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Recent advances in tree improvement for agroforestry species

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ABSTRACT: Agroforestry as the name suggests, involves tree and crop interaction, hence tree improvement for agroforestry species slightly differs as that of the forest tree species. In addition to maximizing the economic yield, these species are selected for their social, economical and environmental sustainability. The breeding objectives vary as their use in different agroforestry systems according to suitability of different agro-climatic regions. The breeding programs are being implemented for the genetic improvement starting from the classical breeding to the use of modern biotechnology tools. The prioritization of the breeding tools varies with the knowledge of the species, their breeding objective and the economic returns upon the improvement. Thus in the improvement of agroforestry species, we can find the basic reproductive biology to the recent omics study depending on the species. The present review focuses on the recent trends in the tree improvement for some of these agroforestry important species.

Key words: Agroforstry species, classical breeding, clonal propagation, genomics, transgenics.

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1. INTRODUCTION

Agroforestry is a collective name for land use system and technologies in which woody perennials are deliberately used on the same land management units or agricultural crops and animals in some form of spatial arrangement or temporal sequence. In the 21st century, when land holdings are reducing in size and natural resources are depleting because of excessive use, agroforestry promises an economic and environmental sustainable livelihood. Agroforestry is uniquely suited to address the need to grow more food and biomass for fuel while sustainably managing agricultural landscapes for the critical ecosystem services they provide. Selection of the tree species for agroforestry systems depends on several criteria but the most important of them is their maximum economic yield suitable to that region. Thus improvement in agroforestry species includes broadening the range and diversity of trees that can be integrated into farming systems and maximizing the productivity of agroforestry systems through improved tree germplasm.

2. BREEDING OBJECTIVES

Improvement of agroforestry important tree species differs slightly from the regular tree breeding programs, as in agroforestry systems; the interactive effect of the tree component with the associated crop becomes important. In case of pure stands, the maximizing of yield in terms of wood or any other product and fast growth are the major concern. In agroforestry species, for some farmers, reliability of production may be more important than mean annual yield (Simons 1992) i.e., livelihood security in terms of economical, social and environmental.The most common objective for the woody tree species to be incorporated in the agrisilviculture system is the clean bole height (to minimize the shade effect). Depending on the purpose and the species involved, the objective of breeding program varies. In case of the most commercially propagated trees for timber yield like Teak, Eucalyptus, Poplar, etc., fast growing is the selection criteria in addition to the wood quality & ability to coppice. In the alley farming systems, incorporating the use of trees such as *Gliricidia* or *Leucaena* for protection and mulch production, the biomass yield is important. In shade tree systems, the wide canopy with short height is preferred. In arid regions, trees are grown principally for fodder and fuelwood in agroforestry systems, *e.g. Prosopis* and arid zone acacias. Here fodder yield is the primary selection criteria, followed by fast growth, timing,reliability of leaf production and the quality of the wood. In some trees like Mahua, where the trees are valued for the long productive life than their wood yield, the high yield in terms of flowers and fruits gains value. In *Pongamia*, high yield is coupled with short height for the ease in collection of fruits. In *Jatropha* stability of the high yielding genotypes is a major national concern for improvement.

As the commercial value for the species increases the breeding objectives also varies, for *e.g.*, in tree improvement programs for paper and pulp industry, the high cellulose and low lignin contents in addition to fast growth, good form and wood qualityare the important selection criteria. While species where the major improvement is for fodder, foliage biomass, thornlessness, fodder quality and the year round availability is the major criteria. In tree borne oilseeds, which are recently gaining importance as biofuel crops, the yield and oil content, in addition to convenient plant height is the major criteria for selection. In agroforestry tree species, breeding for abiotic stress like tolerance to salt, drought, frost, high temperature and degraded soil conditions takes prominence than the selection for biotic stresss. Pest and disease resistance are taken as important when it is affecting the industrial quality of wood yield as in case of *Eucalyptus*, *Dalbergia*, Poplar, *Acacia mangium* for which the defined market system is available. In some species, breeding for cytoplasmic male sterile lines are also important where the prolific seed production is a hinderance like for Leucerne.

3. CLASSICAL BREEDING PROGRAM

Advances in tree improvement are relatively few in comparison to the vast development achieved in agri-horticultural crops. The reason may be that these are recently domesticated for economic yields. The reproductive biology, geographic patterns of genetic variation in each species, maintaining adaptive genetic variation, and maintaining wild forest reserves are all issues that add to the complexity of tree improvement. Other major limitations to rapid improvement are;1.Long generation intervals, related to poor juvenile-mature correlations and the long juvenile phase with respect to flowering.2.Low effectiveness of selection for many characters, due to low heritability or difficulty in assessment.3.Through the use of open-pollinated orchards, the exploitation of only a part of the genetic variation available.4. Breeding for tree species is labour and land intensive.

Classical breeding approaches developed for domesticated plant species have been adapted to accommodate the unique biological features of forest trees. Till date more than 400 tree species are brought into the breeding program, one way or the other. The major implementation revolves around the evaluation of the provenance selection & clonal selection of the superior ones and in few commercial successful species intraspecific hybridization. In many other potentially valuable species testing of their potentiality, characterization of mating systems, provenance collection, establishment of trials, and commencement of other breeding work are still in nascent stage. Broader implementation of good improvement programmes is an important priority for these species. In species where natural stands are exploited for their potential resources and in species where few clonal selections are grown in large plantations, implementation of gene conservation measures and management of genetic resources is an urgent task to avoid the genetic erosions.

Application of the recent biotechnological interventions is feasible for very few crops where the economic returns are very high. Though the unconventional breeding techniques are being applied in the agroforestry tree species, the major works are limited to tissue culture and somaclonal variation and the marker analysis study for diversity. In very few species, genomics and transgenics are being developed like in *Eucalyptus*, Poplar, *Jatropha*, *Pongamia*, etc. (Table.2).

Genetic improvement offers clear benefits for planting programmes with agroforestry species. Breeding programmes incorporating provenance and progeny testing is continuing for most of the species like *Azadirachta*, *Acacia*, *Albizia*, *Pongamia*, *Dalbergia*, *Jatropha*, Alder, *Ailanthus*, *Grewia*, *Prosopis*, *Melia*, *Gmelina* and some of the fruit trees like Amla, ber, bael, etc. Mass selection works well for highly heritable traits especially where phenotype is good reflection of genotype. Family selection works best with traits of low heritability, but this is rarely used in forestry. Thus in tree breeding, selections of family and within family are combined in tree breeding – a two stage method: selection of families followed by selectionof individuals within the families.

Broad provenance collections have been made and trials planted, and seedling seed orchards established at several centres. Gene pools are relatively secure for major tree species. Trials in several places demonstrates wide provenance variation phenotypically.

The primary consideration for any genetic improvement is its base, i.e., utilization of genetic variability. Selection of superior trees has been of primary importance even today and is being taken up in most of the important species at various research institutes. Evaluation of candidate plus trees and/or provenance selections have helped in identification of best performing plus trees in *Eucalyptus, Dalbergia, Azadirachta indica, Acacia,* Leucaena, *Ailanthus, Pongamia, Casuarina, Anogeissus, Bamboo, Mangium*hybrids, Poplar, teak, Pines, *Albizia* sp., *Cedrus, Gmelina, Tecomella, Commiphora, Pinus, Santalum, Moringa,* etc. Registration of the elite germplasm of Dalbergia by National Research Centre for Agroforestry, Jhansi (Bundel-1 and Bundel-2) and GB Pant University of Agriculture and Technology (PS 52), Poplar clones (L-48/89, L-47/88) by Panjab Agricultural University, Ludhiana, teak clone (PDKV/A/1) by College of Agriculture, Nagpur and Eucalyptus (SRY-16) by Mahatma Phule Krishi Vidhyapeet, Rahuri are some of the examples (Dhyani *et al.,* 2013). A detailed list of varietal notification in India is provided in the Table 1.

Genetic variation with respect to a range of characters is substantial in terms of inter and intra-specific population. Hybridisation and clonal selection in much narrower populations has been in progress for some of the important characters like timber yield, wood quality, coppicing ability and insect and pest resistance. Significant genetic gains are being achieved in these breeding programmes on the genetic quality of plantations.

In indigenous species, generating base line information for tree improvement programmesis still a priority for many of the species which has high potential. In this view, studies on reproductive biology, phenology, floral biology, pollination and reproductive success has been initiated in species like *Azadirachta indica*, *Dalbergia sissoo*, *Acacia auriculiformis*, *Gmelina arborea*, *Bruguiera* spp., tamarind, etc.

The morphological variations are studied across the geographical areas and between the populations in most of the species to represent their germplasm assemblage by surveying different agro-climatic zones. Evaluation of the germplasm diversity will help in identificaiton of phenotypically superior types. Maintenance of germplasm and their multiplication is very essential to conserve the broad base of the gene pool, which forms the national genetic diversity of the species.Gene conservation collections are being undertaken where the populations in danger of extinction or genetic erosion because of over exploitation of the natural stands.

4. CLONAL PROPAGATION

Clonal propagation is another indispensable components in tree improvement programme by means of mass multiplication of superior genotypes for clonal multiplication and thereby improvement of productivity. Successful clonal propagation and rooting is still far to achieve in many of the tree species. Advances are being made in terms of modifications of propagation techniques like, mini cutting technique in enhancement of rooting percentage in eucalyptus. *Ex vitro* propagation techniques is also being developed in species like *Aquilaria malaccensis*, *Dipterocarpus retusus*, *Jatropha curcas* and *Commiphora wightii*, etc. In *Shorea* & *Scleichera oleosa*, besides stem cutting, other macro propagation methods like air layering and grafting were carried out. Grafting techniques was standardized for propagation of selected adult male and female plants of *Ailanthus excelsa*. Success rate of grafting increased up to 50% over the earlier method. The plants produced were also used for field evaluation.

Plantations of many important species raised from seed have extremely large genetic variation and poor productivity. Clonal plantations exploit existing natural variation for fast and immediate genetic gains taking full advantage of superior genetic qualities of field tested clones. Vegetatively propagated clonal plants, developed from a single mother tree having most desirable genetic qualities, are uniform and true to type with all of the genetic qualities of the mother plant. Clonal plantations of many conifers including pines and spruce and hardwoods like gamhar (*Gmelina arborea*), casuarinas, poplars, *Acacia mangium* and eucalypts etc. have been raised in many countries with major improvements in productivity and quality of timber. However in India, Poplars and clonal Eucalyptus, mainly *Eucalyptus tereticornis* and *Eucalyptus camaldulensis*, plantations are the two most outstanding success stories of clonal plantations.

Table 1. Released/notified varieties/hybrids/clones in some important agroforestry tree species in India

Sl.No	Botonical name	Variety Name	Type of material	Institute name	Year of release/ registered
1	*Moringa oleifera*	Periyakulam 1 (PKM 1)	Selection	HCRI, Periyakulam, TNAU	1989
2	*Popolus deltoides*	PL-1	Clone	PAU, Ludhiana	1997
3	*Popolus deltoides*	PL-2	Clone	PAU, Ludhiana	1997
4	*Popolus deltoides*	PL-3	Clone	PAU, Ludhiana	1997
5	*Popolus deltoides*	PL-4	Clone	PAU, Ludhiana	1997
6	*Popolus deltoides*	PL-5	Clone	PAU, Ludhiana	1997
7	*Popolus deltoides*	PL-6	Clone	PAU, Ludhiana	1997
8	*Popolus deltoides*	PL-7	Clone	PAU, Ludhiana	1997
9	*Popolus deltoides*	Pant Poplar 5	Clone	GBPUAT, Pantnagar	1998
10	*Eucalyptus tereticornis*	SYR-16	Clone	MPKV, Rahuri	1997
11	*Leucana leucocephala*	K-636	Introduction	UAS, Dharwad	1998
12	*Tamarindus indicus*	DTS-1	Selection	UAS, Dharwad	2000
13	*Tamarindus indicus*	DTS-2	Selection	UAS, Dharwad	2000
14	*Moringa oleifera*	PKM 2	Selection	HCRI, Periyakulam, TNAU	2001
15	*Azadirchta indica*	DOR Neem 2	Selection	DOR, Hyderabad	2003
16	*Jatropha curcas*	Soumya	Interspecific hybrid selection	DOR, Hyderabad	2005
17	*Jatropha curcas*	Swetha	Interspecific hybrid selection	DOR, Hyderabad	2005
18	*Jatropha curcas*	Chhatra-pati	Selection	SDAU, Sardar Krushinagar	2006
19	*Jatropha curcas*	BAAS-51	Selection	NBPGR Reg. Stn, Hyderabad	2008
20	*Jatropha curcas*	SNES-45	Selection	NBPGR Reg. Stn, Hyderabad	2008
21	*Jatropha curcas*	FC	Interspecific hybrid selection	FCRI, TNAU, Mettupalayam	2009
22	*Jatropha curcas*	RI-HC 32	Interspecific hybrid selection	FCRI, TNAU, Mettupalayam	2009
23	*Populus deltoids*	L-48/89	Clone	PAU, Ludhiana	2009
24	*Populus deltoids*	L-47/88	Clone	PAU, Ludhiana	2009
25	*Dalbergia sissoo*	PT-1	Selection	NRCAF, Jhansi	2011
26	*Dalbergia sissoo*	PT-2	Selection	NRCAF, Jhansi	2011
27	*Dalbergia sissoo*	FRI-DS-14	Clone	ICFRE, Dehradun	2011
28	*Eucalyptus hybrid*	FRI-EH-001	Clone	ICFRE, Dehradun	2011
29	*Melia dubia*	MTP 1	Selection	TNAU, Coimbaore	2011
30	*Casuarina*	MTP 2	Hybrid Clone	TNAU, Coimbaore	2011
31	*Casuarina*	MTP CA1		TNAU, Coimbaore	2011
32	*Dalbergia sissoo*	PT-52	Selection	GBPUAT, Pantnagar	2013
33	*Populus deltoids*	PP-5	Mutant Selection	GBPUAT, Pantnagar	2013
34	*Tamarindus indicus*	Goma Prateek	Selection	CHES under CIAH, Bikaner	2013
35	*Tectona grandis*	PDKV/A/1	Clone	Coll. of Agri, Nagpur	
36	*Eucalyptus sp*	FCRI EC 48	Clone	FC&RI, TNAU, Coimbaore	
37	*Eucalyptus sp*	FCRI 103	Clone	FC&RI, TNAU, Coimbaore	
38	*Eucalyptus sp*	FCRI 53	Clone	FC&RI, TNAU, Coimbaore	
39	*Eucalyptus sp*	FCRI 56	Clone	FC&RI, TNAU, Coimbaore	
40	*Eucalyptus sp*	NGL 2	Clone	FC&RI, TNAU, Coimbaore	

The huge domestic demand for timber and wood based products (344 m mt. of firewood and charcoal, 37 m m^3 of industrial round wood, 33 m m^3 of sawn timber, 5.7 m mt. of paper/boards, 1.3 m mt. wood based panel) has given way for private sectors to play a major role in forest plantations. In case of seed propogated plantations for industrial purpose, the effect of biotic and abiotic factors will pose a huge risk in investments. Hence many private players have initiated their own R & D and their main focus is on candidate plus tree and clonal selections. Some of the pioneer players in the industry are Wimco limited, ITC limited for eucalyptus in Andhra Pradesh, followed by Ballarpur Industries limited in Orissa, Maharashtra and Andhra Pradesh, J.K. Paper mills in Orissa, Pragati biotechnologies in Punjab, etc (Lal, 2010).

5. *IN VITRO* REGENERATION

Among the unconventional breeding techniques, the most frequently used is the *in vitro* studies. As in many tree species seed propagation is a problem owing to the seed dormancy, short viability period and poor germination, number of studies is reported for the standardization and *in vitro* regeneration in many of the tree species. Yet there are many species which are difficult to propagate *in vitro* and rooting is major problem, thus tissue culture studies are still the prirortiy. In addition protocols are being standardized for gene transformation studies owing to the many genes of interest being identified and cloned in many other crops. Also tissue culture is used for enhancement of root-ability and planting stock production of selected high yielding tree species. Evaluaton of the tissue cultre raised plants are also in progress for their stability. Variation induced during cell or callus cultures has been reported for many species but their persistence of modification through subsequent sexual generations has not been demonstrated. Thus somaclonal variation is not a field of study for these species.

Cell culture protocol for e.g., in *Aegle marmelos* are being developed and tested the efficacy of the metabolites production. Also, assuming the stability of characteristics, the approach is of most value where selection can be done at the cell level, thus enabling screening of large numbers of genotypes, where screening for the trait lies outside the range available naturally in the species. The traits such as cold tolerance, salt tolerance, tolerant to phytotoxin or high mineral levels can be effectively screened for large number of genotypes *in vitro*.

6. DNA PROFILING

Developing appropriate strategies for tree improvement and integrating them with the tree genetics are essential to speed up the tree improvement programs.In recent times, the development in the fields of genetics and molecular biology are being applied to realize the objectives at a faster pace.

To understand the complex genetic structure of trees with respect to their genetic diversity, molecular characterization of germplasm, inheritance pattern and establishment of genotype and species specific makers DNA fingerprinting is carried out in many tree species. RAPD and ISSR markers are the most commonly used DNA markers for characterization of tree resources. With the evolution of genomics and the availability of the large molecular data on the public domain, numbers of reports are published using SSRs, STS, AFLP, DArTs & SNP markers. Molecular characterization of Himalayan pines, *Cedrus deodara, Tectona grandis, Eucalyptus, Dalbergia, Jatropha*, *Pongamia* and many more germplasm is in progress. Based on the polymorphism exhibited by the markers, Species specific RAPD markers, SCAR markers are being developed with a view to detecting natural hybrids between the species.

7. MOLECULAR CHARECTERIZATION FOR BREEDING

As large diversity exists for the economic trait in the germplasm and selection at juvenile stage is difficult to impossible for the tree produce, chararterization and establishment of DNA marker association has taken importance. Marker diversity is used for marker trait association like in *Pinus roxbughii* population for resin yield diversity, genetic determinants of salt stress tolerance in *Casuarina* and many more. The DNA marker trait association was also extended to the *Casuarina* for their holocellulose and lignin content and distinct gender specific isoenzyme markers were developed. Also gender specific peroxidase profiles were generated in important bamboo species like *Bambusa balcoa*, *B. tulda* and *B. nutans*.

Association Analysis of Traits Using STS Markers is also attempted for identifying potential markers for rooting in *Eucalyptus tereticornis*, where the rooting variability varies from 10-90%. Mapped SSRs and EST-SSRs linked to rooting were used for association analysis approach, to generate the basic information to select candidate STS makers for identifying marker-trait associations. Similar attemps are also in the process of identification of molecular markers associated with wood quality traits in *Tectona grandis* L., etc. Allelic Diversity of Cinnamoyl CoA Reductase Enzyme Gene were used to associatate marker for lignin and holocellulose content in in *Casuarina equisetifolia* and around four CCR (forward & reverse) specific primers were identified. SNP markers are used in developing the significant association for wood and pulp traits in *Corymbia citriodora*. Identification of pulping trait markers through candidate gene association in Eucalyptus has been initiated. It is also extended to the study on sex determination in Date palm, for developing molecular diagnosis of sex in plantlets at seedbed stage. SCAR markers are developed based on the Single RAPD primer, OPA-02 which showed polymorphism between male and female plants of Date Palm. Efforts are on to identify suitable biochemical markers for sex determination and also for screening of clones for salinity tolerance by differential expression of isozyme patterns (ICFRE Annual report).

Apart from genotyping, high throughput multienvironment phenotyping of the population is equally important in marker trait association. One such initiative has been started at IFGTB, Coimbatore for adventitious rooting and woody property traits in Eucalyptus.

Markers are also useful to develop core collections of the germplasm for functional utilization of the accessions in the pre-breeding programs. Using DArTs, SSRs, SNPs markers in association with agronomic traits, the core size of 68 accessions (19%) and 36 accessions (10%) were developed from the whole collection of 361 olive accessions in IFAPA, Spain. Due to its high efficiency at capturing all the alleles/traits found in the whole collection, this would be of special interest for genetic conservation applications in olive and forms the working collection for olive breeders.

8. QUANTITATIVE TRAIT LOCI (QTL) MAPPING

The long reproductive cycles and the long time to expression of mature traits makes marker-assisted selection particularly attractive for tree breeding. In such situations, development of linkage maps with traits and markers will be of great help. Identification of useful markers for application in tree breeding is mainly based on two approaches, 1. quantitative trait locus (QTL) mapping and association analysis & 2. Genome wide selection. First approach depends on identification of QTLs and trait and QTL association studies. While several studies have identified significant markers, effect of the individual markers is low making it difficult to utilize them in breeding programs. Recently, genomic selection (GS) was proposed for overcoming some of these difficulties. In GS, high density markers are used for predicting phenotypes from genotypes. GS based on large numbers of random markers or small numbers of associated markers is poised to make marker-assisted selection a reality in forest tree breeding.

Quantitative traits, such as wood yield, wood quality or pulp yield, are usually controlled by many genes, termed quantitative trait loci (QTL). By using molecular markers closely linked to, or located within, one or more QTL, information at the DNA-level can be used for early selection. The potential benefits of MAS are greatest for traits that are difficult, time-consuming or expensive to measure (for example, wood quality traits or pulp yield). Mapping and MAS tend to be used only in species of high economic value and have most potential in clonal breeding programmes, where additional genetic gains can be rapidly multiplied (Carmenza *et al.,* 2013).

Generating mapping population is one of the important prerequisite for QTL mapping. Attempts for controlled pollination are being made in *Eucalyptus*, *Jatropha*, *Prunus*, *Corymbia*, etc by inter specific crosses to generate mapping population. Traits such as seed dormancy, salt tolerance, adventitious rooting and wood property were chosen in this hybridization work to generate genetic linkage maps in *Eucalyptus*, where as in *Jatropha* & *Elaeisoleifera*, fatty acid synthesis genes were targeted. A dense microsatellite linkage map of 362 loci spanning 1.485 cM, representing the 16 pairs of homologous chromosomes in the *Elaies* genus were developed and from which a total of 19 QTLs associated with fatty acid composition were evidenced. Further using intra-gene SNPs related to oleic acid C18:1, the information of QTLs linked to FATA and SAD enzymes were evidenced, which are valuable resource for marker assisted breeding.

Seed dormancy is one of the major obstacles in propagating many of the tree species, for which different strategies are being developed to break dormancy. Marker association has also been useful in seed dormancy studies in peaches. As the genetic factors involved in regulation of seed dormancy and their location on the peach genome has been well established, SSR markers were selected form the *Prunus* reference map at an average resolution of 20cm and used in the segregating F_2 population of high x low chill F1 peach hybrid. Seed dormancy QTLswere discovered in the same region of the QTLs associated with floral bud chilling requirement and bloom time in peach, which can be further utilized in marker assisted breeding for breaking dormancy for seed, floral and vegetative buds in peaches simultaneously (Blaker *et.al.,* 2013).

9. GENOMICS/FUNCTIONAL GENOMICS

Post whole genome sequencing, functional genomics and comparative genomics have evolved in many crop plants and thus have been extended to many of the tree species. In silico analysis for the target traits is generated to catalogue, assemble nucleotide and protein sequences of desired trait of interest and primers are synthesized to evaluate allelic diversity and generate trait specific markers. This has practical utility in marker assisted breeding and also for development of transgenics.

One such study has been initiated for cellulose synthase (CesA) genes and primer pairs were designed and synthesized targeting the CSR II domain of the CesA. The primer pairs were screened and six transcripts representing the six families of CesA were identified in the developing secondary xylem tissues of *E. tereticornis*. Studies are in progress to identify the 3' downstream sequences of the CesA genes. Similarly, Class I chitinase gene with approximate size of 1.4 kb was identified with ORF size of 867 bp, 3'UTR of 240 bp and 5'UTR of 293 bp encoding a protein of 289 amino acid with Glycoside hydrolase & family 19 chitinase domain was characterized.Six transcript representing six classes of cellulose synthase were characterized from *Eucalyptus tereticornis*. For rapid functional analysis of genes involved in salt tolerance, parameters critical for development of *E. tereticornis* composite plants with transgenic hairy roots were also identified.

Large variability is displayed in tropical rainforest trees for life history traits related to biomechanics which has a unique importance with the evolutionary perspective. Thus from sequences and gene expression data available in model species, specific primers for six candidate genes for mechano-sensing in five tropical species were developed. Most of the gene sequences were polymorphic in most species.

Two Pathogen defense related genes, chitinase and glucanase were cloned to *Casuarina equisetifolia* and their expression during pathogen elicitation was studied using Transcriptome analysis. Further, major transcripts including cytochrome oxidase, proteasome, signal recognition particle and unknown transcripts with similarity to drought tolerant ESTs were demonstrated to be over expressed during pathogen elicitation Web Enabled Database and Analysis of Gene.

Web enabled database was used for the analysis of gene sequences implicated in abiotic stress tolerance for screeing gene homologues in salt tolerant species. Using the comparative genomic studies, sodium transporter gene homologues, viz., NHX1 and HKT gene from salt tolerant tree species were sequenced. Transcriptome analysis of salt tolerance is also inititated in *Casuarina* and*Eucalyptus*, in addition to their transformation in *Casuarina* for their functional analysis.

Transcript profiling using differential display of mRNA is underway for identification and functional characterization of novel pathogen defense related genes from *Casuarina*. Another initiative on identification of differentially expressed cellulose synthase (CesA) genes from the developing secondary xylem tissues of *Eucalyptus tereticornis* is under progress. One of the transcript amplified only in the secondary xylem tissues implicating its specific expression during wood formation. This study will open a new dimension of research using CesA as a candidate gene in association analysis for pulping trait.

Trees altered in their lignin profile with reduced amounts of lignin or increased extractable syringyl are desirable in the pulp and paper industry. Cinnamoyl-CoA reductase (CCR) and caffeic acid O-methyltransferase (COMT) are two enzymes likely to regulate lignin content and composition in the syringyl (S)- and guaiacyl (G)-related monolignols at specific branches of the lignin biosynthetic pathway. There is large potential for selection of high quality parents especially with characteristics favourable for some end products like pulp and fibre. The genetic variation in the trait related genes using SNPs were studied and the alleles were isolated, sequenced, analysed and allelic diversity was revealed by genotyping in *Acacia*. This can further be used in exploitation through the candidate gene-based LD mapping to identify QTLs for the *Acacia* marker-assisted breeding (Paula *et al.,* 2007).

For the first time, EST-derived SSR markersshowing polymorphism was developed from the expressed sequence tags (ESTs) of *Jatropha curcas* from National Center for Biotechnology Information species/genera by

NBRI, Lucknow. Apart from being a valuable resource for future genetical studies, like construction of linkage maps, diversity analysis, quantitative trait locus/ association mapping, and molecular breeding of *J. curcas* it has shown 47% transferability across genera in *Ricinus communis* (Yadav *et al.,* 2011).

10. TRANSGENICS

Sophisticated breeding programmes are unlikely for most of the agroforestry species, but in certain species, it is possible that "quantum leap" approach to breeding may be more appropriate than breeding programmes offering continuous incremental gains. Genetic engineering options are increasingly contributing to development of improved plant varities worldwide. Breeding integrated transgenic programme is ongoing in few tree species for genetic modification of desired traits and functional analysis of genes. More than 100 reported trails involving 24 tree species, most of which are timber-producing are ongoing. Traits for which genetic modification can realistically be contemplated in the near future include insect and virus resistance, herbicide tolerance, lignin content and oil contentor yield.

Trangenics can be of different types. One, it may be transformation with novel genes to confer a new trait, or with modified genes to enable more effective performance of an existing function e.g., herbicide tolerance, insect resistance and cold tolerance. Two, it may be transformation with genes which lead to the blocking of protein synthesis, through either antisense or ribozyme technology. With the antisense approach, a gene is transcribed in reverse direction, and an mRNA is produced that is complementary to, and thus able to bind, the normal message - blocking protein synthesis, eg. RNAiapproach for incorporation of resistance against the Eucalyptus pest, *Leptocybe invasa*; silencing of a toxic gene to increase the fodder value or viral resistance by targetting viral gene expression and/or replication. Third transformation may be genetic engineering of the promoter genes for over expression of the existing genes. Eg.FAD2 gene involved in oilbiosynthesis for higher production of oleic acid in *Jatropha*.

A preliminary review list of trangenics in tree R&D efforts reveals that hundreds of field trials, notifications and genetic engineering laboratory efforts are under way around the world to commercially develop genetically engineered trees for large scale monocultured plantations, food crops, ornamental trees, essential oils, biopharmaceutical crops, reforestation of endangered trees like the American chestnut, soil erosion protection in places like China, toxic hot spot soil remediation, and a variety of other uses (http://www.nwrage.org). The most common GE tree traits are faster growth, herbicide tolerance, sterility (terminator), reduced lignin biosynthesis, resistance to pests and insects, improvement of fiber/product uniformity, genetic markers, toxic absorption from the soil and more. Cold tolerance genes are likely to be of some commercial value in many species, in particular the eucalyptus. The reduction of lignin biosynthesis by antisense technology is a very valuable objective for the pulp species. At present, the genetic engineering of lignin biosynthesis appears to be a promising forest biotechnological application to improve wood quality. Because the economy of pulping processes depends almost entirely on the efficiency of removing lignin which encrusts the wood fibers. Thus, one way to improve the efficiency of pulping is to genetically reduce the quantity or to alter the quality of lignin in pulpwood species. Lignin biosynthesis was the most peculiar biological process involving various components of wood. Genetic manipulation of lignin is largely a matter of manipulating genes encoding lignin pathway-specific enzymes. It takes a long time to reach the breeding goals in traditional breeding program, because multi-genes controlled most wood traits. At present, transgenic method is of great expectations. So, the genetic engineering of lignin biosynthesis has bright prospects in its application in tree breeding for pulping and papermaking industries.Some of the tree species which are being manipulated with transgenic technology are Poplar, *Eucalyptus*, etc.

The availability of effective transformation techniques remains an obstacle, but improved techniques are being developed. Regeneration is a difficulty for some tree species, but the problem may be over-rated - the non-competence of mature material is not necessarily an obstacle to effective application of genetic engineering, provided that juvenile material responds satisfactorily. An often overlooked research component is the testing which would be required before a responsible recommendation for large scale deployment of trangenic plants could be made. Such testing could be extensive and prolonged, depending on the species and genes involved.

Transgenic projects on using gene silencing techiniques for functional validation of the genes cloned from other species through comparative genomics are also ongoing.Initiatives are underway in using RNAi for incorporation of resistance against the Eucalyptus pest, Leptocybeinvasa and other traits like improved productivity under drought conditions and improved pulping traits.Prevention of the escape of genes into wild populations is likely to become an important

Table 2. Different Tree Improvement methods in some of agroforestry tree species

Sl.No.	Tree Species	RB	PS	CPT	CP	CH	IH	GD	MM	IR	SMP	G/FG	M	T	Tr
1	*Acacia sp.*	√	√	√	√	√	√		√	√		√			
2	*Ailanthus excelsa*		√	√	√										
3	*Albizia*		√	√				√	√						
4	*Alnus sp.*		√	√	√										
5	*Emblica officinalis*		√												
6	*Anogeissus pendula*			√		√				√					
7	*Artocarpus heterophyllus*				√			√	√	√					
8	*Aegle marmelos*		√								√				
9	*Azadirachta indica*	√	√	√	√			√	√	√	√				
10	*Butea monosperma*							√	√						
11	*Buchanian lanzan*		√		√			√		√					
12	Bamboo sp.			√	√			√	√	√					
13	*Bruguiera* spp	√													
14	*Casuarina equetifolia*			√	√			√	√	√		√		√	
15	*Cedrus deodara*			√				√	√						
16	*Commiphora wightii*			√	√			√	√	√	√		√		
17	*Corymbia citriodora*						√		√	√					
18	*Dalbergia sissoo*	√	√	√				√	√	√					
19	*Dipterocarpus retusus*				√										
20	*Eucalyptus sp.*		√	√	√	√	√	√	√	√		√		√	√
21	*Elaeis spp.*								√						
22	*Garcinia indica*							√	√				√		
23	*Gliricidia sepium*	√	√												
24	*Gmelina arborea*	√	√	√	√										
25	*Grewia sp.*		√												
26	*Jatropha curcas*	√	√	√	√		√	√	√	√		√	√	√	√
28	*Leucaena leucocephala*			√											
29	*Madhuca latifolia*		√					√							
30	*Melia dubia*		√												
31	*Moringa oleifera*		√	√											
32	*Paulownia tomentosa*				√										
33	*Pongamia pinnata*		√	√	√			√	√	√		√			
34	*Populus spp*			√	√			√	√	√		√			
35	*Pinus roxbughii*		√	√	√		√	√	√	√		√			
36	*Prosopis pallida*		√							√					
37	*Pterocarpus marsupium*									√					
38	*Santalum album*			√				√	√	√					
39	*Spondias sp.*		√		√										
40	*Sapindus trifoliatus*									√					
41	*Syzygium cuminii*									√					
42	*Tamarinus indicus*	√	√					√	√						
43	*Tectona grandis*			√	√	√		√	√	√					√
44	*Tecomella undulata*			√	√					√					
45	*Terminalia chebula*							√	√	√					

RB-Reproductive biology; PS-Provenance Selection; CPT-Candidate Plus tree selection; CP-Clonal Propagation/Selection; CH- Clonal hybrids; IH-Interspecific hybrids; GD-Genetic diversity; MM-Molecular Marker studies; IR-In vitro Regeneration; SMP-Secondary Metabolite production; G/FG-Genomics/Functional Genomics; M-Metabolomics; T-Transcriptomics; Tr-Transgenics.

concern and sterility should be an early target of genetic engineering work with forest tree species. The major factor limiting application of genetic engineering in forest tree species is the state of knowledge of molecular control of the traits which are of most interest - those relating to growth, adaptation and stem and wood quality.

It is important that genetically engineered genotypes be of high quality with respect to other traits as well. The clonal test is the most logical basis for integration of genetic engineering into traditional tree improvement programmes. For these reasons, genetic engineering is most appropriately conducted with species where breeding programmes are advanced and clonal forestry can be realistically contemplated. Research on this subject should not assume a high priority with species for which natural variation available within the taxon remains poorly investigated.

11. CONCLUSION

To summarise, the recent advances in agroforestry tree species involves application of breeding tools both conventional and unconventional. As agroforestry systems are being developed for sustainable livelihood, many tree species are being evaluated for their performance in different agro-climatic zones, and their breeding programs revolves around their basic study on mating systems, reproductive biology and the morphological variaitons for the triats of interest. Collection and evaluation of provenance selections is a continuous process and in addition, marker analysis is being introduced for diversity analysis. In few species where long term breeding programs have resulted in superior clones for large scale production and in industrially important species the advanced genomics and transgenic studies are being implement for trait specific modifications. A summary of the recent advances in some of the agroforestry tree species are listed in the Table 2.

The basic phenology and reproductive biology studies are relatively sparse and the emphasis has to laid on study the biology of tree species. Provenance variation studies should be high lighted in feature with respect to the important useful traits of the tree species which needs the stress on the quality traits, both at morphological and molecular level. This will help in utilization of the species with proper selection for economic traits.Clonal forestry which is restricted to few of the species has to be extended to other for agroforestry species. A number of research is concentrated on *in vitro* regeneration studies and molecular diversity analysis, the result of which has to be reaped. For this, more studies has to focus on the field analysis of the *in vitro* established plants for its stability and performance. The scattered studies on molecular diversity have to be concised to develop trait specific markers, which can be of more practical use in tree improvement.

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