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# Variability for seed oil content and seedling traits in *Pongamia pinnata* (L.) Pierre

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**Abstract:** Twenty three CPTs (Candidate Plus Trees) of *Pongamia pinnata* were selected from different agro-climatic conditions of Haryana state of India and were assessed to identify the elite planting material for improvement of the species in terms of oil content. The differences among CPTs of *P. pinnata* were significant for seed oil content and all growth parameters of the progenies of these CPTs at the seedling stage. Oil content in *P. pinnata* varied from 27.07 (P12) to 38.17% (P2). The estimates of genotypic coefficients of variation for the characters studied were less as compared to the phenotypic coefficients of variation for all the characters examined. The highest phenotypic coefficient of variation (49.33) and genotypic coefficient of variation (28.56) was recorded for the germination percentage followed by height of the first branch. Number of leaves (0.5551**), inter-nodal length (0.5580**) and number of branches (0.6182**) showed high and positive correlation with the seed oil content. The progeny number 9, 21 and 2 were found to be the best on basis of oil content (36. 37, 36.83 and 38.17 %, respectively), and other characters examined. $D^2$ analysis grouped the CPTs into 5 clusters. The highest numbers of progenies were included in the cluster I followed by cluster III and least number of progenies i.e., two were observed in cluster II. The intra cluster distances ranged from 4.12 (cluster V) to 5.96 (cluster II). The maximum inter-cluster distance was observed between cluster II and III (10.02) followed by I and III and minimum was between clusters I and cluster V. The crosses between clusters II and III may result sufficient segregation for further improvement of the species. Therefore, the progenies belonging to the clusters II and III could be taken as parents for a successful hybridization program.

**Keywords:** Heritability, *Pongamia pinnta,* progeny, seed oil content, variability

## INTRODUCTION

Karanja (*Pongamia pinnata*), a medium sized glabrous tree with a short bole and spreading crown, is indigenous to Indian sub-continent and south-east Asia and is a member of leguminosae family. It is widely distributed throughout India along roadsides and railway tracks. The tree has been introduced to several countries with humid tropical lowlands including parts of Australia (Scott *et al.,* 2008). Historically, this plant has been used in India as a source of traditional medicines, animal fodder, green manure, timber, fish poison and fuel (Scott *et al.,* 2008). This tree species has recently drawn considerable attention for its oil as a viable resource for the bio-fuel industry. It is emerging as an important biouels feedstock. It produces about 30 kg per tree per year of seeds containing 55% oil (Biswas *et al.,* 2013) This species has the potential of about 0. 2 M MT seeds, which can yield about 0.05 M MT, oil (Punia *et al.,* 2006). The tree can be cultivated on marginal lands which not only eliminate the competition between bio-fuel and food crops for arable land and water use but also provide the bio-diesel on a sustainable basis.

For future success of *P. pinnata* as a sustainable source of bio-fuels, research should be targeted to maximize the plant growth as it relates to oil biosynthesis (Scott *et al.,* 2008). The survival and establishment of a plant depends upon its genetic potential for germination, growth, development and reproduction. The effective tree improvement programme depends upon the nature and magnitude of existing genetic variability and also on the degree of transmission of the desired traits. Knowledge about the nature and extent of source variation in relation to seed oil content, germination and seedling characters is very useful for the production of good quality planting stock. Present study is aimed at evaluation of different progenies of *P. pinnata* for estimation of genetic parameters for seed oil content and different growth characters for further improvement of the species for higher oil yield.

## MATERIALS AND METHODS

**Experimental material:** The survey was done in 12 districts of Haryana state of India and 115 Candidate Plus Trees (CPTs) were identified based on the morphometric and qualitative traits from different locations/sites of the state as given in Table 1. The young CPTs having 160-180 cm girth and height ranging

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from 10-15 m almost of the same age (15-16 years) were selected. The superiority of plus trees were confirmed after comparing with five co-dominant trees within a radius of 25-50 m and out of 115 CPTs, 23 plus trees were selected for present study. Care was taken to collect the dry pod directly from the marked trees. Sufficient pods were collected from each CPT for the study.

**Experimental site and methodology:** The study was conducted in nursery area of Department of Forestry & Natural Resources, Punjab Agricultural University, Ludhiana (India), situated at an elevation of 244 meters above mean sea level with a latitude of 30° 56' North and longitude of 75° 48' East in the sub-tropical region. The site is characterized by hot and dry summers and cold winters. The average annual rainfall is 700 mm, 75 per cent of which is received from July to September. The soil was silt loam in texture. Polythene bags of size 22 × 10 cm were filled with equal proportion of farm yard manure, sand and soil. The soil collected from the university farm area was first dried and then passed through 2 mm sieve. Uniform quantity of 1.25 kg soil was filled in each polythene bag. Experiment was laid out in a randomized block design with three replications. Fifty seeds were sown in each replication with a total of 150 seeds in each CPT. Measurements were recorded for seed germination percentage, plant height, collar diameter, number of branches, number of leaves, inter-nodal length and height of the first branch above ground level for each progeny. Only normal seedlings were considered for calculating percent germination.

**Oil content:** The dry, cleaned kernels were crushed in a Waring blender and extracted (AOAC 1984) with n-hexane (60-80°C) in a Soxhelt extractor for 8 h (at this stage, a drop of hexane extract when evaporated on a filter paper left no residual oily spot). Hexane was removed in a rotary evaporator under reduced pressure at 60°C to yield oil.

**Statistical analysis:** Analysis of variance was carried out as per Panse and Sukhatme (1978).

**Estimation of genetic parameters:** Genotypic and phenotypic Coefficients of variations were estimated by the formula suggested by Burton (1952) for each character as follows:

$$\text{Genotypic coefficient of variance (GCV)} = \frac{\sqrt{\sigma^2 g} \times 100}{X}$$

$$\text{Phenotypic coefficient of variance (PCV)} = \frac{\sqrt{\sigma^2 p} \times 100}{X}$$

Where X was mean of that particular character

Heritability in broad sense was calculated according to the formula suggested by Johnson *et al.* (1955) for each character.

$$\text{Heritability (broad sense) in percent} = h^2 = \frac{\sigma^2 g}{\sigma^2 p} \times 100$$

where $\sigma^2 g$ = Genotypic variance

$\sigma^2 p$ = Phenotypic variance

Estimates of appropriate variance components were substituted for the parameters to predict the expected genetic gain as suggested by Lush (1949). The expected genetic gain was calculated at 5% selection intensity for each character as:

$$\text{Genetic advance (\%) of mean} = \frac{K \times \sigma p \times h^2}{X}$$

Where:

K = selection differential (2.06)

$\sigma p$ = phenotypic standard deviation

$h^2$ = heritability in broad sense

X = general mean

The genetic divergence was calculated by using non-hierarchical Euclidian cluster analysis (Spark, 1973)

## RESULTS AND DISCUSSION

**Oil content and seedling traits:** In the present study wide variability was recorded for oil content, germination (%), plant height, collar diameter, number of branches and inter-nodal length(Table 2). Oil content varied from 27.07 to 38.17% .Maximum oil content (38.17%) was recorded in progeny number 2 followed by progeny number 21 and 9. Maximum plant height (37.75 cm) and collar diameter (5.71 mm) were observed in progeny number 9. Highest number of

**Table 1.** Seed collection sites and their geographical parameters.

| Progeny No. | Seed collection site | Latitude | Longi-tude |
|---|---|---|---|
| 1 | Suthana | 27° 41’ N | 76° 05’E |
| 2 | Rewari | 28° 40’ N | 76° 50’ E |
| 3 | Hisar | 29° 10’ N | 75° 46’E |
| 4 | Hisar | 29° 10' N | 75° 46' E |
| 5 | Agroha | 29° 31' N | 75° 30' E |
| 6 | Fatehabad | 29° 51' N | 75° 23' E |
| 7 | Mugalpura | 29° 10' N | 75° 46' E |
| 8 | Narwana | 29° 48 N | 76° 23 E |
| 9 | Thana | 29° 48' N | 78° 26' E |
| 10 | Thana I | 29° 48' N | 78° 23' E |
| 11 | Thana II | 29° 48' N | 78° 26' E |
| 12 | Pehowa | 29° 52’N | 76° 26’ E |
| 13 | Jalbera | 29° 52’N | 76° 26’ E |
| 14 | Materi Sekha | 30° 21' N | 76° 47' E |
| 15 | Balana | 30° 21' N | 76° 47' E |
| 16 | Dhurkara | 30° 21' N | 76° 52' E |
| 17 | Ambala | 30° 21' N | 76° 47' E |
| 18 | Kurukshetra | 29° 58' N | 76° 56' E |
| 19 | Thanesar | 29° 58' N | 76° 56' E |
| 20 | NDRI | 29° 42' N | 77° 02' E |
| 21 | Madhuban | 29° 50’ N | 77°45’ E |
| 22 | Panipat | 29° 32’ N | 76° 58’ E |
| 23 | Rohtak | 28° 54' N | 76° 38' E |

branches (9.68) was recorded in progeny number 21 closely followed by progeny number 5. Seed germination ranged from13.33% in progeny number 11 to 66.67% in progeny number 20 (Table 2). As regard the inter-nodal length, highest (2.76 cm) was recorded in progeny number 11 closely followed by progeny number 10 (2.75 cm). Height of the first branch ranged from 5.17 in progeny number 22 to 11.08 cm in progeny number 9.

Seed oil content variation is more widely reported not only in annual crops but also in a wide variety of trees borne oil seed (Divakara *et al.,* 2010; Sunil *et al.,* 2010). These findings are in agreement with Kaushik *et al.,* 2007 and Mukta *et al.,* 2009. Kesari *et al.* (2008) also observed seed oil variability in different progenies of *P. pinnata*. The tree (plus tree) is selected on the basis of phenotypic traits, therefore, it is necessary to test the progeny of plus tree to confirm that the tree is a good genotype, and is capable of transmitting the good traits to the progeny. In the present study the progeny raised in the nursery showed significant ($P>0.05$) variation between different progenies for germination percentage, plant height, collar diameter, number of leaves, inter-nodal length, height of the first branch and number of branches in *P. pinnata*. Progeny no. 2 was better assessed on the basis of germination and seedling characters. The present findings showed that the performance of seedlings in nursery was under strong genetic control as seeds from all the progenies were raised under similar conditions. Divakara and Das (2011) also reported diversity in 24 candidate plus trees of *P. pinnata* for seed and seedling characters, which are in line with the present study and Rao *et al.* (2011).

**Table 2.** Mean performance of different progenies.

| Progeny | Seed oil content (%) | Germination (%) | Plant height (cm) | Collar Diameter (mm) | No. of leaves | No. of Branches | Inter- nodal length (cm) | Height of first branch (cm) |
|---|---|---|---|---|---|---|---|---|
| 1 | 34.17 | 43.66 | 33.50 | 4.52 | 16.62 | 7.15 | 2.47 | 6.33 |
| 2 | 38.17 | 61.67 | 35.80 | 4.60 | 18.24 | 7.55 | 2.56 | 7.10 |
| 3 | 30.30 | 15.00 | 21.39 | 4.33 | 14.89 | 7.03 | 2.56 | 7.66 |
| 4 | 35.00 | 46.67 | 30.20 | 4.96 | 16.50 | 6.72 | 2.68 | 7.37 |
| 5 | 34.97 | 32.48 | 30.32 | 4.71 | 14.16 | 8.62 | 2.29 | 8.43 |
| 6 | 28.30 | 31.67 | 17.26 | 4.07 | 16.52 | 6.25 | 1.98 | 7.21 |
| 7 | 30.00 | 28.33 | 29.44 | 5.26 | 15.88 | 7.05 | 2.64 | 10.53 |
| 8 | 29.10 | 41.67 | 33.53 | 5.29 | 17.36 | 7.74 | 2.48 | 7.47 |
| 9 | 36.37 | 26.84 | 37.75 | 5.71 | 11.83 | 7.03 | 3.20 | 11.08 |
| 10 | 33.67 | 41.67 | 28.35 | 4.39 | 17.84 | 6.68 | 2.75 | 10.63 |
| 11 | 30.33 | 13.33 | 32.72 | 5.32 | 15.63 | 7.16 | 2.76 | 8.46 |
| 12 | 27.07 | 46.67 | 27.83 | 4.11 | 17.60 | 6.97 | 2.57 | 6.30 |
| 13 | 32.00 | 31.67 | 26.54 | 3.36 | 12.44 | 6.99 | 2.55 | 8.57 |
| 14 | 35.47 | 40.46 | 25.43 | 4.21 | 9.47 | 5.31 | 2.70 | 8.47 |
| 15 | 31.53 | 48.33 | 30.14 | 4.23 | 13.84 | 6.29 | 2.31 | 9.40 |
| 16 | 28.33 | 46.67 | 28.78 | 4.16 | 13.46 | 6.62 | 2.25 | 10.23 |
| 17 | 33.18 | 30.00 | 30.10 | 4.42 | 12.77 | 6.92 | 2.63 | 9.63 |
| 18 | 28.00 | 50.00 | 29.53 | 3.93 | 16.03 | 6.74 | 2.39 | 8.87 |
| 19 | 33.87 | 22.06 | 32.01 | 4.50 | 13.46 | 8.11 | 2.50 | 7.33 |
| 20 | 35.43 | 66.67 | 30.83 | 4.14 | 12.68 | 7.25 | 2.47 | 7.10 |
| 21 | 36.83 | 35.00 | 29.93 | 4.79 | 16.90 | 9.68 | 2.22 | 7.43 |
| 22 | 30.37 | 25.37 | 26.11 | 4.88 | 11.03 | 7.97 | 2.50 | 5.17 |
| 23 | 32.00 | 25.00 | 36.00 | 4.95 | 10.33 | 7.83 | 2.25 | 9.12 |
| ($P<0.05$) | 1.90 | 9.64 | 4.05 | 0.61 | 2.62 | 1.48 | 0.435 | 1.860 |

**Table 3.** Estimates of genetic parameters for germination and seedling characters

| Characters | Coefficient of variation (%) | | Heritability (%) | Genetic advance as (%) of mean | Range |
|---|---|---|---|---|---|
| | Phenotypic | Genotypic | | | |
| Seed Oil content | 10.19 | 9.54 | 87.72 | 18.41 | 27.07-38.17 |
| Germination | 49.33 | 28.56 | 33.50 | 34.05 | 13.33-66.67 |
| Plant height | 16.78 | 14.61 | 75.79 | 26.20 | 17.26-37.75 |
| Collar Diameter | 13.44 | 10.89 | 65.67 | 18.18 | 3.36-5.71 |
| No. of leaves | 19.54 | 16.22 | 68.90 | 27.74 | 9.47-18.24 |
| Inter- nodal length | 12.98 | 7.62 | 34.49 | 9.22 | 1.98-2.76 |
| Height of first branch | 21.60 | 16.75 | 60.11 | 26.75 | 5.17-11.08 |
| Number of Branches | 15.88 | 9.86 | 38.57 | 12.62 | 5.31-9.68 |

**Table 4.** Correlation coefficients among germination and seedling traits of *P. pinnata*.

| Characters | Germination | Plant height | Collar Diameter | No. of leaves | Inter-nodal length | Height of first branch | No. of branches | Seed Oil content |
|---|---|---|---|---|---|---|---|---|
| Germination | 1 | | | | | | | |
| Plant height | 0.1584 | 1 | | | | | | |
| Collar Diameter | 0.1957 | 0.6748** | 1 | | | | | |
| No. of leaves | 0.3626 | 0.0512 | - 0.0770 | 1 | | | | |
| Inter- nodal length | 0.2986 | - 0.0154 | - 0.0394 | 0.7918** | 1 | | | |
| Height of first branch | 0.3581 | 0.1926 | 0.0661 | 0.7216** | 0.7676** | 1 | | |
| Number of Branches | 0.0948 | - 0.4068 | - 0.5198 | 0.5771** | 0.3886 | 0.3001 | 1 | |
| Seed Oil content | 0.1168 | - 0.3210 | - 0.3538 | 0.5551** | 0.5580** | 0.4485* | 0.6182** | 1 |

** significant at 0.01 level of significance (P<0.01)

**Table 5.** Path coefficients showing direct and indirect effects of selected characters on seed oil content of *P. pinnata*.

| | Germination | Plant height | Collar Diameter | No. of leaves | Inter-nodal length | Height of first branch | Number of branches |
|---|---|---|---|---|---|---|---|
| Germination | -0.0311 | -0.0260 | -0.0094 | 0.0139 | 0.0860 | 0.0499 | 0.0335 |
| Plant height | -0.0049 | -0.1643 | -0.0324 | 0.0020 | -0.0044 | 0.0269 | -0.1438 |
| Collar Diameter | -0.0061 | -0.1108 | -0.0480 | -0.0030 | -0.0114 | 0.0092 | 0.1837 |
| No. of leaves | -0.0113 | -0.0084 | 0.0037 | 0.0384 | 0.2280 | 0.1007 | 0.2040 |
| Inter-nodal length | -0.0093 | 0.0025 | 0.0019 | 0.0304 | 0.2880 | 0.1072 | 0.1373 |
| Height of first branch | -0.0112 | -0.0316 | -0.0032 | 0.0277 | 0.2211 | 0.1396 | 0.1061 |
| Number of branches | -0.0030 | 0.0667 | 0.0250 | 0.0221 | 0.1120 | 0.0419 | 0.3534 |

**Table 6.** Clustering for genetic divergence in pod and seed traits of *Pongamia pinnata.*

| Clusters | No. of accessions in cluster | Accessions (IC numbers) |
|---|---|---|
| Cluster I | 8 | 1, 4, 10, 5, 19, 20, 21, 2 |
| Cluster II | 2 | 9, 23 |
| Cluster III | 5 | 3, 6, 12, 16 18 |
| Cluster IV | 4 | 8, 11, 7, 22, |
| Cluster V | 4 | 15, 17, 13, 14 |

**Table 7.** Intra and inter cluster distances for pod and seed traits in *P. pinnata*

| Clusters | Cluster I | Cluster II | Cluster III | Cluster IV | Cluster V |
|---|---|---|---|---|---|
| Cluster I | 4.95 | 7.47 | 8.50 | 7.67 | 5.16 |
| Cluster II | | 5.96 | 10.02 | 7.45 | 6.54 |
| Cluster III | | | 5.41 | 6.42 | 6.55 |
| Cluster IV | | | | 4.68 | 6.57 |
| Cluster V | | | | | 4.12 |

**Estimation of genetic parameters:** The estimates of genotypic coefficients of variation for the characters studied were less as compared to the phenotypic coefficients of variation. The highest phenotypic coefficient of variation (49.33) and genotypic coefficient of variation (28.56) was recorded for the germination percentage followed by height of the first branch. Estimation of broad sense heritability for germination and seedling characters showed that heritability was highest for the seed oil content (87.72%) followed by the plant height. The genetic gain was highest for germination (34.05%) followed by height of the first branch in *P. pinnata* (Table 3).

Genetic coefficient of variation which indicates the range and magnitude of genetic variability existing between the traits was in the vicinity of phenotypic coefficient of variation for most of the characters examined. The genotypic coefficient of variation was much less than the phenotypic coefficient of variation for germination percent indicating the influence of

**Table 8.** Cluster mean values for seed oil content and seedling traits in *P. pinnata.*

| Clusters | Seed oil content (%) | Germination (%) | Plant height (cm) | Collar Diameter (mm) | No. of leaves | No. of branches | Inter- nodal length (cm) | Height of first branch (cm) |
|---|---|---|---|---|---|---|---|---|
| Cluster I | 35.26 | 41.44 | 31.37 | 4.18 | 15.80 | 2.43 | 7.71 | 7.72 |
| Cluster II | 30.55 | 25.92 | 36.88 | 5.33 | 11.09 | 2.73 | 10.10 | 7.43 |
| Cluster III | 28.55 | 36.67 | 24.96 | 4.12 | 15.70 | 2.53 | 8.05 | 6.72 |
| Cluster IV | 29.95 | 27.18 | 30.42 | 5.19 | 14.98 | 2.60 | 7.91 | 7.48 |
| Cluster V | 33.05 | 36.53 | 28.05 | 4.06 | 12.13 | 2.55 | 9.18 | 6.38 |

environment on this trait. GCV alone is no indication of the magnitude of heritable variation. Heritability has an important place in tree improvement programmes as it provides index of the relative strength of heredity versus environment (Dorman, 1976). In the present study, the heritability estimates were recorded higher than 60.00% for seed oil content, plant height, collar diameter, number of leaves and height of the first branch above ground level in *P. pinnata* with highest estimates for the seed oil content (87.72%). Reasonably high estimates of heritability were observed for the number of branches, inter-nodal length and germination percent *P. pinnata*. Maximum genetic advance (34.05 %) was achieved in germination percent. In the present study, high magnitude of heritability (>60.00%) for most of the characters also envisaged that environment has comparatively low influence for seed oil content, plant height, collar diameter, number of leaves and height of the first branch above ground level. High heritability coupled with moderately high genetic advance for plant height, number of leaves and height of the first branch above ground level suggested the presence of an additive gene effect for these characters. Thus, individual plant selection for these characters would be satisfactory. High heritability accompanied by moderate genetic advance for several growth parameters have earlier been reported in *Pongamia pinnata* (Ali *et al.,* 2009).

**Correlation and path analysis:** Seed oil content showed positive and highly significant correlations (P< 0.01) with number of leaves, inter-nodal length and number of branches (Table 4). The component characters are not independent in their action but are interlinked. The Path coefficients analysis was used in the present study for understanding the complex traits (Table 5). Number of branches had highest direct effect on the seed oil content. The study revealed that number of branches, inter-nodal length, height of the first branch and number of leaves can be collectively used as selection criteria for improving the seed oil content in *P. pinnata*.

In the present study, seed oil content showed positive and highly significant correlations with number of leaves (5551**), inter-nodal length (5580**) and number of branches (6182**) (Table 4). The component characters are not independent in their action but are interlinked. In this interlinked complex system, selection practices for an individual character might subsequently bring about a simultaneous change in the other. Similar results on inter trait correlations in *Dalbergia sissoo* have been reported by Gera *et al.* (1999) and Vakshasya (1992).

**Genetic divergence and association studies:** Hierarchical Euclidean cluster analysis ($D^2$ statistics) grouped the progenies into 5 clusters (Table 6). The highest numbers of progenies were included in the cluster I followed by cluster III and least number of progenies i.e., two were observed in cluster II. The intra cluster distances ranged from 4.12 (cluster V) to 5.96 (cluster II). The maximum inter-cluster distance was observed between cluster II and III (10.02) followed by I and III and minimum was between clusters I and cluster V (Table 7). The cluster mean also varied among different cluster groups for germination and seedling traits (Table 8). The highest cluster mean for seed oil content (35.26%), germination (41.44 %) and height of first branch (7.72 cm) was recorded in cluster I while cluster II recorded maximum height (36.88 cm), collar diameter (5.33 mm), no. of branches (2.73) and inter-nodal length (10.10 cm). In divergence study conducted by Divakara and Das (2011), 24 accessions of *P. pinnata* were grouped into 6 clusters on the basis of non-hierarchical euclidian cluster analysis and similarly in our study 23 progenies have been grouped into 5 clusters and revealed that geographical diversity was not necessary to be related to genetic diversity

Maximum intra-cluster distance (5.96) shown by cluster II indicates wide divergence with in cluster itself which may be due to environmental factors and thus, suggests that selection of parents for hybridization within cluster should be based on genetic diversity rather than geographic diversity. Maximum inter-cluster distance between cluster II and III (10.02) followed by cluster I and III (8.50) indicates greater divergence between genotypes belonging to these clusters and an attempt to cross the genotypes in these clusters should bring out desirable gene combinations. The lowest inter cluster distance observed between clusters I and V suggests that progenies originating from these clusters were not genetically much diverse and thus, selection of parents from these clusters should be avoided. Similar results have been reported in *P. Pinnata* by Divakara and Das (2011) and Rao *et al.* (2011).

## Conclusion

Considerable differences were found among the progenies of *P. pinnata* for seed germination, oil content, and seedling characters. Progeny number 9, 21 and 2 were found to be the best on basis of most of the characters examined. These progenies showed considerable potential which can be judiciously tapped for planting and selecting the improved varieties. Maximum inter-cluster distance between cluster II and III indicated greater divergence between genotypes belonging to these clusters and an attempt to cross the genotypes in these clusters should bring out desirable gene combinations. Therefore, the progenies belonging to these clusters could be taken as parents for a successful hybridization program.

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