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) [27, 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) [7]. 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) [28]. 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, Mettupalayam (11°32’N and 76°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

\[
\% \text{Total carotenoid (Bixin)} = \frac{A}{3090} \times \frac{100000}{\text{seed weight (mg)}} \times 100
\]

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]

\[
\% \text{Total carotenoid (Norbixin)} = \frac{A}{2570} \times \frac{100000}{\text{seed weight (mg)}} \times 100
\]

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°C at room temperature. The oil content was calculated using the equation given by Sharma et al. (2016) [25].

\[
\text{Seed oil content} \% = \frac{W_{fb} - W_{ib}}{W_s} \times 100
\]

Where

\[
W_{fb} = \text{Final beaker weight}
\]

\[
W_{ib} = \text{Initial beaker weight}
\]

\[
W_s = \text{Sample weight}
\]

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) [31] 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 % (Kafa et al., 2015; Kumaran, 2014) [11, 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 [28] 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         |
|                | Green pericarp and maroon bristles | Maroon pericarp and bristles | General mean | SEd | CD (p < 0.05) |
|----------------|-----------------------------------|-----------------------------|-------------|-----|---------------|
| Ovate          | 3.059                             | 2.993                       | 2.227       | 35  | 2.453         |
|                 |                                   |                             |             | 1.276| 1.950         |
| Ovate          | 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         |
| SEd            | 0.408                             | 0.435                       | 0.195       | 4.893| 0.518         |
| CD (p < 0.05)  | 0.049                             | 0.058                       | 0.017       | 0.699| 0.127         |

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 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

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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