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Effects of Xenia on Fruit and Seed in *Vernicia fordii*

Shifei Qin[1,2], Huijuan Zhang[1,2], Shuai Liu[1,2], Qingwei Zhang[1,2*], Keming Luo[1,2*]

[1]Chongqing Key Laboratory of Plant Resource Conservation and Germplasm Innovation, School of Life Sciences, Southwest University, Chongqing, China
[2]Key Laboratory of Eco-environments of Three Gorges Reservoir Region, Ministry of Education, Southwest University, Chongqing, China
Email: *qwzhang18@swu.edu.cn, *kemingl@swu.edu.cn

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Abstract

Tung tree, *Vernicia fordii*, is a plant species producing industrial oil (tung oil). Although the cultivation of the tung tree produces great economic value, some important genetic and physiological traits in *V. fordii* have not been fully recognized. As one of them, the effect of pollen on the maternal plant (xenia) is unknown in *V. fordii*, which is an important part of the efficient cultivation system of many crops. This study performed hybridization with three important tung cultivars (Dami, Xiaomi and Putao) to evaluate the influence of pollen source on fruit and seed development. The results revealed that xenia is present in *V. fordii*, which influences fruit setting, fruit size, seed weight and oil content. Among the cultivars investigated, the hybridization combination with Putao as a female parent and Dami as male parent showed significant improvement of seed yield and oil content than self-pollination, which could be considered to apply in practice.

Keywords

Tung Tree, Xenia, Fruit Development, Oil Content

1. Introduction

Vernicia fordii (Hemsl) Airy Shaw, namely tung tree, is a woody plant species in the family Euphorbiaceae. *V. fordii* seed can be used to produce tung oil that is excellent dried oil with characteristics of insulation, acid and alkali resistance, and anti-corrosion [1]. Besides, tung oil is a kind of raw material to produce biodiesel [2]. In recent years, the international demand for tung oil is constantly increasing, leading to a promoted industrial interest in improving yield and chemical/physical properties of tung oil [3]. However, many important traits of *V. fordii* have not been understood in terms of breeding and utilization, in which xenia has not been paid attention to for a long time.

It has been well known that pollen sources could have a direct influence on fruit and seed features in maternal plants, which is defined as xenia (effects on the endosperm and embryo or even effects on tissues around embryo) [4] [5]. The effects of xenia on fruit and seed characteristics have been revealed in a large number of plant species, such as maize [6], cotton [7], cucumber [8], grape [5], and almond [9], etc. Furthermore, there are available reports demonstrating that xenia could improve oil content in maize [10] and almond [9]. Whether xenia affects oil content and oil quality in *V. fordii* is an interesting question and is of great importance in the application.

V. fordii, Dami, Xiaomi and Putao, to conduct experiments in Chongqing, one of the main plantation areas of *V. fordii* in China. The results demonstrated that pollen sources significantly affected various characteristics of fruit and seed in the maternal plant of the specific cultivar.

2. Materials and Methods

The *V. fordii* trees (planted in 2012) were growing in a plantation in Kaizhou District, Chongqing, China (N31˚07', E108˚23'), which is one of the natural distribution areas of *V. fordii*. The trees with uniformed height (3.0 - 3.5 m), stem diameter (7.0 - 8.0 cm) and crown diameter (3.0 - 4.0 m) were chosen to prepare for hybridization experiment. In 2018 and 2019, when the corolla of male flower was open but that of female flower was not (April 10th to 15th), pollens from male flower were collected and painted on stigma of female flower, then the female flower was wrapped around by plastic bags to avoid the influence of other pollens. The pollen activity was assayed according to the report of Trognitz [11], specifically, iodine-potassium iodide test, 2-3-5-triphenyle tetrazolium chloride test and in vitro pollen germination were performed.

In 2018, the cultivar Putao acted as female parent and Putao, Xiaomi and Dami as male parents. In the hybridization experiment of 2019, six combinations were set with the cultivars Xiaomi and Putao as female parents and Xiaomi, Putao and Dami as male parents. Each combination included at least 400 flowers and was repeated for three times. One month after the hybridization operation, fruit setting rate was counted. On October 30th, fruits were collected to measure fruit size (the transverse diameter and longitudinal diameter), fresh fruit weight, number of seed in fruit, dry weight of single seed. The kernel ratio was calculated with seed weight divided by fruit weight.

Lipids in seed were extracted according to the method of Shockey *et al.* [12]. Specifically, kernels were dried at 80˚C for 48 h, then were ground into powder. 100 mg powder was extracted by 5 ml 2-propanol at 87˚C for 10 min, and mixed with solvent to achieve a final extract ratio of hexane/2-propanol/H_2O, 6:4:0.5 (v/v/v). The phases were separated by adding 15 ml of 6.6% (w/v) aqueous sodium sulphate. The organic phase was collected and the aqueous phase was back-extracted with 15 ml hexane: 2-propanol (7:2, v/v). Kernel oil content was calculated as the quality of oil extracted divided by dry weight of seeds. In addition, the acid value, saponification value and iodine value of extracted oil were determined by standard procedures described by Ajiwe *et al.* [13].

3. Results

3.1. The Pollen Viability of Different Cultivars

Pollen quality is important for successful fertilization in plants. In this study we examined the pollen viabilities and germination rates of three different cultivars (Table 1). Although the results of I_2-KI staining and TTC staining varied, there was no significant difference of pollen viability among the cultivars Xiaomi, Putao and Dami shown by certain a method (Table 1). It is noteworthy that the pollen germination rate of Dami was lower than those of Xiaomi and Putao (Figure 1).

3.2. The Influence of Pollen Source on Fruit Setting in *V. fordii*

According to long-term practical experience, Putao produces most fruits, but

Table 1. The pollen viability examined by I_2-KI test and TTC test.

	Cultivar	Pollen viability (%)
	Xiaomi	75.39 ± 7.45a
I_2-KI test	Putao	75.58 ± 7.11a
	Dami	70.68 ± 6.55a
	Xiaomi	61.2 ± 9.28a
TTC test	Putao	60.49 ± 7.10a
	Dami	57.44 ± 4.59a

Note: The data demonstrate the means ± standard derivation from 3 biological repeats. Each biological repeat includes 3 samples. The different letters indicate significant difference from ANOVA (LSD-test, p value < 0.05) within a staining method.

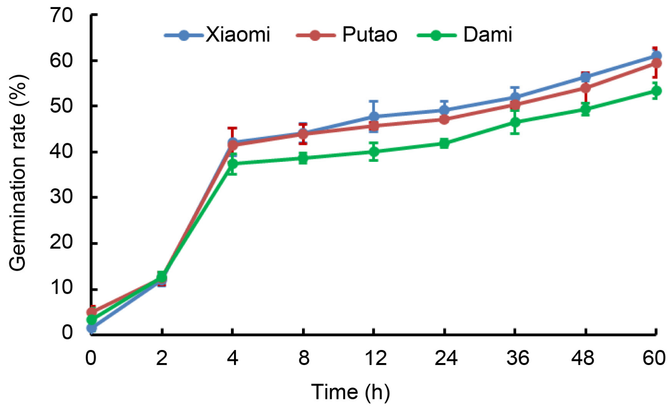


Figure 1. The pollen germination rates of three cultivars. The data demonstrate the means ± standard derivation from 3 biological repeats.

fruit weight was lowest among the three cultivars. In contrast, Dami produces the biggest fruit, but the number of fruit is lowest. The performance of Xiaomi is moderate in terms of fruit number and fruit weight. Therefore, this study chose the cultivars Putao and Xiaomi as female parents because of satisfactory fruit number, to investigate the effect of xenia on fruit and seed development, and oil content.

After fertilization, we counted fruit setting rates of various hybridization combinations. The results showed that the pollens from Putao and Dami led to higher fruit setting rates in Xiaomi (female parent) (**Figure 2**). Similarly, the pollens from both Xiaomi and Dami significantly improved fruit setting rates in plants of Putao. It seems that heterogenous pollen could promote fruit setting rate in *V. fordii*.

3.3. The Influence of Pollen Source on Fruit Development

In 2018, we observed that pollen source had influence on fruit in female parent plant (**Figure S1**), specifically, the pollen from the cultivar Dami led to increase of fruit size and fresh weight in plant of Putao. The experimental results in 2019 demonstrated that different pollen sources did not affect the size and fresh weight of fruit in plant of Xiaomi (**Figures 3(a)-(c)**). In contrast, the pollens from the cultivar Dami significantly promoted the size and fresh weight of fruit in plant of Putao (**Figures 3(a)-(c)**).

3.4. The Influence of Pollen Source on Seed Development

The number of seed in single fruit and the dry weight of single seed are important indicators for oil production. In 2018, we found that the number of seed in fruit was not affected by pollen source when only Putao was used as female parent (**Figure S2**). However, the dry weight of single seed was promoted by the

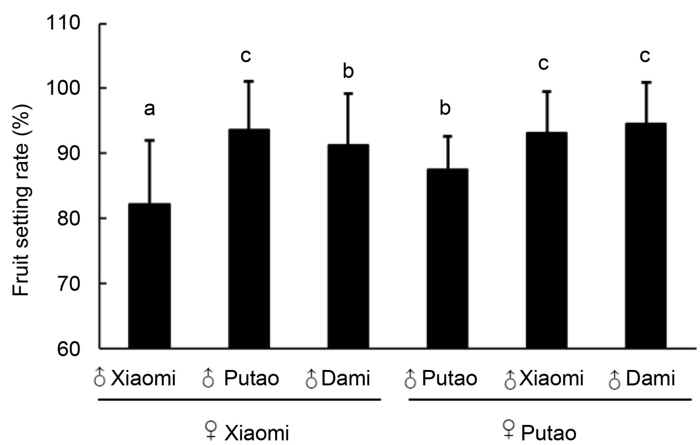


Figure 2. The fruit setting rate from various hybridization combinations in 2019. The data demonstrate the means ± standard derivation from 3 biological repeats. Each biological repeat includes 50 samples. The different letters indicate significant difference from ANOVA (LSD-test, p value < 0.05).

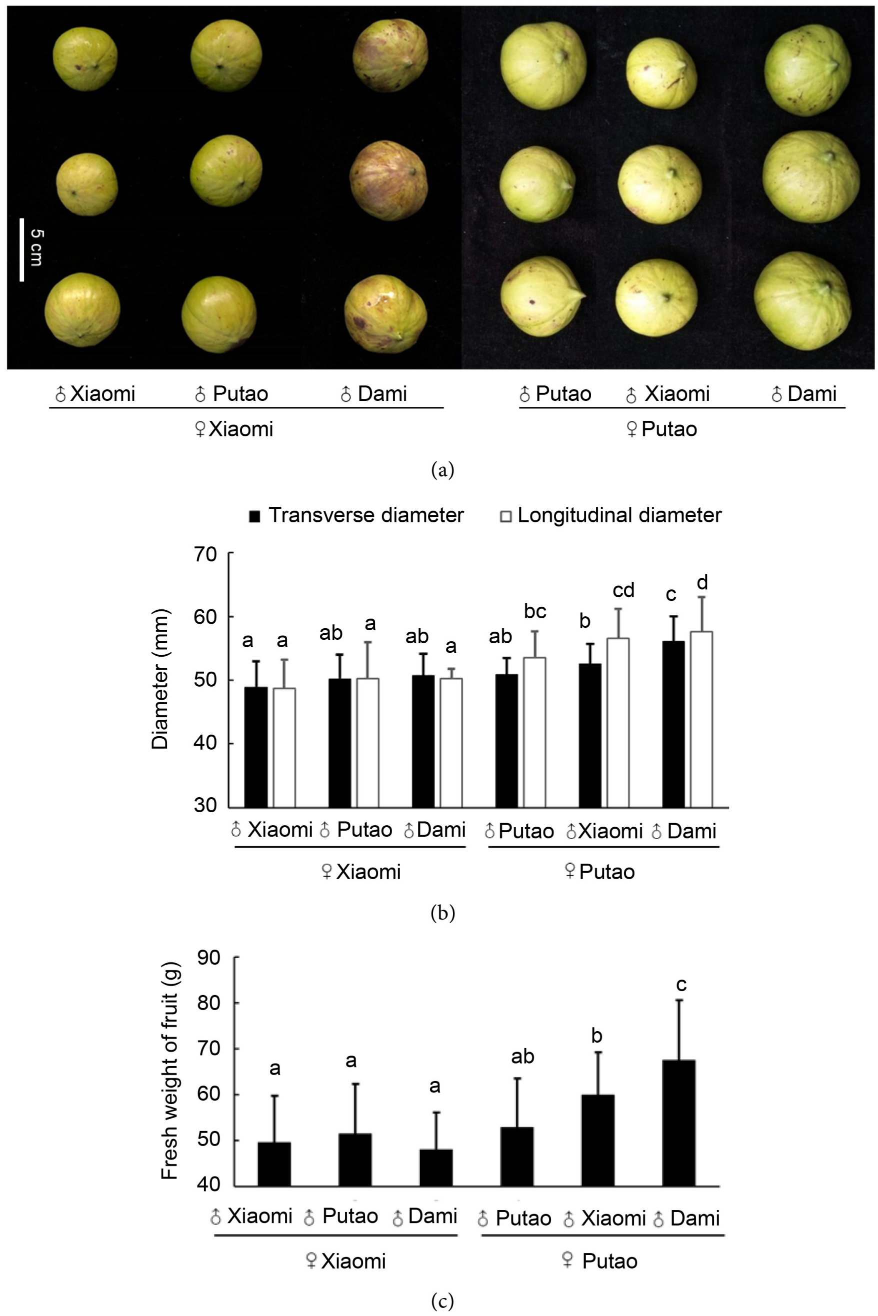
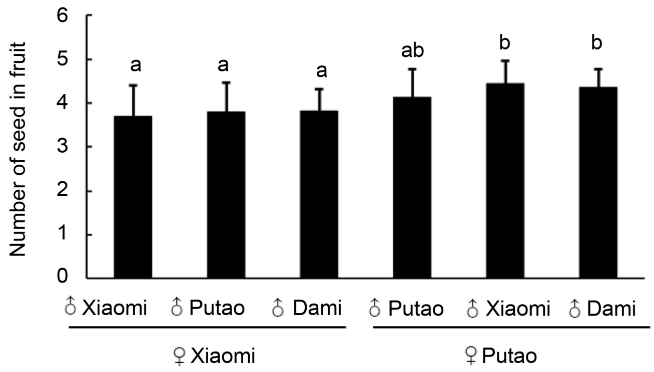
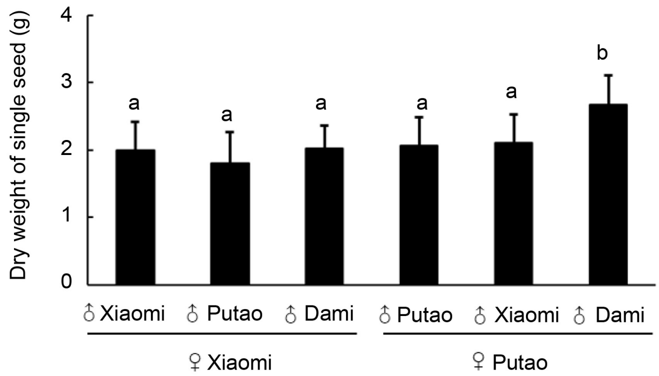


Figure 3. The photos of fruits (a) and fruit sizes (b) and fruit fresh weight (c) from various hybridization combinations in 2019. The data demonstrate the means ± standard derivation from 3 biological repeats. Each biological repeat includes 20 samples. The different letters indicate significant difference from ANOVA (LSD-test, p value < 0.05).

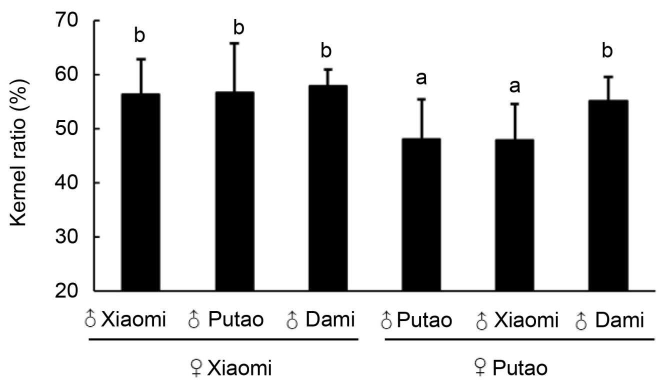
pollen from Dami. In 2019, it was found that heterogenous pollen did not affect the number of seed in single fruit in both plants of Xiaomi and Putao (**Figure 4(a)**). However, the cultivar Putao tended to produce more seeds in fruit than Xiaomi. In terms of dry weight of each seed, there was no change resulted from pollen source in plant of Xiaomi, but a significant increase of dry weight of single seed was observed in plant of Putao pollinated by Dami (**Figure 4(b)**).



(a)



(b)



(c)

Figure 4. The seed quantity (a), dry weight (b) and kernel ratio (c) from various hybridization combinations in 2019. The data demonstrate the means ± standard derivation from 3 biological repeats. Each biological repeat includes 20 samples. The different letters indicate significant difference from ANOVA (LSD-test, p value < 0.05).

Furthermore, we analyzed the kernel ratio (kernel weight/seed weight) to find that different pollen source did not affect the kernel ratio in plant of xiaomi, while the pollen from Dami led to a higher kernel ratio in plant of Putao (**Figure 4(c)**).

3.5. The Influence of Pollen Source on Kernel Oil Content and Oil Characteristics

Whether oil content is affected by pollen source is the most interested question. The experimental results in 2018 showed that the pollen from Dami could improve the oil content in seed in plant of Putao (**Figure S3**). In 2019, we found that the pollen from Putao declined the oil content in kernel in plant of Xiaomi, while the pollen from Dami promoted it (**Figure 5(a)**). In the plants of Putao, both the pollens from Xiaomi and Dami improved the oil content in kernel compared to the pollen from Putao itself (**Figure 5(a)**).

Acid value, iodine value and saponification value are important indicators for evaluating oil quality. The results in 2018 showed that these values were not changed by different pollen sources. In 2019, it was found that pollen source did not affect the acid value of oil extracted from kernels (**Figure 5(b)**). For iodine value which reflects the unsaturated degree of fatty acid, we observed that in the plants of Xiaomi, the iodine values of oil did not change regardless of pollen resource, while in the plants of Putao, the pollens from Dami resulted in a higher iodine value, indicating that the content of unsaturated fatty acid of oil extracted

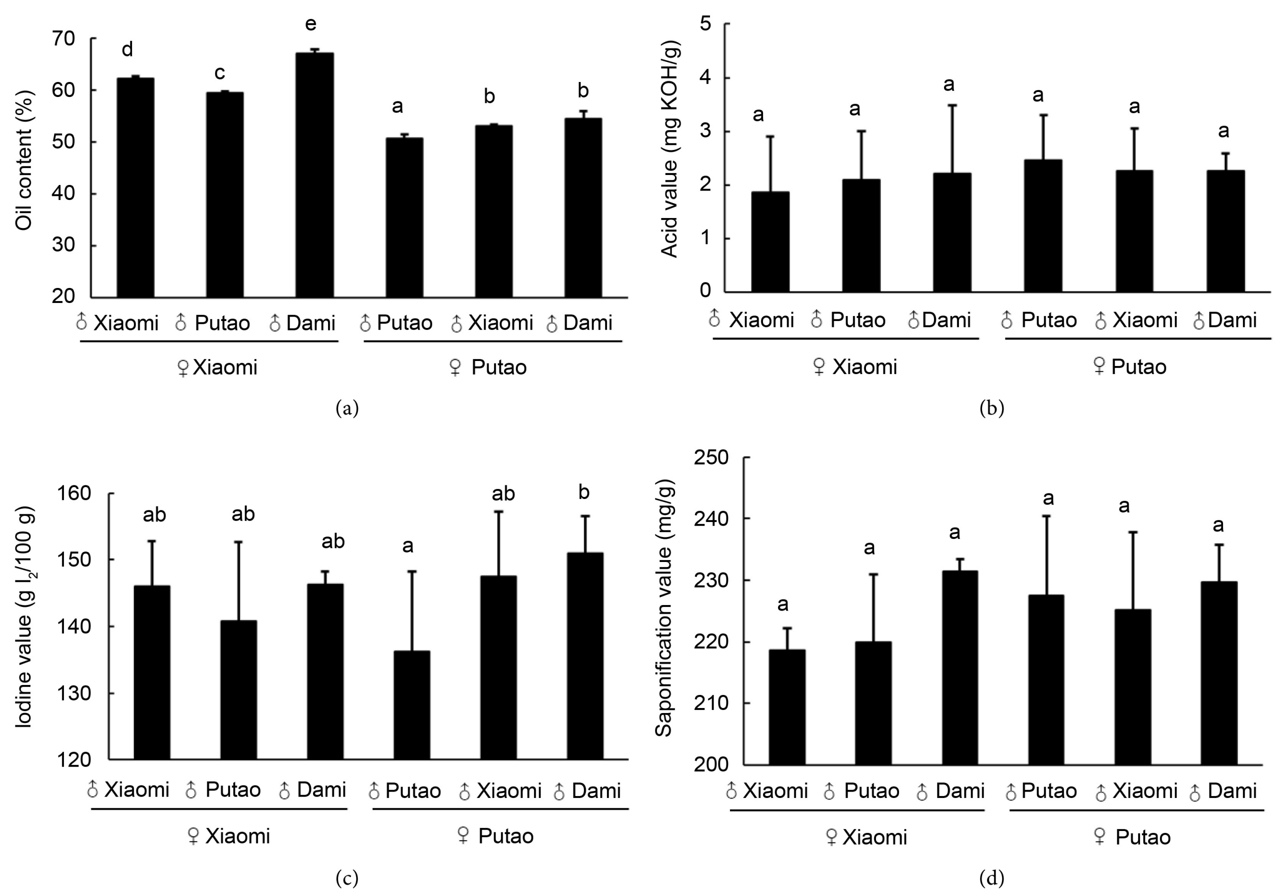


Figure 5. The kernel oil contents (a) and their acid values (b), iodine values (c) and saponification values (d) from various hybridization combinations in 2019. The data demonstrate the means ± standard derivation from 3 biological repeats. Each biological repeat includes 3 samples. The different letters indicate significant difference from ANOVA (LSD-test, p value < 0.05).

from kernel increased (**Figure 5(c)**). Saponification value of oil demonstrates the average molecular mass of fatty acid. We found that the saponification value was not influenced by pollen source in plants of both Xiaomi and Putao (**Figure 5(d)**). Taken together, the results in 2018 and 2019 demonstrated that the oil content was affected by pollen source, while the oil characteristic was not.

4. Discussion and Conclusion

To date, it has been well known that xenia, an important genetic phenomenon, is widespread in planta, which brings about changes in various aspects. To our knowledge, so far there is no effect of xenia found in plants of the Euphorbiaceae family. This study revealed that xenia is present in tung tree for the first time, which affects fruit setting, fruit size, seed formation and seed oil content. In this study, the effect of xenia was demonstrated only by a specific combination of male and female parent, although it is unknown why xenia affects fruit and seed in a species-dependent manner in tung tree, similar phenomena were reported in many plant species, e.g. *Citrus mangshanensis* [14], *Actinidia arguta* [15] and Macadamia [16]. In this study, we found that the pollen germination rate of the cultivar Dami was lower than those of the cultivars Xiaomi and Putao, but pollens from Dami did improve fruit setting rates in plans of Xiaomi and Putao. The reason is probably that excessive pollens were used in the hybridization operation, which offset the drawback of pollen germination rate. The study showed that heterologous pollens are beneficial for promoting fruit setting rate, which has been revealed in many plant species, such as pummel [14]. There are two hypotheses to explain the effect of xenia on fruit setting: the hormonal hypothesis and the mobile mRNAs hypothesis. They both propose that heterologous pollens induce more physiological processes associated with seed formation (pollen tube growth and pollen-pistil interaction, etc.) [17]. Whether the positive processes to enhance pollination and embryo development are also launched by heterologous pollens in *V. fordii* needs further investigation.

In this study, both the experimental results in 2018 and 2019 showed that the pollen from Dami boosted fruit size in plants of Putao, along with the improvement of fruit size, the weight of single seed was also promoted in plant of Putao pollinated by Dami, resulting in a higher kernel ratio in the plants of Putao. Substantial studies have revealed that different pollen source brings about varied influence on fruit and seed trait [15] [16] [18], but our understanding on the mechanism of xenia is still lacking in *V. fordii*.

Previous studies have demonstrated that the change of oil content and oil characteristics is a part of xenia in various plant species [16] [19] [20]. This study showed that oil content in the kernel could be improved by heterologous pollens in plants of the cultivars Xiaomi and Putao. The studies on tree peony demonstrated that the heterologous pollens from the cultivars producing higher oil content would promote oil content in kernels in female parent plants and enhance the expression of genes involved in fatty acid and triacylglycerol biosynthesis in the process of rapid oil accumulation in seeds [21]. So far, the process of oil synthesis in *V. fordii* has been well revealed [22] [23], whether it is enhanced by heterologous pollens in *V. fordii* needs to be proven in future.

In this study, we observed that only one or a few traits of fruit or seed were influenced by pollen source, this phenomenon is also shown by a lot of studies in various plant species. It could be affirmed that the newly-formed embryo after pollination with heterogenous pollen could bring about stimulus to seed and fruit whose development are controlled by the genetic character of cultivar. However, the mechanism underlying it is still not clear.

When considering the application of xenia in practice, we found the pollen from Dami significantly improved seed oil content in both plants of Xiaomi and Putao, meanwhile, it also improved the number of seed in fruit and single seed weight in the plant of Putao. As mentioned before, the cultivar Putao natively produces much more fruits than the cultivars Xiaomi and Dami. Therefore, the hybridization combination of Putao (female parent) and Dami (male parent) showed enormous application potential.

Acknowledgements

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Conflicts of Interest

No interest conflict to declare.

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Supplementary Material

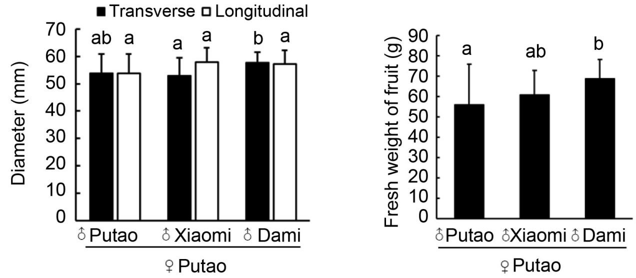

Figure S1. The fruit sizes (left) and fruit fresh weight (right) from various hybridization combinations in 2018. The data demonstrate the means ± standard derivation from 3 biological repeats. Each biological repeat includes 20 samples. The different letters indicate significant difference from ANOVA (LSD-test, p value < 0.05).

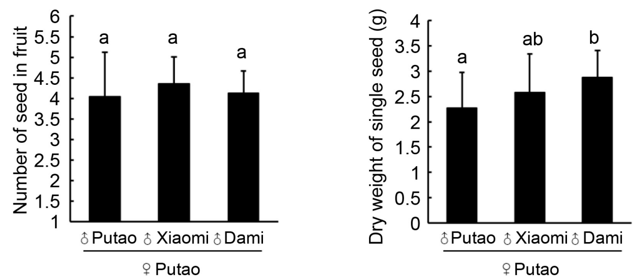

Figure S2. The seed quantity in fruit (left) and dry weight of single seed (right) from various hybridization combinations in 2018. The data demonstrate the means ± standard derivation from 3 biological repeats. Each biological repeat includes 20 samples. The different letters indicate significant difference from ANOVA (LSD-test, p value < 0.05).

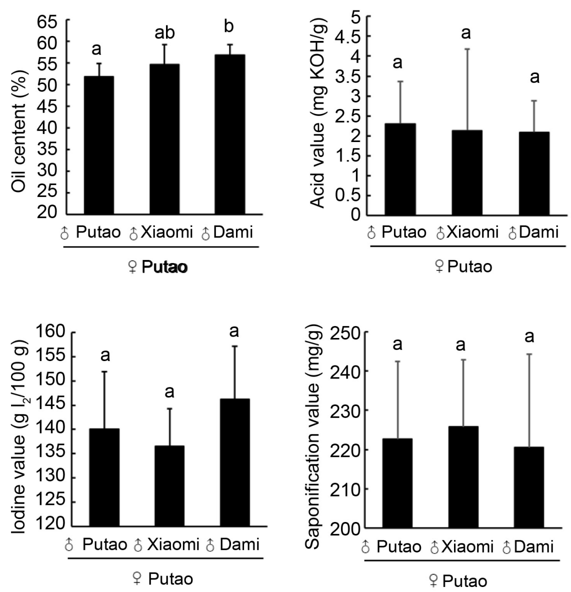

Figure S3. The kernel oil contents (top-left) and their acid values (top-right), iodine values (bottom-left) and saponification values (bottom-right) from various hybridization combinations in 2018. The data demonstrate the means ± standard derivation from 3 biological repeats. Each bio-logical repeat includes 3 samples. The different letters indicate significant difference from ANOVA (LSD-test, p value < 0.05).