

**National Program for
Conservation and Development of Forest Genetic Resources**

Pilot Project

***On*
Creation of Centre of Excellence on
Forest Genetic Resources (FGR) of India
(CoEFGR)**

**Funded under
National CAMPA Fund
Ministry of Environment, Forest & Climate Change
(2016-2022)**



BRIEF PROGRESS

**Submitted by
Forest Research Institute (FRI),
New Forest P.O., Dehradun 248 006**

BRIEF PROGRESS

Title of the Project:	National Program for Conservation and Development of Forest Genetic Resources:Pilot project on 'Creation of Centre of Excellence on Forest Genetic Resources (CoFGR)'at FRI Dehradun
Funding Agency:	National CAMPA Fund Ministry of Environment, Forest & Climate Change, Govt. of India
Project Outlay:	Rs. 861.20 lakhs (January 2016 – 31 March 2022)
Project Period:	6 years
Executing Agency:	FRI Dehradun

Objectives/Deliverables

1. Establishment of Centre of Excellence on Forest Genetic Resources (CoE-FGR) at FRI Dehradun
2. Upgradation of Dehradun (DD) Herbarium
3. Comprehensive inventory with population and threat status of 250 FGRs of Uttarakhand in place.
4. Priority list of FGRs, based on assessment of their conservation status prepared. Eco-distribution maps of 50 priority FGRs in place
5. Seed of 90 species of FGRs collected with passport data and deposited in Seed Banks for medium and long-term conservation and use in FGR improvement programs.
6. Evaluation and molecular characterization done for 5 important FGRs of commercial importance for various traits
7. Genetic diversity & population structure of 5 important FGRs studied & their *in situ* conservation strategy put in place.
8. Germplasm of a minimum of 10 important FGRs conserved in the form of Field Gene Banks.
9. A comprehensive computerized database on FGRs of Uttarakhand developed.

Achievements

Background Information

Forest Genetic Resources (FGRs) constitute a very important sub-set of biodiversity. Conserving FGR is vital, as they are unique and irreplaceable resources for the future. In

India alone, more than 340 million people are estimated to be dependent upon the FGRs for their livelihoods. There is a definite need to address the FGR related issues through a comprehensive FGR conservation and development strategy and implementation plan.

As per present state of knowledge, 18,236 higher plant species (18,159 Angiosperms and 77 Gymnosperms) documented from India so far (*BSI, 2015: Plant Discoveries 2014*). More than 80% of this higher plant diversity is contained in the forest habitats (\approx 14,500 species). About half of this forest plant diversity constitutes FGRs (\approx 7,250 species), the remaining being herbaceous flora including soft climbers, twiners, herbs, and grasses. FGRs contain a huge potential in ensuring food and health security of the country's burgeoning human population and its livestock.

To generate understanding and knowledge on FGR, and to develop and strengthen in situ and ex situ FGR conservation programmes, the National CAMPA Advisory Council (NCAC) of Ministry of Environment and Forests & Climate Change, Govt. of India has sanctioned a pilot project entitled "National Program for Conservation and Development of Forest Genetic Resources: Pilot Project to be implemented at FRI on Creation of Centre of Excellence on Forest Genetic Resources (CoFGR)".

The activities of the projects are being executed through four Working Groups created within the strength of FRI viz. FGR Documentation, FGR Seed and Germplasm Storage, FGR Characterization and FGR Conservation. The progress made under these working groups in last one year is summarized hereunder:

A. FGR Documentation

1. Upgradation of DD Herbarium : Target Completed

Herbarium of the Forest Research Institute, Dehra Dun which is internationally known as Dehra Dun Herbarium (DD) houses approximately 3,30,000 specimens, the oldest collection dates back to 1807. The system of classification of plant specimens followed is that of Bentham & Hooker. Besides collections from the Indian region, Herbarium contains specimens from all over the world. To safeguard and expend this valuable genetic resource and for its better access, modernization of herbarium was executed during the year 2016 and 2017 under the CAMPA grant. The DD herbarium building has been renovated through expert's consultation. Mobile herbarium compactors have been procured and installed in the herbarium building for the better access and long-term safeguard of specimens. A total facelift to the existing herbarium has been given.

Dicotyledonous floral specimens following the Bentham & Hooker classification has been initiated.



A view of New Herbarium hall with state of art Compactors after furnish



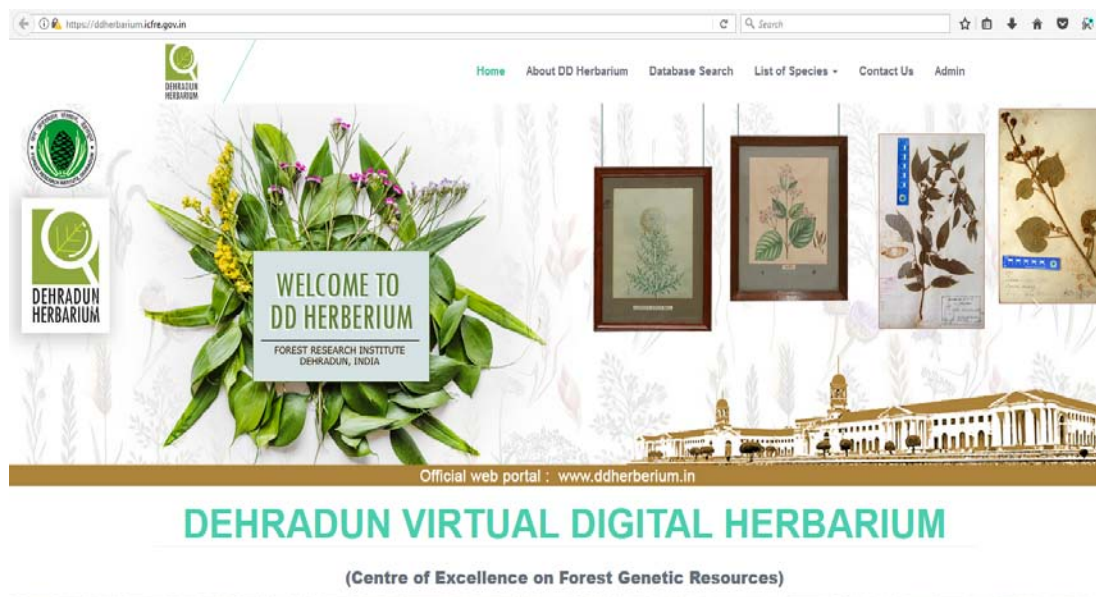
Shifting of Herbarium specimens

2. Digitization of DD herbarium : Target Completed

Being the second largest herbarium of the country, the collection offers a unique insight into the flora of the Indian subcontinent. To make the herbarium data available to taxonomists worldwide, an urgent need for digitizing and developing a database was

felt. Accordingly a herbarium database was developed during 2008-2009 with the digitization of one specimen/species. Under the CAMPA funded Co-FGR project, further expansion of DD Herbarium digital database has been undertaken. As per the expert panel review, it was suggested that one specimen/ species may not be sufficient to cover all the morpho-variations associated with the species distribution range. Thus, the current project activity envisages digitizing additional two specimen/species (67974 specimens) so as to purge the associated apprehensions. Having this information available through the Web once the project gets completed, shall definitely obviate the need to borrow DD specimens or travel to examine them as the information will be accessible for ready reference to a national / international audience more quickly, with less expense and without causing potential damage to preserved materials.

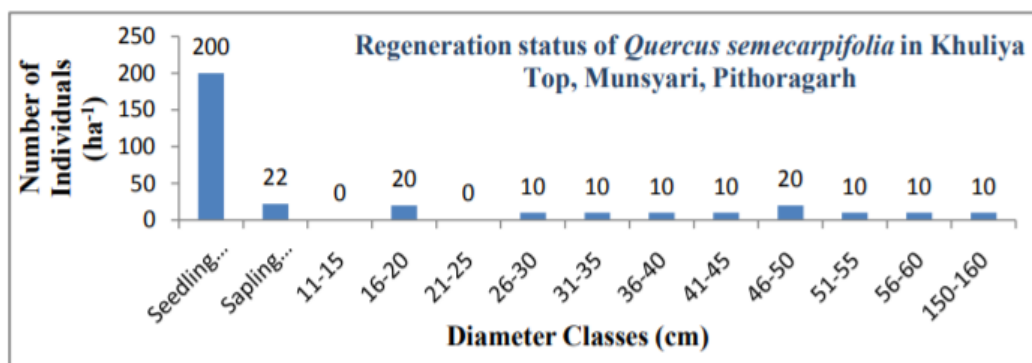
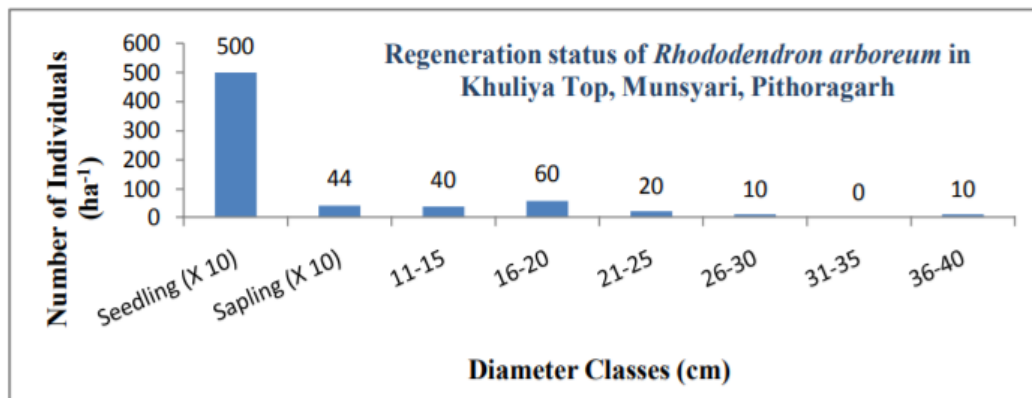
Hence, a herbarium FGR database was developed. The task of the digitization of 67974 specimens has been completed. The herbarium database has been hoisted through a web portal and the online access has been provided to the users. At present the database has record of 1,02,000 specimens belonging to 39,916 species of flowering plants representing approximately 1/3rd of total Herbarium collection.



Screen shot of the Dehradun virtual Digital Herbarium Database

3. Documentation of FGR diversity : Target 250 species, Completed analysis ongoing

A list of 250 priority species (141- tree species, 29 shrubs, 15 lianas/woody climbers and 65 RET species) has been prepared for Uttarakhand. Out of which 50 species have been selected for the preparation of eco-distribution maps. In order to document the FGR diversity, record population size and assess regeneration status of different FGR species in Uttarakhand; distribution of 250 species has been traced from DD Herbarium, BSI Herbarium (Northern Circle), working plans and records from the literature. For ground verification, field surveys of all 250 species in all 44 forest divisions of Uttarakhand has been carried out to check the present status of presence of key species their distribution and regeneration. The GPS coordinates of the selected species and their populations has been recorded and the population structure and regeneration status of the species in various forest areas has been worked out.

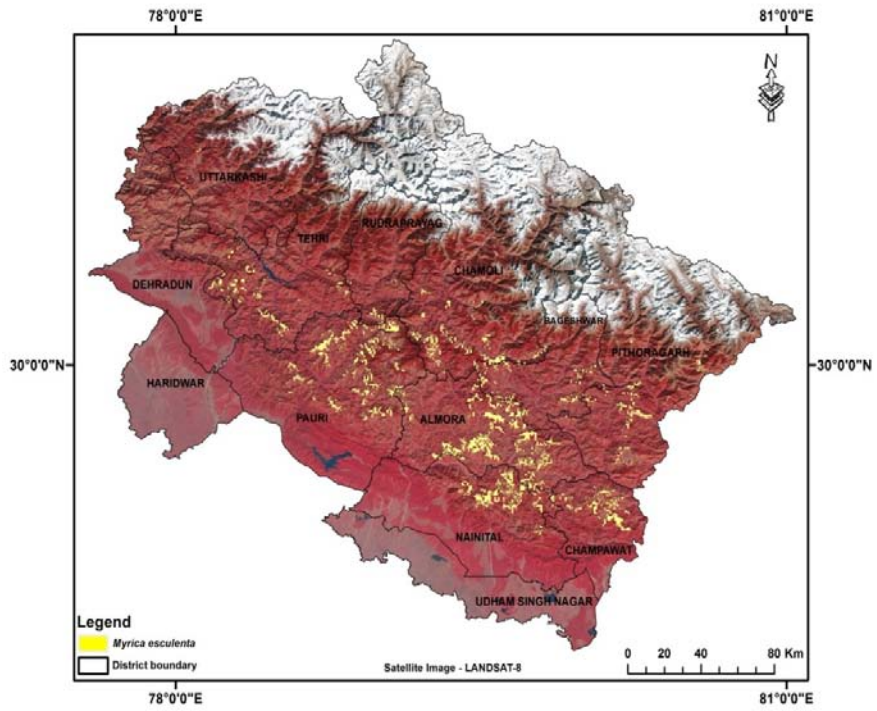




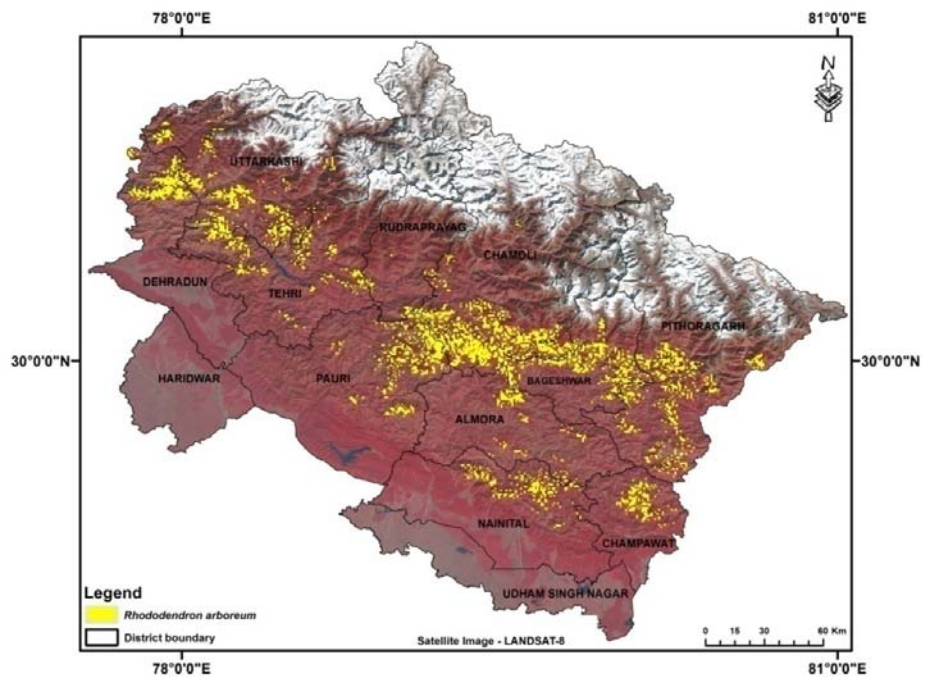
Exploration and collection of field data from Champawat Forest Division

4. Development of Eco-distribution : Target 50 sp. Completed

Eco-geographical mapping of forest tree species is important aspects of germplasm conservation program as the conservation efforts are best aided by vegetation and land use maps. This is particularly important for those species which are co-dominant in occurrence, have sparse distribution and are under some kind of threat. For development of eco-distribution maps of important FGR species, mapping methodology has been developed. The methodology was tested in the Mohand and Sukhblock of Chilllawalii Range, Rajaji National Park, Dehradun (Uttarakhand). The estimation was quite similar to the FSI Forest Type Report (Satellite Image LISS III used) and working plan for Rajaji National Park, indicating the reliability and accurateness of the developed methodology. At present, 50 FGRs species of Uttarakhand has been mapped through RS and GIS based tools and the targets has been achieved. Some of the important species being mapped are *Betula utilis*, *Quercus semicarpifolia*, *Rhododendron arboreum*, *Taxus wallichiana*, *Myrica esculenta*, *Diploknemabutyraceae* etc.



Myrica esculenta



Q. semecarpifolia

Fig. Eco-Distribution map of *Myrica esculenta* and *Q. semecarpifolia*

B. FGR Seed and Germplasm Storage : Target 90 species, Likely to complete

It is intended to collect seeds of 90 important FGR species and their populations under this project for their long term storage and conservation. A list of prioritized FGR species of Uttarakhand district was prepared based on the socio- economic, ecological status of forestry species. The list contains Rare, Endangered, and Threatened (RET) species; fuelwood, food and fodder species; economically and ecologically important species.

1. Collaboration with NBPGR, New Delhi in germplasm conservation

- Memorandum of Agreement (MoA) signed between Forest Research Institute, Dehradun and National Bureau of Plant Genetic Resources, New Delhi regarding long-term conservation of seed germplasm of FGRs at -18°C in their Seed Bank.
- As per the request of FRI, National Bureau of Plant Genetic Resources (NBPGR) New Delhi organised a training course on “Techniques for of Conservation of Plant Genetic Resources” from 27th June to 2nd July, 2016. Ten Scientists and research personnel working in various components of CoFGR-CAMPA project, participated in the training.



Training of FRI Scientists at NBPGR New Delhi



Signing MOU between FRI and NBPGR New Delhi

2. Survey of populations for seed collection

- Reconnaissance survey of FGR species conducted and information about their phenology, various morphological parameters of trees like height, crown size, fruit maturity were recorded. Populations of prioritized species were identified and geo-referenced for fruit/seed collection (passport data generated). Fruits/Seeds of FGR species were collected based on their maturity indices. Presently, seeds of 86 species have been collected and processed according to the standard procedures suitable for FGR conservation.
- Seeds of the collected species were cleaned and processed, initial viability determined, carefully desiccated to safe moisture content levels (using desiccant/cool air seed dryer) then stored in air-tight containers at 5⁰C. Quarterly the viability of seeds being monitored.
- Currently seeds of 86 forestry species have been processed for long-term conservation in consultation with team of scientists from NBPGR.

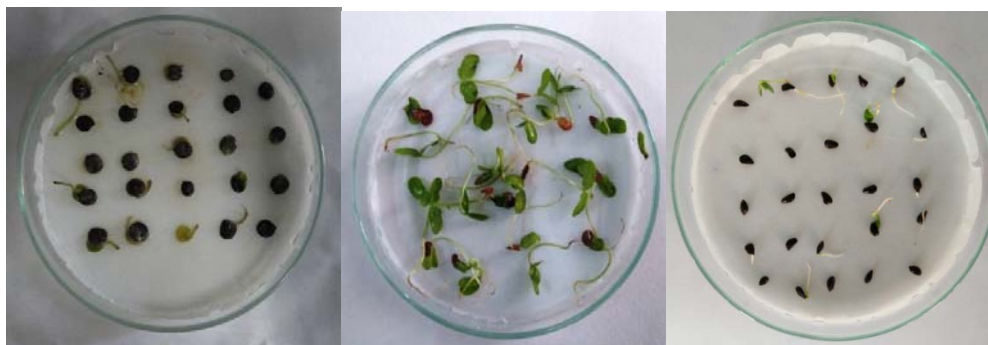


Seed collection of Alnus nepalensis

Seed collection of Berberis vulgaris



Aristolochiaelegans BischofiajavanicaPinuswallichiana

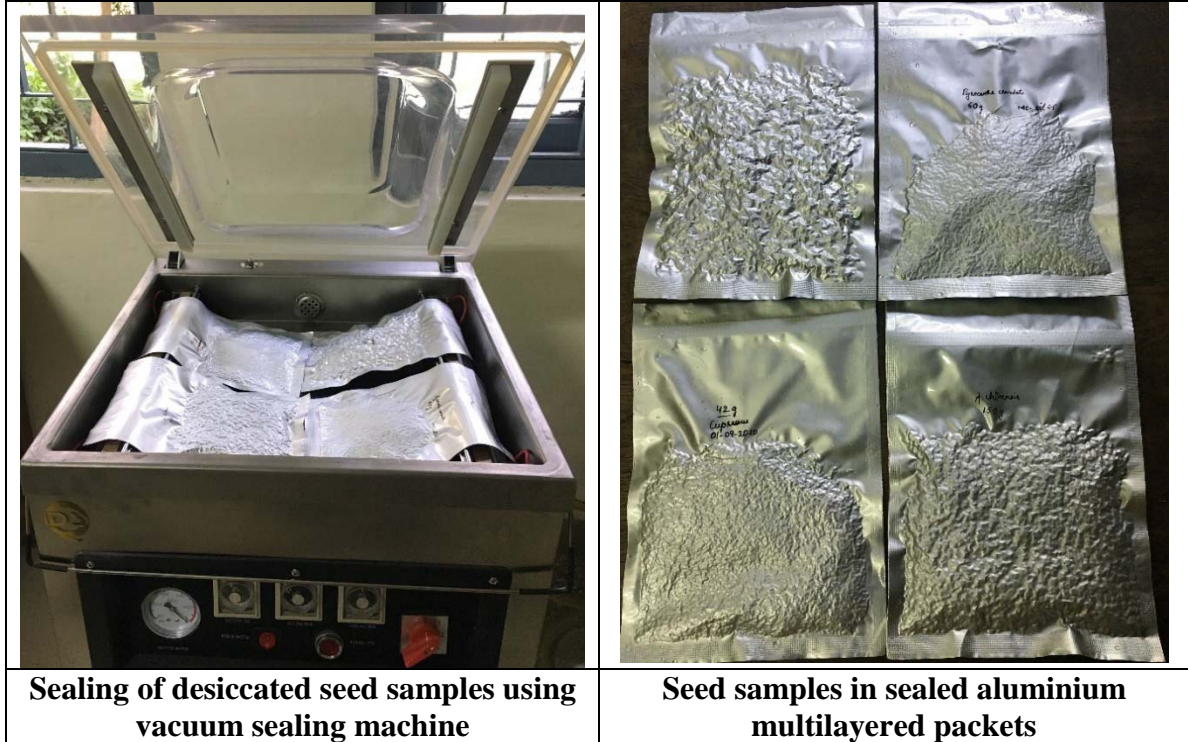


Pyruspaschia Dalbergia sissooAcacia catechu

Seed Germination Test of FGR species

3. FGR species deposited at NBPGR Seed bank, New Delhi, for long-term conservation

The processed, viability determined seeds of 86 forestry species were desiccated up to safe moisture level and deposited in the Gene bank of ICAR-NBPGR at -18°C along with passport details for *ex-situ* conservation of seed germplasm.



4. *In-vitro* storage of FGR species : Target completed

In vitro storage in the form of slow growing and/or cryopreserved cultures is one of the ways of conserving valuable germplasm in medium and long term, respectively. This objective can be fulfilled once the protocols for whole plant regeneration from these stored cultures i.e. callus, shoot tips, slow growing shoot cultures etc is developed. With an aim to conserve forest genetic resources (FGRs) of very high conservation concern or those having recalcitrant seeds or both, *in vitro* regeneration protocols have been developed for ten species in order to achieve whole plant regeneration as well as medium term storage.

Species : *Desmodium oojeinense*, *Taxus contorta*, *Hippophae salicifolia*, *Albizia julibrisin*, *Aristolochia punjabensis*, *Oroxylum indicum*, *Hymenodictyon orisense*, *Dysoxylum gotadhora*, *Catamixis baccharoides*, *Rhus parviflora*, *Butea pellita*.

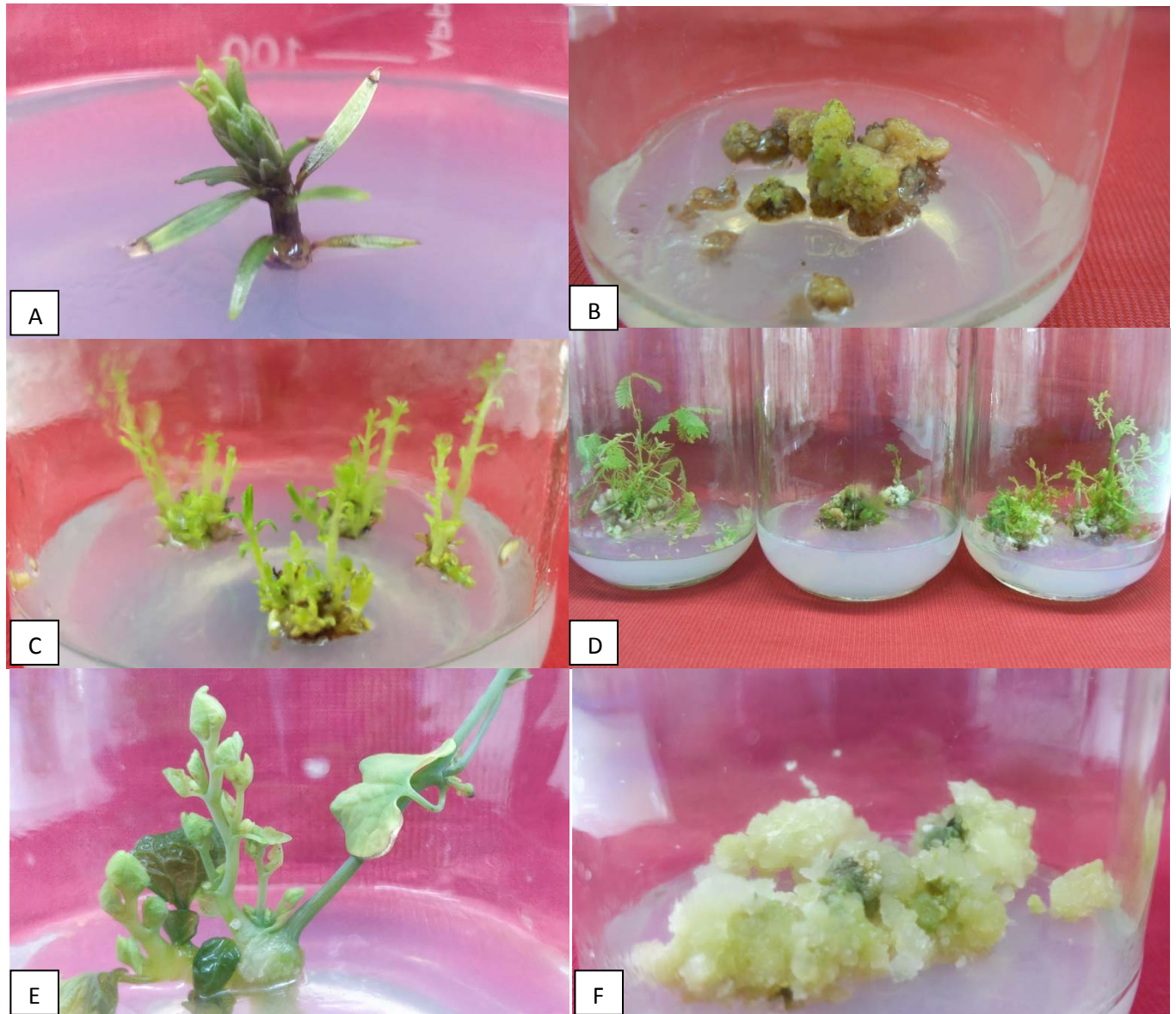


Fig. A- *In vitro* shoot elongation in *T. contorta*, B- Callus regrowth in *D. oojeinense*, C- proliferation of *in vitro* cultures in *H salicifolia*, D- proliferation of *in vitro* cultures in *A.julibrisin*, E- proliferation of *in vitro* cultures in *A. punjabensis*, F-Callus culture in *P.eriocarpum*.

5. Pollen Storage of Species of High Conservation Concern – Target completed

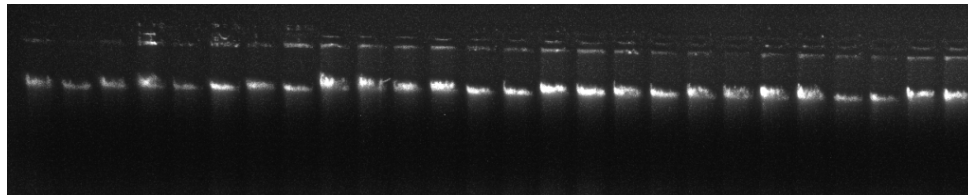
Protocols for storage of FGR germplasm in the form of ‘pollens’ for the species of high conservation concern of ten targeted species has been developed.

Species : The pollens of *Heteropanax fragrans*, *Oroxylum indicum*, *Diploknema butyraceae* and *Sterculia colorata*, *Butea pellita*, *Rauwolfia serpentina*, *Cratevaadansonii*, *Alstoniascholaris*, *Mahonia jaunsarensis*, *Sapium insigne*, *Buxus wallichiana* and *Sophora mollis*.

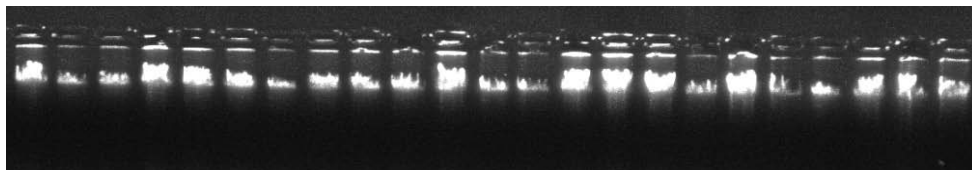
5. FGR Characterization : Target 6 species , Likely to complete

Five species have been prioritized for molecular characterization and genetic diversity estimation viz. *Rhododendron arboretum* (Burans), *Taxus wallichiana* (Thuner), *Quercus semecarpifolia* & *Q. lanuginosa* (Kharsu oak and Rianj Oak), *Betula utilis* (Bhojpatra) and *Myrica esculenta* (Kafal)

A better understanding of the diversity of these species is crucial for their sustainable use and conservation. Monitoring of patterns of distribution and genetic diversity of these species allows the prioritization of populations for in situ conservation and identification of populations and species most at risk and existing gaps in genebank collection. Extensive survey and sampling work has been carried out in Uttarakhand hills for the selected species. Samples of the selected species have been collected from their natural zone of occurrence and stored at -80°C. A total of 30-35 samples/trees were collected from each population in all the species. The samples of these populations were segregated for chemical examination and DNA fingerprinting. DNA extraction protocol has been standardized for *Diploknemabutyraea*s, *Rhododendron arboretum*, *Taxus wallichiana*, *Myrica esculenta* as well as *Betula utilis*. Genotyping of the extracted DNA of the various populations is being carried out using robust polymorphic SSR markers. Chemical characterization/chemical marker(s) assisted screening of *Rhododendron arboretum* and *Myrica esculenta* is under process.

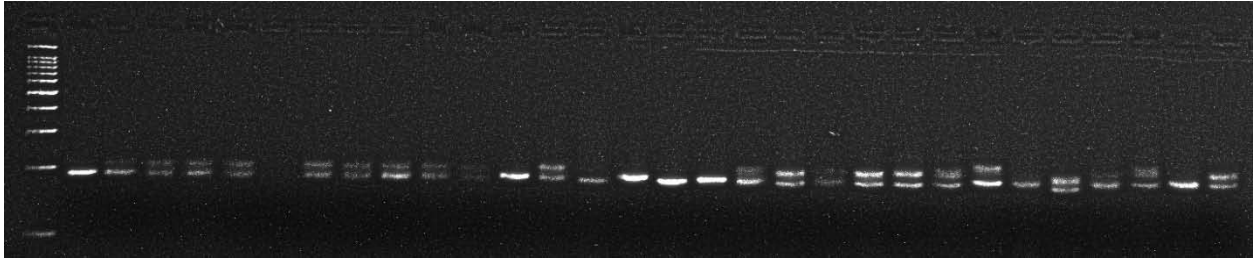


Rhododendron arboretum

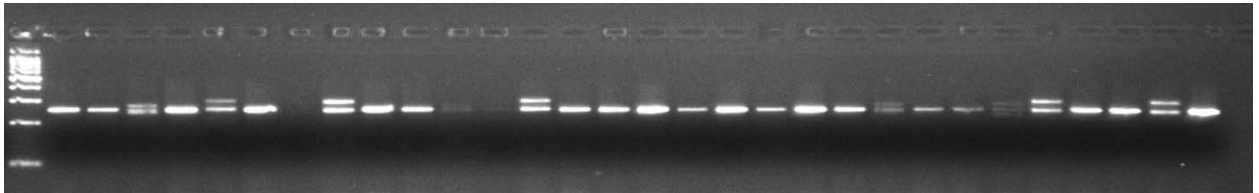


Taxus wallichiana

Fig. Gel photograph showing genomic DNA isolated from *R. arboretum* and *T. wallichiana*



PCR profile of *R. arboreum* genotypes using SSR primer R460



PCR profile of *Q. semecarpifoliagenotypes* using SSR primer CN627959

Fig. SSR genotyping of samples

- a) Genetic diversity analysis **completed in three species viz. *Rhododendron arboreum* (Burans), *Taxus wallichiana* (Thuner), *Quercus semecarpifolia* (Kharsu oak), *Betula utilis* (Bhojpatra), *Myrica esculenta* (Kafal) and genotyping work is progressing in other two species viz. *Taxus wallichiana* (Thuner), and *Betula utilis* (Bhojpatra).**

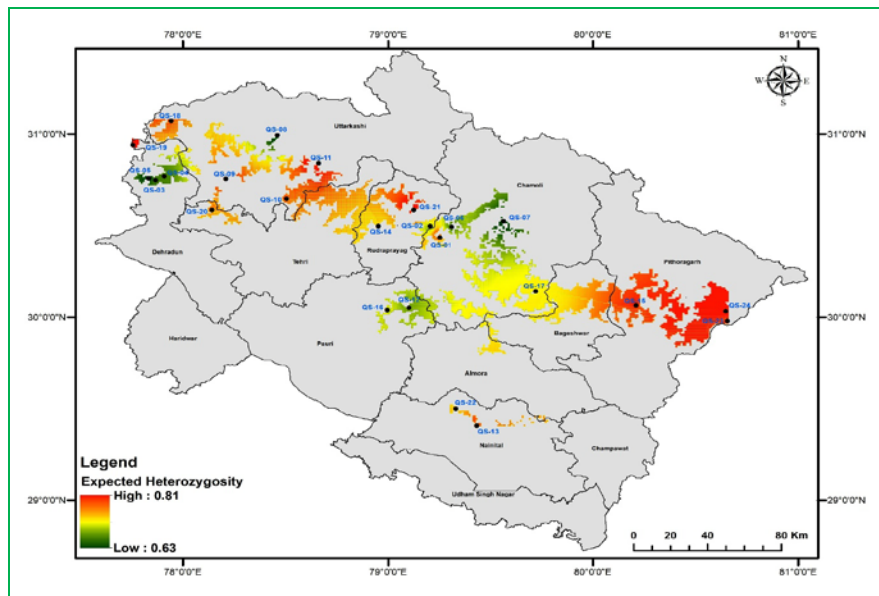







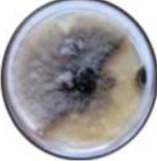









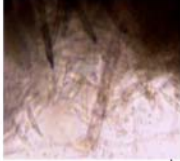


Fig. Spatial distribution of genetic diversity, in *Quercus semecarpifolia* populations for identification of genetic diversity hotspots for conservation and development of in-situ forest field gene banks

b) Diseased survey in selected species and their populations completed in Uttarakhand region. Isolation and identification of the fungal entity and pure fungal cultures were maintained in fungal repository of FRI.

Species	Disease symptoms (Causal fungus)	Fungal morphology (culture and spores)	
RA (a)	Leaf spot (<i>Alternaria</i>) 		
RA (b)	Leaf blight (<i>Colletotrichum</i>) 		
	Leaf blight (<i>Nigrospora</i>) 		
RA (c)	Leaf blight (<i>Gleosporium</i>) 		
RA (f)	leaf blight (<i>Colletotrichum</i>) 		
RA (g)	leaf spot (<i>Colletotrichum</i>) 		

Diseases in *Rhododendron arboreum*

c) Biochemical characterization of the selected species in various population lines has been carried out with respect to the total flavonoid contents (TFCs), total phenolics content (TPCs), Tri-terpenoid Content (TTCs).

C. FGR conservation : likely to be completed

Five priority species were sort listed for FGR Conservation as per the target of the project. The species are *Cinnamomum tamala*, *Diploknema butyracea*, *Rhododendron arboretum*, *Myrica esculanta* and *Taxus wallichiana*. Information on distribution and status of prioritized species has beenextracted through the existing records. Preliminary survey of the selected species for conservation has been for the identification of the source populations for the conservation works.

1. Develop propagation techniques : target 5 species, completed

Propagation techniques have been standardized for *Rhododendraon arboreum*, *Diploknema butyracea*, *Cinnamomum tamala*and *Taxus wallichiana*. Germplasm of these species collected from different populations and propagated in the nursery. Air layering has also proved effective in *Cinnamomum tamala* and *Diploknema butyracea* for their propagation. The source populations were propagated through the standardized methods for the establishment of field gene banks.



Fig. Propagation of *Diploknema butyracea* through seeds as well as cuttings has been standardised



Cinnamomum tamala

Taxus wallichiana

Fig. Successful air layering in *Cinnamomum tamala* Successful rooting in cutting of *Taxus wallichiana*

2. Establishment of Field Gene banks : Target 10 species, likely to be completed

The ex-situ field gene banks of six prioritized species viz. *Diploknema butyracea*, *Cinnamomum tamala*, *Myrica esculenta*, *Taxus wallichiana*, *Rhododendron arboretum*, and *Toona ciliata* has been established at different sites where the land was provided by Uttarakhand Forest Department. In-situ field gene banks of four species will be demarcated in existing forest areas based on genetic diversity results.

D. Notification for Centre of Excellence

A Centre of Excellence on Forest Genetic Resources (CoE-FGR) was created in FRI in the year 2016 involving the existing strengths of scientists. Proposal for the notification of Centre of Excellence on Forest Genetic Resources already submitted to DIG (RT), MoEF&CC vide letter no. 3-11/ICFRE(R)/RP/FGRMN/ PartII/407 dated 21-07-2020.



Diploknemma butyraceae



Myrica esculenta

Established Ex-situ Field Gene Banks

Financial Progress (Rs. in lakh)

Budget Outlay	Total released	Total Utilization (as on date)	Balance
861.20	861.20	810.00 <i>(Rec. 600 + Non R. 200)</i> approx.	51.20

*CA audited Utilization Certificate and the Progress reports up to March 2021 already submitted to MoEF&CC

