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IMPACT OF SEED WEIGHT ON GERMINATION PARAMETERS OF *CALOPHYLLUM INOPHYLLUM* L.: A POTENTIAL BIODIESEL TREE SPECIES OF COASTAL REGION

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ABSTRACT

We studied the variation in seed physical and biochemical attributes in different weight classes and to find out the influence of seed weight on germination parameters of *Calophyllum inophyllum* L. The fruits were obtained from the coastal areas of Thiruvananthapuram, Kerala during May-June 2012. Prominent size variation was observed among the fruits of the same lot and the seeds were extracted after drying. The seeds were graded based on their weight in to classes 2-5, 5-8 and 8-11 g. Number of fruits per kg of 8-11, 5-8 and 2-5 g weight classes was 116.0 ± 17.7, 166.7 ± 18.7 and 262.0 ± 65.6 and thousand seed weight was $8.55 \pm .24$, 6.60 ± 0.30 and 5.07 ± 1.53 kg, respectively. With regards to individual seed characters, seeds belonging to 8-11 g class recorded a higher weight (8.62 ± 0.59 g), length (3.24 ± 0.18 cm), breadth (2.99 ± 1.17 cm) and volume (0.12 ± 0.01 cm^3) compared to other classes. The biochemical constituents also decreased with decreasing weight class and the oil content of large seeds were 63 %. However, the germination percentage were the highest in medium sized seeds (68.00 ± 3.00) followed by large seeds (68.00 ± 2.00). Hence, the medium sized seed can be advocated for planting stock production in the nursery and followed by large seeds and small seeds.

INTRODUCTION

Seed traits such as seed size, seed coat thickness, shape and moisture content are already known to affect seed germination, dormancy and early seedling establishment (Sudhakara and Jijeesh 2008; Singh and Saxsena, 2009; Thapa *et al.,* 2012; Jijeesh and Sudhakara, 2013). Of these traits, seed size has been studied the most (Shankar, 2006); seed size variation may be a considered as a fitness trait contributing to competition among plants as well as a determinant for successful establishment of seedlings (Zhang, 1998).

Calophyllum inophyllum L. belonging to *Clusiaceae* is an evergreen, medium to large sized tree distributed mainly in the coastal areas and adjacent lowland forests and occasionally in inland at higher elevations. It is one among the most important oil yielding trees (Dalvi *et al*., 2012) with its seeds containing 60-75% oil which is extracted by cold expression. Generally, a well grown tree produces up to 100 kg of fruits and about 18 kg of oil (Venkanna and Reddy 2009). *C. inophyllum* is a potential biodiesel tree as it meets the specifications of biodiesel (Azam *et al*., 2005). Recently Dalvi *et al*. (2012) reported the biodiesel preparation from *Calophyllum* seeds with *in situ* transesterification. Seed oil has some medicinal properties (Chanda *et al.,* 2013) and other uses such as illuminant, lubricant, for soap making etc. Since the alternate energy sources plays an important role in mitigating the global climate change, the large scale cultivation of *C. ionophyllum* has to be initiated for oil extraction and the biodiesel production. Quality planting stock production is the major constraint of any tree planting programmes. Grading of seeds based upon their size and weight is a common practice in a majority of plant species and a better understanding of seed size variation will give an edge to nursery man to produce superior quality seedlings. Perusal of literature indicated that the studies on variation in seed physical and biochemical character with grading for lacking in *C. ionophyllum*. Hence, present investigation was undertaken to study the variation in seed physical and biochemical attributes in different weight classes and to find out the influence of seed weight on germination parameters of *C. ionophyllum*.

MATERIALS AND METHODS

The present investigation was carried out at College of Forestry, Kerala Agricultural University, Thrissur, Kerala during May to December 2012. *C. inophyllum* seeds were collected during May- June 2012 from the mature trees with well developed crowns in the coastal areas of Thirupuram Panchayath (8°5′ N and 76°9′ E), Thiruvananthapuram, Kerala. Seed lots were thoroughly hand mixed to improve the homogeneity and graded into weight classes 2-5, 5-8 and 8-11 g. Number and weight of seeds and thousand seed weight were recorded in each weight class (x10). Moisture and pulp content of the seeds as well as moisture content of the pulp were determined (x10). From each weight class, 25 seeds were randomly selected to record weight, length, width, volume, density and specific gravity. Specific gravity was determined by specific gravity module (Schimadzu AUY220). The shell and kernel separated using modified seed cutter (Dabral, 1976) and weight of each grade was determined. Biochemical analysis of both shell and kernel of the seeds belonging to different weight classes was carried out. The oil content of the seeds was determined by Soxhlet method and total carbohydrate content determined by Phenol Sulphuric acid method (Sadasivam and Manickam, 1996). Protein was extracted using tris HCl buffer (pH = 7.8) and estimated by the Lowry's method (Sadasivam and Manickam, 1996). Seeds belonging to each weight class were soaked in water for 24 hours and sown in polythene bags filled with medium soil, sand and cow dung in the ratio 2:1:1 to record daily germination counts. From these observations, Germination Percentage, Mean Daily Germination (MDG), Peak Value of germination (PV) and Germination Value (GV) were calculated (Czabator, 1962).

Statistical analysis

The data was analysed using the IBM SPSS Statistics 20. One-way ANOVA was used compare the between weight class variation in all parameters and Person's bivariate correlation coefficient was obtained among individual seed characters and biochemical characters. The variation in seed biochemical constituents was analysed using Two-way ANOVA with seed part as one factor and size class as second.

RESULTS

Fruit characteristic

The seeds of *Calophyllum ionophyllum* were born in clusters at the terminal end; fruits collected were spherical in shape. The light green fruits were turned yellow and then brown and wrinkled when the fruit is ripe and cover the thin layer of pulp, the shell is a corky inner layer and a single seed as kernel. Prominent variation in fruit size was obvious and its diameter ranged from 2 to 5 cm. Number of fruits per kg of 8-11, 5-8 and 2-5 g weight classes was 116.0 ± 17.7, 166.7 ± 18.7 and 262.0 ± 65.6 and thousand seed weight was 8.55 ± .24, 6.60 ± 0.30 and 5.07 ± 1.53 kg respectively. Moisture content of fruits at the time of collection was above 50% in all weight classes and the highest value was recorded in the smallest class. Pulp content of the fruits varied from 5.59 ± 0.18 to 2.04 ± 1.56% which decreased with decrease in weight class. The moisture content of the pulp was as high as 82 to 88% at the time of collection and seeds belonging to 5-8 g class recorded the highest value (Table 1). Analysis of variance revealed significant variations in all fruit parameters due to weight class at 1 per cent level.

The seeds were extracted from the fruits and the individual seed characteristics also varied significantly ($p = 0.01$) among the different weight classes. Higher weight (8.62 ± 0.59 g) length (3.24 ± 0.18 cm), breadth (2.99 ± 1.17 cm) and volume (0.12 ± 0.01 cm^3) was recorded in 8-11 g class and all these values decreased with decrease in weight (Table 2). But the density and specific gravity were the highest in seeds belonging to 2-5 g class. The weight, length and breadth of the seeds in 8-11 g class were almost double compared to 2-5 g class. The volume of seeds of 8-11 g class was 12 times more compared to 2-5 g class. Whereas, density and specific gravity of 2-5 g class seeds was 4.8 and 1.3 times respectively higher compared to 8-11 g class. Shell and kernel content of the seeds also varied significantly ($p = 0.01$) with weight class. A greater shell weight (5.02 ± 0.77), kernel weight (5.09 ± 0.15) and shell: kernel ratio (0.98 ± 0.12) were recorded in 8-11 g class and values decreased in lower weight classes. However the shell: kernel ratio doesn't statistically vary among weight classes.

Relationship among seed characters

The correlation matrix laid out among seed characters of different weight classes is given below (Table 3). The volume of the seeds was strongly correlated with length and breadth. Density of the seeds was strongly and positively correlated

Table 1: Variations in fruit attributes of *Calophyllum inophyllum* among different weight classes

Seed attributes	Weight class (g)			Mean
	8-11	5-8	2-5	
No of seeds per kg	116.0 ± 17.4^{c}	166.7 ± 18.7^{b}	262.0 ± 65.5^{a}	181.56 ± 0.24
Thousand seed weight (kg)	$8.55 \pm .26^{a}$	6.60 ± 0.30^{b}	5.07 ± 1.53^{c}	6.74 ± 1.52
Moisture content (%)	52.19 ± 0.22^{c}	54.65 ± 0.30^{b}	57.39 ± 0.43^{a}	54.74 ± 2.27
Specific gravity	0.73 ± 0.01^{b}	0.75 ± 0.03^{b}	0.93 ± 0.04^{a}	0.80 ± 0.10
Pulp content (%)	5.59 ± 0.18^{a}	3.71 ± 0.24^{b}	2.04 ± 1.56^{c}	3.78 ± 21.08
Pulp moisture content (%)	82.91 ± 0.69^{c}	84.09 ± 0.22^{b}	87.82 ± 2.25^{a}	84.94 ± 7.00

Values with same superscript with in a row are homogenous

Table 2: The individual seed characteristics of *Calophyllum ionophyllum*

Seed characters	Weight class (g)			Mean
	8-11	5-8	2-5	
Weight (g)	8.62 ± 0.59^{a}	6.11 ± 0.47^{b}	4.09 ± 0.47^{c}	6.27 ± 1.93
Length (cm)	3.24 ± 0.18^{a}	2.87 ± 0.11^{b}	1.58 ± 0.13^{c}	2.56 ± 0.72
Breadth (cm)	2.99 ± 1.17^{a}	2.60 ± 1.14^{b}	1.42 ± 0.14^{c}	2.34 ± 0.68
Volume (cm^3)	0.12 ± 0.01^{a}	0.08 ± 0.009^{b}	0.01 ± 0.003^{c}	0.07 ± 0.003
Density (g/cm3)	0.06 ± 0.009^{a}	0.07 ± 0.09^{a}	0.29 ± 0.08^{b}	0.14 ± 0.11
Specific gravity	0.73 ± 0.01^{b}	0.75 ± 0.03^{b}	0.93 ± 0.04^{a}	0.80 ± 0.10
Shell weight (g)	5.02 ± 0.77^{a}	2.63 ± 0.60^{b}	1.60 ± 0.20^{b}	3.08 ± 1.60
Kernel weight (g)	5.09 ± 0.15^{a}	2.74 ± 0.88^{b}	2.29 ± 0.64^{b}	3.37 ± 1.41
Shell: kernel ratio	0.98 ± 0.12^{a}	1.05 ± 0.50^{a}	0.73 ± 0.19^{a}	0.92 ± 0.31

Values with same superscript within a row do not vary significantly

Table 3: Coefficient of correlation among seed characteristics in *Calophyllum ionophyllum*

	Length	Breadth	Volume	Density	Specific gravity
Weight	0.75*	0.77*	0.81**	0.81**	-0.59
Length		0.99**	0.98**	0.98**	-0.96**
Breadth			0.98**	0.98**	-0.95**
Volume				1.00**	-0.93**
Density					-0.93**

*Significant at 5 % level ** Significant at 1 % level

Table 4: Variation in the biochemical parameters of *Calophyllum inophyllum* seeds in different weight classes

Weight class (g)	Oil content (%)		Total carbohydrate (%)		Protein (%)	
	Shell	Kernel	Shell	Kernel	Shell	Kernel
8-11	0.00	62.71±0.64[a]	5.31±0.03[a]	5.66±0.06[a]	2.90±0.17[a]	6.05±0.21[a]
5-8	0.00	56.97±2.33[b]	4.60±0.07[b]	5.22±0.04[b]	2.54±0.16[a]	5.10±0.27[b]
2-5	0.00	50.25±0.26[c]	4.14±0.07[c]	4.88±0.21[b]	1.75±0.20[b]	3.82±0.29[c]
Mean	0.00	56.64±5.53	4.68±0.51	5.25±0.35	2.40±0.53	4.99±0.99

The values with same superscript with in a column do not vary significantly

Table 5: Correlation among seed characters and biochemical constituents of *Calophyllum inophyllum*

	Oil content in kernel		Total carbohydrate (%)	Protein (%)	
		Shell	Kernel	Shell	Kernel
Weight	0.827**	0.846**	0.869**	0.882**	0.820**
Length	0.950**	0.876**	0.897**	0.936**	0.950**
Breadth	0.946**	0.901**	0.905**	0.951**	0.952**
Volume	0.973**	0.918**	0.943**	0.953**	0.954**
Density	0.973**	0.918**	0.943**	0.953**	0.954**

** Significant at 1 % level

Table 6: Germination parameters of *Calophyllum inophyllum* seeds as affected by weight

Weight class (g)	Germination %	PV	MDG	GV
8-11	68.00±2.00[a]	0.30±0.02[a]	1.07±0.25[b]	0.32±0.10[ab]
5-8	68.00±3.00[a]	0.32±0.06[a]	1.34±0.33[a]	0.44± 0.17[a]
2-5	44.00±4.00[b]	0.24±0.02[b]	0.83±0.08[b]	0.20±0.03[b]

with all other seed characteristics except specific gravity. There existed high negative correlation among specific gravity and other seed traits (Table 3). This must be due to higher volume found in large size classes.

Biochemical composition

The biochemical constituents of seed varied among different weight classes. Analysis of variance revealed significant ($p=0.01$) variation in oil, carbohydrate and protein due to part of the seed x weight class interaction. Of the seed parts, kernel recorded higher biochemical constituents than shell. Of the three weight classes a higher oil, carbohydrate and protein content was observed in 8-11 g class followed by lower size classes (Table 4).

The Pearson's bivariate correlation coefficient was laid out among seed dimension and biochemical constituents of seed. Seed morphological characters was strongly and positively ($p=0.01$) correlated with biochemical characters (Table 5). This was in conformity with earlier reports indicating a higher biochemical chemical content in the large size grades.

Prediction of biochemical composition of seeds

The strong positive correlation between seed dimensions and biochemical constituents showed their closeness. Multiple linear regression equations were fitted linking biochemical constituents and seed dimensions. All the regression equations fitted were significant and had a higher regression coefficient. Hence reliable prediction of seed biochemical composition using seed dimensions is possible

Oil content (Kernel) = 36.088 + 1.171 (Weight) + 2.044 (Length) – 1.688 (Breadth)

(R^2 = 0.95)

Total carbohydrate (Kernel) = 41.354 + 0.351 (Weight) - 1.761 (Length) + 2.302 (Breadth) (R^2 = 0.92)

Total carbohydrate (Shell) = 28.357 + 1.312 (Weight) + 0.438 (Length) – 0.060 (Breadth) (R^2 = 0.89)

Protein (kernel) = 14.998 + 1.617 (Weight) – 1.836 (Length) + 3.036 (Breadth) (R^2 = 0.96)

Protein (shell) = 5.117 + 0.775 (Weight) + 0.789 (Length) - 0.277 (Breadth) (R^2 = 0.93)

Germination parameters

Germination parameters of the seeds were significantly influenced by weight at one per cent level. The germination percentage and peak value of germination of 8-11 and 5-8 g of classes were at par but significantly differed from 2-5 g class (1.5 times). A higher MDG and GV were observed in 5-8 g class and the lowest was recorded in 2-5g class (Table 6).

DISCUSSION

In the present study, prominent variation was observed in fruit weight and on an average 545 fruits per kilogram was observed. Of which, the number of seeds per kg was the highest in 2-5 g class which was 1.5 and 2.2 times more compared to 5-8 and 8-11 g classes. Whereas, thousand seed weight of 8-11 g class was 1.3 and 1.7 times lower compared to 5-8 and 2-5 g classes. The fruit recorded higher moisture content at harvest and with increase in weight class, the moisture content decreased. The pulp content of the seeds was higher in 8-11 g class. A higher moisture content (82- 88 %) was observed in the pulp and the highest value was obtained for 2-5 g class. Higher moisture content in 2-5 g class seeds might be due to higher moisture content in the pulp. Within tree variation in seed size or weight is explained by different authors. Warringa *et al*. (1998) attributed the factors contributing to early development of inflorescence and partitioning of assimilates at anthesis to the variations in seed size. Schmidt (2001) had opined that the seeds exhibit variation in size but this is neither due to the less accumulation of reserve food material nor earlier abscission of seeds. Environmental influences during the development of the seeds combined with genetic variability are reported to cause variations in seed dimensions.

With regards to individual seed attributes, with the exception of density and specific gravity an increase was observed with increasing weight class. Length and width of the 8-11 g weight class was 2.0 and 2.2 times more compared to 2-5 g class. The density and specific gravity was higher in 2-5 g class which might be due to the higher volume recorded in larger weight classes. A negative and highly strong correlation was obtained among other seed parameters and specific gravity. The high density may an indication of a hard endospermic structure. It indicates that the large weight class seed have a hard endospermic structure. Jijeesh and Sudhakara (2007) also observed variation in drupe characteristics of teak due to seed size in different plantations of teak. Similarly, studies on *Pongamia pinnata* (Hooda *et al*., 2009), *Azadirachta indica* (Kaushik *et al*., 2012) and *Aegle marmelos* (Venudevan *et al*., 2013) also confirmed the same trend.

The biochemical composition of the seeds decreased from 8-11 to 2-5 g class. Biochemical constituents of seed varied between the kernel and shell and among weight classes. Shell was lacking in oil whereas oil was the major constituent of the kernel. Earlier reports indicated an average oil content of 65% for the seeds (Azam *et al.,* 2005; Sahoo *et al.,* 2006). In the present study the oil content varied from 50- 62 % with increasing weight class. Oil content of the seeds was reported to increase with maturity and higher temperature at the time of fruit development seems to increase the oil content and the amount of saturated fatty acids (Hathurusingha *et al.,* 2011). The total carbohydrate in kernel was higher and equal or less in the case of shell. The protein in the kernel was high and in the case of shell was half of that of kernel. Seed characters were positively and strongly correlated with chemical constituents indicating higher food reserves in large seeds. Jijeesh and Sudhakara (2009) also obtained positive correlation among the seed physical and biochemical parameters of teak.

In our study, grading of the seeds based on weight also influenced the germination parameters with 5-8 as well as 8-11 g classes recording higher values. This may be ascribed the low vigour of small seeds (Baalbaki *et al*., 2007; Kaydan *et al*., 2008; Wahid and Lahourai, 2013). Our results are in conformity with studies in many other tree species (Manonmani and Vanangamudi, 2002, Sudhakara and Jijeesh 2008, Gunaga *et al*. 2010, Ellison 2001, Dlamini 2011, Mwase and Mvula, 2011, Jijeesh and Sudhakara, 2013) that the larger seed size gives a better germination. The higher germination of the seeds in higher weight class might be due to the variation in seed biochemical components which increased with seed weight.

CONCLUSION

From the present investigation it can be concluded that prominent variations in seed size was obvious in *C. inophyllum*. The seeds with large size have higher seed characterisation and biochemical constituents. Which in turn was reflected in the germination parameters. Oil content in the seeds was higher in large and medium weight classes which can be used for more economic oil extraction. However further investigation is to be carried out to confirm the higher germination of the seeds and to know whether this enhanced performance is continued under field conditions.

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