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Effect of light on seed germination of some tropical and temperate species of Nepal

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Abstract

Seed germination is a fundamental process in plant development, influenced by both intrinsic and extrinsic factors. Light and dark have quite diverse effects on seed germination in different plant species under different environmental conditions. This study aimed to determine the lag period and germination speed (t_{50}) of seeds of 37 plant species under light and dark conditions with a standard temperature of 25°C in light/alternating dark at 15°C with a 12-hour alternating photoperiod and 75% relative humidity in a plant growth chamber. The statistical analysis did not reveal any significant impact of light treatment on seed germination percentage across different plant life forms. However, a higher percentage of species (52%) exhibited the germination class >75% in light, compared to 43% in dark. The lag period of 38% of species was very short (<8 days), and 30% of the species had very fast (<8 days) germination speed (t_{50}). In addition, the lag period and germination speed (t_{50}) values were found to be comparable. This study may contribute to unveiling the viability and regeneration potential of seeds, which is a prerequisite for storage in the seed bank.

Keywords: Germination speed (t_{50}), Lag period, Restoration, Seed bank, Viability

1. Introduction

Seed germination is a critical phase in plant development, marking the transition from seed to seedling (Smolikova & Medvedev, 2022). Several physiological, biochemical, and environmental variables affect this process, and these elements collectively determine the success of germination and the subsequent growth of the plant (Fu et al., 2024). Seed germination variability across populations is significantly influenced by environmental cues experienced both before and after seed dispersal (Abley et al., 2024).

Native seeds are crucial for ecosystem restoration because they control soil seed banks, which act as a reservoir of regeneration potential, stabilizing populations and promoting resilience during environmental fluctuations (Heenan et al., 2024; Rebelo et al., 2024). For instance, the population of an endemic Nepalese banana, i.e., *Ensete nepalense*, and the CITES Appendix II-listed species *Dalbergia sissoo* is declining and under threat (DPR, 2022; Parmar, 2023); therefore, seed ecological knowledge is essential for conservation and restoration of threatened species. Their significance extends beyond improving biodiversity to supporting the restoration of degraded ecosystems, which can meet the Global Goals for 2050 of the Convention on Biological Diversity (CBD Secretariat, 2022). The germination of the seeds is influenced by factors such as temperature, moisture, and light. More effective seed management is possible for both direct seeding (*in-situ* restoration) and native plant cultivation in nurseries (*ex-situ* restoration) if dormancy and germination patterns in native species are controlled.

The effect of light and dark on seed germination varies significantly across different plant species and conditions. Research indicates that some seeds require light to germinate, while others thrive in darkness, depending upon the species (Baskin & Baskin, 2014). Considering the lack of species-specific information, this study aimed to unveil the seed germination pattern of native species in light and dark conditions and the lag period and germination speed (t_{50}) of seeds. The outcomes of this study will contribute to the broader understanding of seed biology, plant ecology, and ecosystem restoration, especially in the face of climate change and habitat loss.

2. Materials and Methods

Standards for the collection, processing, and conservation methodology of wild seeds were according to the Wild Millennium Seed Bank Project (MSBP, 2015, with updated version 2022). All the data of seeds presented in this study were from primary sources.

2.1 Seed collection and processing

In the time period between 2022 and 2024, seeds of 37 native annual and perennial species were gathered from different locations of tropical to temperate bioclimatic zonation around Nepal. In this study, shrubs are dominant (16 species), followed by herbs (10 species), trees (7 species), and climbers (4 species), respectively (Table 1). Herbarium specimens were prepared for each collection to ensure future reference based on Bridson and Forman (1992). Matured seeds were collected from each taxon in air-drying nylon-net bags and cotton bags and registered in the seed bank. Seed collection was done according to the seeds' size and number, i.e., 200 gm of seeds for a size less than 2 mm, while 500 gm of seeds for a size of 2–5 mm and a minimum of 200 seeds were collected for seeds larger than 5 mm. Color changes, fruit dehiscence, and the hardening and drying of seeds are indicators of physiological maturity in seeds (Pedrini et al., 2020). Manual cleaning of collected seeds was done to ensure the effectiveness of the seed processing (Rao et al., 2006). The processed seeds were weighed on a digital balance (SF-400C), and before being tested for viability, the seeds were cleaned and placed in air-sealed bottles in an ambient temperature room. Additionally, seed measurement was done by taking 2-3 seed samples representing the smallest and largest sizes.

2.2 Seed germination test

Seeds of 37 sampling taxa were carried out in standard environmental conditions that might support a large number of species, i.e., a temperature of 25°C with light/alternating dark at 15°C with a 12-hour alternating photoperiod, ca. 3000 lux light, and 75% relative humidity in a plant growth chamber (Faithful FPG-250). In most of the species, incubation of seeds at 20/10 °C and 25/15°C will result in a higher germination percentage, according to Baskin and Baskin (2014).

For the germination, a varied number of seeds were placed on the moist sheets of Whatman No. 1 seed testing paper in the sterilized 9-cm diameter petri dishes that were sealed by Parafilm to stop moisture evaporation. Seed germination tests were performed soon after seeds were collected and brought to the seed bank. Different species require different substrates and numbers of seeds; for large seeds (>1 cm in diameter), sterilized sand and 5/10 seeds per plate were used with 3/6 replica number of petri plates, while for medium-sized seeds (5-10 mm), 20/30 seeds per plate were used with 2/3 replica number of petri plates, and for smaller seeds (<5 mm), filter paper and 50/100 seeds per plate were used with 2 replica number of petri plates (modified from Rao et al., 2006).

A radicle that protrudes >1 mm is a certain sign that the seed is germinating; consequently, all of the germination-initiating seeds were counted and removed on a daily basis till the end of the experiment. However, germination was only observed once at the end of the experiment for seeds that were incubated in conditions of continuous darkness following Baskin and Baskin (2014). Germinated seeds were transferred to the polyhouse of the National Botanical Garden (NBG) for their germplasm conservation. Although seeds with 75% germination are suitable for storage (Rao et al., 2006), those seeds having >50% germination were also stored in the seed bank of the NBG (Chaudhary et al., 2022). Seed germination percentage was calculated by following Baskin and Baskin (2014).

2.3 Categorization of germination behavior for comparative analysis

Germination percentage of the species was classified into five categories: i) d”10%, ii) 11-25%, iii) 26–50%, iv) 51–75%, and v) >75% germination. The lag period, the time duration between the start of the experiment and the first germination, is categorized into i) very short (<8 days), ii) short (8-14 days), iii) moderate (15–28 days), and slow germination (>28 days). Similarly, we categorized germination speed (t_{50}), the time period from the start of the experiment until 50% of observed germination occurred, into four categories: i) very fast (<8 days), ii) fast (8-14 days), iii) moderate (15–28 days), and slow germination (>28 days) (modified form of Morgan, 1998).

2.4 Data analysis

An independent sample t-test was conducted to assess the impact of light treatment (light and dark) on the mean germination percentage within different plant life form categories (climber, herb, shrub, and tree), while the Mann-Whitney U test was conducted for a total of 37 species, as data were not normally distributed. All statistical analyses were conducted using R (Version 4.3.3; R Core Team, 2024).

Table 1. Total species showing life form (H-Herb; S-Shrub; T-Tree and C-Climber), seed size, germination percentage (%) in light and dark with lag period and t_{50} (days)

Family & Scientific Name	Life Form	Seed Size	G_{Light} (%)	G_{Dark} (%)	Lag (days)	t_{50} (days)	Collection Place
Acanthaceae							
Pseuderanthemum latifolium (Vahl) B.Hansen	H	Diam. 3–4 mm	80	100	13	14	Jhalthal, Jhapa
Amaryllidaceae							
Allium hypsistum Stearn	H	Diam. 3–4 mm	20	83	2	11	Mulpani, Salyan
Anacardiaceae							
Dobinea vulgaris Buch.-Ham. ex D.Don	S	Diam. 2–3 mm	80	100	22	24	Phulchowki, Lalitpur

Apocynaceae							
Calotropis acia Buch.-Ham.	S	5.5–7×3.5–5 mm	96	96	5	5	Dudhauli, Sindhuli
Calotropis gigantea (L.) W.T.Aiton	S	5–6.5×3–4.5 mm	86	86	4	4	Dudhauli, Sindhuli
Asparagaceae							
Asparagus racemosus Willd.	C	Diam. 4–5 mm	50	70	28	28	NBG, Lalitpur
Asteraceae							
Jurinea auriculata (DC.) N.Garcia, Herrando & Susanna	H	4–4.5×1.5–2 mm	95	30	13	13	Chandragiri, Kathmandu
Jurinea deltoidea (DC.) N.Garcia, Herrando & Susanna	H	3.5–4×1–1.5 mm	100	100	6	7	Chandragiri, Kathmandu
Berberidaceae							
Mahonia napaulensis DC.	S	3.5–5×1.5–3 mm	100	100	8	9	NBG, Lalitpur
Bignoniaceae							
Oroxylum indicum (L.) Kurz	T	18–20×15–18 mm	80	40	30	30	Dudhauli, Sindhuli
Coriariaceae							
Coriaria napalensis Wall.	S	1.5–2×1–1.5 mm	94	92	8	8	NBG, Lalitpur
Ericaceae							
Gaultheria fragrantissima Wall.	S	0.5–0.6 mm	52	50	10	10	Dhap Dam, Kathmandu
Fabaceae							
Albizia julibrissin Durazz.	T	5.5–9×4–6 mm	10	20	10	10	Motichaur, Lalitpur
Albizia lebbeck (L.) Benth.	T	6–10×5–8 mm	30	60	3	4	NBG, Lalitpur
Butea buteiformis (Voigt) Grierson	S	15–22×12–15 mm	87	100	12	12	Chure, Kailali
Dalbergia sissoo Roxb. ex DC.	T	7–10×4–6 mm	90	100	3	4	Ghorahi, Dang
Lotus corniculatus L.	H	Diam. 0.8–1 mm	80	92	4	4	NBG, Lalitpur
Piptanthus nepalensis (Hook.) Sweet	S	6–8.5×4.5–5 mm	16	33	22	22	Chandanbari, Rasuwa
Gentianaceae							
Swertia multicaulis D.Don	H	Diam. 1.5–2.5 mm	52	76	21	23	Cholangpati, Rasuwa
Hydrangeaceae							
Hydrangea febrifuga (Lour.) Y.De Smet & Granados	S	0.5–0.9×0.2–0.6 mm	70	68	6	10	Bhatkepati, Kathmandu
Hypericaceae							
Hypericum podocarpoides N.Robson	S	1.5–2×0.5–1 mm	47	50	18	24	NBG, Lalitpur
Lamiaceae							
Ocimum tenuiflorum L.	H	0.8–1.2×0.5–0.8 mm	60	24	4	4	Rupandehi
Lythraceae							
Woodfordia fruticosa (L.) Kurz	S	0.5–0.8×0.2–0.4 mm	73	31	37	41	NBG, Lalitpur
Malvaceae							
Abelmoschus manihot (L.) Medik.	S	3.5–4×3–3.5 mm	26	33	31	56	Phulchowki, Lalitpur
Melastomataceae							
Osbeckia nepalensis Hook.	S	0.5–0.7×0.2–0.4 mm	82	78	6	7	Bhatkepati, Kathmandu
Musaceae							
Ensete nepalense (Wall.) G.Parmar & Trias-Blasi	H	3–8×4–8 mm	10	20	369	-	NBG, Lalitpur
Phytolaccaceae							
Phytolacca acinosa Roxb.	H	Diam. 2.5–3 mm	96	93	46	47	NBG, Lalitpur

Pinaceae							
Larix griffithii Hook.f.	T	4–5×2.5–3 mm	53	20	12	16	Ghunsa, Taplejung
Piperaceae							
Piper longum L.	C	Diam. 1.5–2 mm	83	56	34	44	Ranigunj, Sarlahi
Poaceae							
Coix lacryma-jobi L.	H	8–12×6–8 mm	90	90	3	3	NBG, Lalitpur
Primulaceae							
Embelia floribunda Wall.	C	Diam. 2–2.5 mm	53	100	29	42	Phulchowki, Lalitpur
Maesa chisia D.Don	S	0.4–0.6 mm	73	66	6	10	Bhatkepati, Kathmandu
Rosaceae							
Photinia integrifolia Lindl.	T	2.5–3×1.5–2 mm	90	66	17	23	Dhap Dam, Kathmandu
Pyracantha crenulata (D.Don) M.Roem.	S	2–2.2×1.2–1.4 mm	90	50	6	6	NBG, Lalitpur
Rubiaceae							
Rubia manjith Roxb.	C	2–3 mm	33	46	13	13	NBG, Lalitpur
Ulmaceae							
Holoptelea integrifolia (Roxb.) Planch.	T	7–10×4–6 mm	85	70	4	6	Godawari, Kailali
Urticaceae							
Debregeasia longifolia (Burm.f.) Wedd.	S	0.3–0.7 mm	52	32	20	22	Chandragiri, Kathmandu

3. Results and Discussion

3.1 Effect of light on general germination behavior of seeds

In this study, the statistical analysis did not indicate any significant impact of light treatment on seed germination percentage within different plant life forms (herb: t = 0.12497, p-value = 0.9021; tree: t = -0.48342, p-value = 0.6372; climber: t = 0.84468, p-value = 0.4311; t = -0.35092, p-value = 0.7278) and there were no significant differences in germination percentage of the total 37 plant species that were subjected to the experiment (W = 793, p-value = 0.95). This suggests that the seed germination response of plants for various life form categories was similar under both light and dark conditions (Fig 1). The non-significant results in preliminary studies involving diverse plant taxa with small sample sizes can often be attributed to several factors, including inadequate sampling strategies and inadequate predictivity (Morgan, 1998; Goodall, 1970). More importantly, seed germination ability is a function of seed traits, phylogeny, and environmental adaptation rather than plant life form (Zhao et al., 2021).

Research indicates that no single set of conditions can optimize germination for all species due to their unique physiological and ecological requirements (Morgan, 1998). The characteristics of germination can differ between species and populations consisting of the same species, and germination is extremely sensitive to climatic conditions (Hsu et al., 2024). Species with high germination rates are often adapted to environments where conditions are predictably favorable for seedling establishment shortly after dispersal, especially in the case of exotic or invasive species (Wainwright & Cleland, 2013; Baskin & Baskin, 2014; Kharel et al., 2023). Higher percentages of seeds germinate in environments in which seedlings have better survivability with respect to light, temperature, ion concentrations, terpene type, or simply geographic location. Increment in the range of germination conditions could provide the opportunity for the adaptation of post-germination traits to a vast range of conditions (Donohue, 2010; Baskin & Baskin, 2014). The germination percentage of the total species did not vary statistically based on light and dark treatment (Fig 1). A higher percentage of species, i.e., 52% in light and 43% in dark, fell under the category of germination class >75%. Only 5% of species had d"10% germination percentage in light, while no species exhibited germination percentage below 10% in dark (Table 1; Fig 2).

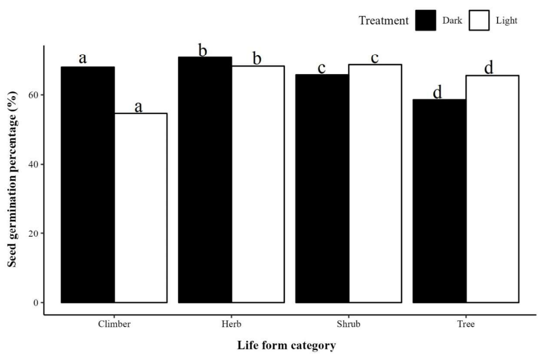

Fig 1. Germination percentage across different plant life forms in two light responses (light and dark) (similar letters represent no statistical significance at the 0.05 significance level, and NS: non-significance)

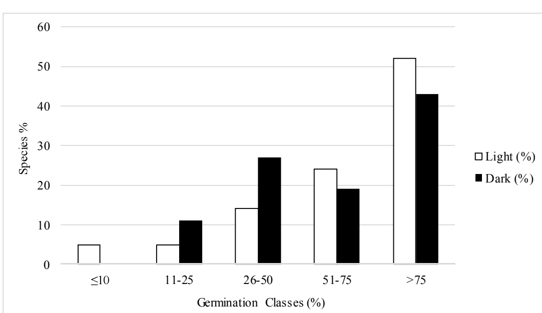

Fig 2. Total species germination percentage in light and dark

Species' germination percentage of five species (14%), i.e., *Calotropis acia*, *C. gigantea*, *Coix lacryma-jobi*, *Jurinea deltoidea*, and *Mahonia napaulensis*, were unaffected by light and darkness. Altogether, 17 species (46%) preferred darkness, while 16 species (43%) had germination promoted by light treatment, also reported by Clarke et al. (2000), and their study suggested that light can further enhance immediate germination.

Similarly, *Allium hypsistum* also had a lower germination percentage (20% in light) in this study, which might be due to a physiological barrier as discussed by Kirmizi (2017) in other *Allium* species but found to be significantly higher in the dark condition (83%) than in the light condition, as was revealed by Washa (2015). However, *A. hypsistum* showed a very short lag period (2 days) and fast germination speed (11 days). In this study, the germination percentage of *Gaultheria fragrantissima* was slightly higher in light, which is also reported in some other species of *Gaultheria* where germination is stimulated by light (Bannister, 1990).

Larix griffithii, which showed a peculiar result that the germination was higher in light (53%) than in dark (20%), and germination speed was found to be fast, and also the lag period was short (8-14 days). *Larix* seeds exhibit photoblastic behavior, germinating more readily in light conditions compared to darkness (Laudi & Bonatti, 1967). Similarly, two species of Rosaceae, i.e., *Photinia integrifolia* and *Pyracantha crenulata*, showed positive photoblastism, which is similar to other species of *Rosa* reported by Stoian-Dod (2023). All of the freshly collected seeds of *Mahonia napaulensis* that were treated with distilled water for 24 hrs. germinated in both light and dark. However, a study by Kim et al. (2023) suggested the light and cold stratification of *Berberis* seeds can influence seed germination. Freshly collected seeds had 100% viability; however, as the storage period increased, viability gradually decreased in the *Berberis* species reported by Krishan et al. (2023).

The impact of light treatment on seed germination percentage varies significantly across different plant life forms, particularly influenced by seed size and environmental conditions (Bu et al., 2017). *Holoptelea integrifolia* had higher germination in light (85%) than in dark (70%) with a very short (<8 days) lag period and germination speed (t_{50}). However, for the better germination rate and seedling ratio, pretreatment by GA3 has been suggested by Ragula et al. (2024). According to Singh et al. (2014), *Oroxylum indicum* seeds may germinate without pretreatment, which is similar to the results reported in this study, i.e., a higher germination rate in light than in dark (80% light, 40% dark) when treated with distilled water for 24 hrs. They also recommended cold water pretreatment to increase the germination rate and shorten the dormancy period.

Freshly collected seeds of a climber species, *Embelia floribunda*, showed a higher germination percentage in the dark (100%) than in the light (53%) with a long lag period and germination speed (> 28 days). A similar result was found in *Embelia ribes*, which has small seeds with an abortive embryo and slow germination time; however, freshly collected seeds showed a higher germination rate when pre-treated with cow dung slurry, and also seed viability will decrease with the storage time because of its recalcitrant nature (Raghu et al., 2016). The tropical shrub *Abelmoschus manihot* exhibited a poor germination percentage i.e. 26% in light and 33% in dark with a long lag period and germination speed >28 days. Studies by Chawan (1971) on different *Sida* species and Rodrigues et al. (2014) on *Hibiscus sabdariffa* reported that higher temperature pretreatment is required for the fast germination of seeds.

3.2 Lag period and germination speed (t_{50})

The germination lag period was found to be short in most of the species; i.e., 38% fell under the very short (<8 days) category. Similarly, 24% of species showed a short (8-14 days) lag period, while 19% of species had both a moderate (15-28 days) lag period and were slow germinators (>28 days) (Fig 3). Similarly, the germination speed (t_{50}) was found to be more or less similar to the germination lag period. Out of the total species, 30% of the species had t_{50} in both less than 8 days and 8-14 days. However, 21% of species fell under the moderate germination (15-28 days), and 19% of species were slow germinators (>28 days) (Fig 4).

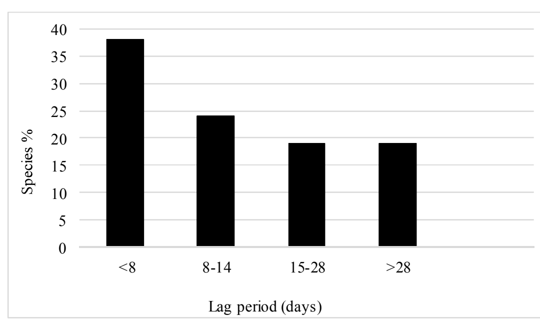

Fig 3. Lag period of total species in various classes

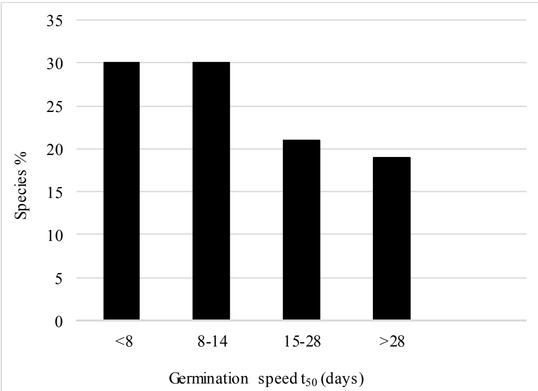

Fig 4. t_{50} of total species in various classes

A wide variety of germination behaviors were shown by numerous plant species; some are easily germinable, while others have complicated dormancy or need particular conditions for germination. For instance, *Ensete nepalense*, an endemic Nepalese banana, has fast germination (6 months) in the field, while in the laboratory it takes around a year with a low germination rate. In the case of *E. ventricosum* (Karlsson et al., 2013), using a non-sterilized medium, intact seeds and seedlings performed better than micro-propagation and also did not exhibit physical or morphological seed dormancy.

Seeds are non-dormant and neutral photoblastic in the case of *Calotropis procera*, as revealed by Navarrete-Sauza (2023). Similar results were found in this study, i.e., *C. acia* and *C. gigantea* exhibited very short germination periods (<8 days) with a germination percentage of more than 75%. While germination speed is an important factor, it may not directly correlate with the final germination percentage, as indicated by the need to consider multiple parameters for a comprehensive understanding of germination patterns (Seo et al., 2009).

3.3 Seed size and other seed attributes

Amongst the total species in this study, poor germination shown by some species of Fabaceae, i.e., *Albizia julibrissin*, *A. lebbeck*, and *Piptanthus nepalensis*, might possess physical dormancy because of their hard seed coat, which is breakable by various scarifications (Rolston, 1978; Kwakye & Blankson, 2022). However, *Butea buteiformis* and *Dalbergia sissoo* (Fig 5) show higher germination rates in light and dark with fast germination speed in this study, which is supported by the study of Ahmad et al. (2015).

The findings of this study on the effects of seed size on germination rate were not very remarkable because the tested species belonged to diverse families and genera. *Debregeasia longifolia, Gaultheria fragrantissima*, *Hydrangea febrifuga*, *Hypericum podocarpoides*, *Maesa chisia*, *Ocimum tenuiflorum, Swertia multicaulis*, and *Woodfordia fruticosa*, having small seed sizes (d"2 mm), showed a moderate germination rate compared to medium and larger seed species, i.e., *Butea buteiformis, Calotropis acia, C. gigantea*, *Dalbergia sissoo*, *Holoptelea integrifolia*, *Jurinea auriculata*, *J. deltoidea*, and *Mahonia napaulensis* (Table 1). Despite these, in another study, the species having small seed size (d"1 mm) showed fast germination. However, this cannot be generalized in all species; smaller seeds tend to germinate more quickly than larger seeds. Although the larger seeds may not always lead to higher seed germination, such larger seeds may germinate when buried at a deeper soil compared to the smaller seeds (Dangol et al., 2024). Besides, as seeds get bigger, they presumably need less light to germinate (Baskin & Baskin, 2014), and Milberg et al. (2000) suggested the light

Fig 5. Germination of seeds of different plant species in controlled laboratory conditions

response and the seed mass have a coevolution relationship.

Annual species such as *Allium hypsistum, Coix lacryma-jobi, Jurinea auriculata, J. deltoidea, Lotus corniculatus*, and *Ocimum tenuiflorum* exhibited a fast germination rate in this study. The finding was supported by Scott & Morgan (2012), where they found many annual species with rapid germination, showing that 90% of certain species achieved 50% germination within 10 days.

Consequently, the generalizations presented in this paper may be limited to certain species, and caution is advised when interpreting these results in other regions with differing soil types and climates. Larger seeds do not consistently exhibit faster germination; instead, smaller seeds may show quicker germination under certain conditions. Germination traits and seed mass have a complex relationship. This study's inadequate predictivity might be due to the small sample size that was employed with diverse species from different locations. However, if additional species within a genus or the same family were included in the seed germination study, or if both annuals and perennials had been included, greater predictivity of germination behavior might have been achievable in this study (Morgan, 1998; Martínez-Villegas et al., 2018; Zhao et al., 2021).

4. Conclusion

This preliminary study shows the variability in seed germination behavior, which may indicate the diversity present in the studied species. Effective conservation methods for native and rare species depend on an understanding of seed germination behavior because it guides activities that improve seed viability and restoration. An endemic species, *Allium hypsistum*, showed a higher germination percentage in the dark than in the light and has a short lag period with a very fast germination speed. Similarly, *Dalbergia sissoo*, a CITES Appendix II-listed species, shows good germination rates in light and dark with fast germination speed. Therefore, this type of information can be beneficial for understanding the germination characteristics, improving propagation protocols, and supporting conservation efforts. Moreover, this directly connects the seed ecology and seed storage at the seed bank, which is crucial for preserving plant genetic diversity, especially for rare and endangered species.

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