PROJECT PROFILE

Title:	**Genetic Improvement of Eucalyptus through Mapping and Tagging of QTLs/Genes**
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Duration:	2010 -2015 (5 years)
Sub projects:	• Development of full-sib families for important species of eucalypts through controlled pollination for QTL studies • High throughput multi environmental Phenotyping of Mapping of Eucalypts for Adventitious rooting and wood Property traits • Development of Genetic Linkage Maps and QTL analysis in *Eucalyptus* for Adventitious Rooting and Wood Property traits • Candidate Gene Association for Identification of Pulping Trait Markers in *Eucalyptus tereticornis*
Funding Agency:	Department of Biotechnology, Govt of India

SUMMARY

Development of full-sib families for important species of eucalypts through controlled pollination for QTL studies

The seed parent was selected from the clonal trial at Karunya nagar, Tamil Nadu. The trial consists of three ramets each of the *E. tereticornis* clones (Palmer River - ET217 & Helenvale-

ET285) and *E. camaldulensis* (Victoria River and Gilbert River) in four replications. Ramets were selected on the basis of reproductive performances.

The *E. grandis* provenance stand of 13017 Lorne, New South Wales, Australia (31°37'S; 152°43'E) was selected as pollen parent resource. The said population was developed by IFGTB (Eucalyptus Research Centre-ERC) during 1982 at Hosamund, Ooty District, Tamil Nadu.

The following 6 mapping populations were developed targeting pulp and rooting properties:

Mapping populations for Wood property:

1. Mapping Population 1 (Ec111xEt86-PULP)

2. Mapping Population 2 (Ec17xEg9-PULP)

3. Mapping Population 3 (Ec17xEg14-PULP)

4. Mapping Population 4 (Et86xEg9-PULP)

Mapping populations for Adventitious Rooting :

1. 5. IFGTB-EUC-MP5 (Et 86x Eg14 ROOT)

2. IFGTB-EUC-MP6 (Et217x Ec17ROOT)

Multi Location Hybrids Clonal Trials (MLHCT) were established in different locations in Tamilnadu. Muthupet Thiruvarur (10o28'18.50:"N, 79o 30'22.81" E), Pudhukottai (10o23'2.76" N, 78o51'32.67"E) and Karur (11o02'32.00"N, 78o01'11.31"E) during the year 2013. During the year 2014 three trials have been established. During October, 2014 two trials at a wet zone Hosanagara (13o 55' 12.21" N; 75 04' 11 90" E) and Hosakoppa (14o 10 '06 49" N; 74 58' 40 31" E) at Shimoga district, Karnataka and one trial at Kothur, Nellore (14 o 23' 57 91" N; 80 o 02 04 92"E), Andhra Pradesh were established with the field of inputs of Messrs. MPM Paper Mills Ltd., and Andhra Pradesh Forest Department respectively.

High throughput multi environmental Phenotyping of Mapping of Eucalypts for Adventitious rooting and wood Property traits

Precision phenotyping for adventitious rooting traits including percent rooting, root length, number of roots was conducted in bi-parental population of Eucalyptus (*E. tereticornis* X *E. camaldulensis*) for four setting across 209 hybrid progenies and their parents.

Digital imaging platform was used to document derived root parameters like volume and surface area in hybrid progenies and parent. A combined Near infrared spectroscopy (NIRS) model for non destructive estimation of holocellulose and klason lignin content in Eucalypts was developed.

Multi-environment phenotyping for wood property traits in the mapping population derived from *E. tereticornis* X *E. grandis* will be conducted in phase II of the project. High throughput multi environmental Phenotyping of Mapping of Eucalypts for Adventitious rooting and wood Property traits

Development of Genetic Linkage Maps and QTL analysis in *Eucalyptus* for Adventitious Rooting and Wood Property traits

Totally 320 (269 Genomic SSRs and 51 EST-SSRs) SSR loci were screened for polymorphism between parents in the following two crosses *E.tereticornis* (Et86) X *E.grandis* (Eg9) and *E.tereticornis* (Et217) X *E. camaldulensis* (Ec17). In the Et86 x Eg9 cross, 144 polymorphic loci have been selected and only 130 were genotyped in 98 F1 hybrid individuals. All the hybrids were confirmed for hybrid purity. Only 19% loci were segregated as expected Mendelian ratio.

Linkage map was generated for each parent separately and consensus map was developed with an LOD of above 3.0. Total map length of female parent (Et 86) was 961.8 cM and male parent (Eg9) was 1230.1 cM. The consensus map had 130 SSR loci with the map length of 1563.8 cM having average marker distance of 11.8 cM. Similarly, in Et217 x Ec17 cross, 100 SSR loci were selected having clear polymorphism between parents and all 100 loci were genotyped with 100 F1 individuals. Eighty five percent of the markers segregated as per Mendelian segregation ratio. Parent specific linkage maps were generated, wherein the length of female map was 1573 cM and male map was 2179.6 cM. Consensus map showed a map length of 2312.2 cM. QTL identification for rooting percentage alone was taken up under this objective.

Three types of QTL association analysis showed that the chromosome 2 has a region for QTL associated with rooting percent. The region between markers Embra65 and Embra43 was associated with the trait of interest. Phenotypic variation explained by the markers was about 51% in Interval mapping (IM) and inclusive composite interval mapping (ICIM) analysis and single marker (SM) analysis showed about 16% variation. In single marker analysis carried out using ICIM software the Embra43 loci showed an association with rooting percent at LOD of 3.07. However, these results need additional confirmation of rooting data and inclusion of more number markers in the linkage groups.

Candidate Gene Association for Identification of Pulping Trait Markers in *Eucalyptus tereticornis*

The holocellulose and klason lignin content was determined in wood core samples using NIR spectroscopy in 40 *E. tereticornis* individuals belonging to 15 provenances. Two full length genes (EtCesA3 and EtHB1) were isolated and sequenced. SNP discovery was conducted in a subset of population with extreme phenotypes. No marker-trait correlation could be detected.

Hence, to increase the probability of identifying markers, the strategy of multi-gene association in bi-parental mapping population was developed.

Ninety four genes involved in secondary development were selected for target capture and deep sequencing across three parents used for developing mapping populations. Variants (SNVs and InDels) were documented demonstrating the feasibility of the technique in

identifying high throughput markers In-solution target capture and deep sequencing was conducted in 766 genes presumably involved in xylogenesis in *E. tereticornis* and *E. grandis* and 30 hybrid progenies. A total of 32,204 polymorphic SNPs and 2,348 polymorphic InDels were recorded.