Use of RAPD and ISSR Markers for Molecular Genetic Analysis of *Eucalyptus tereticornis*

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***Abstract*—** Random Amplified Polymorphic DNA (RAPD) and Inter Simple Sequence Repeat (ISSR) polymorphism was employed to assess the genetic variations in the germplasm of *Eucalyptus tereticornis*. 15 trees under cultivation were analysed with 10 RAPD primers and 4 ISSR Primers of which all RAPD Primers and only 2 ISSR primers showed reproducible results. Marker attributes like Polymorphism Information Content (PIC), Effective Multiplex Ratio (EMR), and Marker Index (MI) and Informative bands (Ib) values were calculated to assess the discriminatory power of RAPD and ISSR primers. For RAPD primers the PIC values ranged from 0.34 to 0.50, the EMR ranged from 34 to 201, the MI value ranged from 17 to 88.44 with an average of 9.119 per primers. For ISSR primers the PIC values ranged from 0.36 to 0.41 with an average of 0.38 per primer. The EMR ranged from 122 to 129.6 and the MI values ranged from 50.02 to 46.65 with an average of 46.65 per primer. The Ib value ranged from 50 to 56 with an average of 53 per primer combination. The UPGMA-phenogram categorized the 15 trees into two major clusters based on genetic similarity and dissimilarity.

***Keywords*—** RAPD marker, UPGMA clustering, Polymerase Chain Reaction, EMR, PIC, MI and ISSR Markers.

I. INTRODUCTION

Eucalyptus tereticornis, known as Mysore gum in India and forest gum in Australia, is one of the most extensively planted eucalypt species in India. It is planted to meet the ever increasing demand for pulp wood and solid wood requirements of the Industry. ITC, Bhadrachalam Paper Boards Ltd., Andhra Pradesh has come out successfully, after a number of trails, with some commercial clones of this species with improved productivity [1]. There are only a few studies made on assessment of wood quality of *Eucalyptus tereticornis* from India belonging to different ages and localities of ordinary seed source [2]-[8] initiated work on the assessment of the wood quality of *Eucalyptus tereticornis* clones. RAPD markers are inherited in a Mendelian manner & can be generated for any species without prior DNA sequence information [9]. One advantage of RAPD markers is the increased speed of analysis & dramatic reductions in the amount of DNA required for analysis. Furthermore when haploid megagametophyte tissue from conifers is used as the source to carry out linking mapping [10-11] detected fine-scale genetic structure in Eucalyptus globules sub species globules native forest using 69 random amplified polymorphic DNA (RAPD) markers. This study is unique in demonstrating the congruence between fine-scale genetic structure as revealed by molecular data and quantitative genetic data.

II. MATERIALS AND METHODS

In the present study the total genomic DNA was isolated using CTAB method [12] from the leaves of *Eucalyptus tereticornis*. The isolated DNA was quantified and the concentration were optimize to 55ng/µl for RAPD-PCR and ISSR-PCR reaction. Optimized concentration of RAPD-PCR reaction Mixture and ISSR PCR reaction mixture was used is of 55 µl reaction volume containing the following reagents: 3 µl of dNTPs, 5 µl of Taq polymerase buffer, 2.0 µl of primer, 5.0 µl of template DNA, 1 µl of Taq Polymerase and 39 µl of sterile dd H_2O. PCR reaction condition used were denaturation at 94ºC for 1 min, annealing at 37ºC for 1 min., extension at 72ºC for 1 min. and final extension at end for 7 min at 72ºC. These band pattern obtained was analysed using molecular data analysis software. Thus data obtained was further used for statistical analysis.

TABLE I

Primers used for amplification of *Eucalyptus tereticornis*

S. No.	Primers	Base sequence (5'-3')	Molar conc.(µM)
1	Primer 1	TGCCGAGCTG	20
2	Primer 2	AGGGGTCTTG	20
3	Primer 3	CAATCGCCGT	20
4	Primer 4	TCGGCGATAG	20
5	Primer 5	CTGACGTCAC	20
6	Primer 6	CCGGCCTTAC	20
7	Primer 7	CCGGCCTTCC	20
8	Primer 8	CCGGCTGGAA	20
9	Primer 9	ATTGGGCGAA	20
10	Primer 10	GTGCGTCCTC	20

II. Results and Discussion

RAPD Analysis

In case of RAPD markers, 10 random primers were screened and all the primers showed reproducible and polymorphic bands between different samples of *Eucalyptus tereticornis.*

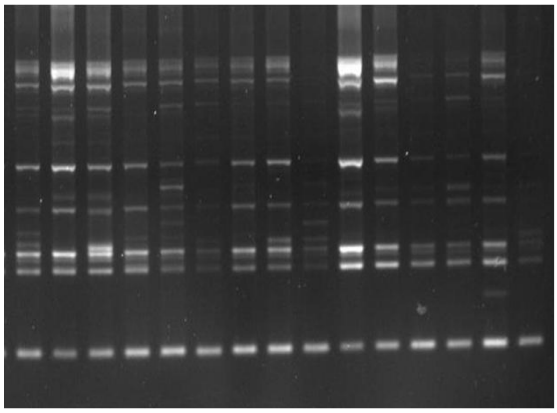

Fig. 1 RAPD marker profiles of 15 samples of *Eucalyptus tereticornis* generated by primer 1 (TGCCGAGCT) in 1.5 % Agarose gel.

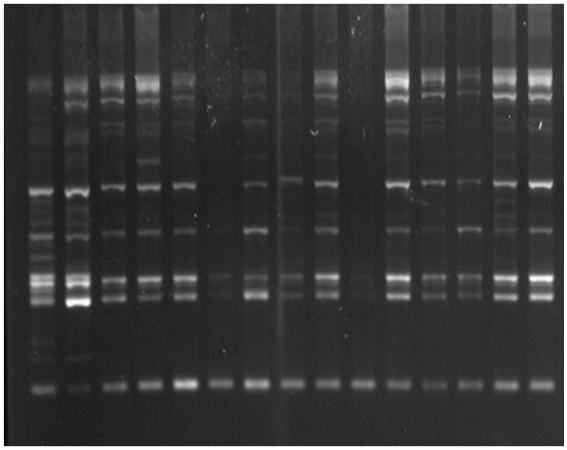

Fig. 2 RAPD marker profiles of 15 samples of *Eucalyptus tereticornis* generated by primer 2 (TGCCGAGCTG) in 1.5 % Agarose gel

For the RAPD primers the statistical analysis was done and the data obtained revealed the information about the percentage of polymorphism for the primers. The PIC values for RAPD ranged from 0.34 to 0.5 with an average of 0.45 per primer combination. Highest value (0.5) was scored with the primer 10 and the lowest value (0.34) was scored with the primer 6.The EMR values for RAPD ranged from 34 to 201 with an average of 102.69 per primer combination. Highest value (201) was scored with the primer 1 and the lowest value (34) was scored with the primer 10. The MI values for RAPD ranged from 17.0 to 88.44 with an average of 46.13 per primer combination. Highest values (88.44) were scored with the primer 1 and the lowest value (17.0) was scored with the primer 10. The Ib values for RAPD ranged from 24 to 78 with an average of 42.1 per primer combination. Highest value (78) was scored with the primer 1 and the lowest value (24) was scored with the primer 10.

TABLE 2

PIC, EMR, MI and Ib values for 10 RAPD primers

Primer	PIC	EMR	MI	Ib
1	0.44	201	88.44	78
2	0.48	103	49.44	34
3	0.49	112.5	55.12	40
4	0.48	119.4	57.32	42
5	0.44	170	74.8	68
6	0.34	68	23.12	36
7	0.42	100	42	47
8	0.48	59	28.32	26
9	0.43	60	25.8	26
10	0.50	34	17	24
Average	0.45	102.69	46.13	42.1

*PIC: Polymorphism information contents; EMR: Effective Multiplex Ratio; MI: Marker index; Ib: Band Informativeness

TABLE 3

RAPD primers used to detect polymorphism, number of bands for polymorphism per primer

Primer	NSB	NPB	PPB
1	39	39	100
2	17	17	100
3	20	18	90
4	21	19	90.47
5	34	34	100
6	18	18	100
7	24	24	100
8	13	13	100
9	13	13	100
10	12	10	83.83

**NSB: Number of scored band; NPB: Number of polymorphic band; PPB: Percentage of polymorphic band*

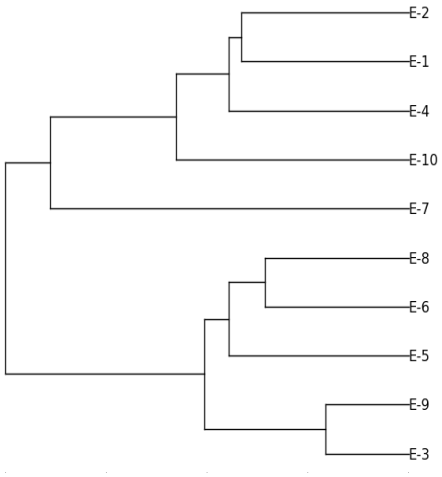

Fig 3 Dendrogram generated using UPGMA analysis, showing relationships between *Eucalyptus tereticornis*, using RAPD data

ISSR Analysis

For the ISSR primers the statistical analysis was done and the data obtained revealed the information about the percentage of polymorphism for the primers. Four Primers of ISSR marker was used, Result came with only two primers. The PIC values for ISSR ranged from 0.36 to 0.41 with an average of 0.38 per primer combination. Highest value (0.41) was scored with the primer 1 and the lowest value (0.36) was scored with the primer 2.The EMR values for ISSR ranged from 122 to 129.6 with an average of 125.8 per primer combination. Highest value (129.6) was scored with the primer 2 and the lowest value (122) was scored with the primer 1. The MI values for ISSR ranged from 46.65 to 50.02 with an average of 48.33 per primer combination. Highest values (50.02) were scored with the primer 1 and the lowest value (46.65) was scored with the primer 2. The Ib values for ISSR ranged from 50 to 56 with an average of 53 per primer combination. Highest value (56) was scored with the primer 1 and the lowest value (50) was scored with the primer 2.

TABLE 4
PIC, EMR, MI and Ib values for ISSR primers

Primer	PIC	EMR	MI	Ib
1	0.41	122	50.02	56
2	0.36	129.6	46.65	50
Average	0.38	125.8	48.33	53

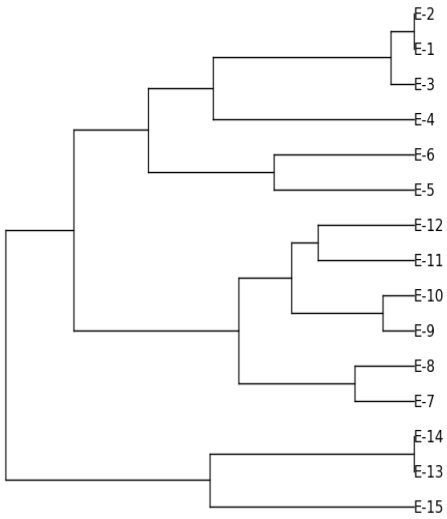

Fig 4 Dendrogram generated using UPGMA analysis, showing relationships between *Eucalyptus tereticornis*, using ISSR data

The study thus revealed the existence of sufficient amount of genetic variability among the *Eucalyptus tereticornis* species samples, which could be exploited further. A close genetic similarity was found in some of the cultivars analysed as shown by high values of similarity index and the dendogram obtained by UPGMA clustering.

Conclusions

The study aimed at estimating the genetic variation in the selected *Eucalyptus tereticornis* species based on the 10 RAPD primers & 4 ISSR primers. All 10 RAPD primers produced reproducible bands and only 2 out of 4 ISSR primers produced reproducible bands. The PIC values for RAPD ranged from 0.34 to 0.5. The EMR values ranged from 34 to 201 and MI values ranged from 17.0 to 88.44. The Ib values ranged from 24 to 78 with an average of 42.1 per primer combination. The PIC values for ISSR ranged from 0.36 to 0.41, the EMR values ranged from 122 to 129.6, the MI values ranged from 46.65 to 50.02 and the Ib values ranged from 50 to 56. The UPGMA clustering method was employed to construct the dendrogram. Keeping in view the markers which have wide ranging applications in the field of genetics including population studies will help in analyzing variation & relationship within species of Eucalyptus populations. The information will be useful in breeding & management program so that the population with high specific variability could be conserved which is better adapted to respond to selective pressure & ensure long term survival of the species.

ACKNOWLEDGMENT

I am thankful to the Director (Admin), CytoGene Research & Development, Lucknow for providing me the facilities and kind support throughout the research work, I am also thankful to the Almighty without whose consent nothing is possible.

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