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Variability Studies for Seed and Seedling Traits in *Calophyllum Inophyllum* (L.) at South India

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ABSTRACT

The present investigation was carried out at Forest College and Research Institute, Mettupalayam, Tamilnadu to identify the best half sibs of *Calophyllum inophyllum* across its natural distribution of south India for further collection of seeds for afforestation or breeding purpose. The seeds were collected from different climatic zones of south India. Seeds were measured for its length, width, Pod length, Width and then sown in nursery to study the variation in germination and initial growth parameters *viz.*, germination percent, germination value, peak value, mean daily germination of seedlings. Seeds collected from Western Ghats of Karnataka were superior compared to seeds from other parts in all the traits considered for the study. These seed sources can be further screened for tree improvement traits considering their immense value in yielding bio diesel.

1. Introduction

Calophyllum inophyllum is a multipurpose tree belonging to the family Clusiaceae, commonly known as mangosteen family. This plant has multiple origins including East Africa, India, South East Asia, Australia, and the South Pacific. Calophyllum inophyllum is known by various names around the world. Shows different vernacular names of Calophyllum inophyllum in some selected countries of the world. Shows the distribution map of Calophyllum inophyllum around the world. As can be seen this tree is widely available in India, South East Asia and Australia. It grows in areas with an annual rain of 1000-5000 mm at altitudes from 0 to 200 m. Calophyllum inophyllum is allowbranching and slow-growing tree with two distinct flowering periods of late spring and late autumn. But sometimes its flowering may occur throughout the year. Calophyllum inophyllum grows best in sandy, well drained soils. However it tolerates clays, calcareous, and rocky soils. The tree supports a dense canopy of glossy, elliptical, shiny and tough leaves, fragrant white flowers, and large round nuts. Its size typically ranges between 8 and 20 m (25-65 ft) tall at maturity, sometimes reaching up to 35 m (115ft). The growth rate of the tree is1m (3.3ft) in height per year on good sites.

Its leaves are heavy and glossy, 10–20 cm (4–8 inch) long and 6–9 cm (2.4–3.6 inch) wide, light green when young and dark green when older. Fruits are spherical drupes and arranged in clusters. The fruit is reported to be pinkish-green at first. However, it turns later to be bright green and when ripe, it turns dark grey- brown and wrinkled. The tree yield is100–200fruits/kg. In each fruit, one large brown seed 2–4 cm (0.8–1.6in.) India meter is found. The trees yield 3000–10,000 seeds /tree/ season. The seed is surrounded by a shell and a thin layer of pulp of 3–5 mm. *Calophyllum inophyllum* Oil is non-edible and dark green. Traditionally, its oil has been used as a medicine, soap, lamp oil, hair grease and cosmetic in different parts of the world. Recently, *Calophyllum inophyllum* has been proposed as a source of biodiesel.

2. Materials and methods

The present study was under taken during the year 2014-15 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 °C.

The extensive survey was under taken across three different state and one union territory of India. A distance of at least 200 mts was maintained between two trees and at least 25 kms between two seed sources. The individual tree was identified based on their phenotypical characteristics and the individual tree identity was also maintained. Seeds were extracted from 100 pods after sun drying for ten days for assessment of seed characteristics. Further same seeds were used for assessing germination and seedling characters. Seed parameters such as seed length, seed width, seed thickness, seed volume and 100 seed weight were recorded for each seed source. The experiment was laid out in completely randomized design with five replications of 100 seeds each. The seeds were sown in the standard nursery bed and regular watering was done. Observations on daily germination were recorded up to 31 days from date of sowing. Germination percentage, peak value, mean daily germination, germination rate and germination value were recorded for each seed source. Data collected was analysed statistically using Mstatc program.

Number of seeds germinated

Germination per cent = X 100

Number of seeds sown Germination value (GV) = PV X MDG, where, PV- Peak Value of germination.

MDG- Mean Daily Germination

Total germination per cent

Peak Value = ----
Total number of days

Final germination per cent

Mean Daily Germination = ----
Number of days that took to reach

Peak Germination

Estimation of oil content using Soxhlet method

For estimating oil, the seeds were depulped, the kernels dried at 50°C for 16 hrs and allowed to cool in a desiccator. Five grams of seeds were pulverized to a fine powder in a porcelain mortar. Ground samples were placed in a filter paper and fastened in such a way to prevent escape of the meal and then carefully transferred to an extraction thimble. The thimble was then placed in a Soxhlet extractor to which sufficient quantity of solvent petroleum ether (40 - 60°C) was added and heated until eleven siphonings were completed. The oil content was recorded by evaporating the petroleum ether at 60°C. The entire extraction process was carried out in

Soxhlet extractor according to AOAC (1970). The percentage of oil content was then calculated by using the formula.

Table.1 Calophyllum inophyllum seed source collection from different places in South India

Tamilnadu	Source	Latitude	Longitude	Altitude						
FCRICI 2 (Nagapattinam) 10°45'N 79°49'E 5 FCRICI 3 (Velankanni) 10°41'N 79°50'E 21 FCRICI 4 (Thiruvarur) 10°45'N 79°37'E 38 FCRICI 5 (Pudhucherry) 11°54'N 79°47'E 25 FCRICI 6 (Tindivanam) 12°13'N 79°39'E 140 FCRICI 7 (Cuddalore) 11°44'N 79°42'E 35 FCRICI 8 (Nagercoil) 08°09'N 77°22'E 148 FCRICI 9 (Pechiparai) 08°26'N 77°18'E 326 FCRICI 10 (Mettupalayam) 11°19'N 76°58'E 1036 FCRICI 11 11°22'N 77°53'E 1405 (Thiruchencode) FCRICI 12 (Coimbatore I) 10°59'N 76°55'E 1373 II) Karnataka FCRICI 14 (Honnavara) 14°15'N 74°26'E 43 FCRICI 15 (Hubli) 15°22'N 75°04'E 2127 FCRICI 16 (Sirsi) 14°39'N 74°52'E 2049 FCRICI 17 (Bhatkal) 13°59'N 74°31'E 51 FCRICI 19 (Udupi) 13°20'N 74°43'E 49 FCRICI 20 (Mangalore) 12°54'N 74°51'E 79 FCRICI 21 (Talugoppa) 14°12'N 74°54'E 1961 FCRICI 22 (Shimoga) 13°53'N 75°33'E 1922 FCRICI 23 (Sagar) 14°09'N 76°54'E 2219 FCRICI 25 (Mandiya) 12°29'N 76°54'E 2219 FCRICI 26 (Mysore) 12°22'N 76°59'E 81 FCRICI 28 (Thirissur) 10°29'N 76°51'E 2161 FCRICI 29 (Kottayam) 9°33'N 76°32'E 21	Т	amilnadu								
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FCRICI 22 (Shimoga) 13°53'N 75°33'E 1922 FCRICI 23 (Sagar) 14°09'N 75°00'E 1982 FCRICI 24 (Tumkur) 13°20'N 76°09'E 2974 FCRICI 25 (Mandiya) 12°29'N 76°54'E 2210 FCRICI 26 (Mysore) 12°22'N 76°40'E 2299 Kerala FCRICI 27 (Tiruvandrum) 8°29'N 76°59'E 81 FCRICI 28 (Thirissur) 10°29'N 76°17'E 161 FCRICI 29 (Kottayam) 9°33'N 76°32'E 21	FCRICI 20 (Mangalore)	12°54'N	74°51'E	79						
FCRICI 23 (Sagar) 14°09'N 75°00'E 1982 FCRICI 24 (Tumkur) 13°20'N 76°09'E 2974 FCRICI 25 (Mandiya) 12°29'N 76°54'E 2210 FCRICI 26 (Mysore) 12°22'N 76°40'E 2299 Kerala FCRICI 27 (Tiruvandrum) 8°29'N 76°59'E 81 FCRICI 28 (Thirissur) 10°29'N 76°17'E 161 FCRICI 29 (Kottayam) 9°33'N 76°32'E 21	FCRICI 21 (Talugoppa)	14°12'N	74°54'E	1961						
FCRICI 24 (Tumkur) 13°20'N 76°09'E 2974 FCRICI 25 (Mandiya) 12°29'N 76°54'E 2210 FCRICI 26 (Mysore) 12°22'N 76°40'E 2299 Kerala FCRICI 27 (Tiruvandrum) 8°29'N 76°59'E 81 FCRICI 28 (Thirissur) 10°29'N 76°17'E 161 FCRICI 29 (Kottayam) 9°33'N 76°32'E 21	FCRICI 22 (Shimoga)	13°53'N	75°33'E	1922						
FCRICI 25 (Mandiya) 12°29'N 76°54'E 2210 FCRICI 26 (Mysore) 12°22'N 76°40'E 2299 Kerala FCRICI 27 (Tiruvandrum) 8°29'N 76°59'E 81 FCRICI 28 (Thirissur) 10°29'N 76°17'E 161 FCRICI 29 (Kottayam) 9°33'N 76°32'E 21	FCRICI 23 (Sagar)	14°09'N	75°00'E	1982						
FCRICI 26 (Mysore) 12°22'N 76°40'E 2299 Kerala FCRICI 27 (Tiruvandrum) 8°29'N 76°59'E 81 FCRICI 28 (Thirissur) 10°29'N 76°17'E 161 FCRICI 29 (Kottayam) 9°33'N 76°32'E 21	FCRICI 24 (Tumkur)	13°20'N	76°09'E	2974						
Kerala FCRICI 27 (Tiruvandrum) 8°29'N 76°59'E 81 FCRICI 28 (Thirissur) 10°29'N 76°17'E 161 FCRICI 29 (Kottayam) 9°33'N 76°32'E 21	FCRICI 25 (Mandiya)	12°29'N	76°54'E	2210						
FCRICI 27 (Tiruvandrum) 8°29'N 76°59'E 81 FCRICI 28 (Thirissur) 10°29'N 76°17'E 161 FCRICI 29 (Kottayam) 9°33'N 76°32'E 21	FCRICI 26 (Mysore)	12°22'N	76°40'E	2299						
FCRICI 28 (Thirissur) 10°29'N 76°17'E 161 FCRICI 29 (Kottayam) 9°33'N 76°32'E 21	Kerala									
FCRICI 29 (Kottayam) 9°33'N 76°32'E 21	FCRICI 27 (Tiruvandrum)	8°29'N	76°59'E	81						
` '	FCRICI 28 (Thirissur)	10°29'N	76°17'E	161						
FCRICI 30 (Upala) 12°41'N 79°54'E 35	FCRICI 29 (Kottayam)	9°33'N	76°32'E	21						
	FCRICI 30 (Upala)	12°41'N	79°54'E	35						

Table 2. Seed characteristics as influenced by various place in South India

Seed source	Pod length	Pod width	Seed length	Seed width	100 Seed	
	(mm)	(mm)	(mm)	(mm)	weight	
					(g)	
FCRICI 1 (Vedaranyam)	29.13	22.38	19.50	18.76	102.13	
FCRICI 2 (Nagapattinam)	34.39*	25.18	21.34	20.66*	146.10*	
FCRICI 3 (Velankanni)	30.64	26.92	19.10	17.54	107.09	
FCRICI 4 (Thiruvarur)	24.96	25.61	21.34	20.38*	124.19*	
FCRICI 5 (Pudhucherry)	30.27	24.06	21.90	19.68*	100.17	
FCRICI 6 (Tindivanam)	29.86	25.67	20.99	14.77	108.27	
FCRICI 7 (Cuddalore)	29.75	24.51	21.34	15.11	102.02	
FCRICI 8 (Nagercoil)	29.39	25.03	20.81	13.89	111.24	
FCRICI 9 (Pechiparai)	33.77*	23.38	20.42	17.91	133.30*	
FCRICI10(Mettupalayam)	29.56	22.06	21.56	14.99	96.30	
FCRICI 11 (Thiruchencode)	24.30	24.31	20.05	14.53	89.20	
FCRICI 12 (Coimbatore I)	28.97	23.88	20.36	13.26	128.30*	
FCRICI 13 (Coimbatore II)	27.56	25.97	20.06	14.26	68.08	
FCRICI 14 (Honnavara)	39.57*	34.44*	27.15*	23.50*	164.27*	
FCRICI 15 (Hubli)	25.07	25.65	18.77	13.31	134.07*	
FCRICI 16 (Sirsi)	26.51	24.08	18.99	14.38	125.32*	
FCRICI 17 (Bhatkal)	28.61	24.07	17.71	12.44	96.13	
FCRICI 18 (Kumta)	30.75	25.25	21.98	13.49	134.04*	
FCRICI 19 (Udupi)	28.32	23.15	21.74	15.05	127.17*	
FCRICI 20 (Mangalore)	26.20	29.10*	21.48	12.85	125.26*	
FCRICI 21 (Talugoppa)	27.68	25.08	20.07	12.81	117.15	
FCRICI 22 (Shimoga)	28.42	24.04	20.99	14.12	131.09*	
FCRICI 23 (Sagar)	34.65*	23.02	21.31	12.89	120.12*	
FCRICI 24 (Tumkur)	33.35*	28.14	22.27	21.44*	115.33	
FCRICI 25 (Mandiya)	29.94	26.81	25.29*	21.92*	120.27*	
FCRICI 26 (Mysore)	27.73	27.00	23.13	15.09	126.16*	
FCRICI 27 (Tiruvandrum)	26.93	25.64	21.60	14.49	119.15*	
FCRICI 28 (Thirissur)	34.15*	24.69	20.72	14.88	121.16*	
FCRICI 29 (Kottayam)	29.01	25.76	18.87	14.21	126.15*	
FCRICI 30 (Upala)	25.38	25.88	22.13	15.51	134.25*	
Mean	29.49	25.36	21.10	15.94	118.45	
SEd =	1.895 1.516	1.478 1	.624 0.12	<u> </u>		

 SEd
 =
 1.895
 1.516
 1.478
 1.624
 0.125

 CD (0.05)
 =
 3.794
 3.035
 2.960
 3.252
 0.250

Table 3. Seed germination attributes in different place in South India

Seed source	Germinatio n percent	Germination value	Peak value	Mean daily germination	Oil %	
FCRICI 1 (Vedaranyam)	63.00	4.41	2.30	1.92	44.9	
FCRICI 2 (Nagapattinam)	68.00	4.71*	2.85*	1.69	58.3	
FCRICI 3 (Velankanni)	61.67	3.68	1.92	1.93	47.8	
FCRICI 4 (Thiruvarur)	56.67	3.87	2.18	1.79	42.6	
FCRICI 5 (Pudhucherry)	58.00	3.32	2.09	1.59	49.6	
FCRICI 6 (Tindivanam)	58.00	3.32	1.98	1.67	41.0	
FCRICI 7 (Cuddalore)	59.33	3.07	1.93	1.59	38.2	
FCRICI 8 (Nagercoil)	68.33	3.80	2.17	1.74	52.4	
FCRICI 9 (Pechiparai)	64.00	4.45	2.55	1.56	57.4	
FCRICI10(Mettupalayam)	59.67	3.10	1.97	1.57	45.8	
FCRICI 11 (Thiruchencode)	54.00	3.64	2.06	1.77	44.4	
FCRICI 12 (Coimbatore I)	64.67	3.50	2.04	1.70	43.7	
FCRICI 13 (Coimbatore II)	58.33	3.48	2.20	1.57	50.6	
FCRICI 14 (Honnavara)	78.00*	5.11*	3.06*	1.79	64.6*	
FCRICI 15 (Hubli)	56.00	3.64	2.31	1.56	39.3	
FCRICI 16 (Sirsi)	53.00	3.63	1.99	1.83	44.8	
FCRICI 17 (Bhatkal)	54.67	3.25	1.92	1.68	51.4	
FCRICI 18 (Kumta)	59.00	3.48	2.02	1.73	45.5	
FCRICI 19 (Udupi)	55.67	2.88	1.98	1.43	43.5	
FCRICI 20 (Mangalore)	46.67	2.54	1.77	1.44	43.0	
FCRICI 21 (Talugoppa)	54.67	2.53	1.68	1.51	43.6	
FCRICI 22 (Shimoga)	52.33	3.26	1.89	1.72	46.3	
FCRICI 23 (Sagar)	56.00	3.62	1.98	1.82	48.2	
FCRICI 24 (Tumkur)	56.00	2.63	1.68	1.73	37.9	
FCRICI 25 (Mandiya)	52.00	2.96	1.87	1.66	48.3	
FCRICI 26 (Mysore)	55.67	2.90	2.09	1.40	35.7	
FCRICI 27 (Tiruvandrum)	57.33	2.24	1.70	1.33	47.7	
FCRICI 28 (Thirissur)	54.67	2.72	1.98	1.52	44.4	
FCRICI 29 (Kottayam)	57.33	2.63	1.74	1.53	44.7	
FCRICI 30 (Upala)	54.33	3.29	2.06	1.58	46.3	
Mean	58.23	3.39	2.06	1.64	46.4	

Table 4. Genotypic correlation of seed and seedling attributes of *Calophyllum inophyllum* in South India (** Significant at 1 % level; * Significant at 5 % level)

Characters	Pod length	Pod width	Seed length	Seed width	Seed weight	Germina tion percent	Germina tion value	Peak value	MDG	Oil %
Pod length	1.000	0.396*	0.566**	0.547**	0.405*	2.403**	0.663**	0.803**	0.533**	0.853**
Pod width		1.000	0.822**	0.525**	0.480**	1.274**	0.196	0.450**	0.067	0.389*
Seed			1.000	0.753**	0.530**	1.520**	0.283	0.576**	-0.124	0.408*
length										
Seed width				1.000	0.295	1.639**	0.557**	0.704**	0.498**	0.639**
Seed wt					1.000	0.928**	0.365*	0.575**	-0.076	0.373*
G.percent						-1.000	1.758**	2.179**	-0.700	2.793**
G.value							1.000	0.991**	0.607**	1.217**
Peak value								1.000	0.346	1.336**
MDG									1.000	0.639**
Oil %										1.000

Table 5. Phenotypic correlation of seed and seedling attributes of *Calophyllum inophyllum* in South India (** Significant at 1 % level; * Significant at 5 % level)

Characters	Pod length	Pod width	Seed length	Seed width	Seed weight	Germina tion percent	Germinati on value	Peak value	MDG	Oil %
Pod length	1.000	0.230	0.343*	0.406*	0.325*	0.226	0.198	0.236	0.074	0.370*
Pod width		1.000	0.389*	0.326*	0.357*	0.048	0.065	0.165	-0.053	0.150
Seed length			1.000	0.438**	0.344*	0.027	0.005	0.137	-0.090	0.171
Seed width				1.000	0.243	0.173	0.375*	0.342*	0.229	0.195
Seed wt					1.000	0.164	0.210	0.296	-0.030	0.187
G.percent						1.000	0.552**	0.511**	0.459**	0.269
G.value							1.000	0.848**	0.638**	0.318*
Peak value								1.000	0.232	0.339*
MDG									1.000	0.060
Oil %										1.000

3. Results and Discussion

Data from the Table. 2 revealed that seed traits for all seed sources showed significant differences. The seeds collected from Honnavara region were longest, thickest and had higher mass as well as seed volume compared to all other seed sources. Seed length amongst various seed sources varied from 17.71 mm to 27.15 mm, seed width from 12.44 mm to 23.50 mm. Pod length and pod width varied from 25.07 mm to 39.57 mm and 22.06 mm to 34.44 mm respectively. The 100 seed weight ranged between 68.08 g to 133.30 g. These variations may be due to the fact that, this species grows over a wide range of climatic conditions as well as soil types and altitudes. Similar findings were revealed by Sudhir Kumar (2003) in Jatropha curcas and Vasanth Reddy et al. (2007) in Pongamia pinnata. Analysis of variance (ANOVA) revealed that the results were statistically significant for all the germination attributes (Table. 3). Overall germination per cent was on/or above the 50 per cent. Maximum germination per cent was found in Honnavara region (78.00 %), owing to higher mean daily germination (1.79), Germination value (5.11) and peak value of germination (3.06). It was followed by Nagapattinam region (68.00 %) and Pechiparai region (81.67 %) seed sources. Whereas, minimum germination per cent was recorded on Dharapuram region (64.00 %). The size and shape of seeds is variable depending on the structure and form of the ovary and environmental conditions under which plant is growing. It is evident from the result that seeds from Honnavara region was found to be superior with respect to germination percentage. This is in line with study made by Dwivedi (1993) in Azadirachta indica and Devagiri et al. (1998) in Dalbergia sissoo. They found that the variation observed in the seed characters may be attributed to adverse environment and differences in their distribution range this in turn affect the germination of seeds. Seedlings of Honnavara region higher oil content (64.6 %). It was followed by Nagapattinam (58.30%) and Pechiparai region (57.30%).

Genotypic correlation

Pod length (0.853), Pod width (0.389), Seed length (0.408), seed width(0.639) Seed weight (0.373), Germination percent (2.793), Germination value (1.217),

Peak value (1.336) and Mean daily germination (0.639) showed positive significant correlation with oil content (Tables 4).

Phenotypic correlation

Pod length (0.370), Germination value (0.318), Peak value (0.339) showed positive but significant correlation with oil content. Pod width (0.150), seed length (0.171), seed width (0.195), Seed weight (0.187), Germination percent (0.269), Mean daily germination (0.060), showed positive but nonsignificant correlation with oil content (Tables 5 and 6).

A highly significant and positive correlation existed between Pod length (0.370), Germination value (0.318), Peak value (0.339). Significant correlation among various seed germination and seedling traits suggests that test weight may prove to be important criteria in selection of geographic seed sources for raising stock for bulk commercial plantations. This study identifies two best sources for Calophyllum inophyllum L based upon seed and seedling traits for those place of Honnavara and Nagergoil which were sampled. On a short term basis, breeding zones may be set up in these environmentally homogeneous areas. However, this may be preliminary as only seedling traits have been considered. Hence, seed source screening provides a great opportunity to the tree breeder to screen and capture natural variation for success of afforestation, besides providing information on the raw material for breeding and evolving improved planting stock within a seed source.

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