*' and 16, 40, 102 for '*Amigdalolia*') distributed around the canopy of the selected trees. White paper pollination bags were placed 2–3 days before flower opening to avoid unwanted cross-pollination, except for the free pollination treatment where no bags were used. A shoot derived from the pollinizer cv. carrying open and ready to open flowers was included into the bag twice: once when the specific tree was at flowering stage "63" and once at stage "65" (Sanz-Cortes et al., 2002). Flowers were not added for the self-pollination treatments since preliminary trials showed no significant difference between either including or not a pollinizer branch effect. Free-pollination shoots were allowed to receive pollen presumably from all cvs present in the field.

The percentage of fruits/hermaphrodite flowers was recorded about 30 days after full bloom in 10 replicate shoots for each treatment and year for the 4 cvs following:

(1) self pollination, (2–4) cross-pollination with pollen of one of the cvs '*Koroneiki*', '*Kalamata*', '*Mastoidis*', '*Amygdalolia*', separately, (5) free multi-cv. pollination.

Self-compatibility of olive cvs was determined according to thresholds: ISI > 0.3 = self-compatible, >0.29 to 0.1 = partially self-incompatible; and 0.1 to 0 = completely self-incompatible. Cross-compatibility was determined according to Inter-compatibility Index threshold: ICOI > 0.6 = mate success, <0.6 = mate failure.

2.3. Microscopy

Observation of stigma for pollen adhesion following artificial pollination through fluorescence and confocal scanning laser microscopy was employed in 10 flowers per cv., year and treatment. In vivo pollen tube growth observations were implemented through the aniline blue fluorescence staining technique according to Cuevas et al. (1994a). Images were obtained on a Leica TCS-NT Laser Scanning microscope using the 20× and 40× objectives.

2.4. Statistical analysis

Two-way analysis of variance (ANOVA) was performed for flower/inflorescence and hermaphrodite flower with genotype and year using SPSS (SPSS Inc., Chicago, USA). Fruit set data (standardized to hermaphrodite flowers) were subjected to one-way analysis of variance (ANOVA). Percentages were transformed using the arcsin transformation before statistical analysis. The least significant difference test at $P = 0.05$ was used to distinguish treatment differences. Standard errors of each mean were calculated and presented in graphs as error bars.

3. Results

3.1. Flower/inflorescence

Significant interactions through variance analysis were observed for number of flowers/inflorescence (Table 1). The three-year average numbers of flowers per inflorescence for the