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Effect of Seed Fortification With GA_3 for Quality Seedling Production in Annatto *(Bixa orellana* L.)

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ABSTRACT

Pre-sowing seed treatments study was designed to evaluate impact of soaking hours and role of GA_3 on quality seedling production in *Bixa orellana*. In the present study seeds soaked in cold water for 18 hours soaking which took 15 days for quick seed vigour in seed germination Accordingly it indicated that 18 hours seed soaking duration treatment has enhanced seed softening, quick seed germination and seedling quality traits than other treatments. Among the treatments, the seeds soaked in GA_3 200 ppm for 18 hours recorded the highest values for seedling per cent and other seed vigour parameters and it was followed by GA_3 150 ppm. Among the treatments studied, T_4 (200 ppm) and T_5 (250 ppm) recorded early germination, took 9 days to initiate germination, whereas control (T_6)) took maximum (23.8) days to germinate. Maximum germination registered with GA_3 200ppm (T_4 - 64.74 %) while minimum was recorded with control (T_6 - 13.67 %). It enhance seedling vigour parameters at nursery studied through like root length, shoot length, collar diameter, dry matter production and vigour index of seed fortified with GA_3 200 ppm escalated over control. Hence, seed fortification with GA3 200 ppm for 18 hours duration is highly useful and easily adoptable for enhanced quick and high quality seedling production especially by commercial nursery growers on *Bixa orellana.*

Key words: *Annatto, Bixa orellana, Edible Dye, Seed Fortification, Vigour Index, Quality Seedling Production, Commercial Nursery.*

Introduction

Colouring of food items is inevitable step in culinary of Indian food history and culture veracity in Indian and Indians have been considered as forerunners in the art of natural dyeing. Annatto dye is basically a red orange pigment known as Bixin (natural carotenoid) extracted from the seed coat of *Bixa orellana.* Annatto seeds are the world's second most important natural colorant after caramel yielding yellow to red colours (Mercandampe and Ptander, 1998). The colours may reach up to 7 per cent of the seeds dry mass (Kutzer, 1999). Being a safe, economical and easy-to-use product, among naturally occurring colorants, annatto ranks second in economic importance, with an average world consumption of 10,650 tons every year (Satyanarayana *et al*., 2003). Bixin is a seed - specific pigment widely used in foods and cosmetics since pre-Columbian times. Natural dyes are biodegradable, non-toxic, aesthetically appealing and may serve a better alternative to generate employment and utilize the wastelands (Kala and

Kumaran, 2012). Natural dyes are obtained from plant, animal and mineral resources. Nowadays, fortunately, there is increasing awareness among people towards natural products. Due to their non-toxic properties, low pollution and less side effects, natural dyes are used in day-to-day food products. Restriction placed by WHO for production and use of toxic dyes possessing carcinogenic properties that has resulted in a search for an alternative by major dyestuff manufacturers.

Bixa orellana is commonly known as Annatto or Lipstick plant. It belongs to Bixaceae family; it is native to tropical Central and South America but has become naturalized in many countries of Africa and Asia. Indian climate and soils are highly favourable for its naturalization and it is well distributed in Karnataka, Andhra Pradesh, Tamil Nadu, Orissa, West Bengal, Gujarat, Maharashtra, Madhya Pradesh and Chhattisgarh and also reported to be cultivated commercially since last five years. Annatto being tropical plant can adapt well under 28^0 to 44^0C temperature and 800-1500 mm annual rainfall. It cannot withstand severe cold climates. The economic life of plants is about 20-25 years. Annatto can be easily propagated through seeds. The crop is now fetching importance in food industry as the seeds are directly powdered and mixed with food products for their attractive colour. Dye derived from seeds is also used for body paint by South American Indians; chemically similar to beta carotene and may protect skin from *UV* light. Seeds are eaten cooked in butter. Bark provides fiber. It is also a good hedge and honey plant.

Recent interest in natural dyes has intensified the evaluation of genetic variability in physical and biochemical properties of annatto seeds. The knowledge of mother tree genetic seed traits is considerable to provide good quality seedling of the species. The study of seed characters with seed bixin content of natural populations is often considered to be useful step in the study of the genetic variability. Therefore, mother trees having more seed weight and bixin content may be used for further improvement programme (Kala *et al.,* 2017). Annatto can propagate both by seed and stem cutting. But seeds are the principal means of propagation. Seed is the basic unit of well-defined nursery and plantations. The quality characters of seed have to be brought by proper seed handling techniques. Raising quality seedlings also requires technical expertise and careful planning for all the key elements, including high-quality seeds, nursery management practices (Srivasta *et al*., 2023). Appropriate pre-sowing techniques for seed germination can improve germination rate and over all process (Koirala *et al.,* 2000). For production of quality seedlings and also for elite planting, pre-sowing management techniques are highly essential in Annatto. While extending the planting of annatto trees under various agro forestry systems and wasteland afforestation projects large quantity of good quality seeds and seedlings are needed. Even the fresh seeds have germination problems as only 60 per cent of seeds are able to produce normal seedlings. Bixa seeds possess lesser germination (10 %) due to physical dormancy (Nelson *et al*., 2008). The present investigation was therefore taken up in order to identify the better pre-sowing treatment and the optimum length / duration of treatment in enhancing the germination of seeds. In many of the forestry species, seed germination and seedling growth are enhanced by externally applied organic nutrients and growth hormones. Due to lack of sufficient literature, an attempt has been made to increase the seed germination percentage and seedling growth in *Bixa orellana* with externally applied growth regulators. The present study was designed to find out cheap and effective pre-sowing seed treatments to enhance the germination potential of *Bixa orellana*. Since only very limited studies are available, the present investigation was taken up to study the different seed fortification techniques, standardize the suitable treatment to increase germination and quality seedling production. Keeping in view the above, the present study was designed with following objective to evaluate the germination and growth performance of annatto seeds using laboratory and nursery experiments.

Materials and Methods

Standardization of seed pre-sowing and seed fortification treatments

The bulk sample seeds which were collected identified mothers trees and soundful pre-sowing treatment based detailed studies were undertaken at seed laboratory cum nursery were at Forest College and Research Institute, Mettupalayam, Tamil Nadu with aim to raise high quality seedling production in *Bixa orellana* at large scale for easy adaptation and cost-efficient method by commercial nursery growers.

Standardization of soaking hours

The bulk seeds collected from superior mothers trees were soaked in water in equal volume of seed for following duration and given in table and were dried back to their original moisture content of 10 ± 0.5 per cent. The treated seeds along with control were sown in 100 seeds of 4 replicates in raised nursery bed and evaluated after 30 days after sowing for the following characters.

Table 1. Standardization of seed soaking duration for *B.orellana*

Treatments	Seed soaking duration in cold water
T_1	10 hours
T_2	12 hours
T_3	14 hours
T_4	16 hours
T_5	18 hours
T_6	20 hours
T_7	22 hours
T_8	24 hours
T_9	Control

Influence of seed fortification treatment with GA_3

The bulk seeds were soaked in different concentrations of GA_3 (50, 100, 150, 200 and 250 ppm) in solution for a duration of 18 hours and were dried back to their original moisture content of 10 ± 0.5 per cent. The treated seeds were sown @ 100 seeds of four replicates were raised in plastic containers and after one month they were evaluated for their seed quality characters *viz.,* seedling percent (%), root length (cm), shoot length (cm), dry matter production 10 seedlings^{-1} (g) and vigour index (I) as detailed in given Table 2.

Table 2. Details of seed fortification treatment for *B.orellana*

Treatments	Concentration of GA_3
T_1	50 ppm
T_2	100 ppm
T_3	150 ppm
T_4	200 ppm
T_5	250 ppm
T_6	Control

Parameters was observed in the study

Days to initial germination

This study was conducted with help of seed germination paper at laboratory condition with ample moisture, light and areation. Days taken for initiation of germination identified through visual hypocotyls bent was observed for each of the treatment replication wise and the mean expressed as days to initial germination.

b. Germination (%)

Germination test was conducted in seed germination paper method and plastic tray methods using sand medium prepared as per ISTA (1999) using 4 x 100 seeds (without seed treatment) and were kept in germination room maintained at 25 ± 2°C and 95 ± 2 per cent RH. After the germination period of 11 days, based on the normal seedling to the total seeds placed for germination was calculated and the mean expressed in percentage.

c. Root length (cm)

After the germination period, 5 normal seedlings were selected at random and the distance between the collar regions to tip of the primary root was measured and the mean value expressed in centimeter.

d. Shoot length (cm)

The seedlings used for root length measurement were again measured for the distance between collar region to tip of the primary shoot and the mean expressed in centimeter.

e. Dry matter production seedlings^{-5} (mg)

The five seedlings used for the growth measurements were dried under shade for 24 hours and then dried in a hot air oven maintained at 85 ± 2°C for 24 hrs and cooled in desiccator for 30 minutes weighed and the mean weight expressed in milligram.

f. Vigour index

Vigour index values were calculated as per the following formula (Abdul-Baki and Anderson, 1973) and the mean value expressed as whole number.

Vigour index = Germination percentage X Total seedling length (cm)

The data of the laboratory tests and nursery observations were analysed using 'F' test of significance following the methods described by Panse and Sukhatme (1978).

Results and Discussion

In seed invigoration techniques involves pre-sowing and seed fortification treatments which improves seed germination and seed vigour and seedling per-

formance. Likewise, soaking duration played a major role to enhance the seed germination and seedling vigour. Germination was maximum with 18 hours soaking (68.50 per cent) followed by 20 hours soaking (53.00%). The seedling quality parameters *viz*., root length, shoot length, collar diameter, dry matter production and vigour index recorded the maximum value with 18 hours soaking and the minimum values with control (Table 3 and Fig.1). Highly significant variation was recorded among the soaking durations with cold water for all the evaluated seed and seedling quality characters (Table 3). Among the treatments, seeds soaked in water for 18 hours (T_5), 20 hours (T_6) 22 hours (T_7), took 13 days for initial germination. But control (T_9) took 26 days for initial germination. Days for complete germination also followed the same trend. Germination was maximum with 18 hours soaking (T_5 - 68.50 %) followed by 20 hours soaking (T_6 - 53 %) whereas control (T_9) recorded 11.50 per cent. The hard seed per cent was found maximum in control (T_9).

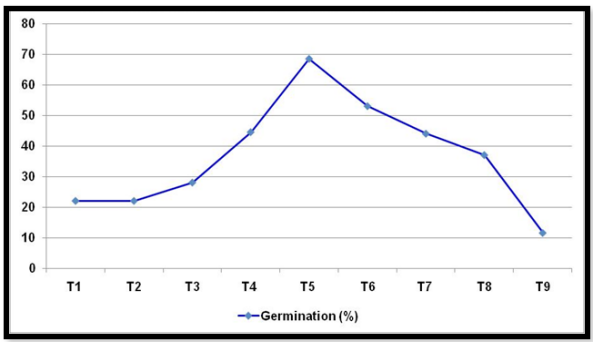

Fig. 1. Influence of seed soaking duration an seed and seedling traits

The seedling quality parameters *viz*., root length, shoot length, collar diameter, dry matter production and vigour index recorded the maximum values with 18 hours (T_5) soaking recording 7.75 cm, 8.55 cm, 0.97 g and 1116.78 respectively. This was followed by 20 hours (T_6) soaking for all evaluated parameters, while the minimum values recorded with control (T_9). The cold water soak for 24 hours yield better than cold water soak for 48 hours in *Albizia lebbeck* (Bhardwaj *et al*., 2007) which lend support to the present study.

Thus the study expressed that seed soaking in water for 18 hours seed soaking enhanced seed germination and seedling quality characters of fresh seeds of *Bixa orellana* at nursery. The pre-sowing treatments initiated early germination and reduced period of germination by facilitating enhanced imbibitions of water in to cotyledons and hastened the bio-chemical reactions which intern increased the mean daily germination (Krishna *et al.,* 2011). Kathiravan (2004) attempted the germination improvement techniques in *Jatropha curcas* and revealed that, soaking of seeds in cold water for 12 hours and in hot water for 45 min. improved the germination by 10 to 12 per cent compared to control.

Germination potential of the seeds differed significantly due to the plant growth regulator and its concentration. In general, the treated seeds recorded higher and earlier germination compared to control. Highly significant variation was recorded among the seed fortification treatment for all the evaluated seed and seedling quality characters (Table 4). Among the treatments, seeds soaked in GA_3 irre-

Table 3. Influence of soaking duration on seed and seedling traits

Treatments	Number of days to initiate germination	Germination (%)	Root length (cm)	Shoot length (cm)	Dry matter production/ seedlings^{-5} (mg)	Vigour index (I)
T_1 – 10 h	23.50	22.00	2.98	4.28	0.80	158.88
T_2 – 12 h	21.50	22.00	3.43	4.73	0.86	179.78
T_3 – 14 h	19.00	28.00	3.85	5.48	0.90	261.65
T_4 – 16 h	18.50	44.50	4.88	6.38	0.93	500.88
T_5 – 18 h	15.00	68.50	7.75	8.55	0.97	1116.78
T_6 – 20 h	13.00	53.00	6.45	7.35	0.95	732.25
T_7 – 22 h	13.00	44.00	5.28	6.93	0.87	536.95
T_8 – 24 h	13.00	37.00	4.00	5.98	0.80	369.05
T_9 – Control	26.00	11.50	2.88	3.55	0.75	71.18
SEd	0.91	2.01	0.23	0.16	0.01	26.34
CD (0.05)	1.87	4.13	0.47	0.33	0.02	54.05

spective of concentration, recorded early germination and 200 ppm of GA_3 took nine days to initiate germination and control took maximum of 23.8 days to germinate. Germination registered maximum with GA_3 200 ppm (64.74 %) followed by GA_3 150 ppm (59.88 %) in stored seeds while minimum was recorded with control (13.67%) (Table 4). The root length recorded was the shortest (3.94 cm) with control which, was followed by GA_3 50 ppm (9.86 cm) while and it was the longest (20.60 cm) with GA_3 200 ppm followed by GA_3 250 ppm (16.99 cm). The shoot length was the longest (13.47 cm) with GA_3 200 ppm while the shortest (4.95 cm) with control (T_6). Accumulated dry matter production and computed vigour index was also maximum with GA_3 200 ppm (1.74 g and 2205.26) (Table 4, Fig. 2 and Photo 1).

The increase in germination due to GA_3 fortification had been claimed to be due to the breakdown of starch and other substrates that induced the enzyme action, the first step of the germination process which created an ability to overcome a metabolic block in the embryonic axis and endosperm (Pandey and Sinha, 1999). The higher seedling quality characters *viz.*, root length, shoot length, drymatter production and vigour index values observed with seeds fortified with GA_3 also could be attributed to the increase in cell division and proliferation to root and apical meristem tissues as discussed by Das *et al*. (1989).

Thus the present study on evaluation of pre-sowing seed treatment for seedling emergence expressed that the enhancement of germination and vigour parameters was made possible due to the softening of the seed and the differential conducive osmatic pressure created by those solution on soaking the seeds in the specific solution. The other possible reason might be the antagonistic effect of growth regulating substances on the growth inhibiting substances present in the seeds and the enhancement in the rate of germination metabolism reported by Jerlin, 1998. Similar results were also revealed by Masilamani and Dharmalingam (1995) in *Grevillea robusta*, that the germination and vigour evaluation had beneficial effects in GA_3 (250 ppm) over the water. Bahuguna *et al.* (1988) also attained maximum germination in *Michelia champaca* seeds due to GA_3 application.

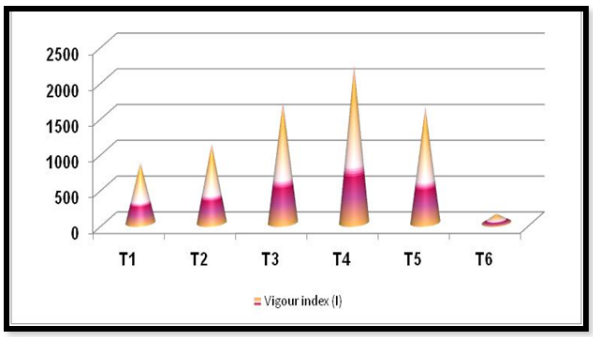

Fig. 2. Influence of seed fortification treatments with GA3

Conclusion

Considering the restrictions placed on the use of synthetic dyes by the World Health Organization, focus on natural dye based products are getting attracted and demand for product is also increasing now a days. Recent research has been intensified on genetic variability, propagation methods and other biochemical properties and its extraction in dye yielding species especially in annatto plant. Our experiment results on pre-sowing seed treatment

Table 4. Influence of seed fortification with GA_3

Treatments	Number day to initiate germination	Germination (%)	Root length (cm)	Shoot length (cm)	Dry matter production/ seedlings^{-5} (mg)	Vigour Index (I)
T_1 – 50 ppm	19.3	46.63	9.86	8.23	1.46	843.25
T_2 – 100 ppm	15.8	51.38	12.01	9.50	1.55	1104.88
T_3 – 150 ppm	12.8	59.88	16.55	11.73	1.61	1692.86
T_4 – 200 ppm	9.0	64.74	20.60	13.47	1.74	2205.26
T_5 – 250 ppm	9.0	55.05	16.99	12.38	1.59	1616.23
T_6 – Control	23.8	13.67	3.94	4.95	0.90	122.07
SEd	0.87	1.28	0.31	0.32	0.03	36.42
CD (0.05)	1.82	2.69	0.65	0.68	0.05	76.52

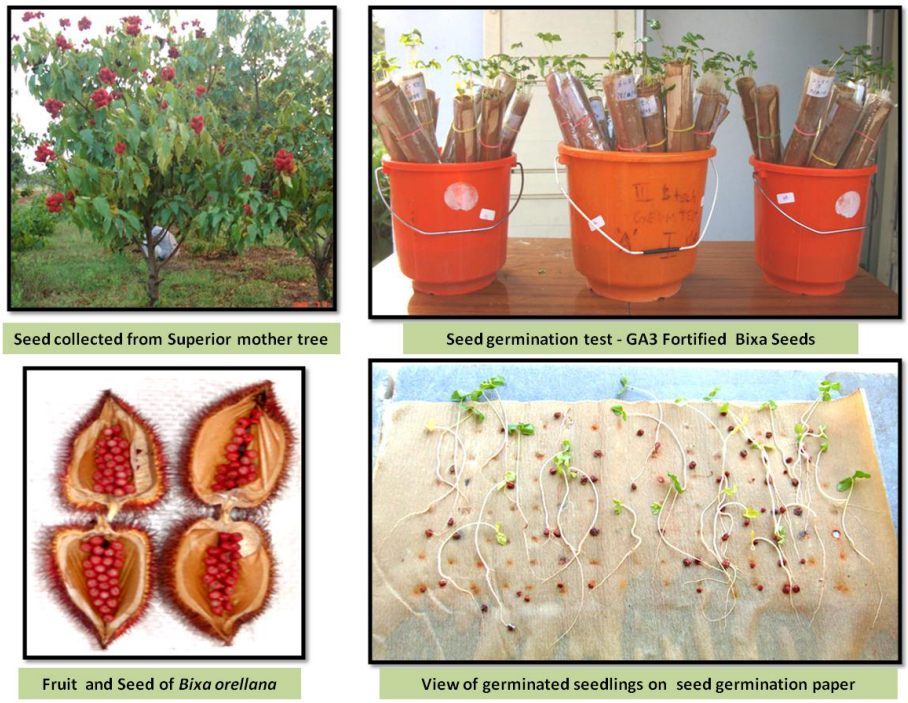

Photo 1. Impact of GA_3 fortified seed germination studies in *Bixa orellana*

evaluation study clearly confirmed that seed germination and seed vigour potential were differed significantly due to the plant growth regulator and its concentration. This was made possible due to the softening of the seed and the conducive osmatic pressure created by those duration on soaking the seeds in the GA_3 - growth promoting specific solution. The lab and nursery studies were confirmed that 18 hours seed soaking duration which enhanced seed germination and seedling quality traits than other treatments. Germination registered maximum with GA_3 200 ppm (64.74 %) followed by GA_3 150 ppm (59.88 %) in stored seeds while minimum was recorded with control (13.67%). Hence, seed fortification with GA_3 @ 200 ppm for 18 hours was enhanced seed germination, seedling vigour and seedling quality traits of fresh seeds of *Bixa orellana*.

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