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Study of self-incompatibility in some Iranian olive cultivars

M. R. Taslimpour[a*] and E. Aslmoshtaghi[b]

[a]Agricultural and Natural Resources Research Center of Fars Province, Shiraz, Iran.
[b]Faculty of Agriculture, Shiraz University, Shiraz, Iran.
* Corresponding author's E-mail address: rtaslimpour@yahoo.com.

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ABSTRACT

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Despite the profusion of flowers that develop on olive trees, only a few set fruit during the growing season and only about 1-2% of the fruit remains on the trees at maturity. Therefore, to maximize fruit set and yield, it is important for growers to understand the cross-compatibility between cultivars when planning to establish an olive orchard. This study was carried out in Shiraz, Fars Province, in 2005 and 2006 to identify the best pollinizers for Zard, Roghani, Fishomi and Shiraz olive cultivars. Flowers were pollinated using pollen from cultivars Dezfoul, Roghani, Zard, Shengeh, Shiraz, Fishomi and Fishomi-Roudbar and/or the flowers were self-pollinated or open-pollinated. Initial and final fruit set, fruit yield and self-incompatibility were assessed. Results showed that cultivars Zard and Fishomi are self-compatible, whereas Roghani and Shiraz are relatively self-incompatible (self-incompatibility index = 0.47 and 0.34, respectively). Cultivars Fishomi-Roudbar and Dezfoul were identified as suitable pollinizers for Roghani and Shiraz, respectively.

Keywords: cross pollination, fruit set, fruit yield, pollinizer, self-incompatibility index

INTRODUCTION

The efficiency of the olive industry greatly depends on harvesting economic fruit yields that can be used for producing oil and table olives. For successful cross-pollination to occur, adequate amounts of compatible pollen have to be available when flowers are in bloom (Fernandez-Escobar and Gomez-Valledor, 1985; Cuevas and Rallo, 1990; Marco *et al*., 1990; Martin, 1990; Taslimpour *et al*., 2008). This is possible when compatible cultivars grown in the orchard have overlapping flowering times.

Some olive cultivars are self-incompatible, which means that flowers cannot be fertilized by pollen from the same cultivar, whereas some cultivars are cross-incompatible, indicating that flowers cannot be fertilized by pollen from certain other cultivars. Therefore, when planning an olive orchard, it is important for olive growers to understand the cross-compatibility between cultivars in order to maximize fruit set and yields (Moutier, 2000; Lavee and Datt, 1978).

Ugrinovik and Stampar (1996) evaluated olive cultivars Leccino, Pendolina and Istrska Belica under self-pollination, cross-pollination and open pollination conditions, and showed that Leccino and Pendolina were self-sterile, while Istrska Belica had very low self-fruiting. Among Istrska Belica olives, fruit set (%) under self-pollination was 0.21% and 4% under the best conditions (i.e., cross pollination with Leccino). Cultivar Pendolina showed the best results when cross-pollinated with Leccino and when open-pollinated, with fruit set of 1.77% and 1.78%, respectively.

When open-pollinated or cross-pollinated by Pendolina and Istrska Belica, cultivar Leccino had fruit set of 6.88, 5.75 and 5.45, respectively. Pinillos and Cuevas (2009) reported that Picual behaved as a self-incompatible cultivar with reduced fruit set under self-pollination. However, cross-pollination rarely increased fruit set compared with open-pollination. Cuevas and Polito (1997) showed that Manzanillo is a self-incompatible olive cultivar. Its fruit set (%) when cross-pollinated with Sevillano was four times greater than when self-pollinated. Manzanillo was cross-incompatible with cultivars Mission and Ascolano.

Díaz *et al*. (2006) studied self-incompatibility by testing seeds obtained from mother trees Picual and Arbequina using paternity analysis with data obtained from four microsatellite markers. In the 90 seeds tested, they found only three incidences of selfing and concluded that cultivars Picual and Arbequina were self-incompatible. In fact, several other studies have also found that some olive cultivars are self-incompatible (Porlingis and Voyiatzis, 1976; Androulakis and Loupassaki, 1990; Cuevas and Rallo, 1990; Sibbett *et al*., 1992; Iannotta *et al*., 1999).

Therefore, the objective of this study was to determine self-compatibility and identify suitable pollinizers among some Iranian olive cultivars in Shiraz, Fars Province, southern Iran.

MATERIALS AND METHODS

This study was carried out in 2005 and 2006 in a commercial olive orchard in Shiraz, Fars Province, southern Iran. Trees of olive cultivars Zard, Roghani, Fishomi and Shiraz that were of the same age, and size and fruiting state were chosen in both seasons. From each cultivar, we selected 16 shoots almost 1 cm in diameter, with equal height above the ground and similar configuration on the tree. These shoots were randomly assigned to three treatments: self-pollination, open pollination and cross-pollination with pollen from cultivars Dezfoul, Roghani, Zard, Shengeh, Shiraz, Fishomi and Fishomi-Roudbar.

In both the 2005 and 2006 seasons, 100 complete flowers were selected from each shoot before the onset of blooming (i.e., at the balloon stage, when blooms are completely swollen, white and about to open). To avoid unwanted pollination, all shoots (except those in the open pollination treatment) were covered by paper bags. Pollen was collected from the parts of the trees facing south, where flowers open earlier, and kept in glass containers tightly covered with cotton. The containers were placed in desiccators, next to moisture-absorbent material (potassium permanganate) in a refrigerator at 4 °C.

The shoots that were to be cross-pollinated were pollinated at the beginning and middle of flowering and at full bloom. The paper bags were removed and pollen was placed on the stigmas of the complete flowers with a soft paint brush (size 0000). After pollination, the paper bags were immediately put back on the shoots. Some flowers had blackened stigmas (due to physical damage or some other reason) and were not pollinated. The number of blackened stigmas were recorded and later deducted from the initial number of complete flowers.

Twenty days after full bloom, the paper bags were removed from the entire shoot and the initial fruit set (%) on each shoot was measured. At this stage, fruit set included normal and parthenocarpic fruits, and distinguishing the morphological differences between these two kinds of fruit was difficult. Fruit set was measured separately for all treatments at both mid-summer and harvest, and only normal fruits were counted. At harvest, fruits from each treatment were harvested separately and fruit yield was measured for each treatment. The self-incompatibility index was calculated following Zapata and Arroyo (1978):

$$\text{Self-incompatibility index (\%)} = \frac{\text{Fruit set by self-pollination}}{\text{Fruit set by cross-pollination}}$$

Self-incompatibility index categories by Zapata and Arroyo (1978) are presented in Table 1.

Table 1. Self-incompatibility index categories.

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

Source: Zapata and Arroyo (1978).

In another part of this study, in both the 2005 and 2006 seasons, a 15% sucrose + 1% agar culture medium at 24 °C was used to examine pollen germinability (%) of different cultivars. To this end, 10 ml of the prepared culture medium was poured into each petri dish and then the petri dishes were divided into groups of four. Pollen from one particular cultivar was cultured in every group (Sunzui and Biltui, 1984). After 24 h, germinated pollen grains were counted under the microscope.

Statistical analysis: In the pollination study, the trial was conducted using a randomized complete block design with three replications. Each tree was considered a block and its shoots the experimental plots. Combined analyses of variance were performed. Data collected as percentages were transformed according to the arcs prior to the analysis of variance. Means were compared using Duncan's Multiple Range Test at the 1% probability level.

The germination experiment was conducted using a completely randomized design with four replications. Combined analyses of variance were performed. Means were compared using Duncan's Multiple Range Test at the 1% probability level.

RESULTS

Results for cultivars Zard and Fishomi indicated there were no significant differences among treatments for fruit set and yield (Tables 2 and 3). In cultivar Roghani, initial fruit set increased when it was cross-pollinated with Zard in comparison with self-pollination. Compared with self-pollination, fruit set was significantly higher after open pollination and cross-pollination with Fishomi-Roudbar, Fishomi, Zard and Shengeh, and also at harvest after open pollination and cross-pollination with Fishomi-Roudbar, Zard, Fishomi, Shengeh and Shirazthan (Table 4). Since the self-incompatibility index for Fishomi-Roudbar cultivar was 0.47, it was identified as relatively self-incompatible (Table 4).

Table 2. Effect of different pollen sources on fruit set of cv. Zard.

Treatments	Fruit set 20 days after full bloom (%)	Fruit set in mid-summer (%)	Fruit set at harvest (%)	Self-incompatibility index	Fruit yield (g treatment^{-1})
Self pollination	8.62	6.36	6.02	---	21.66
Dezfoul	13.12	7.24	7.24	0.83	24.64
Roghani	9.98	7.59	7.28	0.83	22.51
Shengeh	11.85	6.98	6.78	0.89	19.72
Shiraz	11.61	7.84	7.84	0.77	25.96
Fishomi	13.55	7.93	7.31	0.82	23.82
Fishomi-Roudbar	13.33	8.57	8.31	0.72	26.99
Open pollination	10.45	7.20	6.66	0.90	23.92

Table 3. Effect of different pollen sources on fruit set of cv. Fishomi.

Treatments	Fruit set 20 days after full bloom (%)	Fruit set in mid-summer (%)	Fruit set at harvest (%)	Self-incompatibility index	Fruit yield (g treatment-1)
Self pollination	9.92	8.79	8.26	---	37.68
Dezfoul	11.30	10.79	9.24	0.89	43.78
Roghani	14.11	9.38	9.05	0.91	40.03
Zard	9.57	8.68	8.11	1.02	41.03
Shengeh	11.26	9.56	9.56	0.86	44.65
Shiraz	10.45	9.11	7.95	1.04	34.90
Fishomi-Roudbar	10.46	9.79	8.14	1.01	39.21
Open pollination	15.08	11.67	10.31	0.80	48.81

Table 4. Effect of different pollen sources on fruit set of cv. Roghani.

Treatments	Fruit set 20 days after full bloom (%)	Fruit set in mid summer (%)	Fruit set at harvest (%)	Self-incompatibility index	Fruit yield (g treatment^{-1})
Self pollination	12.27 b	8.35 d	7.84 d	---	18.17 c
Dezfoul	14.76 ab	10.72 cd	10.72 cd	0.73 a	26.57 bc
Zard	27.15 a	14.65 ab	14.65 ab	0.53 bc	39.76 ab
Shengeh	16.89 ab	13.05 bc	12.54 bc	0.62 ab	29.63 abc
Shiraz	18.92 ab	11.95bcd	11.95 bc	0.66 ab	31.79 abc
Fishomi	19.68 ab	14.71 ab	14.25 ab	0.55 bc	38.63 ab
Fishomi-Roudbar	24.36 ab	17.14 a	16.65 a	0.47 c	44.54 a
Open pollination	20.02 ab	15.66 ab	14.33 ab	0.55 bc	45.61 a

In each column, means followed by the same letter(s) are not significantly different at the 1% probability level using Duncan's Multiple Range Test.

Results for cultivar Shiraz showed that initial fruit set increased after open pollination and cross-pollination with Roghani and Dezfoul (Table 5). The highest fruit set in mid-summer and at harvest was observed when Shiraz was open-pollinated or cross-pollinated with Roghani, Dezfol and Fishomi Roudbar (Table 5). Cultivar Shiraz had a self-incompatibility index of 0.34 and was identified as relatively self-incompatible (Table 5).

Results indicated that the highest germination occurred in Fishomi-Roudbar (70.5%), followed by Shiraz (69.63%) and Shengeh (69.38%); the lowest was recorded for cultivar Fishomi (52.13%) (Table 6).

Table 5. Effect of different pollen sources on fruit set of cv. Shiraz.

Treatments	Fruit set 20 days after full bloom (%)	Fruit set in mid summer (%)	Fruit set at harvest (%)	Self-incompatibility index	Fruit yield (g treatment^{-1})
Self pollination	10.60c	9.03b	9.03b	---	23.69c
Dezfoul	32.11ab	22.88a	22.88a	0.39 b	80.21a
Roghani	41.81a	26.75a	26.75a	0.34 b	89.96a
Zard	19.79bc	13.11b	11.55b	0.78 a	43.11bc
Shengeh	19.14bc	10.43b	10.43b	0.86 a	40.56bc
Fishomi	19.79bc	10.29b	10.28b	0.88 a	40.24bc
Fishomi-Roudbar	26.33abc	20.10a	20.10a	0.45 b	73.21ab
Open pollination	28.48ab	23.31a	22.51a	0.40 b	99.28a

In each column, means followed by the same letter(s) are not significantly different at the 1% probability level using Duncan's Multiple Range Test.

Table 6. Germinability of applied pollen grains.

Pollen source	Germination (%)
Dezfoul	55.38ab
Roghani	60.75ab
Zard	64.38ab
Shengeh	69.38a
Shiraz	69.63a
Fishomi	52.13b
Fishomi-Roudbar	70.50a

Means followed by the same letter(s) are not significantly different at the 1% probability level using Duncan's Multiple Range Test.

DISCUSSIONS

Results showed that cultivars Zard and Fishomi behaved as self-compatible cultivars, while Roghani and Shiraz behaved as relatively self-incompatible cultivars. The olive is a wind-pollinated species and, although there are self-compatible and sterile male cultivars, self-incompatibility has been reported in many cultivars including Picual, which is one of the most important cultivars for oil production in Spain (Ghrisi *et al*., 1999; Cuevas *et al*., 2001; Wu *et al*., 2002; Díaz *et al.*, 2006).

The genetic basis of self-incompatibility and the detailed mechanisms controlling self-incompatibility in *O. europaea* L. are unknown but the olive belongs to the Oleaceae family and has a wet stigma and bicellular pollen grains (Ateyyeh *et al*., 2000; Reale *et al.,* 2006; Serrano *et al.,* 2008). Self-incompatibility could result in low fruit yields in orchards consisting of a single cultivar (Lavee and Datt, 1978).

Sibbett *et al*. (1992) observed that cross-pollination could improve the quality of the crop by reducing the number of shotberry (seedless) fruit, and a topical application of Sevillano pollen on Manzanillo reduced the number of small or parthenocarpic fruit. Similar observations were made by Fernandez-Escobar and Gomez-Valledor (1985) and Cuevas and Polito (1997). Even cultivars that exhibit some level of self-fertility have been observed to produce higher fruit yield following cross-pollination (Lavee *et al*., 2002).

Porlingis and Voyiatzis (1976) reported that in self-incompatible olive cultivars, pollen tubes grew slowly and most of them either did not reach the embryo sacs or reached them when the sacs were defunct. Cuevas and Polito (1997) observed that during self-pollination, most pollen tubes were unable to grow through the style and reach the ovules for fertilization, while pollen tubes arising from cross-pollination grew faster and reached the ovule. This indicates that a self-incompatibility system operates in olives. Wu *et al.* (2002) tested self-incompatibility by hand-pollinating and observing pollen tube growth, and found that olive cultivars Frantoio, Kalamata and Verdale were self-incompatible. Moutier (2000) studied compatibility relationships in 16 olive cultivars and found that most needed cross-pollination for adequate fruit set.

Our pollen germination study of the test cultivars revealed differences among them (Table 6). Pinney and Polito (1990) also found that pollen viability varies among cultivars. They found that Ascolano had the highest pollen viability and Mission the lowest. Wu *et al.* (2002) observed that pollen viability ranged from as low as 14% in Pendolino to as high as 79% in Frantoio.

Occasionally, olive cultivars have been found to be male sterile (Moutier, 2000; Villemur *et al.*, 1984). Lavee *et al.* (2002) suggested that since blooming time and pollen viability of cultivars vary from year to year, fruit yield may benefit from having more than one pollen donor in an orchard to ensure sufficient pollination. Pollen vitality was also observed to vary between years in Arbequina clones (Rovira and Tous, 2000).

In conclusion, based on the results of this study, cultivars Fishomi-Roudbar and Dezfoul were identified as suitable pollinizers for Roghani and Shiraz, respectively.

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