Available online at www.elixirpublishers.com (Elixir International Journal)

Bio Technology

Elixir Bio Tech. 38 (2011) 4143-4149

ISSN: 2229-712X

Genetic pairwise distance matrix analysis in the clones of *Casuarina equisetifolia* L. using RAPD Markers

M. Ramakrishnan[1], A. Shanthi[1], S. A. Ceasar[2], Melvin A. Daniel[2] and S. Ignacimuthu[2]

[1]Division of Plant Biotechnology, Institute of Forest Genetics and Tree Breeding, Coimbatore, India-641 002.
[2]Division of Plant Biotechnology, Entomology Research Institute, Loyola College, Chennai, India-600 034.

ARTICLE INFO

Article history:
Received: 26 June 2011;
Received in revised form:
22 August 2011;
Accepted: 27 August 2011;

Keywords
RAPD,
Casuarina equisetifolia,
Distance matrix,
NTSYSpc.

ABSTRACT

Development of molecular markers for various desirable traits related to disease resistance, drought tolerance, compact canopy, higher yield, etc hold great potential for crop improvement of *Casuarina equisetifolia* L. This work focuses on the analysis of genetic relationship between 24 first generation colones of *C. equisetifolia*. Total genomic DNA was isolated by CTAB method and amplified with random oligonucleotide primers (Ten arbitrary 10-mer primers from Oberon Technologies Primer, USA) using PCR. The polymorphic bands were detected on agarose gels. Each random primer used in this study produced distinct bands. These were used for the final analysis. Six primers (OPM-05, OPM-13, OPB-18, OPE-06, OPE-07 and OPE-08) yielded 39 scorable bands. These bands were then constructed using the RAPD distance v1.04 package. Following this, SHAN method in NTSYSpc v2.02 was used to generate a pair wise distance matrix between various clones of *C. equisetifolia*. The highest genetic similarity co-efficient value (0.91) was observed between clones APSKLM-25 and APVSP-14 while the lowest co-efficient value (0.04) was seen between clones APKKD-11 and APKKD-9. The RAPD study was helpful to establish clonal identify.

Introduction

The members of the Family Casuarinaceae are commonly known as casuarinas. They are monoecious or dioecious trees or shrubs having unique needle like branchlets with many articles. Currently casuarinas are grouped under four genera (Wilson and Johnson 1989), which encompass over 90 species (Moncur et al. 1997). Among the four genera, the species of *Casuarina* and *Allocasuarina* are commercially cultivated in many tropical and sub-tropical regions of the world while the other two genera *Gymnostoma* and *Ceuthostoma* occur as wild species (Chonglu Zhong et al. 2010; Sergio Svistoonoff et al. 2010).

Casuarina (2n = 18) comprises about 17 species distributed throughout Southeast Asia and Australia (Pinyopusarerk and House 1993), whereas *Allocasuarina* consisting of 59 species are endemic to Australia (Wilson and Johnson 1989). They are considered to be the nearest relatives of the genus *Casuarina* since they share many common morphological features. The basic chromosome number of *Allocasuarina* varies from n = 10 to n = 14. Polyploidy, particularly tetraploidy is known to be prevalent in some species like *A. luehmanii* (2n = 56) and *A. littoralis* (2n = 44). Phylogenetic and taxonomic relationships within the species of casuarinas have been studied using the size and number of chromosomes, pattern of geographical distribution (Barlow 1959; Barlow 1983) and diversification in the morphological characteristics (Wilson and Johnson 1989) to distinguish the members of *Allocasuarina* and *Casuarina*. Recently, rbcL and plastid matK sequences were used for the genetic analysis of Casuarinaceae (Sogo et al. 2001) and the study was further extended to decipher the phylogeny of 76 species demonstrating the monophyly of the four genera and examining the relationships within the family (Steane et al. 2003).

Although about 15 species of *Casuarina* and *Allocasuarina* are recognized for multiple utilization, only *C. equisetifolia* is cultivated widely in many countries (Doran and Hall 1983; Pinyopusarerk and House 1993; Krishnamoorthy 1989) accounting for about 1.4% of tree plantations of the casuarinas (FAO 1995). This commercial and silvicultural importance of *C. equisetifolia* has led to the establishment of multinational provenance trials co-ordinated by Doran and hall (1983); Pinyopusarerk et al. (1996). The species is propagated by cladode cuttings (Gurumurthi and Bhandari 1988) and yield gain is achieved through clonal plantations using genetically divergent and productive clones (Ahuja and Libby 1993). In India, major effort is directed towards production of improved planting stocks through selection of superior performers from plantations/provenances and their vegetative propagation. Additionally, seed orchard raised with selected clones can provide good quality seeds for plantations if vegetative propagules fall short of demand. The optimal utilization of diversity requires genetic characterization of the stocks and identification of the selected clones in the early stage. Morphological characters have been used to estimate genetic divergence of clonal selections of *C. equisetifolia* (Kumar and Gurumurthi 2000). Genetic variation at the population level has been studied using RAPD markers in *C. equisetifolia* (Ho et al. 2002). RAPD variation in casuarinas of Taiwan has revealed that most plants in Taiwan were closely related but not typical *C. equisetifolia* indicating introgressive hybridisation among *C. equisetifolia*, *C. glauca* and *C. cunninghamiana* (Ho et al. 2002a). The evaluation of genetic diversity would promote the

Tele: 91-44-28178348, 91-44-28175566
E-mail addresses: entolc@hotmail.com

efficient use of genetic variations in the breeding program (Paterson et al. 1991) and inbreeding depression has occurred in *Lentinula edodes* (Hasebe 1991), so the evaluation of genetic diversity between breeding materials takes on additional importance.

The scope of the present study was to generate a reliable DNA based marker system for the characterization of Casuarinaceae in general and *C. equisetifolia* in particular. Among many different types of DNA markers, the markers based on Random amplified polymorphic DNA (RAPD) provide a co-dominant, highly reproducible and genetically informative marker system (Van et al. 1995; Fracaro et al. 2005; Venkatachalam et al. 2004; Pedro and José 2000; Campbell 1999). However information on RAPD loci is not available in Casuarinaceae clones. The Random amplified polymorphic DNA-PCR (RAPD-PCR) takes advantage of the ubiquitously distributed RAPD in the eukaryotic genomes. So the present work was taken up with the objective of establishing an efficient and reproducible marker system based on RAPD-PCR for the identification of clonal selections and plant breeding of *C. equisetifolia* for forest development.

Materials and methods

Plant martial

Twenty four parent clones (cuttings) of Casuarina equisetifolia were collected from different plantations in the districts of Andhra Pradesh, India (Table 1). The clonal cuttings were planted in the silviculture nursery, Institute of Forest Genetics and Tree breeding (IFGTB), Coimbatore, India. Again the collected clonal cuttings were grown in the nursery to get the F1 Generation. These were used as samples for DNA fingerprinting studies.

Reagents and chemicals

The following solutions and chemicals were used: CTAB 3% (w/v), 1M Tris-Cl (pH 8), 0.5 M EDTA (pH 8), 5 M NaCl, absolute ethanol (AR grade), chloroform-IAA (24:1 [v/v]), polyvinylpyrrolidone (PVP) (Sigma) and ß-mercaptoethanol. All the chemicals used in the experiments were of analytical grade. The extraction buffer consisted of CTAB 3% (w/v), 100 mM Tris-Cl (pH 8), 25 mM EDTA (pH 8), and 2 M NaCl. The PVP and ß-mercaptoethanol were prepared fresh.

DNA extraction and purification

DNA was isolated from growing tips of juvenile needles of each clone using a modified Cetyl Trimethyl Ammonium Bromide (CTAB) method (Yasodha et al. 2005). The plant materials were ground to powder in sterilized pestle and mortar in liquid nitrogen. The fine powder was transferred to microcentrifuge tubes containing freshly prepared 600 µl of extraction buffer (100 mM Tris buffer pH 8, 25 mM EDTA, 2 M NaCl, 3% CTAB and 3% polyvinyl pyrrolidone). The suspension was mixed gently and incubated at 65ºC for 20 min with occasional mixing. The suspension was then cooled to room temperature and an equal volume of chloroform: isoamyl alcohol (24:1) was added. The mixture was centrifuged at 12000 rpm for 10 min. The clear upper aqueous phase was then transferred to a new tube; a 2/3 volume of ice-cold isopropanol was added and incubated at -20ºC for 30 min. The nucleic acid was collected by centrifuging at 1000 rpm for 10 min. The resulting pellet was washed twice with 80% ethanol and air-dried under a sterile laminar hood, and the nucleic acid was dissolved in TE (10 mM Tris buffer pH 8, 1 mM EDTA) at room temperature. The contaminating RNA was eliminated by treating the sample with RNase A (10 mg/ml) for 30 min at 37ºC. DNA concentration and purity were determined by measuring the absorbance of diluted DNA solution at 260 nm and 280 nm. The quality of the DNA was determined using agarose gel electrophoresis stained with ethidium bromide

RAPD-PCR amplification

The RAPD-PCR was performed according to the method of McClelland et al. (1995). The reactions were carried out in 25 µL volume in a tube using ten random decanucleotide primers separately,OPM-02, OPM-05, OPM-13,OPB-04, OPB-12,OPB-15, OPB-18, OPE-06, OPE-07 and OPE-08 (Arbitrary 10-mer primers from Oberon Technologies Inc., Alameda, California, USA) (Williams et al 1990). Each reaction tube contained 30 ng template DNA, 1.5 mM MgCl2, 300 µM of dNTPs, 2.5 µL of 1x *Taq* DNA polymerase buffer, 25 pM decanucleotide primer and 1.5 units of *Taq* DNA polymerase (Bangalore Genei, India). Amplification was performed in a DNA thermal cycler (Techne Thermal Cycler, England) using the following conditions: 95ºC for 3 min; 36 cycles at 94ºC for 1 min, 35.6ºC for 30 s and 72ºC for 1 min; final extension at 72ºC for 2 min. PCR products were resolved on 1.2% agarose gel in 1xTAE buffer. The DNA was stained with 0.5 mg/mL ethidium bromide, visualized and photographed under a UV transilluminator. A sample without template DNA was included as a negative control in each experiment to check contamination. Electrophoretic profile was visualized under UV radiation and photographed with a UV transilluminator. The sizes of DNA fragments were estimated by comparison with standard ladder (1kb and 100 bp; Bangalore Genei, India)

Designation for RAPD marker

Each RAPD marker was given a two-part name. The first part corresponded to the primer with which the polymorphism was observed (one or two letters followed by a two-digit number which corresponded to an Operon Technologies primer), while the second part corresponded to the approximate size (in bp) of the band.

Data collection and analysis

The amplification products were scored separately for each primer. The presence or absence of band for each clone was assessed. The binary code 1 for the presence and 0 for absence of the band were used. Only intensely stained polymorphic bands were used in the statistical analysis. Pair-wise similarity matrices were generated by Jaccard's coefficient of similarity (Jaccard 1908) using the SIMQUAL format of NTSYS-pc (Rohlf 2002). A dendrogram was constructed using the unweighted pair group method with arithmetic average (UPGMA) with the SAHN module of NTSYS-pc to show a phenetic representation of genetic relationships as revealed by the similarity coefficient (Sneath and Sokal 1973). The binary data were also subjected to principal component analysis (PCA) using the EIGEN and PROJ modules of NTSYS Pc.

Results and Discussion

Marker polymorphism

C. equisetifolia L species were analyzed using 10 random primers of which 6 produced reproducible polymorphic banding patterns. A total of 39 bands were scored of which 35 (89.6%) were polymorphic and 4 were monomorphic bands (10.24%). The number of bands generated per primer varied from 6 to 9 and a minimum of 6 bands was generated by the primers OPM-13, OPM-06 and OPM-07 while the maximum of 9 bands were observed with primers OPM-05 and OPE-08. The primer OPB-20 also generated 7 bands. The size of the amplified products varied from 900 to 2,900 bp in different primer (OPM-05(1000

bp-2500 bp), OPM-13(900 bp-2500 bp), OPB-20(1200 bp-2500 bp), OPE-06(1500 bp-2900 bp), OPE-07(1350 bp-2500 bp), and OPE-08 (1250 bp-2500 bp)). The minimum size (900bp) of the amplified products was from primer OPM-13 and maximum size (2900bp) of the amplified products was from primer OPE-08. Three primers (OPM-05, OPE-06 and OPE-08) generated 100% polymorphic bands and primers OPM-13 (83.3%), OPE-20 (71.4%) and OPE-06 (83.3%) also generated higher polymorphic bands. Only primer OPE-20 alone generated less polymorphic bands of 71.4% (Table 2).RAPD profiles of a representative primer OPM-05 are shown in Fig 1.

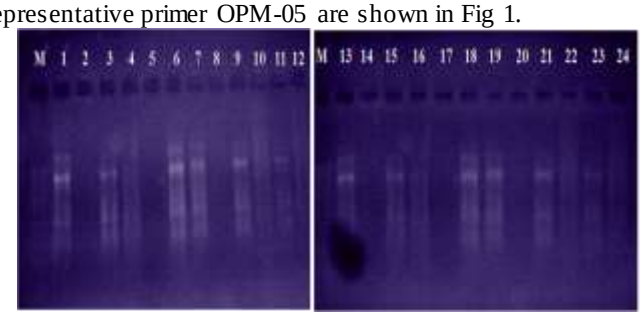

Fig. 1 RAPD profiles of the primer OPM-05. M-DNA size Marker (Samples 1–24 as in Table 1)

The high level of polymorphism detected by the RAPD markers in this study is comparable with the other perennial species (Chalmers et al. 1992; Gillies et al. 1999; Izumi et al. 1997; Ganesh et al.2008). Similar levels of polymorphic bands were generated in these studies by same primers.

Clones of *C. equisetifolia* specific diagnostic markers

The primers such as OPM-05 (APPKD-1, APVSP-14 and APVSP-16), OPM-13 (APVJM-33), OPB-20 (APPKD-5, APPKD-10, APVSP-15, APVSP-23 and APVJM-35), OPE-06 (APSKLM-25 and APVJM-39), OPE-07 (APPKD-4, APPKD-6, APPKD-12, APVSP-22, APSKLM-27 and APVJM-31) and OPE-08 (APKKD-3, APPKD-7, APPKD-9, APPKD-11, APVSP-18, APSKLM-26 and APVJM-32) detected clones-specific diagnostic markers suitable for discriminating 24 clones of *C. equisetifolia*. These clones-specific RAPD markers could potentially be used for identifying clones of *C. equisetifolia* from any mixed population comprising other clones or species of *Casuarina* complex. Similar approach has been successfully used for molecular diagnosis of species and cultivars by many workers (Sosinski and Doucher 1996; Yamamoto and Duich 1994; Trujillo Y Cabrera and María Valdés 2010).

Genetic diversity

The DNA banding patterns *of C. equisetifolia* from the investigated sites provide evidence for both genetic homogeneity and diversity within individual clones, as well as differences between geographically separate clones. Andhra Pradesh clones of *C. equisetifolia* produced genetic similarity co-efficient (pairwise distance matrix) which varied from 0.00 to 0.91 (Table 3). The highest genetic similarity co-efficient (0.9) was observed between clones APSKLM-25 and APVSP-14 of *C. equisetifolia* while the lowest value (0.04) was measured between APKKD-11 and APKKD-9. Similar wide range in similarity values had also been observed in many other perennial species (Nair et al. 1999; Sarmah et al. 2007). UPGMA cluster analysis the clones of *C. equisetifolia* similarity co-efficient generated a dendrogram (Fig. 2) which illustrated the overall genetic relationship among the genotypes surveyed. Cluster analysis indicated 10 major distinct clusters and 12 subclusters comprising all the accessions. Among twenty four Andhra Pradesh clones of *C. equisetifolia*, clones APKKD-11 and APKKD-9 are genetically very similar while clones APVSP-15 and APVJM-35 are highly divergent among the rest of the clones; APVJM-35 has a wider geographic distribution in Andhra Pradesh as compared to the other clones of *C. equisetifolia* assessed, apart from its morphological distinctiveness. This clone has a smaller plant spread and dichotomously branched narrow leaves with serrated margin which makes it morphologically distinct from the other clones of *C. equisetifolia*. Similar wide range of similarity values has also been observed on studies of seed weight and seedling growth within *C. equisetifolia* species (Yang et al. 1995). The genetic variations of 70 individual samples of *C. equisetifolia* growing along the northern coast of Senegal were studied using 160 primers; they generated 1396 reproducible bands and 61 polymorphic bands. This result showed a narrow genetic variation among (4.36%) and within (5.90%) *C. equisetifolia* subsp *equisetifolia* and *C. equisetifolia* subsp *incana* plantation sites (Ndoye et al. 2011). 456 individual samples of *C. equisetifolia* were evaluated to assess the genetic diversity and to identify hybridization in *Casuarina* grown in Taiwan. 11 primers were used and 81 polymorphic bands were scored. The average Nei's gene diversity of *Casuarina* grown in Taiwan (0.198) was significantly higher ($p<0.001$) than those of native provenances of *C. equisetifolia* (Ho et al. 2002). RAPD markers were also used for the identification of genetic diversity of 142 individual samples belonging to 12 native accessions of *C. equisetifolia* grown in an international Provenance trial garden in Taiwan (Ho et al. 2002a). RAPD markers were used in *C. equisetifolia* to characterize closely related genotypes (Yasodha et al. 1999).

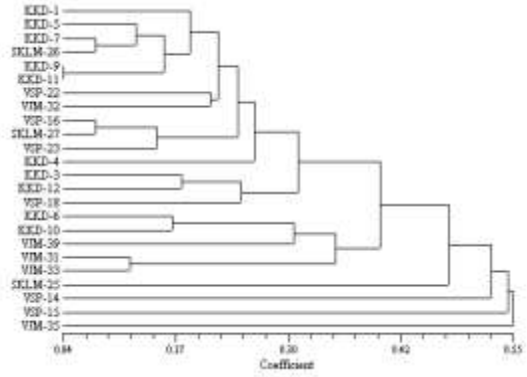

Fig. 2 Dendrogram showing the clustering between different clones of Casuarina equisetifolia L

RAPD-PCR has been used widely in plants for the analysis of genetic relationships between and within species (Bassam 1992; Beekman et al. 1990; klein-Lankhorst 1991; Kuen et al. 2002; Kuen et al 2004; Nicodemus et al. 2006; Rogstad and Willams 1988; Reiter et al. 1992; Sanchez et al. 1998; Varshney et al. 2000; Weising et al. 1995) and assessment of hybridisation in natural populations and of analysis germplasm (Williams et al. 1990). Further RAPD-PCR is useful in fingerprinting and characterisation of accessions and identification of cultivars and varieties.

The technical simplicity of the RAPD technique has facilitated its use in the analysis of genetic relationship in several genera (Wilikie et al. 1993; Demeke 1992; Nair et al. 1999). The major concerns regarding RAPD-generated phylogenies include homology of bands showing the same rate of migration and variation in fragment mobility (Stammers et al. 1995). In spite of this limitation, RAPD markers have the greatest advantage to scan across all regions of the genome;

hence they are highly suited for phylogeny studies at species level (Wilikie et al. 1993; Demeke 1992). Recent developments in DNA marker technology together with the concept of marker assisted selection provide new solutions for selecting and maintaining desirable genotypes.

Once molecular markers closely linked to desirable traits are identical, than a marker assisted selection can be performed in early segregating populations and at early stages of plant development. Marker assisted selection or identification can be used to pyramid the major genes including resistance genes, with the ultimate goal of producing varieties with more desirable characters.

In the present investigation, RAPD markers have been used to clones of *C. equisetifolia* accessions at intra- and inter-specific levels. This study will be helpful for the selection and improvement of *C. equisetifolia* in future.

Acknowledgements

The authors gratefully acknowledge Department of Biotechnology, ICFRE, Govt. of India for Financial support. We also thank Director, Student co-ordinate and Head of the Plant Biotechnology Department of IFGTB-Coimbatore for providing infrastructural facilities for this work.

References

1. Ahuja MR, WJ Libby. Clonal Forestry I; Genetics and Biotechnology. Berlin Heidelberg. New York; Springer; 1993.
2. Barlow BA. Chromosome numbers in Casuarinaceae. Aust J Bot. 1959; 7: 230–237.
3. Barlow BA. The casuarinas – a taxonomic and biogeographic review, In; SJ Midgley, J Turnbull, RD Johnston (eds) Casuarina Ecology, Management and Utilization. CSIRO, Australia. 1983: 10–18.
4. Bassam BJ, Caetano-Anolles G, Gresshoff PM. DNA amplification fingerprinting of bacteria. Applied Microbiology and Biotechnology; 1992; 38: 70–76.
5. Beekman LS, Soller MEN, Simon PW. Effects of plant tissue and DNA purification method on RAPD-based genetic fingerprinting analysis in carrot. Amer J Soc Hort Sci; 1990; 124: 32-38.
6. Campbell CS, Alice LA, Wright WA. Comparisons of within population; 1999.
7. Chalmers KJ, Waugh R, Sprent JI, Simons AJ, Powell W. (1992) Detection of genetic variation between and within populations of Gliricidia sepium and G. maculata using RAPD markers. Heredity; 1992; 69: 465–472.
8. Chonglu Zhong, et al. Casuarina research and applications in China. Symbiosis; 2010; 50:107–114.
9. Doran J, N Hall. (1983) Notes on Fifteen Australian Casuarina species. In; SJ Midgley, J Turnbull, RD Johnston (eds) Casuarina Ecology, Management and Utilization. CSIRO, Australia. 1983: 19–52
10. FAO. Forest Resource Assessment-1990. Tropical forest plantation resources. FAO Forestry paper. 1995: 128 Rome.
11. Fracaro F, Zacaria J, Echeverrigaray S. RAPD based genetic relationships between populations of three chemotypes of *Cunila galioides* Benth. Biochemical Systematics and Ecology. 2005; 33: 409-417.
12. Ganesh Ram S, Parthiban KT, Senthil Kumar R, Thiruvengadam V, Paramathma M. Genetic diversity among Jatropha species as revealed by RAPD markers. Genet Resour Crop Evol. 2008; 55:803–809.
13. Gillies AC, Navarro C, Lowe AJ, Newton AC, Hernandez M, Wilson J, Cornelius JP. Genetic diversity in meso- American populations of mahogany (*Swietenia macrophylla*) assessed using RAPDs. Heredity. 1999; 83: 722–732.
14. Gurumurthi K, HCS Bhandari. Induction of rooting in cladode cuttings of Casuarina equisetifolia. Curr Sci. 1988; 57: 958–959.
15. Hasebe K. Genetic studies on mutants and agronomic characters in Shiitake, *Lentinula edodes*. Rep Tottori Mycol Inst. 1991; 29:1–69.
16. Ho KY, CH Ou, JC Yang, JY Hsiao. An assessment of DNA polymorphisms and genetic relationships of Casuarina equisetifolia using RAPD markers. Bot Bull Acad Sin. 2002; 43: 93–98.
17. Ho KY, JC Yang, JY Hsiao. (2002a) An assessment of genetic diversity and documentation of hybridization of Casuarina grown in Taiwan using RAPD markers. Int J Plant Sci. 2002 a; 163: 831–836.
18. Izumi ME, Kojima M, Matsuda T, Shimizu A, Murakami T, Tanabe, Murakami K. Random amplified polymorphic DNA observed in Eucalyptus by PCR study with random primers. Nucleic Acids Symp. 1997; 37: 167–168.
19. Klein-Lankhorst AR. Use of random amplified polymorphic DNA markers in the discrimination and verification of genotypes in Eucalyptus. Theor Appl Genet. 1991; 89: 442-450.
20. Krishnamoorthy AVRG. A multipurpose species in farm forestry economics. National Seminar on Casuarinas. Neyveli, India. 1989; 13: 69-73.
21. Kuen-yih Ho, Chern-Hsiung Ou, Jenq-Chuan yang, Ju-Ying Hsiao. An Assessement of Casuarina equisetifolia using RAPD markers. Botanical Bulletin of Academia Sinica. 2002; 43: 93-98.
22. Kuen-Yih Ho, Ju-Ying Hsiao, Shu-lin Deng. Genetic Variation in International Provenances of *Casuarina equisetifolia* (Casuarinaceae) based on RAPD Figerprinting. Taiwan Journal of Forest Science.2004; 18: 47-51.
23. Kumar A, K Gurumurthi. Genetic divergence studies on clonal performance of *Casuarina equisetifolia*. Silvae Genetica. 2000; 49: 57–60.
24. McClelland M, Mathieu-Daude F, Welsh J. RNA fingerprinting and differential display using arbitrarily primed PCR. Trends in Genetics. 1995; 11: 242-246.
25. Moncur MW, DJ Boland, JL Harbard. Aspects of floral biology of *Allocasuarina verticilliata* (Casuarinaceae). Aust J Bot. 1997; 45: 857–869.
26. Nair NV, Nair S, Sreenivasan TV, Mohan M. Analysis of genetic diversity and phylogeny in Saccharum and related genera using RAPD markers. Genet Resour Crop Evol. 1999; 46: 73–79.
27. Ndoye AL, O Sadio, D Diouf. Genetic variation of *Casuarina equisetifolia* subsp *equisetifolia* and *C. equisetifolia* subsp *incana* populations on the northern coast of Senegal. Genet Mol Res. 2011; 10 (1): 36-46.
28. Nicodemus A, Nagarajan B, Narayanan C, Varghese M, Subramanian K. RAPD Variation in Indian Teak Populations and its Implications for Breeding and Conservation. Journal of Indian Forest.2006: 321-330.
29. Paterson Ah, Tanksley SD, Sorrells Me. DNA markers in plant improvement. Adv Agron. 1991; 46: 39-90.
30. Pedro Jordano*, José A Godoy†. RAPD variation and population genetic structure in *Prunus mahaleb* (Rosaceae) an animal-dispersed tree. Molecular Ecology. 2000; 9: 1293–1305.

31. Pinyopusarerk K, APN House. Casuarina: An annotated bibliography of Casuarina equisetifolia, C. junghuhniana and C. oligodon. International Center for Research in Agroforestry, Nairobi, Kenya. 1993.
32. Pinyopusarerk K, ER Williams, V Luangviriyasaeng, B Puriyakorn. Geographic variation in growth and morphological traits of Casuarina equisetifolia. In: K Pinyopusarerk, JW Turnbull, SJ Midgley (eds) Proc. of 3rd International Casuarina Workshop on Recent Casuarina Research and Development, Vietnam. 1996: 143–151.
33. Reiter RS, JG Williams, KA Feldmann, JA Rafalski, SV Tingey, PA Scolnik. RAPD Markers. Plant Mol Biol. 1992; 15: 18-15.
34. Rogstad RS, Willams KA. DNA finger printing in forest tree species. Genetic. 1988; 2: 357-363.
35. Sanchez, JM Grau, JA Manzanera, MA Bueno. RAPD markers for the identification of *Populas* species. Silvae Genetica. 1998; 47: 2-367.
36. Sarmah P, Barua PK, Sarma RN, Sen P, Deka PC. Genetic diversity among rattan genotypes from India based on RAPD marker analysis. Genet Resour Crop Evol. 2007; 54: 593–60.
37. Sergio Svistoonoff, et al. Contribution of transgenic Casuarinaceae to our knowledge of the actinorhizal. 2010.
38. Sogo A, H Setoguchi, J Noguchi, T Jaffre, H Tobe. Molecular phylogeny of Casuarinaceae based on rbcL and matK gene sequences. J Plant Res. 2001; 14: 259–464.
39. Sosinski B, Douches DS. Using polymerase chain reaction based DNA amplification to fingerprint North American potato cultivars. Hort Sci. 1996; 31:130–133.
40. Steane DA, KL Wilson, RS Hill. Using matK sequence data to unravel the phylogeny of Casuarinaceae. Mol Phylogenet Evol. 2003; 28: 47–59.
41. Trujillo Y Cabrera, María Valdés. Different genetic groups of frankia within the root nodules of *Casuarina* growing in Mexico. Symbiosis. 2010; 50: 45–50.
42. Van de Yen, WTG, R Me Nicol. The use of RAPD markers for the identification of Sitka Spruce (*Picea sitchenis*) clones. Heredity. 1995. 75: 126–132.
43. Varshney P, Joshi, Prabhakar, K Ranjekar, Vidya S, Gupta. Molecular markers in plant genome analysis. Plant mol Biol. 2000; 15: 110-115.
44. Venkatachalam P, Priya P, Saraswathyamma CK, Thulseedharan A. Identification, cloning and sequence analysis of a dwarf genome specific RAPD marker in rubber tree(*Hevea brasiliensis*). Plt Cell Rep. 2004; 23:327-332.
45. Weising K, Nybom H, Wolff K, Meyer W. DNA Fingerprinting in Plants and Fungi (ed. Arbor A). CRC Press, Boca Raton. 1995: 1–3.
46. Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Research. 1990; 18: 6531-6535.
47. Wilson KL, LAS Johnson. Casuarinaceae. Flora of Australia. 1989; 3: 100–175.
48. Yamamoto I, Duich JM. Electrophoretic identification of cross-pollinated bentgrass species and cultivars. Crop Sci. 1994; 34: 792–798.
49. Yang JC, Chang TR, Chen TH, Chen ZZ. Provenance trial of Casuarina equisetifolia in Taiwan I. Seed weight and seedling growth. Bulletin of Taiwan Forestry Research Institute. 1995; 10: 195–207.
50. Yasodha R, Sumathi R, Gurumurthi K, Ravi N. Optimisation of RAPD assay in *Casuarina equisetifolia*. Indian J Pl Physiol. 1999; l4: 158-60.
51. Yasodha, Modhumitha Ghosh, Ramasam Sumathi, Krish Gurumurthi. Cross-Species Amplification of Eucalyptus SSR Markers in Casuarinaceae. Journal of Acta Bot Croat. 2005; 64(1): 115-120.

Table 1: List of Andhra Pradesh Clones of *Casuarina equisetifolia* L selected for the RAPD studies

S.No	CLONAL IDENTITY	Origin
1	APPKD-1	Kakinada
2	APKKD-3	Kakinada
3	APPKD-4	Kakinada
4	APPKD-5	Kakinada
5	APPKD-6	Kakinada
6	APPKD-7	Kakinada
7	APPKD-9	Kakinada
8	APPKD-10	Kakinada
9	APPKD-11	Kakinada
10	APPKD-12	Kakinada
11	APVSP-14	Visakhapatnam
12	APVSP-15	Visakhapatnam
13	APVSP-16	Visakhapatnam
14	APVSP-18	Visakhapatnam
15	APVSP-22	Visakhapatnam
16	APVSP-23	Visakhapatnam
17	APSKLM-25	Srikakulam
18	APSKLM-26	Srikakulam
19	APSKLM-27	Srikakulam
20	APVJM-31	Vijayanagaram
21	APVJM-32	Vijayanagaram
22	APVJM-33	Vijayanagaram
23	APVJM-35	Vijayanagaram
24	APVJM-39	Vijayanagaram

Note: All the clones were collected in Andrapradsh- India

Table 2 List of primers, number of amplified products, polymorphism percentage and polymorphism information content

S.No	Primer	No. of bands generated	o. of monomorphic bands	No. of polymorphic bands	% of polymorphic bands (%)	% of monomorphic bands (%)	Fragment (size range in bp)
1	OPM-05	9	0	9	100	0	≈ 2500-1000
2	OPM-13	6	1	5	83.34	16.66	≈ 2500-900
3	OPB-20	7	2	5	71.42	28.57	≈ 2500-1200
4	OPE-06	6	1	5	83.34	16.66	≈ 2900-1500
5	OPE-07	6	0	6	100	0	≈ 2500-1350
6	OPE-08	9	0	9	100	0	≈ 2500-1250

Table 3 Pairwise distance matrix for the selected clones of *Casuarina equisetifolia* L

{	APPKD-1	APKKD-3	APPKD-4	APPKD-5	APPKD-6	APPKD-7	APPKD-9	APPKD-10	APPKD-11	APPKD-12	APVSP-14	APVSP-15	APVSP-16	APVSP-18	APVSP-22	APVSP-23	APSKLM-25	APSKLM-26	APSKLM-27	APVJM-31	APVJM-32	APVJM-33	APVJM-35	APVJM-39
APPKD-1	0.000																							
APKKD-3	0.2432	0.000																						
APPKD-4	0.2222	0.4286	0.000																					
APPKD-5	0.2381	0.1707	0.3000	0.000																				
APPKD-6	0.2667	0.3103	0.5000	0.4118	0.000																			
APPKD-7	0.2174	0.2000	0.2273	0.1200	0.4737	0.000																		
APPKD-9	0.1707	0.3000	0.1795	0.2444	0.4545	0.1429	0.000																	
APPKD-10	0.375	0.4194	0.4667	0.4444	0.1617	0.4500	0.3714	0.000																
APPKD-11	0.1628	0.2857	0.2195	0.1915	0.4286	0.9800	0.0435	0.3514	0.000															
APPKD-12	0.2571	0.1765	0.3333	0.3333	0.2593	0.2558	0.2105	0.2414	0.2000	0.000														
APVSP-14	0.3548	0.6000	0.3793	0.4286	0.5652	0.4359	0.4118	0.6000	0.4444	0.5714	0.000													
APVSP-15	0.5714	0.5556	0.5385	0.5625	0.5000	0.5000	0.4839	0.3636	0.4545	0.3600	0.9048	0.000												
APVSP-16	0.2273	0.3023	0.2857	0.2083	0.3889	0.1538	0.2766	0.4211	0.2653	0.4146	0.4595	0.5882	0.000											
APVSP-18	0.3846	0.2632	0.4054	0.3488	0.4194	.02766.	0.1905	0.3333	0.2273	0.2222	0.6250	0.5172	0.3333	0.000										
APVSP-22	0.2105	0.2973	0.2222	0.2381	0.4667	.02174.	0.2195	0.5625	02558.	0.3714	0.4194	0.6429	0.2273	0.3846	0.000									
APVSP-23	0.3659	0.3500	0.3333	0.2000	0.4545	.01837.	0.3636	0.3714	0.3043	0.3684	0.5294	0.4194	0.1489	0.3810	0.3171	0.000								
APSKLM-25	0.5484	0.3333	0.7241	0.4286	0.4783	0.4872	0.6471	0.5200	0.6111	0.5714	0.9167	0.5238	0.3514	0.4375	0.5484	0.3529	0.000							
APSKLM-26	0.1429	0.1707	0.3000	0.1304	0.4118	0.0800	0.1556	0.5000	0.1064	0.2821	0.4286	0.5625	0.2083	0.4388	0.1905	0.2889	0.4857	0.000						
APSKLM-27	0.2727	0.3023	0.2381	0.2083	0.4444	0.1154	0.2340	0.4211	0.2245	0.3659	0.5135	0.5294	0.0800	0.3333	0.2273	0.1489	0.4054	0.2083	0.000					
APVJM-31	0.5000	0.3143	0.4118	0.3500	0.3571	0.2727	0.4359.	0.3333	0.4146	0.3333	0.6552	0.5385	0.2381	0.2973	0.4444	0.2308	0.3793	0.4000	0.1905	0.000				
APVJM-32	0.2558	0.2381	0.3171	0.1489	0.5429	0.1765	0.2609.	0.5135	0.2500	0.4000	0.3889	0.6970	0.2245	0.3636	0.2093	0.2609	0.4444	0.1915	0.1837	0.3659	0.000			
APVJM-33	0.5556	0.3714	0.4706	0.3500	0.4286	0.3636	0.4872.	0.3333	0.4634	0.3939	0.7241	0.5385	0.3333	0.3574	0.5000	0.2821	0.3793	0.5000	0.2381	0.1176	0.3171	0.000		
APVJM-35	0.5172	0.7143	0.3333	0.5152	0.7143	0.4595	0.5000	0.6522	0.5294	0.6923	0.5455	0.5789	0.4857	0.6667	0.4483	0.4375	0.6364	0.5758	0.4286	0.5556	0.4706	0.5556	0.000	
APVJM-39	0.4000	0.2414	0.5000	0.3529	0.2727	0.4211	0.5172	0.3333	0.4857	0.3333	0.5652	0.6000	0.4444	0.4194	0.6000	0.3939	0.3913	0.4118	0.4444	0.2857	0.3714	0.3571	0.6190	0.000