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Seed Germination of Tree and Herbaceous Peonies: A Mini-Review

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ABSTRACT

Tree and herbaceous peonies are beautiful and vigorously-growing plants commonly employed in the ornamental industry in China and around the world. Germinating peony seeds is a pre-requisite for peony breeding. Much attention has recently been attracted by researchers to the seed germination of peonies for most of them have the habit of double dormancy in hypocotyls and epicotyls. This paper provides a short review of this field. The review covers several themes: 1) the effect of seed collecting time on germination; 2) the effect of different storage conditions on seed germination; 3) physical and chemical pretreatments on seed germination; 4) changes in metabolite content during seed germination; 5) embryo culture of peony seed.

Keywords: epicotyl dormancy; hypocotyl dormancy; *Paeonia*

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INTRODUCTION

In China, peonies symbolize wealth and nobleness due to their large flowers. Many people regard the peony as the "king of flowers" and as China's national flower. It was most favored by noble women during Tang dynasty and enjoyed the supreme position, and it's still honorable after thousands of years. Nowadays, the growing and trading of peonies have become a pillar industry in the middle area of China, such as Shandong and Henan Provinces.

Peony seed (from herbaceous plants or trees) remains dormant for a period of time before its germination because of the double dormancy. It means both the epicotyl and the radicle require chilling, but are released from dormancy at different times. Seeds in this group require a chilling period to relieve radicle dormancy, followed by a warm period to allow the radicle to grow, then a second cold period to release the epicotyl from dormancy. In nature, such seeds require at least two full growing seasons to complete germination. For different species or varieties and different germinating stages, the temperature and its length are variable.

It has reported that the seed germinating temperature of *Paeonia suffrucicosa* 'Feng Dan' varied in three phases (Zheng 1995): 1) After harvesting, the after-ripening of the seed required 40 days for the embryo to fully develop; 2) at 25°C, the radicle began to develop after 30 days (warm rooting stage); 3) at 5°C, the cotyledon began to develop after 60 days (low temperature sprouting stage).

The existence of inhibitors also makes peony seed germination difficult. They are concentrated in the embryo and endosperm. It has been documented that some cotyledon-promoting substances formed after the radicle began to grow (Jing *et al*. 1995). Endogenous ABA is the main inhibitor in peony seed. It reached 157.6 ng/g in dormant seeds and along with seed germinating it declined to 32.2ng/g in one month (Deng 2001; Shi *et al*. 2004).

In this paper, we aim to review the effect of peony seed collecting time, different storage conditions and physical and chemical pretreatments on its germination. The changes of metabolite content in the process of germination will also be discussed.

THE EFFECT OF SEED COLLECTING TIME ON GERMINATION

The maturity stage of peony seeds varies greatly according to the variety and cultivating area. If harvested too early, the seeds can not reach maturity, or if too late, the seed coat turns black and hard, which would reduce germination capacity. Fengxiang Xue, who lived in the Ming Dynasty (1644-

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1911 AD), concluded from practical experiences and wrote in his literature '*MuDan Ba Shu*', that the seeds of tree peony "prefer tender to old…it's the proper harvest time when the seeds become yellow." and "The tender seed will come into bud in one year, the little older need two years, the much older need three years" (Xue 1983). The seeds can not be harvested until the fruit begins to crack and the seeds take on an olive-brown or slightly black appearance. Sowing must be immediately after harvest for the seed will dry quickly. It can also be temporarily (1-2 years) stored in cool, wet sand (moisture content 10%, temperature varies depending on the outside season) and in a ventilated site with 25-50% relative humidity. It was reported that more than 80% seeds of tree peonies in Beijing Botanic Garden harvested before September had decayed a great; seeds collected from the beginning of September to the end of October resulted in a 90% germination rate; seed vigor declined gradually if harvested after November (Jing *et al*. 1995).

THE EFFECT OF ENVIRONMENTAL CONDITIONS ON SEED LIFE AND ITS GERMINATION

In its natural environment, the longevity of peony seed is 3 years, and its vigor apparently weakens along a temporal gradient. Proper storage conditions can extend seed longevity, but cannot keep it from senescing. Zhang *et al*. (2005) treated old tree peony seeds (collected a year earlier) in four ways: dry seeds in a refrigerator (2-4°C); seeds soaked in water in a refrigerator; dry seeds at room temperature (20-25°C); seeds soaked in water at room temperature. They concluded the germination rates were 48%, 55%, 25% and 5%, respectively. The germination rate could be increased by storing in either a wet or dry refrigerator.

Storage conditions and temperature

In most cases, seeds with endogenous physiological dormancy respond in a similar manner with regard to stratification temperature. A temperature near 4°C has maximum effect, while below freezing temperatures or conditions for the radicle and epicotyl (Barton 1944; Baskin and Baskin 1998). The response of the epicotyl to chilling varies with the size of the radicle (Barton and Chandler 1957). For peony, 85% of the epicotyls exposed to 7 weeks of chilling grew if the radicle had reached 4 cm in length. In contrast, only 40% of the epicotyls were released from dormancy under the same conditions with smaller (2-3 cm) radicles.

When the storage temperature range is 10-15°C, the proportion of cultivated peony seeds which retain their vigor for one year is 88%, but it reduced to zero in the fourth year. However, when seeds were placed at -20°C storage temperature, the proportion of seed vigor was 57% until the fourth year (Jing *et al*. 1996). Studies on wild tree peonies (*P. zechuanica*, *P. rockii*) showed that the most favorable temperature for germination was 10-15°C. A warmer temperature, over 20°C, would inhibit the growth of hypocotyls (Jing *et al*. 1996).

In addition, Bewley (1997) observed that 50% of *P. anomala* seeds could survive for up to 3 years and germinate if appropriately stored.

Water and moisture

Water and moisture play important roles in seed germination. The embryo vigor test of P. lutea showed that if the water content of fresh seeds was 63.7%, the germination rate was 100% (He 2001). If seeds were dried naturally in a ventilated or cool site, the water content declined to 35.6% by the third day, but the germination rate could still reach 100%. By the fourth day, the water content decreased to 29.5%, the endosperm became very hard and separated from the embryo. By the seventh day, the water content decreased to 17.1%, the germination rate was 15%. By the eighth day, the water content decreased to 14.1%, the embryo had dried and the seed totally lost its vigor (Gong 1993).

Air humidity is another important factor which effects seed longevity. An excessively wet environment reduces seed longevity. The appropriate relative humidity for germination was suggested to be 30-60% (Yuan 2000; Wang LY 2005; Wang JG 2006).

PHYSICAL AND CHEMICAL PRETREATMENTS ON SEED GERMINATION

In order to improve the germination rate of peony seeds, physical and chemical pretreatments can be made. If the two methods are combined then epicotyl dormancy can be effectively broken.

Physical pretreatments

It has reported that warm water soaking can improve tree and herbaceous peony seed germination. Li *et al*. (2004) studied that after seed soaking under a constant temperature (15°C) embryonic roots grew rapidly but epicotyl dormancy did not break. If the seeds had some roots and were, however treated at 3-5°C for one month, then cultivated at a constant 15°C, then seedlings sprouted immediately.

The hard seed coat of peony is impervious to water and gases which also make germination difficult unless the seed coat is altered physically. Scratching a cross on the seed coat hilum of *Paeonia lactiflora*, then soaking the scraped and intact ones at 25°C for 70 d demonstrated that the germination rate of scraped seeds was 66.7% while that of controls was only 35% (Tao 2005). The paper concluded that herbaceous peony seed coats maybe have some special substance which hindered the inflow of oxygen to the inner part of the seed, resulting in dormancy.

One study indicated that peony seeds stored in sand for 22 d at 4°C had an up to 82.5% germination rate. If cultivated first in the dark for 7 d then in the light for 12 h/d, the germination rate reached the highest level (81.3%), but had no significant difference with that in complete darkness (80.2%). So it was summarized that culture in the dark benefits the germination of peony seeds (Yang *et al*. 2006).

Chemical pretreatments

Some studies revealed that 200 mg/L gibberellic acid (GA_3) could increase the germination rate of *P. suffruticosa* 'FengDan' seed more than low temperature (Zheng *et al*. 1994; Zhou *et al*. 2002). Soaking seeds with a 4 cm radicle in GA_3 (100 ppm) for 24 hours and at a low temperature (2-4°C) for 25 days could eradicate epicotyl dormancy (Zhang *et al*. 2004). Research on the regulation of *P. veitchii* seed germination (Deng 2001; Hu 2002) showed that by soaking in cold water, then treating with gibberellin and taking notice of the control of humidity and temperature, could effectively break the dormancy and increase the germination rate and seedling formation rate.

Concentrated sulfuric acid and ethanol could also break seed dormancy. Treatment of *P. suffruticosa* and *P. lactiflora* seeds with thick sulfuric acid for 3 min, or a soak in 95% ethanol, the germination rates of both plants treated by thick sulfuric acid were higher than ethanol, 94.5% and 68.67%, respectively (Li *et al*. 2004).

Rare-earth (0.15%) has also been used to effectively promote seed germination of some peony varieties (*P. suffruticosa* 'Zhaofen', 'Fengdanbai', 'Erqiao'; Zhang *et al*. 2005).

CHANGES OF METABOLITE CONTENT DURING SEED GERMINATION

In the process of using low temperature to break dormancy, the content of GA_3 in the cotyledon increases remarkably while that of ABA apparently decreases. The different hormones in the endosperm change only a little, and this change in the hypocotyls is irregular.

The results of biochemical analysis of four wild species of tree peonies (*P. rockii*, *P. szechuanica*, *P. spontanea* and *P. delavayi* var. *lutea*) indicated that the content of GA_3 increased and the content of ABA decreased in cotyledons during the breaking of epicotyl dormancy, and the contents of endogenous growth regulators changed only slightly in the endosperm. They suggested that cotyledons might be a key part controlling the epicotyl dormancy in peony seeds (Jing *et al*. 1999).

Jin *et al*. (2006) used herbaceous peony seeds (*Paeoni alactiflora* 'Yingsuzi') as the experimental material to study the changes of the contents of the inner IAA, ABA, GA_{1+3}, and ZR (zeatinriboside) in the dormancy process of seeds by sowing them in the ground and storing them at low temperature (5°C). They concluded that by sowing them in the ground on August 29, 2006, the content of ABA in the seed decreased rapidly when the hypocotyls began to extend, and that the content of GA_{1+3} reached its peak 6.465 nmol/g after three months, then decreased after the extension of the cotyledon. It showed GA_{1+3} had the greatest influence on hypocotyl dormancy breaking. The content of both IAA and ZR showed an increasing tendency. The herbaceous peony seeds could not germinate when stored at a low temperature 5°C. ABA reached its peak at 1352.8 nmol/g after two months but decreased to its lowest concentration at 380.3 nmol/g and then increased again. IAA and ZR in the seeds that were in cold storage were notably lower than in seeds sown in the ground.

Deng (2001) showed that a treatment with exogenous GA_3 could change seed metabolism, decrease the content of endogenous ABA in seeds from 157.6ng/g to 38.5ng/g, and release dormancy. In terms of hormone balance, GA_3/ABA and IAA/ABA ratios were high, and the alteration in concentrations varied widely. In the process of herbaceous peony seed germination, the endogenous hormones and their balance affect the development of seeds.

EMBRYO CULTURE OF PEONY SEED

Seed propagation is often used for breeding new cultivars. But it takes a very long time from sowing to obtaining seedlings, probably one year. Embryo culture of seed is an effective method for improving germination rate and accelerating breeding course.

Studies on the *in vitro* culture of ovules and immature embryos of two tree peony cultivars (*Paeonia rockii* 'Shusheng Pengmo', *Paeonia ostii* 'Fengdanbai') showed that 1.0 $mg{\cdot}L^{-1}$ IAA could accelerate their sprouting. 1.0 $mg{\cdot}L^{-1}$ BAP also made the epicotyl thicker and resulted in more cotyledons, but if it was more than 2.0 $mg{\cdot}L^{-1}$, then germination would be restrained. GA_3 at 0.5-1.0 $mg{\cdot}L^{-1}$ broke epicotyl dormancy and produced more shoots. And a high ratio of IAA/BAP favored rooting and seedling establishment (He *et al*. 2006).

Peony seed embryos were cultured *in vitro* under different light and medium conditions. It was concluded that peony seed embryos germinated very quickly when seeds were stored in sand at 4°C, with a germination rate as high as 82.5%. The best medium for embryo culture was MS + 0.5 $mg{\cdot}L^{-1}$ 6-benzyladenine (BA) + 0.2 $mg{\cdot}L^{-1}$ α-naphthalene acetic acid (NAA), and the optimal medium for subculture of plantlets was MS + 1.0 $mg{\cdot}L^{-1}$ BA + 0.1 $mg{\cdot}L^{-1}$ NAA. MS + 0.5 $mg{\cdot}L^{-1}$ BA + 2.0 $mg{\cdot}L^{-1}$ 2,4-D was optimum for inducing callus, and the medium MS + 0.5 $mg{\cdot}L^{-1}$ IAA was the best for inducing roots from shoots (Yang 2006).

Epicotyl dormancy was broken in cultured peony (*Paeonia lactiflora* Pall.) embryos after topical application of agarose gels containing GA_3, with optimum growth at 1.5 mmol/L GA_3. The addition of 100 μmol/L ABA to the medium resulted in complete inhibition of GA_3-stimulated promotion of dormant epicotyls. Epicotyl dormancy was also broken in embryos by culture on media containing 1 or 10 μmol/L benzylaminopurine, or BAP (Buchheim *et al*. 1994).

CONCLUSION

When treating peony seeds we should pay attention to two aspects: one is the breaking of dormancy in hypocotyls, while promoting the growth of roots. The other is the breaking of dormancy in epicotyls, while promoting seed germination. In order to increase the germination rate of seeds, the two aspects must be carefully considered.

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