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BIOLOGY

Cross pollination of different peony cultivars with 'Feng dan'

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ABSTRACT: *Paeonia ostii 'Feng dan' is recommended as a new promising oil crop because of the nutrient elements in its seed; however, the development of this new oil crop is now limited due to its low seed setting rate. This study identified ways to improve the cross pollination of Feng dan as the maternal parent, which has been proved to be a suitable oil crop, so as to improve the seed setting rate of 'Feng dan', and to predict the functional relationship between pollen vigor and seed setting rate. improving the efficiency of cross pollination. Three major steps were conducted, first, to screen the appropriate method of measuring pollen vigor of the paternal parent, which could have a strong relationship with seed setting rate. Second, to obtain the corresponding seed setting rate by cross pollination. Third, the functional relationship between two indexes was founded which could be used in cross pollination practice. Results indicated that the best paternal parents of 'Feng dan' were 'Feng dan' cultivars from other different cultivation populations; The most suitable medium concentration for pollen germination was 100~150 g/L sucrose, 0.06~0.08 g/L boric acid, and 50~200 g/L PEG-6000. Both the seed setting rate and the number of seed grains were positively correlated with the pollen activity, and the seed setting rate was negatively correlated with the local precipitation.*
Key words: *sucrose, boric acid, PEG-6000, pollen germination, seed setting rate.*

Polinização cruzada de diferentes cultivares de peônia com 'Fengdan'

RESUMO: *Peonia, Feng dan' (Paeonia ostia) do género Paeonia (o grupo lenhoso) é recomendado como uma nova cultura promissora na produção de óleos, devido aos elementos nutritivos na semente, no entanto, o desenvolvimento desta nova cultura está agora limitado devido às baixas taxas de fixação de sementes. Este estudo tem por objetivo identificar meios de melhorar a polinização cruzada de 'Feng dan' como o parental paterno, por ser uma cultura de óleo adequada, de modo a melhorar as taxas de fixação de sementes de 'Feng dan', e para prever a relação funcional entre o vigor do pólen e as taxas de fixação de sementes para melhorar a eficiência da experiência de polinização. Em primeiro lugar, foram realizados três passos para analisar o método de medição do vigor do pólen do pai paternal, que poderia ter uma relação forte com as taxas de fixação das sementes. Em segundo lugar, obter as taxas de fixação de sementes correspondentes por polinização cruzada. Em terceiro lugar, foi estabelecida a relação funcional entre dois índices que poderiam ser utilizados na prática da polinização cruzada. Os resultados indicaram que os melhores parentais paternos de 'Feng dan' eram cultivares de 'Feng dan' de outras populações de cultivo diferentes. A concentração de meio mais adequada para a germinação do pólen foi 100~150 g/L de sacarose, 0,06~0,08 g/L ácido bórico, e 50~200 g/L PEG-6000. Tanto a taxa de estabelecimento das sementes como o número de sementes foram positivamente correlacionados com a atividade do pólen, e a taxa de estabelecimento das sementes foi negativamente correlacionada com a precipitação local.*
Palavras-chave: *sacarose, ácido bórico, PEG-6000, germinação de pólen, taxa de fixação de sementes.*

INTRODUCTION

Paeonia ostii has been an outstanding ornamental plant with medicinal and oil value. Especially in recent years, it is recommended as a new type of promising oil crop because of the nutrient elements in its seed, such as trace elements, high-concentration fatty acids, and peony flavonoids, which are beneficial to human health. Accordingly, the crop is widely used in health products, cosmetics, beverages, and edible oils. *Paeonia ostii* 'Feng dan' is a main cultivar of oil peony because of its seed yield and oil content (GUO AN et al., 2014). However, 'Feng dan' belongs to an outcrossing cultivar with very low yield in a single cultivation, and its fruit only produces few seeds (YA YUN et al., 2017; HU LIANG et al., 2017). Therefore, this study selected suitable cultivars as paternal plants to improve the seed setting rate of 'Feng dan' by cross pollination. The pollen vigor of each cultivar must be measured accurately as an important index for artificial pollination and hybrid breeding in peony production

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(TANGMITCHAROEN & OWENS, 1997; MIAO et al., 2018). Previous studies have shown that sucrose, boric acid, polyethylene glycol (PEG), and plant growth regulators all have significant effects on pollen germination in other species (AND et al., 2003; FEI & NELSON, 2003; GUI RONG, 2010; CHUN-YAN et al., 2010; TAYLOR & HEPLER, 1997; SHAO LING et al., 2005). Thus, we obtained the pollen germination rate of the pollinated cultivars and then determined a cubic function relationship between pollen germination and seed setting rate. This relationship should be regarded as an important index for cross pollination, which could improve the efficiency of the cross test and save time in practice.

MATERIALS AND METHODS

The experiment was conducted from April to early August in 2018. Pollen germination of nine peony cultivars and cross pollination with 'Feng dan' were both carried out during April. Seed setting rate was monitored from May to August, and mature seeds were harvested in August. Nine peony cultivars were selected from three plantations in Luoyang City, Henan Province, namely, 'Feng dan', 'Luoyang hong' , 'Yinhong qiaodui', 'Xiang yu', 'Zipao Jindai, 'Japan Huangguan', 'Sheng dai' , 'Hai huang', and 'Jin ge' (FU HUI et al., 2005). The early-blooming cultivars were around March 28, and the late-blooming cultivars were around April 15 (Table 1). The pollination experiment was carried out in three plantations: East Garden (112°40′ E, 34°39′ N, Alt. 119-121 m), Henan University of Science and Technology (112°24′ E, 34°36′ N, Alt. 149-151 m), and Zhou Mountain (112°22′ E, 34°38′ N, Alt. 197-215 m) which all located in Luoyang City. The soil conditions of the three plantations were shown in table 2, and the monthly average temperature and precipitation were shown in table 3. All the peony materials are robust plants without pests and diseases.

Screening the optimum content of liquid medium for pollen germination

When the bud of 'Feng dan' was soft and the anther was not cracked, the anthers were taken out and then stored in an airtight container at 20 °C for germination test (SHIVANNA & RAN GASWAMY, 1992). Suspension drop method was used to determine the optimum content of liquid medium for pollen germination, and monofactor analysis was applied. First, a drop of culture solution was added on the cover-slips and spread out, and then the pollen was spread on the culture solution by approximately same amount. Next, the cover glass was turned over and the concave glass was put on; it was cover and sealed by wet cotton balls. Finally, they were culture in an incubator at 25 °C for several hours. The optimum concentration of sucrose from a series (0, 50, 100, 150, and 200 g/L), boric acid from a series (0, 0.04, 0.06, 0.08, and 0.1 g/L) and PEG-6000 from a series (0, 50, 100, 150, and 200 g/L) were selected respectively.

Pollen germination was observed under a microscope, and pollen germination rate was counted after 5 h of incubation at 25 °C. The standard of pollen germination is that the length

Table 1 - Flowers phenology of each peony cultivars in 2018.

Cultivars	Early flowering	Maximum flowering	Late flowering
Campus 'Feng dan'	03-28—04-02	04-02—04-10	04-10—04-15
East garden 'Feng dan'	04-07—04-08	04-08—04-15	04-15—04-20
Zhou mountain 'Feng dan'	03-29—04-03	04-03—04-11	04-11—04-16
'Luoyang hong'	04-02—04-03	04-03—04-09	04-09—04-11
'Yinhong qiaodui'	04-03—04-04	04-04—04-08	04-08—04-10
'Xiang yu'	04-03—04-04	04-04—04-09	04-09—04-11
'Zipao jindai'	04-05—04-06	04-06—04-10	04-10—04-12
'Japan Huangguan'	04-05—04-06	04-06—04-11	04-11—04-13
'Sheng dai'	04-10—04-11	04-11—04-13	04-13—04-15
'Hai huang'	04-11—04-13	04-13—04-18	04-18—04-21
'Jin ge '	04-12—04-13	04-13—04-17	04-17—04-20

Table 2 - General situation of 'Feng dan' planting land for oil in different areas of Luoyang City.

Site	Soil type	PH	Geomorph-ological type	Soil thickness/cm	Irrigation	Fertilization
Henan University of Science and Technology	red clay	8.17	plain	＞100	yes	no
Zhou mountain	red clay	8.29	hilly	＞80	yes	no
East Garden	red clay	8.31	plain	＞100	yes	no

of pollen tube is more than twice the diameter of the pollen grain. Each variety was randomly examined by three visual fields, and the average value was determined as the germination rate of the cultivars.

Cross pollination test

'Feng dan' flowers in three locations were pollinated with nine different cultivars, including 'Feng dan' from three different cultivated populations. Five peony with uniform growth were selected, and 30 flowers were randomly selected from the upper, middle, and lower parts of each tree. Another 30 'Feng dan' flowers were randomly selected without any treatments and allowed to be pollinated naturally as the control. When the bud of 'Feng dan' was soft and the anther was not cracked, the petals were unfolded and carefully emasculated with tweezers. Then, the pollens were dipped onto the stigma with a brush. The pollination experiment was carried out from 09:00 to 12:00 a.m every hour. All the cultivars were tagged after the pollinating treatments. At early August, matured fruits were harvested according to the combination of pollination. The number of fruits was counted, then the seed setting rate and average quality of a single grain were calculated. The percentage of pollen germination (%) was equal to the number of germinated pollen grains/the total number of pollen grains (GUO AN et al., 2014).

Data processing and analysis

We processed and analyzed the experimental data by SPSS 20.0, the mainly statistics method included one-way ANOVA, regression analysis. We made relevant maps by Origin 2018.

RESULTS

Effect of sucrose concentrations on pollen germination rate

At 0-150 g/L sucrose concentration, the pollen germination rate of most peony cultivars gradually increases with sucrose concentration increase (Figure 1-A) and reach a maximum value at 150 g/L mass concentration. For 'Xiang yu' and 'Japan Huangguan', the optimum concentration was 100 g/L. The highest germination rate of 'Feng dan' was 23.25 %, and the 'Feng dan' cultivars in the East Garden and Zhou Mountain had slightly lower germination rate than the 'Feng dan' cultivar in Henan University of Science and Technology. The cultivars which had the lowest germination rate was 'Luoyang hong' and 'Japan Huangguan', 3.80 % and 2.44 % respectively. When the sucrose concentration was more than 150 g/L, the pollen germination rate showed a downward trend as sucrose concentration rises. Variance analysis showed that the difference between 150 g/L sucrose treatment and control groups was significant, and the suitable sucrose concentration ranged between 100~150 g/L for most cultivars.

Table 3 - Changes of meteorological factors during the growth and development of 'Fengdan' pods in different locations in 2018.

Site	----------Monthly average temperature (°C) /Monthly average precipitation(mm)----------			
	April	May	June	July
Campus	21.9/41.7	27.4/27.2	30.1/70.1	32.7/203.4
Zhou Mountain	20.8/41.9	26.2/28.3	26.4/71.8	27.2/204.5
East Garden	18.4/42.6	26.9/33.4	26.5/73.3	29.4/204.9

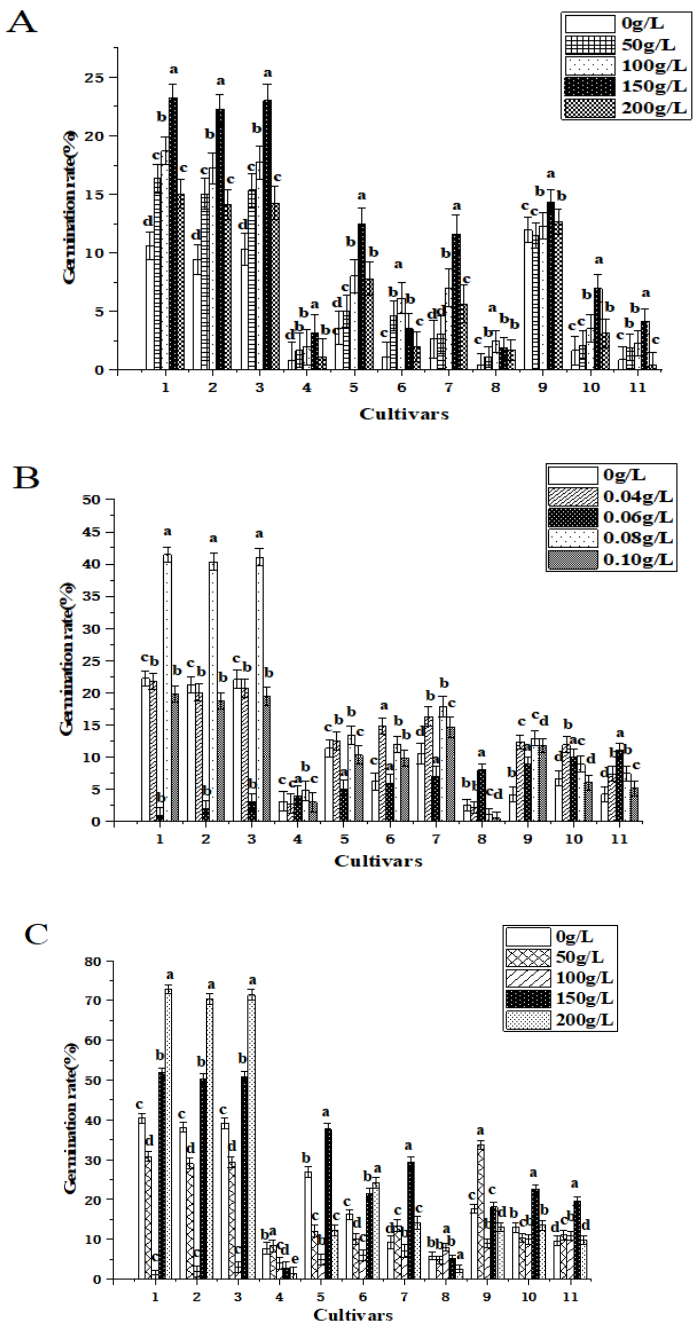

Figure 1 - (A) Effect of sucrose of different mass concentrations on pollen germination rate. (B) Effects of boric acid of different mass concentration on pollen germination rate. (C) Effects of PEG-6000 of different mass concentrations on pollen germination rate.

1 - (1:Campus 'Feng dan' 2: East Garden 'Feng dan' 3: Zhou Mountain 'Feng dan' 4: 'Luoyang hong' 5: 'Yinhong qiaodui' 6: 'Xiang yu' 7: Zipao jindai 8: 'Japan Huangguan' 9: 'Hai huang' 10: 'Sheng dai' 11: 'Jin ge').
2 - The same index in the figure, different lowercase letters indicate significant differences at 0.05 (the same as in the following table). Please return figures . tiff with at leas 300 dpi.

Effects of boric acid on pollen germination rate

Boric acid had a significant effect on the increase of pollen germination rate (Figure 1-B). When the concentration of boric acid was 0.06 g/L, the germination rate of all pollens except 'Feng dan' increased and reached maximum values. The highest germination rate of 'Feng dan' was 0.08 g/L. The germination rate of 'Yinhong qiaodui' and 'Zipao jindai' increased significantly which were 2.44 and 2.66 times of the control group, respectively. When the concentration of boric acid was between 0.08 and 0.10 g/L, it inhibited pollen germination significantly. Results showed that 0.06~0.08 g/L boric acid was beneficial for peony pollen germination.

Effects of PEG-6000 of different concentrations on pollen germination rate. PEG-6000 had a significant effect on pollen germination rate (Figure 1-C). When the concentration of PEG-6000 ranged from 0 g/L to 50 g/L, the pollen germination rate of 'Luoyang hong' and 'Hai huang' reached maximum values. The germination rate of 'Japan Huangguan' reached its maximum at 100 g/L. The germination rate of 'Hai huang', 'Luoyang hong', 'Sheng dai', and 'Jin ge' reached their maximum values at 150 g/L, whereas those of 'Feng dan' and 'Xiang yu' reached their maximum values at 200 g/L (Table 2). In conclusion, the mass concentration of PEG-6000 suitable for pollen germination was 50~200 g/L, but the optimal mass concentration of PEG-6000 of different cultivars still vary within this range.

Analysis on the results of cross pollination of 'Feng dan' showed significant differences among different 'Feng dan' cultivation populations in these three locations. As shown in Figure 2-A, the highest seed setting rate was 56.67%, which was obtained from the cultivars between campus 'Feng dan' and Zhou Mountain 'Feng dan' and higher than the natural seed setting rate of a single cultivation population. From samples of these three locations, we could conclude that the 'Feng dan' cultivation populations are the best pollination cultivars for each other and could effectively improve the seed setting rate of 'Feng dan'. However, the seed with the highest 100-seed weight were the hybrid seed of 'Yinhong qiaodui' and 'Feng dan' in Henan University of Science and Technology (Figure 2-B). The 100-seed weight per seed was 82.27 g, which could increase the quality of single peony grain due to heterosis. From figure 2-C, we reported that there existed a significant difference in the number of pollen grains, and the 'Feng dan' pollens from three different cultivation areas were still the paternal parent with higher number of grains. This outcome demonstrated that in pollen oil production, the most suitable paternal parent for 'Feng dan' is still 'Feng dan' cultivars own pollen from different cultivation populations.

Table 4 indicates that both the seed setting rate and the number of seed grains had a very significant positive correlation with the pollen activity, while the number of seed grains also had a very significant positive correlation with the seed setting rate. Table 5 indicates that there was no significant correlation between the pollination result index and the accumulated temperature. Table 6 indicates that the seed setting rate has a significant negative correlation with the average precipitation of April, may, June and July, and the number of seed grains had a significant negative correlation with the average precipitation of July.

DISCUSSION

Pollen vitality refers to the ability of pollen to survive and germinate and directly affects the results of pollination test, so it is an indispensable factor in the determination of pollen viability before artificial pollination. Germination, dyeing, and inorganic acid methods are the three main methods for determining pollen viability, and germination determination method can be divided into *in vitro and in vivo* germination determination methods (DE YI, 2009). This experiment adopts the *in vitro* germination determination method, which is the closest method to determine the true viability of pollen because it accurately observes pollen germination under a microscope (KAI ZHI et al., 2008). Results showed that the pollen viability of the nine peony cultivars varied significantly. 'Feng dan' cultivars had the highest pollen viability, with pollen germination rate reaching 72.3 %. 'Luoyang hong' cultivars had the lowest pollen viability, with pollen germination rate of only 8.32 %. Such peculiar result was also reported in pear (SHAO LING et al., 2005) and jujube (LING et al., 2006), indicating genetic variation in these species. The proportions of suitable liquid medium for pollen germination of different cultivars had significant differences. Sucrose is an essential component for the pollen culture of most plants *in vitro* and provides nutrition and suitable osmotic pressure for pollen germination and pollen tube growth. The optimum concentration of sucrose for most peony cultivars is 150 g/L. Boron is a unique essential element of higher plants. It binds with free sugar, facilitates the diffusion of sugar across the plasma membrane, and promotes sugar transport

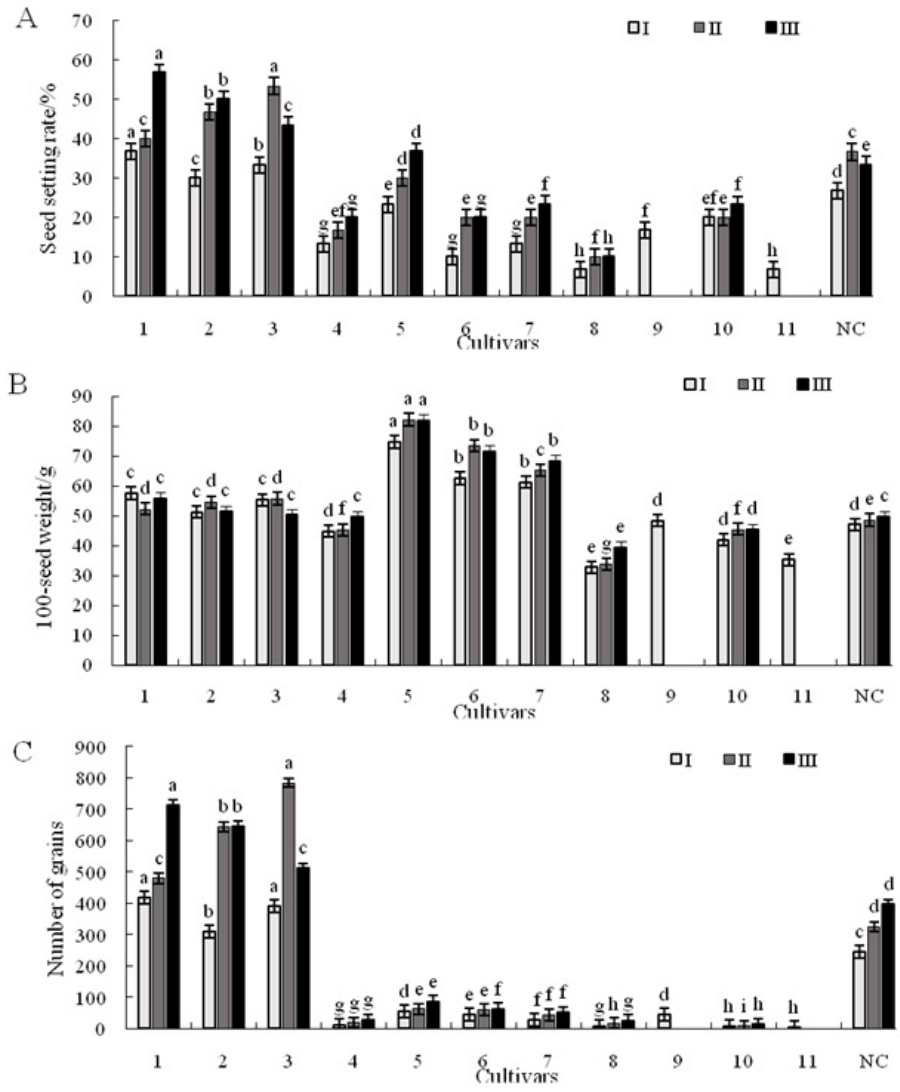

Figure 2 - (A) Seed setting rate of the cross-pollination by different male parents with 'Feng dan' in different plantations. (B)100-seed weight of the cross-pollination by different male parents with 'Feng dan' in different plantations.(C) The number of grains of the cross-pollination by different male parents with 'Feng dan' in different plantations.

1 - Maternal parent: (I, East Garden 'Feng dan'. II, Campus 'Feng dan'. III, Zhou Mountain 'Feng dan'.
2 - Paternal parent: 1, Campus 'Feng dan'. 2, East Garden 'Feng dan' .3, Zhou Mountain 'Feng dan'. 4,'Luoyang hong'. 5, 'Yinhong qiaodui'. 6, 'Xiang yu'. 7, 'Zipao jindai'. 8, 'Japan Huangguan'. 9, 'Hai huang'. 10, 'Sheng dai'. 11,'Jin ge').
3 - NC: Natural control.
4- The same index in the figure, different lowercase letters indicate significant differences at 0.05, n=1.

and pollen tube elongation (GUI RONG, 2010). The optimum boric acid concentration for pollen germination of most peony cultivars was 0.06 g/L, while that of 'Feng dan' was 0.08 g/L. PEG-6000 has good water solubility and good compatibility with many organic components. It mainly promotes the elongation of pollen tube to improve pollen germination rate (GUO AN et al., 2014).

The results showed that 'Fengdan' pollens from other different locations were the best male

Table 4 - Correlation analysis of the indexes and the germination rate of pollen.

	Seed setting rate	100-grain weight	Number of grains	Pollen germination rate
Seed setting rate	1			
100-grain weight	0.289	1		
Number of grains	0.915**	0.036	1	
Pollen germination rate	0.882**	0.185	0.905**	1

**There was significant correlation at 0.01 level (bilateral).
*At the 0.05 level (bilateral), there was a significant correlation.

parents for ‘Fengdan’ itself as an outcrossing species. Previous studies have shown that ‘Fengdan’ from different places not only has higher pollen vitality than other ornamental varieties, but also, as a relative of wild Peony species ‘Yangshan Peony’, has stronger stress resistance than other ornamental peonies. Moreover, its pollen survives longer under natural conditions, which is more conducive for successful cross pollination based on flowering phenology (CHANG WEI et al., 2019; YU & QIANG, 2006).

Nearly 1000 cultivars of peony are reported in China, and thus finding suitable paternal parents for ‘Feng dan’ is quite difficult. Moreover, the flowering of peony occurs only once a year and is very short, so convenient and quick methods to screen parental breeds are required. Both the seed setting rate and the number of seed grains had a significant positive correlation with the pollen activity, and the seed setting rate has a significant negative correlation with the local precipitation. Therefore, we could choose parents with higher pollen activity and areas with relatively less precipitation before pollination test to achieve better results. To sum up, suitable paternal parents could be determined by pollen pollination test in advance. If the selected pollinating cultivars and ‘Feng dan’ have good seed setting characteristics, the new pollinating cultivars not only can be used as the main pollinating cultivars of ‘Feng dan’ but also can be used as the accompanying cultivars of oil ‘Feng dan’ to increase yield and ornamental value conveniently.

Table 5 - The correlation analysis of each index and the average temperature of each month.

	Seed setting rate	100-grain weight	Number of grains	Average temperature in April	Average temperature in May	Average temperature in June	Average temperature in July
Seed setting rate	1						
100-grain weight	0.289	1					
Number of grains	0.915**	0.036	1				
Average temperature in April	0.336	0.179	0.214	1			
Average temperature in May	-0.121	-0.033	-0.027	0.19	1		
Average temperature in June	0.118	0.085	0.11	0.732**	0.808**	1	
Average temperature in July	-0.036	0.01	0.024	0.405*	0.975**	0.919**	1

**There was significant correlation at 0.01 level (bilateral).
*At the 0.05 level (bilateral), there was a significant correlation.

Table 6 - Correlation analysis of each index and monthly average precipitation.

	Seed setting rate	100-grain weight	Number of grains	Precipitation in April	Precipitation in May	Precipitation in June	Precipitation in July
Seed setting rate	1						
100-grain weight	0.289	1					
Number of grains	0.915**	0.036	1				
Precipitation in April	-0.409*	-0.177	-0.28	1			
Precipitation in May	-0.403*	-0.181	-0.269	0.999**	1		
Precipitation in June	-0.432*	-0.143	-0.345	0.956**	0.944**	1	
Precipitation in July	-0.426*	-0.113	-0.372*	0.881**	0.862**	0.981**	1

**There was significant correlation at 0.01 level (bilateral).
*At the 0.05 level (bilateral), there was a significant correlation.

CONCLUSION

The best paternal parents of 'Feng dan' were 'Feng dan' cultivars from other different cultivation populations; The most suitable media concentration for pollen germination was 100~150 g/L sucrose, 0.06~0.08 g/L boric acid, and 50~200 g/L PEG-6000. Both the seed setting rate and the number of seed grains were positively correlated with the pollen activity, and the seed setting rate was negatively correlated with the local precipitation.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

Ting Li wrote the main manuscript. Qi Qiao designed experiments and revised paper. Jianfeng Li and Xingfei Guo collected test date. Xiaogai Hou provided test materials. All authors critically revised the manuscript and approved of the final version.

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