Keywords: Inoculation, Medicinal herb, Seed treatment, Tissue culture

Plants are one of the most important natural sources of medicine. Kashmir, the valley of flowers in Jammu & Kashmir, India, is a naturally rich bed of large number of medicinal plant wealth. Some of the important medicinal herbs of Kashmir are Aesculus hippocastanum, Allium sativum, Artemisia dracunculus, Cannabis indica, Crocus sativus, Dioscorea deltoidea, Eurphobia heliscopia, Nymphaea stellata, Sassurea, Valeriana, etc. However, many of them are now endangered and a considerable number of them are at the verge of extinction, due to various factors such as deforestation, destructive collection techniques, etc. Efforts have been made by the authorities from time to time to save these medicinal plants by systematic cultivation of medicinal plants adopting various propagation methods.

Valeriana is a hardy perennial flowering herb from the family Valerianaceae, grows up to 1.5 m, and usually found among herbaceous vegetation on humus-rich soils at altitudes, 1900-3100 m. In Kashmir, it is locally called Mushk-e-Bala. Valerian herb is well known for its medicinal value. It is used to treat insomnia and other sleep related disorders and can be used as herbal alternative to benzodiazepine drugs. It is also known to exhibit anti-spasmodic and blood pressure lowering properties. Biochemical composition and active constituents in valeriana are valepotriates, volatile oils, valeric acid and gamma-aminoButyric acid (GABA). Tang et al. has reported two new flavone glycosides from the rhizomes and roots of Valeriana jatamansi Jones. Valepotriates from valeriana (20%) are useful tranquillizers and sedatives similar to meprobromate. Valerian has been shown to have no adverse effects on fertility or fetal development. Valerene acid from Valeriana officinalis extract is reported to ameliorate D-galactose-induced reductions in memory, cell proliferation, and neuroblast differentiation inaged mice.

Methanolic extract of rhizomes of V. officinalis, in particular, has been reported to have demonstrated anticonvulsant effects against chemically and electrically induced convulsions in mice. V. officinalis root is reported to be a good source of valerianic acid. Cricosta et al. have established that the ethanolic and aqueous extracts of V. officinalis roots possess significant coronary spastic, antihypertensive and antibronchospastic properties. Earlier workers have shown that cultivated valeriana yield significantly higher content of valepotriates and essential oils compared to their wild counterparts. Caqstiilo et al. has stressed that large scale propagation of this
endangered plant may offer an alternative for its production for medicinal purpose\textsuperscript{19}. Sood et al. has successfully demonstrated in-vitro propagation of \textit{V. jatamansi} by induction of shoot proliferation from shoot buds on nutrient medium supplemented with BAP and IAA or NAA\textsuperscript{20}. Similarly, Reza et al. has proposed a method was developed for rapid micropropagation of \textit{Valeriana officinalis} through shoot regeneration from calli derived from leaf and petiole explants suitable for its conservation and mass propagation\textsuperscript{21}. In this study, we attempted in-vitro micro propagation of cultivated \textit{V. officinalis} and also explored influence of light and cold on its seed germination.

Materials and Methods

Initially, we attempted culture with explants from greenhouse of CSIR-Indian Institute of Integrative Medicine (IIIM), Srinagar, Jammu & Kashmir, India. However, we encountered the problem of contamination and media browning due to release of phenolic compounds from the explants. To remove contamination, we grew plantlets aseptically in laboratory and used different seed treatments to enhance the germination rate. Browning of media was removed by adding activated charcoal.

Seeds of \textit{V. officinalis} (Fig. 1) were collected from the field gene bank of CSIR-IIIM, Srinagar, and divided into 3 groups of 10 each and kept in light, dark and control. The seeds were washed with 0.1-1.0\% mercuric chloride for 5-7 min and then with 70\% alcohol for 1 min, thoroughly rinsed with double distilled water, and then subjected to various treatments and placed on a moist filter paper in a petri-plate of 4 inches diameter. Various treatments given are as under:

(i) Gibberellic acid: \textit{GA}$_3$ @ 200 ppm was prepared by dissolving 200 mg of \textit{GA}$_3$ in 1 litre distilled water. The seeds were washed in autoclaved double distilled water and kept soaked in the \textit{GA}$_3$ solution for about 24, 48 and 120 h;

(ii) Chilling: Seeds were subjected to chilling at 4 °C for 48, 72 and 120 h using refrigeration. The control group was not put to any chilling treatment.

In-vitro micro propagation—\textit{V. officinalis} explants were taken from greenhouse of IIIM and later from aseptically grown plantlets on Murashige and Skoog medium (MS) supplemented with BAP, Kinetin and BAP+IAA in 10 replicates. Surface sterilization of explants was carried out various sterilizing agents. After washing explants thoroughly under running water, the explants were left in laboratory detergent (2-3 drops of Tween-20) dissolved in distilled water for 2-3 min. Again a thorough washing was given with distilled water to remove the traces of detergent. Further sterilization with done with bleaching powder (90g/L) for 3-5 min. The efficiency of sterilization was improved by treating explants with 0.1-1.0\% \textit{HgCl}$_2$ for 1-2 min. Finally, the tissue was thoroughly washed with sterilized double distilled water for 4-5 min. The morphogenetic response of shoot tips such as initiation of shoots, shoot multiplication and elongation, and stunted growth were observed with different phytohormone combinations.

Results and Discussion

Breaking seed dormancy—The germination of seeds has shown a clear pattern depending upon the treatment. The un-chilled seeds of treated with \textit{GA}$_3$ for 24 h and kept in dark have shown maximum germination (48\%) followed by the group treated for 120 h under light exposure (44\%). The control group that did not have \textit{GA}$_3$ pre treatment exhibited only 10 and 15\% germination rate for dark and light conditions, respectively. In general, number of days taken by the seeds exposed to light to germinate was

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Fig. 1—\textit{Valeriana officinalis}, its flowers, roots and seeds
less in all the groups when compared to the seeds kept in dark (Table 1 and Fig. 2).

Effect of pre-chilling—Pre-treatment with reduced temperature (4 °C) also had considerable impact on seed germination. Similar to GA\(_3\) treatment of un-chilled seeds, the number of days taken by chilled seeds to germinate under light exposure was less compared to those kept in dark condition (Table 2). Maximum germination was observed in seeds pre-chilled for 72 h and exposed to light (60%) followed by 40% germination in seeds chilled for same period but kept under dark. Forty-eight hour pre-chilling also exhibited 40% seed germination when exposed to light and showed 25% germination under dark (Fig. 3).

Effect of light exposure—Exposure to light did enhance germination rate as was evident in all the test groups including the controls, but for the 24 h GA\(_3\) treated ones. Similarly, number of days taken by the seeds to germinate with exposure to light, in both, treated and untreated seeds, were less compared to their counterparts kept in dark (Table 1 and 2).

The relevance and significance of different seed treatments for breaking dormancy and improving seed germination has been discussed earlier by Shanmugavalli et al.\(^{22}\). Treatments such as soaking before sowing, acid wash, chilling, GA\(_3\), etc., have been found to induce and enhance germination of dormant seeds\(^{23}\). Similar results were obtained in Atropa belladonna seed germination by Andarabi\(^{24}\). These studies are in tune with our present study wherein, both, pre-chilling and treatment with gibberellic acid did break the seed dormancy.

Tavili et al.\(^{25}\) reported a positive germination rate and mean germination time but decreased germination percentage for medicinal plants, Foeniculum vulgare and Cuscuta epithymum on pre-chilling for 10 days. However, Farajollahi et al.\(^{26}\) did not find any such effect on germination in Calotropis persica seeds pre-chilled for 10 days. Hartmann and Kester\(^{27}\) attributed the improvement in germination percentage in the achene to the activation of embryo by chilling temperature (4 °C). Nadeem et al.\(^{28}\) recommended 30 days of warm temperature followed by 60 days of cold stratification and 30% sulphuric acid treatment for 10 min for higher seed germination, seed vigour

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**Table 1—In-vitro seed germination of un-chilled seeds of Valeriana officinalis subjected to gibberellic acid treatment**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Days taken for first seed to germinate (L)</th>
<th>Days taken for last seed to germinate (D)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10</td>
<td>19</td>
</tr>
<tr>
<td>Group I</td>
<td>4</td>
<td>15</td>
</tr>
<tr>
<td>GA(_3) 24 h</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>Group II</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>GA(_3) 48 h</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>Group III</td>
<td>4</td>
<td>6</td>
</tr>
</tbody>
</table>

GA, gibberellic acid; L, Light; D, Dark

**Table 2—In-vitro seed germination of chilled seeds of Valeriana officinalis subjected to prechilling**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Days taken for first seed to germinate (L)</th>
<th>Days taken for last seed to germinate (D)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>15</td>
<td>26</td>
</tr>
<tr>
<td>48 h</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td>72 h</td>
<td>2</td>
<td>9</td>
</tr>
</tbody>
</table>

L, Light; D, Dark. Data for 120 h could not be recorded.

Fig. 2—Effect of gibberellic acid (GA) pretreatment of un-chilled seeds of V. officinalis on germination [GI, GA\(_3\) 24 h; GII, GA\(_3\) 48 h; GIII, GA\(_3\) 120 h].

Fig. 3—Effect of prechilling of seeds of V. officinalis on germination.
On the influence of photoperiod over germination of seeds, there are various interpretations available in literature. Some find light a hindrance and some, a boost. Most cultivated plants prefer to germinate in the dark. However, exceptions such as few greenhouse perennials, epiphytes, many grasses, and even tobacco prefer light. Other factors viz., storage, fertilizers, etc. also matter.\(29,30\)

Study on the seeds of Carex spp., (8 species) on germination responses after exposure to different lengths of white light varied widely. Carex brevior and C. stipata exhibited >25 % germination in continuous darkness. Carex brevior took <15 min of white light for ≥50 % germination, whereas C. hystericina, C. comosa, C. granularis and C. vulpinoidea required ≥8 h.\(^{31}\)

**Morphogenetic response**—The morphogenetic responses such as initiation of shoots, shoot multiplication and elongation of shoot tips were observed in the explants grown on MS medium with various phytohormones after six weeks of culture period. Explants grown on MS medium enriched with BAP (1mg/L) have shown the maximum initiation of shoots (60%) followed by the MS medium enriched with BAP (1mg/L) + IAA (0.1 mg/L) with 50 % response in shoot multiplication and elongation. Explants grown on MS + BAP (1mg/L) + NAA (0.1 mg/L) showed 40 % shoot multiplication but followed by stunted growth (Table 3). Our results are more or less in alignment with similar reports on other medicinal plants such as Artemisia pallens and Dioscorea floribunda.\(^{32,33}\) Multiple shoot regeneration from shoot apices was recorded with BAP (1mg/L) and BAP (1 mg/L) + IAA (0.1 mg/L). Similar results of multiple shoot regeneration in shoot tip cultures in various BAP concentrations and combinations have been reported for Lavandula officinalis.\(^{34}\) Camen et al.\(^{35}\) who studied the regenerative possibilities from cell suspension from callus and roots of V. officinalis has also observed that the results vary according to the hormone balance in the medium.

**Table 3**—Morphogenetic response of shoot tips of Valeriana officinalis to various phytohormones.

[Data scored after 6 weeks of cultural period]

<table>
<thead>
<tr>
<th>Medium*</th>
<th>Response</th>
<th>Nature</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>MS Basal medium</td>
<td>Nil</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>MS + BAP (1mg/L)</td>
<td>Initiation of shoots</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>MS + Kn (1mg/L)</td>
<td>Initiation of shoots</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>MS + BAP (1mg/L) + IAA (0.1mg/L)</td>
<td>Shoot multiplication and elongation</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>MS + BAP (1mg/L) + NAA (0.1mg/L)</td>
<td>Initiation of shoot multiplication followed by stunted growth</td>
<td>40</td>
<td></td>
</tr>
</tbody>
</table>

*10 replicates/treatment

[Kn, Kinetin; MS, Murashage and Skoog (1902); IAA, Indole acetic acid; BAP, Benzylamino purine; NAA, Naphthallena acetic acid]

index of the progenies, and lower germination period in *Rosa × hybrid*.

The present study demonstrated breaking seed dormancy in *Valeriana officinalis* seeds by pretreatments with gibberellic acid and prechilling, both individually, as well as combined with photoavailability (light and dark). Further, the *in vitro* propagation potential of *V. officinalis* explants and plantlet regeneration have also been evidently shown using an appropriate MS medium enriched with suitable phytohormones, viz., BAP, IAA and NAA in required concentrations. Thus, the present investigation forms an important preliminary step for *in vitro* micro-propagation of *Valeriana officinalis*.

**Conclusion**

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**Acknowledgment**

References

5. Gilani AH, Khan AU, Jabeen U, Subhan F & Ghafar, Antispasmodic and blood pressure lowering effects of Valeriana wallachi are mediated through K+ channel activation. *J Enthopharmacol*, 100 (2005) 347
Yu-Ping Tang, Xin Liu & Biao Yu, Two New Flavone Acids from Müürisepp, Variation in the composition of the essential oil of *Müürisepp*. 
Ain Raal, Anne Orav, Elmar Arak, Tiiu Kailas & Mati Müürisepp, Variation in the composition of the essential oil of *Müürisepp*. 


Yu-Ping Tang, Xin Liu & Biao Yu, Two New Flavone Glycosides from *Müürisepp*. 


J Ethnopharmacol, 113 (2007) 204.

Sung Min Nam, Jung Hoon Choi, Dae Young Yoo, Woosuk Kim, Hyo Young Jung, Jong Whi Kim, Soo-Yong Kang, Jaekil Park, Dong-Woo Kim, Wan Jae Kim, Yeon Sung Yoon & In Koo Hwang, *Valeriana officinalis* extract and its main component, valerenic acid, ameliorate D-galactose-induced reductions in memory, cell proliferation, and neuroblast differentiation by reducing corticosterone levels and lipid peroxidation. 


Dharmarata HWR, Nanayakkara MPD & Khan IA, 3-beta, 6213770). 


Cricosta C, De Plasquale BD, Pino A & Occhiuto F, Biological and analytical characterization of two extracts from *Valeriana officinalis*. 


Bos R, Hendricks, Pars D, Stojanavo & Georgiev EV, Essential oil composition of *Valeriana officinalis* ssp collina cultivated in Bulgaria. 


Ghaderi N & Jafari M, Efficient plant regeneration, genetic fidelity and high level accumulation of two pharmaceutical compounds in regenerated plants of *Valeriana officinalis* L. 


Caqstilo P, Zamilpa A, Marquez, Hernandez, Lara & Alvarez, Comparative study on different on differentiation levels and Valepotriate content of *in vitro* cultures and regenerated and wild plants of Valeriana edulis ssp. Proceria. 


Reza AG, Morteza KK, Akhtar S, Rapid micropropagation through shoot regeneration of *Valeriana officinalis* L. 


M Shamsugavalli, PR Renganayaki & C Menaka, Seed dormancy and germination improvement treatments in fodder solghurn. 


Bose B & Sharma MCS, Influence of maize seed treatment with nitrates on nitrogen economy and nitrogen pollution. 


Asghar Farajollahi, Bahram Gholinejad & Hamed Jonaodi Jafari, Effects of different treatments on seed germination improvement of *Calotropis persica*. 


Tavili A, Farajollahi A, Pouzesh H & Bandak E, “Treatment induced germination improvement in medicinal species of *Foeniculum vulgare* Miller and *Cuscuta epithymum* (L.). 


Muhammad Nadeem, Atif Riaz, Adnan Younis, Masum Akond, Amjad Farooq & Usman Tariq, Improved technique for treating seed dormancy to enhance germination in *Rosa x hybrid*. 


David Batty, The Effect of Light on Germination and Seedlings. 


Karin M Kettenring, Gary Gardner & Susan M. Galatowitsch, Effect of light on seed germination of eight wetland *Carex* Species. 


Plant cell tissue cult, 21, 159.


Plant cell tissue organ cult, 3 (4), 325.


Karin M Kettenring, Gary Gardner & Susan M. Galatowitsch, Effect of light on seed germination of eight wetland *Carex* Species. 


Plant cell tissue cult, 21, 159.