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MOLECULAR VARIABILITY IN PONGAMIA ECOTYPES FROM NORTH KARNATAKA, INDIA

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ABSTRACT
Pongamia pinnata (L.) Pierre is identified as a potential biofuel plant species due to its high non-edible oil yield and wide adaptability to arid conditions. Pongamia is an out breeding species and causes variability in vegetative and productive traits like yield, oil content, etc. It is important to identify the potential of Pongamia plant via molecular tools. The present study emphasizes to access the variability and relatedness among the ten identified candidate plus trees of Pongamia ecotypes from North Karnataka (Hyderabad Karnataka) region, India. A total of 27 RAPD and 13 ISSR primers were used for screening the genetic diversity among the CPT's of Pongamia, totally 48 loci and 179 bands were produced and 22.35 % of polymorphism was studied by RAPD marker analysis. ISSR marker produced 88 loci and 141 bands with a size ranging from 100 kb to 2.5 kb and 15.6 % polymorphism. Both the primers varied in detecting the variations within and between the Pongamia ecotypes. Dendrogram was constructed using unweighted pair group method with arithmetic mean (UPGMA) and genetic distances were studied by Nei's method to analyze the variation using RAPD and ISSR markers.

KEYWORDS: Biodiesel, ISSR, Molecular markers, Molecular variability, Pongamia, RAPD.

INTRODUCTION

Pongamia pinnata (L.) Pierre is a fast growing, glabrous, deciduous tree commonly known as Indian beech, pongam tree and karanja tree, which is a member of the subfamily Papilionideae more specifically the tribe Millettieae. It is indigenous to the Indian subcontinent and South East Asia. It is well adapted to arid and semi-arid zones; it is tolerant to a wide range of abiotic stress like drought, frost, heat, salinity, *etc*. (Meera *et al*., 2003). It grows fast and mature after 4-7 years, yielding fruits which are flat, elliptical, long and each fruit contains 1-2 kidney shaped brownish red kernels. The oil content in the kernel is about 30-40% and Pongamia seed oil has similar properties to that of petro-diesel have gained the importance as biodiesel which is a fast emerging, viable alternative to fossil fuel. The natural constraints that limit its large-scale production and availability, to meet the demand for biodiesel production are its long gestation period (4– 7years), plant height, seed storage behavior, insect pests and the seed oil yield and quality (NOVOD 2010). The cross-pollinating nature of *P. pinnata* contributes to its wide germplasm biodiversity. Thus, it becomes an important step to examine the genetic variations among naturally growing elite individuals of *P. pinnata* at inter and intra population levels and to prepare strategies for its specific exploitation by plant breeders in promoting it as a versatile biodiesel plant. The information on identification of elite quality in North Karnataka, India will enable the successful cultivation and management of identified elite quality Pongamia in the region. Until recently, the identification of elite individuals of *P. pinnata* was mainly described in terms of morphological and agronomic traits which are known to be deeply influenced by environmental factors (Kaushik *et al*., 2007; Kesari *et al*., 2008). 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 (Meudt and Clarke 2006; Simmons *et al*., 2007; Li *et al*., 2008). In the last decade, several polymerase chain reaction (PCR)-based DNA marker classes, *viz*. random amplified polymorphic DNA (RAPD), inter-simple sequence repeat (ISSR) contributed to this goal, and may extend and complement the assessment of genetic variation within the species based on morphological and polypeptide descriptions, providing more speed, accurate and detailed information (Williams *et al*., 1990; Zabeau and Vos 1993; Zietkiewicz *et al*., 1994; Surekha and Larson, 2005). RAPD markers have been widely used in plant research for phylogenetic studies, genome mapping and genetic variation analysis both at intra and inter population levels (Li *et al*., 2008). The technique has several advantages including simplicity, low cost, speed and lack of requirement for DNA sequence information (Williams et al. 1990; Lopes *et al*., 2007). However, RAPD technology has several limitations including dominance, uncertain locus homology, sensitivity and reliability. Inter-simple sequence repeat represents the marker of choice for varietal identification studies as they are transferable, hyper variable, highly polymorphic, multiallelic dominant markers, relatively simple to interpret and show high information content (Souframanien and Gopalkrishna, 2004).

There is a strong need to assess and document the extent of genetic diversity in naturally growing, systematically characterized *P. pinnata* populations to expedite its use in different germplasm-related studies and breeding programs. In continuation of our studies on candidate plus trees (CPTs), the present study was undertaken with the

objective of screening the untapped genetic variability or relatedness among the 10 CPTs of *P. pinnata* tagged from North Karnataka, India at the inter and intra population levels using PCR-based DNA markers (RAPD, ISSR). Candidate plus trees are individual trees of *P. pinnata* possessing superior morphological and reproductive characters compared with other individuals of the same species identified on the basis of morphometric, biochemical marker and also by progeny growth studies (Patil *et al*., 2015; Patil and Naik, 2015).

MATERIALS & METHODS

Plant material

The young seeds of Pongamia were used to study the genetic diversity between the Pongamia accessions which were collected from different regions of North Karnataka, India. The Pongamia accessions (GRP4, 8, 9, 13, 14, 16, 20, 24, 28 and GRP29) were selected as elite trees based on our previous studies on vegetative and productive (morphological and progeny) traits (Patil *et al*., 2015 and Patil and Naik, 2015) and biochemical markers and oil traits (Patil and Naik, 2015) . The young Pongamia seeds were stored at -20ºC for further analysis.

Extraction of genomic DNA

The genomic DNA from young Pongamia seed sample were extracted using CTAB method followed by Doyle and Doyle 1990 with necessary modification in extraction buffer (100 mM Tris-HCl pH 8.0; 25 mM EDTA pH 8.0; 1.5 M NaCl; 3 % CTAB; 2% activated charcoal; 1 % PVP and 0.2% β- mercapto ethanol). The quality and quantity of the genomic DNA were checked by spectrophometric method and 0.8 % agarose gel electrophoresis respectively.

RAPD analysis

PCR amplification of RAPD fragments was performed according to Williams *et al*., 1990 using decamers. The reaction mixture (20 µl) contains a final concentration of 50 ng of DNA, 1X PCR buffer, 0.2 mM dNTP's, 5 pM primer and 0.5 U Taq polymerase (Bangalore Genei, India). The PCR amplification was performed in a thermo cycler (Corbet, USA) programmed for 35 cycles with a initial denaturation step at 94ºC for 5 min, followed by 34 cycles of denaturation at 94ºC for 1 min, annealing at 32-36ºC for 1 min and extension at 72ºC for 1 min 30 s and with a final extension step at 72ºC for 7 min. The PCR products were separated on 1.5% agarose gel using 1X TAE buffer by electrophoresis at 100V for 3 hr and visualized and photographed using gel documentation system.

ISSR analysis

PCR amplification was carried out using ISSR primers using a similar method used for RAPD analysis. the PCR steps for initial denaturation was at 94ºC for 5 min, followed by 34 cycles of denaturation at 94ºC for 1 min, annealing for 1 min at varied temperatures as per melting temperature of ISSR primers and extension at 72ºC for 1 min 30 s and with a final extension step at 72ºC for 7 min. The PCR products were separated on 1.5% agarose gel and photographed for further analysis.

Data analysis

The molecular markers (RAPD and ISSR) were studied in duplicates for each individual and only clear, unambiguous and reproducible bands amplified in both cases were considered for scoring the data. A binary matrix was used for scoring the gel as 1 (presence of band) and 2 (absence of band). To avoid taxanomic weighing, the intensity of the band was not considered and only presence and absence of band was scored. The number of polymorphic and monomorphic amplified products was determined for 10 Pongamia samples using each primer.

The efficiency of the primers, polymorphic information content (PIC) as a marker discrimination power was calculated by the formula $PIC=1-\sum P_i^2$, where P_i is the frequency of i^{th} allele at a locus (Anderson *et al*., 1993). Marker index (MI) was calculated to estimate the overall utility of each marker system and was calculated using the formula MI= POL (%) X PIC as according to Sarkhesh *et al*., 2007. The similarity between the indexes was computed with the genetic distances calculated as per the formula by Nei 1978 using TFPGA software to generate a pair wise genetic distance matrix

$$Sab = \frac{2Nab}{Na + Nb}$$

Where, S*ab* is the similarity index between a and b genotypes, N*ab* is the total of positive matching or common DNA bands between a and b genotypes and N*a* and N*b* are the total of DNA bands present in each genotype a and b respectively. A phylogenetic tree was constructed to representing the genetic relationship among the Pongamia ecotypes from North Karnataka, India.

RESULTS

Genomic DNA was extracted from the Pongamia seed samples and run on 0.8 % agarose gel; the extracted DNA was quantified using nanodrop (Eppendrof). The genomic DNA bands after electrophoresis are depicted in Fig. 1 and the quantity and purity of the DNA is as given in Table 1. A good quantity and quality of genomic DNA was extracted from all the Pongamia ecotypes which were further used for PCR fingerprinting studies via RAPD and ISSR.

RAPD analysis

A total of 31 primers were used and only 27 primers generated results for screening the genetic diversity among the CPT's of Pongamia, the remaining primers did not generate any amplification products or banding pattern. The number of amplification products and the number of polymorphic fragment for the primers, percentage of polymorphism are given in Table 2.

TABLE 1. Quantity of genomic DNA extracted from Pongamia seeds ecotypes from North Karnataka region.

CPT's /Provenance	Quantity	260/280
GRP 04 (Yadgiri)	140 µg/ml	1.81
GRP 08 (Koppal)	75 µg/ml	1.80
GRP 09 (Koppal)	110 µg/ml	1.92
GRP 13 (Raichur)	160 µg/ml	1.74
GRP 14 (Bidar)	240 µg/ml	1.80
GRP 16 (Bidar)	83 µg/ml	1.78
GRP 20 (Bellary)	154 µg/ml	1.81
GRP 24 (Gulbarga)	175 µg/ml	1.80
GRP 28 (Gulbarga)	184 µg/ml	1.65
GRP 29 (Gulbarga)	120 µg/ml	1.76

TABLE 2. Degree of polymorphism by RAPD markers, where POL is polymorphism; PIC is average polymorphic information content for polymorphic bands; MI is marker index.

Markers	Primer code	Total number of bands	Number of polymorphic bands	POL (%)	PIC	MI
RAPD	OPC 07	8	2	25	0.34	8.5
	OPAH 15	9	4	44.45	0.38	16.89
	OPAN 01	5	1	20	0.45	9
	OPD 08	6	2	33.34	0.34	11.34
	OPAP 10	8	2	25	0.41	10.25
	OPAB 01	7	2	28.57	0.41	11.71
	OPAF 02	6	1	16.67	0.34	5.67
	OPX 20	4	0	-	-	-
	OPE 08	7	2	28.57	0.34	9.71
	OPE 12	8	3	37.5	0.42	15.75
	OPE 14	6	2	33.34	0.38	12.67
	OPE 16	8	3	37.5	0.36	13.5
	OPI 01	5	0	-	-	-
	OPI 04	7	2	28.57	0.39	
	OPL 11	6	1	16.67	0.42	7
	OPAM 20	7	1	14.29	0.36	5.15
	OPAP 20	8	2	25	0.27	6.75
	OPAN 05	5	0	-	-	-
	OPAA 01	7	1	14.29	0.37	
	OPAB 14	8	2	25	0.23	5.75
	OPAJ 19	4	0	-	-	-
	OPE 07	6	1	16.67	0.42	7
	OPE 09	8	1	12.5	0.48	6
	OPE 13	3	0	-	-	-
	OPE 15	6	1	16.67	0.45	7.5
	OP3 20	8	2	25	0.23	5.75
	OPI 02	9	2	22.23	0.25	5.8
	Total	179	40	22.35	0.26	5.81
	Mean	6.63	1.48	-	-	-

The scored data was used for calculating similarity indices by using the formula of Nei 1978 to generate pair wise matrix. The pair wise matrix of genetic distances was then employed to draw the dendrogram produced by Unweighed Pair Group Method with Arthimetic Averages (UPGMA) to Tool for population genetic analysis (TFPGA) in order to study the precise relationships between the Pongamia ecotypes and also for cluster analysis for grouping the Pongamia ecotypes based on their traits. The primers OPD 08, OPAB 01, OPAJ 19, OPE 15, etc (Fig. 4.2) presented considerable polymorphism when compared to the other primers used for screening the RAPD analysis produced 48 loci among 10 populations of Pongamia which has produced a total of 179 bands were produced and the polymorphism percentage was 22.35 %. The primers varied in detecting the variations among within and between the species as shown in Table 2. Only 5 primers produced monomorphic bands which are OPX 20, OPI 01, OPAN 05, OPAJ 19 and OPE 13, interestingly the primer which produced monomorphic bands have the least number of bands in total and the others have produced polymorphic bands. Bands per primer range from 3 (OPE 13) to 9 (OPAH 15 and OPI 02) with a mean value of 6.63 bands per primer, the moderate valued primers produced 8 bands were OPC 07, OPAP 10, OPE 12, OPE 16, OPAP 20, OPAB 14, OPE 09 and OPE 20 and the primers producing 7 bands were OPAB 01, OPI 04, OPAM 20 and OPAA 01. The number of polymorphic bands ranged with a minimum value of 0 (OPX 20, OPI 01, OPAN 05, OPAJ 19 and OPE 13) and a maximum value of 4 (OPAH 15) while the moderate value of 3 were OPE 12 and OPE 16, there are 11 primers which valued at 2 polymorphic bands and totally they have a mean value of 1.48 bands per primer.

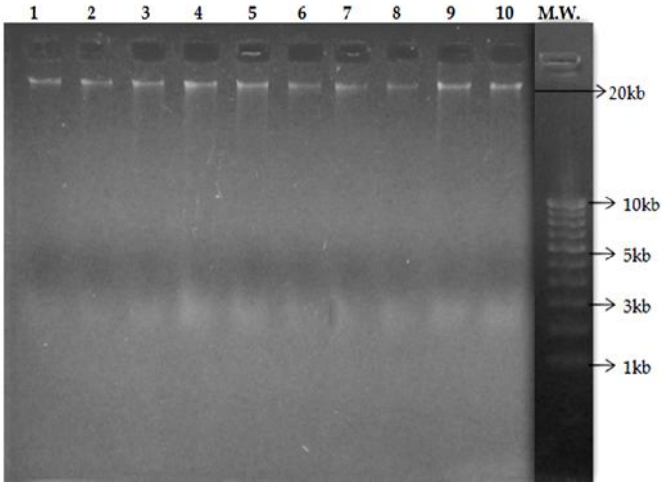

FIGURE 1. Agarose gel electrophoresis of genomic DNA extracted from young seeds of CPT's of Pongamia, where lane 1: GRP 04; lane 2: GRP 08; lane 3: GRP 09; lane 4: GRP 13; lane 5: GRP 14; lane 6: GRP 16; lane 7: GRP 20; lane 8: GRP 24; lane 9: GRP 28; lane 10: GRP 29 and the lane M.W. is 1kb ladder.

The highest percentage of polymorphism was ranged from a minimum of 12.5 % (OPE 09) and a maximum value of 44.45 % (OPAH 15), the moderate value ranging from 25-37.5 % has the highest degree of polymorphism (OPC 07, OPD 08, OPAP 10, OPAB 01, OPE 08, OPE 12, OPE 14, OPE 16, OPI 04, OPAP 20, OPAB 14 and OPE 20, the mean percentage of polymorphism between the 10 CPT's of Pongamia has a value of 22.35 % (Table 2). The least PIC value was 0.23 (OPAB 14 and OPE 20) and the highest was 0.48 (OPE 09) where as the marker index (MI) ranged from 5.67 (OPAF 02) to 16.89 (OPAH 15), most of the primers with moderate MI were valued between 6-12 (OPC 07, OPAN 01, OPD 08, OPAP 10, OPAB 01, OPE 08, OPE 12, OPE 14, OPE 16, OPL 11, OPAP 20, OPE 09 and OPE 15). The amplified product was ranging from 0.2 kb to 1.6 kb in size as shown in Fig. 2.

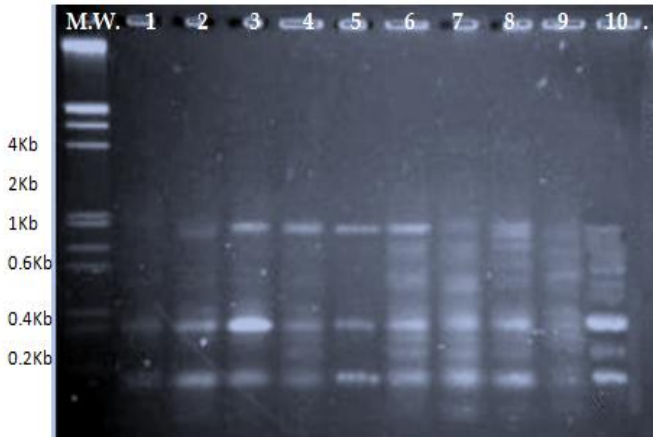

FIGURE 2. RAPD polymorphism profiles of 10 CPT's of Pongamia ecotypes from North Karnataka region, India using OPD 08 RAPD primer.

A phylogenetic tree was constructed using genetic diversity studies by RAPD showed the similarity among the Pongamia ecotypes. The most similar ecotypes were attached by a single node. The Pongamia ecotype GRP 2 and GRP 5 are in a single node representing similarity among the ecotypes. However, the ecotypes have difference compared to other ecotypes. The ecotype GRP 6 and GRP 7 also have least difference and the ecotypes GRP 2, 5, 1 and GRP 3 are the least diverse and meet at a distance of 0.6 in the phylogenetic tree (Fig. 3). The population distance was studied according to Nei, 1978 shows the distance between the Pongamia ecotypes. High similarity was studied between the GRP 5 and GRP 2 with a reading of 0.9583, which showed close relationship according to matrix. However, the most of the other ecotypes have moderate similarity between the ecotypes like GRP 6, 7, 8 and GRP 9 with a value ranging from 0.7 to 0.875. The others were least similar as depicted in the Table 3.

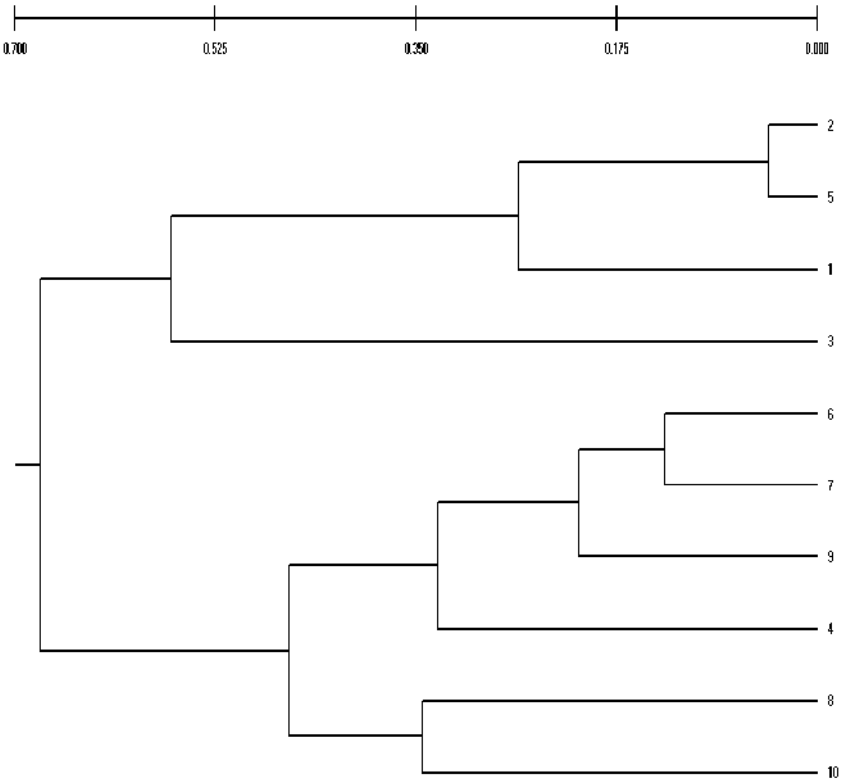

FIGURE 3. Phylogenetic tree constructed based on the RAPD analysis of 10 CPT's of Pongamia from North Karnataka region, India. Where the numbers 1-10 are the Pongamia ecotypes.

TABLE 3. Genetic distances generated by Nei's method using the RAPD data set containing 10 CPTs Pongamia ecotypes from different places of North Karnataka region, India. Where the numbers 1-10 are the Pongamia ecotypes

Population	1	2	3	4	5	6	7	8	9	10
1	1.0									
2	0.75	1.0								
3	0.5833	0.5833	1.0							
4	0.5417	0.7083	0.4583	1.0						
5	0.7917	0.9583	0.5417	0.6667	1.0					
6	0.5417	0.625	0.375	0.75	0.6667	1.0				
7	0.5	0.5833	0.4167	0.7917	0.625	0.875	1.0			
8	0.5	0.5	0.5	0.625	0.4583	0.7083	0.75	1.0		
9	0.5833	0.6667	0.3333	0.625	0.7083	0.7917	0.8333	0.6667	1.0	
10	0.4583	0.375	0.375	0.5	0.3333	0.6667	0.625	0.7083	0.5417	1.0

ISSR analysis

A total of 19 primers were used and only 13 primers produced genetic diversity with good quality banding pattern while the remaining primers did not produce good or stable amplification. The ISSR primers produced 88 loci among 10 CPTs of Pongamia ecotypes with a total of 141 bands were produced with a size ranging from 100 kb to 2.5 kb, only 2 primers HB 12 and HB 13 have produce monomorphic bands, while the other primers have produced polymorphic bands with a mean value of 10.85. The maximum number of bands produced were ranging from 8-15 bands, the least number of bands were produced by the UBC 808, while the highest bands were produced by UBC 811, others primers were ranging between the values of 9-13 which were UBC 809, UBC 815, UBC 818, UBC 836, UBC 840, UBC 841, HB 12, HB 13, HB 14 and HB 15 and most of the primers have a value of 9, however the total number of bands were 22 with a mean value of 1.69 as depicted in Table 4.

TABLE 4. Degree of polymorphism by ISSR markers. Where POL is polymorphism; PIC is average polymorphic information content for polymorphic bands; MI is marker index.

Markers	Primer code	Total number of bands	Number of polymorphic bands	POL (%)	PIC	MI
ISSR	UBC-808	8	2	25	0.23	5.75

UBC-809	9	3	33.34	0.34	11.34
UBC-811	15	3	20	0.43	8.6
UBC-815	11	2	18.18	0.39	7.09
UBC-818	13	3	23.07	0.42	9.69
UBC-836	12	2	16.67	0.47	7.83
UBC-840	9	1	11.11	0.43	4.78
UBC-841	13	2	15.38	0.38	5.84
UBC-848	11	2	18.18	0.41	7.45
HB 14	12	1	8.33	0.48	3.99
HB 13	9	0	-	-	-
HB 15	10	1	10	0.42	4.2
HB 12	9	0	-	-	-
Total	141	22	15.6	0.43	6.7
Mean	10.85	1.69	-	-	-

The percentage of polymorphism ranged between 8-34 % with a mean value of polymorphism was 15.6 %, the least polymorphism was exhibited by primer HB 14 and the highest was exhibited by the primer UBC 809, the other primer with moderate values were UBC 808, UBC 20 and UBC 818 with the values of 25, 20 and 23.07 respectively. The PIC value was ranging between the values of 0.23 to 0.48 and a mean value of 0.43, the least value of 0.23 was exhibited by the primer UBC 808 while the highest value of 0.48 was exhibited by the primer HB 14. The primers UBC 809, UBC 811, UBC 815, UBC 818, UBC 836, UBC 840, UBC 841 and HB 15 are the moderate valued primers ranged at 0.3 to 0.45. The MI value was exhibited to be ranging between the values of 3.99 to 11.34 and a mean value of 6.7, the least value was exhibited by the primer HB 14 while the highest value was exhibited by the primer UBC 809 (Table 4). The other primers with moderate values were ranging between the values 7-10 with the primers UBC 811, UBC 815, UBC 818, UBC 836 and UBC 848. The amplified product was ranging from 0.1 kb to 2.5 kb in size as shown in Fig. 4.

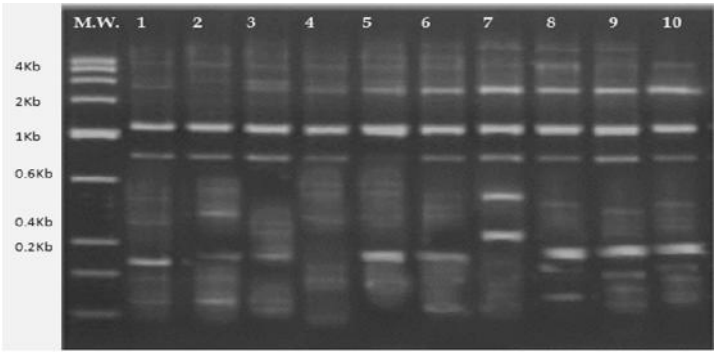

FIGURE 4. ISSR polymorphism profiles of 10 CPT's of Pongamia ecotypes from North Karnataka region, India using UBC 809 ISSR primer.

According to the phylogenetic tree analysis constructed based on ISSR marker studies, the Pongamia ecotype GRP 4 and GRP 5 are most similar thus are in a single node and are close to GRP 2. The populations GRP 4, 5 and GRP 2 are attached to next close group GRP 1, 3 and GRP 6 at a distance of ~0.2 in the phylogenetic tree. The ecotypes GRP 9, 10 are attached by a single node and close to GRP 8 and are grouped by other group of GRP 4, 5 and GRP 2 at a distance of ~0.3. However, all the ecotypes are similar and meet at a distance of ~0.5 as depicted in Fig. 5. The genetic distances scored according to Nei's 1978 showed similarity values among the Pongamia ecotypes as depicted in phylogenetic tree based on ISSR marker system. A high similar value was read between GRP 1and GRP 3 and between GRP 4 and GRP 5 at 0.9091, there are many moderate values ranging from 0.89 to 0.7 where most of the ecotypes coincide in the value range. However, there are few values showing low similarity among the Pongamia ecotypes such as GRP7 shows low similarity with all the other ecotypes (horizontally) with a value ranging from 0.5 to 0.64 as depicted in Table 5.

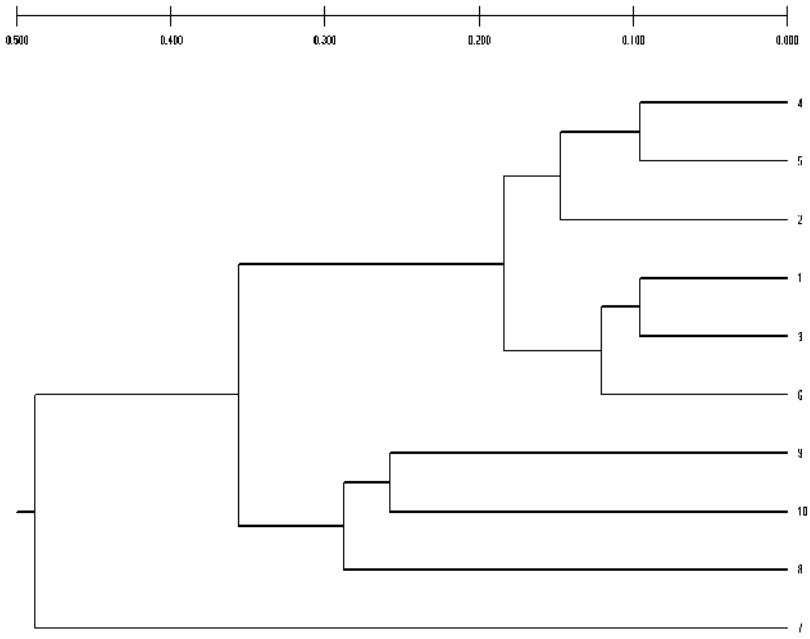

FIGURE 5. Phylogenetic tree constructed based on the ISSR analysis of CPT's of Pongamia from North Karnataka region, India, where the numbers 1-10 are the Pongamia ecotypes.

TABLE 5. Genetic distances generated by Nei's method using the ISSR data set containing 10 CPTs Pongamia ecotypes from different places of North Karnataka region, India. Where the numbers 1-10 are the Pongamia ecotypes.

Population	1	2	3	4	5	6	7	8	9	10
1	1.0									
2	0.8864	1.0								
3	0.9091	0.7955	1.0							
4	0.8636	0.8864	0.7727	1.0						
5	0.8182	0.8409	0.7727	0.9091	1.0					
6	0.8864	0.8636	0.8864	0.8409	0.8864	1.0				
7	0.6136	0.5	0.6136	0.5682	0.6136	0.6364	1.0			
8	0.7955	0.7273	0.7955	0.6591	0.7045	0.8182	0.7273	1.0		
9	0.6818	0.7045	0.6364	0.6818	0.6364	0.7045	0.6136	0.75	1.0	
10	0.7273	0.6591	0.7273	0.6818	0.6364	0.6591	0.6591	0.75	0.7727	1.0

DISCUSSION

The genetic diversity and degree of heritability are the major factors in a tree improvement program as genetic variation is the fundamental requirement for maintenance and long term stability of forest ecosystems. A wide range of genetic diversity was studied among the Pongamia ecotypes in North Karnataka region with respect to seed and oil traits (Patil *et al*., 2015). Morphological and biochemical markers are limited to number as they are interacting with environment, epistasis, and are largely controlled by genetic traits (Smith and Smith, 1989). But, the dominant marker systems will reveal the genetic relationships at early stage of plant growth, which are not linked with environmental or development stages thus genetic markers make an ideal tool for studying genetic relationship (Reddy *et al*., 2002). Similar studies of genetic diversity have been studied in various other places in India and in other countries. The genetic improvement program of Pongamia by maintaining the high genetic diversity is the most important issue as the species has multipurpose importance. The RAPD analysis produced 48 loci among 10 CPTs of Pongamia which has produced a total of 179 bands were produced and the polymorphism percentage was 22.35%. The ISSR primers produced 88 loci among 10 populations of Pongamia ecotypes with a total of 141 bands were produced with a size ranging from 100 kb to 2.5 kb and a polymorphism of 15.6 %. Sahoo *et al*., 2009 used ISSR markers to access the genetic structure using 226 individuals of Pongamia encompassing 7 populations from different geographical regions of Orrisa. Sharma *et al*., 2010 have assessed about 20 random individuals of Pongamia using 2 dominant markers. The use of seeds instead of leaves for studying the genetic diversity saves time and is less expensive, the used of seeds for RAPD marker study is also a stable manner of varietal identification and genetic diversity as already been reported (McDonald *et al*., 1994 and Fu *et al*., 2004). A high level of variability in Pongamia ecotypes was studied on the basis of morphological characters such as pod and seeds traits, biochemical and oil traits in seeds in North Karnataka region, however, low level of genetic diversity has been studied by RAPD (22.35 %) and ISSR (15.6 %) markers. Similar kind of low level of genetic variation was detected in other woody perennial species such as *Populus* and *Isotoma* (Yeh *et al*., 1995 and Bussell 1999).

The screening of Pongamia using ISSR primers result in repeated motifs of dinucleotide at higher frequencies along the genome similar to crops such as bean, maize, soybean and rice, similar observations were studied in *Swertia chirayita* (Joshi and Dhawan, 2007) and *Gerbera* (Bhatia *et al*., 2009). PIC analysis can also be used to evaluate markers to select most appropriate marker for genetic mapping and phylogentic analysis (Anderson *et al*., 1993). PIC values with MI values are used to assess the information of the markers earlier in Jatropha (Tatikanda *et al*., 2009). A low level of polymorphism was revealed using UPGMA by studying the RAPD and ISSR marker system at DNA level and least difference was observed in dendrogram topologies. However, both the markers amplify different regions of genome thus revealed differences among the two dendrograms based on the individual sets. Similarity was observed between the neighbor joining tree with similar traits in morphological and biochemical trait studies.

Duminil *et al*., 2007 reported the population genetic structure and species level trait study in seed plants were key variables in evolutionary biology. Kaushik *et al*., 2007 reported the gene flow of Pongamia is limited to basis of morphological characters such as pod and seed traits. However, the UPGMA phylogenetic tree correlates the genetic and geographical distances among the Pongamia populations. Similar kind of moderate results were used for studying the attributes of a number of factors including adaptation of genetic systems in small populations, genetic systems in small populations. These are limited to gene flow because of the combination of wind pollination and high inbreeding rate (Maguire and Sedgley, 1977).

In our present study, the identified 10 CPTs of Pongamia ecotypes were studied for molecular diversity using RAPD and ISSR marker system. The RAPD analysis produced 48 loci among 10 populations of Pongamia which has produced a total of 179 bands were produced and the polymorphism percentage was 22.35%. The ISSR primers produced 88 loci among 10 populations of Pongamia ecotypes with a total of 141 bands were produced with a size ranging from 100 kb to 2.5 kb and a polymorphism of 15.6%. However, the studies showed least similarity among the Pongamia ecotypes, but the phylogenetic tree showed variable distances among the ecotypes. This study can be used for tree improvement in Pongamia via molecular marker system.

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