

Diversity and variability in seed characters and growth of *Pongamia pinnata* (L.) Pierre accessions

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Abstract A thorough and extensive wild germplasm exploration survey was undertaken and 50 high yielding candidate plus trees (CPTs) of *Pongamia pinnata* (L.) Pierre from different locations from a latitudinal and longitudinal spread between 12°41' and 22°E longitude and 77° and 84°40'N latitude covering 11 locations in an area spread of 150,000 km² were collected for evaluating genetic association and variability in seed and growth characters. There were significant differences observed in seed morphology and oil content as was in plant height, and number of branches in the progeny trial. Plant height and number of branches exhibited much higher values of both phenotypic and genotypic variance than observed in the seed characters. Among seed characters oil content exhibited highest broad sense heritability of more than 93% followed by seed length (90.0%). In contrast seed width showed the second highest genetic advance of 5.64% following the highest genetic advance of 10.15% exhibited by oil content. Hierarchical clustering by Ward's Minimum

Variance Cluster Analysis method showed phylogeographic patterns of genetic diversity. K means clustering revealed that trees from different geographic regions were grouped together in a cluster and as were trees from the same geographical area placed in different clusters suggesting that geographical diversity did not go hand in hand with genetic diversity. In addition clustering identified promising accessions with favourable traits for future establishment of orchards.

Keywords Biodiesel · Heritability · Genetic advance · Cluster analysis · Tree breeding · Germplasm

Introduction

Pongamia pinnata (L.) Pierre is a fast growing, glabrous, deciduous, tree reaching up to a height of 25 m on full growth (Allen and Allen 1981). It is a nitrogen-fixing tree and a member of the subfamily Papilionoideae, more specifically the Fabaceae: tribe Millettieae. This plant has been synonymously known as *P. pinnata* Merr., *P. glabra* Vent., *Derris indica* (Lam) Bennett and *Millettia novoguineensis* Kane & Hat (Scott et al. 2008). It is a hardy tree with tolerance to a wide range of abiotic stresses like drought, frost, heat, limestone, salinity, sand and shade. It grows fast and matures after 4–7 years yielding fruits which are flat, elliptic and 7.5 cm long. Each fruit contains 1–2 kidney-shaped brownish red kernels. The tree is a native of India and is now also found extensively in Australia, USA (Florida and Hawaii), Malaysia, Oceania, Philippines, and Seychelles. There is a debate as to its probable origin outside India although conclusive evidence to it is yet to be provided. The tree has numerous medicinal uses ranging from time tested folk medicine for human and

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animal aliments like; abdominal tumours skin ailments, liniment for rheumatism, colds, coughs, diarrhoea, dyspepsia, flatulence, gonorrhoea and leprosy to clinically tested antiseptic, antimicrobial, antiviral and antihyperglycemic activity (Rameshthangam and Ramasamy 2007; Punitha and Manoharan 2006; Elanchezhian et al. 1993). The seeds contain pongam oil, bitter, red brown, thick, non-drying, non-edible oil, which is used for tanning leather, soap, as a liniment to treat scabies, herpes, and rheumatism and as an illuminating oil (Burkill 1966). The oil content of the kernel is 30–40% and the fatty acid composition of the seed oil has been reported to be—oleic (44%), linoleic (17.4%), erucic (15.9%), palmitic (7.2%), linolenic (5.5%), 11-eicosenoic (3.4%), stearic (3.3%), behenic (2.5%) and eicosanoic (0.78%) acids (Bala et al. 2010). A single tree yields between 9 and 90 kg seed per tree, indicating a yield potential of 900–9,000 kg seed ha⁻¹ (assuming 100 trees ha⁻¹), and 25% of which might be rendered as oil. In general, mills extract 24–27.5% oil, and the village crushers (indigenous custom made low cost crushers and expellers) extract 18–22% oil. More recently the potential of the oil has been rediscovered as an effective alternative/additive for fossil derived diesel. This has fuelled research in the area of cultivation aspects of the tree which was largely neglected earlier as the tree's economic potential was limited to unmanaged forest by product. Although the *Jatropha* seed oil is a better feed stock for biodiesel as compared to pongamia in terms of viscosity and combustion characteristics, the numerous uses of the tree and low cost production, pest resistance, deep rooted nature, drought hardiness and management outweighs *Jatropha* in terms of overall economic potential.

In order to bring out the full potential of the tree there is an urgent need to standardize cultivation practices (for block plantations) and agro techniques (for agroforestry systems) for the tree. In order to achieve this, the need for planned conventional breeding programs is paramount. The very fact that pongamia has adapted itself to a wide range of edaphic and ecological conditions suggest that there exists considerable amount of genetic variability to be exploited for potential realization. There are a few reports of genetic variability and divergence studies in pongamia in terms of PCR amplification of the genomic DNA; biodiesel traits; pod and seed traits (Kesari et al. 2009; Mukta et al. 2009; Kaushik et al. 2007), on the other hand, comprehensive work on collection of germplasm and evaluating of growth, morphology, seed characteristics and yield traits is still in its infancy world over.

Knowledge of genetic variability of this species is necessary, more so because it affects the evolutionary potentialities of the species. Considering the multi various

uses of the tree, evaluation of provenance progenies is an essential aspect which could play an important role in developing standard agro techniques for this tree which is particularly lacking when it comes to agroforestry (von Carlowitz 1986). Seed characters are important when evaluating trees of unknown genetic potential especially in a tree like pongamia where little work has been done in terms of progeny evaluation. Yield stabilization in this tree is expected to be seen only after the 8th year of establishment, hence it would take a minimum of 10 years to identify superior germplasm in terms of seed yield, to circumvent this problem we in this study analyzed the initial growth characteristics of this tree for the first 3 years which could have a bearing on the quality of the tree in later years of growth and yield (León et al. 2004). Considerable genetic variation in growth, chemical composition of seed and seed traits at the level of provenance, variety or progeny has been reported in several multipurpose tree species such as *Albizia*, *Acacia* and *Prosopis* (Wanyancha et al. 1994; Raddad and Luukkanen 2006; Goel and Behl 2001), which are widely used in agroforestry systems. The knowledge on this type of variation would be useful for genetic selection especially in a tree where the desired ideotype in terms of ideal growth pattern, phenology and yield component distribution is yet to be defined due to its wide adaptability and end use pattern. Genetic variation in seed characteristics, growth and branching pattern, and yield attributes and components of pongamia can be of great potential in tree improvement programmes, particularly selection of genotypes having more oil content and yield in addition to suitability in agroforestry systems.

The necessity to obtain adequate information on these aspects and to assess the magnitude of genetic variability and their corresponding heritability is imperative for any breeding program to succeed. Hence a large scale collection wild germplasm, conservation and evaluation program of various pongamia accessions was taken up with objectives of progeny trial, genetic analysis of variability, broad sense heritability and genetic advance in order to estimate the extent to which improvement is possible through selection with ultimate aim of establishing seedling/clonal seed orchard with elite material.

Materials and methods

A thorough and extensive wild germplasm exploration survey was undertaken to identify high yielding candidate plus trees (CPTs) of pongamia from different locations in Andhra Pradesh, India. Targeted districts were Adilabad, Chittoor, Cuddapah, Anantapur, Kurnool, Nellore, Prakasam, Guntur, Visakhapatnam, Vizianagaram and Srikakulam

covering an latitudinal and longitudinal spread between 12°41' and 22°E longitude and 77° and 84°40'N latitude. Collection of seeds/cuttings from selected plus trees were done during the fruiting season of pongamia in 2004–2005 and 2005–2006. Criteria advocated by Cornelius (1994) were followed for selection of plus trees. The selection was made on phenotypic assessment of characters of economic interest, i.e. yield potential, Number of flowers per raceme, branching pattern, crown spread, girth, disease resistance, etc. Trees of more than 10 years of age and yielding above 20 kg of seed/tree were identified as plus trees. Care was taken to avoid trees infested with pests and diseases and isolated trees or wolf trees (a large heavy-branched tree that is usually larger and older than the surrounding forest). The accession data normally followed for selection of plus trees was adhered to. Freshly collected pongamia seed were tested for germination. In general, the seed germination percentage of different accession ranged from 45 to 50. Whereas, germination in 1-year-old pongamia seed was 30%. Seed characteristics was analysed after seeds were separated from the fruit, cleaned and stored in muslin bags at ambient conditions. All seed lots were dried under similar temperature and humidity conditions to reach a constant weight. Five samples were drawn from each seed lot and 100 random undamaged seeds (total 500 seeds) were measured for their length, width and thickness in millimetres. For seed weight five samples of seeds including 100 seeds each, were taken from each seed lot and measured for weight and expressed in grams. The oil content of seeds was estimated by soxhlet method according to Kaushik et al. (2007) using three replicates for each seed lot. After collection the germplasm was brought to Central Research Institute for Dryland Agriculture (CRIDA), Hyderabad, India and the seed/cuttings of germplasm of pongamia collected during 2004–2005 and 2005–2006 were raised in the nursery for further evaluation trials in nursery as well as in field conditions to identify elite germplasm. Progeny trial with 28 progenies selected from the 50 collected germplasm accessions on the basis growth in terms of germination percentage, seedling height and colour diameter and also on the basis of seed oil content (30% oil content and more were selected for progeny trial) is underway under a randomized block design with 3 replications. Nine plants of each accession were planted at a spacing of 3 m × 3 m.

Recording of data

Data were recorded on all the plants in each germplasm line in each replication for the following morphological traits:

- (a) Plant height (cm): the average height from the ground level to the tip of the inflorescence on the main stem at the time of harvesting was measured.
- (b) Branches/plant: the total number of branches growing from the main stem at different node positions, including the basal branches. Although this would confound data to a certain extent it will be indices for agroforestry related interests.
- (c) Collar diameter: diameter at collar height was measured using an measuring tape.

Statistical analysis

The raw data was compiled by taking the means of all the plants taken for each line and replication for different traits in the experiment. The means of were subjected to further statistical and biometrical analysis. Mean, standard error, variance and coefficient of variation were analyzed statistically using Systat for windows (Wilkinson et al. 1996). Correlation analysis was performed to determine the relationships between oil content and seed characteristics in seeds and growth, reproductive phenology and yield in progeny trials and all the component traits, both at genotypic and phenotypic levels according to Johnson et al. (1955). The variability, genetic advance as percent of mean, phenotypic and genotypic variance, phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) were worked out for seed and oil content and growth traits as suggested by different workers (Yoshida et al. 2007; Pliura et al. 2007; Baenziger et al. 2004). Broad sense heritability in all the accessions was estimated by partitioning the variance in measurements into between-accessions and within-accessions components and applying these in the following function (Falconer and MacKay 1996).

$$H = \frac{\sigma^2 g}{\sigma^2 p}$$

$\sigma^2 g$ is genotypic variance = (variance between-accessions – variance within-accessions)/ n

$\sigma^2 p$ is phenotypic variance = [(variance between-accessions – variance within-accessions)/ n]
+ variance within accessions

n number of replicates per treatment (Ali et al. 2007).

Genetic advance was calculated as the difference between the genotypic mean of selected lines and genotypic mean of population. Genetic advance as percentage of mean was calculated by the following formula (Bhargava et al. 2007):

Table 1 Seed characteristics and oil content variability of Candidate Plus Trees (CPT) *Pongamia pinnata* germplasm

Acc. No	Length (cm)	Width (cm)	100 seed weight (g)	Oil content (%)	District
CDRP1	2.03	1.21	110.38	34.20	Adilabad
CDRP2	1.97	1.52	119.27	35.30	Adilabad
CDRP3	1.54	1.34	102.87	35.30	Adilabad
CDRP4	2.00	1.43	105.54	35.60	Adilabad
CDRP5	2.28	1.59	163.56	35.85	Adilabad
CDRP6	2.11	1.32	160.23	35.00	Adilabad
CDRP7	2.02	1.62	167.25	38.16	Adilabad
CDRP8	1.53	1.20	129.38	34.00	Adilabad
CDRP9	1.68	1.24	171.92	35.30	Adilabad
CDRP10	1.91	0.47	106.86	35.50	Adilabad
CDRP11	2.09	1.32	153.00	34.85	Adilabad
CDRP12	1.96	1.47	131.00	34.65	Adilabad
CDRP13	1.83	1.24	123.00	34.35	Adilabad
CDRP14	2.35	1.37	160.00	33.70	Adilabad
CDRP15	2.04	1.45	106.00	30.70	Adilabad
CDRP16	1.99	1.12	115.00	33.85	Chittor
CDRP17	2.15	1.58	142.00	34.80	Chittor
CDRP18	1.69	1.41	117.20	35.10	Chittor
CDRP19	1.91	1.32	105.40	32.10	Chittor
CDRP20	1.87	2.01	129.00	33.60	Chittor
CDRP21	2.04	1.30	121.13	32.75	Chittor
CDRP22	2.11	1.76	74.00	33.70	Chittor
CDRP23	1.81	2.06	146.43	34.85	Chittor
CDRP24	1.90	1.40	123.82	31.80	Chittor
CDRP25	2.24	1.70	115.75	30.85	Chittor
CDRP26	1.74	1.31	131.66	31.30	Kadapa
CDRP27	1.92	1.43	146.86	34.95	Kadapa
CDRP28	2.21	1.41	125.41	34.95	Kadapa
CDRP29	1.94	1.28	129.66	32.40	Kadapa
CDRP30	1.68	0.82	124.12	31.05	Kadapa
CDRP31	1.69	1.32	117.11	32.65	Kadapa
CDRP32	2.07	1.67	122.91	32.05	Kadapa
CDRP33	2.28	1.44	119.64	33.95	Kadapa
CDRP34	2.18	1.44	117.10	30.95	Kadapa
CDRP35	2.13	1.29	115.31	31.05	Kadapa
CDRP36	2.08	1.15	123.11	31.10	Anantapur
CDRP37	2.50	1.47	112.46	30.80	Anantapur
CDRP38	2.01	1.47	133.06	34.15	Kurnool
CDRP39	1.83	1.33	88.04	30.60	Chittor
CDRP40	2.31	1.43	71.00	30.20	Chittor
CDRP41	2.34	1.32	102.00	31.25	Kadapa
CDRP42	1.96	1.10	170.00	34.30	Kadapa
CDRP43	2.06	1.32	108.00	30.65	Anantapur
CDRP44	2.32	1.25	88.00	31.10	Anantapur
CDRP45	2.39	1.64	150.00	33.66	Anantapur
CDRP46	1.96	1.46	106.00	34.56	Anantapur
CDRP47	2.21	1.59	75.00	30.95	Kurnool

Table 1 continued

Acc. No	Length (cm)	Width (cm)	100 seed weight (g)	Oil content (%)	District
CDRP48	2.04	1.19	76.00	31.21	Kurnool
CDRP49	2.05	1.33	118.00	30.20	Kurnool
CDRP50	1.91	1.34	168.00	33.69	Kurnool
LSD at 0.05	0.0387	0.0318	7.83	1.54	

$$\text{Genetic advance (\%)} = \frac{\text{Genetic advance (trait)}}{\text{Mean (trait)}} \times 100$$

Euclidean distance cluster analysis captures the kinetic styles of patterns of variation in the parameters observed and recorded. Both hierarchical Euclidean cluster analysis wherein divisive method is used and non-hierarchical (K means) were agglomerative method is used was done in both initially collected wild germplasm and progeny trial. Intra- and inter-cluster distances were calculated to identify accessions that can be used as parents for desired traits in future breeding programmes. The broad genetic divergence was calculated by using non-hierarchical Euclidean cluster analysis (Sachan et al. 2004). The shortest Euclidean distances were calculated by the method of the closest neighbourhood according to Ward, and dendrogram was constructed to examine the relationships within and between populations (Karoński and Caliński 1973; Sokal and Rohlf 2003).

Results

Variability in seed and growth characters

Analysis of variance in the seed characters revealed that there was significant variation among all the wild accession studied in all the characters at $P < 0.05$. CDRP5, CDRP6, CDRP7 and CDRP9 showed highly significant values for 100 seed weight as compared to other accession. The low ranking accession differed significantly from the rest. With regard to oil content percentage it was seen that CDRP2, CDRP3, CDRP4, CDRP5, CDRP6, CDRP7, CDRP9 and CDRP10 had more than 35% oil in seeds. The low ranking ones with less than 32% oil were CDRP15, CDRP25, CDRP37, CDRP39, CDRP40 and CDRP47. Highest variance was seen in 100 seed weight among all the characters recorded followed by oil content.

Significant variation was observed in plant height recorded during the initial 6 months of growth of all the accessions and also during the 1 year growth period of progeny trial of 28 seeds selected from the initial seed sources (Tables 1, 2). A few low ranking accessions like CDRP1, and CDRP4 from the initial trial out performed medium ranking ones in the progeny trial after 1 year of

Table 2 Growth and branching after 1 year in progeny trial of selected superior germplasm *Pongamia pinnata* germplasm

Acc. no	Plant height (cm)	Collar diameter (cm)	No. of branches
CDRP1	37.9	0.58	2.9
CDRP2	30.7	0.48	2.4
CDRP3	25.0	0.57	3.0
CDRP4	31.5	0.49	2.2
CDRP5	39.3	0.48	2.2
CDRP6	41.8	0.59	2.6
CDRP7	44.8	0.60	2.9
CDRP8	28.8	0.45	2.5
CDRP9	47.0	0.59	9.6
CDRP10	29.0	0.41	6.5
CDRP11	37.0	0.41	8.8
CDRP12	17.3	0.39	5.7
CDRP13	51.6	0.46	9.3
CDRP14	46.8	0.64	7.5
CDRP16	35.0	0.46	3.4
CDRP17	36.4	0.47	9.8
CDRP18	31.0	0.53	8.4
CDRP20	35.5	0.41	7.9
CDRP21	39.6	0.47	6.7
CDRP22	49.4	0.50	7.0
CDRP23	56.5	0.73	8.8
CRDP28	70.0	0.68	7.0
CRDP33	70.2	0.65	15.1
CRDP38	53.0	0.60	7.6
CRDP42	63.7	0.43	8.6
CRDP45	68.4	0.57	7.7
CRDP46	55.9	0.57	6.3
CRDP50	51.9	0.42	7.9
CV %	10.7	22.7	14.1
LSD	9.64	NS	1.86

growth in plant height as well as collar diameter. CDRP12 recorded the lowest plant height after 1 year of growth. Collar diameter too exhibited significant difference among the accession after 1 year of growth with CDRP23 exhibiting the highest value of more than 0.7 cm. A median of 0.495 with a minimum variance of 0.008 was seen in collar diameter as against higher values in the rest of the parameters. Number of branches per tree exhibited the highest range of only 2 branches in CDRP4 and CDRP5 as compared to high branching accessions CRDP33 with 15 branches. Most accessions had more than 6–7 branches as seen by the mean value of 6.4.

Genetic associations in seed and growth characters

The amount of genetic variations and association was evident from the study of PVC, GCV and correlation

analysis. Hundred Seed weight exhibited a significant positive correlation (Table 3) at 0.01 probability level with seed oil content. Whereas there no evident correlation (genotypic and phenotypic) with either seed length or width and oil content. The rest of the seed characters did not show a strong correlation with the initial plant growth. On the other hand, seed characters did show an impact on the initial plant growth. Hundred seed weight showed a positive significant correlation with plant height at 0.05 probability level. This was seen at phenotypic and genotypic levels. Among the growth parameters recorded namely plant height and collar diameter it was seen that these two parameters exhibited a highly significant positive correlation between themselves at both genotypic and phenotypic levels. Seed length and seed width did not show any relation with early growth of the plants.

The values for variance, coefficient of variation, heritability and genetic gain for seed characters are presented in Table 4. The magnitude of PCV was higher than the corresponding GCV for all the characters studied though they were only small differences. Phenotypic variance denoting total variance was maximum for 100 seed weight (32.84) among the seed the characters studied which was followed by oil content. Plant height and number of branches exhibited much higher values of both phenotypic and genotypic variance than observed in the seed characters. Broad sense heritability was high in general and exceeded 80% for all the traits studied. Among seed characters oil content exhibited highest broad sense heritability. Among the growth parameters Collar diameter and plant height showed a heritability of nearly more than 90% as against a low heritability of 66.45 by number of branches. A high genetic advance of 58.12 was seen in plant height followed by number of branches and collar diameter.

Diversity in seed and growth characters

Composition of Euclidean clusters and cluster mean values obtained by K-Means non-hierarchical clustering for seed characters, oil traits and initial plant growth in *Pongamia pinnata* germplasm are given in Table 5. Fifty accession of pongamia was placed in 5 clusters on the basis of K-Means clustering, the maximum number of accession were in cluster II (19 accessions) and the minimum number were placed in cluster V (5 accessions). Clusters II and IV which contain maximum accession (19 and 13, respectively) did not exhibited high means for any of the characters studied. The above clusters (II and IV) showed lowest means for seed length, plant height and collar diameter (cluster II) and seed width (cluster IV). Inter-cluster and intra-cluster distances are shown in Fig. 1. The dendrogram obtained

Table 3 Genotypic (G) and Phenotypic (P) correlation coefficient between seed characteristics and growth traits in *Pongamia pinnata* germplasm

Character	Genotypic/Phenotypic	Seed width	100 seed weight	Plant height ^a	Collar diameter ^a	Oil content
Seed length	Genotypic	0.245	−0.117	0.235	0.218	−0.242
	Phenotypic	0.218	−0.113	0.219	0.225	−0.211
Seed width	Genotypic		0.168	0.083	−0.060	0.098
	Phenotypic		0.204	0.105	0.012	0.115
100 seed weight	Genotypic			0.342*	0.051	0.589**
	Phenotypic			0.311*	0.069	0.543**
Plant height	Genotypic				0.614**	0.187
	Phenotypic				0.564**	0.169
Collar diameter	Genotypic					0.056
	Phenotypic					0.119

^a Plant height and Collar Diameter after 6 months of growth in nursery of all the collected germplasm and in the field at 6 monthly intervals up to 3rd year

* Significant at 5% level

** Significant at 1% level

Table 4 Estimation of genetic variables for seed characteristics and growth traits in *Pongamia pinnata* germplasm

Traits	Range	Variance		Co-efficient of variation		Heritability (broad sense)	Genetic advance as % of mean
		Genotypic	Phenotypic	Genotypic	Phenotypic		
Seed length	1.53–2.50	0.27	0.30	5.65	5.99	90.00	4.23
Seed width	0.47–2.06	0.05	0.07	11.79	12.51	71.42	5.64
100 seed weight	71.00–171.92	28.46	32.84	7.63	8.20	86.35	4.19
Oil content	30.20–38.16	11.14	11.89	12.13	12.60	93.69	10.15
Plant height ^a	17.30–70.20	105.05	117.84	27.77	29.56	89.14	58.12
Collar diameter ^a	0.39–0.73	7.81	8.19	19.99	20.96	95.36	12.37
Number of branches ^a	2.20–15.10	121.76	183.23	42.76	46.34	66.45	39.06

^a Plant height and Collar Diameter after 6 months of growth in nursery of all the collected germplasm and in the field at 6 monthly intervals up to 3rd year

Table 5 Composition of Euclidean clusters and cluster mean values obtained by K-Means Non-hierarchical Clustering for seed characters, oil traits and initial plant growth in wild *Pongamia pinnata* germplasm

Cluster	Accessions	Accession names	Seed length (cm)	Seed width (cm)	100 seed weight (g)	Oil content (%)	Plant height (cm)	CD (cm)
I	7	CDRP-5, CDRP-6, CDRP-7, CDRP-9, CDRP-14, CDRP-42, CDRP-50	2.04	1.37	165.85	35.14	25.37	1.13
II	19	CDRP-2, CDRP-8, CDRP-12, CDRP-13, CDRP-18, CDRP-20, CDRP-21, CDRP-24, CDRP-26, CDRP-28, CDRP-29, CDRP-30, CDRP-31, CDRP-32, CDRP-33, CDRP-34, CDRP-36, CDRP-38, CDRP-49	1.93	1.38	123.98	32.96	23.05	1.08
III	6	CDRP-22, CDRP-39, CDRP-40, CDRP-44, CDRP-47, CDRP-48	2.14	1.43	78.67	31.29	23.20	1.17
IV	13	CDRP-1, CDRP-3, CDRP-4, CDRP-10, CDRP-15, CDRP-16, CDRP-19, CDRP-25, CDRP-35, CDRP-37, CDRP-41, CDRP-43, CDRP-46	2.05	1.30	108.58	32.80	23.66	1.10
V	5	CDRP-11, CDRP-17, CDRP-23, CDRP-27, CDRP-45	2.07	1.61	147.66	34.62	25.20	1.11

Fig. 1 Estimates of intra-cluster and inter-cluster distance (five clusters) by K-Means non-hierarchical clustering for seed characters, oil traits and initial plant growth in wild *Pongamia pinnata* germplasm

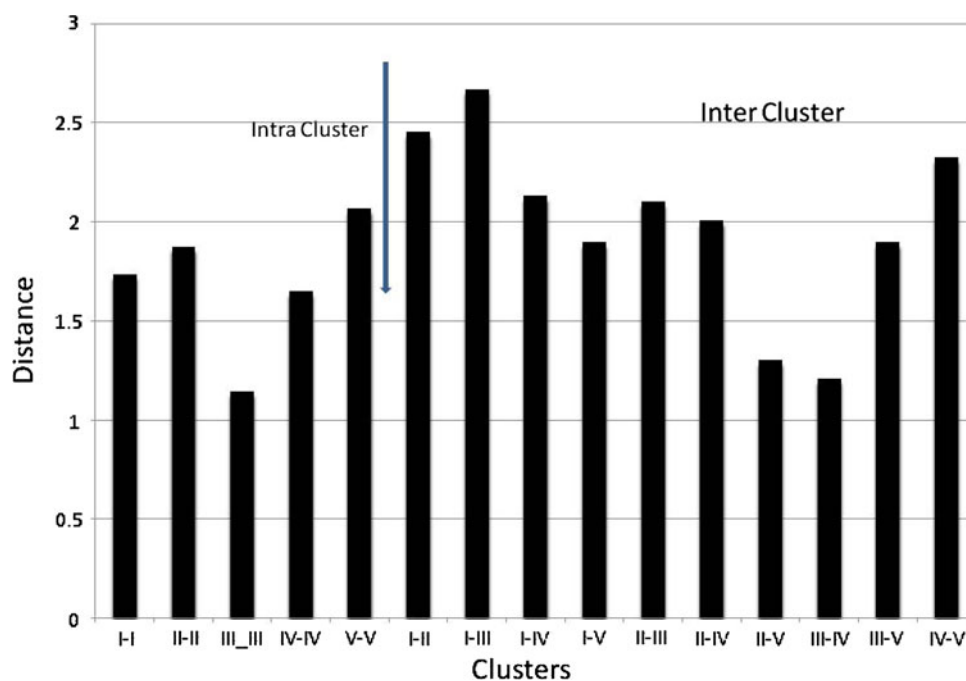
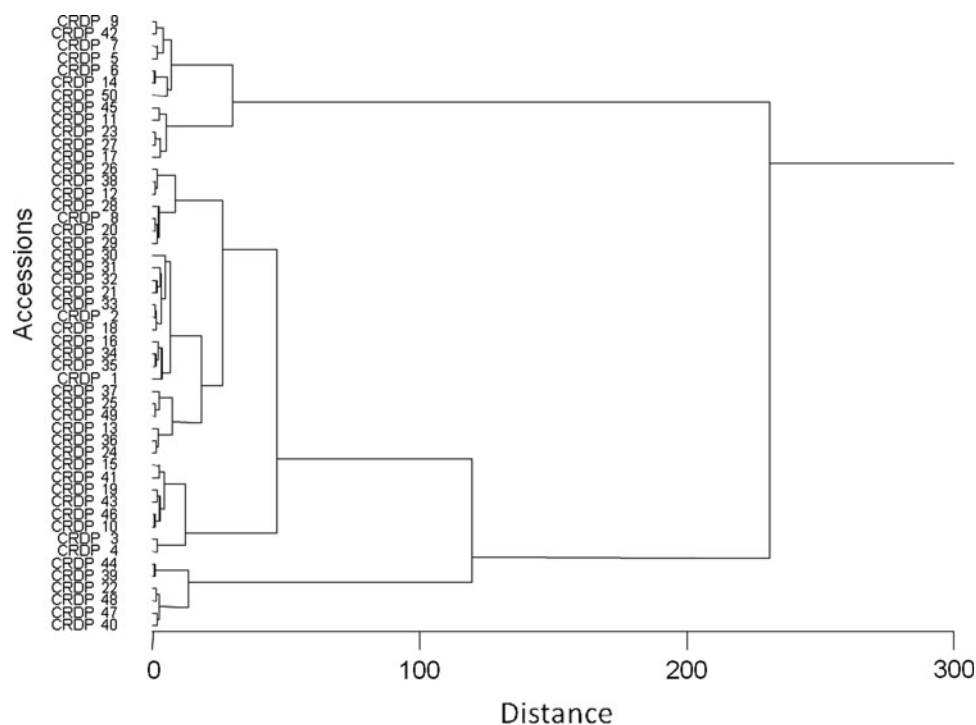


Fig. 2 Dendrogram constructed by Ward Method of Cluster Analysis on the Euclidean distances by Hierarchical Clustering of growth and reproductive phenology in progeny trial of selected *Pongamia* germplasm. The location of the accession has been listed in Table 1



hierarchical Euclidian cluster analysis according to Ward's Method is shown in Fig. 2. Length of the horizontal branches between clusters indicates that there is a high degree of dissimilarity between clusters. The maximum number of members (apart from the super cluster containing all the accession was populated by 50 accessions was 38 when cluster containing CDRP26 and CDRP47 were joined at distance of 119.629.

Discussion

Genetic associations and variability

Seed morphological characters of the wild accessions are considered to be the first step in ascertaining genetic variability of the population. On the other hand, selection based on the variability in seed characters alone is not a

sure step for selection for high yield. Thus, it is imperative to follow up this step with selection based on growth in the initial years for faster operation of breeding programs especially in tree crops. Variation in morphological characters of the seeds among the wild accession of *Pongamia pinnata* could be due to fact that the species is widely adapted to a broad range of edaphic conditions. Seed character variations in pongamia has been reported although from different geographical locations and much smaller expanse of sampling area than that was undertaken in our studies (Kaushik et al. 2007). Similarly, it has been show that other trees have wide variations in relation to habitat have also been reported in *Azadirachta indica* by Kundu and Tigerstedt (1997). Multiple factors may induce and maintain variation in seed size some of which could be that large seeds may be favoured because they produce larger and more vigorous seedlings with better chances of survival than small seeds and conversely the small seeds may have a selection advantage due to wider and more effective dispersal. Therefore, contradictory evolutionary pressures mediated by biotic as well as abiotic agents potentially act upon plants to produce the seed size distributions observed in natural plant populations (Eriksson 1999). Seed oil content variation is more widely reported not only in annual crops but also in a wide variety of trees (Vollmann et al. 2007; O'Neill et al. 2003; Johansson et al. 1997; Kaura et al. 1998). The variation found in oil content in the present study along with other seed morphological attributes presents us with a viable selection alternative at a very early stage (collection of germplasm) from base seed material. This could be of use in improvement programmes especially considering the fact the pongamia is a new tree crop where crop breeding is still in its infancy.

Variation in growth was clearly evident from the study. All the parameters studied exhibited considerable amount of variation in the nursery as well as the progeny trial of the trees. Understanding the sources of phenotypic and genotypic variation in organisms is central to the understanding of natural variation and the responses of organisms to their environment apart from the objective of evolving a new plant type to suit ones needs. The marginal difference between PCV and GCV of almost all the character studied in both the seed characters and initial growth suggested that there was high heritability of variation among the characters. Heritability tells the breeder how much confidence to place in the phenotypic performance of a crop when selecting for improvement. To exploit the relative role of heredity and environment to the fullest extent possible in the expression of various traits the knowledge of heritability is important. Mahmood et al. (2003) reported that heritability estimate is important in tree improvement programme. It is also useful for ranking importance of each trait in crossbreeding programmes. Gains from tree

breeding programmes depend on the type and extent of genetic variability. In the present study all characters excepting seed width showed distinctively higher heritability in the broad sense. Hence these characters especially 100 seed weight and oil content in seeds and collar diameter during initial growth can be viewed as best gain characteristics for pongamia improvement more so because of its strong genetic control and a wide range of variability.

However, it should be noted that estimation of heritability is of little significance in coherent selection breeding programs unless accompanied by sufficient genetic gain (Tefera et al. 2003). Due to large differences in the phenotypic variation between different traits, genetic advance is not directly related to heritability values. In the present study, low to moderate genetic gain values for seed characters indicate that improvement could be made in the these characters. Genetic advance was considerably larger in the growth traits as compared to the seed characteristics. This could be due to extreme variation in the material investigated, and smaller values for genetic advance are expected in further selection cycles in a more improved material. Within the growth traits plant height showed maximum advance indicating that the progress in shifting the genotypic mean and gene frequencies of plant height in the population could be achieved in the desired direction by selection.

Correlation coefficients revealed interesting relationship in the seed characters as well as growth parameters studied here. Degree of correlation coefficient at genotypic level was higher than their corresponding phenotypic coefficient of correlations in all the parameters indicating the genetic association among the characters. The correlation matrix revealed that statistically significant correlation of seed weight existed with oil content, therefore, seed weight can be considered as important trait for early selection of seed sources. Similar results have been reported other tree-borne oils seeds (Kaura et al. 1998). Selecting on the basis of these characters is all the more useful because of the difficulty in selecting plants solely based on yield as the full potential of yield is reached only after several years of growth in pongamia.

Genetic diversity

Accurate assessment of the levels and patterns of genetic diversity can be invaluable in crop breeding for diverse applications including (i) analysis of genetic variability in cultivars (Smith 1984; Cox et al. 1986), (ii) identifying diverse parental combinations to create segregating progenies with maximum different levels: individual genotypes such as inbred genetic variability for further selection. Analysis of genetic diversity in germplasm collections can facilitate reliable classification of accessions and identification of core subsets of the accessions with future utility

for specific breeding purposes. Hierarchical clustering by Ward's Minimum Variance Cluster Analysis method (Ward 1963) for both seed characters and growth data for all the accessions revealed the genetic relatedness between-accessions. More truncations were seen in both seed characters and growth with 2 members in a cluster indicating high degree of genetic diversity among all the parameters studied. The dendrogram also were indicative of the relative involvement of different constituent traits to the total diversity and quantity the degree of divergence between populations. Other the hand K means clustering pattern in this study revealed that trees from different geographic regions were grouped together in a cluster and as were trees from the same geographical area placed in different clusters. K means clustering is done to understand the trend of evolution and choose genetically diverse parents for obtaining desirable recombination (Tams et al. 2006). High intra-cluster distance in seed characters and growth attributes shown by cluster V indicated that of selection of parents for hybridization should be within these clusters in event of future hybridization programs with expectations for vigour in 100 seed weight and growth, respectively, as the means for these values were high in these clusters. The maximum inter-cluster distance between cluster I and III indicates extensive genetic differences between the trees in these groups and selecting of parents from these clusters would prove useful in developing novel hybrids with characters like increased growth, higher oil content and higher yield. Selection of trees as parents from clusters with low inter-cluster distance should be avoided.

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