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MORPHOLOGICAL AND PHYSILOGICAL STUDIES ON FLOWERING, POLLINATION AND FRUITING OF "PICUAL" OLIVE TREES

III- FLUORESCENCE MICROSCOPY STUDY ON POLLINATION OF PICUAL OLIVE FLOWERS

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ABSTRACT

Picual olive flowers were examined under fluorescence microscope after being exposed to different pollination treatments. Cross, self and open pollination were examined. Also, the effect of pollination stage of Picual flowers was studied. Investigation of Picual flowers by means of fluorescence microscope showed that percentages of Kronaki and Coratina pollen germination on the surface of Picual stigmas, percentage of penetrated styles and number of pollen tubes at the end of the stylar canal were higher when pollination was carried till three days after anthesis compared to delayed pollination and ovules viability started to decline from the 4th day after anthesis. Cross pollination by Kronaki pollinizer showed the highest significant percentages of pollen germination on the stigma, pollen tube growth through the styles and number of pollen tubes at the end of the penetrated style compared to other pollen donors. Thus, it can be recommended to use Kronaki as a pollinizer for Picual to ensure efficient pollination and fertilization.

Keywords: Olive- Picual- pollination- stigma- ovules- pollen grains

INTRODUCTION

Olive tree (*Olea europea* L.) is widely grown in many arid zones native to the Mediterranean region. In Egypt the total acreage grown with olive cvs. reached 104 000 Feddans**.** Picual cv. -which was introduced from Spain to Egypt- represents about 60% of the total olive cultivation (Ministry of Agriculture, A.R.E. 1999).

Most olive cvs. have variable degrees of self incompatibility and require compatible cross pollination for successful cropping (Androulakis and Loupassaki, 1990. Pollen germination and pollen tube growth were used to assess compatibility levels of olive cvs. (Lavee and Datt, 1978). Pistil extracts were added to the germination media to study pollen- pistil incompatibility in six olive cvs., it was found that pistil extracts of any cv. enhanced pollen germination and pollen tube growth for the same or other cvs. (Fernandez *et al.,* 1983). In investigating self and cross pollination of olive trees under varying conditions, it was concluded that pollen tube growth was faster and percentage of embryo sac penetrated by pollen tubes was higher in cross than in self pollination (Bradley and Griggs 1963). It was demonstrated that pollen tube growth of Manzanillo olive cv. after selfing stopped shortly after tubes had penetrated the stigma surface (Cuevas and Polito 1997). The time between pollination and fertilization may range from hours to days and it was

found that stigma is probably receptive up to 48 hrs (Fernandez, 1993). Olive pollen germination ranged from 30- 50% and usually occurred one to two days after anthesis with cross pollinated flowers, pollen tube growth was vigorous and competitive (Cuevas *et al.,* 1994 a, b and c).

Ovular penetration by a pollen tube always occurs through the micropyle and usually in a single ovule flower (Cuevas *et al.,* 1994 a). In addition, olive viable ovule stained with aniline blue showed an orange- colour with a light yellow fluorescence limited to the micropylar area. While senescent ovules exhibited an intense yellowish fluorescence over their entire surface (Cuevas *et. al.,* 1994 c).

Thus, the present investigation was carried out to distinguish between cross and self pollination of Picual olive flowers by means of fluorescence microscope. Also, the effect of pollination stage related to anthesis was studied by the same technique.

MATERIALS AND METHODS

The present investigation was carried out on Picual olive trees grown in a private orchard in 'El-Mansoria' Giza Governorate. The trees were about 8-10 years old, planted in a sandy soil, six meters apart mixed with Coratina, Manzanillo and Toffahi cvs. The trees were under drip irrigation and received the normal cultural practices.

Self, cross and open pollination regimes were studied on five replicated trees of Picual olive cv. In each replicate tree, after inflorescence appearance, 40 one year old shoots were labeled and divided randomly into four groups (each of ten shoots) examined for self, cross and open pollination.

Self pollination was carried on 10 shoots on each replicate tree, where the shoots were enclosed with pergamin bags which were wrapped to prevent entrance of unwanted pollen. This was done at the balloon stage and the bags were removed at the 14th day after bloom. Cross pollination was done by testing two pollen donors (Kronaki and Coratina) 10 shoots/ each. When the inflorescence were at the balloon stage, flowers were hand emasculated, covered with pergamin bags and pollinated when the flowers on the indicator inflorescences reached the stage of anthesis. On the other side, the last 10 shoots per replicate tree were used to assess open pollination and left under the natural conditions in the orchard.

The effect of pollination time was determined by selecting twenty shoots on another four replicate Picual trees. On ten shoots, flowers were divided into ten groups and cross pollinated with Kronaki in ten stages related to days from anthesis as follows: -2, -1, 0 (anthesis), +1, +2, +3, +5, +6, +7 days. Similarly, the other ten shoots were divided and cross pollinated with Coratina pollen.

Samples of twenty pistils from each replicate for each of the previous pollination treatments were collected daily for seven successive days. Pistils were fixed in FAA (Formalin1: acetic acid 2: ethanol 70% 17). Samples of pistils were softened in 0.8 n Na OH for 6 hrs at room temperature, then

washed in running tap water for 24 hrs and stained in 0.1% aniline blue dissolved in 0.1 n K_3 PO_4 and examined under Leica fluorescence microscope according to methods of Kho and Baer (1968) and Cuevas *et al., (*1994 a, b &c).

Pollen germination on the stigma and pollen tube growth through the style of Picual olive flowers were investigated after pollination from different sources and at different stages according to Cuevas *et al.* (1994 a). Number of pollen tubes at the end of the penetrated style of olive cv. was investigated after pollination treatments according to Cuevas *et al.* (1994 b). Ovule viability of Picual olive pistils was studied at different stages from anthesis in the sampled flowers according to Cuevas *et al.* (1994 c).

The obtained data were statistically tested for analysis of variance in simple and factorial design using MSTAT package (1998) and significant differences among the various treatments were compared using L.S.D values at 0.05 according to Waller and Duncan (1969).

RESULTS

I- Pollination treatments

Samples of Picual flowers were collected daily for 7 successive days after each pollination treatment. Flowers were microscopically examined in order to detect percentage of pollen germination on the surface of stigma, follow pollen tube penetration through stylar length, notice the number of pollen tubes that reached the end of style and study ovules viability in each sample after different pollination treatments.

1- Effect of pollination treatments on percentages of pollen germination on stigma of Picual olive cv.

Table (1) and plate (1a and b) showed that pollen germination of Kronaki and Coratina cvs. was significantly higher compared to open pollination and selfing, it averaged 32.57, 28.14, 17.94 and 10.43% respectively. Meanwhile the lowest percentages of pollen germination was noticed seven days after pollination, it recorded 18.95% at this stage.

2- Effect of pollination treatments on percentages of penetrated styles:

Seven days after pollination, the average percentage of style length penetrated by pollen tubes reached its maximum as a results of using Kronaki pollen (Table 2). Investigated styles of Picual olive cv. during 7 successive days after pollination showed that style penetration was significantly higher in the period from 5-7 days after pollination compared to the period 1-3 days after pollination.

Five days after pollination, it was noticed that Kronaki pollen tubes recorded the maximum style penetration and was significantly higher than that of Coratina, open and selfing as it reached at this stage 86.40, 52.80, 46.20 and 46.20 respectively.

من الأصلTable1,2

plate

3-Effect of pollination treatments on number of pollen tubes at the base of the stylar canal:

Number of pollen tubes at base of the stylar canal was affected by the source of pollen (Table 3). Seven days after pollination, the general average of Kronaki cv. pollen tubes reached the maximum number (2.71) and was significantly higher than that of Coratina (1.49). Meanwhile Coratina was significantly higher than open pollination (1.11) and selfing was the lowest (0.71), during the period from 3-7 days after anthesis significantly higher number of pollen tubes was noticed at the base of the stylar canal compared to the period 1-2 days after anthesis.

4- Effect of pollination treatments on percentage of viable ovules:

Table (4) showed that ovules viability was not affected by pollen donor but it was only affected by aging. Ovules viability started to decline 4 days after anthesis and was significantly lower 6-7 days after anthesis as it averaged 86.25 and 88.75 than during the period from 1-5 days after anthesis (Plate 2 a and b).

II- Pollination stages

Picual olive flowers were cross pollinated by Kronaki and Coratina at ten different stages starting from 2 days before anthesis till 7 days after anthesis. Picual olive flowers were sampled daily for 7 successive days after each pollination stage. In the sampled flowers pollen germination, pollen tube growth, number of pollen tubes at the end of style and ovules viability were microscopically examined.

1-Efefct of pollination stages on the percentage of pollen germination on stigma of Picual cv. after cross pollination by pollen grains from kronaki and coratina cvs.

Tables (5 and 6) showed that percentage of pollen grains germination of Kronaki and Coratina cvs. on the stigma surface of Picual olive flowers was significantly affected due to pollination stages and days after pollination. The highest percentage was noticed when pollination was carried 2-3 days after anthesis for Kronaki cv, it average 40.91 and 41.34% respectively . while in Coratina cv. was noticed when pollination was at 1-2 days after anthesis and averaged 33.03 and 33.63 respectively.

As for the effect of days after pollination, it is noticed that pollen germination started to decline 5-6 days after pollination for Kronaki and Coratina cvs. and was significantly lower at 6-7 days after pollination compared to the period 1-5 days after pollination.

2- Effect of pollination stages on percentages of Picual styles penetrated by pollen tubes of Kronaki and Coratina cvs.

Data presented in Table (7) showed that average percentage of style length penetrated by pollen tubes of Kronaki cv. was significantly higher when pollination was carried at the stage of anthesis and recorded 59.66% while the significantly lower penetration was 42.49% when pollination was carried 7 days after anthesis.

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Investigated styles of Picual olive cv. at 7 successive days after pollination showed that style penetration was significantly higher in the period from 6-7 days after pollination compared to the period from 1-4 days after pollination .

Concerning Coratina cv. Table (8) showed that percentage of Picual style penetrated by pollen tubes did not affect by pollination stage. Meanwhile investigated styles of Picual olive cv. at 7 successive days after pollination showed that it was significantly higher in the period at 7 days after pollination (79.02%) compared to the period 1-2 days after pollination (0.66 and 31.68%) .

3- Effect of pollination stages on number of Kronaki and Coratina pollen tubes at the base of penetrated style:

Tables (9 and 10) showed that number of Kronaki and Coratina pollen tubes at the base of penetrated styles was significantly affected by pollination stages and days after pollination.

The highest number of pollen tubes for Kronaki and Coratina cvs. was recorded when pollination was carried at the stage of anthesis and recorded 2.51 and 2.03 while the lowest number was recorded when pollination was carried 7 days after anthesis and 2 days before anthesis it recorded (1.17 and 0.97) for Kronaki and Coratina cvs.

Investigated number of Kronaki and Coratina pollen tubes at 7 successive days after pollination showed that the highest number of Kronaki and Coratina pollen tubes was in the period from 5-7 days after pollination meanwhile the lowest number was in the period 1-2 days after pollination.

4- Effect of pollination stages on percentages of viable ovules pollinated with Krnoaki and Coratina cvs.

Data presented in Tables (11 and 12) showed that ovules viability was significantly affected by stages and days after pollination. Investigated ovules of Picual olive flowers pollinated by pollen grains from Kronaki and Coratina cvs. showed that ovules viability started to decline 4 days after pollination.

Concerning the effect of the time of pollination, it was noticed that ovules started to decline when pollination was carried at 5 days after anthesis and recorded its minimum level when pollination was delayed to the 7th day after anthesis.

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DISCUSSION

The present study showed that percentages of pollen germination of Kronaki and Coratina cvs. were significantly higher as compared to open pollination and selfing. Also, the percentages of pollen germination of Kronaki and Coratina cvs. were significantly affected by pollination time and days after pollination. These findings come in agreement with previous findings reported by (Cuevas *et al*., 1994a) who found that olive pollen germination ranged from 30 to 50% and usually occurred one to two days after anthesis.

The results of the present study showed that average percentage of style length penetrated by pollen tube after seven days from pollination reached its maximum as a result of using Kronaki pollen grain. Moreover average percentage of style length penetrated by pollen tubes of Kronaki and Coratina cvs. was significantly higher when pollination was carried at the stage of anthesis. These results are in line with findings of Bradly *et al.* (1963) who reported that pollen tube growth was faster in cross than in self pollinated olive flowers. Similarly (Cuevas 1994) found that pollen tube growth of cross pollinated flowers was very vigorous and competitive compared to those resulted from self pollination. Moreover Cuevas and Polito (1997) recorded that pollen tube growth of Manzanillo olive cv. after selfing stopped shortly after tubes had penetrated the surface of the stigma.

The present investigation showed that ovules viability was not affected by pollen donor but it was affected by aging, as ovules viability started to decline 4 days after pollination and 5 days after anthesis. These results are in harmony with those found by Williams (1970) who found that ovules longevity ranged from a few to many days in various species under different microclimate conditions. Similarly, (Cuevas *et al.* 1994c) reported that viable olive ovules stained with aniline blue showed orange colour with a light yellow fluorescence limited to mciropylar area. While senescent ovules exhibited an intense yellowish fluorescence over their entire surface. Moreover it was found that differences in longevity between ovules in the same flowers were smaller than those between ovules of different flowers. However, viable and senescent ovules could be found within the same ovary and ovules.

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دراسات مورفولوجية و فسيولوجية على التزهير و التلقيح و الإثمار لأشجار الزيتون "صنف البيكوال"

٣- دراسة على تلقيح أزهار الزيتون صنف البيكوال باستخدام الميكروسكوب الفلورسنتى

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****المركز القومى للبحوث**

أجريت هذه الدراسة على أزهار الزيتون صنف البيكوال بإستخدام طريقة الفحص بالميكروسكوب الفلورسنتي بعد أجراء معاملات تلقيح مختلفة. أشتملت معاملات التلقيح على دراسة تاثير التلقيح الذاتي والتلقيح الخلطي بإستخدام صنفي الكروناكي والكوراتينا. كما تم دراسة تأثير التلقيح في عشرة مواعيد مختلفة بداية من ٣ أيام قبل التفتح وحتى ١٠ أيام بعد التفتح.أظهرت نتائج الدراسة أن التلقيح الخلطي بإستخدام صنف الكروناكي أدى إلى زيادة معنوية في نسبة إنبات حبوب اللقاح على مياسم أزهار الصنف البيكوال وكذلك نسبة أختراق أنابيب اللقاح للاقلام وعدد أنابيب اللقاح في نهاية القلم مقارنة بالتلقيح الخلطي بالصنف كوراتينا وكذلك التلقيح الذاتي. كما أوضحت النتائج عند التلقيح الخلطي بالكروناكي والكوراتينا أن نسبة إنبات حبوب اللقاح وإختراق أنابيب اللقاح لأقلام البيكوال وعدد أنابيب اللقاح في نهاية القلم كان أكثر معنوية عند إجراء التلقيح بداية من التفتح حتى ثلاثة أيام بعد التفتح الكامل. بينما بدأت هذه القياسات في الأنخفاض التدريجي عند تأخير التلقيح إلى أربع أيام بعد التفتح حتى عشرة أيام بعد التفتيح.

يمكن التوصية من خلال نتائج هذه الدراسة باستخدام صنف الكروناكي كملقح له كفاءة عالية مع صنف البيكوال حيث أظهر كفاءة عالية فى إنبات حبوب اللقاح و اختراق أنابيب اللقاح. كما يجب مراعاة عدم تأخير التلقيح عن اليوم الثالث بعد التفتح حيث تنخفض كفاءته فى إحداث الإخصاب نظرا لفقد البويضات لحيويتها.