ISSN 1682-296X (Print)
ISSN 1682-2978 (Online)

# Bio Technology

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Biotechnology 10 (2): 215-219, 2011
ISSN 1682-296X / DOI: 10.3923/biotech.2011.215.219


# Influence of Pre-Sowing Treatments on *in vitro* Seed Germination of Ativisha (*Aconitum heterophyllum* Wall) of Uttarakhand

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**Abstract:** In the present study *in-vitro* seed germination protocol using various pre-sowing treatments for *Aconitum heterophyllum* Wall intended for its *ex-situ* conservation was optimized. Temperature requirement for seed germination in this species was optimized under controlled conditions which revealed that low temperature of 15°C ($p<1.0\%$) was obligatory. Two *in-vitro* presowing treatments: PST-1 (pre-treated seeds kept at normal room temperature) and PST-2 (pre-treated seeds kept at 15°C) were also studied. Highest seed germination was recorded with PST-2 ($p<0.1\%$) with 0.5 mg $L^{-1}$ IAA (97.17%) as compared to PST-1 ($p<1.0\%$). As seed embryo in *A. heterophyllum* Wall is small, it requires low temperature to grow and also $GA_3$ (100 and 200 μM) could not improve the seed germination even at low temperature; so it may be assumed that it exhibit deep complex morphophysiological dormancy (MPD).

**Key words:** *Aconitum heterophyllum*, seed embryo, germination, presowing treatments, dormancy, conservation, endangered

## INTRODUCTION

ATIS, *Aconitum heterophyllum* Wall (Ranunculaceae), a high altitudinal medicinal plant (2800-4500 m asl) of alpine and sub-alpine regions of Himalayas is famous for its medicinal and pharmaceutical value and is being used in the traditional medicine system in the region. Alkaloids isolated from *A. heterophyllum* Wall have been reported to show significant antibacterial, antipyretic, enzyme inhibition activity etc. (Ahmad *et al*., 2008; Ameri, 1998; Anwar *et al*., 2003; Nisar *et al*., 2009). Aconitine and Atisine are the marker components that are extracted from *A. heterophyllum* Wall and are commercially used. The demand for medicinal plant species has been increased globally due to the resurgence of interest in herbal medicine due to which high value medicinal plant species are threatened their status ranging from low risk, near threatened to critically endanger (Srivastava *et al*., 2010a). Indiscriminate and unscientific extraction of tubers of *Aconitum heterophyllum* in large quantities has reduced this species towards rarity and is now identified as critically endangered (CAMP, 2003; IUCN, 1993; Nautiyal *et al*., 2002). Organized cultivation of *Aconitum* is therefore necessary to ensure the quality and continuous supply of drugs (Jabeen *et al*., 2006). The potential of medicinal plants out of their natural habitat is poor. Vegetative propagation for this species is slow and time consuming to achieve large scale production of propagules while the propagation through seeds is limited with seed dormancy and depends upon rainfall, soil moisture, time of sowing etc. Germination is said to be complete when the structure called as radicle penetrates the area surrounding the embryo, rest of the events including mobilization of major storage reserves are associated with the growth of seedlings (Bewley, 1997). But seed germination in many medicinal plants of Ranunculaceae is restricted by different kinds of environmental factors or due to underdeveloped embryo which lead to morphological and or physiological dormancy. Seed germination is a complex process involving various physical and biochemical cues such as water, light and phytohormones (Sen, 2010).

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Previously 6-BAP or hot water treatment has shown reasonable effect on the germination of *Aconitum heterophyllum* Wall (Pandey *et al*., 2000; Pandey *et al*., 2005). Chilling has been found be the crucial factor for germination *Aconitum heterophyllum* Wall (Beigh *et al*., 2005). Other than these studies, no extensive study on other factors influencing seed germination and dormancy break has been documented for the genus Aconitum. Although, many research works had been carried to conserve the *Aconitum spp.* through cultivation but then also no complete effective method has been primed to bring it in conservation programmes (Srivastava *et al*., 2010b). Plant tissue culture provides a viable alternative practice for maintaining this valuable plant in a sustainable manner. Using *in-vitro* seed germination, requisite conditions can be provided and also the mass propagation can also be achieved in short interlude. Till date no study has been documented on *in-vitro* seed germination of the species under study. Therefore, the present study has been performed to analyze the best treatment by determining the effect of temperature and different pre-sowing treatments on seed germination and dormancy break *in vitro*.

## MATERIALS AND METHODS

**Seed collection and viability studies:** The present work is the part of project funded by Uttarakhand Council of Science and Technology (UCOST) carried out at Sardar Bhagwan Singh P.G. Institute of Biomedical Sciences and Research, Balawala, Dehradun from 01-01-2008 till 01-01-2011. Seeds of *Aconitum heterophyllum* Wall (Acc. No. IC-567646, National Bureau of Plant Genetic Resources, Pusa Campus, New Delhi) were procured from Forest Nursery, Munsiyari (alt-2290 m, Lat.-30°13'12", Long.-80°25'12") Uttarakhand, India. Seeds were packed in plastic bags and taken to the laboratory where the study was conducted. Seeds were washed and allowed to dry on filter paper at room temperature and stored at 4°C in a sealed plastic bag until use. Only mature seeds were used for further experimentation. Viability studies were conducted using the float test method, to ensure that only the healthy and high quality seeds were used for the study. After sterilization (Srivastava *et al*., 2010c) 20 seeds were inoculated in Phyta jars with sterile filter paper lined above absorbent cotton moistened with 1/4th strength MS (Murashige and Skoog 1962) media. All the experiments were conducted in triplicates.

**Low temperature requirement for seed germination:** To study the effect of temperature on the seed germination, twenty sterile seeds in three replicates were inoculated in Phyta jars with sterile filter paper lined above absorbent cotton moistened with 1/4th strength MS media. These were then exposed to different temperature regimes: 0, 10, 15 and 20°C. Seeds kept at normal room temperature were taken as control. Seed germination profile was recorded for 12 weeks. Observation for the seeds were recorded when radicle pierced the seed coat.

Table 1: Pre-sowing Treatments given to seeds of *Aconitum heterophyllum* Wall

| | Time | |
|---|---|---|
| Treatments (concentrations) | $T_1$ | $T_2$ |
| Water soaking | 12 h | 24 h |
| NAA (0.5 mg $L^{-1}$) | ,, | ,, |
| NAA (1.0 mg $L^{-1}$) | ,, | ,, |
| IAA (0.5 mg $L^{-1}$) | ,, | ,, |
| IAA (1.0 mg $L^{-1}$) | ,, | ,, |
| $GA_3$ (100 µM) | ,, | ,, |
| $GA_3$ (200 µM) | ,, | ,, |
| $KNO_3$ (0.5%) | ,, | ,, |
| $HNO_3$ (0.1N) | 3 min | 5 min |
| HCl (0.1N) | 3 min | 5 min |
| $H_2SO_4$ | 30 sec | 60 sec |

**Effect of pre-sowing treatments:** After standardizing the temperature requirement, sterile seeds of *Aconitum heterophyllum* Wall were subjected to various pre-sowing treatments using pair of time intervals for each experiment accordingly as shown in Table 1. All the seeds were then washed with the sterile distilled water to remove the traces of chemicals. Twenty seeds in triplicates were inoculated in Phyta jars with sterile filter paper lined above absorbent cotton moistened with 1/4th strength MS media. One batch of pre-treated seeds was kept under the normal room temperature condition (PST-1) and another batch of pre-treated seeds was exposed to constant standardized temperature (PST-2). Un-treated seeds were taken as control. Radicle emergence was the criterion for germination. Seed germination was recorded for 8 weeks.

**Statistical analysis:** Data collected for various treatments was subjected to ANOVA to find out the significance of difference observed in germination of seeds of *Aconitum heterophyllum* Wall.

## RESULTS AND DISCUSSION

Seeds of *A. heterophyllum* Wall showed varied responses that revealed significant differences in response to each treatment. It was observed that low temperature induces *in-vitro* seed germination in *A. heterophyllum* Wall, as highest percentage (66%) of *in-vitro* seed germination was achieved at 15°C in 8th week ($p<1\%$). Seeds of *A. heterophyllum* Wall exposed to low temperature regime showed parabolic response

towards it (Fig. 1). From 0 to 10°C the germination rate improved as compared to control, however, it was insignificant. At 12th week no further germination was observed. The effect of pre-sowing treatments under two conditions PST-1 and PST-2 revealed that pre-sowing treatments differ significantly with respect to germination of seeds in both cases, whereas the time intervals showed insignificant variation between themselves in case of PST-2. It is also found that the pre-sowing treatments, except the HCl and $GA_3$ treatment, are better than control in both the batches. It was observed that PST-2 showed highly significant ($p<0.1\%$) effect on the seed germination as compared to the PST-1 ($p<1\%$) (Fig. 2). Highest seed germination was induced at 15°C by IAA (0.5 mg $L^{-1}$) to about 97.17% at time $T_2$ and then 0.5% $KNO_3$ (94.40%) closely followed by Water Soaking (93.83%) at $T_2$ (Fig. 3). In its natural habitat *Aconitum heterophyllum* Wall multiplies by seeds as well as through root tubers. However, the level of difficulty in its cultivation is very high due to poor seed germination, lack of superior germplasm (Nautiyal *et al*., 2009). To germinate seeds of *A. heterophyllum* Wall require a constant moist and low temperature regime (15°C) and thus bear a high loss of viability (Giri *et al*., 1993). Cold stratification has been found to be the effective treatment for breaking dormancy mostly in Ranunculaceae, growing in temperate and alpine climates (Baskin and Baskin, 1994; Forbis, *et al*., 2002; Frost, 1974; Walck *et al*., 1999). In *A. lycoctonum,* embryo has to grow to a critical size before the seeds can germinate indicating that it has an underdeveloped embryo (Grushvitzky, 1967). Similarly, in seeds of *A. heterophyllum* Wall also embryo lies in small vicinity of the seed. Seeds with an underdeveloped embryo can germinate within 30 days in case no physiological dormancy is present (Baskin and Baskin, 1998). However, in the present study, it was observed that merely giving a cold treatment is not satisfactory enough. Although, seeds of *A. heterophyllum* Wall germinated at low temperature but in a protracted time period. So, it can be assumed that it may have physiological dormancy also besides having morphological dormancy i.e., the seeds are morphophysiological dormant (MPD). Soaking of the seeds for treatment with hormones and chemicals like IAA, NAA, $KNO_3$ etc. was found be better stimulator of germination rather than incorporating them in the moistening medium. Presowing treatment with IAA was found to be the best for *in- vitro* seed germination at low temperature. IAA has also been recommended by Hilhorst and Karssen (1992) and Iglesias and Babiano (1997), to break seed dormancy and enhance germination. $KNO_3$ and Water Soaking also showed promotive effect on seed germination but only at low temperature. Bao and

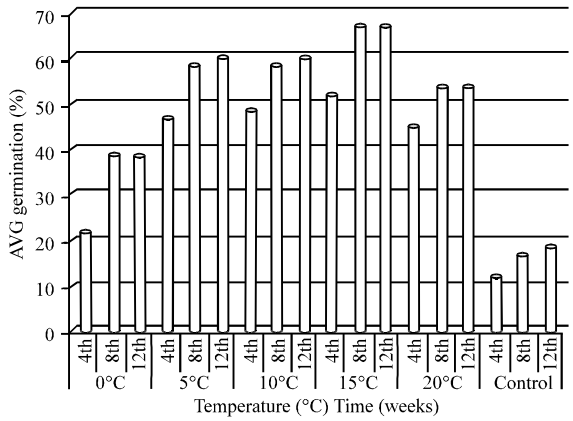


Fig. 1: Low temperature requirement for the seed germination in *Aconitum heterophyllum* Wall

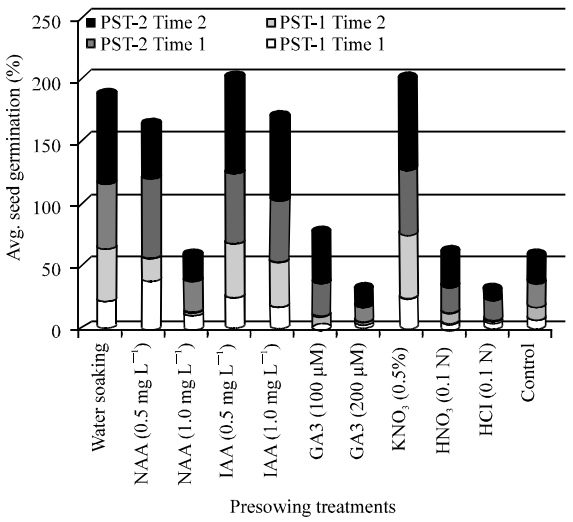


Fig. 2: Average germination percentage after the Presowing treatments: PST-1 (Pre-treated seeds at normal temperature) and PST-2 (Pre-treated seeds at low temperature)

Zhang (2010) also observed the promotive effect of $KNO_3$ on the intact and IAA on the naked seeds of *Pyrus betulaefolia* Bge. and *Pyrus calleryana* Dcne. PST-1 could not enhance the seed germination which indicates that low temperature is crucial factor for seed germination. Although, the untreated seeds sowed at low temperature showed promotive effect on the germination but the results obtained with PST-2 were much satisfactory. Therefore the present study suggests that pretreatment of the seeds before sowing at low temperature can enhance the seed germination significantly. Germination in *A. lycoctonum* was also found to be enhanced after the low temperature treatment

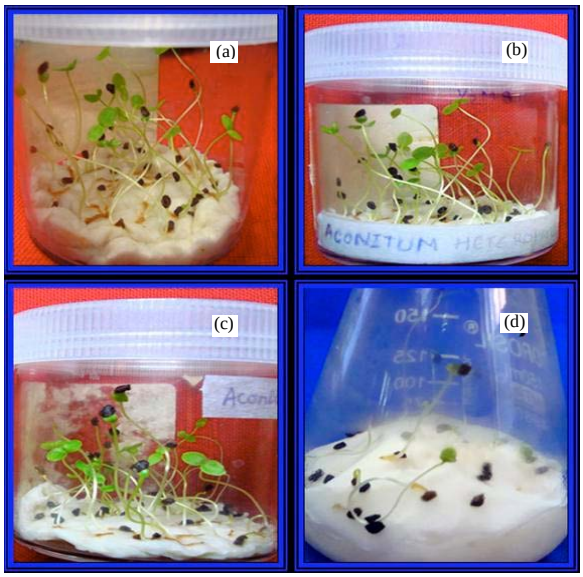


Fig. 3: Seed Germination in *A. heterophyllum* Wall with PST-2. (a) IAA (b) $KNO_3$ (c) Water Soaking and (d) Control

and based on the observation with $GA_3$, it was assigned to have deep complex MPD (Vandelook *et al*., 2009). Similarly, in view of the fact in the present study that $GA_3$ (100 and 200 μM) could not improve the seed germination even at low temperature; seeds of *A. heterophyllum* Wall may exhibit deep complex MPD. Instead treatment with $GA_3$ was found to be inhibitory in the present study which was also observed by Pandey *et al.* (2000) in the same species. Exogenous $GA_3$ was also found completely preventing in seed germination of *Verbascum bithynicum, V. wiedemannianum* and *Salvia dicroantha* (Senel *et al*., 2007). Pandey *et al.* (2000) also observed that $KNO_3$ (50 and 100 μM) could not enhanced the seed germination, in contrary to this report, in the present study $KNO_3$ (0.5%) at PST-2 was found to enhance the seed germination in *A. heterophyllum* Wall significantly. Higher concentration of $KNO_3$ was also found to be effective in seed germination *Swertia chirayita* (Pradhan and Badola, 2010).

## CONCLUSION

As Presowing treatments coupled with requisite environmental factors can enhance *in-vitro* seed germination significantly, utilizing such protocols may prove to be an outstanding contrivance for their mass production concurrently conserving the endangered medicinal species in their wild habitats.

## ACKNOWLEDGMENT

The authors are thankful to Management, Sardar Bhagwan Singh (P.G.) Institute of Biomedical Sciences and Research Balawala, Dehradun, Uttarakhand, India for providing necessary research facilities. The Uttarakhand Council of Science and Technology (UCOST), Uttarakhand, India is thanked for financial assistance.

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