NMHS Progress Report

(Period *from* ...April 2017..... *to* .....March 2018.....)

1. Project Information

Project ID:	**NMHS/SG-2016/011**	**Sanction Date:**	**31st March,2016**

Project Title:	**Conservation strategies for *Taxus wallichiana* and *Ulmus wallichiana* by DNA markers and geospatial technologies.**
BTG:	
PI and Affiliation (Institution):	**Dr. Pankaj Bhardwaj, Asst. Professor, Centre for Plant Sciences, Central University of Punjab, Bathinda.** Email: pankajihbt@gmail.com Phone No. 9501686709
Name & Address of the Co-PI, if any:	**Dr. Puneeta Pandey, Asst.Professor, Centre for Environmental Sciences and Technology, Central University of Punjab, Bathinda** Email: puneetapandey@gmail.com

Structured Abstract - detailing the current year progress [Word Limit 250 words]:	The SSR markers were mined from the denovo assembled transcriptome. The SSRs were *insilico* characterized for the repeat types and distribution. CDS region was investigated for tri repeats and possible codons, the amino acid type they specify and the usage bias in the codons. After positional distribution of SSRs, majorly, primers were designed from the SSRs at 5'UTR and synthesized. The characterization of the synthesized primers so far have led to the identification of 9 polymorphic and 16 monomorphic loci in *T. wallichiana*. The marker characterization will take about 2 months and the polymorphic markers will be applied in population and landscape genetic analysis. Sampling for DNA isolation was accomplished from Western Himalayas, while from eastern Himalayas, sampling is in progress. The geospatial mapping is in progress.

	Project Partner Name	Affiliations	Role & Responsibilities
1	**Dr. Pankaj Bhardwaj**	**Centre for Plant Sciences, School of Basic and Applied Sciences, Central University of Punjab, Bathinda**	Sample collection and nucleic acid isolation Transcriptome sequencing for *T. wallichiana* and *U. wallichiana* Assembly, annotation, Prediction of microsatellites, primer-designing and Characterization on the populations
2	**Dr. Puneeta Pandey**	**Centre for Environmental Science and Technology, Central University of Punjab, Bathinda**	Micro-level and macro-level spatial mapping of *T. wallichiana* and *U. wallichiana* Change detection studies to ascertain the changes in vegetation pattern and temperature variations in the last 40 years.

2. Project Site Details

Project site	Central University of Punjab, Bathinda
IHR states covered	
Lat./Long.	
Sitemaps	
Site photographs	

3. Project Activities Chart w.r.t. Timeframe [Gantt or PERT]

PROJECT ACTIVITIES	WORK UNDERTAKEN April 2017 to March 2018 Qtr 1	Qtr 2	Qtr 3	Qtr 4	OUTPUT
Sampling	Samples for isolation of RNA for *Ulmus wallichiana* was collected from Rajori J&K under senescent condition				RNA sample under senescent condition for Ulmus and under summer condition for Taxus.
RNA sequencing, Assembly and Functional Annotation	Sequencing was performed at Genotypic Technology Pvt. Ltd, Bangalore	Raw reads were processed and denovo assembled.	Functional annotation was carried out using Annocript and Trapid.	Identification of SSRs containing sequences using MISA and Primer designing was carried using BatchPrimer3 200 Primers synthesized. Characterization of the synthesized primers is in progress. So far we have identified 16 monomorphic and 9 polymorphic SSRs in *T. wallichiana.*	7041 SSRs for Taxus and 16570 SSRs for Ulmus. 9000 designed primers for Ulmus and 4958 designed primer pairs for Taxus. 9 Polymorphic SSR identified for *T. wallichiana*.

				Sampling from Eastern Himalayas is in progress. Geospatial mapping is in Progress.	

4. Financial and Resource Information

Total Grant:	34,94,600.00	Grant Received Date:	709115.00

Project Partner(s)	Affiliations/ Institution	Budget Allocated to	Work Done
Dr. Puneeta Pandey	**Centre for Environmental Science and Technology, Central University of Punjab, Bathinda**	**Dr. Puneeta Pandey**	Micro-level and macro-level spatial mapping of *T. wallichiana* and *U. wallichiana*

Project Staff Information:

S. No.	Name	Qualification	Designation	Fellowship/wages paid	Remarks
1.	Amandeep Singh	M.Sc., M.Phil.	JRF	Rs. 16750 pm	

5. Equipment and Asset Information

S. No.	Equipment Name (Qty)	Details (Make/ Model)	Cost	Date of Installation	Photographs of Equipment	Lowest Quotation, IF NOT purchased
1.						
2.						
3.						
[Add]						

6. Expenditure Statement and Utilization Certificate

Please update the annual Expenditure Statement and Utilization Certificate (UC) periodically.

Expenditure Information:

S. No.	Financial Position/Budget Head	Funds Sanctioned	Expenditure	% of Total cost
I	Salaries/Manpower cost	330000.00	100500.00	32%
II	Travel	100000.00	71000.00	71%
III	Expendables &Consumables	300000.00	149772.00	49%
IV	Contingencies	30000		
V	Activities & Other Project cost			
VI	Institutional Charges			
VII	Equipment's			
	Total	709115.00		
	Interest earned			
	Grand Total	709115.00		

Period	Expenditure Statement	Utilization Certificate (UC)
Annual		

7. Project Beneficiary Groups

Beneficiary Groups [Capacity Building]	Target	Achieved
No. of Beneficiaries with income generation:		
No. of stakeholders trained, particularly women:		
No. of capacity building Workshops/ trainings:		
No. of Awareness & outreach programmes:		
No. of Research/ Manpower developed:		

8. Project Progress Summary (as applicable to the project)

Description	Total (Numeric)	Description
IHR States Covered	North Western states have been covered. Sampling from Eastern Himalayas is in progress.	
Project Site/ Field Stations Developed:		
No. of Patents filed (Description):		
Article/ Review/ Research Paper/ Publication:		
New Methods/ Modellings Developed (description in 250 words):		
No. of Trainings (No. of Beneficiaries):		
Workshop:		
Demonstration Models (Site):		
Livelihood Options:		
Training Manuals:		
Processing Units:		
Species Collection:		
Species identified:		
Database/ Images/ GIS Maps:	Attached file gives complete description of the work done.	

Note: Photos/ maps should be attached in high quality in compatible formats *viz*., JPEG, .JPG, .PNG, .SHP, etc. along with a suitable figure legend/ caption.

9. Project Linkages (with nearby Institutions/ State Agencies)

S. No.	Institute/ Organization	Type of Linkages	Brief Description

10. Additional (publication, recommendations, etc.)

Time Period	Publications (Research Papers, Information Material, Policy drafts, Patents, etc.)
Annual [Year .....]	[Attach]

11. Project Concluding Remark

Kindly update the following Progress Parameters for the Reporting Period:

Project Objectives	Project Output against each objective	Progress made against Monitoring Indicators (specified in Sanction Letter)	Remarks
Sample collection and nucleic acid isolation	RNA	Samples for isolation of RNA under senescence for Ulmus and Summer conditions for Taxus was carried out. Data processed, functional annotation done, SSRs identified and Primers designed for both Ulmus and Taxus. SSR primers synthesized.	
Transcriptome sequencing for *T. wallichiana* and *U. wallichiana*	Sequencing done at Genotypic Technology pvt. Ltd. Bengaluru	Characterization is ongoing. 9 polymorphic and 16 monomorphic loci identified.	

Methodology in brief	Samples for RNA isolation were collected from Saloni, Himachal Pradesh in liquid nitrogen. RNA was isolated using CTAB method with some modifications. Sequencing was performed on Illumina HiSeq 2000 platform. The raw reads were processed by using Trimmomatic to remove adapter sequences and low quality bases. The cleaned reads were denovo assembled using Trinity followed by removal of sequence redundancy and generation of unigenes using CD-HIT at 95% sequence identity threshold. The completeness of the assembly was analysed using BUSCO version 2. Further, the raw reads were mapped back to the assembly using Bowtie2 for quality assessment. The non-redundant assembly was annotated by using the annotation pipeline Annocript. Further, the transcripts were assigned to gene families using the pipeline TRAPID. For the identification of transcription factors, PlantTFDB was used. SSRs were identified using MISA and primers were designed using

	BatchPrimer3. Positional distribution of the SSRs in the transcripts was analysed by predicting the ORFs from the SSR containing sequences using orfPredictor (Min et al, 2005) followed by correlating the SSR start and end positions with the start and stop positions of the predicted ORFs. The primers were designed by BatchPrimer3 and synthesized. The synthesized primers were characterized for their polymorphic value through UREA PAGE on 30 genotypes.
Major Achievements	• SSRs identified • Primers designed • Primers Synthesized • Characterization of SSRs (inprogress) • 9 polymorphic loci identified.
Brief conclusion, current year progress- during the reporting period (point wise)	We successfully sequenced transcriptomes of Taxus and Ulmus and screened them for SSRs containing sequences. We identified the SSR regions and designed primers for them. Further, we functionally annotate the transcriptomes of both species. The designed primers were successfully synthesized and characterized on 30 genotypes through UREA PAGE. So far 9 polymorphic loci have been identified.
Progress achieved (%)	
Remaining work to be done	• Marker characterization (partially). • Marker application in Population and Landscape genetic analysis. • Geospatial mapping.