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Substitute of Light Requirement for Germination in Positive Photoblastic Seeds of *Inula racemosa* - A Critically Endangered Medicinal and Aromatic Plant of North-Western Himalaya, India

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ABSTRACT

Inula racemosa, commonly known as pushkarmool, has high economic value due to its medicinal and aromatic properties. The species has been over-exploited for commercial purposes and become threatened (critically endangered) in NW Himalaya. The seeds exhibited photo-sensitivity and were complete positive photoblastic. They germinated (80%) only when exposed to light; no germination was recorded in dark. The hydrated seeds required only a brief exposure of light to initiate germination, thus can be categorized under Low-Fluence Responces (LFRs). Under natural field conditions the seeds do not germinated when sown below 1.5 cm soil depth, as the light became limiting factor. Interestingly, GA_3 (1 mM) substituted the requirement of light for germination; 70% germination was recorded under complete darkness due to GA_3. These observations are completely new for seeds of *I. racemosa* and discussed in detail. The findings have practical applications for seed-based multiplication of the species.

Key words: Gibberellic acid, Light, Phytochrome, Pushkarmool, Threatened plant, Seed germination.

INTRODUCTION

Seed germination is controlled by various environmental factors such as moisture or humidity, temperature regime, nitrate concentration, and quality/ quantity of light (Baskin and Baskin 2014). Seeds response to light for germination can be neutral (non-photoblastic seeds), positive (positive photoblastic seeds) or negative (negative photoblastic: light inhibiting the germination). Seeds sense the depth or canopy shade and/or day length through the quality and quantity of light they receive (Pons 2000, Chen et al. 2014). Generally, smaller seeds require light for germination (positive photoblastic) as they have to start photosynthesis quickly due to lack of enough reserve food or avoid germination in deep underground or under thick canopy as the limited resources would be insufficient for seedling to reach the surface (Seo et al. 2009). In contrast, light also inhibit seed germination in many species (Takaki 2001, Yang et al. 2020). The latter is an adaptive trait to avoid germination in open dry habitats (Pons 2000, Carta et al. 2017).

In the present study, we examined such feature in seeds of *Inula racemosa* Hook f. (Family: Asteraceae) commonly known as manu or pushkarmool; a critically endangered, economically and medicinally important plant from Lahaul, Himachal Pradesh, India (Fig. 1). *I. racemosa* has been extensively used in Indian, Chinese, Tibetan and other systems of traditional medicine (Wangchuk et al. 2023). The roots and rhizomes reported to exhibit hepatoprotective, cardioprotective, hypoglycemic, antioxidant, anti-inflammatory, anti-nociceptive, anti-cancerous, cytotoxic, anti-asthmatic, anti-mutagenic, anti-apoptotic, anti-bacterial, anti-fungal, larvicidal and aphrodisiac (seeds) activities (Chauhan 1999, Rathore et al. 2022). The said effects have been confirmed by various authors on animal models or through in-vitro studies and the latter can be attributed to the presence of many bioactive molecules including, alantolactone, isoalantolactone, inunolide, dihydroisoalantolactone, β-sitosterol, dihydroxinunolide, neoalantalactone, inunolide, sesquiterpene lactone, alantodiene, phytosterols and glycosides (Rathore et. al. 2022,

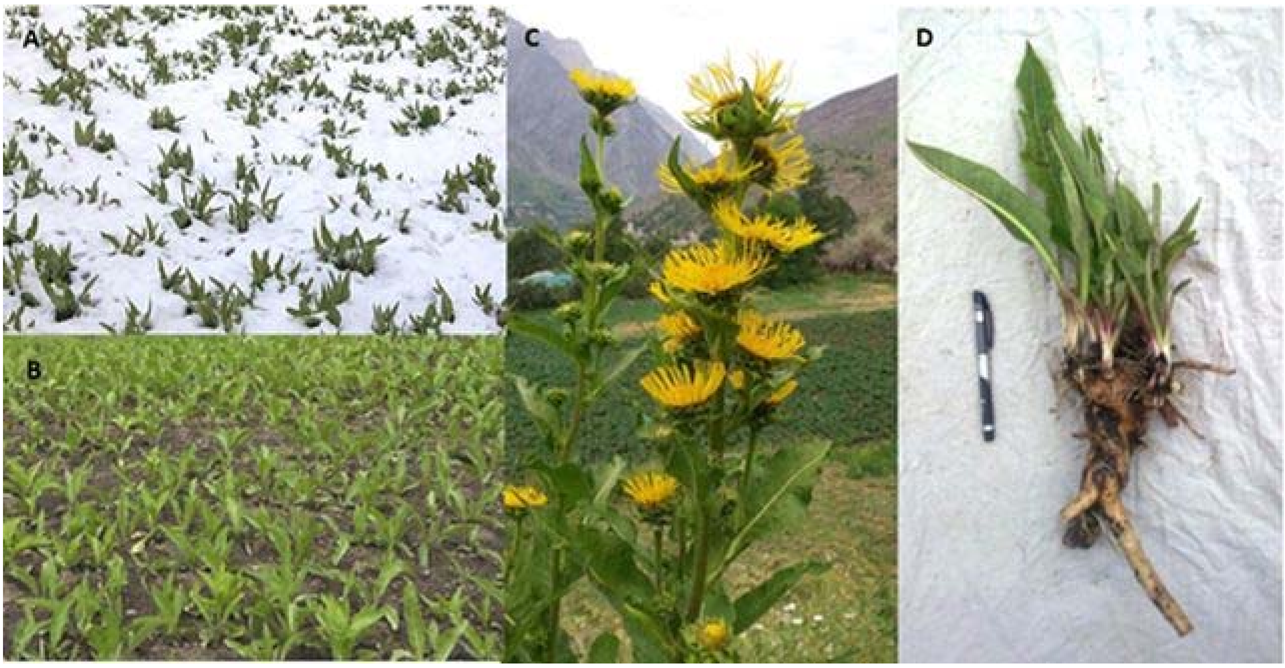

Figure 1. *I. racemosa* in cultivated fields (A) during early spring under snow, (B) during summer season, (C) three years old flowering plants, and (D) harvested plants with root

Wangchuk et al. 2023). In addition, the roots are aromatic and have high demand and value for the preparation of incense (Aswal and Mehrotra 1994). The restricted distribution, high demand from the pharmaceutical industries and resulted unsystematic over-exploitation has caused rapid reduction in *I. racemosa* population. According to the red data book of Indian plants (Nayar and Sastry 1990) the species has vulnerable status globally, and as per the conservation assessment and management prioritization (CAMP) workshop, Lucknow (Molur and Walker 1998) the species has been categorized as a critically endangered (North Himalaya). Further, commercial use of the species has been restricted until or unless legal procurement certificate obtained from the concerned forest officers (Anonymous 2003). Therefore, efforts are needed for conservation and/or multiplication of the species. *I. racemosa* can be propagated vegetatively (rhizome cuttings) as well as through seeds and both methods have certain advantages and disadvantages. However, the latter is generally preferred as it is natural, easy, realistic and having many advantages over other methods (Sharma and Sharma 2022). The literature revealed many reports on seed germination behaviour of *I. racemosa* (Sharma et al. 2006, Jabeen et al. 2007, Shabir et al. 2010, Sharma and Sharma 2010, Siddique and Jeelani 2015, Singh et al. 2018, Bano et al. 2019). Surprisingly, none of them reported about positive photoblastic nature of *I. racemosa* seeds except, Sharma and Sharma (2010) where, only a brief account about the involvement of light in seeds germination was given. Since, most of the reported experiments have been done on moist substratum in Petri plates under different photo-period, therefore such feature was remained unnoticed. However, the germination would have been significantly affected if the same has been performed under field conditions, where such seeds will never germinate under soil, until or unless they get exposed to light. Therefore, such seed features are very crucial for seed-based cultivation and propagation of the species. We reported here in detail about the photoblasicity of *I. racemosa* seeds, their response to quality and quantity of light. In addition, we tried to find out substitute for light requirement in *I. racemosa* seeds. The outcome will have definite impact on cultivation and conservation of *I. racemosa* through seeds.

MATERIALS AND METHODS

Plant Material

Mature seeds of Pushkarmool or Manu (*Inula *racemosa* Hook f.) were collected from fully ripened, partially dried capitulum of the three years old domesticated plant population (Fig. 1) from Chander-Bhaga valley of Lahaul, Himachal Pradesh, India (latitude: 32°36’56" N, longitude: 76°54’54" E and altitude: 2980 m asl) in the month of September, 2022. The pappus were removed manually and seeds were dried under shade at room temperature for about 10 days and kept in completely insulated plastic container in a refrigerator at 5±1°C till further use.

Seed viability and germination tests

The viability of non-germinated seeds was analyzed through triphenyl tetrazolium chloride (TTC) reduction test as followed by Krishan et al. (2022) where, the seed testa was removed (to allow the easy uptake of TTC) and incubated in aqueous solution of 0.5% TTC at 25°C for 24 hr. The completely pink stained embryos were considered viable. Germination tests were carried out in Petri plates with four layers of wet filter paper as substratum. All the tests were carried out in three replicates, placing 50 seeds in each Petri plate. The Petri plates were then placed in a growth chamber under controlled conditions at 25°C with a 10 hr-light/12 hr-dark photoperiod. Germination was considered when radicle protrusion was more than 2 mm and recorded at alternate days till the completion of 14 day experiment.

Effect of light and darkness on seed germination

To examine the effect of different light regime (white, red, green and darkness), on seed germination, seeds were sown in Petri plates. For white light, cool white fluorescent tubes were used and for darkness, Petri plates were wrapped in two layers of aluminum foil, and germination was recorded at the end of the 14 day experiment. Red and green light was provided by covering the Petri plates with two layers of red and green plastic light filters, respectively. Additionally, seeds were also sown in pots at different soil depth (0.5, 1.0. 1.5, 2.0, 2.5 and 3.0 cm) and kept under natural conditions.

Seed pre-treatments

To find out pre-treatments that can substitute the requirement of light for germination, various concentrations of GA_3 (0.01, 0.1 and 1 mM), KNO_3 (5, 10 and 15 mM), and Kinetin (0.001. 0.01 and 0.1 mM) were used. The substratum was moistened with respective solutions and were incubated for germination. Two sets (light and dark) were used for each treatment and germination was recorded at the end of the 14 day experiment.

Photoblastic Index and Relative Light Germination

The photoblastic responses of seeds were measured by using Photoblastic Index (PI) (Castillo et al. 2013) and Relative Light Germination (RLG) (Milberg et al. 2000) as following:

$$PI = (GD - GL)/(GD + GL)$$

$$RLG = (GL)/(GD + GL)$$

Where, GD (germination percentage in darkness), GL (germination percentage under light).

The PI value deviates between 1 and -1 (1: negative photoblastic; 0: germination independent of light; -1: positive photoblastic). The RLG values ranges from 0 to 1: 0 (negative photoblastic), 1 (positive photoblastic), and 0.5 (non-photoblastic).

Data analysis

The experiments were conducted in three replicates (50 x 3) and the results were presented as mean percentage. The significance of difference between means were calculated based on the Student’s *t*-test.

RESULTS AND DISCUSSION

Seed germination of *Inula racemosa* in the present study was strongly promoted by light. The seeds germinated only under light and no germination was recorded in dark: 82, 76 and 3% germination were recorded under white, red and green light, respectively, as compared to no germination in dark after 14 days of incubation in growth chamber (Fig. 2). The Photoblastic Index (PI) and Relative Light Germination (RLG) values calculated were found to be -1 and 1, respectively, indicating complete positive photoblastic nature of the seeds. The involvement of phytochromes and possibly other photoreceptors in such process are known. Plants perceive light signals through various photoreceptor molecules like, phytochromes (red and far-red: 600-750 nm); cryptochromes, phototropins, Zeitlupe (320-500 nm),

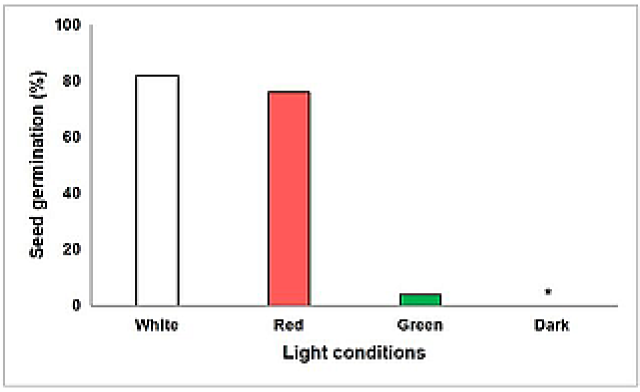

Figure 2. Influence of light quality on seed germination of *I. racemosa*. All treatments are significant with their respective control (n=3, $P \leq 0.05$). *no seed germination occured

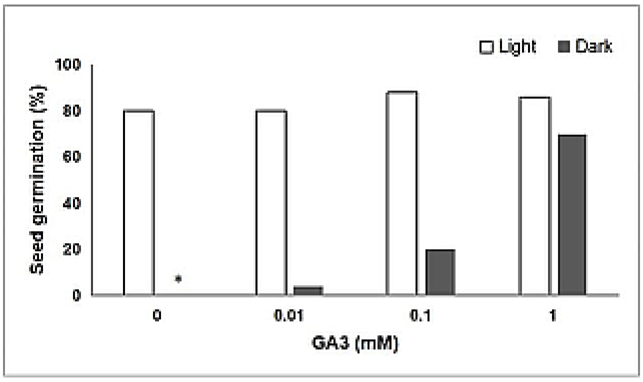

Figure 3. Effect of different concentrations GA_3 on seed germination of *I. racemosa* under light and dark conditions. All treatments are significant with their respective control (n=3, $P \leq 0.05$). *no seed germination occured

UV Resistance Locus 8 (282-320 nm) and influence various light sensitive physiological processes including seed germination (Gyula et al. 2003, Rizzini et al. 2011, Christie et al. 2015). Sharma and Sharma (2010) reported various seed physiological aspects of *I. racemosa*. However, only a brief account was presented about photoblastic nature of *I. racemosa* seeds. Various authors also have reported seed germination features of *I. racemosa* (Sharma et al. 2006, Jabeen et al. 2007, Shabir et al. 2010, Siddique and Jeelani 2015, Singh et al. 2018, Bano et al. 2019) and none of them have noticed the positive photoblastic nature of seeds as most of the experiments were conducted under light exposure. Further, the poor seed germination (20%) of *I. racemosa* under open field conditions as reported by Rinchen et al. (2019) could be due to the positive photoblastic nature of the seeds, where, the seeds under soil do not received sufficient quantity of light to initiate the process of germination. Otherwise, maximum germination may have been achieved. The complete absence of germination in seeds sown below 1.5 cm soil depth in present study (data not shown) also confirms the same. Quite interestingly, *I. racemosa* has been successfully cultivated in Lahaul (Himachal Pradesh, India) for long period of time through rhizome cuttings, and seed-based cultivation/ propagation known to have certain constrains including poor and erratic germination (Rathore et. al. 2022, Sharma and Sharma 2010). These observations signify the importance of such seed features (photoblasticity) in germination where, light requirement will become a limiting factor. Therefore, screening of seeds for photo-sensitivity should be done before any conclusion about seed germination and dormancy.

Positive photoblastic seeds, depending upon the species, require different fluence rate (mol m^{-2} s^{-1}) or quantity of light to initiate the process of germination and are categorized under: Very-Low-Fluence Responses (VLFRs); Low-Fluence Responses (LFRs) and High-Irradiance Responses (HIRs) (Baskin and Baskin 2014). In the present study, seeds of *I. racemosa* required only a brief exposure (5 to 10 seconds) of light to initiate germination thus, showing Low-Fluence Responses (LFRs). Quite remarkably, in our experiment GA_3 substituted the requirement of light for germination. Thus, GA_3 (1 mM) treated seeds germinated in complete darkness: 70 % germination was recorded against 0 % in control (Dark, without GA_3) (Fig. 3 and 4). Under white light, with or without GA_3 the germination was 86 and 80 % respectively. The effect of GA_3 was concentration dependent, due to GA_3 0.01, 0.1 and 1 mM; 3, 20 and 70 % germination respectively, was recorded in dark (Fig. 3).

GA regulates seed germination and dormancy in various ways (Sharma et al. 2021). The involvement of GA in phytochrome dependent germination and/ or phytochrome mediated activation of GA via, expression (*GA2OX1*, *GA2OX3*) and repression (*GA2OX2*) of GA related transcripts is known in some species (Cho et al. 2012, Arana et al. 2014).

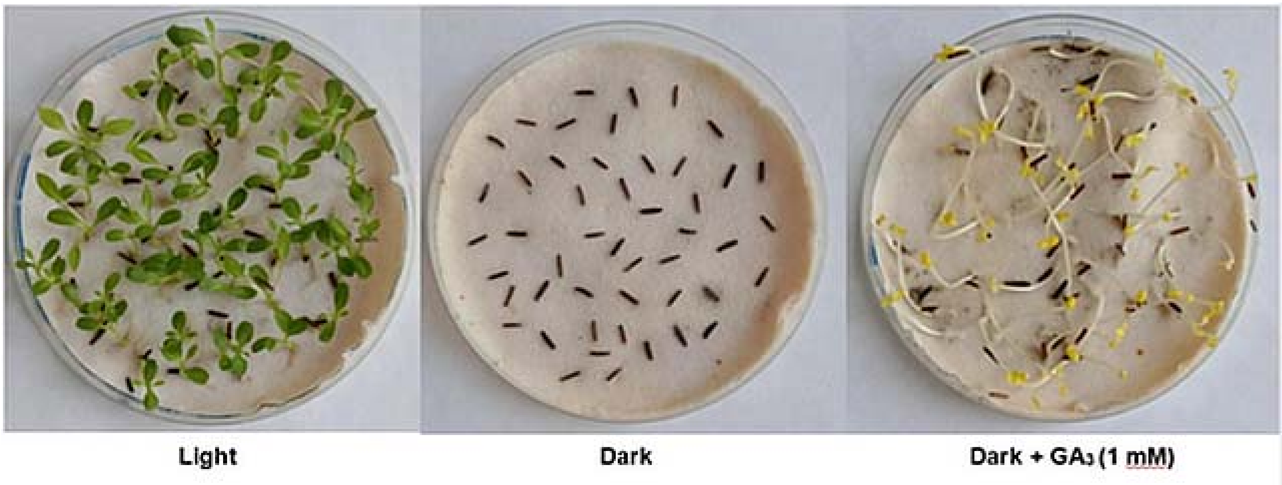

Figure 4. Complete positive photoblastic nature of *I. racemosa* seeds and their response to GA_3 under dark condition

Other seed pre-treatments tried (KNO_3, Kinetin) completely failed to substitute the requirement of light for germination. The seedlings resulted from the GA_3 dependent germinated seeds under dark, were etiolated and exhibited elongated hypocotyl, inhibition of epicotyle growth, maintenance of plumular hook, an absence of primary leaf expansion, and lack of chlorophyll (Fig. 5 B). However, when exposed to light, these seedlings undergo deetiolation, a process under complete control of phytochromes and cryptochromes.

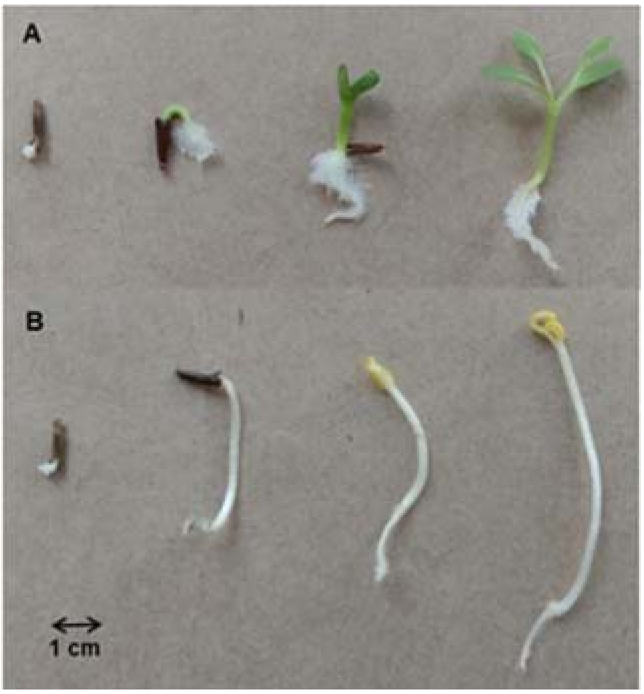

Figure 5. Different stages of seed germination and seedling growth in *I. racemosa* under light (A) and Dark (B) conditions

CONCLUSION

The seeds of *I. racemosa* exhibited positive photoblasticity and were categorized under Low-Fluence Responces (LFRs) based on the quantity of light required to initiate germination. GA_3 substituted the requirement of light for germination. The latter is reported for the first time in *I. racemosa* and has high significance for achieving maximum and uniform seed germination under field conditions where, light becomes the limiting factor due to sowing of seeds at variable soil depth. These observations have direct implications for seed-based propagation and cultivation of the species.

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Author contributions: RKS collected seeds, conceptualized idea, designed the study and wrote the initial draft of the manuscript. RKS, SM, R, I and KK performed the experiment. BB and AKC edited the final version of manuscript.

Conflict of interest: The authors declare no conflict of interest.

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