Status of Perennial Tree Germplasm Resources in India and their Utilization in the Context of Global Genome Sequencing Efforts

Kunasekaran Rajarajan, Alka Bharati, Hirdayesh Anuragi, Arun Kumar Handa, Kishor Gaikwad, Nagendra Kumar Singh, Kamal Prasad Mohapatra, Puran Chandra, Sangram Bhanudas Chavan, Lal Chand, Veena Gupta, Ravinder Singh Dhillon, Chhavi Sirohi, Fatima Shirin, Sengodan Saravanan, Kundapura R Ravishankar, Murugan Sankaran, Harsh Mehta, Govindarajan Venkatesh, Archana Verma, Rajwat K Kalia, Modhumita Ghosh Dasgupta, Ramasamy Yasodha, Harish Singh Ginwal, Rupesh Deshmukh, Surendra Kumar Malik, Suryanarayan Bhaskar, Altafhusain Nadaf, Devashree Nayak, Shiv Kumar Dhyani, Prasad Suresh Hendre, Javed Rizvi

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The Organizations

The Central Agroforestry Research Institute (CAFRI) is a national institute and India's only dedicated research institution on agroforestry under the umbrella of the Indian Council of Agricultural Research (ICAR). It promotes and works on improving the quality of life of rural people through integration of perennials in agricultural landscapes for economic, environmental and social benefits.

The Indian Council of Agricultural Research (ICAR) is the research wing of the Ministry of Agriculture & Farmers Welfare, Government of India. It harnesses the power of science and innovation for food security, food safety and farmer's prosperity, as well as enhances the natural resource base to promote inclusive growth and sustainable development.

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LIST OF ABBREVIATIONS AND ACRONYMS

ACC	Accession
AF	Agroforestry
AFLP	Amplified fragment length polymorphism
AGL	Agamous-Like
AICRPAF	All India Coordinated Research Project on Agroforestry
AMOVA	Analysis of Molecular Variance
AOCC	African Orphan Crops Consortium
AREB	Absciscic acid Responsive Element-Binding protein
ARF	Auxine Responsive Factor
ARM	Armadillo
BAC	Bacterial Artificial Chromosome
BGI	Beijing Genomics Institute
BI	Bax Inhibitor
CAFRI	Central Agroforestry Research Institute
CAP	Citrus Annotation Project
CC	Coiled coil
cDNA	Complementary DNA
CeSA	Cellulose synthase
CGIAR	Consortium of International Agricultural Research Centres
Chi1	Chitinase1
CIMAP	Central Institute of Medicinal and Aromatic Plants
Cp	Chloroplast
cpDNA	Chloroplast DNA
cpSSR	Chloroplast Simple Sequence Repeats
CPT	Candidate Plus Trees
CRIDA	Central Research Institute for Dryland Agriculture
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
CSIR	Council for Scientific and Industrial Research
CSO	Clonal Seed Orchard
DAMD	Directed Amplification of Minisatellite DNA
DArT	Diversity Arrays Technology
DBBT	2-Debenzoyl-7,13-diacetylbaaccatin III-2-O-benzoyl transferase

DEGs	Differentially expressed genes
DEmiRs	Differentially Expressed miRNAs
DNA	Deoxyribonucleic Acid
DPPH	1,1-diphenyl-2-picrylhydrazyl
DREB	Dehydration Responsive Element-Binding protein
DUS	Distinctness Uniformity and stability
EMA	Enzymatic Maceration and Air Drying
EMS	Ethyl Methane Sulphonate
ERF	Ethylene Responsive Factors
ESTs	Expressed Sequence Tag
EYE	Embryo Yellow
F5H	Ferulate 5-Hydroxylase
FAO	Food and Agriculture Organization of the United Nations
FISSR	Fluorescent-ISSR
FMCG	Fast-Moving Consumer Goods
FRAP	Ferric Reducing Antioxidant Power
FRI	Forest Research Institute
FT	Flowering locus T
GABA	Gamma Amino Butyric Acid
Gb	Giga bases
GBS	Genotyping-by-sequencing
GC	Guanine-Cytosine
GC-MS-FID	Gas Chromatography–Mass Spectrometry– Flame Ionization Detection
GI	Gigantea
GLP	Germin Like Protein
GO	Gene Ontology
GRAS	Gibberellic Acid Insensitive (GAI), Repressor of GA1 (RGA), and Scarecrow (SCR)
GS	Genome Sequence
GS	Genomic Selection
GWAS	Genome Wide Association Studies
HB2	Hemoglobin 2
H _e	Expected Heterozygosity Mean
HiC	Hypermethylated in Cancer

H _o	Observed Heterozygosity Mean
HSP	Heat Shock Protein
HS-SPME	Headspace Solid-Phase Microextraction
HT	Total heterozygosity
HUL	Hindustan Unilever Limited
IARI	Indian Agricultural Research Institute
ICAR	Indian Council of Agricultural Research
ICFRE	Indian Council of Forestry Research and Education
ICGC	International Citrus Genome Consortium
ICRAF	World Agroforestry
IFGTB	Institute of Forest Genetics and Tree Breeding
IIHR	Indian Institute of Horticultural Research
InDels	Insertion Deletions
IR	Inverted Repeat region
ISSH	Interspecies Suppression Subtractive Hybridization
ISSR	Inter Simple Sequence Repeat
ITS	Internal Transcribed Spacer
JMV	Jatropha Mosaic Virus
KASP	Kompetitive Allele Specific PCR
Kb	Kilo base
LEA	Late Embryogenesis Abundant
LSC	Large Single-Copy region
MAPKs	Microtubule Associated Protein Kinase
MCSU	Molybdenum Cofactor Sulfurase
miRNA	micro RNA
MNC	Multinational Company
MPTS	Multi-Purpose Tree Species
MSL	Mean Sea Level
mtDNA	Mitochondrial DNA
MYB	Myeloblastosis
NABI	National Agri-Food Biotechnology Institute
NBPGR	National Bureau of Plant Genetic Resources
NCBI	National Centre for Biotechnology Information

NCU	Neem Coated Urea
NEB	N-Ethylbuphedrone
NGO	Non-Governmental Organization
NGS	Next Generation Sequencing
NRM	Natural Resource Management
nSSR	Nuclear Simple Sequence Repeats
ONT	Oxford NanoPore Technologies
OP	Open Pollinated
PAM	Protospacer Adjacent Motif
PCA	Principle Component Analysis
PCR	Polymerase Chain Reaction
PEG	Polyethylene Glycol
PIC	Polymorphic Information Content
PIP	Plasma membrane Intrinsic Protein
PISSR	Pongamia Inter-Simple Sequence Repeats
PR	Pathogenesis-Related
q-PCR	Quantitative-Polymerase Chain Reaction
qRT-PCR	Quantitative Reverse Transcriptase PCR
QTL	Quantitative Trait Loci
RAD	Restriction site Associated DNA
RAPD	Random Amplification of Polymorphic DNA
RNA	Ribonucleic Acid
rRNA	ribosomal RNA
SAU	State Agricultural Universities
SBS	Sequencing by Synthesis
SCAR	Sequence Characterized Amplified Regions
SDS	Sodium Dodecyl Sulfate
SGS	Second Generation Sequencing
SMRT	Single Molecule Real-Time
SND	Secondary Wall-Associated NAC Domain
SNP	Single Nucleotide Polymorphism
SNVs	Single Nucleotide Variants
SPME	Solid-Phase Micro-Extraction

SRAP	Sequence-Related Amplified Polymorphism
SRS	Short Read Sequencing
SSC	Small Single-Copy region
SSR	Simple Sequence Repeat
StLFR	Single tube Long Fragment Read
TAT	Taxadienol acetyltransferase
TGS	Third Generation Sequencing
TNPL	Tamilnadu Newsprint & Papers Limited
TPS	Tandem Duplicated Terpene Synthase
tRNA	Transfer RNA
UHS	University of Horticulture Science
USA	United States of America
VND	Vascular-Related NAC-Domain
WIP	Walnut Improvement Program

ABSTRACT

Tree species are characterized by their perennial growth habit, woody morphology, long juvenile period phase, mostly outcrossing behaviour, highly heterozygosity genetic makeup, and relatively high genetic diversity. The economically important trees have been an integral part of the human life system due to their provision of timber, fruit, fodder, and medicinal and/or health benefits. Despite its widespread application in agriculture, industrial and medicinal values, the molecular aspects of key economic traits of many tree species remain largely unexplored. Over the past two decades, research on forest tree genomics has generally lagged behind that of other agronomic crops. Genomic research on trees is motivated by the need to support genetic improvement programmes mostly for food trees and timber, and develop diagnostic tools to assist in recommendation for optimum conservation, restoration and management of natural populations. Research on long-lived woody perennials is extending our molecular knowledge and understanding of complex life histories and adaptations to the environment, enriching a field that has traditionally drawn its biological inference from a few short-lived herbaceous species. These concerns have fostered research aimed at deciphering the genomic basis of complex traits that are related to the adaptive value of trees. This review summarizes the highlights of tree genomics and offers some priorities for accelerating progress in the next decade. We begin by introducing some of the key agroforestry (AF) tree species used in genomic research by various institutions/organizations. The publication then briefly reviews the progress in genomics and other related areas in selected species like *Populus deltoides* (poplar), *Eucalyptus tereticornis* (eucalyptus), *Tectona grandis* (teak), Bamboo species (bamboo), *Dalbergia sissoo* (sissoo), *Azadirachta indica* (neem), *Casuarina equisetifolia* (casuarina), *Leucaena leucocephala* (Leucaena), *Acacia nilotica* (Indian babool), *Prosopis cineraria* (khejri), *Tamarindus indica* (tamarind), *Syzygium cumini* (jamun), *Citrus species* (citrus), *Moringa oleifera* (drumstick) and *Mangifera indica* (mango). Germplasm of these species is maintained in the form of seeds as *in-situ* field genebank collections and also as *ex-situ* seed collections under appropriate conditions largely by various affiliate institutes of the Indian Council of Agricultural Research (ICAR), headquartered in New Delhi, India; Indian

Council of Forestry Research and Education (ICFRE), located in Dehradun, India and various state agricultural universities (SAUs) spread all over India. We describe how the basic genomic-based research has been applied for tree breeding with these available resources. Finally, we conclude by highlighting the potential future direction and expected outcomes of genomic research in these tree species which will provide the necessary tools to elucidate the past evolutionary success history and to understand their future response to environmental changes, thus hastening the deployment of breeding populations that can satisfy major economic and livelihood needs.

Keywords

Agroforestry species, genetic improvement, genome sequencing, national genebank, tree biotechnology, tree genomes, woody perennials

1. INTRODUCTION

Trees are the source of many human needs such as food, fodder, building materials, pulp and paper products, firewood for heating and cooking, medicines and pharmaceutical compounds, and more recently, as a non-conventional renewable bioenergy source. Trees also provide various ecological services such as biodiversity conservation, acting as carbon sinks, climate change adaptation and mitigation, and maintaining water quality. Thus, these trees with diverse functional uses have formed an indispensable part of human life and livelihood from time immemorial. The system where such trees are grown along with field crops on agricultural landscapes is referred to as agroforestry. More precisely, agroforestry is a collective term used to describe land-use systems and technologies where woody perennials (trees, shrubs, palms, bamboos, etc.) are deliberately integrated into the same land-management units with crops and/or animals, in some form of spatial arrangement or temporal sequence. India has a very long historical tradition of agroforestry with important tree species such as *Populus deltoides* (poplar), *Eucalyptus tereticornis* (eucalyptus), *Tectona grandis* (teak), Bamboo species (bamboo), *Dalbergia sissoo* (sissoo), *Azadirachta indica* (neem), *Casuarina equisetifolia* (casuarina), *Leucaena leucocephala* (Leucaena), *Acacia nilotica* (Indian babool), *Prosopis cineraria* (khejri) etc. All these have multiple uses. Besides, horticultural tree species such as *Tamarindus indica* (tamarind), *Syzygium cumini* (jamun), *Citrus* species (citrus), *Moringa oleifera* (drumstick) and *Mangifera indica* (mango) are used as fruits, vegetables and/or spices and condiments. Lately, they have been referred to as 'food trees'. Species such as poplar, eucalyptus, melia, casuarina and leucaena (*L. leucocephala*) are the major industrial species mainly used in the timber, paper and pulp industry.

Tree improvement has generally been lagging in comparison to annual field crops due to inherent biological limitations such as very long seed-to-seed generation cycle, slow growth and delayed economic returns, high heterozygosity, high inbreeding depression and under-studied floral and reproductive biology. Genomics and genomics-assisted

crop improvement and selection are promising tools which offer a great opportunity to tackle some of these issues. They could result in expediting genetic gains, high returns on investment, faster upscaling and turn-around time for varietal development, and also enhance understanding of basic biology of economic and reproductive traits. Besides, tree genomics research supports genetic improvement programmes and develops diagnostic tools, thus enabling development of recommendations for optimum conservation, restoration and management of natural populations. Research progress on trees is very limited due to genome complexity, lack of reverse genetic approaches and more importantly, lack of investment in research and development activities. The scope of genomic research in trees primarily aims to:

- i. Understand the structure and evolution of various trees and their genomes
- ii. Discover biology, genes and pathways behind phenotypes, economic traits and understand the genotype-phenotype interactions using genomic tools
- iii. Translate discovered genomics knowledge into developing genomic-based tools for genetic enhancement of forest trees with special reference to the changing climate scenario
- iv. Develop landscape genomic approaches to understand population structures of tree species for recommendation in conservation and restoration strategies
- v. Develop diagnostic tools for assessment of tree and forest health and understand epidemiology of tree diseases

These objectives can be accomplished by the genome sequencing of trees and finding genomic diversity present in different populations of these trees. They can further help in comprehensive understanding of the structure of tree genomes in the context of evolution. In addition, the inter-relation of gene functions and the phenotypes, with an emphasis on longevity, perennial and woody habit, and phenotypic plasticity in domesticated and natural populations will help to manage forest tree populations in the face of a changing climate scenario and other challenges (degradation and loss due to disease, fires, unstructured felling, etc.).

2. GENOME SEQUENCING TECHNOLOGIES

2.1. Capillary-based first-generation sequencing technologies

Genome sequencing based on Sanger sequencing approach performed in individual capillaries was the only possible option for around three decades (1975-2000) (Shendure et al 2017). The sequencing space was primarily dominated by Applied Biosystems Inc. through its different instrumentation platforms such as ABI Prism 377, ABI Prism 3100, ABI Prism 3700, ABI Prism 3730 and to a lesser extent, by Amersham's Megabace 1000. Both of these could sequence DNA to a length of up to 700-900 bases in a single reaction. Due to technological limitations, the output was very low and expensive, but it provided a reasonably good sequence quality to undertake initial plant genome sequencing projects for *Arabidopsis* (Arabidopsis Genome Initiative 2000) and rice (Sasaki 2005).

The traditional Sanger sequencing approach could sequence up to 96 samples in tandem in a single or multiple capillary setting at a time, restricting upscaling and data generation capabilities. The advancements in robotics, mechanics, nanotechnology, optical physics and computational capacity enabled the development of the next level of instrumentation that followed, which was capable of sequencing billions of DNA fragments in parallel at the same time. Thus, these massively parallel sequencing technologies are referred to as high throughput sequencing technologies. All these new and innovative sequencing approaches, which used these developments were sequentially referred to as second- or third-generation sequencing technologies (Karger and Guttman 2009; Durney et al 2015).

2.2. Second-generation sequencing (SGS) technologies

The SGS technologies were able to conduct tens of millions of microscale sequencing reactions in parallel on a small glass slide or similar equipment using the traditional Sanger sequencing approach, thereby producing data at a scale that was unprecedented. Although it revolutionized the genome sequencing space, the SGS

technologies had a major drawback of sequencing length, where only up to a maximum of 600 bases (on MiSeq platform) could be sequenced in a single reaction. However, in most cases, the sequencing length was restricted to 250 bases (for other HiSeq platforms). These included technologies by companies such as Illumina (Sequencing by synthesis – SBS approach through their GAIIx, MiSeq, NextSeq, HiSeq 2000, HiSeq 2500, HiSeq 4000, HiSeq X Five, HiSeq X Ten, NovaSeq 6000 platforms) and Thermo Fisher Scientific which was earlier used by various companies like Roche, and then Life Technologies (Sequencing by Oligonucleotide Ligation and Detection- SOLiD or 454-pyrosequencing approach through their platforms like GS-FLX, IonTorrent, IonProton, S5, etc.). However, all these technologies could sequence only a few hundred bases at a time due to limitations on sequencing chemistry and optics at that scale (Reuter et al 2015; Levy and Myers 2016; Heather and Chain 2016).

The plant genomics era where basic genomics-assisted breeding tools were developed for genome-wide association mapping (GWAS) and genomic selection (GS) used these SGS technologies (Bhat et al 2016). The SGS technologies contributed significantly to discovering the most fundamental and important variations present in the genomes, i.e., single nucleotide polymorphisms (SNPs) (Koboldt et al 2013). This resulted in breakthroughs in the advancement of plant breeding and crop improvement toolkits for important field crops.

2.3. Third-generation sequencing (TGS) technologies

The limitations of sequencing length of a DNA fragment were improved upon by the third-generation sequencing (TGS) technologies, which can sequence a few Kbs of individual strands of DNA at a very high speed by reading the original DNA molecule; thus, they were also known as single molecule real-time (SMRT) sequencing technologies. These were developed by sequencing companies like Pacific Biosciences, known as PacBio (instrument platforms: RS, RS II, Sequel, Sequel II) (Eid et al 2009), Oxford NanoPore Technologies also referred to as ONT (instrument platforms: MinION, GridION, PromethION) (Clarke et al 2009; Eisenstein 2012), and BioNano Genomics. Apart from

these actual long-read sequencing technologies, there are synthetic long-read technologies by 10X Genomics and BGI (StLFR) (Ott et al 2018; Peters et al 2014; Wang et al 2019), which use an innovative method where long reads are generated from short reads using barcoded pools of long DNA molecules. Besides, at least two more companies are developing new approaches of TGS, Quantapore, and Stratos Genomics for which information is not yet available. The length of DNA that can be sequenced using TGS technologies is limited only by the length and quality of DNA used to prepare the sequencing library. One of the main downsides of these technologies is that they have higher error rates compared to the earlier SGS technologies. However, it opens up the possibilities for understanding genome reorganizations, structural variations and chromosomal rearrangements apart from SNP variations. Along with SGS technologies, they provide a good option to develop long and reliable genome assemblies and scaffolds (Dijk et al 2018).

2.4. Chromatin conformation capture and sequencing

Isolation of DNA in its native chromatin form can help to build chromosome length scaffolds from the sequencing assemblies. Also referred to as HiC, it crosslinks DNA-protein complexes with formaldehyde, followed by DNA fragmentation, ligation, digestion, and then sequencing. This builds chromosome level sequencing scaffolds, which could otherwise be almost impossible. These different sequencing technologies have been widely reported by Levy and Myers (2016); Leggett and Clark (2017); Nakano et al (2017); and Pal et al (2019).

3. GLOBAL STATUS OF GENOME SEQUENCING OF WOODY PERENNIAL AND TREE SPECIES

Arabidopsis thaliana was the first plant genome to be sequenced in the year 2000 (Arabidopsis Genome Initiative 2000) using the Sanger shotgun sequencing approach. It took nearly five more years before the second plant genome, rice, (Eckardt, 2000; Sasaki 2005) could be published using a similar approach through an international consortium. It was the year 2006 that the genome sequence for poplar (Tuscan et al 2006), a fast-

growing model tree species also used for timber, was published using a similar shotgun sequencing approach. The tree genome sequencing scenario was dominated by a similar shotgun approach where DNA fragments were cloned into vectors like plasmids, cosmids, bacterial artificial chromosomes (BACs), etc. and further sequenced via capillary sequencers using the Sanger sequencing approach. Then a real jump in genome sequencing efforts happened only after 2013, where the SGS approach became affordable and available on a large scale.

At the time of writing this review, 45 perennial woody tree species had been sequenced and published (10 timber and wood species, 23 fruit and 12 multipurpose species). Table 1 provides information along with a brief description about the sequencing technology employed by each study. It can be observed that most of the studies have used short read sequencing (SRS) technology, primarily the Illumina-based sequencing platforms, whereas the most recent genomes have used long read SMRT as well as chromatin-based sequencing technology to attain a high quality, chromosome-level genome assemblies. There are 15 studies which have used PacBio SMRT technology, but only five have used the HiC approach to build chromosome-level assemblies (*Rubus occidentalis* (Black raspberry) – VanBuren et al 2018; *Mangifera indica* (Mango) – Wang et al 2020; *Diospyros oleifera* (Persimmon) – Zhu et al 2019a; *Actinidia chinensis* (Golden kiwifruit) – Wu et al 2019; *Hevea brasiliensis* (rubber) – Liu et al 2020).

Table 1 presents a list of 45 perennial woody species with diverse functional uses for which genome sequences are publicly available. It provides a short description of different next-generation sequencing (NGS) technologies used for sequencing different tree genomes.

Table 1. A list of perennial woody species with diverse functional uses for which genome sequences are available in the public domain

Sl. No.	Name of species, reference	Sequencing Approach
1	<i>Populus trichocarpa</i> (Black cottonwood tree) Tuskan et al (2006)	Whole genome shotgun strategy based on random plasmid cloning and sequencing using the first-generation Sanger sequencing technology on ABI PRISM 3700 DNA Analyzer. The shot gun genome assembly was aligned using BAC end sequencing.
2	<i>Picea abies</i> (Norway Spruce) Nystedt et al (2013)	Multiple paired end, mate pair and fosmid DiTag libraries of different sizes sequenced using Illumina's GAll, HiSeq 2000 and MiSeq.
3	<i>Picea glauca</i> (White Spruce), Birol et al (2013)	Multiple paired end, mate pair and fosmid DiTag libraries of different sizes sequenced using Illumina's GAll, HiSeq 2000 and MiSeq.
4	<i>Pinus taeda</i> (Loblolly pine) a. Neale et al (2014) b. Zimin et al (2014)	a. Multiple paired end, mate pair and fosmid DiTag libraries of different sizes sequenced using Illumina's GA II, HiSeq 2000 and MiSeq. b. Multiple paired end, mate pair and fosmid DiTag libraries of different sizes sequenced using Illumina's GA II, HiSeq 2000 and MiSeq.
5	<i>Eucalyptus grandis</i> (Rose gum) Myburg et al (2014)	Multiple BAC, plasmid and fosmid libraries of different sizes sequenced using first-generation Sanger sequencing

		and use of linkage maps for chromosome level assembly.
6	<i>Betula pendula</i> (Silver birch), Salojärvi et al (2017)	Multiple paired end small-insert libraries sequenced using HiScan SQ, MiSeq and NextSeq 500. In addition, mate pair sequencing for mid-sized libraries done using SOLiD 5500xl Sequencer, and long reads generated using PacBio RSII sequencer.
7	<i>Quercus robur</i> (English oak), Plomion et al (2018)	Multiple paired end small-insert libraries sequenced using HiSeq 2000 sequencer.
8	<i>Tectona grandis</i> (Teak), Yasodha et al. (2018)	A combination of short read sequencing using HiSeq 4000 of small, medium and long insert paired end libraries and long read sequencing using Oxford NanoPore's MinION sequencer.
9	<i>Casuarina equisetifolia</i> (Beefwood tree), Ye et al (2019)	Multiple paired end small to large insert libraries sequenced using HiSeq 2000 sequencer in combination with PacBio SMRT.
10	<i>Castanea mollissima</i> (Chinese chestnut), Xing et al (2019)	Small insert paired end libraries were sequenced using HiSeq 2500 and long read sequencing using PacBio SMRT technology.
11	<i>Vitis vinifera</i> (Grapevine), Jaillon et al (2007)	Whole genome shot gun sequencing using ABI3730xl and assembly using BAC end sequencing

12	<i>Carica papaya</i> (Papaya), Ming et al (2008)	Whole genome shotgun sequencing of plasmids and BAC end sequencing using capillary sequencer.
13	<i>Malus domestica</i> (Apple), Velasco et al (2010)	Small to large sized shot gun libraries in plasmids sequenced using Sanger sequencing on ABI 3730XL DNA Analyzer and mid-sized shot gun and paired end libraries using 454 sequencing in GS FLX.
14	<i>Phoenix dactylifera</i> (Date palm), Al-Dous et al (2011)	Small insert shotgun paired end libraries were sequenced using GA IIx from Illumina.
15	<i>Prunus mume</i> (Chinese plum), Zhang et al (2012)	Small to large insert sized paired end libraries sequenced using Illumina's HiSeq2000 platform.
16	<i>Pyrus bretschneideri</i> (Chinese white pear), Wu et al (2012)	A heterogenous approach of combining sequencing data from small insert shotgun paired end libraries from individual BACs, large to very large insert shotgun mate pair and small sized paired end libraries, all sequenced using HiSeq 2000 from Illumina.
17	<i>Prunus persica</i> (Peach), Verde et al (2013)	Small to large sized shot gun libraries in plasmids, fosmids were sequenced using Sanger sequencing on ABI 3730XL DNA Analyzer and BAC end sequencing on the same platform used for assembly.
18	<i>Actinidia chinensis</i> (Kiwifruit), a. Huang et al (2013)	a. Small insert paired end sequenced on HiSeq2000

	b. Wu et al (2019)	b. Small insert paired end libraries on HiSeq X Ten and SMRT sequencing over PacBio Sequel and chromosome level assembly using HiC.
19	<i>Pyrus communis</i> (European pear), Chagne et al (2014)	Small to large insert shotgun paired end library sequenced on Illumina's GA II sequencer and BAC end sequencing using capillary sequencer.
20	<i>Citrus sinensis</i> (Sweet orange), Xu et al (2013)	Small shotgun library and mid and large insert paired end libraries, both sequenced using GS FLX+ from Roche.
21	<i>Citrus clementina</i> (Clementine), Wu et al (2014)	Medium to large insert shotgun plasmid libraries and Bac and fosmid ends sequenced using Sanger sequencing on ABI 3730 XL and in addition shotgun single end sequencing using 454 technology.
22	<i>Ziziphus jujuba</i> (Jujube), Huang et al (2016)	Small to very large insert shotgun libraries sequenced using HiSeq 2000 from Illumina.
23	<i>Cocos nucifera</i> (Coconut), Xiao et al (2017)	Multiple small insert paired end and medium to very large insert mate pair libraries were sequenced using HiSeq 4000
24	<i>Ficus carica</i> (Fig), Barghini et al (2017)	Multiple small insert paired end libraries sequenced using MiSeq and HiSeq2000.
25	<i>Rubus occidentalis</i> (Black raspberry), a. VanBuren et al (2016)	a. Small to medium insert paired end libraries sequenced using HiSeq 2000.

	b. VanBuren et al (2018)	b. PacBio and HiC used to improve over VanBuren et al 2016.
26	<i>Prunus avium</i> (Sweet cherry), Shirasawa et al (2017)	Small insert paired end and small to large insert mate pair libraries sequenced on HiSeq2000
27	<i>Moringa oleifera</i> (Drumstick) Chang et al (2018), African Orphan Crops Consortium	Multiple small insert paired end libraries sequenced using HiSeq 4000.
28	<i>Persea americana</i> (Avocado), Rendón-Anaya et al (2019)	Multiple accessions sequenced using small insert paired end and small to medium sized long read mate pair libraries on 454-FLX (Roche) and HiSeq2000. A BAC library was also generated and sequenced using shot gun sequencing and end sequenced using traditional Sanger sequencing. SMRT long read sequencing using PacBio and genome anchored using genetic linkage map.
29	<i>Sclerocarya birrea</i> (Marula), Chang et al (2018), African Orphan Crops Consortium	Multiple small insert paired end libraries and medium insert size mate pair libraries sequenced using HiSeq 4000.
30	<i>Diospyros</i> spp. (Persimmon) a. <i>Diospyros oleifera</i> (Persimmon), Zhu et al (2019a)	a. Multiple small insert libraries and small to large insert mate pair libraries sequenced using HiSeq2500, SMRT sequencing using PacBio RS II and chromosome level assembly using HiC. b. Multiple small insert paired end libraries sequenced using HiSeq2500 and HiSeq4000 and large insert libraries

	b. <i>Diospyros lotus</i> (Persimmon), Akagi et al (2020)	sequenced using PacBio RSII. The chromosome level maps were assembled using genetic linkage maps created using RAD-seq markers.
31	<i>Artocarpus altilis</i> (Breadfruit), Sahu et al (2020), African Orphan Crops Consortium	Multiple small insert paired end libraries sequenced using HiSeq 4000.
32	<i>Artocarpus heterophyllus</i> (Jackfruit), Sahu et al (2020), African Orphan Crops Consortium	Multiple small insert paired end libraries sequenced using HiSeq 4000.
33	<i>Mangifera indica</i> (Mango), Singh et al 2018, Wang et al (2020)	Small insert paired end and small to medium sequenced using HiSeq2000 and MiSeq, SMRT sequencing using PacBio platform, HiC and mate pair sequencing for scaffolding and high-density linkage map used for anchoring contigs onto chromosomes.
34	<i>Theobroma cacao</i> (Cocoa) a. Argout et al (2011) b. Motamayor et al (2013) c. Cornejo et al (2018)	a. Single end and paired end reads from Roche's GS FLX, paired end reads from Illumina's GA IIx, and BAC ends sequenced using ABI 3730XL b. Small to very large insert paired end libraries were sequenced using GA IIx from Illumina. c. Multiple small insert paired end libraries sequenced using HiSeq 2000/2500.
35	<i>Azadirachta indica</i> (Neem) a. Krishnan et al (2012)	a. Small insert paired end and medium to large insert mate pair libraries sequenced using Illumina's GA IIx; small insert

	b. Krishnan et al (2016)	shotgun libraries sequenced by IonTorrent PGM, and shotgun sequencing on ABI 3500 capillary sequencer. b. The earlier assembly from Krishnan et al. (2012) was improved using large insert mate pair libraries sequenced using HiSeq and PacBio sequencing.
36	<i>Elaeis guineensis</i> (African oil palm), Singh et al (2013)	Small and very large insert paired end libraries were sequenced using GS FLX Titanium and BAC ends were sequenced using Sanger sequencing on ABI 3730.
37	<i>Morus notabilis</i> (Mulberry), He et al (2013)	Multiple small insert paired end and long insert mate-pair libraries sequenced on Hiseq2000.
38	<i>Hevea brasiliensis</i> (Rubber tree) a. Rahman et al (2013) b. Lau et al (2016) c. Pootakham et al (2017) d. Liu et al (2020)	a. Multiple shotgun small-insert paired end libraries sequenced using Illumina, Roche/454, and SOLID platforms. b. Shotgun paired end and mate pair libraries sequenced using Illumina's HiSeq2500 and MiSeq and in addition SMRT sequencing using PacBio RS II sequencer. c. Small insert single end and paired end sequencing on 454 GS-FLX+, Illumina's HiSeq2000, and long read sequencing using PacBio RS II. d. Long read sequencing using PacBio and chromosome level assembly using HiC.

39	<i>Ginkgo biloba</i> (Ginkgo), Guan et al (2016)	Small to very large insert paired end libraries were sequenced using HiSeq 2000/4000 from Illumina.
40	<i>Macadamia integrifolia</i> (Macademia nut), Nock et al (2016)	Small insert paired end and medium insert mate pair libraries sequenced using GA IIx and HiSeq2000.
41	<i>Juglans</i> spp. (Walnut) a. <i>Juglans regia</i> , Martinez-Garcia et al (2016) b. <i>Juglans regia</i> × <i>J. microcarpa</i> by Zhu et al (2019b) c. <i>Juglans mandshurica</i> , <i>J. regia</i> , <i>J. nigra</i> , Bai et al (2018)	a. Mate-pair and paired-end small insert genomic libraries sequenced using Illumina's HiSeq2500 and SMRT using PacBio RS II b. SMRT sequencing using PacBio RS II and optical sequencing c. Paired end small insert sequenced on HiSeq2500 and long insert jumping libraries sequenced on HiSeq X ten.
42	<i>Faidherbia albida</i> (White acacia), Chang et al (2018) also African Orphan Crops Consortium	Multiple small insert paired end libraries and medium insert size mate pair libraries sequenced using HiSeq 4000.
43	<i>Polygonum cuspidatum</i> (Japanese Knotweed), Zhang et al (2019)	Small insert paired end libraries and small to long insert mate pair libraries sequenced using HiSeq2500.
44	<i>Santalum album</i> (Sandalwood) a. Mahesh et al (2018) b. Dasgupta et al (2019)	a. Multiple small insert paired end and small to medium size mate pair libraries sequenced using HiSeq1000. b. Small insert paired end libraries sequenced using MiSeq and NextSeq500.

4. APPLICATION OF GENOMIC-BASED TECHNOLOGIES FOR PERENNIAL WOODY AND TREE SPECIES

Many of the tree species have not undergone extensive domestication processes as seen in field crops such as wheat, rice, maize and edible legumes. The domestication processes in these field crops have corrected the 'wild traits' primarily by developing shattering resistance, improving plant stature, cooking quality, reproductive biology quality, and by enhancing field manageability. However, this was followed by the process of selection and crop improvement which made these crops more productive and economical under crop husbandry practices; this improved their nutritional quality by enriching them with energy-rich carbohydrates (for cereals) and proteins (for legumes). Though there are many similarities between the domestication of annual crops and tree crops at morphological level, tree domestication processes have some important differences such as diversity of morphology, growth habits and physiology, among many other traits. Hardwood trees show extensive variation in wood anatomy, leaf morphology, whole-tree architecture, secondary metabolism and numerous adaptive traits (Groover and Crook 2017). Reflecting their evolutionary history, hardwood tree genomes display divergent chromosomal architectures, with multiple ancestral whole-genome duplications and rearrangements, gene family expansions, and many segmental deletions across lineages (Tuskan et al 2006), besides, some changes in vegetative forms like thorny to thorn-less, ploidy level, and plant stature from large to dwarf. Further improvements in productivity and quality traits resulted from combinations of clonal propagation and human selection.

A high genetic diversity, clonal mode of propagation, and perennial behaviour of the trees do put some bottlenecks for employing traditional crop breeding methodologies. Nevertheless, they also offer some specific advantages. A high genetic diversity provides a wider spectrum of genetic variability available for selection; clonal propagation helps to multiply true-to-type seedlings; and perennial behaviour allows recording of observations in different spatial and time scales. As the role of genetics in these traits is now well known either in model systems and/or cultivated crops and trees, it is possible

to expedite improvement activities by using modern approaches like genomics and its application in QTL mapping, genomics-assisted breeding, genome-wide association studies (GWAS), and genomic selection (GS) (Hickey et al 2017). The first step towards this is to sequence their genomes, develop SNP assaying technologies, and then use them for genomics-assisted or genomics-based improvement processes. However, substantial investment is required to develop suitable mapping populations, experimental designs, analytical tools, and to translate these findings to develop long-term breeding programmes and reproducible genetic gains.

Some studies in tree species like eucalyptus, poplar, pine, spruce, apple, peach, oil palm, citrus, cocoa, grape and macadamia used genomics-based technologies to link or associate a few economically-important traits with SNP variants. These studies indicate that a sufficient amount of groundwork has already been carried out to start the genomics-assisted selection. Hence, the era of field application in tree improvement is not too far away.

5. CURRENT SCENARIO OF TREE GENOMICS RESEARCH GLOBALLY

In India, trees have historically been an important part of the agricultural landscape. While a number of tree species have been traditionally grown, a few of them gained momentum because of their economic, social or cultural values. Here, we give a global research account of a few species that have been systematically collected, curated and maintained in genebanks across India.

i. Azadirachta indica (Neem)

Azadirachta indica A. Juss (Neem) belongs to the family Meliaceae and order Rutales. It is an evergreen tree native to the Indian sub-continent. Neem is an excellent multi-purpose evergreen, tall and fast-growing species, with copious traditional values. It has medicinal and biological properties, seed oil which is used as bio-pesticide, and is an excellent source of animal feed and timber. Further, it consists of $2n=28$ of chromosome. Neem timber is durable even in extended exposed conditions and is resistant to termites

and wood worms. Its wood is used for firewood and charcoal, and is of excellent quality. The Government of India also promotes Neem Coated Urea (NCU), a fertilizer from neem oil among various agricultural schemes initiated to boost the growth of wheat and paddy. ICAR-Central Agroforestry Research Institute (CAFRI), Jhansi has 170 provenances of neem collected from various states of India. They have conducted studies on reproductive biology, vegetative propagation techniques, variability on fruit and seed traits as neem seed oil yield and azadirachtin content, and evaluated neem germplasm for straightness and other growth-related traits (Gupta et al 2011). Neem is a cross-pollinated species, mainly pollinated through insects and wind.

Neem possesses more useful non-wood products (leaves, bark, flowers, fruits, seed, gum, oil and Neem cake) than any other tree species. Seed kernels constitute 50-60% of seed weight and 25% of the fruit. Percentage of kernel fat ranges from 30-50%. The oil is brownish yellow, non-drying with an acrid taste and unpleasant odour. Several valuable bioactive substances from limonoids class of triterpenoids were found in Neem oil. Azadirachtin is the most important bio-active compound, having effective biological functions and huge commercial value. Its commercial formulations are toxic against a large range of insect species, whilst retaining very low mammalian toxicity. The genome size of Neem is 384 Mbp (Ohri et al 2004).

The Neem population was predominantly allogamous and polycarpy was found to be a possible way of avoiding inbreeding (Kundu 1999). Randomly amplified polymorphic DNA (RAPD) analysis was employed to assess genetic divergence among 29 neem accessions collected from two agro-ecological regions of India (11 agro-climatic sub-zones) (Deshwal et al 2003). The first attempt to sequence the Neem genome was made by Krishnan et al (2012). The draft genome sequence released was assembled to Scaffold N50 of 452,028 bp and Scaffold N90 of 56,222 bp. They reported about 20,000 genes with an average transcript length of 1.69 Kbp and *A. indica*'s evolutionary closeness to the citrus species was verified by both molecular phylogenetic analyses and sequence similarity. An improved genome assembly was generated by the same lab

(Krishnan et al 2016) using additional short reads from Illumina and long reads from Pacific Biosciences SMRT sequencer. The updated genome assembly (v2.0) yielded a 3- and 3.5-fold increase in N50 and N75, respectively. Kuravati et al (2015) also performed sequencing in Illumina and 454 sequencing platforms. They assembled 267 Mbp, which accounts for 70% of the estimated size of the neem genome and predicted 44,495 genes. Some transcriptome studies related to transcriptomics for identifying genes related to the terpenoid biosynthesis pathway are also documented in neem (Krishnan et al 2012, Wang et al 2016, Bhambhani et al 2017). Hodgson et al (2019) identified key enzymes responsible for protolimonoid biosynthesis thus opening the door for understanding azadirachtin synthesis in neem. In their study on 3 limonoid-producing species (*A. indica*, *Melia azedarach* and *Citrus sinensis*), they characterized tirucalla-7, 24-dien-3 β -ol synthase OSCs (AiOSC1, MaOSC1, CsOSC1) as enzymes responsible for early-stage protolimonoid synthesis.

ii. *Dalbergia sissoo* (North Indian rosewood)

The genus *Dalbergia*, which comprises approximately 250 species of trees, shrubs and woody climbers is widely distributed in tropical and sub-tropical regions of the world, with Amazonia, Madagascar, Africa and Indonesia being considered centres of high diversity. Among various species, *Dalbergia sissoo* commonly known as North Indian rosewood or sissoo, is a fast-growing, hardy deciduous tree native to the Indian sub-continent and Southern Iran. It is one of the important multipurpose tree species primarily distributed from the foothills of the Himalayan tract of India and Pakistan. It is used for high-quality furniture, cabinets, decorative veneer, marine and aircraft grade plywood, ornamental turnery, carving, engraving, tool handles and sporting goods (Chaturvedi et al 2017). Due to its multipurpose use, coupled with wider adaptation in different climate and soil conditions, this species should be prioritized for agroforestry systems.

ICAR-CAFRI, Jhansi, and its network All India Coordinated Research Project on Agroforestry (AICRPAF) and ICFRE-Forest Research Institute (FRI), Dehradun have done much tree improvement work on shisham. ICAR-CAFRI has collected 14 accessions

from different parts of central India, which have been evaluated for rapid growth, clear bole and canopy. It has further identified superior genotypes- shisham bundle 2 (PT-2) and shisham bundle 6 (PT-6) for straightness and higher bole volume, which has been registered as genetic stock at ICAR-NBPGR, New Delhi. AICRPAF Centre at GBPUA&T, Pantnagar proposed Shisham PS-52 for registration as this genotype had unique features of straight bole, higher height and diameter growth. ICFRE-FRI, Dehradun released the Shisham variety FRI-DS-14, which shows resistance to the devastating disease of die-back. Besides, the variety has an excellent bole form which increases its value in the timber industry. Further improvement of these species can be effected using recent biotechnological approaches, particularly genomics-based, next generation sequencing (NGS) and genotyping-by-sequencing (GBS) techniques.

Dalbergia belongs to the sub-family Mimosoideae within the family Fabaceae and includes several valuable timber-yielding species of economic importance. *Dalbergia sissoo* is a diploid species with $2n=20$ and is mainly self-pollinated by honey bees, beetles, butterflies and thrips. Genomics study in this species is at a very initial level. Some research related to genetic diversity has been carried out with molecular markers. Javaid et al (2014) assessed genetic diversity in *D. sissoo* in vegetations from Lahore, Gujranwala and Sialkot districts of Pakistan and discovered that polymorphism ranged from 27.27% to 89.50%. Random amplified polymorphic DNA (RAPD) markers were used to study the genetic relationships among 10 species and genetic diversity in 49 genotypes of *Dalbergia sissoo* in India (Hiremath and Nagasampige 2004, Dobhal et al 2019). Complete chloroplast genome sequenced and compared in nine *Dalbergia* species including *Dalbergia sissoo* and chloroplast genomes were conserved to genome size, structure and gene content, and showed low sequence divergence. Eight mutation hotspots, namely, six intergenic spacer regions (*trnL-trnT*, *atpA-trnG*, *rps16-accD*, *petG-psaJ*, *ndhF-trnL*, and *ndhG-ndhI*) and two coding regions (*ycf1a* and *ycf1b*) were also identified (Song et al 2019).

iii. *Tectona grandis* (Teak)

Tectona grandis L. (Teak) belonging to the mint family *Lamiaceae* (earlier member of *Verbenaceae*) is one of the world's highly valued tropical timber species that occurs naturally in India, Laos, Myanmar and Thailand. In addition to this, teak is commercially grown in many countries, such as Sri Lanka, Bangladesh and China in Asia; Ghana, Nigeria, Ivory Coast, Senegal, Togo and Benin in West Africa; Sudan and Tanzania in East Africa; Trinidad, Puerto Rico and Panama in Central America; and Brazil and Ecuador in South America. The timber is highly valued because of its extreme durability, strength, stability as well as resistance to pests. Quinones and other extractives found abundant in the teakwood are responsible for its anti-termite and anti-fungal properties, conferring the longevity of timber. Thus, the wood is used in building ships, railway carriages, sleepers, construction, furniture, veneer, and carving owing to its admirable timber qualities and aesthetical properties. Teak has been an out-crossing species, but to some extent, up to 1-2% self-pollination is possible. It is a diploid species with $2n=26$ chromosomes. Zhao et al (2019) identified 14 clusters of tandem-duplicated terpene synthases (TPSs), genes central to the biosynthesis of terpenes, which are involved in plant defense and pollinator attraction. Transcriptome analysis revealed 10 TPSs highly expressed in woody tissues, of which eight were in tandem, revealing the importance of resolving tandemly duplicated genes and the quality of the assembly and annotation. Galeano et al (2019) studied the physiological and molecular responses to drought stress in teak and revealed that *TgTPS1*, *TgDREB1*, *TgAREB1* and *TgPIP1* along with *TgHSP1*, *TgHSP2*, *TgHSP3* and *TgBI* (other stress-related genes) showed that with moderate treatment, *TgTPS1*, *TgDREB1*, *TgAREB1*, *TgPIP1*, *TgHSP3* and *TgBI* genes had higher expression than the control treatment. However, with severe treatment, only *TgTPS1* and *TgDREB1* showed higher expression than the control treatment. Thus it was concluded that these physiological and biochemical adjustments in leaves and genetic changes in roots under severe and prolonged water shortage situations can be a limiting factor for teak plantlet growth. Galeano et al (2015) presented a widely expressed gene catalog for *T. grandis* using Illumina technology and the *de novo* assembly. A total of 462,260 transcripts were obtained, with 1,502 and 931 genes differentially expressed for

stem and branch secondary xylem, respectively, during age transition. Analysis of stem and branch secondary xylem indicates substantial similarity in gene ontologies including carbohydrate enzymes, response to stress, protein binding, and allowed us to find transcription factors and heat-shock proteins which are differentially expressed. TgMYB1 displays an MYB domain and a predicted coiled-coil (CC) domain, while TgMYB2, TgMYB3 and TgMYB4 showed R2R3-MYB domain and grouped with MYBs from several gymnosperms and flowering plants. TgMYB1, TgMYB4, and TgCES presented a higher expression in mature secondary xylem, in contrast to TgMYB2, TgHsp1, TgHsp2, TgHsp3 and TgBi whose expression is higher in young lignified tissues. TgMYB3 is expressed at a lower level in secondary xylem. Yasodha et al (2018) assembled a draft genome of 317 Mbp at 151 coverage and annotated 36,172 protein-coding genes. Approximately 11.18% of the genome was repetitive. Microsatellites or simple sequence repeats (SSRs) are undoubtedly the most informative markers in genotyping, genetics, and applied breeding applications. They generated 182,712 SSRs at the whole genome level, of which, 170,574 perfect SSRs were found; 16,252 perfect SSRs showed *in silico* polymorphisms across six genotypes, suggesting their promising use in genetic conservation and tree improvement programmes. Genomic SSR markers developed in this study could make a significant contribution to the conservation and management of teak genetic resources.

Mohammad et al (2017) studied the effect of discrete (individual) and mixed (bulk) genomic DNA on genetic diversity estimates and population structure in Teak. They recommended strict guarding of identities of genotypes within the collected samples for obtaining precise estimates and drawing accurate conclusions about the genetic diversity and clustering of populations. They compared the genetic variation detected and genetic relationships inferred in five teak populations via 10 genomic DNA samples per population each of either single-seed or bulk of 3- or 5-seed with the help of ISSR markers.

Teak populations from Western Ghats and Central India were reported to be genetically distinct as revealed by RAPD markers. The intra-population variation was found to be substantially higher (78%) when compared to the inter-population variation (Nicodemus et al 2005).

Vaishnav et al (2015) used AFLP markers for analysis of genetic diversity and structure of teak in India. Five AFLP primer combinations were employed for analysis of genetic diversity, differentiation and structure of 96 genotypes of teak from 10 natural locations in India. The primer combinations detected maximum polymorphism in the teak genome. The population genetic structure revealed two distinct centres of teak diversity, i.e., central India and peninsular India. The very high proportion of genetic diversity residing within locations encourages the intensive selection and collection of diverse superior genotypes from each location for the conservation of germplasm and genetic improvement of teak.

Narayanan et al (2008) assessed the comparative efficacy of different DNA extraction methods for PCR-based assay in teak. Four extraction methods and sample types were evaluated for yield, quality and suitability of genomic DNA for ISSR (inter simple sequence repeats) marker amplification by PCR in teak. Using identical PCR conditions, DNA extraction methods, and sample types affected amplifications of ISSR markers with a pattern: seedling > graft > tree leaves. Genomic DNA of seed samples contained a high amount of protein and showed no ISSR amplification, which was overcome by the inclusion of proteinase K in SDS-based extraction method.

Vaishnav and Ansari (2018) investigated the genetic differentiation of teak meta-population in India to geographical and climatic variations employing dominant ISSR markers followed by Bayesian statistical analysis to understand the adaptability of the species. Ansari et al (2012) utilized ISSR markers for analysis of molecular diversity and genetic structure of Indian teak populations. Twenty-nine populations of teak were collected from central and peninsular India and five ISSR primers were used for the

analysis of genetic diversity and structure. The cluster analysis distinguished the drier teak populations of central India from the moist teak populations of south India, which was also confirmed by Principal Coordinate Analysis. The findings advocate the need not only for enhancing selection intensity for a large number of plus trees, but also for laying out a greater number of in situ conservation plots within natural populations of each cluster for germplasm conservation of teak. The aim is to improve teak productivity and quality in future.

iv. *Leucaena leucocephala* (Leucaena)

Leucaena leucocephala (Lam.) de Wit. belongs to the Mimosoideae family and is indigenous to Colombia, Costa Rica, El Salvador, Guatemala, Honduras, Mexico, Nicaragua, Panama, Spain and USA. It is widely distributed throughout the world and has become naturalized. *Leucaena* is an evergreen species; however, under frost or prolonged drought it sheds its leaves, but regains them once normal conditions return. It has multiple uses and is, therefore, referred to as a “miracle tree” worldwide. The leaves are highly nutritious for ruminants. The species is widely utilized for fuelwood, pulpwood, small timber, green manure, charcoal, parquet flooring and fencing. The genus *Leucaena* comprises 19 diploid and five tetraploid species. *L. leucocephala* is an allotetraploid species ($2n=104$) which originated from natural hybridization two diploid species *L. pulverulenta* as the maternal parent and *L. cruziana* as the paternal parent (Govindarajulu et al 2011). It is mostly self-pollinated and consists of bisexual flowers (each flower of each individual has both male and female structures). Boff and Schifino-Wittmann (2003) also argued segmental allopolyploid nature and did not rule out autotetraploid nature just based on cytological data. Genomic research in this species is still at the initial stages. Few studies related to transcriptomics have been documented to date. Negi et al (2011) identified some species-specific genes using Interspecies Suppression Subtractive Hybridization (ISSH). They use cDNAs from *leucaena* and *Acacia confusa* (acacia) as the tester and driver, respectively, and identified 96 *leucaena* genes showing homologies to various plant genes with known functions and 46 other genes-encoding hypothetical proteins. Among these, 22 genes

were related to various stress-related proteins including chitinase, PR-10 protein, type 2 metallothionein, thaumatin-like protein, germin-like protein (GLP), molybdenum cofactor sulfurase (MCSU), aldehyde dehydrogenase, cysteine proteinase and Kunitz trypsin inhibitor.

An improved method for RNA extraction from *Leucaena leucocephala* is also standardized. This method can be extended to woody leguminous species, *Acacia koa* and *Acacia gray* to facilitate genetic studies (Ishihara et al 2016a). Ishihara et al (2016b) did Transcriptome Analysis by illumina sequencing followed by SOAPdenovo assembly and generated over 120,000 unigenes from roots and shoots combined. Approximately 80% of identified genes were annotated of Identification of Highly Expressed Genes in Roots and Shoots. Microarray and q-PCR are used for further validation of the expression of unigenes. Root and shoot-specific sequences were also identified. Up-regulated sequences in the root were mostly represented by unigenes that were related to secondary metabolism, while in the shoot, up-regulated sequences were mostly represented by unigenes that were involved in carbohydrate and lipid metabolism. The unigenes sharing homology with terpenoid biosynthesis genes and a nicotianamine synthase gene were up-regulated more than 100-fold in the root, which indicates that these genes may have important roles in high-stress tolerance of *Leucaena*.

v. *Melia dubia* (Malabar Neem)

Melia dubia Cav. syn. *M. composita* Willd. (Family: Meliaceae), commonly known as Burma dek, white cedar, Malabar neem or drek, is a deciduous tree, native to the Indian sub-continent. *Melia dubia* is endemic to certain pockets of the Western Ghats in India. It is found in tropical moist and tropical dry deciduous forests. Naturally, it is distributed in India, Sri Lanka, Malaysia, Bhutan, Myanmar, Australia and Africa. However, in India, it is mostly found in small pockets of Sikkim Himalaya, Upper Assam, Khasi Hills, Orissa, North Bengal, Deccan Plateau and Southern India at elevations ranging from 600-1,800 m above MSL. The species is widely used for pulp & paper, veneer making, packing cases, ceiling planks, building purposes, agricultural implements, pencils, matchboxes,

splints, kattamarans, musical instruments and tea boxes. Although the species is reported to be predominantly self-fertilized, some cases of open pollination have been reported. The chromosome numbers of *Melia dubia* is $2n=28$. Some studies related to genetic diversity using different morphological traits (Kumar et al 2013) and molecular markers has been reported in this species. Results from the RAPD used to evaluate the genetic diversity in populations of *Melia dubia* from different agro-climatic regions of India showed similarity indices ranged from 0.80 to 0.90. Similarly, genetic variation was also assessed using ISSR markers in 11 natural populations and seven plantations across eight districts of Karnataka, comprising 232 samples through 15 Markers (Rawat et al 2018). Phenotyping using pilodyn as a tool was also carried out followed by selective genotyping and preliminary genetic diversity estimation for wood basic density mainly using wood stiffness as a trait and SSR markers (Joshi et al 2018, 2019).

vi. *Gmelina arborea* (White teak)

Gmelina arborea Roxb is a fast-growing indigenous multipurpose tree species which produces one of the best quality timbers in India. It is a medium- to large-sized deciduous tree and can grow up to 40 m in height. It is endemic to Bangladesh, Cambodia, China, India, Japan, Laos, Myanmar, Nepal, Pakistan, Sri Lanka, Thailand and Viet Nam. In India, it is found in the eastern sub-Himalayan tract, Indo-Gangetic plains, Aravali Hills, central India, western Peninsula and western Himalayas. It grows well in eastern sub-Himalayan belt but extends into mixed-deciduous forests of central India and sal forests of Uttar Pradesh, Bihar and Orissa, and occasionally in wet evergreen forests of the Western Ghats (Chaturvedi et al 2017).

The tree has been commercially grown due to its woody timber used for pulp, paper, medium density fibre board, laminated veneer lumber, wood cement composites, particle board, furniture, furniture interiors, plywood interiors, finger jointed lumber, door panelling, pallets, pencils and match sticks. The breeding system of white teak involves both self- and cross-pollination. Pollination is entomophilous, i.e., by insects. Naik et al (2009) reported 41% and 59% genetic diversity within and between *Gmelina arborea*

population using ISSR primers and observed positively correlated genetic differentiation among six Indian populations to geographic distance ($r=0.626$, $P=0.029$).

Shankar et al (2009) described a modified protocol based on Dellaporta et al (1983) for isolating high-quality DNA from dried leaves of *Gmelina arborea*, which is suitable for molecular analysis. Manokar et al(2018) designed genetic markers for *G. arborea* and *G. asiatica* based on the nuclear ribosomal DNA-internal transcribed spacer 1 (*ITS1*) region to distinguish them from substitutes and adulterants.

vii. *Eucalyptus grandis* (Rose gum)

Eucalyptus grandis Hill ex Maid. belongs to the Myrtaceae family and is commonly known as red gum, rose gum and flooded gum. It is native to Australia but was introduced to several countries including Australia, Angola, Argentina, Brazil, Ethiopia, India, Kenya, South Africa, Tanzania, Uganda, Uruguay, Zambia and Zimbabwe. The flowers are bisexual, with fertile male and female organs on the same flower. Pollination is dependent on insects or animal vectors. Like many *Eucalyptus* species, it has a tendency to out-breed. The species is used for pulpwood, plywood, poles, timber, honey production, packaging cases and fuelwood.

Eucalyptus species and hybrids represent the most widely cultivated hardwood biomass crop globally, and *Eucalyptus grandis* (rose gum) clonal genotype 'BRASUZ1' was selected as the reference for the genus. Published in 2014, the *E. grandis* genome was the first of the order Myrtales and the family Myrtaceae. Key features of the genome included a very high proportion (34%) and many genes (over 12,500) in tandem duplicate arrays, an ancient genome-wide duplication event and high diversity of gene families encoding plant-specialized metabolites such as phenylpropanoids and terpenes which are important for plant defense and pharmaceutical uses such as eucalyptus oils (Mewalal et al 2017).

BRASUZ1 was one of the last plant genomes produced exclusively with Sanger technology combined with extensive BAC-end coverage. The result was a high-quality assembly (currently v2.01) comprising 691 Mbp in 4,943 scaffolds with a scaffold N50 of 57.5 Mbp, contig N50 of 67 Kbp and 94% of the estimated 640 Mbp genome in 11 pseudomolecules containing 288 scaffolds longer than 50 Kbp. A total of 36,349 protein-coding loci are annotated and the assembly has served as a reference for extensive transcriptomics studies targeting the regulation and biosynthesis of lignocellulosic biomass (Mizrachi et al 2014; Carocha et al 2015; Hussey et al 2015; Soler et al 2015) and in comparative analyses with *Arabidopsis* (Davin et al 2016) and *Populus* (Hefer et al 2015; Pinard et al 2015). Other research targets have included terpene biosynthesis (Myburg et al 2014; Kulheim et al 2015), reproductive biology (Vining et al 2015), plant defense (Christie et al 2015; Mangwanda et al 2015; Oates et al 2015; Meyer et al 2016) and abiotic interactions (Plett et al 2015; Spokevicius et al 2017). High-throughput transcriptome sequencing in 100s of segregating interspecific hybrids of *E. grandis* × *E. urophylla* have been used to perform the first comprehensive systems genetic analysis of wood development in Eucalyptus (Mizrachi et al 2017).

The *E. grandis* genome has also served as a reference for whole-genome analysis of the causes of inbreeding depression (Hedrick et al 2016) and development of a commercial, multi-species 60K SNP genotyping chip tagging 96% of the genome with 1 SNP every 12-20 Kbp (Silva-Junior et al 2015). The EuCHIP60K has been a key resource in investigating genome-wide recombination, linkage disequilibrium and nucleotide diversity (Silva-Junior and Grattapaglia 2015), carry out genome-wide association (Resende et al 2016; Muller et al 2019) and drive genomic selection in different Eucalyptus species (Duran et al 2017; Tan et al 2017, 2018). Eucalyptus transcriptomics resources are available at <https://eucgenie.org>. Ongoing and future efforts are focused on understanding genome diversity and evolution in this species-rich genus and other members of Myrtaceae (Grattapaglia et al 2012), including sister genera such as *Corymbia* for which a genome assembly, soon to be released, revealed conservation but dynamic evolution of terpene genes relative to Eucalyptus (Butler et al 2018).

Steane et al (2011) tested a set of >8000 DArT markers across a range of *Eucalyptus* species for its utility in high-resolution population and phylogenetic studies which could help in identifying interspecific hybrids and resolve biogeographical disjunctions within species. Myburg et al (2014) have sequenced and assembled more than 94% of the 640 Mbp genome of *Eucalyptus grandis* and predicted 36,376 protein-coding genes. Dasgupta et al (2015) identified a total of 2294 SNVs and 479 InDels in *E. camaldulensis*, 2383 SNVs, and 518 InDels in *E. tereticornis*, and 1228 SNVs and 409 InDels in *E. grandis* which could be used for the development of markers supposedly related to wood property traits in *Eucalyptus*. Sumathi and Ramasamy (2017) amplified 55 alleles from six microsatellite loci in two inter-specific hybrid populations (*E. tereticornis* × *E. grandis* and *E. tereticornis* × *E. camaldulensis*) followed by their sequencing and reported the amplification for target repeat types, except for loci Eg61 and Embra2002.

The genetic diversity estimations using RAPD, ISSR and SSR were conducted at different levels of the population including species, provenances, seed orchards and clones assembled by IFGTB in *Eucalyptus* spp. (Balasaravanan et al 2005; Chezian et al 2010) and species-specific ISSR markers were reported in different *Eucalyptus* species (Balasaravanan et al 2006). Generation of high throughput markers (SNPS and InDels) using the strategy of target capture and deep sequencing of 94 genes involved in wood formation across three *Eucalypt* species was reported for QTL/Association studies (Dasgupta et al 2015).

viii. Isolation and characterization of cell wall related genes in *Eucalyptus tereticornis*

Three full-length developing xylem specific cellulose synthases (*EtCesA1*, *EtCesA2*, and *EtCesA3*) were isolated and characterized from *Eucalyptus tereticornis*. The differential expression analysis of the three *EtCesA* genes using qRT-PCR revealed 49- to 87-fold relative expression in developing secondary xylem tissues. All three *EtCesA*

transcripts were up-regulated by indole acetic acid and gibberellic acid (Sundari and Dasgupta, 2014).

The homeodomain-leucine zipper class III transcription factor gene (*EtHB2*) was isolated from the leaf tissues of *Eucalyptus tereticornis*. The complete coding domain sequence was 2535 bp and harbored the signature domains including Homeodomain, Leucine Zipper, START and MEKHLA. Tissue-specific expression profiling of *EtHB2* revealed its predominant expression in developing xylem tissues (Dharanishanthi and Ghosh Dasgupta, 2017).

System approach to understanding complex traits in *Eucalyptus*

A customized array representing 3,359 water stress-responsive genes were designed to document their expression in *E. grandis* leaf cuttings subjected to -0.225 MPa of PEG treatment. The co-expression network was constructed with 915 nodes and 3,454 edges with a degree ranging from two to 45. Ninety-four (94) GO categories and 117 functional pathways were identified in the network. MCODE analysis generated 27 modules and module 6 with 479 nodes and 1005 edges was identified as the biologically relevant network. The major water-responsive transcripts represented in the module included dehydrin, osmotin, LEA protein, expansin, arabinogalactans, heat shock proteins, major facilitator proteins, ARM repeat proteins, raffinose synthase, tonoplast intrinsic protein and transcription factors like DREB2A, ARF9, AGL24, UNE12, WLIM1 and MYB66, MYB70, MYB55, MYB16 and MYB103. (Ghosh Dasgupta and Dharanishanthi 2017).

The natural variation in expression patterns of 18,987 transcripts expressed in the developing xylem tissues was documented across four phenotypes of *Eucalyptus tereticornis* with contrasting holocellulose/klason lignin content. The differentially expressed genes across all the phenotypes were used to construct co-expression networks and sub-network 2 with 380 nodes and 17,711 edges were determined as the network of relevance, including 30 major cell wall biogenesis-related transcripts with 2394 interactions and 10 families of transcription factors with 3360

interactions. *EYE* [EMBRYO YELLOW] was identified as a major hub transcript with 173 degrees which interacted with known cell wall biogenesis genes. K-mean clustering was also performed for differentially expressed transcripts and two clusters discriminated against the phenotypes based on their holocellulose/klason lignin content. The cluster-based networks were enriched with GOs related to cell wall biogenesis and sugar metabolism (Dharanishanthi and Ghosh Dasgupta 2016).

The expression patterns of 2,651 transcripts representing different pathways governing secondary development were documented across four genotypes of *E. tereticornis*. A co-expression network was constructed with 330 nodes and 4,512 edges and the degree ranged from 11 to 53. The network documented 75 (22%) transcription factors with a high degree of interaction. A secondary wall-associated NAC domain transcription factor (*SND2*) was identified as the top hub transcript with 53 interactions (Dharanishanthi and Ghosh Dasgupta 2018a). Co-expression network was developed for transcription factors expressed during wood formation in *E. tereticornis* and ethylene-responsive element-binding factor, *EtERF2*, was identified as the major hub transcript which co-expressed with other secondary cell walls biogenesis-specific TFs such as: *EtSND2*, *EtVND1*, *EtVND4*, *EtVND6*, *EtMYB70*, *EtGRAS* and *EtSCL8* (Dharanishanthi and Ghosh Dasgupta 2018b).

ix. *Populus* species (Poplar)

Populus species are the first perennial components to secure recognition as model tree crops for forest tree breeding programmes because of the groundbreaking work in species hybridization, polyploid breeding, and investigations into pathogen resistance during the early part of the twentieth century (Pauley 1949). Poplar is one of the most widely planted species after eucalyptus and pinus for industrial purposes. It is found in temperate regions such as Europe, the Mediterranean basin, Central Asia, Ukraine, Russia, northwest China, North America (between the Canadian prairie and Gulf of Mexico, and over 40° of longitude between the Atlantic seaboard and the Great Plains), and the Japanese Islands of Sakhal. Stanton et al (2014) reported that the poplar species

was domesticated in European countries through Eastern cottonwood (*P. deltoides*) in the late eighteenth century. The total area of planted poplar reported in 2016 was 31.4 million ha, of which 18.3 million ha (58%) are managed for multiple purposes, 9.4 million ha (30%) are primarily for wood production, 2.9 million ha (9%) for environmental protection and the balance of 0.9 million ha (3%) is managed for biomass production for fuelwood (FAO, 2016). The genus *Populus* Linn. includes 44 species in five sections like white poplars (Leuce), black poplars (Aigeiros), balsam poplars (Tecamahaca), Leucoides and Turanga. These are widely distributed in the temperate and sub-tropical regions of the Northern Hemisphere (Naithani and Nautiyal 2012).

When the *Populus trichocarpa* genome, 'Nisqually-1' was released in 2006, it was the first plant genome to use the shotgun sequencing assembly approach, the first genome to create chromosome-level assemblies based on genetic mapping, the first woody perennial genome to be assembled and annotated, as well as the first metagenomic assembly of associated endophytic bacteria and fungi (Tuskan et al 2006). Today, v3.2 contains 423 Mbp [of the ~485 Mbp genome] in 1,446 scaffolds with 2,585 gaps. The scaffold N50 metric is 8 Mbp and contig N50 is 205 Kbp, with roughly 98% of the genome in 181 scaffolds. Trained on deep RNAseq data from the Gene Atlas project, there are 41,335 predicted loci and 73,013 protein coding transcripts. Methylation maps are available for 10 tissue types, including bud tissue, callus tissue, female catkins, internode explants, male catkins, phloem, xylem and roots (Vining et al 2012). Over 28 million single nucleotide polymorphisms (SNPs) from re-sequenced data representing over 880 native *P. trichocarpa* genotypes covering the core distribution of the species range are publicly available (Geraldes et al 2013). This SNP resource has been used to characterize the geographic structure and linkage disequilibrium (Slavov et al 2012), detect signatures of selection across the genome (Evans et al 2014) and identify genetic loci associated with various phenotypes based on genome-wide association approaches (Porth et al 2013; Evans et al 2014; McKown et al 2014; Muchero et al 2015; Fahrenkrog et al 2017; Liu et al 2018). The re-sequenced data is currently being assembled into a *Populus* pan genome, with preliminary data suggesting that there may

be as many as 20,000 additional gene models that are not included in the current *P. trichocarpa* reference genome annotation (Pinosio et al 2016). The first draft assemblies of *Populus deltoides*, 'WV94' and *Populus tremula* × *P. alba* hybrid, '717-1B4,' have been sequenced, assembled, and annotated. The assembly for WV94 is approximately 446 Mbp in 1,375 scaffolds, with scaffold N50 of 21.7 Mbp and contig N50 of 590 Kbp and there are 44,853 protein-coding loci. CRISPR-related PAM (protospacer adjacent motif) sites for *P. tremula* × *P. alba* 717 have been published online at <http://aspendb.uga.edu/s717>.

Highly contiguous, de novo genome assemblies have also been produced for *P. euphratica* (Ma et al 2014) and *P. tremula*, which greatly widen the phylogenetic sampling of the genus. Ongoing resource development work in *Populus* includes efforts to expand the tissue type and experimental conditions in the Gene Atlas, enlarge the number of re-sequenced genotypes from the southern and eastern portions of the range, and release of V4.0 of the *P. trichocarpa* genome based on long-read sequencing and dense genetic maps derived from re-sequencing 1000 progeny from 49 half-sib families. Dozens of genome-wide association studies continue across a broad array of phenotypes and efforts to estimate the somatic mutation rate found in old-growth *P. trichocarpa* are currently underway. Methods to integrate SNP, gene expression, transcription factor binding, gene dosage, methylation, metabolite expression, phenome, and co-evolutionary relationships have been developed to generate a systems biology view of the molecular and regulatory interactions that lead to organismal scale traits (Henry et al 2015; Liu et al 2015; Joubert et al 2017, 2018; Weighill et al 2018). New algorithms are being developed to use signal processing techniques as well as explainable artificial intelligence to build better systems biology models. These include methods to discover and model genome-wide epistasis and pleiotropy. Efforts are also underway to gain a comprehensive view of chromatin structure and transcription factor-binding sites (Rao et al 2014).

x. *Acacia* species

The genus *Acacia* is one of the largest genera in the world, consisting of about 800 species of trees and shrubs in the sub-family Mimosoideae of the pea family, Fabaceae. It is native to tropical and sub-tropical regions of the world. It is mainly found in Australia (where they are called wattles) and Africa, where they are well-known landmarks on the veld and savanna. Acacias are commonly cultivated throughout temperate, tropical and desert regions, and include numerous distinctive growth forms with profusion of yellow or white flower clusters. Acacias also provide us with many useful products, from beautiful hardwoods to water-soluble gums used as a thickening agent in your favourite frozen dessert. Several acacia species are used commercially for tannins, fuelwood, timber, gum, adhesives, pharmaceuticals, inks, confectionary, and other products. Self-incompatibility is considered an important outbreeding mechanism in *Acacia*, with many species being highly self-incompatible (Fleming et al 2007).

A total of 37,786 putative SNPs were identified in *Acacia auriculiformis* × *Acacia mangium* hybrids using next-generation transcriptome sequencing. Most SNPs were converted to successful assays which are not only important for linkage map construction, but are also very useful for hybrid discrimination and genetic diversity assessment of natural germplasms in the future (Wong et al 2012). Genes involved in lignin biosynthesis and secondary cell wall formation in *Acacia* hybrids were identified by sequencing transcriptomes of non-normalized cDNA libraries synthesized from the pooled young stem and inner bark tissues using paired-end libraries and a single lane of an Illumina GAII machine (Wong et al 2011). The role of microRNAs was demonstrated and characterized by acting as sequence-specific guides expressed during secondary wall biosynthesis in *Acacia mangium*. The involvement of six highly-conserved amg-miRNA families viz., amg-miR166, amg-miR172, amg-miR168, amg-miR159, amg-miR394 and amg-miR156 in secondary cell wall biosynthesis (Ong and Wickneswari 2011, 2012) was reported. Williams et al (2015) reported the first cp genome of *A. ligulata* with 158 Kbp size which comprised inverted repeats of 25,925 bp and single-copy

regions of 88,576 bp and 18,298 bp, and revealed *clpP1* gene which is highly divergent and an essential part of cp genomes.

xi. *Casuarina equisetifolia* (Casuarina)

Casuarina equisetifolia L. (beefwood) is an evergreen, dioecious or monoecious tree, 6-35 m tall, with a finely branched crown. It belongs to the casuarinaceae family and originated from Australia, Bangladesh, Brunei, Cambodia, Fiji, Indonesia, Malaysia, New Zealand, Papua New Guinea, Philippines, Samoa, Solomon Islands, Thailand, Tonga, Vanuatu and Viet Nam. However, due to its fast-growing nature, it has been introduced to more than 60 countries and is now a common feature of the coastal landscape of most tropical and sub-tropical countries, where it is often naturalized. The species was introduced for firewood, beautification and other purposes to India, Pakistan, East, Central and West Africa, West Indies, Florida (USA) and the Gulf of Mexico. It is commercially planted under farm forestry, agroforestry or as windbreaks for poles, fuelwood, bioenergy, pulpwood and plywood purposes. It comprises nine pairs of chromosome ($2n=18$). The work on reproductive biology suggests that *C. equisetifolia* is self-compatible and that the breeding system should be classified as facultative xenogamy.

The molecular mechanism during *Casuarina equisetifolia*-*Trichosporium vesiculosum* was assessed and transcript profiling revealed the expression of resistance genes; cytochrome oxidase; trans membrane proteins; genes involved in programmed cell death like 26S proteasome and ubiquitin-activating enzyme; early nodulin gene, wound inducible metallocarboxy peptidase inhibitor, glucanase, metal binding protein and signal recognition particle (Ghosh Dasgupta et al 2013).

A class I chitinase (*CeChi1*) belonging to PR-3 family was cloned and characterized from the needle tissues of *C. equisetifolia* challenged with fungal elicitor. The *CeChi1* open reading frame comprised 966 nucleotides harboring a cysteine-rich chitin-binding domain and lysozyme-like domain. Quantitative real-time PCR analyses at different time

points showed up-regulation of the transcript during pathogen elicitation and salicylic acid signaling (Veluthakkal and Ghosh Dasgupta 2012). *CeChi1* was ectopically expressed in tobacco and *in vitro* antifungal bioassay using the total proteins from leaves of transformed plantlets revealed the characteristic lysis of hyphal tips of pathogenic fungi including *Trichosporium vesiculosum*, *Fusarium oxysporum* and *Rhizoctonia solani* (Veluthakkal and Ghosh Dasgupta 2015).

The transcriptome of the developing secondary tissues (15 years old) of *Casuarina equisetifolia* sub sp. *equisetifolia* was sequenced, *de novo* assembled, annotated and mapped to functional pathways. A total of 26,985 transcripts were predicted and mapped to 31 pathways. Mining of the annotated data identified nine genes involved in the lignin biosynthesis pathway and relative expression of the transcripts in four tissues including scale-like leaves, needle-like brachlets, wood, and root were documented. The expression of *CeCCR1* and *CeF5H* were significantly high in wood tissues, while the maximum expression of *CeHCT* was documented in the stem. Additionally, *CeTUBA* and *CeH2A* were identified as the most stable reference transcript for normalization of qRT-PCR data in *C. equisetifolia* (Vikashini et al 2018). ISSR and FISSR markers were used for the genetic analysis of the six species of *Allocasuarina*, five species of *Casuarina* and 12 superior-performing selections of *C. equisetifolia* (Yasodha et al 2004). Further, Ghosh et al (2011) and Nicodemus et al (2011) reported the development of species diagnostic ISSR-SCAR markers for *Casuarina equisetifolia* and *C. junghuhniana*.

xii. *Santalum album* (Sandalwood)

Sandal (*Santalum album* L.), one of the most primitive, precious and useful plants species, belongs to the santaleaceae family. Botanically, there are 16 species in the genus *Santalum* and one of them is *Santalum album* – Indian Sandalwood which is widely exploited for its heartwood oil “santalol”. Historically, the Western Ghats region is home to Indian sandalwood trees. The species is presently distributed in Australia, Indonesia, Malaysia, Africa and China. In traditional medicine, sandalwood oil has been used as an

antiseptic and astringent. It has also been used to treat headaches, stomachaches, and urinary and genital disorders. It is widely used in perfumes and pharmaceuticals.

Studies have revealed that flowers of *Santalum album* are self-incompatible and are strictly adapted for cross pollination by insects. The cytological studies in sandal states $2n=20$ and $2n=40$ chromosomes numbers. Rani et al (2013) isolated the sequences encoding farnesyl diphosphate synthase and santalene synthase from sandalwood using suppression subtraction hybridization and 2D gel electrophoresis technology. Mahesh et al (2018) established a draft map of *Santalum album* with 221 Mbp genome sizes and predicted 38,119 protein coding genes and 27.42% repetitive DNA elements. Proteomics revealed 72,325 unique peptides, which confirmed 10,076 predicted genes, while transcriptomic studies identified 53 novel proteins and 1,348 potential noncoding RNAs. Dasgupta et al (2019) have sequenced the *S. album* representing the natural population of Kerala and presented its draft genome with 286 Mbp size containing 37,500 genes, including 30 genes from the terpene synthase gene family, and predicted a total of 1,191,838 (0.42%) SNPs and 98,312 (0.034%) InDels. In addition, 147.25 Kbp chloroplast genome has been sequenced. Yang et al (2020) determined the first complete cp genome sequence of *S. album* using Illumina HiSeq pair-end sequencing data and reported 144,101 bp size containing 123 genes, including 80 protein-coding genes, 8 rRNA genes, and 35 tRNA genes. Mohammad et al (2012) evaluated the transferability of *Santalum austrocaledonicum* and *Santalum insulare* SSRs to *Santalum album* to examine the molecular diversity of 20 accessions from two diverse localities comprising germplasm of Madhya Pradesh and Karnataka.

xiii. *Litsea glutinosa* (Indian laurel)

Litsea glutinosa (Lour.) C.B. Rob. is an evergreen species of the tropical rainforest, and belongs to the family, Lauraceae. It is commonly known as bollygum, bolly beech, brown beech or Indian laurel, and grows 3-15 m tall in height. It is native to India, South China, Malaysia, Philippines, Australia and the western Pacific islands. The species was commercially introduced to La Réunion, Mauritius and Mayotte, and is principally used

as a binding agent in the incense-stick industry (Agarbatti), in tablet formulations and as plaster for fractured limbs. The wood is used for making agricultural tools, and the root fibre for making ropes and paper pulp (Arunachalam et al 2019). *Litsea glutinosa* is traditionally used to treat various human ailments and diseases.

It is a dioecious plant comprising separate staminate and pistillate in flowers. The florets display myophilous pollination syndrome and are pollinated by flies, as well as other insects. The genetic diversity and population structure of *Litsea glutinosa* in Central India using ISSR markers was first assessed by Mohammad et al (2018). The species is one of the fastest dwindling forest species in central India, and is represented by highly fragmented populations that have been drastically reduced in the last 40 years; its extraction has been banned by the government.

The sampled populations harbored a high level of genetic diversity (mean $h = 0.294$ and $I = 0.424$) that was partitioned more within populations (73%) than between populations (27%). Bayesian structure analysis revealed the existence of four admixed genetic pools in *L. glutinosa*. The dioecious nature of the species advocates *in-situ* conservation as the most suitable approach – Chhindwara, Jagdalpur, Balaghat and Jabalpur populations are appropriate.

xiv. *Pongamia pinnata* (Karanj)

Pongamia pinnata L. Pierre is a multipurpose versatile Leguminosae (papilionaceae) member and native to Bangladesh, India, Myanmar, Nepal and Thailand. It has been introduced to parts of Australia, New Zealand, China and the USA, as well as other humid tropical regions of the world. The natural distribution of the species is along coasts and river banks in India and Myanmar. It is mostly used for biodiesel, as fodder for cattle and goats, blended with nitrogenous fertilizers, and as fuelwood. The timber is moderately strong and used for cabinet making, cartwheels, posts, agricultural implements, tool handles and combs (Chaturvedi et al 2017).

Bees and wasps promote cross pollination in pongamia. The number of chromosomes are $2n=22$. However, to date, there has been little genomic research aimed at exploitation of the biotechnological potential of this species. Choudhury et al (2013) studied the genome size of *P. pinnata* which was estimated by flow cytometry to two standards (*Zea mays* and *Pisum sativum*), and compared with that of in vitro-raised plants (nodal segment, in vitro-rooted plantlets and acclimatized in vitro plants). Jin et al (2019) identified 236 conserved miRNAs within 49 families and 143 novel miRNAs via deep sequencing of pongamia seeds sampled at three developmental phases. For these miRNAs, 1,327 target genes were computationally predicted. Furthermore, 115 differentially expressed miRNAs (DEmiRs) between successive developmental phases were sorted out. The DEmiR-targeted genes were preferentially enriched in the functional categories associated with DNA damage repair and photosynthesis.

Many nuclear genes associated with oil biosynthesis and nodulation in Pongamia have been characterized. Kazakoff (2012) assembled and annotated pongamia chloroplast and mitochondrial genomes; the cpDNA contains 77 unique protein-coding genes and is almost 60% gene-dense. Comparison of *Pongamia organelle* genome with other legumes revealed many similarities among species (Biswas et al 2013). It contains a 50 Kbp inversion common to other legumes, as well as a novel 6.5 Kbp inversion that is responsible for the non-disruptive, re-orientation of five protein-coding genes. Additionally, two copies of an inverted repeat firmly place the species outside the subclade of the Fabaceae. The *Pongamia* and *L. japonicus* mtDNA contain just 33 and 31 unique protein-coding genes, respectively, and like other angiosperm mtDNA, have expanded intergenic and multiple repeat regions. Through comparative analysis of *P. pinnata* L. japonicus and *Vigna radiata*, the average synonymous and non-synonymous divergence was measured in protein-coding genes of mitochondrial (1.59% and 2.40%, respectively) and chloroplast (8.37% and 8.99%, respectively) protein-coding genes. The utility of the organellar genome sequences was unveiled by mapping transcriptomic data to identify up- and down-regulated stress-responsive gene candidates and confirm in silico predicted RNA editing sites.

Sreeharsha et al (2016) performed the whole transcriptome analysis of pongamia through the Illumina NextSeq platform and generated 2.8 Gbp of paired-end sequence reads. The *de novo* assembly of raw reads generated 40,000 contigs and 35,000 transcripts, representing leaf, flower and seed unigenes. Spatial and temporal expression profiles of photoperiod and floral homeotic genes in pongamia, identified GIGANTEA (GI) - CONSTANS (CO) - FLOWERING LOCUS T (FT) as an active signal cascade for floral initiation. Besides, four prominent stages of seed development were selected in a high yielding pongamia accession (TOIL 1) to follow the temporal expression patterns of key fatty acid biosynthetic genes involved in lipid biosynthesis and accumulation. They provide insights into an array of molecular events, from flowering to seed maturity, which will provide a substantial basis for modulation of fatty acid composition and enhancing oil yields which could serve as a potential feedstock for biofuel production.

Jiang et al (2012) studied the variation for seed mass, oil content and oil composition. To evaluate genetic diversity and to lay the basis for a molecular breeding approach, we developed second-generation sequencing (2GS)-derived ISSR markers (*Pongamia* Inter-Simple Sequence Repeats; PISSR). The special feature of PISSRs is that the number of nucleotide repeats and the 5' and 3' nucleotide extensions were not arbitrarily chosen, but were based on pongamia genomic sequences obtained from an NGS (Illumina®) database. They demonstrated: 1) an abundance of nucleotide core repeats in the genome, 2) large genetic and phenotypic diversity among randomly sampled pongamia trees, 3) restricted diversity in progeny derived from a single mature tree; 4) stability of PISSR markers in clones, and 5) genomic DNA sequences within PISSR markers. The genetic diversity in 37 wild species of *P. pinnata* has been assessed using 18 RAPD and 23 SRAP primers which generated 150 polymorphic bands (96.8% polymorphism) and 122 polymorphic (84.1% polymorphism) bands. The mean PIC for both the markers was 0.77 and 0.68. This, along with PCA, confirmed the existence of genetic diversity among the accessions.

xv. *Morus* species

The scientific name of mulberry is *Morus* spp., a genus belonging to the Moraceae family of the Urticales sub-class. There are about 68 species of the genus *Morus*, and majority of them occur in Asia (Datta 2000). In China, there are over a thousand varieties under cultivation. They originated from four main species, the White mulberry (*Morus alba*), the Lu mulberry (*M. multicaulis*), the Mountain mulberry (*M. bombycis*) and the Guangdong mulberry (*M. atropurpurea*) (Yongkang 2000). Mulberry, a perennial deciduous plant is reported to have originated from China, the primary centre of the plant origin, apart from the Indian species namely, *M. indica*, *M. alba*, *M. serrata* and *M. laevigata*, which are considered indigenous to India. The species is utilized for silk production, fodder, fuelwood and food. The Central Silk Board–Central Sericulture Research and Training Institute’s Mysore campus possesses 350 mulberry accessions (CSRTI 2019) while Pampore, Srinagar campus possesses 47 temperate origin mulberry accessions including 16 indigenous and 31 exotic (Saini et al 2018). *Morus* consists of diploid chromosome number ($2n=28$) and monoecious flowers. The mode of pollination is through wind, i.e., anemophilous pollination.

In *Morus* sp. identification of drought-regulated genes and the generation of a large number of ESTs was reported by Gulyani and Khurana (2011). Thumilan et al 2013 identified and characterized 222 genomic and 136 genic SSRs from genomic clones and ESTs in this species. Besides, Krishnan et al (2014) reported around 217,312 SSRs from genome sequence and 961 SSRs from EST sequences of *M. notabilis*. However, Saeed et al (2016) have generated 87.0 and 80.3 Mbp of transcriptomic data from *M. laevigata* and *M. serrata*, assembled them into 95,181 and 85,269 transcripts, respectively, and identified 24,049 SSRs, 1,201,326 SNPs and 67,875 InDels. Further, 206 EST-driven SSRs has been developed and validated from transcriptomic data of drought-tolerant mulberry cv. Dudia white (Thumilan et al 2016). Recently, 248 genomic-SSR and 490 genic-SSR (EST-SSR) primers were designed and used in marker-assisted breeding programmes (Sarkar et al 2017).

xvi. *Boswellia sacra* (Frankincense)

Boswellia sacra is commonly known as frankincense or olibanum-tree. It belongs to the Burseraceae family and is considered an economically important species of the genus *Boswellia*. The species is endemic to Ethiopia, northern Somalia, south-western Oman and southern Yemen. It is considered an economically important species, and is a good source of high quality frankincense and bioactive compounds. Medicinally it can be used as an analgesic, hepato-protective, antioxidant, diuretic, anti-coagulant, and to treat tumors and skin disorders (Al-Harrass et al 2018). The species has chromosome numbers of $2n=22$ and is open-pollinated mainly through bees and wind. It is self-incompatible and the selfed pollen tubes are inhibited soon after their entry into the stigma. The chloroplast genome of *Boswellia sacra* has been sequenced and the size is about 160 Kbp with 37.61% GC content (Khan et al 2017). Further, *De novo* assembly and annotation showed the presence of 114 unique genes with 83 protein-coding regions. Based on this, a phylogenetic relationship was assessed with *Azadirachta indica* and *Citrus sinensis*.

xvii. *Moringa oleifera* (Drumstick)

Moringa oleifera Lam. belongs to the family Moringaceae. It is a single genus family comprising 13 species distributed in India, Kenya, northeastern and southwestern Africa, Arabia and Madagascar (Saini 2015). *M. oleifera* is native to the sub-Himalayan Mountains of northern India and cultivated throughout the world in tropical and sub-tropical regions (Leone et al 2015). It has been considered as a super food, and miracle tree as every part of it is filled with minerals, vitamins and antioxidants, and has the potential to treat over 300 diseases. Its tender roots are used to make pickle, especially in the Bundelkhand region of India. Its seed oil is equivalent in quality to olive oil, and has the potential to be used as biodiesel. The chromosomes number in *Moringa* is $2n=28$. *Moringa* flowers are dichogamous and follow xenogamy and cross-pollination mainly through bees and other insects. *Moringa* leaves, flowers, and pods are commonly used as food due to its richness in vitamins and ions.

Pasha et al (2020) have developed a bioinformatics pipeline to assemble transcriptome, along with the previously published *M. oleifera* genome, to predict 17,148 gene models. Few candidate genes related to the biosynthesis of secondary metabolites, vitamins and ion transporters were identified. Expressions were further confirmed by real-time quantitative PCR experiments for a few promising leads. Quantitative estimation of metabolites, as well as elemental analysis, was also carried out to support our observations. Enzymes in the biosynthesis of vitamins and metabolites like quercetin and kaempferol are highly expressed in leaves, flowers and seeds. The expression of ion transporters and calcium storage proteins was observed in roots and leaves. In general, leaves retained the highest amounts of small molecules of interest. This study showed that the leaves contain 30 times more iron and 100 times more calcium than spinach. Indeed, iron, zinc and magnesium transporters were found to be highly abundant in the roots and stem. A total of 36 candidate genes were tracked in this study from all five tissues. These include the synthesizing enzymes for compounds (flavonoids, terpenoids, vitamins and alkaloids like moringine) and transporters of minerals. Among these, the bioactive compounds of medicinal value such as quercetin (effective in metabolic disorders) and its synthesizing enzymes are observed more in leaves and flowers. Kaempferol (effective as anti-cancer agent) was observed to be abundant in the flowers.

Tian et al (2015) report a high-quality draft genome sequence of *M. oleifera*. This assembly represents 91.78% of the estimated genome size and contains 19,465 protein-coding genes. Comparative genomic analysis between *M. oleifera* and related woody plant genomes helps clarify the general evolution of this species, while the identification of several species-specific gene families and positively selected genes in *M. oleifera* may help identify genes related to the species' high protein content, fast-growth, heat and stress tolerance. Liu et al (2019) reported the complete chloroplast genome sequence of *M. oleifera* for the very first time (accession number of MH778650), and it was also the first reported chloroplast genome of Moringaceae. The chloroplast genome is 160,599 bp long and includes 113 genes. Its LSC, SSC and IR regions are 88,933, 19,482 and

26,092 bp long, respectively. Phylogenetic tree analysis revealed that *M. oleifera* was clustered with other Moringaceae species with 100% bootstrap values.

xviii. *Albizia procera* (White siris)

Albizia genus comprises over 160 species in the sub-family Mimosoideae of the family Fabaceae. The genus is pantropical, occurring in Asia, Africa, Madagascar, America and Australia, but is more common in the Old World tropics. It is a valuable timber and is used in general construction (house posts, beams, scantlings, planks, boards), carriage and carts, lorry and bus bodies, agricultural implements, tool handles, packing cases and crates (Sivakrishnan and Swamivelmanickam 2019). The species is self-pollinated through insects and consists of $2n=26$ numbers of chromosomes. Aparajita et al (2010) used 12 AFLP primers to study the genetic diversity of *Albizia* species. Mohammad et al (2016) used RAPD and ISSR markers to authenticate the true-to-type nature of the in vitro-raised plantlets of *Albizia procera*. Aparajita et al (2016) studied the structure of 102 individuals representing six natural populations of wider range using ISSR markers and observed 34.16% and 65.84% variances between and within the population, respectively.

xix. *Tamarindus indica* (Tamarind)

Tamarindus indica L. (Leguminosae: Caesalpinioideae), is an example of an economically important but little studied tropical fruit tree. The seeds in its indehiscent pods are enveloped by dark brown pulp with a pleasantly sweet and acid flavor, much appreciated in condiments and used as juice. Breeding system and pollination biology of the semi-domesticated tamarind tree has been examined (Diallo et al 2008). They observed that open- and cross-pollination give better quantitative seed than self-pollination. It is a diploid species with chromosome number of $2n=24$ as quoted by Gunasena and Hughes (2000). A high rate of seed abortion in the self-pollination system has been observed. Results revealed that there is a post-zygotic self-incompatibility and other factors, not related to the pollination system, intervened in seed development after fertilization of ovules. Genetic diversity was also assessed by RAPD markers (Diallo et al

2007). In Vivo Biochemical and Gene expression analyses of the antioxidant activities and hypo-cholesterolaemic properties of *Tamarindus indica* fruit pulp extract were also analyzed.

xx. *Ailanthus excelsa* (Tree of heaven)

Ailanthus excelsa Roxb. is a member of the Simaroubaceae family and is commonly known as Ardu, Mahaneem or tree of heaven. It is considered native to the Indian Peninsula and distributed throughout the tropical and sub-tropical regions of India. The tree is multi-purpose and is used in production of match splints, packing cases, fishing floats and sword sheaths. It can also be used as fodder. The species favours xenogamous pollination. The wood transcriptome of a 22-month old tree was sequenced and *de novo* assembled. A total of 48,493 transcripts were predicted with N50 of 1,606 bp. The chloroplast genome was re-constructed and 160,804 bp genome assembled, encoding 116 genes. Phylogenetic analysis using the complete chloroplast genome revealed that members of Simaroubaceae form sister clade with a 95% bootstrap support to Rutaceae family (Saina et al 2018). Mining for microsatellite repeats predicted the presence of 5,501 repeats in the wood transcriptome. This study presents the first genomic resource in the genus *Ailanthus*. The information generated can be used for subsequent research in diversity assessment, population structure analysis, and accelerated breeding targeting the improvement of wood property traits in this species (unpublished data from IFGTB Coimbatore, India).

xxi. *Korthalsia laciniosa* (Rotan Dahan)

Korthalsia laciniosa is commonly known as Rotan dahan. It is a climbing, evergreen shrub, producing a cluster of slender; woody stems around 20-30 mm in diameter. It is grown in Southeastern Asia – Thailand, Cambodia, Laos, Viet Nam, Malaysia, Indonesia and the Philippines. The durable canes are highly sought after at village level, and used to make baskets, for weaving, etc. The cytological study reveals that the species has 2n=32 chromosomes. Mode of pollination is through bees. Draft genome sequence of *Korthalsia laciniosa* (Griff.) Mart.: a clustering rattan was generated and the reads

assembled into 243,005 scaffolds with N50 of 23,613 bp. A total of 21,833 genes were predicted and assigned to 6,627 gene families. Taxonomic distribution based on homology search with monocots revealed the highest similarity with *Elaeis guineensis*. Cataloging of functional pathways mapped the predicted genes to 391 pathways. Additionally, the draft genome was mined for microsatellite repeats and 267,240 SSRs were predicted (unpublished data from IFGTB Coimbatore, India).

xxii. *Annona squamosa* (Custard Apple)

Annona squamosa L., (custard apple) belongs to the Annonaceae family. It is a popular fruit tree which originated in America and is a widely cultivated species both within its native range and in tropical regions around the world. This plant is also known as sugar apple or sweetsop and has many other regional names such as custard apple (India), anon (Portuguese), and noi-na (Thailand). The fruits are consumed widely and the tree is also a source of medicinal and industrial products. The fruits contain vitamin C and minerals such as calcium, phosphorous and potassium. They are also an excellent source of energy, being rich in carbohydrates. Its chromosome number is $2n=14, 16$ and flowers are protogynous, pollinated mainly by wind and honeybees. Custard apple *de novo* assembly and characterization of transcriptomes of early-stage fruit from two genotypes of *Annona squamosa* L. with contrast in seed numbers through massive pyrosequencing were reported by Gupta et al (2015). The data reported here is the first source of genome-wide differential transcriptome sequence in two genotypes of *A. squamosa* and identifies several candidate genes related to seed development. Liu et al (2017) studied the comparative transcriptomic analyses of normal and malformed flowers in sugar apple (*Annona squamosa*) to identify the differential expressed genes between normal and malformed flowers. In this study, a comprehensive perspective on the differences between normal and malformed flowers of sugar apple, was prepared independently by Illumina sequencing. The data generated a total of 70,189,896 reads that were integrated and assembled into 55,097 unigenes with a mean length of 783 bp. A large number of differentially expressed genes (DEGs) were identified. Among these DEGs, 701 flower development-associated transcript factor encoding genes were

included. Furthermore, a large number of flowering- and hormone-related DEGs were also identified, and most of these genes were down-regulated expressed in the malformation flowers. The expression levels of 15 selected genes were validated using quantitative-PCR. The contents of several endogenous hormones were measured. The malformed flowers displayed lower endogenous hormone levels compared to the normal flowers. Anuragi *et al.* (2016) suggested the existence of significant genetic diversity among 20 *Annona* genotypes representing five different species of *Annonaceae* based on 11 RAPD and 12 SSRs markers which yielded 152 bands (80.01% polymorphism), 0.89 PIC for RAPD, 39 amplicons (93.05% polymorphism) and 0.339 PIC for SSR primers.

xxiii. *Syzygium cumini* (Jamun)

The family of Myrtaceae consists of about 121 genera with 3800-5800 species of shrubs and trees mainly distributed in tropical and sub-tropical regions of the world (Stefanello et al 2011). The genus *Syzygium*, a leading member of this family, embrace 1,100 species with deserving attention to *Syzygium cumini* (L.), an important indigenous fruit tree in India. It is a good source of iron apart from being the source of minerals, sugars and other phytochemicals. *S. cumini* is diploid, consists of $2n=66$ chromosomes, and is open-pollinated by wind and honey bees. Only few genomics-related studies have been conducted in the Myrtaceae family so far. The 23 wild jamun genotypes comprising Andaman and Nicobar Islands and the mainland were analyzed for genetic diversity with the help of 30 ISSR and 20 RAPD primers (Shakya, 2010). Asif *et al.* (2013) estimated that the size of the IR region in *S. cumini* cpDNA is 26,392 bp with 20 genes. They also compared *S. cumini* cpDNA sequence with previously reported partial sequences of *S. cumini* as presented in GenBank and found a range of sequence polymorphisms.

xxiv. *Commiphora wightii* (Guggul)

Commiphora wightii [(Arnot) Bhandari] is a medicinal shrub of the family Burseraceae. It is xerophytic in nature and grows naturally in the arid and rocky zones of India, that is, Gujarat, Karnataka, Madhya Pradesh and Rajasthan, and also in Pakistan. Guggul

consists of $2n=26$ chromosomes. It is valued for its medicinal oleo-resin gum rich in guggulsterone and reproduces through sporophytic apomixes. Further, it is dioecious or polygamous, apomictic and pollinated by birds. The main economic product from this tree is gum-resin. The Central Institute of Medicinal and Aromatic Plants (CIMAP), Lucknow has developed a good oleo-gum-resin-yielding cultivar called 'marusudha'. Guggul is enlisted under the 'data deficient' category in the IUCN Red Data Book (<http://www.iucnredlist.org/details/31231/0>). Geetha et al (2013) carried out a comprehensive investigation of guggul reproduction to understand histology, controlled pollination, flow cytometry and conducted RAPD analyses of progeny to assess the occurrence and extent of sexual reproduction. They reported obligate sexual female plant and documented a unique pollen–pistil incompatibility that prevents all but one pollen tube growth into the style to effect fertilization. Harish et al (2014) estimated the genetic diversity to ascertain the conservation priorities and strategies, and reported a total of 155 amplified products using RAPD and ISSR primers with overall 86.72% polymorphism across 45 individuals representing eight populations. Mantel's test revealed no correlation between genetic and geographical distances of populations ($R^2=0.122$).

xxv. *Prosopis cineraria* (Khejri)

Prosopis cineraria (L.) Druce, widely known as Khejri or the king of desert, belongs to the sub-family Mimosoideae of Leguminosae family, and originated from Afghanistan, India, Iran, Pakistan and Sri Lanka. It is commonly found in the Thar Desert of India and Pakistan. *P. cineraria* pods are used as a vegetable, both in dried and green forms, in many parts of the Thar Desert. The tree provides fodder, fuelwood, pods and timber. The species is mainly planted for sand dune stabilization and reclamation. It also fixes atmospheric nitrogen (Kreethika et al 2015). The species consists of $2n=28$ chromosomes and non-synchronous flowering to facilitate cross-pollination. Sharma et al (2011) assessed genetic diversity within and between *Prosopis cineraria* accessions collected from different districts of Rajasthan (India) and generated a total of 83 bands from eight ISSR and five DAMD primers with 95.18% polymorphism.

Nei's gene diversity (h) was 0.185-0.301, while Shannon's information index (I) values ranged from 0.253 to 0.438, demonstrating the higher genetic variation within the population. Sivalingam et al (2016) developed markers for varietal identification among Thar Shobha and other *Prosopis cineraria* resources in which 80 RAPD primers were tested. Out of these, nine were polymorphic between 'Thar Shoba' and trees in a natural population of khejri. Phenetic analysis revealed that 'Thar Shoba' formed a separate cluster from the natural population with similarity coefficients of 0.57–0.78. The 10-mer primers OPBE05, OPBA13, OPA12, and OPA14 were each found to differentiate 'Thar Shoba' from the natural population by one amplicon. Transcriptome analysis of *P. cineraria* using RNA_Seq technology was first analyzed by Rai et al (2017). The study concluded that about 142,711 unigenes assembled de novo from leaf transcriptome and 60,583 CDS predicted from unigenes. However, 57,249 CDS were annotated by blast searches against public database and 950 CDS had a high expression level of > 100 rpkm.

xxvi. *Sapindus emarginatus* (Soap-nut tree)

Sapindus emarginatus Wahl, commonly known as soap nut tree or reetha, belongs to the sapindaceae family. It is andromonoecious and has both bisexual and male flowers on the same plant. This species is native to south India, mainly around the peninsular region, and is globally distributed in Sri Lanka and Myanmar. The fruits are used to cure asthma, cholera and diarrhea, and to ease delivery during childbirth. The fruit paste is applied in scorpion stings, snakebites and used as a leech repellent.

Sapindus emarginatus is cross-pollinated by a wide variety of insects; the species has a chromosome number of $2n=30$. Mahar et al (2011) studied the genetic variability within and between 41 accessions representing five populations using RAPD, DAMD and ISSR primers, and observed 82.32% polymorphism across all the accessions of *Sapindus emarginatus*. AMOVA revealed 72% variation among individuals of populations followed by 16% among regions and 12% among populations. Mantel's test revealed a highly

significant correlation between cumulative vs RAPD, and showed the maximum (0.93) correlation coefficient, followed by cumulative vs ISSR (0.78) and cumulative vs DAMD (0.91), respectively.

xxvii. *Jatropha curcas* (Jatropha)

Jatropha curcas L. belongs to the Euphorbiaceae family and is traded as jatropha or physic nut. It originated from Belize, Costa Rica, El Salvador, Guatemala, Honduras, Mexico, Nicaragua and Panama. From the Caribbean, *Jatropha curcas* was probably distributed by Portuguese seafarers via the Cape Verde Islands and former Portuguese Guinea (now Guinea Bissau) to other countries in Africa and Asia. It is mainly used for biodiesel, feedstock, organic manure and live hedges. The genus *Jatropha* is a diploid with a chromosome number of $2n=22$. It comprises about 175 species worldwide. In India, 12 species are found scattered in various states. The species is monoecious, with male and female flowers on the same plant (Chaturvedi et al 2017). *Jatropha* is a cross-pollinated species and is mainly pollinated by insects.

Kancharla et al (2019) have generated the hybrid *de novo* genome assembly with 265.7 Mbp in *J. curcas* using Illumina and PacBio sequencing technologies. Besides, they identified three minor QTLs for *Jatropha* Mosaic Virus (JMV) resistance in F_2 population of a resistant and susceptible crosses using 207 polymorphic SSR markers. Ha et al (2019) reported ~339 Mbp draft whole-genome sequence in Chai Nat variety using both the PacBio and Illumina sequencing platforms and identified differentially expressed genes relevant to the biosynthesis of lipids and toxic compounds during seed development. Tian et al. (2017) sequenced the transcriptome and developed a large number of microsatellite and SNP markers from the transcriptome sequences to facilitate the *jatropha* genetic improvement programme. Wang et al (2018) performed genome-wide identification and transcriptional expression analysis and identified 12 JcMAPKs, 5 JcMAPKKs, and 65 JcMAPKKKs gene families which are fundamental signal transduction modules for controlling cell division, hormone signaling and overall growth and are activated under a variety of biotic and abiotic stresses. Kole et al (2015a) studied

the genetic variability and relationship among 182 accessions of *J. curcas* collected from 19 Indian states. 30 RAPD markers generated 254 polymorphic bands (96.82%) with average PIC of 0.791. All this confirmed a high level of genetic variation among the accessions.

xxviii. *Mangifera indica* (Mango)

Mango (*Mangifera indica* L.) is an evergreen fruit tree belonging to order Sapindales of the family Anacardiaceae. It is known as the king of fruits due to its cultural value and taste, large production volume, and diverse end usage. However, mango breeding is challenging due to the long juvenile phase, high heterozygosity and heavy fruit drops. Flowers are either male (staminate) or hermaphroditic, containing both stamens and carpels. Mango has self-, cross-pollination, self-incompatibility and self-sterility systems; however, some cultivars are either semi-compatible or fully compatible. Wind is important in mango pollination. It has 20 pairs of chromosomes ($2n=40$) with an estimated genome size of 439 Mbp. A major limiting factor for mango improvement has been the paucity of genomic resources. Total 44.8 Gbp of genomic sequence (102x coverage) was generated using PacBio platform. The genome was assembled and polished using FALCON and Quiver software, respectively. The resulting 9,500 contigs (401 Mbp) were arranged into 4,312 scaffolds (403 Mbp), covering 91.8% of the genome with the largest scaffold of 2.1 Mbp and N50 of 282 Kbp. Over 80% of the scaffolds were anchored to 20 mango chromosomes with 6,242 markers of a recently published high density genetic map of 6,594 markers (94.66% coverage). A total of 53,018 genes were predicted, annotated and manually curated.

Further, 168,484 SSRs were identified, including 30,821 Type 1 and 12,855 highly variable SSR. We also identified 5.2 million high-quality SNPs in 19,656 single copy genes by re-sequencing 24 diverse mango cultivars and developed 80K SNP chip. Mango showed the highest similarity with *Citrus sinensis* among the published fruit genomes. The reference genome would fast track mango varietal improvement for stress tolerance, higher productivity, and superior quality. The genome sequence of mango

variety Amrapali was deposited in the NCBI GenBank public repository in 2015 and upgraded in 2017 (Acc no. LMWC000000001), and now maternal and paternal phase-separated reference genome assemblies of the two parental lines of Amrapali, namely Dashehari and Neelam have been submitted to NCBI. Recently, genome sequences of two more varieties were assembled based on PacBio SMRT sequencing (Li et al 2020; Wang et al 2020).

xxix. *Quercus leucotrichophora* (Banj oak)

Among the broad leaf species, oaks (*Quercus* spp.) are important, dominant vascular plants of the Himalayas, ranging from the sub-tropical to the sub-alpine zones. Banj oak (*Quercus leucotrichophora* A. Camus) is the most common broadleaf tree in the mid-elevation central Himalaya in India. It is the most preferred tree species in the temperate region, mainly used for fodder, fuel and small timber. *Q. leucotrichophora* forms an extensive belt along the middle elevation (1200-2200 m) facing excessive pressure for existence. The replacement of oak by pine has become a common and ever-increasing phenomenon in this region. Basic chromosome number $x=8$ with various levels of ploidy, monoecious (individual flowers are either male or female, but both sexes can be found on the same plant) and is pollinated by wind. Very few studies have been reported on this species. Research has been confined to genetic diversity analysis with the help of random primers. Four populations collected from Jhungi Suket, Taradevi Shimla, Bhatwari-Taknor Range and Nohra-Shimla were studied using RAPD markers out of 10 primers, five polymorphic markers were selected which gave total mean diversity (H_t) value of 0.2253 (Saha 2018).

xxx. *Cedrus deodara* (Himalayan cedar)

Cedrus deodara (Roxb. ex D. Don) G. Don (deodar or Himalayan cedar) also known as Indian cedar is one of the most important temperate timber species of the Western Himalayas and is considered one of the endangered conifer species in this region. It grows right from Afghanistan to Garhwal at an elevation of 1200-3050 m. The strongest of Indian coniferous woods, it is used for door and window frames, furniture packing-

cases, beams, masts, spars and shingles, and also for bridges and railway sleepers. Wood yields an oleoresin and dark coloured oil used for ulcers and skin diseases. It is a monoecious, anemophilous species where male and female cones typically occur on the same tree but on separate branches. RAPD markers were used to study genetic variation in three natural populations of western Himalaya using 10 primers. The study indicated that *C. deodara* populations maintained moderate level of genetic diversity (mean $H_t=0.233$) with more variation among the populations when compared to within population (Rai 2020). Karyotype investigation showed a simple monosomatic haploid level in the megagametophyte, as compared to the diploid level of the corresponding embryo. Cytogenetic studies showed a diploid chromosome number of $2n=24$ in 11 populations sampled over the four species including *Cedrus deodara*. Eight GC-rich chromomycin A3 bands were observed in *Cedrus deodara* chromosomes (Dagher-Kharat 2001). Complete chloroplast genome of 53,775 kb is available in the public domain (Lin et al 2010).

xxxi. *Myrica esculenta* (Chinese Bayberry)

The *Myrica* genus is a large group comprising more than 97 species in the Myricaceae family. Among several species, *Myrica esculenta* Buch.-Ham. ex. D. Don dubbed 'Hairy Bayberry' or Chinese Bayberry, is also widely known as Kaiphal or Kataphala in the Indian sub-continent (Kabra et al 2019). It is a popular medicinal plant distributed in the sub-tropical Himalayas. It is also found in the sub-tropical and temperate zones of China, Taiwan, Japan, Western Highland of Cameroon, North America, South Africa, Australia, Brazil, Ethiopia and Nepal. All the parts of the *M. esculenta* plant have huge nutritional and therapeutic importance. Fruits are used for syrups, jams, pickles and in the preparation of refreshing drinks (Makdoh et al 2014). Basic chromosome number throughout the family is eight, with various levels of ploidy. The dioecious flowers are cross pollinated mainly through wind. This tree is well recognized in ayurvedic pharmacopeia and is widely used as traditional medicine to treat several ailments such as asthma, cough, chronic bronchitis, ulcers, inflammation, anemia, fever, diarrhea, and ear, nose and throat disorders. Recent upsurge in *M. esculenta* use and demand has led

to illicit harvesting, thus pushing the plant to the brink of extinction. A 158-simple sequence repeat (SSR) markers have been developed by surveying of whole genome shotgun data (Jiao et al 2012). Sharma et al (2020) carried out DNA barcoding of *Myrica esculenta* for its molecular identification done through sequence analysis with three barcode loci – *rbcL*, *matK* and ITS genes. The nucleotide sequence of internal transcribed spacers (ITS2), chloroplast maturase kinase gene (*matK*) and *rbcL* have determined a close relationship among 12 other species of *Myrica*.

xxxii. *Taxus wallichiana* (Himalayan yew)

Taxus wallichiana Zucc. (*Taxaceae* yew family) is an evergreen tree which is mostly distributed in the temperate zone of the Northern Hemisphere, extending into the tropics and south of the Equator into Malaysia. In Asia, *T. wallichiana* Zuccarini (Himalayan Yew) is mostly distributed in the Himalayas stretching into Afghanistan, Bhutan, China, India, Indonesia, Malaysia, Myanmar, Nepal, Pakistan, the Philippines and Viet Nam. In the Indian Himalayan region, occurrence of the species has been reported in the states of Arunachal Pradesh, Himachal Pradesh, Uttarakhand, Jammu and Kashmir, Manipur, Meghalaya, Nagaland, Sikkim and West Bengal. It is the only *Taxus* species occurring in India. The yew has important economic value and has thus been subjected to severe exploitation. Moreover, as compared to other species, yew has some congenital deficiency such as insufficient seedlings, weak competitiveness and rigid habitat requirements (Ru et al 2006). Taxol is one of the most potent and effective anti-cancer drugs and is originally isolated from *Taxus* species. It has $2n=24$ chromosomes and follows cross pollination.

A combination of transect study, population genetic analyses based on chloroplast DNA sequences and nuclear microsatellite data, and ecological modelling used to study patterns of genetic variation, levels of diversity and structure in the endangered allopatrically distributed *Taxus contorta*, *T. mairei* and *T. wallichiana* was undertaken in the highly fragmented and degraded habitats of the Himalayas. Results showed low genetic diversity and high inbreeding (Poudel et al 2014). Transcriptome sequencing of

Taxus wallichiana var. *Mairei* (high taxol content Jinxishan variety) revealed that high expressions of the genes taxadienol acetyltransferase (*TAT*), taxadiene 5- α hydroxylase (*T5H*), 5- α -taxadienol-10- β -hydroxylase (*T10OH*), and 2-debenzoyl-7,13-diacetylbaccatin III-2-O-benzoyl-transferase (*DBBT*), which catalyze a series of key acetylation and hydroxylation steps, are the main cause of high taxol content in Jinxishan (Wang et al 2019).

xxxiii. *Dendrocalamus hamiltonii* (Hamilton's bamboo)

Dendrocalamus hamiltonii is an economically important bamboo species used to produce fermented bamboo shoots which is a popular traditional food item of the northeast state of India. This species has been experiencing population depletion due to heavy extraction from natural forests. Genetic variability of this bamboo species in Chandel and Imphal-East (commercial production districts) was assessed using AFLP molecular markers. Principal coordinate analysis based on the AFLP data clearly separated the populations according to their genetic diversity and antioxidant activity. Association of DNA markers with antioxidant activities and total cyanide content was also found (Waikhom et al 2012). Meena et al (2019) used nuclear simple sequence repeats (nSSRs) to study the genetic diversity and population genetic structure of 19 natural stands of *D. hamiltonii* distributed across the northeast Himalayas. Transcriptome analysis of rhizome and shoot during the major vegetative developmental transitions of *D. hamiltonii* is performed to dissect factors governing growth. It signifies the role of environmental cues, predominantly rainfall, decreasing day length and high humidity for activating dormant buds to produce new shoot, possibly through complex molecular interactions among phosphatidylinositol, calcium signaling pathways, phytohormones, circadian rhythm and humidity responses (Bhandawat et al 2017).

xxxiv. *Citrus* species

Citrus is a large genus that includes several cultivated species and is grown in more than 100 countries. The most widely cultivated citrus species under domestication and selective breeding include sweet orange (*Citrus sinensis*), mandarin (*Citrus reticulata*), pummelo (*Citrus grandis*),

grapefruit (*Citrus paradisi*) and lemon (*Citrus limon*) (Li et al 2020). Citrus fruits possess unique botanical features, i.e., nucellar embryony nucellus cells that can develop into apomictic embryos that are genetically identical to the mother plant. Diploidy is the general rule in citrus and related genera of Aurantioideae with a basic chromosome number $x=9$. The seedless citrus are produced through parthenocarpy. With self-incompatible varieties, self-pollination does not work because the pollen tube grows down the style into the ovary (fruit) too slowly and sexual fertilization does not actually occur. The International Citrus Genome Consortium (ICGC) which was organized in 2003 aimed at developing useful genomic resources for citrus genetics and breeding research. Draft genome sequences of several citrus species have been released in genomic databases through the Citrus Annotation Project (CAP) (Wang et al 2014) and Phytozome (Wu et al 2014, 2018) with the availability of reference genomes of sweet orange, wild mandarin etc. (Xu et al 2013; Wang et al 2018; Wu et al 2014; Wang et al 2017).

Genome-wide association study and genomic prediction for fruit quality traits was also studied in citrus (Minamikawa, 2017). Indian citron (*Citrus medica* L.) is one of the important citrus fruits worldwide, with a production that stands at 122 million tons. It is grown in a wild or semi-wild state in the forests of the Himalayan and North Eastern states in India. Uchoi et al (2017) studied the genetic diversity of 46 representative accessions using 17 RAPD and 11 ISSR markers and found 82% and 73% polymorphism, respectively. This suggests the existence of huge genetic variability among citron species. *Citrus macroptera* Montr. (*Melanesian papeda*) is wild, endangered but an economically important species of northeastern India. In order to understand the genetic variability among 30 *C. macroptera* accessions from Tripura and Mizoram states, Malik et al (2012) generated 91 polymorphic bands (98.8%) using 12 RAPD primers. They reported 0.26-1.00 pair-wise genetic similarity value between the accessions, indicating the existence of high level genetic diversity among these accessions. Comparative transcriptome analysis in ovules of *C. sinensis* revealed the stress-related genes and three novel ORFs associated with nucellar polyembryony (Kumar et al 2014). The Citrus Genomic Variation Database V1.0 (CitGVD) contains 1,493,258,964 non-redundant SNPs and INDELs and 84 phenotypes from 346 citrus individuals (<http://citgvd.cric.cn/home>).

xxxv. *Juglans* sps. (Walnut)

Walnut (*Juglans* spp.) belongs to the family Juglandaceae, order Fagales and is a diploid species ($2n=32$). The genus *Juglans* broadly comprises 21 species. Persian walnut (*J.*

regia) originated from Iran (Persia) and is particularly known for its high quality nut production. The species of *Juglans* genus are valued for high quality timber production worldwide. Walnut has been cultivated since the Greek era, and is native to the region from the Balkans eastward to the Western Himalaya. It is cherished for its nutritious kernel and wood (Fernandez-Lopez et al 2000). In India, *J. regia* is commonly found in the Himalayan states. The North Western Himalayan region is known for walnut (*J. regia*) production and has maximum variability (Chand et al 2017). In walnut, natural cross pollination rules due to monoecism and heterodichogamy and it is a wind-pollinated tree with $2n=32$ chromosomes.

Marrano et al (2019) carried out GWAS for development of SNPs and developed a high-density 700K SNP array in Persian walnut. Over 609K high-quality SNPs have been selected from a set of 9.6 M genome-wide variants, previously identified from the high-depth re-sequencing of 27 founders of the Walnut Improvement Program (WIP) of University of California, Davis. The effectiveness of the array was validated using a genotyped collection of 1,284 walnut trees, including 1,167 progeny of 48 WIP families and 26 walnut cultivars. Unravelling the genetic architecture of agronomic traits in walnut such as budbreak date and bearing habit, is crucial for climate change adaptation and yield improvement. Bernard et al (2020) conducted GWAS using multi-locus models in a panel of 170 walnut accessions genotyped using the Axiom™ *J. regia* 700 K SNP array, with phenological data from 2018, 2019 and legacy data. Using GWAS, they reported strong associations of SNPs located at the beginning of chromosome 1 with both budbreak and female flowering dates. Highly significant associated SNPs were also detected using GWAS for heterodichogamy and lateral bearing habit, both on chromosome 11. A Kompetitive Allele Specific PCR (KASP) marker for budbreak date in walnut was developed, and validated using plant material from the Walnut Improvement Program of the University of California, Davis. Candidate genes involved in flowering events in walnut identified, including a gene related to heterodichogamy encoding, a sugar catabolism enzyme and cell division-related genes linked to flowering.

Table 2. Major achievements of tree biotechnology studies at global level

	Tree species	Major findings / achievements	Reference
1	Neem (<i>Azadirachta indica</i>)	RAPD analysis to assess genetic divergence among 29 <i>Azadirachta indica</i> accessions collected from two agro-ecological regions of India	Deshwal et al (2003)
		Chromosome number estimated to be 2n=28 by enzymatic maceration and air drying method (EMA)	Jha (2014)
		Draft genome (v1.0) and four transcriptomes	Krishnan et al (2012)
		Improved Genome Assembly (v2.0)	Krishnan et al (2016)
		Transcriptome study for terpenoid production	Kuravadi et al (2015); Wang et al (2016)
		Identified key enzymes responsible for protolimonoid biosynthesis	Hodgson et al (2019)
		High quality PCR usable DNA isolation from neem	Rajarajan et al (2015)
2	Shisham (<i>Dalbergia sissoo</i>)	Complete chloroplast genome sequencing by comparison of nine <i>Dalbergia</i> species	Song et al (2019)
		Genetic polymorphism in <i>Dalbergia sissoo</i> genotypes Using RAPD markers	Dobhal et al (2019)
		Genetic diversity in <i>Dalbergia sissoo</i> through ISSR markers	Javaid et al (2014)

		Species phylogentic genetic relationship among <i>Dalbergia</i> species through molecular markers	Hiremath et al (2004)
3	Teak (<i>Tectona grandis</i>)	Efficacy of DNA extraction methods for PCR-based assay	Narayanan et al (2008)
		Evaluation of genetic diversity and population structure in Indian teak populations	Nicodemus et al (2005)
		Diversity and genetic structure analysis using ISSR marker to distinguish drier teak populations of central India from the moist teak populations of south India.	Ansari et al (2012)
		Genetic diversity and structure analysis using AFLP marker.	Vaishnav et al (2015)
		The genetic differentiation of teak meta-population to geographical and climatic variations using ISSR	Vaishnav et al (2018)
		Developed a widely expressed gene catalog using Illumina technology and the <i>de novo</i> assembly	Galeano et al (2015)
		Physiological and molecular responses to drought stress	Galeano et al (2019)
		Identification of 156 SNP markers and validation in 20 teak population of Myanmar	Dunker et al (2019)
		Draft genome of 317 Mbp at 151 coverage and annotation of 36,172 protein-coding genes.	Yasodha et al (2018)

		A chromosomal-scale genome assembly of <i>Tectona grandis</i>	Zhao et al (2019)
4	Subabul (<i>Leucaena leucocephala</i>)	Identification of species-specific genes using inter-species suppression subtractive hybridization	Negi et al (2011)
		Transcriptome analysis and identification of highly expressed genes in root and shoot	Ishihara et al (2016b)
5	Malabar Neem (<i>Melia dubia</i>)	Evaluation of genetic diversity using RAPD markers	Johar et al (2017)
		Genetic variations among open pollinated families of selected trees	Kumar et al (2013)
		Genetic diversity assessment ISSR markers among different	Rawat et al (2018)
6	Gamhar (<i>Gmelina arborea</i>)	Standardization of protocol for isolating high quality DNA from dried leaves	Shankar et al (2009)
		Genetic diversity assessments using ISSR markers	Naik et al (2009)
		Development of genetic markers for <i>G. arborea</i> and <i>G. asiatica</i> based on the nuclear ribosomal DNA- internal transcribed spacer 1 (<i>ITS1</i>) region	Manokar et al (2018)
7	<i>Eucalyptus</i> Species	Chromosome level assembly of <i>E. grandis</i> genome	Myburg et al (2014)
		Genomic selection for growth and wood quality in Eucalyptus	Resende et al (2012)
		DArT markers used for GWAS for growth and wood traits over <i>E. globulus</i> population	Cappa et al (2013)

		Multi-species SNP chip designed	Silva-Junior et al (2015)
		GWAS for wood traits in <i>E. grandis</i> x <i>E. urophylla</i> hybrids	Resende et al (2016)
		Genomic selection studies in <i>Eucalypts nitens</i>	Suontama et al (2019)
		Independent and joint GWAS for growth traits across four breeding populations using EUCAChip60KSNP platform	Muller et al (2019)
		Transcriptome study in 100s of segregating interspecific hybrids of <i>E. grandis</i> × <i>E. urophylla</i> for comprehensive systems genetics analysis of wood development in Eucalyptus.	Mizrachi et al (2017)
		Effect of inbreeding depression by whole-genome analysis.	Hedrick et al (2016)
		Genetic diversity estimations using RAPD, ISSR and SSR assessed in different population and species.	Chezhian et al (2010); Balasaravanan et al (2005; 2006)
		Generation of high throughput markers using target capture and deep sequencing strategies.	Dasgupta et al (2015)
		Isolation characterization of xylem development related genes (cellulose synthases: <i>EtCesA1</i> , <i>EtCesA2</i> , <i>EtCesA3</i> ; homeodomain- leucine zipper class III TF: <i>EtHB2</i>) in <i>E. tereticornis</i> .	Sundari and Dasgupta (2014); Dharanishanthi and Ghosh

			Dasgupta (2017)
		Assessment of water stress responsive genes and their expression in leaves of <i>E. grandis</i> under water stress	Ghosh Dasgupta and Dharanishanthi (2017)
		Study of co-expression network for xylogenesi s-related, secondary cell wall biogenesis-related transcripts	Dharanishanthi and Ghosh Dasgupta (2016; 2018a)
		Study of terpene biosynthesis, reproductive biology, plant defense and abiotic interactions	Myburg et al (2014); Kulheim et al (2015); Vining et al (2015); Christie et al (2015); Mangwanda et al (2015); Oates et al (2015); Meyer et al (2016); Plett et al (2015); Spokevicius et al (2017)
8	Poplar (<i>Populus</i> species)	Physical map of <i>Populus trichocarpa</i> using shotgun strategy	Tuskan et al (2006)
		Identification of candidate genes/SNPs for lignin and cellulose biosynthesis traits in <i>P. trichocarpa</i>	Wegrzyn et al (2010)

		Methylation maps for 10 tissue types in <i>Populus trichocarpa</i>	Vining et al (2012)
		GWAS for wood related traits in <i>P. trichocarpa</i>	Porth et al (2013)
		QTL mapping and candidate genes for cell wall properties.	Muchero et al (2014)
		GWAS for adaptive traits in <i>P. trichocarpa</i> and bioenergy traits in <i>P. deltoids</i>	Evans et al (2014) Fahrenkrog et al (2017)
		GWAS and QTL analysis in <i>Populus</i> to identify regulation of chlorogenic acid	Zhang et al (2018)
		Development of highly contiguous, <i>de novo</i> genome assemblies in <i>P. euphratica</i>	Ma et al (2014)
		Methods to integrate SNP, gene expression, transcription factor binding, gene dosage, methylation, metabolite expression, phenome, and co-evolutionary relationships have been developed to generate a systems biology view of the molecular and regulatory interactions that lead to organismal scale traits.	Henry et al (2015) Liu et al (2015); Joubert et al (2017, 2018); Weighill et al (2018)
9	<i>Acacia</i> species	Studied the involvement of six highly conserved amg-miRNA families viz., <i>amg-miR166</i> , <i>amg-miR172</i> , <i>amg-miR168</i> , <i>amg-miR159</i> , <i>amg-miR394</i> and <i>amg-miR156</i> in secondary cell wall biosynthesis in <i>Acacia mangium</i>	Ong and Wickneswari (2011, 2012)

		Identification of genes involved in lignin biosynthesis and secondary cell wall formation in <i>Acacia</i> hybrids	Wong et al (2011)
		Identification of 37,786 putative SNPs in <i>Acacia auriculiformis</i> × <i>Acacia mangium</i> hybrids	Wong et al (2012)
		First <i>cp</i> genome of <i>A. ligulata</i> with 158,724 bp and revealed <i>clpP1</i> gene which is highly divergent and essential part of it	Williams et al (2015)
10	Sandalwood (<i>Santalum album</i>)	Isolation of sequences encoding <i>farnesyl diphosphate synthase</i> and <i>santalene synthase</i> enzyme	Rani et al (2013)
		Draft genome sequence with 221 Mbp genome size. It includes 38,119 protein coding genes, 27.42% repetitive DNA elements, 72,325 unique peptides, 53 novel proteins and 1,348 potential noncoding RNAs	Mahesh et al (2018)
		Draft genome sequence with 286 Mbp size containing 37,500 genes (30 genes from terpene synthase gene family) along with 1,191,838 (0.42%) SNPs and 98,312 (0.034%) InDels. Also reconstruction of consensus <i>cp</i> genome with 147.25 Kbp size	Dasgupta et al (2019)
		First complete <i>cp</i> genome sequence of <i>S. album</i> with 144,101 bp size containing 123 genes, including 80 protein-coding genes, 8 rRNA genes, and 35 tRNA genes.	Yang et al (2020)

		The transferability of <i>Santalum austrocaledonicum</i> and <i>Santalum insulare</i> SSRs to <i>Santalum album</i> was evaluated to examine molecular diversity of 20 accessions of two diverse localities comprising germplasm of MP and Karnataka.	Mohammad et al (2012)
11	Karanj (<i>Pongamia pinnata</i>)	Genome size variation through somoclonal variation in <i>Pongamia pinnata</i>	Choudhury et al (2014)
		Identified 236 conserved miRNAs within 49 families and 143 novel miRNAs via deep sequencing of <i>Pongamia</i> seeds sampled at three developmental phases	Jin et al (2019)
		sequencing and annotation of chloroplast and mitochondrial genome (152,968 and 425,718 bp, respectively)	Biswas et al (2013)
		Whole transcriptome analysis of <i>Pongamia</i> to unravel genes underlying floral initiation to lipid biosynthesis	Sreeharsha et al (2016)
		Genetic diversity in 37 wild species of <i>P. pinnata</i> using 18 RAPD and 23 SRAP markers	Kole et al (2015b)
12	<i>Casuarina equisetifolia</i>	300 Mb draft genome sequence.	Ye et al (2019)
		Genetic diversity assessment in <i>Allocasuarina</i> , and <i>Casuarina</i> species.	Yasodha et al (2004)
		Development of species diagnostic ISSR-SCAR markers for <i>Casuarina equisetifolia</i> and <i>C. junghuhniana</i> .	Ghosh et al (2011); Nicodemus et al (2011)

		Molecular mechanism during <i>Casuarina equisetifolia</i> – <i>Trichosporium vesiculosum</i> was assessed and transcript profiling during elicitation revealed expression of resistance genes; cytochrome oxidase; trans membrane proteins; genes involved in programmed cell death like 26S proteasome and ubiquitin activating enzyme; early nodulin gene, wound inducible metallocarboxy peptidase inhibitor, glucanase, metal binding protein and signal recognition particle	Ghosh Dasgupta et al (2013)
		A class I chitinase (<i>CeChi1</i>) belonging to PR-3 is identified to be upregulated during pathogen elicitation and salicylic acid signaling	Veluthakkal and Ghosh Dasgupta (2012)
		<i>CeChi1</i> results in characteristic lysis of hyphal tips of pathogenic fungi including <i>Trichosporium vesiculosum</i> , <i>Fusarium oxysporum</i> and <i>Rhizoctonia solani</i>	Veluthakkal and Ghosh Dasgupta (2015)
		The transcriptome of the developing secondary tissues of 15 years old <i>Casuarina equisetifolia</i> sub sp. <i>equisetifolia</i> was sequenced, <i>de novo</i> assembled, annotated and mapped to functional pathways.	Vikashini et al (2018)
13	Tree of Heaven (<i>Ailanthus excelsa</i>)	Wood transcriptome of a 22 month old tree was sequenced and <i>de novo</i> assembled. A	Unpublished data from IFGTB

		total of 48,493 transcripts were predicted with N50 of 1,606 bp.	Coimbatore, India
14	Rotan Dahan (<i>Korthalsia laciniosa</i>)	Draft genome sequence of <i>Korthalsia laciniosa</i> (Griff.) Mart., a clustering rattan, was generated and the reads were assembled into 243005 scaffolds with N50 of 23,613 bp.	Ghosh Dasgupta et al (2020) unpublished data
15	<i>Morus</i> species	Draft genome sequence of <i>Morus notabilis</i> (Mulberry)	He et al (2013)
		Mining of 217,312 SSRs from genome sequence and 961 SSRs from EST sequences of <i>M. notabilis</i>	Krishnan et al (2014)
		Generation of 87.0 and 80.3 Mbp of transcriptomic data from <i>M. laevigata</i> and <i>M. serrata</i> , and identification of 24,049 SSRs, 1,201,326 SNPs and 67,875 InDels	Saeed et al (2016)
		Identified and characterized 222 genomic and 136 genic SSRs from genomic clones and ESTs of <i>M. alba</i>	Thumilan et al (2013)
		Development and validation of 206 EST driven SSRs from transcriptomic data of drought tolerant mulberry cv. Dudia white	Thumilan et al (2016)
		Identification of drought-regulated genes and large number of ESTs through suppression subtractive hybridization of K2 (drought susceptible) × AR12 (drought tolerant)	Gulyani and Khurana (2011)
		248 Genomic-SSR and 490 genic-SSR (EST-SSR) primers designed	Sarkar et al (2017)

15	Frankincense (<i>Boswellia sacra</i>)	Sequencing of cp genome and revealed of 160,543 bp size with 37.61% GC content and 114 unique genes with 83 protein-coding regions	Khan et al (2017)
17	Drumstick (<i>Moringa oleifera</i>)	Draft genome sequence	Chang et al (2018); African Orphan Crops Consortium
		Developed a bioinformatics pipeline to assemble transcriptome, along with the previously published <i>M. oleifera</i> genome, to predict 17,148 gene models.	Pasha et al (2019)
		Report a high-quality draft genome sequence of <i>M. oleifera</i> and represents 91.78% of the estimated genome size and contains 19,465 protein-coding genes.	Tian et al (2015)
		The first complete chloroplast genome sequence of <i>M. oleifera</i> (accession number of MH778650)	Liu et al (2019)
18	White siris (<i>Albizia procera</i>)	Study of genetic diversity through AFLP markers	Aparajita et al (2010)
		RAPD and ISSR markers to authenticate the true to type nature of <i>in vitro</i> raised plantlets	Mohammad et al (2016)
		Genetic variations between and within the populations white siris using ISSR markers	Aparajita et al (2016)
19		Breeding system and pollination biology	Diallo et al (2007)

	Tamarind (<i>Tamarindus indica</i>)	Differential gene expression and biochemical activities of antioxidant enzymes in Tamarind	Lim et al (2013)
		Assessment of genetic diversity using RAPD markers	Diallo et al (2007)
20	Custard Apple (<i>Annona squamosa</i> L.)	Transcriptome analysis of early-stage fruit of two genotypes with contrast in seed number	Gupta et al (2015)
		Transcriptome analyses of normal and malformed flowers	Liu et al (2017)
		Genetic diversity among 20 <i>Annona</i> genotypes representing five different species of <i>Annonaceae</i> using 11 RAPD and 12 SSRs markers	Anuragi et al (2016)
21	Jamun (<i>Syzygium cumini</i>)	Molecular characterization and diversity analysis by RAPD, ISSR and SSR markers	Ahmad et al (2012); Shakya et al (2010)
		Chloroplast genome sequencing	Asif et al (2013)
24	Guggul (<i>Commiphora wightii</i>)	Genetic diversity using RAPD and ISSR markers	Harish et al (2014)
		Study of genetic relationships among 24 genotypes of <i>C. wightii</i> using 60 RAPD and 27 ISSR markers	Samantaray et al (2011)
		Genetic diversity assessments among 7 populations of <i>C. wightii</i> using RAPD markers.	Haque et al (2009b)
		Identification of sex-specific molecular markers in dioecious and hermaphrodite plants of <i>C. wightii</i> using RAPD markers.	Samantaray et al (2009)

		Evaluated genetic relationship among different accessions of Rajasthan and Gujarat states using 40 RAPD markers.	Suthar et al (2008)
		Conducted molecular phylogenetic analysis of <i>Commiphora</i> , predominantly growing in tropical Africa using ETS and ITS sequences	Weeks and Simpson (2007)
		Phylogenies based on nuclear and chloroplast data were reconstructed for 13 of the 18 genera (50 spp. total) of Burseraceae family.	Weeks et al (2005)
		Used ITS sequences of <i>C. wightii</i> plants for population fragmentation and habitat loss	Haque et al. (2009a)
		Analyzed the extent of genetic variability among 52 <i>C. wightii</i> accessions of Rajasthan and Haryana using ISSR and	Kulhari et al (2015)
		Analyzed genetic diversity among six accessions of <i>C. wightii</i> collected from southern states using three fluorescent-labeled RAPD primers	Ch. Mohan et al (2017)
		Developed SCAR markers for identification of closely related species <i>C. wightii</i> and <i>C. myrrha</i>	Sairkar et al (2016)
25	Khejri (<i>Prosopis cineraria</i>)	Genetic diversity in accessions of Rajasthan using ISSR and DAMD primers	Sharma et al (2011)
26	Reetha (<i>Sapindus emarginatus</i>)	Genetic diversity assessemnet using RAPD, DAMD and ISSR markers	Mahar et al (2011)
27	Jatropha (<i>Jatropha curcas</i>)	Identified 12JcMAPKs, 5 JcMAPKKs and 65 JcMAPKKKs gene familes involved in	Wang et al (2018)

		cell division, hormone signaling and overall growth and get activated in under variety of biotic and abiotic stresses	
		Reported ~339 Mbp draft whole genome sequence in Chai Nat variety and identified genes relevant to the biosynthesis of lipids and toxic compounds during seed development.	Ha et al (2019)
		Generated hybrid <i>de novo</i> genome assembly with 265.7 Mbp using Illumina and PacBio sequencing technologies	Kancharla et al (2019)
		genetic variability and relationship among 182 accessions collected from 19 Indian states 30 RAPD markers	Kole et al (2015a)
28	<i>Mango</i> (<i>Mangifera indica</i>)	Phase separated maternal (Dashehari) and paternal (Neelam) genome assemblies of highly heterozygous mango genome (<i>Mangifera indica</i> cv. Amrapali) have been developed using trio-binning of Illumina and PacBio SMRT sequencing. Total 53,018 genes were predicted from the mosaic Amrapali genome assembly of 403 Mbp.	Singh et al (2016, 2018)
		Leaf transcriptome analysis of Dashehari, Neelam and Amrapali was done for genic-SSR markers and estimation of heterozygosity.	Mahato et al (2016)
		Genome wide association study (GWAS) has been conducted of pomological traits in mango using genic-SSR markers	Lal et al (2017)

		Mango genomic SNP database (MiSNPdb)	Iquebal et al (2017)
		Mango fruit peel transcriptome analysis was done to study the genes of anthocyanin pigmentation pathways.	Bajpai et al (2018)
		Transcriptome analysis of flowering genes in relation to floral malformation	Yadav et al (2019)
29	Pines (<i>Pinus</i> species)	High density SNP chip design for Maritime pine (<i>Pinus pinaster</i>)	Plomion et al (2016)
		Genomic selection predictions in Maritime pine	Bartholome et al (2016)
		Stochastic simulation study of SNP-based genomic selection in conifers	Li and Dungey (2018)
30	Spruces (<i>Picea</i> species)	Association genetics on ~550 candidate genes for wood physical traits	Beaulieu et al 2011
		GWAS for productivity and wood quality traits in White Spruce and Norway Spruce, respectively.	Beaulieu et al (2014a); Beaulieu et al (2014b) Baison et al (2018)
31	Apple (<i>Malus domestica</i>)	Genomic selection for fruit and key traits in apple	Kumar et al (2012) Muranty et al (2015)
		Genome wide association studies (GWAS) for mapping flowering, ripening traits and polyphenol content.	Urrestarazu et al (2015); McClure et al (2019)

32	Peaches (<i>Prunus persica</i>)	GS for yield and quality traits using IPSC 9k SNP array in <i>Prunus persica</i>	Biscarini et al (2017)
		GWAS for fruit shape in <i>Prunus persica</i>	Tan et al (2019)
33	Date palm (<i>Phoenix dactylifera</i>)	GWAS for fruit traits	Hazzouri et al (2019)
34	Oil Palm (<i>Elaeis guineensis</i>)	GWAS for productivity traits	Cros et al (2015)
35	Citrus species	GWAS for fruit quality traits in multiple Citrus species	Minamikawa et al (2017)
		Genetic diversity of forty-six representative accessions using RAPD and ISSR markers.	Uchoi et al (2017)
		Genetic variability among 30 <i>C. macroptera</i> accessions from Tripura and Mizoram states using RAPD markers.	Malik et al (2012)
		Comparative transcriptome analysis in ovules of <i>C. sinensis</i>	Kumar et al (2014).
36	Cocoa (<i>Theobroma cacao</i>)	15k SNP chip development	Ill et al (2017)
		GWAS and genomic prediction for fruit yield and resistance to black and frosty pod and disease resistance	Navarro et al (2017) McElroy et al (2018)
37	Grapes (<i>Vitis vinifera</i>)	GWAS for important fruit traits	Guo et al (2019)
38	Macadamia nut (<i>Macadamia integrifolia</i>)	GWAS for yield components in <i>Macadamia integrifolia</i>	O'Connor et al (2020)

39	Banj Oak (<i>Quercus leucotrichophora</i>)	Genetic diversity analysis in 4 natural population from Himachal Pradesh using RAPD markers	Saha (2018)
40	Deodar (<i>Cedrus deodara</i>)	Study of genetic variation in three natural populations of western himalaya using RAPD markers.	Rai et al (2020)
		Karyotype investigation and cytogenetic study	Dagher-Kharrat (2001)
		Complete chloroplast sequence	Lin et al (2010)
41	Chinese Bayberry (<i>Myrica esculenta</i>)	Development of 158 SSR markers by genome survey	Jiao et al (2012)
42	Himalayan Yew (<i>Taxus wallichiana</i>)	Combination of transect study, population genetic analyses based on chloroplast DNA sequences and nuclear microsatellite data, and ecological modelling used to study Patterns of genetic variation	Poudel et al (2014)
		Transcriptome study for taxol synthesis	Wang et al (2019)
43	Hamilton's bamboo (<i>Dendrocalamus hamiltonii</i>)	Genetic diversity analysis by molecular markers	Waikhom et al (2012); Meena et al (2019)
		Genome-wide transcriptional profiling to elucidate key candidates involved in bud burst and rattling Growth	Bhandawat et al (2017)

The National Bureau of Plant Genetic Resources (NBPGR) aims to plan, coordinate and conduct explorations for collecting germplasm. It has the prime responsibility of characterization and evaluation of all the indigenous and exotic germplasm collections for their field performance and other important traits like resistance to biotic/abiotic

stresses and phytochemical attributes along with maintenance and regeneration. The Bureau maintains the collections in its genebank.

Tables 3 and 4 present major agroforestry tree species (exotic and indigenous collections at the National Genebank in India). Table 5 lists Tree Genomics-focused MPTS at NBPGR, New Delhi.

Table 3. List of available tree genetic resources at different ICAR, ICFRE and allied centers in India

S.No.	Name of the Institute	Name of the species	Number of germplasms	Details of research conducted
1	CAFRI, Jhansi (ICAR)	Neem (<i>Azadirachta indica</i>)	170	Genetic variability through morphological traits, kernel yield, oil content and azadirachtin content estimation, DNA extraction protocol optimization
		Subabul (<i>Leucaena</i> species)	33	Fodder quality assessment, molecular genetic diversity and wood properties studies
		North Indian Rosewood (<i>Dalbergia sissoo</i>)	14	Genetic variability through morphological traits, early abiotic stress tolerance (drought and waterlogging stress)

		Malabar neem (<i>Melia dubia</i>)	02	Germplasm collection and evaluation for industrial traits
2	IIHR, Bangalore (ICAR)	Curry leaf (<i>Murraya koenigii</i>)	100	Morphological characterization is done at 1 year after planting, initiated work on development of SSR markers for this species and Carotenoids, Flavonoids, Ascorbic acids, alkaloids, Phenols are being estimated
		Tamarind (<i>Tamarindus indica</i>)	460	In situ evaluation and total sugars and acidity has been assessed in 150 genotypes
		Jamun (<i>Syzygium cumini</i>)	95	28 accessions done at flowering and fruit set (4-5 Years) as per the DUS parameters, developed SSR markers and presently work on molecular characterization and diversity analysis, 34 accessions, basic biochemical parameters like anthocyanin, Phenols, DPPH, FRAP has been

				estimated and Estimation of antidiabetic activity is under progress, mutant population has been generated.
		Pomegranate (<i>Punica granatum</i>)	280	Molecular characterization done for 100 accessions and remaining under progress, 154 accessions, Biochemical analysis for anthocyanins, flavonoids, anti-oxidants and phenols are done and for rest it is in progress, SSR has been developed at IIHR, Biochemical analysis of value added products of pomegranate are being done and F1 of Triple cross , Mutation progenies (M0) of EMS and Gamma irradiation, Colchiploids.
		Drumstick (<i>Moringa oleifera</i>)	167	Morphological characterisation has been completed for 90

				germplasm lines at one-year old crop, molecular characterisation is initiated using SSR markers, A total of 82 accessions have been assessed for total carotenoids, minerals, oxalates, antioxidant capacity, phenols and flavonoids.
		Custard apple (<i>Annona sp</i>)	6 species	Morphological characterization has been done at 10 years old bearing trees, Interspecific hybridization has been attended between atemoya and custard apple. About 2000BC1 of population of Arka Sahan is available in the field and Ph.D thesis completed on characterization on <i>Annona</i> varieties and species (UHS, Bagalkot)
		Guava (<i>Psidium guajava</i>)	62	Morphological characterisation has been carried out and was

				done after the plants attained 5 years of age and Crossing work has been carried out using Arka Mridula x Purple local and Purple local X Arka Mridula, Arka Poorna x Purple local, Purple local X Arka Poorna and F1 are available in the nursery
		Manago (<i>Mangifera indica</i>)	767	Morphological characterization has been done for more than 400 genotypes, SSR markers were employed, the volatile profiling for selected germplasm and the breeding methods such as clonal selection, OP, halfsibs, full sibs and mutant populations are being evaluated
		Jackfruit (<i>Artocarpus heterophyllus</i>)	150 accessions 500 Half sibs	IIHR has developed SSR markers and diversity analysis using Pan-India genotypes with SSR markers is under progress

3	IFGTB, Coimbatore	<i>Eucalyptus tereticornis</i> and <i>E. camaldulensis</i>	400 including clones, half sib, full sib and provenances	Different levels of morphological characterization have been carried out. Some of the clones were characterized for DUS descriptors development and IFGTB is the DUS testing centre for Eucalypts, Wood characterization like lignin and cellulose content were estimated, RAPD, ISSR, AFLP, SSRs and SNP markers were used since the year 2000, wood characterization like lignin and cellulose content were estimated, F2, back cross or any segregating generation, mapping population etc is available.
		<i>Casuarina junghuhniana</i>	5 Clones	IFGTB has released five superior windbreak tree varieties of <i>Casuarina junghuhniana</i> viz. IFGTB-WBC-1, IFGTB-WBC-2, IFGTB-WBC-3, IFGTB-

				<p>WBC-4 and IFGTB-WBC-5.</p> <p>Windbreak tree varieties of IFGTB show high level of branch persistence with 40 to 50 thick and horizontally spreading branches within 3 m height from the base of the tree and also having greater height and diametrical growth rate.</p>
		<i>Thespesia populnea</i>	139 Plus Trees	Germplasm Bank cum Vegetative Multiplication Garden and Clonal trial has been established.
		<i>Haldina cordifolia</i> (<i>Adina cordifolia</i>)	89 Candidate Plus Trees	Selected 89 CPTs from Kerala.
		<i>Ailanthus excelsa</i>	Progeny trial cum germplasm assemblage	<p>64 families of half-sib families established. Growth parameters (tree height, basal diameter, diameter at breast height (dbh) and bole volume) were recorded.</p> <p>Fodder quality assessment also been done.</p>

		<i>Calophyllum inophyllum</i>	40 clones and 30 half-sib progenies	<p>Seed oil was content was estimated for all accessions.</p> <p>Three clonal trials, one germplasm assemblage and one half-sib progeny trial have been established. The clones were evaluated for growth parameters (height, DBH, crown circumference and fruit yield).</p>
		<i>Sapindus emarginatus</i>	30 families	<p>The accessions were evaluated for Saponin content.</p> <p>Three half-sib mulilocation progeny trials have been established.</p>
		<i>Acacia leucophloea</i>	30 families	One germplasm assemblage comprising 30 accessions from 15 locations has been established
		<i>Casuarina</i> spp. (<i>C. equisetifolia</i> and <i>C. junghuhniana</i>)	450 (provenances, landraces, families and clones)	<p>Breeding populations and seed orchards established.</p> <p>Outstanding clones released for planting</p>

				programmes. Interspecific hybrids developed, validated through DNA markers and released for commercial planting.
		<i>Leucaena leucucocephala</i>	35	Seed sources from India, USA and south America were evaluated in diverse planting environments. Outstanding individuals are undergoing field testing for clonal release. Seed production areas have been formed to produce genetically improved seeds.
		<i>Acacia</i> spp. (<i>Acacia auriculiformis</i> and <i>A. mangium</i>)	100	Second generation seed orchards have been developed using a diverse collection of family accessions. Outstanding clonal selections are under amplification testing for release and commercial planting.
4	Savitribai Phule Pune University	Kewda (<i>Pandanus odorifer</i>)	10 accessions and 7 species	Molecular characterization has been done using cpDNA,

				ISSR and RAPD markers and volatiles imparting aroma in bracts, stamens of male and female flowers have been qualitatively and quantitatively analysed following HS-SPME-GC-MS/FID, Gamma amino butyric acid (GABA) estimation has been done
		<i>Pandanus</i> species	-	<p>Genomic DNA estimation of 8 species: Leaf tissues DNA content variation recorded in eight <i>Pandanus</i> species which includes <i>P. odorifer</i>, <i>P. unipapillatus</i>, <i>P. dubius</i>, <i>P. nepalensis</i>, <i>P. kaida</i>, <i>P. furcatus</i>, <i>P. sanderi</i> and <i>P. amaryllifolius</i>. The estimated DNA content (2C) ranged from 0.69 pg to 0.91 pg.</p> <p>It was observed that three <i>Pandanus</i> species viz: <i>P. unipapillatus</i>, <i>P. nepalensis</i> and <i>P.</i></p>

				<p><i>furcatus</i> showed very closed genome content, however, species <i>P. dubius</i> was with maximum genome content, while <i>P. amaryllifolius</i>, with minimum genome content. Thus, from the 2C measurements obtained for the eight <i>Pandanus</i> species from leaf tissues, their genome size ranged from 678.71 Mbp to 886.10 Mbp.</p>
5	ICAR Research Complex, Tripura	<p><i>Acacia auriculiformis</i> (Akashmoni), <i>Morus alba</i> (Tut), <i>Leucaena leucocephala</i> (Subabul), <i>Dalbergia sissoo</i> (Shisam), <i>Gliricidia maculate</i> (gliricidia), <i>Azadirachta indica</i> (Neem),</p>	Each species one germplasms is available	<p>Growth parameters (tree height, basal diameter, diameter at breast height (dbh) and bole volume) were recorded. The data were recorded for the age of 8 and 16 years for all the species mentioned in the table.</p>

		<i>Michelia champakca</i> (Champa) , <i>Eucalyptus hybrid</i> (Safeda), <i>Tectona grandis</i> (Teak), <i>Gmelina arborea</i> (Gamahar), <i>Samania saman</i> (Raintree) <i>Albizzia procera</i> (White sirish)		
6	NEH Region, Meghalaya (ICAR)	<i>Parkia roxburghii</i>	7 provenances	Morphological characterization done
		<i>Jatropha (Jatropha curcas L.)</i>	10	Seed oil content was estimated (28.79% - 42.89%). Fatty acid profile of 5 germplasm was analysed. Bio-chemical characterization with special reference to bioactivity guided screening of different plant parts to evolve genotype/varieties of phytoceutical importance.

7	TFRI, Jabalpur (ICFRE)	<i>Tectona grandis</i>	40 clones	40 grafted clones of teak from all over India are assembled as CSO.
		<i>Dalbergia latifolia</i>	12 families	Progeny Trial has been established using half-sib seeds from 12 families.
		<i>Pterocarpus marsupium</i>	25 families	Progeny Trial has been established using half-sib seeds from 25 families.
		<i>Litsea glutinosa</i>	90 accessions	Germplasm Bank has been established using 90 accessions from 10 locations.
		<i>Bamboo</i> spp. (<i>Bambusa vulgaris</i> var. green, <i>B. bambos</i> , <i>B. tulda</i> , <i>Dendrocalamus strictus</i>).	4 species	Germplasm Bank of 4 species of bamboo has been established.
8	CCS, Hisar Haryana	<i>Melia composite</i>	24 plus trees	Molecular genetic diversity through RAPD markers. The cluster analysis indicated two distinct clusters, one comprising of MCP10 and MCP11 while the second cluster included

				rest of the genotypes. Cluster II was further divided into two subgroups, the first subgroup consisted of six genotypes and second subgroup had sixteen genotypes.
9.	FRI, Dehradun (ICFRE)	<i>Pinus kesiya</i> Royle ex. Gordon	-	Genetic Diversity and Population Structure of <i>Pinus kesiya</i> through Trans-specific Amplification of Nuclear SSR Markers. Transfer rate was 10.6%. Expected heterozygosity mean (H_E) was 0.540 and observed heterozygosity mean (H_o) was 0.342. (Rai et al 2018). Genetic diversity also studied using chloroplast SSR markers in 250 individuals of 10 populations. (Rai and Ginwal 2018)
		<i>Pinus roxburghii</i> Sarg		Comparative assessment of SSR, ISSR and AFLP markers for characterization of selected genotypes

				<p>based on resin yield. AMOVA by collection site revealed no significant variation among the populations. Whereas, AMOVA by resin yield markers revealed genetic differentiation among the groups. (Rawat et al 2014)</p> <p>Marker trait association for resin yield using 238 DNA markers in 240 genotypes of a natural population (Rawat et al 2015)</p> <p>Cross species transferability of SSR marker tested in 275 trees of a natural population. The number of alleles in case of nSSRs was up to 5 per locus and the expected heterozygosities ranged from 0.409 to 0.562 (Chauhan et al 2010).</p> <p>Genetic diversity analysis has been done by RAPD markers.</p>
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		<i>Pinus wallichiana</i>		Cross species transferability of microsatellite markers showed 46.25% transfer rate from 6 pinus species. Phylogenetic analysis of Plastid DNA showed applicability in barcoding
		<i>Quercus leucotrichophora</i>		27 natural populations from Uttarakhand and Himachal Pradesh were studied for genetic diversity using RAPD markers. AMOVA analysis showed 83.49% and 16.51% of diversity within and among population respectively
		<i>Cedrus deodara</i>		Transferability of 49 microsatellite markers (cpSSR and nuSSR) from pinus in C. deodara. Out of which 21 cpSSR markers amplified and 20 was polymorphic. Screening of natural population by polymorphic marker found 64 variants

			combined in 70 haplotypes (Ginwal et al 2011)
		<i>Dalbergia sissoo</i>	Optimization of genomic DNA extraction method (Ginwal and Mourya 2010). Genetic diversity analysis by RAPD marker on 67 cultivated clones of north India.
		<i>Eucalyptus species</i>	Differential profile of 17 RAPD markers is assessed in eucalyptus F1 natural hybrid FRI-4 (<i>E. tereticornis</i> x <i>E. camaldulensis</i>), FRI-5 (<i>E. camaldulensis</i> X <i>E. tereticornis</i>) and controlled reciprocal crosses FRI-14 (<i>E. torelliana</i> X <i>E. citriodora</i>) along with their parental combinations
		<i>Myrica esculenta</i>	Population genetic analysis of the <i>Myrica esculenta</i> populations from entire Uttarakhand state is under way using SSR markers

		<p><i>Taxus wallichiana</i> Zuccarini</p>		<p>As a part of a major project, CAMPA Co-FGR, <i>T. wallichiana</i> populations from 18 locations in Uttarakhand, have been sampled. Polymorphic SSR markers from loci developed in relative <i>Taxus</i> species has been amplified. The amplified products of all the loci are presently being genotyped in order to characterize the individual genotypes for further analysis.</p>
		<p><i>Dendrocalamus hamiltonii</i></p>		<p>Meena et al (2019) used nuclear simple sequence repeats (nSSRs) to study the genetic diversity and population genetic structure of 19 natural stands of <i>D. hamiltonii</i> distributed across the northeast Himalayas. The marker analysis indicated that <i>D. hamiltonii</i> populations have maintained a low</p>

				level of genetic diversity ($h=0.175$, $I=0.291$) in northeastern region of India.
10	CRIDA, Hyderabad (ICAR)	<i>Pongamia pinnata</i>	39 accessions	Accessions of <i>Pongamia</i> were collected from Southern India Growth parameters (tree height, and diameter at breast height (dbh)) were recorded and established in field. Genotype versus year interaction of pod and seed mass and stability of <i>Pongamia pinnata</i> families in a semi-arid region was studied for biofuel parameters.
		<i>Leucaena leucocephala</i>	32 accessions	Germplasm lines of advanced breeding material were collected and maintained at field.
		<i>Tamarindus indica</i>	40 accessions	Germplasm of released varieties from southern India were maintained at field and studied for fruit and growth characters

11	ICAR-CITH, Srinagar (J & K)	<i>Juglans regia</i>	136 accessions	Germplasm largely consist indigenous collections from North- Western Himalayas, institute released varieties (10) and seven exotic varieties. DUS guideline development, morphological characterization and genetic variability, correlation and path- coefficient studies (Chand et al 2020), pollination compatibility studies and genetic diversity study in 82 accessions through SSR and RAPD markers (Ahmed et al 2012) were carriedout.
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Table 4. Exotic collection of major agroforestry species in the National Gene Bank of India

S. no.	Crop/Species	No. of accessions
1	<i>Sesbania</i>	16
2	<i>Sesbania rostrata</i>	9
3	<i>Prickly sesban</i>	8
4	<i>Sesbania cannabina</i>	8

5	<i>Sesbania aculeata</i>	6
6	Common Sesban	3
7	<i>Sesbania sesban</i>	3
8	<i>Sesbania tetraptera</i>	1
9	<i>Simarouba</i>	1
10	<i>Simarouba glauca</i>	1

Table 5. Indigenous collection major agroforestry species in the national gene bank of India

S.No.	Crop name/Botanical name	Total no of accessions
1	<i>Sesbania</i>	649
2	<i>Acacia</i>	239
3	<i>Albizia</i>	166
4	<i>Prosopis cineraria</i>	48
5	<i>Tamarindus indica</i>	45
6	<i>Salvadora oleoides</i>	30
7	<i>Bauhinia spp.</i>	19
8	<i>Dalbergia sissoo</i>	19
9	<i>Semecarpus anacardium</i>	18
10	<i>Butea monosperma</i>	17
11	<i>Sesbania cannabina</i>	17
12	<i>Erythrina indica</i>	15
13	<i>Sterculia</i>	15
14	<i>Pterocarpus marsupium</i>	13
15	<i>Bauhinia variegata</i>	13
16	<i>Anogeissus spp.</i>	12
17	<i>Decalepsis</i>	10
18	<i>Pterocarpus santalinus</i>	9
19	<i>Nyctanthes arbor-tristis</i>	8

Table 6. Tree genomics focused multipurpose tree species (MPTS) at ICAR-NBPGR, New Delhi, India

S.No.	Name of species	No. of accessions
1	<i>Prosopis cineraria</i>	48
2	<i>Tamarindus indica</i>	45
3	<i>Moringa oleifera</i>	8
4	<i>Dalbergia sissoo</i>	6
5	<i>Commiphora wightii</i>	3
6	<i>Melia dubia</i>	2
7	<i>Schleichera oleosa</i>	2
8	<i>Parkia roxburghii</i>	2
9	<i>Casuarina equisetifolia</i>	2
10	<i>Punica granatum</i>	1
11	<i>Syzygium cumini</i>	1
12	<i>Litsea glutinosa</i>	1
13	<i>Tectona grandis</i>	1
14	<i>Azadirachta indica</i>	1

6. INTERNATIONAL EFFORTS TOWARDS GENERATING GENOMICS RESOURCES FOR AGROFORESTRY TREE SPECIES

Different aspects of application-oriented translational genomics research have become extremely specialized due to different skill-sets needed to address problems associated with each part. Thus, it can be observed that many such efforts are following the consortium- or group-type approach for developing genomics resources. Some of the well-known international consortia are avocado genome consortium (<https://www.fabnet.up.ac.za/index.php/arp-projects/avocado-genome-consortium>), mango genome sequencing consortia in India, Australia and USA (Kuhn et al 2019; Singh et al 2018), and informal groups working on breadfruit, jackfruit, coffee, cacao, etc. Another international multi-crop consortium promoted by World Agroforestry (ICRAF) is the African Orphan Crops Consortium.

6.1. African Orphan Crops Consortium

The African Orphan Crops Consortium (AOCC, <http://africanorphancrops.org/>) is a consortium of public-private partners that was built to develop genomics resources for Africa's under-utilized, under-researched, and under-funded food crops and trees (Hendre et al 2019; Jamnadass et al 2020). The AOCC framework provides a robust skeleton that can be used to build a similar multi-tree species consortium in India. Various functional aspects of the consortium have been partitioned into five broader areas where the 27 partners fall into one or more roles (<http://africanorphancrops.org/partners-and-network/>).

The consortium relies on its partners who bring an interdependent web of services and tools on board, thus ensuring sustainability. The consortium has already sequenced five tree species (*Moringa oleifera*, *Faidherbia albida*, *Sclerocarya birrea*, *Artocarpus heterophyllus*, *Artocarpus altilis*) and is ready with genomic data for nine more tree species (*Vitellaria paradoxa*, *Annona cherimola*, *Vangueria madagascariensis*, *Balanites aegyptica*, *Tamarindus indica*, *Irvingia gabonensis*, *Passiflora edulis*, *Annona reticulata*, and *Ricinodendron heudelotii*).

Figure 1: Five major areas of partnership under the AOCC (Hendre et al 2019)

6.2. Indian partnership on tree genomics and improvement

India has a huge potential for development and application of modern tools, especially high throughput genomics and phenomics due to a strong base of supporting genomics and bioinformatics infrastructure in both the public and private sectors. As majority of these tools are generated for annual crops, the perennial crops need a major boost to reap the benefits and see tangible benefits to the farmers. Here, we propose formation of an India-centric partnership to achieve these goals along the lines of the AOCC, where a diverse and broad partnership would provide a strong foundation and functional complementaries. Thus, the following major groups of partners are being proposed for a possible Indian partnership on tree genomics and improvement:

1. **Advocacy partners:** This would constitute government bodies, secretaries from various government departments (Department of Agriculture, Department of Science & Technology, Department of Bio-Technology, etc.), nodal bodies of universities, vice-chancellors, NGOs, philanthropic organizations to lobby and support the cause.
2. **Philanthropic trusts:** Philanthropic trusts such as Tata Trust, Infosys Foundation, Azim Premji Foundation, MS Swaminathan Research Foundation will be vital for cash support to run such an organization.
3. **Research institutes:**
 - a. *Indian Council of Agricultural Research (ICAR):* India has one of the largest and oldest national agricultural research systems in the world with several regional/centralized institutes, national-level coordinated programmes, which are working on a number of crops (annuals and trees), some of which are under-utilized. This can provide established processes and methods to conduct part of the above activities like I.1 to I.5. Some national-level organizations like the Indian Agricultural Research institute (IARI) can support activities like II.1 to II.4.
 - b. *Council for Scientific and Industrial Research (CSIR):* CSIR has a high potential to help in undertaking some basic research like bioavailability of food nutrients and also capacity to help in systems and synthetic biology

of these crops. These are some potential areas that could arise after working on these crops.

- c. *Indian Council of Forestry Research and Education*: ICFRE with nine regional research institutes and five centres in different bio-geographical regions of the country is involved in genetic improvement of several forestry tree species and manages genetic resources of tree species for conservation and sustainable utilization. ICFRE-IFGTB is presently involved in generating genomic resources of several indigenous tree species through whole genome and transcriptome sequencing.
 - d. *State Agricultural Universities*: India has many agricultural universities and affiliated colleges which have knowledge about local/niche crops through their regional centres and can work alongside ICAR organizations to support activities like I1 to I.5 and to some extent, II.1 to II.4.
 - e. *Others*: Other regional and local institutes which work on focused crops or areas.
4. *International and national instrumentation-technology partners*: This is particularly important for instrumentation necessary for high throughput nutrient analysis, and next-generation sequencing. There are various companies like Illumina, Thermo Fisher Scientific, Oxford Nanopore, PacBio, Affymatrix, LGC, Perkin Elmer, NEB, Roche, etc. which can be approached for in-kind donation or to form a fund to sponsor some activities.
5. *International and national data analytics and logistics partners*: These partners could offer secured, safe and easy data access, storage and analytics services. Google has already entered into the space of publicly accessible genomics services. Likewise, there will be providers such as Amazon, Microsoft which are also coming with their own offerings. Some local partners are also good at customized data analytics which just require minor tweaking of the analysis pipelines.

6. *Private seed industry*: India has a strong seed industry with expertise in seed production, supply chain, distribution and feedback mechanisms. Some of them are MNCs but there are several local seed companies which produce seeds of niche or specialized crops to satisfy regional needs. These can be encouraged to invest in these orphan or new crops, but it may need some state sponsorship until these new crops sustainably settle in the market.
7. *Private processed or raw food producers* (infant formula, powdered foods, oil producers, vitamin, protein & health supplements, FMCG products, specialty food supplements, ready-to-eat, food colours etc.): Private sector food industry is always searching for new tastes, delicacies and value-added products and can be roped in during the product profiling stage and trait prioritizations. Nutralite, Amway, Himalaya, ITC, HUL, Ashoka, Tata, etc. are some partners working in this area.
8. *Timber, paper and pulp companies*: This forms a major beneficiary group of agroforestry-based industries and include national players like Ballarpur Industries, ITC Ltd, Tamilnadu Newsprint & Papers Limited (TNPL), JK Paper Ltd, etc. Almost all of these have been practicing responsible sourcing of wood and can form a very important group of partners to further the goals of sustainable agroforestry practices.
9. *Development agencies, community and farmer's organizations*: Various development organizations, NGOs, farmer's groups, local cooperative societies, women's groups, self-help groups and microfinancing groups will be very important for local ground-level operations. These will help in gender balance, youth inclusivity, popularization, demonstrations, agricultural finance, extension, knowledge dissemination and feedback mechanisms.

7. SCOPE OF TREE GENOMIC RESEARCH

Trees are valued sources of food (nuts, vegetables, fruit) pulp, timber, fodder, medicinals, biofuels etc., and play an essential role in CO₂ sequestration, biodiversity maintenance and watershed stability, erosion control, host to pollinators, etc. which needs to be deeply understood and improved for meeting the specific demands of present-day challenges. Genomics is among the promising tools that can bring revolutions in future healthcare and environmental protection. The first step is to enrich the tree genomic resources which will help in a deeper understanding of structural and functional genomics. Capturing wide genetic variation residing in natural populations, developing mapping population, molecular markers, gene mapping, transcriptomics, and genome sequencing to identify potential candidate genes involved in biotic, abiotic stresses and quality parameters are the prioritized area in practical tree genomic research in India. Database development, structural and functional genomics will help in dissecting genetic control of various economically important traits of tree species. Population scale studies through comparative genomics, ecological genomics, and quantitative genetics can give a deeper understanding of forest tree populations.

8. FUTURE PERSPECTIVES

Assembly and annotation of multiple hardwood tree genomes will facilitate an increase in: (1) functional characterization of genes and gene networks related to tree habit, (2) GWAS and genomic selection investigations and applications, and (3) comparative genomic efforts among various tree species. The rapidly expanding new technologies will add an even greater number of hardwood species to these efforts. The power of comparative genomics will increase our understanding of how these highly dynamic genomes have evolved and resulted in the amazing array of phenotypic diversity found among and within hardwood species.

In sum, most hardwoods are undomesticated long-lived plants that provide many ecological and commercial benefits, whose management, conservation and domestication for economic and ecological purposes will benefit from a set of rich

genomics resources. Ultimately such resources will favourably impact the pressing problems of climate change, soil and water conservation, bioenergy and biomaterials production, and maintenance of healthy ecosystem functions. The genomic studies in reference to the fruit and nut trees have a wider scope and application to hasten the breeding programme. It will help in identification and characterization of candidate genes and gene families related to important traits viz., tree architecture, yield governing traits like regular bearing, bearing habit, fruit and nut weight, quality traits (fresh and processed fruits), resistance and/or tolerance to biotic and abiotic stresses.

Tree genomics will ease the understanding of phylogenetic structure and introgression of genes from wild relatives for development of climate-smart varieties of fruits and nuts. It has opened up doors for germplasm conservation, characterizations and utilization by use of high-throughput genetic structure analysis throughout wild relatives, land races and commercial cultivars in many fruit crops. Use of genomic tools will help in understanding the genotype-phenotype interactions, discover physiological pathways for nutrient uptake, assimilations, and in synthesis of biomolecules in fruit and nut crops. The breeding for nutrient (iron, zinc, iodine, selenium, vitamin A, etc.)-enriched genotypes in fruit and nut crops is the key through bio-fortification using genome-editing approach like CRISPR/Cas 9 to tackle malnutrition.

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