Postprint: Dormancy Type and Germination of Epimedium sagittatum Seeds

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Abstract

Epimedium sagittatum possesses significant medicinal value and substantial market demand; however, the dormancy and germination characteristics of its seeds remain insufficiently understood, severely impacting commercial-scale seedling production and cultivation. To elucidate the seed dormancy type and identify the optimal dormancy-breaking method, this study utilized mature seeds of Epimedium sagittatum as experimental material to investigate seed water absorption, desiccation tolerance, and the effects of temperature, stratification, and plant hormones on seed dormancy and germination. The results demonstrated that Epimedium sagittatum seeds: (1) lack physical dormancy; (2) exhibit desiccation tolerance; (3) show zero germination at 4–25 ºC under dark conditions, indicating dormancy characteristics; (4) have a very small embryo/seed ratio, with stratification at 4 ºC, 10 ºC, and variable temperatures significantly promoting embryo growth and development and increasing the rate and percentage of germination; and (5) respond to gibberellin and fluridone with significant increases in germination rate and percentage. In conclusion, the dormancy type of Epimedium sagittatum seeds is morphophysiological dormancy, and the optimal method for dormancy release and germination promotion is stratification at 10 ºC for 30 days followed by germination at 4 ºC.

Full Text

Dormancy Type and Germination of *Epimedium sagittatum* Seeds

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Abstract

Epimedium sagittatum possesses important medicinal value and substantial market demand, yet the characteristics of seed dormancy and germination remain poorly understood, severely impacting its industrial-scale seedling production and cultivation. To determine the seed dormancy type and optimal methods for breaking dormancy, we investigated water uptake and desiccation tolerance in mature *E. sagittatum* seeds, as well as the effects of temperature, stratification, and phytohormones on seed dormancy and germination. The results demonstrated that *E. sagittatum* seeds: (1) lack physical dormancy; (2) exhibit desiccation tolerance; (3) show zero germination at 4–25 ºC under dark conditions, indicating dormancy characteristics; (4) have a very small embryo-to-seed ratio, with stratification at 4 ºC, 10 ºC, and fluctuating temperatures significantly promoting embryo growth and development while increasing germination rate and percentage; and (5) respond to gibberellin and fluridone with significantly increased germination rate and percentage. We conclude that *E. sagittatum* seeds exhibit morphophysiological dormancy, and the optimal method for releasing dormancy and promoting germination is stratification at 10 ºC for 30 days followed by germination at 4 ºC.

Keywords: dehydration tolerance, *Epimedium sagittatum* seed, germination, morphophysiological dormancy, stratification

Introduction

Seed dormancy is a mechanism whereby viable seeds temporarily cannot germinate under favorable environmental conditions, playing a critical role in the survival and reproduction of seed plants (Gao & Ayele, 2014). Research has established that seed dormancy represents an important component of plant environmental adaptation (Donohue et al., 2010; Huang et al., 2010). High levels of seed dormancy delay germination and cause uneven emergence, shortening the growing season and reducing growth uniformity. Conversely, low dormancy may lead to germination before the favorable growing season begins, risking seedling mortality (Donohue et al., 2010) and thereby reducing crop yield and quality.

Dormancy is a complex trait determined primarily by genetic and environmental factors, with diversity and ubiquity existing among plant species (Baskin & Baskin, 2014). Seed dormancy can be classified into physiological dormancy (PD), morphological dormancy (MD), morphophysiological dormancy (MPD), physical dormancy, and combinational dormancy (Baskin & Baskin, 2004, 2014;

Iwasaki et al., 2022). PD occurs in seeds with fully developed and mature embryos, primarily caused by the presence of inhibitors in seed components and/or lack of plant growth substances (Chen et al., 2010). PD is the most common dormancy form, which can be divided into deep, intermediate, and shallow PD, and is widespread in gymnosperms and all major angiosperm groups (Baskin & Baskin, 2004, 2014; Finch-Savage & Leubner-Metzger, 2006). Seeds with MD have small (underdeveloped) but differentiated embryos; germination does not require pretreatment to release dormancy but needs extended time for the embryo to grow to sufficient volume before germination. MPD seeds have both underdeveloped embryos and physiological dormancy, requiring pretreatment to release dormancy. In MPD seeds, embryo growth/radicle protrusion through the seed coat takes much longer than in MD seeds.

Plant hormones abscisic acid (ABA) and gibberellin (GA) participate in controlling seed dormancy and germination (Weitbrecht et al., 2011; Iwasaki et al., 2022). Dong et al. (2012) found that fluridone (an ABA biosynthesis inhibitor) and GA_3 significantly reduced thermodormancy in lettuce seeds, while diniconazole (an inhibitor of ABA catabolic enzyme ABA 8' -hydroxylase) and paclobutrazol (a GA biosynthesis inhibitor) increased thermodormancy. In reducing lettuce seed thermodormancy, paclobutrazol clearly antagonized fluridone, while fluridone significantly enhanced GA_3 effects. Particularly, the balance between ABA and GA levels and their respective signaling pathways play important roles in regulating dormancy induction and maintenance as well as germination promotion (Finkelstein et al., 2008; Graeber et al., 2012; Song et al., 2020a, b). Additionally, seed dormancy can be released by environmental factors such as after-ripening, temperature fluctuation, cold/warm stratification, and alternating light, depending on the species (Baskin & Baskin, 2004, 2014; Black et al., 2006; Finch-Savage & Leubner-Metzger, 2006).

Epimedium L. plants are perennial herbs in the Berberidaceae family, comprising approximately 55 species worldwide, with 45 species in China. *Epimedium brevicornum*, *Epimedium sagittatum*, *Epimedium pubescens*, *Epimedium koreanum*, and *Epimedium wushanense* are included in the *Pharmacopoeia of the People' s Republic of China* (National Pharmacopoeia Committee, 2020). *Epimedium* is a commonly used traditional Chinese medicine first recorded in *Shennong Bencaojing*, with functions of tonifying kidney yang and dispelling wind-dampness. Clinically, it is also used to treat osteoporosis, menopausal syndrome, breast lumps, hypertension, and coronary heart disease, and possesses immune-enhancing, anti-aging, anti-tumor, and anti-HIV effects. Research shows that *Epimedium* contains various physiologically active components, mainly including flavonoids, alkaloids, polysaccharides, lignans, and essential trace elements (Kang et al., 2012; Li et al., 2020). Due to difficult seed germination, *Epimedium* cultivation has traditionally relied on digging wild plants for division planting, resulting in low propagation coefficients and severe damage to wild resources. In recent years, with increasing market demand, wild *Epimedium* resources have dwindled sharply, approaching depletion. Therefore, using seeds for seedling propagation has become a critical issue that must be

resolved for industrial cultivation.

Studies have shown that when *Epimedium* fruits mature, the embryo is underdeveloped and requires a period of cold stratification or warm-cold stratification to germinate, exhibiting clear dormancy (Fan et al., 2010; Fu et al., 2012; Tian et al., 2015; Su et al., 2016; Rhie & Lee, 2020). GA_3 treatment promotes embryo growth in *E. wushanense* (Su et al., 2016) and *E. koreanum* (Rhie & Lee, 2020), while fluridone treatment significantly increases germination percentage in *E. wushanense* seeds (Su et al., 2016). Tian et al. (2015) preliminarily studied germination characteristics of *E. sagittatum* seeds using conventional germination tests and direct measurement and paraffin sectioning techniques. To date, the dormancy and germination characteristics of *E. sagittatum* seeds remain insufficiently clear. This study used mature *E. sagittatum* seeds to investigate water uptake, desiccation tolerance, and the effects of temperature, stratification, and phytohormones on seed germination, aiming to clarify the dormancy type and identify suitable methods for dormancy release and germination promotion to provide references for industrial seedling production.

Materials and Methods

1.1 Experimental Materials

Mature *Epimedium sagittatum* fruits were collected in June 2020 from Badagong Mountain in Zhangjiajie (29°77 N, 110°07 E, altitude 1,302 m). The collection site has an average annual temperature below 9.9 °C, with the coldest month (January) averaging 3.5–4.3 °C and the hottest month (July) averaging 26.3–28 °C. Annual rainfall is 2,300 mm, with spring and summer (April–June) accounting for approximately 48% of precipitation. After collection, fruits were naturally dried at $(25\$\pm3)°C and(50\pm5)\pm\$0.49)\%$. Some seeds were used for desiccation tolerance analysis, while others were stored at 4 °C for future use.

Maize (*Zea mays* L. 'Zhengdan 958') and rice (*Oryza sativa* L. 'japonica') seeds were provided by the Germplasm Resources Laboratory, Agricultural Biological Gene Center, Guangdong Academy of Agricultural Sciences.

1.2 Desiccation Tolerance Analysis

Seeds with 9.7% moisture content were placed in mesh screens positioned over desiccators containing dry silica gel. After dehydration for 0, 2, 4, 8, 12, 24, and 36 hours, some seeds were used for moisture content determination, while others were stratified at 4 °C for 60 days and then germinated at 10 °C under dark conditions.

1.3 Moisture Content Determination

Seed moisture content was determined following the International Seed Testing Association method (International Seed Testing Association, 1999) and expressed as a percentage of fresh weight.

1.4 Seed Water Uptake

Epimedium sagittatum, maize, and rice seeds were sown in petri dishes lined with two layers of filter paper, supplied with 7 mL distilled water, and allowed to imbibe at 25 °C in darkness for various durations before measuring seed moisture content, expressed as a percentage of fresh weight.

1.5 Stratification Treatment

The stratification substrate was perlite. Perlite was first rinsed with tap water to remove dust, sterilized at 121 °C for 30 minutes, cooled, placed in self-sealing bags with appropriate sterile water, and left at room temperature for 20 minutes before removing excess water with a pipette. *Epimedium sagittatum* seeds with 9.7% moisture content were thoroughly mixed with moist perlite (V/V = 1:10) in self-sealing bags and stratified at 4 °C, 10 °C, (4+10) °C, (10+4) °C, (4+10+4) °C under dark conditions. After various stratification time combinations, samples were taken. Some seeds were observed for embryo morphological changes using an OLYMPUS (SZ61) stereomicroscope, photographed, and the embryo-to-seed ratio (embryo length/seed length) was calculated. Other seeds were subjected to germination tests to determine germination rate and percentage.

1.6 Seed Germination

Seeds subjected to different treatments were sterilized with 0.1% (V/V) NaClO for 15 minutes, sown in petri dishes lined with two layers of filter paper, supplied with 6 mL distilled water or different concentrations of phytohormone GA_3 or fluridone, and germinated under specified temperatures and dark conditions. Radicle protrusion of 2 mm was used as the germination criterion.

1.7 Statistical Analysis

All data were analyzed using the one-way ANOVA model in SPSS 20.0 software. Significance of mean differences was determined using Student-Newman-Keuls test (S-N-K, $P = 0.05$).

Results

2.1 Seed Water Uptake

To determine whether seeds possess physical dormancy, freshly harvested *E. sagittatum*, maize, and rice seeds were imbibed at 25 °C in darkness for 0–72 hours. *E. sagittatum* seeds exhibited two-phase water uptake: Phase I (0–4 h) rapid water absorption and Phase II slow water absorption until reaching a plateau (4–72 h) [Figure 1: see original paper]A. During Phase I, seed moisture content increased from 9.7% to 52%; during Phase II, it increased from 52% to 56.7%. Maize and rice seeds showed three-phase water uptake: Phase I rapid absorption, Phase II slow absorption, and Phase III rapid absorption after germination [Figure 1: see original paper]A.

No *E. sagittatum* seed germination was observed within 72 hours of imbibition, whereas maize and rice seeds gradually germinated with water uptake [Figure 1: see original paper]B. Maize and rice seeds reached 10% germination at 22 and 33 hours, respectively, and 50% germination at 34 and 42 hours. At 72 hours, germination percentages were 97% and 93% for maize and rice, respectively [Figure 1: see original paper]B.

2.2 Effect of Desiccation on Seed Germination

To investigate desiccation tolerance, *E. sagittatum* seeds with 9.7% moisture content (fresh weight basis) were further dehydrated on dry silica gel surfaces for various durations. Initially, seed moisture content decreased rapidly, then slowly. For example, after 2 hours of dehydration, moisture content decreased by 18.9%; from 8 to 12 hours, it decreased by only 4.2% [Figure 2: see original paper]A.

When moisture content decreased from 9.7% to 7.3% and 5.7% after 4 and 8 hours of dehydration, seed germination percentage (after 4 °C stratification for 60 days) increased. With further dehydration, germination percentage decreased slightly but remained higher than the control without significant differences [Figure 2: see original paper]B. Dehydration accelerated germination rate; for instance, non-dehydrated seeds began germinating at day 155, while dehydrated seeds began at day 105 (data not shown).

2.3 Effect of Temperature on Seed Germination

To identify suitable germination temperatures, freshly collected dry seeds were imbibed at 4, 10, 15, 20, and 25 °C under dark conditions for 30 days, showing zero germination (data not shown). However, at 4 °C and 10 °C, seeds began germinating at 115 and 145 days, respectively, with germination percentages increasing over time to 55% and 44% at 240 days [Figure 3: see original paper]A. At 15, 20, and 25 °C, germination percentages were only 2%, 0%, and 0% at 240 days, respectively. Seeds could partially germinate at 4 °C and 10 °C, with

significantly higher germination rate and percentage at 4 °C [Figure 3: see original paper]A.

To further determine optimal germination temperature, seeds stratified at 10 °C for 60 days then transferred to 4 °C for 30 days were germinated at different temperatures under dark conditions. Germination percentages were 88%, 85%, 3%, 2%, and 0% at 4, 10, 15, 20, and 25 °C, respectively, confirming 4 °C and 10 °C as suitable germination temperatures. Initially, germination rate was slightly earlier at 10 °C, but with extended germination time, both rate and percentage were higher at 4 °C [Figure 3: see original paper]B.

2.4 Effect of Stratification on Embryo Growth and Seed Germination

Stratification promotes embryo growth. When *E. sagittatum* seeds detached from pods, embryos were small and spherical, with an embryo-to-seed (E/S) ratio of only 0.07 [Figure 4: see original paper]A and B. With increasing stratification time at 4 °C and 10 °C, embryos gradually developed from spherical to heart-shaped, torpedo-shaped, and mature embryos, with continuously increasing volume [Figure 4: see original paper].

During stratification at 4 °C, embryo growth was slow from 0–60 days (small E/S values), reaching an E/S ratio of 0.09 at 60 days. Embryos grew gradually from days 61–90, reaching an E/S ratio of 0.14 at 90 days. After 90 days, embryos grew rapidly, achieving an E/S ratio of 0.88 at 165 days [Figure 4: see original paper]C.

During stratification at 10 °C, embryo growth increased gradually, reaching an E/S ratio of 0.8 at 165 days [Figure 4: see original paper]D. The E/S ratio of seeds stratified at 10 °C was significantly greater than those at 4 °C. For example, after 60 days stratification at 10 °C, the E/S ratio (0.24) was 167% higher than at 4 °C (0.09) [Figure 4: see original paper]D.

When seeds stratified at 4 °C for 30 days were transferred to 10 °C, embryo growth rate was faster than continuous 4 °C stratification. For instance, seeds transferred from 4 °C (30 days) to 10 °C (60 days) had an E/S ratio (0.25) approximately 74% higher than seeds stratified only at 4 °C for 90 days (0.144). Seeds transferred from 4 °C (60 days) to 10 °C showed similar E/S ratios to continuous 4 °C stratification initially, but ratios increased with extended 10 °C stratification [Figure 4: see original paper]C. When seeds stratified at 10 °C for 30 or 60 days were transferred to 4 °C, their E/S ratios were significantly greater than continuously 10 °C-stratified seeds. For example, continuously 10 °C-stratified seeds for 90 days had an E/S ratio of 0.4, while seeds stratified at 10 °C for 30 days then 4 °C for 60 days reached 0.83, and those stratified at 10 °C for 60 days then 4 °C for 30 days reached 0.67 [Figure 4: see original paper]D.

Seeds stratified at 4 °C for 30 days then 10 °C for 90 days, or at 4 °C for 60 days then 10 °C for 60 days, showed similar embryo growth (E/S) trends. Growth was slightly faster in the former from days 75–135, with no significant differences

from days 136–165. At 165 days, both E/S ratios exceeded 0.8 [Figure 4: see original paper]E.

Stratification promotes seed germination. Seeds stratified at 4 °C for 30 or 60 days and germinated at 10 °C in darkness showed significantly increased germination rate and percentage with extended stratification time [Figure 5: see original paper]A. Similarly, seeds stratified at 10 °C for 30 or 60 days and germinated at 4 °C in darkness also showed significantly increased germination rate and percentage, with similar effects for 30- and 60-day stratification [Figure 5: see original paper]B.

Notably, seeds stratified at 10 °C for 30 or 60 days and germinated at 4 °C had much higher germination rates and percentages than seeds stratified at 4 °C for 30 or 60 days and germinated at 10 °C [Figure 5: see original paper]A and B. For example, seeds stratified at 4 °C for 30 and 60 days began germinating at 125 and 90 days at 10 °C, with 22.5% and 67% germination at 150 days, respectively. In contrast, seeds stratified at 10 °C for 30 and 60 days began germinating at 45 days at 4 °C, with 93% and 96% germination at 150 days, respectively. Seeds stratified at 4 °C for 60 days required 135 days to reach 50% germination at 10 °C, whereas seeds stratified at 10 °C for 30 days needed only 60 days to reach 50% germination at 4 °C [Figure 5: see original paper]A and B.

Both alternating temperature stratification treatments (4 °C for 30 days + 10 °C for 90 days, or 4 °C for 60 days + 10 °C for 60 days) significantly increased germination rate and percentage at 4 °C, with 50% germination reached at 70 days [Figure 5: see original paper]C. Compared to seeds stratified at 10 °C for 30 or 60 days then germinated at 4 °C, germination rate and percentage were slightly reduced but remained much higher than seeds stratified at 4 °C for 30 or 60 days then germinated at 10 °C [Figure 5: see original paper].

2.5 Promotional Effects of Gibberellin and Fluridone on Seed Germination

To understand *E. sagittatum* seed germination responses to phytohormones GA_3 and fluridone, seeds were first stratified at 10 °C in darkness for 120 days to allow embryo maturation, then germinated in different concentrations of GA_3, fluridone, and GA_3 + fluridone. Results showed that 1 and 10 $\mu mol \cdot L^{-1}$ GA_3 had no effect on germination, while 100 $\mu mol \cdot L^{-1}$ to 10 $mmol \cdot L^{-1}$ GA_3 significantly promoted germination, though 10 $mmol \cdot L^{-1}$ GA_3 was less effective than 1 $mmol \cdot L^{-1}$, and germination percentage remained below 70% [Figure 6: see original paper]A. Fluridone at 5–500 $\mu mol \cdot L^{-1}$ significantly promoted germination, with percentages exceeding 90%, representing increases of over 46% compared to the control [Figure 6: see original paper]B.

When 10 or 100 $\mu mol \cdot L^{-1}$ GA_3 was combined with 10 or 100 $\mu mol \cdot L^{-1}$ fluridone, both germination rate (data not shown) and percentage were much higher than with GA_3 alone, reaching approximately 95% germination [Figure 6: see original paper]C.

Discussion

3.1 Water Uptake in *Epimedium sagittatum* Seeds

Seed germination begins with water uptake and ends when the radicle breaks through the seed coat (Bewley et al., 2013). *E. sagittatum* seeds showed two-phase water uptake: rapid absorption (Phase I) and slow absorption (Phase II), lacking Phase III [Figure 1: see original paper]A. During Phase I, moisture content rapidly increased to 52%, indicating that the seed coat is permeable and lacks physical dormancy. Phase I water uptake is driven by matrix potential because dry seeds have very low water potential (Obroucheva & Antipova, 1997). The slow water absorption during Phase II until 72 hours (or longer, data not shown) represents a lag phase. These results are similar to water uptake in *E. wushanense* seeds (Fan et al., 2010). Phase III water uptake is caused by seed germination; within 72 hours, *E. sagittatum* seed germination was zero, thus no Phase III was observed. At 25 °C, maize and rice seeds showed Phase I rapid uptake, brief Phase II slow uptake, and Phase III rapid uptake [Figure 1: see original paper]A because germination percentage increased with water uptake, reaching 50% germination at 34 and 42 hours, respectively [Figure 1: see original paper]B.

3.2 Effect of Desiccation on Seed Germination

Maturation drying is the terminal event in orthodox seed development and plays a critical role in plant seed (germplasm) resource conservation (Song et al., 2022). *E. sagittatum* seed moisture content decreased from 9.7% to 4.6%, yet germination percentage remained at 56.3% after 4 °C stratification for 60 days [Figure 2: see original paper], indicating desiccation tolerance. Notably, germination percentages below 60% were not due to desiccation damage but because 60 days of 4 °C stratification could not completely release dormancy [FIGURE:3-5]. These findings provide a basis for drying and long-term conservation of *Epimedium* germplasm resources.

3.3 Effect of Temperature on Seed Germination

Temperature is one of the most important environmental factors affecting seed germination, determining germination capacity and rate and breaking primary and/or secondary dormancy (Brándel, 2004; Baskin & Baskin, 2014). *E. sagittatum* seeds imbibed at 4, 10, 15, 20, and 25 °C in darkness for 30 days showed zero germination, indicating dormancy characteristics. However, at 4 °C and 10 °C, seeds began germinating at 115 and 145 days, reaching 55% and 44% germination at 240 days, respectively. At 15, 20, and 25 °C, germination percentages were only 2%, 0%, and 0% at 240 days [Figure 3: see original paper]A, indicating deep dormancy and significant temperature effects, with 4–10 °C being the suitable germination temperature range. Su et al. (2016) reported that

E. wushanense seeds could only germinate at low temperature (4 °C), not at fluctuating temperatures (10 °C/20 °C).

Seeds stratified at 10 °C for 60 days then transferred to 4 °C for 30 days showed germination percentages of 88%, 85%, 3%, 2%, and 0% at 4, 10, 15, 20, and 25 °C, respectively [Figure 2: see original paper]B, also confirming 4–10 °C as the suitable germination temperature range [Figure 3: see original paper]B.

3.4 Effect of Stratification on Embryo Growth and Seed Germination

Graeber et al. (2012) proposed that dormancy is a quantitative trait whose depth changes during development. Primary dormancy forms during seed maturation, reaching maximum levels at physiological maturity, then gradually decreasing during subsequent dry storage (after-ripening) or stratification (Finch-Savage & Leubner-Metzger, 2006; Holdsworth et al., 2008). When *E. sagittatum* seeds detached, embryos were small (E/S = 0.07). With increasing stratification time at 4 °C and/or 10 °C, embryos gradually developed from spherical to heart-shaped, torpedo-shaped, and mature embryos (E/S > 0.8) [Figure 4: see original paper], indicating morphological dormancy and progressive embryo development during stratification. Tian et al. (2015) observed similar results, finding that *E. sagittatum* embryos were undifferentiated and remained at the spherical stage, with structures gradually developing into cotyledonary embryos during after-ripening. *E. koreanum* (Wang et al., 2013) and *E. wushanense* (Fan et al., 2010) seeds share similar characteristics, requiring stratification to promote embryo growth. These results align with phenological patterns of embryo growth, germination, and seedling emergence in *E. koreanum* under field conditions, where embryos were underdeveloped and grew little from June to early September, but grew rapidly from late September to November, completing growth by early December with a 16-fold length increase, followed by 84.4% germination in March of the next year (Rhie & Lee, 2020).

When seeds stratified at 4 °C for 30 days were transferred to 10 °C, embryo growth rate was faster than continuous 4 °C stratification [Figure 4: see original paper]C. Seeds stratified at 10 °C had significantly greater E/S ratios than those at 4 °C [Figure 4: see original paper]C and D, indicating that 10 °C stratification favors embryo development. Furthermore, seeds stratified at 10 °C for 30 or 60 days then transferred to 4 °C had significantly greater E/S ratios than continuously 10 °C-stratified seeds [Figure 4: see original paper]D, suggesting that certain growth-inhibiting substances may exist in the embryo and/or endosperm that are more effectively released at 4 °C than at 10 °C, favoring embryo growth. The identity and mechanism of these inhibitory substances require further investigation. [Figure 4: see original paper]E also shows that alternating temperature stratification benefits *E. sagittatum* embryo development. Rhie and Lee (2020) found that *E. koreanum* embryos grew minimally when cultured at constant 20 °C or 15 °C.

At 10 °C germination temperature, *E. sagittatum* germination rate and percent-

age increased significantly with extended 4 °C stratification time [Figure 5: see original paper]A. Similarly, at 4 °C germination temperature, germination rate and percentage increased significantly with extended 10 °C stratification time [Figure 5: see original paper]B. However, seeds stratified at 10 °C for 30 or 60 days then germinated at 4 °C had much higher germination rates and percentages than seeds stratified at 4 °C for 30 or 60 days then germinated at 10 °C [Figure 5: see original paper]A and B. These germination results parallel the effects of stratification temperature on E/S ratios [Figure 4: see original paper]. Fu et al. (2012) reported that *E. pseudowushanense* seeds treated at 15 °C for 1–7 months then transferred to 4 °C showed significantly increased embryo growth and germination percentage, though germination rate decreased with extended 15 °C treatment. Alternating temperature stratification of 4 °C for 30 days + 10 °C for 90 days or 4 °C for 60 days + 10 °C for 60 days both significantly increased germination rate and percentage at 4 °C, similar to their effects on E/S ratios [Figure 4: see original paper]E.

3.5 Promotional Effects of Gibberellin and Fluridone on Seed Germination

ABA is a positive regulator of dormancy induction and maintenance and a negative regulator of germination, while GA can release dormancy, promote germination, and antagonize ABA effects. Their levels and signal transduction play important roles in regulating dormancy release and germination (Hauvermale et al., 2012; Song et al., 2020a, b; Iwasaki et al., 2022). For *E. sagittatum* seeds stratified at 10 °C in darkness for 120 days (embryo matured), 1 and 10 $\mathrm{mol \cdot L^{-1}}$ GA_3 had no effect, while 100 $\mathrm{mol \cdot L^{-1}}$ to 10 $\mathrm{mmol \cdot L^{-1}}$ GA_3 significantly promoted germination, though percentages remained below 70% [Figure 6: see original paper]A, indicating partial GA_3 promotion. Fluridone at 5–500 $\mathrm{mol \cdot L^{-1}}$ significantly promoted germination, with percentages exceeding 90% [Figure 6: see original paper]B. Additionally, 10 or 100 $\mathrm{mol \cdot L^{-1}}$ fluridone had additive effects with GA_3 on germination [Figure 6: see original paper]C. Su et al. (2016) used *E. wushanense* seeds stratified at fluctuating temperatures (10 °C/20 °C) for 90 days to study fluridone and gibberellin effects on dormancy release, finding stronger fluridone effects and weaker GA effects, with fluridone promoting germination of seeds that could not germinate at fluctuating temperatures. Rhie and Lee (2020) also observed that GA_3 treatment increased embryo growth in *E. koreanum* seeds but had minor promotional effects on germination (<10%). These results suggest that ABA may play an important role in inhibiting *E. sagittatum* seed germination.

Epimedium sagittatum seeds have permeable seed coats without physical dormancy, exhibit desiccation tolerance for storage at low moisture and temperature, show zero germination at 4–25 °C in darkness, have very small E/S ratios, and respond to 4 °C, 10 °C, and alternating temperature stratification with significantly promoted embryo development and increased germination rate and percentage. GA_3 and fluridone significantly increase germination rate and

percentage. Based on Baskin and Baskin (2004, 2014) and Finch-Savage and Leubner-Metzger (2006), we conclude that *E. sagittatum* seeds exhibit morpho-physiological dormancy, and the optimal method for dormancy release and germination promotion is stratification at 10 °C for 30 days followed by germination at 4 °C.

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Note: Figure translations are in progress. See original paper for figures.