Pollination Compatibility and Xenia in *Camellia oleifera*

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Abstract. *Camellia oleifera*, a major woody oil plant, has a low oil yield because of self-incompatibility. For commercial oil production, compatible pollen and optimal cross-pollination combinations are required. To evaluate the effects of pollination compatibility and pollen source on oil yield and quality, four *C. oleifera* cultivars—Huashuo (HS), Huajin (HJ), Huaxin (HX), and Xianglin XLC15 (XL)—were subjected to self-, cross-, and natural pollination. Pollen compatibility, oil yield, and quality indices were analyzed. There were no significant differences in pollen germination and tube growth between self- and cross-pollination. Following self-pollination, fertilization was unsuccessful, resulting in severe ovule dysplasia; cross-pollination decreased the ovule abortion rate. Pollen source significantly affected the fruit set, fruit traits, seed traits, and fatty acid content, implying xenia in *C. oleifera*. In cross-pollinated plants, HX pollen produced more seeds, and HJ pollen increased linoleic acid content relative to naturally pollinated plants. For the XL and HS combinations, linolenic acid contents were significantly higher than other pollination combinations. However, oleic acid content was not significantly affected by pollen source, in any of the cultivars. Cultivar HX was, therefore, the most effective pollen donor, and HS × HX was the optimal cross-pollination combination for improving oil yield and sustainability.

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Xenia is defined as the effect of foreign pollen on the development of fruit tissue. It involves the interaction between a nuclear gene from the male gamete and either two polar nuclear genes from the endosperm or one nuclear gene from the egg, during double fertilization in angiosperms (Pozzi et al., 2019). This interaction results in the genetic influence of the paternal parent on traits such as the size, shape, color, and chemical composition of seeds and fruits (Denny, 1992; Yan et al., 2019). The use of xenia has been proposed mostly for improving the yield and quality of many tree species, including *Macadamia* sp., *Cydonia oblonga*, *Vitis vinifera*, and *Prunus dulcis* (Herbert et al., 2019a; Sabir, 2015; Sanchez-Perez et al., 2012; Tatari et al., 2018). Use of different pollen sources has been demonstrated to affect the fruit size, seed weight, seed quality, and ripening time of *Phoenix dactylifera* (Maryam et al., 2015). In

Paeonia suffruticosa, pollen source was found to affect seed yield and even the fatty acid composition of seed oil (Xie et al., 2017).

Self-incompatibility usually refers to the phenomenon in which a fertile hermaphrodite seed plant cannot produce zygotes after self-pollination. Based on the type of genetic control of pollen recognition, self-incompatibility can be divided into gametophytic self-incompatibility (GSI) and sporophytic self-incompatibility (SSI) (Takayama and Isogai, 2005; Zhou and Zheng, 2015). For instance, citrus (Jahromi et al., 2019), plum (Jia et al., 2008), and pear (Wang et al., 2017) have GSI, whereas Brassicaceae (Higashiyama, 2010) and Asteraceae species (Faehrich et al., 2015) have SSI. Fertilization can be unsuccessful even if the pollen tube manages to enter the style or ovule; this is called late-acting self-incompatibility or ovarian self-incompatibility (Asatryan and Tel-Zur, 2013; Gao et al., 2015a; Seavey and Bawa, 1986). For instance, Chen et al. (2012) observed that in *Camellia sinensis* self-pollination, pollen tubes successfully elongated through the style, but they had difficulty in entering the ovule, causing fertilization to fail.

Camellia oleifera, one of the world's four major woody oil plants, produces edible tea oil with ≈75% to 83% oleic acid and 7% to 13% linoleic acid; these acids are able to soften blood vessels, lower blood lipids, and reduce blood pressure (Cheng et al., 2014). The area under *C. oleifera* cultivation in China exceeds 4.6 million ha; however, its cultivation is limited by several problems, including low fruit set and poor fruit quality (Wen et al., 2018). The low yield of *C. oleifera* has long hampered the development of this industry in China (Gao et al., 2015a), and therefore it needs to be addressed urgently. Several studies have been conducted on pollination and embryo development (Liao et al., 2014a), self-compatibility (Gao et al., 2015a), and the pollination effectiveness of various insects for this species (Wei et al., 2019). However, the effects of using different pollen donors on *C. oleifera* oil yield and quality have not been explored. Selection of the appropriate pollen may improve the fruit set and fruit quality of *C. oleifera*.

The four *C. oleifera* cultivars—Huashuo (HS), Huajin (HJ), Huaxin (HX), and Xianglin XLC15 (XL)—are widely grown in China, and they show the greatest potential for oil production. To screen them for compatible pollen and optimal pollination combinations, their pollination compatibility, pollen germination, and pollen tube growth were observed. In addition, the effects of using different pollen sources on *C. oleifera* ovule abortion, fruit set, fruit traits, seed traits, and fatty acid content were compared. Finally, principal component analysis (PCA) was used to evaluate the influence of different pollen on *C. oleifera* oil yield and quality, and to screen compatible pollen and determine the optimal pollination combinations.

Scientific selection of pollination cultivars (pollen donors) could improve *C. oleifera* oil yield and quality. This study provides a basis for the scientific selection of pollination cultivars of these four *C. oleifera* cultivars.

Materials and Methods

Study site and plant material. The study used 6-year-old, high-crown grafted *C. oleifera* cultivars (HS, HJ, HX, and XL) that had reached their peak production stage and showed similar growth potential and consistent management. The trees were planted in the experimental field of the Central South Forestry University of Science and Technology, Changsha, China (lat. 28°05' N, long. 113°21' E). This area belongs to the subtropical, humid, monsoon climate zone, with rainy springs and sunny autumns. The annual average precipitation, temperature, and accumulated temperature of the experimental site are 1380 mm, 19.3 °C, and 5463 °C, respectively.

Experimental design. Cross-, self-, and natural pollinations were carried out using the four *C. oleifera* cultivars. Twenty trees of each cultivar were randomly selected as female parents. At the full flowering stage, ≈80 buds from the middle and upper crown of each tree were selected for the pollination experiment, using specific pollination combinations (Supplemental Table 1). Anthers were manually removed, pollinated, bagged, and labeled in cross- and self-pollinating plants. Untreated flowers that opened on the same day were selected for natural pollination. Each pollination combination included at least 100 buds and was repeated three times. After 7 d, the pollination isolation bags were removed. The number of fruits on each branch of each experimental tree remained about the same during the experiment. The pollen tube growth and ovule penetration of cross- and self-pollinating plants were observed. Ninety days after pollination, the fruit set of each pollination combination was investigated. Mature fruits were randomly selected to compare fruit traits, seed traits, and fatty acid contents (Fig. 1).

Pollen collection and germination test. The buds of four *C. oleifera* cultivars were harvested at the bell stage. The anthers were collected and dried at 25 °C until the pollen was released (usually 24 h). Then the pollen was placed on culture medium consisting of 10 g·L⁻¹ agar, 0.1 g·L⁻¹ boric acid, and 100 g·L⁻¹ sucrose at 25 °C. After 4 h, pollen germination was observed under an Olympus BX-51 microscope (Olympus, Tokyo, Japan). If the length of the pollen tube exceeded the diameter of the pollen grain, the pollen was defined as germinating.

Observation of pollen tube growth, ovule penetration, and fruit development. The pistils were collected 2, 4, 6, 12, 24, 28, 32, 36, 40, 44, 48, 54, 60, 66, and 72 h after self- and cross-pollination. The collected pistils were immediately fixed in Carnoy's fixative [95% ethanol:glacial acetic acid (v/v, 3:1)] for 12 h. After undergoing vacuum treatment,

the material was transferred to a solution of 70% ethanol and stored at 4 °C.

Fluorescence microscopy was used to observe pollen tube growth. Briefly, this involved scraping off the ovary wall of the pistil, and then cutting each style with the lower ovary along the central axis (placenta). This was soaked in NaClO solution (effective chlorine content, 9000 mg·L⁻¹) for 2 h. After rinsing with distilled water, styles were transferred to a 8-mol·L⁻¹ NaOH solution and soaked for 2 h. The styles were rinsed with distilled water three times, and then stained with 0.5% water-soluble aniline blue dyeing solution for 6 h. Finally, the style was spread onto a thin glass slide (using a few drops of aniline blue staining solution), and observed and photographed under a glass slide cover, using an Olympus BX-51 fluorescence microscope.

To observe ovule penetration, conventional paraffin sections with a thickness of 13 μm were used. After dewaxing and rehydration, ovules were stained with 0.3% aniline blue solution for 2 h, and then observed and photographed under an Olympus BX-51 fluorescence microscope.

At 180 d after pollination, the self- and cross-pollinated fruits were harvested, and ovule development was observed and photographed under an Olympus SZX16 stereomicroscope (Olympus, Tokyo, Japan).

Fruit yield, fruit quality, and fatty acid analysis. Ninety days after pollination, the fruit set of each pollination combination was recorded. Mature fruits were randomly selected from each combination to assess fruit yield and quality. The single fruit weight, hundred-grain weight, fresh seed weight, dry seed weight, and kernel weight were measured using an electronic balance; and fruit transverse diameter, vertical diameter, and peel thickness were measured using an electronic digital caliper as described by You et al. (2019). Seed number (the number of seeds produced) was the average number of normally developing seeds in 10 fruits, and seed shell color was described according to Zhuang (2008). Oil extraction for the analysis of seed-kernel oil concentration was performed using Soxtec 2050 (Foss Analytical, Hillerød, Denmark) following the manufacturer's manual.

Yield indices were evaluated as follows: fresh seed rate (%) = (fresh seed weight/fresh fruit weight) × 100; dry seed rate (%) = (dry seed weight/fresh seed weight) × 100; kernel rate (%) = (kernel weight/dry seed weight); and kernel oil rate (%) = (oil weight/kernel weight) × 100.

Fatty acid composition was analyzed using a gas chromatograph (Shimadzu GC-2014; Shimadzu, Kyoto, Japan); the relative content of each fatty acid was calculated according to the method of Zhang et al. (2019). The gas chromatograph parameters were as follows: FID detector temperature 250 °C; sample inlet temperature 250 °C; chromatographic column 60 m × 0.25 mm × 0.2 μm; carrier gas: nitrogen split ratio 1:50; sample injection volume 1 μL. The heating

process was as follows: 50 °C for 2 min, increased to 170 °C at 10 °C·min⁻¹, and maintained for 10 min; increased to 180 °C at 2 °C·min⁻¹, and maintained for 10 min; and finally, increased to 220 °C at 4 °C·min⁻¹, and maintained for 22 min.

Principal component analysis. PCA is defined as an orthogonal linear transformation system that transforms the data onto a new coordinate system, such that the largest amount of variance explained by some scalar projection of the data comes to lie on the first coordinate (called the first principal component, PC1), the second largest amount of the variance on the second coordinate (PC2), and so on (Upadhyayula et al., 2006). PCA was performed using SPSS 21.0 (SPSS Inc., Chicago, IL); and the fruit trait, seed trait, and fatty acid content values were standardized using the procedure "Save standardized values as variables" in the Descriptive Statistics module. The correlation matrix, composition matrix, eigenvalues, and variances were generated using the "Factor Analysis" tool in the Data Reduction module, and the PCs were extracted when a factor's eigenvalue was more than one. The eigenvectors (coefficients) of each PC were calculated using the procedure "Compute Variable" in the Transform module, as follows: eigenvector = composition matrix/sqrt(eigenvalue). The weights used to calculate coefficients of comprehensive PC score model were the ratios of each PC's eigenvalue, to the sum of the eigenvalues of the extracted PCs.

Statistical analysis. The experiments were performed in triplicate. SPSS version 21.0 was used for data processing. To identify statistically significant differences in the fruit set, fruit traits, seed traits, and fatty acid contents for each cultivar obtained using the different pollen donors, the data were analyzed using one-way analysis of variance (ANOVA), at a significance level of $P < 0.05$. This was followed by a least significant difference (LSD) multiple comparisons test,

where $LSD = t_{0.05/2; DFw} \sqrt{MSw \left(\frac{1}{n_A} + \frac{1}{n_B} \right)}$;

and MSw is the mean square within groups determined by ANOVA. Figures were drawn using Origin 9.1 (OriginLab, Northampton, MA).

Results

Pollen germination and pollen tube growth in self-pollination and cross-pollination experiments. *Camelia oleifera* has a long flowering period, ranging from late October to early December. The flowering periods were mostly the same for the four cultivars (Supplemental Fig. 1). The pollen germination rates of HS, HJ, HX, and XL were 82.95%, 72.23%, 87.47%, and 81.60%, respectively (Fig. 2, Supplemental Fig. 2), indicating that they have good pollen vitality. The pollen tubes in self-pollination (Fig. 3A–D) and cross-pollination experiments (Fig. 3E–H) germinated easily at the stigma and grew inside the style 2 h after pollination.

Fig. 1. Schematic representation of the experimental design. The main objective of the study was to screen compatible pollen and optimal pollination combinations of four *C. oleifera* cultivars (HS, HJ, HX, and XL). Cross-pollination, self-pollination, and natural pollination were carried out in the four cultivars. Anthers were manually removed, pollinated, bagged, and labeled in cross- and self-pollinating plants. Untreated flowers that opened on the same day were selected for natural pollination. Pollen tube growth of cross- and self-pollinating plants were observed. To analyze pollen compatibility, pollen tube growth and ovule penetration were observed in the pistils collected 2, 4, 6, 12, 24, 28, 32, 36, 40, 44, 48, 54, 60, 66, and 72 h after self- and cross-pollination. Ninety days after pollination, the fruit set of each pollination combination was investigated. Mature fruits were randomly selected to compare fruit traits, seed traits, and fatty acid contents. PCA was used to evaluate the influence of different pollen on *C. oleifera* oil yield and quality, and to screen compatible pollen and determine the optimal pollination combinations.

Fig. 2. Pollen germination of four *C. oleifera* cultivars on the culture medium. The data are the mean $\pm$ SD of three biological replicates, and different lowercase letters indicate significant differences at 5% probability level in a least significant difference (LSD) test.

About 40 h after pollination, the pollen tubes reached the base of the style in both the self-pollination (Supplemental Fig. 3A–D) and

cross-pollination experiments (Supplemental Fig. 3E–H). After 40–48 h, the pollen tubes of the cross-pollinated plants continued to grow

downward to the ovule (Fig. 4E–H). However, the growth of the pollen tubes of the self-pollinated plants slowed down, and the tubes eventually stagnated at the ovary (Fig. 4A–D).

Fruit set and ovule development in self- and cross-pollinated combinations. Using pollen from different cultivars had a significant influence on fruit set. The fruit set was significantly lower in self-pollinated plants than in naturally pollinated and cross-pollinated plants (Fig. 5). In the self-pollination experiment, the cultivar HJ had the highest fruit set (14.04%) among the four cultivars. In the cross-pollination treatment, the fruit set of HS ranged from 72.60% to 81.85%. In addition, the cultivars HJ (pollinated by XL and HX) and XL (using HS pollen) had significantly higher fruit set values than the other cultivars. In the cross-pollination treatment, the highest fruit set values of HS, HJ, HX, and XL were 50.67%, 39.82%, 41.72%, and 37.36% higher, respectively, than those of naturally pollinated plants.

The fruits of the self- and cross-pollinated plants were dissected 180 d after pollination. In the fruits of the self-pollinated plants, only one to two ovules developed normally, and most of the ovules turned brown (Fig. 6A–L). However, the ovules of the fruits of the cross-pollinated plants developed normally (Supplemental Fig. 4A–L). These results suggest that cross-pollination significantly decreased the ovule abortion rate in *C. oleifera*.

Effects of pollen source on seed shell color, single fruit weight, vertical and horizontal diameter, and peel thickness. Pollen source had a significant effect on seed shell color in the four cultivars (Supplemental Fig. 5). In all four cultivars, cross-sectioning of fruits from plants pollinated by HX showed that seed shell color was distinctly black, while that of plants pollinated by HS was brown.

The single fruit weight of self-pollinated plants was significantly lower than that of cross-pollinated and naturally pollinated plants (Table 1). Compared with natural pollination, pollination with HX as pollen donor led to the single fruit weight of HS, HJ, and XL being higher by 141.43%, 74.31%, and 53.57%, respectively. Pollination using HJ and XL pollen led to the single fruit weight of HX being higher by 27.02% and 32.12%, respectively, than that of naturally pollinated plants. In contrast, the single fruit weights of the combinations HJ $\times$ HS, HJ $\times$ XL, HX $\times$ HS, and XL $\times$ HS did not differ notably from that of naturally pollinated plants. The effects of the different pollen sources on fruit diameter and peel thickness were almost the same as their effects on single fruit weight.

Effects of pollen source on seed number, hundred-grain weight, fresh seed rate, dry seed rate, kernel rate, and kernel oil rate. Among the self-pollinated plants, each cultivar had two to three seeds per fruit. In cross-pollinated plants, HJ, HX, and XL produced different numbers of seeds when

Fig. 3. Pollen germination in the style at 2 h after self- and cross-pollination. Pollen grains germinated from the stigma and pollen tubes (PT) grew into the interspaces between the papillar cells in self-pollinated HS (A), XL (B), HJ (C), and HX (D) plants and cross-pollinated HS × XL (E), XL × HS (F), HJ × HX (G), and HX × HJ (H) plants. Bars A–H: 1000 μm.

Fig. 4. The pollen tube growth in style after self- and cross-pollination. At 40 h after self-pollination of HS (A), XL (B), HJ (C), and HX (D), the pollen tubes (PT, indicated by arrows, fluorescence micrograph ×100) grew to the styler base. At 48 h after cross-pollination of HS × XL (E), XL × HS (F), HJ × HX (G), and HX × HJ (H), the pollen tubes entered the ovary ovule (OV).

Fig. 5. The fruit set of different pollination combinations. The data are the mean ±SD of three biological replicates, and different lowercase letters indicate significant differences at 5% probability level in a least significant difference (LSD) test.

they were pollinated using different pollen donors, whereas HS was unaffected by the pollen source (Table 2). Moreover, the HS pollen reduced the number of seeds produced in all the cultivars. Using the HX pollen, HJ and XL produced more seeds than the cultivars pollinated using pollen from other cultivars. Therefore, pollen source affected the number of seeds produced by the *C. oleifera* cultivars.

The hundred-grain weight of self-pollinated plants was low; in cross-pollinated plants, however, it depended on the cultivar combination (Table 3). HX pollen effectively increased the hundred-grain weights of HS, HJ, and XL by 30.47%, 49.10%, and 56.40%, respectively. The hundred-grain weights were generally lower when XL was used as the pollen donor in cross-pollination combinations.

Pollen source affected the fresh and dry seed rates differently (Fig. 7A and B). The fresh seed rates of HS pollinated by HJ, HX, and XL were significantly higher than those of fruits of the self-pollinated and naturally pollinated plants, but the differences among the three pollen donors were not significant. Pollen from HX and XL increased the fresh seed rates of the cross-pollinated plants relative to those of naturally pollinated plants. However, the dry seed rates of self-pollinated HX and XL were significantly higher than those of naturally and cross-pollinated plants.

The kernel rate of HS pollinated by XL was significantly higher than that of naturally pollinated plants, whereas it was significantly lower when pollinated with HX (Fig. 7C). In addition, HS pollen effectively increased the kernel rate of XL. Unexpectedly, self-pollinated HJ and HX plants had higher kernel rates than the corresponding cross-pollinated plants, even though self-pollination usually leads to seed dysplasia, resulting in a lower kernel yield.

Pollen source had both positive and negative effects on kernel oil contents (Fig. 7D). Compared with naturally pollinated plants, the kernel oil rates of HS and XL pollinated with HX were significantly lower, whereas HS pollen caused the kernel oil rate of HJ to be higher than that of naturally pollinated plants. However, the oil rate in cross-pollinated HX plants was unaffected by pollen source.

Effects of pollen source on fatty acid contents. The palmitic, stearic, linoleic, and linolenic acid contents of the seeds varied with the pollen source. The variation in oleic acid content was not significant (Table 4). The palmitic acid contents of HS and HJ were not significantly affected by pollen source; palmitic acid content was slightly lower in HX and XL, but only when HS was used as the pollen donor. HJ pollen increased linoleic acid content in all cross-pollinated plants relative to naturally pollinated plants. Compared with naturally pollinated plants, the linoleic acid content was significantly higher in cross-pollinated HS, by 40.90%, 30.09%, and 36.76%, when

pollinated by HJ, HX, and XL, respectively. The linolenic acid content was significantly higher in XL and HS combinations. How-

ever, oleic acid content was not significantly affected by pollen source, in any of the cultivars. These results show that pollen

source had variable effects on the fatty acid content of *C. oleifera*.

PCA and evaluation of the effect of different pollen types. The fruit set, fruit weight, vertical and horizontal diameter, peel thickness, seed number, hundred-seed weight, and linoleic acid contents were the dominant contributors to PC1, with a variance contribution of 39.74%. The variance contribution of the PC2 was 18.47%; this PC mainly reflects the contribution of kernel rate and linolenic acid. PC3 denotes fresh seed rate, kernel oil rate, palmitic and stearic acid content, with a variance contribution of 12.79%. Dry seed rate was the largest contributor to PC4, which contributed 8.15% of the variance. The variance contribution of PC5 was 6.56%, with oleic acid as the dominant contributor. The cumulative variance contribution of the first five components was 85.71%. This indicates that these five principal components effectively summarize the factors affecting *C. oleifera* yield and quality (Supplemental Table 2).

To determine the optimal pollinating male parent among the four *C. oleifera* cultivars, PCA was used to evaluate 20 pollination combinations. The comprehensive principal component score model was $S = 0.19ZX_1 + 0.17ZX_2 + 0.19ZX_3 + 0.21ZX_4 - 0.13ZX_5 + 0.20ZX_6 + 0.19ZX_7 + 0.18ZX_8 + 0.11ZX_9 + 0.02ZX_{10} - 0.06ZX_{11} + 0.06ZX_{12} - 0.01ZX_{13} + 0.01ZX_{14} + 0.04ZX_{15} - 0.06ZX_{16}$. Pollination combinations with a higher score are more suitable pollinators. All self-pollinated plants exhibited lower comprehensive scores than cross-pollinated and naturally pollinated plants (Table 5). Among the cross-pollination combinations, HS × HX had the highest comprehensive score, followed by HS × HJ and HS × XL. Pollen from HX exhibited a higher comprehensive score in HJ and XL cross-pollinated plants, indicating

Fig. 6. The development of young fruits and ovules at 180 d after self-pollination. The cross section, equatorial, and polar view of young fruits and ovules of self-pollinated HS (A–C), HX (D–F), HJ (G–I), and XL (J–L) are shown.

Table 1. The effects of pollen source on the fruit traits of *C. oleifera*.

Female	Male	Fruit wt (g)	Vertical diam (mm)	Horizontal diam (mm)	Peel thickness (mm)
HS	HS	27.24 ± 2.77 d	24.63 ± 1.26 c	26.61 ± 1.01 c	5.23 ± 0.32 c
	HJ	78.42 ± 2.70 b	44.03 ± 3.38 a	54.49 ± 5.07 a	6.54 ± 0.46 b
	HX	88.46 ± 2.49 a	44.10 ± 3.81 a	56.97 ± 3.51 a	7.50 ± 0.50 a
	XL	79.21 ± 2.57 b	50.16 ± 5.74 a	46.07 ± 4.65 b	6.86 ± 0.67 ab
	CK	36.64 ± 2.75 c	33.09 ± 2.57 b	41.49 ± 3.13 b	6.14 ± 0.52 bc
LSD _{0.05}		4.84	6.66	6.82	0.92
HJ	HS	29.88 ± 2.15 b	40.09 ± 3.53 ab	38.30 ± 2.84 ab	5.95 ± 0.65 ab
	HJ	23.13 ± 2.19 c	35.58 ± 2.36 b	34.24 ± 3.54 b	5.78 ± 0.71 b
	HX	48.67 ± 2.59 a	46.02 ± 3.68 a	43.59 ± 5.58 a	6.97 ± 0.51 a
	XL	29.92 ± 2.97 b	41.83 ± 1.63 a	37.81 ± 1.94 ab	6.09 ± 0.54 ab
	CK	27.92 ± 2.02 b	41.20 ± 3.61 ab	36.87 ± 4.72 ab	6.37 ± 0.54 ab
LSD _{0.05}		4.38	5.59	7.17	1.08
HX	HS	33.49 ± 2.11 b	33.56 ± 2.24 ab	40.32 ± 1.91 a	4.78 ± 0.22 b
	HJ	40.15 ± 2.90 a	35.94 ± 3.74 a	44.53 ± 4.12 a	5.93 ± 0.88 a
	HX	15.33 ± 2.04 c	26.31 ± 4.28 b	30.01 ± 6.93 b	3.54 ± 0.23 c
	XL	38.60 ± 2.73 a	35.25 ± 3.33 a	43.74 ± 4.13 a	4.94 ± 0.51 ab
	CK	30.39 ± 0.72 b	35.01 ± 5.79 a	38.18 ± 3.51 a	4.52 ± 0.86 bc
LSD _{0.05}		4.29	7.36	8.05	1.11
XL	HS	26.37 ± 1.69 c	33.98 ± 2.55 ab	38.32 ± 2.72 ab	4.65 ± 0.72 b
	HJ	34.17 ± 2.34 b	33.35 ± 2.27 ab	40.46 ± 1.94 ab	5.14 ± 0.58 ab
	HX	40.13 ± 1.47 a	37.39 ± 1.94 a	43.01 ± 1.79 a	6.13 ± 0.63 a
	XL	16.57 ± 1.96 d	24.32 ± 3.56 c	26.58 ± 4.79 c	3.51 ± 0.64 c
	CK	26.13 ± 2.38 c	31.85 ± 2.96 b	37.34 ± 2.60 b	4.58 ± 0.44 bc
LSD _{0.05}		3.63	4.94	5.40	1.11

Data represent the mean ±SD of three replications. Mean values followed by different lowercase letters in each column indicate significant differences at $P < 0.05$ in a least significant difference (LSD) test.

Table 2. The effects of pollen source on the seed number of *C. oleifera*.

Male	Female			
	HS	HJ	HX	XL
HS	2.30 ± 0.48 b	9.70 ± 1.05 c	8.90 ± 0.99 b	9.60 ± 0.97 b
HJ	19.50 ± 1.08 a	2.30 ± 0.48 d	14.80 ± 1.03 a	11.50 ± 0.71 a
HX	20.20 ± 1.03 a	14.90 ± 0.74 a	2.70 ± 0.67 c	11.90 ± 0.74 a
XL	19.50 ± 1.17 a	11.10 ± 1.10 b	15.50 ± 0.71 a	2.10 ± 0.57 c
CK	19.40 ± 1.34 a	10.40 ± 0.97 bc	9.10 ± 0.73 b	9.80 ± 0.92 b
LSD _{0.05}	0.96	0.81	0.76	0.71

Data represent the mean ±SD of three replications. Mean values followed by different lowercase letters in each column indicate significant differences at $P < 0.05$ in a least significant difference (LSD) test.

Table 3. The effects of pollen source on hundred-grain weight of *C. oleifera* (g).

Male	Female			
	HS	HJ	HX	XL
HS	153.23 ± 9.59 d	213.89 ± 17.45 b	206.68 ± 22.67 b	207.84 ± 18.07 b
HJ	275.98 ± 21.52 a	135.17 ± 7.15 d	258.69 ± 12.87 a	157.36 ± 8.97 c
HX	248.68 ± 12.34 b	257.67 ± 20.99 a	135.22 ± 8.65 d	247.99 ± 16.94 a
XL	231.08 ± 14.10 b	185.12 ± 16.17 c	164.01 ± 10.21 c	125.07 ± 8.06 d
CK	190.60 ± 6.53 c	172.82 ± 11.03 c	172.17 ± 10.19 c	158.56 ± 11.64 c
LSD _{0.05}	25.06	27.94	25.24	24.33

Data represent the mean ±SD of three replications. Mean values followed by different lowercase letters in each column indicate significant differences at $P < 0.05$ in least significant difference (LSD) test.

Fig. 7. Comparison of the fresh seed rate (A), dry seed rate (B), kernel rate (C), and kernel oil rate (D) of different pollination combinations. The data are the mean ±SD of three biological replicates, and different lowercase letters indicate significant differences at 5% probability level in a least significant difference (LSD) test.

that HX had a positive influence on *C. oleifera* yield and quality when it was used as the pollen donor.

Discussion

In flowering plants, pollination is the key event in the development of the fruit set, influencing fruit quantity and quality (Jahromi et al., 2019; Selak et al., 2014). The development of self-incompatibility systems in plants is a long-term evolutionary process that ensures genetic diversity (Perez

et al., 2016; Wang et al., 2017). In this study, there was no significant difference in pollen germination and pollen tube growth in the style between the cross-pollination and self-pollination treatments. The cross-pollination tube was able to penetrate the ovule, but the self-pollination tube stagnated at the base of the style and finally failed in fertilization. Although self-pollination was able to produce a fruit set, the fruit set was much lower than in cross-pollinated plants. Furthermore, the aborted ovules and seeds that occurred in the self-pollinated fruits are examples of late-

acting self-incompatibility, which is consistent with previous studies (Gao et al., 2015b; Gibbs, 2014; Liao et al., 2014b; Liu et al., 2014).

There were significant differences in fruit set and fruit traits of the four cultivars when pollen from different cultivars was used for cross-pollination. The four *C. oleifera* cultivars pollinated by different pollen sources showed significant differences in fruit traits such as fruit longitudinal diameter, fruit transverse diameter, and peel thickness. These findings are like those of previous studies that report xenia in cherimoya (Kahn et al., 1994), pistachio (Acar and Eti, 2011), and date palm (Rezazadeh et al., 2013). Xenia has also been observed in the development of tomato and sweet cherry fruits (Piotto et al., 2013; Radunic et al., 2017).

Pollen source influenced the seed number, hundred-grain weight, fresh seed rate, dry seed rate, kernel rate, and kernel oil rate of the four *C. oleifera* cultivars to different extents. HJ and XL exhibited a certain degree of xenia regarding these six traits; in contrast, HS and HX exhibited no direct xenia regarding seed number and kernel rate. Xenia was positive for the dry seed rate of HS, HJ, and HX, but it was negative for XL. Xenia has also been reported in other plants regarding seed traits. For instance, Herbert et al. (2019b) found that pollen source had a positive impact on fruit growth, nut size, and kernel size in macadamia. According to Fattahi et al. (2014), hazelnut nut weight was significantly affected by pollen source, whereas self-pollination reduced its nut and kernel trait values and increased the proportion of blank nuts. Xenia typically positively influences the yield and quality of economic crops, but it sometimes has a negative impact. Ismail et al. (2016) found that xenia negatively affected coconut nut yield. Therefore, studies on xenia in *C. oleifera* regarding fruit and seed characteristics are necessary to improve its oil yield.

Table 4. The effects of pollen source on fatty acid contents of *C. oleifera* (%).

Female	Male	Palmitate acid	Stearic acid	Oleic acid	Linoleic acid	Linolenic acid
HS	HS	7.14 ± 0.070	2.36 ± 0.050	82.43 ± 0.04	7.35 ± 0.003 a	0.72 ± 0.002 b
	HJ	7.51 ± 0.060	1.98 ± 0.040	81.86 ± 0.03	7.82 ± 0.005 a	0.82 ± 0.002 b
	HX	7.13 ± 0.050	2.58 ± 0.030	82.30 ± 0.04	7.22 ± 0.003 a	0.78 ± 0.001 b
	XL	7.75 ± 0.050	2.45 ± 0.030	81.23 ± 0.03	7.59 ± 0.005 a	0.98 ± 0.001 a
	CK	7.42 ± 0.070	2.43 ± 0.050	83.94 ± 0.05	5.55 ± 0.004 b	0.71 ± 0.001 b
LSD _{0.05}		NS	NS	NS	0.93	0.14
HJ	HS	7.62 ± 0.004	2.73 ± 0.003 ab	83.54 ± 0.01	6.11 ± 0.006 a	—
	HJ	8.14 ± 0.009	2.39 ± 0.005 b	82.89 ± 0.02	6.04 ± 0.006 a	0.54 ± 0.0005 b
	HX	7.08 ± 0.007	3.04 ± 0.005 ab	84.14 ± 0.02	5.74 ± 0.005 ab	—
	XL	7.36 ± 0.005	3.51 ± 0.004 a	82.86 ± 0.03	5.72 ± 0.004 ab	0.55 ± 0.0007 b
	CK	6.90 ± 0.009	3.30 ± 0.003 a	84.22 ± 0.02	4.89 ± 0.004 b	0.69 ± 0.001 a
LSD _{0.05}		NS	0.84	NS	0.88	0.11
HX	HS	6.61 ± 0.080 b	2.70 ± 0.004 ab	84.32 ± 0.03	5.72 ± 0.004 b	0.65 ± 0.001
	HJ	7.07 ± 0.060 ab	2.45 ± 0.005 b	82.99 ± 0.03	6.78 ± 0.006 a	0.71 ± 0.001
	HX	7.31 ± 0.070 ab	2.79 ± 0.004 ab	83.77 ± 0.03	5.51 ± 0.004 b	0.62 ± 0.002
	XL	7.04 ± 0.070 ab	3.35 ± 0.004 a	83.85 ± 0.03	5.13 ± 0.003 b	0.63 ± 0.002
	CK	7.77 ± 0.060 a	1.97 ± 0.004 b	82.82 ± 0.03	6.66 ± 0.005 a	0.78 ± 0.001
LSD _{0.05}		0.97	0.88	NS	0.76	NS
XL	HS	6.93 ± 0.005 c	3.54 ± 0.003 a	83.16 ± 0.05	5.49 ± 0.005 c	0.88 ± 0.005 a
	HJ	7.46 ± 0.004 ab	2.57 ± 0.003 b	81.77 ± 0.03	7.35 ± 0.003 a	0.84 ± 0.007 a
	HX	7.41 ± 0.003 ab	3.38 ± 0.002 a	81.89 ± 0.04	6.70 ± 0.008 ab	0.62 ± 0.004 b
	XL	7.33 ± 0.003 ab	3.96 ± 0.002 a	83.41 ± 0.03	4.68 ± 0.005 c	0.62 ± 0.004 b
	CK	7.73 ± 0.003 a	1.93 ± 0.004 b	84.23 ± 0.04	5.54 ± 0.007 bc	0.57 ± 0.005 b
LSD _{0.05}		0.45	0.73	NS	1.19	0.16

Data represent the mean ±SD of three replications. Mean values followed by different lowercase letters in each column indicate significant differences at $P < 0.05$ in least significant difference (LSD) test. NS = not significant.

Table 5. Comprehensive score and ranking of different pollination combinations.

Female	Male	Comprehensive score	Ranking
HS	HS	-2.08	19
	HJ	1.79	2
	HX	2.01	1
	XL	1.76	3
HJ	CK	-0.25	12
	HS	0.26	8
	HJ	-1.48	17
	HX	1.37	4
	XL	0.22	9
HX	CK	-0.31	13
	HS	0.18	10
	HJ	0.89	5
	HX	-1.51	18
XL	XL	0.67	7
	CK	-0.45	15
	HS	-0.44	14
	HJ	-0.07	11
	HX	0.75	6
	XL	-2.28	20
	CK	-1.03	16

The comprehensive score and ranking reflect the pros and cons of each pollination combination (the higher the score, the better the effect).

Xenia significantly affects oleic and linoleic acid contents in almond (Kodad et al., 2009), and pollen source possibly affects fatty acid content and composition in tree peony oil (Xie et al., 2019). In the present study, compared with the fruits from naturally pollinated plants, fruits originating from cross-pollination of HJ with HS as the pollen donor had higher linoleic acid content; whereas this content was significantly lower in HX when HS and XL were used as pollen donors. Compared with naturally pollinated HJ plants, the linolenic acid content in the cross- and self-pollinated HJ plants was significantly lower. However, in the pollination combinations XL × HS, XL × HJ, and HS × XL, the linolenic acid content

was significantly higher than in under natural pollination. These results show that pollen source can affect linoleic and linolenic acid contents, thus affecting the quality of tea oil.

Conclusions

This study reports pollination compatibility and xenia regarding fruit and seed traits in four *C. oleifera* cultivars. In the cross-pollination treatment, the pollen tubes were able to penetrate the ovules, whereas in the self-pollination treatment, they remained at the base of the style. The stagnated growing pollen tubes, severe ovule abortion, and low fruit set of the self-pollination combination reflect the self-incompatibility of *C. oleifera*. Pollen source significantly affected the oil yield and quality indices, including single fruit weight, seed number, hundred-grain weight, fresh seed rate, dry seed rate, kernel rate, kernel oil rate, and the fatty acid content, to varying degrees, implying xenia in *C. oleifera*. HX was the most effective pollination cultivar, and HS × HX was the optimal cross-pollination combination, resulting in the best oil yield and quality. This research indicates that the scientific selection of pollination cultivars has great potential to improve the yield and oil quality of *C. oleifera* and may help growers to enhance profitability and sustainability.

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Supplemental Table 1. *Camellia oleifera* pollination combinations.

Female	Male				Natural pollination
	HS	HJ	HX	XL	
HS	HS × HS	HS × HJ	HS × HX	HS × XL	HS CK
HJ	HJ × HS	HJ × HJ	HJ × HX	HJ × XL	HJ CK
HX	HX × HS	HX × HJ	HX × HX	HX × XL	HX CK
XL	XL × HS	XL × HJ	XL × HX	XL × XL	XL CK

Supplemental Table 2. The composition matrix and explanation of the total variance.

Traits	Component				
	1	2	3	4	5
Fruit set (%)	0.8	0.32	0.23	-0.11	-0.16
Fruit weight (g)	0.92	-0.06	-0.02	0.01	0.12
vertical diameter (mm)	0.8	0.44	-0.15	0.16	-0.07
horizontal diameter (mm)	0.94	0.1	0.11	-0.03	0.23
Peel thickness (mm)	-0.76	-0.35	0.38	0.11	0.02
Seed number	0.87	0.13	0.11	-0.06	0.28
Hundred-seed weight (g)	0.84	0.33	0.02	-0.08	0.01
Fresh seed rate (%)	0.52	-0.1	0.69	0.29	-0.03
Dry seed rate (%)	-0.26	0.54	0.22	0.67	0.13
Kernel rate (%)	-0.12	0.69	-0.15	0.13	-0.54
Oil rate of kernel (%)	-0.11	0.49	-0.71	-0.4	0.06
Palmitate acid (%)	-0.03	0.36	0.60	-0.57	0.06
Stearic acid (%)	0.27	-0.48	-0.49	0.22	0.45
Oleic acid (%)	-0.58	0.54	0.16	-0.13	0.54
Linoleic acid (%)	0.69	-0.46	-0.28	0.15	-0.2
Linolenic acid (%)	0.25	-0.73	0.23	-0.35	-0.18
Eigenvalues	6.36	2.96	2.05	1.31	1.05
Variance	39.74	18.47	12.79	8.15	6.56
Accumulative variance	39.74	58.21	71	79.15	85.71

Supplemental Fig. 1. Flowering periods of four *C. oleifera* cultivars. The first flowering stage is 5% to 25% the length of the flowering phase of the whole tree, full flowering stage is 25% to 75% the length of the flowering phase of the whole tree, last flowering stage is more than 75% the length of the flowering phase of the whole tree.

Supplemental Fig. 2. Pollen germination of Four *C. oleifera* cultivars on the culture medium. HS (A), HJ (B), HX (C), and XL (D).

Supplemental Fig. 3. A complete view of pollen tube growth at 40 h after pollination in the styles and ovaries of *C. oleifera*. The pollen tube growth in self-pollination of HS (A), XL (B), HJ (C), and HX (D); the pollen tube growth in cross-pollination of HS $\times$ XL (E), XL $\times$ HS (F), HJ $\times$ HX (G), and HX $\times$ HJ (H). Bars A–H: 800 μ m.

Supplemental Fig. 4. The development of young fruits and ovules at 180 d after cross-pollination. The cross section, equatorial, and polar view of young fruits and ovules in cross-pollination of HS $\times$ XL (A–C), XL $\times$ HS (D–F), HJ $\times$ HX (G–I), and HX $\times$ HJ (J–L) are shown.