Original Research Article

Effect of Various pre-sowing treatments on germination and seedling performance of *Bixa orellana* L. at low seed moisture content

ABSTRACT

In today's world, there is a renewed interest in natural compounds derived from plants for various applications. *Bixaorellana*L. commonly known as Annatto is one such plant that has been used by indigenous communities in Brazil and other tropical countries for its diverse biological properties.The seed coat of Annatto is especially sought-after as it is commercially used to produce a vibrant red-orange dye called bixin. This pigment finds extensive usage in the dairy, medicine, textile, and even animal feed industries. This plant has immense potential to be used as agroforestry species throughout the tropical countries.However, there are conflicting findings regarding its germination behavior in the literature. Most of the studies on germination were conducted at high seed moisture content i.e. above 40%.To fill this research gap we haveconducted a complete studyon seed morphology, germination and seedling performance at low seed moisture content of 10%.This study showed highest germination (52%) and highest Mean Daily Germination (4.6) in 24hrs Water-soaked seeds whereas,Mechanical scarification in combination with 24hrs water soaking increasedthe germination percentage from 19% (Control) to 47%. In addition to this highest Seed Vigour Index(411.25), was also observed in Mechanical scarification in combination with 24hrs water soaking. These findings of germination and vigour at low seed moisture content will certainly help in formation of desiccation protocols for medium to long term conservation of *Bixaorellana* L. seeds.

Keywords:*Bixaorellana*L., Annatto, Achiote,Germination, Vigour, Scarification.

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1. INTRODUCTION

Bixaorellana L. (Family: Bixaceae) commonly known as achiote, annatto or lipstick tree is a valuable, non-toxic dye yielding bushy treenative to Brazil. The Spaniards were the first to introduce it to Southeast Asia in the 17th century (Ulbricht et al. 2012). It is now found in every tropical and subtropical region of the world, including India (Ambika and Poornima 2004, Elias et al. 2002).The bixin concentration of annatto seeds is a key determinant of the seed's price.After caramel, annatto is the second-most significant natural colorant in the world (Kala and Kumaran2015).Annatto finds utility in the pharmaceutical, textile, dairy, food and beverage, paint, and cosmetic sectors (Hirko and Getu 2022,Dequigiovaniet al.2017). Due to its wide utility in different sectors, the plant has immense potential to be used as agroforestry species throughout the tropical countries.

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This plant has a normal economic life of 20-25 years (Castello et al. 2012), making it a good 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 (Kumar et al. 2012). Seed sowing is widely used as a mode of propagation and one of the main propagations issue is poor and delayed seed germination due to dormancy (Eira and Mello 1997, Goldbach 1979, Idu 1994, Sharon and D'Souza 2000).There is very little information available on germination combined with seedling performance of this species.Most of the studies on germination were conducted at high seed moisture content i.e. above 40%. Hence to overcome this gap present study were 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 *Bixaorellana* L. collected from Gomarda wildlife sanctuary, Chattisgarh, India (N 21^{o}33'51" and E 83^{o}16'08")in the month of December 2022.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^{o}28'11.72" and E 78^{o}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 seeds that were randomly chosen in 4 replications for biometric determination. The width was measured in the midline of the seeds, and the length was measured from the base to the apex(Cheib 2009). 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

The moisture content of seed was determined by using hot air oven method. In which 4 replications (2 gm per replication) of seeds were Ovendried at a temperature of 103±2^{o}C for 17±1 hours (ISTA 2010).Moisture content was calculated by using below mentioned formula:

$$\text{Moisture Percentage} = \frac{\text{Fresh seed weight} - \text{Dry seed weight}}{\text{Fresh seed weight}} \times 100$$

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 following six pre-sowing treatments: T1 (Control), T2 (Water Soaking 24h), T3 (mechanical Scarification), T4 (Mechanical Scarification + Water Soaking 24h), T5 (Acid scarification 10 min) and T6 (Acid scarification 20 min). Double-distilled water was used for water soaking, sandpaper for scarification, and H_2SO_4 (5%) for acid treatment. These treated seeds were set for germination in petri-dishes (Figure 1) using top of the paper method and thesepetri-dishes were then placed inside the germinator at temperature 25±1^{o}C and relative humidity 80%. Six pre-sowing treatments were tested in four replications (25 seeds each replication). Germination data were recorded on daily basis 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 measuredfor each treatment.Since all these experiments were conducted in a homogenous laboratory condition,

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hence Completely Randomized Design (CRD) was used and data was statistically analyzed using WASP 2.0 software of ICAR (Indian Council of Agriculture Research).

Figure 1: Germinated seeds of *Bixa orellana* L.

2.4 Mean daily germination (MDG) and Peak Value (pv)

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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 (Czabator 1962). Peak value was calculated as the maximum mean daily germination reached at any time during the period of test (Czabator 1962).

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

2.5 Seed Vigour Index

This is calculated by determining the germination percentage and seedling length of the same seed lot. 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 (Abdul-Baki and Anderson 1973)

3. RESULTS AND DISCUSSION

3.1 Morphological parameters and moisture content

Figure 2. *Bixa orellana* tree bearing fruits.

The annatto fruit is a soft echinate capsule (Figure 2) that is ovoid or subglobose in shape. Each capsule contains 30 to 50 seeds (Figure 3) 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 were 4.53±0.31mm,3.69±0.27mm and 2.82±0.35mm respectively, with a seed index(100 seed weight) of2.83±0.06 gm. The moisture content of seed was found to be 10%. Seed parameters can be considered as important traits for early selection of seed sources. Sharma et al. (2024) observed considerable difference in seed length, seed width and 100 seed weight of *Boswellia serrata* collected from three different sources.In addition to this, significant positive correlation between seed size and seedling growth has earlier been reported in*Acacia catechu* Willd. and *Acacia nilotica* Willd. in laboratory as well as in nursery conditions (Khera et al. 2004).

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Figure 3. Seeds of *Bixa orellana* L.

3.2 Effect of pre-sowing treatments

Table 1: 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

3.2.1 Germination

As per data shown in Table 1, Germination percentage was found to be significantly highest in T2 (Water soaking 24h), as compared to T3 (Mechanical scarification) treatment.The results were statisticallyanalyzed ($CD_{0.05}$= 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)reported82%germination in water soaked seeds of*Bixaorellana*L. at 45% moisture content.Whereas, in present study we found about 52% germination in water-soaked seeds at a low moisture content of 10%.

3.2.2 Mean Daily Germination (MDG) and Peak Value (pv)

As per present study and data shown in Figure 4, the initial germination started in about 3 days after sowing for T2 (Water soaking 24h) and T4 (Mechanical Scarification + Water Soaking 24h) whereas it was 5 days for T1 (Control) and T6 (Acid scarification 20 min); 6 days for T3 (mechanical Scarification) and T5 (Acid scarification 10 min);. MDG was significantly highest for T4 initially whereas at the end of germination test, T2 treatment exhibit highest Mean Daily Germination (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. In

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addition to this highest peak value was found in T4 (10) which was followed by T2 (6.8), T6 (4), T5 (3.57), T1 (2.71), whereas lowest peak value was found in T3 (1.85).

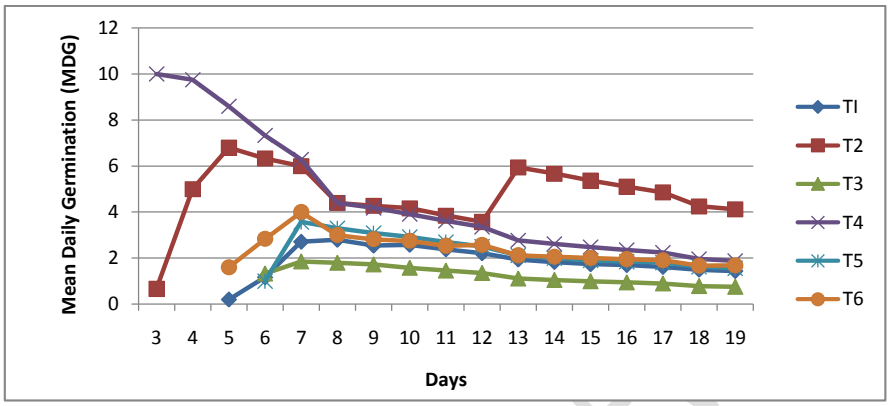

Figure 4: Mean Daily Germinationacross different treatments

3.2.3 Seedling Performance

3.2.3.1 Effect on Root length

Data presented in Table1, reveal a difference between pre-sowing treatments for Root length of *Bixaorellana*L.The highest Root length was exhibited by the T4 treatment. However, this was followed by T5, T6, T1 and T2 treatments in descending order. The least root length was exhibited by the T3 treatment.

3.2.3.2 Effect on shoot length

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

3.2.3.3*Effect on Seedling length*

Data presented in Table1,reveals a difference between pre-sowing treatments for seedling length of *Bixaorellana*L.The highest Plant length was exhibited by the T5 treatment. However, this was followed by T4, T1, T6 and T2 treatments in descending order. The least seedling length was exhibited by the T3 treatment.

Figure 5: Measurement of seedling growth parameters across different treatments

3.2.4 Seed Vigour Index (SVI)

The highest Seed Vigour Index (411.25) was recorded for T4 (mechanical scarification + water soaking) treatment. However, this was followed by T2 (water soaking), T5 (acid scarification for 10 minutes), T6 (Acid Scarification 20min) and T1(Control) treatments in descending order. The lowest seed vigour index (132.81) was recorded for T3 (mechanical scarification) treatment. Similar results were reported by Sharma et al., (2020) on*Pinus gerardiana*, where mechanical scarification + water soaking treatment shows the highest SVI as compared to non-scarified seeds.

4. CONCLUSION

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From present study we can conclude that at low moisture of 10% *Bixaorellana* L. seeds shows good germination i.e. above 50 percent if seeds were soaked in water for 24 hrs. However simple mechanical scarification with sand paper showed least germination and vigour. The possible reason for this could be attributed to various factors, including embryo decay or drying. Hence we recommend using mechanical scarification in combination with water soaking to enhance the germination and seedling performance of *Bixaorellana* L. These finding at low seed moisture content of 10% will be helpful in developing desiccation and seed storage protocols for medium to long term conservation of Annatto seeds.However further investigation is necessary to determine the exact reasons behind the lower germination rate in T3 treatment (Mechanical scarification). Conducting additional studies can help identify the specific factors contributing to the observed germination challenges and provide insights into potential solutions to improve germination in the mechanical scarification treatment.

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