EVALUATION OF TEAK CSO AT WALAYAR USING DNA PROFILING

1. Principal Investigator:	Dr. D. Thangamani, Scientist-C
2. Duration of the Project:	2 years (2007 – 2009) (Reduced the period to one and half year)
3. Total Budget:	Rs.2.5 Lakhs

4. Objectives

- To assess the genetic make-up of stock and scion of bud-grafted standing trees in the teak clonal seed orchard at Walayar, Kerala using RAPD markers
- To assess the genetic diversity of the teak clonal seed orchard at Walayar

5. Overall progress since the implementation of the project

- The project was aimed to check the identity of bud grafted teak CSO; it was suspected that the standing trees might have come up from the stock part, but not through the scions.
- The CSO was developed in Walayar, Kerala with 2.6 ha planted with 722 ramets of 20 clones through bud grafting during the year of 1974 to 1978. These 20 clones originated from Karulai (KLK), Nilambur (KLN), Sungam (KLS) and Thunakadavu (TNT) regions in Western Ghats, and South Bhadrachalam (SBL) in Andra Pradesh.
- The list of clones studied were

Clone No.	**Number of ramets**
1. TNT-10	**26**
2. TNT- 6	**26**
3. TNT-3	**16**
4. KLS -1	**17**
5. KLS – 4	**16**
6. TNT – 1	**12**
7. TNT – 11	**20**
8. TNT – 20	**20**
9. KLN – 2	**20**
10. KLK – 1	**25**
11. KLK – 2	**20**
12. TNT – 15	**18**
13. KLS – 3	**16**
14. TNT – 5	**23**

15. TNT – 16	**9**
16. KLS – 2	**28**
17. KLN – 4	**23**
18. KLN-1	**24**
19. SBL – 1	**32**
20. TNT – 4	**23**

- Young tender leaves from scion portion of 454 ramets of teak were used for the DNA profiling. The leaves were stored at -20^{O}C .
- CTAB based modified Doyle and Doyle (1990) maxi preparation protocol was followed for DNA extraction.
- DNA quality and quantity were determined by running the DNA samples on a 0.8% agarose gel using bromophenol blue as the tracking dye. The intensities of bands were compared with the Lambda DNA marker.
- RAPD amplification procedure was followed according to the method of Williams *et al*. (1990) with some minor modifications. The cycles were as follows: 94°C for 5 min; 36 cycles of 92°C for 1 S, 37°C for 1 S, 72°C for 1 min; a final extension step of 72°C for 5 min and a final incubation at 4°C. The amplifications were carried out on a Thermal Cycler Controller (JH Bio, Corbett Life Sciences). Reproducible polymorphic bands were used for statistical analysis. The amplification products were separated by electrophoresis with a 1.5% agarose gel in 1× TBE (Tris-EDTAborate) buffer and stained with ethidium bromide.
- Profiling was done with 7 RAPD primers (decamers) for 20 clones.
- It was observed that the 20 clones are genetically distinct based on SM and DICE co-efficient. In SM co-efficient based UPGMA dendrogram, Tamil Nadu and Kerala clones clustered distinctly. In DICE co-efficient based UPGMA dendrogram clustering analysis showed six major clusters. The clone KLS-3 was not grouped into any one of these clusters indicating high divergence with respect to the other clones. Also SBL varied from others with the difference of 31.4%. The clone KLS-3 was not grouped into any one of these clusters indicating high divergence with respect to the other clones. TNT clones resembled each other by 80% whereas, KLN-4 and TNT-11 were related by 88 %.