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European Journal of Experimental Biology, 2012, 2 (1):174-179

ISSN: 2248 –9215
CODEN (USA): EJEBAU

In Vitro Culture studies of *Bixa orellana* L: IV-*In Vitro* and *In Vivo* Trials for Breaking the Dormancy of Seeds of *Bixa orellana*

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ABSTRACT

Harvested seeds of Bixa orellana L. show dormancy due to impermeability of water through its tegument. Wet thermal treatment (soaking seeds in warm water at 70^0 C for one hour and then continue soaking in the same water at room temperature for six hour – ten days), soaking treatment in autoclaved cow-dung water (six hours – ten days) and different plant growth regulators (Kinetin, BAP, IAA, Triacontanol and GA) were given to different sets of seeds to break the dormancy. Warm water treatment for six days showed 90% germination within thirteen days. Whereas autoclaved cow-dung water treatment for six days showed 80% germination within eleven days only. But most of the cow-dung water treated seeds showed heavy bacterial infection and perished. Soaking seeds with 500 ppm Kinetin caused maximum seed germination (90%) in minimum time (eight days). All the tried treatments increased seed germination.

Keywords: *Bixa orellana,* Seed dormancy, Annatto, Kinetin, BAP, IAA, Triacontanol and GA.

INTRODUCTION

Bixa orellana L. (annatto) is a small tree native to Tropical America. It is widely distributed through out the tropics. It has become naturalized in hotter parts of India [1 & 2]. It is a unique plant having massive storage of bixin, a carotenoid, in the aril layer of seeds. Bixin is used as a food colorant for dairy products, in cosmetics and as dye in textiles. Moreover all parts of *B. orelana* have medicinal value and are widely used in Ayurvedic medicine [3 & 4] . Fruits of *B. orellana* contain seeds of different sizes and all of them do not germinate. The seeds are harvested during winter season. *B. orellana* is propagated by seeds [5]. The seeds have thin papery green cotyledon and albuminous endosperm. During seed germination the albuminous endosperm swells and attracts microbial and fungal infection and often fails to germinate. The germination is epigeal and progenies from seed grown plants are mostly homozygous.

It has been reported [6] that mature seeds of *B. orellana* are dormant when harvested and this dormancy is caused by impermeability of seed coat to water. They used thermal, mechanical and chemical methods to break dormancy and found that heat treatment caused loss in seed viability. Hence in present work to break the dormancy a wet thermal treatment, cow-dung water treatment and plant growth regulator treatment have been tried and reported. Germination of treated seeds has been checked under *in vitro* conditions.

MATERIALS AND METHODS

Seeds collected from Dapoli (Maharashtra, India) were pre-treated with 1% Bavistin (a broad-spectrum fungicide) along with 400 ppm Chloramphenicol (an anti-bacterial agent) for three hours, on rotary shaker at 100 rpm and then

washed with autoclaved water under sterile conditions. The pre-treated seeds were further sterilized with 2% sodium hypochlorite and few drops of Tween 20 for 10 min, followed by three rinses with autoclaved distilled water. Final sterilization was done by treating the seeds with 0.2% mercuric chloride for 7 min and then three thorough rinses with autoclaved double distilled water.

For breaking the dormancy, sterilized seeds were given three types of soak treatments. Hundred seeds were used for each treatment.

i. *Wet Thermal Treatment:* Seeds were soaked in autoclaved warm water (70^0C) for one hour and then kept in same water at room temperature for different duration ranging from six hours to ten days.

ii. Cow-Dung Water Treatment: Cow-dung was dried under sun for three days then autoclaved at 121^0C and 15 lb./sq.inch for 20 min.100 g of sterilized cow-dung was added to 1000 ml autoclaved water. It was swirled and mixed thoroughly. Cow-dung was then allowed to settle, water was decanted and re-autoclaved. Sterilized seeds were soaked in autoclaved cow-dung water for different time duration ranging from six hours to ten days.

iii. Plant Growth Regulator Treatment: Seeds were soaked in different concentrations (0 – 1000 mg/l) of Gibberellic Acid (GA), Benzyl Adenine (BA), Kinetin (Kin) and Triacontanol (Tri) for one day.

Treated seeds were initially inoculated on three different culture media i.e. ½ MS, MS [7] and B5 [8] and incubated in dark at 25^0C ± 2^0C till the emergence of radicle. Once the radicle emerged from the seeds, they were transferred to 3000-Lux light intensity with a sixteen hour h photoperiod followed by an eight hour dark period and allowed to grow. Percentage seed germination, time taken for germination and seedling growth was recorded. Since MS medium showed best germination, all the results reported below are of the seedlings grown on MS medium only.

RESULTS AND DISCUSSION

Wet Thermal Treatment: With increase in soaking time there was an increase in germination percentage till sixth day after that it started declining. Seeds soaked in water for six days showed 90% germination, while seeds soaked for ten days showed 70% germination (figure 1). The radicle emerged after thirteen days when the seeds were treated for six days while the radicles emerged after twenty-one days from seeds soaked for ten days. Moreover better seedling growth i.e. length of radicle and hypocotyl and number of nodes, leaves and lateral roots; was also observed from seeds soaked for six days (figure 3). In all the treatments lateral roots appeared seven days prior to emergence of shoots. It has been reported [6] that thermal scarification was not effective in breaking dormancy as it caused loss of seed viability. Hence, in the present work wet thermal treatment was given, which could break the seed dormancy.

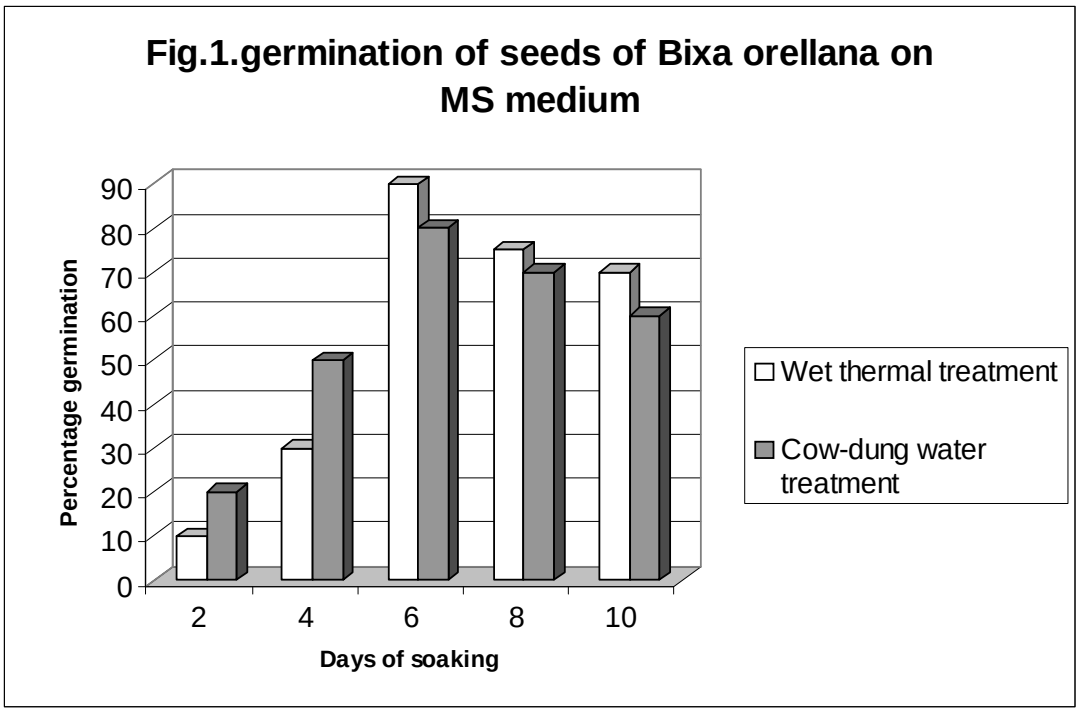


Fig.2. Germination of seeds of Bixa orellana treated with various PGRs for 24 hours, on MS medium

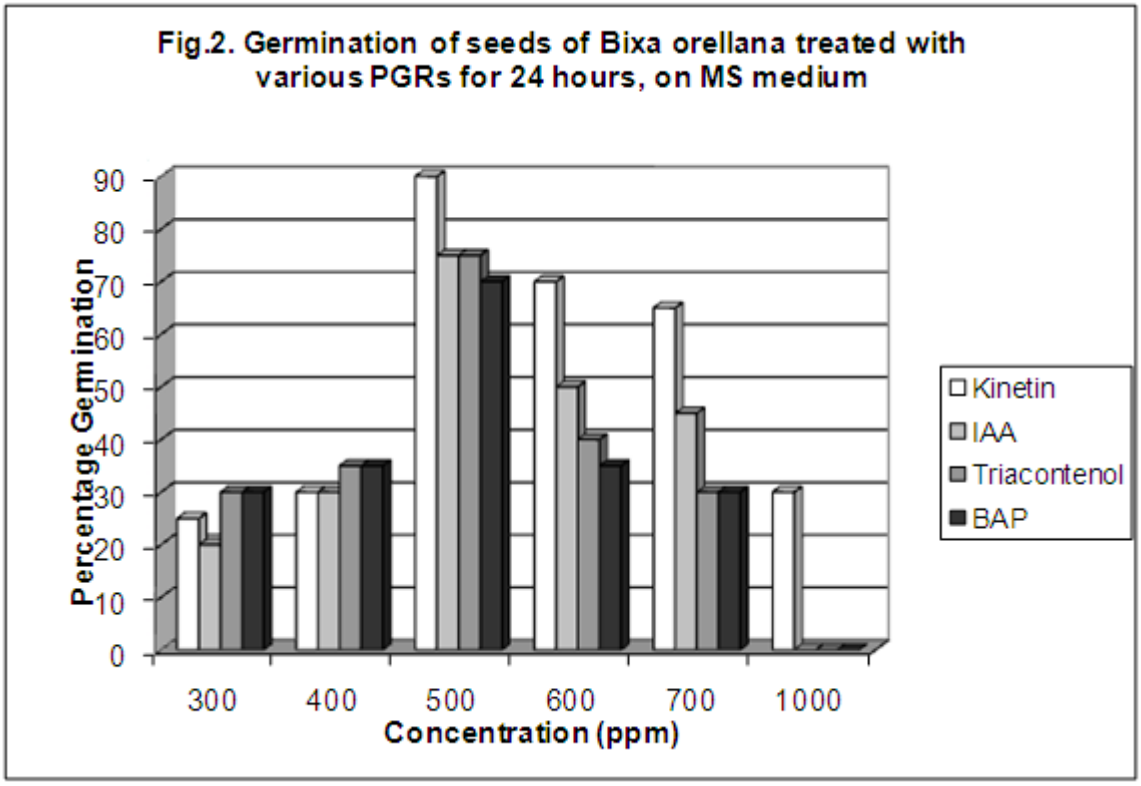


Fig.3. Length of hypocotyl and radicle of 42 days old seelings of Bixa orellana

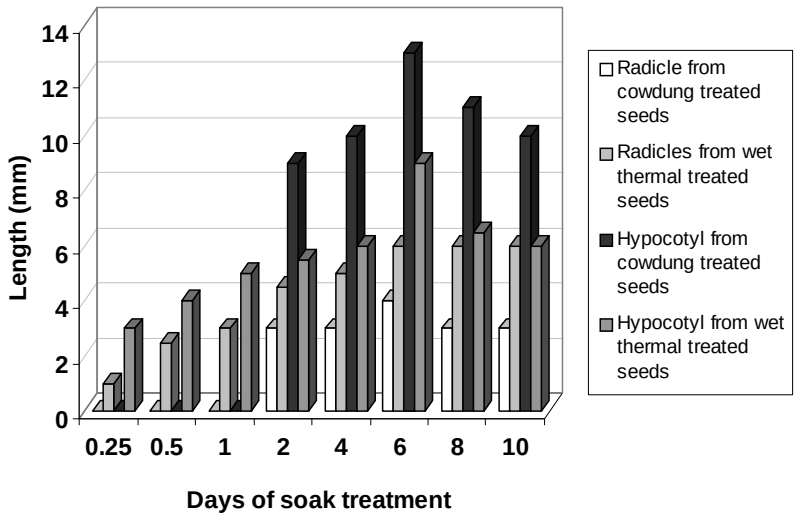


Fig. 4. Hypocotyl and radicle growth of 42 days old Bixa orellana seedlings. (1-hypocotyl & 2-radicle from kinetin, 3-hypo. & 4-rad. from BAP, 5-hypo & 6-rad. from IAA and 7-hypo & 8-rad. from Triacontenol treated seeds).

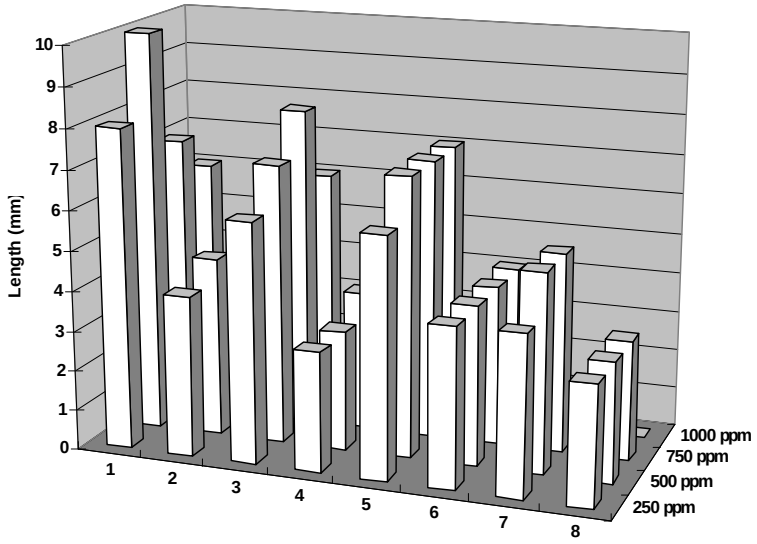
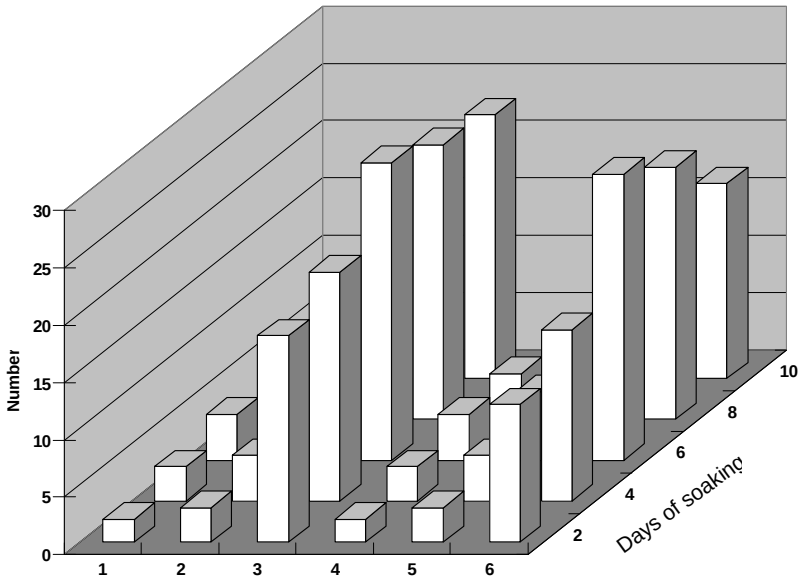


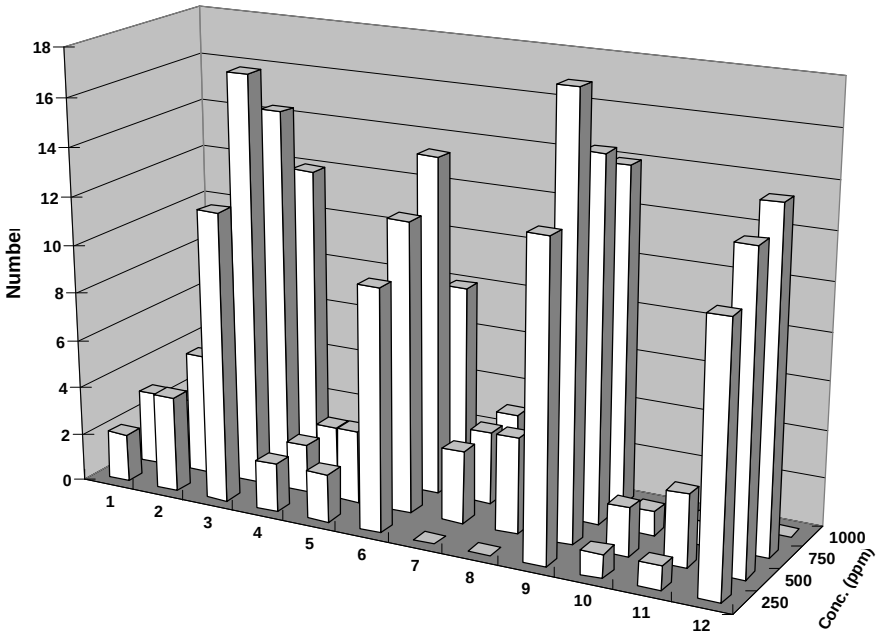
Fig.5. Growth of 42 days old seedlings of Bixa orellena
(No. of 1-nodes, 2-leaves & 3- lateral roots from wet thermal treated & 4-nodes, 5-leaves & 6-lateral roots from Cow-dung water treated seeds)



Cow-Dung Water Treatment: Although cow-dung water was autoclaved, seeds soaked in it had heavy contamination during *in vitro* germination. Infected seeds did start germination and showed 80% germination (figure 1), but soon perished. Radicles emerged after eleven days whereas seeds soaked with warm water for the same period of time took thirteen days. Growths of arial parts of seedlings, which survived and grew, were better than the seedling from wet-thermal treatment (figure 3 and 5). Cow-dung, though is an undefined medium, however it is a rich source of nitrogenous compound; and many nitrogenous compounds have been found to be effective in breaking dormancy such as thiourea [9] and potassium nitrate [10].

Plant Growth Regulator Treatment: GA is known to be an initiator of biochemical activities of seed germination [11]. Moreover there are reports that GA has been effective in breaking dormancy [11]. But in *B. orellana* it did not break dormancy at all. None of the seeds could germinate.

Fig.6. 42 days old Bixa orellana seedlings (No. of 1-nodes, 2-leaves, 3-lat.roots of KIN; 4-nodes,5-leaves, 6-lat.roots of BAP; 7-nodes, 8-leaves, 9-lat.roots of IAA; 10-nodes, 11-leaves, 12-lat.root of Triacontenol treated seeds).



The maximum percentage of germination (90%), amongst all the tried PGRs was observed in seeds soaked with 500 ppm Kinetin (figure 2), which also showed best seedling growth (figure 4 and 6). Cytokinins have been known to stimulate germination [12] and even break dormancy in some plants [13]. Concentrations of Kinetin higher than 500 ppm started declining the growth of seedlings. Moreover it caused senescence of apex in some plants, which resulted in death of seedlings.

500 ppm BA showed maximum (75%) seed germination (figure 2) and number of nodes and leaves (figure 6). Whereas 750 ppm BA treatment of seeds induced best growth of radicle and hypocotyl (figure 4).

IAA could break dormancy and germinated seeds at 500 – 1000 ppm (figure 2); at 500 ppm IAA seed germination was as much as 75%. But about 25% of germinated seedlings showed abnormal swelling at one or more points of the roots and callus formation were also observed from these points. Auxins when applied externally at higher concentrations are known to be associated with swelling of roots [13]. Seedlings grown from seeds treated with 500, 750 and 1000 ppm IAA did not show much difference in length of hypocotyl or radicle (figure 4). As suggested [6]

impermeability of seed coat to water is the cause of dormancy in *B. orellana* and the auxin stimulated growth is closely associated with changes in the plasticity of cell wall [14]. It could be possible that seed coat of *B. orellana* might have responded to auxin treatment with an increase in plasticity, thus allowing the imbibition of water to take place and help in breaking the dormancy and seed germination.

Soaking seeds with 500 ppm Triacontanol resulted in 70% germination of seeds (figure 2). Like IAA, Triacontanol also showed swelling of radicle and hypocotyl, causing abnormal growth of seedlings. Maximum number of nodes and leaves were observed in seedlings grown from 500 ppm Triacontanol soaked seeds and for lateral roots 750 ppm was found to be the best (figure 6) concentration of Triacontenol.

It can be concluded that Kinetin was the best PGR for breaking the seed dormancy as well as seedling growth of *B. orellana.* However at commercial level wet thermal treatment can also be used, as it is much cheaper, though it needs six days of prolonged soak treatment.

Acknowledgements
Authors wish to acknowledge Shr i Jayesh Choksi and Shri Pankaj Pandya of GUFIC BioSciences for the laboratory facilities and financial assistance

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