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

PROGRESS REPORT

October, 2018 – March, 2019

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By

BOTANICAL SURVEY OF INDIA

KOLKATA

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Title of the Project

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

Objectives of the Project

1. To develop species recovery mechanism for 50 targeted species (50 species will be selected out the list of 72 species after consulting with local forest departments).
2. Develop germplasm banks, mass multiplications (through micro- and macro-propagation) and rehabilitate in conversation priority areas across the Himalayan landscape.
3. Assessment of genetic variability in the populations existing in different ecological conditions using appropriate markers.
4. Chemical profiling of medicinally important species
5. Developing geo-referenced database and mapping them.
6. To develop management strategies for long-term conservation of those species through community engagement.
7. To enhance capacities of different stakeholders (including Forest & Wildlife department staff, local Institutions/ colleges, local NGOs and local communities regarding monitoring and sustainable utilisation of threatened flora in the IHR through capacity building programs and use of modern science, technological tools and approaches for livelihood generation.

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PROGRESS MADE

(October 2018 - March 2019)

1. Tissue culture work progress (plant-wise)

1.1. *Rhododendron wattii* Cowan

Methodology

Plant material and ex-plant preparation

Shoots of the *in vitro* raised cultures of *Rhododendron watti* from tissue culture laboratory of BSI, ERC were taken as starter plant material for *in vitro* multiplication.

Media preparation and culture conditions

Shoots were revived and routinely sub-cultured on plain Murashige and Skoog (MS) media with 3% sucrose + 0.1% Activated Charcoal (AC). The medium was solidified with 0.8% plant tissue grade agar; pH of the medium was adjusted to 5.8 before autoclaving at 121°C at 1.05kg/cm2 pressure for 20mins. To study the effect of various auxins on the induction of rooting in these cultures three growth regulators namely Indole acetic acid (IAA), α-Napthalene acetic acid (NAA) and Indole butyric acid (IBA) were supplemented individually in the medium. All the cultures were incubated at controlled temperature of 25±2ºC and kept under culture conditions of 14h photoperiod and photosynthetic photon flux of

60µmol$m^{-2}s^{-1}$provided by cool-white fluorescent lamps. The survival percentage and the response of the plants are regularly monitored and recorded.

Results

Survival percentage of about 40% was recorded (Fig.1.1). Contamination in few of the cultures was also observed which could be due to old cultures and culture handling issues. Difficulties in rooting induction of *Rhododendron watti* was earlier reported, therefore, standardisation of protocol for rooting experiments in MS medium was studied. After 4 weeks of rooting experiments, multiple shooting was observed in some of the IBA treated medium (Fig.1.1) while no observable results have been recorded in the rest of the experiments.

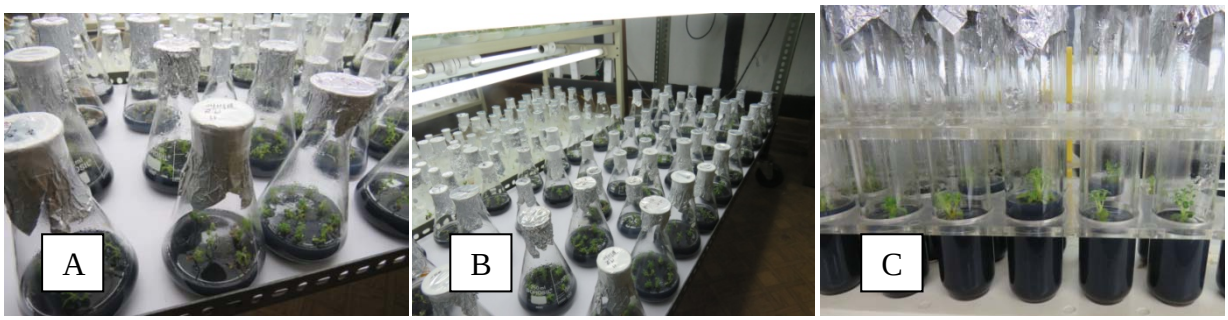


Figure 1.1: *Rhododendron watti*. A) Close up of culture, B) Culture shelf and C) IBA treated cultures

1.2 *Nepenthes khasiana* Hook. f.

Plant material and ex-plant preparation

Shoots of the *in vitro* raised cultures of *Nepenthes khasiana* from tissue culture laboratory of BSI, ERC and seed pods of *N. khasiana* collected from BSI Botanical Garden, Barapani were taken as starter plant material for *in vitro* multiplication.

Media preparation and culture conditions

Nodal sections were sub-cultured on fresh ½ MS medium incorporated with 6-Benzylaminopurine (BAP) @ 2.0mg/l + Kinetin@2.5mg/l for induction of multiple shooting. The medium was solidified with 0.8% plant tissue grade agar; pH of the medium was

adjusted to 5.6 before autoclaving at 121°C at 1.05 kg/cm2 pressure for 20mins. The same medium without growth regulators were used for inoculation of seeds. All the cultures were incubated at culture conditions described above. The survival percentage and the response of the plants are regularly monitored and recorded.

Protocol for seed sterilisation

Mature seed capsules were collected, the seeds were removed and carried inside the Laminar Hood. The seeds were surface sterilised with 10% Sodium Hypochlorite with 10% Sodium hypochlorite with 2-3 drops of Tween-20 for 10 mins, filtered on filter paper using a funnel and followed by rinsing with sterile water till the filtrate is clean and clear. The sterilised seeds were then inoculated on ½ MS plain media. All the cultures were incubated in light inside the culture room at controlled conditions described above. The survival percentage and the response of the plants are regularly monitored and recorded.

Results

After 4 weeks of shoots inoculated on ½ MS medium with BAP+Kn, the shoots have started responding and recorded a percentage response in shooting of about ~60% (Fig. 1.2). While, seed germination on plain ½ MS media, an approximate percentage survivability of 50% was recorded.

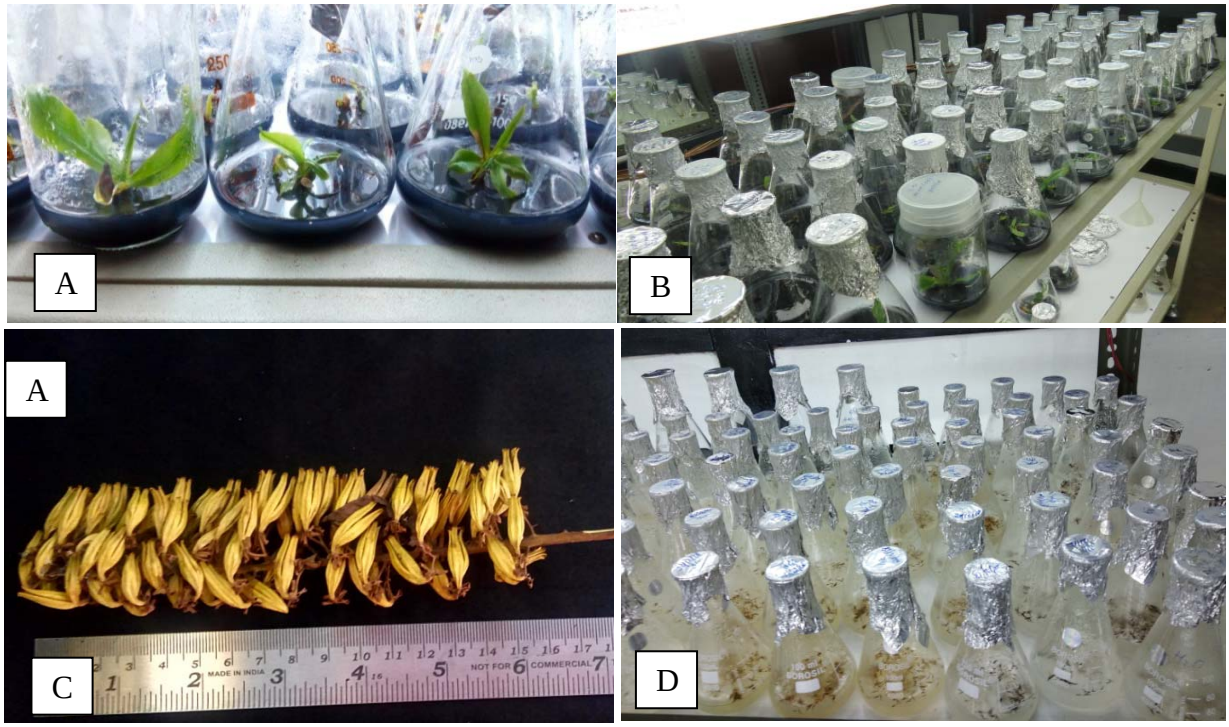


Figure 1.2: *Nepenthes khasiana*. A) Close up view of nodal culture generating new shoot, B) Shoots in culture, C) Seeds and D) Inoculated seeds in culture.

1.3 *Cymbidium tigrinum* C.S.P.Parish ex Hook.

Plant material and explant preparation

Protocorms of *in vitro* raised cultures of *Cymbidium tigrinum* from tissue culture laboratory of BSI, ERC were taken as starter plant material for *in vitro* multiplication.

Media preparation and culture conditions

Protocorms of *C. tigrinum* were routinely subcultured on plain MS medium for multiplying and generation of enough cultures for further scale-up these cultures with incorporation of growth regulators. The medium was fortified with 3% sucrose, solidified with 0.8% plant tissue grade agar; pH of the medium was adjusted to 5.8 before autoclaving at 121°C at 1.05 kg/cm2 pressure for 20mins. Cultures were kept in culture conditions same as described earlier. The survival percentage and the response of the plants are regularly monitored and recorded.

Hardening of plantlets

Tissue culture raised plantlets with well developed roots and shoots were transferred for acclimatisation in net house. The plantlets were hardened in coco peat with a layer of sphagnum moss on top. The hardened plants were initially covered with a polythene bag for maintaining adequate humidity. Monitoring and watering was done regularly or as and when required.

Results

After 6 weeks of protocorms in culture 86% survival rate of the inoculated cultures were recorded. Shoot regeneration of the inoculated explants was recorded with an average of 1.3 shoots/protocorms (Fig. 1.3). In addition, formation of protocorm like bodies (PLBs) were also observed. The hardened plants showed ~43% survival rate after 1 month of field transfer as of now and is under observation.

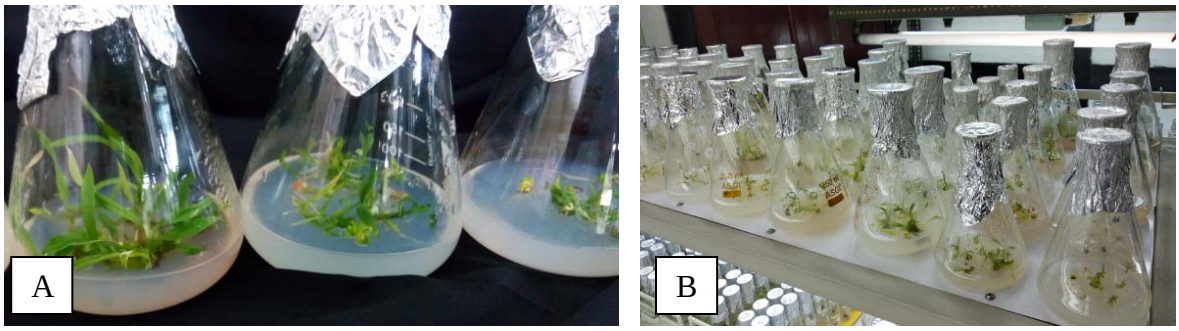


Figure 1.3: *Cymbidium tigrinum*. A) Shoots regeneration from protocorms and B) Culture shelf.

1.4 *Cymbidium eburneum* Lindl.

Plant material and explant preparation

Seed capsule of *Cymbidium eburneum* collected from the orchidarium of BSI, ERC were taken as starter plant material for *in vitro* multiplication.

Media preparation and culture conditions

Seeds of *C. eburneum* were cultured on plain MS medium containing 3% sucrose, solidified with 0.8% plant tissue grade agar; pH of the medium was adjusted to 5.8 before autoclaving at 121°C at 1.05kg/cm2 pressure for 20mins. The cultures were kept in dark condition initially for a week and later on kept in light culture conditions described above. The survival percentage and the response of the plants are regularly monitored and recorded.

Seed capsule sterilisation protocol

The capsule was first washed with clean water, blot dried and taken inside the Laminar Hood. It was flame sterilised by dipping in 70% alcohol and flamed sterilised; care was taken not to expose the capsule to too much heat. The seed capsule was slit open with a scalpel blade longitudinally, seed exposed and inoculated on plain MS medium.

Results

The cultures are under observation for further response. So far, no contamination was observed in the inoculated cultures (Fig.1.4).

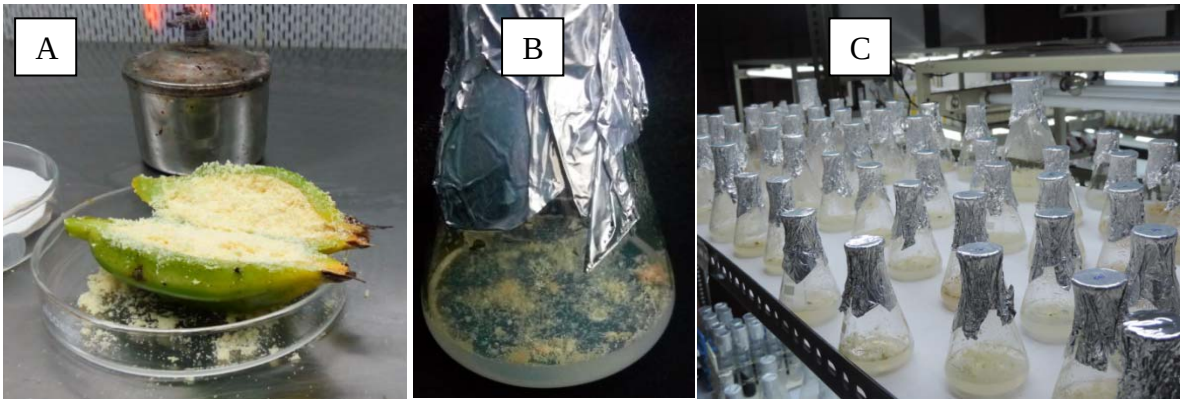


Figure 1.4: *Cymbidium eburneum.* A) Open seed capsule, B) Close up of Inoculated seeds and C) Seeds in culture

1.5 *Cymbidium whiteae* King & Pantl.

Plant material and explant preparation

Protocorms of *in vitro* raised cultures of *Cymbidium whiteae* from tissue culture laboratory of BSI, ERC were taken as starter plant material for *in vitro* multiplication.

Media preparation and culture conditions

Protocorms of *C. Whiteae* were routinely sub-cultured on plain MS medium for multiplication with 3% sucrose, solidified with 0.8% plant tissue grade agar; pH of the medium was adjusted to 5.8 before autoclaving at 121°C at 1.05 kg/cm2 pressure for 20mins. Cultures were kept in conditions same as described earlier. The survival percentage and the response of the plants are regularly monitored and recorded.

Results

After 1 month in culture, nearly 74% shooting was observed in the inoculated *C. Whiteae* protocroms. The rest multiplied into PLBs (Fig. 1.5).

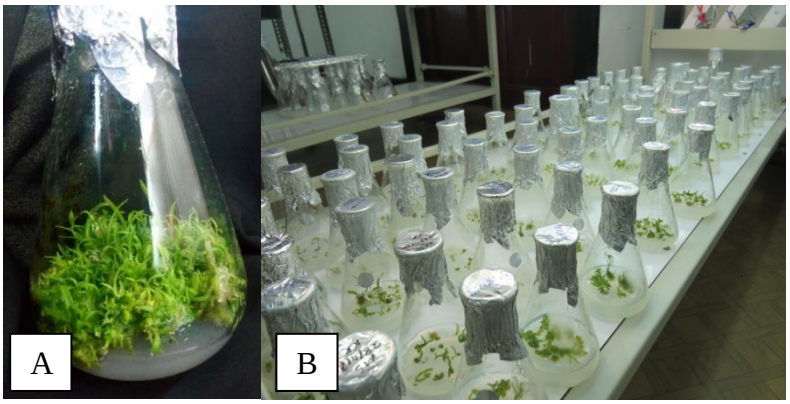


Figure 1.5: *Cymbidium whiteae*. A) Old cultures and B) Subcultured cultures

1.6 *Paris polyphylla* Sm.

Plant material and explant preparation

Seed capsule and rhizomes collected from the BSI, ERC garden were taken as plant material. Seeds and rhizome sections as explants were utilised for micropropagation studies.

Media preparation and culture conditions

Seeds were inoculated on ½ strength MS medium with 3% sucrose and solidified with 0.8% plant tissue grade agar. For rhizome sections, the culture medium was supplemented with 0.5 mg/lBAP and 2-Isopentyl (2iP) in combination. The pH of the medium was adjusted to

5.8 before autoclaving at 121°C at 1.05kg/cm^2 pressure for 15min. All the cultures were initially incubated in dark inside the culture room at controlled temperature of 25±2ºC. The cultures were routinely monitored for occurrence of responses.

Seeds and rhizomes sterilisation protocol

Seed capsule were collected and washed with fungicide for about 10 minutes after which it was washed and kept under running tap water for 20 mins. Blot dried blot dried and taken inside the Laminar Hood; surface sterilised with 15%sodium hypochlorite for 15 mins followed by 3-4 times wash with sterile water. Seeds were removed and re-sterilised 0.1% mercuric chloride for 5 mins followed by rinsing. After which seed coat was removed and kept soaked overnight for 48 hours. Zygotic embryos were removed and inoculated on media.

Rhizomes were sterilised with 15% sodium hypochlorite for 10 mins and washed with sterile water. Rhizomes sections about 0.5-1.0mm thick sections weighing about 0.15g were made and inoculated onto the medium.

Results

The inoculated zygotic embryos after 3-4 weeks in culture started swelling and showed the initial stages of leaf emergence with a record of 60% percentage response was observed (Fig. 1.6). For rhizome sections experiments, the set up was kept in dark and still under observation.

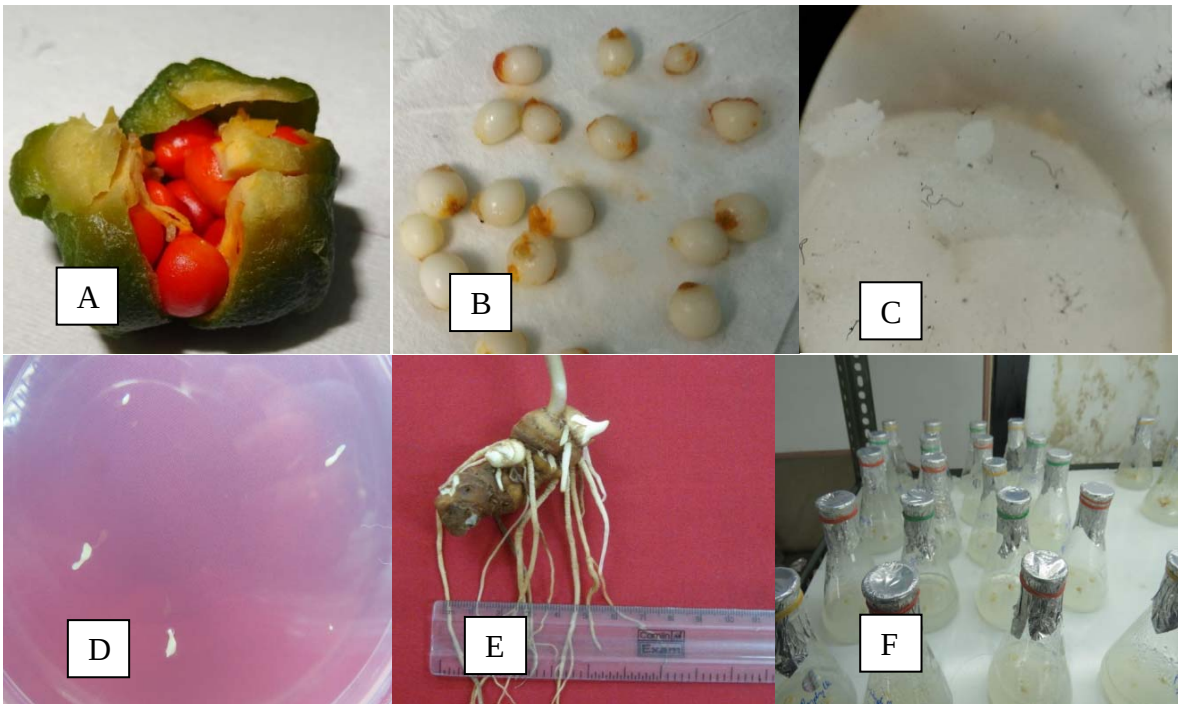
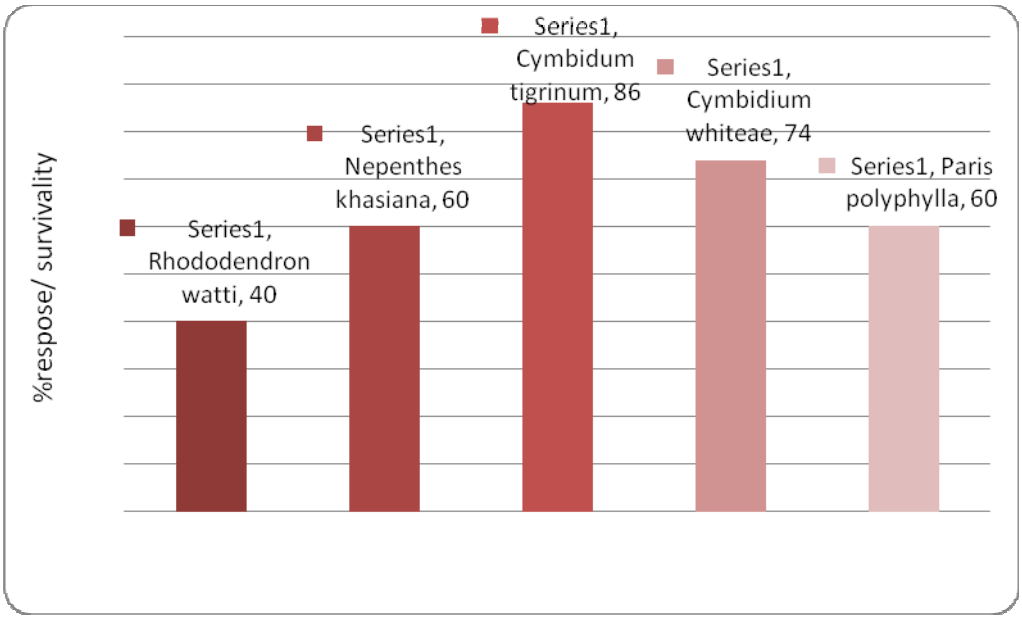


Figure 1.6: *Paris polyphylla.* A) Seed capsule, B) Decoated seeds, C) Zygotic embryo dissection, D) Inoculated embryos showing response, E) Rhizomes and F) Rhizome sections in culture.



Bar diagram showing the percentage response/ survival rate of the plants in culture

1.7 *Calanthe biloba* Lindl.

Plant material and explant preparation

Seed capsule of *Calanthe biloba* collected from the orchidarium of BSI, ERC, Shillong were taken as starter plant material for *in vitro* multiplication.

Media preparation and culture conditions

Seeds of *Calanthe biloba* were cultured on plain MS medium. Culture conditions were same as discussed for *C. eburneum* cultures.

Seed capsule sterilisation protocol

Same as described above for *C. eburneum.*

Results

The cultures are still under observation; no contamination has been observed so far (Fig.1.9).

Figure 1.9: *Calanthe biloba* inoculated seeds in culture condition.

1.8 *Arundina graminifolia* (D. Don.) Hochr.

Plant material and explant preparation

Seeds of *A. graminifolia* were collected from the BSI, ERC, Shillong and the same was taken as explant for futher *in vitro* propagation.

Media preparation and culture conditions

Seeds of *A. graminifolia* were cultured on plain MS medium. Culture conditions were same as discussed for *C. eburneum* cultures.

Seed sterilisation protocol

Same as described above for *C. eburneum.*

Results

The cultures are still under observation; no contamination has been observed so far.

2. Molecular Diversity work progress:

Genomic DNA extraction protocols has been standardised for nine selected plants. PCR conditions using primers ITS2 and ITS4 have also been standardised in the selected three plants. The selected plants are listed as under:

1. *Rhododendron wattii*
2. *Phiaus flavus*
3. *Cephalantheropsis longipes*
4. *Calanthe biloba*
5. *Cymbidium elegans*
6. *Asplenium nagalandicum*
7. *Cymbidium whiteae*
8. *Nepenthes khasiana*
9. *Areca triandra*
10. *Phaius longipes*
11. *Deplezium nagalandicum*

Methodology

Sample collection

For the purpose of genomic DNA extraction, young, uninfected leaves samples were collected from *Phaius flavus*, *Cephalantheropsis longipes*, *Cymbidium elegans*, *Nepenthes khasiana*, *Calanthe biloba* and *Asplenium nagalandicum* growing in the garden of BSI, ERC, Shillong. Leaf samples of *Areca triandra* were collected from the Pynursla, East Khasi Hills District, Meghalaya while leaf samples of *Rhododendron watti* and *Cymbidium whiteae* were collected from *in vitro* plants from tissue culture laboratory of BSI, ERC, Shillong. Leaves were collected and cleaned first with water and then with 70% alcohol. Fresh leaf are always preferred, however, field collected plants can be cleaned and kept stored at -20º until use.

DNA Extraction protocol

CTAB method of DNA extraction described by Doyle and Doyle, 1990 were followed for all the plant species with few minor modifications. The steps for extraction of DNA followed are described below:

1. Finely crushed leaf powder was transferred to 50ml falcon tubes, containing 10ml of extraction buffer and kept in water bath at 60ºC. To the solution, 20µl of β-mercaptoethanol was added prior to incubation.
2. The tubes were kept incubated at 60ºC for 1hr with occasional mixing of the tubes to facilitate even mixing of the reagents.
3. Equal volume of freshly prepared chloroform: isoamyl alcohol (24:1) was added in the tubes and allowed to mix for 10 min.
4. The tubes were centrifuged at 6000-8000rpm in centrifuge for 10 min at RT.
5. DNA was precipitated by adding 2/3rd volume of pre-chilled isopropanol.
6. Precipitated DNA was pelleted out by centrifuging at 4000rpm for 5min, collected and air-dried to remove the alcohol.
7. DNA was dissolved in 1ml of 1X Tris-EDTA (TE) buffer.
8. The dissolved DNA was transferred to 15 ml falcon tube; to it 3µl of RNAse (10µg/ml) was added and kept for incubation at 37°C for 45min.
9. After incubation, equal volume (1ml) of solution containing phenol and chloroform: isoamyl alcohol was added and allowed to mix for 10min.
10. The tubes were centrifuged at 4000-6000rpm for 10min at RT.
11. The aqueous phase were aspirated in another fresh tube, and 1/10th of 3M sodium acetate was added along with 1/4th volume of pre-chilled ethanol.
12. DNA was peletted out by centrifuging at 4000rpm for 5 min.
13. DNA collected was air-dried, dissolved in TE buffer and kept stored at -20ºC for long-term use.

Quality check of the isolated DNA

The quality of isolated DNA was checked in 0.8% agarose gel for all the samples extracted and visualized in Gel Documentation System and photographed.

2.3 Optimization of PCR protocol for ITS region

Different genomic DNA concentrations, $MgCl_2$ concentrations and Taq DNA polymerase were tested in multiple combinatorial approach to precisely identify the most preferable PCR optimization conditions along with annealing temperature gradient setting from 50ºC-60ºC.

ITS2 and ITS4 were used as forward and reverse primer

Primer sequence for ITS2-S2F: 5'ATGCGATACTTGGTGTGAAT3' ' '

Primer sequence for ITS4: 5'TCCTCCGCTTATTGATATGC3'

Results

All extracted DNA were found to be of good quality without RNA contamination. Positive PCR amplification was observed for the given region for three plants namely *Cymbidium whiteae*, *Phaius flavus* and *Cephalantheropsis longipes.* The amplicon size ranged between 300-400bp.

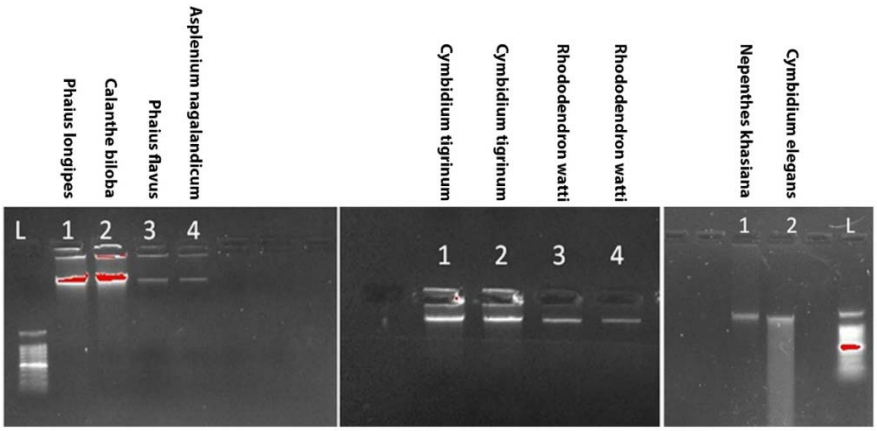


Fig.2.1: Genomic DNA extracted from selected plants

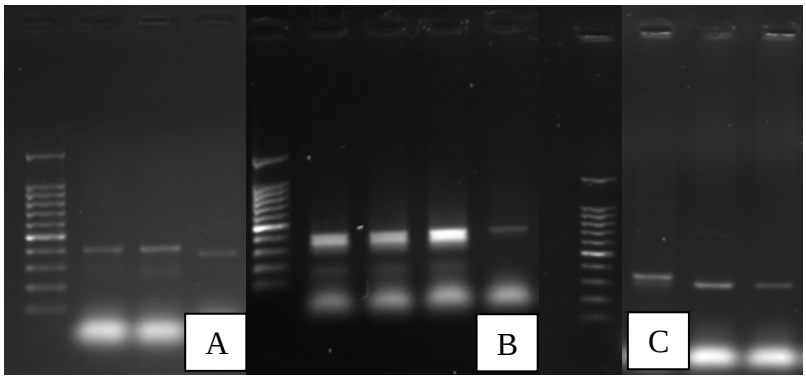


Fig. 2.2: PCR amplification using primers targeting ITS region in A) *Cymbidium whiteae* B) *Phaiuslongipes* and C) *Phaius flavus*

2.4 Optimization of PCR protocol for mitochondrial (matK) and ribosomal chloroplast (rbcL) region.

Plants DNA used were:

1. *Phaius flavus* (Blume) Lindl.
2. *Phaius longipes* (Hook.f.) Holttum
3. *Calanthe biloba* Lindl.
4. *Cymbidium elegans* Lindl.
5. *Cymbidium whiteae* King & Pantl.

Optimization of PCR protocol

Different genomic DNA concentrations, MgCl2 concentrations, Go Taq buffer and Taq DNA polymerase were tested in multiple combinatorial approaches to precisely identify the most preferable PCR optimization conditions along with annealing temperature gradient.

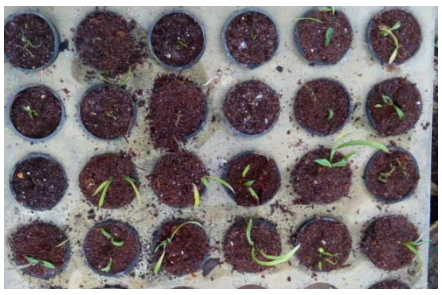
3. Macro propagation/hardening undertaken at BSI-ERC garden

3.1 *Nepenthes khasiana* Hook. f.

Seed germination of *Nepenthes khasiana* in glass house has been initiated (Fig.3.1).The seeds were sprinkled on compost bed of moistened coco peat and layered with sphagnum moss on top. To maintain high humidity the set up was covered with a plastic sheet or polythene sheet. It is watered timely and routinely monitored. No response observed yet.

Figure 3.1: Seed germination of *Nepenthes khasiana* in glass house.

3.2 *Cymbidium tigrinum* C.S.P. Parish ex Hook.



Hardening of tissue cultured raised seedlings of *Cymbidium tigrinum* initiated (Fig. 3.2)

Figure 3.2: Hardening of *Cymbidium tigrinum*

3.3 *Ilex khasiana* Purkay.

Seeds of *Ilex khasiana* collected from Upper Shillong, Meghalaya have been used to study the effect of germinating medium and effect of temperature on the germination of the seeds. Four germination medium viz., germination paper, humus rich soil, coco peat and soil

were tested in four temperature condition like 15ºC, 19ºC, 25ºC and room temperature. Three replicates of 100 seeds each have been applied (Fig.3.3). Watering is done regularly and timely monitored.

B

Figure 3.3: *Ilex khasiana.* A) Seed size measurement and B) Seed germination studies on different germination media.

3.4 *Areca triandra* Roxb. ex Buch.-Ham.

Seeds of *Areca traindra* were collected from Mawtongreng and Thangkyrta village in East Khasi Hills District, Meghalaya. About 800 seeds and about 400 saplings were collected andmaintained at BSI-ERC garden, Shillong. Seeds were also planted at BSI ERC Garden (Fig.3.4)

Figure 3.4: *Areca triandra*. A) Plot for seed plantation, B) A single sapling and C) Saplings in the net house.

3.5 *Prunus nepaulensis* Hook.f.

About 3000 germinated saplings of *Prunus nepaulensis* have been transferred from the garden into polythene bags and are ready for field transfer (Fig. 3.5).

Figure 3.5: *Prunus nepaulensis* saplings in the net house.

3.6 *Prunus* sp.

Seeds and seedlings of two different species of *Prunus* (i) species 1 from Nagaland (250 seeds) and (ii) species 2 from Laitmawsiang, Meghalaya (720 saplings) were collected. The seeds have been sown in the garden and the saplings have been planted on polythene bags. Both are maintained at the garden of the BSI-ERC, Shillong (Figure 3.6).

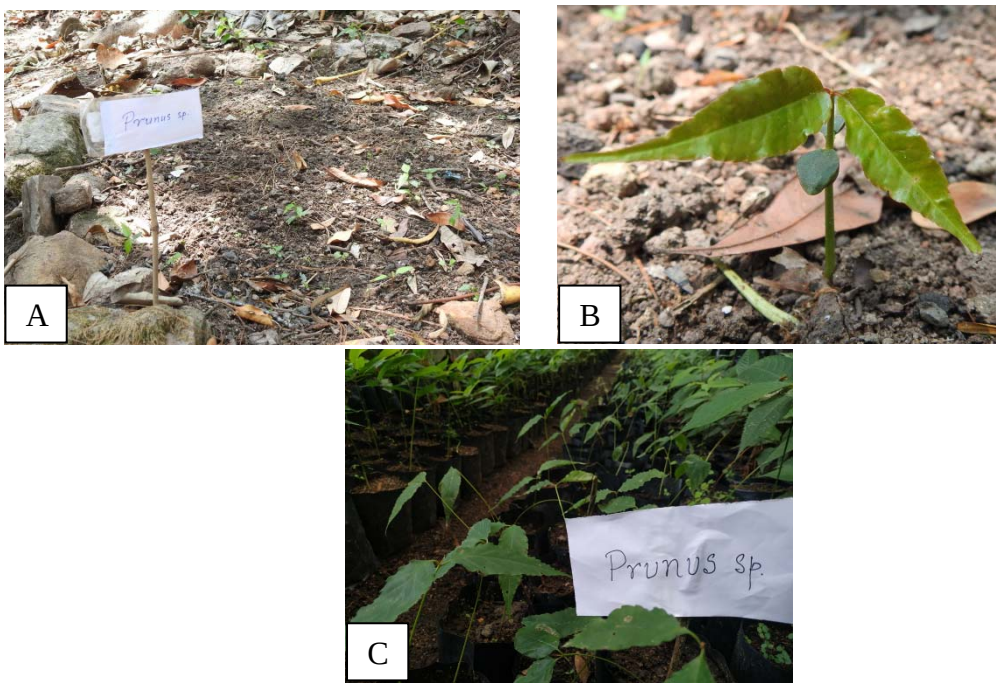


Figure 3.6: *Prunus* sp. A) Seed bed and B) Germinated seedling from Nagaland, C) Saplings from Meghalaya.

3.7 *Prunus cerasoides* Buch.-Ham.ex D.Don

About 1000 seeds of *Prunus cerasoides* were collected from the BSI-ERC campus, Shillong. The seeds were sown and maintained at the garden of the BSI-ERC, Shillong (Fig.3.7).

Figure 3.7: Seed bed of *Prunus cerasoides.*

3.8 *Gynocardia odorata* R.Br

Seeds of *Gynocardia odorata,* around 5000 in number were collected from Laitmawsiang, Meghalaya and sown in the garden of BSI-ERC, Shillong in a span of three months (January-

March). Around 117 seeds sown in January have germinated and transferred in polythene bags. The germinated seedlings are kept maintained in the net house (Fig. 3.8).

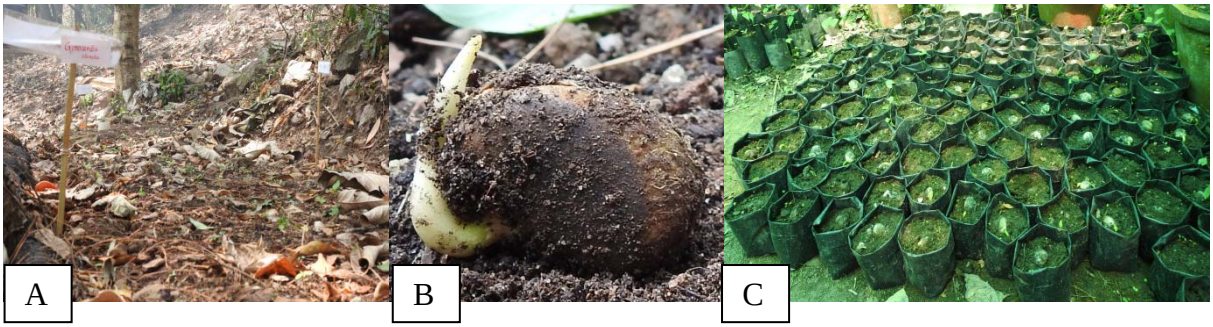


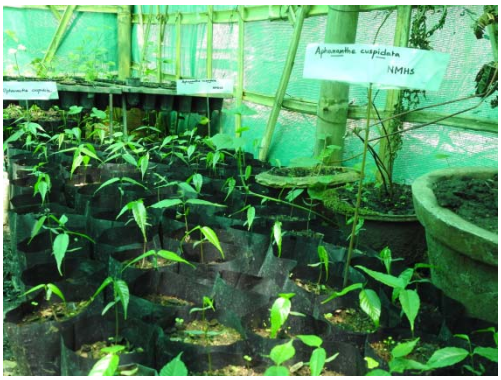
Figure 3.8: *Gynocardia odorata.* A) Seed bed, B) Germinated seedling and C) Seedlings transferred to polythene bags.

3.9 *Garcinia xanthochymus* Hook. F. ex. Anderson

About 3400 seeds of *Garcinia xanthochymus* seeds were collected from Laitmawsiang, Meghalaya were sown in the garden of BSI-ERC, Shillong in the month of January and Ferbruary, 2019. Till date the seeds have not yet germinated (Fig.3.7).

Figure 3.7: Seed bed of *Garcinia xanthochymus.*

3.10 *Aphananthe cuspidata* (Blume) Planch.



Aphananthe cuspidata seeds of about 400 in number were sown in soil on germination trays (250 nos) inside glass house and 150 seeds in garden of BSI-ERC, Shillong. A total 113 seeds from the germination trays have germinated which have been transferred into polythene bags and kept inside net house (Figure 3.8).

Figure 3.8: Saplings of *Aphananthe cuspidata* in polythene bags.

3.11 *Gnetum* sp.

Seeds of *Gnetum* sp. (280 nos) collected from Nagaland have been sown at the garden of BSI-ERC, Shillong. No germination has been observed till date (Figure 3.9).

Figure 3.9: Seed bed of *Gnetum* sp.

3.12 *Rhododendron arboreum* Sm.

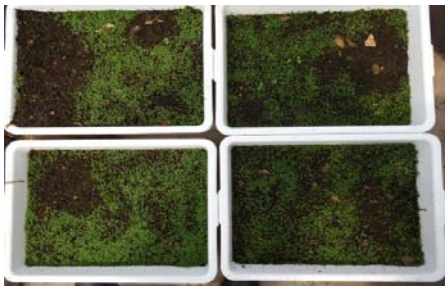
Seeds of *Rhododendron arboreum* were collected from the trees grown at SBI-ERC, Shillong campus. The seeds were sown in germination trays containing garden soil which was kept inside glass house. After 20 days of sowing, the seeds have started germinating (Figure 3.9).

However, the seeds are very minute and the germination is at the initial stages, so the data of the number of germinated seedlings have not been reported as of now.

Figure 3.9: Germinated seedlings of *Rhododendron arboretum* (arrows showing the seedlings).

3.13 *Betula alnoides* Buch.-Ham. ex D.Don

Seeds of *Betula alnoides* were collected from SBI-ERC, Shillong campus were sown in soil on 7 germination trays (1.7x1.2ft). After 20 days of sowing the seeds have started germinating. Since, the young germinated seedlings are very delicate, hence they will be transferred after they attain a healthy transferrable stage (Fig. 3.11).



A

B

Figure 3.11: *Betula alnoides*. A) Germinating seeds on germination trays and B) Close –up view of the seedlings.

3.14 *Brucea mollis* Wall. ex Kurz

Around 125 seeds of *Brucea mollis* collected from the garden of BSI-ERC, Shillong were sown in 1 germination tray on soil and maintained at the glass house. No response yet and is still under observation.

3.15 *Mahonia napaulensis* DC.

Seeds of *Mahonia napaulensis* were collected from SBI-ERC, Shillong campus. Around 15607 seeds were sown in 2 germination trays on soil and kept inside glass house. Germination has been observed after 15 days of sowing (Figure 3.13). The germinated seedlings are ready to be transferred to polythene bags for further growth and development.

Figure 3.13: *Mahonia napaulensis* germinated seedlings in germination trays.

3.16 *Quercus griffithii* Hook.f.&Thomson ex Miq.

Around 518 saplings of *Quercus griffithii* collected from the garden of BSI-ERC, Shillong has been transferred to polythene bags and maintained at the garden.

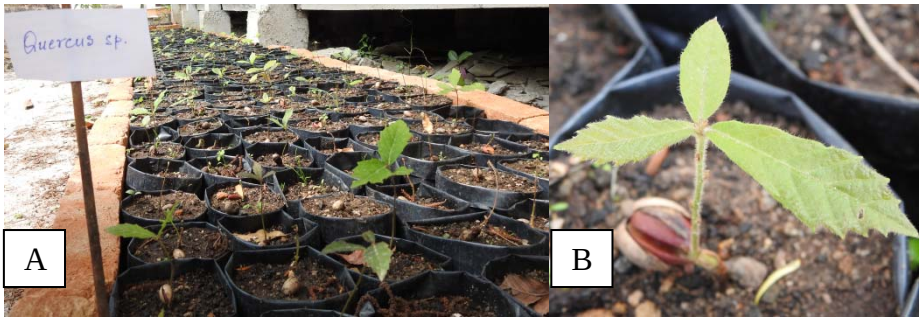


Figure 3.14: *Quercus griffithii*. A) Seedlings in polythene bags and B) Single sapling.

3.17: *Oroxylum indicum* (L.) Kurz.

Around 150 seeds of *O. indicum* were transferred to seed bed and maintained at the BSI, ERC, Shillong garden for germination.

3.18 *Betula alnoides* Buch-Ham. *ex* D. Don.

Around 100 seeds of *B. alnoides* were transferred for seed germination in the green house.

4. Phytochemical analysis of selected plants

Initial phytochemical screening of the following plants were carried out in selected four plants.

Sl. No.	Plant Name	Plant part used
1	*Illigera grandiflora* (Harnandiaceae)	Leaves
2	*Coptis teeta* (Ranunculaceae)	Leaves
3	*Nepenthes khasiana* (Nepenthaceae)	Leaves
4	*Areca triandra* (Arecaceae)	Leaves

Methodology:

Plant extract preparation:-

The collected plant samples were air-dried in shed at room temperature for 3 weeks, after which they were grinded to a uniform powder. The methanol extracts were prepared by macerating each of the dry powder in methanol and kept at room temperature. The solution obtained was filtered through a Whatmann filter paper No. 1. The filtrate was Vacuum dried (Rotalab, Lab India). Then the crude extract of each plant material was stored at 4 °C.

Qualitative phytochemical analysis:-

Qualitative phytochemical analysis were carried out to detect the presence of the some bioactive groups (alkaloids, tannins, saponins, flavonoids, cardiac glycosides and polyphenols) from extract using different standard methods.

Test for alkaloids: Sample was stirred with aqueous HCl on a steam bath and then filtered. After that filtrate was treated with a few drops of Mayer's reagent and a second portion was treated similarly with Dragendorff reagent.

Test for tannins: Dried extract was stirred with distilled water. This was filtered and ferric chloride ($FeCl_3$) reagent was added to the filtrate.

Test for saponins: Plant extract was shaken with water in a test tube.

Test of flavonoids: Extract solution was added to concentrated HCl and a stiff of pink magnesium.

Test for cardiac glycosides: Glacial acetic acid, $FeCl_3$ and concentrated H_2SO_4 was respectively added into the extract solution.

Test for polyphenols: Plant extract was heated in a water bath. $FeCl_3$ was added to the mixture then followed by the addition of potassium ferrocyanide. The mixture was filtered and the formation of polyphenols.

Test for steroids and triterpenoids:-Extract was added to chloroform along with a few drops of conc. sulphuric acid. The mixture was shaken well and kept aside for some time.

Results

Alkaloids are present in all the plant leaf extracts except *N. khasiana* whereas Tanins are present in *I. grandiflora* and *N. khasiana*. Saponins and cardiac glycosides are absent in the methanolic extract of all leaf extract. Flavonoids and steroids and triterpenoids are present in *I. grandiflora* and *N. khasiana* (Fig. 4.1 – 4.3; Table 1)

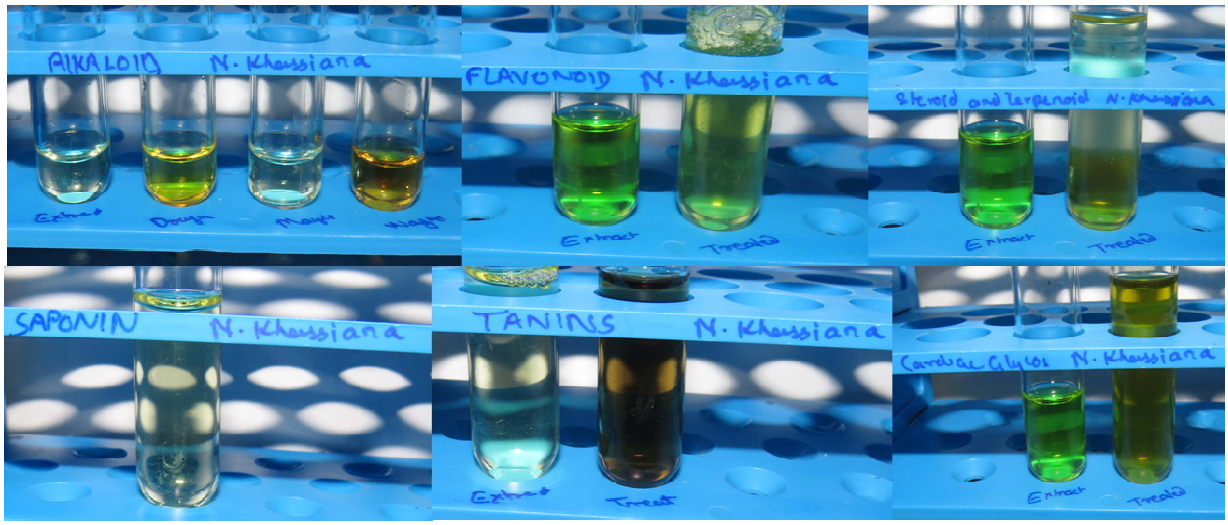


Figure 4.1: Phytochemical screening of *Nepenthes khasiana*.

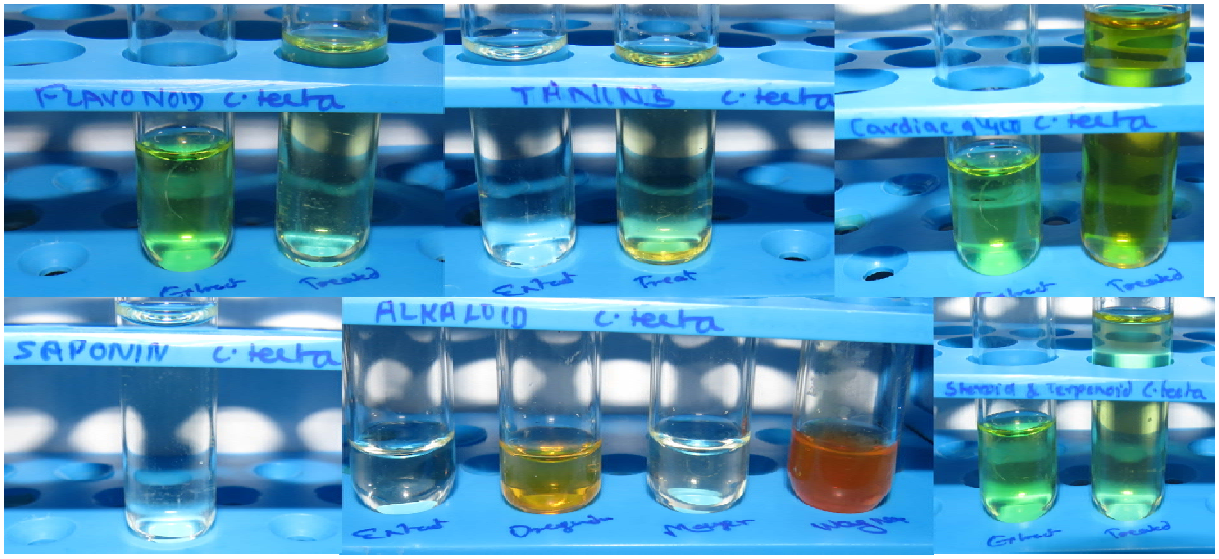


Figure 4.2: Phytochemical screening of *Coptisteeta*.

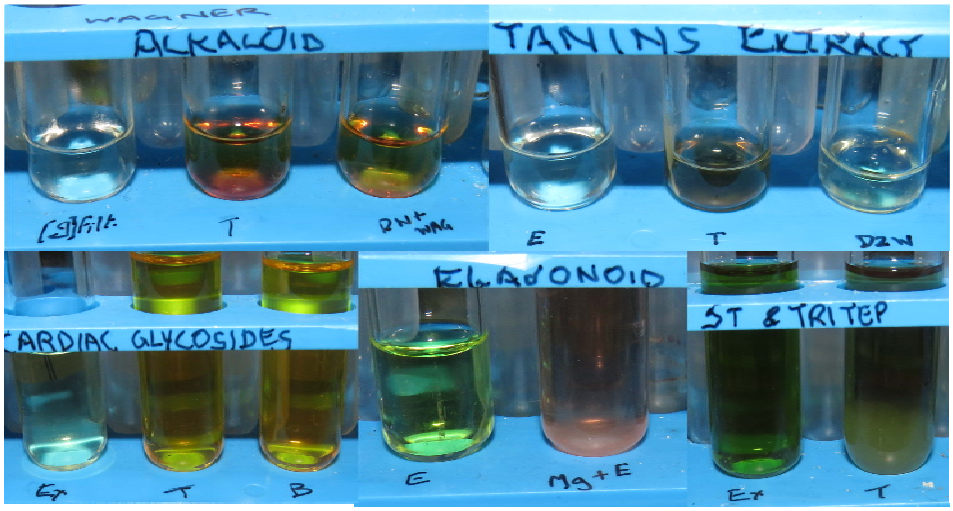


Figure 4.3: Phytochemical screening of *Illigera grandiflora*.

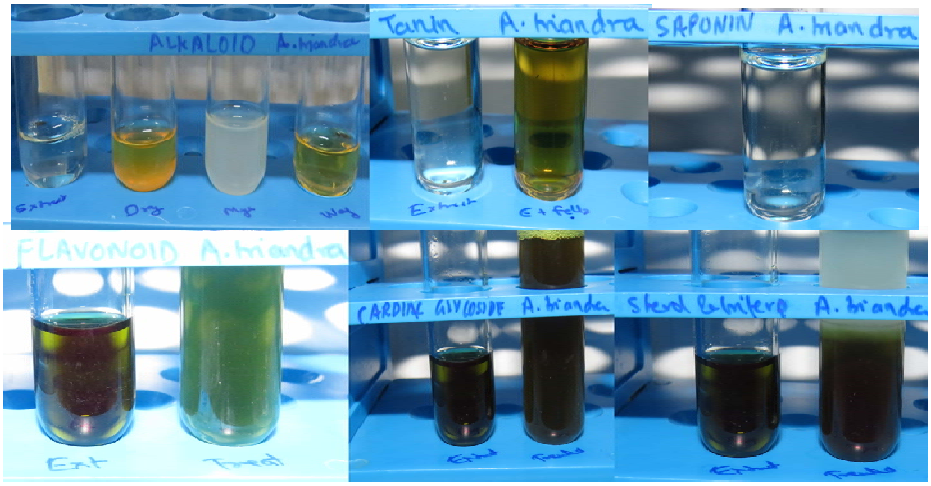


Figure 4.4: Qualitative analysis of *Areca triandra*.

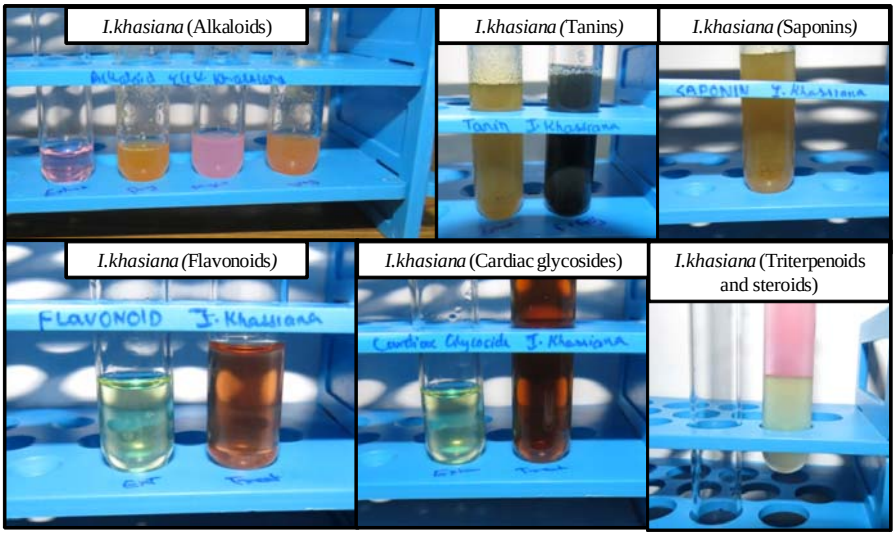


Figure 4.5: Qualitative analysis of *I. khasiana*.

Table 1: Classes of compounds present in the selected plants.

Name of plant	Alkaloids	Tanins	Saponins	Flavonoids	Cardiac glycosides	Steroids & Triterpenoids
I. grandiflora	+	+	-	+	-	-*
C. teeta	+	-	-	-	-*	-
N.khasiana	-	+	-	-	-	+
A. triandra	+	- *	-	-	-	-
I. khasiana Leaves	+	+	+	*	*	-
I. khasiana Fruits	+	+	+	+	-	-

* needs to be performed once again because may be present in small amount.

5. Field Tours

For the purpose of collection of the selected plant materials, field tours were undertaken at various places in and around Shillong during the period.

5.1. Tour to Upper Shillong on 03/01/2019

A local field tour was undertaken to Upper Shillong for the purpose of collecting *Ilex khasiana.* Seeds were collected for germination at BSI ERC Garden.

5.2. Tour was carried out during 07.01.2019 to 08.01.2019 to Kongthong, Thangkarta and Mawtongreng East Khasi Hills District, Meghalaya.

A total of 400 sapling and 800 seeds of *Areca triandra* were collected during the tour.

5.3. A tour was carried out during 17.01.2019 to Mawmluh Forest and Lad Mawphlang East Khasi Hills District, Meghalaya.

Five species of Orchids and one Tutcheria sp. (Theaceae) were collected during the tour.

5.4. A tour was carried out during 01.02.2019 to Tyrshi falls, Jaintia Hills District, Meghalaya.

5.5. A tour was carried out during 18.02.2019 to Mawlyindiar and Laitmawsiang East Khassi Hills, Meghalaya.

5.6. A tour was carried out during 22.02.2019 to Umtyngar and Upper Shillong East Khasi Hills, Meghalaya.

6. GIS Training Conducted

A three day "Hands-on Training in GIS mapping, database management, and spatial analysis" was organised at BSI, ERC Shillong from 23 – 25 January, 2019 with special emphasis on Ecological Niche Modelling of plants. Dr. Dibyendu Adhikari, Research Associate, Department of Botany, NEHU was the resource person during the training. The training was attended by 28 participants including Scientific staffs, Research Associates, Junior Project Fellows of BSI, ERC Shillong and BSI, NRC, Dehradun. At the end of the three day training, a power point presentation on different GIS mapping models was solicited from the participants to assess their knowledge and to comprehend the positive impacts of the training program.

7. Future Plan of Work

1. Micro-propagation and rehabilitation of selected species
2. Field tours for collection of selected species
3. Molecular diversity studies and analysis of selected species
4. Phytochemical screening and analysis of selected species
5. Ecological Niche Modelling for rehabilitation and recovery of selected species
6. Capacity building and awareness workshop to be conducted at selected places

8. Budget

a. Expenditure Information: Combined expenditure for the year 2018-2019

S. No.	Financial Position/Budget Head	Total Amount Received	Expenditure	Balance
1	**Salary**	2418240/-	1120865/-	1297375/-
2	**Travel**	750000/-	37500/-	712500/-
3	**Expendables**	625000/-	625000/-	NIL
4	**Contingency**	428571/-	262936/-	165635/-
5	**Activities**	2571429/-	17000/-	2554429
6	**Equipments**	3616776/-	3579000/-	37776/-
7	**Total Budget**	10410016/-	5642301/-	4767715/-

b. Expenditure details of NMHS project 2018-2019

Heads	Amount Received	Amount Carried forward	Total Budget 2018-19	Expenditure 31-01-2019	Committed Expenditure 31-03-2019	Total Expend 2018-19	Balance
Salary	2418240/-	**NIL**	2418240/-	**740625/-**	**380240/-**	**1120865/-**	**1297375/-**
Travel	750000/-	**NIL**	750000/-	**37500/-**		**37500/-**	**712500/-**
Expendables	625000/-	**NIL**	625000/-	**22341/-**	**602659/-**	**625000/-**	**NIL**
Contingency	428571/-	**NIL**	428571/-	**262936/-**		**262936/-**	**165635/-**
Activities	2571429/-	**NIL**	2571429/-	**17000/-**		**17000/-**	**2554429/-**
Equipments	3616776/-	**NIL**	3616776/-	**NIL**	**3579000/-**	**3579000/-**	**37776/-**
Total Budget	10410016/-	**NIL**	10410016/-	**890282/-**	**4561899/-**	**5642301/-**	**4767715/-**

Botanical Survey of India, Northern Regional Centre, Dehradun

Principal Investigator
Dr. A.A.Mao

Co-Principal Investigator
Dr. Giriraj Singh Panwar
Dr. Puneet Kumar

Research Associates
Dr. Amber Srivastava

Junior Project Fellows
Ms. Aakriti Bhandari
Mr. Harminder Singh

PROGRESS MADE

(October 2018 - March 2019)

QUANTIFIABLE DELIVERABLES

1. Spatial distribution maps to identify areas of occurrence in respective states (UK, H.P. and J&K).

2. Prediction models to reveal potential sites of occurrence for identifying undiscovered populations.

3. Database for delisting species from the red list category through efficient recovery programs and monitoring survival of the species.

4. The development of ecosystem threat indicator status.

5. Development of baseline geospatial and threatened species database focusing endemic/threatened/overexploited and rare species in the respective states (UK, H.P. and J&K).

6. Capacity building of local stakeholders in selected states (UK, H.P. and J&K).

METHODOLOGY

The selection of the species was made on the basis of earlier reports on their threats, endemism, exploitation, population deplete etc. The data related to their distribution and occurrence was compiled from pertinent literature and herbarium consultation. Extensive and intensive field surveys were made to locate the plants in their natural population on the basis of this secondary data. Different propagating materials required for the propagation of a particular species *viz.,* seeds, stem cuttings, suckers, bulbs, rhizome etc. were collected in a sustainable way from the wild habitat for mass propagation of these species. The data related to specific habitat requirements (altitude range, slope aspect, soil pH, moisture etc.) of the selected species were collected during the field surveys to make necessary arrangements for their conservation under *ex-situ* and selection of suitable habitat for reintroduction. The population status of each species at particular location is also evaluated to know the stability of population. This data will be further used in the reassessment of the species as per IUCN guidelines.

Different propagation techniques both macro and micro were applied and experimented with on the collected materials and a propagation protocol is prepared for each species. Attempts were made to adopt the more economical methods for the propagation and conservation of these species.

For cytological studies, root tip and flower buds were also collected and fixed immediately for further study. The GPS data will be used for Ecological Niche Modeling and marking the suitable habitat and regions for reintroduction.

SELECTED SPECIES

The following species were selected on the basis of their rarity, low regeneration potential and depleting population rate in their wild populations. All these species are mainly distributed in the different altitudinal regions of Indian Western Himalaya. These are:

1. *Aconitum heterophyllum* Wall. ex Royle
2. *Berberis osmastonii* Dunn
3. *Ceropegia macrantha* Wight
4. *Cypripedium cordigerum* D.Don
5. *Dactylorhiza hatagirea* (D.Don) Soó
6. *Gentiana kurroo* Royle
7. *Indopiptadenia oudhensis* (Brandis) Brenan
8. *Jasminum parkeri* Dunn
9. *Lilium wallichianum* Schult. & Schult.f.
10. *Meizotropis pellita* (Prain) Sanjappa
11. *Sophora mollis* (Royle) Baker
12. *Trachycarpus takil* Becc.

1. Secondary data collection: The secondary data (literature & herbarium data) were collected from different floras, research papers, online available dataset and herbarium specimens. Within this period, five recognized herbaria *viz.,* BSD, DD, GUH, IIIM and JUH were visited to record the earlier occurrence data of the concerned species. This dataset will be helpful in further relocation of the species, conservation status assessment and mapping of the species distribution range.

2. Primary data collection: Field visits were made to different regions of IWH to locate the targeted species and to collect the propagating material along with population and ecological data. A total number of seven tours were conducted within a period of four months, the details of which are as follows:

S. No.	Localities surveyed	Duration	Collected species
1.	Neelkanth glacier and adjoining areas	25.10.2018 to 28.10.2018	*Aconitum heterophyllum, Dactylorhiza hatagirea,*
2.	Deoban, Chakrata	15.11.2018	*Aconitum heterophyllum, Gentiana kurroo*
3.	Dhanolti, Tehri	13.12.2018 to 14.12.2018	*Gentiana kurroo*
4.	Holi, Chamba, Himachal Pradesh	14.12.2018 to 17.12.2018	*Jasminum parkeri, Aconitum heterophyllum, Dactylorhiza hatagirea*
5.	Sangrah, Sirmour, Himachal Pradesh	15.11.2018	*Gentiana kurroo*
6.	Jammu	2.1.2019 to 4.1.2019	Herbarium consultation at IIIM and JUH
7.	Srinagar, Uttarakhand	16.1.2019 to 18.1.2019	Herbarium consultation at IIIM and GUH

A. Collection of *Dactylorhiza hatagirea* from Neelkanth glacier

B. Collection of seeds of *Gentiana kurroo* from Sangrah, H.P.

C. Collection of *Jasminum parkeri* from Holi, H.P.

D. Collection of *Gentiana kurroo* from Suwakholi

3. Species propagation: Different propagation methods were used for the propagation of the targeted species from the collected propagating materials. These are:

A. *In-vitro* propagation: *Ex-situ* conservation approach by using *in-vitro* techniques is therefore critical for the conservation of germplasm to ensure the propagation of valuable provenance material through micro-propagation and its mass multiplication. It is the rapid method to conserve and propagate the RET species just to fulfill the same. Different ex-plants were used with respect to different species and availability of material. The species-wise details of *in-vitro* method is as follows:

i. *Dactylorhiza hatagirea*

Ex-plant used: seeds and root

Seeds were collected from the wild and inoculated into MS0 medium (without any plant growth regulator). Root explants were also inoculated into MS media supplemented with 0.5-1.0 mg/l.0 BAP and 0.5 mg/l NAA for shoot initiation and MS media supplemented with 0.5 mg/l BAP and 0.5 mg/l 2,4-D for callus induction.

ii. *Aconitum heterophyllum*

Ex-plant used: seeds and root

Seed and root explants were collected from the wild in the month of December. Seeds were inoculated into MS0 medium (without any plant growth regulator). Root explants were also

inoculated into MS media supplemented with 0.5-1.0 mg/l.0 BAP and 0.5 mg/l NAA for shoot initiation and MS media supplemented with 0.5 mg/l BAP and 0.5 mg/l 2,4-D for callus induction.

iii. *Sophora mollis*

Ex-plant used: seeds and Nodal Segments

The seeds, nodal segments and shoot tip explants were collected from the Experimental Botanical Garden, Dehradun. Seeds were inoculated into MS0 medium (without any plant growth regulator). Nodal segment and shoot tip explants were inoculated into MS medium supplemented with 0.5-2mg/l BAP and 0.1-0.5 mg/l NAA. Preliminary shoots were started developing in the shoot tip explants.

iv. *Jasminum parkeri*

Ex-plant used: seeds and Nodal Segments

Seeds and nodal segment explants were collected from Chamba, Himachal Pradesh in the month of December. Seeds were inoculated into MS medium without any plant growth regulator while nodal segments were inoculated into MS media supplemented with 0.5-2.0 mg/l BAP and 0.1-0.5 mg/l NAA.

v. *Gentiana kurroo*

Ex-plant used: Nodal segment and Leaves

Shoot tip and leaf explants of *Gentiana kuroo* were collected from the wild habitat and were inoculated into shoot and callus induction medium. Shoot tip explants were inoculated into MS media supplemented with 0.5-2 mg/l BAP and 0.1-0.5 mg/l NAA while leaves were inoculated into MS media supplemented with 0.5-1.0 mg/l BAP with 1-3 mg/l NAA and MS media with 0.5-1mg/l BAP with 0.5-1mg/l 2,4-D.

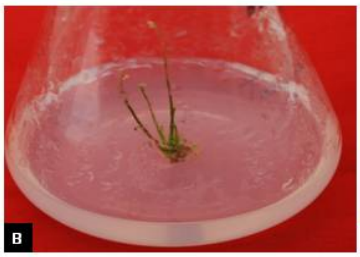
Table 1: Details of explants and different combinations of plant growth regulators used for each plant.

Plant	Explant used	Media combination
Dactylorhiza hatagirea	Seed	MS medium without PGR
	Root	MS+1BAP+0.5NAA
		MS+0.5 BAP+0.5 2,4-D
		MS+0.5 BAP+1 2,4-D
Aconitum heterophyllum	Seed	MS medium without PGR
	Root	MS+1BAP+0.5NAA
		MS+0.5 BAP+0.5 2,4-D
		MS+0.5 BAP+1 2,4-D
Sophora mollis	Shoot tip	MS+1 BAP+0.5NAA
		MS+1.5 BAP+0.5NAA

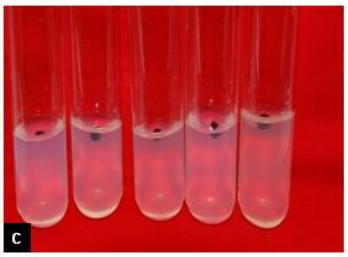
		MS+2 BAP+0.5NAA
		MS+2.5 BAP+0.5NAA
	Nodal segments	MS+1 BAP+0.5NAA
		MS+1.5 BAP+0.5NAA
		MS+2 BAP+0.5NAA
		MS+2.5 BAP+0.5NAA
		MS+1 BAP+0.1NAA
		MS+2 BAP+0.1NAA
		MS+1.5 BAP+0.1NAA
Jasminum parkeri	Nodal segments	MS+1 BAP+0.5NAA
		MS+1.5 BAP+0.5NAA
		MS+2 BAP+0.5NAA
		MS+2.5 BAP+0.5NAA
Gentiana kurroo	Nodal segments	MS+1 BAP+0.5NAA
		MS+1.5 BAP+0.5NAA
		MS+2 BAP+0.5NAA
		MS+2.5 BAP+0.5NAA
	Leaves	MS+1 BAP+1NAA
		MS+1 BAP+2NAA
		MS+1 BAP+3NAA
		MS+0.5 BAP+0.52,4-D
		MS+0.5 BAP+1 2,4-D

Note: MS- Murashige and Skoog medium and PGR concentrations are in mg/l

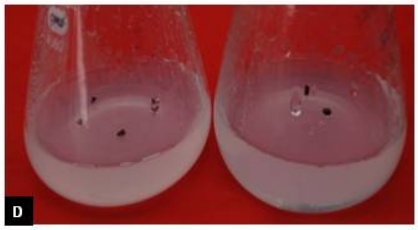
A. Inoculated nodal segment ex-plants of *Sophora mollis*



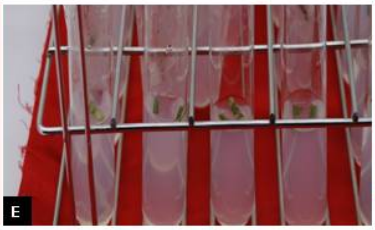
B. Development of shoots in shoot tip ex-plant of *Sophora mollis*



C. Seeds of *Jasminum parkeri* inoculated in MS0 medium



D. Seeds of *Aconitum heterophyllum* inoculated in MS0 medium



E. Leaf ex-plant of *Gentiana kurroo* inoculated in callus induction Medium

F. Rhizome of *Dactylorhiza hatagirea* inoculated in callus induction medium

B. Macro propagation:

Various conventional methods were applied for the propagation of the selected species using nursery techniques.

i. Through cuttings: Cuttings of *Jasminum parkeri* were treated with rooting hormone and planted in pure sand medium for rooting.

ii. Through seeds: Seeds of *Jasminum parkeri, Aconitum heterophyllum* and *Sophora mollis* were planted in suitable medium for germination.

iii. Through rhizome/bulb: Collected rhizome and bulbs of *Aconitum heterophyllum* and *Dactylorhiza hatagirea* were planted in cocopeat and vermiculite medium.

iv. Through suckers: Plants of *Gentiana kurroo* were propagated through offshoots and suckers collected from Suwakholi and Sangrah localities.

A. *Jasminum parkeri* cuttings treated with rooting hormone

B. Cuttings of *Jasminum parkeri* planted in pot for rooting

C. Seeds of *Jasminum parkeri* sown for germination

D. *Gentiana kurroo* flowering under *ex-situ* conservation

4. Cytological studies

Methodology:

1. Preparation of fixatives: Carnoy's fixative (60% ethanol, 30% chloroform and 10% glacial acetic acid) was prepared for fixation of sample for meiotic and mitotic studies. Carnoy's fixative is preferred over other fixatives for its better results in flowering plants.
2. Pretreatment: For pretreatment of roots for mitotic studies, solutions of various concentration (0.1 %, 0.3 %, 0.4% and 0.5%) of Colchicine were prepared.
3. For meiotic studies of *Sophora mollis* (Royle) Baker (Leguminosae), unopened floral buds were collected on different days and fixed in Carnoy's fixative for 24 hours after

which the buds were transferred into 70% alcohol and refrigerated till further analysis.

4. Due to non-availability of seeds of S*. mollis*, apical buds were collected for mitotic studies. Two types of pretreatment were given to the apical buds 1) 0.05 % Colchine for 1-2 hours and 2) Cold water treatment (0-4 °C) for 24 hours, after which buds were fixed in Carnoy's fixative for 24 hours. The apical buds of *S. mollis* were heated in 1N HCL (hydrochloride acid) at 60 °C for 10 minutes and later stained with acetocarmine following the standard squash technique.
5. For karyotypic studies of *Jasminum parkeri* Dunn (Oleaceae) seeds were sowed in sand and cocopeat for germination and root tip collection. The seed have not yet geminated. The plant cuttings of *J. parkeri* along with adventitious roots collected from Holi, Chamba are also potted in cocopeat for growth of new roots tips and further studies can be carried on.

Results:

1. The pollen mother cells (PMCs) of *S. mollis* when worked out for meiotic chromosome count ('n') by standard acetocarmine squash technique were observed at different meiotic stages (Prophase-I, Metaphase-I, Anaphase-I & II, Telophase-I & II, Tetrad stage etc.). During meiosis, all the PMCs unequivocally show the presence of 9 bivalents at Metaphase -I and 9:9 chromosomes distribution at 2 poles during anaphase-I and metaphase-II, respectively. Based on the basic chromosome number, x=9, the species exist at diploid level. Present chromosome count of 2n=18 is the first report form Indian Himalaya.
2. *S. mollis* showed meiotic abnormal behavior (*cytomixis*). The floral buds are still being collected on different days and at different time intervals for determination of the meiotic behavior of the species.
3. Both the pretreatments given to apical buds of *S. mollis* for karyotypic studies failed to give desired results.
4. Nearly all of the selected of plants under the project do not flower during this period of the year so further meiotic studies are to be success during the flowering period of these plants (January to October).

5. Budget details:

The details of expenditure incurred from the total allotted budget as per different heads is summarized as under:

Sl. No	Heads	Amount Sanctioned	Expenditure	Balance
1.	Salary	10,91,520 /-	4,56,000/-	6,35,520/-
2.	Travel	3,00,000 /-	58,000/-	2,42,000/-
3.	Expendables	2,50,000/-	25,000/-	2,25,000/-
4.	Contingency	1,71,429/-	36,000/-	1,35,429/-
5.	Activities	10,28,571/-	NIL	10,28,571/-
6.	Equipments	14,46,710/-	NIL	14,46,710/-
	Total	**42,88,230/-**	**5,75,000/-**	**37,13,230/-**

Overall progress:

Since the project was started in October 2018, and due to winter season only limited number of tours were conducted. However, few field tours were conducted to the possible localities and the period is mainly utilized in data collection and other activities.

Remaining work and future targets:

1. Data collection from various renowned herbaria and literature for knowing the species occurrence records.
2. Extensive and intensive field surveys to locate the species in earlier reported localities and searching newer localities to get the present population count of a particular species in each location and to evaluate the prevalent threats.
3. Collection of propagating material for each species and developing a nursery.
4. Collection of material and cytological analysis in selected species.
5. Developing different propagation protocol for the mass propagation of these species.
6. Mass propagation of species using micro/macro techniques and through collaboration with forest department and NGOs.
7. Ecological Niche Modeling on the basis of collected data to predict the suitable niche for species reintroduction
8. Reintroduction of propagated plants in identified niche and encouraging local people and forest staff for their in-situ conservation as well.
9. Preparing an updated database of species occurrence, habitat range and conservation status to design the further conservational works required for its restoration.

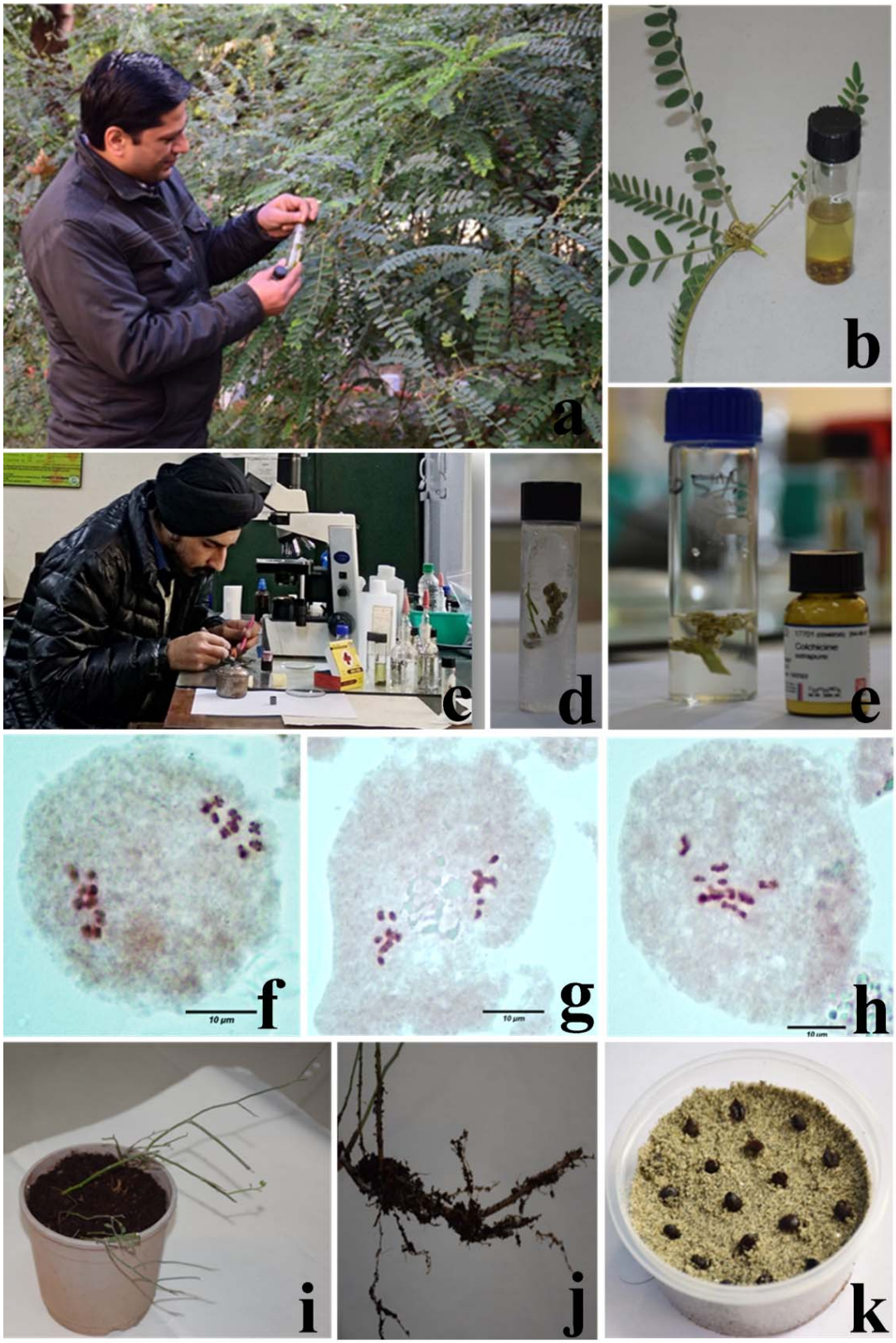


Figure 1. (a-h *Sophora mollis*) a) Collection of young floral buds b) Fixation of young floral buds c) Working out meiotic chromosome number d) Cold pre-treatment of apical buds e) Colchine pre-treatment of apical buds f) 9:9 chromosome distribution at Metaphase-II g) 9:9 chromosome distribution at Anaphase-I h) n=9 bivalents at Metaphase-I (i-j *Jasminum parkeri*) i) Potting of stem cutting for root tip growth j) Adventitious roots k) Seed set in sand for germination.

Botanical Survey of India, BSI Kolkata

Principal Investigator
Dr. A.A.Mao

Co-Principal Investigator
Dr. B.K.Sinha

Dr. S.S.Dash

Research Associates
Dr. Kishor Deka

Junior Project Fellows
Mr. Ashish Uday Zagade
Mr. Sujit Rambhau Jejurkar
Mr. Rohan Maity

PROGRESS MADE

(December 2018 - March 2019)

A. RECRUITMENT OF MANPOWER

REASEARCH ASSOCIATE:

Dr. Kishor Deka -Joining Date: 21/12/2018

JUNIOR PROJECT FELLOW(S):

1. Ashish Uday Zagade -Joining Date: 11/12/2018
2. Sujit Rambhau Jejurkar - Joining Date: 11/12/2018
3. Rohan Maity - Joining Date: 17/12/2018

B. LITERATURE SURVEY:

During this period, we have studied -

- Several literatures on floral diversity of Indian Himalayan region majorly focusing on Arunachal Pradesh, Sikkim and Darjeeling Himalayan Region at CNH library.
- Herbarium study of available selected species deposited at CAL.
- Distribution and specific locations of selected species from published scientific literatures.
- Macro and micro-propagation methods of medicinal and RET plants for conservation purpose.
- Literature studied about reinforcement/reintroduction of RET plants into the suitable wild habitat to improve conservation status using Ecological Niche Modelling.

C. SELECTED PLANT LIST FOR CONSERVATION:

AREA OF STUDY	Scientific name	Flowering / Fruiting	FAMILY	Reported location/ Elevation
Darjeeling	*Aesculus assamica* Griff.	Fl: 02-03 Fr: 05	Sapindaceae	900 m
	Castanopsis indica (Roxb. ex Lindl.) A.DC.	Fl: 09-10 Fr: 10-12	Fagaceae	900-1500 M
	Oroxylum indicum (L.) Kurz	Fl: 05-06 Fr: 12-01	Bignoniaceae	400- 1500 m
	Pterocarpus marsupium Roxb.	Fl: 05-08	Leguminosae	
	Quercus glauca Thunb.	Fl: 03-06 Fr: 09-01	Fagaceae	1100-2000 m
Arunachal Pradesh	*Arenga micrantha* C.F.Wei	Fl: 08	Arecaceae	1400-1500 M
	A. westerhoutii Griff.	Fl: 08	Arecaceae	Below 1400 m
	Cinnamomum bejolghota (Buc h.-Ham.) Sweet	Fl: 03-07 Fr: 09-11	Lauraceae	400-2000 m
	Corylus jacquemontii Decne.	Fl: 03-04	Betulaceae	Mainly in western Himalaya up to Western Nepal, 1800-3000 m
	Ormosia robusta Baker	Fl: 04-05	Leguminosae	Itanagar, Papumpare district
	Quercus lamellosa Sm.	Fl: 04-05 Fr: 11	Fagaceae	1500-2500 m
	Schima wallichii Choisy	Fl: 04-05 Fr: 11-12	Theaceae	600-2000 m
Sikkim	*Cinnamomum impressinervium* Meisn.	Fl: 05-08 Fr: 12	Lauraceae	1800-2500 m
	Magnolia doltsopa (Buch.-Ham. ex DC.) Figlar	Fl: 02-04 Fr: 10-11	Magnoliacea e	1000-2500
	Rhododendron falconeri Hook.f.	Fl: 04-06 Fr: 10-12	Ericaceae	2500-3200 m
	Rhododendron thomsonii Hook. f.	Fl: 03-05 Fr: 04-07	Ericaceae	2000-3400
	Taxus wallichiana Zucc.	Fl: 03-04	Taxaceae	1800-3500 m
	Ulmus wallichiia Planch.		Ulmaceae	800-3000 m

Budget details:

The details of expenditure incurred from the total allotted budget as per different heads is summarized as under:

Sl. No	Heads	Amount Sanctioned	Expenditure	Balance
1.	Salary	14,46,720 /-	2,51,961/-	11,94,759/-
2.	Travel	4,50,000 /-	1,01,000/-	3,49,000-
3.	Expendables	3,75,000/-	NIL	3,75,000/-
4.	Contingency	2,57,143/-	2,30,447/-	26,696/-
5.	Activities	15,42,857/-	NIL	15,42,857/-
6.	Equipments	21,70,065/-	NIL	21,70,065/-
	Total	**62,41,785/-**	**5,83,408/-**	**56,58,377/-**

Botanical Survey of India, Botanic Garden of India Republic (BGIR), NOIDA.

Principal Investigator
Dr. A.A.Mao

Co-Principal Investigator
Dr. Paramjit Singh
Dr. Sandeep K. Chauhan

Research Associates
Dr. Mayank D. Dwivedi
Junior Project Fellows
Damini Sharma
Kusum Upadhyay
Priya
Deepakshi Babbar

PROGRESS MADE

(October 2018 - March 2019)

A. Work Done So Far:

- Identified and listed the focused taxa from three states - viz. UK, HP and J&K (29 spp.).

Trachycarpus taxil Becc. (Arecaceae); *Ceropegia bulbosa* Roxb. (Asclepiadaceae); *Berberis kunawurensis* Royle (Berberidaceae); *Berberis osmastonii* Dunn (Berberidaceae); *Sinopodophyllum hexandrum* (Royle) T. S. Ying (Berberidaceae); *Ephedra gerardiana* Wall ex Stapf (Ephedraceae); *Allium auriculatum* Kunth; *Allium loratum* Baker; *Allium roylei* Stearn; *Allium stracheyi* Baker; *Fritillaria cirrhosa* D. Don (= *Fritillaria roylei* Hook); *Lilium polyphyllum* D. Don ex Royle; *Lilium wallichianum* Schultes; *Paris polyphylla* Smith; *Polygonatum verticillatum* (L.) All (Liliaceae); *Jasminum parkeri* Dunn (Jasminum); *Calanthe alpine* Hook. f.; *Cypripedium cordigerum* D. Don; *Dactylorhiza hatagirea* (D.Don) Soo (Orchidaceae); *Rheum spiciforme* Royle (Polygonaceae); *Primula minutissima* Jackex Duby (Primulaceae); *Aconitum balfourii* Stapf ; *Aconitum chasmanthum* Stapf ex Holmes; *Aconitum dimorphism* Stapf; *Aconitum ferox* Wall. ex Ser.; *Aconitum heterophyllum* Wall. ex Royle; *Aconitum violaceum* Jacq. ex Stapf (Rananculaceae); *Atropa acuminate* Royle ex Lindl. (Solanaceace); *Nardostachys grandiflora* DC. (Valerianaceae).

- Distributed taxa among Junior Project Fellows and Research associate [colour coded].

- Developed morphological understanding for 29 spp. for identification purpose and field visits clarified.
- Protologue information and type localities were collected for the enlisted species.
- Phenology of the identified taxa were studied and documented on the calendar for field visits.
- Systematic position for *Allium* spp. and *Aconitum* spp. taxa were undertaken by downloading all barcodes deposited on GenBank.
- Threats to the selected taxa were enumerated to take up conservation strategies after field collections.
- GIS Map for *Lillium polyphyllum* was prepared.
- Ecological Niche Modelling map for *Ephedra gergardiana* and *Lillium polyphyllum* were laid.

B. Work to be done:

- To visit leading Herbaria in Himalayan regions for collecting localities and ascertaining species boundary of selected species.
- To undertake extensive field trips to identified localities and collection of germplasm from identified localities.
- To study taxonomy of the collected specimens which include preparation of diagnostic keys and Ethnobotanical uses.
- Frequency, intensity of fruiting and flowering; inflorescence parameters and floral biology of all taxa will be closely monitored.
- To make GIS and ENM maps for remaining species.
- Barcoding of identified specimens will be done by using standard selected markers.
- Ex-Situ conservation of selected species through traditional and biotechnological methods at WHHARC (Western Himalaya high altitude regional centre) BSI, Solan (H.P.) and BGIR, Noida (U.P.).
- To raise community awareness programmes.

Time Frame (December 2018- 30 March 2019)

December 2018

- Appointment of JPF RA and Field assistant starting 12th December 2018.
- Study of all the 72-plant species their distribution.

January 2019

- Short listing of species (total no.= 29)
- Literature survey on (i) Protologue (ii) Distribution (iii) Phenology (iv) relevant Pictures and illustrations
- Herbarium visit to NBRI, Lucknow from 15 Jan to 19 Jan 2019
- Sorting key morphological features for field identification

February 2019

- Retrieving distribution locality (lat. & long.)
- Literature survey on (i) Protologue (ii) Distribution (iii) Phenology (iv) relevant Pictures and illustrations for remaining species from January 2019
- Ecological Niche Modelling using MAXENT and GIS software for selected species
- Download Molecular barcode markers for DNA barcoding from GenBank.
- Project presentation - 4-7 February 2019 at Almora

March 2019

- Download GenBank Barcode sequences of selected species and preparation of Phylogram.
- Ecological Niche Modelling using MAXENT and Diva GIS for remaining selected species.
- Field trip to Himachal Pradesh from 15-24 March 19 for seed collection from seed banks of NBRI, Lucknow; Kashmir University; GB Pant University

April 2019

- Literature survey on (i) Protologue (ii) Distribution (iii) Phenology (iv) relevant Pictures and illustrations
- Shortlisting the collection localities for visiting

May to **September 2019**

- Field collection trip to identified and marked localities in Himachal Pradesh and Jammu & Kashmir.

UTILIZATION CERTIFICATE

For six monthly periods (from 12 December, 18 to 31st March of the next calendar year).

1. Title of the Project: **Conservation of Threatened Plants in Indian Himalayan Region: Recovery and Capacity Building**

2. Name of the organization/Network Partners: **Botanic Garden of India Republic, Noida, U.P.**

3. Principal and Lead Investigators: Dr. A.A. Mao and Dr. P. Singh & Dr. S.K. Chauhan

4. Ministry of Environment and Forests letter: **No. GBPNI/NMHS-2017-18/LG-03/570dt.26/02/2018** and date of sanctioning of the Project **27/08/2018**

5. Amount brought forward from the: previous six monthly periods: **NIL**

6. Amount released in respect of the: six month period to which this Utilization Certificate relates (Please give No. and dates of sanctions showing the amount) **Rs. 80, 75,341/-**

7. Total amount available: for expenditure for the period to which this Utilization Certificate relates **Rs. 80, 75,341/-**

8. Actual expenditure (excluding: commitments) incurred in the period to which this Utilization Certificate relates : **2,51,578.00**

9. Unspent balance: **78,23,763.00**

Certified that the expenditure of Rs. 2,51,578.00 (Rupees two lakh fifty one thousand and five hundred seventy eight only). Was actually incurred on the Project for the purpose for which it was sanctioned.

D Budget [Expected Expenditure Statement until 31st March 2019

Statement showing expenditure for period from 12 December 2018 to 31st March of the next calendar year 2019.

1. Total outlay of the project Rs. 80, 75,341/-.

2. Date of start of the project: 12 December 2018.

3. Duration 3 years Date of completion 12 December 2021.

a. Amount released in respect of six-month period to which the utilisation certificate relates Rs. 80, 75,341/-.

b. Amount brought forward from previous six-monthly period: NIL

c. Total amount available for expenditure (a+b) Rs.: 80,75,341/-.

Expenditure Heads	Amount sanctioned 1st year (Rs.)	Actual amount spent 31 January, 19 (Rs.)	Proposed amount spent (31st March, 19) (Rs.)	Balance (Rs.)
Salaries/Wages of staff	**16,81,920.00**	**1,73,128.00**	**3,15,520.00**	**13,66,400.00**
Permanent Equipment purchased	**28,93,421.00**	**00.00**	**28,93,421.00**	**00.00**
Travel	**6,00,000.00**	**00.00**	**50,000.00**	**5,50,000.00**
Contingency	**3,42,857.00**	**00.00**	**42,857.00**	**3,00,000.00**
Activities	**20,57,143.00**	**00.00**	**00.00**	**20,57,143.00**
Expenditure	**5,00,000.00**	**78,450.00**	**00.00**	**5,00,000.00**
Total	**80,75, 341.00**	**2,51,578.00**	**33,01,789.00**	**47,73,552.00**

BOTANICAL SURVEY OF INDIA

EASTERN REGIONAL CENTRE

LOWER NEW COLONY, LAITUMKHRAH

SHILLONG – 793003 MEGHALAYA