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REVIEW

A review of the seed biology of *Paeonia* species (Paeoniaceae), with particular reference to dormancy and germination

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Abstract

Main conclusion **Most *Paeonia* species have epicotyl dormancy. Germination of peony seeds requires warm stratification for embryo growth and radicle protrusion followed by cold stratification for epicotyl growth.**

The genus *Paeonia* (Paeoniaceae) includes many popular ornamentals, has colorful flowers and contains several Chinese medicinal species. The germination protocol for seeds of *Paeonia* species is complex and impedes the breeding of new cultivars and contributes to the rarity and high cost of the plants. Although numerous reports on seed dormancy/germination in peonies are scattered throughout the literature, most of them are in Chinese. The primary aims of this paper are to provide a general overview of the available information on seed dormancy/germination in peonies and to make some suggestions regarding propagation for the peony industry and breeders. Most *Paeonia* species have epicotyl dormancy. The embryo is differentiated into organs, but it is underdeveloped (small) and must grow inside the seed before the radicle can emerge. Germination of peony seeds requires warm stratification for embryo growth and radicle protrusion followed by cold stratification for epicotyl growth. In addition, the epicotyl is sensitive to cold stratification only after the root has grown to a certain length. GA_3 treatment enhances embryo growth and subsequent germination percentages. Further investigations on the physiology, genetics and proteomics would contribute to a better understanding of seed dormancy in *Paeonia*.

Keywords Cold stratification · Endogenous plant hormones · Epicotyl morphophysiological dormancy · *Paeonia* · Seed dormancy/germination · Warm stratification

Introduction

Paeonia, the only genus of the Paeoniaceae and previously included in the Ranunculaceae, has been cultivated in China since the Han dynasty, for more than 2000 years (Liu et al. 1987; Sang et al. 1997; Hong et al. 2017). It is among the most popular garden plants in temperate regions and has been named the "king of flowers" and "flowers of richness and honor" in China (Liu et al. 1987; Sang et al. 1997). The genus is taxonomically complex, consisting of 25–33 species (15 in China including 10 endemic species) (Rogers 1995; Hong et al. 2001) and more than 3000 cultivars (Fig. 1) (Liu et al. 1987). Three sections are recognized within *Paeonia* (Stern 1946): section *Oneapia*, two herbaceous species endemic to Pacific North America; section *Moutan,* six shrub species in central and western China; and section *Paeonia*, about 27 herbaceous species distributed disjunctly in eastern Asia, central Asia, the western Himalayas and the Mediterranean region (Sang et al. 1997). Sections *Oneapia* and *Moutan* contain only diploid species, while one-third of the species in section *Paeonia* are tetraploids (Stern 1946). Hong et al. (2017) reviewed the status of wild peony species in section *Moutan* based on the history of field surveys and investigations. They found that *P. cathayana* and *P. ostii* each survive as only one individual; *P. decomposita*, *P. qiui*, *P. rockii* and *P. rotundiloba* are endangered; and *P. jishanensis* and *P. ludlowii* are vulnerable.

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Fig. 1 Flowers of various cultivars of *Paeonia lactiflora*, *P. ostii* and *P. suffruticosa*. **a** *P. lactiflora* 'Changfengyu'; **b** *P. lactiflora* 'Chunxiao'; **c** *P. lactiflora* 'Fenlanxiuqiu'; **d** *P. lactiflora* 'Foguangzhuying'; **e** *P. lactiflora* 'Furongjinhua'; **f** *P. lactiflora* 'Xiangyangqihua'; **g** *P. ostii* 'Fengdan'; **h** *P. suffruticosa* 'Daojin''; **i** *P. suffruticosa* 'Roufurong' Photographs by Jun Tao

Many peony species have a wide range of uses and thus have been subjected to unsustainable rates of harvest. The root contains bacteriostatic, antipyretic and anticonvulsant agents that have been used in traditional medicines for convulsions and analgesic uses (Liu et al. 1987; Zhang 2003; Hong et al. 2017; Meng et al. 2017). The esthetically desirable flowers have been extensively used as cut flowers and as a teatime delicacy (Liu et al. 1987; Lu 2016; Meng et al. 2017). The seeds contain unsaturated fatty acids, especially α-linolenic acid, which is one of the most promising healthy edible oils (Liu et al. 1987; Zhang 2003; Yang 2009; Meng et al. 2017).

Based on an investigation in southwest China, Cheng et al. (1997) divided reproduction of wild peony species into two categories: facultative vegetative and obligate seed. In the field, *P. delavayi*, *P. lutea*, *P. potaninii*, *P. qiui* and *P. spontanea* reproduce by rhizomes and fleshy storage or tuberous roots, with seeds playing only a supplementary role. On the other hand, *P. decomposita*, *P. lutea* var. *ludlowii*, *P. ostii* and *P. rockii* reproduce in the field only by seeds (Cheng et al. 1997). In horticulture, herbaceous peony cultivars are propagated by root division and sometimes by seed (Meyer 1976). Tree peony cultivars can be propagated by grafting, cutting, division and seeds (Meyer 1976; Liu et al. 1987), but vegetative propagation is more expensive and labor intensive than propagation from seeds. As a result, the demand for plants on the open market, especially for some rare varieties, cannot be met by vegetative propagation (Liu et al. 1987).

Propagation by seeds is an important method for tree peonies and is required to breed new cultivars. This method is always used for producing a supply of rootstocks in grafting for large-scale production of oil-producing tree peonies (Krekler 1962; Liu et al. 1987). However, dormancy in peony seeds typically is complex, requiring sequentially breaking dormancy of root and shoot (Barton 1933; Barton and Chandler 1957; Zhang 2003; He et al. 2008; Lou 2008; Wang 2008; Zhang 2008; Ni 2009; Yang 2009; Yue and Yang 2009; Hao et al. 2014; Yuan and Yu 2014). It usually takes 8–9 months for seeds to germinate under natural conditions, and seedling survival percentage is low (He et al. 2008; Lou 2008; Guo 2016).

Conventional methods of propagation by seeds are time consuming and slow, contributing to the rarity and high cost of the plants (Wister 1962; Liu et al. 1987). Therefore, knowledge of seed dormancy/germination of peony species will be useful for the peony horticultural industry and species conservation. The aims of this paper are to review: (1) characteristics of peony seeds; (2) seed dormancy; (3) methods used to break the dormancy of root and epicotyl; (4) embryo culture; (5) physiological and molecular aspects of seed dormancy/germination; and (6) suggest future research needs for peony seeds. In particular, this review provides English-speaking scientists a detailed overview of research on peonies that has been published in Chinese.

Breeding system in relation to seed dormancy

Many peony species have been reported to be partially self-incompatible (Zhou et al. 1999; Andrieu et al. 2007). No seeds were produced after self-pollination in *P. jishanensis*, while cross-pollination yielded 2.4 seeds per carpel (Zhou et al. 1999). Seeds of *P. broteroi* obtained from self-pollinated flowers had significantly lower mass than those obtained from open- and cross-pollinated flowers (Sanchez-Lafuente and Parra 2009). Artificial pollination of *P. spontanea* significantly increased fruit set and seed germination percentages, especially using pollen from a different population. Germination of seeds of open-pollinated flowers was 0–42.4% and for hand cross-pollinated flowers 44.1–95.8% (Jing and Zheng 1999). Jing and Zheng (1999) concluded that the absence of effective pollination in wild peonies, especially between those in different populations, was an important cause for the high ovule abortion rate, the low fruit set of flowers and the low seed germination percentages of wild peonies.

Characteristics of peony seeds

Seed size and color

Seeds of peonies mature slowly, ripening in late summer and dispersing in autumn, mainly by barochory (Fig. 2) (Liu et al. 1987; Hong et al. 2001). The fruit is a spindle- or oval-shaped follicle of one to eight carpels, and the seeds are black or dark brown and globose or ovoid globose (Hong et al. 2001). Species and cultivars differ in seed number and

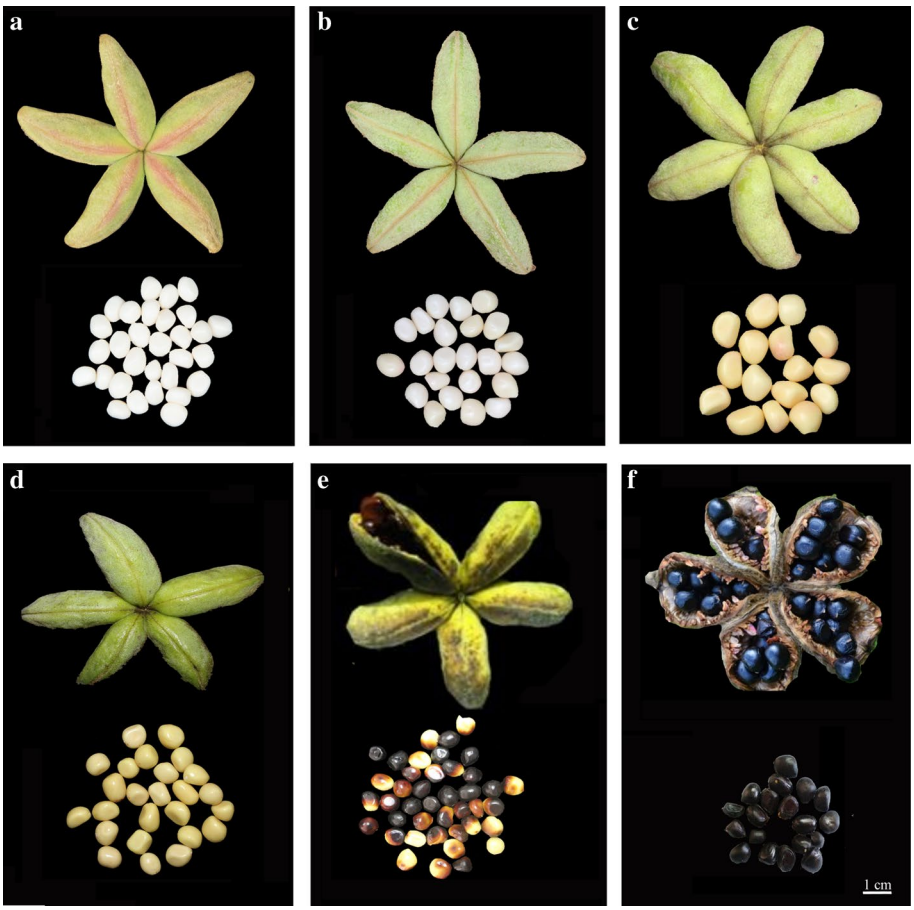

Fig. 2 Morphological and color changes in developing fruits and seeds of *Paeonia ostii* 'Fengdan'. Fruits and seeds in **a**–**f** are 31, 60, 75, 90, 115 and 129 day after pollination, respectively Photographs by Jun Tao

size (Fig. 3). Seeds of *P. spontanea*, *P. rockii* and *P. szechuanica* are smaller than those of the cultivated *P. suffruitcosa*, while seeds of *P. delavayi* var. *lutea* are three times as large as those of the cultivated *P. suffruitcosa* (Jing and Zheng 1999). *P. delavayi* produces two to five follicles, each with nine oval- or ovoid-shaped seeds. Immature seeds of *P. delavayi* are reddish brown, and mature seeds collected in the mountains of western Henan were black with an average seed mass 0.62 g (Ni 2009). Seed length, width and thickness of *P.ludlowii* collected in Yunnan Province were 1.52, 1.18 and 1.13, respectively, and seed mass was 1.64 g (Zhang 2008). Seed mass of *P. anomala* collected in the Altai mountains (Guan et al. 2009) and *P. ostii* 'Fengdan' collected in Tongling country (Qian 2009) was 0.32 g and 0.85 g, respectively. The thousand-seed mass of *P. decomposita* for different wild populations collected in Sichuan Province varied significantly, with an average maximum of 398.94 g and an average minimum of 213.09 g (Yang 2009).

Seed collection (maturation) time

The time of seed collection for peony significantly affects seed germination; collection too early or too late can be detrimental to seed germination (Jing et al. 1995b). The proper time for collecting peony seeds is when the follicle begins to open. At which time the fruits have turned yellow, and the seeds are brown yellow or beginning to turn black (Jing et al. 1995a; Wang 2008). Radicle emergence of *P. ostii* 'Fengdan' and *P. rockii* was > 90% for seeds collected when the follicle began to open in early August (Fig. 2e), and seed moisture content was 30–60% at physiological maturity (i.e., maximum dry mass) (Wang 2008). However, the percentage of radical emergence of *P. ostii* 'Fengdan' and *P. rockii* seeds collected in mid-July when the fruits were green was only 41% and 30.5%, respectively (Wang 2008). Germination of *P. suffruticosa* seeds collected in early September at the Institute of Botany, Chinese Academy of Science in Beijing, was > 90%, while > 80% of the seeds collected in late July and early August decayed when incubated at room temperature. Seed vigor had decreased for seeds collected later than November (Jing et al. 1995b).

Fig. 3 Freshly matured seeds of various cultivars of *Paeonia lactiflora*, *P. ostii* and *P. suffruticosa*. **a** *P. lactiflora* 'Furongjinhua'; **b** *P. lactiflora* 'Hangbaishao'; **c** *P. lactiflora* 'Hongyanzhenghui'; **d** *P. lactiflora* 'Zifengyu'; **e** *P. ostii* 'Fengdan'; **f** *P. suffruticosa* 'Zhihong'

Methods of seed dispersal

Seeds of wild peony (*P. brownii*) in California (USA) are dispersed by seed-caching rodents such as chipmunks, deer mice and pocket mice. These rodents harvest seeds from the dehiscent, pendant pods, transport them short distances (most < 20 m) and cache one or a few seeds 0–15 mm deep in soil (Barga and Vander-Wall 2013). Seed rain density of *P. delavayi* in September was 13–65/m^2, while the seed bank in November was 6–48/m^2; most seeds were removed from the pendant pods by rodents or decay (Li 2013).

Seed storage and longevity

Within a *Paeonia* species, differences in seed size and morphology significantly affect viability and germination percentages. Most seeds of the cultivated tree peonies are plump, lustrous and well-developed, while those of wild species of tree peonies are shrunken and dull (Jing and Zheng, 1999). The viability of freshly matured *P. lutea* (Zhang 2008), *P. ostii* 'Fengdan' (Guo 2016), *P. ostii* 'lishizhenii' (Wang et al. 2002) and *P. rockii* (Guo 2016) seeds was 100%, 96.67%, 85% and 84.67%, respectively. During dry storage, most seeds of wild tree peonies lost viability within 1 year (Jing et al. 1995a). Gong and Wu (1993) used tissue culture to test viability of *P. lutea* seeds, and they found that the moisture content of freshly matured seeds was 63.7% and germination (radicle emergence) 100%. However, when the moisture content decreased to 29.5 and 14.1%, radicle emergence decreased to 85% and 0%, respectively. Air relative humidity (RH) is another important factor that affects seed longevity in *Paeonia,* and storage at an excessively high RH reduces seed longevity. The appropriate RH for storage of most peonies was suggested to be 30–60% (Liu et al. 1987; Yu et al. 2014).

Storage temperature had a significant effect on seed viability. The viability of freshly matured peony seeds was 88%. After storage for 1 and 4 years at 10–15 °C, viability decreased to 25% and 0%, respectively. However, viability during storage for 4 years at − 20 °C decreased only about 50% (Jing et al. 1995b).

Seed dormancy in peonies

Kind of seed dormancy

At seed maturity, the linear-shaped embryos of *Paeonia* species are differentiated but underdeveloped (small), and thus they must grow inside the seed before the radicle emerges (Baskin and Baskin 2014). The embryo length/seed length (E/S) ratio of *P. ostii* 'Fengdan' was 0.33 (Lin 2007) and that of *P. ludlowii* was 0.13 (He et al. 2008). The embryo of *P. corsica* seeds was small and underdeveloped at seed dispersal, and it doubled in length before radicle emergence was possible (Porceddu et al. 2015). If embryo growth and radicle emergence in seeds with underdeveloped embryos require warm (≥ 15 °C) and/or cold (0–10 °C) stratification treatment(s) to germinate (i.e., to break dormancy), they have morphophysiological dormancy (MPD; Baskin and Baskin 2014). Nine kinds of MPD have been identified, based on cold and/or warm stratification requirements for germination, temperature requirements (warm vs. cold) for embryo growth, timing of root and shoot emergence and response to gibberellic acid (GA) (Baskin and Baskin 2014). When freshly matured peony seeds are sown in autumn, the radicle develops into a root system in winter after a period of warm stratification, but the epicotyl (shoot) remains dormant. About 60–90 days at 0–10 °C breaks dormancy of the shoot (Zhang 2003; Yu et al. 2014).

The required temperature and its duration for dormancy break differs among *Paeonia* species (Jing et al. 1995a; Jing et al. 1995b; Zhang 2003; He et al. 2008; Porceddu et al. 2015). If the required dormancy-breaking sequence is out of order, the seeds will not germinate, and thus they remain dormant in the soil seed bank until exposed to the proper sequence of dormancy-breaking conditions (Jin et al. 2006).

Deep simple epicotyl morphophysiological dormancy has been observed in seeds of *P. albiflora* (Nikolaeva et al. 1985), *P. anomala* (Nikolaeva et al. 1985), *P. corsica* (Porceddu et al. 2015), *P. delavayi* (Ni 2009), *P. delavayi* var. *lutea* (Jing and Zheng 1999), *P. intermedia* (Nikolaeva et al. 1985), *P. jishanensis* (Zhang 2003; Wang 2008), *P. lactiflora* (Yang 2009; Yuan and Yu 2014); *P. ludlowii* (Zhang 2008; Ni 2009), *P. lutea* (He et al. 2008; Lou 2008), *P. oreogeton* (Nikolaeva et al. 1985), *P. ostii* (Cheng and Du 2008; Wang et al. 2002; Ren 2016a), *P. ovata* (Nikolaeva et al. 1985), *P. qiui* (Ren 2016a), *P. rockii* (Jing and Zheng 1999), *P. spontanea* (Jing and Zheng 1999), *P. suffruticosa* (Barton 1933; Gao et al. 2008; Lu 2016), *P. szechuanica* (Jing and Zheng 1999), *P. tenuifolia* (Nikolaeva et al. 1985) and *P. wittmanniana* (Nikolaeva et al. 1985). To break this kind of dormancy requires warm stratification for the loss of physiological dormancy (PD) of the root. Then, after PD of the root is broken and grows to a certain length, growth of the epicotyl requires cold stratification. Thus, the root emerges in autumn and the shoot the following spring.

Seeds of *P. officinalis* have intermediate simple MPD (Nikolaeva et al. 1985). These seeds require warm stratification for loss of PD, and this occurs during summer. After PD is broken, embryo growth occurs in autumn at 15–20 °C. However, seeds with fully developed embryos require cold stratification before they can germinate; thus, germination occurs in the field in spring.

The seed coat as a barrier to germination

Several researchers have reported that the hard (but water-permeable) seed coat of peony seeds makes it difficult for the seeds to germinate (Liu et al. 1987; Jing and Zheng 1999; Meng et al. 2017). Immersing seeds in water at 50 °C for 24 h, concentrated H_2SO_4 for 2–3 min or 95% ethanol for 30 min softened the seed coat and promoted germination of *P. suffruticosa* seeds (Liu et al. 1987). The radicle of excised embryos of *P. rockii* elongated in 2 weeks on a MS medium at 20 °C, while whole seeds did not germinate under the same conditions even after 2 months (Jing and Zheng 1999). Tao et al. (2005) also found that mechanical scarification of the seed coat of *P. lactiflora* increased germination (radicle emergence) from 35% to 66.7%. Seedlings of intact seeds of *P. rockii* required 45 days to develop roots at room temperature (10–25 °C), but those with seed coat removed did so in 19 days (Guo 2016).

Since both intact and scarified seeds of peonies can imbibe water, scarification of the seed coat only results in an increased rate (speed) of water absorption for *P. lactiflora*, *P. ostii* and *P. rockii* (Sun et al. 2012; Guo 2016; Meng et al. 2017). Sun et al. (2012) used SEM to study the anatomy of mature *P. lactiflora* seeds, and they found that the outer seed coat is composed of palisade cells. Although the cells are tightly packed, gaps exist between them. They concluded that the seed coat cannot prevent, but can slow, the rate of entry of water and air into the seed. The respiration rate of *P. anomala* var. *intermedia* seeds with the seed coat removed is higher than that of intact seeds. However, with an increase of warm stratification time the respiration rate of both intact and scarified seeds increased (Qiu et al. 2016). Thus, although the seed coat of peonies can impede germination it is permeable to air and water.

Chemicals inhibitors of germination

Peony seeds are reported to contain chemical substances that inhibit germination (Ding 2015; Qiu et al. 2016). Extracts of the seed coat and endosperm of *P. rockii* and *P. decomposita*

significantly decreased germination percentage and seedling growth of *Brassica campestris* (Yang 2009; Zhang et al. 2017). The average root length of *B. campestris* in water, endosperm extracts and seed coat extracts of *P. rockii* was 2.55, 1.26 and 0.68 cm, respectively (Yang 2009). The seed coat extracts of *P. ludlowii* had a stronger inhibitory effect on germination of *B. campestris* than endosperm or embryo extracts, and this effect decreased after the radicle emerged. Therefore, radicle emergence of *P. ludlowii* might be related to a decrease of inhibiting substances in the seed coat (He et al. 2008; Qiu et al. 2016).

Ding (2015) used gas chromatography–mass spectrometry and identified 55 organic compounds, including organic acids, ketones and aldehydes, in seeds of *P. ostii* 'Fengdan'. The most significant inhibitors of germination of *B. campestris* seeds were 1,3-dihydroxyacetone, acetone, furfural, phenol, acetic acid and formic acid. Extracts of *P. ludlowii s*eeds significantly decreased catalase and peroxidase activity of *B. campestris* seedlings, and tests on seeds of *P. ludlowii* of a different population had a similar effect (Zhang et al. 2017).

To decrease the chemical substance that inhibits germination in *Paeonia* species, presoaking seeds in tap water for 1–7 days is recommended (Liu et al. 1987; Zhao et al. 2010; Ma et al. 2015; Guo 2016). However, based on these studies there was no way to know if the inhibitor would prevent embryo growth of nondormant peony seeds from which it was extracted. In many studies, the effects of peony seed inhibitors have been tested on seeds of *Brassica campestris* and not on seeds of peonies.

Endogenous hormones

There is considerable evidence that hormones play important roles in the regulation of seed dormancy and germination. Abscisic acid (ABA) induces dormancy during seed maturation, and gibberellins (GA) play a key role in breaking dormancy and promoting germination (Finch-Savage and Leubner-Metzger 2006). Indole-3-acetic acid (IAA) and indole-3-butyric acid (IBA) promote root initiation and lateral root development, and zeatin riboside (ZR) promotes cell division (Finch-Savage and Leubner-Metzger 2006). Maintaining dormancy also depends on high ABA/GA ratios, and dormancy release involves a net shift to increased GA biosynthesis and ABA degradation resulting in relatively low ABA/GA ratios (Finch-Savage and Leubner-Metzger 2006).

Seeds of *P. ostii* 'Fengdan' reached physiological maturity 104 days after pollination, and ABA level peaked at this time (Zhang 2014). In the field, ABA content of the *P. lactiflora* seeds declined rapidly, while GA_{1+3} (combined content of GA_1 and GA_3) increased when radicles began to grow (Jin et al. 2006). During radicle emergence in *P. ostii* 'Fengdan', GA_3, IAA and ZR increased, while ABA decreased (Qian 2009; Ma et al. 2015). Exogenous GA_3 increased the ratios of GA/ABA and ZR/ABA, which accelerated radicle emergence (Qian 2009). After imbibition was completed, GA_3 content increased by 12% and ABA content decreased by 5.6% compared with freshly matured seeds. IBA content remained stable during imbibition, and it increased significantly with radicle elongation, revealing that IBA played an important role in radicle elongation (Ma et al. 2015).

As seeds of *P. rockii* with emerged roots were being cold stratified to break epicotyl dormancy, there was a substantial increase in GA_3, a decrease in ABA and little or no change in IAA or ZR in the cotyledons. Little or no change occurred in concentrations of GA_3, ABA, IAA or ZR in the embryo axis or endosperm. The authors concluded that the cotyledons played a dominant role in controlling epicotyl dormancy in *P. rockii* (Jing and Zheng 1999).

Methods of breaking dormancy in peony seed

Effects of temperature on breaking root and shoot dormancy

Temperature has a significant effect on the germination of peony seeds. In seeds of most peony species, warm stratification breaks dormancy of the root, while cold stratification breaks dormancy of the epicotyl (Table 1). Seeds of *P. ostii* 'Fengdan' were incubated at 25 °C to break the dormancy of the root and then at 5 °C to break dormancy of the shoot (Lin 2007). Without growth of root of *P. suffruticosa* and *P. lactiflora*, the shoot will not grow regardless of the temperature (i.e., the epicotyl will only emerge after the root emerges to a certain length) (Li et al. 2004). Jin et al. (2006) also found that regardless of how much time passed after *P. lactiflora* seeds were sown at 5 °C, the radicle did not emerge.

As temperature increased from 10 to 25 °C, days to first radicle emergence decreased, and radicle emergence percentage increased in *P. ostii* 'Fengdan', *P. qiui*, *P. rockii* and *P. suffruticosa* (Ren 2016a). The suitable temperature range for germination of wild *P. delavayi* var. *lutea*, *P. ludlowii*, *P. rockii*, *P. spantanea* and *P. szechuanica* seeds is narrower than that for cultivated species (Jing and Zheng 1999; He et al. 2008). Radicle emergence after 3 months warm stratification at 15 °C was 85% for wild *P. ludlowii,* and temperatures higher or lower than 15 °C were not beneficial in breaking dormancy of the root (He et al. 2008). Radicle emergence from seeds of wild *P. delavayi* var. *lutea*, *P. rockii*, *P. spantanea* and *P. szechuanica* at 10–15 °C was 19, 32, 53 and 31%, respectively (Jing and Zheng 1999). Temperatures > 20 °C were unfavorable for seed germination of wild *P. delavayi* var. *lutea*, *P. rockii P. spantanea* and *P. szechuanica*, which led to a much longer time for germination

Table 1 Methods of breaking seed dormancy of *Paeonia* species

Species	Root dormancy break	Root length (cm)[a]	Shoot dormancy break	References[b]
P. corsica	250 mg/L GA_3 + stratification at 25 °C for 90 days	–	250 mg/L GA_3 + stratification at 5 °C for 60 days	Porceddu et al. (2015)
P. delavayi	500 mg/L GA_3 (48 h) + stratification at 15-20 °C for 25 days	3	200 mg/L GA_3 (2 h) + stratification at 4 °C for 20 days	Ni (2009)
P. delavayi var. *lutea*	Stratification at 10–15 °C for 180 days	–	Stratification at 4 °C for 90 days	Jing and Zheng (1999)
P. jishanensis	Stratification at 10–15 °C for 60 days	2	Stratification at 4 °C for 90 days	Zhang (2003)
P. lactiflora	300 mg/L GA_3 (1 h) + stratification at 25 °C for 30 days	3-4	100 mg/L GA_3 (24 h) + stratification at 15 °C for 90 days	Yue and Yang (2009)
P. ludlowii	Presoaking in water for 7 days + 500 mg/L GA_3 (48 h) + stratification at 10–15 °C for 32 days	3	200 mg/L GA_3 (2 h) + stratification at 4 °C for 60 days	Zhang (2008)
P. lutea	Presoaking in 50 °C water for 24 h + 200 mg/L GA_3 (24 h) + stratification at 15 °C for 60 days	3-4	200 mg/L GA_3 (24 h) + stratification at 15 °C for 50 days	Lou (2008)
P. ostii 'Fengdan'	100–200 mg/L GA_3 (24 h) + stratification at 20–25 °C for 40 days	3 ± 1	100–200 mg/L GA_3 (24 h) + stratification at 5 °C for 52 days	Cheng and Du (2008)
P. qiui	300 mg/L GA_3 + stratification at 20 °C for 37 days	3	300 mg/L GA_3 + stratification at 4 °C for 50 days	Ren (2016a)
P. rockii	500 mg/L GA_3 (48 h) + stratification at 25 °C for 100 days	4	300 mg/L GA_3 (1 h) + stratification at 5 °C for 30 days	Ren (2016a)
P. spontanea	Stratification at 10–15 °C for 180 days	–	Stratification at 10–15 °C for 90 days	Jing and Zheng (1999)
P. suffruticosa	300 mg/L GA_3 + stratification at 15–5 °C for 30 days	3	300 mg/L GA_3 + stratification at 4 °C for 40 days	Ni (2009)
P. suffruticosa 'Xiangdan'	Presoaking in water for 30 min + 350 mg/L GA_3 (12 h) + stratification at 15 °C for 25 days	5	400 mg/L GA_3 (6 h) + stratification at 4 °C for 27 days	Lu (2016)
P. szechuanica	Stratification at 10–15 °C for 180 days	–	Stratification at 10–5 °C for 90 days	Jing and Zheng (1999)

– no data

[a]Root length required for epicotyl growth

[b]If different people conducted research on the same species, we chose to cite the one with the best or fastest method of propagation

and an increase in frequency of abnormal seedlings (Jing and Zheng 1999).

If germination conditions do not change after the radicle of peony seeds emerges, the root will grow and produce lateral roots, but the shoot will not grow until after the seeds with an emerged root overwinters (Jing et al. 1995a). Roots of embryos excised from *P. lactiflora* and *P. suffruticosa* seeds and placed in modified Linsmaier–Skoog medium without a plant growth regulator grew to 8–10 cm in length in 6–8 weeks, but a moist cold treatment at 4 °C for 4–6 weeks was required for shoot emergence (Zilis and Meyer 1976). A period of cold stratification broke epicotyl dormancy in seeds of *P. corsica* (Porceddu et al. 2015), *P. delavayi* (Ni 2009), *P. jishanensis* (Zhang 2003; Wang 2008), *P. ludlowii* (Zhang 2008; Ni 2009), *P. ostii* 'Fengdan' (Lin 2007; Cheng and Du 2008), *P. qiui* (Ren 2016a), *P. suffruticosa* (Gao et al. 2008; Ni 2009) and *P. rockii* (Ren 2016a) (Table 1).

Epicotyl dormancy of cultivated *P. suffruticosa* seeds was broken over a temperature range of 4–10 °C, while in seeds of wild *P. delavayi* var. *lutea*, *P. rockii*, *P. spontanea* and *P. szechuanica* it was broken only at 4 °C (Jing and Zheng 1999). Zhao et al. (2010) reported that 4 °C and 7 °C promoted emergence of the shoot of *P. lactiflora*. Seeds with roots emerged moved to 4 °C needed a longer period of cold stratification to break epicotyl dormancy than those moved to 7 °C, but seedling growth rate was higher at 7 °C than that at 4 °C (Zhao et al. 2010). A temperature of 4 °C also broke epicotyl dormancy in *P. ostii* 'Fengdan' and *P. rockii.* With an increase in cold stratification time from 14 to 28 days, epicotyl emergence increased from 26.5 to 100% in *P. ostii* 'Fengdan' and from 36.5 to 100% in *P. rockii* (Wang 2008). Germination of *P. szechuanica* reached > 60% when the duration of the chilling period exceeded 90 days, but it was nearly 0% when duration of chilling was < 60 days (Wang 2008). After 2 months of cold stratification of *P.*

corsica seeds at 5 °C, 92% and 58% of the epicotyls–plumules emerged at 10 and 15 °C, respectively (Porceddu et al. 2015).

Although cold stratification can break epicotyl dormancy of *P. ostii* 'Fengdan', *P. rockii* and *P. qiui* seeds, long periods of cold stratification did not benefit horticultural production of these species of peony. Thus, Ren (2016a) suggested that the best length of cold stratifications should be 30, 40 and 50 days in *P. ostii* 'Fengdan', *P. rockii* and *P. qiui*, respectively. Using these cold stratification times, the time for shoot development was < 20 days, and the percentage of seedlings developing shoots > 60% (Ren 2016a).

Effects of hormones on breaking root and shoot dormancy

GA_3 has been reported to break root dormancy of *P. corsica* (Porceddu et al. 2015), *P. delavayi* (Ni 2009), *P. lactiflora* (Yang 2009; Yue and Yang 2009; Yuan and Yu 2014), *P. ludlowii* (Zhang 2008; Ni 2009; Hao et al. 2014), *P. lutea* (He et al. 2008; Lou 2008), *P. ostii* 'Fengdan' (Lin 2007; Cheng and Du 2008; Ren 2016a), *P. qiui* (Ren 2016a), *P. rockii* (Ren 2016a), *P. suffruticosa* (Krekler 1962; Gao et al. 2008; Ni 2009) and *P. suffruticosa* 'Xiangdan' (Lu 2016) seeds. However, the most suitable concentration of GA_3 for breaking dormancy varies from 0 to 500 mg/L among different species (Table 1). A high concentration of GA_3 increased the time to root emergence and also increased seed mortality (Cheng and Du 2008; Ren 2016a). For *P. ostii* 'Fengdan' and *P. rockii*, 0–200 mg/L GA_3 and 0–300 mg/L GA_3, respectively, increased the speed of root and shoot emergence (Wang 2008). In *P. ostii* 'Fengdan', exogenous GA_3 increased IAA content, decreased ABA content and increased the IAA/ABA, and GA/ABA ratios, thus shortening the time needed for warm stratification (Qian 2009). However, a higher concentration of GA_3 decreased the proportion of roots longer than 40 mm and decreased the number of lateral roots > 10 mm long (Wang 2008; Qian 2009). The optimal concentration of GA_3 for radicle emergence of *P. ostii* 'Fengdan' is 100–200 mg/L (Cheng and Du 2008) and for *P. lactiflora* 300 mg/L (Yang 2009). GA_3 released root dormancy and promoted radicle emergence of *P. lactiflora* (Yuan and Yu 2014), and 100 mg/L GA_3 was optimal when considering both radicle emergence percentage and quality; radicle emergence was as high as 41.33% (Yuan and Yu 2014).

GA_3 also broke epicotyl dormancy in seeds of *P. corsica* (Porceddu et al. 2015), *P. delavayi* (Ni 2009), *P. lactiflora* (Yang 2009; Yue and Yang 2009; Yuan and Yu 2014), *P. ludlowii* (Zhang 2008; Ni 2009; Hao et al. 2014), *P. lutea* (He et al. 2008; Lou 2008), *P. ostii* 'Fengdan' (Lin 2007; Cheng and Du 2008; Ren 2016a), *P. qiui* (Ren 2016a), *P. rockii* (Ren 2016a), *P. suffruticosa* (Krekler 1962; Gao et al. 2008; Ni 2009) and *P. suffruticosa* 'Xiangdan' (Lu 2016). However, the most suitable concentration of GA_3 for breaking epicotyl dormancy also varies among species. Epicotyl dormancy of *P. delavayi* var. *lutea* seeds collected in Yunnan and Tibet Provinces, *P. rockii* seeds collected in Gansu, Shananxi and Hubei Provinces, *P. spontanea* seeds collected in Shanxi Province and *P. szechuanica* seeds collected in Sichuan Province was broken by 100 mg/L GA_3 (Jing and Zheng 1999). GA_3 at 300 mg/L was the most suitable concentration for breaking epicotyl dormancy of *P. ostii* 'Fengdan' collected in Tongling, Anhui Province (Lin 2007). However, 200 mg/L GA_3 was the most suitable concentration for breaking epicotyl dormancy of *P. ostii* 'Fengdan' collected in Luoyang city, Henan province (Gao et al. 2008). GA_3 at 500 mg/L was optimal for epicotyl emergence and seedling growth of *P. lactiflora* 'Zhu Sha Pan' if it was applied when root length was < 3 cm; seed germination was as high as 96.67% with this treatment (Yuan and Yu 2014). There is also a report of no effect of GA_3 on seeds of *P. szechuanica* (Wang 2008). The only way to break the epicotyl dormancy of *P. szechuanica* is by exposing seeds with an emerged radicle to 4 ± 2 °C for ≥ 90 days (Wang 2008). The reason for this discrepancy between the results of the two studies on the dormancy-breaking effect of GA_3 on seeds of *P. szechuanica* is not known.

Root length requirements for breaking epicotyl dormancy

The shoots of seeds with epicotyl dormancy are sensitive to cold stratification only after the root has elongated (Baskin and Baskin 2014). Many researchers have found that epicotyl dormancy of cultivated *P. suffruticosa* seeds cannot be broken until the root length is ≥ 3 cm (Krekler 1962; Gao et al. 2008; Ni 2009). Barton (1933) found that epicotyl dormancy could be broken by exposing *P. suffruticosa* seeds with emerged roots 4–5 cm long to temperatures ranging from 1 to 10 °C. Eighty-five percent of *P. suffruticosa* seedlings with roots 4–5 cm long produced emergent cotyledons after 7 weeks of cold stratification at 5 °C, whereas only 40% of those with roots 2–3 cm long produced emergent cotyledons (Barton and Chandler 1957).

The suitable root length for breaking epicotyl dormancy varies among species of peonies. For epicotyl dormancy break, root length needs to be 2 cm for *P. jishanensis* (Zhang 2003; Wang 2008); 3 cm for *P. delavayi* (Ni 2009), *P. qiui* (Ren 2016a) and *P. suffruticosa* (Krekler 1962; Gao et al. 2008; Ni 2009); 4 cm for *P. ostii* 'Fengdan' (Lin 2007) and *P. rockii* (Ren 2016a); 5 cm for *suffruticosa* 'Xiangdan' (Lu 2016); and 6 cm for *P. ludlowii* (Hao et al. 2014) and *P. lutea* (He et al. 2008; Lou 2008). Cotyledon emergence of *P. suffruticosa* 'Xiangdan' was 100% when seeds with 5 cm long

roots were cold stratified at 4 °C and treated with 100 mg/L GA_3 for 27 days (Lu 2016).

The different root lengths for breaking epicotyl dormancy seem to depend on the hormone content in the epicotyl of radicle-emerged seeds. Hao et al. (2014) showed that the GA_3 content of *P. lutea* seeds with a root length of 6 cm was significantly higher than that of seeds with root lengths of 1.5, 3.0 and 4.5 cm, but the ABA content was lowest for seeds with root lengths of 1.5, 3.0 and 4.5 cm. The authors conclude that the underlying reason for root length affecting epicotyl dormancy release is the difference in the GA_3/ABA ratio in the epicotyl of radicle-emerged seeds, which is mainly the result of the difference in ABA accumulation before cold stratification (Hao et al. 2014).

Effects of radiation and magnetism on seed germination

Much research has been done on the effects of ^{60}Co-γ ray radiation on peony seeds in relation to germination (Wang 2008; Li et al. 2010). Li et al. (2010) used ^{60}Co-γ to radiate *P. lutea* seeds and found that with an increase in radiation dose radicle and epicotyl emergence percentages decreased. Compared with the control, 100 gray (Gy) of radiation prolonged the root emergence from 45 to 71 days and shoot emergence from 102 to 155 days. However, a low dose of ^{60}Co-γ ray radiation increased germination of *P. suffruticosa* (Li 2014). For example, 8.76 Gy of radiation increased root emergence from 14.3 to 32.7%, but 26.28 Gy decreased root emergence to 10.33%.

For wild and cultivated seeds of *P. lactiflora* exposed to a 3000 gauss magnetic field for 1.5 h, 72.8% and 43.3%, respectively, of the radicles and 62.2% and 5.6%, respectively, of the shoots emerged (Jian et al. 2009). For wild and cultivated seeds of *P. lactiflora* not exposed to a magnetic field, 63.4% and 7.8%, respectively, of the radicles and 11% and 0%, respectively, of the shoots emerged. The authors concluded that a proper magnetic field can break dormancy and promote germination of *P. lactiflora* seeds.

Embryo culture of peony seeds

Embryo culture is an effective way to accelerate germination of peony. Both GA_3 and cold stratification at 4 °C have been found to be useful in breaking epicotyl dormancy of seeds of *P. lactiflora* during embryo culture (Stanys et al. 2007). Cold stratification at 2 °C for 30–40 days broke epicotyl dormancy of radicle-emerged seeds of *P. lactiflora* (Meyer 1976). Torpedo-shaped embryos of *P. lactiflora* that were 1.8 mm in length produced two times more roots in MS medium than in White medium (Stanys et al. 2007). Radicle and epicotyl emergence of mature embryo explants were best for *P. ostii* in MS + 1.0 mg/L 6-BA + 1.0 mg/L GA_3 (Liu et al. 2015), while 1/2 MS + 1.0 mg/L IAA + 1.0 mg/L GA_3 was best for *P. rockii* (Wang et al. 2012).

Physiological aspects of seed dormancy and germination

For seeds of *P. ostii* 'Fengdan' incubated at 25 °C for 100 days, starch, soluble sugars and ABA content decreased; soluble protein content increased; and GA_3 content initially increased then decreased (Qian 2009). For seeds of *P. ostii* 'Fengdan' incubated at 4 °C, starch content remained stable, but the trends of soluble sugar, soluble protein and GA_3 content were similar to those at 25 °C (Qian 2009). When the seed coat ruptured, GA_3 and ABA contents were significantly higher and lower, respectively, at 25 °C than at 4 °C (Qian 2009; Ren 2016b).

Cold stratification of *P. ostii* 'Fengdan' and *P. suffruticosa* seeds with an emerged root resulted in a large increase in alanine, globulin and glutamine, first in the endosperm and then in the embryo (Fine and Barton 1958). The increase in glutamine during cold stratification may indicate that this compound is involved in breaking epicotyl dormancy, but it did not play a role in the morphological differentiation of the shoot since its increase occurred at warm temperatures (Fine and Barton 1958; Lin 2007).

Total protein content decreased in the endosperm and increased in the embryo of root-emerged seeds of *P. suffruticosa* incubated at 5 °C but not when they were incubated in a heated greenhouse (Barton and Bray 1967). At 5 °C, the amount of all amino acids and amides, except aspartic, asparagine and arginine, increased in the endosperm, and alanine, histidine, and glutamine increased in the embryo (Barton and Bray 1967). Thus, it appears that nitrogen metabolism is involved in breaking epicotyl dormancy after the root emerges. However, exogenous application of glutamine did not promote shoot emergence of *P. suffruticosa* (Barton and Bray 1967).

Ren (2016b) studied proteomics of seed germination of *P. ostii* 'Fengdan'. For freshly matured seeds incubated at 25 °C and 4 °C for 100 days, 39 and 27 specific proteins were found at 25 and 4 °C, respectively. Further analysis showed that many proteins appeared when the radicle ruptured the seed coat at 25 °C, and most of them were involved in carbohydrate metabolism. However, a large number of stress-related proteins appeared at 4 °C. In addition, a GA synthesis-associated protein and an ABA-induced protein kinase were found at 25 and 4 °C, respectively (Ren 2016b). The results suggested that 4 °C delayed the oxidation pathway and inhibited radicle emergence of *P. ostii* 'Fengdan'. Furthermore, expression of the ABA-related gene *PobZIP* decreased, while

the GA-related genes *PoGAMYB4*, *PoGID1*a and *PoGAI* increased (Ren 2016b). Therefore, ABA can inhibit radicle emergence by regulating the expression of *PobZIP*, and GA can promote radicle emergence by regulating the expression of *PoGAMYB4*, *PoGID1a* and *PoGAI*. Ma et al. (2017) used transcriptome analysis to identify genes related to seed dormancy and germination in *P. lactiflora* by comparing seeds incubated for 0 and 40 days. They found that 1794 genes were differentially expressed in the functional enrichment analysis. The key genes for seed germination and dormancy, *GAI1* and *ARF*, were upregulated in seeds incubated for 40 days.

Future research needs

The results of numerous studies indicate that epicotyl morphophysiological dormancy is prevalent in peonies (Fig. 4). Although previous studies provided information on methods of breaking dormancy in peony seeds, several important aspects of the seed germination biology have been overlooked, such as seed aging and the maintenance of a soil seed bank. Further research on these aspects will provide additional insights into the germination and conservation of wild peony species.

Previous studies provided information on seed dormancy and germination of a single peony species collected in a single place, but little is known about variation in dormancy-breaking requirements between populations. Species in

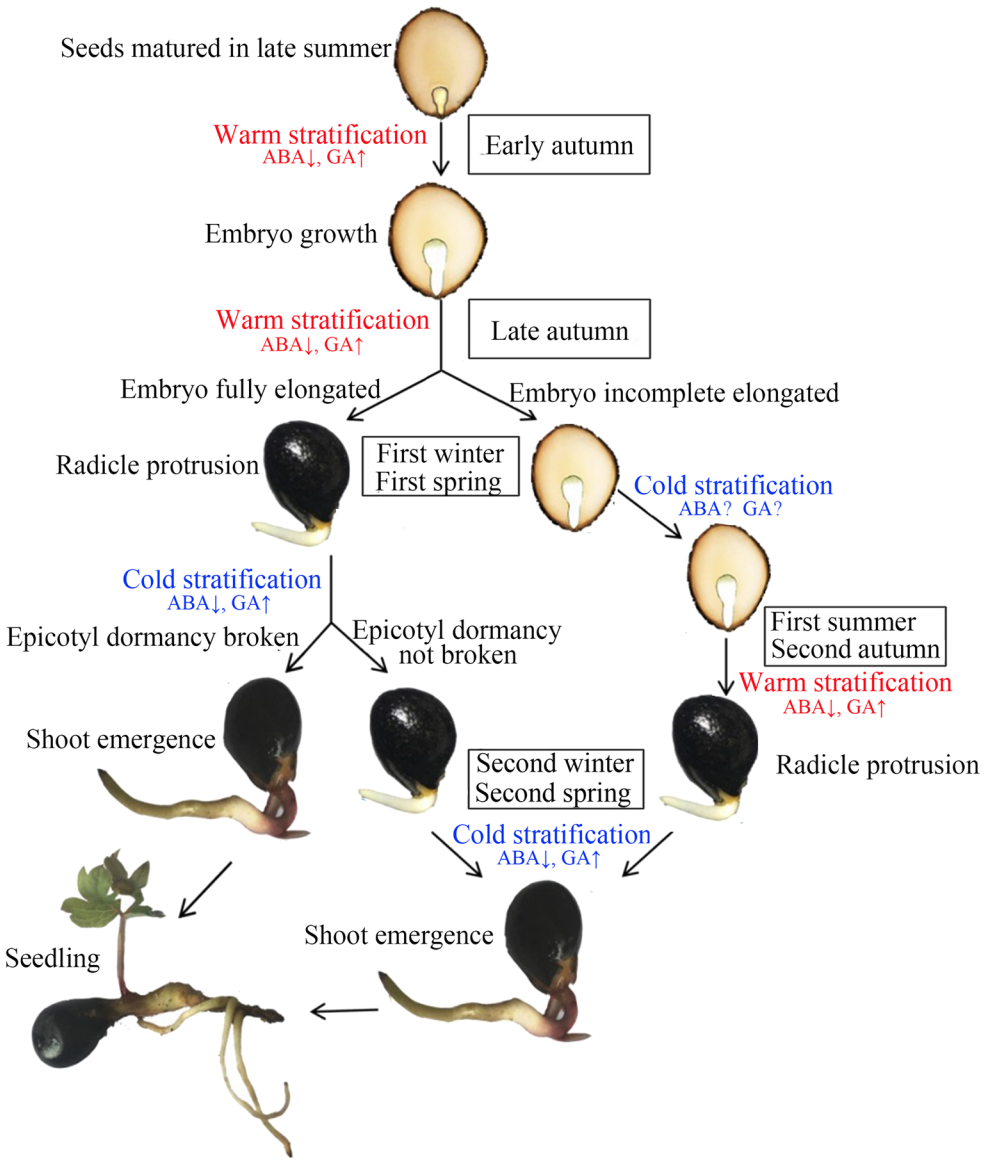

Fig. 4 A conceptual model of dormancy breaking in seeds of *Paeonia*

section *Paeonia* are distributed disjunctly in Asia and the Mediterranean region. Has any divergence developed?

Numerous studies have demonstrated that peony seeds contain chemical substances that inhibit germination (Yang 2009; Ding 2015; Qiu et al. 2016; Zhang et al. 2017). However, the extracts of peony seeds were tested on seeds of *Brassica campestris*. From these studies, there is no way to know if the inhibitor would prevent embryo growth of nondormant peony seeds from which it was extracted. We suggest that the effects of peony seed extracts need to be tested on nondormant peony seeds.

The embryos of *Paeonia* species are differentiated into organs, but they are underdeveloped (small) and must grow inside the seed before the radicle can emerge. However, neither the ontogeny of the embryo nor acquisition of epicotyl dormancy has been studied previously. It is necessary to combine additional physiological, genetic and proteomic tools (e.g., expression profiling technology and immunohistochemistry) to explain the ontogeny of the underdeveloped embryo and mechanisms underlying this special kind of dormancy.

Barton and Chandler (1957) found that although the epicotyl differentiated in *P. suffruticosa* in root-emerged seeds, a shoot was not produced, i.e., dormancy of the epicotyl was not broken. Although the shoot remains inside the seed all winter, little is known about its growth and morphological/histological development prior to emergence in spring. Investigations also would benefit greatly from the application of molecular biology techniques to identify the genes responsible for the two-step process of germination. Although a period of warm + cold stratification provides an important signal for dormancy release for seeds, the related detailed molecular mechanisms remain unclear. Why do root and shoot need different temperatures to break physiological dormancy (warm + cold is effective but cold + warm is not) from the physiological and molecule perspectives?

Empirical data are also needed to understand the selective pressures and ecological factors favoring the evolution of epicotyl dormancy. The delay of shoot emergence in epicotyl dormancy until spring was suggested to be related to intolerance of shoots to temperatures below freezing, but this has not been tested (Baskin and Baskin 2014).

Paeonia is an economically important plant genus. Emergence of seedlings depends on a range of environmental factors, of which water and temperature are the most important (Baskin and Baskin 2014). The hydrothermal time model has been used to quantify the breaking of physiological dormancy using after-ripening in several species. However, this concept has not been used for the explanation of stepwise epicotyl dormancy-breaking processes. A hydrothermal time model is needed to provide an accurate description the two-step process of *Paeonia* seed germination to dormancy-breaking factors; such information will provide a useful reference for propagation for the peony industry and breeders.

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Compliance with ethical standards

Conflict of interest The authors declare no conflicts of interest.

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