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RESEARCH

Effect of elevated temperature and water stress on seed germination of the Himalayan medicinal herb *Aconitum spicatum*

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Background: Seed germination studies of high mountain plants across environmental gradients are potentially important for understanding the impacts of climate and other environmental changes. In this study, we analyzed the variation in seed germination patterns of the Himalayan medicinal herb *Aconitum spicatum* across temperature, light, and water stress gradients. Seeds of *A. spicatum* collected from three different elevations (low: 3,315, mid: 3,910, high: 4,200 m asl) were germinated in a growth chamber under different temperatures (low: 25/15°C; high: 30/20°C), light conditions (12-hour photoperiod and complete dark), and water potentials (–0.10, –0.25, –0.50, –0.75, –1 MPa).
Results: Seed mass and germination traits such as germination percentage (GP), mean germination time (MGT) and Timson's index (TI) did not vary consistently with elevation. While light did not affect germination, high temperatures significantly reduced GP and TI, and increased MGT when compared with low-temperature conditions. The GP declined from approximately 80% at control to less than 20% under mild water stress (–0.50 MPa) with complete inhibition at higher water stress levels. The MGT increased and TI declined with increasing water stress.
Conclusions: Our study demonstrated a significant negative impact of elevated temperatures and increased water stress on the germination of *A. spicatum*, an important medicinal herb of the Himalaya. These findings highlight the species' high vulnerability to the effects of climate change, particularly of the temperature increases and declining precipitation. We suggest incorporating potential impacts of warming and drought into strategies for the sustainable harvest and conservation of *A. spicatum* in future.

Keywords: alpine plants, climate change, elevation gradient, germination traits, seed mass

Introduction

The distribution of plants in mountainous areas is primarily influenced by the environmental conditions associated with the elevational gradient (Körner 2021). Human-mediated global environmental changes such as warming and increasing drought due to a decline in precipitation are expected to alter growth performance and population dynamics of the high mountain plant species (Dolezal et al. 2021; Steinbauer et al. 2018). Given these potential impacts, understanding how these species respond to changing environmental conditions is crucial. To effectively estimate the effects of climate change on these plant species, it is essential to comprehend the patterns of variation in sensitivity both within populations and across different developmental stages including seed germination (Dawson et al. 2011).

Among different life history traits, successful germination of a plant species under strong environmental selection pressure is important for determining its survival capacity in a particular area (Bhatt et al. 2021; Klupczyńska and Pawłowski 2021). Seed germination is considered a critical indicator of plant growth and tolerance to the global change in alpine habitats (Fernández-Pascual et al. 2021). Additionally, seed germination is regarded as a key driver of vegetation regeneration and species distribution (Baskin and Baskin 2014). Furthermore, temperature and moisture content significantly influence plant seed biology, particularly germination performance (Walck et al. 2011). These environmental cues, along with plant traits, often vary among populations of mountain plants due to the high microhabitat heterogeneity (Opedal et al. 2015). Therefore, understanding the variation in plant traits such as the germination performance across populations is crucial for assessing the impact of environmental changes on plant species (Dawson et al. 2011).

In heterogeneous landscape such as mountains, seed traits and germination behavior are expected to vary among populations growing under different habitat conditions. Seed mass tends to increase with rising elevation (Pluess et al. 2005) and seed dormancy is higher at high elevations where temperature and rainfall are low (Fernández-Pascual et al. 2013). Germination rates (germinability), timing, and dormancy are seed properties that may differ between plant populations of the same species (Pérez-García 2009). Larger seeds generally exhibit better germination and establishment success compared to smaller seeds (Hodkinson et al. 1998) while smaller seeds tend to germinate more quickly (Howell 1981). Variation in seed traits including germination performance has been studied in several plant species across different regions (Bhatt et al. 2021; Cochrane et al. 2015). Such variation among populations is expected to attenuate the negative impacts of environmental changes such as climate shifts (Cochrane et al. 2015). Although the variation in several functional and life history traits of mountain plants along elevation gradients has been examined (e.g., Chapagain et al. 2019; Pandey et al. 2021), studies examining variation of seed germination among populations along elevation gradients are very scarce in the Himalaya (e.g., Saklani et al. 2012; Wang et al. 2021).

The genus *Aconitum* is typically found in the mountainous regions of the Nepal Himalaya and is represented by 28 species in Nepal (Shrestha et al. 2022). With approximately 400 species worldwide, primarily in the mountain regions of the Northern Hemisphere, many *Aconitum* species possess significant pharmacological properties and therapeutic values (Ali et al. 2023). One of such medicinal species is *Aconitum spicatum* (Brühl) Stapf, found in the high mountain regions of the Himalaya (Shrestha et al. 2022). This species is one of seven *Aconitum* species traded in Nepal, where it is exclusively collected from wild populations (Pyakurel et al. 2019). Destructive harvesting practices have already threatened the wild populations of this species (Chapagain et al. 2019), and climate change may further exacerbate these threats by altering the species' habitat and reducing its resilience. This indicates the urgent need for sustainable harvesting and conservation planning to prevent the further decline. Such planning requires adequate biological and ecological knowledge of the species, yet few studies have examined *A. spicatum* in this context (e.g., Chapagain et al. 2019). In this study, we examined (i) how seed mass and germination patterns vary among populations at different elevations and (ii) how the germination of seeds from populations at different elevations responds to variations in light, temperature, and water stress gradients. The results of this study can contribute to understanding the population status of *A. spicatum* in the wild and predicting the potential impacts of climate change on seed germination of this species.

Materials and Methods

Study species

Aconitum spicatum (Brühl) Stapf (Family: Ranunculaceae; Syn: *Aconitum lethale* Griff.), commonly known as monkshood, is a herbaceous, perennial, medicinal plant native to the Himalaya, with an erect stem reaching up to two meters in height (Ghimire et al. 1999). It is distributed across the Himalayan countries including Nepal, India, Bhutan and China at elevations between 1,800–4,800 meters above sea level (m asl) (Shrestha et al. 2022). Each monkshood plant produces about 1–110 fruits, and each fruit contains an average of 41 seeds (Chapagain et al. 2019) (Fig. 1). As one of the ten most marketed medicinal plants from Nepal's Himalaya, *A. spicatum* had a trade volume exceeding 300 kg/year for its rhizome (DoF 2017; Olsen and Larsen, 2003). In Nepal, *A. spicatum* is commonly used for treating fever, headache, cuts and wounds, as well as lymphatic, nerve and lung disorders after detoxification (Ghimire et al. 2021). The tuber of this plant is rich in norditerpenoid alkaloids (Gao et al. 2006) and is known for its antimicrobial and cytotoxic activities (Ali et al. 2023).

Seed collection and processing

Seeds were collected from the Annapurna Rural Municipality in the Kaski district of Gandaki Province, which lies within Annapurna Conservation Area (ACA) in the central Nepal. Three sites were selected along Modi River Valley on the route to the Annapurna Base Camp for seed collection, based on the abundance of individuals (sufficient for seed collection) and the elevation range of distribution (low: 3,315, mid: 3,910, high: 4,200 m asl) (Table 1). Seasonal sheepfolds were located close to all three collection sites.

Mature and healthy fruits of *A. spicatum* were collected in November 2022. The fruits containing seeds were placed in cloth bags and hung outside for air drying during the field work period. Upon return to the laboratory, seeds were extracted from the fruits, air-dried in the shade for a week, cleaned, and kept in airtight plastic bottles with silica gel (mesh size: 6–20). The bottles were kept in a refrigerator at 4°C until the germination experiments were initiated.

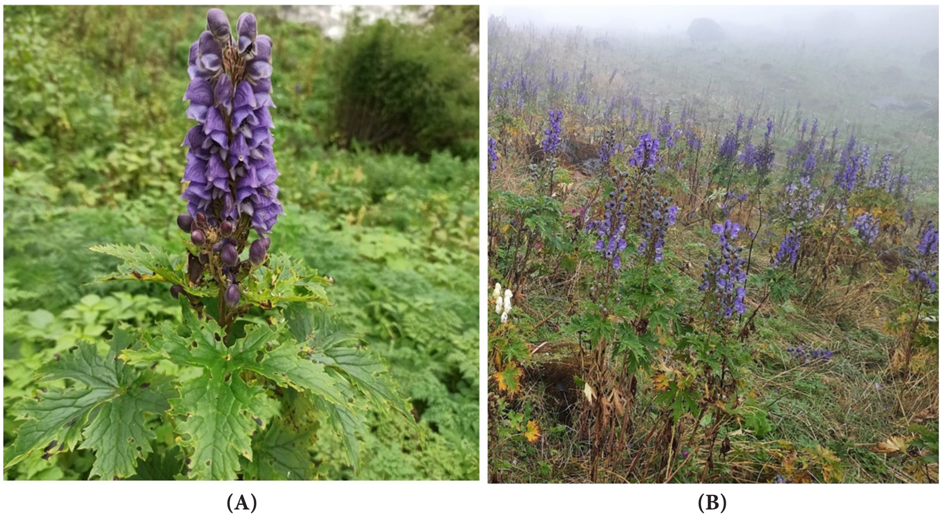

Fig. 1 (A) Individual plant with flower. (B) Habitat of *Aconitum spicatum*.

Table 1 Details of the seed collection sites of *Aconitum spicatum*

Elevation (m asl)	Locality	Latitude	Longitude	Habitat
Low (3,315)	Deurali	28.50935	83.90481	Forest margin along river bank
Middle (3,910)	Between Machhapuchre Base Camp and Annapurna Base Camp	28.52886	83.89433	Alpine grassland
High (4,200)	Annapurna Base Camp	28.53037	83.87641	Alpine grassland

These sites are located in Annapurna Rural Municipality of Kaski district within Annapurna Conservation Area.
m asl: meters above sea level.

Seed mass measurement

For seed mass, three lots of mature seeds (50 seeds in each) from each elevation were oven-dried at 60°C for 72 hours (Baskin and Baskin 2014). Seed mass was measured using a digit weighing balance (0.0001 g) (Model: Mg214Ai; Bel Engineering, Monza, Italy).

Germination experiments

After six days of cold storage, a preliminary germination test was performed. Seeds began to germinate after 30 days of incubation indicating a physiological or morphophysiological dormancy. Therefore, the seed germination experiment was initiated after 36 days of cold storage. Germination experiments were carried out in Petri dishes incubated under different light (12-hour/12-hour photoperiod and complete darkness), temperature (low: 25/15°C and high 30/20°C; light/dark) and water stress conditions within a growth chamber (Model: GC-300TLH; Jeio Tech, Daejeon, Korea). Inside the growth chamber, white fluorescent light with an intensity of 44.78 μ mol/m^2/s was maintained with a 12-hour/12-hour light-dark cycle and 70% humidity. To ensure complete darkness, the Petri dishes were wrapped by double-layered aluminum foil. The low temperature regime closely corresponds to the mean maximum and minimum air temperatures during the early summer months (April–May), as indicated by the meteorological data recorded at the nearest weather station (Fig. S1). The high temperature regime aligns with the predicted global temperature increase (+4.4°C) under the extreme greenhouse gas emission scenario (Shared Socio-economic 5–8.5, IPCC 2023). Given the declining precipitation reported in the high mountain regions of the Himalaya (Pepin et al. 2022), understanding the impact of water stress on seed germination in high mountain plants is crucial. Thus, in this experiment, seeds were incubated at different levels of water stress (−0.10, −0.25, −0.50, −0.75 and −1 MPa) using polyethylene glycol (PEG 6000) solutions. The PEG solution of −1 MPa was prepared by dissolving 296 g of PEG in 1 L of distilled water (Michel and Kaufmann 1973). This served as a stock solution and solutions with higher water potential (low concentration) were prepared by serial dilution method. The effect of water stress on germination was examined only under the low-temperature regime.

There were five replicates (Petri dishes) for each treatment, with 30 seeds in each replicate, resulting in a total of 150 seeds incubated under each environmental condition. Each Petri dish (9 cm diameter) was lined with a double-layer of Whattman No.1 filter paper and 30 seeds were placed at equal distances (Baskin and Baskin 2014; Prakash et al. 2011). For the control and complete darkness treatment, only distilled water (3 mL) was used to moisten the filter paper. Except for the complete darkness treatment, seeds were observed daily to record the number of seeds that had germinated. Seeds were considered germinated when a radicle of ≥ 1 mm emerged (Fig. S2) (Baskin and Baskin 2014). Germinated seeds were removed during each observation. The germination experiment was conducted over 20 days. For seed incubated under complete darkness, germination was recorded on the last day (day 20) of the experiment (Bhatt et al. 2021; Herranz et al. 2010).

Data analysis

Daily records of seed germination in Petri dishes were used to calculate germination percentage (GP), Timson's index (TI), and mean germination time (MGT) following Baskin and Baskin (2014). The GP was calculated as the number of seeds germinated expressed as a percentage of the total number of seeds incubated. TI, a measure of germination rate, was calculated as the sum of cumulative daily GPs obtained for each Petri dish (Baskin and Baskin 2014). Since the germination experiment lasted 20 days, the maximum possible value for TI was 2,000. Finally, MGT, a measure of time it takes for the majority of seeds to germinate, was calculated using the formula $\sum_{i=1}^{k} \mathrm{n}it i/\sum_{i=1}^{k} \mathrm{n}i$, where, t_i is the time from the start of the experiment to the i^{th} observation (day), n_i is the number of seeds germinated at time i, and k is the final day of the germination experiment (Baskin and Baskin 2014).

Statistical analyses were performed using the Statistical Package for Social Science (SPSS), version 25 (IBM Corp. 2017). Data were tested for normality (Shapiro–Wilk test) and homogeneity of variance (Leven's test) prior to the parametric test. To meet the assumptions of analysis of variance (ANOVA), the GP was first square root transformed and then arcsine transformed (Ahrens et al. 1990). A one-way ANOVA along with Tukey's test was conducted to assess differences in GP, MGT and TI among populations from different elevations. Additionally, independent samples t-tests were performed to compare GP, MGT and TI within each elevation between temperature (low and high) and light (12-hour photoperiod and complete darkness) conditions. We used two-way ANOVA to assess the effect of the interactions between elevation and temperature on germination traits.

Results

Seed mass

Among the seeds of *A. spicatum* collected from the three different elevations, highest seed mass was found at the low elevation which was twice as high as the seed mass collected from the middle elevation (Table 2).

Germination pattern

Effect of light and temperature

There was no difference in GP between photoperiod and complete darkness conditions both at low (25/15°C) and high (30/20°C) temperatures (t-test; $p > 0.05$; Table S1). Within each elevation, a paired t-test revealed that germination was significantly higher at low temperature compared to high temperature under both photoperiod and complete darkness condition ($p \leq 0.05$) (Fig. 2). Under the 12-hour photoperiod, seed germination did not vary significantly among the three elevations at low temperature, however, it was significantly higher at the middle elevation under high temperature (Fig. 2A). In complete darkness, germination did not differ significantly among the three elevations at both high and low temperatures (Fig. 2B).

Table 2 Seed mass (mean ± deviation; $n = 3$) of *Aconitum spicatum* collected from different elevations

Elevation (m asl)	Seed mass (mg/seed)
Low (3,315)	1.21 ± 0.07
Middle (3,910)	0.64 ± 0.05
High (4,200)	1.04 ± 0.06

m asl: meters above sea level.

MGT was longer at high temperature compared to low temperature for seeds collected from each elevation (Fig. 3A). However, ANOVA result revealed that MGT did not vary significantly among the three elevations at either high or low temperatures. The TI, a measure of germination speed, was significantly higher at low temperature than at high temperature across all three elevations (Fig. 3B). Among the elevations, seeds from the middle elevation exhibited a significantly higher TI at high temperature, whereas no significant difference in TI was observed among elevations at low temperature.

Effect of water stress

Germination was observed only up to −0.50 MPa water potential; no germination occurred at −0.75 and −1 MPa water potentials. For seeds from all elevations, increased water stress (i.e., decreased water potential) resulted in a significant decrease in GP. Within each elevation, a one-way ANOVA followed by post-hoc Tukey's tests indicated that GP was reduced to its lowest value at −0.50 MPa for seeds collected from all three elevation sites (Fig. 4A). The absence of germination at −0.75 and −1 MPa highlights the sensitivity of *A. spicatum* seeds to severe water stress.

MGT increased with increasing water stress (Fig. 4B). The MGT was longest at −0.50 MPa water potential and shortest under control conditions. For seeds collected from each elevation, the TI generally declined as water stress increased, with the highest TI observed under control conditions and the lowest at −0.50 MPa (Fig. 4C). The decline in TI with increasing water stress was more pronounced in seeds collected from high and low elevations compared to those collected from the middle elevation.

The results of two-way ANOVA revealed that elevation had a significant impact on the TI but not on the GP and MGT (Table 3). However, water potential significantly af-

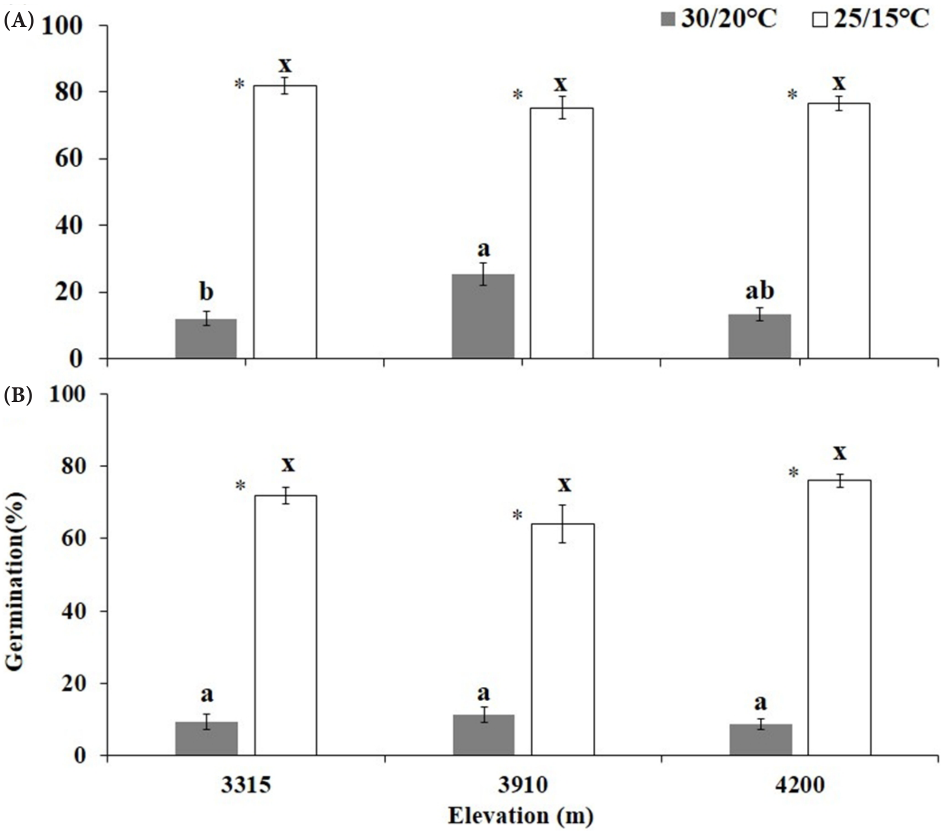

Fig. 2 Effect of temperature on germination percentage at (A) photoperiod and (B) complete dark. Error bars represent (± standard error) of the mean ($n = 5$). Different letters (a, b for high temperature and x for low temperature) above bars indicate significant difference ($p \leq 0.05$) among elevations (ANOVA) and '*' represents significant difference ($p \leq 0.05$) between high and low temperature within each elevation (t-test).

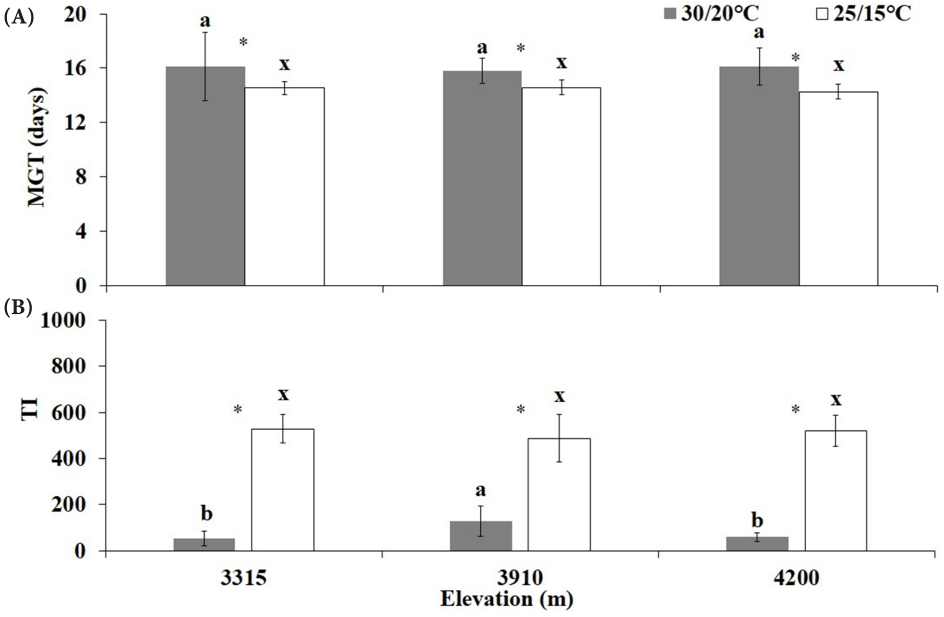

Fig. 3 Effect of temperature on (A) mean germination time (MGT) and (B) Timson's index (TI) of the seeds incubated under photoperiod condition. Error bars represent standard error of the mean ($n = 5$). Different letters (a, b for high temperature and x for low temperature) above bars indicate significant difference ($p \leq 0.05$) among elevation, and '*' represents significant difference between two temperature regimes ($p \leq 0.05$) within each elevation (t-test).

fected all three parameters (GP, MGT and TI). The interactions between elevation and water potential had a significant effect on TI indicating that the effect of elevation on TI varies with water potential. However, no significant interaction effects were observed for GP and MGT.

Discussion

Seed mass

Our results showed substantial variation in seed mass among populations of *A. spicatum* without a consistent pattern of change along the elevation gradient suggesting that microhabitats may have a greater effect on seed mass than elevation itself. The higher seed mass observed at the lowest elevation did not translate into higher germination

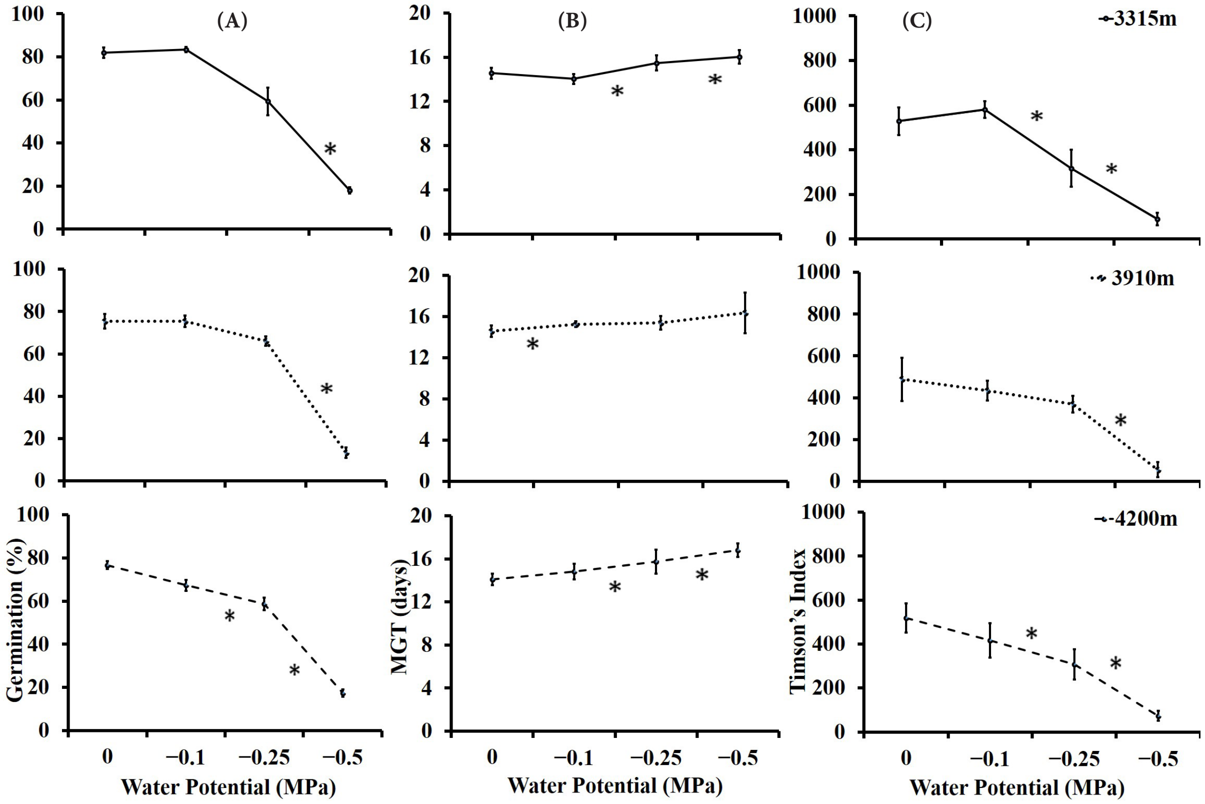

Fig. 4 Effect of water stress on (A) germination percentage, (B) mean germination time (MGT) and (C) Timson's index of three elevations under different water potential (MPa) at photoperiod at low temperature (25/15°C). Error bars represent standard error of the mean (*n* = 5) and '*' represents significant difference ($p \leq 0.05$) among different levels of water potential.

Table 3 Result of two-way ANOVA showing the effect of interactions between elevation (3,315 m, 3,910 m and 4,200 m) and water potential (MPa) on germination percentage, mean germination time and Timson's index

Variables		Df	F-value	*p*-value
Germination percentage	Elevation	2	2.03	0.142
	Water potential	3	114.93	0.000
	Elevation × water potential	6	1.14	0.355
Mean germination time	Elevation	2	1.40	0.256
	Water potential	3	15.52	0.000
	Elevation × water potential	6	0.94	0.474
Timson's index	Elevation	2	3.861	0.028
	Water potential	3	146.79	0.000
	Elevation × water potential	6	3.13	0.011

rates, a finding consistent with the observations reported by Cuyckens et al. (2021) and Walder and Erschbamer (2015). Seed mass is strongly influenced by environmental factors such as soil moisture, temperature and nutrient concentrations (Tremayne and Richards 2000). The absence of a consistent seed mass pattern along the elevation gradient has been also reported in the studies of four alpine species in the Swiss Alps (Pluess et al. 2005) and four *Polylepis* species in northwest Argentina (Cuyckens et al. 2021). Such elevation-independence of seed mass is often attributed to multiple factors such as genetic homogenization among populations due to pollen and seed exchange (Ohsawa and Ide 2008; Pluess et al. 2005), and random variations in microhabitat characteristics such as edaphic factors (e.g., moisture, nutrients), microclimate, and disturbances in high mountain environments (Opedal et al. 2015).

Germination pattern along elevation gradients

We did not find a consistent pattern within-species variation in seed morphological and germination traits of *A. spicatum* along the elevation gradient, a result that aligns with the findings from studies on plants in eastern Tibet (Wang et al. 2021). While elevation is generally expected to influence seed germination traits in mountain plants, no consistent pattern has emerged across different studies (Bauk et al. 2017; Giménez-Benavides and Milla 2013; Wang et al. 2021). For instance, higher GPs for seeds collected from higher elevations have been reported in two species of *Saxifraga* endemic to northern Spain (Giménez-Benavides and Milla 2013) and in *Gymnocalycium monvillei* from Cordoba mountains, Argentina (Bauk et al. 2017). It is likely that the selection pressures associated with elevation gradients are overridden by variations in other microhabitat characteristics prevalent in high mountain environments (Opedal et al. 2015).

Germination patterns across environmental gradients

The result of the present study revealed that the seeds from three elevation germinates both in dark and light. The seeds of this plant species can germinate whether they are below the soil's surface or exposed to light, if both the temperature and moisture content are suitable. This suggests that they have the ability to germinate in the gravelly soils of the alpine habitats (Peng et al. 2017). Similar results was observed in *Aconitum napellus* where, high percentage of the seeds of this plant species even germinating while buried in the soil (Herranz et al. 2010). Such light independency during germination has also been reported in some members of Ranunculaceae, *Thalictrum mirabile* (Thompson et al. 1997).

High temperature (30/20°C) and water stress (< −0.25 MPa) had a profound negative effect on the germination traits of *A. spicatum* seeds collected from all three elevations. Similar to our results, *G. monvillei* from Cordoba mountains in Argentina showed reduced germination at 32°C compared to 25°C, with further reductions as water stress increased (Bauk et al. 2017). High temperature and water stress may negatively affect enzyme activities and embryo survival of the germinating seeds (Fenner and Thompson 2005; Hawker and Jenner 1993). Several previous studies have reported such negative effects of warming on seed germination of mountain plants such as *Salvia officinalis* (Žutić and Dudai 2008), *Rhinanthus minor*, *Rhinanthus alectorolophus* and *Rhinanthus glacialus* (Ter Borg 2005), *Quercus ilex* (Amimi et al. 2023) and several species of treeline ecotone of the central Alps in Austria (Walder and Erschbamer 2015). The results from the present study along with these previous findings suggest that relatively high temperatures may negatively impact the germination performances of species adapted to cold regions. This also implies that global warming may alter the regeneration and population dynamics of high mountain plants by reducing their germination success.

Drought stress is an important environmental factor affecting seed germination (Channaoui et al. 2017; Evans and Etherigntion 1990). Inhibition of seed germination incubated under mild to severe water stress has been reported in several species such as *Nepeta persica* (Mohammadizad et al. 2013), *Trachyspermum ammi* (Rohamare et al. 2014), and *Lavandula mairei* (Hamdaoui et al. 2021). The significant reduction in seed GP at mild water stress (−0.5 MPa) and complete inhibition at lower water potentials (≤ −0.75 MPa) observed in *A. spicatum* suggests high vulnerability to drought stress. This is unsurprising given that the seed collection site in the present study receives very high precipitation (> 5,000 mm/year; Fig. S1) and remains moist throughout the year. However, drought episodes are expected to increase in future, particularly during pre-monsoon due to the predicted increases in temperature and reduced precipitation in the high mountain regions of the Himalaya (Pepin et al. 2022). As most high mountain plants including *A. spicatum* germinate during pre-monsoon summer, increased drought during this period could negatively affect germination in natural habitats. This indicates the low germination potential of these species under drought conditions and suggests a low germination adaptation and survival potential for these species under water stress or drought conditions (Srivastava et al. 2010). The sensitivity of alpine species to drought could have major implications for their future survival if extreme weather events like drought become more frequent with climate change (Zubler et al. 2014).

Conclusions

Our study demonstrated a significant negative impact of rising temperatures and increased water stress on the germination of *A. spicatum*, an important medicinal herb of the Himalaya. These findings highlight the species' high vulnerability to the effects of climate change, particularly in the face of temperature increases and declining precipitation, trends already observed in the high mountain regions of the Himalaya (Pepin et al. 2022). Negative impacts of elevated atmospheric CO_2, a primary cause of climate change, on the secondary metabolites content in *Aconitum* species have already been reported in the Indian Himalaya (Chandra et al. 2022). The lack of consistent variation in seed mass and germination traits across elevations suggests that microhabitat characteristics may play a more dominant role than elevation itself in shaping these traits. This highlights the importance of incorporating considerations of climate change impacts particularly of warming and drought into strategies for the sustainable harvest and conservation of *A. spicatum*. Future studies that account for microhabitat variability (e.g., soil moisture, nutrient, biotic interactions) into the experiment could offer deeper insights into the environmental factors driving seed and germination dynamics in high mountain plants. In situ germination experiments involving the monitoring of seed germination and seedling survival at future climate analogue regions (i.e., warmer region at elevation lower than the current distribution range of the species) can provide better understanding of the impacts of warming high mountain plant species.

Supplementary Information

Supplementary information accompanies this paper at https://doi.org/10.5141/jee.24.077.

Table S1. Comparison (independent sample t-test, *n* = 5) of germination percentage (mean ± deviation) between 12 h photoperiod and complete darkness at low and high temperatures. **Fig. S1.** Mean of maximum and minimum temperature as well as precipitation recorded between 2012 and 2022 at Lumle station of Kaski, Nepal. **Fig. S2.** (A) Seeds. (B) Seeds under experiment. (C) Germinated seeds.

Abbreviations

ACA: Annapurna Conservation Area
ANOVA: Analysis of variance
DoF: Department of Forest
GP: Germination percentage
MGT: Mean germination time
MPa: Mega pascal
PEG 6000: Poly ethylene glycol 6000
TI: Timson's index

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Authors' contribution

BBS, AD, AT, UBS, and BRP conceptualized and designed the study. Material preparation and data collection were performed by BSG and SD. Data analyses were performed by BSG under the supervision of AD and BBS. The first draft of the manuscript was written by BSG and all authors commented/edited on the draft and prepared the manuscript. The manuscript was edited and finalized by BBS, UBS, and AD. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable requests.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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