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Genetic variability studies among candidate plus trees of *Pongamia pinnata* L. for seed and oil traits

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Abstract
The present investigation was carried out at Forest College and Research Institute, Mettupalayam, Tamil Nadu to identify the best CPTs of *Pongamia pinnata* across its natural distribution areas in Coimbatore and Dharmapuri for further collection of seeds for afforestation or breeding purpose. Seeds were measured for its length, width, 100 seed weight and oil content. Significant was observed among 20 CPTs for seed and oil content. Maximum hundred seed weight (124.36 g) were recorded in PP 17, while PP 7 showed maximum oil content (36.54 %) followed by PP 3 (35.64 %). High heritability (broad sense) and genetic gain were observed for all seed and oil content respectively. Seed length-width ratio and seed length showed significant correlation with oil content. Among twenty CPTs, PP 7, PP 3, PP 4, PP 2 and PP 1 were found superior in terms of oil content. Hence these seed parameters could be considered as selection criteria for early and positive exploitation of high oil yielding genotypes. These CPTs can be further screened for tree improvement traits considering their immense value in yielding bio diesel.

Keywords: CPTs, *Pongamia pinnata*, Seed length, Seed width, 100 seed weight, oil content, Genetic estimation

Introduction
Energy security has an important bearing on achieving national economic development goals and improving the quality of life of the people. The level of per capita energy consumption has for long been considered as one of the key indicators of economic growth. In this way, economic development of many developing countries has lead to huge increase in the energy demand. As most of the countries now enjoying rapid development are also large petroleum importers, their dependence on external energy sources from highly unstable regions would increase to uncomfortable levels. Energy security has thus become a key issue for many countries. With the constant increase in population and in the requirements, the situation in the future is going to get even worse. Thus all countries including India are grappling with the problem of meeting this ever-increasing demand of transport fuel within the constraints of international commitments, legal requirements, environmental concerns and limited resources.

India consumes about 194 million tonnes of petroleum products in fiscal year 2021. At present, 5 million barrels of petroleum is being consumed in our country every day and it is also increasing by 3%, which is higher than the global average of around 1%. India is the world's third-largest importer of crude oil after the US and China, which imports about 55% of its natural gas requirements and 85% of the crude oil it processes. The demand for petroleum products in India is supported by the healthy economic growth of 7.1 per cent, continuing economic reopening amid ease of COVID restrictions and easing of trade-related bottlenecks supporting both mobility and industrial sector activity (Indian Petroleum & Natural Gas Statistics 2021-22) [5]. As the demand and price for crude oil skyrocket, India is becoming more and more vulnerable in the matter of energy security. In the light of this energy quandary, the importance of biofuels (bio-diesel and ethanol) is rapidly increasing with growing anxiety over crude oil supply and fast climatic changes.

Biofuel has also been more attractive recently because it is renewable, socio economically viable and environmentally beneficial. Biofuel is an eco-friendly, alternative diesel fuel prepared from domestic renewable resources i.e. vegetable oils (edible or non-edible oil) and animal fats. India has about hundreds of species which could yield oilseeds that have enough potential for use in biodiesel production. Since India is deficient in edible oils, therefore, the non-edible oils could be the desirable source for production of bio-diesel.

In such case, tree borne oilseeds (TBO's) are the best and potential alternative to mitigate the current and future energy crisis and also to transform the vast stretch of wastelands to green oil fields (Vigya Kesari and Latha Rangan, 2010) [20].

The Pungam tree is known as one of the richest and brightest trees which have big potential for that and can be planted all over tropics. The tree is scientific named as '*Pongamia pinnata*'. It is synonymously known as *Derris indica* (Lam) Bennett. Is an arboreal legume, belonging to the subfamily Papilionoideae. It is an Indo-Malaysian species, a medium-sized evergreen tree, common on alluvial and coastal situations from India to Fiji, from sea level to 1200 m. Pungam is a nitrogen fixing and drought tolerant species that survive varies climatic and edaphic conditions. It is an important source of fuel, fodder and small timber in rural India. However, it is economically valued for its seeds, which provide commercially important oil, popularly known as pongam oil is utilized in tanning, pharmaceutical and soap industries (Karoshi & Hegde, 2002) [9]. The oil is also used in illumination and as a lubricant. It has low viscosity and excellent fuel properties almost comparable with petro-diesel (Parthiban *et al*., 2009) [17]. Therefore, a lot of emphasis is given in recent years to explore the potential of pungam oil as an alternate source of petro-diesel in India.

The demands for petrol and diesel are rapidly increasing in developing countries. The rate of increase is very high in India (Katwal and Soni, 2003) [10]. The consumption was 37 million metric tonnes in 1970, which increased up to 222.79 million metric tonnes during 2020-2021 (Gyanaranjan Sahoo *et al*., 2020) [4]. The country is importing about 78 % of crude oil and investing a huge amount as a foreign exchange for fulfilling oil requirements. The prices of crude oil are enormously increasing in the international market over the last few years. On the other hand, the excessive use of fossil fuels are releasing a huge quantity of greenhouse gases (GHGs) in the atmosphere and altering the world climate. Growing concern over increasing prices of fossil fuels, depleting oil reserves coupled with environmental degradation has prompted to search alternative, renewable, economically viable and environmentally safe fuels derived from plant origin usually termed as biofuels (Kesari *et al*. 2009) [12]. Emphasis is given in India to exploit the potentials of pungam oil as a source of bio-diesel because it has low viscosity, better fuel properties and could be safely blended with petro-diesel even up to 20 % without any modification of existing diesel engines (Shrinivasa, 2001) [19]. The pungam oil has proved as an excellent feedstock for bio-diesel production in the country. The planning commission of India has set to blend 5-10 % of bio-diesel from the year 2010 to meet Euro III norms as per the agreement of Kyoto protocol. In this background, pungam by virtue of its broad existence and commercial exploitation over the years has proved to be real alternative for fossil fuels. In this background an attempt has been made to carry out the study.

Materials and Method

The study was under taken at Forest College and Research Institute (Mettupalayam), Coimbatore. Which is situated at 11°19'N latitude and 77°56'E longitude and an altitude of 350 m above MSL. The average annual rainfall is 945 mm, most of which is received between June to September. The temperature varies from 15 to 34.9^0 C. The extensive survey was under taken two districts of Tamil Nadu *viz*., Coimbatore and Dharmapuri. The individual tree was identified based on their morphometric characteristics and oil content of trees. Seeds were extracted from 100 pods after sun drying for ten days for assessment of seed characteristics. Seed parameters such as seed length, seed width, 100 seed weight and oil content were recorded for each CPTs. The oil content of seeds was estimated by soxhlet method using three replicates for each CPTs.

Observed data was analyzed using SPSS statistical package 'version 2000'. Duncan Multiple Range Test (DMRT) was performed at 5% significance level to observe the homogeneous sub-set between the CPTs. Analysis of variance was carried out following the procedure given by Panse and Sukhatme (1976) [16]. The variability, heritability in broad sense, genetic advance as percent of mean, phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) were worked out for seed traits and oil content as suggested by Lush (1940) [13], Johnson *et al*., (1955) [7] and Burton (1952) [1].

Results and Discussion

Nature has provided some wonderful plants that possess immense energy potential. *Pongamia pinnata* is a medium sized leguminous tree which finds most significant utilization in the form of biodiesel. The basic aim for any improvement programme is the exploitation of available natural variability within the species. The tree improvement programme must always be preceded by the estimation of the extent of variability available in the natural population. The assessment of genetic variability is vital to tree improvement (Zobel, 1971) [21]. 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. Since most of the plant characters of economic importance are polygenic in nature and are highly influenced by environmental fluctuations, it is difficult to judge whether the observed variability is heritable or due to environment. This suggests the imperative need to partition the phenotypic variation into heritable and non-heritable components. The largest, cheapest and fastest gains in most forestry tree improvement programmes will accrue if use of suitable species and seed sources within species is assured (Zobel and Talbert, 1984) [22].

Variability studies in seed parameters

The present study revealed that significant amount of variability existed among different candidate plus trees of *Pongamia pinnata* in all the seed characters investigated *viz.*, seed length, seed width, seed length- width ratio, 100 seed weight and seed oil content (Table.2). Six progeny tress *viz.*, PP 1, PP 2, PP 3, PP 7, PP 8 and PP 18 recorded the highest and significant values for seed length, seed width, seed length- width ratio, 100 seed weight and seed oil content. However, the candidate trees PP 5 and PP 11 recorded high and significant values for seed length, seed width, 100 seed weight and seed oil content. This type of variability in seed morphology and germination was attributed to the out-breeding nature of the species. Genetic control of seed size traits has been observed in several tree species like *Tectona grandis* (Jayasankar *et al*,. 1999) [6], *Pongamia pinnata* (Sharma *et al*., 2016 [18] and Palanikumaran *et al*., 2016 [14]) and *Bixa orellana* (Kala *et al*., 2017) [8].

Genetic variability and association studies

The amount of genetic variation expressed by different seed and oil traits could be judged through the study of PCV and GCV. Variance components for various traits are presented in Table 3. The magnitude of PCV was higher than the corresponding genotypic ones for all characters. The seed length and width ratio had recorded high PCV and GCV followed by seed length, seed oil content, seed width and 100 seed weight. Seed length width ratio also recorded the maximum heritability estimate of 0.99 followed by seed length, seed width, 100 seed weight and seed oil content.

Panse (1957) [15] reported that the heritability is mainly due to non-additive genetic effects. The genetic gain would be low while on the other hand if the heritability is chiefly due to additive gene effects, a high genetic advance is expected. In this study, Pungam expressed high heritability for seed length/ width ratio followed by seed length, seed oil content and seed width and 100 seed weight which were governed partly by additive gene actions. Also, seed length width ratio and seed oil content recorded high genetic advance as percentage of mean (Table 3). Hence, these seed physical parameters could be considered for selection in tree improvement programmers.

In the present investigation, highly positive and significant phenotypic correlation existed between seed length width ratio and seed oil content while highly positive and significant genotypic correlation existed between seed width and seed oil content (Table 4). Similarly, positive and significant correlation seed length width ratio with oil content and other growth parameters were reported in *J. Curcas* (Ginwal *et al.*, 2004) [3]. The other seed parameters *viz.*, seed length and seed length- width ratio also exhibited positive and significant association with seed oil content. The correlation of seed physical parameters with seed oil content in the current study was found to be significant. This indicates that these physical characters may be used to the advantage of the breeder for bringing simultaneous improvement of these traits easily (Ginwal *et al.*, 2004 [3] and Kaushik *et al*., 2007 [11]).

In the study, all the four traits *viz*., seed length, seed width, seed length-width ratio and hundred seed weight exercised positive direct effect on oil content. The maximum positive direct effect was exerted by seed width (0.531) on seed oil content followed by, seed length- width ratio (0.295), seed length (0.256) and hundred seed weight (0.053). Also the parameters *viz.*, seed length, seed width and seed length-width ratio recorded maximum positive indirect effect through 100 seed weight on oil content (Table 5). The present investigation envisaged that high and positive association coupled with intensive direct effect of seed width on oil content could be used as selection criterion in Pungam improvement programmer and also for utilization of Pungam for oil and biodiesel recovery (Divakara and Das, 2011) [2].

Conclusion

It was concluded that, potentially huge genetic variability existed in seed and oil content among the selected plus trees of *Pongamia pinnata*. Among the 20, five candidate plus trees *viz.*, PP 7, PP 3, PP 4, PP 2 and PP 1 were found superior for all the traits studied including oil content. Hence, selection, mass propagation and popularization of these superior candidate plus trees for industrial plantations would help to improve the overall productivity of pungam in terms seed, and oil content. Higher genotypic correlation coefficient of seed characters revealed that the traits are genetically controlled and selection can be very effective in further tree improvement programmer.

Table 1: Location details of *Pongamia pinnata* CPTs selected in Tamil Nadu

CPTs	Locations	District	Latitude	Longitude	Height (m)	Girth (m)	Volume (m^3)
PP 1	Mettupalayam	Coimbatore	11°9'N	76°56'E	8.50	0.22	0.032
PP 2	Mettupalayam	Coimbatore	11°9'N	76°56'E	6.50	0.32	0.051
PP 3	Mettupalayam	Coimbatore	11°9'N	76°56'E	7.50	0.10	0.040
PP 4	Mettupalayam	Coimbatore	11°9'N	76°56'E	7.00	0.25	0.026
PP 5	Annur	Coimbatore	11°9'N	76°56'E	6.00	0.23	0.021
PP 6	Annur	Coimbatore	11°9'N	76°56'E	6.50	0.24	0.031
PP 7	Annur	Coimbatore	11°9'N	76°56'E	6.00	0.22	0.023
PP 8	Therampalayam	Coimbatore	11°9'N	76°56'E	8.00	0.16	0.110
PP 9	Moothepalayam	Coimbatore	11°9'N	76°56'E	7.50	0.33	0.035
PP 10	Moothepalayam	Coimbatore	11°9'N	76°56'E	6.50	0.21	0.011
PP 11	Mettupalayam	Coimbatore	11°9'N	76°56'E	6.50	0.11	0.031
PP 12	Mettupalayam	Coimbatore	11°9'N	76°56'E	7.50	0.15	0.020
PP 13	Mettupalayam	Coimbatore	11°9'N	76°56'E	6.50	0.19	0.019
PP 14	Palacode	Dharmapuri	12°9'N	78°20'E	7.00	0.28	0.642
PP 15	Palacode	Dharmapuri	12°9'N	78°20'E	8.50	0.31	0.064
PP 16	Palacode	Dharmapuri	12°9'N	78°20'E	6.50	0.27	0.036
PP 17	Dharmapuri	Dharmapuri	12°9'N	78°20'E	8.00	0.25	0.038
PP 18	Dharmapuri	Dharmapuri	12°9'N	78°20'E	6.50	0.22	0.025
PP 19	Dharmapuri	Dharmapuri	12°9'N	78°20'E	7.40	0.27	0.040
PP 20	Kunjapanai	Coimbatore	11°9'N	76°56'E	6.00	0.21	0.020

Table 2: Variations in seed physical parameters and oil content

CPTs	Seed Length (mm)	Seed Width (mm)	Seed l/w Ratio (mm)	100 Seed Weight (g)	Oil Content (%)
PP 1	21.80**	13.60**	1.60*	110.25	32.53**
PP 2	20.60*	13.20**	1.56*	104.58	34.09**
PP 3	14.40	13.70**	1.02	100.32	35.64**
PP 4	14.30	11.20	1.27	093.65	35.44**
PP 5	19.30	14.50**	1.33	117.43**	24.79
PP 6	16.40	18.00**	0.91	106.88	21.31
PP 7	21.20*	09.30	2.27**	099.37	36.54**
PP 8	22.20**	11.40	1.94**	116.34**	30.52**
PP 9	17.60	10.40	1.69*	106.48	22.57
PP 10	18.90	10.60	1.78*	101.69	26.58
PP 11	18.00	8.30	2.16**	115.47*	30.51**
PP 12	18.50	11.80	1.56*	093.67	28.28*
PP 13	18.10	10.80	1.67*	099.64	22.41
PP 14	18.80	12.10	1.55*	111.33	23.54
PP 15	21.30**	12.80	1.66*	112.58*	24.30
PP 16	18.60	14.30**	1.30	099.82	21.41
PP 17	28.80*	13.80**	2.08**	124.36**	29.61**
PP 18	27.60**	14.00**	1.97**	124.33**	25.62
PP 19	20.60*	15.00**	1.37	113.58*	27.50
PP 20	17.65	15.10**	1.16	100.23	23.52
Mean	19.73	12.69	1.59	107.71	27.83
S.E.D	0.36	0.17	0.02	2.16	0.42
CD(0.05)	0.73	0.37	0.02	4.38	0.66
CD(0.01)	0.93	0.53	0.29	8.14	0.91

*Significance at (5%) **Significance at (1%)

Table 3: Estimation of genetic variables for seed and oil traits in *Pongamia pinnata*

Traits	PCV (%)	GCV (%)	Heritability (%)	GA (%) of mean
Seed length	18.43	18.29	91.00	37.39
Seed width	18.05	17.97	84.08	36.86
Seed length-width ratio	23.38	23.27	99.10	47.82
100 seed weight	8.70	8.34	74.83	16.49
Seed oil content	18.19	17.98	82.21	36.61

Table 4: Phenotypic and Genotypic correlation coefficient between seed and oil traits of *Pongamia pinnata*

Traits		Seed length	Seed width	Seed length- width ratio	100 Seed weight	Seed oil content
Seed length	P	1.000**	0.083	0.626**	0.117	0.012
	G	1.000**	0.079	0.634**	0.121	0.010
Seed width	P		1.000**	0.685**	0.373**	0.348**
	G		1.000**	0.693**	0.388**	0.353**
Seed length-width ratio	P			1.000**	0.167	0.252**
	G			1.000**	0.177	0.257**
100 seed weight	P				1.000**	0.237**
	G				1.000**	0.245**
Seed oil content	P					1.000**
	G					1.000**

** Significant at 1% level

Table 5: Path coefficient analysis of seed parameters on seed oil content

Traits	Seed length	Seed width	Seed length- width ratio	100 Seed weight	Correlation (r) with seed Oil content
Seed length	<u>0.256</u>	0.044	0.187	0.015	0.992
Seed width	0.021	<u>0.531</u>	0.204	0.047	1.011
Seed length-width ratio	0.162	0.367	<u>0.295</u>	0.021	0.617
100 seed weight	0.031	0.206	0.052	<u>0.122</u>	0.969

Residual effects = 0.1960 (Diagonal values are direct effect)

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