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Short Communication

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

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Genetic variation between 10 trees of *Eucalyptus tereticornis* was evaluated using inter simple sequence repeats (ISSR) markers. PIC (polymorphism information content), EMR, MI value and Rp value was obtained for per primer combination. These values for ISSR suggested that the marker system were effective in determining polymorphisms. The similarity coefficient and the UPGMA clustering method were employed to construct the dendrogram. ISSR marker system was found to be useful for the genetic diversity studies in *E. tereticornis*.

Keywords: *Eucalyptus tereticornis,* ISSR marker, UPGMA dendogram, PCR, etc.

The genus *Eucalyptus* is native to Australia, has more than seven hundred species. *Eucalyptus* is widely used species for plantation establishment in tropical and subtropical regions of the world due to their fast growth and wide range of adaptability (Sartorelli *et al.,* 2007). They are valued for some valuable sources of proteins, tannins, gum, and dyes. The leaves *Eucalyptus* are distilled for essential oil and their flowers attract honey-bees to produce honey. Its oil can be used for cleaning and functions as a natural insecticide, and it is sometimes used to drain swamps to reduce the risk of malaria (Nei, 1987). It has strong, hard and durable heartwood; it is used for construction in heavy engineering, such as for railway sleepers. It is used for fuel, pulp, pilings, fiberboard, construction and fence posts. *Eucalyptus species* can reach 30 to 45 m in height and 1 to 2 m in diameter. The species grows in open forests or as scattered trees in alluvial plains and along streams, including brackish waters (Varshney *et al.,* 2005). It grows better in deep, light textured, neutral, or slightly acid soils. Outside its natural range, the tree has been planted in a great variety of places, including alluvial, muddy, and sandy clay soils. For short time it tolerates seasonal floods and can endure up to 15 freezes per year in the southern part of its natural range (Trivedi and Hotchandani, 2004). In spite of its commercial importance and world wide effort in breeding and propagation research, very little effort has been devoted to the development and use of molecular genetic markers for species of this genus. ISSRs are DNA fragments of about 100-3000 bp located between adjacent, oppositely oriented microsatellite regions. The complementary sequences of two neighboring microsatellites are used as PCR primers; the variable region between them gets amplified (Williams *et al.,* 1990). The

limited length of amplification cycles during PCR prevents excessive replication of overly long contiguous DNA sequences, so the result will be a mix of a variety of amplified DNA strands which are generally short but very much in length (Prevost and Wilkinson, 1999).The objective of this study was to estimate the genetic variation in 10 trees of *Eucalyptus tereticornis* based on ISSR markers, in support of improvement and hybridization studies.

Materials and Methods

Collection of plant material and Genomic DNA extraction

Ten different *Eucalyptus tereticornis* species fresh leaves were collected for isolation of genomic DNA. Before isolation the leaves were washed with distilled water and wiped with spirit in order to sterilize leaves surface. Genomic DNA isolation was done by following the modified procedure described by Stange *et al. (*1998). DNA quality was estimated by measuring the 260:280 UV absorbance ratios (Csaikl *et al*., 1998).

PCR amplification of isolated products

DNA amplification protocol was performed as described by Williams *et al*. (1990) with some modifications. A total of 3 primers (ISSR-3, ISSR-4 and ISSR-11) were used in the present study. The sequences and molar concentration of primers are given in Table 1.

Table-1: ISSR Primer sequences used for amplification

S. No	Primer name	Base sequence (5′-3′)	Annealing temp.
1	ISSR-3	GAG AGA GAG AGA GAG AC	27.6 ^{o}C
2	ISSR-4	GAG AGA GAG AGA GAG AA	44.3 ^{o}C
3	ISSR-11	TGT GTG T GT GTG TGT GRT	54 ^{o}C

The 355 µl double distilled water, 50 µl Taq buffer, 30 µl $MgCl_2$, 40 µl dNTPs, 5.0 µl BSA and primers (20 µl) each were thawed, vortexed (except primers) and spinned and were kept in PCR tray in the ice tray. Required amount of these components were taken in a 0.6 ml centrifuge tube (master mix).After adding all the components Taq DNA polymerase (4.0 µl) was thawed and spinned and immediately added to the master mix. Then the master mix was vortexed properly and spinned again. After that diluted DNA samples (1 µl) were added in 0.2 ml PCR tubes. The DNA amplifications were conducted in 25 µl reaction volumes containing 24 µl from master mix and 1µl DNA sample in PCR tubes. The first cycle was performed as denaturation for 7 min at 94°C, annealing for 45 sec at 27°C to 54°C and extension 2 min at 72°C and the final extension at the end of the cycle for 7 minutes at 72°C. A total of 44 cycles were carried out to obtain the amplification product.The amplification products were electrophoresed using 1.5 % Agarose gel with TBE Buffer for 1 hour. Bands were visualized by Ethidium bromide and photographed under UV light using Gel Documentation System.The similarity coefficient and the un-weighted Pair Group Method Based on Arithmetic Mean (UPGMA) clustering method were employed to construct the dendrogram.

Results and Discussion

The isolated DNA was quantified and the concentration were optimize to 10ng/µl for ISSR -PCR reaction.

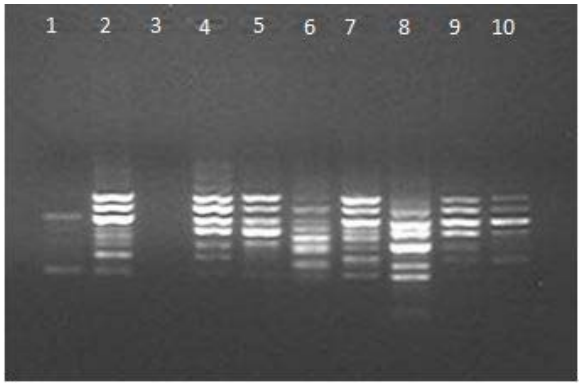

Figure 1: ISSR marker profiles of 10 samples of *Eucalyptus tereticornis* generated by primer 3

Table-2: ISSR Primer- PIC, EMR, MI &Rp values

S.No.	Primer Name	PIC	EMR	MI	Rp
1	ISSR-3	0.29	38	17.21	66
2	ISSR-4	0.28	26	12.94	60
3	ISSR-11	0.32	46	13.24	60
	Average	0.29	36.6	14.46	62

The PIC values for ISSR ranged from 0.29 to 0.32 with an average of 0.29 per primer combination. Highest value (0.32) was scored with the primer ISSR-11 and the lowest value (0.28) was scored with the primer ISSR-4.The EMR values for ISSR ranged from 38 to 46 with an average of 36.6 per primer combination. Highest value (46) was scored with the primer ISSR-11 and the lowest value (26) was scored with the primer ISSR-4.MI values for ISSR ranged from 17.21 to 13.24 with an average of 14.46 per primer combination. Highest value (17.21) was scored with the primer ISSR-3 and the lowest value (12.94) was scored with the primer ISSR-4.Rp values for ISSR ranged from 66 to 60 with an average of 62 per primer combination.

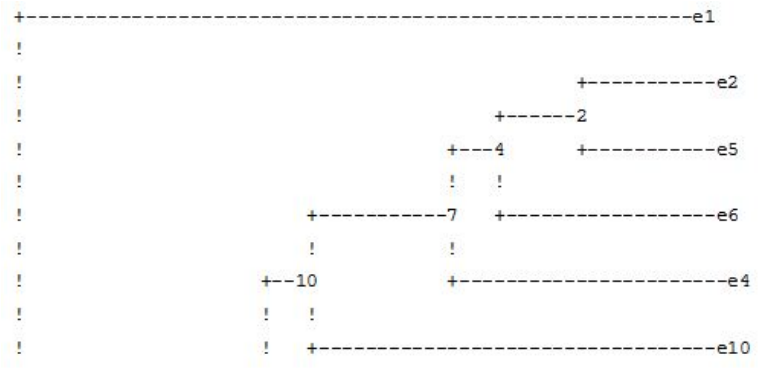

Figure 2: UPGMA clustering dendrogram of *Eucalyptus tereticornis* population based on average genetic distance by using ISSR.

The matrix of genetic distance was used to generate a dendrogram (Figure 2) in which the samples were grouped by UPGMA method using bootstrap analysis, where each group was formed by individuals of smaller genetic distance.The cluster contains e-2, e-5, e-6, e-4 and e-10 in which e-2 and e-5 are similar with the value of 1.Similar type of result was obtained by Okun et al., 2008, in which Five ISSR primers generated 41 scorable polymorphic bands which were used to analyse genetic diversity between and within the seed sources and for construction of neighbour-joining pheno-gram. Mean Genetic Diversity per each primer loci based on Nei (1987) statistics indicated significant genetic variation between seed sources with 26.4%, (Gst = 0.264) of the total variation attributed to differences between seed sources. The variation between populations could be due to ecological, geographical association and gene flow rates and hence they should be conserved to retain the full breadth of genetic variation of the species.

Conclusion

Genetic variation between 10 trees of *Eucalyptus tereticornis* was evaluated using inter simple sequence repeats (ISSR) markers. PIC (polymorphism information content) value: 0.29, EMR value: 36.6, MI value: 14.46and Rp value: 62wereobtained for per primer combination. These values for ISSR suggested that the marker system were effective in determining polymorphisms. The

similarity coefficient and the UPGMA clustering method were employed to construct the dendrogram. ISSR marker system was found to be useful for the genetic diversity studies in *E. tereticornis*.

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