PROJECT PROFILE

Title:	**Genetic transformation of Eucalyptus and Casuarina to enhance salinity tolerance**
Principal Investigator:	Dr. N.V. Mathish, Scientist E
Co Investigators:	Dr. Santan Barthwal, Scientist D
	Dr. Modhumita Dasgupta, Scientist E
Associates:	Mr. Kannan C. Warrier, Scientist D
	Dr. A. Shanthi, Scientist B
	Smt. Lalitha, R.A. I
	Mr. A. Balasubramanian, R.A. I
	Smt. N. Sudha, TA I
Start and Completion dates:	7 Years (2002 to 2009)
Objectives:	
	1. Evolving a standard protocol for routine transformation of *Eucalyptus tereticornis* and *Casuarina equisetifolia.*
	2. Transforming *Eucalyptus tereticornis* and *Casuarina equisetifolia* with candidate genes conferring salt tolerance.
	3. Confirming the gene transfer using GUS assays and PCR
Funding Agency:	Indian Council of Forestry Research and Education (ICFRE)
Total Budget:	Rs. 17.13 Lakhs

Summary

Eucalyptus and *Casuarina* are among the most widely planted tree species in India. Development of genetic transformation methods would therefore, contribute to understanding and engineering for enhanced salt tolerance in addition to opening up avenues for improving wood properties and pest tolerance. The project therefore, envisaged evolving a standard protocol for routine transformation of *Eucalyptus tereticornis* and *Casuarina equisetifolia*, transforming them with candidate genes conferring salt tolerance, confirming the gene transfer using GUS assays and PCR. Genetic transformation studies mainly involve *Agrobacterium* mediated and direct gene transfer techniques,

both of which rely on development of appropriate tissue culture regeneration system. For transformation of elite clones, leaves of micropropagated Eucalyptus clones were found to be suitable explants for regeneration. However, cotyledonary leaves of germinated seedlings showed higher regeneration efficiency. In *C. equisetifolia*, the most suitable tissue for regeneration was determined to be epicotyls and further refinement of regeneration methods is necessary for making it amenable for transformation. Alternative methods not using the tissue culture route need to be attempted. *A. rhizogenes* mediated transformation experiments in *Eucalyptus* and *Casuarina* were also initiated. Generation of composite plants derived from *A. rhizogenes* mediated transformation provides for a rapid method for genetic analyses in transformation recalcitrant species. Important parameters determining transformation efficiency, like the optimum distance for particle bombardment using the pCAMBIA1305.2 vector, were assessed by GUS assays. The *AtNHX* gene known to confer salt tolerance was used for *A. tumefaciens* mediated transformation studies. Manual injury and sonication treatments showed a greater percent of regenerants when compared to carborundum treatment. Silver nitrate improved regeneration from explants co-cultivated with *Agrobacterium.* PCR confirmation of putative *AtNHX* transformed plantlets regenerating in kanamycin media were carried out. The leads are being continued in a follow-up ICFRE project in which *AtNHX* transgenics are being characterized, and composite plant strategy is being used for functional analysis of the *EcHKT1* gene. The project led to establishing collaboration with IRD, France, through a one-year DBT associateship, for *A. rhizogenes* - RNAi based functional analysis of the *Casuarina glauca CCaMK* gene. The project has thus also led to development of expertise in transgenics, international collaboration, and a specialized genetic transformation lab for taking up advanced studies that would help breeding for better trees by understanding gene functions, using them for marker assisted selection, and by development of transgenic trees with improved productivity.