PROJECT PROFILE

Title of the Project: **Genome evaluation and characterization in Casuarinas and *Eucalyptus* for improving productivity and conservation**

Principle Investigators: Dr. K. Gurumurthi (March 2003 to August 2004)
Dr. Modhumita Ghosh (September 2004 to June 2007)

Co Investigators: Dr. R. Yasodha
Dr. Mohan Varghese

Duration: 2003 - 2007

Objectives:

1. Quantify the level and distribution of genetic variability in sub-specific taxa and between species of Casuarina and Eucalyptus.
2. Develop specific DNA markers for species, provenances and clones.
3. Identify markers related to desirable traits.

Funding Agency: Department of Biotechnology, Govt. of India

Summary

- The genetic diversity between and within six eucalypt species including *E. tereticornis, E. camaldulensis, E. grandis, E. urophylla, E. pellita* and *E. citriodora* were estimated using ISSR marker system. Results indicated that 53.32% of the variation was attributable to variation among species within groups, while within species diversity was 31.03%.
- The genetic diversity was also assessed within and between three *Casuarina* species (*C. equisetifolia, C. glauca,* and *C. junghuhniana*) and two *Allocasuarina* species (*A. littoralis* and *A. heugeliana*). The analysis grouped *C. equisetifolia*, *C. glauca* and *A. littoralis* while *C. junghuhniana* and *A. huegeliana* formed separate cluster.
- Eighteen morphometric parameters were analyzed to estimate the diversity existing between three *Casuarina* and two *Allocasuarina* species and compared across the ISSR profile generated genetic diversity. Both procedures revealed high genetic distance between *C. equisetifolia* and *C. junghuhniana*.

- Nine species-diagnostic markers were identified for *Casuarina* and *Allocasuarina* species and twenty one diagnostic markers were identified for four species (*E. camaldulensis, E. citriodora, E. grandis, E. tereticornis and E. urophylla*).
- Developed species diagnostic SCAR marker of 500 bp for *C. equisetifolia* (designated as IFGTB27CE-01) and 190 bp for *C. junghuhniana* (designated as IIFGTB23CJ-02) and validated them in thirty individuals of *C. equisetifolia* and twenty individuals of *C. junghuhniana*. The absence of the markers in the other four species were ascertained.
- The genetic diversity within and between sub specific taxa of *Casuarina* and *Eucalyptus* was assessed where in fifteen provenances of *E. tereticornis*, six progenies of seed orchards of *E. camaldulensis* and *E. tereticornis*, four SSOs of *C. equisetifolia* and two SSOs of *C. junghuhniana* were analyzed for their diversity using ISSR marker system.
- Forty superior performing clones of *E. tereticornis* and *E. camaldulensis* were profiled using ISSR - PCR and clonal discrimination using a single or combination of markers was achieved.
- Three putative markers were identified using RAPD and four markers using SSRs for non-rooting clones of *E. tereticornis*. Two RAPD specific bands were observed in clones with 100% rooting. The DNA data was correlated with parameters like rooting %, number of roots, endogenous free and bound root IAA.
- The SSR marker (EMBRA 13) generated three specific alleles in non rooting clones. This was further validated in the provenance Orobay of *E. tereticornis* which showed maximum variation in rooting percentage. The families showing non adventitious rooting trait was showed similar allelic pattern.
- In an attempt to obtain leads for developing early selection markers for pulping trait in *E. tereticornis*, an unstructured population in the provenance trail of the species was identified showing a wide variation in the lignin and cellulose content. Representative individuals from the population were genotyped using gene specific primers for cellulose synthase (*CesA*), CCR, CAD and CCoMT. One of the primer pair targeting *CesA* (*CesA 2)* amplified two alleles at 176 bp and 174 bp in all individuals having high cellulose content in Orobay and other provenances. The marker data was correlated with lignin, pentosan and holocellulose content of the wood. A significant correlation was observed with all the three parameters. The

alleles positively correlated with holocellulose (0.950) and negatively correlated with penstosan (-0.845) and lignin (-0.925) with significance level up to 0.01. Similarly, the primer pair targeting CCR gene amplified a 265 bp allele in all individuals with high cellulose content and showed positive correlation with holocellulose (0.564) and negative correlation with lignin (-0.575) and pentosans (-0.520). The data was validated at the family level in provenance Orobay.