



Journal of **Plant Sciences**

ISSN 1816-4951



Academic
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Research Article

Pretreatments Comparison of Seed Germination of *Heracleum candicans* Wall

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Abstract

Background and Objective: *Heracleum candicans* belongs to the family apiaceae. In Kashmir Himalaya, this plant grows at an altitude of 1700-5000 masl. The present study was carried out to enhance seed germination using different physical, chemical and hormonal treatments. **Materials and Methods:** For germination studies, seeds were subjected to different physical, chemical and hormonal treatments. Seeds were surface sterilised with 2% aqueous sodium hypochlorite (Hi-Media) for 5-10 min. The disinfected seeds were placed in sterilized Petri plates containing sterilized absorbent cotton moistened with 10 mL of treatment solution. Statistical analysis was carried out using SPSS (Version 22.0) statistical software and the results were examined according to Tukey's test. Different letters on the values indicate that the means are significantly ($p < 0.05$) different. **Results:** Among the physical treatments, wet chilling at 4°C for 20 days proved to be most effective for seed germination. Among the three chemical treatments used, sodium hypochlorite treatment proved to be effective. **Conclusion:** Among the two hormonal treatments used, kinetin showed effectiveness in breaking seed dormancy as compared with GA₃.

Key words: Apiaceae, chilling, dormancy, germination, *Heracleum candicans*

Citation: Jan, M., F. Maqbool, S. Singh and I.A. Nawchoo, 2022. Pretreatments comparison of seed germination of *Heracleum candicans* Wall. J. Plant Sci., 17: 62-65.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Poor germination of seeds is of common occurrence in family Apiaceae¹. Probably the most important reason for this is the presence of non-viable seeds that have no embryo. Furthermore, dormancy occurs in seeds of most species of family apiaceae when temperature and light are favourable². This dormancy can be overcome by exposing the seeds to light and cool or alternating cool and warm temperatures^{3,4}. Low temperature seems to favour germination by promoting the breakdown of reserve proteins in the seeds releasing particular amino acids which are necessary for the growth of the embryo^{5,6}. Pre-sowing treatments have been used to enhance seed germination of several wild-sourced medicinal plants of the Himalayan region^{1,7-9}. *Heracleum candicans* Wall., a perennial herb endemic to the Northwest Himalayas, exhibits poor (42-46%) seed germination¹⁰. Poor and erratic seed germination coupled with overharvesting of plants from wild habitats for commercial use has put this species under severe threat. Consequently, it has been categorised as vulnerable in the state of Jammu and Kashmir. The development of protocols for enhancing seed germination and seedling survival is an important step to meet the increasing demand for the species from the pharmaceuticals industry. In this regard, the present study was carried out to compare the effect of different physical, chemical and hormonal treatments on seed germination.

MATERIALS AND METHODS

Study-area: For the present study, mature seeds were collected in August, 2017 from four different populations growing at different altitudes (Kashmir University Botanical Garden, Verinag, Ferozpora and Sonmarg) in Kashmir valley in Table 1.

Cleaning procedure: Seeds were picked, cleaned manually and subjected to air-drying at room temperature.

Research Protocol: For calculating seed weight, ten replicates of 100 air-dried seeds collected from all four populations were weighed and the mean 100 seeds weight was calculated. Seed

weight varied significantly among seeds from the different populations showing a gradual increase with the increase in altitude. For germination studies, seeds were subjected to different physical, Chemical and hormonal treatments. Physical treatments involved dry and wet chilling for 20, 40 or 60 days. Chilling experiments were performed by storing the seeds at 4°C for different time durations. Chemical treatments involved exposing seeds to 2% NaHClO₃ for 20, 40 or 60 min, germinating seeds with 0.25, 0.5 or 1.0 mg L⁻¹ KNO₃, or germinating seeds with 0.25, 0.5 or 1.0 mg L⁻¹ thiourea. For the hormone treatments, seeds were germinated with 0.25, 0.5 or 1.0 mg L⁻¹ GA₃ or kinetin. Prior to chemical and hormonal treatments, seeds were surface sterilized with 2% aqueous sodium hypochlorite (Hi-Media) for 5-10 min. The disinfected seeds were placed in sterilized Petri plates containing sterilized absorbent cotton moistened with 10 mL of treatment solution.

RESULTS

All the experiments were conducted in a completely randomised block design. Ten replicates of seeds were used for each treatment and the control sets were retained in each case using double distilled water. The germination count was prepared daily wherein the appearance of the radical was used as the measure to determine germination and total germination was recorded after germination in all the treatments. Germination was not observed in freshly collected seeds. The seeds of *H. candicans* had a chilling requirement for germination. Among different chilling treatments, chilling for 20 days proved to be effective for stimulating seed germination with 51-61 to 62-83% germination for dry and wet chilled seeds respectively. However, prolonged chilling (60 days) was ineffective concerning the germination of seeds from all four populations. Moreover, germination was higher (67-84%) for seeds given 60 minutes of sodium hypochlorite treatment. Compared with control, germination was improved by exogenous application of kinetin and GA₃ for seeds from all four populations. Among three different concentrations of kinetin and GA₃ used, 0.5 mg L⁻¹ was the most effective for improving the germination of seeds from all four populations in Fig. 1.

Table 1: *Heracleum candicans* collection sites in India

Collection site	District	Latitude N	Longitude E	Altitude (masl)	100-seed weight (g)
*KUBG	Srinagar	34°07'52"	74°49'58"	1609	137.4±3.2 ^a
Verinag	Anantnag	33°32'2"	75°14'21"	1920	147.6±3.3 ^b
Ferozpora	Baramulla	34°03'08"	74°25'18"	2200	157.1±2.9 ^c
Sonmarg	Ganderbal	34°33'58"	75°33'3"	2800	169±1.5 ^d

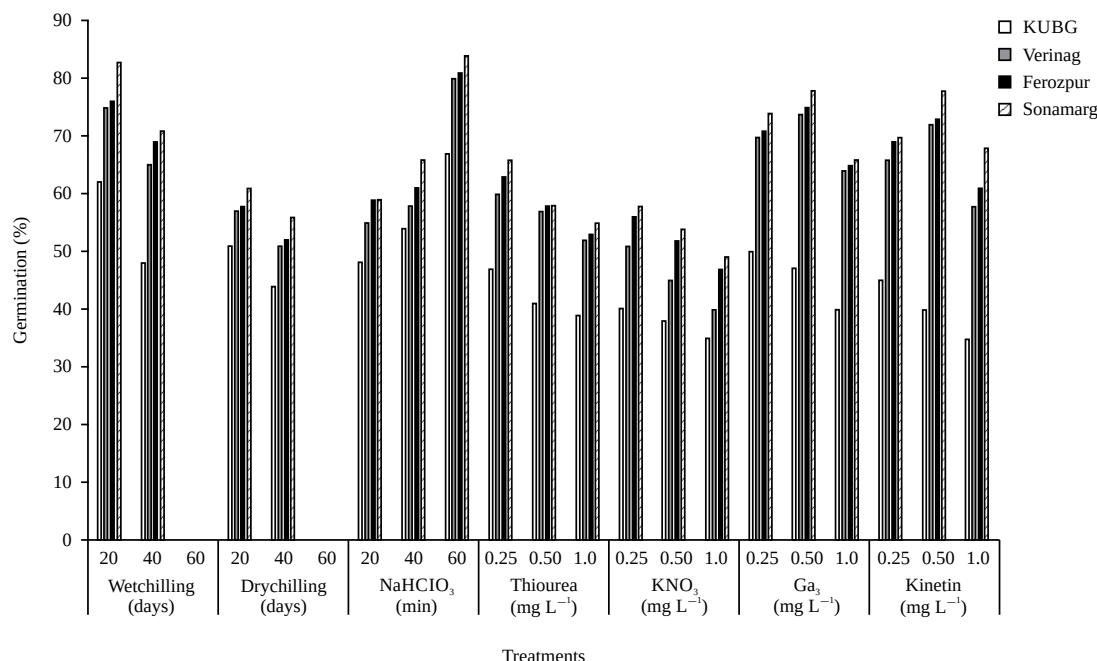


Fig.1: Effect of different treatments on germination of *Heracleum candicans* seeds from four populations

DISCUSSION

Experiments conducted during the present study showed that among the physical treatments, wet chilling at 4°C for 20 days proved to be most effective for seed germination. Among the three chemical treatments used, sodium hypochlorite treatment proved to be effective. It was also found that percentage seed germination correlates with altitude. Seeds collected from low altitude population i.e., KUBG shows less percentage seed germination compared with the other three populations. The results obtained suggest that both physical, as well as chemical treatments, were effective in breaking dormancy and stimulating seed germination in *H. candicans*. The effect of cold stratification in breaking dormancy may be due to the decrease in the level of inhibitors or due to increase in the seeds capacity for production of high level soft promotive hormones. The chemical treatments with thiourea and KNO₃ also enhanced seed germination. Improvement in seed germination by physical and chemical treatments has also been reported in *H. candicans*¹, *Ferulaassa-foetida*¹, *F. ovina*¹², *F. jaeschkeana*¹³. The results obtained during the present study suggest that cold stratification is essential for enhancing percentage seed germination. For obtaining the best results sodium hypochlorite treatment for 60 min and wet chilling at 4°C for 20 days is recommended.

CONCLUSION

During the present study, it was observed that seed germination is very low if not treated with different physical and chemical treatments. Wet chilling treatment and treatment with sodium hypochlorite showed best results for enhancement of seed germination followed by Kn, GA₃, KNO₃ and thiourea. During the present study, it was also observed that percentage seed germination in this plant species correlates with the altitude. Seeds collected from low altitude populations show less percentage germination rate with a high mean germination time as compared to seeds collected from populations at high altitudes.

SIGNIFICANCE STATEMENT

This study discovered that seed germination in *H. candicans* can be enhanced by treating seeds with different physical, chemical and hormonal treatments that can be beneficial for the propagation of this medicinal plant. This study will help the researchers to uncover the critical areas of seed biology and seed propagation.

ACKNOWLEDGMENT

The authors take this opportunity to thank all the scholars whose articles are cited and included in references of the manuscript.

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