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Physiological and Biochemical Variability Studies of *Melia dubia*

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ABSTRACT

Melia dubia is a large perennial deciduous tree with medicinal and economic importance. The present study aimed to evaluate physical and biochemical variability of *Melia dubia* collected from three different states viz., Karnataka, Kerala and Tamil Nadu in India. One hundred seeds of *Melia dubia* were randomly collected from Chamraj nagar, Chikkahole, Pannampalli, Thalamalai and Theni region of three states. The variability of physical parameters was analyzed by Lecia QWin image analyzer and also determined biochemical parameters. The results revealed that *Melia dubia* from Chickkahole region showed maximum amount of area, length, perimeter, equivalent diameter, curve width and convex area was compared to other sampling areas. According to the biochemical proximity studies, Chamraj nagar and Theni showed the highest level of amino acids and protein content. This study concluded that physical and biochemical character plays a part in the selection and migration of seeds for improved germination and afforestation.

Key words: Malai vaembu, Karnataka, image analyzer, protein

INTRODUCTION

India is a treasure of biodiversity which hosts a large variety of plants. It is estimated that there are over 7800 medicinal drug manufacturing units in India which consumes about 2000 tons of herbs annually (Satyavani *et al*., 2010). Fast growing plantations with multipurpose tree species offer large benefits to the growing economy of the country. Selection of suitable tree species, that meet specific requirements of the region, is one of the important factors which determine the maximum utility. *Melia dubia* (Tamil: Malai vembu) is a large perennial tree belongs to Meliaceae family growing from 6-25 m in height. It is commonly found in the hills at elevations ranging from 600-1800 m also native to Southern Asia, India, Indonesia, Malaysia, Myanmar, Australia, China, Bangladesh, Philippines, Thailand and Mexico. Traditionally used to treat mumps, thrombosis, goiter, insect bite, blood sugar treatment, stomach pain, scabies and economically valuable for plywood, cabinet making (Mohan, 2010). It is very lightweight (0.34 weight at 12.5 moisture content of 336 kg m^{-3}), used for small boat constructions. It has drupaceous, ovoid or ellipsoid fruit and yellowish when ripe. However, seedling production is low as the species shows poor seed germination rate.

Hence improvement in the crop yield depends on the progress of variability in the seed traits. Generally, the polymorphism can be detected at three levels and each is interlinked such as; phenotype, biochemical variation and molecular variation. The appropriate analysis of various parameters of seed and fruit is difficult by visual observation. To overcome these problems, image processing is widely used technique in the area of horticulture (Gonzalez and Woods, 1993). It consists of computer loaded with suitable software processing, digital image grabber card and a macro viewer stage fitted with a CCD camera light source which is a tool for gathering data from an image. For this task, numerous image processing algorithms are available, which complemented with classification methods, make a field of machine vision suitable for seed identification. Based on the issue, the present study evaluate the physical variability and biochemical parameters of *Melia dubia* collected from three different states of India.

MATERIALS AND METHODS

Sources: Fruits of *Melia dubia* were randomly collected from the three states of India such as Tamil Nadu, Kerala and Karnataka (Chamraj nagar, Chikkahole, Pannampalli, Thalamalai and Theni). The seeds were cleaned, dried and stored at room temperature in the air tight container for further analysis.

Analysis of physical parameters: One hundred fruits from each source were randomly selected and weighed using a physical balance.

Image analysis: The seeds and fruits per accession were taken randomly and the measurement of physical characters made by image analyzer (Leica Quantimet-QWin 500). The 2D surface area was calculated as the area of seed occupied in the calibrated 2Dimage. Diameter was measured as the average breadth of the seed in two different perpendicular angles. Perimeter was the total length of the boundary of the seed. Roundness was a shape factor, which gives minimum value of unity in a circle. This is calculated from the formula given below:

$$\text{Roundness} = \frac{\text{Perimeter}}{4\text{XPX2D surface areas}\times 1.064}$$

An adjustment factor of 1.064 corrects the parameter for the effect of the corner produced by the digitization of the image. Fullness ratio is also a shape factor, equal to the square root of the ratio of area to circumscribe area as given below:

$$\text{Fullness ratio} = \frac{\text{2D surface area}}{\text{Convex area}}$$

Proximity parameters: Seed extracts were prepared by depulping the ripened fruits, dried and powdered. The powdered material was used for the biochemical assays.

Estimation of protein: Different concentration of BSA (0.2, 0.4, 0.6, 0.8 and 1 mL), 0.1 and 0.2 mL of the seed extract were taken in the various test tubes. All the tubes were made upto 1 m L^{-1} with distilled water. Added alkaline $CuSO_4$ and 0.5 mL Folin to all the tubes, blank was prepared simultaneously. The intensity of blue color was measured at 660 nm after 30 min of incubation (Lowry *et al*., 1951).

Estimation of reducing sugars: Pipetted out 0.5 to 3 mL of the seeds to extract in test tubes and equalized the volume to 3 mL of dinitrosalicylic acid reagent and boiling in water bath for 5 min. Added 1 mL of 40% Rochelle salt solution. The intensity of dark red color was measured at 510 nm (Miller, 1959).

Estimation of total carbohydrates: Various concentration of glucose (0.2, 0.4, 0.6, 0.8 and 1 mL), 0.5 and 1 mL of the aliquots were taken in various test tubes. All the test tubes were made upto 1 mL with distilled water and 4 mL of anthrone reagent was added to all tubes. Cool rapidly and the intensity of dark green color was measured at 630 nm (Yemm and Willis, 1954).

Extraction of amino acids: The 0.1 mL of seed extract added to 1 mL of ninhydrin and made up the volume to 2 mL with distilled water. Heated the tube in a boiling water bath for 20 min. Added 5 mL of the diluents and mixed the contents. 0.1 mL of 80% ethanol was used as a blank. After 15 min, read the intensity of the purple colour against a reagent blank in a colorimeter at 570 nm.

Estimation of phenols: The 0.1-0.5 g of the sample ground with 80% ethanol. Centrifuge at 10,000 g for 20 min and the supernatant were mixed with known volume of distilled water. The 0.2-2 mL of different aliquots into test tubes and made up the volume to 3 mL in each tube. 0.5 mL of Folin ciocalteau reagent was added and 2 mL of 20% Na_2CO_3 solution to each tube after 3 min prepared a standard curve using catechol. Simultaneously placed the tubes in boiling water for exactly 1 min and measured the absorbance at 650 nm against a reagent blank.

RESULTS

Image analysis of fruit: Improvement of the crop yield depends on the progress of variability in the desired characters of the seed traits. The weight of fruits 0.458±0.01, 0.788±0.02, 0.633±0.03, 0.932±0.01, 0.588±0.02 (g kg^{-1} of seeds) and drupe consist of 0.153±0.01, 0.222±0.02, 0.144±0.01, 0.183±0.01, 0.161±0.02 (g kg^{-1} of seeds) weight, respectively (Fig. 1). The highest weight of *M. dubia* fruit were observed in Thalamalai and was the lowest in Chamraj nagar. Also, the highest drupe weight observed in Thalamalai and lowest in Chikkahole than compared to sampling sites. Among these, the fruit and seeds of *Melia dubia* at Chikkahole region showed maximum

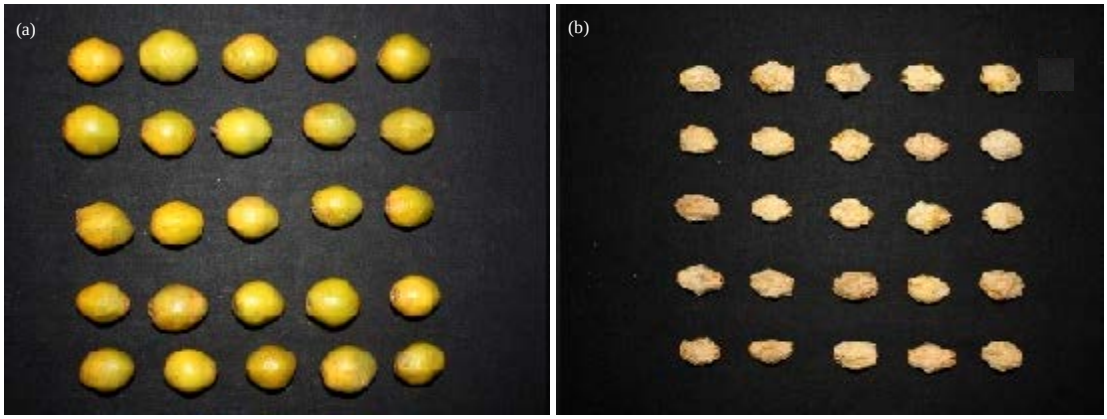

Fig. 1(a-b): Image analysis of five different sources of *M. dubia* (a) Fruits and (b) Drupes

Table 1: Image analysis of fruits and drupes from different sampling sites

Sample	Sampling site	Area (cm^2)	Length (cm)	Breadth (cm)	Perimeter (cm)	Full ratio	Convex perimeter (cm)	Roundness	Aspect ratio	Equiv. diam. (cm)	Curve length (cm)	Curve width (cm)	Convex area (cm)
Fruits	Chamraj nagar	3.93	2.73	1.91	8.560	0.96	7.31	1.41	1.42	2.23	2.89	1.43	4.19
	Chikkahole	5.02	3.08	2.18	10.080	0.96	8.28	1.54	1.42	2.52	3.56	1.54	5.38
	Pannam palli	3.91	2.53	2.82	7.458	0.85	7.24	1.51	1.30	2.23	2.72	1.21	4.13
	Thalamalai	5.05	2.90	2.24	10.620	0.97	8.16	1.72	1.29	2.50	4.03	1.27	5.31
	Theni	4.41	2.91	2.00	9.454	0.95	7.73	1.74	1.49	2.33	3.39	1.34	4.79
Drupes	Chamraj nagar	1.93	2.37	1.18	7.090	0.89	5.65	2.01	2.01	1.56	2.84	0.69	2.40
	Chikkahole	2.33	2.45	1.36	7.440	0.91	6.12	1.82	1.80	1.72	2.86	0.85	2.79
	Pannam palli	1.93	2.26	1.21	7.180	0.90	5.38	1.67	1.75	1.55	2.81	0.63	2.30
	Thalamalai	1.88	2.06	1.25	7.120	0.93	5.28	2.05	1.65	1.55	2.88	0.67	2.16
	Theni	2.16	2.22	1.37	7.430	0.92	5.78	1.96	1.63	1.66	2.95	0.77	2.52

amount of area, length, breadth, perimeter, convex perimeter, equivalent diameter, curve width and convex area than compared to others sampling sites (Table 1). The area of fruit collected from Chikkahole was found to be the highest whereas the area of the fruit collected from Chamraj nagar had the lowest. The length of fruit collected from Chikkahole was found to be the highest whereas the length of fruit collected from Chamraj nagar was lowest. The breadth of fruit collected from Thalamalai was found to be the highest compared to fruit collected from Chamraj nagar. The perimeter of fruit collected from Thalamalai was found to be the highest and Chamraj nagar had the lowest. The full ratio of the fruit collected from Thalamalai was found to be the highest and Theni had the lowest. The convex perimeter of fruit collected from Chikkahole was found to be the highest compared to Chamraj nagar. The roundness of fruit collected from Theni was found to be the highest whereas Chamraj nagar had the lowest. The aspect ratio of fruit collected from Theni was found to be the highest whereas Chamraj nagar had the lowest. The equivalent diameter of fruit collected from Chikkahole was found to be the highest compared to Chamraj nagar had the lowest. The curve length of fruit collected from Thalamalai was found to be the highest whereas the curve length of the fruit collected from Chamraj nagar had the lowest. Fruit collected from Chikkahole was found to be the highest whereas the curve width of the fruit collected from Thalamalai had the lowest. The convex area of the fruit collected from Theni was found to be the highest whereas Chamraj nagar had the lowest value.

Image analysis of drupe: The area of the drupe collected from Thalamalai was found to be the highest whereas the area of the drupe collected from Chamraj nagar had the lowest area. The length of the drupe collected from Chikkahole was found to be the highest whereas the length of the drupe collected from Thalamalai was lowest. The breadth of the drupe collected from Theni was found to be the highest whereas the breadth of the drupe collected from Chamraj nagar had the lowest. The perimeter of the drupe collected from Theni was found to be the highest whereas the perimeter of the drupe collected from Chamraj nagar had the lowest. The full ratio of the drupe collected from Thalamalai was found to be the highest whereas the full ratio of the drupe collected from Chamraj nagar had the lowest. The convex perimeter of the drupe collected from Chikkahole was found to be the highest whereas the convex perimeter of the drupe collected from Thalamalai had the lowest. The roundness of the drupe collected from Thalamalai was found to be the highest whereas the roundness of the drupe collected from Chikkahole had the lowest. The aspect ratio of the drupe collected from Chamraj nagar was found to be the highest whereas the aspect ratio of the drupe collected from Theni had the lowest. The equivalent diameter of the drupe collected from

Table 2: Biochemical analysis of seeds from various sources

Sources	Total free amino acids (μg g^{-1})	Proteins (mg g^{-1})	Total carbohydrate (mg g^{-1})	Reducing sugars (mg g^{-1})	Phenols (mg g^{-1})
Chamraj nagar	0.83	130.82	100.22	9.78	1.09
Chikkahole	0.30	36.12	20.51	8.06	0.58
Panampalli	0.27	18.23	14.62	11.34	1.50
Thalamalai	0.82	40.08	89.80	19.61	1.10
Theni	0.83	63.23	120.30	16.67	1.07

Chikkahole was found to be the highest whereas the equivalent diameter of the drupe collected from Thalamalai had the lowest. The curve length of the drupe collected from Theni was found to be the highest whereas the curve length of the drupe collected from Chamraj nagar had the lowest. The drupe collected from Chikkahole was found to be the highest whereas the curve width of the drupe collected from Thalamalai had the lowest. The convex area of the drupe collected from Chikkahole was found to be the highest whereas the convex area of the drupe collected from Thalamalai had the lowest value (Table 1).

Biochemical parameters: The biochemical parameters of seeds of various regions were presented in Table 2. The total free amino acid concentration was found to be the highest in Theni and chamraj nagar seeds whereas it was lowest in Panampalli seeds. Theni seeds showed a high carbohydrate content and Chikkahole seeds showed the lowest carbohydrate content. Total protein content was observed to be high in Chamraj nagar seeds and low in Panampalli seeds. The phenol concentration was found to be the highest in Panampalli seeds whereas it was lowest in Chikkahole seeds. Thalamalai seeds possessed high reducing sugar content while Chikkahole seeds showed a lower content. In conclusion, study demonstrates selection and location of seeds to improve germination and afforestation.

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