

*Full Length Research Paper*

# Effect of pre-sowing, temperature and light on the seed germination of *Arnebia benthamii* (Wall. ex G. Don): An endangered medicinal plant of Central Himalaya, India

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*Arnebia benthamii* is under intensive utilization because of its wide use as medicinal and aromatic plant resource. Self-propagation of this species is by both seed and vegetative propagation. In an effort to improve and promote the cultivation of this over-exploited medicinal herb, the effect of temperature and light on the germination of seeds was investigated with various presowing treatments of water and GA<sub>3</sub>. Germination was found to be temperature and light dependent. Though the seed viability was 82% as determined by tetrazolium staining, maximum germination of 100% was obtained only when the seed was soaked in 100 ppm GA<sub>3</sub> solution for 24 h and incubated for germination at 25°C constant temperatures in 12 h light conditions. Barring this treatment all other presowing treatments, incubation temperatures and photoperiod treatments showed less germination. Mean germination time (MGT) was lowest at 25°C both in light and continuous dark conditions. The present study indicates that constant 20°C temperature incubation and light have a positive relationship with seed germination of the species even under no pretreatments. All the treatments at 25°C and presoaking in 100 ppm GA<sub>3</sub> and incubation at 15 and 25°C seems to be effective treatments and could be easily adopted by the potential farmers for economic cultivation of this species.

**Key words:** *Arnebia benthamii*, seed germination, pre-soaking treatments, Himalaya, medicinal and aromatic plants.

## INTRODUCTION

The Himalaya covers 18% of the Indian sub continent, accounts for more than 50% of Indian forest, and contains 40% of India's endemic species (Maikhuri et al., 2000). The Indian Himalayan Region (IHR) is a rich reservoir of biological diversity in the world. Over 1748 species of medicinal and aromatic plants (MAPs) reported from IHR are used in different systems of medicine (Samant and Joshi, 2005). Central Himalayan region

(Uttarakhand) is a treasure house for several medicinal and aromatic plants (MAPs). Most demands for these (MAPs) are met through extractions from wild and the increasing demands of medicinal species in the international market. It will certainly have serious implications to the survival of such plant species that are listed as rare and endangered. There is thus an urgent need to develop and implement regeneration/conservation strategies for over exploited medicinal plant species. Several medicinal plants have slow growth rate and low population density and narrow geographical ranges and more prone to extinction (Kala, 1998; Nautiyal et al., 2002). There are

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several other causes such as narrow range of distribution, land use changes, introduction of non-native species, habitat alteration, climate changes, fragmentation and degradation of the plant populations and genetic drift (Kala, 2000, 2005; Weekley and Race, 2001). Among them *Arnebia benthamii* is one of the important high value species, which has huge economic potential, being used in pharmaceutical industries as well as in traditional health care system (Anonymous, 1985). But due to heavy anthropogenic pressure its natural population is threatened, which in turn has affected the natural regeneration. While different workers classify the status of the species as rare (Jain and Sastry, 1984), vulnerable and critically endangered (Ved and Tandon, 1998), officially this species has been listed under the endangered category (Tewari, 2000).

*A. benthamii* (Wall.ex G.Don) Johnston Syn. *Macrotomia benthamii* (Wall). DC, belongs to family, Boraginaceae, an erect herbaceous perennial species, 30-90 cm in height and found in the alpine areas of Western and Central Himalaya at an altitude of 3,000-3,900 m asl. The roots are used as hair tonic and considered to be good to control hair loss/baldness (Anonymous, 1985). This plant is a major ingredient of a drug called Gaozaban. The flowering shoots are used in the preparation of sherbet (syrup) and jam, used for treatment of various diseases of the tongue and throat, fever and cardiac disorders (Anonymous, 1985). The roots of this genus are sold in the market under the trade name 'Ratanjot' (Kirtikar and Basu, 1984). Use of this species and allied species is extensively studied by several workers (Maikhuri et al., 2000; Ganai and Nawachoo, 2002; Javidnia et al., 2003; Manjkhola et al., 2003, 2005; Kala et al., 2006).

Presowing chemical treatments were used to enhance and improve seed germination in Himalayan medicinal plants (Pandey et al., 2000; Joshi and Dhar, 2003; Gupta, 2003; Shivkumar et al., 2006) and from other regions (Plummer and Bell, 1995; Yamaguchi and Kamiya, 2000; Kambizia et al., 2006; Kulkarni et al., 2007). Nautiyal et al. (2002) also reported seed and vegetative means of agrotechnology for some high altitude medicinal plants. Seed germination studies on several Himalayan medicinal plants have proved useful in developing appropriate conservation strategies (Nautiyal et al., 1987; Raina et al., 1996; Kandari et al., 2007).

Use of plant growth regulators (PGRs) and other chemicals to stimulate and to synchronize seed germination have been reported by several workers (Pandey et al., 2000; Manjkhola and Dhar, 2002; Ghimire et al., 2006). There are limited studies on *A. benthamii* (Maikhuri et al., 2000; Ganai and Nawachoo, 2002; Manjkhola et al., 2003, 2005). In our previous ethnomedical study in Niti valley of Garhwal Himalaya (Maikhuri et al., 2000; Kandari, 2005) we observed that the populations of this plant have become low and scattered in the wild, which may be attributed to its intensive harvesting for traditional

medicine use coupled with its low potential for natural regeneration. Self-propagation of the plant is through both seeds and vegetative buds (Kandari, 2005; Manjkhola et al., 2005). However, natural regeneration was found low in nature due to over exploitation of plants for root system much before flowering (Kandari, 2005). Seeds collected by the graziers from the few plants flowering generally reach the propagation centers in about 5 to 6 months as these graziers move with the animals for prolonged periods. These seeds used in the next growing season generally have less viability due to improper processing and storage. Propagation by natural reseedling or through vegetative propagules is thus no longer sufficient to guarantee the survival of this plant. It has become necessary, therefore, to develop rapid methods of cultivating the plant in order to ensure its sustainable utilization in the Himalayan region. As the species is having high commercial value, utilisation of its potential through commercial propagation require basic information on the best local conditions for the germination of its seeds and vegetative propagation. Though we conducted studies on both seed germination and vegetative propagation of *A. benthamii*, we report here the effects of temperature, light and plant growth regulator (GA<sub>3</sub>) on the germination of seeds *only as propagules* obtained from seeds will have better genetic variability required for these plants to adopt to varying conditions.

## MATERIALS AND METHODS

### Seed collection

Mature seeds of *A. benthamii* were collected from two natural population areas ie., Suki (3200 m asl) and Khambdhar (3700 m asl) in Nanda Devi Biosphere Reserve (NDBR) of Uttarakhand Himalaya during the first week of October, 2002. Seeds were collected in small cotton bags. Immediately after collection, seeds were separated and dried at room temperature for 1 week, then stored at 4°C. The various trials were conducted eight weeks after collection. Only mature seeds were used in the experiments.

### Seed viability assessment

To ensure that the seeds used for the experiment were viable and of high quality, the sample lots were subjected to viability test using the tetrazolium technique (Peters, 2000). Ninety seeds (3 replicates) were subjected to 2,3,5, triphenyl tetrazolium chloride (TTC) test after 1, 6 and 12 months of storage at 4°C. In this method, seeds were longitudinally sectioned and the sections were immersed in a 0.5% aqueous solution of TTC (pH 6.5) for 24 h at room temperature (25°C) under dark conditions. The TTC solution was drained and sections were rinsed 2-3 times with water. The topographical staining pattern of the embryos (plumule and radicle) and cotyledons were studied under a dissection microscope (ISTA, 2003).

### Water imbibition assessment

To verify if the species have water impermeable seed coat, one

hundred seeds (3 replicates) of *A. benthamii* were weighed and allowed to imbibe water in a beaker (50 ml) at room temperature (25°C) under dark condition. At regular intervals (10 h) seeds were removed, wiped dry with blotting paper and weight was recorded. The seeds were transferred back to beaker containing water. The process was repeated till the seed weight remained constant (Baskin and Baskin, 1998).

### Seed germination assessment

To examine whether *A. benthamii* seeds possess physical, physiological, or chemical dormancy, a variety of presowing treatment was applied. The presence of physical dormancy was investigated by soaking seeds in water at room temperature (25°C) for 24 h and given heat shock (seeds were placed in beaker of 70°C water, allowed to cool to room temperature for 24 h). Optimal conditions for seed germination were determined by subjecting the seeds to varied concentration of plant growth regulator-GA<sub>3</sub> (100, 200 and 300 ppm), different temperature regimes and light conditions. Seeds were kept in the treatment solutions for 24 h for imbibition. After standard soaking, seeds were incubated for germination in Petri plates (9 cm diameter) of two-layer Whatman No 1 filter paper with about 5 ml of distilled water. The moisture levels of filter paper were maintained by adding distilled water as required. To determine the effect of different temperatures, the seeds were incubated at 15, 20 and 25°C constant temperatures. These treatments were conducted in light (12 h light and 12 h dark) and continuous dark (24 h dark) photoperiod conditions. Cool white fluorescent lamps were used for providing light conditions in the incubation chambers. Control indicates no pretreatment of seeds. Germination was monitored daily from the date of seed sowing. Germination for seeds of continuous dark treatment was monitored under low green light. Seed with radical emergence (>2 mm) was considered as germination. Germination percentage was recorded every day until no further germination was found.

### Data analysis

For each experiment 50 seeds with 3 replicates were used. The germination percentage and mean germination times (MGT) were calculated following the formulas of Ellis and Roberts (1981). All data were analyzed using IRRISTAST statistical software for windows (IRRI, 1997). Least significance difference (LSD) at the 5% level was used to test difference between means of germination percentage (Steel and Torrie, 1960).

## RESULTS AND DISCUSSION

### Seed viability

Tetrazolium chloride test has been shown to be generally in close agreement with germination test results. The viability of the seeds of *A. benthamii* as determined by the tetrazolium test was 82%. This high percentage of viability is probably due to the short period between the harvesting and the trials of the seed (8 weeks). Seed viability in *A. benthamii* is reported to decrease fast when stored at room temperature (25°C) (Kandari, 2005) but even storing at 4°C did not help much as the viability of seed stored at 4°C was 64% after six months and 50% after 12 months (Figure 1). As compared to other medici-

nal and aromatic plants (MAPs) growing in the area along with this species such as *Angelica glauca* and *Pleurospermum angelicoides* (Kandari et al., 2007), *A. benthamii* seed had greater viability at the end of 12 months storage at 4°C. High inter population variation of seed viability was also reported for *A. benthamii* (Manjkhola et al., 2003, 2005). Therefore, to obtain maximum germination results current season seeds are recommended for cultivation of this species.

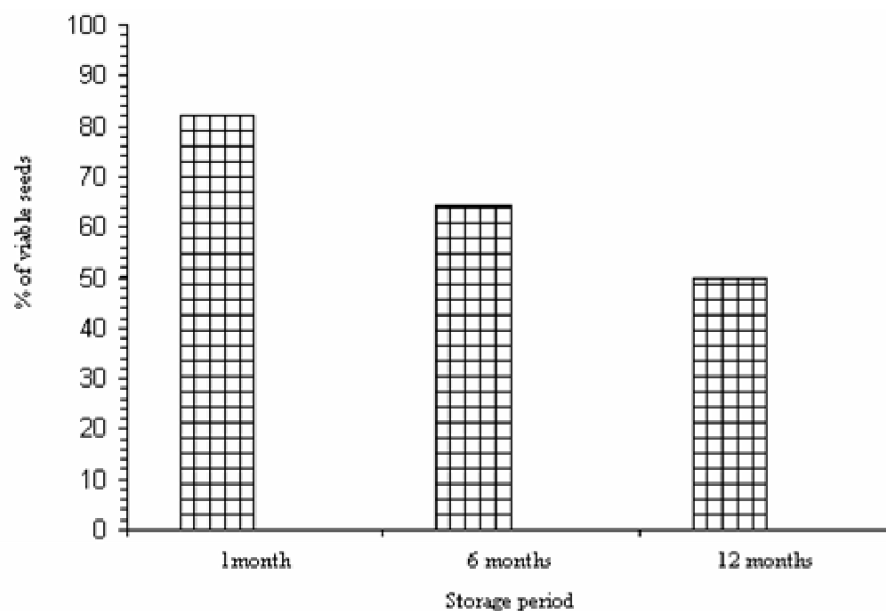
### Imbibition capacity

Seeds of *A. benthamii* absorbed water quite rapidly within the first 24 h and show 1.7-1.9% increase over initial weight. Water imbibition reached maximum (2.5% over initial weight) after 80 h and thereafter attained saturation (Figure 2) Thus, a presoaking treatment of about 24 h used in the present study is sufficient to have imbibition of growth promoting hormones. The low water imbibition by seeds of *A. benthamii* in comparison to other MAPs occurring in similar habitat (Kandari et al., 2007) might be related to its seed coat characteristics. Baskin (2003) reports that species with water impermeable seeds have physical dormancy and such seed generally have a water gap in seeds, which open in response to an appropriate environmental signal. More critical studies will be required to understand the environmental conditions that facilitate seed germination in nature (Kaye, 1997).

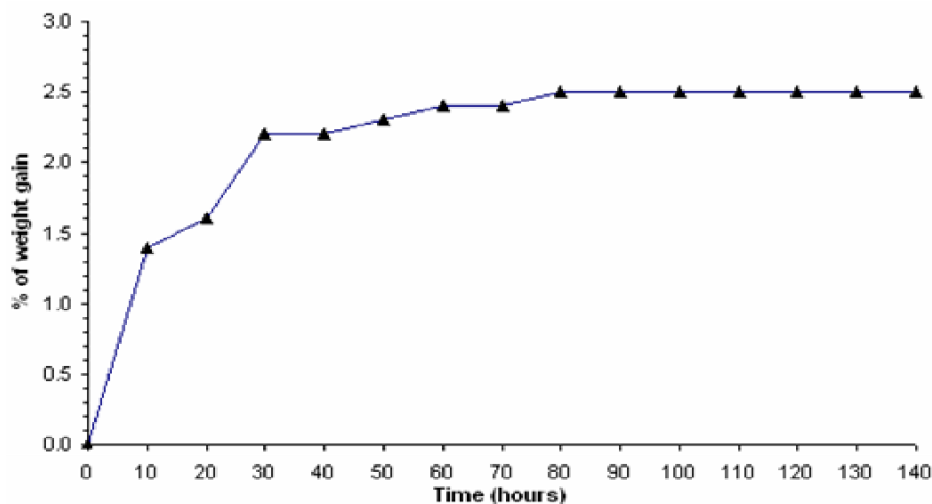
### Effects of presowing treatments

Germination of *A. benthamii* seed significantly varied among the various presowing treatments ( $P < 0.05$ ). Freshly harvested seeds had 5% germination at 15°C and presowing treatments applied to break physical dormancy did not improve germination over the control (Figure 3, treatments T2, T3) at 25°C in light conditions. Among the treatments applied to overcome physiological dormancy, soaking in 100 ppm GA<sub>3</sub> for 24 h and incubation at 25°C in 12 h light photoperiod conditions resulted in maximum germination (100%) compared to control and all other treatments. This combination of presoaking treatment and temperature had highest germination even in continuous dark conditions and the mean germination time (MGT) is also lowest.

The result of this study clearly showed that temperature and photoperiod affected the germination of *A. benthamii* seeds the most. The influence of these two conditions is significant when considered in isolation or when the interaction between them is taken into account (Table 1). Germination under light (12 h) was significantly higher than continuous dark. This type of light controlled germination was associated with phytochrome. The sensitivity of seeds to the spectral quality of the light mediated by phytochrome is a frequent natural process within species



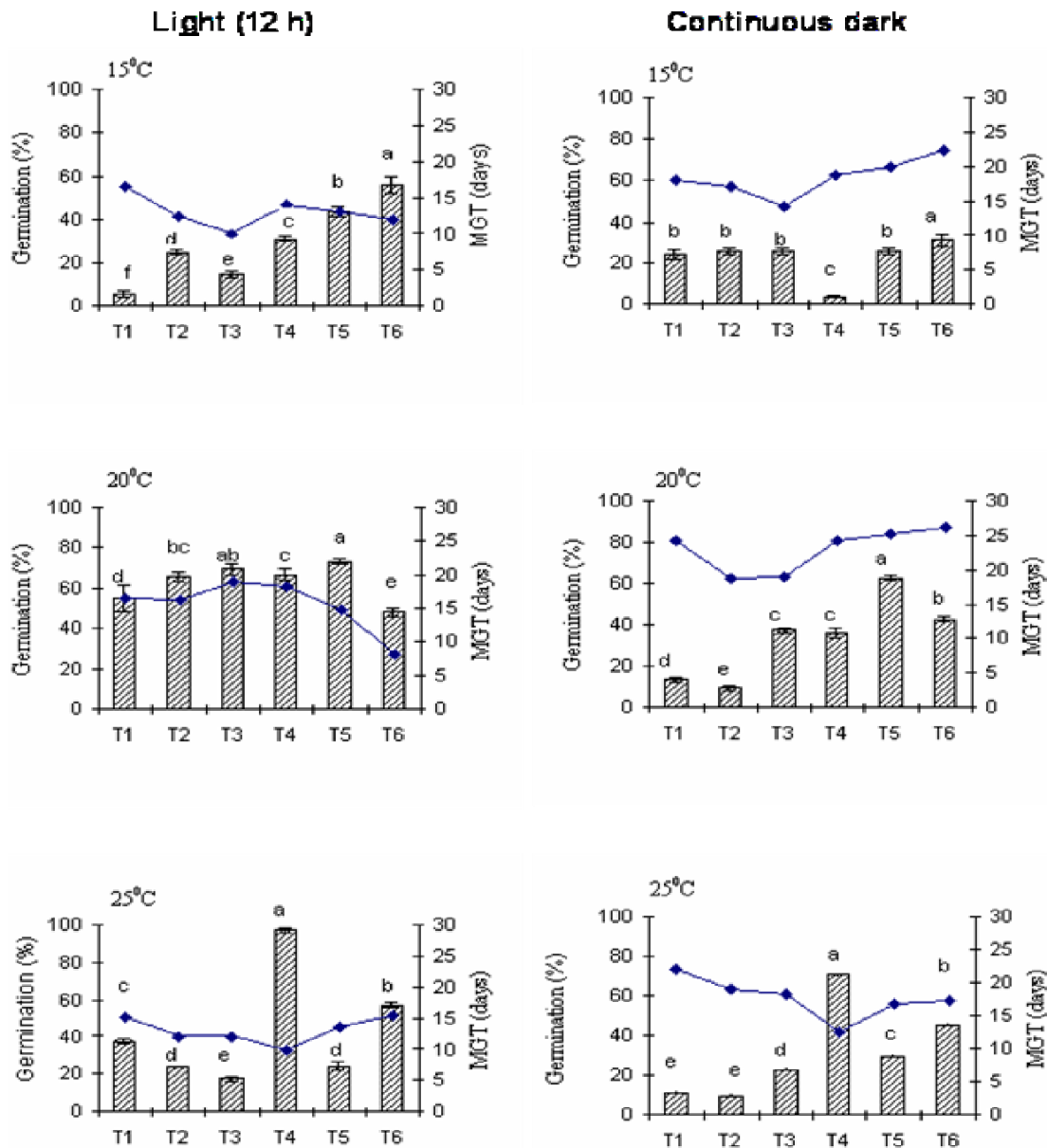
**Figure 1.** Changes in seed viability of *A. benthamii* during storage at 4°C



**Figure 2.** Water imbibition by seeds of *A. benthamii* with time at 25°C under dark conditions.

that grow in open areas. The results of this study imply that the seeds of *A. benthamii* are light dependent for germination but their performance showed high degree of variation to various presowing treatments both at constant 15°C and 25°C as compared to 20°C. Other workers also reported that some species have responded better at alternating day and night temperatures better than constant temperatures (Kambizi et al., 2006). We do not have sufficient information to confirm or disagree to such possibility in *A. benthamii* at present. Previous studies

have shown that GA<sub>3</sub> enhances the germination of seeds exhibiting physiological, morphological or morphophysiological dormancy (Ganai and Nawchoo, 2002; Shivakumar et al., 2006). The efficacy of GA<sub>3</sub> treatment in breaking dormancy depends on the concentration and length of incubation. In the present study, increased GA<sub>3</sub> concentrations have positive effect on seed germination at low temperatures. However, as the incubation temperatures increased the germination decreased significantly with increase in GA<sub>3</sub> concentration both in light and continuous



**Figure 3.** Effect of pre sowing treatments and incubation temperature regimes on germination (bars) and mean germination time (MGT) (lines) for seeds of *A. benthamii* under light (12 h) and continuous dark conditions (T1-Control, T2-Hot water, T3- Cold water, T4-GA<sub>3</sub> 100 ppm, T5-GA<sub>3</sub> 200 ppm, T6-GA<sub>3</sub> 300 ppm and n = 90). Bars with the same letters do not differ at 5% level of significance.

dark photoperiods. Even the MGT also increased with increased concentration of GA<sub>3</sub> in both light and continuous dark photoperiods at 25°C incubation. Contrary to the present study, Ganai and Nawchoo (2002) recorded decrease in germination of *A. benthamii* seeds at 100 and 200 ppm GA<sub>3</sub> as compared to 25 and 50 ppm. Their study also reported the percentage of germination has direct correlation with seed mass and this might be a major reason for the contrasting results observed from

current study.

Barring for soaking in 100 ppm GA<sub>3</sub> for 24 h and incubation at constant 25°C in 12 h light photoperiodic conditions all other treatments showed less germination than the viability recorded in TTC test. This discrepancy, in addition to overexploitation, explains why there is low natural regeneration with consequent low plant population of *A. benthamii* in the wild. Therefore to conserve this species in wild, effective human intervention is required

**Table 1.** Analysis of variance of seed germination in *Arnebia benthamii*

Source of variation	Degree of freedom	Sum of squares	Mean square	Computed F	Remark
Photoperiod conditions (A)	1	2240.44	2240.44	4.80	S
Incubation temperature (B)	2	3037.55	1518.78	3.33	S
Presowing treatments (C)	5	3894.89	778.98	1.65	NS
A X B	5	6137.56	1227.51	3.08	S
B X C	17	12598.90	741.11	2.43	S
A X C	11	6715.22	610.48	1.29	NS
A X B X C	10	1815.11	181.51	1.00	NS
Total	35	18093.90	516.97		

NS – not significant; S – significant at  $P < 0.05$

to cultivate the species to meet the market demands to reduce extractions from wild which may allow some natural regeneration of the species. All the treatments at 25°C and presoaking in 100 ppm GA<sub>3</sub> and incubation at 15 and 25°C seems to be effective treatments and could be easily adopted by the potential farmers for economic cultivation of this species.

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