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Enhanced seed germination by some pre-sowing treatments in endangered medicinal Herb *Swertia chirayita*

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Abstract

High value and threatened medicinal plant *Swertia chirayita* needs attention for conservation and was therefore studied for germination enhancement. Seeds of *Swertia chirayita* were treated with phytohormones/chemicals and cold stratification. The present study reveals that combination of incubation at low tempreture followed by pre sowing is more effective for seed germination of *Swertia chirayita.* Among all treatments, incubation at 15^{0C} for 12 weeks followed by pre-sowing with GA_3 (100 µM) found to be most effective (>80%) in germination of *Swertia chirayita* seeds.

Keywords: *Swertia chirayita,* seeds, presowing, cold stratification, GA_3.

Introduction

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The widespread use of *Swertia chirayita* in traditional medicine and support of modern scientific validation reflects its pharmacological importance. In traditional systems of healthcare this plant has been used in cure of chronic fevers and liver disorders. The whole plant has been reported to be used for hepatitis, inflammation, and digestive disorder treatment by local populations [1]. The use of plant or its parts in the treatment of variety of diseases including hepatotoxic disorders, liver disorders, ulcers, hepatitis, gastritis, constipation, dyspepsia, skin diseases, worms, epilepsy, scanty urine, hypertension, melancholia, chronic fever, malaria, anemia, bronchial asthma, and certain types of mental ailments, bile secretion, blood cleansing, and diabetes reflects its widespread medicinal usage [2-6].

The various bioactive compounds such as lignans, alkaloids, flavonoids, xanthones and their derivatives, terpenoids, secoiridoids, iridoids, and phytoconstituents such as chiratin, ophelic acid, palmitic acid, oleic acid, and stearic acid are responsible for the wide range medicinal and pharmacological properties of *S. chirayita* [7,8]. These tremendous medicinal and pharmaceutical potential has resulted in constant increase in its demand at national and international level. This has led to the unauthorized and unsustainable extraction of its natural populations. Moreover, destruction of native habitat has also resulted in the reduced number of natural population. Large scale seed based propagation of *Swertia chirayita* is generally discouraged due to unavailability of seeds or due to harvesting of plants before seeds mature, further seed based propagation is limited because of dormancy and decided by many factors like cold temperature, rainfall, moisture and sowing time etc. The species is, therefore, deprived of natural regeneration. The problems of seed dormancy and the necessary delicate field handling of the seedlings discourage commercial cultivation of the species. National Medicinal Plant Board, Govt. of India, NEW Delhi has prioritized *Swertia chirayita* and also designated as critically endangered by International Union for Conservation of Nature and Natural resources (IUCN) (http://www.nmpb.nic.in). Sustainable propagation and extraction of *Swertia chirayita* is therefore necessary to facilitates the continuous supply and ensuring the quality of raw material without threatening its natural population.

The objective of sustainable propagation via seeds for maintaining this valuable plant can be achieved using the Plant tissue culture methodologies. Requisite treatments and conditions can

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be manipulated in *in-vitro* seed germination protocols and enhanced multiplication can be achieved in minimum of time. Seed germination being a complex process depends upon many environmental and biochemical factors such as plant growth regulators, water and photoperiod [9]. Plant tissue culture can provide us the stage to manipulate this factors to enhance the germination potential. In past attempts have been made by the researchers for enhancing the seed germination percentage in the said plant species but they have used only chemical treatments [10] also very limited literature is available on cold stratification [11,12] and others have studied the effect of storage conditions and time period on germination of seeds [13]. Therefore, the current study has been done to analyze the best combination of treatments of cold stratification and different chemical/PGRs pre-sowing on *in vitro* germination of *Swertia* seeds and dormancy break.

Material & Methods

Swertia chirayita seeds were procured from Hi-tech Forest Nursery, Patalthod, Munsiyari, Uttarakhand. For identification and germplasm conservation procured samples were submitted to National Bureau of Plant Genetic Resources, Pusa Campus, New Delhi and assigned the accession number (VS-03-IC- 567642). Seeds for the subsequent experimentation were sterilized with 1% NaOCl for 5 minutes as per the protocol standardized by [14].

Pretreatments

Cold stratification and different pre-sowing chemical treatment was tested for breaking seed dormancy by incubating seeds at low temperature (0°C-20°C) for varying time intervals (4-12 weeks) and different pre-sowing chemical treatments of GA_3, IAA, NAA and KNO_3, HNO_3 and HCl were assessed for seed germination. The sterilized seeds were soaked in solutions of IAA (0.5-1.0 mg/L), 1-Naphthaleneacetic acid (0.5-1.0 mg/L) and GA_3 (100-200 µM), KNO_3 (0.5%), HNO_3 (0.1N) and HCl (0.1N) for 12-24 hrs. After washing the seeds for 2-3 times with double distilled water, they were placed in petri dishes (90 mm), lined with single layer of moistened Whattman filter paper no. 1. Double distilled water was used for moistening the filter paper and further moistening of the seeds. In control sets only double distilled water was used. Seeds were

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subjected to regular monitoring and double distilled water was used for moistening when required. Radical emergence was recorded for seed germination.

Results

There was a significant stimulation of seed germination in *Swertia chirayita* seeds after cold stratification and chemical/PGR pre-sowing in comparison to control where very low seed germination was recorded. There were morphogenic variations among different treatments. Among all the temperatures tested incubation of seeds at 15^{0C} for 12 weeks resulted in highest percentage of germination (70-75%) (Figure 1A). In the pre-sowing chemical treatments applied GA_3 (100 µM) was most effective in stimulating seed germination. Germination percentage varied from 53%-80%. IAA also stimulated the seed germination effectively and 0.5 mg/L IAA for 24 hrs recorded 68% seed germination. KNO_3 was effective in stimulating seed germination as compare to control but it showed less effectiveness in comparison to GA_3 and IAA. Interestingly NAA (0.5 mg/L) resulted in germination comparable to that of KNO_3 when incubation was given for 12 hrs. HNO_3 and HCl resulted in very poor germination (Figure 1B). Among all treatments, incubation at 15^{0C} for 12 weeks followed by pre-sowing with GA_3 (100 µM) reported to be most effective (>80%) in *Swertia chirayita* seed germination.

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A B

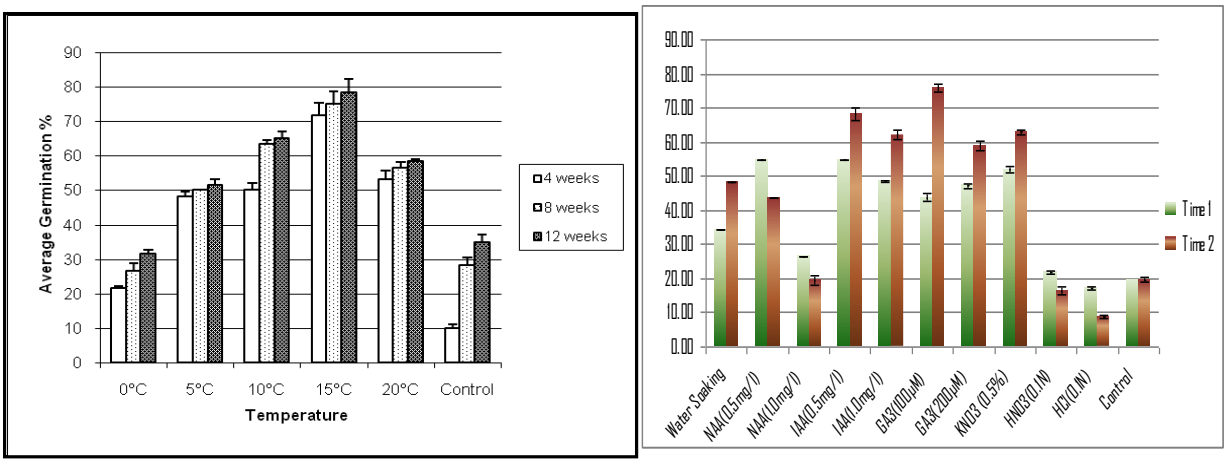

Figure 1A: Cold stratification treatment of seeds of *Swertia chirayita*.

Figure 1B: Pre sowing chemical treatment of seeds of *Swertia chirayita*.

Discussion

It has been observed that the seed germination potential is very poor in *S. chirayita* resulting in its poor cultivation and also additional problem of unavailability of superior germplasm hinders its large scale propagation, which has also been reported in other medicinal plants [15]. Present investigation suggested that a constant moisture and administration of low temperature serves as a good alternative for better seed germination. There are reports in other plants of commercial importance where cold stratification has resulted in significant dormancy breaking [16-18]. The treatment of seeds by soaking with plant growth regulators like IAA, GA_3, NAA and chemicals like HNO_3, HCl and KNO_3 etc. resulted in superior germination compared to moistened medium method. Out of PGRs used GA_3 was optimum in germinating seeds at low temperature. Auxin IAA was also effective has also been suggested in earlier reports [19,20]. NAA and KNO_3 were less effective comparatively. HNO_3 and HCl soaking was not effective in seed germination but only at low temperature. In our work with chirayita, it was found that simply a low temperature treatment alone or pre-sowing alone was not effective enough in enhancing seed germination. But

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combination of incubation at low temperature followed by pre sowing gave best results (incubation at 15^{0C} for 12 weeks followed by pre-sowing with GA_3). The report is in accordance with other workers also where exogenous application of gibberellic acid and chilling was used to overcome the problem of dormancy and increased the germination in seeds of Asafoetida (*Ferula assafoetida* L.) [21]. Therefore the uniform and prompt seed germination in higher altitudinal plants requires the low temperature exposure along with hydration is also established in the present work [22].

Conclusion

As Cultivation of medicinal plants as a renewable resource and non-food crops will provide stable supply of raw materials and could lower the pressure on natural habitats [23]. As environmental This is evident from the research that the pre-sowing treatments coupled with essential environmental factors causes significant enhancement in seed germination *in-vitro*. These protocols can be used for the large scale production of the propagules and thus can help in conservation of this very important endangered medicinal herb in the natural habitats. This work also opens the gate for the combinatorial strategies of chilling and chemical pretreatments for seed germination enhancement in other medicinal plants/herbs also.

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