

Analysis of Cross-compatibility among Wild Species of *Paeonia* subsect. Delavayanae and with Cultivated Varieties

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Abstract [**Objectives**] To provide a reference for the efficient use of wild *Paeonia suffruticosa* germplasm resources and the scientific selection of parents. [**Methods**] This study systematically evaluated cross-compatibility by conducting interspecific crosses among the major wild species subsect. Delavayanae (*Paeonia delavayi*, *Paeonia lutea*, *Paeonia potaninii*, and *Paeonia ludlowii*) as well as reciprocal crosses between these species and cultivated varieties from the subsect. Vaginatae. [**Results**] Crosses among *P. delavayi*, *P. lutea*, and *P. potaninii* generally exhibited good seed set, with the resulting seeds showing a high emergence rate. In contrast, crosses involving *P. ludlowii* as a parent produced only a limited number of seeds, none of which germinated. In crosses with cultivated varieties where the wild species served as the maternal parent, the average seed set ranked as follows: *P. lutea* > *P. delavayi* > *P. ludlowii* > *P. potaninii*. When wild species were used as paternal parent, the average seed set ranked as follows: *P. potaninii* > *P. lutea* > *P. delavayi* > *P. ludlowii*. On the whole, most crosses between wild species of subsect. Delavayanae and cultivated varieties achieved normal seed set, demonstrating a certain degree of cross-compatibility. However, when the wild species served as the maternal parent, both the seed set and seedling emergence rate were superior to those in the reciprocal crosses (with wild species as the paternal parent). [**Conclusions**] Significant differences in cross-compatibility were observed among species within *Paeonia* subsect. Delavayanae. In addition, a significant maternal effect was identified in crosses between wild species of this subsection and cultivated varieties. These findings suggest that by selecting highly compatible combinations, there remains considerable potential for utilizing these wild species in peony breeding.

Key words *Paeonia suffruticosa*, *Paeonia* subsect. Delavayanae, Wild species, Cross-compatibility, Seed set, Seedling emergence rate

0 Introduction

Paeonia suffruticosa is a perennial deciduous shrub belonging to Section Moutan of the genus *Paeonia* in the family Paeoniaceae. This section includes nine wild species and thousands of cultivated varieties^[1]. China is the native range of all wild tree peony species and serves as the global center of origin, evolution, and cultivation for domesticated tree peonies^[2]. The cultivated peonies currently grown worldwide all originate from the natural evolution, domestication, or systematic breeding of wild types. Among traditional Chinese varieties, all cultivated forms are derived from subsect. Vaginatae^[3]. In contrast, the wild germplasm of subsect. Delavayanae has not yet been incorporated into the breeding backgrounds of the four major cultivar groups; the Central Plains, Northwest, Southwest, and Jiangnan groups^[4]. However, since the early 20th century, wild species of subsect. Delavayanae such as *Paeonia delavayi* and *Paeonia lutea*, which were introduced to regions like France and the United States, have been extensively used in crossing-breeding. This effort has successfully developed a range of new cultivars characterized by unique flower colors (*e. g.*, pure yellow, deep red), high adaptability, and excellent

disease resistance. These cultivars have been widely promoted and applied globally^[5].

The wild species of *Paeonia* subsect. Delavayanae possess unique floral colors such as pure yellow and deep red, aesthetically pleasing leaf shapes, along with excellent stress tolerance and adaptability, making them important raw materials for peony germplasm improvement and cultivar innovation. At present, the utilization of these wild species in breeding research in China is still at a preliminary stage, and their genetic potential urgently needs further exploration. Crossing is the primary means for creating new cultivars, while distant crossing plays a crucial role in introducing superior traits^[6]. However, distant crossing often faces technical bottlenecks such as insufficient pollen viability, low parental compatibility, and crossing sterility. In addition, in practical crossing operations, reliance on pollen storage technology is frequently required due to non-synchronized flowering periods of the parents. Under natural conditions, peony pollen has a relatively short lifespan, typically lasting 10 to 20 d. Although low-temperature storage at 4 °C can extend the viability of pollen from most peony cultivars to 1 to 2 months, it remains insufficient to meet the demands of cross-year crossing^[7]. Cryopreservation technology, as a novel method for the long-term preservation of germplasm resources, has achieved success in pollen storage for various plant species^[8]. Meanwhile, cross-compatibility is significantly influenced by parental genotypes, and screening crossing combinations with high compatibility serves as an important foundation for distant cross-breeding^[9].

In view of these, we used wild species and cultivars of *Paeo-*

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nia subsect. Delavayanae as crossing parents. We conducted interspecific crossing among the wild species within this subsection, as well as reciprocal crossing trials between wild species and cultivated varieties within the section *Paeonia*. We systematically evaluated their cross-compatibility, aiming to screen for parental combinations or types with good seed set. It is intended to provide a reference for further utilizing the superior genes of wild species in *Paeonia* subsect. Delavayanae and realizing their breeding potential.

2 Materials and methods

2.1 Experimental materials Wild species of *Paeonia* subsect. Delavayanae were planted in National Peony and Peony Germplasm Resource Bank of Luoyang Academy of Agriculture and Forestry Sciences and Luoyang Luanchuan Peony Wild Species Resource Nursery. Wild species include *Paeonia delavayi*, *Paeo-*

nia lutea, *Paeonia potaninii*, and *Paeonia ludlowii*. Their main traits are shown in Table 1, and the flower type and color are shown in Fig. 1. The 25 cultivars involve *Paeonia xsuffruticosa* Zhongyuan Group, *Paeonia rocki* Rock Group, *Paeonia Xsuffruticosa* Japan Group, and *Paeonia xsuffruticosa* Jiangnan Group.

The crossing experiments within the wild species of *Paeonia* subsect. Delavayanae and those using wild species as the maternal parent were conducted at the Luoyang Luanchuan Peony Wild Species Resource Nursery, using freshly collected pollen from the same year. The crossing experiments using *Paeonia* subsect. Delavayanae wild species as the paternal parent were conducted at the National Peony and Peony Germplasm Resource Bank of the Luoyang Academy of Agriculture and Forestry Sciences, utilizing wild species pollen that had been cryopreserved for one year. The crossing experiments were carried out from 2021 to 2023.

Table 1 Main traits of wild species in *Paeonia* subsect. Delavayanae

Species name	Plant	Leaf	Flower	Seed	Propagation
<i>P. delavayi</i>	The plant is very tall, about 1.5 m.	Leaves are biternately compound with a broadly ovate or ovate outline, pinnately divided; the lobes are lanceolate to oblong-lanceolate, and the leaf underside is grayish-white.	2–5 flowers on branch tips and leaf axils; 9–12 petals, red to purplish-red; filaments dark purple; floral disc fleshy, sheathing the base of carpels; 2–5 carpels, glabrous, stigmas purple.	Low seed set, black seed	Root suckering, with facultative vegetative propagation.
<i>P. lutea</i>	The plant is tall, 1.0–1.5 m.	Leaves are biternately compound, alternate, papery in texture, pinnately divided with lanceolate lobes that are entire or serrated.	2–5 flowers (rarely solitary) at branch tips, often also in the upper 2–3 leaf axils; petals yellow, sometimes edged red or with a purple patch at base; filaments pale yellow; usually 3 carpels.	Low seed set, black seed	Having rhizomes and exhibiting facultative vegetative propagation.
<i>P. potaninii</i>	The plant is tall, 1.0–1.5 m.	Leaves are opposite or nearly opposite, biternately pinnate; leaflets are deeply divided into 3–5 narrow lanceolate lobes; the upper surface is green, the lower surface is pale green and glabrous.	Flowers red to purplish-red, small-sized; anthers yellow; 2–3 carpels, glabrous.	Low seed set, black seed	Developed rhizomes and having facultative vegetative propagation.
<i>P. ludlowii</i>	The plant exhibits a tall stature, with a height of 2.0 m, reaching up to 2.4 m under cultivated conditions.	Leaves are biternately compound, large in size, yellowish-green, and deeply lobed.	Each branch bears 2–5 flowers, large and yellow with 5–8 petals; 1–2 or 2–3 carpels, glabrous.	Low seed set, large seed, oblate, black	Seed propagation



NOTE a. *Paeonia ludlowii*; b. *Paeonia potaninii*; c. *Paeonia delavayi*; d. *Paeonia lutea*.

Fig. 1 Flower morphology and color of wild species in *Paeonia* subsect. Delavayanae

2.2 Experimental method

2.2.1 Crossing combinations. Two types of crossing combinations were established; interspecific crosses among wild species

within *Paeonia* subsect. Delavayanae, and crosses between wild species of subsect. Delavayanae and cultivars of subsect. Vaginatae. All parental plants possessed normal stamens and pistils along

with stable seed-setting ability, ensuring the feasibility of the crossing experiments.

2.2.2 Pollen collection. To avoid contamination from the external environment, pollen used in the experiments was collected during the bud-breaking stage. Flowering shoots with three leaves retained were cut, brought back to the laboratory, placed separately by variety in containers with water, and kept isolated. When the flowers fully opened, anthers were removed using forceps. The collected anthers were placed on sulfuric acid paper labeled with their identification number and collection date, and air-dried in a shaded area until pollen was naturally released. The pollen was then further dried in a constant-temperature air-blowing oven at 30 °C. Fully dried pollen was stored in airtight containers for later use.

2.2.3 Pollen storage. Pollen intended for use within the same year of collection was stored at -20 °C while pollen reserved for use in the following year was stored at -80 °C.

2.2.4 Determination of pollen viability. Pollen viability was assessed using the *in vitro* germination method. A solid culture medium with a standardized formulation was employed, consisting of 60 mg/L boric acid, 0.04 g/L CaCl₂, 100 g/L sucrose, and 5 g/L agar. An appropriate amount of the medium was poured into Petri dishes. After the medium solidified, a fine brush was used to evenly distribute a suitable quantity of pollen onto the surface. Each dish was labeled with the pollen type and the time of inoculation. The dishes were then placed in a constant-temperature incubator at (25 ± 1) °C for cultivation. Pollen germination was observed after 3 h. For each test, three random fields of view were examined under a microscope to calculate the germination rate. Pollen was considered germinated when the pollen tube length was at least twice the diameter of the pollen grain.

2.2.5 Crossing experiment. Prior to crossing, the buds of the maternal parent were emasculated when the petals began to show color. The emasculated buds were then bagged for isolation. When the maternal plants reached the full-bloom stage and the stigmas began to secrete mucus, pollination was performed. Pollination was repeated three times between 9:00 AM and 11:00 AM. One week after the flowering period of the peony population in the crossing area had ended, the bags were removed. When the seeds were fully mature but before the seed pods split, they were harvested

and placed indoors in a cool location for post-harvest ripening. The seed set was then calculated based on the total number of seeds obtained.

2.3 Data processing and calculation Data were statistically analyzed using Excel 2021. The formulas were as follows:

Germination rate (%) = (Number of germinated pollen grains/Total number of pollen grains) × 100%; Seed set per flower (seeds/flower) = (Total number of seeds obtained/Number of pollinated flowers); Seed germination rate (%) = (Number of germinated seeds/Total number of seeds sown) × 100%.

3 Results and analysis

3.1 Statistical results of pollen germination rate in wild species of *Paeonia* subsect. *Delavayanae* Because pollen viability directly affects the success and efficiency of cross breeding^[10], pollen vitality tests were conducted on both freshly collected and one year stored pollen before initiating crossing work. Pollen viability serves as a prerequisite for successful cross breeding, as pollen carries part of the genetic material from the paternal parent, and its activity significantly influences the outcome of plant breeding^[11]. In peony cross breeding, selecting pollen with higher viability and creating suitable conditions for pollen germination are fundamental to achieving successful crossing. The *in vitro* pollen germination method was used to determine the pollen germination rates of the tested wild species, and the results are presented in Table 2. As shown in Table 2, all wild species of *Paeonia* subsect. *Delavayanae* exhibited relatively high pollen germination rates, though significant variation was observed among different species. The highest germination rate in freshly collected pollen was recorded in *P. ludlowii*, reaching 89.3%, followed by *P. delavayi*, *P. potaninii*, and *P. lutea*.

After one year of storage at -80 °C, the pollen of wild species in *Paeonia* subsect. *Delavayanae* still maintained a germination rate of over 50%. Among them, *P. ludlowii* showed the highest pollen germination rate at 59.7%. The results of the pollen germination tests indicate that both freshly collected and one year stored pollen of wild *Paeonia* subsect. *Delavayanae* species retain relatively high germination rates, which can support the normal progress of crossing work.

Table 2 Pollen germination rates of wild *Paeonia* subsect. *Delavayanae* species

Wild species	Fresh pollen germination rate	Pollen germination rate after one year of storage	Wild species	Fresh pollen germination rate	Pollen germination rate after one year of storage
<i>P. delavayi</i>	80.4	56.8	<i>P. ludlowii</i>	89.3	59.7
<i>P. lutea</i>	74.2	52.4	<i>P. potaninii</i>	78.6	55.3

3.2 Seed set and seedling emergence of crossing combinations in *Paeonia* subsect. *Delavayanae*

3.2.1 Crossing in *Paeonia* subsect. *Delavayanae* groups. As shown in Table 3, the crossing combinations among wild species within *Paeonia* subsect. *Delavayanae* generally demonstrated good crossing compatibility, with an average seed set of 5.18 seeds per

flower and an average seedling emergence rate of 37.16% for the harvested crossing seeds. In particular, reciprocal crosses between *P. delavayi*, *P. lutea*, and *P. potaninii* exhibited relatively high seed set and seedling emergence rates, indicating good crossing compatibility and suggesting a close genetic relationship among these species, which are considered closely related. However,

when implementing the ‘*Paeonia delavayi* × *Paeonia ludlowii*’ and ‘*Paeonia ludlowii* × *Paeonia delavayi* Franch’ (mixed pollen; *P. delavayi* Franch is a collective term for *P. delavayi*, *P. lutea*, and *P. potaninii*, with pollen generally collected and mixed in equal proportions) combinations, seeds could be obtained, but none of the seeds germinated normally. This indicates

that although *P. ludlowii* and *P. delavayi* Franch both belong to subsect. Delavayanae, they exhibit significant reproductive isolation and prominent cross-incompatibility. This is also the reason why the wild species *Paeonia ludlowii* has remained relatively pure under natural environmental conditions.

Table 3 Crossing results among wild species of *Paeonia* subsect. Delavayanae

Maternal parent	Paternal parent	Number of flowers crossed	Number of seeds harvested	Seed set seeds/flower	Seedlings emerged//plant	Seedling emergence rate//%
<i>P. lutea</i>	<i>P. delavayi</i>	20	123	6.15	43	34.96
<i>P. delavayi</i>	<i>P. lutea</i>	20	180	9.00	63	35.00
<i>P. delavayi</i>	<i>P. potaninii</i>	20	307	15.35	151	49.19
<i>P. potaninii</i>	<i>P. delavayi</i>	20	88	4.40	37	42.05
<i>P. lutea</i>	<i>P. potaninii</i>	20	97	4.85	43	44.33
<i>P. potaninii</i>	<i>P. lutea</i>	30	135	4.50	48	35.56
<i>P. delavayi</i>	<i>P. ludlowii</i>	30	103	3.43	0	0.00
<i>P. ludlowii</i>	<i>P. delavayi</i> Franch (mixed in the same proportion)	40	3	0.08	0	0.00

3.2.2 Crossing between wild species of *Paeonia* subsect. Delavayanae (as maternal parent) and cultivars of subsect. Vaginatae. The crossing results between wild species of *Paeonia* subsect. Delavayanae (as maternal parent) and cultivars of subsect. Vaginatae are listed in Table 4. A total of 973 flowers were pollinated, yielding 2 632 seeds. The average seed set was 2.71 seeds per flower, and the average seedling emergence rate for the harvested seeds was 18.43%. The average seed set, from high to low, was: *P. lutea* (3.95 seeds/flower), *P. delavayi* (3.37 seeds/flower), *P. ludlowii* (1.62 seeds/flower), and *P. potaninii* (1.15 seeds/flower). The seedling emergence rate of harvested seeds, from high to low, was: *P. potaninii* (39.86%), *P. lutea* (26.07%), *P. delavayi* (11.34%), and *P. ludlowii* (4.62%). These data indicate that, in crosses using wild species of subsect. Delavayanae as the maternal parent and cultivars, the average seed set and the number of seedlings emerged are not directly proportional. Although *P. lutea* had a higher average seed set than *P. potaninii*, the seedling emergence rate of *P. potaninii* seeds was significantly higher than that of *P. lutea*. This suggests that the seed germination rate depends on the proportion of healthy seeds. Any issue or overall developmental disharmony in the post-fertilization development of the embryo, endosperm, seed coat, or pod can affect seed health, thereby influencing subsequent seedling emergence.

When *P. delavayi* served as the maternal parent, the paternal parents showing better compatibility and higher seed sets included ‘Yumiantaohua’, ‘Baiyuanhongxia’, and ‘Huawang’. For *P. lutea* as the maternal parent, paternal parents with good compatibility and relatively high seed sets were ‘Riyuejin’ and ‘Cengzhongxiao’; additionally, ‘Yeguanglei’, ‘Sihelian’, and ‘Baiyuanhongxia’ also exhibited good compatibility. When *P. ludlowii* and *P. potaninii* were used as maternal parents in crosses with cultivars, seed set was achieved, but the quantity was consistently low. This indicates the presence of significant crossing

Table 4 Crossing results between wild species of *Paeonia* subsect. Delavayanae (as maternal parent) and cultivars of subsect. Vaginatae

Maternal parent	Paternal parent	Number of flowers crossed	Number of seeds harvested	Seed set seeds/flower	Seedlings emerged plant	Seedling emergence rate//%
<i>P. delavayi</i>	Yeguanglei	30	83	2.77	8	9.64
	Fengdanbai	50	113	2.26	23	20.35
	Huawang	30	129	4.30	36	27.91
	Riyuejin	30	66	2.20	3	4.55
	Cengzhongxiao	30	87	2.90	6	6.90
	Baiyuanhongxia	30	143	4.77	11	7.69
	Yumiantaohua	25	138	5.52	2	1.45
<i>P. lutea</i>	Baiyuanhongxia	30	142	4.73	48	33.80
	Cengzhongxiao	40	266	6.65	87	32.71
	Fengdanbai	20	44	2.20	0	0.00
	Sihelian	40	187	4.68	33	17.65
	Yeguanglei	40	196	4.90	37	18.88
	Xueyingtaohua	30	42	1.40	22	52.38
	Fangji	30	55	1.83	8	14.55
	Yumiantaohua	30	87	2.90	29	33.33
	Huadachen	8	0	0.00	0	0
	Huawang	20	31	1.55	0	0
<i>P. ludlowii</i>	Riyuejin	20	166	8.30	53	31.93
	Daodachen	30	42	1.40	2	4.76
	Fangji	20	33	1.65	1	3.03
	Huangjiamen	30	68	2.27	4	5.88
	Huajing	30	62	2.07	0	0
	Baqiandaichun	30	58	1.93	6	10.34
	Baiwangshizi	20	22	1.10	1	4.55
	Lianhe	30	38	1.27	2	5.26
	Riyuejin	30	53	1.77	2	3.77
	Huadachen	20	19	0.95	0	0.00
	Fengdanbai	30	66	2.20	3	4.55
	Yeguanglei	30	49	1.63	3	6.12
	Heihuakui	20	9	0.45	0	0
<i>P. potaninii</i>	Yeguanglei	20	23	1.15	9	39.13
	Heixuanfeng	10	14	1.40	5	35.71
	Fangji	20	29	1.45	14	48.28
	Taiyang	30	48	1.60	21	43.75
	Fenli	20	16	0.80	6	37.50
	Duohualuohan	20	8	0.40	0	0

barriers in crosses using *P. ludlowii* or *P. potaninii* as the maternal parent with cultivars. However, certain specific combinations still retained some degree of compatibility. In particular, for *P. potaninii*, despite its low seed set, the average seedling emergence rate of the harvested seeds was remarkably high at 39.86%, suggesting that most crossing embryos developed healthily following successful fertilization. In contrast, when *P. ludlowii* was the maternal parent, a small number of seeds could be harvested, but the seed germination rate was only 4.62%. This clearly indicates pronounced post-fertilization developmental obstacles in the crosses involving *P. ludlowii*.

3.2.3 Crossing between cultivars of subsect. *Vaginatae* (as maternal parent) and wild species of *Paeonia* subsect. *Delavayanae* (paternal parent). A total of 36 crossing combinations were set up using peony cultivars as the maternal parent and wild species of subsect. *Delavayanae* as the paternal parent (Table 5). Pollination was performed on 940 flowers, resulting in the harvest of 1 405 seeds, with an average seed set of 1.49 seeds per flower. Among these, 170 seedlings emerged, yielding an overall seedling emergence rate of 12.10%. As can be seen from Table 5, the average seed set for different wild species used as the paternal parent, from highest to lowest, was *P. potaninii*, *P. lutea*, *P. delavayi*, and *P. ludlowii*, at 2.21, 1.52, 0.98, and 0.17 seeds per flower, respectively. The seedling emergence rate of the harvested seeds, from highest to lowest, was for *P. lutea*, *P. delavayi*, *P. ludlowii*, and *P. potaninii*, at 19.64%, 17.90%, 15.00%, and 5.62%, respectively. From the above data, it can be seen that when wild species of subsect. *Delavayanae* are used as the paternal parent in crosses with cultivars, there is also no proportional correlation between the average seed set and the seedling emergence rate. In particular, when *P. potaninii* was used as the paternal parent in crosses with cultivars, the average seed set was the highest at 2.21 seeds per flower, yet the seedling emergence rate was the lowest, at only 5.62%.

When *P. delavayi* was used as the paternal parent, the maternal parents exhibiting good compatibility and higher seed set were ‘Fengdanbai’ and ‘Yeguangbei’. When *P. lutea* was used as the paternal parent, the maternal parents with good compatibility and higher seed set were ‘Yeguangbei’, ‘Sihelian’, and ‘Yumiantaohua’; besides, ‘Fangji’ and ‘Caiyelanju’ also showed good compatibility. When *P. potaninii* was used as the paternal parent, the maternal parents demonstrating good compatibility and higher seed set were ‘Yeguangbei’, ‘Huadachen’, and ‘Huangjiamen’; furthermore, Japanese cultivars such as ‘Fangji’, ‘Lianhe’, and ‘Huajing’ also exhibited good compatibility. Compared to other wild species with fleshy disc, using *P. ludlowii* as the paternal parent in crosses with cultivars resulted in a prominent phenomenon of crossing failure (no seed set). Although a few seeds were occasionally obtained in individual combinations, the overall average seed set was only 0.17 seeds per flower. Crosses with ‘Yeguangbei’ and ‘Duohualuohan’ yielded a small number of seeds, but none germinated normally, indicating

very obvious defects in seed development.

Table 5 Crossing results between subsect. *Delavayanae* and cultivars of subsect. *Vaginatae*

Maternal parent	Paternal parent	Number of flowers crossed	Number of seeds harvested	Seed set seeds/flower	Seedlings emerged plant	Seedling emergence rate//%	
Fengdanbai	<i>P. delavayi</i>	30	68	2.27	13	19.12	
Yeguangbei		20	35	1.75	8	22.86	
Yumiantaohua		30	22	0.73	3	13.64	
Cengzhongxiao		20	23	1.15	4	17.39	
Baiyuanhongxia		25	7	0.28	0	0	
Taiyang		20	0	0	0	0	
Fangji		20	7	0.35	1	14.29	
Caiyelanju		<i>P. lutea</i>	15	22	1.47	3	13.64
Hongbanbai			15	7	0.47	2	28.57
Hongyangfei			30	8	0.27	3	37.50
Fengdanbai	25		11	0.44	3	27.27	
Sihelian	40		126	3.15	17	13.49	
Yeguangbei	30		99	3.30	24	24.24	
Xueyingtaohua	30		18	0.60	6	33.33	
Fangji	30		66	2.20	9	13.64	
Yumiantaohua	30		89	2.97	27	30.34	
Daodachen	30		0	0	0	0	
Huawang	30	22	0.73	0	0		
Xiangyu	20	26	1.30	3	11.54		
Daodachen	<i>P. potaninii</i>	30	43	1.43	4	9.30	
Fangji		30	55	1.83	2	3.64	
Huangjiamen		30	92	3.07	6	6.52	
Huajing		30	51	1.70	0	0	
Baqiandaichun		30	55	1.83	7	12.73	
Baiwangshizi		30	28	0.93	1	3.57	
Lianhe		30	66	2.20	2	3.03	
Riyuejin		30	23	0.77	0	0	
Huadachen		30	102	3.40	8	7.84	
Fengdanbai		30	48	1.60	2	4.17	
Yeguangbei	<i>P. ludlowii</i>	30	166	5.53	9	5.42	
Yeguangbei		20	3	0.15	0	0	
Heixuanfeng		20	5	0.25	1	20.00	
Fangji		20	0	0	0	0	
Taiyang		20	0	0	0	0	
Fenli		20	8	0.40	2	25.00	
Duohualuohan		20	4	0.20	0	0	

4 Conclusions

4.1 Significant differences in cross-compatibility among species within *Paeonia* subsect. *Delavayanae* In reciprocal cross experiments between *P. delavayi*, *P. lutea*, *P. potaninii*, and *P. ludlowii*, the crossing combinations among *P. delavayi*, *P. lutea*, and *P. potaninii* generally exhibited higher seed set and seedling emergence rates, indicating a closer genetic relationship and strong cross-compatibility among these species. However, in combinations involving *P. ludlowii* as a parent (*e.g.*, "*P. delavayi* × *P. ludlowii*" and "*P. ludlowii* × *P. delavayi*"), although a

small number of seeds were obtained, none germinated, reflecting a significant reproductive barrier between *P. ludlowii* and the other three wild species. These results support the classification of subsect. Delavayanae into two groups; the *P. delavayi* complex^[12] (comprising *P. delavayi*, *P. lutea*, and *P. potaninii*) and *P. ludlowii*. They also provide corroborating evidence for the observation and claim that natural hybrids, such as *Paeonia* × *suffruticosa* ‘Yinlian Mudan’, exist in wild populations where *P. delavayi* and *P. lutea* distributions overlap.

4.2 Significant maternal parent effect in crosses between wild species of *Paeonia* subsect. Delavayanae and cultivars

Distant crossing between wild germplasm and cultivated varieties plays a crucial role in improving cultivars and breeding novel germplasm^[13]. Based on phylogenetic distance, distant crossing within *Paeonia* sect. Moutan mainly refers to crossing between different sections or, within the section, between different species or subsections. The crossing between wild species of subsect. Delavayanae and cultivated varieties of subsect. Vaginatae is a form of inter-subspecific distant crossing. Any failure in the process (from pollen grain adhesion, germination and pollen tube growth to the completion of double fertilization, and further to the development of the zygote and endosperm) can directly lead to a crossing failure.

The experimental results indicate a certain level of compatibility in these crosses. Most combinations produced normal seed set, but the crossing direction had a significant effect on the outcome. In crosses with wild species of subsect. Delavayanae as the maternal parent and cultivars as the paternal parent, the average seed set was 2.71 seeds per flower, and the average seedling emergence rate for the harvested seeds was 18.43%. In contrast, in the reciprocal crosses with cultivars as the maternal parent, the average seed set was only 1.49 seeds per flower, with a seedling emergence rate of 12.10%. Both the seed set and seedling emergence rate were higher when wild species served as the maternal parent compared to when they acted as the paternal parent. This suggests that in distant crossing, using wild species of subsect. Delavayanae as the maternal parent facilitates greater success. Furthermore, while *P. ludlowii* exhibited more prominent crossing barriers as a parent, resulting in an overall low level of seed set, it still showed a degree of compatibility in specific combinations. This indicates that by screening for high-affinity combinations, there remains potential to explore its utility in breeding programs.

5 Discussion

5.1 Screening combinations with strong cross-compatibility is a prerequisite for successful breeding The study and breakthrough in the mechanism of distant crossing incompatibility are the core issues for fully exploiting the breeding potential of wild tree peonies from subsect. Delavayanae and promoting the process of distant cross-breeding. Western countries have made significant progress in breeding using wild species from this subsection. They have not only successfully overcome the problem of sterility in hy-

brid offspring but have also achieved the selection of advanced-generation hybrids, having developed eighth- and ninth-generation hybrids as early as the beginning of the 21st century^[14]. In contrast, domestic breeding work utilizing wild species of subsect. Delavayanae started relatively late. Although there have been breakthroughs in recent years, most research still remains at the stage of F₁ generation selection, and the advantages in terms of flower form and growth vigor are not yet pronounced. This study confirms that, under the premise of ensuring a certain level of compatibility in the crossing combinations, further screening for specific combinations with higher compatibility is the key foundation for successfully obtaining hybrid seeds and breeding new cultivars. This conclusion is consistent with the findings of He Guimei^[15] and Wang Yuelan^[16].

5.2 Overcoming pre-fertilization barriers is key to enhancing seed set Regarding crossing combination types, identifying the causes of crossing incompatibility and proposing reasonable solutions are fundamental to achieving major breakthroughs in the distant crossing of tree peonies. In this study, we found that although wild species with fleshy floral discs exhibit relatively high pollen viability, their seed set is generally low when crossed with cultivated species of subsect. Vaginatae. This indicates that the hybridization barriers primarily occur during the stage from pollen germination on the stigma to fertilization, representing typical pre-fertilization barriers. To effectively mitigate such barriers, it is recommended to actively explore and apply physical and chemical treatments during pollination. These include stigma cutting, moderate heating, ultraviolet irradiation, as well as the application of gibberellin, boric acid, or sucrose solutions, aiming to improve the stigmatic environment and promote pollen germination and pollen tube growth. In addition, active efforts should be made to attempt or refine pollination methods, such as mixed pollination, mentor pollination, bud pollination, and repeated pollination. These approaches seek to develop effective technical pathways for overcoming pollen-stigma recognition barriers and ensuring the successful progression of the fertilization process.

5.3 Improving embryo rescue technique is key to overcoming post-fertilization barriers In distant crossing, many combinations can yield a high seed set and produce hybrid seeds, yet the overall seedling emergence rate remains generally low or even negligible. This phenomenon indicates the existence of post-fertilization barriers, whereby during seed development, the embryo, endosperm, seed coat, or fruit pod may undergo arrested or uncoordinated growth, ultimately leading to structurally defective seeds that lose their germination capacity. Moreover, no positive correlation is observed between seed set quantity and seedling emergence rate, demonstrating that both pre- and post-fertilization barriers are critical constraints on the success of crossing. Embryo rescue technique stands as one of the most effective approaches to overcome embryo abortion and has been widely employed in field crops such as wheat and rapeseed, as well as in various horticultural species. Therefore, upon completion of crossing in tree peo-

nies, it is essential to monitor the developmental progress of hybrid embryos, for instance, through consecutive observation of ovule development via paraffin sectioning, to precisely determine the optimal timing for embryo rescue. Advancing the refinement and systematic application of embryo culture technology in tree peonies, and closely integrating it with the entire distant crossing process, constitutes vital technical support for ensuring hybrid seedling establishment and enhancing breeding efficiency.

5.4 Research methods and calculation indicators to be optimized Due to long-term differences in natural selection and cultivation domestication, significant variation exists between wild and cultivated tree peony species in floral organ structure, particularly in traits that determine reproductive capacity, such as carpel number and ovule count per carpel. Research has shown that crossing combinations with high seed set tend to exhibit lower rates of ovule abortion after pollination^[17]. In this experiment, when calculating the seed set of crossings, the number of pollinated flowers was used as the basis (*i. e.*, number of mature seeds per pollinated flower), without fully accounting for differences in pistil structure among different maternal parents. Although this method can objectively reflect compatibility trends in comparisons of the same type, calculations based on ovule number (*e. g.*, number of developed seeds per total ovules) would yield more precise results. In future studies, optimizing this calculation indicator will contribute to more scientific and detailed evaluation results.

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(From page 38)

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