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Analysis of Genetic Diversity in Pongamia [*Pongamia pinnata* (L) Pierrre] using AFLP Markers

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In recent years, Pongamia has been considered as important renewable source of biodiesel, however not much molecular information is available in this species. Molecular characterization of this legume tree will enhance our understanding in improving the optimal yields of oil through breeding and enable us to meet the future demands for biodiesel. To assess the molecular genetic diversity in 48 *Pongamia pinnata* accessions collected from six different states of India, amplified fragment length polymorphism (AFLP) marker system was employed. Five AFLP primer combinations produced 520 discernible fragments, of which 502 (96.5%) were polymorphic. AFLP primer informativeness was estimated evaluating four parameters namely polymorphism information content (PIC), effective multiplex ratio (EMR), marker index (MI) and resolving power (RP). In total, 51 unique fragments were detected of which 19 unique fragments were observed with primer combination E-ACG / M-CTA. Although neighbour joining (NJ) method did not group accessions strictly according to their region of collection, a good level of genetic diversity was observed in examined germplasm. However, accessions collected from Karnataka showed comparatively higher diversity than accessions from other states. The diverse accessions identified in this study may be useful in *Pongamia pinnata* improvement to meet the future demands of biodiesel.

***Key words*:** *Pongamia pinnata*, AFLP, molecular diversity, biofuel, polymorphism.

Pongamia [*Pongamia pinnata* (L) Pierrre] is a leguminous deciduous tree, commonly known as Indian Beech, Pongam, Honge and Karanj, grows about 15-25 m tall and is well-adapted to semi-arid and humid zones and can be grown up to 1000 m above sea level. *Pongamia pinnata* is indigenous to the Indian subcontinent and has been successfully introduced to humid tropical regions of the world as well as parts of Australia, New Zealand, China and the United States (1, 2). Cytogenetics studies have suggested a chromosome number for *Pongamia pinnata* of either $2n = 20$ (3) or $2n = 22$ (4). Various parts of *Pongamia pinnata* have been considered as valuable source and are used for a variety of applications in agriculture and medicine. For instance, extracts from bark, leaves and seeds are effective against the cluster caterpillar (*Spodoptera litura*) and the insect pests of stored products, *Trogoderma granarium* and *Tribolium castaneum* (1, 5). A methanolic fraction from the seed oil exhibited the greatest toxicity towards *S. litura* and *T. granarium*, while leaf extract was most toxic towards *T. castaneum*. Nematicidal activity from seed extracts has been demonstrated against the root-knot nematode *Meloidogyne incognita* (6, 7). In traditional medicine systems like Ayurvedha and Siddha, *Pongamia pinnata* was used for the treatment of clinical lesions of skin and genitalia. It was also evaluated for antiviral properties against herpes simplex virus type-1 (HSV-1) and type-2 (HSV-2) by *in vitro* studies in Vero cells (8).

Rapid depletion of fossil fuels necessitates the search for alternative potential sources of energy. Among the alternative non-conventional energy sources, one of the renewable energy sources is non-edible vegetable oils. Pongamia is a promising oil-crop with immense potential for exploitation as a source for biodiesel (9-11). Pongamia oil is non-edible due to the presence of toxic flavonoids like karanjin, pongapin and pongaglabrin (10). The seeds contain 30 to 40% oil (12-14), which can be converted into biodiesel (fatty acid methyl esters; FAMEs) by esterification

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Abbreviations: AFLP- Amplified fragment length polymorphism, EMR- Effective multiplex ratio, FAME- Fatty acid methyl esters, MI- Marker index, PIC- Polymorphism information content, RP- Resolving power, SVO- Straight vegetable oil.

with methanol in the presence of KOH or straight vegetable oil (SVO) is also used as fuel in diesel generator sets (1, 9).

Several molecular markers have been widely used to assess the genetic diversity and phylogenetic studies in a number of legume taxa like *Medicago* (15), *Atylosia-Cajanus* complex (16) and *Acacia* (17). However, there is no information available on the genetic diversity of *Pongamia pinnata,* although biological and biochemical properties and genetics of the plant have been extensively reviewed (2). AFLP marker system (18) is the most suitable marker system for conducting genetic diversity analysis for tree species like *Pongamia pinnata,* where no molecular genetics research has been done and not much sequence information is available. Genetic improvement for enhanced biomass, oil yield and stress tolerance depends on selection and maintenance of elite *Pongamia pinnata* accessions. A number of *Pongamia pinnata* accessions have been collected from several states of India and the present study was undertaken to assess the genetic diversity among 48 selected *Pongamia pinnata* accessions using AFLP marker system.

Materials and Methods

***Plant material* —** Forty-eight accessions of *Pongamia pinnata* representing different geographical locations from six states of India were analyzed for genetic diversity study (Table 1). Further, these accessions also differ for the oil content in the seeds and the seed weight.

***DNA isolation* —** Three grams of young leaf tissue was harvested from ten individual plants and bulked for each accession. DNA was isolated from the leaf tissue as per Tatikonda *et al* (19). The quantity of the DNA was estimated using UV-spectrophotometer and quality was checked on 0.8% agarose gel.

AFLP analysis — AFLP analysis was performed using standard protocol (18) with minor modifications. Restriction digestion, ligation, pre- and selective amplification was carried out as per Tatikonda *et al* (19).

Data analysis — To analyse AFLP marker data, we assumed that each AFLP amplified product, regardless of its relative intensity, corresponds to a dominant allele at a unique locus. Polymorphic amplified fragments were scored manually as '1' for the presence and '0' for the absence of an allele at a particular locus across all the 48 genotypes for each primer combination. Only reproducible and clearly distinct bands were scored for data analysis.

Binary data obtained for the AFLP primer combinations was used for assessing the discriminatory power of AFLP primer combinations by evaluating four parameters as follows:

a) Polymorphism Information Content (PIC) for each AFLP fragment was calculated as proposed by Roldan-Ruiz *et al* (20), b) Number of polymorphic fragments in the germplasm set of interest, analyzed per experiment, called effective multiplex ratio (E), was estimated as mentioned in Varshney *et al* (21), c) Marker index, a product of information content, as measured by PIC, and effective multiplex ratio (E), was calculated following Powell *et al* (22), d) Resolving power (RP) of each primer combination was calculated according to Prevost and Wilkinson (23)

***Construction of dendrogram* —** The 1/0 matrix of the marker was used for the calculation of genetic similarity (24). Further, dendrogram was constructed using neighbour joining (NJ) algorithm in PAUP 4.0 (25) and Dendroscope (26). To find the robustness of dendrogram, bootstrapping was carried out for 48 accessions using 1000 replicates with PAUP Win 32 software (4.0 beta version) (27).

Results and Discussion

To the best of our knowledge, this report is the first successful study for the assessment of genetic diversity employing AFLP technique in *Pongamia pinnata,* a potential biofuel plant. AFLP is an information rich marker system due to its ability to generate a large number of polymorphic/informative loci simultaneously in a single genotype with a single primer combination as compared to RAPDs (random amplification of polymorphic DNAs), RFLPs (restriction fragment length polymorphisms) and microsatellites (22, 27, 28)**.** In the present study, a set of 48 *Pongamia pinnata* accessions representing six states of India (Table 1) were analyzed with five AFLP primer combinations (Table 2). Similarly, AFLP marker system has been successfully used to investigate the diversity studies in biofuel plant, *Jatropha* (19, 29).

***Marker polymorphism* —** An efficient molecular marker can detect high polymorphism among the accessions. Screening of 64 AFLP primer combinations on four accessions has generated quality polymorphic fragments. Among these 64 primer combinations, only 5 AFLP primer combinations were selected and further investigated on

Table 1. Details about forty eight *Pongamia pinnata* accessions used for genetic diversity analysis

	Genotype[a]	Accession identity	Place of collection	State of collection[b]
1	P-1	P-1	Bombay	MH
2	P-2	P-2	Goa	GA
3	P-3	P-3	Orissa	OR
4	P-5	P-5	Adilabad	AP
5	P-6	P-6	Behranguda	AP
6	P-7	P-7	Gurgaon	HR
7	P-8	P-8	Gurgaon	HR
8	P-9	P-9	Gurgaon	HR
9	IPC2A	IPC-2A	Alliguda	AP
10	IPC3A	IPC-3A	Alliguda	AP
11	IPC4A	IPC-4A	Alliguda	AP
12	IPC5A	IPC-5A	Alliguda	AP
13	IPC6A	IPC-6A	Alliguda	AP
14	IPC8A	IPC-8A	Alliguda	AP
15	IPC12A	IPC-12A	Alliguda	AP
16	IPC13A	IPC-13A	Alliguda	AP
17	IPC10A	IPC-10A	Alliguda	AP
18	IPC11A	IPC-11A	Alliguda	AP
19	IPC15A	IPC-15A	Alliguda	AP
20	IPC16M	IPC-16M	Mahabubnagar	AP
21	IPC18K	IPC-18K	Kadiri	AP
22	IPC20M	IPC-20M	Mahabubnagar	AP
23	IPC-19M	IPC-19M	Mahabubnagar	AP
24	Kengal	KN-1	Kengal	KA
25	Maagadi	KN-2	Maagadi	KA
26	Basanthapura	KN-3	Basanthpura	KA
27	Malvalli	KN-4	Malvalli	KA
28	Tumkur	KN-5	Tumkur	KA
29	Ketohalli	KN-6	Ketohalli	KA
30	Maiganhalli	KN-7	Maiganhalli	KA
31	Tarapur	KN-8	Tarapur	KA
32	K.M.Doddi	KN-9	K.M.Doddi	KA
33	Kempnahalli	KN-10	Kempnahalli	KA
34	Kilancha	KN-11	Kilancha	KA
35	M.K.Doddi	KN-12	M.K.Doddi	KA
36	Thalli	KN-13	Thalli	KA
37	Hoskote	KN-14	Hoskote	KA
38	Madapura	KN-15	Madapura	KA
39	Hosur	KN-16	Hosur	KA
40	Kunigal	KN-17	Kunigal	KA
41	APHYHY1	APHYHY 1	Mulugu	AP
42	APHYHY3	APHYHY 3	Mulugu	AP
43	APHYHY5	APHYHY 5	Mulugu	AP
44	APHYHY6	APHYHY6	Mulugu	AP
45	IISC2	IISC 2	Mulugu	AP
46	IISC3	IISC 3	Mulugu	AP
47	BIRD1	BIRD 1	Mulugu	AP
48	BIRD2	BIRD 2	Mulugu	AP

[a]the names of genotypes 'Basanthapura' and 'Kempnahalli' in the dendrogram (Fig. 2) appear/denoted as 'Basanthapu' and 'Kempnahall' respectively; [b]MH=Maharashtra; GA=Goa; OR=Orissa; AP=Andhra Pradesh; HR=Haryana; KA=Karnataka

Table 2. Details about adapters and primers used for AFLP analysis

Name	Code	Sequence[c]
*Eco*RI adapter		5′-AATTGGTACGCAGTCTAC-3′ 3′-CCATGCGTCAGATGCTC-5′
*Mse*I adapter		5′-TACTCAGGACTCAT-3′ 3′-GAGTCCTGAGTAGGAG-5′
*Eco*RI primer		5′-GTAGACTGCGTACCAATTCA-3′
*Mse*I primer		5′-GATGAGTCCTGAGTAAC-3′
*Eco*RI+ 3-ACG	E-ACG	5′-GTAGACTGCGTACCAATTC*ACG*-3′
*Eco*RI+ 3-ACC	E-ACC	5′-GTAGACTGCGTACCAATTC*ACC*-3′
*Eco*RI+ 3-ACT	E-ACT	5′-GTAGACTGCGTACCAATTC*ACT*-3′
*Eco*RI+ 3-ACA	E-ACA	5′-GTAGACTGCGTACCAATTC*ACA*-3′
*Mse*I+ 3-CAT	M-CAT	5′-GATGAGTCCTGAGTAA*CAT*-3′
*Mse*I+ 3-CAA	M-CAA	5′-GATGAGTCCTGAGTAA*CAA*-3′
*Mse*I+ 3-CTA	M-CTA	5′-GATGAGTCCTGAGTAA*CTA*-3′
*Mse*I+ 3-CTT	M-CTT	5′-GATGAGTCCTGAGTAA*CTT*-3′
*Mse*I+ 3-CTA	M-CAG	5′-GATGAGTCCTGAGTAA*CAG*-3′

[c]Nucleotides in italics are for selective amplification

48 accessions. High quality marker profiling was obtained on all 48 *Pongamia pinnata* accessions in the present investigation. These five primer combinations generated an average of 104 fragments, of which 101 fragments showed polymorphism. This indicates the power of AFLP marker system that provides a large number of genetic fragments per experiment, presumably covering the entire genome. Indeed, use of AFLP marker system in those species where no prior sequence information is available, is highly appreciated (18).

The frequencies of the polymorphic fragments for a given AFLP primer combination across 48 accessions, ranged from 0.02 to 0.99 with an average of 0.69. A large proportion (46.34%) of AFLP fragments had frequencies in the range of 0 to < 0.20 (Fig. 1a). Similar results were also reported in *Jatropha* (19) and *Sinapis alba* (30). The level of polymorphism detected by AFLP in the present study (96.5%) is comparable to the other AFLP diversity

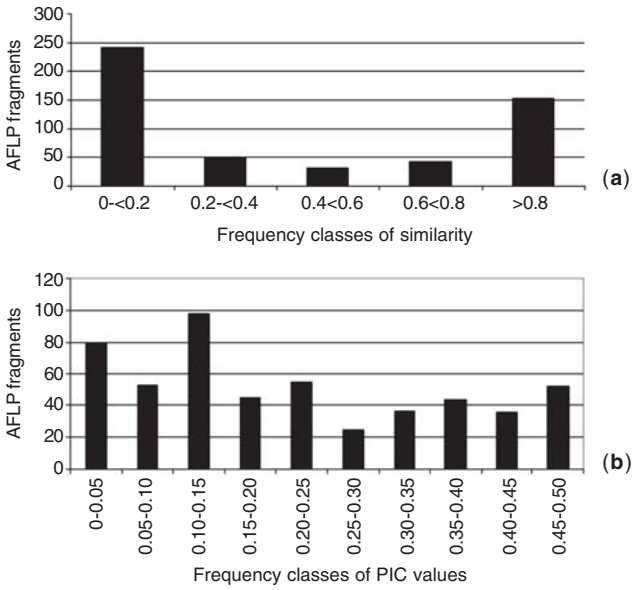

Fig 1. Frequency and PIC distribution of AFLP markers (**a**) Frequency distribution for polymorphic AFLP fragments in *Pongamia pinnata* germplasm collection and (**b**) PIC distribution for polymorphic fragments generated by AFLP primer combinations

study (97.2%) to understand genetic divergence and phylogenetic analysis of genus *Jatropha* (19). The pair wise genetic similarity among 48 *Pongamia pinnata* accessions ranged from 0.28 to 0.90 with an average of 0.61, suggesting that the current collection preserved the vast majority of the natural variation in *Pongamia pinnata*. High percentage of polymorphic fragments (96.5%) with five primer combinations were obtained in the present study compared to *Jatropha* (88.2%; 19) and common bean (74%; 31).

Unique AFLP fragments — Fifty one unique accession specific fragments identified in the present study can serve as genetic markers in identifying these accessions for plant improvement. Two primer combinations namely E-ACT / M-CTT and E-ACG / M-CTA were found to be most effective to detect higher number of unique fragments, 12 and 19, respectively. However, no unique fragments were detected by any of the primer combinations in the germplasm from Goa and Orissa states. Most likely this is due to the use of only single accession from these two states. Interestingly, in total, all primer combinations produced unique fragments in P-8, an accession from Gurgaon of Haryana.

Marker attributes — Different marker attributes like PIC, MI and RP were used in several studies to assess the informativeness or discriminatory power of the primer combinations for genetic diversity studies.

Polymorphism information content (PIC) — The PIC values for 502 polymorphic fragments varied from 0.04 to 0.50 with an average of 0.22 per fragment. Majority of the fragments (96) showed PIC value between 0.10-0.15, while 52 fragments showed the PIC value between 0.45-0.50 (Fig.1b). In terms of the PIC value of the primer combination, it ranged from 0.18 (E-ACT / M-CTT) to 0.26 (E-ACA / M-CAG) with an average of 0.22 (Table 3). PIC was used extensively in majority of diversity/marker studies (18, 19, 27, 31). Moderate levels of PIC values for AFLP primer combinations in the study can be attributed to either the diverse nature of germplasm or highly informative AFLP primer combinations used in this study. Among the different primer combinations, E-ACA / M-CAG (overall PIC value 0.26), is recommended for analyzing genetic diversity of different germplasm collections of *Pongamia pinnata*.

Marker index — Marker index (MI) is the marker attribute used to calculate the overall utility of a marker system and is the product of PIC and effective multiplex ratio. The MI ranged from 14.14 (E-ACT / M-CTT) to 20.23 (E-ACA / M-CAG). A positive correlation was observed between MI and PIC value ($r^2 = 0.99$; $p < 0.005$). Marker index (MI) together with PIC value has been used to assess the discriminatory power of the AFLP primer combinations in several crop species including soybean (PIC = 0.32, MI = 6.14) (20), wheat (PIC = 0.32, MI = 3.41) (31), corn salad (PIC = 0.25, MI = 4.47) (33), etc. In the present investigation, MI values were reported in the range of 14.14 to 20.23 (average 16.83) and PIC in the range of 0.18-0.26 (average 0.22) which are comparable to the earlier diversity studies. Discrimination of as many accessions as possible would be the most important feature of a given primer combination, when the purpose of the study is to distinguish accessions analyzed (24).

Effective multiplex ratio and resolving power — Along with PIC and MI value, Prevost and Wilkinson (23) used another attribute called resolving power (RP) to compare the informativeness of AFLP. The higher number of fragments per primer combination is the feature of AFLP that provides higher EMR to AFLP. There was no significant difference in the effective multiplex ratio of all five primer combinations however this feature varied from 74.40 to 78.57 (Table 3). Besides, to understand the marker informativeness of RFLP, AFLP, RAPD and SSR marker systems, RP was used as potential marker attribute (22). The RP is a feature of the primer combination that indicates the discriminatory potential of the primer combination. The

Table 3. Features of AFLP diversity obtained in 48 *Pongamia pinnata* accessions

Primer combinations	Total fragments	Monomorphic fragments	Polymorphic fragments	Unique fragments	% Polymorphism	PIC[d]	EMR[e]	MI[f]	RP[g]
E-ACG / M-CAT	82	2	80	7	97.56	0.24	78.05	18.73	25.03
E-ACC / M-CAA	100	7	93	8	93.00	0.21	74.40	15.62	27.62
E-ACG / M-CTA	115	4	111	19	96.52	0.20	77.22	15.44	31.04
E-ACT / M-CTT	112	2	110	12	98.21	0.18	78.57	14.14	25.33
E-ACA / M-CAG	111	3	108	5	97.29	0.26	77.84	20.23	38.46
Minimum	82	2	80	5	93.00	0.18	74.40	14. 14	25.03
Maximum	115	7	111	19	98.21	0.26	78.57	20.23	38.46
Average	104	3.6	101.6	10.20	96.52	0.22	77.22	16.83	29.50
Total	520	18	502	51					

[d]PIC=polymorphism information content; [e]EMR=effective multiplex ratio; [f]MI=marker index; [g]RP=resolving power

resolving power varied from 25.03-38.46 with an average of 29.49. The resolving power of the primer combination was high for E-ACA / M-CAG (38.46). A strong and linear relationship between the ability of a primer combination to distinguish accessions and RP was observed (22, 32), hence the primer combination E-ACA / M-CAG with the highest RP value (38.46) should be the most informative primer combination for distinguishing the accessions. The RP during the present study is comparatively higher than earlier AFLP studies (19, 33). Infact as compared to RAPD and SSR markers, the resolving power was found highest for AFLP primer combination in our study (29.5) as compared to RAPD (5.6) and ISSR (7.0) markers (34). The MI and RP are comparatively higher than the earlier reports (21, 33). Although a very strong and positive correlation was observed between PIC value and MI ($r^2 = 0.99$, $p < 0.005$), therefore confirming results of some earlier studies (23, 35), but no correlation was observed between RP and MI.

No specific relation was observed among the PIC, EMR, MI and RP. Lack of correlation among PIC, MI, RP was also reported by Laurentin and Karlovsky (33). This indicates that probably a single parameter is not a good indicator to assess the informativeness of the primer combinations.

Genetic relationships among Pongamia accessions — Genotyping data obtained for all the five primer combinations were used to evaluate and understand genetic diversity in the germplasm analyzed. The neighbour joining tree based on 502 AFLP fragments grouped 48 *Pongamia pinnata* accessions into four major clusters (Cl I, Cl II, Cl III and Cl IV) (Fig. 2). High bootstrap values indicate that the grouping of the accessions is reliable. Variable number of accessions were present in three clusters i.e. Cl I (12 accessions), Cl II (13 accessions), Cl IV (13 accessions) while Cl III has only 8 accessions and 2 accessions were not grouped in any cluster. Further, Cl I is sub-grouped into Cl A with 3 accessions (P-2, IPC-5A and P-9) and Cl B with 9 accessions (P-5, Kengal, M.K.Doddi, Hosur, Maiganhall, Hoskote, Maagadi and IPC13A). While 13 accessions in Cl II were subgrouped into Cl IIA with 8 accessions (IPC-2A, IPC-12A, IPC-11A, IPC-15A, IPC-16M, IPC-4A, IPC-6A and IPC-8A) and Cl IIB with 5 accessions (IPC-3A, IPC-10A, IPC-18K, IPC-20M and IPC-19M).

The Cl III has 8 accessions (P-3, P-7, KN-6, Ketohalli, Thalli, Madapura, Kilancha, BIRD2 and P-6). Cl IV is subgrouped into 3 groups: Cl IVA with 6 accessions (Malvalli, APHYHY6, Tumkur, APHYHY1, APHYHY5 and IISC2), Cl IVB with 3 accessions (APHYHY3, IISC3 and BIRD1) and Cl IVC with 4 accessions (Tarapur, K.M.Doddi, Kempnahalli and Kunigal). Higher bootstrap (99%) was observed between the accessions IISC3 and BIRD1, from Karnataka. Similarly, higher bootstrap values were observed among different clusters are indicated (56-99%) on the nodes of the dendrogram (Fig. 2). Region specific grouping was however, limited to Cl II only, as all the genotypes (13) in this cluster have come from Andhra Pradesh (IPC series) (Table 1). The IPC series accessions shared more homology and were grouped in Cl II collected from Alliguda, Andhra Pradesh, and for instance the genetic similarity between IPC-4 and IPC-6 was 0.84, interestingly more genetic similarity is recorded between KN-8 and KN-9 (0.90) (data not shown) from Karnataka. The remaining 2 IPC series accessions, IPC-5A and IPC-13A, from Andhra Pradesh were grouped in Cl IA and Cl 1B, respectively. All

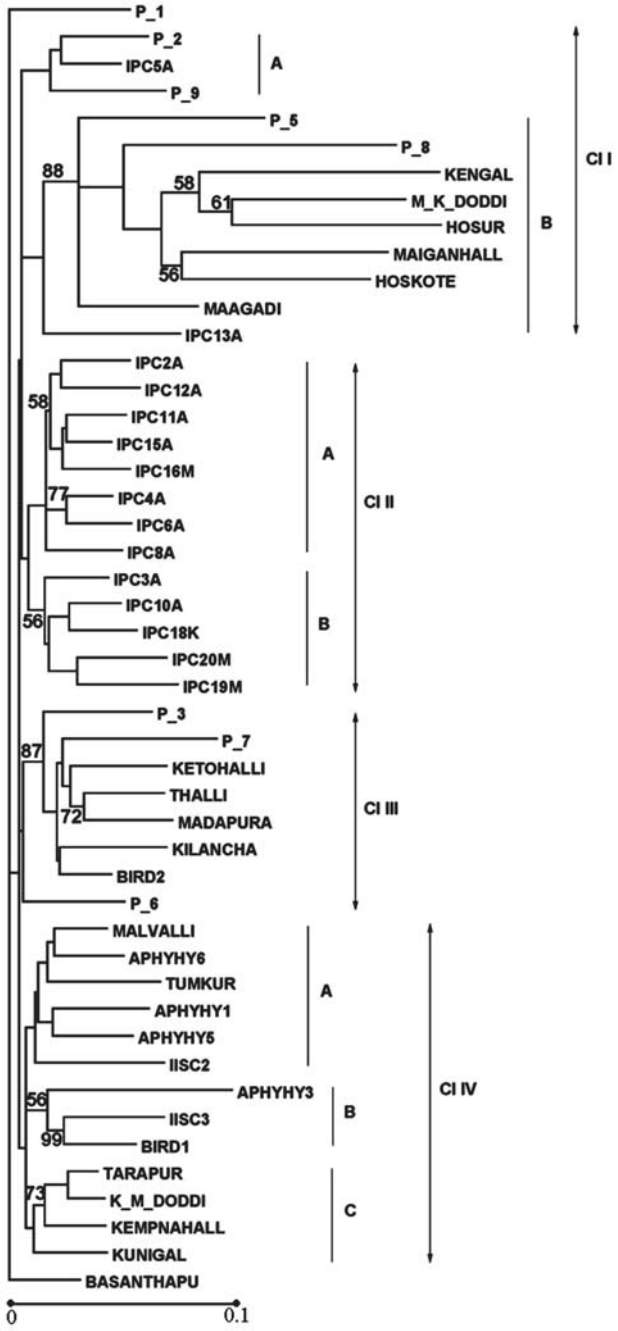

Fig 2. Dendrogram for 48 *Pongamia pinnata* accessions constructed based on neighbour joining algorithm using 502 AFLP fragments

accessions in Cl II B, has relatively low diversity and sharing more degree of similarity among the accessions which have come from Alliguda (Andhra Pradesh). The KN accessions (17) were scattered in all the clusters (Cl I, Cl III and Cl IV) except Cl II. However, majority of KN series accessions coming from Karnataka state were grouped in Cl IVA and Cl IVB. Interestingly, all four APHYHY accessions from Mulugu (Andhra Pradesh) were grouped together with KN accessions (Karnataka) (Fig. 2) that indicates the close relationships of these four accessions with accessions from Karnataka as compared to Andhra Pradesh. Similarly four accessions IISC2, IISC3, BIRD1 and BIRD2 were grouped along with accessions from Karnataka. The dendrogram showed highest genetic similarity (0.90 similarity coefficient) between KN-8 and KN-9 that have come from Karnataka. As the pedigree data are not available on the accessions analyzed, the higher genetic similarity indicates the higher probability of origin of all these accessions (IPC series) from the same source (Alliguda, Andhra Pradesh).

Among three accessions examined from Haryana, P9 was grouped in Cl IA, P8 in Cl IB and P7 in Cl III. This indicates the high genetic diversity among these accessions from Haryana state. Similarly, the Cl IA was found a diverse sub-cluster with accessions grouped from different states i.e. Goa (P2), Andhra Pradesh (IPC5A) and Haryana (P9). It is also interesting to note that out of 48 accessions examined, 2 accessions namely P1 (Maharashtra) and Basanthpura (Karnataka) did not group into any of the four clusters, and they remained on the two extremes of the dendrogram (Fig. 2).

Although comparable number of accessions from Karnataka state were included in the diversity, but these accessions didn't show any significant grouping. This indicates that probably Karnataka accessions are more diverse as compared to Andhra Pradesh accessions. Interestingly, all four APHYHY accessions from Mulugu (Andhra Pradesh) were grouped together with KN accessions (Karnataka), indicating close relationships of these four accessions with accessions from Karnataka as compared to Andhra Pradesh. Similarly four accessions IISC2, IISC3, BIRD1 and BIRD2 were grouped along with accessions from Karnataka. Indeed these accessions were originally present at IISC, Bangalore and later these were planted in the Forest Academy, Mulugu, Andhra Pradesh. It is also interesting to note that out of 48 accessions examined, 2 accessions {P1 (Maharashtra) and Basanthpura (Karnataka)} did not group into any of the four clusters, and they remained on the two extremes of the dendrogram. This indicates that these accessions showed high genetic dissimilarity than the other accessions analyzed during the present study. A high level of genetic polymorphism is expected for an out-crossing species like

Pongamia pinnata and is comparable with those earlier reports (31, 36). The high level of polymorphism suggests the wide genetic diversity among *Pongamia pinnata* accessions analyzed. Results of the present study also showed that only few AFLP primer combinations are sufficient to discriminate various germplasm accessions, if extensive diversity exists among taxa or genus.

The wide range (0.28 to 0.90) of pair wise genetic similarity between the accessions indicates the existence of higher genetic diversity among the accessions examined. The results of genetic diversity study provide estimates on the level of genetic variation among diverse materials that can be used in germplasm management, protection and improvement. Average genetic similarity recorded as 0.61 suggests that the current collection preserved the vast majority of the natural variation in *Pongamia pinnata*. Further, the accessions P1 and Basanthapura with least genetic similarity 0.28 were on the extremes of the dendrogram without being grouped into any of the clusters, indicating that these genotypes are extremely diverse than other accessions analyzed during the present study. Accessions from Karnataka state showed significant diversity among all the 48 accessions, indicating that these accessions are more diverse than the Andhra Pradesh accessions. However, comparatively narrow range of genetic similarities was reported by Gupta *et al* (34) using RAPD and ISSR markers and Sun *et al* (29) using AFLP and SSR markers in *Jatropha*.

In conclusion, this study indicates that AFLP is an efficient marker system for genetic diversity studies in *Pongamia pinnata.* All the five primer combinations showed more or less same informativeness. In general, a good level of genetic diversity was observed in examined germplasm collection of *Pongamia pinnata*. However, accessions from Karnataka showed comparatively higher diversity than accessions from Andhra Pradesh. Some accessions, from Andhra Pradesh showed higher similarity with accessions from Karnataka thus may indicate that these accessions are originally from Karnataka. The diverse accessions identified during present study may be utilized in various crossing programs and therefore, should be useful in *Pongamia pinnata* improvement. Furthermore, some of the AFLP assays, generated genotype-specific fragments in some cases, and therefore, may be useful for precise identification of the corresponding accessions for the establishment of proprietary rights and determination of accession purity. The information generated on unique fragments in different accessions together with genetic dissimilarly data would be very useful for varietal improvement of the species through conventional breeding methodologies as well as molecular breeding approaches such as marker assisted selection (MAS).

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