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Efficient Propagation Methods and Management Implication of *Aconitum chasmanthum* Stapf ex Holmes: A Critically Endangered and Endemic Medicinal Herb of Kashmir Himalaya

G.G. Mohi-Ud-Din, Irshad A. Nawchoo[1], B.A. Wafai[1]

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ABSTRACT

Background: Knowledge about propagation of rare and endangered plant species is very important for their conservation and sustainable utilization. Therefore, it is imperative to understand different reproductive aspects of such species for their conservation and management. It is in this backdrop different propagation method of *Aconitum chasmanthum*- a Critically Endangered and Endemic Medicinal plant species has been undertaken.

Methods: Seed germination was carried out using different media. For each treatment mean germination time (MGT) was also calculated. Different agro-techniques were also developed in different soil textural classes. The fresh underground tubers of the species were collected from natural population and were split longitudinally into 2, 3 and 4 pieces depending upon the size of the parental tuber containing a portion of shoot apex. The split cuttings were treated with 100 ppm IAA, IBA and GA_3 for 48 hours and were sown in sandy loam soils under pots at Herbal Garden. The pots were kept in diffused light to monitor the leafy shoot generation, survival and comparison with the control (untreated cuttings).

Result: The present study revealed that seeds of the species remain dormant for a pretty long period and do not germinate in darker conditions. Among various treatments the species showed 90.0±8.66% germination when chilled for 20 days with mean germination time (MGT) of 13.678±1.874 days as against 10.66±1.154% germination of the control. Although the plants grew nicely on various soils, maximum plant survival and vigorous growth was obtained in loamy textured soils. The plants responded to various nutrient treatments and in loamy soil the maximum dry biomass was shown by the plants treated with nitrogen (300 mg/kg soil) and NPK (500 mg/kg soil). The plants treated with NPK (500 mg/kg soil) produced 14.46±2.50 gm dry biomass as against the control of 5.23±0.750 gm per individual. The experimental manipulations also reveal that the species has a potential to propagate through tuber cuttings in sandy loam soils. The cuttings treated with IAA, IBA and GA_3 showed 68.75% to 75.0% survival rate as against the 62.5% in control.

Key words: *Aconitum chasmanthum*, Critically endangered, Endemic medicinal plant, *Ex situ* conservation, Growth regulators, NPK treatments, Seed germination.

INTRODUCTION

Aconitum chasmanthum (Ranunculaceae) locally called *Mohand*, *Mohand Posh* or *Mohand gurd* is a perennial herb having tuberous root, erect stem, numerous leaves and hermaphrodite flowers aggregated in racemes. The species is restricted to alpine meadowlands of Kashmir and North West Pakistan (Dhar and Kachroo, 1983) and is used for the treatment of fever, rheumatism, cough, asthma and snake bites (Mohi-Ud-Din *et al.* 2009). Pulvarized roots are mixed with butter oil and used as an ointment to cure abscess and boils (Rasool, 1998). The underground tubers are known to have antifungal, insecticidal and antibacterial properties (Anwar *et al.* 2003). Several bioactive compounds including alkaloids, terpenoids, glycosides, flavonoids, saponins, proteins, carbohydrate and fixed oil were detected in the extract of acetone, methanol, chloroform and water solvents from the seeds of the species under study (Perveen *et al.* 2018). The major chemical constituents of *A. chasmanthum* root are aconitine, mesaconitine and hypaconitine and their respective hydrolyzed analogues, called monoester alkaloids, which include benzoylaconine, benzoylmesaconine and benzoylhypaconine (Dubey *et al.* 2009). In view of large-scale exploitation, biotic interferences and increasing demand for *A. chasmanthum* as herbal drug, its assessment as critically endangered species in nature and consequently the need for its cultivation to salvage the species from loss and also raise the germplasm and provide economic avenues for poor, it becomes obligatory to initiate steps for

Department of Botany, Govt. Degree College for Women, Sopore-193 201, Jammu and Kashmir, India.
[1]Department of Botany, University of Kashmir, Srinagar-190 006, Jammu and Kashmir, India.

Corresponding Author: G.G. Mohi-Ud-Din, Department of Botany, Govt. Degree College for Women, Sopore-193 201, Jammu and Kashmir, India. Email: dggmdin@gmail.com

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its large scale cultivation and development of elementary agrotechniques at lower altitudes under *ex situ* conditions. Owing to its endemic nature, restricted ecological niches, limited reproductive potential through vegetative and sexual means and intensive and unabated extraction for pharmaceutical purposes the herb has been declined alarmingly in its natural populations to the extent that it has been declared "critically endangered" (Anonymous 1997). It is imperative to understand the biology of such species and different aspects of reproduction (Primack, 1980; Ayensu 1981; Manjikola *et al.* 2005). Although *Aconitum chasmanthum* has been recently investigated for its pollination mechanisms and breeding behavior (Mohi-Ud-Din *et al.* 2009), however; least is known in respect of its seed germinability, propagation and *ex situ* conservation. In this backdrop, the present studies were under taken which may prove useful in up scaling the cultivation of the herb under *ex situ* conservation.

MATERIALS AND METHODS

Five alpine sites of Kashmir Himalaya ranging in altitude from 3,150 to 3,846 m a.s.l were monitored and used as a source material for seed, soil and propagule collection (Table 1).

Seed germination

For seed germination, mature seeds of *A. chasmanthum* of the same age (seed cohort) were collected from a selected natural population, Apharwat II, 3250 m a.s.l (Kashmir Himalaya). The seeds were treated with 0.1% mercuric chloride for 5 minutes and washed with double distilled water. Seeds were germinated in petriplates on Whatman filter paper (*in vitro*) using different media (Mohi-Ud-Din *et al.* 2007) (Table 2). For each treatment three replicates were used each with a set of control. The mean germination time (MGT) was calculated following Joshi and Dhar (2003) as given below:

$$MGT = (n \times d)/N$$

Where

'n' = Number of seeds germinated on each day.

'd' = Number of days from the beginning of the test.

'N' = Total number of seeds germinated at the termination of the experiment.

Development of plant production technique (*ex situ* conservation)

To make the cultivation of this herb possible in an effective way at lower altitudes, its response was observed in different soil textural classes recommended by Nautiyal *et al.* (2001) and fertilizer application were given to each treatment (Table 3). The following soil textural classes were used: (i) Natural soil (collected from natural habitat). (ii) Loam: organic manure (2:1). (iii). Loam soil (garden soil) (iv) Sandy loam (2:1) (v) sandy loam (1:2) (vi) Sand: silt: clay (1:2:2).

Young seedlings (of the same age) of *A. chasmanthum* were collected from natural habitats in April-May and sown in the above mentioned soil textural classes in pots. Each set of pots (containing equal amount of soil) were irrigated at different intervals for the first year. In the next growing season the stabilized and adapted plants were treated with various inorganic fertilizers (Table 3) in different concentrations each with a set of control. The plants were kept in diffused light and harvested (at full bloom) to determine dry biomass (after oven dried at 80°C for 72 hours; Singh and Purohit 2003).

In vivo propagation

The fresh underground tubers of the species were collected from natural population Apharwat II, 3250 m a.s.l (Kashmir Himalaya) in the first week of May and split longitudinally into 2, 3 and 4 pieces depending upon the size of the parental tuber containing a portion of shoot apex. The split cuttings were treated with 100 ppm IAA, IBA and GA_3 for 48 hours and were sown in sandy loam soils under pots at Herbal Garden (1495 m), University of Kashmir, Srinagar (India). The pots were kept in diffused light to monitor the leafy shoot generation, survival and comparison with the control (untreated cuttings) (Table 4).

RESULTS AND DISCUSSION

The present studies on seed germination reveal that even though the seeds germinate nicely without any treatment (control), the speed of germination is slow. The mean germination time (MGT) in control replicas works to 22.11±0.358 days with only 10.66±1.154% germination.

Among different treatments only chilling (of varying periods) and GA_3 resulted in germination (Table 2, Fig 1). Chilling at 4°C for 20, 40 and 100 days, however, respectively led to 90.0±8.66, 63.33±10.40 and 11.66±7.63% seed germination against the control of 10.66±1.154%. The mean germination time (MGT) also decreased in chilled seeds and varied between 13.67±1.87 to 19.55±1.53 days possibily because of the fact that the seeds of *A. chasmanthum* remain under snow for about five months and requires the long chilling period in order to break the seed dormancy. The seeds chilled for 20 days germinated in 0.25 mM thiourea showing 30.0±22.19 per cent germination with 14.11±1.694 day MGT indicating that the chilling triggers the breaking of seed dormancy.

The studies on agro-techniques (Table 3 and Fig 2) reveal that the application of fertilizers increased the herbage yield/biomass and longevity of growth period as compared to control. The present studies reveal that the total biomass per individual works to 12.37±11.25 gm in natural soil. Among different soil types with different fertilizers used, the plants in loamy soils fertilized with nitrogen (300 mg/kg soil) and NPK (nitrogen: phosphorus: potassium; 3:1:1; 500 mg/kg of soil) showed maximum dry biomass of 13.36±2.13 gm and 18.23±1.16 gm per individual respectively as against the control of 4.50±4.24 gm. The loamy texture offers little or no resistance for rooting and less leaching of nutrients than clayey and sandy soils.

The *in vivo* propagation studies reveal (Table 4, Fig 3) that the cuttings without any treatment showed 62.5%

Table 1: Salient features of some Kashmir Himalayan sites selected for studies on *Aconitum chasmanthum.*

Habitat character	Populations				
	Apharwat I	Apharwat II	Rayil	Posh pathri	Mahaganesh top
Altitude (m)	3,150	3,250	3,450	3,480	3,846
Latitude/	34°04/N	34°04/N	34°02/N	34°30/N	34°30/N
Longitude	74°23/E	74°23/E	75°23/E	75°30/E	75°30/E
Direction with reference to Srinagar	North west	North west	South west	South east	South east
Climatic zone	Alpine	Alpine	Alpine	Alpine	Alpine
Habitat	Open grassy slope	Open meadowland	Open moist meadowland	Open meadowland	Open grassy slope
Threat factor	Extraction, trampling, beetles and pests	Extraction, trampling, beetles and pests	Extraction, trampling	Extraction, trampling	Extraction, trampling

All the populations are exposed to open sun and having loamy clay type soils.

Table 2: Effect of physical and chemical treatments on *in vitro* seed germination of *Aconitum chasmanthum.*

Treatment	Mean germination time (MGT) days†	% germination
Control	22.11±0.358***	10.66±1.154
GA3 (mM)*		
0.12	19.58±2.592	11.66±2.890
0.25	20.866±1.847	11.60±7.630
0.50	19.77±0.582	6.60±5.773
1.0	-	-
Thiourea (mM)*		
0.05	-	-
0.10	-	-
0.20	22.396±1.789	6.60±5.760
0.25	-	-
Kinetin (mM)*		
0.05	-	-
0.10	-	-
0.20	-	-
0.25	-	-
Acid dip	-	-
Chilling (days)**		
20	13.678±1.874	90.0±8.660****
40	15.516±1.117	63.33±10.40****
100	19.556±1.535	11.66±7.633
20 day chilled* seeds germinated in 0.25 mM thiourea	14.115±1.694	30.0±22.19
Seed coat puncturing	-	-

*The seeds were germinated on Whatman filter paper soaked in different concentrations of growth hormones shown in the Table.
**Chilling was given at 4°C.
***Mean±Standard deviation.
****Maximum seed germination; Seed sample taken for each replica = 20; Temperature range = 15°C-20°C
†MGT = (n×d)/N (Joshi and Dhar, 2003).

survival and require 18-24 days to regenerate a leafy shoot. However, the cuttings treated with IAA and IBA (100 ppm) exhibited 75% and 70% survival respectively and required 10-15 days for shoot generation in sandy loam soils. GA_3 (100 ppm) treated cuttings showed shoot regeneration in lesser time, but survival was a little lesser than other treatments. The difference even though marginal clearly demonstrates that the tuber cuttings having a piece of apical shoot have the capability to regenerate and form new plants under *ex situ* conditions. Utilizing this cost effective method of propagation, the species can be multiplied in lesser time with good survival rates.

The seeds of the species remain dormant for a pretty long period of time and do not germinate in darker conditions and seem to be photoblastic in nature. The present studies reveal that only chilling and GA_3 concentrations show desirable response. The species under discussion showed 90.0±8.66% germination when chilled for 20 days with mean germination time (MGT) of 13.678±1.874 days. The seeds chilled for 40 and 100 days, however; showed low germination rates than of the 20 day chilled seeds depicting superiority of 20 day chilling as a dormancy breaking technique to improve seed germination. Similar kind of results was obtained by Yousef and Mahmood (2014) on *Bunium persicum* an important medicinal aromatic plant species of Himalaya. Seeds of alpine plant species mostly hydrated ones commonly germinate when exposed to low temperature (Prakash *et al*. 2011). The overexploitation of important plant species is the basic reason for their extinction. This has necessitated the development and

Table 3: Effect of various treatments on the production of *Aconitum chasmanthum* grown on different soil types.

Soil type and	Dry weight per plant (gm)		Total dry biomass
fertilizer applied	Below ground	Above ground	per plant (gm)
Natural soil	4.25±3.83*	8.125±7.65	12.37±11.25**
Loamy soil			
N-300 mg/kg soil	4.633±0.60	8.73±1.56	13.36±2.13**
N-500 mg	3.366±1.05	9.40±0.98	12.76±1.78**
K-300 mg	1.80±1.229	3.80±2.32	5.60±3.16
DAP-500 mg	1.15±0.494	3.16±1.72	4.26±1.16
NPK-500 mg†	6.56±0.404	11.66±0.763	18.23±1.167**
Control	1.25±1.06	3.25±3.18	4.50±4.24
Loam with organic matter (2 : 1)	5.066±3.156	5.40±3.77	10.46±6.925**
Sandy loam (2:1)			
N-300 mg/kg soil	2.566±1.006	3.40±1.216	5.996±2.193
N-500 mg	1.75±1.06	3.00±0.707	4.75±1.767
K-300 mg	2.85±0.494	3.10±1.55	5.95±2.05
DAP-500 mg	2.166±1.040	5.06±3.108	7.23±4.142
NPK-500 mg†	3.56±0.602	10.90±1.652	14.46±2.250**
Control	2.50±0.707	2.25±1.06	4.75±1.767
Sandy loam (1: 2)			
N-300 mg/kg soil	1.85±0.494	3.35±2.616	5.20±3.111
N-500 mg	5.50±0.866	5.466±1.778	10.966±2.638**
K-300 mg	2.50±1.00	7.50±3.774	10.0±4.769**
DAP-500 mg	3.50±2.00	6.50±2.179	10.0±4.092**
NPK-500 mg†	8.30±1.014	19.56±3.494	27.86±4.508**
Control	1.83±0.288	3.40±1.014	5.23±0.750
Sand: Silt: Clay (1: 2: 2)			
N-300mg/kg soil	3.50±0.707	10.75±8.131	14.25±8.830**
N-500 mg	3.56±0.60	12.56±0.6	16.13±0.115**
K-300 mg	0.90±0.424	1.85±0.494	2.75±0.919
DAP-500 mg	1.60±0.871	3.233±1.939	4.833±2.80
NPK-500 mg†	3.925±0.836	11.82±4.71	15.33±4.82**
Control	1.40±0.360	2.33±1.250	3.733±1.553

*Mean±Standard deviation.

**Showed maximum dry biomass.

†NPK (N-300 mg + P-100 mg + K- 100 mg) = 500 mg/kg soil.

The concentrations of fertilizers given per kg of soil.

N= Nitrogen; P= Phosphorus; K= Potassium.

standardization of agrotechniques for their cultivation and consequent conservation (Joshi *et al.*, 1990). The major conservation strategies recommended are development of germplasm centres, *in situ* and *ex situ* conservation of critically endangered species, establishment of high altitude nurseries, systematic collection and domestication etc. (Joshi and Rawat, 1997; Dwivedi, 1999). Considering the increased demand of herbal drugs and consequent depletion of several species, it is important to urgently initiate proper steps for conservation (Nautiyal *et al.* 2001). The species under discussion showed increased herbage yield/biomass and longevity of growth period as compared to control after application of different fertilizers. The species responded to each nutrient treatment, however, in all the soil textural classes the nitrogen nutrient trials and NPK treated individuals have shown overall best performance and biomass. The plants performed well and gave excellent adaptability when grown on various soils. However, maximum plant survival and vigorous growth was obtained in loamy textured soils, possibly because this texture offers little or no resistance for rooting and less leaching of nutrients than clayey (difficult to root) and sandy (maximum nutrient leaching) soils. Similar kind of results was observed by Ramesh *et al.*, (1989), Khan and Zaidi (1991), Singh and Neoparly (1993), Yugalkishore *et al.* (2019), Adebayo *et al.* (2021) and Senthilkumar and Gokul (2021) on several plant species.

The application of auxins is known to stimulate the activity of the cambium resulting in the mobilization of reserve food materials to the site of root initiation (Gurumurthi *et al.*, 1984). Several species of the genus *Aconitum* inhabiting the alpine zones of Indian Himalayan region have been propagated *in vitro* (Giri *et al.* 1993; Singh *et al.* 1998; Nadeem *et al.* 2001). The experiments on the species under discussion reveal that the species has a potential to propagate through their tuber cuttings in the sandy loam soils. The cuttings treated with IAA and IBA showed best survival and required lesser time duration to develop plantlets as also argued by Giri *et al.* (1993) who observed

Table 4: *In vivo* propagation of *Aconitum chasmanthum.*

Treatment	No. of cuttings	Days required for shoot regeneration	No. of plantlets surviving till senescence	% survival
Control	16	18 - 24	10	62.5
IAA (100 ppm)	20	12 - 15	15	75.0
IBA (100 ppm)	20	10 - 14	14	70.0
GA_3 (100 ppm)	16	10 - 12	11	68.75

Soil used: Sandy loam.

Fig 1: *In vitro* seed germination of *A. chasmanthum.*
1.1-1.2: Germinated seeds in Petri plates inside the lab; 1.3- Transplanted seedlings just after generation.
1.4- Establishment of seedlings in pots, note the adaptability.

Fig 2: Development of agro-technology under *ex situ* conditions.
2.1 - Plants at young stage treated with nitrogen (300 mg/kg soil); 2.2 - Young plants treated with NPK (500 mg/kg soil);
2.3 -Nitrogen (300 mg/kg soil) treated plants at maturity; 2.4 - NPK (500 mg/kg soil) treated plants.

Fig 3: *In vivo* propagation of *A. chasmanthum.*

3.1 - Single tuber; 3.2 - Growth hormone treated longitudinal cut pieces of the single tuber having a portion of shoot apical meristem; 3.3 - Germination of the cut pieces; 3.4 - 3.5: Formation of plantlets; 3.6 - Dug out cutting after shoot regeneration, note the two small tubers generated from one cut piece.

that regenerated embryos were successfully converted to plantlets on addition of IBA to MS medium. GA_3 treated cuttings required least time period to regenerate the leafy shoot but showed lesser % survival as compared to IAA and IBA treated cuttings. Such kind of findings was observed earlier by Nautiyal *et al.* (2001), Naidu *et al.* (2006) and Rathod *et al.* (2021). Utilizing these cost effective techniques of propagation, multiplication and conservation at low altitudes can relieve the pressure of extinction (especially for threatened taxa) to a large extent and also can provide an alternate income-generating resource.

CONCLUSION

Present study demonstrates the efficient methods of seed germination, agro-techniques and vegetative propagation through tubers, therefore, may prove useful for sustainable management of this important and critically endangered medicinal plant species.

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