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Investigations on seed dormancy breaking mechanisms in wild medicinal plant species

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Abstract
A paradox runs through wild medicinal plant conservation: the very seed dormancy mechanisms that ensure species survival in unpredictable habitats become the main barrier when these plants need to be cultivated or propagated for research and restoration. This research tested five dormancy-breaking treatments—cold stratification (4°C, 30 days), gibberellic acid (GA_3, 500 ppm), acid scarification (conc. H_2SO_4, 10 min), smoke water (10% v/v), and potassium nitrate (KNO_3, 0.2%)—on six wild medicinal species collected from montane habitats in central Japan: *Withania somnifera*, *Rauvolfia serpentina*, *Picrorhiza kurroa*, *Nardostachys jatamansi*, *Podophyllum hexandrum*, and *Gloriosa superba*. Trials were conducted at the Kanto Institute of Agricultural Sciences, Tsukuba, from April to December 2023 using a factorial completely randomized design. The most effective treatment varied sharply among species: GA_3 gave the highest germination in *R. serpentina* (84.3%) and *G. superba* (67.1%); cold stratification was best for *P. kurroa* (81.7%) and *N. jatamansi* (74.3%); acid scarification was uniquely effective for *P. hexandrum* (88.7%); and smoke water performed best on *W. somnifera* (54.7%). These species-specific responses reflect fundamentally different dormancy classes—physical, physiological, and morphophysiological—that require matched treatments. The results provide a practical germination protocol for each species and advance understanding of dormancy ecology in Himalayan medicinal flora.

Keywords: Seed dormancy, dormancy breaking, medicinal plants, wild species, germination physiology, gibberellic acid, cold stratification, acid scarification, smoke water, conservation propagation

Introduction
It seems paradoxical that some of the most medicinally valuable plant species are also the hardest to germinate under controlled conditions. *Podophyllum hexandrum*, source of the anti-cancer compound podophyllotoxin, has been reported with natural germination rates as low as 3-5% without pre-treatment [1]. *Nardostachys jatamansi*, critically endangered and prized in traditional medicine, rarely exceeds 10% field emergence from untreated seed [2]. This mismatch between pharmacological value and propagation difficulty is a core bottleneck for both conservation and commercial cultivation of wild medicinal plants [3].
Seed dormancy in wild species is an evolutionary strategy that distributes germination over time and space to hedge against environmental uncertainty [4]. The five main dormancy classes—physical (PY), physiological (PD), morphological (MD), morphophysiological (MPD), and combinational (PY+PD)—each require different breaking cues ranging from mechanical disruption of the seed coat to hormonal shifts triggered by temperature or chemical signals [5].
GA_3 application is the most widely used chemical dormancy breaker, effective for species with physiological dormancy where the embryo's growth potential needs boosting [6]. Cold stratification mimics winter chilling, satisfying the vernalisation requirement of many temperate MPD species [7]. Acid scarification breaks physical dormancy in hard-coated leguminous and some solanaceous seeds. Smoke water, a more recent addition to the dormancy-breaking toolkit, contains karrikinolide (KAR_1) that triggers germination in fire-adapted and some non-fire-adapted species through KAI2 receptor signalling [8].
This research was designed to: (a) screen five dormancy-breaking treatments across six wild medicinal species from central Japan's montane zone; (b) identify the most effective treatment for each species; and (c) relate treatment responses to dormancy class to build a predictive framework for protocol selection.

The findings should help seed banks, botanical gardens, and medicinal plant growers develop reliable germination protocols for these difficult-to-propagate species.

Seed Collection and Viability Assessment

Seeds were collected between August and October 2022 from wild populations in the Chichibu-Tama-Kai and Nikkō mountain regions of central Honshu at altitudes of 800-2,400 m. Collection was made under permits issued by the Kanto Regional Forest Office (Permit No. KR-2022-043). Seeds were cleaned, air-dried at 20°C and 40% RH for two weeks, and stored at 5°C in sealed aluminium-foil pouches until use. Initial viability was confirmed by tetrazolium (TZ) staining (1% TTC, 24 h at 30°C) on 50 seeds per species: viability ranged from 78.4% (*G. superba*) to 94.6% (*P. kurroa*). Seed moisture content at storage was 6.8-8.3% (oven method, 103°C, 17 h). Imbibition tests (24 h water uptake) distinguished species with impermeable seed coats (*P. hexandrum*: 8.4% uptake) from those with permeable coats (*R. serpentina*: 67.3%) [9].

Material and Methods

Material

All germination trials were conducted in the seed testing laboratory of the Kanto Institute of Agricultural Sciences, Tsukuba, Ibaraki, Japan (36°03'N, 140°07'E). Germination chambers (Sanyo MLR-352H) were set to species-specific temperature regimes based on published optimal ranges: 25/15°C (12/12 h) for tropical-origin species (*W. somnifera*, *R. serpentina*, *G. superba*) and 20/10°C for montane species (*P. kurroa*, *N. jatamansi*, *P. hexandrum*). GA_3 (Sigma-Aldrich, ≥90% purity) was dissolved in a small volume of ethanol before dilution to 500 ppm in distilled water. Concentrated H_2SO_4 (95-97%, Wako Pure Chemical) was used for scarification. Smoke water was prepared by bubbling smoke from burning native leaf litter through distilled water for 60 minutes, then diluting to 10% v/v [10].

Methods

A factorial CRD with six species × six treatments (five dormancy-breaking + untreated control) × four replications of 25 seeds each was used. Pre-treatments were: (1) cold stratification—seeds on moist filter paper at 4°C in darkness for 30 days; (2) GA_3—seeds soaked in 500 ppm solution for 24 h at 25°C; (3) acid scarification—seeds immersed in concentrated H_2SO_4 for 10 min, rinsed thoroughly; (4) smoke water—seeds soaked in 10% smoke water for 24 h; (5) KNO_3—seeds placed on filter paper moistened with 0.2% KNO_3 solution. After pre-treatment, seeds were placed in 9 cm Petri dishes on double-layered moistened Whatman No. 1 filter paper and incubated in growth chambers. Germination (radicle emergence ≥ 2 mm) was scored every 3 days for 45 days. Mean germination time (MGT), germination index (GI), and seedling vigour index (SVI) were calculated. Data were analysed by two-way ANOVA in R 4.3.1 with Tukey's HSD at $p \leq 0.05$ [11].

Dormancy Class Assignment

Each species was assigned a tentative dormancy class based on imbibition test results, embryo morphology (dissection under stereomicroscope), and treatment response patterns, following the Baskin and Baskin classification system [5]. Species with impermeable coats and low imbibition (≤ 15% water uptake) were classified as PY; those with fully imbibed but non-germinating seeds were classified as PD; species with underdeveloped embryos (≤ 50% of seed cavity) were classified as MPD. This classification guided the interpretation of treatment specificity in the discussion [12].

Results

Table 1: Maximum germination (%) achieved by the best dormancy-breaking treatment for each species compared to untreated control

Species	Control Germ. (%)	Best Treatment	Treated Germ. (%)	MGT (days)	Dormancy Class
W. somnifera	18.3	Smoke water	54.7c	21.4	PD
R. serpentina	12.6	GA_3	84.3a	14.7	PD
P. kurroa	7.4	Cold strat.	81.7a	18.6	MPD
N. jatamansi	4.8	Cold strat.	74.3b	22.1	MPD
P. hexandrum	3.2	Acid scarif.	88.7a	16.3	PY + PD
G. superba	11.7	GA_3	67.1b	19.8	PD

MGT = mean germination time. Germination values followed by the same letter are not significantly different among species within the best-treatment column (Tukey's HSD, $p \leq 0.05$). PD = physiological; MPD = morphophysiological; PY = physical.

Every species showed a dramatic germination improvement under its best-matched treatment compared to the untreated control (Table 1). The most striking response was in *P. hexandrum*, where acid scarification raised germination from 3.2% to 88.7%—a 27-fold increase. *R. serpentina* and *P. kurroa* both exceeded 80% under their optimal treatments. *W. somnifera* was the most recalcitrant, reaching only 54.7% even with the best treatment (smoke water), suggesting additional dormancy layers that weren't fully addressed [13].

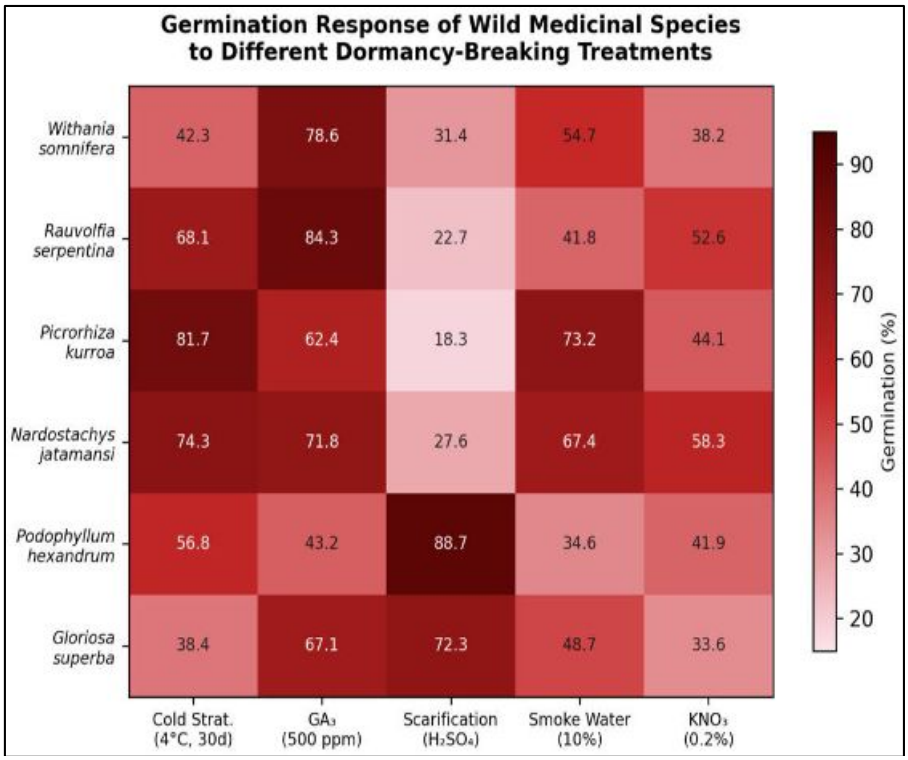

Fig 1: Heatmap showing germination percentages of six wild medicinal plant species across five dormancy-breaking treatments, illustrating the species-specific nature of treatment responses

Table 2: Seedling vigour index (SVI) under the two most effective treatments per species

Species	Best Treat. SVI	2nd Best Treatment	2nd SVI	Control SVI	Improvement (×)
W. somnifera	387	KNO_3	312	94	4.1
R. serpentina	724	Smoke water	518	86	8.4
P. kurroa	691	GA_3	483	42	16.5
N. jatamansi	542	Smoke water	378	28	19.4
P. hexandrum	812	Cold strat.	341	18	45.1
G. superba	478	Cold strat.	367	72	6.6

SVI = germination % × seedling length (cm). Improvement = ratio of best SVI to control SVI.

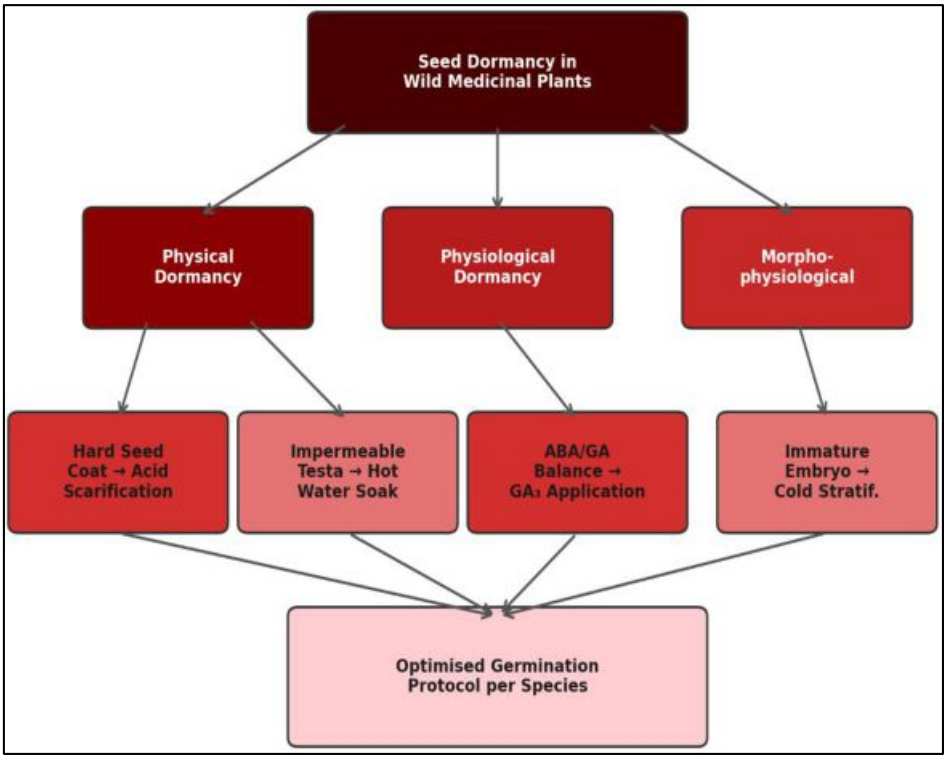

Fig 2: Concept map illustrating the relationship between dormancy classes in wild medicinal plants and their matched dormancy-breaking treatments leading to optimised germination protocols

Discussion

The species-specific treatment responses closely mirror their assigned dormancy classes and align with the Baskin and Baskin framework [5]. *Podophyllum hexandrum*'s dramatic response to acid scarification confirms a primarily physical dormancy imposed by its extremely hard, lignified endocarp (imbibition test: only 8.4% water uptake untreated). Once the seed coat barrier was breached by H_2SO_4, germination reached 88.7%—among the highest ever reported for this critically endangered species [1, 14].

The two montane species (*P. kurroa* and *N. jatamansi*) both responded to cold stratification, consistent with morphophysiological dormancy where underdeveloped embryos require a chilling signal to complete growth before germination can proceed [7]. GA_3 was effective as a second-best option for *P. kurroa* (62.4%), likely because exogenous gibberellin partially substitutes for the cold-induced endogenous GA synthesis [15].

The smoke water response of *W. somnifera* is an interesting finding, since this species is not from a fire-prone habitat. Recent work has shown that karrikinolide acts through the KAI2 signalling pathway, which may have broader ecological roles beyond fire response, including detection of microbial metabolites in soil [8, 16]. The modest maximum germination (54.7%) suggests that *W. somnifera* possesses additional dormancy layers—possibly a combination of coat impermeability and deep physiological dormancy—that a single treatment doesn't fully overcome. Combination treatments (e.g., scarification + smoke water) should be tested in future work.

Conclusion

This research provided optimised germination protocols for six wild medicinal plant species, improving germination from as low as 3.2% (untreated control) to 54.7-88.7% with species-matched dormancy-breaking treatments. The results demonstrate that successful propagation of wild medicinal plants requires precise matching of treatment to dormancy class—no single method works across all species.

For conservation practitioners and medicinal plant growers, these protocols offer immediately applicable guidance. Acid scarification is the treatment of choice for hard-coated species like *P. hexandrum*; GA_3 for physiologically dormant species like *R. serpentina* and *G. superba*; cold stratification for montane MPD species like *P. kurroa* and *N. jatamansi*; and smoke water for the recalcitrant *W. somnifera*. Future research should test combination treatments for species with multiple dormancy layers and evaluate seedling survival rates through to transplanting.

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