

## ROLE OF COMPATIBLE POLLINIZER FOR COMMERCIAL OLIVE PRODUCTION IN POTHWAR, PAKISTAN.

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### ABSTRACT

The final fruit yield is significantly correlated to fruit set which is directly dependent upon self-incompatibility (SI), pollination ability, extent of cross-compatibility among cultivars, and prevailing environmental conditions especially suitable temperature regimes during anthesis. Four Italian cultivars viz. Coratina, Frantoio, Ottobratica, and Leccino under the agro-climatic conditions of Pothwar (Pakistan) were included in these studies during the years of 2017 & 2018. Each variety exhibited different values for the initial fruit set, final fruit set, number of shotberries and extent of self-incompatibility index. The SI resulted through reciprocal crosses depicted that cv. Coratina (0.80), cv. Frantoio (0.67) and cv. Leccino (0.73) possessed partial self-incompatibility while cv. Ottobratica (0.43) was found completely self-incompatible in studied environmental conditions. Cross-compatibility results based on fruit set percentage indicated that the cultivars can efficiently pollinize other cultivars and set good fruit yield, however, the cultivars pairs Coratina/Frantoio was at top and pair of Ottobratica/ Leccino was at the bottom. Thus, the selection and plantation of suitable pollinizers in olive orchards is a prerequisite through which deficiency in fruit setting and attaining commercial yield in olive orchards could be achieved.

**Keywords:** olive, Pakistan, self-incompatibility index, pollinizer, cross-compatibility.

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### INTRODUCTION

Olive is the most important oil producing tree of the Mediterranean region included Turkey, Syria, Palestine, and Israel but the origin of cultivated olive remained unclear (Concepcion *et al.*, 2014). Spain is the largest olive producer with a production of more than 900000 tons of olives per annum. From overall world production, Italy and Spain together account for 50%. In the world, 2713.5 thousand tons of olive oil is being produced on a commercial scale in 47 countries of 5 continents (Mansour *et al.*, 2018).

Olive (*Olea europaea* L.) is an evergreen fruit tree and its oil has a beneficial effect on human health due to its special nutritional values (Estruch *et al.*, 2013). Pakistan is the fourth largest edible oil importing country in the world which imports 70% of the total edible oil by spending a lot of money (2.710 billion US\$) in lieu of import bill that is increasing at an alarming rate (Kiran, 2017). Presently, the increase in per capita consumption of edible oil has jumped from 17 to 18 Kg during the last five years (Rashid, 2018). Olive cultivation and its consumption have extended other than Mediterranean belts such as Pakistan, China, India, Australia, and the Southern part of America during the last 20 to 30 years (Awan *et al.*, 2011). Although Pakistan has a short history of olive cultivation, yet during the last ten years

olive plantation has gained popularity because of its great socio-economic importance. More than 4494.36 hectares area has been brought under olive plantation in the Pothwar region while more than 2,800 acres (280,004 plants) in other parts of the country including Southern KP and Baluchistan (PARC, 2018; BARI, 2019).

The optimum temperature range for the development of the reproductive bud is 2 °C to 19 °C (Malik and Perez, 2011, Torres *et al.*, 2017). However, the temperature higher than 30 °C severely inhibits the pollination process irrespective of the cultivars; thus results in increase self-incompatibility or lower the efficiency of the cross or open pollination. Harsh environmental conditions at blooming, anthesis, fertilization, or just after the fertilization critically influence the pollination and fruit yield (Koubouris *et al.*, 2009; Rapoport, 2012; Rondanini *et al.*, 2014). Self-incompatibility is the characteristics which are associated with many olive genotypes which also depends upon environmental conditions, high temperature, nutrients profile of soil, water availability, pollination ability, percentage of pollinator varieties and failure of pollen tube emergence from pollens, slow or delay the growth of pollen tube in the style (Saumitou-Laprade *et al.*, 2017; Torres *et al.*, 2017).

Pakistan has not true to type Mediterranean climatic conditions however, a limited number of olive

varieties were cultivated in different parts of Pothwar, Khyber Pakhtunkhwa and Balochistan. The data was collected upon only yield and oil content. Fruit yield per tree was significantly affected by cultivars, locations, and various physiological and reproductive processes such as flower differentiation, floral induction and fruit set (Iqbal *et al.*, 2019<sup>a</sup>, Webster, 2002). The yield was found very poor (10 to 12 kg/Tree) in prominent varieties like Coratina, Leccino, and Pendolino (Awan and Rab, 2014).

Thus, before the establishment of the olive industry in Pakistan, the performance of exotic cultivars requires investigations regarding its self-incompatibility, pollinizing efficiency, high-temperature sensitivity and cross-compatibility. Based on these facts, the present study was designed to record the role of pollinator varieties, degree of self-incompatibility of prominent cultivars under the agro-climatic conditions of the Pothwar region, and to determine the best pollinizer among these cultivars to get maximum fruit set.

## MATERIALS AND METHODS

**Experimental site and plant material:** The experiment was carried out in two consecutive years i.e. 2017 and 2018 at 7-8 years old well established commercial olive orchard "Izhar Olive Farm" located at Kallar Kahar, Chakwal, Northern Punjab, Pakistan (32° 46' 33" N and 72° 42' 31" E ) at 460 m above the sea level. The metrological data was recorded through the local weather station (Sensovant, Spain) installed at the site. The detail of the metrological data during the observation period and soil profile analysis were presented in Fig. 1 and Table 1 respectively.

Four olive cultivars including Frantoio, Coratina, Leccino, and Ottobratica were selected. These cultivars constitute the major portion of the orchards planted in the Pothwar region of Pakistan. For the experiment, twelve trees of each cultivar of the same age, height, and growth vigor were selected. All the trees were of seven to eight years old with uniform canopy planted at a distance of 6m x 6m. The trees were pruned to a central open shape. The orchard management practices (irrigation, fertilization, hoeing, weeding, were the same for all the cultivars. Trees were irrigated through high efficient drip irrigation system ten times per year.

**Experimental Layout:** Three different treatments were applied i.e Self-pollination, Controlled cross-pollination and Open pollination. The experiment was laid out according to randomized complete block design (RCBD) with three replications In the process of self-pollination, 20 healthy floral shoots, uniform in length and distribution were bagged with pollination papers randomly on all four sides (east, west, north, south) of canopy at white flower stage, a few days before opening

the flowers. Hence, there was no chance of receipt of foreign pollen and surety of self-pollination at full bloom by falling of self-pollen on stigma. Then at the petal fall stage or when browning of petal occurred data were collected (Selak *et al.*, 2011). To check the cross-compatibility, 20 other flowering shoots were randomly marked on the same plant and were left for free wind-pollination. Then at the petal fall stage or when browning of petal occurred, the data was collected for initial fruit set %, number of shotberries per branch, final fruit set %, and self-incompatibility index (Cuevas and Polito, 2004; Selak *et al.*, 2011).

To check the control cross-compatibility, 30 uniform flowering shoots were randomly marked on the each plant of a cultivar, and isolated by paper bags. Out of them, 10 branches were bagged for pollen collection and 20 for cross-pollination. Flowers in the bags were not emasculated for reflection of existing field conditions. Cross-pollination treatments were performed during the period of full bloom. The floral shoots of pollen donor cultivars with nearly opened flowers were separated from the mother plant. Cross-pollination treatments were done with opening the bags under optimal weather conditions (no wind and rain), placing the branches of donor pollinator, and finally enclosing them in the bags. The bags with both types of floral shoot (mother and donor) were shaken on a daily basis for better pollination. The selected shoots were enclosed until the end of the petal fall (end of stigma receptivity) according to the method of Selak *et al.*, (2011). Normally, it takes 2-3 days in an individual inflorescence, 5-6 days in an individual tree, but 10-15 days in cooler environments (Fabbri *et al.*, 2004). When the temperature is unstable, anthesis may occur in more than one flush, but the first flush usually sets the fruit that reach maturity (Lavee *et al.*, 1985). Each variety was used as a pollen donor for each and every other three varieties (Fig.2).

Data regarding initial fruit set (%) was recorded at petal fall stage one month after anthesis (1<sup>st</sup> week of May), final fruit set (%) was calculated after one month of initial fruit set (1<sup>st</sup> week of June) and the number of shotberries were recorded. The index of self-incompatibility was calculated by using the following formula according to the previous studies (Cuevas and Polito, 2004). The categories of self-incompatibility have been shown in Table 2.

$$ISI = \frac{\text{Final fruit set under self-pollination}}{\text{fruit set under free pollination}}$$

The collected data were analyzed through statistically software 8.1. The two-way ANOVA analysis was carried out and least significant difference (LSD) was utilized to compare the means at  $p \leq 0.05$  (Anonymous, 2005)

## RESULTS

**Initial Fruit Set (%):**The mean maximum initial fruit set was recorded in variety Leccino (13.75 %) followed by Frantoio (9.71 %) and Coratina (8.50 %). While the variety Ottobratica (7.68 %) showed minimum initial fruit set % (Table 3). As for the comparison of self and cross-pollination is concerned, cross-pollination showed a higher initial fruit set (10.62 %) as compared to self-pollination (9.21 %). The interaction between V×T depicted that the maximum initial fruit set was observed in a variety of Leccino in cross-pollination (13.91 %) followed by self-pollination (13.59 %). The variety Frantoio found at 2<sup>nd</sup> position with maximum initial fruit set in cross-pollination (10.47 %). While, variety Ottobratica was found at the lowest level (6.8 %) with a nearby variety Coratina (7.48 %) in self-pollination. The interaction between V×Y, it was concluded that variety Leccino was found at the top with the values of maximum initial fruit set (13.86 %) and (13.64 %) in 1<sup>st</sup> and 2<sup>nd</sup> year of self and cross-pollination process respectively. Variety Frantoio was ranked at 2<sup>nd</sup> position, Coratina at 3<sup>rd</sup>, and variety Ottobratica was found at the bottom level (Table 3). The interaction among V×T×Y, the maximum values for initial fruit set were recorded for variety Leccino in both the years in self and cross-pollination while Ottobratica remained at the lowest level in both the years and in treatments. In this case varieties, Coratina and Frantoio were found at a satisfactory level (Table 3).

The results of controlled cross-pollination depicted that the maximum initial fruit set was obtained in variety Leccino (13.99 % & 13.20 %) in both the years in case of self-pollination closely followed by the cross of Leccino and Frantoio and cross of Leccino and Coratina (Fig.3). All the crosses of varieties Coratina and Frantoio showed satisfactory results. Poor performance was obtained in the crosses of variety Ottobratica. In the case of variety Coratina as a female best cross was found with variety Frantoio in both the years with the values of 9.25 % and 9.49 % in 2017 and 2018 respectively (Fig. 3). In the case of variety Frantoio used as female, maximum initial fruit set was obtained when it was crossed with Ottobratica (9.47 %) in 2018 and cross with variety Coratina (8.76 %) in 2017. As well as the Ottobratica used as a female was concerned, the maximum initial fruit set (9.98 %) was obtained in 2018 when it was crossed with a variety of Frantoio. However, variety Leccino depicted the maximum initial fruit set in self-pollination treatment. Overall, the initial fruit set % was found satisfactory in all crosses except in Ottobratica during self-pollination treatment (Fig. 3).

**Final Fruit Set (%):**In the process of self and cross-pollination, the final fruit set percentage was significantly ( $P \leq 0.05$ ) affected by varieties and treatments. The results

of Table 4 depicted that the maximum final fruit set was obtained in a variety of Coratina (3.35 %) closely followed by Frantoio (3.21 %). The variety Leccino ranked at 3<sup>rd</sup> position (1.46 %) while variety Ottobratica ranked at bottom level (1.07 %). The interaction between V×T depicted that variety Frantoio showed maximum fruit set in cross-pollination (3.99 %) followed by a variety of Coratina (3.78 %). The Ottobratica showed a minimum value in self (0.20 %) but showed satisfactory results in cross-pollination (1.94 %). In comparison of V×Y, it was depicted that all the varieties performed best in 2<sup>nd</sup> year except variety Coratina which showed less fruit set as compared to 2017 (Table 4). In comparison of V×T×Y, variety Frantoio showed maximum value (4.37 %) in cross-pollination in 2<sup>nd</sup> year of production. Overall variety Ottobratica was found at the bottom level in self-pollination in both the year with maximum value in 2<sup>nd</sup> year (0.25 %) than 1<sup>st</sup> year (0.16 %).

In the process of controlled cross-pollination, the results showed that the maximum final fruit set was obtained in a variety of Coratina when it was crossed with the pollens of variety Frantoio (3.68 %) in 2018 (Fig.4). For the variety Frantoio used as a female, the data depicted the maximum final fruit set was obtained in variety Frantoio, when it was crossed with the pollens of variety Ottobratica (3.24 %) and Coratina (2.98 %). The results for controlled cross-pollination in variety Ottobratica portrayed the maximum final fruit set when it was made a cross with the pollens of variety Frantoio and Coratina in both the years (Fig. 4).

In the case of variety Leccino used as a female, the data described maximum final fruit set obtained when it was crossed with the pollens of variety Coratina (2.59 %) in 2017 and with Frantoio in 2018 (2.45 %). In the overall scenario of final fruit set %, the variety Ottobratica and Leccino performed poor in case of self-compatibility while varieties Coratina and Frantoio performed well and found self-compatible. From the glance of Fig. 4, it was also concluded that varieties of Ottobratica and Leccino can play a better role as a pollinator but results for varieties Frantoio and Coratina as a pollinator were found at the top for all the cases.

**Number of Shotberries per Branch:** The maximum number of shotberries per branch was recorded in variety Leccino (22.11) followed by Ottobratica (21.67). Varieties Coratina and Frantoio showed less number of shotberries (Table 5). Maximum numbers of shotberries were found in self-pollination (19.47) as compared to cross-pollination (18.69) with statistically non-significant results. Higher numbers of shotberries were recorded in 2<sup>nd</sup> year (19.48) as compared to 1<sup>st</sup> year (18.61 %). The data regarding the interaction between V×T depicted that, variety Ottobratica showed the maximum number of shotberries in self-pollination (22.65) followed by Leccino in cross-pollination (22.35). In comparison in

between  $V \times Y$ , the data depicted that more number of shotberries were found in Leccino (22.9) in 2<sup>nd</sup> year followed by Ottobratica (21.70) in 1<sup>st</sup> year. While varieties Frantoio and Coratina showed satisfactory results by producing less number of shotberries (Table 5). In comparison of  $V \times T \times Y$ , it was concluded that variety Ottobratica presented the maximum number of shotberries in self-pollination (23.61) at 2<sup>nd</sup> year of production followed by variety Leccino (22.71) in cross-pollination in the same year. The first seven values in this interaction were related to varieties of Leccino and Ottobratica in all treatments and years. The last two positions were occupied by varieties Coratina in self-pollination at 1<sup>st</sup> year (14.43) and Frantoio in cross-pollination at 2<sup>nd</sup> year (13.15). Varieties Frantoio and Coratina could be selected for cultivation in study areas on the basis of minimum numbers of shotberries and maximum final fruit set percentage.

In the process of controlled cross-pollination (Fig. 5), a maximum of three values of the number of shotberries viz 25.43, 25.37, and 24.83 were found where variety Leccino was involved either as a pollinator or as a female. In the second higher-level number of shotberries were found during the process of self-pollination especially in varieties Ottobratica and Leccino. Varieties Frantoio and Leccino showed a satisfactory level by producing less number of shotberries in the self-pollination process. In all the crosses variety Frantoio proved itself a good pollinator for all types of crosses on the basis of less number of shotberries (Fig. 5).

**Self-Incompatibility Index:** For all the olive cultivars under study, self-incompatibility index was calculated to estimate the level of self-sterility in each cultivar grown under the agro-climatic conditions of the Pothwar region. The significant variation was noted between years and varieties. Interactions between olive varieties and year ( $V \times Y$ ) also were found significant against the self-incompatibility index. The results depicted the maximum value was recorded in 1<sup>st</sup> year (0.82) as compared to 2<sup>nd</sup> year (0.49) (Table 6). In comparison of varieties, it was concluded that three varieties Coratina, Leccino and Frantoio showed less self-incompatibility as compared to Ottobratica. The Variety Coratina was found at the top with a value of 0.80 regarding the self-incompatibility index as compared to all other varieties. The interaction between  $V \times Y$  depicted that variety Leccino showed maximum self-incompatibility index (0.91) in 1<sup>st</sup> year followed by a variety of Coratina (0.84) and Frantoio (0.78) in the same year. The variety Ottobratica showed a minimum self-incompatibility index in 2<sup>nd</sup> year (0.10). Ottobratica was found less fruit set in the selfing process in both the years of study which clearly indicated that the variety of Ottobratica behaved as severely self-incompatible genotype. Overall, these results showed that all the studied olive cultivars possessed the phenomenon of self-incompatibility which was varied from cultivar to cultivar. Hence, all these cultivars need pollens from other olive cultivars to produce a high yield in terms of fruit production.

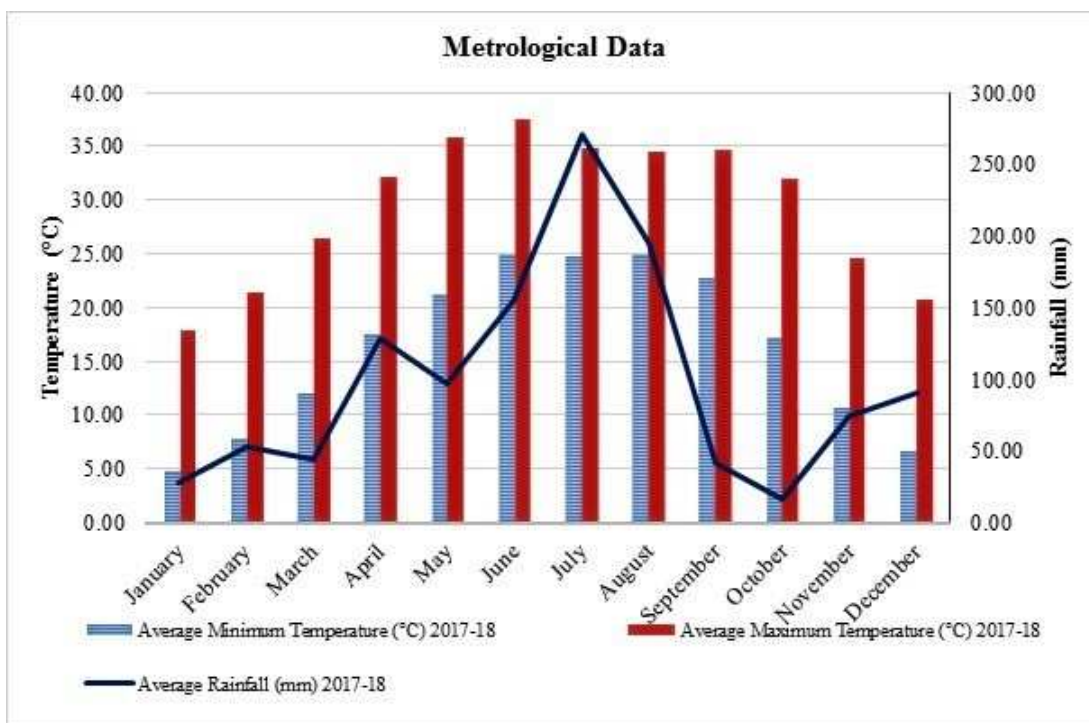


Figure 1. Average minimum temperature (°C), maximum temperature (°C) and rainfall (mm) 2017 and 2018.

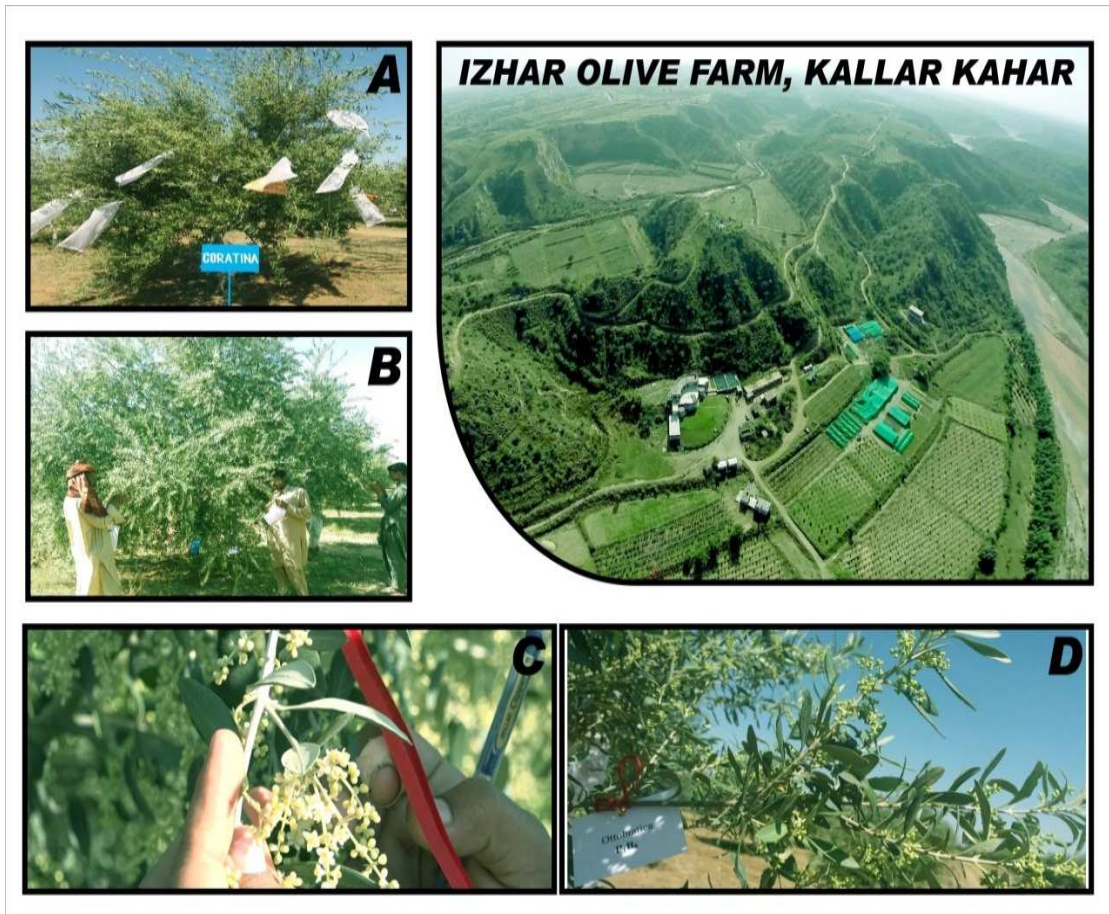


Figure 2. Self, cross and controlled cross treatments at Izhar Olive Farm, Kallar Kahar.

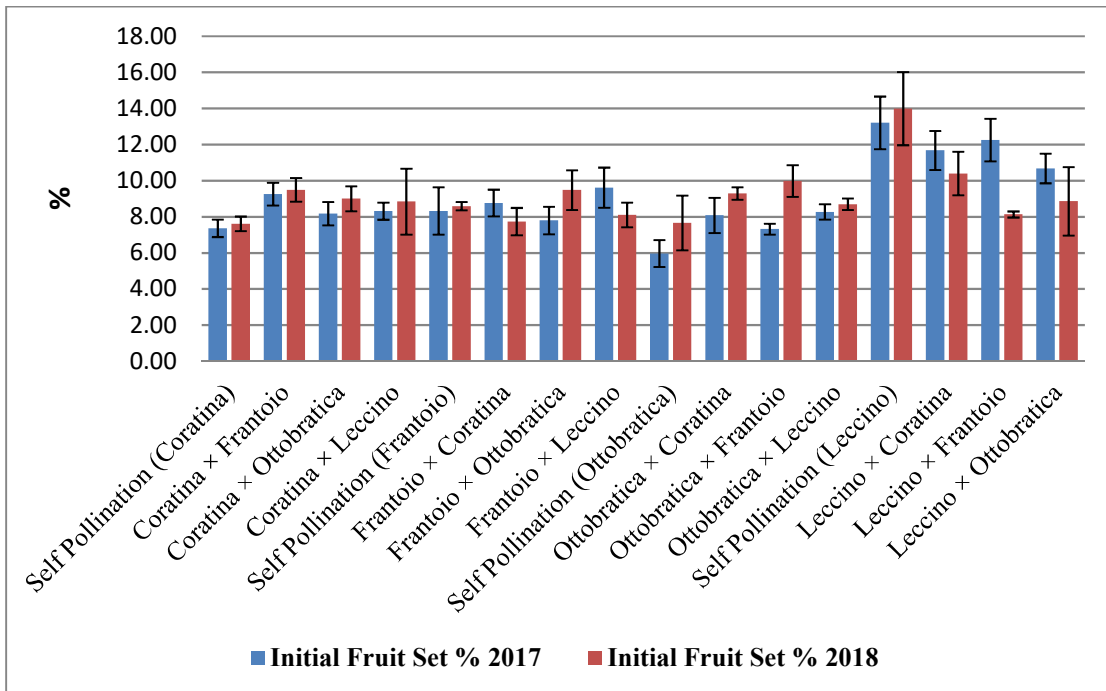


Figure 3. Initial fruit set (%) of four olive varieties during crosses in diallel fashion.

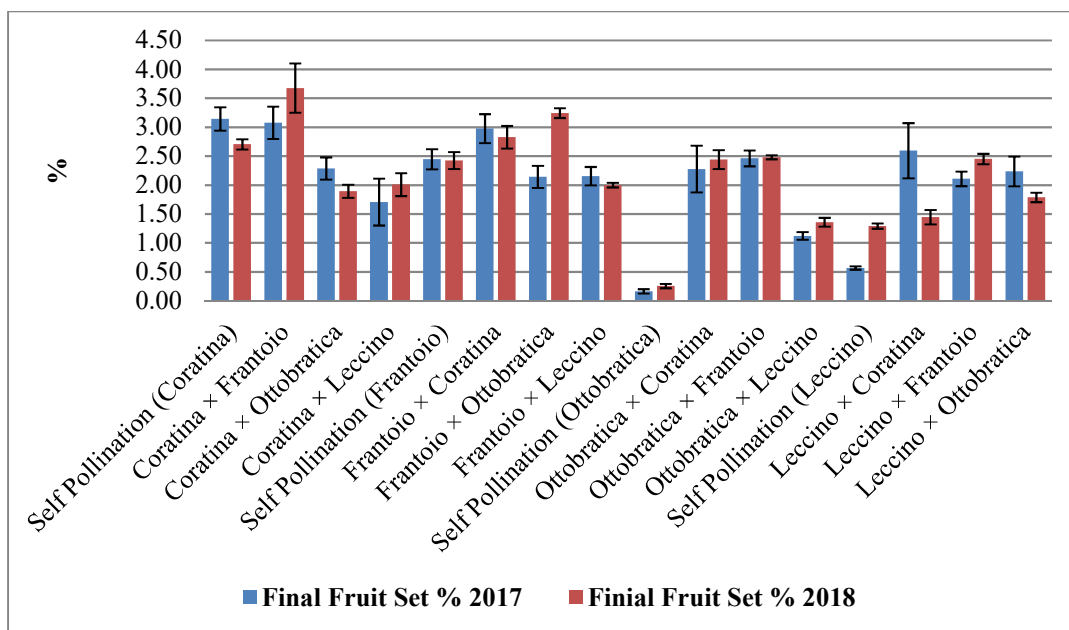


Figure 4. Final fruit set (%) of four olive varieties during crosses in diallel fashion.

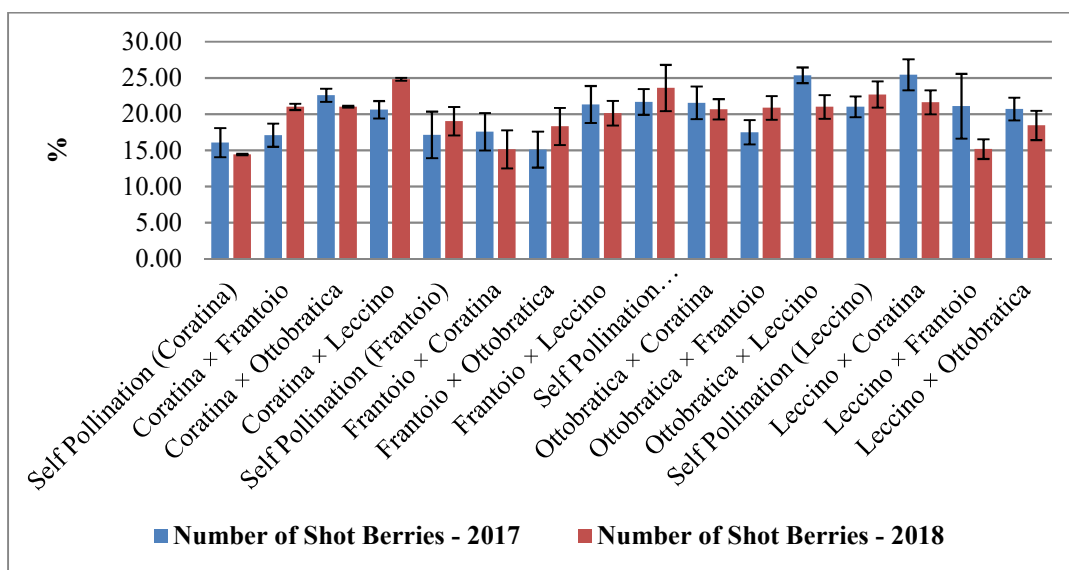


Figure 5. Number of shotberries of four olive varieties during crosses in diallel fashion.

Table 1. Soil physico-chemical status at the location Izhar Olive Farm.

Type/Quantities	Izhar Olive Farm
Texture class	Sandy Loam
pH	8.1
Organic matter (%)	0.55
Nitrogen (%)	0.04
Phosphorus (ppm)	2.6
Potassium (ppm)	110
Electrical conductivity (dSm <sup>-1</sup> )	0.55

Table 2. Categories of self-incompatibility according to Zapata and Arroyo (1978).

Self-incompatibility Index	State
0	Completely self-incompatible
< 0.2	Severely self-incompatible
>0.2 < 1.0	Partially self-incompatible
>1	Self-compatible

**Table 3. Initial fruit set (%) of four olive varieties during the interaction of self and cross pollination in 2017 and 2018.**

Years (Y)	Treatments(T)	Varieties (V)				(T×Y)
		Coratina	Frantoio	Ottobratica	Leccino	
2017	Self-Pollination	7.35 cd	9.32 c	5.96 d	13.19 ab	8.96 b
	Cross Pollination	8.82 cd	10.62 bc	8.28 cd	14.52 a	10.56 ab
2018	Self-Pollination	7.60 cd	8.58 cd	7.65 cd	13.98 a	9.45 ab
	Cross Pollination	10.25 bc	10.32 bc	8.83 cd	13.30 ab	10.67 a
<b>Years (Y)</b>		<b>(V×Y)</b>				<b>(Y)</b>
2017		8.09 bc	9.97 b	7.12 c	13.86 a	9.76 a
2018		8.92 bc	9.45 bc	8.24 bc	13.64 a	10.06 a
<b>Treatments (T)</b>		<b>(V×T)</b>				<b>(T)</b>
Self-Pollination		7.48 cd	8.95 bcd	6.81 d	13.59 a	9.21 b
Cross Pollination		9.53 bc	10.47 b	8.55 bcd	13.91 a	10.62 a
<b>Mean (V)</b>		8.50 bc	9.71 b	7.68 c	13.75 a	

Means having similar letters are not significantly different at 0.05 level of significance using LSD

LSD<sub>0.05</sub> for V = 1.62      LSD<sub>0.05</sub> for V × T = 2.29

LSD<sub>0.05</sub> for T × Y = 1.62      LSD<sub>0.05</sub> for V × T × Y = 3.24

**Table 4. Final fruit set (%) of four olive varieties during the interaction of self and cross pollination in 2017 and 2018.**

Years (Y)	Treatments(T)	Varieties (V)				(T×Y)
		Coratina	Frantoio	Ottobratica	Leccino	
2017	Self-Pollination	3.14 cd	2.44 e	0.16 g	0.56 g	1.58 c
	Cross Pollination	3.98 ab	3.61 bc	1.51 f	1.61 f	2.68 b
2018	Self-Pollination	2.70 de	2.42 e	0.25 g	1.29 f	1.66 c
	Cross Pollination	3.59 bc	4.37 a	2.37 e	2.37 e	3.17 a
<b>Years (Y)</b>		<b>(V×Y)</b>				<b>(Y)</b>
2017		3.56 a	3.02 b	0.83 e	1.09 de	2.13 b
2018		3.14 b	3.39 ab	1.31 d	1.83 c	2.42 a
<b>Treatments (T)</b>		<b>(V×T)</b>				<b>(T)</b>
Self-Pollination		2.92 b	2.43 c	0.20 f	0.92 e	1.62 b
Cross Pollination		3.78 a	3.99 a	1.94 d	1.99 d	2.92 a
<b>Mean (V)</b>		3.35 a	3.21 a	1.07 c	1.46 b	

Means having similar letters are not significantly different at 0.05 level of significance using LSD

LSD<sub>0.05</sub> for V = 0.28      LSD<sub>0.05</sub> for V × T = 0.40

LSD<sub>0.05</sub> for T × Y = 0.28      LSD<sub>0.05</sub> for V × T × Y = 0.57

**Table 5. Number of shotberries of four olive varieties during the interaction of self and cross pollination in 2017 and 2018.**

Years (Y)	Treatments(T)	Varieties (V)				(T×Y)
		Coratina	Frantoio	Ottobratica	Leccino	
2017	Self-Pollination	14.43 de	17.13 bcde	21.70 abc	21.01 abc	18.57 a
	Cross Pollination	15.95 cde	15.35 cde	21.70 abc	21.63 abc	18.65 a
2018	Self-Pollination	16.06 cde	19.03 abcde	23.61 a	22.71 ab	20.35 a
	Cross Pollination	18.50 abcde	13.15 e	19.66 abcd	23.08 ab	18.60 a
<b>Years (Y)</b>		<b>(V×Y)</b>				<b>(Y)</b>
2017		15.19 c	16.24 c	21.70 ab	21.32 ab	18.61 a
2018		17.28 bc	16.09 c	21.64 ab	22.90 a	19.47 a
<b>Treatments (T)</b>		<b>(V×T)</b>				<b>(T)</b>



be considered when planning to establish an olive orchard in any given environment conditions (Moutier, 2002). The present study sheds light on the pollination efficiency of four olive cultivars crossed with each other in all possible combinations. The results revealed that in the reciprocal cross (Frantoio × Coratina) when variety Coratina was used as pollen parent the percentage of fruits set decreased significantly as compared to direct cross (Coratina × Frantoio). Similar results were found between variety Leccino and variety Ottobratica.

Overall, the result of the rest of the cross combinations indicated that all the cultivars can efficiently pollinize other cultivars and set good fruit yield (> 2%). These results are in agreement with the findings of other authors (Al-Kasasbeh *et al.*, 2005; Martin and Sibbett, 2005; Spinardi and Bassi, 2012). It is estimated that 1 to 2 % fruit set in olive is enough to produce good commercial yield (Fabbri *et al.*, 2004). Al-Kasasbeh *et al.*, (2005) reported that variety Frantoio and variety Coratina are efficient pollinizers for one another. Moutier, (2002) demonstrated the beneficial effect of cross-pollination in various olive cultivars and suggested that proper pollinizer should be ensured in olive groves to get maximum yield. In addition to this, some studies reported that cross-incompatibility in some pairs of the olive cultivar is reciprocal like Mission and Manzanilla (Martin and Sibbett, 2005), whereas scientists proposed that the cross-incompatibility is not always bidirectional but depends upon many factors especially environmental conditions (Lavee *et al.*, 2002).

In conclusion, the results of the present study clearly demonstrated that varieties Coratina, Frantoio, and Leccino possessed partial self-incompatibility while Ottobratica was self-incompatible in agro-climatic conditions of Pothwar region. Cross-pollination or open-pollination in varieties Coratina and Ottobratica can enhance the economic yield. All the cultivars can act as good pollinizers as the fruit set percentage in all the crosses were more than 2% except for the cross Ottobratica × Leccino which also produced good fruit set (> 1% fruit set percentage). Taken together, it can be concluded that these cultivars can be used to establish olive orchards in the Arid climate of Pakistan.

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**Author's Contribution:** M.A. Iqbal, A. Mahmood and I.A. Hafiz conceptualized and designed the study. M. Azam and M. A. Khan carried out the methodology and formal analysis. T. Ahmad and A.A. Awan helped in the review, revision and editing. Write up and grammar improvement was made by M. A. Iqbal while M. I. N. Bhatti carried out the statistical analysis.

All authors approved the final version of the manuscript.

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