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Research Article

Altitudinal Variation in Seed Morpho-Physiology and Germination Dynamics of Delphinium denudatum Wall. Ex. Hook. & Thoms. under Different Temperature Regimes

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Soban Prakash

High Altitude Plant Physiology Research Centre, H.N.B. Garhwal University (A Central University), Srinagar, Garhwal, Uttarakhand, India.

Sujeet Pratap Singh

Amity Institute of Biotechnology, Amity University Uttar Pradesh, Lucknow-226028, India

Harish Chandra

Department of Botany and Microbiology, Gurukula Kangri (Deemed to be University), Haridwar, Uttarakhand 249404, India

Ravi Kant Verma

CSIR- Central Institute of Medicinal and Aromatic Plants

Karishma

Department of Forestry and NR, H.N.B. Garhwal University (A Central University), Srinagar, Garhwal, Uttarakhand, India.

Babita Patni

High Altitude Plant Physiology Research Centre, H.N.B. Garhwal University (A Central University), Srinagar, Garhwal, Uttarakhand, India.

Abstract

Delphinium denudatum Wall. Ex. Hook. & Thoms. (Nirbisi or Jadwar), is an important medicinal plant widely used in both Ayurvedic and Unani systems for its diverse therapeutic benefits. Uncontrolled extraction from the wild combined with severely limited natural regeneration has reduced its populations to a precarious state, making scientifically grounded propagation strategies a matter of considerable urgency. The present study characterised seed morpho-physiological attributes across three altitudinal populations in Pauri Garhwal district, Uttarakhand, collected from 850 m, 1550 m, and 2000 m above sea level (asl), and assessed the joint influence of gibberellic acid (GA_3) concentration (100, 200, and 300 ppm) and 4 temperature regimes (15°C, 20°C, 25°C, and greenhouse conditions) on germination percentage (GP%), mean germination time (MGT), and mean daily germination (MDG). Seeds from all three altitudinal populations recorded 100% viability at the time of harvest. Seed size and weight varied significantly across altitudes ($P< 0.01$), with the 1550 m population consistently yielding the largest seeds (length: 1.89 ± 0.18 mm; width: 1.10 ± 0.12 mm; weight: 0.13 ± 0.04 g). Seed moisture content and imbibition capacity also showed marked altitudinal differences. Among all treatment combinations evaluated, seeds from the 1550 m population pre-treated with 300 ppm GA_3 (treatment Gb3) achieved the highest germination percentage

(88.88 ± 9.62%) at 20 °C, alongside the highest MDG (0.29 ± 0.05). These findings establish clear evidence base for developing large-scale propagation protocols and ex situ conservation strategies for this threatened Himalayan species.

Keywords: Delphinium denudatum; gibberellic acid; seed germination; altitudinal variation; TTC viability test; ex situ conservation; Himalayan medicinal plants

1. INTRODUCTION

India is home to one of the most deeply rooted plant-based medicine traditions in the world, a heritage that continues to guide healthcare decisions for millions of people across rural and tribal communities. The country accounts for approximately 20% of all medicinal plant species catalogued by the World Health Organization, a figure that speaks to the extraordinary phytodiversity of the subcontinent [1].

The Himalayan region is one of India's richest centers of medicinal plant diversity. Studies have shown that the Indian Himalaya is home to more than 8,000 vascular plant species, including around 1,748 species of medicinal importance. In many mountain communities, this plant knowledge is still actively practiced and passed down through generations, especially in the western Himalaya, where medicinal plants continue to play an important role in local healthcare traditions. These resources are also deeply connected with classical Indian systems of medicine, particularly Ayurveda and Unani, reflecting the long-standing relationship between Himalayan biodiversity and traditional healing practices [2, 3, 4, 5].

Delphinium denudatum Wall. Ex. Hook. & Thoms. (Ranunculaceae), traded and prescribed under the names Jadwar and Nirbisi, is one of the most medicinally prominent plants of the western Himalayan belt. The drug is derived from its dried tuberous roots, which are rich in norditerpenoid and diterpenoid alkaloids as the principal bioactive constituents [6, 7]. The roots are used ethnobotanically for a wide range of conditions including toothache, rheumatism, diuretic complaints, antipyretic treatment, snakebite, fungal infection, piles, and aconite poisoning. In the Unani system, the plant is additionally prescribed as a tonic and in the management of epilepsy [8, 9]. Its role in Ayurvedic formulations for neurological and inflammatory disorders further underscores both its therapeutic breadth and its continued demand.

The genus Delphinium encompasses approximately 370 species distributed across the temperate zones of the northern hemisphere. In India, 27 species and two infra-specific taxa have been recorded, largely confined to the temperate and alpine belts of Jammu and Kashmir, Himachal Pradesh, and Uttarakhand [10, 11]. D. denudatum is distributed across the western and central Himalayan belt, including adjoining areas of Pakistan and Nepal, at elevations ranging from 1500 to 2700 m asl. Despite its apparent distribution range, the species has been assigned a Critically Endangered status in the northwest Himalayas [12], principally because of unsustainable harvesting of wild roots for commercial trade, ongoing habitat degradation, and markedly restricted natural regeneration.

Seed germination is the most critical phase of plant recruitment and one of the most ecologically sensitive stages in the life cycle of mountain medicinal plants. Temperature is among the most important environmental regulators of germination, governing enzymatic activity, the imbibition of water, and the activation of growth-related hormones [13]. In species distributed along altitudinal gradients, seed quality often varies substantially between populations, with morphometric attributes such as seed size and weight linked to both dispersal capacity and post-dispersal seedling establishment [14, 15, 16]. Smaller seeds, particularly those with morphological adaptations for aerial dispersal, tend to travel further from the parent plant, while larger seeds typically furnish greater nutritional reserves that buffer seedlings against early environmental stress [17].

Gibberellic acid (GA3) is a well-established phytohormone that promotes germination by stimulating the synthesis of hydrolytic enzymes in the aleurone layer, mobilising endosperm reserves, and effectively counteracting physiological dormancy [18]. Its application at varying concentrations, in interaction with temperature, has been shown to improve germination in an endangered Rhododendron species at 15–20 °C and to increase germination speed [19].

Despite the well-recognised conservation urgency and widespread medicinal use of D. denudatum, systematic scientific investigation of its germination ecology and seed physiological responses to altitudinal origin and pre-treatment conditions remains limited. The present study was therefore conducted with three specific objectives: (i) to characterise seed viability and morpho-physiological attributes, including morphometrics, moisture content, and imbibition capacity, across three altitudinal populations in Pauri Garhwal, Uttarakhand; (ii) to evaluate the effects of GA3 concentration and temperature regime on GP%, MGT, and MDG; and (iii) to identify the seed source and treatment combination most suitable for efficient large-scale propagation and ex situ conservation of this Critically Endangered species.

2. MATERIALS AND METHODS

2.1 Study Area and Seed Collection

Seeds of D. denudatum were collected during late May to early June from three altitudinal sites in Pauri Garhwal district, Uttarakhand, India: Site I at 850 m asl (S1), Site II at 1550 m asl (S2), and Site III at 2000 m asl (S3). After collection, seeds were shade-dried to minimise desiccation stress, sealed in airtight polythene zip-lock bags, and stored at 4 °C at the laboratory of the High-Altitude Plant Physiology Research Centre (HAPPRC), HNBGU, Srinagar Garhwal, Uttarakhand, until further experimental use.

2.2 Seed Morphological Characterisation

Seed morphometric attributes, specifically length and width, were measured using a calibrated digital vernier calliper. A minimum of 30 seeds per altitudinal population were individually measured in triplicate. Seed weight was recorded on an analytical balance. Mean values with standard deviations were calculated for each population, and altitudinal effects were assessed using one-way analysis of variance (ANOVA).

2.3 Seed Viability Assessment

Seed viability was assessed by the tetrazolium (TZ) bioassay method of Moore (1962). Thirty seeds from each population, arranged in triplicate, were immersed in a freshly prepared 0.1% (w/v) solution of 2,3,5-triphenyl tetrazolium chloride (TTC) adjusted to pH 6.0, then incubated at 37 °C in darkness for 24 hours. Seeds showing red or pink coloration of the embryo and cotyledon were recorded as viable; seeds remaining colorless were classified as non-viable.

2.4 Seed Moisture Content and Imbibition Percentage

Seed moisture content was determined gravimetrically using the oven-drying method [21]. Fresh weight was recorded before seeds were dried at 103 °C for 17 hours; dried weight was then measured. Moisture content was expressed as a percentage of fresh weight.

For imbibition assessment, pre-weighed seeds from each population were soaked in distilled water at room temperature for 24 hours, then gently surface-dried with filter paper and re-weighed. Imbibition percentage was calculated as the proportion of water absorbed relative to the initial dry seed weight, expressed as a percentage.

2.5 The Impact of Temperature Regimes on the Germination Process of Delphinium denudatum Seeds

The influence of temperature on the germination of seeds and the pace at which germination occurs is of great significance. Specific biochemical reactions governing the metabolism associated with seed germination are activated within a certain temperature range, although this range can vary across different species. Moreover, the process of seed imbibition, which encompasses the uptake of water, is also significantly influenced by temperature variations [13]. To determine the most favorable temperature for the germination of D. denudatum seeds, an empirical study was performed. This experiment encompassed four distinct temperature regimes: 15ºC, 20ºC, and 25ºC. The study was conducted within a controlled environment. Seeds obtained from different altitude (i.e., 850 m (S1), 1550 m (S2) and 2000 m (S3) asl, respectively), prior to the germination experiment, were surface sterilized with 0.5% HgCl2 for 2 minutes. Afterwards, various concentrations of pretreatment were applied to the seeds (100, 200 and 300 ppm) of GA3, for 24 h and evaluated in contrast to the control (distilled water) (Table 1). Each treatment was reproduced in replicates by adding 15 pretreated seeds in each glass petri dish with Whatman number 1 filter paper (90 mm), and each of the Petri dishes was put in a seed germinator (SG-11-D, SR Lab, India) at a temperature of 15°C, 20°C and 25°C, respectively. In Styrofoam trays with a 1:1:1 mixture of soil, forest litter, and sand, similar pretreated seeds were sown (at 1.5 cm depth) and placed in greenhouse conditions. In the greenhouse, the temperature during day time was 30±2.5 °C while the night temperature was 25±1.5°C and during the experiment, the relative humidity ranged from 65 to 80 percent. Subsequently, the germination progress was meticulously observed and recorded. The emergence of the radicle, the embryonic root, was identified as the commencement of germination, and observations were continued until no further germination activity was noted [21].

2.6 Germination Indices

Germination percentage (GP%) was calculated as:

$$GP\ (\%) = (\text{Total germinated seeds} / \text{Total seeds sown}) \times 100$$

Mean germination time (MGT) was determined using the formula of Ellis and Roberts (1981):

$$MGT = \Sigma\ (n_i \times t_i)\ /\ \Sigma n_i$$

where n_i is the number of seeds germinating on day t_i.

Mean daily germination (MDG) was computed using the formula of Czabator (1962):

$$MDG = \text{Total germinated seeds} / \text{Total germination period (days)}$$

Table 1. Seed pretreatment combinations used in germination experiments across three altitudinal populations of Delphinium denudatum

S.No.	Code	Description
1	Ca	Control: S1 seeds (850 m) soaked in distilled water, placed in seed germinator
2	Cb	Control: S2 seeds (1550 m) soaked in distilled water, placed in seed germinator
3	Cc	Control: S3 seeds (2000 m) soaked in distilled water, placed in seed germinator
4	Ga1	S1 seeds treated with GA_3 at 100 ppm, placed in seed germinator
5	Ga2	S1 seeds treated with GA_3 at 200 ppm, placed in seed germinator
6	Ga3	S1 seeds treated with GA_3 at 300 ppm, placed in seed germinator

S.No.	Code	Description
7	Gb1	S2 seeds treated with GA_3 at 100 ppm, placed in seed germinator
8	Gb2	S2 seeds treated with GA_3 at 200 ppm, placed in seed germinator
9	Gb3	S2 seeds treated with GA_3 at 300 ppm, placed in seed germinator
10	Gc1	S3 seeds treated with GA_3 at 100 ppm, placed in seed germinator
11	Gc2	S3 seeds treated with GA_3 at 200 ppm, placed in seed germinator
12	Gc3	S3 seeds treated with GA_3 at 300 ppm, placed in seed germinator

S1 = 850 m asl; S2 = 1550 m asl; S3 = 2000 m asl; GA_3 = gibberellic acid.

2.7 Statistical Analysis

All data were subjected to one-way and two-way ANOVA as appropriate, with post-hoc comparisons conducted using Duncan's Multiple Range Test (DMRT). Results are expressed as means ± standard deviation. Statistical significance was accepted at $P < 0.05$ (*), $P < 0.01$ (**), and $P < 0.001$ (***), with non-significant results denoted ns.

3. RESULTS

3.1 Seed Viability

Seeds of D. denudatum from all three altitudinal populations exhibited 100% viability at the time of harvest, as confirmed by the TTC bioassay. In every tested seed, the embryo and cotyledonary tissues developed a consistent red coloration upon incubation in the tetrazolium solution, confirming active metabolic function and physiological integrity across all altitudes and replicates.

3.2 Seed Morpho-Physiological Attributes

3.2.1 Seed Morphometrics

Altitudinal variation in seed size and weight was statistically highly significant ($P < 0.01$) across the three populations. Seeds from the 1550 m population (S2) were the largest in all three dimensions, with mean seed length of 1.89 ± 0.18 mm, mean width of 1.10 ± 0.12 mm, and the highest mean seed weight of 0.13 ± 0.04 g. The 850 m population (S1) produced seeds of intermediate dimensions (length: 0.553 ± 0.13 mm; width: 0.55 ± 0.36 mm; weight: 0.08 ± 0.02 g), while the 2000 m population (S3) produced the smallest seeds across all parameters (length: 0.38 ± 0.17 mm; width: 0.26 ± 0.15 mm; weight: 0.07 ± 0.02 g). (Fig. 1)

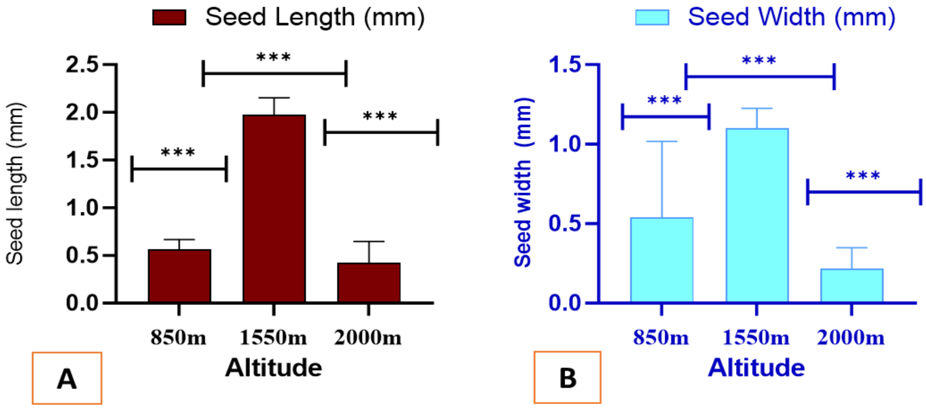

Figure 1. Physiological attributes of Delphinium denudatum seeds collected from three altitudes. (A) Seed length; (B) Seed width. ***p < 0.01 level.

3.2.2 Seed Moisture Content and Imbibition Percentage

Seed moisture content varied considerably across altitudes (P < 0.01). The 1550 m population recorded the lowest moisture content (8.19 ± 1.12%), contrasting with markedly higher values at 850 m (29.38 ± 4.19%) and 2000 m (23.80 ± 1.16%). Imbibition percentage showed a different pattern: the 1550 m population displayed the highest imbibition capacity (201.55 ± 11.95%), followed by the 850 m (161.65 ± 31.28%) and 2000 m (114.92 ± 18.65%) populations (Fig. 2). Particularly, the combination of lowest moisture content with highest imbibition percentage in the 1550 m population suggests a structurally distinct seed coat that restricts passive moisture uptake during storage while enabling efficient active hydration upon wetting.

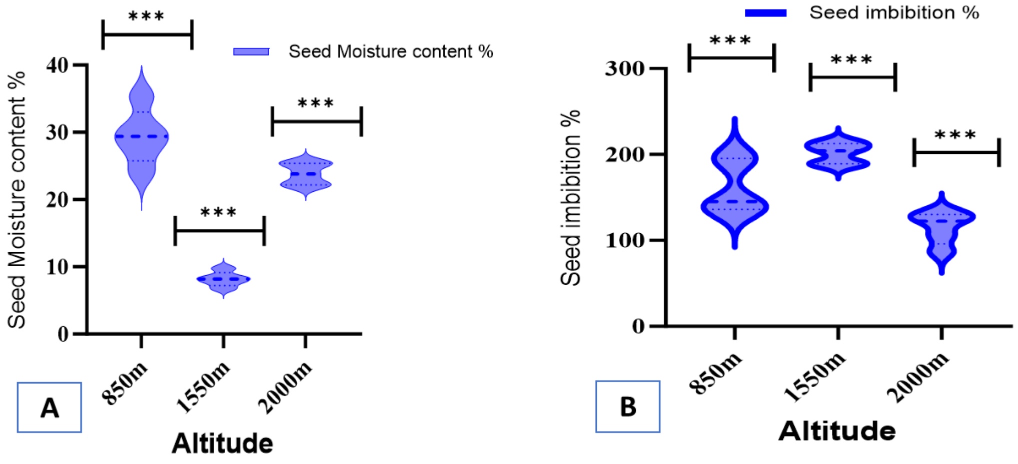

Figure 2. Physiological attributes of Delphinium denudatum seeds collected from three altitudes. (A) Seed moisture content; (B) Seed imbibition. ***p < 0.01 level.

3.3 Effect of Temperature and GA$_3$ on Germination Percentage

The ability of seed germination was considerably affected by temperature regimes. The Among the treatment seeds collected from 1550m altitude (Gb3) demonstrated the highest germination percentage (88.88±9.62%) followed by Gc1 (77.78±9.61 %, p<0.001) and Ga3 (72.22±9.61, p<0.001) at 20 ºC, The treatments Ca comparatively lower germination percentages (11.11±9.62 %) at 15 ºC. Across all temperature regimes, increasing GA*3* concentration was generally associated with improved germination outcomes, particularly at

20 °C (Table 3)

Table 3. Effect of different concentration of GA_3 treatments on percentage of seed germination of Delphinium denudatum under different temperature regimes.

Treatment	15°C	20°C	25°C	GH
Ca	11.11±9.62a	38.89±9.62a	44.44±9.65cb	33.33±0.0bcd
Cb	27.77±9.62a	38.88±9.62a	27.77±9.62abc	16.67±16.65ab
Cc	16.6±16.67a	33.33±16.66a	16.66±16.66a	22.22±9.61abc
Ga1	22.22±9.62a	66.67±0.00bc	50±0.00de	38.89±9.62cd
Ga2	27.77±9.62a	55.55±19.24ab	44.44±9.62cd	50±16.67d
Ga3	27.77±9.62a	72.22±9.61bc	66.66±16.65e	44.44±19.24bcd
Gb1	33.33±9.62a	66.67±0.00bc	33.33±9.62abcd	33.33±0.00bcd
Gb2	33.33±9.62a	61.11±9.62b	27.77±9.62abc	11.11±9.62a
Gb3	27.77±9.62a	88.88±9.62c	33.33±16.66abcd	16.67±0.00ab
Gc1	27.77±9.62a	77.78±9.61bc	38.88±9.62bcd	33.33±0.001bcd
Gc2	27.77±9.62a	33.33±16.66a	16.66±0.00a	16.67±0.00ab
Gc3	16±0.0a	66.66±16.66bc	22.22±9.62ab	11.11±9.62a
F value	1.09ns	6.95**	5.58**	4.90**

Values represent mean ± SD (n = 3). Significance: ** P < 0.01; ns = non-significant.

3.4 Effect of Temperature and GA_3 on Mean Germination Time (MGT)

Across the treatments and temperature conditions, mean germination time (MGT) exhibited varying trends. For instance, "Ga1" displayed the lowest MGT at 20°C (10.23±0.01 days, $p<0.05$), while "Gc3" showcased the highest MGT at 25°C (18±8.66 days) followed by "Gb3" at GH (17 days) (Table 4). The observed differences underscore the distinct response of D. denudatum germination time to varying temperature environments, indicating that temperature plays a role in influencing the temporal aspects of germination.

Table 4. Effect of different concentration of GA_3 treatments on Mean Germination Time of Delphinium denudatum under different temperature regimes.

Treatment	15°C	20°C	25°C	GH
Ca	12.66±10.44a	14.5±0.50abc	14.83±1.04a	15.33±0.58a
Cb	15.66±0.58a	11.9±0.79ab	15.66±1.52a	16.33±3.05a

Treatment	15°C	20°C	25°C	GH
Cc	11±9.54a	16.55±2.88c	12.22±10.68a	14.66±1.52a
Ga1	13.66±1.15a	10.23±0.01abcd	17.66±2.33a	14.78±0.7a
Ga2	14±1.00a	12.33±0.76ab	17.22±6.71a	15.44±2.5a
Ga3	16±0.39a	12.63±0.71ab	12.02±1.23b	16.16±1.9a
Gb1	14.77±3.28a	14±0.00abc	18.72±3.47a	14±0.00a
Gb2	16.50±2.89a	14.88±3.86bc	15±2.00a	10±8.89a
Gb3	16.33±1.53a	11.54±1.26a	14±1.00a	17±0.00a
Gc1	16.66±2.65a	13.20±0.72ab	13.22±1.67a	14±0.73a
Gc2	16±2.65a	13.11±1.83ab	16.33±1.15a	12.33±1.15a
Gc3	16.33±1.53a	13.37±1.22ab	18±8.66a	12.66±10.97a
F value	0.54ns	2.12*	0.69ns	0.80ns

Values represent mean ± SD (n = 3). Significance: * P < 0.05; ns = non-significant.

3.5 Effect of Temperature and GA_3 on Mean Daily Germination (MDG)

The MDG values varied distinctly among treatments under different temperature regimes. Among the observed treatments, "Gb3" demonstrated the highest MDG at 20°C (0.29±0.05 days, p<0.01) followed by "Ga3" at 25°C (0.28±0.05 days, p<0.001), whereas "Ca, Cb" showcased the lowest MDG at 15°C (0.03±0.03 days, ns) (Table 5). These variations highlight the sensitivity of D. denudatum germination to temperature shifts and the potential interplay between specific treatments and temperatures in influencing daily germination dynamics.

Table 5. Effect of different concentration of GA_3 treatments on Mean Daily Germination of D. denudatum under different temperature regimes.

Treatment	15°C	20°C	25°C	GH
Ca	0.03±0.03a	0.12±0.03a	0.15±0.04bc	0.12±0.02a
Cb	0.08±0.03ab	0.13±0.04a	0.10±0.04abc	0.04±0.02a
Cc	0.03±0.03a	0.14±0.08ab	0.07±0.07a	0.08±0.02a
Ga1	0.09±0.02ab	0.23±0.01abcd	0.16±0.10c	0.13±0.03a
Ga2	0.09±0.02ab	0.22±0.08abcd	0.18±0.02c	0.18±0.75a

Treatment	15°C	20°C	25°C	GH
Ga3	0.06±0.05ab	0.25±0.01bcd	0.28±0.05d	0.18±0.08a
Gb1	0.10±0.06b	0.26±0.01bcd	0.11±0.005abc	0.13±0.00a
Gb2	0.10±0.06b	0.15±0.11d	0.10±0.03abc	0.04±0.03a
Gb3	0.06±0.01ab	0.29±0.05cd	0.11±0.02abc	0.06±0.00a
Gc1	0.06±0.01ab	0.26±0.06a	0.16±0.06c	0.12±0.02a
Gc2	0.06±0.01ab	0.12±0.05abcd	0.06±0.004a	0.08±0.005a
Gc3	0.06±0.005ab	0.21±0.07abcd	0.07±0.03ab	0.03±0.03a
F value	1.46ns	3.14**	6.60**	0.49ns

Significant at *P<0.05, **P<0.001, ns—Non-significant.

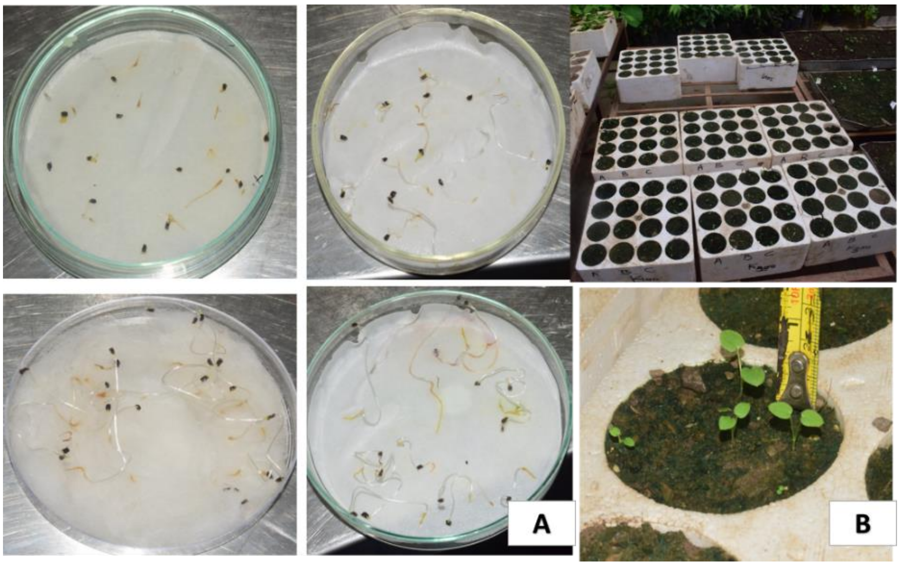

Plate 1: (A) Delphinium denudatum seedling emerging under seed germinator (SG); (B) D. denudatum seedling under Greenhouse condition (GH).

4. DISCUSSION

The results of this study reveal a consistent and interpretable pattern of altitudinal differentiation in seed morpho-physiology and germination performance across populations of D. denudatum. The 1550 m altitude population demonstrated clear superiority across multiple parameters, producing the largest and heaviest seeds, exhibiting the highest imbibition capacity, and achieving the greatest germination percentage under optimal treatment conditions. This pattern is consistent with the broader ecological understanding that larger seeds confer advantages during seedling establishment, particularly through the provision of greater stored nutritional reserves during the critical pre-photosynthetic stage [15, 16]. Seed size has long been recognised as a fundamental ecological trait with far-reaching consequences for dispersal, seedling competition, and community-level dynamics [14, 17].

The 100% seed viability recorded across all three populations at harvest, confirmed by the TTC bioassay [20], is an important and encouraging result for conservation planning. It indicates that D. denudatum seeds maintain strong physiological integrity when collected at the appropriate phenological stage and stored under cold conditions. This is consistent with results reported for other threatened Himalayan medicinal species tested by TTC-based viability assessment [24, 25]. Comparable low-temperature germination responses and GA3-mediated increases in germination speed have been reported in other endangered Rhododendron seed studies [19].

The contrasting combination of low moisture content and high imbibition capacity in the 1550 m population is particularly noteworthy. Seeds with lower initial moisture are generally associated with greater desiccation tolerance and improved storability, while a high imbibition response reflects an efficient hydration mechanism when water becomes available. This combination points to seed coat properties at intermediate altitude that may have been shaped by selection pressures favouring rapid and efficient germination during the relatively narrow seasonal window available at this elevation. Seeds from the 850 m population, by contrast, showed higher initial moisture content that likely reflects the more persistently humid microclimate at lower elevations, where selective pressure for tight water regulation may be less pronounced.

The identification of 20 °C as the optimal germination temperature across the majority of treatments is biologically coherent. This value closely approximates the soil surface temperatures experienced in the sub-alpine Himalayan zone during late spring and early summer, the natural germination window for the species. Below this optimum, at 15 °C, germination was markedly suppressed across all treatment groups, including those receiving the highest GA3 dose, confirming that temperature acts as a threshold factor below which hormonal stimulation cannot adequately compensate for reduced enzymatic efficiency. Comparable low-temperature optima (15–20 °C) and GA3-mediated gains in germination speed have been documented in the endangered Rhododendron protistum var. giganteum [19].

The stimulatory effect of exogenous GA3 on germination, especially at 300 ppm in the 1550 m seed source, is consistent with its established mode of action in breaking physiological dormancy. GA3 promotes the synthesis of alpha-amylase and other hydrolytic enzymes in the aleurone layer of seeds, facilitating the breakdown of endosperm storage reserves and mobilising the substrate necessary for radicle emergence [18]. The dose-response pattern observed here, in which 300 ppm consistently outperformed lower concentrations, aligns with findings for related Ranunculaceae species and suggests that the dormancy mechanism in D. denudatum is genuinely responsive to GA3 but requires a relatively elevated exogenous concentration for complete relief.

The moderate germination percentages obtained under greenhouse conditions, despite broadly comparable temperatures, may reflect the cumulative effects of diurnal temperature fluctuation, greater substrate resistance in the soil-based medium, and increased pathogen pressure relative to the controlled Petri dish environment. These are well-recognised sources of divergence between laboratory and field germination outcomes [13]. Taken together, the results clearly designate the 1550 m seed source pre-treated with 300 ppm GA3 at 20 °C as the most productive and practically viable combination for nursery-scale propagation of D. denudatum.

The literature cited in this context underscores the importance of different concentrations of GA3 (Gibberellic acid) and temperature on seed germination percentages. For instance, studies by Adhikary (2013) have demonstrated that varying concentrations of GA3 and temperature levels can significantly impact germination rates in different plant species, highlighting the intricate interplay between these factors in seed germination.

Additionally, in the case of Lilium polyphyllum, maximum seed weight was attributed to the highest seed moisture content in the Dhanaulti population, further emphasizing the role of moisture in seed weight determination. These findings collectively contribute to our understanding of the germination processes and environmental adaptability of D. denudatum, offering valuable insights for its conservation and propagation strategies [27].

5. CONCLUSION

This study provides the first systematic characterisation of altitudinal variation in seed morpho-physiology and germination ecology of Delphinium denudatum from the Garhwal Himalayas. Seeds from the 1550 m altitude population consistently demonstrated superior quality across all measured parameters: morphometrics, imbibition capacity, and germination performance alike. Pre-treatment with 300 ppm GA3 at a constant temperature of 20 °C was identified as the optimal propagation condition, yielding the highest germination percentage (88.88 ± 9.62%), the lowest mean germination time, and the highest mean daily germination rate. The 100% viability recorded at harvest across all three populations confirms that timely seed collection and cold storage at 4 °C together represent a reliable short-term conservation measure for this species.

These findings offer a practical, evidence-grounded framework for developing propagation protocols and ex situ conservation programmes for this Critically Endangered medicinal plant. Future research should examine long-term seed storability, the potential of vegetative propagation through rhizome division and tissue culture, and the transplantation success of nursery-raised seedlings across altitudinal reintroduction zones. Such integrated research is essential for reversing population decline and ensuring the long-term persistence of D. denudatum in the northwest Himalayan landscape.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS

Soban Prakash: data analysis, experimental work, and manuscript writing. Sujeet Pratap Singh: statistical data analysis. Harish Chandra: experimental work and manuscript finalizing. Ravi Kant Verma: experimental work. Karishma: field work and survey. Babita Patni: conceptualization, supervision, and overall research coordination.

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