Journal of Advances in Biology & Biotechnology

Volume 27, Issue 5, Page 344-351, 2024; Article no.JABB.115538
ISSN: 2394-1081

Effect of Various Pre-sowing Treatments on Germination and Seedling Performance of *Bixa orellana* L. at Low Seed Moisture Content

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JABB/2024/v27i5793

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/115538

Original Research Article

Received: 04/02/2024
Accepted: 08/04/2024
Published: 11/04/2024

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ABSTRACT

Natural plant-based substances are becoming more and more popular in today's society for a variety of uses. Bixin (vibrant red-orange dye) is one such compound derived from *Bixa orellana* L., commonly known as annatto. This plant is primarily propagated through seeds for commercial cultivation. However, there is very little information available on germination combined with the seedling performance of this species. Hence, to fill this research gap, the present study evaluated seed morphological characteristics and various pre-sowing treatments to enhance seed germination and its vigour at a low seed moisture content of 10%. This study used Completely Randomized Design (CRD), and seeds were subjected to six pre-sowing treatments with four replications. The study showed the highest germination (52%) and highest mean daily germination (4.6) in 24hrs Water-soaked seeds, whereas mechanical scarification in combination with 24hrs water-soaking increased the germination percentage from 19% to 47%. In addition, the highest seed vigour index (411.25) was also observed in mechanical scarification combined with 24-hour water soaking. These findings of germination and vigour at low seed moisture content will certainly help in the formation of desiccation and storage protocols for medium to long-term conservation of *Bixa orellana* L. seeds.

Keywords: Bixa orellana L.; annatto; achiote; germination; vigour; scarification.

1. INTRODUCTION

Food colouring is added excessively to colourless meals, such as confections, snacks, drinks, and gelatin desserts, to distinguish the flavour and enhance the palatability. Since many synthetic food colours are dangerous [1], finding more natural ones is crucial. Bixin has been used in cuisine since ancient times as a spice and a dye [2]. As a powerful natural colouring ingredient, bixin is widely utilized in the food and cosmetics sectors [3,4].

This leads to increased demand for bixin, which is naturally obtained from *Bixa orellana* L. (Family: Bixaceae), commonly known as achiote, annatto or lipstick tree. The Spaniards were the first to introduce it to Southeast Asia in the 17th century [5]. It is now found in every tropical and subtropical region of the world, including India [6,7]. The bixin concentration of annatto seeds is a crucial determinant of the seed's price. After caramel, annatto is the second-most significant natural colorant in the world [8]. Annatto finds utility in the pharmaceutical, textile, dairy, food and beverage, paint, and cosmetic sectors [9,10]. Due to its wide utility in different sectors, the plant has immense potential to be used as an agroforestry species throughout tropical countries.

This plant has an expected economic life of 20-25 years [11], making it a suitable species for Agroforestry to improve farmer's socioeconomic conditions. In the Karnataka State (India), 2.6 t seeds/ha were obtained under good fertilizer management [12]. Seed sowing is widely used as a mode of propagation, and one of the primary problems with this method is that dormancy causes poor and delayed seed germination [13,14,15]. Further, the rate of germination was significantly reduced when seeds were dried [16]. Hence to address these two problems, the present study was conducted to determine best pre-sowing treatment for enhancing seed germination and seedling performance at low seed moisture content of 10%.

2. MATERIALS AND METHODS

The mature capsules of *Bixa orellana* L. collected from Village Baramkela, Gomarda Wildlife Sanctuary, Baramkela forest division, Sarangarh-Bilaigarh district, Chhattisgarh, India (N 21°33’51” and E 83°16’08”) in December 2022 (Fig. 1). A total of 1000 fruits were collected from 10 trees (at least 100 m apart). The seeds were extracted, air-dried and stored in air tight plastic container at Forest Tree Seed Laboratory, FRI, Dehradun (N 30°28’11.72” and E 78°03’36.99”). After that, initial morphological parameters were recorded and seeds were subjected to moisture test.

2.1 Morphological Analysis

A digital calliper with a precision of 0.001 mm was used to measure the length and width of 10 randomly chosen seeds in 4 replications for biometric determination. The width was measured in the midline of the seeds, and the

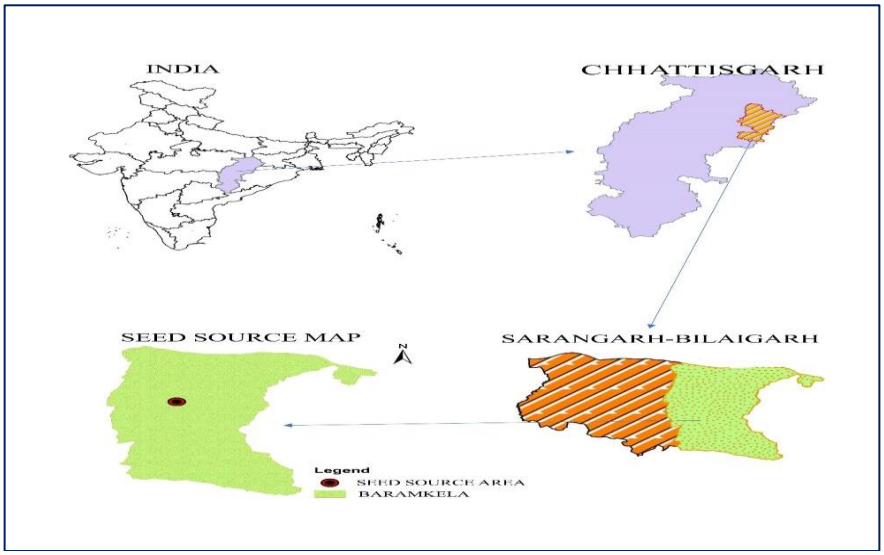

Fig. 1. Showing seed source map

length was measured from the base to the apex [17]. Following length and width measurements, four replications of 100 seeds were weighed on an analytical balance with an accuracy of 0.001 g. The Excel application was used to calculate the arithmetic mean and standard deviation of the seed quantitative characteristic data, which were then submitted for descriptive analysis.

2.2 Moisture Content

About 1000 seeds were desiccated to moisture content of 10% using silica gel drying method. The seed's moisture content was determined using the hot air oven method. In which four replications (2 gm per replication) of seeds were oven dried at a temperature of 103±2ºC for 17±1 hours [18]. Moisture content was calculated by using below mentioned formula:

Moisture Percentage = (Fresh seed weight-Dry seed weight)/(Fresh seed weight)×100

After getting the desired seed moisture content of 10%, the seeds were packed in an airtight plastic jar and kept at +5ºC temperature in a walk-in seed storage chamber.

2.3 Germination Test

For this study seeds were surface sterilized for 1 minute using 5% solution of NaOCl (sodium hypochlorite). After that seeds were given the following six pre-sowing treatments: T1 (control); T2 (water soaking 24h) Joseph et al. [19]; T3 (mechanical scarification) Yogeesha et al. [20], T4 (mechanical scarification + water soaking 24h), T5 (acid scarification 10 min) and T6 (acid scarification 20 min). Mechanical scarification in combination with water soaking for 24h and H2SO4 (5%) acid treatment for 10 and 20 minutes were new treatments incorporated in the current study. These treated seeds were set for germination in petri dishes (Fig. 2) using the top of the paper method, and these petri dishes were then placed inside the germinator at a temperature of 25±1ºC and relative humidity of 80%. Six pre-sowing treatments were tested in four replications (25 seeds for each replication). Germination data were recorded daily for 25 days. After that, germinated seeds were transferred to root trainers for about 1 month, and various growth parameters (root length, shoot length, and seedling length) were measured for each treatment. Since all these experiments were conducted in a homogenous laboratory condition, hence Completely Randomized Design (CRD) was used, and data was statistically analyzed using WASP 2.0 software of ICAR (Indian Council of Agriculture Research).

2.4 Mean daily germination (MDG), peak value (pv), germination index (GI)

Mean daily germination is calculated as the cumulative germination percentage of seeds at the end of the test period divided by the number of days from sowing to the end of the test or total days [21].

$$MDG = \frac{\text{Total number of germinated seed}}{Total\ number\ of\ days}$$

Fig. 2. Germinated seeds of *Bixa orellana* L.

The peak value was calculated as the maximum mean daily germination reached at any time during the period of the test [21]. In order to compute the germination index, the total number of seeds that germinated at the conclusion of the experiment was divided by the amount of time it took for 50% of the seeds to germinate.

2.5 Seed vigour index

Seed vigour index is calculated by multiplying germination (%) and seedling length. The seed lot showing the higher seed vigour index is considered to be more vigorous [22].

3. RESULTS AND DISCUSSION

3.1 Morphological Parameters and Moisture Content

The annatto fruit is a soft echinate capsule (Fig. 3) that is ovoid or subglobose in shape. Each capsule contains 30 to 50 seeds (Fig. 4) that are tightly packed and covered in a reddish pulp-like substance. The seeds vary in shape from pyramidal to conical. The average length, width and thickness of seeds were 4.53±0.31mm, 3.69±0.27mm and 2.82±0.35mm, respectively, with a seed index (100 seed weight) of 2.83±0.06 gm and moisture content of 10%. Seed parameters can be considered essential traits for the early selection of seed sources. Sharma et al., 2024 observed considerable differences in seed length, seed width and 100 seed weight of Boswellia serrata collected from three different sources. In addition, a significant positive correlation between seed size and seedling growth was reported in Acacia catechu Willd. and Acacia nilotica Willd. in laboratory and nursery conditions [23].

3.2 Effect of Pre-Sowing Treatments

3.2.1 Germination

As per data shown in Table 1, the germination percentage was significantly higher in T2 compared to the T3 treatment. The results were statistically analyzed (CD0.05= 10.96) and found significant difference between T1, T2, T3 and T5, whereas no significant difference was found between T2, T4 and T6. Joseph et al., 2010 reported less than 6% germination in *Bixa orellana* L. at 12% seed moisture content. In the present study, we found about 52% germination in water-soaked seeds at a low seed moisture content of 10%.

Mechanically scarified seeds had a very low germination rate of 19%; however, after soaking the seeds in water for 24 hours, the germination percentage jumped to 47%. Our findings are in line with a study on *Pinus gerardiana* by Sharma et al., 2020, which revealed that soaking mechanically scarified seeds in water improved germination from 19% to 46%. Furthermore, Yogeesha et al., 2005 recorded 37.3% germination of acid-scarified *Bixa orellana* seeds in 10 minutes at a 98% H_2SO_4 concentration. Though the concentration of H_2SO_4 was only 5%, we also observed nearly identical findings in the current investigation, notably 39% for 10 minutes and 42% for 20 minutes of acid scarified seeds.

3.2.2 Mean daily germination (MDG), peak value (PV) and germination index (GI)

As per the present study and data shown in Fig. 5, the initial germination started about three days after sowing for T2 and T4, whereas it was five days for T1 and T6 and six days for T3 and T5. MDG was found to be highest for T4 initially

whereas at the end of germination test, T2 treatment exhibit highest MDG (4.6). However, this was followed by T4, T6, T5 and T1 (4.57, 2.36, 2.28 and 1.89 respectively) in descending order. Minimum MDG (1.26) was observed in T3 treatment.

The highest peak value was found in T4 (10), which was followed by T2 (6.8), T6 (4), T5 (3.57), and T1 (2.71), whereas the lowest peak value was found in T3 (1.85). In addition to this, T4 treatment also exhibits the highest germination index (13.48). However, this was followed by T2, T6, T5, and T1 (9.92, 8.37, 4.88, and 4.31, respectively) treatments in descending order. The minimum germination index (2.63) was observed in T3.

The combined effect of scarification and water soaking increased MDG, PV, and GI. These seeds began to germinate sooner and completed germination faster. It could probably be due to the facilitation of cytokinin penetration in the testa and neutralization of inhibitors present in the embryo, thus enabling the embryo to rupture the seed coat [24].

3.3 Seedling Performance

3.3.1 Effect on root length

Data presented in Table 1 reveal a difference between pre-sowing treatments for the root length of *Bixa orellana* L. The T4 treatment exhibited the highest root length. However, this was followed by the T5, T6, T1, and T2 treatments in descending order. The T3 treatment exhibited the least root length.

3.3.2 Effect on shoot length

Data presented in Table 1 reveal a difference between pre-sowing treatments for the shoot length of *Bixa orellana* L. The T1 treatment exhibited the highest shoot length. However, this was followed by the T5, T2, T4, and T3 treatments in descending order. The T6 treatment exhibited the least shoot length.

Fig. 3. Bixa orellana tree bearing fruits

Fig. 4. Seeds of *Bixa orellana* L.

Table 1. Mean ± Standard deviation (SD) for germination percentage, seedling parameters and seed vigour index across different treatments

Treatment	Germination percentage	Root length	Shoot length	Seedling length	Seed vigour index
T1	36±5.65	4.16±0.84	3.83±0.29	7.98±0.77	287.28
T2	52±8.64	3.75±0.50	3.52±0.53	7.27±0.96	378.04
T3	19±6	3.70±0.65	3.28±0.18	6.99±0.42	132.81
T4	47±11.01	5.31±0.57	3.45±0.36	8.75±0.92	411.25
T5	39±6	5.17±0.88	3.67±0.35	8.84±1.06	344.76
T6	42±5.16	4.56±0.74	3.20±0.52	7.76±1.25	325.92
Range	52-19	3.70-5.31	3.20-3.83	6.99-8.84	132.81-411.25

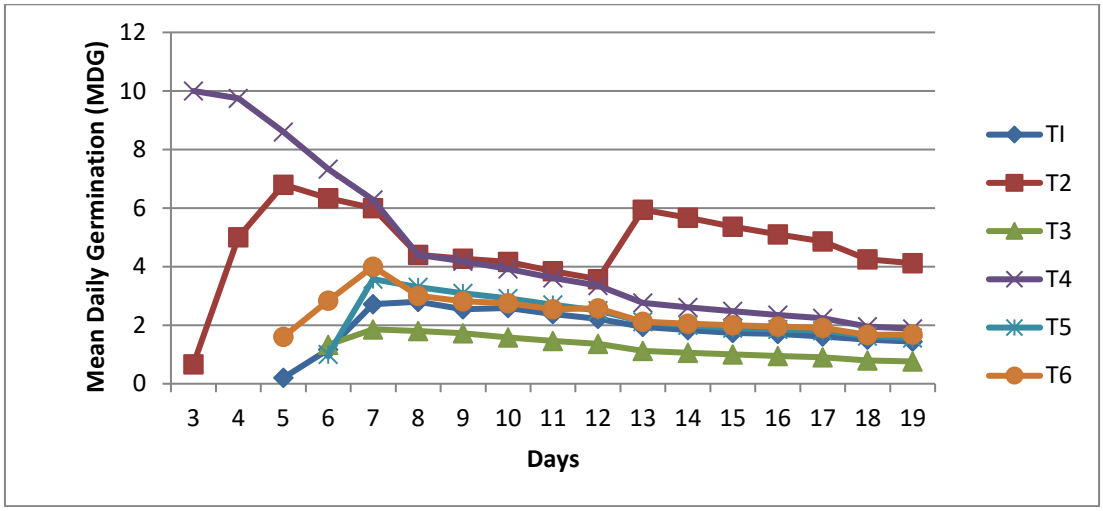

Fig. 5. Mean Daily Germination across different treatments

Fig. 6. Measurement of seedling growth parameters across different treatments

3.3.3 Effect on seedling length

Data presented in Table 1 reveal a difference between pre-sowing treatments for seedling length of *Bixa orellana* L. The T5 Treatment exhibited the highest plant length. However, this was followed by the T4, T1, T6, and T2 treatments in descending order. The T3 treatment exhibited the least seedling length.

3.3.4 Seed vigour index (SVI)

The highest seed vigour index (411.25) was recorded for T4 treatment. However, this was

followed by T2, T5, T6 and T1 treatments in descending order. The lowest seed vigour index (132.81) was recorded for T3 treatment. Similar results were reported by Sharma et al. [25,26] on *Pinus gerardiana*, where mechanical scarification + water soaking treatment shows the highest SVI compared to scarified and non-scarified seeds.

4. CONCLUSION

In conclusion, the study on *Bixa orellana* L. seeds revealed significant findings regarding germination percentage, root length, shoot length, seedling length, and seed vigor index (SVI). T2 treatment exhibited the highest germination percentage, indicating the efficacy of water soaking in enhancing germination rates at a low seed moisture content of 10%. Mechanically scarified seeds showed a considerable increase in germination percentage after water soaking, aligning with previous findings on *Pinus gerardiana*. The combined effect of scarification and water soaking not only improved germination but also accelerated the germination process, possibly by facilitating cytokinin penetration and neutralizing inhibitors in the embryo. Moreover, T4 treatment showed the highest values for root length, shoot length, seedling length, and SVI, highlighting its effectiveness in promoting overall seedling vigor. These results underscore the importance of pre-sowing treatments in enhancing seed germination and seedling growth, which could have implications for agroforestry practices that maximize crop yields. Further, these findings at a low seed moisture content of 10% reveal that annatto seeds are showing orthodox storage behaviour, and these findings will be helpful in developing desiccation and seed storage protocols for medium to long-term conservation of annatto seeds.

ACKNOWLEDGEMENTS

The authors are grateful to the Director of ICFRE-Forest Research Institute, Dehradun, for providing the necessary facilities and infrastructure for the study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Available:https://doi.org/10.9734/arrb/2024/v39i230629

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/115538