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Assessment of Viability, Germination and Efficacy of Pre-treatments in Seeds of *Jurinea dolomiaea*, a Critically Endangered Medicinal and Aromatic Herb of Alpine Himalaya, from the Perspective of Conservation

Research Article

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Abstract

Jurinea dolomiaea Boiss. (Asteraceae) commonly known as dhoop, found in high alpine Himalayas are rich in aromatic resin therefore, used as incense locally as well as in dhoop industry. The species also valued for its medicinal importance to cure colic, puerperal fever, gout, rheumatism, poultice to eruptions, and as stimulant. Further, its antibacterial, antimicrobial, antioxidative and anticancerous properties also have been reported. Currently, *J. dolomiaea* has an endangered population status due to unsystematic collection and overexploitation. Therefore, seed germination features were investigated. The seed showed 96% viability and were non-dormant; 70% germination occurred within 10 days in control. Seed pre-treatments (GA_3, 0.1 mM and cold-stratification) enhanced the germination further and their role in germination improvement especially in alpine species is well known. Surprisingly, we did not find any previous report on seed germination behavior of *J. dolomiaea* and reporting it first time. The findings clearly indicated the suitability of seeds for propagation and conservation of *J. dolomiaea*, an endangered medicinal and aromatic herb.

Keywords: Conservation; Endangered; *Jurinea dolomiaea*; Medicinal-Aromatic Herb; Seed Germination

Introduction

Jurinea dolomiaea Boiss. (Asteraceae) commonly known as dhoop or jari-dhoop or gugul-dhoop, distributed commonly on open gently undulating alpine pastures, dry slopes and glacial moraines of high alpine Himalayas at an altitude range of 3400 to 4500 m asl [1]. The stout tuberous roots are rich in aromatic resin and are used for incense in temples, local houses, religious ceremonies and also sold commercially as a main ingredient of dhoop industry [2]. Besides the aromatic properties, the species has also been valued for its medicinal importance. The roots are, used to cure colic, puerperal fever, as poultice to eruptions and considered as stimulant [3, 4]. The aromatic oil obtained from the roots is beneficial in gout and rheumatism [2]. Further, its antibacterial, antimicrobial, strong antioxidative, DNA protective and anticancerous properties are also reported [5-9]. The presence of high levels of secondary plant metabolites in various parts of the plant signifies its effectiveness [8].

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According to conservation assessment and management plan (CAMP) workshop reports, the species has been categorized as critically endangered in Himachal Pradesh [10] and has endangered status in Uttranchal and J&K [11]. Thus, included in IUCN red data list and considered as an endangered species. The latter is owing to the overexploitation, unsystematic collection and trade of wild population to meet the demand [12]. Therefore, propagation/ multiplication and/or conservation of the species are of urgent need to restore the lost population. *J. dolomiaea* can be propagated through rhizome cuttings [13] and seeds. Surprisingly, we did not find any report on seed germination behavior of *J. dolomiaea*, thus the current study was undertaken. The findings will be undoubtedly helpful for the seed-based propagation and conservation of *J. dolomiaea*, an endangered medicinal and aromatic herb.

Material and Methods

Seed Collection

The seeds of *J. dolomiaea* were collected from the alpine slopes near Tung Nath temple, Chopta, Uttarakhand, India at an altitude of 3800 m asl, during a Botanical excursion (September, 2019), organized by the Botanical society of Deshbandhu College, University of Delhi. The seeds were dried in shade for ten days and then stored in airtight container at room temperature for further study.

Viability Test

Seed viability was assessed through tetrazolium reduction test as followed by Sharma *et al.* [14]. Where, the seeds were first surface sterilized with absolute alcohol for about 30 seconds, followed by through washing with distilled water (DW) and kept submerged in 100 ml of DW for 24h at 25 °C. After 24h the seed coat was removed manually and the embryos were incubated with 0.3% aqueous solution of 2,3,5-triphenyl tetrazolium chloride (TTC) for 24h at 25 °C under complete darkness. The embryos which turned complete pink were considered as viable.

Seed Germination Assays

Seeds with uniform size and shape were selected and surface sterilized with absolute alcohol for 30 seconds, washed, and soaked in distilled water for 24h at 25 °C. Thereafter, the seeds were transferred to Petri plates lined with moist substratum (3-4 layers of filter papers, hydrated with DW) for germination in BOD incubator at 25 °C. The emergence of 3-5 mm radicle was considered as seed germination.

Seed Pre-treatments

To stimulate or enhance the seed germination, seeds were pre-treated with gibberellic acid (GA_3, 0.1 mM), Indole acetic acid (IAA, 0.01 mM) and moist cold-stratification (5 °C), respectively. In brief, surface sterilized seeds were socked in stated concentrations of GA_3 and IAA for 24h at 25 °C and were shifted to Petri plates for germination. For moist cold-stratification, 24h socked seeds (DW) were placed on moist substratum in Petri plates and kept at low temperature (5 °C) in refrigerator for 15 days. Thereafter the Petri plates were transferred in to BOD incubator at 25 °C for germination. In all the cases the moisture level of substratum was maintained by addition of DW.

Germination Speed

Final germination percentage/ Day of completion of germination

Data Analysis

All the experiments were carried out in triplicates. The data were expressed as mean percentage value ± SD. The statistical differences between means were calculated based on the Student's *t*-test.

Results and Discussion

Jurinea dolomiaea is an important species of high altitude Himalaya, and considered as the prime indicator of the alpine region [15]. It is a perennial prostate herb with rosette form, leaves arising at the base, deeply pinnatifid and white tomentose beneath. Flowers in dense deep purple heads, short stalked arising at the center of the leaf rosette (Figure 1A). Seeds are brownish, little curved (Figure 1B) with brown pappus. The plant is extensively utilized for its high medicinal and aromatic properties and become endangered due to overexploitation [16]. Therefore, seed based multiplication or conservation possibilities have been investigated. The plant produces a reasonably good number of healthy and viable seeds per capitulum and the number of capitulum/plant vary depending upon the age of plant. However, we did not count the number of seeds/plant here. The average length and width of seed measured was 5.3 and 3.2 mm, respectively and weight was 23.8 mg (Average of 50 hydrated seeds).

In the present study, seed of *J. dolomiaea* from Uttrakhand Himalaya, showed 96% viability as observed through TTC-reduction assay. The seeds were non dormant and exhibited convincingly good germination in control itself. Seeds germination started on 3rd day of incubation and finally reached to 70% germination within 10 days. GA_3 application significantly enhanced the germination. Due to GA_3 (0.1 mM) pre-treatment 84% germination was recorded against 70% in control (Figure 2). GA_3 not only enhanced the germination percentage but also increased the germination speed or reduced the time required of germination significantly (Table 1). The role of Gibberellins in breaking seed dormancy and enhancing germination rate is well known. GA stimulate germination processes by stimulating

Figure 1: *Jurinea dolomiaea* at its natural habitat (A) and seeds (B).

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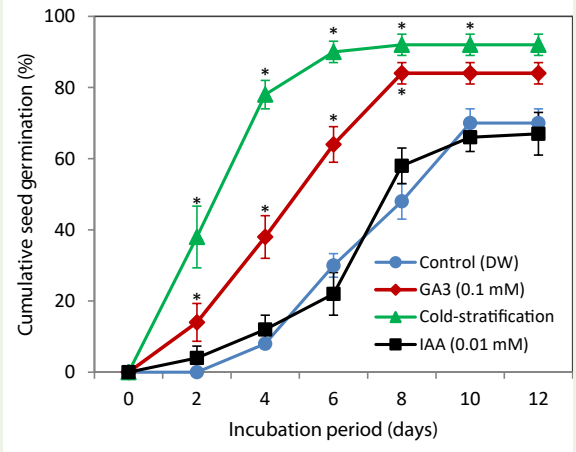

Figure 2: Effect of various seed pre-treatments on seed germination of *Jurinea dolomiaea*. Data are average of 3 replicates each ± SD. *Significantly different from control (p≤ 0.05; Student's t-test).

Table 1: Seed viability, final germination (%) and germination speed (average number of seeds germinated/day) in *Jurinea dolomiaea*. Data are average of three replicates (50 seeds × 3) each ± SD. *Significantly different from control (p≤ 0.05; Student's t-test).

Seed pre-treatments	Seed viability (%)	Seed germination (%)	Germination speed (number of seeds/day)
Control (DW)	96	70 ± 5	7
GA3 (0.1 mM)		84 ± 3.33*	10.5*
Cold-stratification		92 ± 4*	15.33*
IAA (0.01 mM)		67 ± 6.33	6.6

the activities of hydrolytic enzymes [17, 18], enzymatic loosening of seed coat [19], fulfill the requirement of after-ripening, and cold-stratification [20]. The application of IAA seed pre-treatment did not affect the germination significantly. Moist cold-stratification of seeds at 5 °C for 15 days significantly increased the germination speed as well as the final germination percentage (Figure 2, Table 1). Thus, 92% germination was achieved with in a week. The effectiveness of cold-stratification in breaking seed dormancy or germination improvement in alpine species is well reported [19, 21]. Cold-stratification actually simulates the natural conditions essentially experienced by the seeds of alpine region after dispersal. Where, seeds remain under snow or exposed to low temperature during winter months before the initiation of germination. In addition, cold-stratification effectively breaks various forms of seed dormancy (physiological, morphological and morpho-physiological), increase embryo growth potential and sensitivity of seed tissue towards GA, fulfills the requirement of GA and/or after-ripening [21, 22]. Therefore, cold-stratification has been generally utilized to achieve uniform seed germination.

Banday *et al.* [13] reported about the possibility of vegetative propagation of *J. dolomiaea* through rhizome cuttings and find out the efficacy of certain plant growth regulators to improve the same. However, seed based propagation is considered comparatively easy, practical, cost effective, natural and reported to have many advantages over vegetative propagation [23]. The current observations on seed viability and germination of *J. dolomiaea* here also support the above points. Surprisingly, in spite of high demand, economic importance and endangered status of the species, there is complete lack of information about the seed viability and germination aspects of *J. dolomiaea* and we are reporting here first time.

Conclusion

The present findings on seed germination of *J. dolomiaea* clearly indicated the suitability of seeds for propagation and conservation of the species. Since, most alpine perennial plants including *J. dolomiaea* exhibit very sluggish growth, the cultivation cannot be commercially/economically viable. Therefore, in-situ conservation with periodic systematic extraction through skilled workers can be recommended. Further, conservation of species in its natural habitat has ecological significance and ensures local livelihoods, quality of raw drug availability and trade. The realization of the same requires a sincere, realistic and practical approach.

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