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BIOLOGY

Flowering phenology and pollen characteristics of cultivated populations of *Paeonia ostii* 'Feng dan' at different altitudes

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ABSTRACT: From a pollination biology standpoint, this study compared modifications in the flowering phenology, soluble sugar and protein content of pollen, antioxidant enzyme system, and storage traits of five cultivated populations of *Paeonia ostii* 'Feng dan' at various altitudes in Luoyang. The findings indicated that the first blooming phase was delayed at greater altitudes. There was a significant difference in the soluble sugar content of pollen between sites; and there was no discernible relationship between soluble sugar content and altitude. With increasing altitude, there is a tendency for soluble protein content to decrease. The activities of antioxidant enzymes in pollen from cultivated populations at varying altitudes showed SOD and POD enzyme activities generally increasing with decreasing temperature, whereas CAT enzymes showed divergent patterns. Differences existed in the storage time of pollen across populations at different altitudes, and pollen from higher altitudes appeared to be better adapted to low-temperature storage environments. The rate of decrease in pollen viability under dry storage at -74.7 °C was significantly lower than under other storage conditions. The comprehensive analysis concludes that the variations in 'Feng dan' cultivation populations within the same region at different altitude zones can be leveraged for cross-pollination among proximate populations, utilizing low-temperature dry pollen storage technology, with the aim of achieving abundant oil peony production.
Key words: oil peony, 'Feng dan', flowering phenology, pollen storage.

Fenologia da floração e características polínicas das populações cultivadas de 'Feng dan' em diferentes altitudes

RESUMO: Neste estudo, compararmos a fenologia da floração, os açúcares solúveis e as proteínas solúveis do pólen, as enzimas antioxidantes e as características de armazenamento de cinco populações cultivadas de *Paeonia ostii* 'Feng dan' em diferentes altitudes em Luoyang, na perspectiva da biologia da polinização. O pólen é um fruto de uma espécie de árvore, com um teor de proteínas solúveis e de pólen, sistema de enzimas antioxidantes e características de armazenamento. Os resultados mostraram que o início da floração foi mais tardio em altitudes mais elevadas. O teor de açúcar solúvel do pólen variou significativamente entre diferentes locais, não havendo uma relação óbvia com a altitude, enquanto o teor de proteínas solúveis tendeu a diminuir com o aumento da altitude. As atividades das enzimas antioxidantes do pólen de populações cultivadas a diferentes altitudes diferiram entre si, as atividades das enzimas SOD e POD aumentaram basicamente com a diminuição da temperatura, enquanto a enzima CAT apresentou várias tendências. Verificaram-se diferenças no tempo de armazenamento do pólen de populações de diferentes altitudes, e o pólen de populações de altitudes mais elevadas parecia estar mais bem adaptado a ambientes de armazenamento a baixas temperaturas. A taxa de redução da viabilidade do pólen sob armazenamento seco a -74,7 °C foi significativamente menor do que sob outras condições de armazenamento. As análises sugerem que as diferenças entre as populações de cultivo de 'Fengdan' na mesma região a diferentes altitudes podem ser exploradas para efetuar a polinização cruzada entre populações próximas, utilizando a tecnologia de armazenamento de pólen por secagem a baixa temperatura, com vista a atingir o objetivo de produção abundante e em grande escala de peónia oleaginosa.
Palavras-chave: peónia oleaginosa, 'Feng dan', fenologia da floração, armazenamento de pólen.

INTRODUCTION

Paeonia suffruticosa is a perennial deciduous shrub that possesses significant ornamental, medicinal, and nutritional value (DE YUAN & KAI, 1999; TATSUMI et al., 2004). Peony bark (moutan cortex) is regarded as a valuable medicinal herb capable of clearing heat, cooling blood, activating blood circulation, and removing blood stasis. Recent investigations have shown that it contains a variety of active substances (IZUMI et al., 2020), which have anti-tumor (XU et al., 2019) and anti-viral (WANG et al., 2020) effects. Studies have revealed that peony seed oil has a very high content of unsaturated fatty acids and other trace elements that offer various health benefits, contributing to its high market value as a premium edible oil (DENG et al., 2020). Furthermore, products utilizing peony as a raw material have expanded into the food, health care, and cosmetic industries (LIN et al., 1999; AN et al., 2006). Being one of the oil peony varieties, 'Feng dan' has undergone extensive study due to its remarkably high fruiting rate (ZI CHEN et al., 2018). Our findings showed that it belongs to self-incompatible

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varieties, and crossbreeding among populations in distinct locations can significantly enhance the fruiting rate (TING et al., 2020). To achieve higher yields, a prudent approach should be to engage in crossbreeding in proximity at various altitude locations within the same region. Subsequently, it becomes imperative to comprehend the flowering phenology and pollen characteristics of ‘Feng dan’ at different altitude locations. Presently, research has only explored the differences in pollen activity of *Paeonia delavayi* at distinct altitude locations (JING et al., 2022), leaving a research gap regarding ‘Feng dan’ in this context. In this investigation, we explored the flowering phenology among ‘Feng dan’ populations at varying altitudes through the lens of pollination biology and conducted a comparative analysis of the soluble sugar content, soluble protein content, antioxidant enzyme system, and the impacts of low temperature and drying on pollen storage. The objective was to enhance the seed yield of ‘Feng dan’ in practical production. These findings will serve as a foundational reference for enhancing the seed yield of ‘Feng dan’ in practical production and crossbreeding endeavors.

MATERIALS AND METHODS

The test materials were selected from ‘Feng dan’ oil peonies aged over 5 years, exhibiting vigorous growth, and devoid of pests and diseases. These were sourced from five distinct elevation sites in Luoyang, namely East Garden, Henan University of Science and Technology, Zhou Mountain, Kuqu Township, and Luanchuan Diaoyutai (Table 1).

Flowering phenology

Observations of flowering phenology were conducted from mid-March to May at the five sites mentioned above for the respective cultivars. Indicators of flowering phenology, including early flowering, maximum flowering, late flowering, and Single flowering period, were meticulously recorded. In each cultivation group, when more than 1/3 but less than 2/3 of the flowers opened, this was designated as the beginning of the early flowering. When more than 3/2 of the flowers opened, it was acknowledged as the maximum flowering, and when less than 1/3 of the flowers opened, it indicated the commencement of the late flowering.

Pollen collection and storage

The pollen collection occurred between 9:00 and 11:00 a.m. on sunny days at each site. Subsequently, the pollen was collected in 4 mL centrifuge tubes and transported to the laboratory in a vehicle refrigerator. The pollen was then distributed across eight storage environments (25 °C, 25 °C dry, 4 °C, 4 °C dry, -20 °C, -20 °C dry, -74.7 °C, and -74.7 °C dry). Dry storage involved placing silica gel desiccant in a centrifuge tube, with the desiccant occupying 1/3 of the tube.

Soluble sugars, soluble proteins and antioxidant enzymes of pollen

Soluble sugar and soluble protein contents were analyzed on the day of pollen collection using Anthrone colorimetry and the Kjeldahl method (ZHI AN et al., 2003), respectively. Three antioxidant enzyme activities were determined after a 24-hour treatment at 25 °C, 4 °C, and -20 °C. Superoxide dismutase (SOD) was assessed using the nitrogen blue tetrahydrazine method, and peroxidase (POD) was measured using the guaiacol method (ZHI AN et al., 2003). Catalase (CAT) was quantified using hydrogen peroxide (LAN FANG et al., 2011). The experiment was conducted thrice, and the mean values were calculated.

Pollen vitality detection

Pollen viability was assessed using the in vitro germination method, and the liquid medium consisted of 150 g/L sucrose + 0.08 g/L boric acid

Table 1 - Study area.

Site	Coordinates	Elevation [m a.s.l.]	Abbreviation
East Garden	112°40′ E, 34°39′ N	119-121	EG
Henan University of Science and Technology	112°24′ E, 34°36′ N	149-151	HU
Zhou Mountain	112°22′ E, 34°38′ N	197-215	ZM
Kuqu Township	112°7′ E, 34°11′ N	369-371	KT
Luanchuan Diaoyutai	111°55′ E, 33°47′ N	703-710	LD

+ 200 g/L PEG-6000 (TING et al., 2020). Pollen was incubated at 25 °C for 5 hours, then observed under a microscope. For each storage method, three random fields of view were selected to calculate the germination rate. Germination was defined as the pollen tube’s length being 2 times or more than the length of the pollen itself. Vigor tests were regularly conducted for each storage method based on the conditions, continuing until the germination rate of pollen under those conditions reached zero.

Data processing and analysis

The experimental data were processed and analyzed using SPSS Statistics 23 software. And OrijinPro 2021 was used for figure making.

RESULTS

Flowering phenology

There were significant differences in the initial flowering period of ‘Feng dan’ among regions (Table 2). ‘Feng dan’ in the HU region flowered the earliest, on March 27, while ‘Feng dan’ in the LD region flowered the latest, on April 17, with a 21 days difference. The longest flowering period of ‘Feng dan’ in EG was 14 days, while the shortest flowering period of ‘Feng dan’ in HU and ZM was 10 days, with a difference of 4 days.

Soluble sugar and soluble protein content

The soluble sugar and soluble protein contents of ‘Feng dan’ pollen exhibited significant variations among regions, with soluble protein contents being higher at low altitudes than at high altitudes (Table 3). The highest soluble sugar content was 43.86 mg/g in ‘Feng dan’ pollen from KT, and the lowest was only 16.17 mg/g in ‘Feng dan’ pollen from ZM. There was a tendency for soluble protein content to decrease with increasing altitude, with the highest being ‘Feng dan’ pollen from HU at 0.91 mg/g and the lowest being LD at 0.46 mg/g.

Three types of antioxidant enzyme content

‘Feng dan’ pollen antioxidant enzyme activities differed significantly among the three temperatures. The pollen SOD activities at 4 °C and -20 °C were significantly higher than those at 25 °C (Figure 1-A). With decreasing temperature, pollen CAT activity in three regions, ZM, KT, and LD, showed a gradual decrease, pollen CAT activity in EG increased and then decreased, and that of HU, in contrast to that of EG, showed a decreasing and then increasing trend (Figure 1-B). With the decrease of temperature, the pollen POD activity of all five regions showed a gradual increasing trend, and the highest activity was found in ZM at -20 °C, and the lowest in KT and EG (Figure 1-C).

The effect of different storage temperatures

Pollen viability declined the fastest at 25 °C (room temperature), and the addition of desiccant extended the room temperature storage time by 1~2 days (Figure 2-A, B). The region with the longest pollen storage time at 4 °C was LD, in which the pollen was able to be stored for 190 days, and the region with the shortest storage time was EG, which pollen was able to be stored for 150 days (Figure 2-C). Under the condition of drying at 4 °C, pollen from all the five regions was able to be stored for 190 days, and at 190 days LD had the highest pollen activity of 5.26% (Figure 2-D).

Under -20 °C, the pollen of KT had the longest storage time of 300 days. The shortest storage time was for the pollen of ZM and EG, which could be stored for 240 days (Figure 2-E). The longest storage time under dry conditions at -20 °C was the pollen of KT, which could be stored for 330 days. Pollen from the remaining four regions could be stored for 300 days (Figure 2-F).

At -74.7 °C, pollen viability could still exceed 50% after 360 days of storage, with an overall viability decrease of approximately 25% (see Figure 2-G). Under dry conditions at -74.7

Table 2 - Flowering phenology of 'Feng dan' (month- day).

Site	Early flowering	Maximum flowering	Late flowering	Flowering period
EG	04-06—04-07	04-08—04-17	04-17—04-19	14
HU	03-27—03-29	03-30—04-03	04-04—04-05	10
ZM	03-30—03-31	03-32—04-05	04-06—04-08	10
KT	04-01—04-02	04-03—04-11	04-12—04-13	13
LD	04-17—04-18	04-19—04-27	04-27—04-29	13

Table 3 - Contents of soluble sugar and soluble protein in 'Feng dan' pollen from different areas.

Site	Soluble sugars	soluble proteins
EG	28.30±1.15c	0.67±0.05b
HU	39.04±1.87b	0.91±0.08a
ZM	16.17±1.02e	0.61±0.03b
KT	43.86±2.31a	0.51±0.06c
LD	21.56±1.22d	0.46±0.01d

The results are the mean ± SE (n = 3). Different lowercase letters indicate significant differences (P<0.05).

°C, the pollen viability of EG, HU, ZM, KT, and LD decreased by 4.13%, 5.87%, 5.02%, 3.20%, and 4.18%, respectively, after 360 days of storage (Figure 2-H). The decline in pollen viability was less pronounced at higher altitudes, suggesting that pollen from higher altitudes may exhibit greater resistance to low-temperature storage compared to those from lower altitudes.

In summary, it is evident that ‘Feng dan’ pollen can endure storage at all four temperatures, with its vitality gradually diminishing over time. This decline manifests as a slow, followed by a rapid decrease, a pattern that becomes increasingly apparent with decreasing storage temperature. Under dry conditions at -74.7 °C, only the initial phase of the decline in pollen viability, the slow decreasing part, was observed (Figure 2-G). Conversely, at -74.7 °C, it transitioned into a rapid decline at 300 days of storage (Figure 2-H). This implies that dry storage extends the time before reaching the

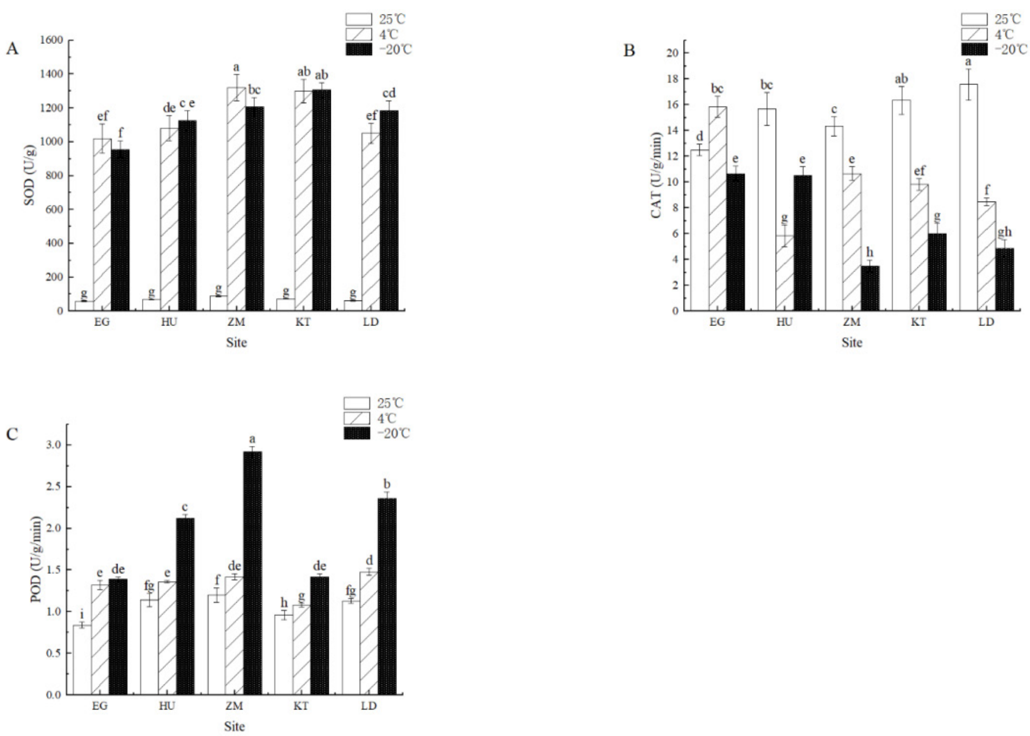

Figure 1 - Three antioxidant enzyme activities of ‘Feng dan’ pollen from different regions at different temperatures. SOD enzyme activity (A), CAT enzyme activity (B), POD enzyme activity (C). The lowercase letters in the figure represent significant differences (P < 0.05) at the same temperature, as shown in the following figure.

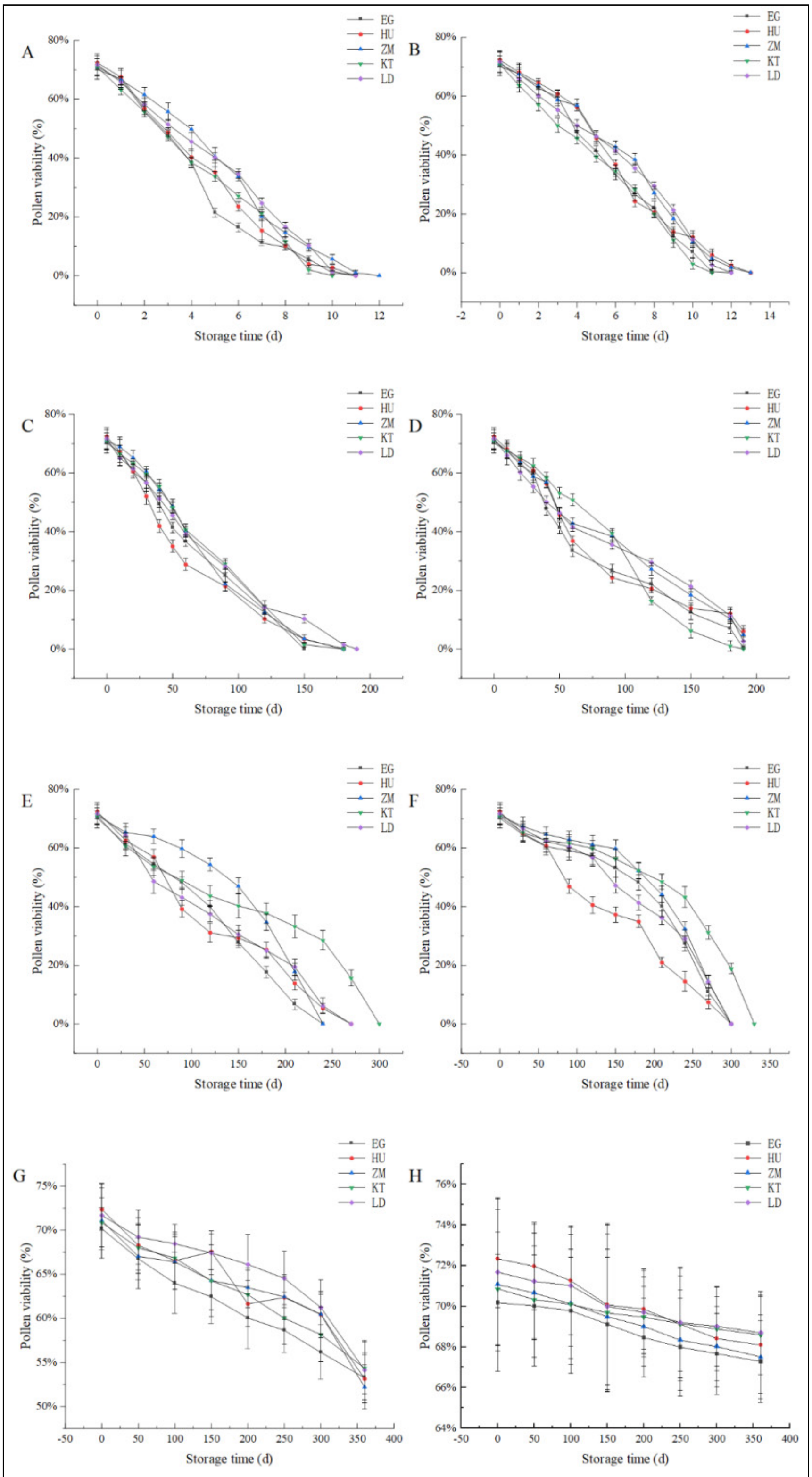

Figure 2 - 'Feng dan' pollen storage from various locations. 25 °C storage (A), 25 °C dry storage (B), 4 °C storage (C), 4 °C dry storage (D), -20 °C storage (E), -20 °C dry storage (F), -74.7 °C storage (G), and -74.7 °C dry storage (H).

inflection point of the decline in pollen vigor, and this capability becomes increasingly pronounced with decreasing temperature.

DISCUSSION

The flowering period is an important phase for observing the climatic phenomenon of peonies, and it varies to some extent among different peony populations at different altitudes or geographical environments. Diverse factors influence the flowering period. First of all, temperature plays a significant role, local temperature changes can profoundly impact the overall blossoming of peonies in the same region. If the blossoming period experiences abnormally warm or cold temperatures, the difference between the early and late blossoming period can be about one week.

Secondly, peony flowering requires reaching a certain cumulative temperature, and this cumulative temperature varies among different cultivated populations (YONG MEI et al., 2017; CUI YING et al., 2017). Therefore, the cumulative temperature can be adjusted through various facilities to increase or decrease the temperature in production practices, aiming to achieve the goal of early or delayed flowering. Another factor related to the flowering period is local rainfall (BAI HONG et al., 2003), and its impact is associated with the amount of rainfall. If the amount of rainfall is low during the peony's flowering period, it does not significantly influence the flowering period; conversely, it enhances colorful blooming. However, in the presence of heavy rainfall, raindrops can exert a strong mechanical destructive effect on the flowers, leading to the disruption of the flower structure and an earlier conclusion to the flowering period.

Altitude changes comprehensively result in corresponding alterations in other environmental factors, such as temperature and light, thereby influencing the flowering period of peonies (BAO et al., 2008). Typically, the flowering period is relatively early at low altitudes and later at high altitudes. Among the five locations in the paper, the onset of flowering of 'Feng dan' in four regions, except EG, was gradually delayed with increasing altitude.

Soluble sugars and proteins, as the primary nutrients of stamens, play a crucial role in pollen viability and serve as vital indicators for assessing the nutritional value of peonies (MENG QIN et al., 2018). The results of this experiment revealed significant variations in the soluble sugar content of pollen from 'Feng dan' at different altitudes, with a maximum difference of 2.7 times. Additionally, the soluble protein content of pollen showed a tendency to decrease with decreasing altitude. This trend may be associated with the temperature difference caused by variations in altitude, as well as the cumulative temperature effect (MAO RUN et al., 2011).

Under low-temperature stress, the metabolic equilibrium between the generation and elimination of free radicals within the cell is disturbed (LI JIN et al., 2016). The cooperative action of protective enzymes facilitates the restoration of free radicals to normal levels, mitigating their damage and safeguarding cells from harsh environments (ALI et al., 2013; SHI et al., 2013). Protective enzyme activities exhibit no consistent pattern within the same stress process. At times, all activities are elevated, while in other instances, some may increase, and others remain unchanged or even decrease (ZHANG et al., 1999; LIU et al., 2005). This study revealed that 'Feng dan' pollen exhibited diverse trends in protective enzyme activity concurrently with variations in pollen genetic background under distinct temperature treatments (JIANHUI et al., 2011). In conjunction with the measurement indices of soluble sugars and soluble proteins, populations cultivated at different altitudes exhibited distinct advantages in physicochemical indices.

Pollen storage is a viable measure to address the significant differences in parental flowering time in crossbreeding. The duration of pollen storage life is influenced by both genetic and environmental factors. In addition to uncontrollable genetic factors, storage temperature and pollen water content emerge as crucial elements in determining pollen longevity among environmental factors (BING LING et al., 2010). A negative correlation exists between pollen moisture content and its storage time within a certain range (JING XING et al., 2001). This correlation arises because low temperatures and low moisture content can reduce the respiration intensity within the pollen (YA ZHEN et al., 2020). In contrast, high temperatures and high moisture content increase the respiration rate, leading to nutrient depletion in pollen (JIA LEI & EN HUI, 2005) and consequently shortening its.

High-altitude populations of the same species typically employ certain strategies to adapt to the challenging environment at high altitude (NEPAL et al., 2023). For instance, high-altitude populations of *Saussurea wellbyi* enhance resource inputs to reproductive organs and decrease the quantity of produced pollen, aiming to enhance pollen quality (WEN MEI et al., 2019). Differences have emerged among 'Feng dan' cultivated populations at different elevations in the paper, and pollen from higher elevations appears to retain activity more effectively at low temperatures. This observation may signify the reproductive adaptation of high-elevation plants to the challenging environment, involving reduced pollen production and improved pollen quality.

CONCLUSION

The flowering period of 'Feng dan' cultivated populations at different altitudes exhibited significant variation, and each demonstrated unique advantages in various physicochemical indexes. Low-temperature drying proved to significantly extend the storage time of peony pollen, and peony pollen from high-altitude regions appeared to be better adapted to low-temperature storage environments. It is recommended that 'Feng dan' cultivation colonies can be established in different altitude zones within the same region for an extended period. Rational cross-pollination among colonies can be facilitated in proximity by leveraging differences in phenology and employing low-temperature drying pollen storage technology, aiming to achieve the goal of high oil peony yield.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest. The funding sponsors played no part in the study's design, data collection, analysis, interpretation, manuscript writing, or the decision to publish the results.

AUTHORS' CONTRIBUTIONS

Xinwei Li wrote the main manuscript. Qi Qiao designed experiments and revised paper. Ting Li, Yuru Shao and Haoang Ma collected test date. Xiaogai Hou provided test materials. All authors critically revised the manuscript and approved of the final version.

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