PROJECT PROFILE

Title:	Assessment of population structure using SSRs and molecular characterization using RAPD in casuarina species
Principal Investigator:	Dr. D. Thangamani, Scientist-E
Project Associate :	Dr. A. Shanthi, Scientist-B
Starts and Completion dates :	From 1.4.2007 to 31.3.2010
Objectives 1.	1. Assessment of population using SSRs markers 2. Generation of DNA profile of superior clones of Casuarina species to help in the clonal identity.
Funding Agency	ICFRE
Total budget outlay:	Rs. 7.85 lakhs

SUMMARY

Sixty Eight Casuarina equisetifolia clones were profiled using ten RAPD primers.. Genomic DNA from Casuarina equisetifolia was extracted using DNA isolation Kit (Quiagen). The DNA extracts were then subjected to PCR amplification with primers purchased from Operon Technologies, USA. Ten different primers were tested on Casuarina samples. The amplified products were resolved through Agarose gel (1.8%) electrophoresis as described earlier. The gel was photographed and documented using Alpha Imager (Gel document system). Later it was scored for the presence or absence of band with 1 and 0 binary codes respectively. A statistical analysis was carried out with the data generated using RAPDistance v1.04 and NTSYSpc v2.02. The dendrogram was derived based on the genetic relationships of the sixty eight clones which were clustered.

For the development of SSR markers, thirty five ISSR primers (both anchored and non anchored) were optimized in Casuarina equisetifolia. ISSR-PCR optimization showed that the annealing temperatures were 500 c, 550C, 580C optimum among the 35 ISSR screened primers Ten ISSR primers were short listed with the range of fragments sizes 100bp-1200bp. The ISSR-PCR fragments were cloned with pTZ57R/T vector. Then it was transformed into DH5a competent cells supplied by chromous biotech Ltd. The cloning experiment was carried out using InsTAclone PCR Cloning kit (Fermentas) .The results obtained from the cloning experiment showed positive clones which were identified in blue/white screening. These cloned colonies of

twenty plates were submitted for sequencing by the outsource service Chromous biotech Ltd, Bangalore.

SSR-PCR parameters and conditions were optimized for the designed primers in Casuarina genome. Initially three populations with five individuals in each population were tested with the four SSR primers. The amplified products were resolved in 6% polyacrylamide gel using 1X TBE buffer, run at 3000V between 2- 2 1/2 h in 410C depending on the size of the expected PCR product and silver stained for visualization. All the four primers were successfully amplified in the Casuarina genome. Nine new genomic sequences were deposited in NCBI Library.