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DORMANCY RELEASE TREATMENTS AND THEIR EFFECTS ON SEED GERMINATION IN *PODOPHYLLUM HEXANDRUM* ROYLE: IMPLICATIONS FOR CONSERVATION AND CULTIVATION

ZAHOOR KHAN[1], BUSHRA KHAN[1*], MIAN AFAQ AHMAD[2], JAVAID IQBAL[4*], SYED TANVEER SHAH, ISRAR UD DIN[2], JAWAD SAEED[5] AND ISHAQ AHMAD MIAN[6]

[1]Department of Environmental Sciences, University of Peshawar, KP, Pakistan
[2]Institute of Biotechnology and Genetic Engineering, The University of Agriculture Peshawar, Peshawar 25130, KP,
[3]Department of Agriculture, Faculty of Biological and Health Sciences, Hazara University Mansehra KP, Pakistan
[4]Department of Environmental Sciences, University of Lakki Marwat, KP 28420, Pakistan
[5]Department of Pharmacy, University of Peshawar, KP, Pakistan
[6]Department of Soil & Environmental Sciences, The University of Agriculture, Peshawar 25000, Pakistan
**Corresponding author's bushraasu@uop.edu.pk; j.iqbal@ulm.edu.pk*

Abstract

Podophyllum hexandrum Royle, an endangered medicinal plant valued for its pharmacological properties, faces propagation challenges due to its strong seed dormancy, limiting its potential for conservation and cultivation. This study systematically evaluates the effectiveness of various physical, chemical, and hormonal pretreatments to overcome seed dormancy and optimize germination. Additionally, the study assesses sterilization treatments with different concentrations of Mercuric Chloride ($HgCl_2$) to minimize contamination while maximizing germination. Among the sterilization treatments, 0.1% $HgCl_2$ emerged as the most effective, achieving a high germination rate (85.43 ± 2.96%) and low contamination (15.67 ± 2.96%). Dormancy-breaking treatments revealed that 5 mg/L GA_3 for 24 hours was the most effective, achieving a maximum germination rate of 90.33%, followed by hot water treatment (80%) and cold-water treatment (68%). These pretreatments effectively counteracted the physical and physiological dormancy barriers of *P. hexandrum* seeds by improving water absorption and gas exchange through the seed coat. This study provides valuable insights for developing optimized germination protocols, supporting both conservation and sustainable cultivation of *P. hexandrum* to ensure its continued availability and medicinal utility.

Key words: Seed dormancy, Seed germination, Chemical Pretreatments, Mechanical scarification.

Introduction

Podophyllum hexandrum Royle, also known as Himalayan mayapple, is an endangered medicinal plant that thrives in the alpine regions of the Himalayas (Kharkwal *et al.*, 2008; Kalam *et al.*, 2021). This perennial plant stands 15-40 cm tall, has a 50 cm long rhizome, multiple roots, and a 30-90 cm stem. It bears 2-3 lobed, dark green branches that open fully during blooming, with a hexa-structure of six petals and stamens (Figs. 1 & 2). Flowering occurs briefly from May to August, producing red berries with embedded seeds weighing around 20 g (Fig. 3). Propagation is possible through seeds or rhizomes, the latter serving as a storage organ for perennial growth and enabling new shoot generation each season (Qazi *et al.*, 2011; Li *et al.*, 2009). *Podophyllum hexandrum* contains podophyllotoxin, a lignan with significant pharmacological activity used in treating cancers, including lung, liver, and verrucous cancers, as well as condyloma acuminatum (Khan *et al*., 2024). Derivatives such as etoposide and teniposide are widely used in clinical cancer treatments (Giri *et al.*, 2000). However, natural populations are declining due to overexploitation, habitat loss, and climate change, leading to its classification as endangered by CITES (Nadeem *et al.*, 2000; Hamayun *et al.*, 2006; Sharma *et al.*, 2022).

Seed germination is a critical stage in the plant life cycle, serving as the foundation for reproductive success and ensuring population persistence and resilience over time. However, species such as *Podophyllum hexandrum* exhibit complex dormancy mechanisms, which prevent germination under normal environmental conditions. These dormancy mechanisms, though beneficial in the wild by allowing seeds to survive unfavorable conditions, create significant challenges for propagation in controlled settings. Thus, overcoming seed dormancy is essential for the conservation and cultivation of this medicinally important species.

Dormancy in *P. hexandrum* seeds is influenced by a combination of environmental and physiological factors, including temperature, light, moisture, oxygen, hormones, and various chemical interactions (Jiu *et al*., 2023). To address these dormancy barriers, traditional and emerging dormancy-breaking methods have been proposed, such as cold and hot stratification, mechanical and chemical scarification, phytohormone application, and solution soaking (Wang *et al*., 2023; Jiu *et al.*, 2023). Despite advancements in these methods, the optimal conditions for efficient dormancy release in *P. hexandrum* remain unclear and require further investigation to improve germination rates and propagation success.

One distinguishing characteristic of *P. hexandrum* is its prolonged dormancy, with germination and seedling establishment often spanning several months to years. This extended dormancy period slows population renewal rates, resulting in resource scarcity and contributing to the species' vulnerability in its natural habitats (Nadeem *et al*., 2000). Among dormancy-breaking techniques, cold stratification has proven to be a widely effective method, significantly enhancing germination in various plant species, including *P. hexandrum*, by simulating natural winter conditions that break down dormancy barriers (Kırmızı *et al*., 2018; Jiu *et al*., 2021). Optimizing these

dormancy-release techniques is thus crucial for conservation and sustainable cultivation practices, which are essential for the survival and medicinal availability of *P. hexandrum* for future generations.

Biomechanically, germination in *P. hexandrum* depends on the balance between the force exerted by the embryo and reduced resistance from external coverings such as the endosperm and seed coat. *P. hexandrum* seeds exhibit cryptogeal morphophysiological dormancy, where the embryo must reach a critical length before germination (Peng *et al.*, 2023). Proteomic and transcriptomic studies suggest that hydrolases play a key role in overcoming germination barriers by weakening the endosperm through increased cell-wall hydrolases (Dogra *et al.*, 2016). Phytohormones, including gibberellins (GA), regulate dormancy by inducing auxin, cytokinin, amylase, and protease production (Matilla *et al.*, 2023). GA transports from the embryo to the endosperm, boosting carbon metabolism and synthesizing proteins essential for cell growth (Penfield *et al.*, 2017).

To ensure the sustainable use and conservation of *P. hexandrum*, understanding its seed dormancy and germination mechanisms is essential. This study focuses on optimizing germination and breaking dormancy to support conservation and cultivation for medicinal and ecological benefits. This research aims to provide practical insights for conservation strategies, sustainable cultivation practices, and deepen our understanding of seed biology in *P. hexandrum*.

Material and Methods

Study area of research: Research was conducted in scenic locations, including the Swat, Gabral, and Utror valleys in the Hindu Kush Mountain range, the remote Astore district in Gilgit-Baltistan, the diverse Hazara region in Khyber Pakhtunkhwa, the historic Dir district bordering Afghanistan, the lush Murree Hills near Islamabad, and the mountainous Chitral district in northwestern Pakistan (Fig. 4).

Plant material collection: The plant materials of *P. hexandrum* were collected from the study areas mentioned above using a random sampling approach. A total of 15–20 *P. hexandrum* plants were selected as representative samples for *In vitro* culture between May and September of 2022. The research was conducted at the Institute of Biotechnology and Genetic Engineering (IBGE), The University of Agriculture, Peshawar. In total, 15 fruits of *P. hexandrum* were collected, yielding approximately 300 seeds. The seeds were separated from the berries, washed under running water, shade-dried overnight, and stored at 4°C in an incubator (Fig. 3). Murashige and Skoog's (1962) MS medium were used as the growth medium for the *In vitro* culture of *P. hexandrum* explants, with the MS medium supplemented with respective growth hormones and additives.

Sterilization of plant materials: Surface sterilization of seeds was performed by washing them with running tap water to remove external contaminants. Seeds were immersed in a 50% aqueous solution of sodium hypochlorite (NaOCl) for 5 minutes, followed by thorough rinsing with autoclaved distilled water. Different concentrations of mercuric chloride ($HgCl_2$) (0.05%, 0.1%, 0.2%) were used, each applied for 5 minutes. After each sterilization treatment, the explants were rinsed three times with double-distilled water (ddH_2O) and gently dried using blotting paper before inoculation in an airflow chamber. The efficacy of various mercuric chloride concentrations was assessed in terms of their ability to prevent fungal and bacterial contamination, while explant survival rates were measured to evaluate the impact of sterilization on explant viability.

Breaking of seed dormancy and seed germination: *P. hexandrum* seeds exhibit dormancy, which prevents germination until suitable environmental conditions are met. To overcome this dormancy, seeds were pretreated using various hormonal, stratification, and scarification methods. For mechanical scarification, seeds were physically abraded to weaken the hard seed coat, thereby alleviating physical dormancy and enhancing water and oxygen absorption. This was achieved using tools such as sandpaper, small blades, or specialized scarification equipment. Care was taken to ensure the seed coat was sufficiently weakened without damaging the embryo. After scarification, seeds were rinsed and soaked in water to further promote germination. This method is precise, cost-effective, and particularly suitable for seeds with hard, impermeable coats.

Additional treatments included gibberellic acid (GA_3), hot water treatment (HWT), cold water treatment (CWT), and low-temperature treatment (LTT), as outlined in Table 1. Chemical scarification was carried out using sulfuric acid, hydrochloric acid, potassium nitrate, and hydrogen peroxide (Table 1). Mechanical scarification was also performed by soaking seeds in tap water for 24 hours, followed by making a 2–3 mm incision opposite the hilum using a sterile scalpel.

Table 1. Treatments for breaking seed dormancy of *P. hexandrum*.

Treatment	Concentration	Duration
GA_3	5 mg L-1	24 hours
H_2SO_4	10% V/V	20 seconds
HCl	10% V/V	20 seconds
KNO_3	50 mM	24 hours
H_2O_2	150 mM	10 minutes
HW (100°C)	100 ml	60 seconds
CWT (40°C)	100 ml	24 hours

Following pretreatment, seeds were rinsed with sterilized water and subsequently sterilized with mercuric chloride ($HgCl_2$). Treated seeds were then inoculated on MS (Murashige and Skoog) medium supplemented with respective growth regulators under *In vitro* conditions. The experiment was arranged in a completely randomized design with three replicates per treatment. Seed germination percentages were recorded at two-week intervals.

Fig. 1. *Podophyllum hexandrum* (Photo taken at Saif Lake, 2022).

Fig. 2. *Podophyllum hexandrum* at flowering and fruiting stage.

Fig. 3. Fruits and seeds of *Podophyllum hexandrum*.

Seed germination in peat moss soil: A total of 15–20 pretreated seeds were sown in peat moss soil, prepared with an equal ratio (1:1:1:1) of vermiculite, farmyard manure, sand, and soil. Replicates were used for each treatment, and seeds were watered daily with 1 mL of water. The planting setup was maintained under shaded and humid conditions at 20°C. Seedlings began to emerge three months after sowing, and seedling percentages were recorded at regular 10-day intervals.

Aseptic transfer of seeds and *In vitro* seed culture conditions: Sterilized and pretreated seeds were transferred aseptically into 250 mL and 500 mL flasks and petri plates containing culture medium with 8 g/L agar, vitamins, and 30 g/L sucrose, in a laminar flow chamber. Cultures were initially kept in the dark for one week and then exposed to a 16-hour photoperiod, at 65–70% relative humidity, with a temperature of 25 ± 2°C, and under 50 µmol m^{-2} s^{-1} fluorescent light.

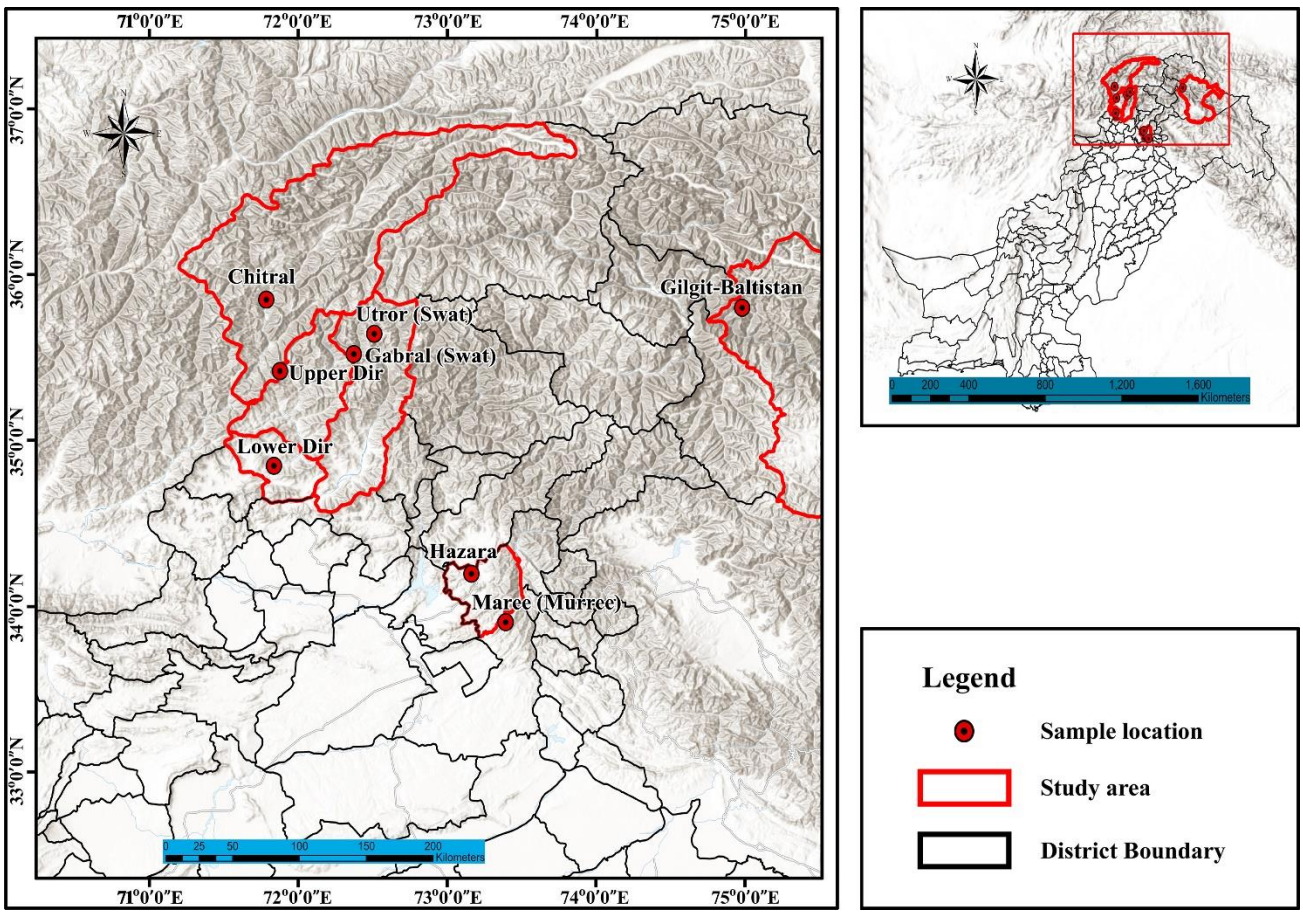

Fig. 4. The distribution and sampling sites of *Podophyllum hexandrum*.in Swat, Gabral, Utror, Astor, Hazara, Dir, Murree Hills, and Chitral areas.

Hardening off and acclimatization of *In vitro* plantlets: *In vitro*-developed plantlets were removed from flasks, rinsed with tap water to remove nutrient medium, and transferred to pots filled with a 1:1 mixture of vermiculite and sterilized soil. To maintain high humidity, the pots were covered with transparent polyethylene bags. Plantlets were placed in a growth chamber set at a 16-hour photoperiod with 65–70% relative humidity, 25°C day temperature, and 18°C night temperature. They were watered every two days with half-strength Hoagland's solution for 3–4 weeks. Survival and growth data were recorded weekly. After one month, successfully acclimatized plantlets were transplanted to larger pots with garden soil and kept in a glasshouse.

Statistical analysis and data visualization: Data were statistically analyzed using MS Excel and Statistix 8.1, and data visualization was conducted with GraphPad Prism. Analysis of Variance (ANOVA) was employed to assess the overall significance of the data, and the Least Significant Difference (LSD) test was applied to determine differences among treatments.

Results

Seed sterilization and growth response: The effects of various $HgCl_2$ concentrations on *P. hexandrum* seed decontamination and germination are shown in Table 1 (Fig. 5). Seeds were sterilized using 0.05%, 0.1%, and 0.2% $HgCl_2$ concentrations for 5 minutes at 25 ± 2°C. Among the three treatments, 0.1% $HgCl_2$ was most effective in reducing contamination and enhancing germination, resulting in an 85.43 ± 2.96% germination rate and a 15.67 ± 2.96% contamination rate. The 0.05% $HgCl_2$ treatment showed moderate germination (63% ± 2.02%) but had a high contamination rate (36.67 ± 2.03%). Conversely, 0.2% $HgCl_2$ negatively affected germination, reducing it to 44.00 ± 2.96%, while contamination was not significantly affected (55.67 ± 2.96%). Thus, the 0.1% $HgCl_2$ concentration was optimal for *P. hexandrum* seed decontamination and germination, as summarized in (Table 2).

Effects of physical, chemical, and hormonal pretreatments on breaking seed dormancy and germination: The germination response of *Podophyllum hexandrum* seeds to a range of physical, chemical, and hormonal pretreatments was systematically evaluated (Figs. 6, and 7). Each pretreatment included 15 seeds to assess its effect on breaking seed dormancy and promoting germination. Among all treatments, seeds exposed to 5 mg L^{-1} gibberellic acid (GA_3) for 24 hours achieved the highest germination rate at 90.33%, indicating GA_3's strong influence on dormancy breakage and germination stimulation. This was followed by the hot water treatment at 60 seconds (80%) and a 24-hour cold-water treatment (68%), underscoring the role of temperature fluctuations in enhancing seed responsiveness. In stark contrast, seeds in the MS_0 (control) treatment showed no germination (0%), indicating the high dormancy level of *P. hexandrum* seeds and the necessity for external treatments to trigger germination. Gradual increases in germination rates were noted for most treatments, except for hydrogen peroxide

(H_2O_2), which resulted in a slight reduction in germination. Intermediate germination rates, ranging from 40.33% to 56.33%, were observed in seeds subjected to MS_0, mechanical scarification (MES), low-temperature treatment (LLT), and H_2SO_4 (20-second exposure).

Notably, pretreatments with plant Growth Regulators (GA_3), Sulfuric Acid (H_2SO_4 at 10% v/v), Hydrochloric Acid (HCl at 10% v/v), Potassium Nitrate (KNO_3 at 50 mM), and Hydrogen Peroxide (H_2O_2) effectively enhanced germination. These treatments appear to counteract the seed's physical and physiological dormancy barriers, such as the hard seed coat and internal biochemical inhibitors. Mechanical scarification (MES) also significantly improved germination by creating minute abrasions in the seed coat, which likely facilitated better water absorption and gas exchange, both essential for initiating the germination process.

Table 2. Effects of different concentrations of $HgCl_2$ on decontamination and germination of *P. hexandrum*.

Treatments × intervals (5 min)	% Of seeds germinated	% Of seeds contaminated
0.01% $HgCl_2$	63.00 ± 2.0276 [b]	36.67 ± 2.03 [a]
0.1% $HgCl_2$	85.43 2.9627 [a]	15.67 ± 2.96 [b]
0.2% $HgCl_2$	44.00 ± 2.9627 [c]	55.67 ± 2.96 [a]

Means within a table followed by the same letter are significantly different by LSD test (p<0.05); ± SE

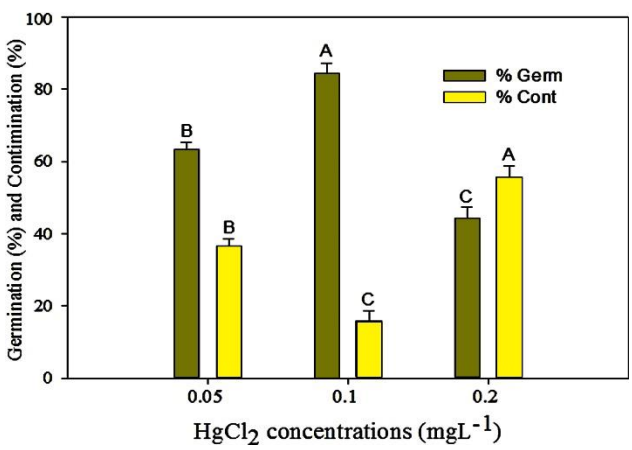

Fig. 5. Effects of different concentrations of Mercuric Chloride on seed sterilization of *P. hexandrum.*

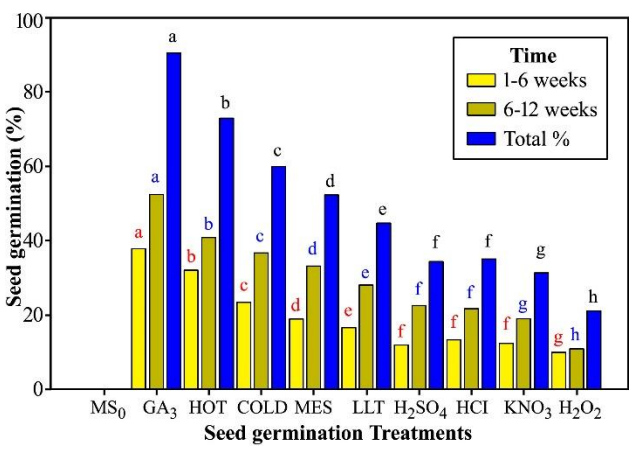

Fig. 6. Shows seed germination after pretreatment with different chemicals (H_2SO_4, HCl, KNO_3, H_2O_2), hormone (GA_3), mechanical scarification (MES), hot water treatment (HW), cold water treatment (CW), and low-temperature treatment (LT).

Discussion

Seed sterilization plays a crucial role in preventing microbial contamination and enhancing germination rates in tissue culture. Maintaining aseptic conditions is essential for avoiding contamination, with mercuric chloride ($HgCl_2$) proving highly effective against bacterial and fungal contaminants. In this study, treating *P. hexandrum* seeds with 0.1% $HgCl_2$ for 5 minutes yielded an 85.43% germination rate, highlighting the effectiveness of $HgCl_2$ in improving decontamination and germination. Concentrations above 0.1% of $HgCl_2$, however, resulted in browning and death of seeds and explants, emphasizing the need for careful optimization. The effectiveness of $HgCl_2$ varies based on exposure time and concentration, where extended exposure and higher concentrations can effectively reduce contamination but may also lead to explant necrosis. These findings align with previous studies on various plant species, confirming the broad-spectrum antimicrobial activity of $HgCl_2$ (Padhi *et al*., 2017). As reported in other studies, $HgCl_2$ penetrates seed coats effectively, with sterilization effectiveness influenced by factors such as exposure duration, explant type, and microorganism type (Torres *et al.*, 2018; Boruah *et al.*, 2020; Babu *et al.*, 2022). While $HgCl_2$ can be an effective disinfectant for *P. hexandrum* seeds, the treatment parameters must be carefully optimized to avoid negative impacts on seed viability, with the ideal concentration varying based on seed characteristics like size, coat thickness, and dormancy level (Sharma *et al*., 2010; Padhi *et al.*, 2017).

The dormancy period of *P. hexandrum* seeds, which extends for approximately 10 months, is an adaptation to the harsh climate of high altitudes (Nadeem *et al*., 2000; Jiu *et al.*, 2023). Dormancy factors, primarily located in the endosperm and seed coat (Khajuria *et al*., 2021), were investigated in this study, and the results align with previous research (Kharkwal *et al.*, 2008; Kumar *et al*., 2017). Various pretreatment methods successfully broke dormancy in *P. hexandrum* seeds, facilitating controlled germination (Nadeem *et al.*, 2000; Singh *et al.*, 2020; Zrig *et al*., 2021; Sharma *et al*., 2022). Among these methods, GA_3 pretreatment for 24 hours yielded the highest germination rate of 93%, consistent with Sharma *et al*., (2006), who also observed peak germination following GA_3 pretreatment.

Mechanical scarification is a physical process of breaking, scratching, or weakening the seed coat to overcome dormancy and promote germination. It is commonly achieved by methods such as rubbing seeds with sandpaper, nicking them with a blade, or using specialized equipment (Singh *et al*., 2020; Zrig *et al*., 2021). Compared to other methods of scarification, such as chemical (using acids) or thermal (hot water treatment), mechanical scarification is often favored for its precision and reduces risk of damaging the embryo. It allows direct control over the extent of scarification and is particularly effective for seeds with hard seed coats, such as legumes or certain medicinal plants (Khajuria *et al*., 2021). However, its manual nature can make it labor-intensive and less suitable for large-scale applications. Integrating this method with other approaches can enhance its efficiency in

a broader context. Hot water treatment at 100°C for 5 minutes was the second most effective method, aligning with Kharkwal *et al*., (2008). Low-temperature exposure and mechanical scarification (MES) further improved germination rates. Low temperatures may induce physiological changes in seeds, while MES enhances water and oxygen absorption by physically disrupting the hard seed coat, which acts as a barrier to germination. This process facilitates the penetration of water and oxygen, essential for initiating the metabolic activities required for seed germination. The observed germination rate of 56.33% demonstrates its effectiveness in reducing dormancy barriers, aligning with findings from Kumar *et al*., (2017–2022), who also reported significant improvements in germination rates with similar scarification methods.

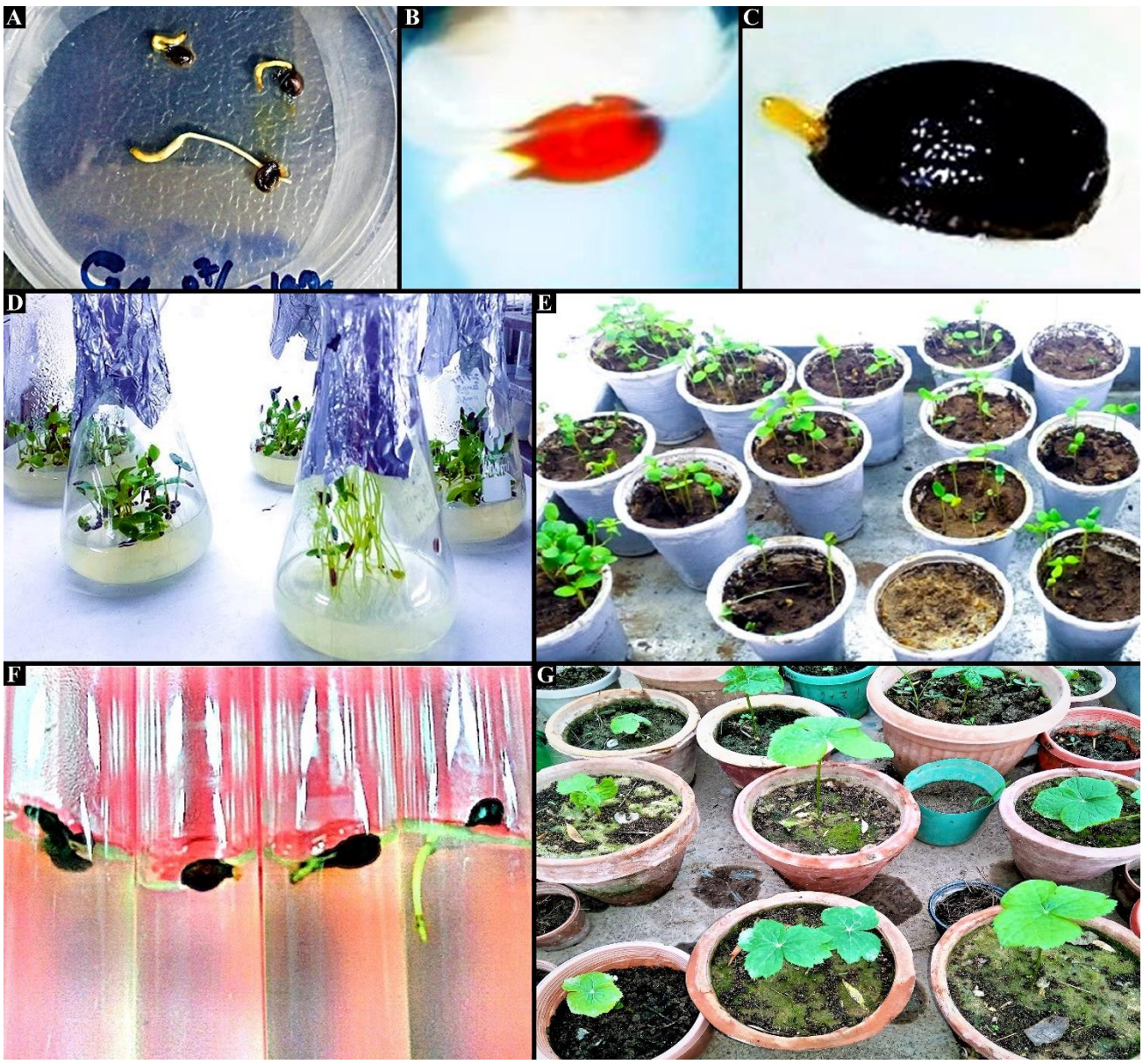

Fig. 7. Panels (a), (b), and (c) show seed germination after chemical pretreatments; Panels (d) and (e) depict seed germination after pretreatment with GA3; Panel (f) shows seed germination following stratification; Panel (g) shows the successful acclimatization of plantlets transferred to larger pots.

Compared to other dormancy-breaking treatments, Mechanical Scarification (MES) demonstrates a moderate yet significant impact, achieving a germination rate of 56.33% (Baskin & Baskin, 2014). While MES primarily targets physical dormancy by enhancing water and oxygen absorption through disruption of the seed coat (Taylor *et al*., 2016), treatments like GA_3 (gibberellic acid) at 5 mg L^{-1} for 24 hours, which achieved a higher germination rate of 90.33%, address physiological dormancy by promoting hormonal balance and metabolic activity (Kucera *et al*., 2005). Similarly, hot water treatment (80% germination) and cold-water treatment (68% germination) are effective in breaking dormancy by mimicking natural conditions that seeds encounter in their environment (Bewley & Black, 2012). The comparative effectiveness of MES lies in its direct, low-cost application and precision for seeds with hard coats (Luna *et al*., 2015). However, its germination rate is lower than chemical or hormonal treatments, which

target multiple dormancy mechanisms simultaneously (Finch-Savage & Leubner-Metzger, 2006). Therefore, while MES is an essential tool, especially for small-scale applications, it is often more effective when integrated with complementary methods to optimize germination outcomes (Hilhorst *et al*., 2010).

Chemical pretreatments revealed that H_2O_2 slightly reduced germination, possibly due to oxidative stress. Understanding these pretreatments is essential for conservation and cultivation, with promising implications for reducing germination times significantly (Kharkwal *et al*., 2008; Sharma *et al*., 2006; Kumar *et al*., 2022; Nadeem *et al*., 2000; Wojtyla *et al*., 2016). The *In vitro* protocol developed in this study reduced *P. hexandrum* seed germination time from one year to approximately three to four months, offering substantial conservation and cultivation benefits. GA_3 pretreatment for 24 hours, hot water, and MES are all effective in overcoming seed dormancy, providing valuable insights for both conservation and cultivation efforts.

Conclusion

This study provides a novel approach to overcoming the significant dormancy barrier in *Podophyllum hexandrum* seeds, a critical step for its conservation and cultivation. Specifically, it assessed an integrated series of pretreatments-physical, chemical, and hormonal to determine optimal dormancy-breaking conditions. This study evaluated sterilization and dormancy-breaking treatments to enhance *Podophyllum hexandrum* Royle seed germination, yielding critical insights for conservation and cultivation. Results indicate that a 0.1% $HgCl_2$ treatment for 5 minutes is optimal, achieving an 85.43% germination rate with only 15.67% contamination. Higher concentrations adversely impacted germination, underscoring the importance of optimizing sterilization protocols. The study also explored various physical, chemical, and hormonal pretreatments for breaking seed dormancy, with GA_3 pretreatment for 24 hours showing the highest germination rate at 90.33%. Cost-effective options, including hot and cold-water treatments, were also effective. MES was particularly beneficial, achieving a 56.33% germination rate. The study's focus on gibberellic acid (GA_3) and various chemicals such as sulfuric acid, hydrochloric acid, potassium nitrate, and hydrogen peroxide to enhance germination rates presents a unique contribution. Additionally, it investigated the effects of different sterilization protocols with mercuric chloride ($HgCl_2$), identifying optimal concentrations for both high germination rates and low contamination. These findings significantly reduce *P. hexandrum* germination time from a year to a few months, supporting efficient conservation and cultivation. This comprehensive analysis of dormancy-breaking techniques provides valuable insights into optimizing germination and seedling establishment, enhancing the understanding of *P. hexandrum* seed biology. It suggests viable strategies for sustainable management, with broader implications for conserving this endangered medicinal species and potentially scaling its cultivation for sustainable pharmacological use.

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References

Babu, G.A., K. Mosa Christas, E. Kowsalya, M. Ramesh, S.I. Sohn and S. Pandian. 2022. Improved sterilization techniques for successful *In vitro* micropropagation. In: *Commercial Scale Tissue Culture for Hort. Plant. Crops*, pp. 1-21. Singapore: Springer Nature Singapore.

Baskin, C.C. and J.M. Baskin. 2014. Seeds: Ecology, biogeography, and evolution of dormancy and germination. Academic Press.

Bewley, J.D. and M. Black. 2012. Seeds: Physiology of Development and Germination. Springer.

Boruah, J. 2020. Effect of 0.1% $HgCl_2$ on surface sterilization of som (*Persea bombycina* King) explant during tissue culture - a major host plant of muga silkworm. *Int. J. Curr. Microbiol. App. Sci*., 9: 954-958.

Dogra, V., R. Sharma and S. Yelam. 2016. Xyloglucan endo-transglycosylase/hydrolase (XET/H) gene is expressed during the seed germination in *Podophyllum hexandrum*: a high-altitude Himalayan plant. *Planta*, 244: 505-515.

Finch-Savage, W.E. and G. Leubner-Metzger. 2006. Seed dormancy and the control of germination. New *Phytol.*, 171(3): 501-523.

Giri, A. and M. Lakshmi Narasu. 2000. Production of podophyllotoxin from *Podophyllum hexandrum*: A potential natural product for clinically useful anticancer drugs. *Cytotech*., 34: 17-26.

Hamayun, M., S.A. Khan, I.J. Lee and M.A. Khan. 2006. Conservation assessment of Hindu-Kush Mountain Region of Pakistan: a case study of Utror and Gabral Valleys, District Swat, Pakistan. *Asian J. Plant Sci*., 725-732.

Hilhorst, H.W.M., W.E. Finch-Savage and J. Buitink. 2010. Seeds: The ecology of regeneration in plant communities. CABI.

Jiu, X., H. Chen, T. Du, X. Jia, D. Liu, J. Meng and X. Xu. 2023. Dormancy release of seeds of *Podophyllum hexandrum* Royle accompanied by changes in phytochemicals and inorganic elements. *Plos One*, 18(11): e0294673.

Jiu, X.J., T. Du, H.G. Chen, F. Li and C.R. Xu. 2021. Changes of embryo morphology, physiology and biochemistry during low temperature stratification of *SinoPodophyllum hexandrum* seeds. *Acta Bot. Boreal. Occident. Sin*., 41(12): 2096-2103.

Kalam, M.A., A.H. Malik, A.H. Ganie and T.A. Butt. 2021. Medicinal importance of Papra (*Podophyllum hexandrum* Royle) in Unani system of medicine. *J. Compl. Integr. Med*., 18(3): 485-490.

Khajuria, A.K., C. Hano and N.S. Bisht. 2021. Somatic embryogenesis and plant regeneration in *Viola canescens* Wall. Ex. Roxb.: an endangered Himalayan herb. *Plants*, 10(4), p.761. https://doi.org/10.3390/plants10040761.

Khan, Z.; B. Khan, S.T. Shah, J. Iqbal, A. Basit, M.S. Khan, W. Iqbal, M.F. Elsadek, A. Jamal and M.A. Ali. 2024. Preserving nature's treasure: A journey into the *In vitro* conservation and micropropagation of the endangered medicinal marvel-*Podophyllum hexandrum* Royle. *Horticulturae*, 10, 809. https://doi.org/10.3390/ horticulturae10080809

Kharkwal, A.C., R. Kushwaha, O. Prakash, R.K. Ogra, A. Bhattacharya, P.K. Nagar and P.S. Ahuja. 2008. An efficient method of propagation of *Podophyllum hexandrum*: An endangered medicinal plant of the Western Himalayas under ex situ conditions. *J. Nat. Med*., 62: 211-216.

Kırmızı, S., G. Güleryüz and H. Arslan. 2018. Effects of pretreatments on seed dormancy and germination in endemic Uludağ flax (*Linum olympicum* Boiss.) (Linaceae). *Hort. Environ. Biotechnol.*, 59: 629-635.

Kucera, B., M.A. Cohn and G. Leubner-Metzger. 2005. Plant hormone interactions during seed germination and seedling development. *Seed Sci. Res*, 15(3): 199-213.

Kumar, J., P. Sandal, A. Singh, A. Kumar, V. Arya, R. Devi, B.P. Sharma and R. Verma. 2022. Conservation status, anticancer compounds and pharmacological aspects of Royle: A review *Podophyllum hexandrum. J. Ecol*., 49(3): 1096-1102.

Kumar, R. and V. Singh. 2017. *Podophyllum hexandrum* Royle: a high value medicinal herb of Himalaya under severe threat. *Int. J. Adv*., 4(1): 206-212.

Li, W., M.F. Li, D.L. Yang, R. Xu and Y.R. Zhang. 2009. Production of podophyllotaxin by root culture of *Podophyllum hexandrum* Royle. *Electron J. Biol*., 5(2): 34-39.

Luna, B., F. Pérez-García and A. Escudero. 2015. Effects of mechanical scarification on seed germination of Mediterranean species. *Seed Sci. Technol*., 43(2): 245-255.

Matilla, A.J. 2020. Seed dormancy: molecular control of its induction and alleviation. *Plants*, 9(10): 1402.

Nadeem, M., L.M.S. Palni, A.N. Purohit, H. Pandey and S.K. Nandi. 2000. Propagation and conservation of *Podophyllum hexandrum* Royle: an important medicinal. *Herb. Biol. Conserv*., 92(1): 121-129.

Padhi, M. and S.P. Singh. 2017. Surface sterilization for reducing microbial contamination in *In vitro* propagation of lasora (*Cordia myxa* Roxb.) using nodal segments. *Int. J. Curr. Microbiol. Appl. Sci*., 6(8): 836-842.

Penfield, S. 2017. Seed dormancy and germination. *Curr. Biol*., 27(17): R874-R878.

Peng, D.L., B.Y. Geng, Y.B. Qin, L.E. Yang, C.C. Baskin and J.M. Baskin. 2023. Ecophysiology of seed dormancy and germination in the alpine-subalpine medicinal plant species *SinoPodophyllum hexandrum* (Royle) T.S. Ying. *J. Appl. Res. Med*., 32: 100448.

Sharma, R., S. Kaur and S.K. Uniyal. 2022. Population and vulnerability assessment of high-value medicinal plants in the alpine regions of western Himalaya. *J. Appl. Res*., 26: 100353.

Sharma, R.K., S. Sharma and S.S. Sharma. 2006. Seed germination behaviour of some medicinal plants of Lahaul and Spiti cold desert (Himachal Pradesh): implications for conservation and cultivation. *Curr. Sci*., pp. 1113-1118.

Singh, H., V. Kaul, V. Wazir and K. Singh. 2020. Direct shoot organogenesis and secondary metabolite analysis in *Podophyllum hexandrum* Royle, a critically endangered medicinal herb. *Physiol. Mol. Biol*., 26(10): 2077-2089.

Taylor, M.B., K. Gba and W.E. Finch-Savage. 2016. Breaking seed dormancy: a review of the role of mechanical scarification. *Seed. Sci. Res*, 26(2): 131-144.

Wang, L., Y. Zhu, J. Jiang, G. Tan, Q. Ma and H. Zhang. 2023. Dynamic changes in the levels of metabolites and endogenous hormones during the germination of *Zanthoxylum nitidum* (Roxb.) DC. Seeds. *Plant Signal. Behav*., 18(1): 2251750.

Zrig, A., A. Saleh, F. Hamouda, M.K. Okla, W.H. Al-Qahtani, Y.A. Alwasel, A. Al-Hashimi, M.Y. Hegab, A.H. Hassan and H. AbdElgawad. 2021. Impact of sprouting under potassium nitrate priming on nitrogen assimilation and bioactivity of three *Medicago* species. *Plants*, 11(1): 71.

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