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Pigment and oil content estimation in seeds of *Bixa orellana* L.

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Abstract
Bixa orellana L. is an economic tree native to the Neotropics. It is perennial in nature growing up to a height ranging from 2 to 8 m. *Bixa orellana* L. shows phenotypic variation in flower color, pod color, pod shape, height as well as girth. The tree gives a natural colorant called 'Annatto' that is second most demanded dye in the world. Selection of trees based on high pigment content becomes a must for this economic tree. Identification of phenotypic character for selection of trees with desirable pigment content was the aim of the study. It was found that cordate pods with green pericarp and maroon bristles gave the highest bixin content, although norbixin content was found to be similar in all pod shapes, cordate pods with maroon pericarp and bristles were advantageous. For selection for oil content conical pods with maroon pericarp and bristles were the most appropriate amongst all.

Keywords: *Bixa orellana* L., Annatto, bixin, norbixin, oil content

Introduction
Bixa orellana L. is an exotic dye yielding tree in India which is commercially cultivated in Kerala, Karnataka, Tamil Nadu, Andhra Pradesh, Orissa, West Bengal, Gujarat, Maharashtra, Madhya Pradesh and Chhattisgarh (Kumaran, 2014, Kala *et al*., 2017, Priyanka *et al*., 2017) [11, 15, 21]. It is native to the Neotropics and belongs to Bixaceae family (Rivera-Madrid *et al*., 2016, Kala *et al*., 2015; Moreira *et al*., 2015; Lombello *et al*., 2014) [23, 18, 12]. The Spaniards first introduced it in Southeastern Asia in 17th century (Ulbricht *et al*., 2012). Now it is naturalized in all tropical and subtropical regions of the world (Ambika and Poornima, 2004) [4]. The tree height ranges from 2 to 8 m (Pandey *et al*., 2019) [19]. The crude pigment extracted from the aril of the seeds is known as 'Annatto', containing bixin, norbixin and other carotenoids in different proportions (Satyanarayana *et al*., 2003; Raddatz Mota *et al*., 2016; Poornima *et al*., 2013) [22, 24, 4]. Annatto is the second most important natural colorant after caramel in the world (Kala *et al*., 2015) [12] and in terms of economic value, it ranks second after saffron with a global market price of $ 5.445 to $ 11.605 (Teixeira da Silva *et al*., 2018) [29]. Biodegradability, cheap availability of raw material, low incidence of allergic reaction and low toxicity are some of the reasons for popularity of natural dyes such as Annatto in Global market (Gajendra *et al*., 2019) [7]. Annatto is used in the pharmaceutical, textile, dairy, food beverage, paint and cosmetic industries (Hirko and Getu, 2022; Dequigiovani *et al*., 2017; Joshep *et al*., 2012) [9, 6]. Bixin is the major constituent of annatto, which makes up to 80% (Stringheta *et al*., 2018) [28]. It is a reddish orange color carotenoid make up to 7% of the dry mass of seeds (Kumaran, 2014) [15]. Bixin is liposoluble whereas norbixin is hydrosoluble (Smith, 2006) [27]. The capsule of *Bixa orellana* L. is echinate and shows wide phenotypic variations in shape and colour (Joshep *et al*., 2011) [13]. The percentage of oil in seeds of Annatto is 0.07 % of the seed weight (Kala *et al*., 2015; Kumaran, 2014) [12, 15] and has the ability to control disease progression with antileishmanial potentialities (Monzote *et al*., 2014) [17]. The present study is carried out with an aim to understand the variation of pigment and oil content in seeds collected from different pod shape and colour.

Materials and Methods
The study was conducted in the Annatto Field Gene Bank in Forest College and Research Institute, Mettupalyalam ($11^0$32'N and $76^0$94'E). Five years old plantation with 3 x 3 m spacing was selected for the study.

To estimate the variation in pigments and oil content, seeds were collected from pods of different shape and color. Observed pod shapes were cordate, conical and ovate, while the pod colors were maroon pods with maroon spines, green pods with green spines and green pod with maroon spines (Singh *et al*., 2016; Akshatha *et al*., 2011) [26, 1].

Bixin

For estimation of oil soluble carotenoid, assay was conducted using method given by Smith, 2006 [27]. 1 gram of seeds was taken in a 100 ml beaker and mixed with 10 ml of Tetrahydrofuran. The volume was made up to 100 ml with acetone. 1 ml of the solution was diluted using acetone to 100 ml. The absorbance was measured at 487 nm in spectrophotometer. Bixin content was calculated by using the equation given below

$$\%\, Total\ carotenoid\ (Bixin) = \frac{A}{3090} \times \frac{100000}{seed\ weigth\ (mg)} \times 100$$

Norbixin

For norbixin estimation, 1 gram of seeds was dissolved in 0.5% potassium hydroxide in a 100 ml beaker. 1 ml of the solution was further diluted to 100 ml. Using a spectrophotometer, the absorbance was measured at 482 nm (Smith, 2006) [27]. Norbixin content was calculated using the following equation given by Smith (2006) [27]

$$\%\, Total\ carotenoid\ (Norbixin) = \frac{A}{2870} \times \frac{100000}{seed\ weigth\ (mg)} \times 100$$

Oil

Solvent method was used for seed oil estimation using n-hexane in Soxhlet apparatus. 5 grams of powdered seed sample were taken in a butter paper cover and placed in the thimble of the Soxhlet apparatus. 350ml of n-hexane was used as solvent and extraction was done for 6-8 hours at an extraction temperature of 15^0C at room temperature. The oil content was calculated using the equation given by Sharma *et al*. (2016) [25].

$$Seed\ oil\ content\ (\%) = \frac{W_{fb} - W_{ib}}{W_s} \times 100$$

Where

W_{fb} = Final beaker weight

W_{ib} = Initial beaker weight

W_s = Sample weight

Statistical analysis

R-studio was used to analyze the bixin, norbixin and oil content using MANOVA and ANOVA.

Results and Discussion

From the present study it was evident that the heart shaped pods (cordate) had the highest number of seeds per pod (41 seeds per pod) (Table 1). It was also observed that cordate pod gave the highest bixin content of 2.899% (Fig 1). However Akshatha *et al*. (2011) [1] and Pandey *et al*. (2019) [19] reported that ovate pods had the highest number of seeds per pod (46) as well as bixin content of 2.13%. Kiruba (2012) reported highest seed bixin content of 3.13%. Valdez-Ojeda *et al*. (2008) [31] found that larger and narrow capsules with red to dark red pericarp gave the highest bixin content. The variation in the Bixin content of the current study as against the results of the referred articles was in accordance as well.

Bixin content in the various pod shapes of conical, cordate and ovate with different pericarp and bristle colours *viz*., maroon pericarp with maroon bristles, green pericarp with green bristles and green pericarp with maroon bristles, showed a wide variation. Amongst all cordate pods, pods of green pericarp with maroon bristles gave the highest bixin content (Fig 4).

In terms of the percentage of hydrosoluble carotenoid, present study found that, norbixin content was similar in all pod shape *viz* conical, cordate and ovate, although cordate pods gave a little higher than the other two (1.689%) (Fig 2). Similar findings in the norbixin content was observed by many authors with no significant difference among all varieties and at a range of 0 to 4% (Raddatz-Mota *et al*., 2016; Smith, 2006; Alves *et al*., 2006) [22, 27]. The norbixin content in cordate pods with maroon pericarp and bristles was found to be slightly higher than the cordate pods with green pericarp and maroon bristles (Fig 5). Castello *et al*., 2004 [5] concluded that the varying amount in in bixin and norbixin content were due to the different solvent used for extraction and impact of phytogeographic conditions respectively.

Present study found that seed oil content in of the Annatto to be in the range of 0.982 to 2.476 %, with maximum in conical pods with maroon pericarp and bristles (Table 1, Fig 3 and Fig 6) though it was reported by many author to be 0.07 % (Kala *et al*., 2015; Kumaran, 2014) [12, 15].

Maximum pod length 4.147 cm and pod width 3.769 cm was found in ovate pod with green pericarp and bristles, while cordate pod with maroon pericarp and bristles was with maximum pod thickness of 2.585 cm and pod width 3.769 cm. The study found that cordate pod with maroon pericarp and bristles had the maximum number of seeds per pod (41). Hence, the pod width and pod thickness influenced the number of seeds per pod. Akshatha *et al*. (2018) [1] concluded that ovate pods were with highest number of seeds per pod (46). While Singh *et al*., 2016 [26] reported green pod plants has maximum number of seed per pod of 36.6.

Table 1: Variation in pigment and oil content with pod morphological characteristics

Pod shape	Pod colour	Pod length (cm)	Pod width (cm)	Pod thickness (cm)	Number of seeds per pod	Bixin content %	Norbixin content %	Oil content %
Conical	Green pericarp and bristles	3.442	3.268	2.360	31	1.551	0.951	1.550
Conical	Green pericarp and maroon bristles	3.232	2.657	2.042	27	1.259	1.412	1.590
Conical	Maroon pericarp and bristles	3.232	2.657	2.042	27	1.657	1.523	2.476
Cordate	Green pericarp and bristles	3.722	3.477	2.133	37	2.269	1.410	0.982
Cordate	Green pericarp and maroon bristles	3.529	3.554	2.330	37	2.899	1.668	1.550
Cordate	Maroon pericarp and bristles	3.673	3.769	2.585	41	2.457	1.689	1.300
Ovate	Green pericarp and bristles	4.147	3.769	2.522	38	2.176	1.157	1.200

Ovate	Green pericarp and maroon bristles	3.059	2.993	2.227	35	2.453	1.276	1.950
Ovate	Maroon pericarp and bristles	2.758	2.993	2.203	35	2.225	1.268	1.165
General mean		3.421	3.237	2.271	34.22	2.105	1.372	1.529
SEd		0.408	0.435	0.195	4.893	0.518	0.239	0.457
CD ($p < 0.05$)		0.049	0.058	0.017	0.699	0.127	0.042	0.136

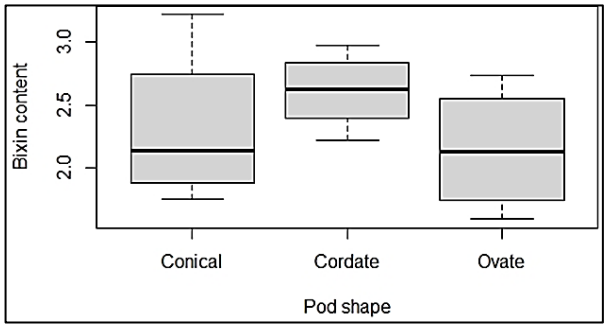

Fig 1: Variation Bixin content in different Pod shape

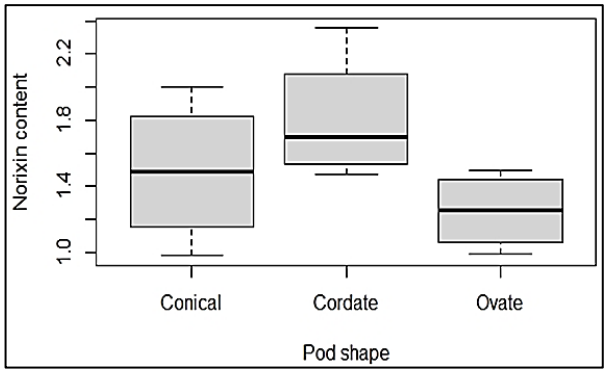

Fig 2: Variation Norbixin content in different Pod shape

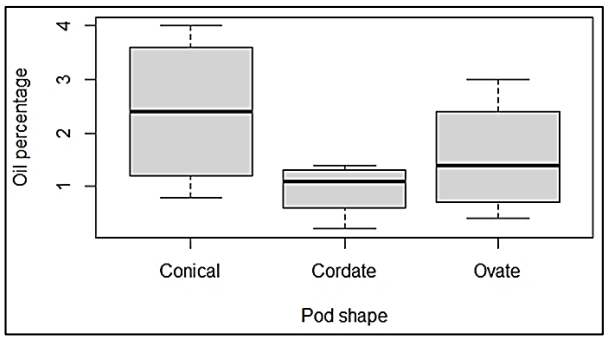

Fig 3: Oil percentage variation in different Pod shape

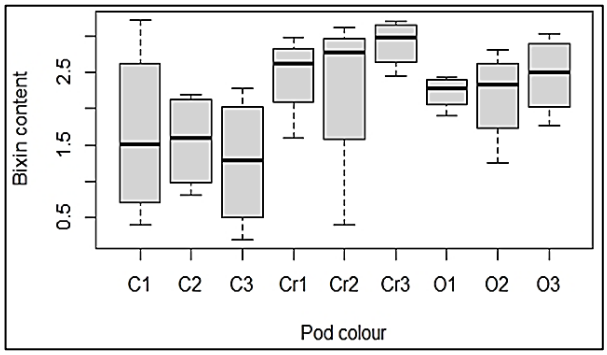

Fig 4: Bixin content variation in different Pod colour

C1- Conical with green pericarp and bristles, C2 – Conical with green pericarp and maroon bristles, C3 – Conical with maroon pericarp and bristles, Cr1 – Cordate with green pericarp and bristles, Cr2 – Cordate with green pericarp and maroon bristles, C3 – Cordate with maroon pericarp and bristles, O1 – Ovate with green pericarp and bristles, O2 – Ovate with green pericarp and maroon bristles, O3 – Ovate with maroon pericarp and bristles

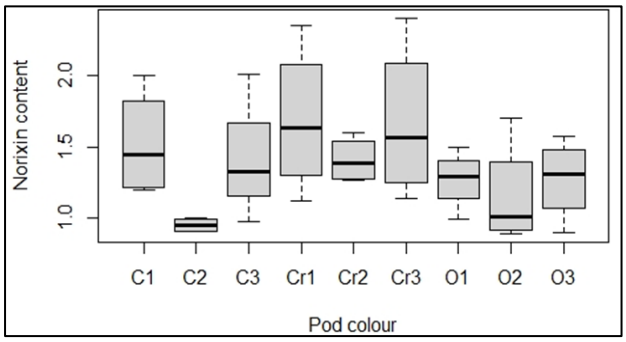

Fig 5: Norbixin content variation in different Pod colour

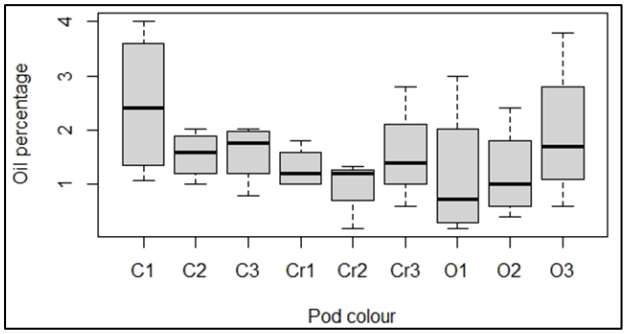

Fig 6: Oil percentage variation in different Pod colour

Plate 1: Variations in pod shape and colour

Plate 2: Annatto field

Conclusion

From the study conducted in *Bixa orellana* L. it was found that number of seeds, pigment and oil content varied with pod shape and pod color. The cordate pods were recorded with highest number of seeds, bixin and norbixin content, while conical pods gave maximum oil content. Therefore, cordate pods with green pericarp and maroon bristles can be utilized as selection indices for high bixin content, while for norbixin content, cordate pods with maroon pericarp and bristles are desirable. Similarly conical pods with maroon pericarp and bristles were advantageous for high oil content.

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