

Variability in pod and seed traits of *Pongamia pinnata* Pierre ecotypes in North Karnataka, India

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Abstract An evaluation of 30 naturally available candidate-plus trees (CPTs) of *Pongamia pinnata* was carried out to elucidate the genetic variability and relationship of pod and seed traits and progeny growth traits to select the best planting material with higher productivity. Significant variations were observed in pod and seed characteristics, oil content, and during the progeny trial studies. The highest values were found in 100-pod weight (410.4 gm) and 100-seed weight (195.92 gm) and the phenotypic and genotypic variance was found maximum in 100 pod weight (4364.71 and 4289.93) and 100 seed weight (813.8 and 768.93), respectively. Estimates of the phenotypic coefficient of variance were higher than the genotypic coefficient of variance for all the pod and seed traits and progeny growth traits, which depict the predominant role of the environment. Approximately 99 % of broad-sense heritability was revealed in oil content, followed by 100-pod weight (98.28 %), and canopy growth (96.93 %). Pod thickness shows the highest genetic advance of 56.61 %, followed by pod width (53.43 %) and canopy growth (49.3 %). Pod and seed traits have proven a positive correlation between each other and with progeny growth traits, except a few negative values. Hierarchical clustering by

Ward's minimum variance cluster analysis showed phylogeographical patterns of genetic diversity. K-means clustering revealed that trees from different geographical regions were grouped together in a cluster where as trees from the same geographical area are placed in a different cluster. Seven *Pongamia* ecotypes (GRP-8, 9, 13, 14, 18, 28, and 29) are found superior in traits, namely 100-pod weight, 100-seed weight, oil content, germination rate, plant height, canopy growth, and collar diameter. The traits 100-pod weight and 100-seed weight are highly correlated with the progeny growth traits and even have higher heritability and genetic advantages.

Keywords Biodiesel · Morphological diversity · Pod and seed traits · *Pongamia pinnata* · Progeny trial

Introduction

India is the 11th largest economy in the world, fourth in terms of purchasing power and fifth largest energy consumer in the world, importing around 80 % of its total petroleum oil and lubricants (POL) (Indian Foreign Trade 2012). Fluctuating global prices, depletion of oil reserves, and environmental pollution are the major concerns associated with the use of fossil fuels. To sustain a clean environment and to meet future demands, India has to be committed to conduct research on alternative energy resources, such as utilization of biodiesel and bioethanol replacing the petrol and petro-diesel for transportation. Biodiesel plants have created tremendous interest all over the world for the use of seed oil as a commercial source of biodiesel. India has suitable climatic conditions and has much waste or barren land for cultivation of biodiesel plant species, mainly *Pongamia pinnata*, which is known to

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grow in these conditions. It has also been identified as a promising plant resource for the production of biodiesel. The challenging task as of today is to screen the naturally available wild *P. pinnata* plant resources for the best plant material with high productivity.

Pongamia pinnata (L.) Pierre is a fast-growing, glabrous, deciduous tree, commonly known as Indian beech, pongam tree, or karanja tree. It is a member of the sub-family *Papilionideae*, more specifically the tribe *Millettieae*. It is indigenous to the Indian subcontinent and South East Asia, and has been successfully introduced to humid and tropical regions of the world (Scott et al. 2008). It is tolerant of a wide range of abiotic stresses like drought, frost, heat, and salinity. It grows fast and matures after 4–7 years, yielding fruits that are flat, elliptical, and long; each fruit contains one or two kidney-shaped brownish red kernels. The oil content in the kernel is about 30–40 % and the fatty acid composition has been reported to be oleic (44 %), linoleic acid (17.4 %), erucic (15.9 %), palmitic (7.2 %), linolenic (5.5 %), stearic (3.3 %), 11-eicosenoic (3.4 %), behenic (2.5 %) and eicosanoic (0.78 %) acids (Bala et al. 2010). *Pongamia* seed oil, which has similar properties to that of petro-diesel, has gained importance as a biodiesel, which is a fast-emerging, viable alternative to fossil fuel.

Pod and seed traits are important in evaluating trees of unknown genetic potential, especially a tree like *Pongamia*, where little work has been done in terms of progeny evaluation. Yield stabilization of this tree is expected to be seen only after 7–8 years of establishment, and so it would take a minimum of 10 years to identify superior germplasm in terms of seed yield. Genetic variation in seed characteristics, growth, branching pattern, and yield can be of great potential in tree improvement programmes, particularly for the selection of genotypes having more oil content and yield.

Hence in our present investigation, the CPTs of *Pongamia* are evaluated by phenotypic and genotypic analysis of variability, broad sense heritability, genetic advance, and progeny trial to identify the best planting material.

Similar studies have been carried out by several researchers across India for the selection of suitable quality plants for the respective ideotypes for afforestation programme. (Kaushik et al. 2007; Sunil et al. 2009; Divakara et al. 2010; Divakara and Rameshwar 2011; Rao et al. 2011; Deebe et al. 2011 and Pavithra et al. 2013).

Materials and methods

A wild germplasm exploration survey was conducted to identify the elite germplasm with high-yielding CPTs of *Pongamia* from different agro-climatic conditions in North Karnataka (Hyderabad Karnataka), India as given in Table 1. The survey covers an area of 45,582.88 km² and the region has approximately 1.5–2 lakh hectares of waste or barren land. Collection of seeds and stem cuttings from CPTs was carried out over a two-year period from May to June 2011–2012 and 2012–2013. The selection criteria for CPTs was made by phenotypic assessment of characteristics as advocated by Cornelius (1994) and Kaushik et al. (2007).

A total of 76 *Pongamia* trees were identified as disease free, healthy, and high yielding plants. All plants were 15 years or older and out of these, 30 CPTs were selected based on their morphological and qualitative traits (e.g., yield potential, branching pattern, number of flowers per raceme, crown spread, girth, and disease resistance). A total of 300 healthy pods and seeds (hundred in each replication) were evaluated for their length, width, and thickness using a vernier caliper. The 100-pod weight and 100-seed weights were evaluated, using a scientific weighing scale in a randomized manner. Seed characteristics were analyzed after separation from pods, and the seeds were then cleaned and stored in muslin bags at ambient conditions. All seed lots were dried under ambient temperature and humidity to attain a constant weight. The oil content (percent) in seeds was estimated by soxhlet method using n-hexane as a solvent. The progeny trial study was conducted in the nursery of Biodiesel

Table 1 Different agro-ecological regions of *Pongamia pinnata* candidate plus trees (CPT's) collected from North Karnataka (Hyderabad Karnataka) region, India

Provenance	No. of CPT's collected	Latitude	Longitude	Altitude (m)	Rainfall (mm)	Temperature (°C)	Humidity (%)	Soil type
Bidar	5	17° 35'–18° 25'N	76° 42'–77° 39'E	586–715	<850	36–39	40–75	Red, black cotton
Gulbarga	8	17° 12'–17° 46'N	76° 04'–77° 42'E	391–472	<750	38–42	35–62	Black soil
Yadgir	4	16° 77'N	77° 13'E	383–454	<650	32–40	70–85	Black clayed, brown loamy
Raichur	4	16° 21' N	77° 35' E	358–508	<500	36–43	50–68	Sandy loam, clayey loam
Koppal	5	15° 35'N	76° 15'E	472–601	<750	32–41	63–84	Red, black cotton, red sandy
Bellary	4	15° 30'–15° 50'N	75° 40'–77° 11'E	384–485	<639	35–44	40–70	Red, medium black

Technology Park, Gulbarga University campus. The germplasm accessions were raised under a randomized block design with three replications and planted at a spacing of 3×3 m, and the growth readings were recorded for germination percentage or germination rate (GR), seedling height or plant height (PH), collar diameter (CD), canopy growth (CG), and number of branches per plant (NBP) as suggested by Mukta et al. (2009), Divakara et al. (2010), NOVOD (2010), Deebe et al. (2011), and Rao et al. (2011). The canopy growth was measured in both north-south and east-west directions of the crown spread of the plant and its average was expressed in cm.

Statistical analysis

The raw data of all the parameters with replications was compiled and analyzed using analysis of variance (ANOVA) to calculate the mean, standard error of mean, variance, and coefficient of variation using statistical software SPSS (version 21). The correlation coefficient was analyzed to determine the relationship between pod and seed characteristics and progeny traits, both at the phenotypic and genotypic level. Phenotypic and genotypic variance and phenotypic and genotypic coefficient of variation were performed as suggested by different researchers (Baenziger et al. 2004; Pliura et al. 2007; Yoshida et al. 2007). Broad sense heritability was estimated by the variations in between and within the accessions by calculating the ratio of genotypic variance to the phenotypic variance (Falconer and MacKay 1996; Allard 1999).

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

σ^2g is genotypic variance = (variance between accessions-variance within accessions)/n

σ^2p is phenotypic variance = [(variance between accessions-variance within accessions)/n] + variance within accessions.

$$PCV = \frac{\sqrt{\sigma^2p}}{X} \times 100; GCV = \frac{\sqrt{\sigma^2g}}{X} \times 100$$

where PCV is the phenotypic coefficient of variation, GCV is the genotypic coefficient of variation, n is the number of replicates per treatment and X is the grand mean of each pod and seed trait and progeny trait respectively. Genetic advance (GA) and genetic advance as percent of mean were estimated in accordance with Johanson et al. (1955) as: $GA = K \cdot h^2B \cdot \sqrt{\sigma^2p}$; Genetic advance (as percent of mean) = $(GA/X) \times 100$

The association and variation in-between and within the pod and seed parameters was observed by hierarchical

euclidean cluster analysis by divisive method and non-hierarchical (K-means) by an agglomerative method for initially collected *Pongamia* accessions. The shortest Euclidean distances in the close neighborhood was calculated by Ward's method (Ward 1963) and a dendrogram was constructed to prove the relationship within and between the population (Sokal and Rohlf 2003).

Results

Variability in pod and seed traits

Analysis of variance of pod and seed traits revealed a significant variation among all the wild accessions studied for all the characters at $P < 0.05$. Variability studies revealed that the accession GRP-29 has recorded maximum values for pod width (2.96 cm), 100-pod weight (410.4 gm), 100-seed weight (195.92 gm), and oil content (43.53 %). The accession GRP-18 has shown maximum values for pod thickness (1.74 cm), seed length (2.71 cm), and seed width (1.74). The accession GRP-22 has higher values in pod length (5.97 cm) and pod width (2.96 cm). The accessions GRP-8, GRP-14, GRP-18, and GRP-29 showed highly significant values for 100-pod weight and 100-seed weight compared to other accessions.

Accessions having pod lengths greater than 5 cm was recorded in GRP-5, GRP-6, GRP-8, GRP-10, GRP-15, GRP-16, GRP-18, and GRP-22. Pod width of greater than 2.6 cm was recorded in GRP-4, GRP-5, GRP-21, GRP-22, GRP-28, GRP-29, and GRP-30. Seed length and seed width was recorded highest in GRP-2, GRP-8, GRP-12, GRP-18 and GRP-22. Oil content of more than 38 % was observed in accessions GRP-29, GRP-28, GRP-18, GRP-16, GRP-13, GRP-8, and GRP-27. Overall, 19 accessions have an oil content of more than 35 %. Accessions GRP-2, GRP-3, GRP-4, GRP-21, and GRP-25 have minimum values for pod and seed traits (e.g., 100PW and 100SW as shown in Table 2).

Genetic association and diversity among pod and seed traits

Genetic association and variability was evident from the study of phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), broad sense heritability (h^2B) and genetic advance (GA). The magnitude of PCV was greater than the corresponding GCV for all of the pod and seed characteristics, indicating a predominant role of environmental factors. The phenotypic and genotypic coefficients of variance were closer to each other for all traits. The phenotypic variance (σ^2p) and genotypic

Table 2 Pod and seed characteristics and oil content variability of candidate plus trees (CPT's) of *Pongamia pinnata* germplasm from different ecotypes of North Karnataka (Hyderabad Karnataka) regions

CTP's	Pod traits				Seed traits				PSR	OC (%)	Place of sample collected (District)
	PL (cm)	PW (cm)	PT (cm)	100PW (gm)	SL (cm)	SW (cm)	ST (cm)	100SW (gm)			
GRP-1	4.56	2.12	0.68	248.96	2.04	1.42	0.92	131.8	1.89	33.4	Yadgir
GRP-2	4.63	2.13	1.22	239.84	2.46	1.64	0.96	126.8	1.88	32.72	Yadgir
GRP-3	3.55	1.55	1.11	227.2	2.16	1.2	0.86	125.58	1.74	32.44	Yadgir
GRP-4	4.63	2.65	0.88	242.8	2.16	1.55	0.81	130.82	1.94	33.3	Yadgir
GRP-5	5.18	2.61	0.91	295.6	2.08	1.55	0.77	149.04	2.23	35.46	Koppal
GRP-6	5	2.29	1.26	288.22	1.81	1.31	0.49	144.78	1.86	34.68	Koppal
GRP-7	4.52	2.34	1.12	336.4	2.24	1.46	0.73	175.8	2.02	37.48	Koppal
GRP-8	5.11	2.49	1.19	372.34	2.54	1.59	0.87	186.6	1.95	38.62	Koppal
GRP-9	4.57	2.51	1.18	329.4	2.62	1.47	1.13	170.72	1.67	37.68	Koppal
GRP-10	5.06	2.07	1.23	283.76	2.22	1.2	1.03	140.32	2.14	35.36	Raichur
GRP-11	4.49	1.78	1.13	290.28	2.17	1.2	0.98	146.94	2.2	36.22	Raichur
GRP-12	4.85	2.35	1.25	317.86	2.49	1.74	1.05	159.28	1.88	37.62	Raichur
GRP-13	4.19	2.13	1.28	333.18	2.32	1.5	1.11	177.12	1.97	38.66	Raichur
GRP-14	4.27	1.79	1.18	370.24	2.24	1.4	0.93	186.86	1.73	39.3	Bidar
GRP-15	5.09	2.22	1.15	266.16	2.38	1.6	0.87	139.18	2.09	35.62	Bidar
GRP-16	5.16	2.11	1.2	332.22	2.11	1.48	0.8	159.36	2.25	39.62	Bidar
GRP-17	4.95	2.43	1.52	335.68	2.35	1.46	0.9	176.18	2.33	34.56	Bidar
GRP-18	5.12	2.54	1.74	396.4	2.71	1.74	0.83	189.06	2.12	41.45	Bidar
GRP-19	3.73	1.78	1.38	328.2	1.85	1.43	1.14	166.38	1.95	32.48	Bellary
GRP-20	4.27	2.17	1.21	254.86	2.02	1.43	0.84	132.32	1.92	35.36	Bellary
GRP-21	4.67	2.82	1.21	227.88	2.38	1.69	0.78	126.38	2.16	33.64	Bellary
GRP-22	5.97	2.96	1.18	248.54	2.52	1.65	0.87	131.4	2.59	32.84	Bellary
GRP-23	4.97	2.42	1.29	318.54	2.32	1.46	1.09	154.82	2.32	35.76	Gulbarga
GRP-24	4.81	2.16	1.23	321.88	2.2	1.36	1.02	156.42	1.76	36.6	Gulbarga
GRP-25	4.98	2.33	1.4	218.1	2.36	1.44	0.97	120.26	1.52	34.3	Gulbarga
GRP-26	4.16	2.19	1.59	260.2	2.4	1.43	0.93	135.1	1.65	33.94	Gulbarga
GRP-27	4.47	2.18	1.45	319.82	2.1	1.38	1.02	155.38	2.16	38.54	Gulbarga
GRP-28	4.76	2.68	1.32	322.58	2.19	1.61	0.89	164.28	1.99	41.73	Gulbarga
GRP-29	4.82	2.96	1.35	410.4	2.21	1.59	0.88	195.92	2.02	43.53	Gulbarga
GRP-30	4.35	2.77	1.3	321.24	2.31	1.51	0.78	158.84	1.96	36.66	Gulbarga
Mean	4.697	2.317	1.238	301.884	2.265	1.483	0.908	153.595	1.996	36.319	
SEM	0.086	0.063	0.036	9.289	0.037	0.026	0.025	3.96	0.042	0.533	
CD 5 %	0.156	0.077	0.041	10.062	0.075	0.049	0.03	5.119	0.066	1.21	

variance (σ^2g) as higher for 100PW (4364.71 and 4289.93) and 100SW (813.8 and 768.93) respectively, moderate for germination rate, plant height, canopy growth, and oil content and low for the remaining traits.

Pod width and seed thickness have exhibited much higher value for both GCV and PCV in comparison to pod and seed traits and the canopy growth (CG) was observed the highest with a PCV value of 24.69 and GCV values of 24.31 in plant growth studies. Broad sense heritability was high in general and exceeded 70 % for all the pod and seed

traits studied and the highest value of 99 % was observed in OC followed by 100PW (98.28 %), 100SW (94.48 %), PW (92.29 %) as given in Table 3.

The characteristics 100PW, 100SW, OC, GR, PH, CG, and CD showed a similar degree of variation in PCV and GCV, which may be due to maternal or environmental factors (Gutterman 2000). Among the progeny trial, h^2B was highest in CG (96.93 %) followed by GR (96.14 %), CD (95.91 %), and PH (95.7 %). Genetic advance as percent of mean was observed highest in PT (56.61 %)

Table 3 Estimation of genetic variables for pod and seed characteristics and progeny traits in *Pongamia pinnata* germplasm

Traits	Variance		Coefficient of variation (%)		Heritability broad sense (%)	Genetic advance (%)
	Genotypic	Phenotypic	Genotypic	Phenotypic		
Pod traits						
PL (cm)	0.72	0.79	18.12	19.03	90.66	35.54
PW (cm)	0.39	0.42	27	28.1	92.29	53.436
PT (cm)	0.13	0.14	29.21	31.05	88.5	56.619
100PW (gm)	4289.93	4364.71	21.69	21.88	98.28	44.307
Seed traits						
SL (cm)	0.13	0.16	15.91	18.15	76.83	28.74
SW (cm)	0.06	0.08	17.08	19.23	78.87	31.356
ST (cm)	0.05	0.07	26.14	30.38	74.11	46.382
100SW (gm)	768.93	813.8	18.05	18.57	94.48	36.148
PSR	0.08	0.1	14.17	16.30	75.5	25.362
OC (%)	8.567	8.621	8.059	8.084	99.375	16.549
Progeny traits						
GR (%)	150.76	156.8	16	16.32	96.14	32.334
PH (cm)	122.53	128.03	15.08	15.41	95.7	30.391
CD (cm)	0.01	0.01	11.72	11.96	95.91	23.646
CG (cm)	96.28	99.32	24.31	24.69	96.93	49.307
NBP	0.83	1.48	13.93	18.57	56.3	21.542

PL pod length, PW pod width, PT pod thickness, 100PW 100 pod weight, SL seed length, SW seed width, ST seed thickness, 100SW 100 seed weight, PSR pod seed ratio, OC oil content, GR germination rate, PH plant height, CD collar diameter, CG canopy growth and NB number of branches per plant

followed by PW (53.43 %) and CG (49.3 %) and other traits and least was recorded in OC (16.54 %).

The correlation coefficient of pod and seed traits and progeny growth traits shows the contribution of each component to the yield and an indirect effect in association with the other components. The correlation coefficient (along the horizontal and vertical axes) between PL, PW, SL, ST, PSR, OC, GR, and PH were prominent and are considered as the main components in tree breeding programs. The association of pod, seed, and progeny growth traits are significantly correlated with each other, except for few negative values in both phenotypic and genotypic levels as in Table 4.

Euclidean clusters and cluster mean values, obtained by K-means non-hierarchical clustering for pod and seed characters of 30 accessions of *Pongamia*, are placed in five clusters, as depicted in Table 5. The maximum number of accessions are in cluster V (12 accessions); they have shown good mean values with respect to seed thickness, pod seed ratio, and number of branches. Cluster III with minimum accessions (3 accessions) has reliable mean values in almost all the traits studied except ST and CG. The dendrogram obtained from hierarchical Euclidian cluster analysis, according to Ward's method, is shown in Fig. 1. The length of horizontal branches between clusters

proved a high degree of dissimilarity between clusters. The maximum turn of members, apart from the super cluster containing all the accessions are populated among 30 accessions. The *Pongamia* ecotypes GRP-23 and GRP-29 coincide at a distance of 25 with 23 accessions between their values.

Progeny trial characters

Significant variations are found between and within the growth of 30 CPTs of *Pongamia* from the initial seed sources. Seven accessions have a germination rate of more than 80 % and only four accessions have a germination rate less than 70 %. Similar results was recorded in PH, CD, CG, and NBP in accession GRP-21, GRP-22, and GRP-29. A few low-ranking accessions are GRP-1, GRP-7, and GRP-26 from the initial trials; medium-ranking accessions are GRP-6, GRP-18, GRP-24, GRP-25, and GRP-28 (see Table 6). The lowest plant height was recorded in GRP-1 (57.76 cm) and the highest collar diameter is recorded in GPR-22 (1.12 cm). The collar diameter was observed to be more than 1.1 cm in four accessions and only one accession (GRP-1) has a value less than 0.9 cm. The highest number of branches per plant the range of only 5.7–5.9 branches in GRP-8, GRP-11, GRP-12, GRP-13, GRP-14

Table 4 Genotypic (G) and phenotypic (P) correlation coefficient between pod and seed characteristics and progeny growth in *Pongamia pinnata* germplasm

		PW (cm)	PT (cm)	100 PW (gm)	SL (cm)	SW (cm)	ST (cm)	100 SW (gm)	PSR	OC (%)	GR (%)	PH (cm)	CD (cm)	CG (cm)	NBP
PL (cm)	G	0.906	0.693	0.787	0.986	1.000**	0.904	0.995	0.972	0.48	0.99	0.993	0.95	0.94	0.893
	P	0.971	0.996	0.631	0.961	0.551	0.846	0.674	0.98	0.792	0.731	0.827	0.693	0.745	0.977
PW (cm)	G		0.322	0.451	0.823	0.9	0.637	0.86	0.98	0.807	0.835	0.95	0.728	0.707	0.617
	P		0.945	0.799	0.866	0.335	0.693	0.832	0.904	0.622	0.874	0.668	0.5	0.563	0.896
PT (cm)	G			0.99	0.803	0.703	0.935	0.761	0.503	-0.299	0.79	0.602	0.884	0.897	0.944
	P			0.558	0.982	0.625	0.891	0.604	0.994	0.844	0.666	0.875	0.756	0.803	0.922
100 PW (gm)	G				0.878	0.795	0.975	0.844	0.618	-0.163	0.868	0.708	0.94	0.95	0.981
	P				0.391	-0.299	0.121	0.998*	0.466	0.026	0.991	0.087	-0.121	-0.047	0.449
SL (cm)	G					0.988	0.962	0.998*	0.919	0.329	1.000*	0.96	0.988	0.983	0.955
	P						0.961	0.443	0.997	0.93	0.513	0.951	0.866	0.901	0.998*
SW (cm)	G						0.909	0.996	0.968	0.468	0.991	0.991	0.954	0.945	0.899
	P						0.911	-0.245	0.705	0.946	-0.166	0.925	0.983	0.967	0.718
ST (cm)	G							0.941	0.776	0.058	0.956	0.846	0.992	0.995	1.000*
	P							0.177	0.935	0.995	0.255	0.999*	0.971	0.986	0.941
100 SW (gm)	G								0.944	0.392	0.999*	0.976	0.976	0.969	0.932
	P								0.515	0.082	0.997	0.143	-0.065	0.01	0.499
PSR	G									0.674	0.927	0.993	0.849	0.833	0.76
	P									0.897	0.582	0.922	0.822	0.862	1.000*
OC (%)	G										0.349	0.581	0.182	0.153	0.033
	P										0.162	0.998*	0.989	0.997*	0.905
GR (cm)	G											0.965	0.985	0.979	0.978
	P											0.222	0.016	0.09	0.567
PH (cm)	G												0.906	0.893	0.832
	P												0.978	0.991	0.929
CD (cm)	G													1.000*	0.989
	P													0.997*	0.832
CG (cm)	G														0.993
	P														0.871

* Significant at the 5 % level; ** Significant at the 1 % level

PL pod length, PW pod width, PT pod thickness, 100PW 100 pod weight, SL seed length, SW seed width, ST seed thickness, 100SW 100 seed weight, PSR pod seed ratio, OC oil content, GR germination rate, PH plant height, CD collar diameter, CG canopy growth and NBP number of branches per plant

Table 5 Composition of Euclidean clusters and cluster mean values obtained by K-means non-hierarchical clustering for pod and seed characteristics and progeny traits in wild germplasm of *Pongamia pinnata*

Cluster accessions	Accession No.	PL (cm)	PW (cm)	PT (cm)	100 PW (gm)	SL (cm)	SW (cm)	ST (cm)	100 SW (gm)	FSR (%)	OC (%)	GR (%)	PH (cm)	CD (cm)	CG (cm)	NBP	
I	7	GRP-1, GPR-2, GRP-4, GRP-15, GRP-20, GRP-22, GRP-26	4.76	2.35	1.13	251.62	2.28	1.53	0.89	132.49	1.99	33.88	74.33	71.43	1.02	37.43	6.84
II	4	GRP-8, GRP-14, GRP-18, GRP-29	4.83	2.45	1.37	387.35	2.43	1.58	0.88	189.61	1.96	40.73	77.8	75.24	1.02	43.59	6.33
III	3	GRP-3, GRP-21, GRP-25	4.4	2.23	1.24	224.39	2.3	1.44	0.87	124.07	1.81	33.46	74.89	75.32	1.06	37.78	6.63
IV	4	GRP-5, GRP-6, GRP-10, GRP-11	4.93	2.19	1.13	289.47	2.07	1.32	0.82	145.27	2.11	35.43	79.49	72.69	0.97	39.27	6.63
V	12	GRP-7, GRP-9, GRP-12, GRP-13, GRP-16, GRP-17, GRP-19, GRP-23, GRP-24, GRP-27, GRP-28, GRP-30	4.61	2.32	1.29	326.42	2.26	1.49	0.97	164.55	2.02	37.28	77.26	73.7	1.01	42.01	6.42

PL pod length, PW pod width, PT pod thickness, 100PW 100 pod weight, SL seed length, SW seed width, ST seed thickness, 100SW 100 seed weight, PFSR pod seed ratio, OC oil content, GR germination rate, PH plant height, CD collar diameter, CG canopy growth and NBP number of branches per plant

and GRP-19 and the highest of 7.7 branches in GRP-18. Most accessions have more than 5.8–6.9 branches with an intermediate value of 6.5.

The accessions with greater values in pod and seed traits (GRP-8, 9, 13, 14, 18, 28, and GRP-29) have shown a good germination rate of more than 70 %. Except for GRP-13, plant height ranged between 70–86 cm, where the accession GRP-29 showed high values for germination rate, plant height, and moderate values for collar diameter, canopy growth and number of branches per plant which indicates direct relationship between the seed size and oil content to germination rate and plant height.

The accessions from Raichur (GRP-10, 11, 12, and GRP-13) and Bellary (GRP-19, 20, 21, and GRP-22) having 100SW ranging from 131 to 177 gm and oil content ranging from 32 to 38 % when grown in Gulbarga region showed good germination rate, plant height, collar diameter, canopy growth and number of branches per plant depicts the role of the environment plays moderate role in the growth of the progenies.

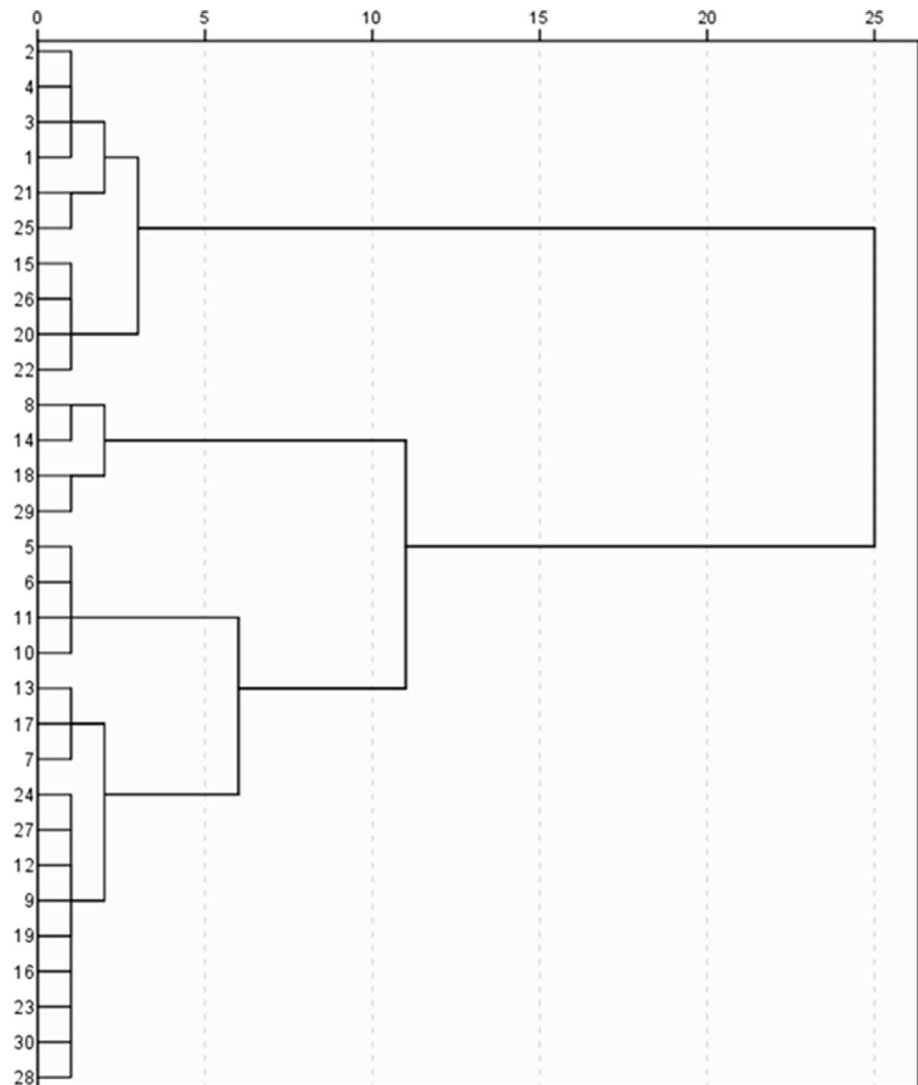
The accessions (GRP-14, 15, 16, 17, and GRP-18) collected from Bidar district shows good values in all pod and seed traits and even in progeny growth studies. While Bidar has the highest rainfall and is at a higher altitude in North Karnataka region (Table 1), the *Pongamia* accessions showed a constant performance during progeny studies. The *Pongamia* ecotypes GRP-8, 14, and 18 are grouped with GRP-29 (superior quality based on high 100 seed-weight and oil content) in the non-hierarchical Euclidean cluster (Table 5). The dendrogram (Fig. 1) depicts the relevance of similar characters among these accessions, which are from different geographical regions.

Discussion

Pongamia is undoubtedly the most prominent non-edible oil-yielding plant species for combating the global fuel crisis through the production of biodiesel. In an agricultural country like India, having much barren/waste land and suitable climatic conditions for cultivation of biofuel plant species like *Pongamia* could produce feedstock of approximately 12–18 tons of seeds per year per hectare, which could produce 3000–4000 l of biodiesel per hectare at the least productive level.

But *Pongamia* has not yet been explored in terms of identifying elite quality plants through morphological and reproductive markers, which could be the beginning stage of breeding elite genotypes in relation to oil content and productivity. It is a highly cross-pollinated species and exhibits a wide spectrum of variation in pod and seed traits. The growth of *Pongamia* wild accessions under diverse conditions causes morphological, biochemical, and genetic

Fig. 1 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 accession has been listed in Table 2



adaptation because multiple factors may stimulate and maintain variations in the pod and seed size. The larger seeds may be chosen because they produce bigger and more vigorous seedlings with a better chance of natural selection as compared to small seeds (Kaushik et al. 2007; Kesari et al. 2008).

Variability in pod and seed characteristics

Pod and seed morphological characteristics of the wild accessions are considered to be the first step in ascertaining genetic variability of the population. Identification of better genotypes or superior phenotypes having better yield and oil content is the initial step in any tree improvement strategy. On the other hand, selection based on the variability in pod and seed characteristics alone is not a sure step in the selection of high production. Thus, it is imperative to follow up with the selection based on growth

in the initial years for faster operation of breeding programmes especially in tree crops. Several researchers have studied variations in morphological (pod and seed traits), progeny growth for the identification of high quality planting material in different geographic regions across India (the data is represented in Table 7).

Variations in seed oil content are more widely reported in a wide variety of trees (O'Neill et al. 2003). Similar variations in morphological characteristics were evident in our present study, namely attributes that are a viable selection alternative at a very early stage (collection of germplasm) from the base seed material. Variation in growth of *Pongamia* ecotypes was clearly evident from the present study: pod width, pod thickness, and seed thickness were recorded with the highest values when compared to the other components as given in Table 7.

All the parameters exhibited a considerable amount of variability in the nursery as well in progeny growth study.

Table 6 Growth and branching after 1 year in progeny trials of selected superior germplasm of *Pongamia pinnata*

Place of sample collection (District)	GRP's	GR (%)	PH (cm)	CD (cm)	CG (cm)	NBP
Yadgir	1	68.14	57.76	0.875	33.21	6.7
Yadgir	2	74.59	70.2	1.05	30.22	7.2
Yadgir	3	71.06	68.26	1.105	29.27	6.6
Yadgir	4	73.28	70.61	1.08	29.74	6.4
Koppal	5	76.28	68.16	0.977	31.47	6.3
Koppal	6	79.46	72.12	0.911	33.53	7.4
Koppal	7	69.78	60.12	1.03	42.35	6.7
Koppal	8	71.26	70.41	1.019	44.06	5.7
Koppal	9	82.47	74.16	0.931	39.9	6.5
Raichur	10	90.73	76.94	1.018	51.09	6.9
Raichur	11	71.5	73.52	0.991	40.97	5.9
Raichur	12	70.64	79.03	1.058	41.99	5.8
Raichur	13	69.71	75.96	0.933	41.15	5.7
Bidar	14	71.31	69.94	0.96	39.29	5.9
Bidar	15	71.67	71.05	0.969	43.77	6.6
Bidar	16	80.6	76.51	1.097	40.58	7.2
Bidar	17	74.98	79.51	1.034	41.01	6
Bidar	18	76.09	73.9	0.975	42.25	7.7
Bellary	19	80.03	78.68	0.971	41.33	5.8
Bellary	20	74.87	73.1	1.014	45.01	6.9
Bellary	21	78.11	83.34	1.108	43.54	7.1
Bellary	22	88.98	85.21	1.126	41.13	7.2
Gulbarga	23	87.39	71.07	1.045	40.62	6
Gulbarga	24	75.18	73.98	0.973	40.6	6.9
Gulbarga	25	75.49	74.37	0.958	40.52	6.2
Gulbarga	26	68.76	72.06	1.038	38.94	6.9
Gulbarga	27	70.81	70.57	1.061	45.8	7.1
Gulbarga	28	83.04	71.59	1.041	46.68	6.9
Gulbarga	29	92.54	86.69	1.113	48.75	6
Gulbarga	30	82.43	73.24	0.937	42.05	6.4
Mean		76.7	73.4	1.013	40.36	6.553
SEM		1.236	1.115	0.011	0.986	0.102
CD 5 %		2.556	2.446	0.033	1.345	0.218

GR germination rate, PH plant height, CD collar diameter, CG canopy growth and NBP number of branches per plant

The association between seed size and germination efficiency in *Pongamia* has been reported by Manonmani et al. (1996). Environmental factors cause the phenotypic and genotypic variations in plants to evolve a new plant type, hence the seeds good characteristics may be selected for producing better progenies in addition to 100 pod weight and 100 seed weight (Jaishankar et al. 2014).

The ability to estimate heritability is important because it makes it possible to rank the importance of each trait in a crossbreeding program, which depends on the type and extent of genetic variability. The study of GCV and heritability will give an idea for selection of candidate plus trees, as the level of heritability may be controlled by

additive gene action in the expression of the characteristics. The marginal differences between PCV and GCV of all the characteristics studied suggest high heritability of variation among the characters and high values of genetic advance. These are indicative of additive genes that are actively involved in the expression of various polygenic traits and low values of non-additive gene action. The correlation coefficient analyses the relationship of variables but cannot quantify the effect of genotypes on dependent variables.

The phenotypic and genotypic correlation of pod and seed characteristics offers an opportunity to select phenotypes based on these traits. The genetic correlations are more reliable estimates for examining the relationship

Table 7 Comparison of pod and seed characteristics and progeny growth of wild germplasm of *Pongamia pinnata* from different agro-climatic conditions of India studied by several workers

Authors	PL (cm)	PW (cm)	PT (cm)	100 PW (gm)	SL (cm)	SW (cm)	ST (cm)	100 SW (gm)	OC (%)	GR (%)	Area of survey
Kaushik et al. 2007	5.703	2.79	1.27	403.93	2.416	1.803	0.993	186.8	41.74	-	Haryana
Sumil et al. 2009	-	-	-	-	2.49	1.78	1.02	273	47.3	-	Northern coastal regions of Andhra Pradesh, Southern coastal regions of Orissa, Rayalseema and South coastal regions of AP and in Telengana
Divakara et al. 2010	6.573	2.706	1.227	542.35	2.793	1.755	-	202.89	44.33	94.33	Jharkhand
Deebe et al. 2011	6.604	2.702	1.163	488.2	2.815	1.755	0.896	177.4	-	-	Orissa
Rao et al. 2011	-	-	-	-	2.5	2.06	-	171.92	38.16	-	Andhra Pradesh
Divakara and Rameshwar 2011	6.56	2.701	1.27	542.4	2.79	1.75	-	217.9	-	-	Jharkhand
Pavithra et al. 2013	4.87	2.31	1.04	337	2.25	1.58	0.78	164.3	34.86	76.2	Southern Karnataka, Transition zones between Andhra Pradesh and Tamil Nadu
Present study	5.97	2.96	1.74	410.4	2.71	1.74	1.14	195.92	43.53	92.54	North Karnataka (Hyderabad Karnataka) region

PL pod length, PW pod width, PT pod thickness, 100PW 100 pod weight, SL seed length, SW seed width, ST seed thickness, 100SW 100 seed weight, OC oil content and GR germination rate

between characteristics. This offers an opportunity to select phenotypes with good quantitative characteristics resulting in improvement programs (Mahmood et al. 2003). In our present investigation, the correlation coefficient studies revealed interesting relationships between pod and seed characteristics to initial growth parameters. The phenotypic coefficient of correlation was higher than the corresponding genotypic coefficient of correlation in most of the parameters, indicating the genetic association among the characters. Correlated quantitative traits are of major interest in any tree-improvement programme, as the improvement of one character may cause simultaneous changes in other characters.

Genetic diversity

Analysis of genetic diversity in germplasm collections can facilitate consistent classification and recognition of elite accessions. Assessment in the level and pattern of genetic diversity can be helpful in crop breeding for analysis of inherited variability in cultivars and identification of diverse parental combinations. Hierarchical clustering of pod and seed characteristics and progeny growth traits, using Ward's Minimum Cluster Analysis method, indicates a high degree of genetic diversity. The dendrogram indicates the relative participation of different constituent traits to the total diversity and quantity divergence between populations. Non-hierarchical (K means) clustering by is done to understand the trend of evolution and to choose genetically diverse parents in order to obtain the desirable recombination as suggested by Tams et al. (2006). Similarly in our present study, *Pongamia* ecotypes GRP-8, 14, 18 and GRP-29 are grouped in same cluster (cluster II, Table 5) and in non-hierarchical clustering (Fig. 1), which indicates these accessions may have similar quality characteristics, where GRP-29 has high values for 100-PW, 100-SW, and oil content and moderate values for progeny growth studies. The maximum distances indicate extensive genetic differences between the trees of these groups. These attributes are helpful for the selection of parents for novel hybrids with increased characteristics like increased growth, oil content, and yield.

The present study also reveals the details of the candidate plus trees necessary in the tree improvement program via the traits like 100-pod weight and 100-seed weight, which were highly correlated with the progeny growth traits. Hence, the identification of elite quality *Pongamia* ecotypes (GRP-8, 9, 13, 14, 28, and 29) are found superior in traits, namely 100PW, 100SW, OC, GR, PH, CG, and CD.

The North Karnataka region has about 1.5 lakh hectares of waste or barren land, where the plantation of *Pongamia* plants could produce 400–500 ml of biodiesel even by

conventional methods. This could fulfil the need of biodiesel in the state and also generate employment in the Hyderabad Karnataka region. It could also increase the forest cover in the region and help to provide a clean environment. Hence, plantation of *Pongamia* trees may be helpful for massive afforestation programs in the semi-arid conditions of North Karnataka. Also, the present study helps as a pointer for taking a molecular biology and genetics approach to characterize genomic studies.

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