

BSI	<b>ANNUAL SCIENTIFIC MEET 2021</b>
<b>PROJECT TITLE:</b>	
In vitro mass multiplication and propagation and rehabilitation in natural habitat of useful and threatened species of North-West Himalaya.	
• <b>Executing officials:</b>	
DR. GIRIRAJ SINGH PANWAR, Sci.-D	
DR. BHAVANA JOSHI, BOTANIST	
• <b>Date of initiation: September, 2021</b>	
• <b>Date of completion: March, 2023</b>	

<b>Objectives &amp; Methodologies</b>	
<b>Objectives</b>	<b>Objective-wise Brief Methodology</b>
Collection of explant/plant propagules (Seeds/live material) from the wild habitat of the selected species.	<ol style="list-style-type: none"> <li>1. Literature survey and Herbarium consultation to examine the locality of the species.</li> <li>2. Collection of explant/plant propagules from the wild</li> </ol>
Standardization of Micropropagation protocol for the species	<ol style="list-style-type: none"> <li>1. Preparation of tissue culture media of different hormonal composition.</li> <li>2. Optimization of media and PGRs for inducing organogenesis/callusing in the explant.</li> <li>3. Proliferation of shoot/root cultures in proliferation medium.</li> <li>4. Sub-culturing at regular time intervals for the maintenance of stock cultures.</li> </ol>
Hardening and reintroduction to the wild habitat	<ol style="list-style-type: none"> <li>1. Properly developed in vitro plantlets were shifted to polyhouse and net house for the acclimatization, respectively.</li> <li>2. Fully acclimatized plants were shifted to their wild habitat under the habitat restoration programme.</li> </ol>

***Eulophia dabia* (D.Don) Hochr**

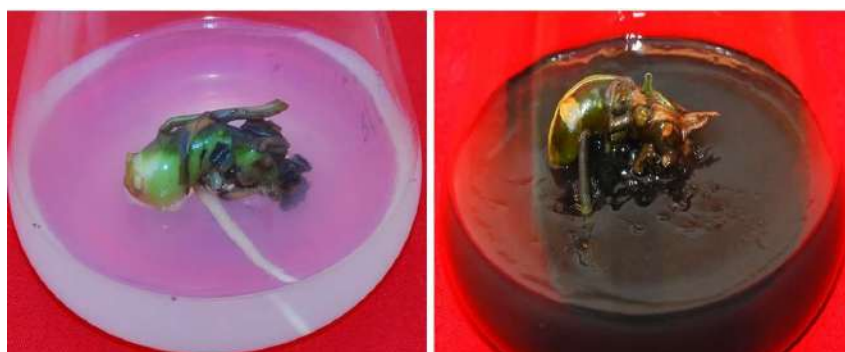
- Threatened, terrestrial orchid species, Saalam mishri.
- **Habitat:** In the banks of seasonal streams in sandy soil in association with grass sp.
- **Distribution:** UK, HP and Haryana
- Collected the capsule and bulbs of *Eulophia dabia* (D.Don) Hochr from Raja Ji National Park, Haridwar.
- Asymbiotic seed germination and development of rhizome in MS medium enriched with CH and AC



**Development of RLBs from seed cultures after 6 months**

**Table:Effect of culture media and organic additives on development of *E. dabia* rhizome after 120 days of incubation.**

Media	Diameter (cm)	Number of nodes
MS	1.7	4.2
<b>MS + CH + Charcoal</b>	<b>2.3</b>	<b>5.0</b>
½ MS	1.5	2.9
½ MS + CH + Charcoal	2.0	3.0
¼ MS	0.9	1.5
¼ MS + CH + Charcoal	1.1	1.63
Knudson	1.9	2.6
Knudson + CH + Charcoal	2.1	3.3
Mitra	1.81	2.53
Mitra + CH + Charcoal	2.0	2.9



**Development of Rhizome in MS medium + PVP or Charcoal after 120 days of Incubation.**

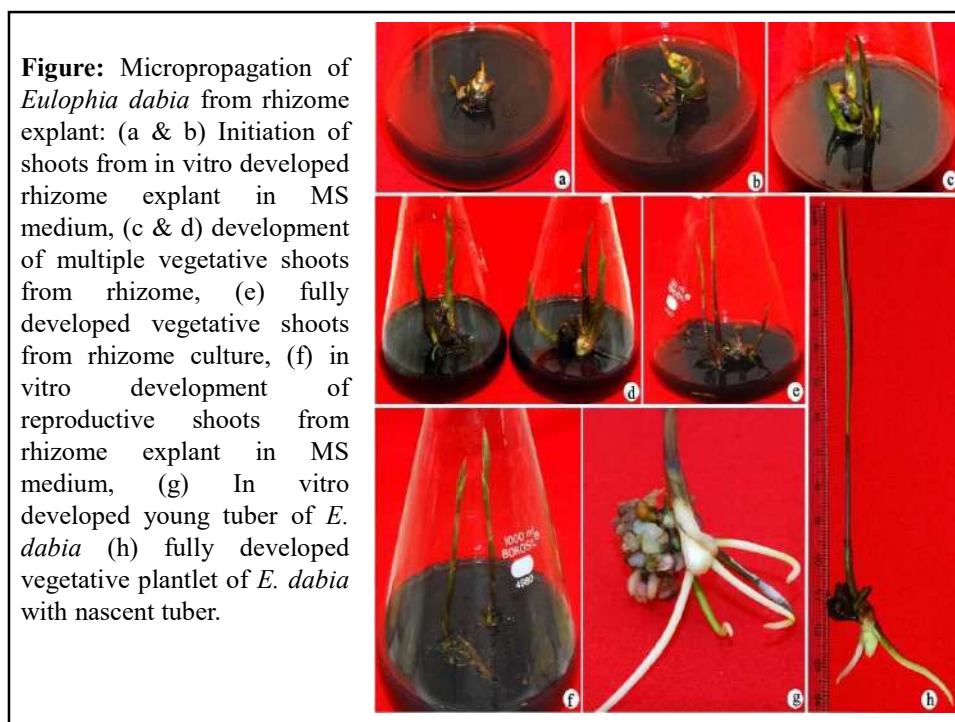
**Table:** Effect of cytokinins and NAA on shoot development from axenic rhizome culture of *E. dabia* inoculated in MS medium after 8-weeks of culture.

Plant growth Hormones ( $\mu\text{M}$ )	Response (%)	Shoot number (Mean $\pm$ SD)	Shoot length (cm) (Mean $\pm$ SD)
MS <sub>0</sub>	-	-	-
<b>BAP</b>			
2.2	46.09	1.37 $\pm$ 0.9 <sup>j</sup>	5.9 $\pm$ 0.8 <sup>g</sup>
3.1	76.87	2.08 $\pm$ 1.2 <sup>h</sup>	8.1 $\pm$ 1.2 <sup>c</sup>
4.4	<b>96.15</b>	<b>4.3 <math>\pm</math> 1.2<sup>f</sup></b>	<b>13.44 <math>\pm</math> 0.8<sup>g</sup></b>
6.6	89.04	3.84 $\pm$ 1.2 <sup>f</sup>	9.89 $\pm$ 0.8 <sup>g</sup>
8.9	75.23	3.71 $\pm$ 1.2 <sup>f</sup>	8.5 $\pm$ 1.4
<b>Kinetin</b>			
2.3	23.48	1.07 $\pm$ 0.8 <sup>j</sup>	4.2 $\pm$ 0.6 <sup>h</sup>
3.2	46.01	1.9 $\pm$ 1.1 <sup>i</sup>	7.3 $\pm$ 1.0 <sup>g</sup>
4.6	71.39	2.55 $\pm$ 1.3 <sup>g</sup>	8.1 $\pm$ 1.2 <sup>c</sup>
6.9	<b>84.23</b>	<b>3.07 <math>\pm</math> 1.2<sup>h</sup></b>	<b>11.2 <math>\pm</math> 1.1<sup>ef</sup></b>
9.3	80.11	3.00 $\pm$ 1.2 <sup>h</sup>	10.97 $\pm$ 1.1
<b>BAP + NAA</b>			
4.4 + 0.53	82.23	3.8 $\pm$ 1.4 <sup>c</sup>	12.36 $\pm$ 1.6 <sup>c</sup>
4.4 + 1.59	<b>85.89</b>	<b>4.0 <math>\pm</math> 2.1<sup>a</sup></b>	<b>12.44 <math>\pm</math> 1.9<sup>a</sup></b>
4.4 + 2.65	84.01	3.9 $\pm$ 2.0 <sup>b</sup>	11.89 $\pm$ 1.8 <sup>b</sup>
<b>Kinetin + NAA</b>			
6.9 + 0.53	68.86	2.44 $\pm$ 1.5 <sup>f</sup>	9.94 $\pm$ 1.5 <sup>cd</sup>
6.9 + 1.59	<b>80.82</b>	<b>2.8 <math>\pm</math> 2.0<sup>b</sup></b>	<b>10.2 <math>\pm</math> 1.6<sup>c</sup></b>
6.9 + 2.65	77.66	2.05 $\pm$ 1.8 <sup>d</sup>	9.96 $\pm$ 1.6 <sup>c</sup>

➤ Axenic Rhizomes cultured on MS medium enriched with BAP (4.4  $\mu\text{M}$ ), CH and AC.



**Shoot induction from rhizome explants of *E. dabia***





### *Nepenthes khasiana* Hook. F.

- *Nepenthes khasiana* is the only insectivorous pitcher plant found in India belongs to monogeneric family Nepenthaceae.
- Endemic to Meghalaya and widely spread in the West Khasi Hills to East Khasi Hills, Jaintia Hills, East to West and South Garo Hills with an altitude range of 1,000 to 1,500m (Mao and Kharbuli, 2002).

- The phyto-chemical screening revealed the presence of various bioactive compounds such as naphthoquinones, plumbagin, droserone and 5 methyl droserone which play curative role against various human ailments.
- The species has been listed as an endangered plant in Appendix-I of CITES and in Negative List of Exports of the Government of India (Ziemer, 2010).

- Non treated seeds: Only 25% germination after 180-200 DAI.



**Germination of GA<sub>3</sub> (500 ppm) treated seeds into MS<sub>0</sub> medium after one month & observed 85% germination.**



**Table:** Effect of cytokinins and NAA on shoot development from shoot tip explants of *N. khasiana* inoculated onto ½ MS medium.

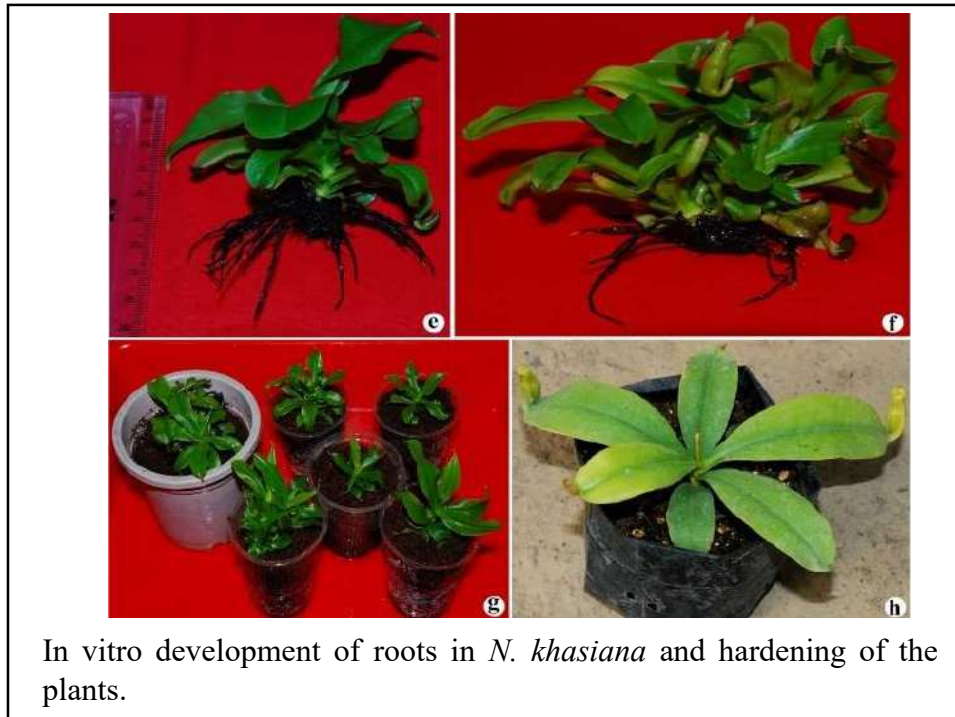
Plant growth Regulators ( $\mu\text{M}$ )	Explants with shoots (%)	No. of shoots per explant	Shoot length (cm)
<b>BAP</b>			
2.2	68.24	10.74 $\pm$ 0.5	6.5 $\pm$ 0.6
4.4	<b>96.81</b>	<b>21.22 <math>\pm</math> 0.78</b>	<b>10.5 <math>\pm</math> 0.5</b>
6.6	91.23	19.32 $\pm$ 0.83	8.7 $\pm$ 0.8
8.9	88.47	17.21 $\pm$ 0.8	8.2 $\pm$ 0.6
<b>Kinetin</b>			
2.32	53.87	7.15 $\pm$ 0.3	4.5 $\pm$ 0.78
4.6	68.18	8.69 $\pm$ 0.28	6.5 $\pm$ 0.42
6.9	74.96	9.86 $\pm$ 0.36	7.8 $\pm$ 0.2
9.3	<b>81.64</b>	<b>13.85 <math>\pm</math> 0.3</b>	<b>7.1 <math>\pm</math> 0.39</b>
11.62	76.09	10.11 $\pm$ 0.5	6.3 $\pm$ 0.49
<b>BAP + NAA</b>			
4.4 + 0.53	88.98	15.26 $\pm$ 0.39	8.1 $\pm$ 0.68
4.4 + 1.59	<b>91.89</b>	<b>18.68 <math>\pm</math> 1.3</b>	<b>10.9 <math>\pm</math> 0.7</b>
4.4 + 2.65	91.23	17.26 $\pm$ 0.4	9.9 $\pm$ 0.6
<b>Kinetin + NAA</b>			
9.3 + 0.53	74.16	10.21 $\pm$ 0.71	6.7 $\pm$ 0.8
9.3 + 1.59	77.25	11.21 $\pm$ 0.29	6.9 $\pm$ 0.7
9.3 + 2.65	<b>80.01</b>	<b>13.00 <math>\pm</math> 0.2</b>	<b>7.3 <math>\pm</math> 0.9</b>





**Table 2.** Effect of auxins on root induction in *in-vitro* regenerated shoots of *N. khasiana* in half-strength MS medium.

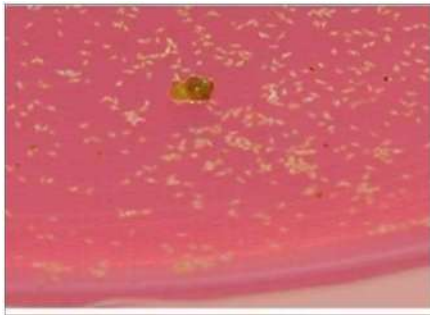
IBA	Auxins ( $\mu\text{M}$ )		Rooting (%)	No. of roots per shoot	Root Length (cm)
	NAA	IAA			
1/4 MS	0	0	10.14	1.9 $\pm$ 0.39	0.5 $\pm$ 0.2
2.46	0		66.13	12.87 $\pm$ 0.42	3.2 $\pm$ 0.9
<b>4.9</b>	<b>0</b>	<b>0</b>	<b>92.11</b>	<b>17.58<math>\pm</math> 0.87</b>	<b>5.5<math>\pm</math> 0.29</b>
7.36	0	0	81.32	15.9 $\pm$ 0.41	4.7 $\pm$ 0.3
9.8	0	0	75.38	14.54 $\pm$ 1.0	4.5 $\pm$ 0.49
0	2.65	0	56.30	8.26 $\pm$ 1.2	2.0 $\pm$ 0.4
0	<b>5.3</b>	<b>0</b>	<b>80.18</b>	<b>14.10<math>\pm</math> 0.92</b>	<b>4.2<math>\pm</math> 0.82</b>
0	7.9	0	78.21	11.56 $\pm$ 0.42	3.5 $\pm$ 0.9
0	10.6	0	72.61	8.14 $\pm$ 0.69	3.3 $\pm$ 0.9
0	0	2.85	54.31	6.23 $\pm$ 0.29	2.6 $\pm$ 0.28
0	0	<b>5.7</b>	<b>79.15</b>	<b>12.21<math>\pm</math> 0.5</b>	<b>3.9<math>\pm</math> 0.35</b>
0	0	8.5	77.59	9.1 $\pm$ 1.0	2.9 $\pm$ 0.4
0	0	11.42	70.47	7.3 $\pm$ 0.9	2.7 $\pm$ 0.39



### ***Rhynchostylis retusa* (L.) Blume**

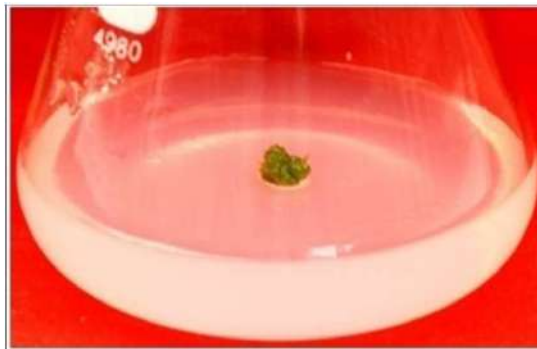
- *Rhynchostylis retusa* native to India and Tropical Asia, and the state flower of Arunachal Pradesh, Andhra Pradesh and Assam (Saxena *et al.*, 2020).
- Important ornamental orchids and commonly known as fox tail orchid because of its long tapering and densely packed inflorescences.
- Distributed in S-E Asian countries viz. India, Bangladesh, Bhutan, Nepal, Sri Lanka, China, Myanmar, Indonesia, Malaysia, Philippines, Thailand and Indo-China.

- In India reported from A & N Islands, Chhattisgarh, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Meghalaya, Odisha, Sikkim, Tamil Nadu, Telangana, Uttarakhand and West Bengal.
- The roots treat wounds, cuts, cramps, rheumatism, vertigo, infantile epilepsy, kidney stone, malarial fever, menstrual troubles, asthma and tuberculosis, while leaf juice relieves rheumatism and asthma Nongdam, 2014.



**Seeds inoculated into  $\frac{1}{2}$  MS medium & development of PLBs after 4 months**

**Development of PLBs**

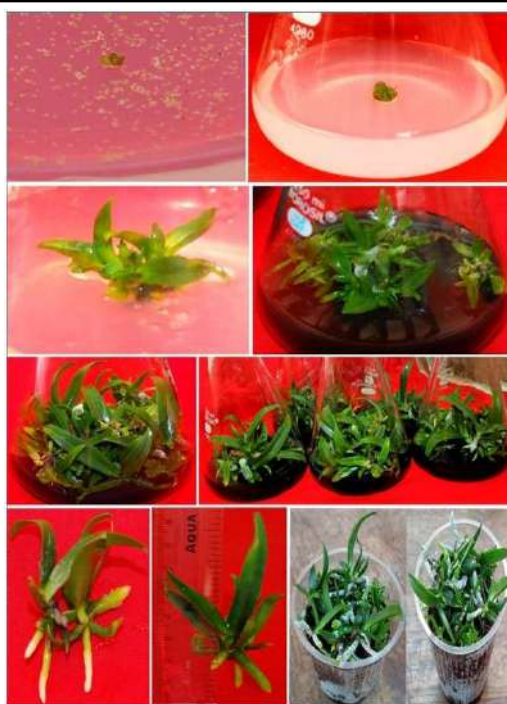


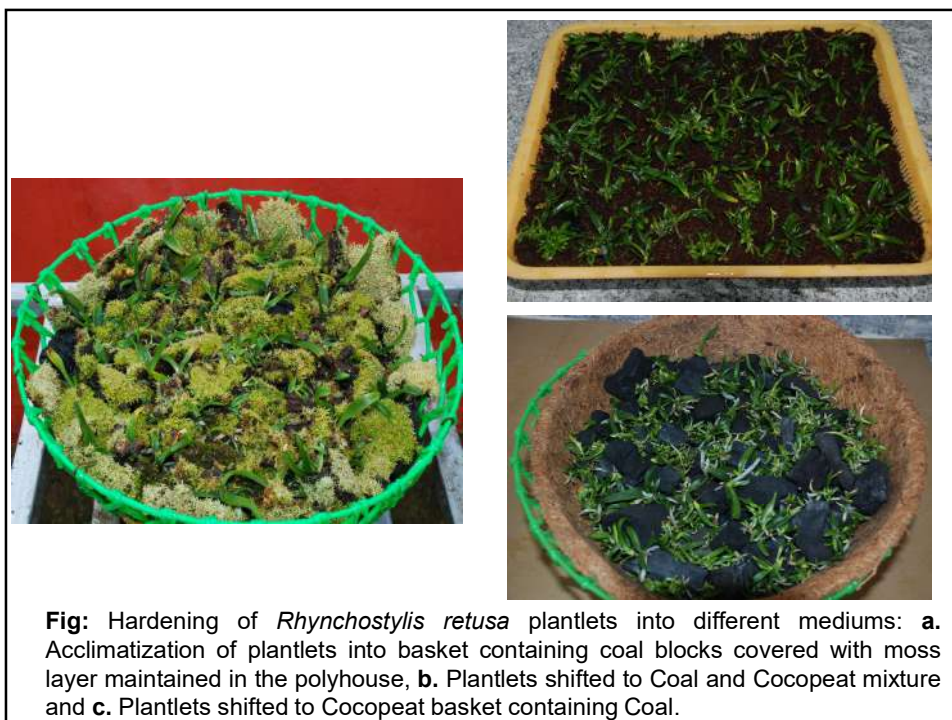
**Table:** Effect of different cytokinins and auxins on shoot proliferation from PLBs of *Rhynchosyilis retusa* inoculated on MS medium after 6 weeks of culture.

Plant Growth Regulators ( $\mu\text{M}$ )	Explants with shoots (%)	No. of shoots per explant	Shoot length (cm)	Rooting %	Root length (cm)
MS <sub>0</sub>	18.27	5.7	1.3	11.2	0.82
BAP					
2.22	49.09	18.36	4.2	33.5	3.2
3.11	72.87	34.21	4.9	42.2	4.8
4.4	<b>95.15</b>	<b>46.84</b>	<b>6.5</b>	<b>55.1</b>	<b>5.3</b>
6.6	77.42	38.21	6.1	44.9	4.9
Kinetin					
2.3	41.11	15.18	4.0	23.2	3.1
3.2	78.61	29.86	5.3	44.8	3.3
4.6	<b>89.27</b>	<b>41.24</b>	<b>5.7</b>	<b>45.9</b>	<b>5.1</b>
6.9	81.24	37.04	5.4	44.4	4.6
BAP + NAA					
4.4 + 0.53	<b>93.48</b>	<b>43.21</b>	<b>6.2</b>	<b>67.1</b>	<b>5.5</b>
4.4 + 1.59	92.29	40.28	6.0	45.9	5.3
4.4 + 2.65	90.89	40.00	6.1	43.8	5.1
Kinetin + NAA					
4.6 + 0.5	87.08	39.89	5.5	42.5	5.3
4.6 + 1.5	88.00	38.66	5.3	43.6	5.0
4.6 + 2.65	88.23	38.22	5.0	46.7	5.2

**Fig.1: Micropropagation of *Rhynchosyilis retusa*:**

**a.** Germination of seeds in  $\frac{1}{2}$  MS medium after 4-months of inoculation and development of protocorm, **b.** Development of protocorm like bodies (PLBs) in MS medium enriched with BAP (4.4  $\mu\text{M}$ ) after 4-weeks of incubation, **c & d.** Development of shoots from PLBs in MS medium enriched with BAP (4.4  $\mu\text{M}$ ), **e & f.** Proliferation of shoots in MS medium enriched with BAP (4.4  $\mu\text{M}$ ) and AC after 8-weeks of incubation, **g & h.** Plantlet with fully developed shoots and roots, **i.** Plantlets shifted for hardening into plastic cups containing coal blocks covered with thin layer of cocopeat and maintained in glass house.





- Seeds of *Zanthoxylum armatum*, *Trachycarpus takil* and *Mezotropis pellita* were collected from the wild and experimental botanical Garden.
- Seeds of the above mentioned species were inoculated onto basal MS medium for the in vitro germination.
- Seeds of *Zanthoxylum armatum* and *Mezotropis pellita* were also sown in soil for the ex-vitro germination assessment.
- Shoot tip and nodal segment explants of *Dendrobium crepidatum*, *Magnolia kisopa* and *Zanthoxylum armatum* were inoculated into MS medium supplemented with different concentration of plant growth regulators.

- Seeds of *Zanthoxylum armatum* were inoculated into MS medium and observed very poor seed germination.
- 27% seed germination was observed in soil.



**PROJECT 2: NMHS-LG PROJECT**

**Conservation of Threatened Plants in Indian Himalayan Region: Recovery and Capacity Building**



- A. *Acer oblongum* Wall. ex DC.
- B. *Aconitum heterophyllum* Wall. ex Royle
- C. *Gentiana kurroo* Royle
- D. *Jasminum parkeri* Dunn
- E. *Indopiptadenia oudhensis* (Brandis) Brenan
- F. *Magnolia kisopa*
- G. *Sophora mollis* (Royle) Baker
- H. *Prunus cerasoides* Buch.-Ham. ex D. Don
- I. *Mahonia jaunsarensis* Ahrendt
- J. *Stereospermum suaveolens* (Roxb.) DC.
- K. *Phlomis superba* (Royle ex Benth.) Kamelin & Makhm.
- L. *Pittosporum eriocarpum* Royle

**Macropropagation of threatened species in Nursery**



**Habitat Rehabilitation under the  
Species Recovery Programme  
(Ex-situ to In-situ)**





***Gentiana kurroo***: Planted 1000 saplings at Khadamba, Dev Van, Chakrata & Bhadraj (Type locality), Mussoorie.

**Followup tour: reported 45% survival**



***Jasminum perkeri***: Extend the distribution of sp. Planted 200 saplings at Dev Van, Chakrata & Cloud End Mussoorie. **reported 42% survival**





*Indopiptadenia oudhensis*: 500 saplings at Raipur Range, Dehradun.

#### PAPER PUBLISHED/COMMUNICATED

- **G.S. Panwar** and Bhavana Joshi. 2020. Micropropagation of *Tricholepis roylei* Hook.f.- a point endemic species of the Western Himalaya. *J of Genetic Engineering and Biotechnology*. 18(40). 1-8. <https://doi.org/10.1186/s43141-020-00051-9> (IF-1.9)
- A. Bhandari, H. Singh, A. Srivastava, P. Kumar, **G.S. Panwar** and A.A. Mao. 2021. *In-vitro* propagation and cytological analysis of *Sophora mollis* Royle: an endangered medicinal shrub. *J. of Genetic Engineering and Biotechnology*. 19(40). <https://doi.org/10.1186/s43141-021-00140-3>. (IF-1.9)
- **G.S. Panwar**, B. Joshi and S.K. Singh. 2021. *Is Tricholepis roylei* Hook. f. can be saved through species specific recovery programme? *Indian forester*. (Accepted)
- A. Srivastava, H. Singh, A. Bhandari, P. Kumar, **G.S. Panwar** and A.A. Mao. 2021. Ornamental potential of *Gentiana kurroo* could be a boon for its survival: a critically endangered species. *Indian forester*. (Accepted)

- A. P. Mishra, A. Srivastava, A. Bhandari, P. Kumar, **G. S. Panwar** and A. A. Mao. Site suitability analysis for the critically endangered *Aconitum heterophyllum* in Alpine regions of Uttarakhand using Analytic Hierarchy Process. *J. of Asia Pacific Biodiversity*. (Communicated)

**THANK YOU**