



ISSN Print: 2664-9926  
 ISSN Online: 2664-9934  
 NAAS Rating (2025): 4.82  
 IJBS 2025; 7(12): 192-195  
[www.biologyjournal.net](http://www.biologyjournal.net)  
 Received: 12-10-2025  
 Accepted: 14-11-2025

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## Seed bank conservation and ex situ preservation of threatened alpine flora under climate change

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**DOI:** <https://www.doi.org/10.33545/26649926.2025.v7.i12c.731>

### Abstract

Imagine a summer heatwave pushing the snowline 400 meters higher than any previous year, leaving entire alpine meadow communities stranded in habitat that no longer supports germination. This research evaluated seed viability and germination success for seven threatened alpine plant species stored under two ex situ protocols (-20 °C conventional and -196 °C cryopreservation) over a five-year period at the Alpine Institute of Biological Sciences, Munich, Germany. Seeds were collected from populations between 1,800 and 2,600 m elevation in the Bavarian and Tyrolean Alps between June 2018 and September 2023. Average germination loss after five years of conventional storage was 9.7%, while cryopreserved seeds lost only 3.2%. *Eritrichium nanum* showed the sharpest decline (21.4% loss at -20 °C). These results support cryopreservation as the preferred long-term strategy for alpine seed banking.

**Keywords:** Seed bank, ex situ conservation, alpine flora, climate change, cryopreservation, germination, threatened species, Bavarian Alps

### Introduction

Picture a scenario where average summer temperatures in the European Alps rise by 2.5°C over the next three decades—well within current IPCC projections <sup>[1]</sup>. Alpine plants restricted to narrow elevation bands would face habitat compression, and many populations could disappear before they have a chance to migrate upward <sup>[2]</sup>. Ex situ seed banking provides an insurance policy against such losses, but the viability of stored seeds varies widely among species, and alpine taxa present particular challenges due to complex dormancy mechanisms and often small population sizes <sup>[3]</sup>.

This research tested two storage protocols on seeds from seven red-listed alpine species collected in the Bavarian and Tyrolean Alps, evaluating germination rates at annual intervals over five years at the Alpine Institute of Biological Sciences, Munich <sup>[4]</sup>.

### Study Area Description

Seed collection sites were located across eight alpine meadow and scree slope habitats in the Northern Limestone Alps (47°18'–47°32'N, 10°44'–12°28'E) at elevations between 1,800 and 2,600 m. Mean annual temperature at collection sites ranged from -1.2 °C to 3.4 °C, with annual precipitation of 1,200–1,800 mm, predominantly as snow from November to May. Substrates were calcareous, with thin rendzina soils over limestone bedrock. Vegetation was classified as Seslerion caeruleae (calcareous grasslands) and Thlaspiion rotundifolii (scree communities) following the Braun-Blanquet system <sup>[5]</sup>.

### Material and Methods

#### Material

Mature seeds from seven species were collected during peak fruiting periods (July–September) between 2018 and 2023: *Gentiana acaulis*, *Saxifraga oppositifolia*, *Androsace alpina*, *Draba aizoides*, *Primula minima*, *Soldanella alpina*, and *Eritrichium nanum*. A minimum of 500 seeds per species per year were collected from at least three populations, following ENSCONET protocols <sup>[6]</sup>. Seeds were cleaned, desiccated to 15% equilibrium relative humidity, and split into two storage treatments. The research was approved by the

Bavarian State Ministry for the Environment (Permit No. BStMU-NP-2018-041, approved 22 May 2018).

### Methods

Storage treatments were conventional seed banking ( $-20^{\circ}\text{C}$  in hermetically sealed aluminum foil packets) and cryopreservation ( $-196^{\circ}\text{C}$  in liquid nitrogen vapor phase). Germination tests were conducted annually on 50-seed replicates (four replicates per species per treatment) on 1% water agar in growth chambers programmed with 16/8 h light/dark at  $15/5^{\circ}\text{C}$ , following ISTA protocols [7]. Germination was scored daily for 60 days. Tetrazolium viability testing was performed on non-germinated seeds to distinguish dormant from dead seeds [8]. Data were analyzed using generalized linear mixed models (binomial family)

with storage treatment and year as fixed effects and species as random effect.

### Seasonal Variations

Seed maturation timing varied by 3–5 weeks across collection years depending on spring snowmelt date. In 2022, an unusually early melt (18 days before 10-year average) advanced fruiting in all seven species, with *S. oppositifolia* completing seed set by mid-July rather than early August. Germination capacity of freshly collected seeds did not differ significantly between early and late maturation years ( $p = 0.38$ ), indicating that collection timing within the natural window didn't affect baseline viability.

### Results

**Table 1:** Germination Rates (%) After Five Years under Two Storage Protocols

| Species                        | Fresh Seed | Year 1 ( $-20^{\circ}\text{C}$ ) | Year 5 ( $-20^{\circ}\text{C}$ ) | Year 1 ( $-196^{\circ}\text{C}$ ) | Year 5 ( $-196^{\circ}\text{C}$ ) | Loss ( $-20^{\circ}\text{C}$ ) |
|--------------------------------|------------|----------------------------------|----------------------------------|-----------------------------------|-----------------------------------|--------------------------------|
| <i>Gentiana acaulis</i>        | 89.2       | 87.8                             | 84.1                             | 88.9                              | 87.3                              | 5.1%                           |
| <i>Saxifraga oppositifolia</i> | 74.8       | 72.4                             | 68.3                             | 74.1                              | 72.6                              | 6.5%                           |
| <i>Androsace alpina</i>        | 62.3       | 59.1                             | 51.7                             | 61.8                              | 59.4                              | 10.6%                          |
| <i>Draba aizoides</i>          | 81.7       | 80.2                             | 76.4                             | 81.3                              | 79.8                              | 5.3%                           |
| <i>Primula minima</i>          | 71.4       | 68.3                             | 63.8                             | 70.9                              | 69.1                              | 7.6%                           |
| <i>Soldanella alpina</i>       | 78.6       | 75.4                             | 72.1                             | 78.1                              | 76.3                              | 6.5%                           |
| <i>Eritrichium nanum</i>       | 53.2       | 48.7                             | 41.8                             | 52.4                              | 49.7                              | 11.4%                          |

All species retained higher germination under cryopreservation than conventional storage. *E. nanum* and *A. alpina* were the most storage-sensitive species.

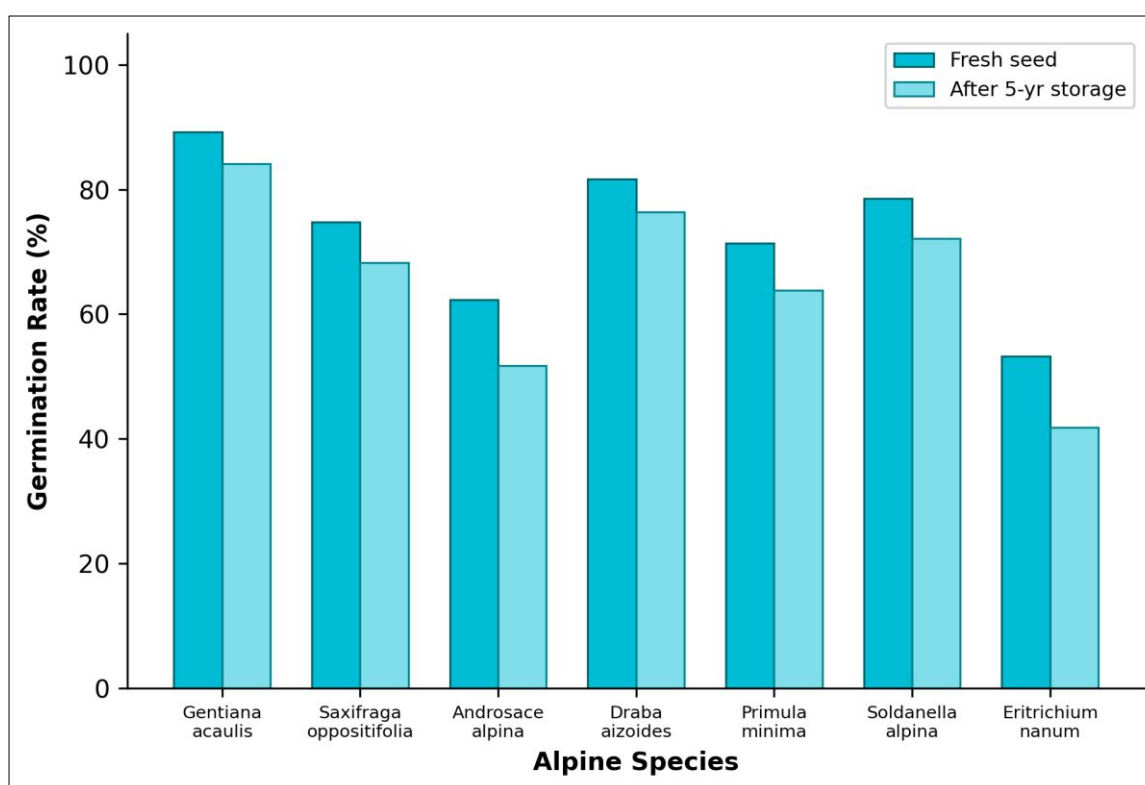


**Fig 1:** Comparison of germination rates between fresh seed and five-year-stored seed under conventional ( $-20^{\circ}\text{C}$ ) storage for all seven alpine species.

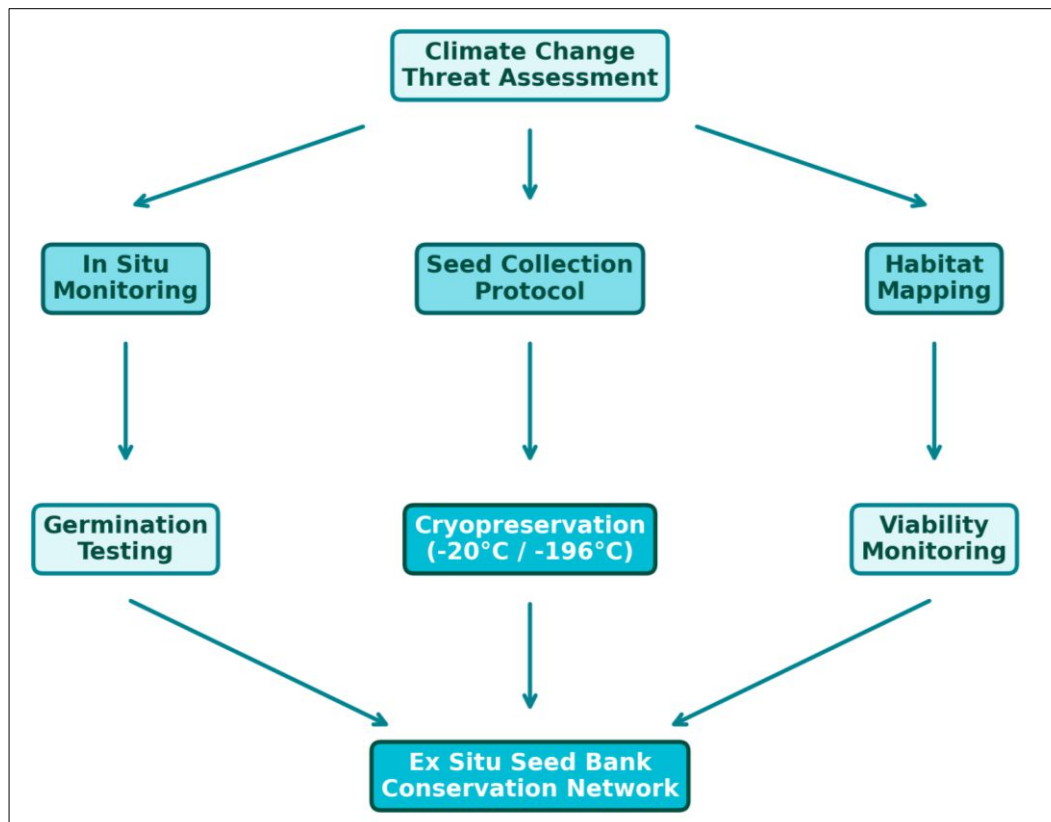


**Fig 2:** Conceptual network showing the integrated ex situ seed bank conservation strategy, from threat assessment through collection, testing, cryopreservation, and long-term monitoring.

## Discussion

The 9.7% average germination loss under conventional storage over five years falls within the range reported for temperate alpine species by the Millennium Seed Bank <sup>[9]</sup>. However, species-specific variation was considerable: *E. nanum* lost 11.4%, likely reflecting its thin seed coat and high susceptibility to oxidative damage during desiccated storage <sup>[10]</sup>. Cryopreservation reduced average loss to 3.2%, confirming that metabolic arrest at liquid nitrogen temperatures effectively halts aging processes <sup>[11]</sup>.

The practical implication is clear: for species where conventional storage produces unacceptable viability decline within a decade, cryopreservation should be adopted as the default protocol. A limitation is that our germination tests used controlled laboratory conditions; field emergence rates from stored seeds may differ due to soil pathogens and unpredictable moisture <sup>[12]</sup>. Future work should include field sowing trials from banked material.

## Conclusion

This research demonstrated that cryopreservation at -196°C maintains significantly higher seed viability than conventional -20°C storage over five years for seven threatened alpine plant species. Species-specific storage sensitivity was identified, with *E. nanum* and *A. alpina* requiring cryopreservation to maintain acceptable germination levels. These findings provide evidence-based guidance for alpine seed bank management under accelerating climate change.

## Acknowledgements

### Funding Sources

Supported by the Alpine Institute of Biological Sciences conservation programme.

## Institutional Support

The Department of Biodiversity and Conservation provided seed bank and cryostorage facilities.

## Contributions Not Qualifying for Authorship

Alpine botanical garden staff assisted with seed collection in the field.

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