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Discrimination of *Calophyllum inophyllum* L. Progenies Through ISSR Markers

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A B S T R A C T

The current study aims at discriminating the thirty *Calophyllum inophyllum* progenies thorough the deployment of Inter simple sequence repeats (ISSR) markers. The thirty progenies of *Calophyllum inophyllum* were collected from the predominant distribution and systematic analysis were carried out to elucidate the genetic divergence at Molecular Biology Lab of Department of Tree Breeding of Forest College and Research Institute, Mettupalayam. Among the ten ISSR markers deployed on the thirty progenies of *Calophyllum inophyllum*, two ISSR markers *viz.*, UBC823 and UBC834 were found to be polymorphic and the number of bands ranged from eight to eleven. The highest polymorphic information content (PIC) value 0.58 was revealed by the primer UBC823 with the average PIC value of 0.45. Cluster analysis using NTSYS generated dendrogram divided all the 30 progenies into ten distinct groups. The progenies A4 and A7, A5 and A8 were closest at the similarity coefficient of 1.000.The lowest PIC value was observed with the primer UBC 834 (0.33). The present investigation has clearly delineated FCRICI 14 progeny genetically diverse and extends scope for further breeding programme through systematic crossing in order to exploit the heterosis.

Introduction

Selection is the most important activity in all breeding programmes (Zobel and Talbert, 1984). Tree yield, *i.e.*, tree volume is a complex and highly variable character which is influenced by many component characters (Deepak Pandey and Hooda, 1997). In the integrated structure of the plant, the overall correlation observed between two variables is a function of a series of direct and indirect relationships between different variables which could be affected through path coefficient studies (Wright, 1921). However, research pertaining to correlation and path analysis in Undi is very scant variable (Divakara *et al.*, 2010; Pavithra *et al.*, 2013) and thus needs intensive research. The nature and degree of divergence in the base population is a pre-requisite for any improvement and conservation strategies (Gradual *et al.*, 1999). The genetic diversity has been assessed traditionally either by provenance testing using Mahalanobis D^2 statistics and Canonical vector analysis or electrophoretic analysis of enzymes. Study of metric characters in field trials was earlier the dominating technique and it is still today the most robust and valid way of assessing genetic variation. Markers also have

important immediate application in supportive research for tropical hardwoods and non-industrial species mainly for quantification of genetic diversity. There are several methods based on DNA markers *viz.,* RFLP (Restriction Fragment Length Polymorphism), RAPD (Randomly Amplified Polymorphic DNA), SSR (Simple Sequence Repeats), ISSR (Inter Simple Sequence Repeats), AFLP (Amplified Fragment Length Polymorphism) etc. Among these techniques, ISSR marker is very significant because this marker in its simplest form, no prior genomic knowledge is required to design arbitrary sequence oligonucleotide primers (Gonzalez *et al.,* 2005). But the use of molecular markers for genetic diversity studies in Undi dismally modest thus underscores the research needs.

Materials and Methods

The predominant *Calophyllum inophyllum* growing areas of Southern India *viz.,* Tamil Nadu, Karnataka and Kerala were identified and Candidate Plus Trees were selected based on the following morphological features *viz.,* Height, Diameter at Breast Height (DBH), and Canopy width by using the method described by Pitcher and Dorn (1966). From the selected 30 candidate plus trees seeds were collected from the crown and the morphometric attributes of selected candidate plus trees were presented in table 1.

The ripened fruits of *Calophyllum inophyllum* were collected from the 30 selected candidate plus trees during the month of September and December. The 30 progenies were raised from the seeds collected from the selected candidate plus trees and One year old saplings were planted in the field by adopting Randomized Block Design.

Young leaf tissues were collected from 30 progenies of one year old saplings of *Calophyllum inophyllum* and the method followed to isolate genomic DNA was chloroform method as described by McCouch lab protocol (1998). The isolated genomic DNA was quantified using fluorometer (Dyna Quant, USA). The isolated DNA was verified for size, intactness, homogeneity and purity.

The isolated genomic DNA was subjected to polymerase chain reaction (PCR) using PERKIN ELMER Gene Amp PCR thermo cycler. The various 10 random ISSR primers *viz.,* UBC808, UBC809, UBC810, UBC811, UBC823, UBC834, UBC835, UBC844, UBC848, UBC881 and UBC890 were used for DNA amplification are given in table 2. Clear and unambiguous bands of ISSR markers were scored based on the presence or absence of the corresponding band among the genotypes. The scores '1' and '0' indicates the presence and absence of bands respectively. Statistical analysis was carried out by using the computer software NTSYSpc version 2.02 developed by Rohif (1998).

The confidence limits for the groupings by dendrograms were computed by using WINBOOT, a program for performing bootstrap analysis of binary data to determine the confidence limits of UPGMA based dendrograms (Yap and Nelson, 1996). Bootstrapping involves repeated sampling with replacement of the characters in matrix of operational taxonomic unit (OUT) x characters to create numerous bootstrap matrices of the same size as the original matrix.

From the binary code matrices obtained from the ISSR markers, the Jaccard's similarity indices Jaccard (1908) were computed for the 30 genotypes of *Calophyllum inophyllum* using the SIMQUAL programme of NTSYSpc version 2.02. Polymorphism information content (PIC) or expected heterozygosity scores for each ISSR marker was calculated as described by Anderson *et al.,* (1993)

Results and Discussion

Assessment of genetic diversity is a prerequisite for efficient conservation and utilization of genetic resources. During the past two decades, several high-throughput PCR-based technologies such as inter-simple sequence repeats (ISSR) and amplified fragment length polymorphisms (AFLP) have been developed to assay genetic polymorphism at the DNA level (Shyam Sundar *et al.*,2014).

An advantage of ISSRs is that in their simplest form, no prior genomic knowledge is required to design arbitrary sequence oligonucleotide primers (Gonzalez *et al.*, 2005). With the large array of molecular analytical techniques available, it has become possible to provide an accurate and unambiguous tool for the evaluation of genetic diversity and identification of germplasm (Li *et al.*, 2008).

Assessment of genetic variation within the species based on morphological and polypeptide descriptions, providing more speed, accurate and detailed information (Zabeau and Vos, 1993). ISSR has been used for diversity assessment by many researchers in tropical and temperate trees.

Extensive studies have already been reported in many tropical trees *viz*., *Pongamia pinnata* (Shyam Sundar *et al.*, 2014); *Casuarinas* (Yasodha and Kathirvel, 2004) ISSR techniques was used to study the genetic structure and relationship between species and populations. Similarly, DNA based ISSR techniques have been successfully utilized in many genera and species of forest trees (Mohapatra and Singhal, 2000) for diversity study. In the present investigation, Out of 10 ISSR primers deployed, only 2primers *viz.*, UBC 823 and UBC 834 exhibited high polymorphism (100 per cent) with all thirty genotypes of *Calophyllum inophyllum*. Nineteen reproducible bands were obtained from these two primers alone and the highest PIC value (0.58) was recorded by the primer UBC823 and the lowest PIC value (0.33) was observed in the primer UBC834 (Table 3).

The present investigation revealed that Undiaccessions used for diversity assessment through ISSR, showed moderate level of genetic variations. Polymorphism in several tree species was documented by several authors *viz*., *Jatropha* spp. (Sunil kumar *et al.*, 2013); and *Tamarindus indica* (Nandini *et al.*, 2011). PCR-based DNA markers were employed to assess genetic diversity in *Jatropha curcas* genotypes (Jubera *et al.*, 2009) and *Casuarina equisetifolia* (Elavazhagan *et al.*, 2009).

The study with DNA markers across species of *Jatropha* has revealed a high level of genetic diversity (Basha *et al.*, 2009). In Undi, the average linkage between the genotypes was used for constructing a phylogenetic tree. The association amongst different genotypes was presented in the form of dendrogram (Fig. 1). The progenies A4 and A7, A5 and A8, were closest at the similarity coefficient 1.000. The progenies A1 and A2, A2 and A19, A2 and A20, A2 and A25, A5 and A15, A8 and A15, A12 and A28 were the farthest or most diverse and dissimilar to all others (Table 4).

Similar grouping pattern within species and related genotypes are evidenced from the earlier reports in *Eucalyptus tereticornis* (Suman Tewari *et al.*, 2013).

Table.1 Morphological characters of selected superior progenies of *Calophyllum inophyllum*

Sl. No.	Name of the CPTs	Height (m)	GBH (m)	Crown diameter (m)
1	FCRICI 1	4.31	0.71	3.07
2	FCRICI 2	5.50	0.83	2.95
3	FCRICI 3	4.16	0.76	2.67
4	FCRICI 4	5.34	0.97	3.21
5	FCRICI 5	5.02	0.75	4.02
6	FCRICI 6	5.96	0.59	3.26
7	FCRICI 7	4.25	0.65	4.50
8	FCRICI 8	5.93	0.73	2.79
9	FCRICI 9	5.14	0.82	3.08
10	FCRICI 10	5.96	0.70	2.99
11	FCRICI 11	5.08	0.67	3.45
12	FCRICI 12	4.13	0.72	2.13
13	FCRICI 13	4.66	0.69	3.05
14	FCRICI 14	7.98	1.02	5.67
15	FCRICI 15	6.97	0.83	4.97
16	FCRICI 16	7.02	0.90	4.06
17	FCRICI 17	7.89	0.79	3.96
18	FCRICI 18	6.48	0.84	4.56
19	FCRICI 19	7.36	0.92	2.13
20	FCRICI 20	8.01	0.86	3.20
21	FCRICI 21	7.00	0.64	4.16
22	FCRICI 22	6.71	0.59	3.99
23	FCRICI 23	6.13	0.96	3.54
24	FCRICI 24	5.67	0.95	2.87
25	FCRICI 25	5.95	0.66	2.40
26	FCRICI 26	6.03	0.76	4.88
27	FCRICI 27	4.05	0.80	2.97
28	FCRICI 28	3.21	0.50	2.18
29	FCRICI 29	5.69	0.72	3.22
30	FCRICI 30	5.01	0.65	2.43
Mean		**5.75**	**0.76**	**3.41**

Table.2 List of primers used for ISSR amplification

S.No	Primers	Primer sequence 5'-3'
1.	UBC808	AGAGAGAGAGAGAGAGC
2.	UBC809	AGAGAGAGAGAGAGAGG
3.	UBC810	GAGAGAGAGAGAGAGAT
4.	UBC811	GAGAGAGAGAGAGAGAC
5.	UBC823	TCTCTCTCTCTCTCTCC
6.	UBC834	AGAGAGAGAGAGAGAGYT
7.	UBC835	AGAGAGAGAGAGAGAGYC
8.	UBC844	CTCTCTCTCTCTCTCTRC
8.	UBC848	CACACACACACACACARG
9.	UBC881	GGGTGGGGTGGGGTG
10.	UBC890	VHVGTGTGTGTGTGTGT

Table.3 Random ISSR primers used for DNA amplification in *Calophyllum inophyllum*

Sl. No	Primers	Sequence 5'-3'	Total number of bands	PIC values
1	UBC823	TCTCTCTCTCTCTCTCC	11	0.58
2	UBC834	AGAGAGAGAGAGAGAGYT	08	0.33
Mean				**0.45**

Table.4 ISSR Similarity matrix of *Calophyllum inophyllum* progenies

Seed	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1	1.0000																													
2	0.3157	1.0000																												
3	0.5789	0.5263	1.0000																											
4	0.4210	0.8947	0.6315	1.0000																										
5	0.3684	0.8421	0.5789	0.9473	1.0000																									
6	0.4736	0.8421	0.4736	0.8421	0.7894	1.0000																								
7	0.4210	0.8947	0.6315	1.0000	0.9473	0.8421	1.0000																							
8	0.3684	0.8421	0.5789	0.9473	1.0000	0.7894	0.9473	1.0000																						
9	0.4736	0.6315	0.5789	0.5263	0.4736	0.5789	0.5263	0.4736	1.0000																					
10	0.5789	0.6315	0.7894	0.7368	0.6842	0.5789	0.7368	0.6842	0.7894	1.0000																				
11	0.4736	0.6315	0.6842	0.7368	0.7894	0.5789	0.7368	0.7894	0.6842	0.8947	1.0000																			
12	0.4210	0.6842	0.4210	0.7894	0.8421	0.6315	0.7894	0.8421	0.4210	0.6315	0.7368	1.0000																		
13	0.6315	0.4736	0.6315	0.4736	0.4210	0.5263	0.4736	0.4210	0.7368	0.7368	0.6315	0.5789	1.0000																	
14	0.6315	0.5789	0.7368	0.6842	0.6315	0.6315	0.6842	0.6315	0.6315	0.7368	0.6315	0.5789	0.7894	1.0000																
15	0.5263	0.3684	0.7368	0.3684	0.3157	0.4210	0.3684	0.3157	0.7368	0.6315	0.5263	0.3684	0.7894	0.6842	1.0000															
16	0.6842	0.5263	0.7894	0.6315	0.5789	0.5789	0.6315	0.5789	0.6842	0.7894	0.6842	0.5263	0.8421	0.9473	0.7368	1.0000														
17	0.5263	0.6842	0.6315	0.7894	0.7368	0.7368	0.7894	0.7368	0.5263	0.6315	0.6315	0.6842	0.6842	0.8947	0.5789	0.8421	1.0000													
18	0.6315	0.5789	0.6315	0.6842	0.6315	0.6315	0.6842	0.6315	0.5263	0.6315	0.6315	0.5789	0.6842	0.8947	0.5789	0.8421	0.8947	1.0000												
19	0.6842	0.3157	0.7894	0.4210	0.4736	0.3684	0.4210	0.4736	0.4736	0.5789	0.5789	0.4210	0.6315	0.7368	0.7368	0.7894	0.6315	0.7368	1.0000											
20	0.5789	0.3157	0.6842	0.4210	0.4736	0.3684	0.4210	0.4736	0.3684	0.5789	0.5789	0.5263	0.5263	0.6315	0.6315	0.5789	0.5263	0.6315	0.7894	1.0000										
21	0.6315	0.4736	0.7368	0.5789	0.5263	0.5263	0.5789	0.5263	0.3157	0.5263	0.5263	0.4736	0.4736	0.6842	0.5789	0.6315	0.6842	0.7894	0.7368	0.8421	1.0000									
22	0.6842	0.5263	0.6842	0.6315	0.5789	0.5789	0.6315	0.5789	0.4736	0.5789	0.5789	0.5263	0.5263	0.7368	0.6315	0.6842	0.7368	0.8421	0.7894	0.6842	0.8421	1.0000								
23	0.4736	0.5263	0.6842	0.6315	0.6842	0.5789	0.6315	0.6842	0.3684	0.4736	0.4736	0.6315	0.5263	0.7368	0.6315	0.6842	0.7368	0.6315	0.7894	0.6842	0.6315	0.6842	1.0000							
24	0.6315	0.4736	0.8421	0.5789	0.5263	0.5263	0.5789	0.5263	0.5263	0.6315	0.6315	0.4736	0.6842	0.7894	0.7894	0.8421	0.7894	0.7894	0.8421	0.6315	0.7894	0.8421	0.7368	1.0000						
25	0.7894	0.3157	0.5789	0.4210	0.3684	0.3684	0.4210	0.3684	0.3684	0.4736	0.3684	0.4210	0.6315	0.6315	0.5263	0.6842	0.6315	0.6315	0.6842	0.4736	0.5263	0.5789	0.5789	0.6315	1.0000					
26	0.5263	0.5789	0.6315	0.6842	0.6315	0.6315	0.6842	0.6315	0.6315	0.7368	0.7368	0.6842	0.7894	0.8947	0.6842	0.8421	0.8947	0.8947	0.6315	0.6315	0.6842	0.7368	0.6315	0.7894	0.5263	1.0000				
27	0.4736	0.5263	0.6842	0.6315	0.5789	0.5789	0.6315	0.5789	0.5789	0.6842	0.6842	0.6315	0.7368	0.8421	0.7368	0.7894	0.8421	0.8421	0.6842	0.6842	0.7368	0.7894	0.6842	0.8421	0.4736	0.9473	1.0000			
28	0.4736	0.5263	0.6842	0.4210	0.3684	0.4736	0.4210	0.3684	0.6842	0.4736	0.3684	0.3157	0.6315	0.7368	0.8421	0.6842	0.6315	0.6315	0.6842	0.5789	0.6315	0.6842	0.6842	0.7368	0.4736	0.6315	0.6842	1.0000		
29	0.5789	0.4210	0.7894	0.5263	0.4736	0.4736	0.5263	0.4736	0.5789	0.6842	0.5789	0.5263	0.7368	0.8421	0.8421	0.7894	0.7368	0.7368	0.7894	0.7894	0.7368	0.7894	0.7894	0.8421	0.5789	0.8421	0.8947	0.7894	1.0000	
30	0.5263	0.5789	0.6315	0.5789	0.5263	0.6315	0.5789	0.5263	0.7368	0.7368	0.7368	0.5789	0.8947	0.7894	0.7894	0.8421	0.7894	0.7894	0.6315	0.5263	0.5789	0.6315	0.5263	0.7894	0.5263	0.8947	0.8421	0.6315	0.7368	1.0000

1	FCRICI 1	11	FCRICI 11	21	FCRICI 21
2	FCRICI 2	12	FCRICI 12	22	FCRICI 22
3	FCRICI 3	13	FCRICI 13	23	FCRICI 23
4	FCRICI 4	14	FCRICI 14	24	FCRICI 24
5	FCRICI 5	15	FCRICI 15	25	FCRICI 25
6	FCRICI 6	16	FCRICI 16	26	FCRICI 26
7	FCRICI 7	17	FCRICI 17	27	FCRICI 27
8	FCRICI 8	18	FCRICI 18	28	FCRICI 28
9	FCRICI 9	19	FCRICI 19	29	FCRICI 29
10	FCRICI 10	20	FCRICI 20	30	FCRICI 30

Fig.1 ISSR profile of *Calophyllum inophyllum*

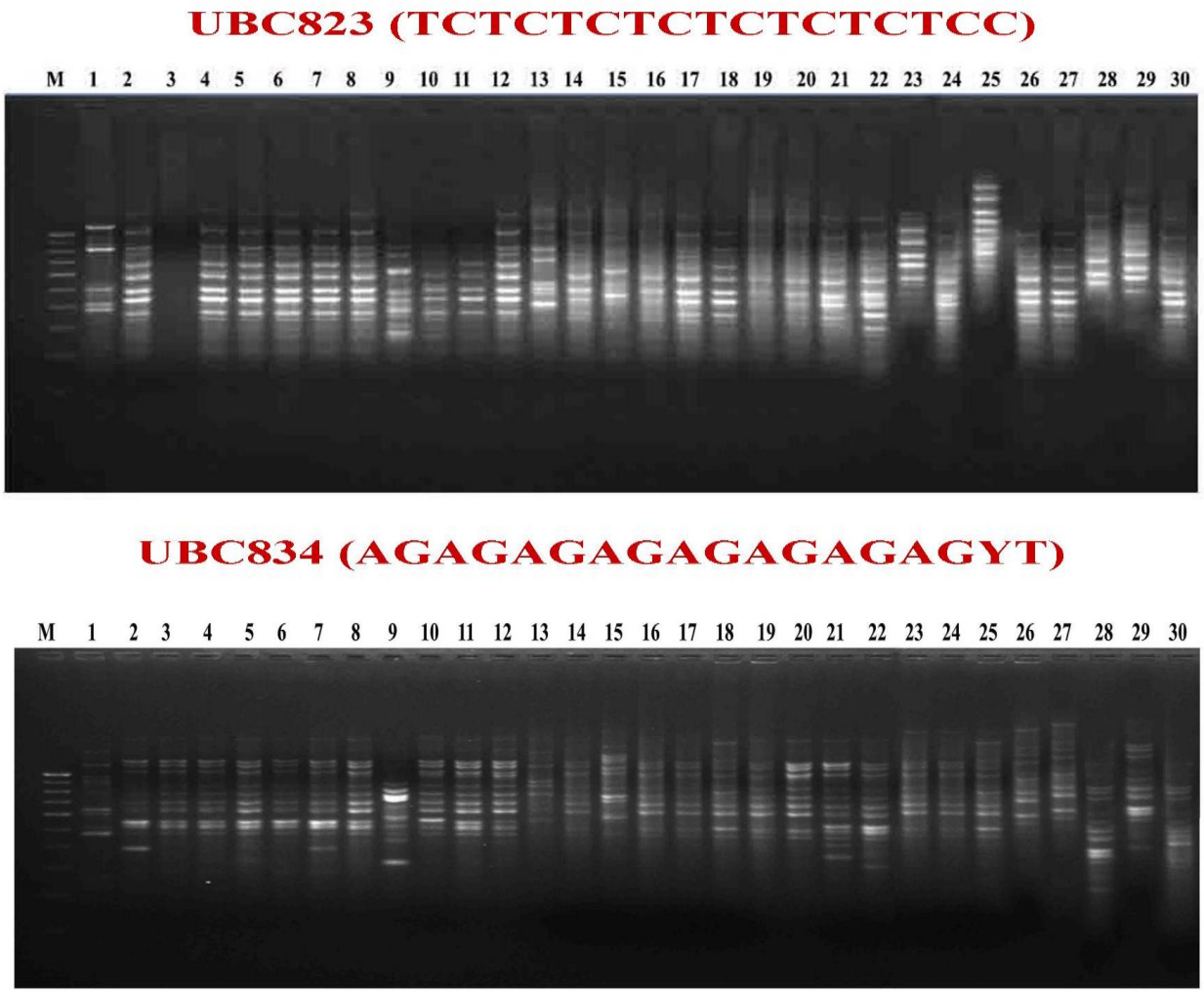
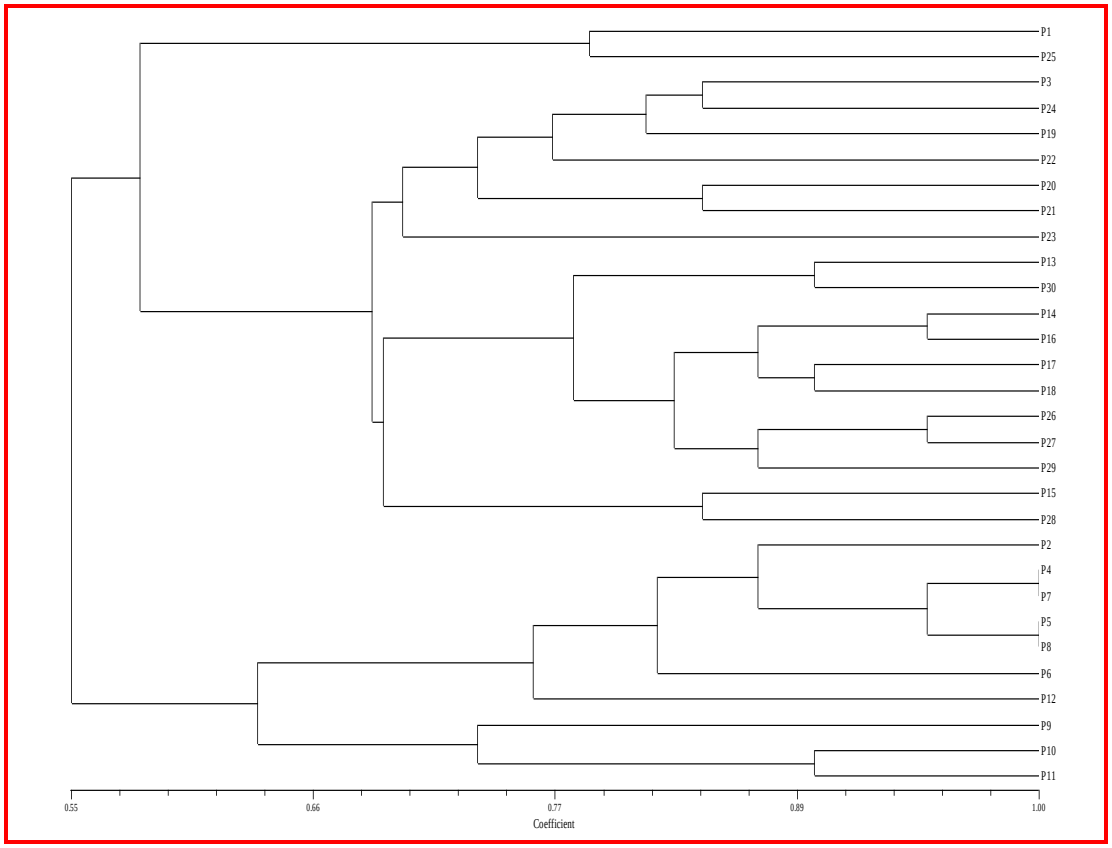


Fig.2 Dendrogram of *Calophyllum inophyllum* progenies based on Jaccard's similarity coefficient



P1- FCRICI 1	P4- FCRICI 4	P7- FCRICI 7	P10- FCRICI 10	P13- FCRICI 13	P16- FCRICI 16	P19- FCRICI 19	P22- FCRICI 22	P25- FCRICI 25	P28- FCRICI 28
P2-FCRICI 2	P5- FCRICI 5	P8- FCRICI 8	P11- FCRICI 11	P14- FCRICI 14	P17- FCRICI 17	P20- FCRICI 20	P23- FCRICI 23	P26- FCRICI 26	P29- FCRICI 29
P3- FCRICI 3	P6- FCRICI 6	P9- FCRICI 9	P12- FCRICI 12	P15- FCRICI 15	P18- FCRICI 18	P21- FCRICI 21	P24- FCRICI 24	P27- FCRICI 27	P30- FCRICI 30

The results revealed that ISSR is an efficient technique to characterize the Undigenotypes and classify different genotypes based on the ISSR markers generated (Fig. 2). It was also indicated that ISSR analysis has determined the genetic relationships and estimated the genetic diversity among the genotypes of Undi. The results of the present study can be used as a stepping stone for evolving well defined approach based on evaluation and characterization of genetic variation in Undi genotypes which can be further used for the improvement of these two species for various traits through different breeding methods.

In conclusion the molecular analysis of Undi progenies through ISSR markers identified the progenies A4 and A7 and A5 and A8 exhibited closest similarity coefficient. In a holistic prospection, the current investigation identified one superior progeny each in Undi (FCRICI 14)for further breeding programme through systematic crossing in order to exploit the heterosis.

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