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RESEARCH ARTICLE

Seed traits, fatty acid profile and genetic diversity assessment in *Pongamia pinnata* (L.) Pierre germplasm

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Abstract Phenotypic variation of important seed traits like seed length, seed breadth, seed thickness, 100 seed weight and seed oil content were recorded in a total of 157 collected accessions of Pongamia. Out of these, fatty acid profiles of 38 accessions selected based on their high and low oil content was analyzed. Fatty acid profile revealed high variability in stearic, oleic and linoleic acid which varied from 0.42 to 10.61 %, 34.34 to 74.58 %, and 7.00 to 31.28 % respectively. Variations in palmitic and linolenic acid were small. Iodine value, saponification number and cetane number (CN) of fatty acid methyl esters (FAME) of seed oil ranges from 186.99 to 201.25, 81.13 to 108.19 and 46.16 to 56.47 respectively. Fatty acid compositions, degree of unsaturation and CN are the important parameters, which are used to determine quality of FAME were used as biodiesel. Some of the Pongamia accessions identified were higher in oil content while some accessions showed higher degree of unsaturation and a few of them had CN values higher than 55. Genetic diversity analysis with six TE-AFLP primers generated a total of 334 bands out of which 174 (52.10 %) were polymorphic. The genetic similarity ranged from 0.11 to 0.47. These findings clearly showed high level of genetic diversity and all economically desirable traits were not present in a single genotype of Pongamia. All these traits could be selected from these CPTs and transfer to a single elite variety through selection and breeding programme and could be utilized for large scale multiplication and plantation to produce high quantity and quality biodiesel in future.

Keywords *Pongamia pinnata* · Oil content · Fatty acid methyl esters · Gas chromatography · TE-AFLP · Genetic diversity

Introduction

Energy security is an important issue all over the world because reservoirs of fossil fuels are depleting day by day which also lead to increase in prices of petroleum products. So, we have to think an alternative and renewable source of energy which also emits less carbon to environment. A number of Tree Borne Oilseed species (TBOs), which can produce oil as a source of energy in the form of biodiesel have been identified in India and other countries (Tewari 2003). *Pongamia pinnata* (L.) Pierre has been found to be one of the most suitable feedstock because its oil can be used as a source of fuel for the biodiesel industry (Karmee and Chadha 2005). Pongamia also have other additional favorable attributes like its hardy nature, capable of fixing atmospheric nitrogen, high yield, high oil recovery and quality of oil. Pongamia tree also has several applications in area of medicine (Elanchezhiyan et al. 1993; Shivanna and Rajakumar 2010) and is also used in insecticides (Pavela 2009). Pongamia is a non-edible oil producing tree legume of India (Lakshmikanthan 1978). The species is found predominantly in Western Ghats of India and Southeast Asia. It is fast growing medium sized tree and starts to yield 7 years after planting (Pavithra et al. 2013). Each mature pod generally encloses 1–2 kidney shaped or elongated brownish red seeds. Oil yield is one of the most important traits determining the commercial viability of

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Pongamia pinnata as an energy crop. The oil content of Pongamia seed is about 32–42 % (Kaushik et al. 2007), which can be converted into biodiesel (FAME) by trans-esterification with methanol in the presence of an alkali catalyst (Karmee and Chadha 2005; Naik et al. 2008).

In recent years, significant research efforts have been made to explore plant-based fuels (Martini and Shell 1998). The potentiality of Pongamia oil as an important source of biodiesel is now well recognized (Karmee and Chadha 2005; Azam et al. 2005). The Pongamia seed oil predominantly contains four fatty acids, namely, palmitic, stearic, oleic and linoleic acid. In addition to these major fatty acids, its oil also contains very less amounts of linolenic, behenic and eicosenoic acid. Oleic acid is the major fatty acid among pongamia trees and generally constitute about half of the oil with a range from approximately one to two thirds (Arpiwi et al. 2012; Sunil et al. 2009). The biodiesel properties of seed oil and FAME are: saponification number (SN), which specifies the relative fatty acid chain length of the FAMEs; the iodine value (IV), measures number of double bonds in respective fatty acids and cetane number (CN), which measures indication of ignition quality of fuel. In the US biodiesel standard (BS 2002), the minimum CN value is 47, where the Pongamia accessions analyzed in this study exhibit CN values from 50 to 59 with a mean of 54.4 (Mukta et al. 2009).

The major bottleneck in harnessing the biofuel potential of this tree is the unavailability of any improved and characterized planting stock. The variation in the pattern of fruiting and flowering was observed in different individuals of this species, also few trees bear high fruit numbers as expected. The high phenotypic diversity was observed in this species, thus providing an opportunity for its genetic improvement (Kaushik et al. 2007). It is important to characterize and select candidate plus trees (CPTs) of Pongamia for the improvement of this species. A plus tree is an individual tree of a species possessing superior morphological and reproductive characters than other individuals of the same species (Kesari and Rangan 2010).

Recently, initiatives have been taken towards molecular characterization and identification of superior genotypes of Pongamia. In the previous work, we attempted to analyze changes in oil and fatty acid profile at various developmental stages of seed in Pongamia (Sharma et al. 2015). The present study was carried out to assess variability and divergence of oil, fatty acids profile and important biodiesel traits for selection of CPTs with more efficient biodiesel yielder and their large scale commercial plantation for its development as an economically viable tree for biodiesel production across India and other tropical countries.

Materials and methods

Plant material

Germplasm collection surveys were conducted during the months of April–June, 2007–2011 from different regions of NCT of Delhi. Some accessions were also collected from other states of India (Assam, Karnataka and Andhra Pradesh) and a total of 157 accessions were collected. Passport data including site of collection, number of pods per bunch, number of seeds per pod and important seed traits were recorded. Global Positioning System (Garmin eTrex Vista Cx) used to mark the location of collected germplasm. Explored area lies between 28°32′8″–28°41′22″N and 77°23′28″–77°07′52″E covering parts of East Delhi, Central Delhi, West Delhi, East Delhi and Noida region of NCT of Delhi, India. Representative samples consisting of 1–2 kg mature pods were collected in cloth bags and seeds were separated from pods by manual dehulling. The mature trees which attained age of 6–10 years were selected. Seeds were dried in an oven at 50 °C till no weight loss was seen on further drying.

Oil content analysis

For oil content analysis, several seeds were pooled to represent an accession. Seed oil content was analyzed by solvent extraction using *n*-hexane as solvent in a Soxhlet apparatus (SOCS PLUS, Pelican Equipments, Chennai). Seed powder (1 g) and 60 mL of *n*-hexane were used per batch. Extraction was carried out at 80 °C for 1 h after which fast solvent recovery was done at 180 °C for 30 min. Two replicates of each accession were used and means were taken. The oil content for each accession was calculated and expressed as percentage (w/w) of dry seed using following equation

$$\text{Percent oil content} = [(W_{fb} - W_{ib})/W_s] \times 100$$

where, W_{fb} is final beaker weight, W_{ib} is initial beaker weight and W_s is the sample weight.

Fatty acid analysis

For fatty acid analysis using gas chromatography (GC), 38 Pongamia accessions were selected from both the tails of the oil content distribution of 157 accessions representing the top as well as the bottom of the distribution table. Analysis was done in two replicates using the method described by Thies (1971) with minor modifications. Dried seed samples of individual accessions were taken and crushed to powder with the help of mortar and pestle. Seed

powder (25 mg) was incubated with 750 µL of sodium methylate for 20 min. Then iso-octane (300 µL) was added and incubated for additional 20 min. The clear upper phase was taken in a GC vial and 400 µL of iso-octane was added. Fatty acid profiles were analyzed using a GC (Shimadzu, Kyoto, Japan) fitted with a flame ionization detector (FID). The oven, injector and detector blocks temperature were maintained at 210, 230 and 250 °C respectively and nitrogen gas was used as the carrier. The proportion of FAME was expressed as percentage of total fatty acids excluding the minor constituents.

Biodiesel properties like SN, IV of oil were calculated using FAMEs composition of oil with the help of following equations (Kalayasiri et al. 1996):

$$SN = \sum(560 \times A_i)/MW_i$$

$$IV = \sum(254 \times D \times A_i)/MW_i$$

where, A_i is the percentage, D is the number of double bonds and MW_i is the molecular weight of each component.

Whereas, Cetane Number of FAMEs was calculated by the following equation (Krisnangkura 1986):

$$CN = \frac{46.3 + 5458}{SN - 0.225 \times IV}$$

Descriptive statistics on oil content and fatty acid data was analyzed. Correlation coefficients between various seed and oil traits of *Pongamia* genotypes were calculated using SPSS software (Version 17.0). *Pongamia* accessions were classified into three categories, namely, low ($<\bar{x} - 1s$), medium ($\bar{x} - 1s$ to $\bar{x} + 1s$) and high ($>\bar{x} + 1s$) based on seed oil and biodiesel traits where $\bar{x}$ and s are the mean and standard deviation respectively (Mukta et al. 2009; Sarkar and Deb 1984).

Three endonuclease (TE)-AFLP assay

The protocol for TE-AFLP marker analysis was based on van der Wurff et al. (2000) with minor modifications. In current study, *Eco* RI, *Pst* I and *Mse* I were used instead of *Bam* HI, *Xba* I and *Rsa* I that were used in the original protocol. The detailed protocol of TE-AFLP has already published by our group (Sharma et al. 2011).

Genotyping and evaluation of molecular marker attributes

A total of 38 accessions were genotyped using six TE-AFLP primers combinations. The amplicons were in the size range of 80–450 bps. Amplified fragments were scored manually for their presence (denoted as '1') or absence (denoted as '0') for each primer combination. For analysis clear, distinct and polymorphic bands were recorded.

The binary matrix was used to estimate genetic dissimilarities using Jaccard's coefficient [GDij = (b + c)/a + (b + c); (Jaccard 1908)], where GD is the genetic dissimilarity measures between individuals i and j, a is the number of polymorphic bands that are shared by i and j, b is the number of bands present in i and absent in j, c is the number of bands present in j and absent in i. The dissimilarity matrix was subjected to neighbor joining clustering in order to construct the phenetic dendrogram DARWin software version 5.0.158 (Perrier and Jacquemoud-Collet 2006). The robustness and reliability of the phenograms were tested by bootstrap analysis for 1000 bootstraps for computing probabilities in terms of percentage for each node of the tree using the DARWin software (Perrier and Jacquemoud-Collet 2006). Different marker attributes like polymorphic information content (PIC), resolving power (RP), marker index (MI) and degree of polymorphism were analyzed (Sharma et al. 2011).

Results and discussion

Seed and oil traits

The mean performance of seed traits like seed width, seed length, seed thickness, seed weight and total seed oil content of 157 *Pongamia* accessions revealed significant differences (Table 1). Maximum value for seed length (25.40 mm) was observed in P276 and minimum (16.40 mm) in P002 accession. Seed thickness also varied significantly among all the accessions. It has been observed that maximum 100-seed weight (250 g) was recorded from P276 accession. In this context of seed trait, *Pongamia* accession P276 from Dwarka region of Delhi has maximum seed length and 100-seed weight. A study on seed and oil traits across twenty four CPTs from Jharkhand revealed that, a genotype CPT-19 recorded maximum values for seed length (27.93 mm), 100-seed weight (202.89 g) and total oil content (44.33 %) (Divakara et al. 2010) which are more similar to results of current study.

Based on seed oil content, 157 *Pongamia* accessions were categorized in five groups (Table 2). Oil content of accessions varied from 20.20 to 40.28 % with a mean of 31.7 %. About 55 % of the accessions had oil content between 30 and 35 %. Ten accessions had oil content less than 25 % whereas one accession had oil content more than 40 %. Other studies also showed oil content was highly variable trait among trees. Across 75 germplasm accessions from Andhra Pradesh oil content ranges from 9.5 to 46 %

Table 1 Mean values for important seed traits in Pongamia accessions

Sr. no.	Accession ID	Seed length (mm)	Seed breadth (mm)	Seed thickness (mm)	100-seed weight (g)	Oil content (%)
1	P001	22.1	16.2	5.6	115	37.80
2	P002	16.4	12.9	7.2	91	29.50
3	P002	18.0	13.5	6.2	98	34.20
4	P003	21.2	13.0	6.8	132	37.93
5	P004	16.8	12.2	5.0	76	33.34
6	P007	17.2	11.3	5.8	120	20.20
7	P008	21.2	12.0	8.2	100	27.83
8	P009	17.4	13.5	5.2	92	33.86
9	P010	21.2	17.4	7.2	118	34.66
10	P012	18.8	13.2	5.8	76	31.81
11	P013	22.2	14.9	7.1	109	38.88
12	P015	23.4	15.0	8.4	117	36.83
13	P016	21.8	12.2	7.1	112	33.13
14	P017	20.8	12.2	6.8	64	27.81
15	P018	18.2	14.4	6.2	63	30.26
16	P019	17.8	16.2	6.8	115	33.53
17	P020	22.2	14.2	7.8	126	30.56
18	P021	23.4	17.2	8.1	165	28.71
19	P022	21.2	11.2	7.2	103	33.60
20	P024	18.2	12.4	7.2	99	30.80
21	P028	20.2	16.5	7.2	120	30.60
22	P031	17.2	12.8	6.8	76	36.69
23	P033	21.1	11.4	7.3	67	25.34
24	P037	18.2	16.8	7.2	117	32.43
25	P039	17.4	16.2	6.2	112	34.58
26	P042	17.7	12.6	6.2	64	35.53
27	P051	21.2	12.0	6.9	111	24.75
28	P053	19.3	12.3	6.5	91	31.85
29	P054	23.2	11.2	7.1	98	30.21
30	P056	17.3	13.6	5.3	69	22.60
31	P057	22.2	12.6	7.3	76	32.47
32	P058	17.8	14.2	5.8	120	33.98
33	P059	19.4	12.6	6.7	100	35.19
34	P060	23.4	11.3	7.8	92	31.54
35	P061	18.2	15.8	7.2	118	31.40
36	P062	20.8	12.0	6.5	76	32.40
37	P065	19.2	13.2	6.4	67	32.74
38	P070	23.8	16.7	8.0	117	35.06
39	P071	19.2	12.2	8.2	112	23.02
40	P075	18.2	12.8	6.6	60	28.77
41	P076	19.1	14.2	5.2	63	26.30
42	P077	17.5	16.2	6.2	113	30.60
43	P078	19.2	14.5	7.4	126	27.20
44	P079	23.1	13.8	8.4	165	31.20
45	P082	22.2	15.2	6.2	103	31.87
46	P083	18.3	13.3	6.6	99	24.95
47	P084	22.4	16.2	6.1	120	36.35
48	P085	19.2	14.4	7.8	70	29.88

Table 1 continued

Sr. no.	Accession ID	Seed length (mm)	Seed breadth (mm)	Seed thickness (mm)	100-seed weight (g)	Oil content (%)
49	P086	23.3	11.4	6.8	90	32.80
50	P089	22.2	12.2	7.1	91	29.20
51	P094	21.3	11.8	7.5	83	29.40
52	P099	20.2	13.8	8.7	133	33.20
53	P101	22.3	12.4	7.8	81	31.74
54	P103	23.2	13.5	7.4	123	33.00
55	P104	21.1	12.2	7.5	105	34.51
56	P105	22.9	12.2	7.3	70	37.99
57	P106	23.3	11.4	7.4	96	32.41
58	P107	22.3	12.4	7.5	91	28.60
59	P108	21.8	11.2	7.2	90	35.22
60	P110	21.6	13.2	8.2	127	34.40
61	P111	21.2	11.2	8.4	113	31.40
62	P124	21.3	11.3	7.8	102	28.00
63	P126	23.7	11.8	8.2	92	30.20
64	P127	20.2	14.2	7.8	128	29.75
65	P130	22.8	14.4	7.2	126	33.75
66	P133	22.0	12.3	7.2	70	29.60
67	P134	23.1	11.5	7.2	96	32.00
68	P135	23.1	11.3	6.5	91	30.80
69	P137	22.8	12.4	7.2	90	30.00
70	P139	22.8	13.4	6.2	94	30.20
71	P140	20.2	14.1	6.2	80	28.00
72	P147	21.5	12.8	6.8	94	29.40
73	P148	22.2	13.3	8.2	87	29.00
74	P157	19.2	13.4	7.8	139	34.73
75	P158	22.2	13.8	8.8	155	27.82
76	P159	20.2	14.1	6.3	86	23.66
77	P160	21.4	12.3	7.8	113	28.06
78	P161	23.1	11.4	7.1	88	37.97
79	P162	21.1	14.2	7.2	99	36.47
80	P164	19.2	13.2	8.2	109	34.19
81	P165	20.1	13.3	5.8	85	23.31
82	P166	17.2	12.1	6.5	51	23.47
83	P167	23.1	11.8	6.4	92	29.00
84	P168	23.3	11.4	6.5	85	34.32
85	P169	22.2	15.0	7.4	154	31.87
86	P170	22.8	12.3	6.8	94	34.19
87	P171	23.3	13.2	5.5	80	29.87
88	P172	23.2	12.8	6.2	90	30.49
89	P173	22.2	12.6	7.2	121	34.49
90	P174	21.8	12.2	8.0	109	31.42
91	P175	22.1	10.2	7.7	114	33.77
92	P176	23.4	11.2	6.8	91	30.20
93	P178	22.3	12.2	6.5	86	34.03
94	P179	18.2	13.2	8.2	107	33.13
95	P185	20.2	14.4	6.2	124	25.46
96	P186	22.2	15.1	6.5	105	32.02

Table 1 continued

Sr. no.	Accession ID	Seed length (mm)	Seed breadth (mm)	Seed thickness (mm)	100-seed weight (g)	Oil content (%)
97	P187	23.4	11.3	6.5	91	30.51
98	P188	21.4	13.0	8.4	134	33.93
99	P189	22.4	12.4	7.2	132	37.98
100	P190	21.4	14.2	7.2	125	31.20
101	P191	21.2	12.3	7.6	110	33.80
102	P192	20.4	14.2	6.2	84	36.60
103	P193	22.2	12.5	6.5	92	34.80
104	P194	21.2	13.2	7.2	88	33.40
105	P195	22.4	12.8	6.8	178	34.40
106	P196	22.2	14.2	9.8	44	29.80
107	P197	20.3	13.4	7.2	114	30.00
108	P198	21.2	14.2	6.8	118	37.00
109	P200	22.2	12.1	8.3	132	30.20
110	P201	19.5	12.8	6.6	96	28.40
111	P202	22.2	12.2	6.2	91	31.80
112	P203	22.4	12.2	6.6	90	35.60
113	P204	21.2	14.2	7.4	127	30.80
114	P205	21.1	11.4	7.5	113	32.20
115	P206	22.2	15.2	6.4	102	25.40
116	P207	21.2	12.4	6.2	92	24.00
117	P208	21.2	15.2	7.1	128	31.60
118	P209	21.2	14.2	7.3	126	37.20
119	P210	21.1	14.2	7.8	117	35.80
120	P211	19.3	13.5	6.5	94	33.20
121	P212	21.2	14.4	7.2	102	30.20
122	P213	22.1	15.2	7.2	163	36.00
123	P214	18.8	13.2	5.8	77	28.37
124	P215	20.2	14.2	8.2	133	32.87
125	P216	23.8	14.2	10.1	224	33.60
126	P217	19.5	14.3	7.4	144	34.72
127	P218	22.2	13.4	6.8	145	33.80
128	P219	21.2	14.2	7.0	168	29.60
129	P220	23.2	16.3	8.2	181	33.40
130	P221	22.6	12.4	7.7	136	29.00
131	P222	19.2	12.3	6.8	101	32.00
132	P223	21.3	16.2	6.3	118	33.80
133	P224	22.2	14.2	7.2	148	34.60
134	P225	19.8	13.6	6.8	93	33.80
135	P226	21.3	12.8	7.2	84	31.00
136	P227	21.4	14.2	9.2	155	36.85
137	P228	21.0	13.8	8.2	135	40.28
138	P229	22.2	14.4	9.2	159	32.00
139	P230	21.8	13.2	7.6	109	37.80
140	P232	20.2	14.2	7.2	120	31.42
141	P233	20.2	14.4	7.4	133	35.00
142	P234	24.4	16.3	8.0	182	32.34
143	P235	21.0	13.5	9.0	135	33.80
144	P236	22.2	14.8	8.2	151	36.00

Table 1 continued

Sr. no.	Accession ID	Seed length (mm)	Seed breadth (mm)	Seed thickness (mm)	100-seed weight (g)	Oil content (%)
145	P237	21.2	15.1	7.8	162	34.20
146	P273	21.0	14.2	6.3	80	31.20
147	P274	22.8	15.2	9.6	180	31.80
148	P275	23.3	15.4	8.5	175	34.00
149	P276	25.4	16.4	9.2	250	37.20
150	P277	23.2	16.2	8.8	192	32.00
151	Assam-1	22.2	14.8	8.2	155	28.20
152	Assam-2	22.4	12.8	8.2	182	29.00
153	Assam-3	NA	NA	NA	104	28.60
154	Assam-4	NA	NA	NA	122	28.40
155	Bangalore-1	23.0	12.2	7.5	133	28.00
156	Eluru-1	NA	NA	NA	102	26.00
157	Eluru-2	21.2	13.4	7.2	113	23.50
	Mean	12.14	13.45	7.21	111.45	31.70
	SD	1.84	1.51	0.93	32.60	3.71

Table 2 Distribution of 157 *Pongamia pinnata* accessions for seed oil content

Phenotypic trait	Range (%)	Number of accession
Seed oil content (%)	>40	1
	35–40	25
	30–35	86
	25–30	35
	<25	10

(Mukta et al. 2009) and across 123 accessions from Andhra Pradesh and Orissa oil content ranges from 15 to 47 % (Sunil et al. 2009).

There was a positive correlation (all at $P = 0.01$) between seed weight and seed length ($r = 0.35$), seed breadth, ($r = 0.46$) and seed thickness ($r = 0.56$). There was no significant correlation these seed traits and oil content (Table 3). The results obtained in this study correlated with the earlier study by Sunil et al. (2009) and Arpiwi et al. (2012).

Fatty acid profile analysis

The fatty acid composition of oil governs its chemical properties and its efficacy as biodiesel. Gas chromatography analysis of the selected accessions exhibited very high variability in palmitic, stearic, oleic, linoleic and linolenic acid content (Table 4). Accessions P001, P003, P189 and P198 had high (>67.75 %) oleic acid content whereas accessions P017, P056, P105, P140, P158 and P161 had high (>22.57 %) linoleic acid content. The oleic acid content observed in these accessions is slightly higher than the previous report (Mukta et al. 2009). Accessions P056, P105, P140, P161 and P166 had high (>12.49 %) palmitic acid content whereas accessions P070, P105, P140, P161 and P165 had high (>7.82 %) stearic acid content

Table 3 Correlation between important seed and oil traits of Pongamia genotypes

	Seed length	Seed breadth	Seed thickness	100-seed weight	Oil content
Seed length	1.00				
Seed breadth	−0.05	1.00			
Seed thickness	0.410**	0.12	1.00		
100-seed weight	0.359**	0.465**	0.568**	1.00	
Oil content	0.201**	0.192**	0.166*	0.239**	1.00

** Correlation is significant at the 0.01 level

* Correlation is significant at the 0.05 level

Table 4 Phenotypic variations in seed oil content, fatty acid profile and biodiesel properties of 38 of Pongamia accessions

Accession	Oil content (%)	Palmitic	Stearic	Oleic	Linoleic	Linolenic	Oleic/linoleic ratio	Saponification number	Iodine value	Cetane number
P001	37.80	8.66	5.01	67.98	13.06	1.07	5.21	191.77	87.72	55.02
P003	37.93	9.69	0.52	70.29	15.61	1.67	4.50	196.07	96.05	52.52
P007	20.20	11.02	5.94	62.33	16.59	1.49	3.76	195.45	90.18	53.94
P008	27.83	12.36	7.44	63.58	10.07	2.09	6.31	191.99	81.13	56.47
P013	38.88	9.02	4.39	63.79	16.02	3.45	3.98	193.73	95.82	52.91
P015	36.83	10.16	4.18	61.67	17.55	1.98	3.51	191.70	92.67	53.92
P017	27.81	11.38	3.74	52.63	27.92	3.76	1.89	199.87	108.19	49.26
P031	36.69	9.04	4.56	60.98	17.94	2.77	3.40	191.00	94.91	53.52
P033	25.34	7.98	7.35	60.71	17	1.61	3.57	189.43	89.79	54.91
P042	35.53	10.45	6.43	56.05	19.53	2.53	2.87	190.68	92.70	54.07
P051	24.75	10.18	4.55	61.68	17.17	2.11	3.59	192.00	92.34	53.95
P056	22.60	13.72	1.46	50.85	31.28	0.68	1.63	197.48	104.25	50.48
P059	35.19	9.96	5.49	60.75	16.66	2.99	3.65	192.27	92.99	53.76
P070	35.06	10.09	8.04	58.81	15.87	2.09	3.71	190.34	87.35	55.32
P071	23.02	10.84	6.52	62.49	16.7	0.32	3.74	194.38	87.32	54.73
P076	26.30	9.94	7.46	58.08	19.24	3.19	3.02	196.36	95.81	52.54
P078	27.20	10.46	4.73	58.34	21.11	3.05	2.76	196.10	99.05	51.85
P083	24.95	12.07	4.91	63.72	15.98	3.02	3.99	200.33	94.51	52.28
P105	37.99	15.49	8.05	45.35	24.96	5.1	1.82	199.67	99.95	51.15
P108	35.22	8.06	6.44	65.6	14.4	1.08	4.56	191.25	88.03	55.03
P124	28.00	9.99	6.46	57.11	18.25	4.28	3.13	192.80	96.13	52.98
P140	28.00	14.41	9.83	34.34	27.13	7.27	1.27	187.68	99.92	52.90
P158	27.82	10.65	5.47	51.01	25.93	2.69	1.97	192.34	100.20	52.13
P161	37.97	13.33	10.02	43.8	24.67	3.86	1.78	192.68	94.64	53.33
P162	36.47	9.33	5.85	64.24	16.07	2.13	4.00	195.62	92.71	53.34
P165	23.31	8.06	10.61	64.1	14.14	0.18	4.53	194.15	83.75	55.57
P166	23.47	14.18	6.1	63.62	14.22	1.87	4.47	201.25	88.09	53.60
P189	37.98	8.9	5.55	74.58	10.8	0.16	6.91	200.10	87.07	53.99
P198	37.00	7.82	5.51	71.42	11.61	0.57	6.15	193.84	86.81	54.92
P203	35.60	9.79	6.71	60.52	14.17	2.84	4.27	188.57	87.86	55.47
P206	25.40	12.18	4.93	62.13	14.07	3.72	4.42	195.05	91.54	53.69
P209	37.20	10.58	5.05	59.93	17.18	2.26	3.49	190.71	91.20	54.40
P210	35.80	10.18	4.79	62.07	16.58	3.14	3.74	194.14	94.44	53.16
P213	36.00	10.5	5.34	59.73	15.54	2.04	3.84	186.99	87.44	55.81
P228	40.28	11.78	4.74	54.75	21.38	3.85	2.56	194.03	98.50	52.27
P230	37.80	12.48	0.42	65.29	16.67	1.39	3.92	193.61	92.71	53.63

Table 4 continued

Accession	Oil content (%)	Palmitic	Stearic	Oleic	Linoleic	Linolenic	Oleic/linoleic ratio	Saponification number	Iodine value	Cetane number
P236	36.00	9.53	5.59	66.42	12.66	2.78	5.25	194.36	90.27	54.07
P276	37.20	8.99	5.18	63.58	18.97	1.23	3.35	196.23	94.90	52.76
Mean	32.06	10.61	5.67	60.11	17.76	2.43	3.70	193.84	92.87	53.57
SD	6.06	1.87	2.15	7.63	4.82	1.43	1.27	3.49	5.58	1.49
Range	20.20–38.88	7.82–15.49	0.42–10.61	34.34–74.58	10.07–31.28	0.16–7.27	1.27–6.91	186.99–201.25	81.13–108.19	46.16–56.47

(Table 5). Total unsaturated fatty acid content in the accessions varied from 68.7 to 87.6 %. Considering high genetic and phenotypic variability observed in Pongamia from India, effect of genetic factors on seed oil composition is very likely (Sharma et al. 2011; Kaushik et al. 2007). Bala and coworkers (Bala et al. 2011) reported the presence of significant levels of erucic acid in Pongamia trees from Western India. The absence of erucic acid in the present accessions may be due to genetic as well as environmental factors. Sharma et al. (2015) reported the significant variation in seed oil and fatty acid composition during seed development in Pongamia.

Assessment of genetic diversity

In the present study, a total of 38 accessions were genotyped using six TE-AFLP primer combinations (Table 6), which were standardized previously for their polymorphism content (Sharma et al. 2011). The representative TE-AFLP gel profile obtained with primer combination E-AG × P-C is shown in (Fig. 1). Six primer combinations produced a total of 334 bands of which 174 (52.10 %) were polymorphic (Table 6). The maximum genetic similarity (Jc = 0.11) was between accessions P070 and P071, both from South Delhi. In contrast, the minimum genetic similarity (Jc = 0.47) was found between accessions P078 (from Eastern NCR) and P209 (from Kerala). The mean genetic distance among accessions was 0.32, which indicates that high level of genetic diversity exists in the analyzed accessions.

For cluster analysis neighbor joining method was used and all accessions were grouped into three major clusters (Fig. 2). The cluster I grouped all accessions belongs to South Delhi and Eastern NCR, cluster II grouped accessions from East and West Delhi region whereas cluster III includes accessions from both clusters. This indicates that these accessions had significant similarity with accessions within Delhi and NCR used in this study. No significant grouping were observed based on oil content and fatty acid profile. The bootstrap values of all major nodes were higher than 40 % and a majority of them were in the range of 50–99 % indicating robustness of data and clustering results.

Marker attributes

Discriminatory power of each TE-AFLP primers combination was studied by calculated PIC, MI and RP with polymorphic bands only. PIC value ranged from 0.26 (E-AC × P-A) to 0.35 (E-AC × P-C) and MI value from 5.58 (E-AG × P-G) to 11.99 (E-AG × P-A) while RP varied from 9.78 (E-AC × P-C) to 17.69 (E-AG × P-A) (Table 6).

Table 5 Distribution of *Pongamia pinnata* accessions based on seed oil content, fatty acid profile and biodiesel properties

Trait	Low ($<\bar{x} - 1s$)	Medium ($\bar{x} - 1s$ to $\bar{x} + 1s$)	High ($>\bar{x} + 1s$)
Seed oil content (%)	007, 033, 051, 056, 071, 083, 165, 166, 206	001, 003, 008, 015, 017, 031, 042, 049, 070, 076, 078, 105, 108, 124, 140, 158, 161, 162, 189, 203, 209, 210, 213, 230, 236, 276	013, 228
Fatty acid content (%)			
Palmitic	001, 033, 108, 165, 198	003, 007, 008, 013, 015, 017, 031, 042, 051, 059, 070, 071, 076, 078, 083, 124, 158, 162, 189, 203, 206, 209, 210, 213, 218, 230, 236,276	056, 105, 140,161, 166
Stearic	003, 056, 230	001, 007, 008, 013, 015, 017, 031, 033, 042, 051, 059, 071, 076, 078, 083, 108, 124, 158, 162, 166, 189, 198, 203, 206, 209, 210, 213, 228, 236, 276	070, 105, 140, 161, 165
Oleic	056, 105, 140, 158, 161	007,008, 013, 015, 017, 031, 033, 042, 051, 059, 070, 071, 076, 078, 083, 108, 124, 162, 165, 166, 203, 206, 209, 210, 213, 228, 230, 236, 276	001, 003, 189, 198
Linoleic	008, 189, 198, 236	001, 003, 007, 013, 015, 031, 033, 042, 059, 070, 071, 076, 078, 083, 108, 124, 162, 165, 166, 203, 206, 209, 210, 213, 228, 230, 276	017, 056, 105, 140, 158,161
Linolenic	056, 071, 165, 189, 198	001, 003, 007, 008, 013, 015, 017, 031, 033, 042, 051, 059, 070, 076, 078, 083, 108, 158, 162, 166, 203, 206, 209, 210, 213, 228, 230, 236, 276	105, 124, 140, 161
Oleic/linoleic ratio	017, 056, 105, 140, 158, 161	003, 007, 013, 015, 031, 033, 042, 051, 059, 070, 071, 076, 078, 083, 108, 124, 162, 165, 166, 203, 206, 209, 210, 213, 228, 230, 276	001, 008, 189,198, 236
Saponification number	033, 070, 140, 203, 213	001, 003, 007, 008, 013, 015, 031, 042, 051, 059, 071, 076, 078, 108, 124, 158, 161, 162, 165, 198, 206, 209, 210, 228, 230, 236, 276	017, 056, 083, 105, 166, 189
Iodine value	008, 165, 189, 198	001, 003, 007, 013, 015, 031, 033, 042, 051, 059, 070, 071, 076, 083, 108, 124, 161, 162, 166, 203, 206, 209, 210, 213, 230, 236, 276	017, 056, 078, 105, 140, 158, 228
Cetane number	017, 056, 078, 105	001, 003, 007, 013, 015, 031, 033, 042, 051, 059, 071, 076, 083, 108, 124, 140, 158, 161, 162, 166, 189, 198, 206, 209, 210, 228, 230, 236, 276	008, 070, 165, 203, 213

Table 6 Summary of the number of TE-AFLP fragments scored for different primer pairs used in selective amplification

Sl no.	Primer combination	Total bands	Polymorphic bands	Polymorphism (%)	PIC	MI	RP
1	E-AG × P-A	63	36	57.14	0.33	11.99	17.69
2	E-AG × P-C	61	37	60.66	0.29	10.82	16.00
3	E-AG × P-G	39	21	53.85	0.31	5.58	10.00
4	E-AT × P-A	73	31	42.47	0.34	10.41	15.34
5	E-AC × P-C	45	18	40.00	0.35	6.36	9.78
6	E-AC × P-A	53	31	58.49	0.26	8.21	11.21
	Total	334	174	52.10	0.31	8.90	13.34

PIC polymorphic information content, *MI* marker index, *RP* resolving power

In current years, studies have been directed to assess molecular diversity in *P. pinnata* using molecular markers such as RAPD, ISSR, AFLP and TE-AFLP (Kesari et al. 2010; Sahoo et al. 2010; Thudi et al. 2010; Sharma et al. 2011, 2014). Both TE-AFLP and AFLP indicated a high level of genetic diversity of *P. pinnata* collected from different locations of NCT of Delhi, India (Sharma et al. 2011) whilst ISSR indicated narrow genetic diversity within the trees from several regions of Orissa, India (Sahoo et al. 2010). AFLP detected higher levels of genetic diversity (100 %) in natural populations of *P. pinnata* on contrary the RAPD and ISSR showed lesser genetic diversity (aprox. 10 %) (Kesari et al. 2010). Another study on genetic diversity of Pongamia populations using AFLP markers in 33 CPT was undertaken in five agro-ecological zones of Southern Peninsular India (Pavithra et al. 2014). This study revealed relatively higher levels of gene diversity and high number of unique bands from eastern dry zone of Karnataka and southern dry and transition zone of Karnataka.

In the recent study a total of 38 Pongamia individuals from different locality of NCT of Delhi genotyped using six TE-AFLP primer combinations. Pongamia exhibited very high genetic diversity even within the accessions from

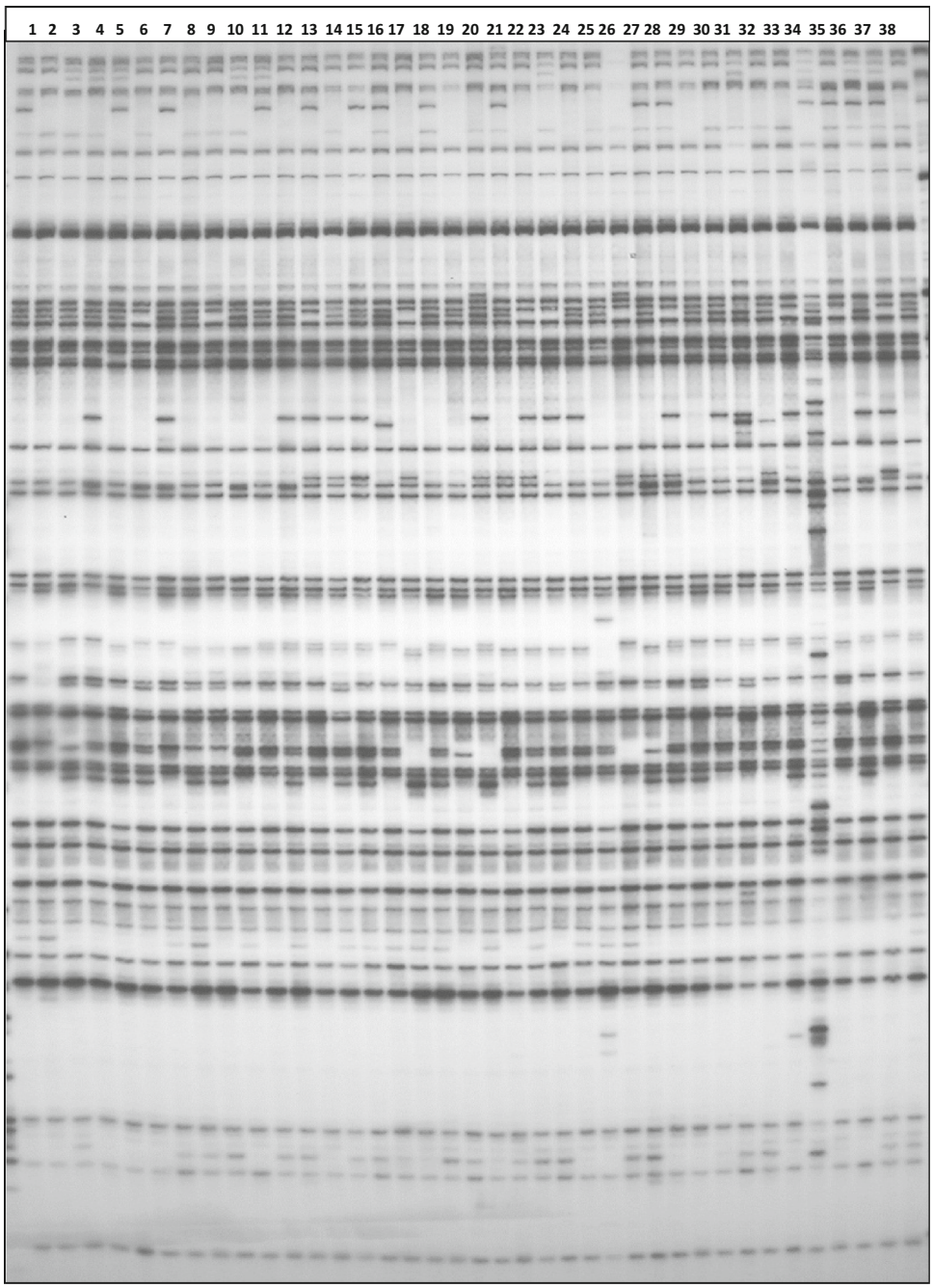

Fig. 1 Representative gel profiles of Pongamia using TE-AFLP primer E-AG × P-C. *Lane numbers* are the same as the serial numbers of the accessions mentioned in Table 4

Delhi and surrounding regions. All accessions grouped into three major clusters and highest genetic similarity was found in the accessions from South Delhi. It may be noted that this area of National Capital harbours the largest number of Pongamia trees. High diversity in pod and fruit traits in Pongamia from a neighboring area of NCT of Delhi has been reported earlier (Kaushik et al. 2007). A high level of genetic diversity in Pongamia is expected because of its presumed Indian origin. Scott et al. (2008) also suggested that *P. pinnata* were probably introduced from India to Australia early in human history.

Conclusions

Currently, no elite variety of Pongamia is available. Therefore, this species has high scope of improvement to make it suitable feedstock for biodiesel production through

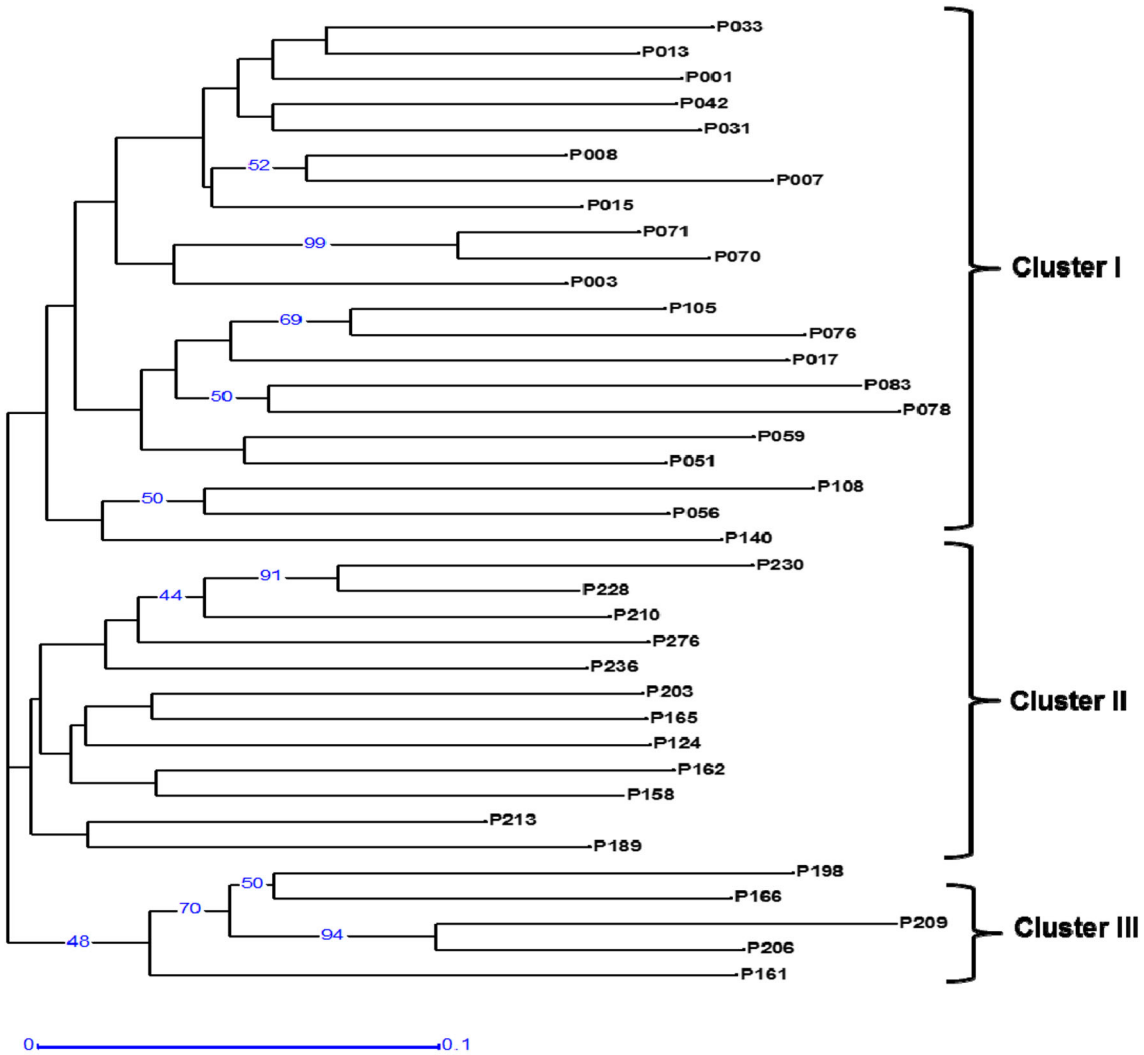

Fig. 2 Dendrogram showing neighbor joining clustering of Pongamia. The *numbers* on nodes indicate % bootstrap values

selection of economically important traits such as high seed yield, high oil content and desirable fatty acid composition. The current study showed that Pongamia accession P276 is high in 100 seed weight and seed yield, the accessions P013 and P228 high in oil content, the accessions P001, P003, P189 and P198 high in unsaturated fatty acids and likewise, accessions P008, P070, P165, P203 and P213 have CN values higher than 55. These findings clearly showed that all economically desirable traits are not present in a single genotype of Pongamia. Further, high level of genetic diversity was observed in collected germplasm of Pongamia and therefore there is a scope for its genetic improvement. In future, these identified CPTs could be used as parental material to combine all desirable traits in a single elite variety though selection and tree breeding programme in this species and further can be utilized for large scale multiplication and plantation to produce high quantity and quality biodiesel production.

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