



RESEARCH ARTICLE

Growth performance and yield of *Bixa orellana* L. (annatto) under organic management

V Shajeeda Banu^{1*}, Awadhesh Kumar Pal², Rajesh Kumar², Mamta Kumari³, Amita Singh⁴, Anil Kumar Singh⁵ & Duniya Ram Singh^{5*}

¹College of Agricultural Biotechnology, Bihar Agricultural University, Sabour, Bhagalpur 813 210, Bihar, India

²Bihar Agricultural College, Bihar Agricultural University, Sabour, Bhagalpur 813 210, Bihar, India

³Krishi Vigyan Kendra Bhagalpur, Bihar Agricultural University, Sabour, Bhagalpur 813 210, Bihar, India

⁴Sabour Agri Incubators, Bihar Agricultural University, Sabour, Bhagalpur 813 210, Bihar, India

⁵Bihar Agricultural University, Sabour, Bhagalpur 813 210, Bihar, India

*Correspondence email - shajeedabanu847@gmail.com; drsinghort66@gmail.com

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Abstract

Bixa orellana L. or Sindoor plant is a perennial shrub renowned for its bixin rich seeds, widely used in food, cosmetics and pharmaceuticals. This study assessed its agronomic performance, seed quality and economic viability under subtropical conditions in Bihar, India, using organic cultivation practices. Among the pre-sowing treatments, scarification combined with warm water hydration achieved the highest germination (94.67 ± 0.93 %) with rapid emergence (12-14 days), followed by warm water hydration alone (80.33 ± 0.77 %). Treated seedlings showed vigorous growth, with the combined treatment producing the tallest seedlings (36.47 ± 0.76 cm), highest leaf number (6.0 ± 0.58) and 95 % survival. Phenological observations showed flowering at 310.0 ± 1.0 day and capsule maturity at 480.0 ± 2.0 days post transplanting. Mature mother plants yielded 3.5 ± 0.25 kg seeds per plant, equivalent to 4.5 - 6.0 t ha⁻¹. Seeds contained moisture (8.53 ± 0.45 %), protein (12.20 ± 0.20 %), fat (5.13 ± 0.15 %), ash (3.43 ± 0.75 %) and fibre (9.20 ± 0.20 %). Bixin content averaged 3.63 ± 0.58 %, translating to 162 - 216 kg ha⁻¹ pigment yield. Economic analysis indicated gross returns of Rs. 36 - 54 lakh ha⁻¹ year⁻¹ with a cost benefit ratio of $45:1$ - $67.5:1$. The present study confirm that *B. orellana* L. performs reliably under subtropical agroecosystems, producing high seed and pigment yield with substantial profitability. The crop demonstrates strong potential as a sustainable, perennial cash crop for natural pigment industries and as a promising diversification option for farmers in organic production management.

Keywords: bixin content; economic analysis; germination enhancement; phenological development; pigment yield; subtropical agroecosystems

Introduction

Bixa orellana L., commonly known as annatto or sindoor plant, is a perennial shrub belonging to the family Bixaceae. Morphologically, *B. orellana* L. is a perennial shrub or small tree that typically grows to a height of 3-5 m (Fig. 1). It exhibits an erect, branched habit with smooth bark that transitions from green in young stems to reddish-brown in mature stems. The leaves are simple, alternate, ovate to broadly ovate, measuring 8-20 cm in length and 6-12 cm in width, with a cordate (heart-shaped) base and acuminate tip supported by long petioles. The plant bears hermaphroditic flowers arranged in terminal panicles, distinctive by 5 pink and numerous yellow stamens, which make it visually distinctive during blooming. The fruit is a spiny, ovoid capsule, 3-5 cm long, which matures from green to reddish-brown and dehisces upon ripening to release 30-50 small angular seeds per capsule. Each seed, approximately 4-5 mm in size, is coated with a red-orange pigment called bixin, it is the principal pigment of commercial and pharmacological importance (1). It holds a prominent place as the primary botanical source of carotenoid pigments, mainly lipid soluble bixin and its

derivative water soluble norbixin. These pigments are extensively utilized in food, cosmetics, pharmaceuticals and textiles, recognized under the food additive code E160b (2-4). Bixin is primarily incorporated into oil-based products such as cheese, margarine, butter and processed meats, whereas norbixin is employed in aqueous systems like beverages, dairy desserts and bakery fillings due to its water solubility (5). Beyond their colorant properties, annatto pigments exhibit significant bioactivity. Studies demonstrate antioxidant, antimicrobial, anti-inflammatory and anticancer effects, making it as functional compounds with dual roles in both health and industrial applications (6, 7). The global natural food colorant market was valued at USD 7.82 billion in 2024, with carotenoid pigments contributing significantly. Annatto accounts for approximately 70 % of natural food colours used in dairy and cheese industries worldwide, particularly in Europe and North America. With increasing consumer awareness, the global annatto pigment market alone is projected to reach USD 12.44 billion by 2034, driven by the shift from synthetic to natural food additives. The market trends emphasize the increasing demand for organic annatto pigments aligned with sustainable agriculture

and residue-free production systems. With annatto pigment extraction and processing facilities established in Latin America and Southeast Asia, there is significant scope for expanding cultivation in India to supply raw seeds for both domestic and export industries (8).

Despite its recognized industrial relevance, *B. orellana* L. has received limited attention in subtropical agro-climatic regions of India. Presently, its cultivation is mostly confined to the southern states such as Tamil Nadu, Kerala and Andhra Pradesh, while subtropical zones like Bihar, characterized by fertile alluvial soils, favourable rainfall patterns and warm temperatures remain largely unexplored for its commercial production. Critical research gaps include the absence of region-specific germination enhancement protocols, limited knowledge on propagation and nursery management practices suited to subtropical conditions and a lack of systematic evaluation of yield potential, pigment concentration and physicochemical properties under organic farming systems. Furthermore, comprehensive guidelines integrating Good Agricultural Practices (GAP) with a standardized Package of Practices (POP) are unavailable for this region and economic feasibility assessments for farmers have not been conducted. These gaps have constrained the wider adoption of *B. orellana* L. as a commercial pigment crop, despite its growing market demand and alignment with national priorities on natural pigment production and sustainable agricultural diversification.

However, the most critical limitation that restricts the expansion of *B. orellana* L. into subtropical regions is the absence of baseline scientific data, such as its germination efficiency, seedling vigour, phenological behaviour, seed yield, pigment concentration and overall adaptability when grown organically in subtropical conditions. This lack of region-specific information restricts the development of reliable nursery protocols, field management practices and economic models, thereby preventing farmers and industries from adopting this crop with confidence. Generating such foundational data is therefore essential for aligning the crop's cultivation potential with the practical requirements of subtropical farming systems.

Addressing these gaps, the present study was undertaken with the objective of establishing *B. orellana* L. as a viable pigment crop in subtropical condition through scientifically validated agronomic interventions. Specifically, it aimed to standardize germination and nursery raising techniques to ensure high-quality seedlings, evaluate plant growth and productivity using benchmark data from mature mother plants and analyze the physicochemical composition and bixin content of seeds to ascertain their industrial suitability. It was hypothesized that *B. orellana* L. can be successfully cultivated as a high value perennial pigment crop under subtropical agro ecosystems through optimized pre-sowing treatments and organic cultivation practices, leading to improved germination, stable growth, high seed and pigment yield and strong economic viability. Accordingly, the present study aimed to standardize germination and nursery raising techniques, evaluate plant growth, phenology and seed productivity, analyse seed physicochemical composition and bixin content, develop an organic cultivation model integrating GAP and region-specific POP and conduct a preliminary economic assessment of its commercial feasibility under subtropical conditions.



Fig. 1. Field grown *B. orellana* L. exhibiting prolific fruit capsule development.

Materials and Methods

Experimental site and agroclimatic conditions

The study was carried out at the experimental fields of Bihar Agricultural University, Sabour, Bhagalpur district, Bihar, India (25.23° N latitude, 87.07° E longitude). The region falls under the humid subtropical agroclimatic zone, characterized by hot summers, cool winters and a distinct monsoonal precipitation pattern. The site receives an average annual rainfall of 1100-1200 mm, with approximately 85-90 % occurring during the southwest monsoon season (June - September). Relative humidity typically ranges from 60-85 % across the year. Mean monthly temperatures vary from 10-15 °C during winter (December - January) to 30-45 °C during summer (April - June). The annual photoperiod fluctuates between 10 and 13 hr, providing adequate light exposure for photosynthetically active radiation.

Soil analysis and site preparation

Prior to planting, composite soil samples were collected from a depth of 0-30 cm across the experimental site using a stainless steel auger. Samples were shade dried, gently crushed and sieved through a 2 mm mesh for laboratory analysis. Soil pH (1:5 soil: water) and electrical conductivity (EC) were determined in aqueous extracts using a calibrated pH meter and conductivity meter, respectively (9). Organic carbon content was quantified using the modified Walkley-Black method with external heating (10). Available nitrogen was determined according to the improved titrimetric method (11). Available phosphorus (P) was extracted using 0.5 M NaHCO₃ (pH 8.5) and estimated colorimetrically, following modern adaptations of Olsen's method (12). Exchangeable potassium (K) was extracted with 1 N ammonium acetate (pH 7.0) and measured using a flame photometer (13). The field was prepared by 2 passes of ploughing to break clods, followed by harrowing and levelling to achieve a fine tilth, ensuring optimal aeration, uniform pit marking and better root establishment after transplanting.

Planting material collection

Mature seed capsules of *B. orellana* L. (Fig. 2) were collected from 5 year old, high yielding mother plants maintained at the Krishi Vigyan Kendra (KVK), Bihar Agricultural University Sabour, Bhagalpur, Bihar, India. Capsules were harvested at the full physiological maturity stage, indicated by a reddish-brown coloration and natural dehiscence. The capsules were sundried for 5-7 days to facilitate moisture reduction and ease of seed release. Seeds were then manually extracted and cleaned to remove debris and inert matter. Only healthy, uniform seeds were selected for use in germination experiments and subsequent nursery establishment.



Fig. 2. Mature, dehiscent capsules of *B. orellana* L. at full physiological maturity.

Pre-sowing treatments, germination enhancement and nursery management

To address the issue of physical dormancy caused by the hard seed coat of *B. orellana* L., pre-sowing treatments were employed to enhance germination. Seeds were subjected to 4 treatments: (i) control (untreated), where seeds were directly sown without any treatment; (ii) mechanical scarification, in which seeds were gently rubbed on fine sandpaper to weaken the seed coat and facilitate water uptake; (iii) warm water hydration treatment, involving soaking seeds in distilled water at room temperature for 24 hr; and (iv) scarification + warm water treatment, where seeds were first scarified and then immersed in water maintained at 65 °C for 1 hr, followed by gradual cooling. These treatments were selected based on previous findings highlighting their effectiveness in breaking seed dormancy and improving germination in hard seeded tropical species (14). Following the treatment, seeds were sown in polyethylene nursery bags filled with a medium comprising soil and vermicompost in a 2:1 ratio, which provided balanced aeration, drainage and nutrient availability. The polyethylene nursery bags were placed under partial shade (50 % shade net) to protect emerging seedlings from direct solar radiation while maintaining an optimal microclimate. Mist irrigation was applied daily to sustain adequate moisture levels essential for germination and early seedling development, as suggested for other perennial tropical species (15, 16). Seedlings were maintained in the polyethylene nursery bags for 8 weeks for further transplantation.

Field layout and transplantation

The field was prepared and laid out following a square planting system with pits spaced at 2.5×2.5 m, accommodating a planting density of 1500 plants per hectare, to ensure adequate light penetration, aeration and canopy development. For the present study, 200 healthy seedlings were raised in the polyethylene nursery bags were transplanted in a demarcated experimental

block. Pits of 45 × 45 × 45 cm were dug and filled with topsoil mixed with well decomposed farmyard manure to enrich soil organic matter and improve moisture retention, along with 50 g of neem cake. Transplantation was carried out during the early monsoon season (July) to coincide with favourable soil moisture conditions, minimizing transplant shock and ensuring better seedling establishment. Light irrigation was applied immediately after transplanting to settle the soil and promote root soil contact.

Phenological observations

The phenological development of *B. orellana* L. was systematically monitored to document key growth and reproductive stages. Germination initiation was recorded as the number of days from sowing until visible radicle emergence, seedling vigor was assessed at 8 weeks after sowing by measuring plant height and counting the number of true leaves, which served as an index of early seedling growth performance (17). The flower initiation stage was recorded as the time interval from field transplantation to the appearance of the first floral bud, while peak flowering and fruiting periods were identified through regular field inspections and visual assessment of maximum floral density and fruit set. Subsequently, the capsule maturity period was determined by tracking the number of days from fruit set to physiological maturity, defined by capsule color change and natural dehiscence, as per the phenological recording protocols for tropical perennial crops (18). All observations were recorded using direct visual methods and standardized field datasheets at weekly intervals. These phenological stages were documented to understand the growth cycle of *B. orellana* L. under subtropical conditions.

Physicochemical analysis of seeds

The physicochemical properties of harvested *B. orellana* L. seeds were analyzed to evaluate their quality attributes, for each parameter, 3 independent seed samples ($n = 3$), each comprising 1 g of powdered seed samples, were analyzed as analytical replicates. Moisture content was determined by oven drying the seed samples at 105 °C to constant weight, as per gravimetric method guidelines (19). Protein content was estimated using the Lowry's method (20). Fat content was quantified through Soxhlet extraction using petroleum ether as the solvent, following standard lipid determination protocols (21). Ash content, indicative of the total mineral composition, was measured by incinerating seed samples in a muffle furnace at 550 °C until a white residue was obtained (19). Crude fibre content was analyzed using the acid-alkali digestion method, which involves sequential treatment with dilute sulfuric acid and sodium hydroxide to remove soluble components, leaving insoluble fibre fractions (22). For pigment estimation, bixin content was quantified using High Performance Liquid Chromatography (HPLC) equipped C-18 column with a UV-visible detector set at 470 nm (23).

Economic implications and cost benefit analysis

The economic viability of *B. orellana* L. cultivation was evaluated by estimating the gross revenue, net returns and cost-benefit ratio (CBR) based on actual yield observations and prevailing market prices. Yield per plant was recorded from 2 mature mother plants maintained at KVK, Sabour and extrapolated to a standard planting density of 1500 plants per hectare. The prevailing market price of seeds, ranging from Rs. 800 to Rs. 1200 per kg, was derived from organic pigment suppliers and herb based industrial sources. Gross revenue was calculated using the formula:

$$\text{Gross revenue (Rs ha}^{-1}\text{)} \\ = \text{Seed yield (kg ha}^{-1}\text{)} \times \text{Seed price (Rs kg}^{-1}\text{)} \quad (\text{Eqn. 1})$$

The total cost of organic cultivation was estimated at Rs. 80000 ha⁻¹ year⁻¹, which included nursery raising, pit preparation, organic inputs, irrigation, manual weeding, labour charges for field operations and miscellaneous expenses such as transport and packaging. Net returns were calculated by subtracting the total cost of cultivation from the gross revenue. The CBR was determined using:

$$\text{CBR} = \text{Gross revenue} / \text{Cultivation cost} \quad (\text{Eqn. 2})$$

In addition to the seed yield potential the medicinal utility of leaves and the potential integration of *B. orellana* L. into agroforestry systems were considered for a broader evaluation of economic sustainability. This approach follows established economic assessment frameworks used in perennial plantation crop research (24).

Statistical design

The experimental data on germination percentage, seedling growth parameters, phenological traits and physicochemical characteristics of *B. orellana* L. were analyzed using a completely randomized design (CRD) with appropriate levels of replication according to the nature of each parameter. Germination and seedling growth experiments were conducted with 20 independent replications per treatment. For phenological observations, 2 plants per replication were selected and 10 branches per plant were tagged, providing 10 replications for recording developmental observations. Physicochemical analyses were performed with 3 independent replications to ensure analytical precision. Data were compiled and processed in Microsoft Excel to compute descriptive statistics, including means and standard deviations. Differences among treatments were tested for statistical significance using one-way Analysis of Variance (ANOVA). Associations among growth, biochemical and yield-related traits were examined using Pearson's correlation coefficients (*r*) and the statistical significance of correlations was tested, *p*-values were calculated and correlations were considered statistically significant when *p* < 0.05. Correlation patterns were visualized using a heatmap generated in BioRender.

Results

Physicochemical properties of the experimental soil

The analysis of experimental soil samples indicated slightly acidic with a mean pH of 6.72 ± 0.04, while the electrical conductivity was recorded at 0.21 ± 0.02 dS m⁻¹ and the organic carbon content was estimated at 0.38 ± 0.03 %, showed moderate level of organic matter. The total nitrogen, phosphorous and potassium content was recorded at 218.40 ± 5.26 kg ha⁻¹, 15.72 ± 1.08 kg ha⁻¹ and 132.84 ± 4.62 kg ha⁻¹ respectively. These soil parameters revealed, the experimental soil samples have low to medium fertility.

Germination percentage and emergence timing

Significant variation was observed in the germination responses of *B. orellana* L. seeds under different pre-sowing treatments (Table 1). Among all treatments, mechanical scarification followed by warm water hydration resulted in the highest germination rate, averaging 94.67 ± 0.93 % across the replications. These seeds initiated radicle emergence within 12-14 days. The warm water treatment group followed with a

mean germination of 80.33 ± 0.77 %, with radicle emergence noted between 13-15 days. Seeds subjected to scarification alone showed a moderate germination rate of 78.33 ± 1.53 with delayed sprouting observed between 14-16 days. The control group, which received no pre-sowing treatment, recorded the lowest germination at 25.33 ± 1.15 %, with late and inconsistent emergence around 20-22 days post-sowing. These results demonstrated not only differences in total germination percentages but also in the speed and uniformity of seedling emergence across treatments.

Table 1. Effect of different pre-treatment methods on seed germination percentage and emergence timing in *B. orellana* L.

| Treatment | Germination (%) (Mean ± SD) | Emergence timing (Days) |
|--------------------------------------|-----------------------------|-------------------------|
| Control | 25.33 ± 1.15 | 20-22 |
| Scarification | 78.33 ± 1.53 | 14-16 |
| Warm water hydration | 80.33 ± 0.77 | 13-15 |
| Scarification + warm water hydration | 94.67 ± 0.93 | 12-14 |

Seedling growth and nursery performance

At the end of 8 weeks, a comparative analysis of seedling growth revealed notable differences in plant height and leaf development (Fig. 3). Seedlings raised from scarified + hydrated seeds achieved a mean height of 36.47 ± 0.76 cm, with 6.0 ± 0.58 true leaves. In contrast, seedlings from scarification only and warm water treated seeds reached 32.83 ± 0.82 cm and 34.23 ± 0.35 cm in height, respectively. True leaf counts for these groups were 5.00 ± 0.21 and 6.0 ± 0.78, respectively. Control seedlings, however, lagged significantly, with an average height of 24.60 ± 0.73 cm and only 3.0 ± 0.43 true leaves. In terms of survival rate during the hardening phase, treated seedlings exhibited high tolerance to field exposure, with survival percentages ranging from 91 % to 95 %, whereas control seedlings showed slightly reduced survival (88 %), attributed to delayed development and lower initial vigor (Table 2).

Phenological development

A distinct pattern of phenological events was recorded in the field grown plants (Fig. 4). The initiation of flower buds occurred at 310.0 ± 1.0 day after transplantation. This was followed by full bloom, identified as the peak flowering period, at 335.0 ± 1.0 day. Fruit set occurred soon after, on average at 360 ± 2.0 days, marking the start of the reproductive phase. The final phenological stage, capsule maturity, was reached at 480 ± 2.0 days, around 4 months after fruit development (Table 3). Plants followed a consistent annual cycle from vegetative growth to reproduction and the variation in days among replications remained minimal, showing that under subtropical agroclimatic conditions, *B. orellana* L. exhibits stable phenology suitable for standardized agronomic planning.

Table 2. Influence of pre-treatment methods on seedling height, leaf development and survival rate of *B. orellana* L.

| Treatment | Height (cm) (Mean ± SD) | True leaves (Mean ± SD) | Survival rate (%) |
|--------------------------------------|-------------------------|-------------------------|-------------------|
| Control | 24.60 ± 0.73 | 3.0 ± 0.43 | 88 |
| Scarification | 32.83 ± 0.82 | 5.0 ± 0.21 | 91 |
| Warm water hydration | 34.23 ± 0.35 | 6.0 ± 0.78 | 93 |
| Scarification + warm water hydration | 36.47 ± 0.76 | 6.0 ± 0.58 | 95 |

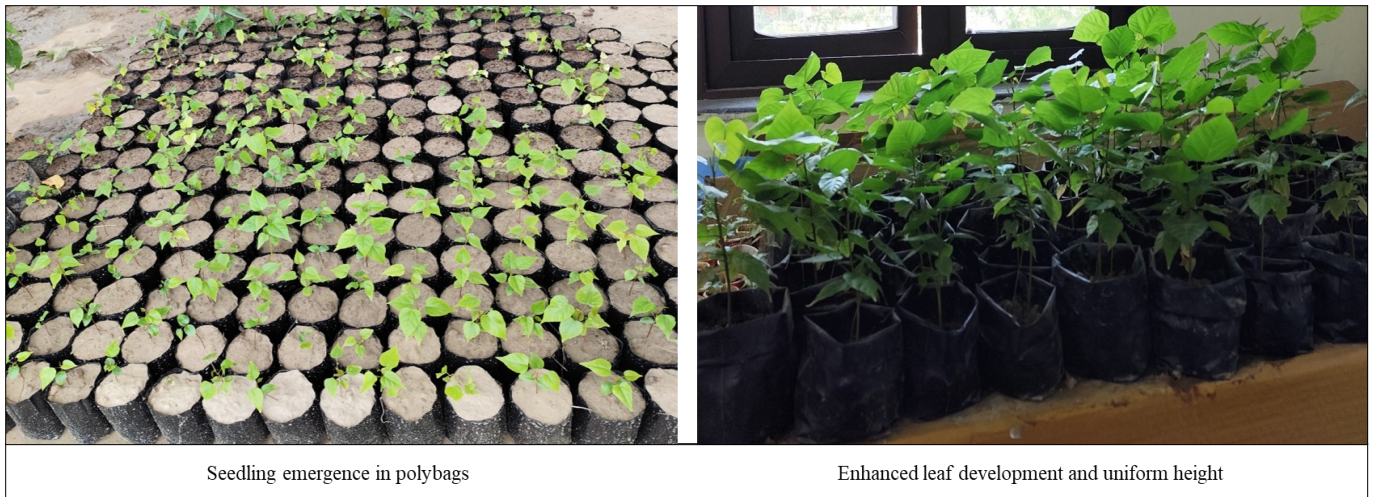


Fig. 3. Seedling growth and nursery performance of *B. orellana* L.



Fig. 4. Phenological progression of *B. orellana* L. observed under subtropical field conditions. a. flower bud initiation; b. peak flowering; c. fruit set; d. immature capsule formation; e. ripening capsule stage; f. mature capsule stage.

Table 3. Phenological development timeline of *B. orellana* L. from transplantation to capsule maturity

| Phenological stage | Days after transplantation (Mean \pm SD) |
|--------------------|--|
| Flower initiation | 310.0 \pm 1.0 |
| Peak flowering | 335.0 \pm 1.0 |
| Fruit set | 360.0 \pm 2.0 |
| Capsule maturity | 480.0 \pm 2.0 |

Yield assessment

Yield observations made from 2 mother plants maintained under field conditions at KVK Sabour revealed a productive performance (Table 4). Each plant produced approximately 1900 ± 50 capsules per year and each capsule typically contained 30 to 50 seeds, with 50 seeds weighing between 1.5 - 2.0 g. Based on these parameters, the average annual seed yield per plant was recorded as 3.5 ± 0.25 kg. When extrapolated to a planting density of 1500 plants per hectare, the projected seed yield per hectare ranged between 4.5 and 6.0 tonnes, depending on plant performance and capsule density. Capsule production was consistent between replications and across seasons.

Table 4. Seed yield potential and agronomic performance of *B. orellana* L. under field conditions

| Parameter | Value (Mean \pm SD) |
|---------------------------|-----------------------|
| Capsules per plant | 1900 \pm 50 |
| Seeds per capsule | 30-50 |
| 50-seed weight (g) | 1.5-2.0 |
| Seed yield per plant (kg) | 3.5 \pm 0.25 |
| Projected yield (t/ha) | 4.5-6.0 |

Physical and chemical properties of seeds

The physicochemical analysis of *B. orellana* L. seeds confirmed consistent quality across samples (Table 5). The moisture content was 8.53 ± 0.45 %, indicating efficient drying. Protein content was 12.20 ± 0.20 % and fat content was measured at 5.13 ± 0.15 %, while ash content and crude fiber were 3.43 ± 0.75 % and 9.20 ± 0.20 %, respectively. These values remained relatively stable across 3 replicates, indicating the homogeneity of seed composition under uniform field conditions. HPLC analysis revealed that the bixin content in seeds ranged between 3.5 % and 3.8 %, with a mean value of 3.63 ± 0.58 %. When this concentration was applied to the projected seed yield (4.5-6.0 tonnes/ha), the estimated bixin production $\text{ha}^{-1} \text{ year}^{-1}$, ranged from 162 -216 kg on dry seed weight basis. Bixin concentration remained consistent across all 3 replications, with low standard deviation, indicating genetic uniformity and environmental stability in pigment expression.

Table 5. Physical and chemical properties of seeds

| Parameter | Value (Mean \pm SD) |
|---------------------------------------|-----------------------|
| Moisture (%) | 8.53 ± 0.45 |
| Protein (%) | 12.20 ± 0.20 |
| Fat (%) | 5.13 ± 0.15 |
| Ash (%) | 3.43 ± 0.75 |
| Crude fiber (%) | 9.20 ± 0.20 |
| Bixin concentration (%) | 3.63 ± 0.58 |
| Pigment yield (kg ha^{-1}) | 162-216 |

Economic implications

The economic analysis of *B. orellana* L. cultivation under organic management revealed exceptionally high profitability. The market price of annatto seeds ranges between Rs. 800-1200/kg, depending on quality, purity and pigment concentration. With a projected seed yield of 4.5 - 6.0 tonnes ha^{-1} , the estimated gross revenue is Rs. 36-54 lakh $\text{ha}^{-1} \text{ year}^{-1}$ (Table 6). The cultivation cost, primarily under an organic system, was estimated at Rs.80 lakh $\text{ha}^{-1} \text{ year}^{-1}$, covering expenses for nursery establishment, organic inputs (farmyard manure and neem cake), irrigation, manual weeding, pest management using neem-based biopesticides, labour for pruning and harvesting and other miscellaneous costs such as transport and packaging. Since *B. orellana* L. is a perennial crop, this cost significantly declines in subsequent years after establishment. Based on prevailing seed prices, the corresponding CBR was 45:1 at Rs. 800 kg^{-1} and 67.5:1 at Rs. 1200 kg^{-1} . This

demonstrates that for every rupee invested, farmers can expect a return of Rs. 45 to Rs. 67.5. Moreover, secondary revenue streams such as bixin pigment extraction (yielding 162-216 kg ha^{-1} annually), the medicinal use of leaves and bark and integration into agroforestry systems provide additional economic benefits. The perennial nature of the crop, with a productive life of 15 - 20 years, further strengthens its potential for long term income stability and low recurring costs. These results indicate that *B. orellana* L. is a highly profitable, sustainable crop suitable for organic cultivation systems and income diversification for farmers.

Table 6. Economic analysis of *B. orellana* L. cultivation

| Parameter | Value/Range |
|---|-------------------|
| Seed yield ($\text{kg ha}^{-1} \text{ year}^{-1}$) | 4500 - 6000 |
| Seed price (Rs kg^{-1}) | 800 - 1200 |
| Gross revenue (Rs $\text{ha}^{-1} \text{ year}^{-1}$) | 3600000 - 5400000 |
| Cultivation cost (Rs $\text{ha}^{-1} \text{ year}^{-1}$) | 80000 (organic) |
| Net return (Rs $\text{ha}^{-1} \text{ year}^{-1}$) | 3520000 - 5320000 |
| Cost-benefit ratio (CBR) | 45:1 - 67.5:1 |
| Bixin yield (kg ha^{-1}) | 162.00 - 216.00 |
| Crop lifespan | 15- 20 years |

Correlation analysis of growth, biochemical traits and yield

A Pearson correlation matrix was constructed to determine the strength and significance of associations among key agronomic, physiological and biochemical parameters of *B. orellana* L., including germination percentage, seedling height, number of true leaves, seed protein content, bixin content and seed yield per plant. The analysis revealed that germination percentage was significantly and positively correlated with all other traits (Fig. 5). Strong correlations were observed with protein content ($r = 0.999$), bixin content ($r = 0.991$) and seed yield per plant ($r = 0.999$), suggesting that better germination enhances both biochemical quality and productivity. Germination also showed high correlation with seedling height ($r = 0.969$) and true leaves ($r = 0.961$), indicating its influence on early seedling vigor. Seedling height showed very high correlations with true leaves ($r = 0.999$), protein content ($r = 0.974$) and seed yield ($r = 0.961$), confirming that strong vegetative growth leads to better productivity. Similarly, the number of true leaves was significantly correlated with seed yield ($r = 0.952$) and protein content ($r = 0.965$), highlighting the importance of early vegetative parameters as predictors of yield and seed quality. Protein content also had a strong positive correlation with bixin content ($r = 0.985$) and seed yield per plant ($r = 0.998$), indicating that higher seed protein levels are associated with greater pigment concentration and productivity. The relationship between bixin content and seed yield was also significant ($r = 0.994$), supporting the economic relevance of high pigment yielding lines. These findings collectively highlight the interconnectedness of seed vigor, vegetative traits, biochemical composition and final productivity. The high correlation coefficients emphasize the potential of using early stage seedling and germination data to predict bixin content and seed yield, aiding in selection and standardization for commercial annatto cultivation under subtropical agroclimatic conditions.

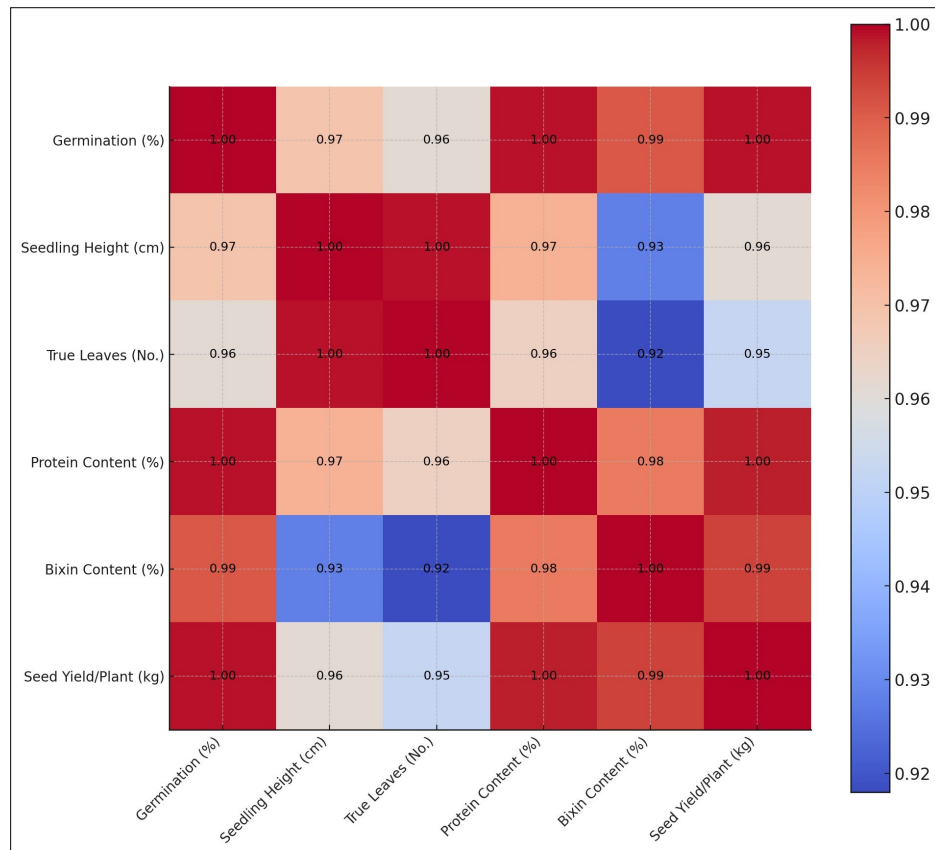


Fig. 5. Heatmap illustrating Pearson correlation coefficients (r) among germination, seedling growth, physiological and yield traits of *Bixa orellana* L. Colour gradients represent the strength of positive correlations and numerical r -values are shown within each cell.

Discussion

The present study provides comprehensive understanding into the germination biology, phenological behaviour, yield potential, seed composition, pigment (bixin) content and economic feasibility of *B. orellana* L. under subtropical agroclimatic conditions in Bihar, India. The results collectively validate the crop's adaptability to subtropical ecosystems and its potential to serve as a high value perennial cash crop under organic and sustainable farming systems. The significantly higher germination percentage observed with mechanical scarification followed by hydration confirms that physical dormancy in *B. orellana* L. is primarily due to the impermeable seed coat, which restricts water imbibition and gas exchange (25). By disrupting this barrier, scarification facilitates rapid hydration and uniform germination, corroborating with the findings, where similar improvements in germination were recorded with pre-sowing treatments (15). This study aligns with the principle that dormancy breaking interventions are crucial for species with hard seed coats, as documented for several other crops (26). The reduced germination in untreated seeds highlights the importance of standardized seed pre-treatment protocols for successful nursery establishment. Hydration treatments alone showed moderate success, while warm water soaking effectively softened the seed coat, promoting faster germination. These findings are similar to the earlier studies on temperature mediated dormancy alleviation in tropical seeds (27, 28). Collectively, these results highlight the need for integrating scarification or thermal hydration as standard pre-sowing practices in commercial *B. orellana* L. propagation.

Seedling vigor directly influences field performance and the highest seedling growth in scarified seeds supports its role in enhancing early plant establishment. Comparable vigor

improvements were reported, linking vigorous seedlings to enhanced nutrient mobilization and photosynthetic efficiency in the early vegetative phase (29). High nursery survival further demonstrates the species suitability for subtropical nursery systems under organic management. Notably, seedlings from untreated seeds displayed stunted growth, indicating delayed metabolic activation and limited root expansion. Such poor vigor can impair field performance and increase transplant shock, as also observed in studies on tropical trees (30). Therefore, implementing pre-germination treatments is essential for uniform nursery output and large-scale commercial planting. The phenological aligns with the established 14 -16 month juvenile phase of *B. orellana* L. reported in tropical zones (31). This synchrony of flowering with subtropical winter conditions suggests favourable pollination timing, as cooler temperatures enhance pollen viability and reduce floral abscission, a factor crucial for perennial crops. The projected seed yield (4.5-6.0 t/ha) significantly exceeds previously reported yields (0.75-1.5 t/ha) in India, likely due to favourable climatic conditions (32). Additionally, capsule production observed in this study reflects global benchmarks for high yielding genotypes. The correlation between plant vigor, capsule number and yield highlights the importance of nursery management and early establishment for long term productivity. The seed composition such as protein, fat and fiber aligns with reported values and confirms the nutritional and industrial quality of seeds grown under organic conditions (33). The observed bixin concentration matches premium pigment standards (1-3 %) documented globally validating the suitability of Bihar grown *B. orellana* L. for high end natural dye industries (6, 34). Bixin yield reported in this study supports its industrial relevance, as pigments derived from annatto seeds are extensively used as food additives (E160b), cosmetics and pharmaceuticals (35). Furthermore, the high

pigment stability in organically grown seeds suggests a superior quality profile. The successful cultivation of *B. orellana* L. under an organic management, with no recorded pest or disease incidence, strengthens its suitability as a low input, eco-friendly crop. Neem-based bioinputs and farmyard manure effectively sustained plant health and soil fertility, corroborating earlier findings that organic amendments improve perennial crop performance (36). Moreover, its perennial nature, requiring minimal replanting for 15-20 years, reduces long term land preparation costs and supports carbon sequestration in plantation systems, contributing to climate-smart agriculture. Its integration into border planting and agroforestry models further enhances biodiversity, soil conservation and ecological resilience.

The cost benefit analysis revealed a CBR of 45:1 to 67.5:1, surpassing most high-value crops. Gross revenues of Rs. 36-54 lakh ha⁻¹ year⁻¹ and net returns of Rs. 35.2-53.2 lakh ha⁻¹ are transformative for smallholder farmers seeking diversification away from cereals or low-value crops. Additionally, the crop's perennial nature ensures stable income for 15 - 20 years post establishment, minimizing recurrent costs. Secondary value chains, including bixin extraction and medicinal applications of leaves and bark, provide further income avenues and align with the emerging demand for natural pigments and plant-based nutraceuticals (37). Given its high pigment yield, organic suitability and economic returns, *B. orellana* L. holds potential for integration into food, cosmetic and textile industries reliant on natural dyes. Establishing localized pigment extraction units could enhance farmer value realization and reduce dependence on imported natural colorants. Strategic policy interventions aimed at incorporating this crop into niche promotion schemes and establishing strong linkages between farmers and value chains can significantly enhance its adoption. The correlation analysis conducted in this study provides significant understanding into the interrelationships among seed germination, seedling vigor, biochemical traits and yield performance in *B. orellana* L. under subtropical conditions. The strong and statistically significant positive correlation between germination percentage and seed yield per plant highlights the fundamental role of early germination success in determining the overall productivity of annatto plants. This aligns with previous report that early germination and uniform seedling emergence are critical indicators of vigor and subsequent field performance in tropical tree crops (38). Similarly, the positive correlation between germination and protein content suggests that physiological quality of seeds influences biochemical potential, an observation consistent with studies in other carotenoid rich species such as safflower (39). Protein metabolism during early seedling establishment is crucial for the synthesis of key enzymes regulating carotenoid biosynthesis pathways, thereby affecting pigment accumulation (40). The association of seedling height and true leaves with seed yield and bixin content further emphasizes the importance of strong vegetative development in pigment crops. Notably, seedling height correlated significantly with bixin content and seed yield, indicating that taller seedlings with higher leaf count contribute to enhanced photosynthetic efficiency and assimilate partitioning factors crucial for capsule formation and pigment synthesis. A similar trend was reported in *Tagetes erecta*, where seedling vigor was linked with higher pigment yield (41). Importantly, the strong correlation between protein content and bixin content may be attributed to the role of proteinaceous

enzymes involved in the methylerythritol phosphate (MEP) pathway of carotenoid biosynthesis in annatto. These enzymes regulate the conversion of geranylgeranyl diphosphate (GGPP) into phytoene, leading to the synthesis of bixin (4). Therefore, protein rich seeds likely possess higher enzymatic activity, contributing to increased pigment accumulation. Furthermore, the significant correlation between bixin content and seed yield is economically relevant. It indicates that selecting high yielding plants can simultaneously result in high pigment productivity, an aspect vital for commercial viability. Overall, the correlation matrix serves as a powerful tool to understand trait interdependencies. It aids in the development of selection indices that can accelerate breeding and cultivation strategies for annatto. The strong linear associations among germination, growth, biochemistry and yield traits observed in this study emphasize that early-stage vigor and physiological parameters can reliably predict final productivity and pigment content under organic, subtropical cultivation systems.

Conclusion

This study shows that *B. orellana* L. performs well under subtropical organic farming and can be developed as a reliable perennial pigment crop. Improved pre-sowing treatments enhanced germination and seedling vigor, while stable phenology and high seed and bixin yields confirmed its agronomic and industrial potential. The correlation matrix serves as a powerful tool for understanding trait interdependencies and aids in the development of selection indices that can accelerate breeding and cultivation strategies for annatto. The crop thrived under low-input organic conditions with minimal pest pressure and maintained good soil health. Its strong economic returns, long productive lifespan and additional value through pigment and medicinal uses make *B. orellana* a promising option for farmer income diversification and sustainable agricultural development.

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Authors' contributions

VSB conceptualized the study, designed and performed the experimental methodology and wrote the first draft of the manuscript. AKP contributed to seed treatment protocols and statistical analysis. RK and MK contributed to germination studies and nursery management. AS contributed to economic analysis. AKS contributed to reviewing the manuscript. DRS supervised the overall research work. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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