PROJECT PROFILE

Title of the Sub-project: **Fingerprinting of economically Important Clones of Eucalypts and Casuarina.**

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Duration of Project: 2000 -2005

Objective:

1. Develop technologies for fingerprinting of economically important clones of eucalypts and casuarina.
2. To study the genetic uniformity of tissue culture raised plants
3. Determine genetic variability within and between provenances of eucalypts and casuarinas
4. Development of SSRs in *C.equisetifolia*

Funding agency: Department of Biotechnology, Govt. of India

Summary:

Clonal fingerprinting: Twenty-two economically important clones of E. tereticornis were selected for DNA fingerprinting using RAPD technique. Thirteen RAPD primers amplified a total of 101 loci of which 15 were monomorphic for all the 22 clones. The minimum and maximum number of bands obtained were 3 (OPB 06) and 16 (OPB 04) respectively. Unique bands were obtained for five clones and the remaining 17 clones were identified by the combination or absence of specific bands. In the case of *C. equisetifolia*, the utility of multi locus PCR-based arbitrary DNA marker techniques like RAPD, ISSR-PCR, FISSR-PCR and AFLP

were examined for genetic characterization of the clones. All the four marker systems successfully discriminated the twelve clonal accessions of casuarina used in the study but differed in determining levels of polymorphism. Among the different genetic measures, Rp influenced the differences observed in efficiency of the marker systems. AFLP obtained the highest Rp value (7.71) as expected, followed by FISSR (3.31) and ISSR (2.72), while RAPD presented the lowest (2.68). Nevertheless, RAPD and ISSR markers are technically simpler, faster and cheaper than AFLP or FISSR and they are the best techniques for large-scale DNA fingerprinting, genetic diversity studies and germplasm evaluation.

Genetic diversity of Provenances: Genetic variability of 15 *E. camaldulensis* provenances forming the part of long term breeding program was estimated using AFLP and RAPD markers respectively. Variability with the range of 0.63 to 0.78 was observed among the provenances of *E. camaldulensis*. Molecular markers grouped the provenances corresponding to proximity of their location in Australia.

Extensive variability was detected within species of *C. equisetifolia* and the variability was partitioned between and within population components. The total genetic diversity existing within the species of *C. equisetifolia* was estimated as 0.4280 by Shannon's index considering the overall frequency of all RAPD loci. On an average most of the variation occurred within *C. equisetifolia* provenances (60.32%), however the RAPD primers differed in their capacity to detect variability between and within provenances. This distribution pattern of genetic variability provides important baseline data for tree breeding activities and conservation of the provenance trial as gene resource population for continuous accession of desirable alleles.

Genetic fidelity of micropropagated plants: Genetic fidelity of in vitro propagated Eucalyptus plants were tested using RAPD and AFLP techniques. Tissue culture raised plants from two groups, group 1 derived directly from SMD7 (a candidate plus tree), and group 2 derived from coppice shoots of trees of group 1, were assessed for their genetic uniformity. The small intra-group genetic variations of 0.02 in the second group were attributed to somaclonal variations induced during long culture periods. However, the genetic distances of 0.20 and 0.31 between SMD7 and the two micropropagated groups were too high to be attributed to somaclonal

variations as axillary bud culture was used for micropropagation. To test the possibility of inadvertent mixing, RAPD profiles of the micropropagated groups were compared with that of other clones in the tissue-cultured Eucalyptus germplasm. The RAPD profiles of group 2 plantlets matched with that of another unrelated clone in the germplasm. The authenticity of this donor was further re-established using AFLP markers.

SSR identification in casuarinas: Cross amplification of *Eucalyptus* SSRs in two species of Casuarina produced locus-specific products with many nonspecific products however no allelic variation was observed among the individuals. Therefore a microsatellite-enriched library was constructed with the PCR products of successful SSR amplifications in *C.equisetifolia*. Selected colonies were sequenced and microsatellites were identified in 13 sequences. Thirteen sequences were found to have tandem repeats with di and tri nucleotides of which dinucleotide repeat (GA)n ((GA)n/(CT)n/(AG)n/(TC)n) was more common. Primers designed and synthesized for microsatellite repeats targeting $(GCT)_5$, $(GCT)_4$, $(CTCCC)_2$ and $(CCACT)_2$ were used to amplify five clones of *C. equisetifolia*. A single monomorphic band at approximately 180 bp was observed in all the five clones of *C. equisetifolia*.