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Low-temperature stratification strategies and growth regulators for rapid induction of *Paris polyphylla* var. *yunnanensis* seed germination

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Abstract

The seeds of *Paris polyphylla* var. *yunnanensis* are deeply dormant, and they remain dormant for 18 months or longer in their natural environment. Periodic exposure of the seeds to a low-temperature of 4 °C broke the dormancy in about 16 weeks (112 days). The most effective temperature stratification scheme was an interval of 14 days at 4 °C and 14 days at 22 °C. Both GA_3 and ethephon significantly enhanced the germination rate during the stratification treatment. The seed coat, particularly the mesophyll outer layer of the seed coat, strongly inhibited the germination. With removal of the seed coat and exposure of the uncoated seeds to 600 mg/l GA_3 for 48 h before the temperature stratification of 14 days at 4 °C and 14 days at 22 °C for 112 days, a germination percentage as high as 95.3% of the seeds was attained in about 160 days.

Abbreviations: ABA – abscisic acid; 6-BA – 6-benzyladenine; GA_3 – gibberellic acid; KT – kinetin

Introduction

Herb-Paris, *Paris polyphylla* Smith var. *yunnanensis* (Franch.) Hand.-Mazz., is a perennial plant belonging to the Trilliaceae family, which is native to the southwest of China, Yunnan Province (Li 1984). The rhizomes of herb-Paris is used in traditional Chinese medicine as a hemostasis, anti-microbial and anti-inflammatory agent for hundreds of years (Song et al. 2001). However, the natural resources of herb-Paris are nearly destroyed due to aggressive harvesting of the herb over the last few decades. In order to reserve the natural resources and to retain a stable and renewable source of herb-Paris for the medicinal industry, domestication and mass cultivation of the herb is imperative (Tang et al. 1998). A basic requirement for mass cultivation is to produce large amounts of seedlings in a short time. Although tissue culture and micropropagation of herb-Paris are possible, seed propagation appears to be more promising and cost-effective for mass production of seedlings. However, the major problems in the propagation of herb-Paris seeds are the long period of seed dormancy and the low rate of seed germination. In the natural environment, herb-Paris seeds have a dormancy period of 18 months or even longer than 2 years and a germination percentage of about 40% (Li 1984).

P. polyphylla var. *yunnanensis* seeds consist of a mesophyll outer layer coat, an inner hardy coat, a large endosperm, and a small undeveloped embryo (Yang 1998). In the natural environment, herb-Paris seeds are released from mature capsules between September (early autumn) and November (early winter). In the next 4 months, the embryos of mature seeds develop into hypocotyl, radicle and cotyledon. This morphological after-ripening process is com-

pleted at the end of the winter season. The seeds will not germinate until after the next winter. This suggests that physiological after-ripening, which may involve endogenous hormone biosynthesis at the low temperature during the second winter season, is needed for the germination of herb-Paris seeds. The local temperature in the winter season has a low of 5 °C and a high of 15 °C on average, and the average local temperature in the summer season is 20–25 °C. It appears that herb-Paris seeds require at least two treatments of low-temperature stratification to complete their morphological and physiological after-ripening.

The main objective of this study was to explore temperature stratification strategies and suitable phytohormones for rapid release of seed dormancy and enhanced seed germination of herb-Paris. The effect of the seed coat on seed dormancy and germination was also assessed.

Materials and methods

Plant material

Mature seeds of *P. polyphylla* var. *yunnanensis* were harvested from plants growing in Kunming, Yunnan Province, China, in October 2000. The seeds were dried after removal of the mesophyll outer layer of seed coat. Each 1000 of the dry seeds weighed about 6 g.

Germination

The mesophyll outer layer of the seed coat was removed in all the germination experiments except in that for testing the effect of seed coat on germination. Seed germination experiments were conducted in temperature-controlled incubators. Each batch of 100 seeds was placed on sands moistened with distilled water in a 9-cm petri dish, which was wrapped with aluminum foil to keep out light. Our preliminary experiments had shown that light had inhibitory effects on herb-Paris seed germination. During the experiments, water was added when needed to keep the substrate moist. Each treatment had five replicates and the percentage germination (%) is the mean from the five replicates of 100 seeds each. A protrusion of 1 mm by the radicle was the criterion for germination. All operations on the seeds during the germination experiments including water addition and germination counting were performed under dim white light.

Table 1 Temperature stratification schemes applied to herb-Paris seeds (overall period: 112 days).

No.	Temperature interval
Scheme 1 (Control)	22 °C for 112 d
Scheme 2	4 °C for 112 d
Scheme 3	4 °C for 56 d → 22 °C for 56 d
Scheme 4	(4 °C for 28 d → 22 °C for 28 d)×2
Scheme 5	(4 °C for 14 d → 22 °C for 14 d)×4
Scheme 6	(4 °C for 7 d → 22 °C for 7 d)×8

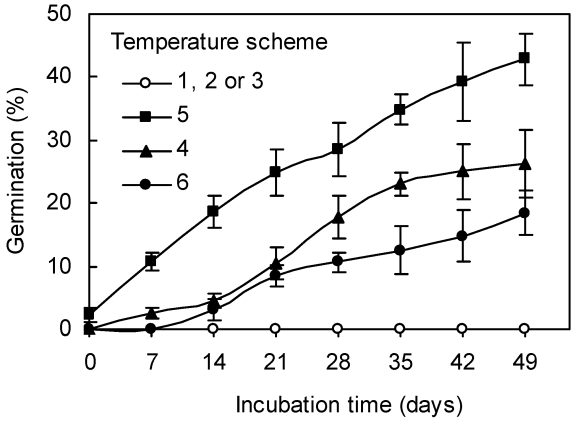

Figure 1. Germination percentage of seeds incubated at 22 °C after treatment for 112 days with various temperature schemes as shown in Table 1.

Simulated seasonal temperature treatment

With a view to partially mimic the seasonal temperature conditions for herb-Paris seeds in the natural environment, we applied different schemes of periodic temperature change from a period of low temperature at 4 °C to a period of high temperature at 22 °C for an overall period of 112 days or 16 weeks (Table 1). The mesophyll outer layer of the seed coat was removed and the seeds soaked in water at 22 °C for 48 h prior to the temperature stratification treatment. After the treatment, the seeds were incubated at 22 °C in darkness for another 49 days (7 weeks) during which the germination counts were taken once a week. Therefore, the overall incubation period (23 weeks) was close to 6 months.

Treatment with growth regulators

Four growth regulators were tested, including gibberellic acid (GA_3), kinetin (KT), 6-benzyladenine (6-BA) and the ethylene-generating compound, ethephon, which were all dissolved in water at selected concentrations before use. After the removal of the mesophyll outer layer of the seed coat, the seeds were soaked in the growth regulator solutions at 22 °C for 48 h. After growth regulator treatment, the seeds were incubated under temperature Scheme 5 in Table 1 for 16 weeks, and then incubated at 22 °C constant temperature for 7 weeks.

Results

Effect of temperature

Figure 1 shows the time courses of germination of herb-Paris seeds after various temperature treatments. Three of the temperature schemes tested, 4, 5 and 6, relieved the seeds from dormancy after 16–18 weeks treatment. Scheme 5, i.e., periodic change of temperature from 4 °C for 14 days to 22 °C for 14 days over 112 days, was the most effective to break the seed dormancy, leading to 42.8% germination after 49 days (7 weeks) further incubation at 22 °C. Treatment of the seeds with a constant low temperature at 4 °C for 56 days (Scheme 3) or 112 days (Scheme 2) did not break the dormancy of herb-Paris seeds.

As temperature Scheme 5, i.e., 14 days at 4 °C and 14 days at 22 °C, was shown to be most effective to break the seed dormancy, it was applied to all the following experiments for testing the effects of growth regulators and seed coat.

Effect of plant growth regulators

Among the four growth regulators tested, GA_3 at concentrations of 100–600 mg/l had the most dramatic effect on the seed germination of herb-Paris (Figure 2). The percentage germination of seeds treated with GA_3 at 400–600 mg/l was about 95%, more than double that of untreated seeds. The ethylene-generating agent, ethephon, at 2000–3000 mg/l also improved the germination percentage significantly, values being 50–70% higher than those of the control, although it was not as effective as GA_3. The other two growth regulators, KT and 6-BA, had only slight but significant stimulating effects at a concentration of 400 mg/l or higher.

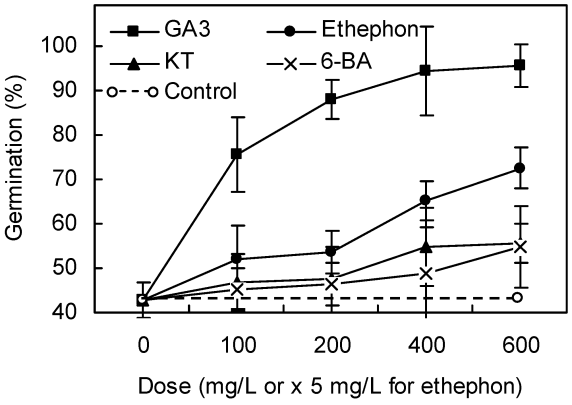

Figure 2. Effect of plant growth regulators on germination (seeds treated with temperature Scheme 5 in Table 1 and then incubated at 22 °C for 49 days).

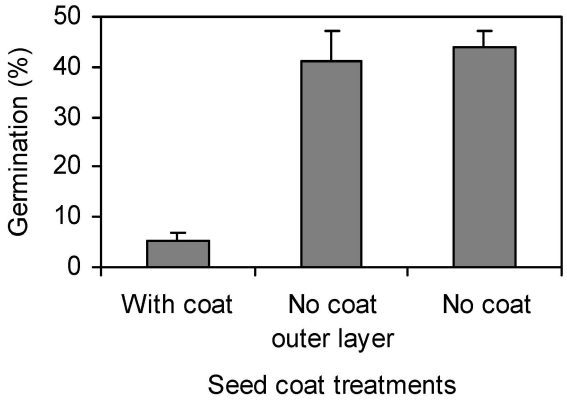

Figure 3. Effect of the seed coat on germination (seeds treated with temperature Scheme 5 in Table 1 and then incubated at 22 °C for 49 days).

Effect of seed coat

In order to test the effect of the seed coat on seed germination, the seed coat was treated in three different ways, reserving the seed coat, removing the mesophyll outer layer, and removing both the outer layer and inner layer of the coat. These seeds were subjected to the same temperature treatment of Scheme 5 for 112 days, and then incubated at 22 °C for 49 days when the germination percentage was determined (Figure 3). The seeds with coat had a low

germination percentage of 5.2%, while the seeds without the mesophyll outer layer coat had a germination of 41.3%. Removal of the inner layer of the seed coat (uncoated seeds) had only slight or negligible effect on the germination (43.9% *versus* 41.3% for seeds with the inner layer). The results show that the seed coat, particularly the mesophyll outer layer, plays an important role in preserving seed dormancy and inhibiting seed germination of herb-Paris.

Discussion

The experimental results have shown that herb-Paris seeds require at least two exposures to low-temperature to break dormancy in a period of 4–6 months, which is a quarter to a third of the normal dormancy period. The temperature change at a frequency of 14 days at 4 °C and 14 days at 22 °C (Scheme 5) has been found to be a more effective stratification scheme than those with less or more frequent temperature change (Scheme 4 or 6) for breaking the seed dormancy. The requirement of a minimum of two low-temperature incubation periods to break the seed dormancy is perhaps a reflection of the dormancy-release process of herb-Paris seeds in the natural environment, which passes through two winters, allowing for germination to occur in spring (Li 1984). Temperature is one of the most important environmental factors regulating seed dormancy and germination of most plant species (Probert 2000). Low-temperature is required to break the seed dormancy of summer annuals and most temperate perennials, which represents a physiological mechanism in the plant seeds to ensure the germination to occur in spring or early summer.

The maintenance and release of seed dormancy is controlled by a balance between inhibitor and promoter hormones, such as the balance between ABA and GA_3 (Ross 1984; Taiz and Zeiger 2002). The hormonal balance can be influenced by changes in environmental conditions, such as chilling and exposure to light, which may trigger the activation of metabolism that leads to seed germination. For example, the content of GA_3 increased and that of ABA decreased in cotyledons during the release of seed dormancy of wild tree peonies (Jing and Zheng 1999). In our present study, plant growth regulators such as GA_3 and ethylene effectively stimulated the germination of herb-Paris seeds during a low-temperature stratification treatment. However, treatment of the seeds with growth regulators alone without the temperature stratification did not break seed dormancy (data not shown). This indicates that temperature stratification is essential for breaking the dormancy of herb-Paris seeds.

The strong inhibitory effect of the seed coat on seed germination may be caused by several possible mechanisms, including mechanical constraint, prevention of water- and oxygen-uptake, and retention or production of chemical inhibitors (Taiz and Zeiger 2002). Since the inhibitory effect of the seed coat on germination is mainly caused by the mesophyll outer layer which is relatively soft and easy to break, but not by the inner hardy layer, mechanical constraint is unlikely a major cause of the seed dormancy of herb-Paris. Further investigation is needed to identify the responsible inhibition mechanisms, permeability and/or chemical inhibition by the mesophyll outer layer. In addition, the long dormancy of herb-Paris seeds in the natural environment may also be partially attributed to embryo dormancy.

In conclusion, the present work has established an effective strategy for breaking seed dormancy and enhancing seed germination of the medicinal herb *P. polyphylla* var. *yunnanensis*, which is mainly through low-temperature stratification and application of growth regulators. Much more work is needed to investigate the causes for the long seed dormancy of this medicinal plant. Particularly, studies on the hormonal balance in the seeds such as that between ABA and GA_3 are deemed important.

Acknowledgements

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