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PC-I

STUDIES ON DNA FINGERPRINTING OF IMPORTANT PAKISTANI TIMBERS FOR THEIR IDENTIFICATION AND ILLEGAL TRAFFICKING CONTROL

ADP No: 541
ADP Code: 220063

Total Cost: Rs. 80.000 million
(Duration: July 2022 to June 2027)

Pakistan Forest Institute, Peshawar
Forestry Environment & Wildlife Department
Government of Khyber Pakhtunkhwa
August, 2022

GOVERNMENT OF PAKISTAN

PLANNING COMMISSION

PC-1 FORM

(SOCIAL SECTORS)

1.	**Name of the Project**:	"Studies on DNA Fingerprinting of Important Pakistani Timbers for their Identification and Illegal Trafficking Control"
2.	**Location**:	Pakistan Forest Institute, Peshawar
3.	**Authorities Responsible for:**	
	i. Sponsoring:	Forestry, Environment & Wildlife Department, Khyber Pakhtunkhwa
	ii. Execution:	Pakistan Forest Institute, Peshawar.
	iii. Operation and Maintenance:	Pakistan Forest Institute, Peshawar.
	iv. Concerned Federal Ministry	N. A

4. **(a)** **Plan provision:** The project is included in ADP 2022-23 under **Serial No.525** and **Code No.220063**

(**b)** Provision for the current year PSDP/ADP: **Rs. 20.000** million.

5. **Project Objectives and its Relationship with Sector Objectives:**

The project has been designed for achieving the following planned objectives:

i. To establish a Forestry Molecular Genetics Lab. at PFI or execution of the envisaged project activities and to combat future challenges of forest resource conservation.

ii. To study molecular identification of important conifers tree species of dry temperate forests.

iii. To develop and establish a DNA barcode library for tracing the origin of illegal timber trafficking.

iv. To preserve gene pool of the studied species for future research work.

v. To build capacity of the researchers and students in the established technology through trainings/workshops/seminars.

Relationship of the Project with the Objectives of the Sector

Recent environmental catastrophes are the hall mark impact of climate change and global warming. Forestry sector of the province is mandated to devise a viable strategy for combating impact of climate change through development of adaptive mechanism and mitigating measures as a tandem approach. Being an active organ of the sector, Pakistan Forest Institute is mandated for Development and Sustainable Management of Forests and allied Natural Resources through Research, Education and Training. The project is of great national importance to promote forestry and allied disciplines research and education and is in line with the National Forest Policy, 2015. It will not only help to control the illegal logging / trafficking of important Pakistani timbers but will also ensure the sustainable conservation of the forestry resources and will help to bridge up the gap in supply and demand of forest products/ services aforementioned problems.

6. Description/Justification and Technical Parameters

6.1 Description

Pakistan is a forest deficit country with forest cover much lesser than the recommended forest area for the economic development while its population continues to grow rapidly at an alarming pace. The key contributors to the decline in forest area include illegal logging, greater rotation period, increased demand of forest products by increasing population, quality issues of forest products, biotic & a-biotic stresses, attack of pathogens, market devaluation global warming and prevailing water crises in the country, socio economic & exogenous factors and managerial decisions etc. Globally millions of hectares of forest are lost every year and this rate is alarmingly high in Pakistan. The major causes are overutilization and illegal logging of natural forest being utilized to meet local demand for energy and timber wood. With the present rate of deforestation Pakistan is expected to lose most of its natural forests during next few years. Beside forest loss and species extinction, deforestation has serious implications on global warming and climate change.

Illegal logging of high valued coniferous species like Deodar (*Cedrus deodara*) and Kail (*Pinus wallichiana*) is a major problem in state owned forests especially in the Distt's. of Chitral and Kohistan of Khyber Pakhtunkhwa and is claimed to be imported from across boarder i.e., Afghanistan or transferred from areas of Gilgit Baltistan. Currently most practical checks being used in the field to monitor the legal

vs illegal timber are still not tamper – proof. Thus, a number of disputes could not be settled or pending due to poor or absence of proper identification system of timber logs. All of these results into major economic losses, affecting wood sustain supply, wood prices as well as severely hampering the forest conservation and management.

Therefore, there is a dare need to introduce such a technology like testing at molecular level into the chain of custody which will not only an effective measure to deter fraud, cutting off illegal supply channels, but also to protect the important forest tree species, and increase transparency in the supply and demand services.

6.2 Justification

Implementing the timber identification methods will help differentiating legal vs illegal log thus will help establishing a framework to fight and regulate illegal logging. Therefore, it is imperative to develop timber identification methods which are difficult or impossible to alter and can be used to submit material evidence against the culprits. Currently a number of academic institutions are conducting the research in the field of molecular biology but lacking focus on forest tree species for such like issues.

Forest Products Research Division and Biodiversity Research Division at Pakistan Forest Institute are conducting research in the field of Wood Sciences and Technology and Forest Tree improvement. The researchers of these divisions also serve as faculty member and supervise the students in thesis work. These divisions also provide facilities of wood testing, supply of quality seed and planting stock to public and private organizations.

Each year Forest products research division deals a number of queries received from public and private organizations regarding wood identification, wood moisture contents, physico-mechanical properties and many mores' properties. Wood anatomy Lab. at this division has a plentiful collection of wood specimen (more than 2500) from 30 countries of the globe. This lab. is carrying out research on anatomical properties of locally grown wood species, compile the basic data and assess their technological properties helpful in improving quality of the wood before utilization. This lab. is also providing services to public and private organizations regarding wood identification of both local and imported timbers.

Likewise, Forest Genetics Branch of Biodiversity Research Division is dealing to improve the productivity of forest species, by studying the population genetics, inter specific & intra specific diversity and genetic parameters such as heritability, genetic

advance and correlations between productivity and adaptability-related traits. This branch has also been mandated to conduct the biotechnological research at molecular level with its application in tree breeding and improvement programs to conserve the forest resources meet the related challenges.

Keeping in view the importance of titled project, Forest Products Research Division and Biodiversity Research Division at Pakistan Forest Institute intend to undertake this initiative of conducting research on this scenario. The financial cost for achieving the objectives of the project cannot met out of the financial resources of Pakistan Forest Institute and therefore it is plausible that the project may be allocated financial resources from ADP.

6.3 Technical Parameters

DNA fingerprinting is a technology used to identify a species using different molecular techniques like RAPD, RFLP, PCR, STR and AFPL analysis etc. This technology also contributing to the monitoring of timber trade and the conservation of forestry resources. Wood identification is extremely important in the modern forest industry and to control the illegal timber trafficking. It also has significant applications in forensics, archeology and ecological research. The most classical and traditional method of wood identification is comparing anatomical features, but a significant drawback associated with anatomical identification is resolution i.e., closely related species have similar or even identical structures which hindering species identification. Further, wood anatomy changes with local environmental condition and therefore it cannot give information about the wood's geographic origin. The advancement of modern molecular biology techniques has significantly improved our ability to accurately identify a wood species as trees exhibit a high level of genetic diversity that can be used to trace timber and even wood products to the population of origin. Currently, tests based on DNA identification method are extensively being used for species identification (DNA barcoding), for verification of place of origin, either at the regional scale (through phylogeographic methods) or concession (population genetic assignment), and for tracking individual logs or wood products (DNA fingerprinting). Therefore, development of fool proof technology based on DNA fingerprint which will be impossible to tamper with, will be a significant improvement over conventional paper-based methods to trace logs back to a legal or sustainable source. DNA fingerprinting is powerful enough that it can successfully differentiate between individual trees that were originated from the same

concession or even from neighboring stumps. It is based upon methods already being used in criminal forensics and paternity testing.

a) Establishment of Forestry Molecular Genetics Lab. at PFI

Pakistan Forest Institute, Peshawar (PFI) is conducting research on various field of forestry and allied disciplines. But since its establishment, there felt a need for the establishment of Biotechnology Lab. to combat the changes of modern era research like molecular identification of trees upto species level for controlling illegal logging and trafficking of important timbers, molecular identification of medicinal plants and their DNA barcoding, availability of gene pool for future tree improvement by adopting tissue culture and DNA recombinant techniques, knowledge of genetic diversity for assessing resilience of studied tree populations to climate change and the availability of database for policy makers regarding forest conservation and management. It is therefore, the first target of this project is set to establish Biotechnology Lab. for the execution of project activities and also to face the future challenges of forest resource conservation.

b) Molecular Identification of Focal Tree Species

i) Field survey

For molecular identification of important coniferous species, research material in the form of wood cores/leaves will be collected from the dry temperate and moist temperate forests of Khyber Pakhtunkhwa. A team including of officers and an expert with supporting staff (3-4) and a driver for which TA/DA will be needed for field survey. PFI vehicles available in the pool are too old (obsolete) and consumed an average of 05-07 km/litter, so required POL will be arranged accordingly. For routine project activities POL will also consumed from this head.

The possible identified areas and species are given as below:

Focused Geographical areas and species.

Forest Types	**Suggested Forest areas**	**Focal Species**
Dry Temperate Forests	Chitral Forest Division	*Cedrus deodara*, *Pinus wallichiana*
	Upper Kohistan Forest Division	*Cedrus deodara*, *Pinus wallichiana*

Samples in the form of wood and leaves will be collected at DBH (Diameter at Breast Height) from healthy trees with the help of Trephor/ increment borer and the holes will be sealed with paraffin wax to avoid infection/ entry of insect. Both cores and leaves will be put into a plastic cover and immediately placed into dried silica gel. After the arrival to the laboratory, the samples will be cleaned and kept for drying at least 3 to 4 days with the change of silica gel which will absorb all moisture content of the samples and these samples will be stored at − 20 °C.

Field Samples intensity for Molecular Studies

S. #	Name of Species	Geograp hical locations	Populations per geographic. location	Each Population				Total No.
				Point-1	Point- 2	Point -3	Point -4	
1.	*Cedrus deodara*	02	4-6	10-15	10-15	10-15	10-15	400-600
2.	*Pinus wallichiana*	02	4-6	10-15	10-15	10-15	10-15	400-600

Note: The number of populations, points and samples may vary in view of availability.

ii) Lab. Activities

Various protocols available will be utilized for isolation total genomic DNA from samples. These protocols will be used *per se* or modified slightly for obtaining quality DNA for PCR. Alternatively, commercially available DNA extraction kits for woody plants may be used. Quantity of extracted DNA will be verified by nanodrop at 260 and 280 nm wavelength and quality as well as suitability for sequencing and barcoding will be determined through gel electrophoresis.

For PCR amplification, a number of primers (e.g., RbcL, matK, and trnH-ITS, SC10-02, SC 10-09, OPA-08, OPA-09, OPA-18 and OPA-19 etc.) will be commercially synthesized and used accordingly. The amplified products will be subjected to gel electrophoresis for scoring of strong visible bands. The amplified bands from each sample will be scored and recorded. PCR amplified products will be purified using PCR purification kits.

c) Development of DNA Barcode Library

Sequencing of the purified PCR products will be carried out. Sequencing of DNA samples will be processed either through open tenders or by signing MoU with well renowned institutions like Centre of Advanced Molecular Biology (CAMB), Lahore. US$15-20/sample will be paid for sequencing. The charges may vary depending on the type of sequencing.

Database search will be carried out using the nucleotide database of the US National Center for the Biotechnology Information (NCBI, www.ncbi.nlm.nih.gov) or the Barcode of Life Database (BOLD, http://www.boldsystems.org/) to identify homologs of the related genes. Gene sequences from primers (e.g., ITS-trnH and rbcL/ others) will be submitted to NCBI sequence data submission bank (http://www.ncbi.nlm.nih.gov/genbank./) to obtain accession numbers. The provenance specific barcode primers from the sequenced data of primers (e.g., ITS-trnH and rbcL/ others) will be designed by Primer3 version 0.4.0 software online (http://bioinfo.ut.ee/primer3-0.4.0).

Multiple sequence alignment comparison will be brought out place of origin identification amongst the studied sites. Nucleotide base FASTA files of all the species will be aligned through online European Bioinformatics Institute (EMBL-EMBI) (Clustal Omega; http://www.ebi.ac.uk/ Tools/msa/clustalo/) program. The nucleotide sequences of primers (e.g., ITS-trnH and rbcL/ others) will be aligned separately and analyzed. Barcode construction for primers (e.g., ITS-trnH and rbcL/ others) region of each selected species will be done.

d) Gene Pool for Future Research

The extracted DNA from the studied species and targeted sites of Khyber Pakhtunkhwa will be preserved for future research like tree improvement, genetic mapping, genetic diversity etc. For the long-term preservation of extracted DNA, standard lab. protocols/procedure will be adopted e.g., storage at -80C^{0} etc.

e) Capacity Building

To execute the project activities in field and Lab., relevant officers of the divisions/ PFI will be trained from the local institute(s) dealing with molecular/ Biotechnology. The concerned officers have gained Basic knowledge through their program but there is quite dearth to have latest knowledge about the use of updated equipment and updated technology particularly applicable in forestry. Trainings will be acquired in Lab. set up and protocols, DNA extraction, PCR amplification, DNA Sequencing, DNA Barcoding etc. from local research organization(s) like

1. Centre of Advanced Molecular Biology (CAMB)-Lahore
2. Alpha Genomics Lab., Islamabad
3. Tree Biotechnology Lab, Department of Forestry and Range Management, University of Agriculture, Faisalabad etc.

Similarly, to create awareness and importance of this new field of study among the Forest scientists, researchers and students who want to conduct research work in this field, trainings/seminars/workshops will be arranged.

7. Capital cost Estimates

a. Local Cost Rs. 80.000 million

b. Foreign Exchange: Nil

c. Total Rs. 80.000 millions

Date of estimation of project cost: 1st August, 2022

8. Annual Operating and Maintenance cost after Completion of the Project

Annual operating and maintenance cost will be met from the regular budget of the Pakistan Forest Institute, Peshawar after completion of the project. Biodiversity Research Division and Forest Products Research Division will be responsible for operation and maintenance of the training and research activities after completion of project.

9. Demand and Supply Analysis

N.A

10. Financial Plan

Source of Financing: Government of Khyber Pakhtunkhwa

Mode of Financing: Forestry Environment & Wildlife Department, Government of Khyber Pakhtunkhwa through ADP.

11. Project Benefits and Analysis

i) Financial

After completion, following facility will be provided to research organizations, students, and wood-based industries as per SOPs.

S.No.	Name of suggested Facility	Tentative Fee/Charges
1.	DNA Extraction	5000/sample
2.	*DNA Amplification	7000/sample
3.	**Molecular Identification of wood species	10,000/sample
4.	***DNA Barcoding	15,000/sample

Note: The fee charged will be consumed as; 80% recycled and 20% workshare.
* Excluding Primer (s) charges only DNA amplification.
** Molecular identification from the available data base.
***Without S.No.1,2&3.

ii) Social benefits with indicators

The project will help to develop interactions among the researchers of Pakistan Forest Institute and other organizations of the concerned department. This will

improve social linkages and ultimately promote the collaborations and technology transfer among PFI and other research community.

iii) Employment generation (direct and indirect)

Through this project employment opportunities will be created for daily labor working in the research labs and in the project area and the persons involved in the proceeding of the procurement of lab. items.

Project Internees Description

Internee	No.'s	Qualification	Job
Research Assistant	01	B.S. Biotechnology	Research Work in laboratory & field

vi) Environmental impact

The project will have positive impacts on the environment as it will provide research inputs for the awareness of researchers, scientists, and policy maker about the impact of illegal logging and trafficking and will help them to prepare for the action of climate change adaptation and climate change mitigation in future. It will also provide research inputs for management and conservation of forest in the scenario of climate change.

v) Impact of delays on project cost and viability

All the project activities are subject to inflation rate. The cost of the project is expected to increase at least by 15% in case of delay and the fulfillment of project objectives will be hampered.

12. Implementation schedule

S. No	Activity	2022-23	2023-24	2024-25	2025-26	2026-27
i.	Purchase/Procurement of Instruments/equipment, Tools etc., for establishment of Biotechnology Lab.					
ii.	Purchase of Chemicals/glassware					
iii.	Survey of field for population identification and sample collection					
iv.	Molecular identification of focal species					
v.	Establishment of Gene pool					
vi.	Development of DNA barcode library					
vii.	Analysis of data.					
viii.	Arrangements of trainings/Seminar/ workshop.					
ix.	Writing of final report.					

b) Result Based Monitoring (RBM) Indicators.

Input	Output	Outcomes		
		Baseline indicator	**Targets after completion of project**	**Targeted impact**
Installation of equipment/instruments /tools etc.	Establishment of Biotechnology lab.	1. Facilities for research at molecular level, inter-specific and intra- specific identification. 2. Studies on diversity of focal species amongst geographical regions, inter populations and intra population. Latest technologies (DNA Barcoding) will be available for molecular identification of important wood upto species level which in turn will help in illegal logging and trafficking.	Facility will be utilized in future for tree improvement, gene mapping, genetic diversity, forest conservation, education, and industrial needs.	Research organizations, Forest Deptt., and academia will be benefitted from the technology in terms of research, forest management, quality monitoring and education respectively.
Research activities.	1. Molecular identification of focal species 2. Availability of DNA barcodes for tracing and controlling illegal timber trafficking.	1.Database regarding DNA fingerprint, DNA barcodes and 2. Knowledge of genetic diversity 3. Availability of gene pool for future tree improvement.	1. Solution of illegal logging and trafficking of focal species at studied sites. 2. Availability of database for policy makers regarding forest conservation and management. 3.To study resilience to climate change of tree species.	Identification of species and origin of transport will help to control illegal cutting and transport of important tree species. Database of the studied species will invite the interest of scientific community in the related field of research.
Capacity building/ Trainings	Capacity building of Forest scientists, researchers, and students.	Improved efficiency/ working of the personals.	Enhancement of skill of personals working in the relevant field.	More scientists/researchers will be interested and involved to conduct research and combat with the illegal logging and trafficking issues.

1. **Management structure and manpower requirements including specialized skills during construction and operational phases.**

The project will be implemented under the overall administration of the Director General, Pakistan Forest Institute, Peshawar. The existing research and technical staff and vehicles of PFI will be utilized for the implementation of the Project. Project allowance will be renumerated to officers/officials (3-4) directly engaged in project activities. A 20% of basic pay will be paid (not exceeding Rs.12000/-) per month. Moreover, the Project In-charge/ Principal Investigator of project will be responsible for timely completion of all the project activities. Forest Products Research Division and Biodiversity Research Division will execute the research activities in a joint action in equal proportion of resources, activities and responsibilities. The project activities will be managed by Forest Geneticist (Biodiversity Research Division) as Project In charge. A committee will be constituted by the Director General, Pakistan Forest Institute for the procurement of equipment/instruments/tools/chemicals etc. Monitoring and evaluation of the project will be carried out by the M&E committee constituted by the DG, PFI.

2. **Additional projects/decisions required to maximize socio-economic benefits from the proposed project.**

   For the establishment of biotechnology Lab. and basic operational guidance, expertise of following scientists will be honored as co-investigator/academic collaborator.

   1. Dr. Kafeel Ahmad, Assistant Professor from Centre of Biotechnology and Microbiology (COBAM), University of Peshawar.
   2. Mr. M. Asif Javeed, PhD (Forest Genetics and Molecular Tree Biotechnology) Assistant Professor, Forestry & Range Management Department, University of Agriculture, Faisalabad.

15. Certified that the project proposal has been prepared on the basis of Instructions provided by the Planning Commission for the preparation of PC-I for Social sector projects.

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