Postprint: Study on Seed Germination Characteristics of Meconopsis horridula, a Tibetan Medicinal Herb

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Abstract

Meconopsis horridula is an annual herbaceous plant belonging to the genus Meconopsis in the family Papaveraceae. It is an alpine plant with high ornamental and medicinal value and is currently endangered. Investigating the germination characteristics of Meconopsis horridula seeds is of great significance for seedling propagation and artificial cultivation. This study used Meconopsis horridula seeds as experimental material to examine the effects of different disinfectants, soaking durations, temperatures, and exogenous plant hormones on seed germination characteristics. The results showed: (1) The optimal disinfection method was 75% ethanol for 1 min + 3% H2O2 for 5 min, the optimal soaking time was 24 h, and the optimal temperature and light condition was 20 ℃/10 ℃ (12 h light/12 h dark). The germination rate of seeds soaked in sterile water was 49.67%. (2) GA3 at 100–600 mg · L ^{1} and NAA at 5–30 mg · L ^{1} increased the germination rate, germination potential, and germination index, shortened the germination initiation time and duration, and promoted seed germination. (3) 6-BA at 5 mg · L ^{1} and 10 mg · L ^{1} had a certain promoting effect on seed germination, but this was not significant; 6-BA concentrations 15 mg · L ^{1} inhibited seed germination. (4) Seeds soaked in GA3 at 500 mg · L ^{1} exhibited the best germination indices, with germination rate, germination potential, and germination index of 69.67%, 33.00%, and 4.51, respectively. The germination initiation time and duration were 10.67 d and 11.67 d, respectively.

Full Text

Seed Germination Characteristics of the Tibetan Medicinal Plant *Meconopsis horridula* Hook. f. et Thoms.

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Abstract

Meconopsis horridula Hook. f. et Thoms. is an endangered annual herb in the family Papaveraceae. This rare alpine plant possesses significant ornamental and medicinal value, and its wild populations are currently threatened. Investigating the germination characteristics of *M. horridula* seeds is crucial for developing effective seed propagation and cultivation techniques. This study examined the effects of different disinfectants, soaking durations, temperature regimes, and exogenous plant hormones on seed germination. The results demonstrated: (1) The optimal disinfection protocol was 75% ethanol for 1 minute followed by 3% H O for 5 minutes, with an optimal soaking time of 24 hours and optimal temperature/light conditions of 20°C/10°C (12 h light/12 h dark). Seeds soaked in sterile water under these conditions achieved a germination rate of 49.67%. (2) GA at 100–600 mg · L ^{1} and NAA at 5–30 mg · L ^{1} enhanced germination rate, germination potential, and germination index while shortening both the germination initiation period and total germination duration, thereby promoting seed germination. (3) 6-BA at 5 mg · L ^{1} and 10 mg · L ^{1} showed modest but non-significant promotional effects, while concentrations 15 mg · L ^{1} inhibited germination. (4) Treatment with GA at 500 mg · L ^{1} yielded the best overall germination performance, with germination rate, germination potential, and germination index reaching 69.67%, 33.00%, and 4.51, respectively, and germination initiation and duration times of 10.67 days and 11.67 days.

Keywords: *Meconopsis horridula* Hook. f. et Thoms., seed, germination, disinfectant, soaking time, temperature, exogenous plant hormone

Introduction

The genus *Meconopsis* Vig. comprises 54 species worldwide, with 43 species found in China, of which 18 have documented medicinal use. *Meconopsis horridula* represents the most characteristic and commonly used Tibetan medicinal material within this genus (Zhao et al., 2017). This annual herb thrives on grassy or rocky slopes at elevations of 3,600–5,100 m, with primary distribution across Tibet (widespread), western Gansu, eastern and southern Qinghai, and western Sichuan (China Flora Editorial Board, 1999). Renowned globally as the "Himalayan Blue Poppy," *M. horridula* is prized both for its ornamental beauty and medicinal properties. In Tibetan medicine, the entire plant or aerial parts, known as "Cier' en," are used to clear heat, mend bones, activate blood circulation, and relieve pain, making it particularly effective for

treating head trauma, fractures, and injuries (Dimaer Danzeng Pengcuo, 2012; Northwest Institute of Plateau Biology, Chinese Academy of Sciences, 1991). The *Drug Standard of Ministry of Public Health of the People' s Republic of China* includes over ten Tibetan patent medicines containing *M. horridula* (Chinese Pharmacopoeia Commission, 1995). Phytochemical investigations have identified alkaloids, flavonoids, terpenoids, and steroids, with pharmacological research focusing on anticancer, antiviral, and cardioprotective activities (Zhao et al., 2017; Guo et al., 2016; Fan et al., 2015).

The specialized high-altitude habitat and limited natural reserves of *M. horridula*, combined with difficulties in domestication, have led to heavy reliance on wild harvesting, causing rapid depletion of wild populations. In 2005, the species was classified as a Grade III endangered Tibetan medicinal material in the Tibet Autonomous Region. Based on regional survey data, Lu et al. (2011; Lu and Lan, 2013a, 2013b) recommended upgrading it to Grade I endangered status. Developing artificial cultivation represents the most critical strategy for conserving wild resources and addressing market shortages (Suonan Renqian, 2017). Seed propagation not only maintains genetic diversity in subsequent generations but also enhances seedling stress resistance and growth potential, making it the most reliable method for artificial cultivation (Qu and Ou, 2012). For high-altitude plants like *Meconopsis* with narrow ecological amplitudes, seed propagation proves more effective than transplantation (Dong et al., 1995).

Meconopsis horridula seeds exhibit compound dormancy (physical + physiological, PY+PD) and naturally low germination rates, posing significant challenges for artificial cultivation (Da et al., 2018). Breaking dormancy to improve germination represents the primary task for successful cultivation, yet research in this area remains scarce. This study systematically investigated the effects of disinfectants, soaking duration, temperature, and exogenous plant hormones on *M. horridula* seed germination to establish optimal protocols for enhancing germination rates and supporting conservation, cultivation, and domestication of this valuable ornamental and medicinal species.

Materials and Methods

1.1 Experimental Materials

Meconopsis horridula seeds were collected in September 2015 from Jiali County, Nagqu City, Tibet Autonomous Region. After natural drying, seeds were stored at 4°C for six months. The thousand-seed weight was (0.0962 ± 0.0085) g.

1.2 Experimental Design

1.2.1 Seed Disinfection Treatments Seeds were rinsed under running water for 10 minutes before transfer to a laminar flow hood for disinfection. The standard protocol involved initial sterilization with 75% ethanol for 1 minute, followed by three sterile water rinses, then treatment with either 0.1% HgCl ,

2% NaClO, or 3% H O for 5 or 10 minutes, and three final sterile water rinses. Prior to inoculation, seeds were soaked in sterile water for 12 hours. Six disinfection treatments were evaluated, each with three replicates of 100 seeds per Petri dish, incubated at 20°C under a 12 h light/12 h dark photoperiod.

1.2.2 Soaking Time and Germination Temperature Treatments Soaking durations were set at 0, 12, 18, and 24 hours. Based on temperature variation data for the Tibet Autonomous Region (Du et al., 2016; Yang et al., 2014), germination temperatures were established as constant 15°C, 20°C, and 25°C, or alternating 20°C/10°C and 25°C/15°C (light/dark). All treatments employed a 12 h light/12 h dark cycle. Using the optimal disinfection method from section 1.2.1 (75% ethanol 1 min + 3% H O 5 min), seeds were soaked for varying durations and then incubated at the designated temperatures. Each treatment comprised three replicates of 100 seeds per Petri dish.

1.2.3 Exogenous Plant Hormone Treatments Based on preliminary studies, three plant hormones were selected: gibberellic acid (GA), 6-benzylaminopurine (6-BA), and naphthaleneacetic acid (NAA). Concentrations were GA at 100, 200, 300, 400, 500, and 600 mg · L ^{1}; 6-BA at 5, 10, 15, 20, 25, and 30 mg · L ^{1}; and NAA at 5, 10, 15, 20, 25, and 30 mg · L ^{1}. After disinfection, seeds were soaked in sterile water (control) or hormone solutions for 24 hours, then rinsed three times with sterile water. Each treatment included three replicates of 100 seeds per Petri dish, incubated at 20°C/10°C (light/dark) with a 12 h light/12 h dark photoperiod. The 24-hour soaking duration and 20°C/10°C temperature regime were determined from results in section 1.2.2.

All disinfectants and hormone solutions were prepared with sterile water, with hormone stock solutions filter-sterilized. The paper bed method was employed: two layers of PhytoTC germination paper (Beijing Qivayixin Technology Co., Ltd.) were placed in Petri dishes, autoclaved, moistened with sterile water, and seeds were evenly sown before sealing with Parafilm.

1.2.4 Measurement Indicators and Calculations Germination was defined as radicle emergence 2 mm. Starting from treatment initiation, germinated seeds were counted and removed every 24 hours. The germination process was considered complete when no additional seeds germinated for seven consecutive days in both control and treatment groups. Contamination rate, germination initiation time, germination duration, germination rate, germination potential, and germination index were calculated (Zheng et al., 2017; Guo et al., 2018):

1. Contamination rate = (Number of contaminated seeds / Total seeds) × 100%
2. Germination initiation time (germination lag): Days from experiment start to first germination

3. Germination duration: Total days from first to final germination
4. Germination rate = (Total germinated seeds / Total seeds) × 100%
5. Germination potential = (Maximum daily germination / Total seeds) × 100%
6. Germination index = Σ(Gt/Dt), where Gt is germination count on day t and Dt is the corresponding day number

1.3 Data Analysis

Data were organized and graphed using Excel 2010. Significance analysis ($P<0.05$) was performed with SPSS 19.0.

Results

2.1 Effects of Different Disinfection Treatments on Seed Sterilization and Germination

Seed-borne pathogens directly impact germination rates. As shown in Table 1 , disinfection treatments significantly affected both sterilization efficacy and germination of *M. horridula* seeds. Three treatments (75% ethanol 1 min + 0.1% HgCl 5 min, 75% ethanol 1 min + 0.1% HgCl 10 min, and 75% ethanol 1 min + 2% NaClO 10 min) achieved 0% contamination, while the remaining three treatments showed contamination rates below 7%. The sterilization efficacy ranked: 0.1% HgCl > 2% NaClO > 3% H O . However, 0.1% HgCl most severely inhibited germination (rate <4%), followed by 2% NaClO, whereas 3% H O showed the least inhibition. The treatment 75% ethanol 1 min + 3% H O 5 min, despite a 6.67% contamination rate, produced the fastest germination (13.33 days), shortest germination duration (13.67 days), and highest germination rate (26.67%).

2.2 Effects of Soaking Time and Germination Temperature on Seed Germination Rate

Figure 1 [Figure 1: see original paper] illustrates the interactive effects of soaking time and temperature on germination rates. At constant 15°C and 25°C, germination rates initially increased then decreased with prolonged soaking. At 20°C, 20°C/10°C, and 25°C/15°C, germination rates increased progressively with soaking duration, with 18 h and 24 h soaking significantly outperforming 0 h and 12 h at each temperature. Alternating temperature regimes consistently produced higher germination rates than constant temperatures at equivalent soaking times, with the 20°C/10°C treatment achieving 46.00% germination at 18 h soaking and 49.67% at 24 h soaking. Consequently, the combination of 24 h soaking and 20°C/10°C germination temperature was identified as optimal and adopted for subsequent hormone experiments.

2.3 Effects of Exogenous Plant Hormones on Seed Germination

2.3.1 Effects of Different GA Concentrations Table 2 demonstrates that GA treatments significantly improved germination rates (51.33–69.67%) compared to the control (48.00%). The 500 mg · L ^{1} treatment achieved the highest germination rate (69.67%), 21.67% higher than control, followed by 600 mg · L ^{1} (19.33% increase), with no significant difference between these two concentrations. GA also enhanced germination potential and index, which increased with concentration up to 500 mg · L ^{1}, peaking at 33.00% and 4.51 respectively (27.00% and 2.17 higher than control). At 600 mg · L ^{1}, both parameters decreased. While GA at 100–400 mg · L ^{1} showed minimal effect on germination timing, 500 mg · L ^{1} significantly shortened both initiation and duration periods, advancing germination by 3 days and reducing total duration to 11.67 days (3.66 days shorter than control). At 600 mg · L ^{1}, these timing parameters increased again. Overall, 500 mg · L ^{1} GA proved optimal.

2.3.2 Effects of Different 6-BA Concentrations Table 3 reveals concentration-dependent effects of 6-BA on germination. Germination initiation time progressively delayed with increasing concentration, extending 0.33 days at 5 mg · L ^{1} and 11.33 days at 30 mg · L ^{1} compared to control. Conversely, germination duration shortened with higher concentrations, decreasing to 5.33 days at 30 mg · L ^{1} (10 days shorter than control). Germination rate, potential, and index initially increased then decreased with concentration. At 5 mg · L ^{1} and 10 mg · L ^{1}, these parameters exceeded control values, though differences in rate and index were non-significant. The 5 mg · L ^{1} treatment produced the highest values: 6.67%, 1.00%, and 0.39 higher than control for rate, potential, and index respectively. Concentrations 15 mg · L ^{1} inhibited germination, with stronger inhibition at higher concentrations. Thus, 5 mg · L ^{1} 6-BA was identified as the optimal concentration.

2.3.3 Effects of Different NAA Concentrations Table 4 shows that appropriate NAA concentrations improved germination rate, potential, and index while shortening initiation and duration periods. The 20 mg · L ^{1} and 25 mg · L ^{1} treatments significantly outperformed the control. At 20 mg · L ^{1}, germination potential (21.00%) and index (3.16) reached maximum values, 15% and 0.82 higher than control respectively. The 25 mg · L ^{1} treatment achieved the highest germination rate (61.67%), 13.67% and 2.00% higher than control and 20 mg · L ^{1} respectively, though potential and index were slightly lower than the 20 mg · L ^{1} treatment. The 25 mg · L ^{1} treatment also produced the fastest germination (2.67 days earlier than control) and shortest duration (5.33 days shorter than control). At 30 mg · L ^{1}, all parameters decreased significantly, with the germination index falling below control values. Therefore, 25 mg · L ^{1} NAA was determined optimal.

2.3.4 Comparative Analysis of Optimal Hormone Treatments Figure 2 [Figure 2: see original paper] compares the optimal treatments identified above.

For germination rate, GA 500 mg·L ^{1}, 6-BA 5 mg·L ^{1}, and NAA 25 mg·L ^{1} all significantly exceeded the control, with GA 500 mg·L ^{1} achieving the highest rate (69.67%, 21.67% higher than control). For germination potential, all three optimal treatments outperformed the control, with GA 500 mg·L ^{1} showing the greatest improvement (33.00%, 27.00% higher than control), followed by NAA 25 mg·L ^{1} (13.33% higher), while 6-BA 5 mg·L ^{1} showed no significant difference. For germination index, only GA 500 mg·L ^{1} differed significantly from control. Regarding germination timing, all treatments except 6-BA 5 mg·L ^{1} accelerated initiation, with GA 500 mg·L ^{1} advancing germination by 3 days. All three optimal treatments significantly shortened germination duration compared to control, with NAA 25 mg·L ^{1} completing germination in approximately 10 days (5.33 days shorter than control), though its rate, potential, and index were lower than GA 500 mg·L ^{1}. Overall, GA 500 mg·L ^{1} demonstrated the most significant promotional effect on *M. horridula* seed germination.

Discussion and Conclusion

Meconopsis horridula is a valuable alpine plant with both ornamental and medicinal significance, and improving its germination rate is essential for successful domestication. Seed germination is influenced by numerous external ecological and internal physiological factors, with primary determinants varying among species (Xu et al., 2014; Qu and Ou, 2012).

Seed-borne diseases significantly affect germination. Preliminary experiments revealed that non-disinfected seeds exhibited 53.00% contamination and only 9.67% germination with poor seedling vigor. In this study, disinfection with 75% ethanol 1 min + 3% H O 5 min effectively reduced contamination and increased germination to 26.67%, demonstrating that seed-borne pathogens inhibit germination and that proper disinfection can substantially improve rates. Compared to 0.1% HgCl and 2% NaClO, 3% H O not only sterilized effectively but also promoted germination, likely by mildly corroding the seed coat to improve permeability and oxygen supply, thereby breaking dormancy (He et al., 2008). Additionally, H O may stimulate the pentose phosphate pathway, which plays a crucial role in dormancy release (Xu et al., 1987).

Germination begins with water imbibition (Xu et al., 2014), and soaking is a common physical method for breaking dormancy. Qu et al. (2018) reported that soaking *Meconopsis racemosa* seeds for 24–36 hours increased both germination rate and potential. Da et al. (2018) found that *M. horridula* seeds reached maximum water absorption (97.80%) after 18 hours and saturation after 20 hours. In this study, soaking for 12, 18, and 24 hours all significantly improved germination rates under optimal temperature conditions. Although 18 h and 24 h soaking produced similar rates, the 24 h treatment yielded the highest germination (49.67%), indicating that the seed coat does not impose permeability barriers, consistent with Da et al. (2018).

Temperature critically influences germination. This study compared three con-

stant temperatures (15°C, 20°C, 25°C) and two alternating regimes (20°C/10°C, 25°C/15°C). The 20°C/10°C alternating temperature proved most favorable, reflecting the natural conditions of high-altitude habitats where *M. horridula* grows (3,600–5,100 m elevation) with low temperatures and large diurnal fluctuations. The 20°C/10°C regime closely matches spring temperature patterns in these regions.

Exogenous hormone application is an important method for elucidating hormonal regulation mechanisms of seed dormancy and germination (Xu et al., 2014). Qu et al. (2018) found that GA at 250 mg · L ^{1} and NAA at 10 mg · L ^{1} effectively promoted *M. racemosa* germination, while 6-BA was inhibitory. In this study, GA at 500 mg · L ^{1} most effectively broke dormancy in *M. horridula*, followed by NAA at 25 mg · L ^{1}. Low-concentration 6-BA (<15 mg · L ^{1}) showed modest, non-significant promotion, while higher concentrations (15 mg · L ^{1}) were inhibitory, similar to findings for *M. racemosa* (Qu et al., 2018).

Integrating the effects of disinfection, soaking time, temperature, and exogenous hormones, the optimal protocol for *M. horridula* seed germination is: disinfection with 75% ethanol 1 min + 3% H O 5 min, soaking in GA 500 mg · L ^{1} for 24 hours, and incubation at 20°C/10°C (12 h light/12 h dark). This combination produces the fastest, most uniform germination with highest rates. However, as this study only examined three hormones, further research is needed to investigate their effects on seedling growth and to identify potentially more effective germination-promoting substances.

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