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Germination Eco-physiology of *Angelica glauca* Edgew Seeds

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Authors' contributions

This work was carried out in collaboration between all authors. Authors JSB and SSS designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors CPK and ARM managed the analyses of the study. Author AA managed the literature searches. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: *Angelica glauca* Edgew is a heavily exploited critically endangered medicinal cum aromatic herb of Himalayan region. Poor and erratic seed germination is one of the constraints in its *in-situ* as well as *ex-situ* conservation and large scale cultivation. Moreover, our understanding of the eco-physiological aspects of the seed germination in this species is very limited. The present study aimed to understand the effect of different temperature regimes, photoperiodic conditions and sowing depths on seed germination in *A. glauca* using laboratory and nursery conditions.
Study Design: Complete Randomized Design was executed.
Place and Duration of Study: G.B. Pant Institute of Himalayan Environment and Development, Mohal-Kullu (HP), India between April 2006 and July 2008.
Methodology: Germinability of the seeds were evaluated under different temperature regimes viz.(5, 15, 25 and 30°C) and photoperiods (light-24 hrs, dark-24 hrs and alternate

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photoperiods-16 hrs dark and 8 hrs light) in laboratory; and at sowing depths (0.5, 1.0, 1.5 and 2.0 cm) in controlled nursery conditions.
Results: In photoperiodic conditions, encouraging results were obtained under alternate light regimes which favored maximum (77.78%; $p<0.05$) mean germination along with minimum MGT (42.08 days) and Germination Potential Index (114.6) under laboratory condition. All these parameters performed significantly better at 25°C as compared to other temperature regimes tested. In nursery condition, seed sown at 1.0cm depth gave comparatively higher ($p<0.05$) seedling emergence. Poor seedling emergence at higher sowing depth clearly indicated the requirement of light for germination in *A. glauca* seeds.
Conclusion: Study concludes that alternate photoperiods (16 hrs dark and 8 hrs light), temperature (25°C) and soil depths not more than 1.0cm are effective treatments to achieve optimum germination in *A. glauca*. These technicalities could be easily adopted by the poor and unskilled farmers for economic cultivation of this species.

Keywords: Angelica glauca; germination; medicinal and aromatic herb; photoperiod; temperature; sowing depth.

1. INTRODUCTION

Angelica glauca Edgew. (Family-Apiaceae), locally known as Chora, Gandrayan or Chippi, is well known for their medicinal and edible values. This 1.2-3.6 m in height glabrous aromatic herb is distributed from temperate to alpine belts of Kashmir, Himachal Pradesh and Uttarakhand and declared as endemic to Indian Himalaya [1]. It mostly grows in moist rocky situations near water springs and forest shades, between 2000-3800 m altitudes [2]

Roots of *A. glauca* contain valeric acid, angelic acid and angelisine resin [3]. It is used as a stimulant, cardio active, carminative and expectorant and yield a pale to brownish yellow essential oil (0.4-1.3% dry basis) [4]. Roots were found to be traditionally used for post delivery weakness, vomiting, dyspepsia, indigestion, stomach pain, headache, dysentery, influenza, loss of appetite and asthma in human being for sheep/goats in cold condition during winter and diarrhea and constipation in Himalayan Musk Deer and as fodder to improve lactation in cattle, particularly in cows and goats [5].

Adopting IUCN criteria in its population assessment, it has been considered as Critically Endangered species for the Himalayan region [6]. Untimely destructive harvesting of the roots has been observed as one of the key reasons of poor regeneration and rapid reduction in sparsely distributed wild populations [7]. Its conservation and sustainable supply of the raw materials can be ensured through wide cultivation in suitable agro-climatic zones [8]. At present, the demand of its raw materials for pharmaceutical industries and of ethno-medicinal utility, are met only through harvesting of their wild populations. *Ex-situ* conservation through cultivation of rare, threatened and endemic medicinal plant species is commonly recommended as a promising tool for conservation purposes. It is also considered most viable option to meet ever increasing demand and ensure consistent supply of certified homogenous quality raw material.

Plant propagation from seed minimizes the loss of genetic diversity while reproducing local ecotypes. However, species have particular requirements for seed germination as a result of adaptive radiation into patchy and changing environments. Moreover, the choice of suitable techniques, based on the requirements of each species, may reduce waste of resources [2,9]. It is evident that environmental conditions (water, temperature, light, oxygen, etc.)

largely affect germination. Pre-sowing chemical treatments are widely used to break seed dormancy to enhance germination [1,2,9,10,11]. Response of seed dormancy breaking treatments varies from species to species depending on actual cause of dormancy [9].

It is important to understand the effect of different temperature regimes, photoperiodic conditions and sowing depths on seed germination both for practical nursery applications and for conservation management [9,10]. In previous studies, seed germination requirements of *A. glauca* has been explored using chemical treatments [1,2,11,12] and growing media and environments [13,14]. However, appropriate temperature regimes, photoperiodic conditions and sowing depths to achieve optimum germination in this species remained unexplored. This communication deals with the germination behavior of *A. glauca* in these lines.

2. MATERIALS AND METHODS

2.1 Seed Collection

Mature seeds of *A. glauca* were collected from Tosh Nala (2350-2400 m asl, East, Along spring, shrubberies, vast slope with high humus), Parvati valley, District Kullu, Himachal Pradesh in the month of October first week. The seeds, after drying at room temperature (20-25°C) for 10 days and retaining 12% moisture, were kept in hermetic plastic containers and placed in refrigerator at 4ºC, until used [2].

2.2 Seed Viability Test

The present study was conducted in the laboratory and nursery of G.B. Pant Institute of Himalayan Environment and Development, Mohal-Kullu (HP), India, between April 2006 and July 2008. Before conducting germination test, viability of the seeds was examined. The seed viability was determined immediately after collection and during the time of germination tests in spring using 30 seeds per replicate (in triplicate) following ISTA [15]. Wings of seeds were excised before immersing in Tz solution (0.5% of 2, 3, 5, triphenyl tetrazolium chloride). Dark red stained embryos were considered as viable.

2.3 Seed Germination and Seedling Emergence

Screening of 3000 seeds of *A. glauca* was done. Only healthy seeds were used for germination test. 50 seeds per replicate (in triplicate) were used for each experiment. All seeds were surface sterilized with 0.04% $HgCl_2$ (1 min) followed by washing thoroughly with redistilled water. Fifty seeds per replicate (in triplicate) were used in each experiment. Three experiments with Complete Randomized Design were undertaken.

1. To evaluate effect of different photoperiodic conditions on seed germination, seeds were placed in Petri dishes (9 cm diameter) lined with Qualigens (615 A) filter paper. The Petri dishes were placed in BOD (Biochemical Oxygen Demand) incubator at 25±2°C under light (24 hrs), dark (24 hrs) and alternate light regimes (8 hrs light and 16 hrs dark).
2. To evaluate effect of different temperature regimes on seed germination, seeds were placed in Petri dishes (9 cm diameter) lined with Qualigens (615 A) filter paper and the Petri dishes were placed at different temperature regimes *viz.,* 5°C, 15°C,

25°C and 30°C inside germinator and room temperature (25-33°C) under continuous (24 hrs) light condition.

3. To evaluate effect of different sowing depths on seedling emergence, seed were sown in Styrofoam seedlings trays containing a mixture of soil (field soil) pure sand (Course): forest litter in equal ratio (1:1:1) at different sowing depths *viz.,* 0.5cm, 1.0cm, 1.5cm and 2.0cm.

For experiment no. 3, raised beds were prepared inside net house (temperature 24.71±14.88°C max., 11.12±5.05°C min; Relative Humidity 81.53±1.93% max., 74.81±4.09% min). Distilled water was used to maintain optimum moisture in Petri dishes. However, experimental beds inside net house were irrigated using sprinkler system. Seed germination and seedling emergence was monitored on daily basis and till the finding constant reading for two weeks. In Petri dishes, the seeds were considered geminated upon radicle emergence (1 mm). However, the seedling in nursery condition was considered emerged on the appearance of seed coat covered cotyledon above the soil.

Mean germination time was calculated by using, $MGT=\Sigma(fx)/\ \Sigma x$, where **x** is the number of newly germinated seeds on each day, and **f** is the number of days after seeds were set to germinate [16]. Germination Potential Index (GPI) was calculated as GPI = Days to start germination + Days to complete germination / Mean germination (%) X 100 as per Butola [17].

2.4 Statistical Analysis

Data were analyzed statistically using MS Excel, 2007. One way analysis of variance (ANOVA) and Fisher's least significant differences (F-LSD; $p<0.05$) was employed [18] to calculate significant difference between the means. Data in percentages were subjected to arcsine transformation before analysis of variance and then converted back to percentage for presentation [19].

3. RESULTS AND DISCUSSION

3.1 Effect of Different Photoperiods

The viability of *A. glauca* seeds at the time of germination test was found to be 82%. Data pertaining to the effect of different photoperiods on seed germination are presented in Fig. 1. Alternate light regime induced (19.0±3.61 days) and highest germination (77.78±18.4%) along with lowest MGT (42.08±4.05 days) and GPI (114.6±6.68). However, germination started after 37.33±5.77 days with maximum MGT (54.13±2.66 days) in continuous light regime. Continuous dark condition did not favor any of the above parameters. Significant variation ($p<0.05$) in mean germination (LSD=14.31; F=10.83), MGT (LSD=8.07; F=6.81) and GPI (LSD=33.27; F=28.185) was found. While working on germination potential of seven alpine and sub-alpine species Nautiyal [20] concluded that almost all the species germinated better in light than in dark condition. Similarly, Semwal and Purohit [21] have observed that seeds of several alpine and sub alpine species from Garhwal Himalaya germinate better under continuous light condition. Sunlight, which is predominantly red light, converts phytochrome to the active Pfr form. Pfr appears to stimulate GA biosynthesis which operates by mobilizing reserved food, weakening endospermic or other surrounding tissues, overcoming inhibitors or stimulating embryo growth [22].

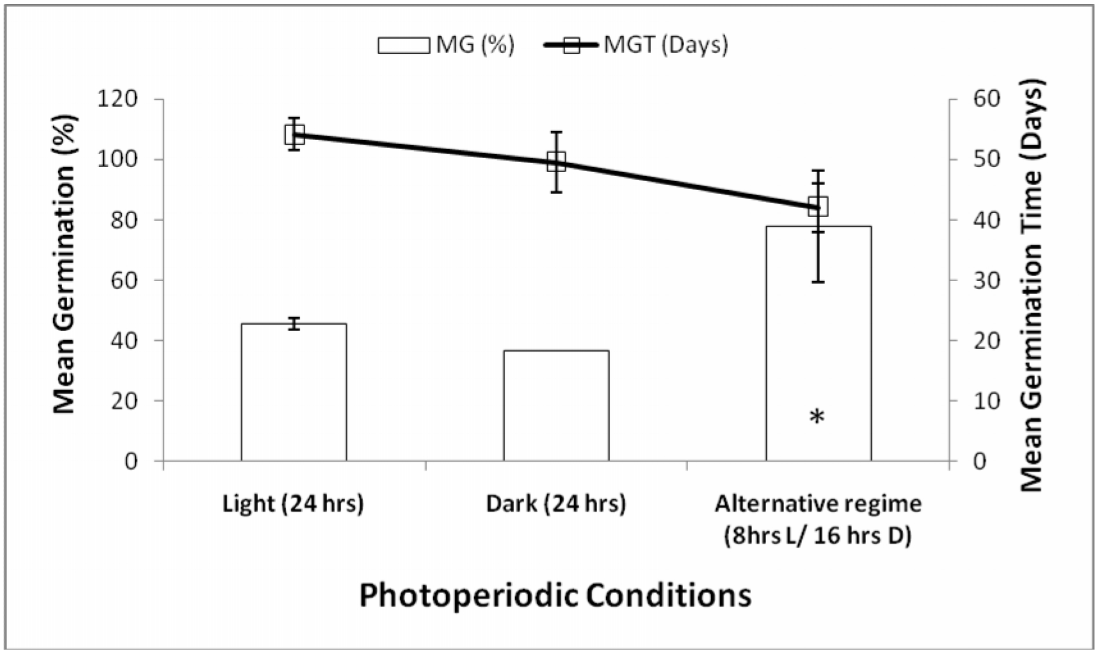

Fig. 1. Effect of different photoperiodic conditions on germination in *Angelica glauca* seeds; *indicates significant difference in mean germination (p<0.05; LSD=14.31; F=10.83) and mean germination time (p<0.05; LSD=8.07; F=6.81) among different photoperiodic conditions

3.2 Effect of Different Temperature Regimes

Data related to the effect of different temperature regimes on seed germination are shown in Fig. 2. Germination was not taken place at 5°C and 15°C. 'Days taken to first germination' (32±9.17 days), MGT (49.55±5.0 days) and GPI (215.6±25.0) were minimum and mean germination (36.67±0.00%) was found maximum at 25°C. 'Days taken to first germination' (LSD=10.97; F=50.72), mean germination (LSD=2.95; F=261.7), MGT (LSD=7.10; F=176.3) and GPI (LSD=424.6; F=13.18) significantly ($p<0.05$) varied at 25°C as compared to 30°C and room temperature. For all these parameters, poorest performance was recorded at room temperature. In general, the effects of temperature on seed germination are quite complex because it affects each stage of the germination process in a different way and is not independent of other factors [23]. Nautiyal et al. [24] concluded that there is poor seed germination in *Aconitum atrox* under low (10°C) as well as under relatively high temperature (30°C). In alpine seeds, optimum germination was observed between 20-30°C [25,26]. Alternate low and high moderate temperature treatments induce and enhance germination of dormant seeds [11]. In nature this will ensure a sufficient growing period allowing seedling establishment at higher altitudes. Therefore, most of the germination takes place soon after the snowmelt in early summers. Osburn [27] concluded that low and fluctuating temperatures hamper seed germination and establishment of seedlings in the alpine areas.

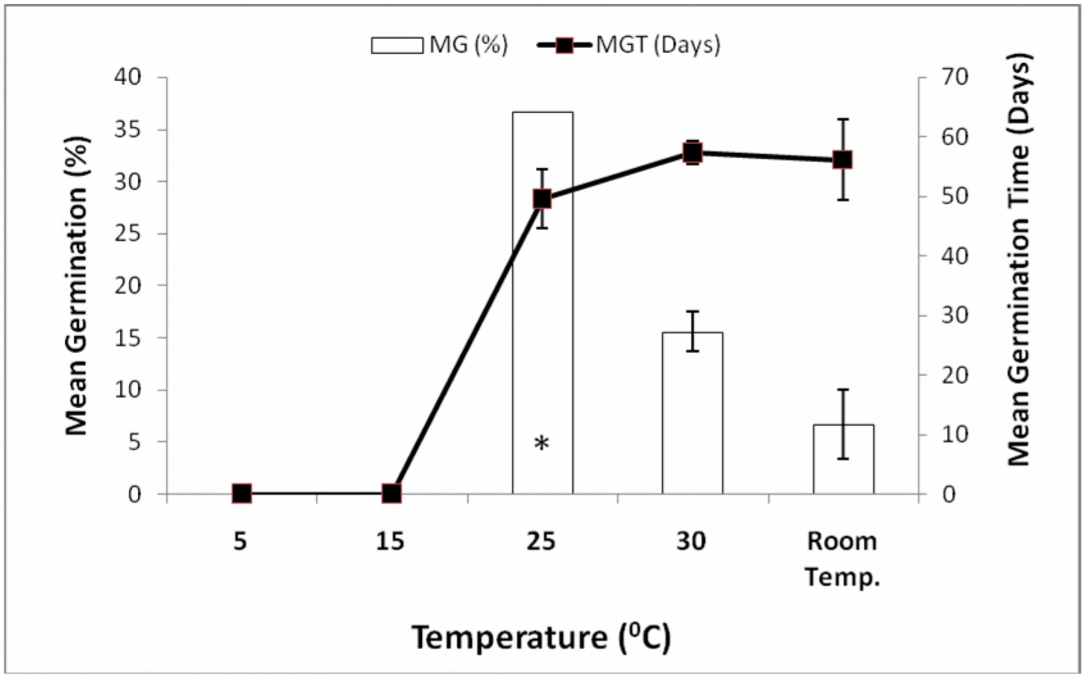

Fig. 2. Effect of different temperature regimes on germination in *Angelica glauca* seeds; *indicates significant difference in mean germination (p<0.05; LSD=2.95; F=261.7) and mean germination time (p<0.05; LSD=7.10; F=176.3) among different temperature regimes.

3.3 Effect of Different Sowing Depths

Data pertaining to the effect of different sowing depths on seedling emergence are summarized in Fig. 3. 'Days taken to first emergence' was found minimum (18±0.00 days) at 0.5cm and maximum (32±8.66 days) at 2.0 cm depth. MGT was least (31.21±2.22 days) at 1.0cm depth and most (34.67±9.50 days) at 2.0cm depth. All these parameters varied non-significantly (p<0.05) among different soil depths. However, significantly higher (p<0.05; LSD=15.70; F=6.38) mean germination (66.70±23.10%) was recorded at 1.0cm as compared to 2.0cm depth (23.33±5.77%). In *A. glauca* and *A. archangelica*, Vashistha et al. [28,29] found better germination at 0.5cm depth than 1.0cm. Poor seedling emergence at higher sowing depth clearly indicates the requirement of light for germination in *A. glauca* seeds.

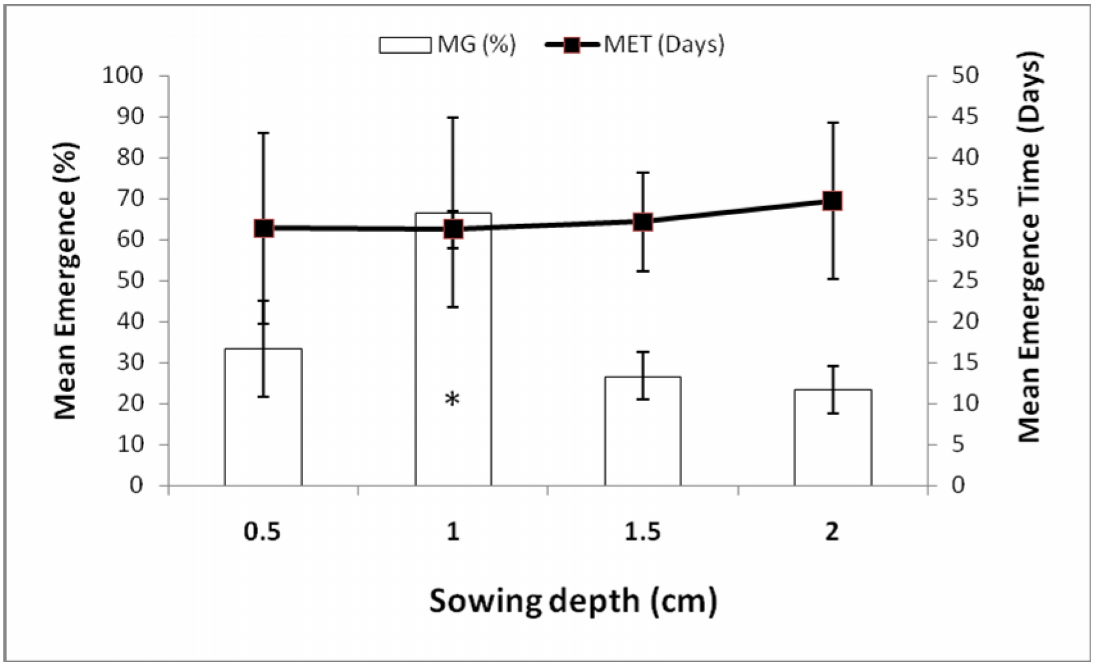

Fig. 3. Effect of different sowing depths on seedling emergence in *Angelica glauca*; *indicates significant difference in mean emergence (p<0.05; LSD=15.70; F=6.38) among different soil depths.

4. CONCLUSION

Study concludes that alternate photoperiods (16 hrs dark and 8 hrs light), temperature (25°C) and soil depths not more than 1.0cm are effective treatments to achieve optimum germination in *A. glauca*. These technicalities could be easily adopted by the poor and unskilled farmers for economic cultivation of this species.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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