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Determining the Best Pollenizer of Olive [*Olea europaea* (L.) ('Dezfoul')] in Fars Province

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Abstract: In order to identify the best pollenizer of olive this study was conducted in Iran, Fars province, Shiraz city during 2002-2003 years. Flowers were hand pollinated using pollens from olive cultivars 'Derak', 'Dezfoul', 'Roghani', 'Shengeh', 'Shiraz', 'Fishomi' and a pollen mixture of all the cultivars used or allowed to get self pollinated or open pollinated. Initial and final fruit set, yield and self-incompatibility index were evaluated. Results indicated that 'Dezfoul' is a severe self-imcompatible cultivar with an index of self-incompatibility of 0.19. The best treatment was the pollination with 'Shiraz' that resulted in a more than 5.21 and 5.73-fold increase in final fruit set and yields over self-pollination, respectively. 'Shiraz' is recommended as an ideal pollenizer for 'Dezfoul'.

Key words: Olive % Pollination % Self-incompatibility % Fruit set

INTRODUCTION

Fars province, under 18000-hectar olive [*Olea europaea* (L.)] cultivation, is ranked the first in the country. 'Dezfoul' olive trees are used widely in the developmental programs of gardens, as a suitable and compatible cultivar. Considering such significance, doing research is necessary regarding this subjecte. Olive trees may not be able to produce enough fruit crop despite having numerous flowers. Cross pollination is needed for maximum yield in most cultivars of olives [1]. Self-incompatibility among olive trees have been reported by many researchers and are affected largely by temperature [2-5]. Porlingis and Voyiatzis [6] reported that among self-incompatible cultivars of olive, pollen tubes grew gradually and most of them either did not reach to the embryo sac or reached when the sacs were defunct. Furthermore, Bini [7] showed a gametophytic incompatibility among 'Moraiolo' olives. According to Fernandez-Escobar and Gomez Valledor [8] self-pollination among 'Sevillano' olive caused shot berries to form 40-50% of the total yield at harvest time. Although, they formed 20 % of the total yield when cross-pollinated with 'Picudo' olive. Cuevas and Rallo [9] reported that cross-pollination versus self-pollination led to increase in fruit set among 'Manzanillo' olives. Sibbett *et al.* [5] used pollens of 'Sevillano' olive for pollination of 'Manzanillo' at the beginning and middle of blooming and at the full bloom stage. The farther 'Manzanillo' olive were from pollens of 'Sevillano' olive, the less was the percentage of normal fruits and the more was the percentage of parthenocarpic fruits. Ugrinovic and Stampar [10] put 'Leccino', 'Pendolina' and 'Istrska belica' olives under self-pollination, cross-pollination and open pollination situations. Results showed that 'Leccino' and 'Pendolina' olives were self-sterile and 'Istrska belica' olive had a very low level of self-fruiting. Among 'Istrska belica' olives, the percentage of fruit-set in the state of self-pollination was 21 % and in the best state, i.e., cross-pollination with 'Leccino' was 4 %. 'Pendolina' olive showed the best results of cross-pollination with 'Leccino' olive and in the state of open pollination, with fruit set percentages of 1.77 and 1.78, respectively. Among 'Leccino' olives, the percentage of fruit set in the state of open pollination, cross pollination with 'Pendolina' and 'Istrska belica' olives were 6.88, 5.75 and 5.45, respectively.Cuevas and Polito [11] showed that 'Manzanillo' is a self-compatible olive cultivar. Its percentage of fruit set when cross-pollinated with 'Sevillano' olive was more than four times the self-pollinated ones. 'Manzanillo' olive was cross-incompatible with 'Mission' and 'Ascolano' olives. Iannotta *et al.* [12] took 'Carolea' olive into consideration and came to the conclusion that 'Carolea' olive was self-incompatible and due to the insufficient development of the ovary, might morphological sterility. 'Leccino' and 'Tundina' olives were the best pollinizers for 'Carolea' olive.

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'Dezfoul' is considered one of the significant commercial olive cultivars in Fars province which is used in either pickling or oil extraction. Considering implementation of the project of improvement and development of olive gardens in Fars province, ideal features of 'Dezfoul' olive for the purpose of planting in this province, lack of sufficient information regarding the manner of pollination of such trees and posing questions on whether this cultivar has to be planted with or without other pollinizing cultivars, warrants a research on the pollination of this cultivar. Therefore, the objective of this study was determining a suitable pollinizer for 'Dezfoul' olive to achieve maximum of the yield.

MATERIALS AND METHODS

Plant Material: This study was carried out in a commercial garden in Shiraz, Fars province, in 2002 and 2003 years. Three 'Dezfoul' olive trees, being the same as each other in the cases of age and size and in fruiting state, were chosen every year. In 2002 year, ten shoots with almost one-centimeter diameter and equal in height above the ground and configuaration on the tree were selected. They were randomly allocated to treatments of self-pollination, cross-pollination with pollens of 'Derak', 'Roghani', 'Shengeh', 'Shiraz', 'Fishomi' and a mixture of the mentoned cultivars pollens and open pollination as well. The purpose of the treatment of pollination with pollens of 'Dezfoul' olive was simulation of the treatment of self-pollination. The purpose of cross-pollination with the mixture of pollens of the mentioned cultivars was to investigate possible effects of combined pollens of several cultivars and to simulate the treatment of open pollination. For the treatment of pollen mixture, equal amounts (by weight) of pollens of the given cultivars were combined.

Pollination: In both 2002 and 2003 years, before the time when flowers would open, i.e., Balloon stage (the stage in which blooms are completely swollen, white and near to open), seventy complete flowers were emasculated using pincers on every branch (except treatments of self-pollination and open-pollination). In order to avoid unwanted pollination, all branches (except in the treatment of open pollination) were coverd by paper bags.

In the treatments of self-pollination and open pollination, seventy complete flowers were chosen but not emasculated. The pollen used in this study was collected from the olive trees in the southern parts of the garden whose flowers would open early and kept in glass containers tightened with cotton. Such containers were in turn put in desiccator, next to moisture-absorbent material (potassium permangenate) inside the refrigerator at 4°C. The branches supposed to be treated by cross-pollination were pollinated with the given pollens during twenty-four, forty-eight and seventy-two hours after the emasculation. At first, the paper bag were removed and the given pollen were put on the stigmas of the emasculated flowers with a soft painting brush (the number 0000) and then, the paper bags were put on the branches, immediately. In this stage, pollination on the blackened stigmas of emasculated flowers was avoided (due to physical damage of emasculation or some other reasons). The number of blackened stigmas were written down to subtract from the initial number of emasculated complete flowers (seventy flowers).

Germination: In another section of this study in both 2002 and 2003 years, Brewbaker and Kwack's culture medium (Table 2) was used to examine the germination ability of pollens of different cultivars at 24°C [14].

In this section of the study, ten ml of the prepared culture medium was poured into each petridish. Petridishes were divided into groups of four. Then, one special cultivar of pollen was poured in every group.

Data Recording: Twenty days after emasculation, paper bags were removed from all the branches and the percentages of their initial fruit set were calculated. In this stage, fruit set includes normal and parthenocarpic fruits. Recognizing morphological difference between these two kinds of fruits was difficult. The percentage of fruit set was calculated separately for all the treatments in both mid-summer and harvest times. At these times, only normal fruits were counted. At the harvest time, fruits of each treatment were harvested separately and the percentage of the final fruit set and the total yield were measured.

To compute the self-incompatibility index, the suggested formula by Zapata and Arroya was used [13]:

$$\text{Self-imcompatibility index} = \frac{\text{Formation of fruit by self-pollination}}{\text{Formation of fruit by cross-pollination}}$$

Zapata and Arroyo have categorized the value of self-incompatibility index (Table 1).

Table 1: Categorization of self-incompatibility index

Self-incompatibility index	State
0	Completely self-incompatible
< 0.2	Severely self-incompatible
0.2 > < 1	Relatively self-incompatible
1 <	Self-compatible

Table 2: Brewbaker and Kwack's culture medium

Chemical material	Chemical formula	Amount
Boric acid	H_3BO_3	0.5 g
Potassium nitrate	KNO_3	0.1 g
Sucrose	$C_{12}H_{22}O_{11}$	15 %
Magnesium sulfate	$MgSO_4$	0.2 g
Calcium nitrate	$Ca(NO_3)_2$, $4H_2O$	0.2 g
Distilled water	H_2O	1 liter

Germinated pollens were counted by flourescent microscope 24 hr later. It is worth mentioning that every petridish was sample four times and the mean of sixteen replications was calculated for every treatment.

Statistical Analysis: In research of pollination, the experiment conducted as a randomized complete blocks design with three replications and each tree was considered as a block and its branches as experimental plot. Analysis of variance was carried out simply and compoundly (after the integration of data of the first and the second year). When the data corresponded to percentages they were arcsintransformed prior to the ANOVA. Means were compared according to Duncan's New Multiple Range Test (DNMRT) at 5 or 1 % levels.

The experiment of germination was conducted in a completely randomized design with four replications. Analysis of variance was carried out simply and compoundly (after integration of the data of the first and second years). The data were arcsintransforned prior to the ANOVA. Means were compared according to DNMRT at 5 and 1 % levels.

RESULTS

Results of combined data of 2002 and 2003 years showed that the percentages of fruit set in all stages in treatments of mixture of pollens, open pollination and 'Shiraz' were significantly more than self-pollination. But this was not the case about other treatments (Table 3).

Pollination with 'Shiraz' enhanced fruit set 5.21 times more than self-pollination treatment. The self-incompatibility index of 'Dezfoul' vs. cross-pollination with 'Shiraz' equalled 0.19. Therefore, this cultivar was categorized as a severe self-incompatible one.

Table 3: Effect of different pollen types on fruit set of 'Dezfoul' cultivar olive in 2002 and 2003 years

Treatments	Fruit set in 20 days after full bloom(%)	Fruit set in summer(%)	Fruit set at harvest time(%)	Yield(g)
Mixture of pollens	2936 A[†]	21.59 A	21.14 A	28.36 A
Open pollination	28.77 A	20.87 A	20.04 A	36.32 A
'Shiraz'	26.98 A	20.72 A	20.35 A	34.24 A
'Shengeh'	19.31 AB	12.77 AB	12.77 AB	18.02 AB
'Fishomi'	8.84 BC	5.20 B	5.20 B	6.20 B
'Selfing'	6.34 BC	4.31 B	3.90 B	5.97 B
'Dezfoul'	5.54 BC	3.00 B	3.00 B	3.89 B
'Derak'	5.07 BC	2.58 B	2.58 B	4.88 B
'Roghani'	3.67 C	3.11 B	3.11 B	3.66 B

[†]In each column, means with the same letters are not significantly different according to DNMRT 1 % level.

Table 4: Percentage of germination of applied pollens on 'Dezfoul' cultivar olive in 2002 and 2003 years

Type of pollen	Germination (%)
'Roghani'	39.94 A[†]
'Dezfoul'	37.64 A
'Fishomi'	33.76 A
'Shengeh'	32.41 A
'Shiraz'	30.22 A
'Derak'	22.55 A

[†]Means in each column with same letters are not significantly different at 5% level using DMRT

Moreover, the results of combined data of the first and second year revealed that the yield of open pollination, 'Shiraz' and mixture of pollens treatment was significantly more than self-pollination treatment while it was not true of the other treatments (Table 3). Having been pollinated with 'Shiraz' pollens, the yield increased 5.73 times more compared to self-pollination treatment.

Combined data of the first and second year showed no significant different among the germination percentage of the applied pollens (Table 4).

Two-years mean of the number of flowers and the percentage of complete flowers in each 'Dezfoul' inflorescence were 22.05 and 25.90, respectively.

DISCUSSION

Results of this study represents self-incompatibility in 'Dezfoul' olive. Cross-pollination with 'Shiraz', open pollination and combination of pollens led to increase in initial and final fruit set and yield compared to self-pollination treatment. It maybe resulted from poor function of pollen tubes in growth towards ovules and fertilization [11].

In self-incompatible olive trees, pollen tubes grow slowly and most of them either do not reach embryo sacs or reach when these sacs are defunct [6]. In some olive cultivars, morphological sterility due to inadequate development of ovary and also gametophytic incompatibility are considered as reasons of self-incompatibility [7, 12]. In addition, in some cultivars it has been reported that stamen filamentes widening causes stamens to move away from stigma, hence, self-pollination is prevented. The latter case was not effective in this study because pollination of 'Dezfoul' olive with 'Dezfoul' pollens was similar to self-pollination treatment. Results achieved in this study regarding self-incompatibility in 'Dezfoul' and increase in fruit set through cross-pollination confirmed other reports with the other cultivars of olive [1, 2, 5, 7-12].

On the other hand, except treatments of 'Shiraz', open pollination and combination of pollens, fruit set and yield were similar to self-pollination treatment. This fact is due to cross-incompatibility of these cultivars with 'Dezfoul' and the reduction in growth of pollen tubes and inability of fertilization using applied pollens [14]. It seems that growth of pollen tubes halts immediately after the penetration of tubes into the surface of stigma. The first callose plates are seen a little below stigma. Formation of the first callose plate shows that bi-nuclear pollen tube has been changed into heterotrophic state and it is a sign of emergence of gametophytic incompatibility. The second and third callose plates are formed at the top and bottom of fillament, severe competition among pollen tubes starts at the top the finishes at the bottom of fillament. Deposited callose on the pollen tube is stable [15, 16].

The treatment of pollination with 'Dezfoul' in order to simulate self-pollination yielded similar to self-pollination and confirmed results of self-pollination. Moreover, the treatment of combining pollens in order to examine possible effects of combined pollens of some cultivars on each other and also simulation of open pollination yielded the same as open pollination. This reveals that except 'Shiraz', existence of pollens of other cultivars are not effective in this combination. Such a case has been reported by Brewbaker and Kwack [14].

It is worth mentioning that self-incompatibility in olive is highly influenced by temperature. Different experiments on a particular cultivar of olive have shown various results in different years and places [3, 4, 8]. In the current study, 'Dezfoul' olive trees were categorized as relatively self-incompatible in the first year and as severe self-incompatabile in the second year (data not shown).

Studying pollen germination of various cultivars reveals difference in germination of them (Table 4). In addition, the manner of collecting and conditions of preserving pollens are important as in the second year of study the percentage of germination improved (data not shown).

One of determining factors in the yield amount of an commercial cultivar is its flowering state. Comparing two-year results of the number of flowers in 'Dezfoul' inflorescence no considerable differences.

It seems that the number of flowers in inflorescences is affected mostly by the cultivar and less by environmental factors. The percentage of complete flowers of this cultivar was effected largely by year. The effect of year was probably in view of changes in temperature and agricultural practices, especially nutrition and water stress [4].

To sum up, considering the presented information and results of this research, 'Shiraz' is introduced as an ideal pollinizer for 'Dezfoul'.

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