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Review Article

SEED GERMINATION OF ANNATTO (*BIXA ORELLANA* L.)- A REVIEW

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ABSTRACT

Annatto (*Bixa orellana* L.) is a valuable medicinal plant popularly known as "sinduri" or "lipstick tree". The seed coat of Annatto commercially produce dye, a red orange pigment known as bixin which is used mostly in dairy industry, medicine industry, and textile industry and for animal feed. This species is used in production of phytochemicals like flavonoid, sterols, tannins, bisulphate and essential oil. It has been reported that sinduri is used to treat skin problem, liver disease, hepatitis, prostrate disorder, as diuretic and as antioxidant. *Bixa .orellana* L. is commonly propagated through seeds for large scale cultivation. But the seed coat is characterized by water impermeable testa. So it is very difficult to germinate and it also contains below 40% moisture which are the main cause of slow germination. Germination may be increased by using several pretreatments with water, hormones, growth regulators cowdung, acids etc.

INTRODUCTION

Bixa orellana, commonly used for yielding not toxic synthetic dye recommended by WHO and more than 60% of consumed bio colorant produced from annatto (Vilar *et al*., 2014). The seeds and pulpy layer of seed coat are orange colour due to the presence of bixin. For the enormous demand of organic colour food supplement sinduri are now purchasing priority for conscious fellows. But this plant showed low germination rate in natural environment by many conventional method. To rescue from this problem many workers worked on it, they tried to give optimum viable condition for germination of *Bixa orellana.*

Annatto seeds soaked six to ten days by wet treatments gave significant increase in germination. Many natural common products, like cow dung were used for germination of the plant, they also noticed positive result. Some plant growth promoter (Indole acetic acid, Gibberelic acid and kinetin) were also used as seed germinating stimulant. It was reported that these plant growth promoter influence the seed germination rate of annatto (Castello *et al*., 2012). Time dependent experiment based on relative humidity and moisture content showed very slow increasing slope on seed germination of *Bixa orellana* (Goldbach, 1979). There were many other tests on seed germination of annatto based on weight and size of the seeds showed the mean time of germination increased compare with its weight respectively (Joseph *et al.,* 2010).

Moisture on Germination of B.orellana

Yogeesha *et al*., 2005 reported that the maximum germination was found with high moisture level i.e. 60.9% in freshly harvested seeds and lower moisture contents below 12% induced a dormant state. The germination was also reduced to 6% in 12% moisture content. They also reported that fully-imbibed and sterilized seeds produced maximum germination. Again it was noticed that the seed coat of Annatto is also permeable in nature. So the water uptake by seed coat was steadily progressed and stabilized within 24 h. (Joseph *et al.,* 2010).

Many author reported that the seeds of *B.orellana* showed physiological dormancy (Amaral *et al*., 2000; Amaral *et al*., 1995; Eira and Mello, 1997; Goldbach, 1979; Yogeesha *et al*., 2005). However, physical dormancy sometimes interacts with physiological dormancy and ultimately modifies the seed germination (Black *et al*., 2006). In this regard studying various aspects of germination is important (Joseph *et al.,* 2010).

If the seeds are allowed to dry below 40% moisture content, it become hard seeded i.e. their seed coat are trends to show

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impermeable to water. Freshly harvested mature seeds with 45% moisture content do not show impermeability.

Seed water content and drying period determines the hard seededness ranged from 10% to over 50%. Unclipped seeds germinate very slowly. 18% of the seeds still remained ungerminated but viable keeping one year in the germinator as compared to subsequent germination test after clipping the seeds (Goldbach, 1979).

Seed soaking on germination

Seeds soaked in water for different durations (6, 12, 24 and 48 h) and hormone treatments at different concentrations showed significant response of germination. Water soaking from shorter (6 h) to longer (24 h) durations gave a sharp increase in germination of *B.orellana*. Seed soaked in water for 24 h produce higher seed germination (82%). Prolonged soaking for 48 h however produced less germination (70%). On the other hand insufficient soaking with 6 h significantly decreased the germination rate (Joseph *et al.,* 2010).

Seed drying up to 4.2% moisture content significantly reduced germination. Again decrease is found after two month of storage. Open storage at 30% and 50% relative humidity also diminished the germination rate within one year (Goldbach, 1979). On the other hand sulfuric acid treatment at elevated moisture content (60 %) enhanced the germination and reduced the mean germination time (MGT). Results are in conformity with several other workers in hard seededness (Das *et al*, 2017).

Pretreatment with acid in germination

Germination of *B.orellana* is an important factor during the life cycle of the plant. In Annatto, different results indicated that all pretreatments reduced the percentage of hard seeds as compared to non treated control seeds. The percentage of hard seed reduced gradually as the increment of pretreatment time.

In Annatto, all pretreatments increased the germination rate markedly. Pretreatment with H_2SO_4 in contrast to the control for 15 mins the seed germinates 50% at 9 days. The germination percentage due to pretreatment was reduced as the pretreatment period decreased from 15 min to 5 sec. Further it was found that sulfuric acid pretreatment kill the seeds or populations. When sulfuric acid was used more than 15 mins (30 mins and 1 hour), severe damage in seed was found which leads to less percentage of germination.

Further hydrochloric acid and acetone also found effective in the germination to a certain extent but not fully. In this case many seeds remained hard and ungerminated (Yogeesha *et al*., 2005).

Cow-Dung Water Treatment on germination

Seeds soaked in autoclaved cow dung water did start germination and showed 80% germination. After eleven days radicles are emerged whereas seeds soaked with warm water. Cow-dung is a rich source of nitrogenous compound which is found effective in breaking dormancy such as thiourea and potassium nitrate (Castello *et al*., 2012).

Seed mass on germination

Seed mass showed a significant role in germination capacity of Annatto seeds. Medium sized seeds (0.0275-0.03 g) played greater role for germination in comparison to light or heavy seeds. In light-weight seeds, the lower rate of germination are found due to poor availability of stored food supply (Fox *et al*., 1994; Siril *et al*., 1998) compared to heavier seeds (Castro *et al*., 2007). Due to the hard seed coat low germination of heavy seeds are found (Khan *et al*., 1999; Susko and Lovett, 2000; Tungate *et al*., 2002, Joseph *et al*., 2010).

Gibberelic acid (GA_3) in seed germination of B. orellana

GA_3 acid is an initiator of biochemical activities of seed germination. It was also reported that GA has capacity in breaking dormancy. But it did not break dormancy at all in *B. orellana.* Considering the hormone treatments to GA_3 at 50 ppm exhibited more germination (93%) than those to all other treatments. As such, GA_3 treatment resulted in the remarkable reduction of mean time to germination (MTG) (3.6) when soaked for 24 h. GA_3 treatment is then considered to decrease physiological dormancy of *B. orellana* seeds.

From many previous reports (Gupta, 2003), it was found that the proportion of viable seeds found to be 20% in an annatto seed lot. Further germination was reported ranging from 5-7% (Sharon and D'Souza, 2000). If dormancy breaking plant hormone (GA_3 at 50 ppm) is used then the germination percentage may increase up to 93%. In GA_3 treated seeds, germination was rapid (MTG 3.6) compared to other soaking treatments. A phytohormone like gibberelin regulates various phases of plant development including seed germination. Gibberellins are synthesized *de novo* during germination (Debaujin and Koorneef, 1999) and it causes its effectiveness (Karseen *et al*, 1989). Gibberellins regulate endosperm degradation by inducing enzyme activity (Groot and Karseen, 1987) and it leads to early germination of seeds. GA_3 effectivity may be attributed to hormone action on cell cycle activation (Groot *et al*., 1988).

At the time of water uptake the tissue surrounding the embryo imposed the mechanical restrain relieved by exogenous gibberellins promote the germination (Toyomasu *et al*., 1994). Germination of seeds with coat-induced dormancy can be stimulated by GA_3 (Black *et al*., 2006). GA_3 showed positive effect on germination and has been commercially used in several crops for raising planting materials in nurseries (Calvo *et al*., 2004; Ayele *et al*., 2006).

Physical dormancy breaking treatments are recommended on germination studies of Annatto by several workers (Custodio *et al*., 2003; Gupta, 2003).It was also noticed that an interaction between physiological dormancy and imbibitions operated and could be tackled by 24 h soaking in a 50 ppm GA_3 solution. This treatment evokes germination by triggering synthesis of different enzymes involved in the process. The heavy, medium and lightweight seeds imbibe and achieve saturation within 24 h of water uptake (Joseph *et al*., 2010).

It may be stated that for Annatto seeds, a practical recommendation is soaking of seeds in GA_3 (50 ppm) for 24 h prior to germination. The light-weight seeds should be eliminated to enhance germination in the seed lot. It indicates the importance of seed quality in maximizing germination (Joseph *et al*., 2010).

IAA on Germination

IAA treatment irrespective of concentration performed poor in accelerating germination of *B. orellana* seeds. However, soaking seeds in 10 ppm IAA for 24 h exhibited higher germination (73%) in comparison to 50 ppm and 100 ppm concentration of IAA (Joseph *et al*., 2010). But about 25% of germinated seedlings showed abnormal swelling of the roots and callus formation by IAA treatment. When auxins were applied externally at higher concentrations swelling of roots were found (Sharon and Kishore, 1975, Amaral *et al.,* 1996, Castello *et al*., 2012)

Other Plant Growth Regulators on germination

Seeds soaked with 500 ppm kinetin gave maximum percentage of germination (90%), amongst all the tried PGRs was observed and produced best seedling growth. Cytokinins also showed better effect to stimulate germination and to break dormancy in different plants (Khan, 1971). Kinetin concentration higher than 500 ppm hampered the growth of seedlings. It also caused senescence of apex in some plants, resulting the total damage of seedlings (Castello *et al*., 2012).

CONCLUSION

It is known that the germination of *B.orellana* is problematic. Therefore, different methods as well as fresh seed stock may be considered and study of various aspects of germination is important. The present discussion on *B.orellana* was done to elucidate behavior and mechanisms operating in the germination process. In brief, it has been discussed on water uptake capacity of seeds, effectiveness of seed soaking in gibberellic acid (GA_3) and indole-3-acetic acid (IAA) on germination, the influence of seed mass on germination, and the extent of variability in germination of seeds collected from different sources.

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