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Growth, Yield and Quality of *Bixa orellana* L. Morphotypes

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Bixa orellana L., commonly known as 'Sinduri' and 'Annatto', is a monotypic genus of the family Bixaceae. It is native to Central America and tropical South America but now-a-days naturalized in Indian-subcontinent. The plant is chiefly valued for its carotenoid pigment, Bixin, the principal coloring matter present in the seeds [1]. Bixin, is a carotenoid carboxylic acid, harm less organic dye, used as a red-orange dye for coloring rice, cheeses, soft drinks, butter, cosmetics and soup, not only in food products but also in the textile, paint, and cosmetic industries. Traditionally *Bixa* is known to treat several ailments, including internal inflammation, gastric ulcers and stomach discomfort [5-7]. Essential oil of *Bixa* seeds possess anti-leishmanial activity [4]. *Bixa* is cultivated mainly for the seeds and is traded in the form of sundried seeds [2]. International food safety restrictions on synthetic colourants in food items, particularly in dairy products have led to the increased use of Bixin. The major world markets are USA (3000 tons/year) and Europe (12,000 tons/year) [2].

Bixa can be propagated by seeds and stem cuttings. The seed germination is very erratic and takes around 30-45 days for some of the seeds to germinate. The seeds require optimum storage conditions for proper germination. There is significant reduction in seed germination with the passage of time. Only 8% germination is found under natural conditions [2] (**Figure 1**). The plant shows flowering from 2nd to 3rd year onwards. In India, *B. orellana* cultivation is mainly restricted to southern states [3].

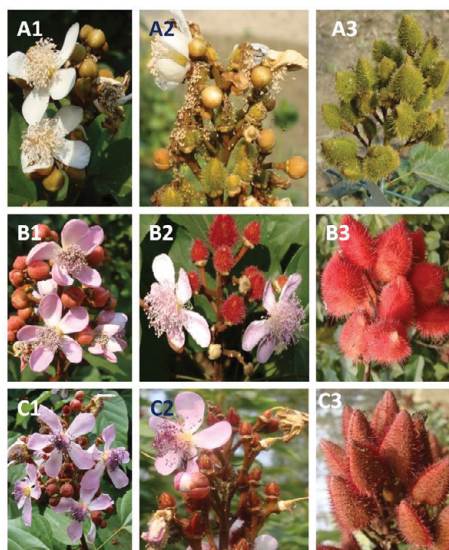


Figure 1. Morphotypes of *Bixa orellana* L. (A1 to A3; B1 to B3 and C1 to C3: buds, flowers, young and mature capsules of white, pink and pinkish-purple flowered plants).

The diverse germplasm collected from various locations of India has been investigated for morphological diversity among the germplasm, effects of biofertilizer treatments on seed germination and plant growth, yield and quality of *B. orellana*.

Seed Collection and Sowing

Matured seeds of *Bixa*, collected from different geographical locations; Tamilnadu, Kerala, Maharashtra, Gujrat, Orissa, Uttar Pradesh and Meghalaya were conserved in the Field Gene Bank at Distant Research Centre of CSIR-National Botanical Research Institute, Lucknow (80° 45'-53'E, 26° 40'-45'N at an elevation of 126 meter above the mean sea level). Total 17 accessions were grown under homogenous agronomic practices in partially reclaimed sodic soil conditions. The soil of the experimental plot was silt-clay loam having a pH of 8.5 and EC 0.3 dS/m. Under good soil conditions, 2 to 5 seeds per hole are sown directly in well prepared field at the beginning of rainy season. After germination, only one healthy seedling is allowed to grow. However, in adverse conditions of sodic wastelands, it is advisable to raise the seedlings in nursery and plant them in field after hardening of about two months. Before sowing, the seeds of *Bixa* were treated with *Trichoderma* and *Bacillus* along with Control. Five seed sets in replication of four (total twenty seeds of each accession) were sown in rooting trays in July 2011. Data on germination was recorded at 30th day of sowing, and seedlings were transferred to perforated polythene bags. The seedlings were kept in nursery for three months before planting them in field in November 2011.

Effect of Bio-Inoculants

The seed treatment with *Trichoderma* and *Bacillus* did not improve the seed germination of *Bixa*. The average seed germinations for 20 seeds among 17 accessions were 9.75 ± 4.41 (*Trichoderma*), 9.56 ± 4.26 (*Bacillus*) and 9.44 ± 4.35 (Control). However, the response to treatment with bio-inoculants was seen in terms of survival of seedlings in field, plant height and number of leaves. Treatment with *Trichoderma* resulted in $95.45\% \pm 5.22\%$ survival, followed by *Bacillus* ($91.81\% \pm 4.04\%$) and control ($67.27\% \pm 7.86\%$). The plant height and number of leaves were highest in *Trichoderma* treated plants ($26.29 \text{ cm} \pm 3.94 \text{ cm}$ and 6.17 ± 1.13), followed by those treated with *Bacillus* ($23.88 \text{ cm} \pm 2.71 \text{ cm}$ and 5.82 ± 1.23) and control ($20.58 \text{ cm} \pm 3.82 \text{ cm}$ and 3.82 ± 0.63), respectively.

Studies on Morphological Variations

Generally *Bixa* is described to have cordate shape leaves with pointed tip while flowers are of white and pink colour. The fruits are capsule, bivalved, covered with bristles and have numerous reddish orange seeds. In our study, few plants started flowering in the second year, while others flowered in third year. On flowering, variations in flower colour (white, pink and pinkish-purple), capsule colour (green, bright red and maroon) and shape (conical, cordate and triangular) were observed. The observations related to the morphological variability, in terms of time of bud initiation, bud colour, bud drop, flower colour, flower diameter and capsule drop were recorded. The post-harvest observations on capsule size, capsule weight, number of seeds/capsule, 100 seeds weight, seed weight/capsule, seed yield per plant were also documented. The biochemical variations in terms of Bixin content were quantified using high performance liquid chromatography photodiode array (HPLC-PDA).

The green, bright red and red color of buds bloomed to white, pink and pinkish-purple colored flowers, respectively. The pink flowers were seen late in October while other groups flowered in September (**Table 1**). Higher bud drop was recorded from plants bearing white (28.1%) and pinkish-purple flowers (27.8%). The bright red buds, producing pink flowers recorded the lowest bud drop (10.2%), the largest flower diameter (6.9 cm) and the highest capsule drop (49.2%). White flowers developed into green capsules, pink flowers developed to bright red capsules whereas pinkish-purple flowers developed to maroon capsules.

Table 1. Variations in morphological characters and phenology of *Bixa orellana* L.

Flower colour	Month of bud formation	Bud colour	Bud drop (%)	Flower diameter (cm)	Capsule colour	Capsule shape	Capsule drop (%)
White	September	Green	28.1 ± 5.0	5.3 ± 0.3	Green	Conical	30.3 ± 6.5
Pink	October	Bright red	10.2 ± 2.3	6.9 ± 0.1	Bright red	Cordate	49.2 ± 4.1
Pinkish- purple	September	Maroon	27.8 ± 3.7	4.8 ± 0.1	Maroon	Triangular	30.4 ± 2.7

The largest capsule size (4.1 cm × 3.4 cm) was documented from pink flowered plants (**Table 2**). Maximum capsule weight (1.91 g), number of seeds per capsule (39.6), 100 seed weight (3.31 g) and seed weight per capsule (1.39 g) were recorded from white flowered/green capsule plants. The highest seed yield per plant was measured from pinkish-purple flowered/maroon capsule plants (3.08 kg) while the pink purple flowered/bright red capsule plants yielded only 0.66 kg seeds per plant. There was large variability in Bixin content of the seeds from different morphotypes. The Bixin content was highest (1.5%) in seeds from maroon capsules, followed by green and bright red capsules.

Table 2. Variations in seed yield and its attributes and Bixin content of *Bixa* morphotypes.

Flower colour	Harvested capsule						Bixin (%)
	Capsule size (L×B) cm	Capsule weight (g)	Number of seeds / capsule	100 seeds weight (g)	Seed weight / capsule (g)	Seed yield per plant (kg)	
White	$3.5 \pm 0.25 \times 2.2 \pm 0.11$	1.91 ± 0.18	39.6 ± 2.70	3.31 ± 0.07	1.39 ± 0.31	1.76	1.15

Pink	$4.1 \pm 0.38 \times 3.36 \pm 0.37$	1.79 ± 0.30	35.6 ± 7.73	1.50 ± 0.12	0.66 ± 0.22	0.66	0.31
Pinkish- purple	$3.26 \pm 0.11 \times 1.7 \pm 0.18$	0.97 ± 0.03	24.6 ± 2.60	2.68 ± 0.22	0.62 ± 0.05	3.08	1.60

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