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Orginal Article

Identification and evaluation of diverse genotypes in *Pongamia pinnata* (L.) Pierre. for genetic improvement in seed traits

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ABSTRACT

Pongamia (*Pongamia pinnata* [L.] Pierre.) Is a highly cross pollinated species and exhibits a wide spectrum of variation in the pod and seed traits. An evaluation of twenty-four Candidate Plus Trees [CPTs] of pongamia was carried out to elucidate the extent of genetic variability, correlation available among the pod and seed traits for diversity analysis and use in breeding. Eleven CPTs were promising for 100-pod weight, seed length, 100-seed weight and oil content. CPT – 19 had good combination for seven traits viz. pod length [65.5 mm], 100-pod weight [541.3 g], 2D surface area [348.7 mm2], seed length [27.7 mm], seed breadth [17.4 mm], 100-seed weight [202.3 g] and oil content [28.5 %]. This would be an important genotype to follow further and use in breeding program. CPT – 14 showed the lowest expression for 100-seed weight [107.6 g] and oil content [28.5 %]. High estimates of heritability and high genetic advance observed for 100-pod weight, 100-seed weight and oil content, indicated the possibility of their improvement by selection. Oil content was particularly correlated at genotypic and phenotypic levels with 100-seed weight [rg = 0.62, rp = 0.58] and 100-pod weight [0.50, 0.46]. The first four Principal Component [PCs] explained large portion [86.4 %] of the total variation. Cluster analysis resulted into two broad clusters with cluster-1 having 6 CPTs and cluster-2 having 18 CPTs. Six CPTs in cluster-1 [CPT – 4, 5, 6, 7, 9 and 19] had combination of desirable traits and can be directly selected for further improvement by breeding.

Keywords: *Pongamia pinnata, co-efficient of variation, heritability, correlation and diversity analysis.*

INTRODUCTION

India consumes approximately 40 million tonnes of diesel and is ranked fifth in the world after the US, China, Russia and Japan in terms of fossil fuel consumption. India consumes petroleum products worth Rs 4 lakh Crores per annum. India is the 5th largest emitter of CO2 in the world after USA [5800 MT], China [4732 MT], Russia [1529 MT] and Japan [1215 MT]. Looming international fossil fuel crisis clubbed with domestic crude oil output reaching plateau is compelling to iead a search for a new, viable and indigenous renewable source of energy especially for biodiesel production. Even though in India 186 tree species are known to contain oil in their seeds, only Jatropha curcas L. and *Pongamia pinnata* [L.] Pierre. Are being considered to gain popularity as non-edible feedstock for bio-diesel production to meet the challenges of steady spurt oil demands and the meeting of national commitment for clean environment. Though *J. curcas* has many advantages, *P. pinnata* is gaining popularity because of it is natural distribution and wide diversity across the country. Added to this, the low-temperature operability of the corresponding methyl esters is superior to that of jatropha oil methyl esters because of the relatively high percentage of oleic acid in karanj oil [Srivastava and Verma, 2008]. Energy plantations involving *P. pinnata* as a microenterprise have already been established in India [www.icrisat.org;www.himalayaninstitute.org].

Pongamia pinnata [L.] Pierre, synonymously known as *P. glabra* Vent., *Derris indica* [Lam] Bennett; *Millettia novo-guineensis* Kane & Hat. And *Cytisus pinnaus* L. is an arboreal legume, belonging to the subfamily Papilionoideae and more specifically the tribe Millettieae. This medium-sized tree being indigenous to the Indian subcontinent and south-east Asia [Malaysia and Indonesia], has been successfully introduced to humid tropical regions of the world as well as parts of Australia, New Zealand, China and the USA [Scott et al., 2008]. Historically, this plant has been used in India and neighboring regions as a source of traditional medicine, animal fodder, green manure, timber, fish poison and fuel. The mature tree can withstand water logging and slight frost, but is highly tolerant to salinity. *P. pinnata* can help in restoration of fertility especially in degraded soils owing to its nitrogen fixing ability. The tree forms the subject of recent reviews covering its chemistry and biological activity [Meera et al., 2003], phytochemical constituents, traditional uses and pharmacological properties [Chopade et al., 2008] and future prospects as a biodiesel yielding species [Scott et al., 2008].

The growing interest in the seed oil of *P. pinnata* and the realization of the need for raising high yielding plantations has led to the search for technologies for its growth and profitable production. Since the species has always been treated as an avenue tree, efforts towards domestication through identification of elite genotypes have been very limited. It has never been exploited as a tree meant for plantations hence only isolated attempts have been made towards genetic improvement. The effectiveness of tree improvement programmes depends upon the nature and magnitude of existing variability and also on the degree of transmission of traits or heritability [Zobel and Talbert, 1984]. *P. pinnata* is an out-breeding species which exhibits high levels of heterogeneity for seed yield [20-200 kg/tree] and oil content varying from 25-40 %. The variation in yield and oil content in *P. pinnata* forms the backbone for exercise on the selection of superior genotypes from natural population. Hence, it is important to screen the naturally available *P. pinnata* genetic resources and to select the best planting material with high oil content for higher productivity. The selection of superior trees based on seed morphology and oil content may have greater impact than that of the conventional breeding. There is no available information on geographical variation and its influence on seed quality and quantity. Added to this, *P. pinnata* is the backbone for the livelihood of tribal community in Jharkhand, and the genetic improvement through selection, evaluation and breeding will definitely have an impact on the socio-economic status of the community and in turn improves the livelihood of the people. Keeping this in view, we investigated genetic variation, correlation and divergence in seed traits and oil content among 24 accessions of *P. pinnata* collected from Jharkhand, India.

MATERIAL & METHODS

An extensive wild germplasm exploration survey was conducted to identify the high yielding Candidate Plus Trees [CPTs] of *P. pinnata* at fruiting stage from different predominant naturalized locations in Jharkhand, India. Since *P. pinnata* is grown as wild and has no definite geometry with neighboring trees for comparison, hence the selection was made by using single tree selection method based on phenotypic assessment of characters of economic importance viz yield potential, crown spread, total height, girth at breast height, age of the tree, free from pest and diseases, seed size and seed weight. A total of 24 CPTs [phenotypically superior trees] covering a latitude and longitudinal range between 22° N to 24° 50' N and 83° 30' E to 87° E, respectively, were selected [Table 1, Fig 1]. From each CPTs, approximately 3 kg mature pods were collected following a random sampling procedure from all the four directions of the crown of each selected tree during fruiting season April-June, 2005. The observations for eleven quantitative traits [4 pod and 7 seed traits] were recorded at Forest Research Centre [latitude: 23° 27' 40 N, longitude: 85° 05' 56 E, altitude 2320 ft msl approx.], Institute of Forest Productivity Mandar, Ranchi district during 2005 – 07.

Pod characters

The pods were cleaned and stored in muslin bags at ambient conditions. All lots were dried under similar temperature and humidity conditions to reach constant weight. A total of 300 healthy pods [hundred in each replication] were collected and observations for four pod traits viz. pod length, pod width, pod thickness and 100-pod weight were measured as mentioned in table 2.

Seed characters

Samples of 300 seeds were randomly collected from each CPT to make three replications of 100 seeds. Measurement of morphometric traits viz. seed length, seed breadth, aspect ratio and 2D surface area, was done using Image analyzer [Leica Quantimet called QWin 500]. Seeds were spread on a glass platform of macro-viewer for each replication and images were captured using

charge coupled device [CCD] camera and taken into the software called Quantimet 500+ or Qwin. The Qwin identifies the object based on our specification for seed colour and calibrates captured images to actual scale. The various 2 dimensional measurements of the detected images and other parameter were measured as mentioned in table 2.

Data analysis

The pod and seed parameters were analysed for Analysis of variance [ANOVA] to understand the significance of differences between the pods, seeds and progenies of CPTs [Gomez and Gomez, 1984]. The phenotypic variation for each trait was partitioned into components due to genetic [hereditary] and non-genetic [environmental] factors and estimated using the following formula [Johanson et al., 1955]:

$Vp = MSG/r$; $Vg = [MSG – MSE]/r$; $Ve = MSE$

where MSG, MSE and r are the mean squares of CPTs, mean squares of error and number of replications, respectively.

The phenotypic variance [Vp] is the total variance among phenotypes when grown over the range of environments of interest, the genotypic variance [Vg] is the part of the phenotypic variance that can be attributed to genotypic differences among the phenotypes, and the error variance [Ve] is part of the phenotypic variance due to environmental effects. To be able to compare the variation among traits, phenotypic [PCV] and genotypic [GCV] coefficients of variation were computed according to the method suggested by Burton, [Burton, 1952]:

$PCV = [\sqrt{Vp}/X] \times 100$; $GCV = [\sqrt{Vg}/X] \times 100$

Vp, Vg and X are the phenotypic variance, genotypic variance and grand mean for each pod and seed-related trait, respectively.

Broad sense heritability [h2B] was calculated according to Allard [1999] as the ratio of the genotypic variance [Vg] to the phenotypic variance [Vp]. Genetic advance [GA] expected and GA as per cent of the mean assuming selection of the superior 5% of the genotypes were estimated in accordance with Johanson et al. [1955] as:

$GA = K \bullet h2B \bullet \sqrt{Vp}$; GA [as % of the mean] = $[GA/X] \times 100$

K is the selection differential [2.06 for selecting 5% of the genotypes].

Phenotypic [rp] and genotypic [rg] correlations were further computed to examine inter-character relationships among seed and seedling traits following Goulden [1952] as:

$rp = Covp [x1, x2]/[Vp[x1] \bullet Vp[x2]]^{½}$

$rg = Covg [x1, x2]/[Vg[x1] \bullet Vg[x2]]^{½}$

Covp and Covg are phenotypic and genotypic covariances for any two traits x1 and x2, respectively, and Vp and Vg are the respective phenotypic and genotypic variances for those traits. The mean observations for all traits were standardized by subtracting from each observation the mean value of the character and subsequently dividing it by its respective standard deviation. These standardized values, with average 0 and standard deviation 1, were used for principal component analysis [PCA] on Genstat 10 to know the importance of different traits in explaining multivariate polymorphism. Cluster analysis was performed using the scores of first three principal components [PCs] following Ward [1963]. Mean, range and variance were computed for each trait and cluster. Means of clusters were compared using Newman-Keuls procedure [Newman, 1939; Keuls, 1952]. The homogeneity of variances among the clusters was tested using Levene's test [1960].

RESULTS & DISCUSSION

Genetic improvement depends upon the nature and magnitude of existing variability and also on the degree of transmission of traits or heritability [Zobel and Talbert, 1984]. Seed size may vary due to both internal [maternal, hereditary] and external [environmental] conditions operating at the time of seed development [Harper et al., 1970]. This differential development might have an adaptive advantage in local edapho-climatic condition.

Variability in pod and seed traits

Seeds from different CPTs of *P. pinnata* varied significantly in respect of pod and seed traits at p = 0.05 level of significance [Table 3, Plate 1 and 2]. Highest coefficient of variation was recorded for 100-seed weight [22.5 %] followed by 100-seed weight [17.6 %]. Variability studies revealed that, more than nine CPTs recorded above average for oil content [34.5 %], 100-pod [341.8 g] and 100-seed [146.6 g] weight. CPT-19 recorded highest for six traits viz. pod length [65.5 mm], 100-pod weight [541.3 g], 2D surface area [348.7 mm2], seed length [27.7 mm], 100-seed weight [202.3 g] and oil content [43.9 %]. CPT-6 was superior for pod thickness [12.7 mm], seed breadth [17.5 mm] and pod/seed ratio [2.8]. The least value for oil content among all CPTs was obtained in CPT-14 [28.5 %], which is also at par with CPT-13 [28.6 %]. In the present study, CPTs differ significantly in size, weight and oil content exhibiting wide range of variation in oil content [28.5 – 43.9 %], 100-pod weight [231.6 – 541.3 g] and 100-seed weight [107.4 – 202.3 g] compared to other traits indicating a good scope for

improvement [Table 3]. The consideration of seed weight in selecting and understanding the geographical variation has been advocated because of the least plasticity in this character [Harper et al., 1970]. The difference recorded may be in response to different intensities of natural selection pressure acting upon these traits in their natural habitat. In a leguminous species the pod, seed and germination traits were considered largely under maternal influences but were strongly controlled by micro and macro habitats, besides the age and general health of the parent trees [Isik, 1986]. Such variations in relation to habitat have also been reported in *P. pinnata* by Kaushik et al., [2007a] in Haryana, Kumar et al., [2003] in Tamil Nadu, and Mukta et al., [2009a; 2009b] in Andhra Pradesh, India. *P. pinnata* being indigenous to Indian subcontinent with wide diversity form the basic resource for further improvement and breeding programme at global level. The results of the present study will be valuable for seed zone delineations, strategies for conservation of genetic variation, prospects of improvement and assessment of the potential of locally adapted seed source.

CPT-19 found far superior oil content and 100-seed weight than the remaining 23 CPTs. Based on the seed oil content, CPT-19 is taken as 100 grades and treated as excellent [Table 4]. The rest of CPTs can be categorised into very good [90 – 99 grades], good [85 – 89 grades], moderate [80 – 84 grades] and low [< 80 grades]. Based on 100-seed weight, CPT-19 is taken as 100 grades and treated as excellent. Thus on the basis of 100-seed weight, CPTs further are classified into very good [90 – 99 grades], good [85 – 89 grades], moderate [80 – 84 grades] and low [< 80 grades]. As per the above said gradation of CPTs based on seed oil content and 100-seed weight, one-third of the selected CPTs ranking excellent, very good and good, sounds better for further improvement [Table 4].

Genetic variability and correlation studies

Though the selection of superior trees was carried out intensively and clonal superiority over seed raised plants was established [Kumar, 1995], genetic superiority per se needs to be determined. The success of tree breeding programme depends largely on the type and extent of genetic variability present in the base population, which is measured by different population parameters including genotypic and phenotypic variations and genotypic and phenotypic coefficient of variation [Subramanian, 1995]. The genetic estimates can be very useful tools in predicting the amount of gain expected in short period. The variation among genotypes is commonly used as an estimate of total genetic variation and to calculate the degree of genetic control for a particular trait [Foster and Shaw, 1988]. Genetic analysis of pod and seed traits of *P. pinnata* is presented in Table 5. In general there were fair differences between the values of genotypic and phenotypic variance [data not given] and genotypic and phenotypic co-efficient of variation for all the traits. Estimates of broad sense heritability of all pod and seed traits were very high and ranged from 82.6 % [seed length] to 98.4 % [100-pod weight]. Genetic advance as percent of mean ranged from 12.3 to 46.0 for seed and 100-pod weight respectively. Trait 100-pod weight and 100-seed weight expressed very high heritability [98.4 %, 96.9 %] accompanied with high genetic advance percent of mean [46.0 %, 34.9 %]. The trait oil content expressed high heritability [87.1 %] accompanied with genetic advance as percent of mean [20.5 %]. Marginal difference between phenotypic coefficient of variation [PCV] and genotypic coefficient of variation [GCV] and high estimates of heritability [broad sense] for all pod and seed traits under study revealed the heritable nature of variability present [Table 5]. Relatively high value of genotypic variance that resulted in high estimates of heritability which contributed to the high genetic advance expected in this material. In the present study the genotypic coefficient of variation and the genetic advance were found to be comparatively higher for an important trait such as oil content, 100-pod and 100-seed weight. Hence these traits may be viewed as best gain characteristic for *P. pinnata* genetic improvement. The high estimates of heritability combined with high genetic advance suggests that population means for traits oil content, 100-pod and 100-seed weight may be changed considerably by selecting the superior 5 % of the genotypes. High heritability for seed traits accompanied by high genetic advance have been reported in *P. pinnata* [Kaushik et al., 2007a] and other tree borne oil seed species like *J. curcas* [Kaushik et al., 2007b; Ginwal et al., 2004; Rao et al., 2008], Madhuca latifolia [Divakara and Krishnamurthy, 2009].

The ultimate goal of the genetic improvement in *P. pinnata* is to improve tree species for economically viable trait seed and oil. Pod and seed traits are complex and the end product depends on the interplay of many physiological and morphological attributes, hence improvement based on per se performance alone might prove to be less effective. In genetic improvement of pod and seed traits of *P. pinnata*, clear understanding of the relationships among pod and seed traits is

very essential. As variation among clones used for estimation of genetic variation and genetic gain, co-variance estimates between traits can be used to estimate genetic correlations between the traits [Foster, 1986]. Correlated quantitative traits are of a major interest in an improvement program, as the improvement of one character may cause simultaneous correlated changes in the other characters. The degree of correlation coefficient at genotypic level was higher than their corresponding phenotypic coefficient of correlations in all the pod and seed traits indicating the genetic association among the characters. The genotypic and phenotypic correlation coefficients [r] among the pod and seed traits are presented in Table 6. In general, the magnitudes of genotypic correlation co-efficient values were higher than corresponding phenotypic values. Of the 100 [50 genotypic and 50 phenotypic] correlations, 23 genotypic and 18 phenotypic combinations were significant at 1 % along with 6 genotypic and 9 phenotypic combinations significant at 5 %. Oil content was found to have significant positive relationship [p = 0.01] with 100-seed weight [rg= 0.62, rp=0.58]. 100-pod weight was also found to have significant positive relationship with oil content [0.50, 0.46] at genotypic [p = 0.01] and phenotypic [p = 0.05] level. However, pod length expressed positive significant relation with oil content [0.43] at genotypic level [p = 0.05]. The genotypic correlation is an estimated value, whereas, phenotypic correlation is a derived value from the genotype and environmental interaction [Chaturvedi and Pandey, 2004]. The genotypic correlation indicates genotypic association among the traits and is, therefore, a more reliable estimate for examining the degree of relationship between character pairs. Among all the correlations, trait aspect ratio expressed significant negative relationship with seed breadth [rg = -0.77, rp = -0.75] at p =0.01 and pod width [rg = -0.46, rp = 0.41] at p = 0.05 at both genotypic and phenotypic levels. The correlation matrix revealed that statistically significant positive relationship among traits oil content, pod length, 100-pod and 100-seed weight, hence it may be used to the advantage of the breeder for bringing simultaneous improvement in these traits. The inter correlations found among seed weight and oil content in present study is in consistent with those of earlier studies in *P. pinnata* [Kaushik et al., 2007a] and other tree borne oil seed species like *J. curcas* [Kaushik et al., 2007b; Ginwal et al., 2004; Rao et al., 2008], Madhuca latifolia [Divakara and Krishnamurthy 2009].

Divergence studies

Genetic diversity in plant species is a gift to mankind as it forms the basis for selection and further improvement. Morphometric traits had been utilized to assess the relationship among the germplasm/cultivars in agriculture [Brown, 1978] and in trees [Surendran, 1982]. The study of relationships is based on the assumption that the difference in the characters reveals their genetic divergence. The information from 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 to realize the potentiality for maximizing seed and oil yield. The clustering pattern in the present study showed that 24 CPTs of *P. pinnata* are grouped into 2 clusters based on the scores of first four principal components [PCs] derived from four pod and seven seed traits, revealing geographical diversity which need not necessarily be related to genetic diversity. This kind of genetic diversity might be due to differential adoption, selection criteria, selection pressure and environment [Vivekananda and Subramaninan, 1993]. This indicated that genetic drift produce greater diversity than the geographic diversity [Singh et al., 1996]. Absence of any relationship between genetic diversity and geographical distribution is in accordance with the findings of Kaushik et al., [2007b], Rao et al., [2008] and Gohil and Pandya [2008] in *J. curcas* and Divakara and Krishnamurthy [2009] in Madhuca latifolia. The trees that originated in one region had been distributed into different clusters indicated that trees with same geographic origin could have undergone change for different characters under selection.

The first three PCs of the total ten explained exhibit large portion [86.6 %] of the total variation for pod and seeds of *P. pinnata*. The first PC alone accounted for 42.9 % of the variation followed by the second, third and fourth PCs which explains 22.3, 11.4 and 9.7 % of the variations respectively [data not given]. Based on the loading for the first four PCs, traits such as pod length, pod thickness, seed breadth, aspect ratio, oil content, 100-pod and 100-seed weight are important and adequate descriptors for pod and seed traits study in this material.

Cluster analysis performed on the scores of the first four PCs resulted into two clusters [Fig. 2] with cluster 1 comprised of 6 CPTs [CPT – 19, CPT – 6, CPT – 9, CPT – 5, CPT – 7 and CPT – 4] and remaining 18 CPTs [CPT – 12, CPT – 10, CPT – 21, CPT – 15, CPT – 11, CPT –

8, CPT – 14, CPT – 18, CPT – 3, CPT – 23, CPT – 20, CPT – 22, CPT – 16, CPT – 13, CPT – 2, CPT – 17, CPT – 24 and CPT – 1] are grouped into second cluster. Since cluster one comprising six CPTs is delineated from cluster two based on significantly high means for majority of pod and seed traits [Table 7], CPTs in cluster 1 can be directly selected and utilized for within group hybridization for maximizing seed and oil yield. Difference between means of different cluster were tested using Newman-Keuls test and found that the, means of cluster 1 was significantly different from cluster 2 for all the pod and seed traits except pod length, pod width, aspect ratio and 100-seed weight at 0.05 level of significance. Since, wide diversity exists between the cluster 1 and 2, crosses between these clusters may result in substantial segregates and further selection for overall improvement of species. This kind of study can help to identify the better genotypes of *P. pinnata* having better yield and oil content. Therefore, the best genotypes selected will improve the poor sites for agroforestry systems and energy plantations in the wastelands. Earlier studies, in crop plant had indicated that inter-mating of divergent groups would lead to greater opportunity for crossing over which would release latent variation by breaking up predominantly repulsion linkage [Thoday, 1960] and utilization of diverse parents in breeding was also stressed by Singh et al. [1981].

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Table 1. Locational details of *Pongamia pinnata* Candidate Plus Trees (CPTs) selected in Jharkhand, India

CPTs	District	Location/Village	Latitude	Longitude	Altitude (m)	Age in years	Height (m)	DBH (cm)	Seed yield (kg Y^{-1})	Crown area (m^2)
CPT-1	Ranchi	Barhe	23°28′36″N	85°01′06″E	610	75	17	125	200	333.1
CPT-2	Gumla	Indrakela Girijatoli	23°07′02″N	84°33′21″E	520	25	12	50	60	162.8
CPT-3	Lohardaga	Chechra Nawadih	23°26′17″N	84°38′36″E	590	80	14	107	300	194.7
CPT-4	Lohardaga	Kandra	23°21′06″N	84°39′16″E	570	85	10	103	250	297.0
CPT-5	Simdega	Piosokra	22°35′47″N	84°40′49″E	370	55	13.6	92	150	193.5
CPT-6	Lohardaga	Bather nawatana	23°33′02″N	84°54′43″E	640	50	13.7	128	100	312.4
CPT-7	Garhwa	Vishrampur	23°55′30″N	83°46′11″E	410	20	10.3	35	50	150.6
CPT-8	Chatra	Utta sangra	24°14′15″N	85°00′15″E	640	60	15.5	92	250	260.0
CPT-9	Hazaribag	Nawakutar	23°54′19″N	85°19′04″E	610	100	17	114	160	289.4
CPT-10	Hazaribag	Gramurwan	24°27′10″N	85°31′42″E	370	20	11.9	70	35	142.0
CPT-11	Koderma	Bariyadi	24°27′21″N	85°46′12″E	380	40	10.2	93	85	239.0
CPT-12	Ranchi	Chuttupallu	23°27′45″N	85°28′39″E	630	60	11.5	77	100	198.5
CPT-13	Saraikela	Hatnada Tal-tola	22°51′42″N	85°56′55″E	390	20	11	55	40	122.7
CPT-14	Dhalbum	Dhalbumghar	22°27′10″N	86°37′09″E	350	20	8.0	50	45	69.4
CPT-15	Ranchi	Pansakam	23°09′04″N	85°28′40″E	500	80	21	98	150	306.2
CPT-16	Gumla	Hutar	23°16′31″N	85°03′21″E	790	70	14.5	90	120	399.2
CPT-17	Gumla	Bishrampur Jhatnitoli	23°08′22″N	84°46′47″E	800	80	12.7	140	140	331.5
CPT-18	Gumla	Bombibary	22°52′39″N	84°53′36″E	500	50	12.4	105	100	323.5
CPT-19	Chaibasa	Murumbura	22°52′35″N	85°18′15″E	690	80	16	140	200	333.1
CPT-20	Khunti	Itae dartoli	23°03′05″N	85°13′40″E	700	50	18.5	122	100	342.9
CPT-21	Ranchi	Jamun Tolli	23°33′55″N	85°05′05″E	650	60	16	158	140	289.4
CPT-22	Giridih	Bangabad	24°17′11″N	86°21′55″E	390	50	9.9	86	150	281.9
CPT-23	Ranchi	Chund	23°28′40″N	85°10′17″E	790	60	12.0	93	100	229.5
CPT-24	Ranchi	Pandu	23°17′07″N	85°10′35″E	810	65	10.3	102	130	248.7

Table 2. Methodology followed for measuring pod and seed traits of *Pongamia pinnata*

Sl. No.	Traits	Method
1.	Pod length	Length of the pod at longest side measured using vernier caliper, average value was computed and expressed in mm.
2.	Pod width	Length of the pod at shortest side measured using vernier caliper, average value was computed and expressed in mm.
3.	Pod thickness	Thickness of the pod measured using vernier caliper, average value was computed and expressed in mm.
4.	100 – pod weight	Weight of 100-pods weighed on electronic balance and average value was calculated and expressed in grams.
5.	2D surface area	2D surface area of the seed in the direction of measurement. This was estimated using either side and expressed in mm^2.
6.	Seed length	Length of the seed at longest side, measured in mm.
7.	Seed breadth	Length of the object at shortest side, measured in mm.
8.	Aspect ratio	Ratio of length divided by breadth.
9.	100 – seed weight	Weight of 100 seeds weighed on electronic balance, measured in grams.
10.	Pod/seed ratio	Ratio of 100-pod weight divided by 100-seed weight.
11.	Oil content	Estimated using soxhlet apparatus following the procedure of Sadasivam and Manickam (1992).

Table 3. Mean performance of *Pongamia pinnata* genotypes for pod and seed traits

CPTs	Pod traits					Seed traits					
	Pod length (mm)	Pod width (mm)	Pod thickness (mm)	100-Pod weight (g)	2D area (mm^2)	Length (mm)	Breadth (mm)	Aspect ratio	100-seed weight (g)	Pod/ seed ratio	Oil content (%)
CPT-1	51.3	26.2	9.7	231.6	278.9	22.0	15.8	1.4	125.3	2.0	32.4
CPT-2	45.2	19.0	11.7	254.7	237.9	24.8	12.8	1.9	142.8	2.4	30.8
CPT-3	55.8	20.4	12.0	358.0	284.9	23.4	15.2	1.5	113.4	2.9	34.2
CPT-4	51.0	26.5	11.5	407.1	343.8	25.5	17.5	1.5	171.2	2.4	34.9
CPT-5	50.0	20.4	11.9	356.3	324.6	24.1	16.6	1.5	167.9	2.2	36.6
CPT-6	58.1	24.9	12.7	473.7	342.4	26.4	17.5	1.5	185.4	2.8	39.0
CPT-7	56.6	26.3	11.5	357.7	313.7	24.5	16.9	1.5	154.8	2.4	34.8
CPT-8	47.8	19.8	12.2	284.9	287.0	23.5	16.2	1.5	135.4	2.1	29.9
CPT-9	50.0	23.7	12.0	352.3	314.8	24.1	17.3	1.4	163.9	2.3	34.2
CPT-10	65.5	23.6	11.0	451.2	330.6	26.6	15.2	1.8	125.1	2.6	35.4
CPT-11	57.6	23.1	12.1	358.8	297.2	25.5	14.4	1.8	149.7	2.0	34.0
CPT-12	58.4	25.9	10.5	337.4	245.7	24.8	15.1	1.6	129.6	2.2	37.5
CPT-13	50.4	23.4	9.7	275.1	271.8	24.0	14.6	1.6	142.3	2.1	28.6
CPT-14	51.5	24.9	11.7	422.7	304.9	24.3	16.5	1.5	107.4	2.4	28.5
CPT-15	48.6	21.4	11.7	303.3	282.2	23.7	15.2	1.6	135.8	2.2	34.2
CPT-16	45.2	18.8	11.1	258.1	257.9	23.7	14.5	1.6	115.2	2.1	31.9
CPT-17	48.9	27.0	10.1	329.3	288.0	22.3	17.4	1.3	151.8	2.2	34.7
CPT-18	47.5	21.1	11.6	366.4	290.3	25.5	14.6	1.8	147.1	2.7	31.2
CPT-19	65.5	23.7	11.7	541.3	348.7	27.7	17.4	1.6	202.3	2.5	43.9
CPT-20	43.3	20.4	10.8	333.3	259.8	23.7	14.3	1.7	184.6	1.8	39.1
CPT-21	48.2	22.8	10.7	343.4	285.0	24.3	14.6	1.7	144.7	2.4	33.4
CPT-22	44.8	18.8	10.0	233.9	273.9	23.7	15.0	1.6	112.9	1.9	34.4
CPT-23	44.5	24.2	11.3	297.0	271.2	22.8	15.3	1.5	181.0	2.1	35.5
CPT-24	49.2	23.2	9.9	276.6	261.9	20.6	16.2	1.3	129.6	1.9	39.3
Mean	51.4	22.9	11.2	341.8	291.5	24.2	15.7	1.6	146.6	2.3	34.5
SEM	0.7	0.4	0.2	5.7	6.5	0.4	0.1	0.0	2.6	0.1	0.8
CD 5%	2.1	1.0	0.5	16.6	18.8	1.2	0.4	0.1	7.6	0.2	2.3
CV (%)	12.0	11.4	7.7	22.5	10.6	6.3	8.1	9.7	17.6	12.4	10.4

Table 4. Gradation of P. pinnata CPTs based on 100-seed weight and seed oil content

		Seed oil content (%)				100-seed weight (g)			
Rank	CPTs	oil content	Gradation (considering CPT-19 as 100)	Difference between two adjacent grades	Rank	CPTs	100-seed weight	Gradation (considering CPT-19 as 100)	Difference between two adjacent grades
1	CPT-19	43.9	100.0	-	1	CPT-19	202.3	100.0	-
2	CPT-24	39.3	89.6	10.4	2	CPT-6	185.4	91.6	8.4
3	CPT-20	39.1	89.2	0.4	3	CPT-20	184.6	91.3	0.4
4	CPT-6	39.0	88.9	0.3	4	CPT-23	181.0	89.5	1.8
5	CPT-12	37.5	85.4	3.5	5	CPT-4	171.2	84.6	4.9
6	CPT-5	36.6	83.3	2.1	6	CPT-5	167.9	83.0	1.6
7	CPT-23	35.5	80.9	2.4	7	CPT-9	163.9	81.0	2.0
8	CPT-10	35.4	80.7	0.2	8	CPT-7	154.8	76.5	4.5
9	CPT-4	34.9	79.4	1.3	9	CPT-17	151.8	75.0	1.5
10	CPT-7	34.8	79.3	0.1	10	CPT-11	149.7	74.0	1.0
11	CPT-17	34.7	79.1	0.2	11	CPT-18	147.1	72.7	1.3
12	CPT-22	34.4	78.4	0.7	12	CPT-21	144.7	71.5	1.2
13	CPT-3	34.2	78.0	0.4	13	CPT-2	142.8	70.6	0.9
14	CPT-9	34.2	77.8	0.1	14	CPT-13	142.3	70.3	0.3
15	CPT-15	34.2	77.8	0.0	15	CPT-15	135.8	67.1	3.2
16	CPT-11	34.0	77.6	0.3	16	CPT-8	135.4	66.9	0.2
17	CPT-21	33.4	76.1	1.4	17	CPT-12	129.6	64.1	2.9
18	CPT-1	32.4	73.8	2.3	18	CPT-24	129.6	64.1	0.0
19	CPT-16	31.9	72.8	1.0	19	CPT-1	125.3	61.9	2.2
20	CPT-18	31.2	71.2	1.6	20	CPT-10	125.1	61.8	0.1
21	CPT-2	30.8	70.2	1.0	21	CPT-16	115.2	57.0	4.9
22	CPT-8	29.9	68.1	2.1	22	CPT-3	113.4	56.0	0.9
23	CPT-13	28.6	65.1	3.0	23	CPT-22	112.9	55.8	0.2
24	CPT-14	28.5	64.9	0.2	24	CPT-14	107.4	53.1	2.7
			Difference between maximum and minimum 35.1					Difference between maximum and minimum 46.9	

able 5. Estimates of variance components and other parameter for pod and seed traits in *P. pinnata*.

Traits	Variance due to genotype	Heritability (%)	Coefficient of variation (%)		Genetic advance as % of mean
			Genotypic coefficient of variance (GCV)	Phenotypic coefficient of variance (PCV)	
Pod traits					
Pod length	38.6	96.2	12.1	12.3	24.4
Pod width	6.9	94.6	11.5	11.8	22.9
pod thickness	0.8	89.4	7.8	8.2	15.2
100 pod weight	5934.3	98.4	22.5	22.7	46.0
Seed traits					
2D area	994.5	88.4	10.8	11.5	21.0
Length	2.5	82.6	6.6	7.2	12.3
Breadth	1.7	96.8	8.2	8.4	16.7
Aspect ratio	0.0	90.4	10.4	11.0	20.4
100 seed weight	670.2	96.9	17.2	17.5	34.9
Pod – Seed ratio	0.1	89.9	12.7	13.4	24.7
Oil content	13.6	87.1	10.7	11.4	20.5

*significant at $P = 0.05$, **significant at $P = 0.01$

Table 6. Genotypic (G) and phenotypic (P) correlation coefficients for pod and seed traits in *P. pinnata*

Seed traits		Pod width	pod thickness	100-pod weight	2D area	Seed length	Seed breadth	Aspect ratio	100-seed weight	Pod – Seed ratio	Oil content
Pod length	**G**	0.44*	0.23	0.74**	0.61**	0.64**	0.36	0.09	0.57**	0.49*	0.43*
	P	0.46*	0.21	0.73**	0.58**	0.61**	0.35	0.11	0.55**	0.48*	0.40
Pod width	**G**		-0.19	0.37	0.39	0.05	0.61**	-0.46*	0.36	0.12	0.19
	P		-0.18	0.37	0.37	0.07	0.58**	-0.41*	0.35	0.12	0.17
pod thickness	**G**			0.51**	0.49*	0.54**	0.20	0.19	0.29	0.55**	0.04
	P			0.49*	0.45*	0.50*	0.18	0.20	0.27	0.51**	0.04
100-pod weight	**G**				0.83**	0.79**	0.52**	0.07	0.82**	0.63**	0.50**
	P				0.77**	0.73**	0.50	0.08	0.81**	0.62**	0.46*
2D area	**G**					0.61**	0.74**	-0.23	0.65**	0.52**	0.34
	P					0.58**	0.71**	-0.18	0.60**	0.46*	0.30
Seed length	**G**						0.08	0.57**	0.59**	0.57**	0.24
	P						0.07	0.61**	0.53**	0.51**	0.20
Seed breadth	**G**							-0.77**	0.45*	0.24	0.38
	P							-0.75**	0.45*	0.20	0.34
Aspect ratio	**G**								-0.01	0.18	-0.17
	P								-0.01	0.19	-0.15
100-seed weight	**G**									0.09	0.62**
	P									0.04	0.58**
Pod – Seed ratio	**G**										0.02
	P										0.00

*significant at $P = 0.05$, **significant at $P = 0.01$

Table 7. Range, mean, and variance for different pod and seed traits in two clusters of selected genotypes of *P. pinnata*

Traits	Range		Mean[1]		Variance[2]		F value	Prob > F
	Cluster 1	Cluster 2	Cluster 1	Cluster 2	Cluster 1	Cluster 2		
Pod length	50.0 – 65.5	43.3 – 65.5	55.2[a]	50.2[a]	37.4	33.9	0.0	0.973
Pod width	20.4 – 26.5	18.8 - 27.0	24.3[a]	22.5[a]	5.1	6.8	0.6	0.457
pod thickness	11.5 – 12.7	9.7 – 12.2	11.9[a]	11.0[b]	0.2	0.7	4.5	0.045
100 pod weight	352.3 – 541.3	231.6 – 451.2	414.7[a]	317.5[b]	6021.0	3713.5	0.4	0.519
Seed 2D area	313.7 – 348.7	237.9 – 330.6	331.3[a]	278.3[b]	241.6	473.4	0.7	0.417
Seed length	24.1 – 27.7	20.6 – 26.6	25.4[a]	23.8[b]	2.1	1.9	0.0	0.962
Seed breadth	16.6 – 17.5	12.8 – 17.4	17.2[a]	15.2[b]	0.1	1.1	1.7	0.208
Aspect ratio	1.4 – 1.6	1.3 – 1.3	1.5[a]	1.6[a]	0.0	0.03	2.4	0.136
100 seed weight	154.8 – 202.3	107.4 – 184.6	174.3[a]	137.4[b]	289.6	452.5	0.5	0.510
Pod – Seed ratio	2.2 – 2.8	1.8 – 2.9	2.4[a]	2.2[a]	0.1	0.1	0.6	0.452
Oil content	34.2 – 43.9	28.5 – 39.3	37.2[a]	33.6[b]	13.8	10.1	0.1	0.747

[1]Difference between means of different clusters were tested using the Newman-Keuls test, Means followed by the same letter are not significantly different at $P = 0.05$ [2]Variance were tested following Levene (1960) test.

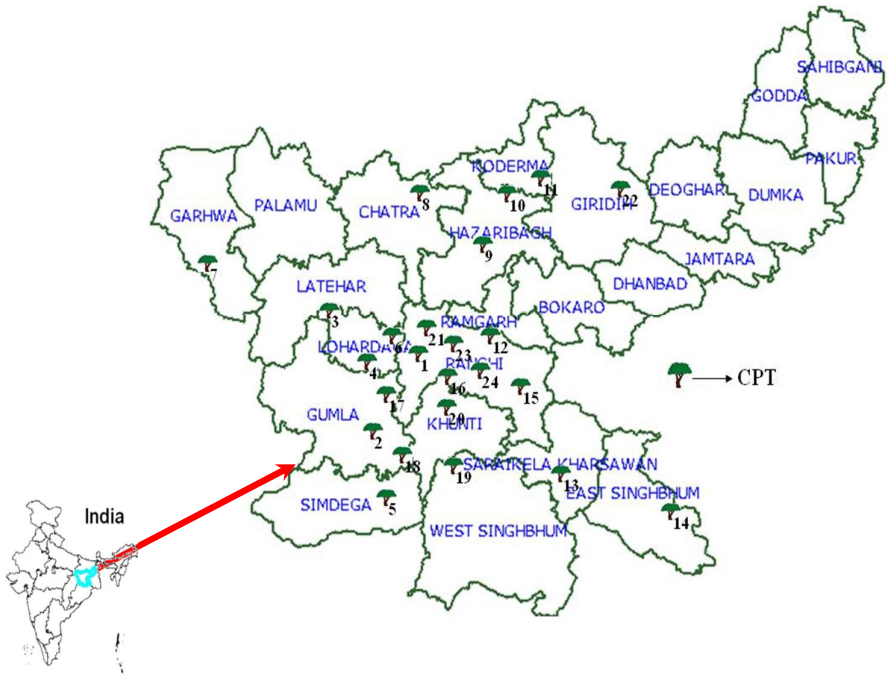

Fig1. Distribution map of candidate plus tree of *Pongamia pinnata* (Details of number representation is in table 1)

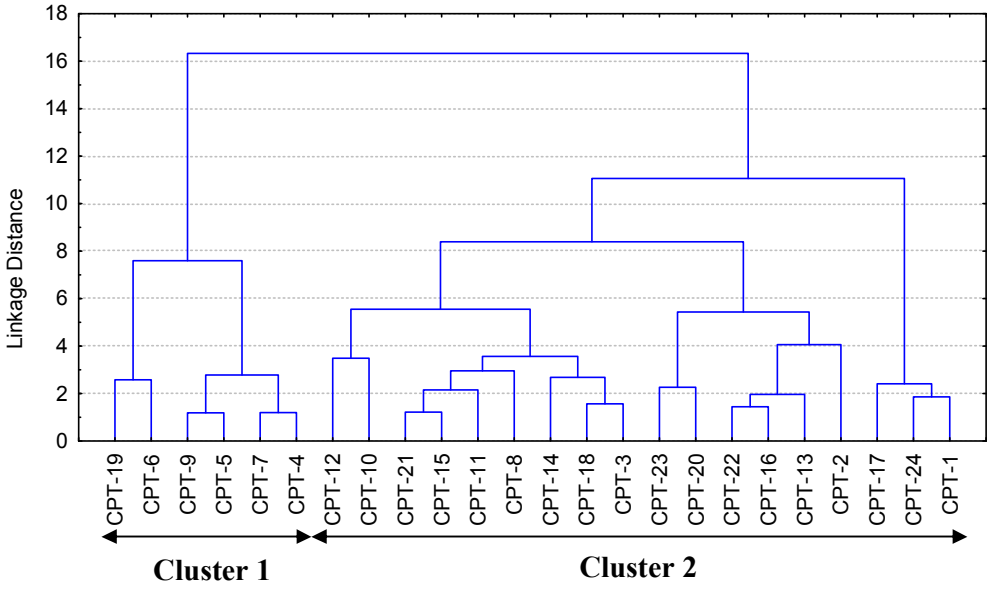

Fig2. Grouping of 24 *P. pinnata* genotypes based on scores of first four principal components derived from four pod and seven seed traits

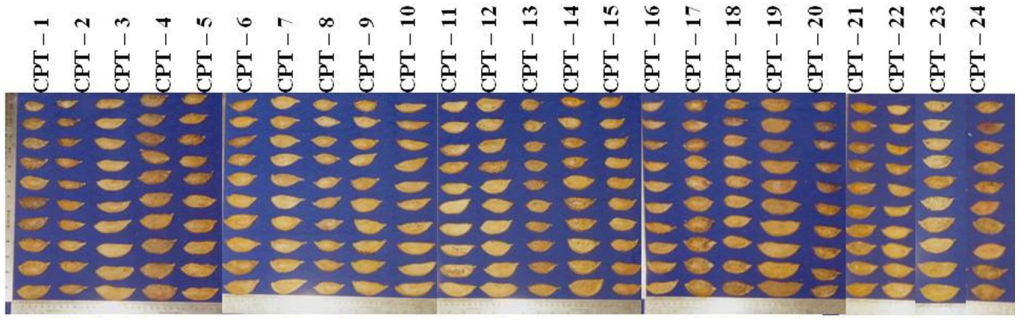

Plate1. *Pongania pinnata* pod diversity among selected Candidate Plus Trees

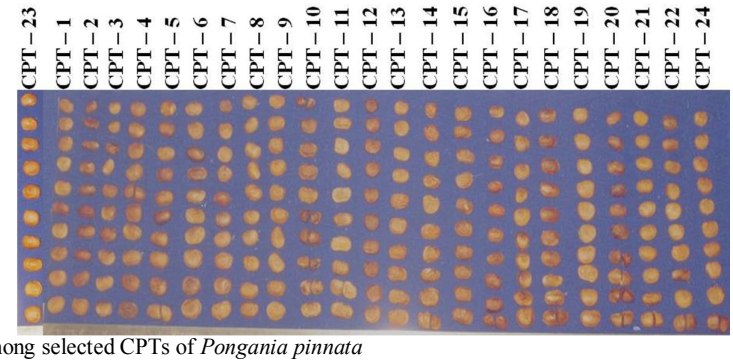

Plate2. Seed diversity among selected CPTs of *Pongania pinnata*