

Full Length Research Paper

# No chilling obligation for germination in seeds of *Arnebia benthamii*: A critically endangered alpine medicinal plant of north-west Himalayas

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*Arnebia benthamii* (Wall. ex G. Don.) Johnst., Boraginaceae, is an important Himalayan alpine herb with tremendous medicinal properties. The species is facing the pressure of overexploitation and is ranked as a critically endangered species. In an effort to develop a strategy to conserve and cultivate the species, the present study of *in vitro* seed germination was carried out. The study depicted that the seeds have a very high viability (98%) and contain oil as the reserve food material. The seeds imbibe water nicely and there is no physical dormancy imposed by the seed coat. Among the many pretreatments used to increase percentage germination and reducing mean germination time (MGT), scarification (seed coat removal) proved most effective. The scarification treatment enhanced seed germination to 96.66% and reduced mean germination time to 4.03 days, followed by Kinetin (50 ppm) with 90.83% seed germination and MGT of 4.15 days, as against control, with 31.66% germination and MGT of 9.18 days. Furthermore, when the scarified seeds were treated with seed coat extract, the percentage germination depleted drastically to 28.33% which is suggestive of the fact that the seed coat contains the chemical inhibitors which do have a regulatory or inhibitory effect on seed germination. The study also revealed that the seeds do not need chilling for witnessing germination.

**Key words:** *Arnebia benthamii*, Kashmir Himalayas, conservation, seed germination, alpine.

## INTRODUCTION

No doubt seeds are produced as units of multiplication, perennation, dispersal and variation (Harper, 1967), processes which keep the plant species going and harmonious with the environment, rather changing environment. However, since plant species started melting under the stress of overexploitation and other threats, seeds became the appropriate and reliable material for developing *ex-situ* conservation strategies (Hawkes et al., 2000; Singh and Ghowse, 1993; Ved and Tandon, 1998; Ganai and Nawchoo, 2002). Seed dormancy and other attributes have been pondered upon minutely, and seeds are pre-treated with different physico-chemical agents to enhance percentage germination and germinability (Kevseroglu, 1993; Prasad, 1999; Stabel, 1989; Laliberte, 1997). Seeds are germinated *in vitro* periodically to analyse influence of

storage period on germination and viability (Toth et al., 1996). Many species of Himalayan medicinal plants require gibberellins or chilling for breaking dormancy (Prasad, 1999).

The present study was conducted with a view to develop a protocol for the *ex-situ* conservation of *Arnebia benthamii* (Wall. ex G. Don.) Johnst., Boraginaceae, which is facing the threat of extinction. The study was carried out to understand the mechanism of dormancy and to analyse the effect of various pre-treatments on enhancement of seed germination.

## MATERIALS AND METHODS

### Seed collection

Seeds were collected at maturity from two alpine natural populations, Munwarsar Pahalgam and Sonamarg of Kashmir Himalayas. Seeds were collected in cotton bags and then dried in the laboratory. They were then stored at 4°C and later used for germination after few weeks.

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**Table 1.** Percentage seed viability of different alpine and sub alpine populations.

S. no.	Population	Percentage viability (X± S.D)
1	Panchal <sup>a</sup>	93 ± 1
2	Munwarsar <sup>b</sup>	98 ± 0
3	Chenpathri <sup>b</sup>	96 ± 2

a - Sub alpine population, b - alpine population, X - mean %age viability, S. D - standard deviation.

### Seed viability assessment

Seed viability was determined using 2, 3, 5, triphenyl tetrazolium chloride (TTC) test. Fifty seeds of each replicate (3) were placed in a moist filter paper at room temperature for 24 h and then longitudinally sectioned. The sections were incubated in the dark in a 1% aqueous solution of 2, 3, 5, triphenyl tetrazolium chloride for 24 h. The seeds were then analyzed for the viability staining pattern.

### Moisture content

Seed moisture content was determined using five replicates of 40 seeds. Seeds were collected from alpine and sub-alpine populations and fresh weight recorded. There after they were kept at 60°C for 24 h in a hot air oven after which they were reweighed and moisture content calculated as follows:

$$[(FW-DW)/FW] \times 100$$

### Seed size and seed endowment

For calculating seed size 1000 seeds were weighed and average seed size calculated as follows:

$$\text{Average seed size (weight)} = \frac{\text{Weight of N seeds}}{\text{N (number of seeds)}}$$

Sudan IV was used to detect oil endowment in the seeds. It was also unraveled by filter paper transparency test.

### Seed germination

Two replicates of 40 seeds were used for each pretreatment to study germination, the seeds were placed on moist filter papers in Petri plates and the following various were pretreatments carried out to determine the seed germination:

1. Scarification: In scarification the seed coat was removed completely to investigate its role in seed dormancy and enhancement of germination. The probability of any physical dormancy was investigated by soaking seeds in water for few hours and then recording the imbibitions. To unfold the presence of dormancy inducing chemical inhibitors in seed coat and their role in dormancy, the seed coat leacheate was prepared and then applied on scarified seeds.

2. Growth regulators: The effect of different growth regulators such as gibberlin, kinetin and auxin on enhancement of germination was also studied. These treatments include, GA<sub>3</sub>-25, 50,100, 200 ppm, Kinetin - 25, 50, 100 ppm and NAA - 25, 50, 100 ppm. Seeds were kept in test solution (growth regulators) for one hour then removed and placed in moist Petri plates at room temperature (12 to 18°C).

3. In addition to the above pre-treatments the effect of different concentrations of KNO<sub>3</sub>, namely, 25, 50, 100 ppm on germination was also investigated.

4. The role of chilling in dormancy and germination was studied by using: (1) seeds which have received no chilling and (2) seeds which have received chilling of different durations such as one week, two weeks, three weeks and one month chilling.

Seeds were considered germinated upon the emergence of the radicle. Germinated seeds were counted and removed from the Petri plates every day. The germination pattern was monitored for 20 to 25 days on daily basis and data recorded accordingly.

### Mean germination time

MGT was calculated as:

$$\text{MGT} = \frac{\sum (n \times d)}{N}$$

where n = the number of seeds which germinated after each period in days (d) and N=the total number of seeds germinated at the end of the experiment (Hartman and Kester, 1989).

## RESULTS

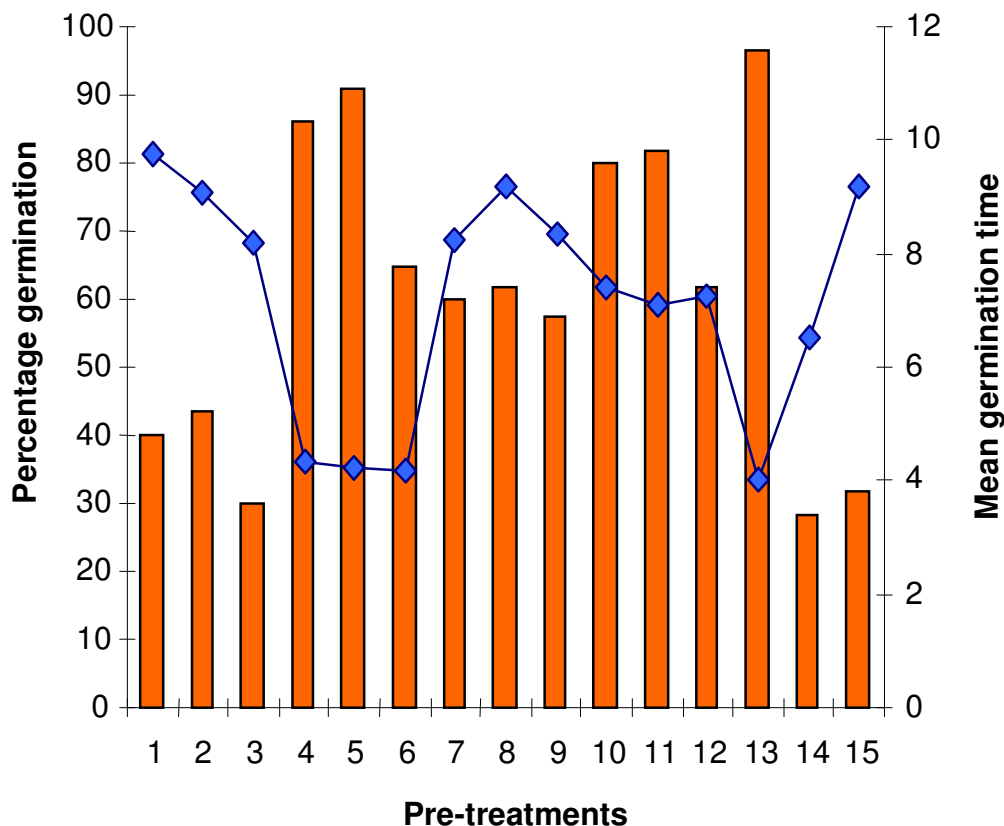
*A. benthamii* produces a very good seed set, with maximum seed output of 235 seeds per plant. The seeds contain oil as reserve food material, with seed mass varying between 6 to 8 mg per seed. The seed viability in the species is very high with values ranging from 93 to 98% as is depicted in Table 1.

### Seed moisture

At maturity the moisture content of seeds varies from one population to another population. The seeds of alpine populations contain higher moisture content when compared to sub alpine populations. The seeds collected from Munwarsar (alpine population) contained a moisture content of 7.8% while as the seeds from panchal (sub-alpine population) contained a moisture content of 5.9%.

### In vitro seed germination

The heart shaped seeds of *A. benthamii* soon after maturation can be induced to germination. The species



**Figure 1.** Effect of various pretreatments on the percentage of *in vitro* seed germination and mean germination time in *A. benthamii*. (Note 1 to 15 pre-treatments as given in Table 2 and blue line shows the MGT values).

however, shows poor seed germination and germinability. Of the different pretreatments used to enhance percentage germination, maximum germination (96.66%) was observed in the case of removing the seed coat. The scarification treatment or seed coat removal enhanced the rate of seed germination, reducing mean germination time to around 4 days against control where the mean germination time is 9.18 days. After scarification, kinetin pretreatment is the most effective treatment to enhance percentage seed germination and also rate of seed germination. The kinetin treatment increases percentage germination to 90.83% as compared to control with 31.33%. The kinetin pre-treatment also decreased the mean germination time to around 4 days as compared to control. The effect of other pre-treatments on seed germination is depicted in Figure 1 and Table 2.

## DISCUSSION

*A. benthamii* is reproducing both sexually as well as vegetatively. The ultimate outcome of the sexual reproduction in plants is the seeds production which besides multiplication governs some fundamental processes: serving as an agent of perennation,

generation of variation and dispersal. In *A. benthamii* amphimixis leads to seed formation. The average seed set varying from population to population, with average maximum seeds per plant witnessed in Munwarser population (185) and lowest value presented by Sonmarg population (80) in Kashmir Himalayas. Seeds of *A. benthamii* possess high viability (96 to 98%), with size ranging from 6 to 8 mg in weight. Despite a very good seed set with efficient viability, the species is critically endangered because of overexploitation for medicinal use. The seeds contain oils as reserve food material. Seeds soon after maturation can be induced to germinate which envisages that seeds do not require any after ripening period. The seeds do require light for germination which depicts that the seeds are positively photoblastic. Shimono and Kudo (2005) are of the same view and advocate that the seeds of most of the alpine plants require light for germination. They also suggest that the seeds of many alpine species require chilling for germination. However in *A. benthamii* seeds do not require chilling for germination. The seeds germinate nicely without receiving any pre-chilling. Our *in vitro* studies are reinforced by the field studies. In the transplanted populations at Botanical Garden K.U (1450 m) seeds formed as a result of manual pollination, mature

**Table 2.** Effect of different pre-treatments on seed germination in *A. benthamii*.

S. no.	Pre-treatment	Percentage germination	MGT(days)
1	Chilling-7 days	40±2.04	9.78
2	14 days	43.33±1.17	9.08
	( 21 days)	42.21±1.31	
	(1 Month)	40.42±2.12	
3	GA <sub>3</sub> -25 ppm	30±4.08	8.18
4	Kinetin-25 ppm	85.83±5.89	4.31
5	50 ppm	90.83±4.24	4.21
6	100 ppm	65±11.36	4.15
7	NAA -25 ppm	60±14.28	8.24
8	50 ppm	61.65±3.11	9.18
9	100 ppm	57.5±8.89	8.35
10	KNO <sub>3</sub> -50 ppm	80±4.08	7.40
11	100 ppm	81.6±1.17	7.10
12	150 ppm	61.66±1.19	7.25
13	Scarification	96.66±2.35	4.03
14	Seed coat removal + coat leacheate	28.33±5.13	6.50
15	Control	31.66±4.24	9.18

in June, and germinate in the 3rd week of August to 2nd week of September, thus making it evident that germination without chilling can be cherished in *A. benthamii* (Ganaie, 2006).

*In vitro* studies reveal a very low percentage of germination 31.66% (control) in *A. benthamii*. Among many pretreatments used to increase percentage germination and reducing mean germination time, scarification (seed coat removal) ranks first, with percentage germination enhanced to 96.33% and MGT reduced to 4.03 days. Similar results were obtained by Jia and He (2009), who found scarification as the most effective pretreatment to enhance germination in *Anisodus tanguticus*, an endangered high altitude medicinal plant of China. In *A. tanguticus*, scarification enhanced germination to 70% against 0% in control.

In *A. benthamii* the seed coat which is papery and thin interferes with seed germination not by impairing water imbibitions and oxygen diffusion (no physical dormancy), but because of chemical inhibitors contained in the seed coat. Seeds imbibe water normally and swell nicely (doubling their weight), seed coat puncturing not affecting seed germination, thus the possibility of water and oxygen impairing seed germination is ruled out. Our results are contrary to the findings of Jia and He (2009) who found that the seed dormancy of *A. tanguticus* is imposed by thick seed coat which prevent water and gaseous diffusion into the seed.

The scarified seeds in *A. benthamii* when treated with seed coat leacheate or extract, witnessed an inhibitory response with reduction in germination and percentage germination, therefore, making it evident that inhibitors

contained in the seed coat interfere with and impair seed germination. This is however of tremendous ecological importance, the significance being that these inhibitors play a great role in regulating seed germination in the alpine natural conditions. Here too the parental care is delivered and displayed by *A. benthamii* species, the parental maternal tissue (seed coat) regulating and controlling the offspring till germination. Removing seed coat serves as a bye pass to remove the effect of inhibitors, therefore furnishing high values of percentage germination. Cytokinins are known antagonistic to ABA and other growth inhibitors- dormancy inducing substances. Thus exogenous supply of cytokinins neutralizes or nullifies the inhibition, therefore relieving dormancy and enhancing percentage germination. Both scarification and kinetin reduce the mean germination time. Among other treatment KNO<sub>3</sub> also enhances percentage germination to 81%.

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## REFERENCES

- Harper JL (1967). A Darwinian approach to plant ecology. J. Ecol., 55: 247-270.
- Hawkes JG, Maxted N, Ford-Lloyd BV (2000). The Ex-situ Conservation of Plant Genetic Resources, Kluwer Academic publishers, Dordrecht.

- He T, Jia JF (2009). Breaking dormancy in seeds of *Anisodus tanguticus*: An endangered medicinal herb of high altitude in the Qinghai-Tibet Plateau. *Seed Sci. Technol.*, 37(1): 229-231.
- Kevseroglu K (1993). The effect of some physical and chemical treatments on germination of *Datura stramonium* seeds collected from natural vegetation. *Doga, Turk, Tarum ve Ormancilik Dergisi*, 17(3): 727-735.
- Ganaie KA, Nawchoo IA (2002). *In vitro* seed germination studies on *Arnebia benthamii*. *Ind. J. Plant Physiol.*, 7(3): 252-255.
- Ganaie KA (2006). Assessment of reproductive strategies of *Arnebia benthamii* and development of a protocol for its *ex-situ* conservation. Ph.D thesis University of Kashmir Srinagar India.
- Hartmann HT, Kester DE (1989). *Plant Propagation: Principles and Practices*. Prentice Hall, New Delhi, India.
- Laliberte B (1997). Botanic garden, seed banks, gene banks worldwide their facilities, collections and network. *Bot. Gardens Conserv. News*, 2: 18-23.
- Prasad P (1999). Enhancement of seed germination of *Podophyllum hexandrum* and *Aconitum heterophyllum* wall by different treatments. *J. Hill Res.*, 12(2): 102-106.
- Shimano Y, Kudo G (2005). Comparisons of germination traits of alpine plants between fellfield and snow bed habitats. *Ecol. Res.*, 20(2): 189-197.
- Singh CK, Ghowse AKM (1993). Plantation of medicinal plants is the need of the day in India. *Medicinal plants, New Vistas of Research*, 10: 203-207.
- Toth E, Nemeth E, Pank F (1996). Recent results in the development of gene bank technologies specialized for medicinal plants. *Beitrage-Zur-Zuchtigforschung-Bundersan at alt-fur-Zuchtigforschung-an-Kulturpflanzen*, 2(1): 72-75.
- Ved, DK, Tandon V (1998). *CAMP Report for High Altitude Medicinal Plants of Jammu – Kashmir and Himachal Pardesh*. FRLHT, Bangalore, India.