

Effect of various pre-treatments for breaking the dormancy of *Bixa orellana* Linn.

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Abstract

In the present investigation, seeds of *Bixa orellana* were subjected to various treatments to achieve early germination by breaking dormancy.

So, the highest germination percentage (87%) was recorded for the seeds treated with IAA followed by 76% which was achieved in the seeds subjected to hot water pretreatment on the 17th day after sowing. The germination percentage was 17, 23, 20, 47, 27, 21, 27, 18, 35, 40, 22, 44, 21 and 64 in the seeds respectively kept as control, subjected to scarification, stratification, alternating high and low temperature, KNO₃, thiourea, kinetin, GA₃, H₂SO₄, pre-soaking, electric current, mechanical injury, coumarin and brassinolide treatments for the same time period. Thus, for achieving higher germination percentage for the seeds of *B. orellana*, treatment with IAA is the best option.

The seeds of some plants easily germinate after sowing in nature but the seeds of a number of plants do not germinate easily and exhibit dormancy for varying period of time. The dormancy may be due to internal factors or may be due to external factors. Certain plants may immediately germinate after the harvest, it can be best exemplified by the seeds of *Pisum sativum*, which sometimes germinate in the fruit itself which is still on the plant, a phenomenon known as vivipary. However, sometimes the dormancy period is very prolonged and can take months together for germination. This is true for the seeds of

Malus domestica which has a hard seed coat and *Entada gigas* which has a very thick seed coat and do not germinate easily.

The plant *B. orellana* which has been chosen for this investigation is a shrub or a small tree with beautiful pink flowers. The seeds are coated with a reddish color often known as *Sindoori*, which is a source of a natural dye. The plant is often grown in gardens for its beautiful flowers, despite producing very large number of seeds, the germination percentage of this plant under natural conditions is very poor.

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According to Berlyn (1972), germination is a sequential series of morphogenetic events that result in the transformation of an embryo into a seedling. The seeds of every plant have the capability to germinate but their germination is affected due to some factors, such as seed coat, hard seed coat, rudiment embryo, over-ripening, presence of plant growth inhibitors, due to absence of water, oxygen and due to unfavourable conditions. Dormancy of seeds is due to external factors or due to internal factors. When it is caused due to internal factors, it is called as true dormancy or innate dormancy or primary dormancy. And when it is caused due to external factors, it is called as imposed dormancy or quiescent dormancy or secondary dormancy. Both of these primary and secondary dormancy influences are mutually dependent and can not be singled out. True dormant seeds do not germinate even if they are provided with suitable environmental factors. Secondary dormant seeds may germinate immediately after shed off. After some storage, they fail to germinate and thus exhibit secondary dormancy. Some seeds such as *Brassica alba*, *Ambrosia tripolia* and *Xanthium pennsylvanicum* exhibit secondary dormancy. Secondary dormancy is opposite to after ripening. Presence of high carbon dioxide concentration, absence of light and very high or low temperature induce the secondary dormancy.

A number of techniques are available for breaking the dormancy of seeds, such as; scarification, exposure to light, alternating high & low temperatures, stratification, impaction, pressure, electric current, pretreatment with coumarins, kinetin, GA₃, H₂SO₄, thiourea, KNO₃ and hot water.

Studies on germination and dormancy of seeds have been carried out by various workers on different types of species. These include; the studies of Shul, (1911) on the oxygen minimum and the germination of *Xanthium* seeds. A detailed account of seed dormancy mechanics was given by Crocker, (1916). Influence of low temperature in improving germination percentage was found out by Conville, (1920). Similarly, alternating temperatures to break the dormancy was used by Harrington, (1923). Morinaga, (1926) has studied the germination of seeds under water.

Davis, (1928) used high pressure to achieve higher seed germination. Denny & Stanton, (1928) suggested chemical treatments for breaking the seed dormancy. Joseph, (1929) investigated the germination and vitality of birch seeds. Barton, (1930) investigated on coniferous seeds. In 1936, Crocker investigated the effect of visible spectrum upon the germination of seeds and fruits. In 1938, Crocker also gave an account of life-span of seeds.

Chouard⁵ has investigated vernalization and its relation to dormancy. Experimental induction of dormancy in *Betula pubescens* was investigated by Eagles & Wareing⁹. Evanari¹⁰, has studied the physiology of seed dormancy, after ripening and germination. Ribosome and enzyme changes during maturation and germination of the castor bean seeds was investigated by Marre¹⁶. Effects of light, temperature and their interaction on the germination of seeds was investigated by Toole²⁷.

Hayes & Klein¹², investigated special quality influence of light during development

of *Arabidopsis thaliana* plants in regulating seed germination. Bewley and Black², studied the physiology and biochemistry of seeds. Isoenzymes of sugar phosphate metabolism in endosperm of germinating castor beans were studied by Nishimura²¹. Seed germination and dormancy have been studied by Bewley³. Improvement of seed germination in *Asparagus racemosus* has been reported by Gupta, *et al.*,¹¹.

Effect of pre-sowing treatment on seed germination of *Babchi* (*Psoralea corylifolia*) and *Senna* (*Cassia angustifolia*) in nursery has been reported by Koppad and Umarbhadsha¹⁴. Seed germination behaviour of *Asparagus racemosus* (*Shatavari*) under *in-vivo* and *in-vitro* conditions has been investigated by Raghav and Kaser²². Siva, *et al.*,²⁵ have studied the enhanced seed germination of *Psoralea corylifolia* L. by heat treatment. Musara, *et al.*,¹⁷ have investigated the evaluation of different seed dormancy breaking techniques on Okra (*Abelmoschus esculentus* L.) seed germination. Asha, and Illa,¹ have studied the effect of seed direction and growth media on *in vitro* seed germination and seedling establishment of *Pterocarpus marsupium*.

Cantoro, *et al.*,⁴ have reported seed dormancy QTL identification across a *Sorghum bicolor* segregating population. Dave, *et al.*,⁶ have investigated the regulation of *Arabidopsis thaliana* seed dormancy and germination by 12-oxo-phytodienoic acid. *Entada phaseoloids* seed dormancy and germination: implications for conservation and restoration has been reported by Deepa, and Shinde,⁷. The effect of the use of temperature

on the breakage of dormancy and the subsequent performance of rice (*Oryza* spp.) has been investigated by Doku, *et al.*,⁸. Transcriptome analysis of seed dormancy after rinsing and chilling in ornamental peaches (*Prunus persica*) has been investigated by Kanjana, *et al.*,¹³.

Effect of different pretreatments and seed coat on dormancy and germination of seeds of *Senna obtusifolia* has been studied by Mensah, and Ekeke¹⁷. Mishra,¹⁸ has investigated the effect of temperature and light on the seed germination of *Sida cordifolia*. Redwood, *et al.*,²³ have reported seed longevity and dormancy state in a disturbance-dependent forest herb, *Ageratina*. Germination pretreatments to break hard-seed dormancy in *Astragalus cicer* L. has been studied by Statwick²⁶.

Effect of various dormancy breaking treatments on seed germination, seedling growth and seed vigour of medicinal plants has been investigated by Warghat, *et al.*,²⁸. Zohra, *et al.*,²⁹ have reported the effect of salicylic acid on germination of *Ocimum gratissimum* seeds induced into dormancy by chlormequat. The release of dormancy, a wake-up call for seeds to germinate has reported by Nee, *et al.*,²⁰.

Healthy seeds of this plant were collected from the seed market (Bhopal). The seeds were washed with running tap water three to four times and once surface sterilized with 0.1% H₂CL₂ solution for 5 minutes to remove the surface adhering microbes. After surface sterilization, the seeds were again washed with double distilled water. Uniform

sized seeds were then transferred to sterilized Petri Plates provided with filter paper pads.

Three replicates of treated and control seeds were kept for germination studies. The filter paper pads were moistened as and when needed. The emergence of radical was taken as germination.

None of the seeds exhibited germination on the 3rd day in control, those stratified and treated with KNO₃ or subjected to electric current and coumarin. The first sign of germination was observed on the 5th day under all treated and untreated seeds except coumarin, however the germination percentage differed. It was 2, 17, 5, 2, 8, 5, 3, 2, 5, 7, 7, 2, 12, 0, 8 and 21 under control, hot water, scarification, stratification, alternating high and

low temperature, KNO₃, thiourea, kinetin, GA₃, H₂SO₄, pre-soaking, electric current, mechanical injury, coumarin, brassinolide and IAA respectively. For this seed, highest germination percentage (87%) was recorded for the seeds treated with IAA followed by 76% which was achieved in the seeds subjected to hot water pretreatment on the 17th day after sowing. The germination percentage was 17, 23, 20, 47, 27, 21, 27, 18, 35, 40, 22, 44, 21 and 64 in the seeds respectively kept as control, subjected to scarification, stratification, alternating high and low temperature, KNO₃, thiourea, kinetin, GA₃, H₂SO₄, pre-soaking, electric current, mechanical injury, coumarin and brassinolide treatments for the same time period. Thus, for achieving higher germination percentage for the seeds of *B. orellana*, treatment with IAA is the best option (table 1).

Table-1 showing the effect of various treatments on the germination percentage of *B. orellana*.

Days after sowing/ Treatment	3rd day	5th day	7th day	9th day	11th day	13th day	15th day	17th day
Control	0	2	5	11	11	17	17	17
Hot water pretreatment	2	17	35	42	58	73	75	76
Scarification	1	5	11	17	17	23	23	23
Stratification	0	2	8	15	16	18	20	20
Alt. High & low temp.	1	8	18	26	33	45	46	47
KNO ₃	0	5	5	12	17	25	27	27
Thiourea	1	3	9	17	20	21	21	21
Kinetin	2	2	7	15	17	26	26	27
GA ₃	1	5	7	12	18	18	18	18
H ₂ SO ₄	2	7	12	17	28	34	35	35
Presoaking	1	7	12	19	27	38	39	40
Coumarin	0	0	5	11	19	21	21	21
Electric current	0	2	8	14	14	20	20	22
Brassinolide	2	18	31	42	51	60	64	64
Mechanical injury	3	12	18	27	27	41	43	44
IAA	5	21	42	56	68	85	87	87

Although, there is abundant seed production in *B. orellana*, the germination percentage under natural conditions is not very high. The plants of *B. orellana* apart from being medicinally important are also in high demand as ornamentals. Among all the treatments to which the seeds of this plant were subjected, IAA was found to be highly stimulant, which resulted in 87% germination on 17th day. Hot water pretreatment and treatment with brassinolide respectively resulted in 76% and 64% germination during the same time span. As pointed out earlier, IAA treated seeds of *Andrographis paniculata* exhibited a higher germination percentage Kumar *et al.*,¹⁵. Thus for *B. orellana* seeds, treatment with IAA is the best option for achieving higher germination percentage (table 1).

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