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Effect of GA_3 treatments and sowing conditions on *ex situ* seed germination of *Oroxylum indicum* (L) Benth. Ex Kurz: A threatened high value medicinal plant

Aseesh Pandey[1,2,3]*, Ngalengshim Ngashangva[2], Sushma Tamta[2,3]

[1]Department of Biodiversity Conservation and Management Group, G.B. Pant National Institute of Himalayan Environment and Sustainable Development, Kosi-Katarmal, Almora, Uttarakhand, India, [2]Department of Biotechnology, Plant Tissue Culture and Molecular Biology Laboratory, Bhimtal Campus, Bhimtal, Kumaun University, Uttarakhand, India, [3]Department of Botany, Plant Tissue Culture Laboratory, D. S. B. Campus, Kumaun University, Nainital, Uttarakhand, India

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ABSTRACT

The objective of this study was to observe the effect of pre-sowing treatments of gibberellic acid (GA_3) and different sowing conditions on *ex situ* seed germination of *Oroxylum indicum*. For this, after pre-sowing treatments, seeds were subjected to three different conditions for their germination. Results revealed that only GA_3 treatments have no significantly different ($P < 0.5$) influence, on seed germination, to control. However, sowing conditions have significant ($P < 0.5$) influences on seed germination, and condition i (*in vitro*) found most suitable for the seed germination. Further, the synergistic influence of treatments and sowing conditions significantly ($P < 0.5$) enhanced the germinationwith rate. The 100 ± 00% seed germination within 7.92 ± 0.58 days having 10.5 ± 0.76 cm seedling height, and 1050 ± 76.38 seedling vigor was recorded in seeds treated with 50 µM GA_3 for 24h and placed under condition (i). Well-developed seedlings were planted in the departmental garden with 90% survival rate. This effort will be helpful for the conservation of this endangered and high-value medicinal tree through *ex situ* reforestation programs, and to harness its potential sustainably.

1. INTRODUCTION

Oroxylum indicum (L) Benth. Ex Kurz (Family: Bignoniaceae) is a medium-sized, deciduous tree with soft and light brown bark [1]. This plant is native to the Indian subcontinent with extended distribution to southeast and south Asian countries [2]. In India, it grows in Himalayan foothills and Eastern and Western Ghats [3], and due to the resemblance of its flowers to the trumpet, it is called "Trumpet tree" or "Syonakh" in India [4]. Nevertheless, it is an endangered tree species in Southern India [5] and cited as IUCN vulnerable medicinal plant [6].

O. indicum possess a diverse range of traditional uses and has been used for centuries to cure various ailments in many Asian countries [7]. The phytochemical investigations of the different parts of the *O. indicum* revealed the presence of approximately 111 compounds, among which flavonoids are the most abundant [8]. Further, each plant part such as root bark, stem bark, leaves, fruits, and seeds of *O. indicum* have high medicinal value [9]. The root bark possesses immunostimulant activity [10]; antiarthritic [9] and the biochanin-α obtained from root bark is known to possesses antifungal and tumor necrosis factor-α (TNF-α) inhibitory action [11]. Moreover, various flavonoids, namely, baicalein, chrysin, Oroxylin A, and scutellarin are also reported from the stem bark and leaves of the plant [12]. Baicalein and chrysin are reported to possess antibacterial, antifungal, anti-inflammatory, antioxidant, antiulcer, antiviral, hepatoprotective, and immunomodulatory activity [13] whereas, Oroxylin A inhibits adipogenesis and induces apoptosis in 3T3-L1 cells [14]. Seeds of *O. indicum* are important constituents of Mu Hu Die (a traditional Chinese medicine), which is widely used in the treatment of cough, acute or chronic bronchitis, pharyngitis, pertussis, and other respiratory disorders [15]. Several Ayurvedic formulations such as Dashmularishta (a compound decoction of 10 roots) and Chyawanprash [16,17] are commercially available that utilize different plant parts of *O. indicum* along with other herbs. These formulations are considered to be effective as anti-anorexic, anti-bronchitis, anthelmintic, anti-inflammatory, anti-leucoderma, and anti-rheumatic elements and also cure viral hepatitis and gallstone disease [18]. Besides, medicinal uses, the young shoots, flowers, and unripe fruits are eaten as vegetables, and the tree is lopped for fodder [19].

At present, a large quantity of raw materials, of this vastly utilized high-value medicinal plant, is being procured from natural forests to meet out the needs of pharmaceutical industries. Due to the indiscriminate collection, overexploitation and uprooting of whole

*Corresponding Author
Aseesh Pandey, G.B. Pant National Institute of Himalayan Environment and Sustainable Development, Kosi-Katarmal, Almora, Uttarakhand, India.
Email: draseeshpandey@gmail.com

plants, this tree has become vulnerable in different states of India such as Karnataka, Andhra Pradesh, Kerala, Maharashtra, Madhya Pradesh, and Chhattisgarh [20,21]. Hence, this species needs domestication, commercialization, and conservation in its natural habitats as well as *ex situ* habitats.

Conventionally, *O. indicum* reproduces through seeds and roots, but the low seed viability and destructive collection of roots limits its natural regeneration [22]. Since this plant has high growth rate and considered good for afforestation purpose, it is needed to study the seed germination behavior of this multipurpose plant in *ex situ* conditions. There are few reports on *in vitro* propagation of *O. indicum* [19,22,23] are available. Nevertheless, a comprehensive and reliable seed germination procedure is lacking for this high-value medicinal plant. Therefore, the present study is an attempt to discourse these issues by evaluating the effect of pre-sowing treatment of gibberellic acid (GA_3) on *ex situ* seed germination of *O. indicum* under different sowing conditions.

2. MATERIALS AND METHODS

Mature fruits containing seeds were collected from elite tree growing in the forests of Ukhrul district, Manipur 978 m asl; 24°55'2.25"N, 94°09'4.9"E (North-East) India and brought to the laboratory situated at Bhimtal, Uttarakhand 1,339 m asl; 29°21'19.52"N, 79°33'5.93"E (North) India. Feathery seeds [Plate 1a] were taken out from the fruit and stored at 4ºC until use. Tetrazolium chloride test was performed to check the viability of seeds [24]. Viable seeds were selected, and surface-disinfected (surface sterilized) by following the method described by Pandey and Tamta [25] with some modifications, the feathery cover of seeds was removed before the treatment, and they were rubbed under tap water until the papery cover removes properly [Plate 1b]. To standardize the concentration of mercuric chloride ($HgCl_2$), seeds were treated with different concentrations (0.01%, 0.05%, and 0.1%) of $HgCl_2$ for the different time interval (3, 5, and 10 min) in the laminar air flow chamber, followed by five rinses of sterilized double distilled water. Seeds were then dried on sterilized blotting paper and used for the experiment.

2.1. Pre-sowing Treatments

Sterilized seeds were subjected to pre-sowing treatments of GA_3 (GA: Duchefa Biochemie, The Netherlands). Seeds were soaked in two different concentrations of GA_3 (50, 100 µM) and introduced to different sowing conditions after 24 h and/or 48 h of GA_3 pre-sowing treatments.

2.2. Sowing Conditions

To identify best *ex vivo* sowing condition, GA_3 pre-treated seeds were transferred into three different sowing conditions; (i) inoculated on to MS (Murashige and Skoog, [26]) basal medium and culture vessels were maintained at 25 ± 2°C under 16/8 h (light/darkness) photoperiod with an irradiance of 60 µ/mol/m^{-2}s outside the culture vessels supplied by cool fluorescent tubes (Philips TL 40 W), (ii) placed over sterilized double layers of Whatman No. 1 filter paper, in sterile Petri plates (90 mm) and wetted with MS medium, at 25 ± 2°C under (16 h light/8 h darkness) photoperiod with an irradiance of 60 µ/mol/m^{-2} s outside the Petri plates supplied by cool fluorescent tubes (Philips TL 40 W), and (iii) sown directly in the soil bed inside ploy house under varying day and night temperature (−1°C-30°C) 60-80% relative humidity and daylight intensity up to 45 µ/mol/m^{-2} s. Observations for seed germination and germination time (days required) were taken every day. Seeds having radicle emergence (≥5 mm) were considered as germinated. Chemicals for MS basal medium and all other add-ons were procured from Himedia Laboratories Pvt. Limited, Mumbai India.

2.3. Seed Germination Parameters Calculation

Total germination percentage (TG) was calculated as TG= (n/N*100), where n= total number of seeds germinated at the end of the experiment and N= total number of seeds used for the germination test. Mean germination time was calculated based on the following equation [27].

Mean germination time=$\Sigma(n\times d)\div N$

Where, n is the number of seeds germinated after each period of incubation in days (d), and N is the total number of seed germinated normally at the end of the experiment.

2.4. Seedling Vigor Index (SVI)

SVI was calculated as per the recommendations of ISTA [28]

Seedling vigor index=GP × SL

Where GP = germination percent and SL = length of seedling at the end of the experiment.

2.5. Statistical Analysis

Experiments were performed in a completely randomized design to determine the effect of treatments and sowing conditions. Each treatment contained 18 explants with three replicates and the data recording time was 60 days for soil condition and 30 days for remaining two conditions. Mean values of the various treatments were subjected to analysis of variance (ANOVA) using SPSS version 20. The level of significance was determined at $P < 0.05$ level, and the means were separated using Duncan's multiple range *post hoc* tests (DMRT) if the values were significantly different.

3. RESULTS AND DISCUSSION

3.1. Seed Viability

Tetrazolium chloride test has consensus with the germination test. The viability of the seeds of *O. indicum* was determined by the tetrazolium test, and it was found 90%. The viability percentage was high probably due to the short gap between the harvesting time and the viability test (3 weeks). Seed viability of *O. indicum* is known to decrease when stored for the longer duration [29].

3.2. Imbibition Capacity

Seeds of *O. indicum* absorbed water rapidly within the first 24 h and reached the maximum after 65 h and thereafter attained saturation (data not shown). Thus, a presoaking treatment time of about 24 h and 48 h used in the present study was considered sufficient for imbibition of GA_3 during pre-sowing treatment.

3.3. Disinfection

Among used concentrations of mercuric chloride ($HgCl_2$) [Figure 1], 5-min exposure of 0.1% $HgCl_2$ found to be the best for seed disinfection and resulted in 100% survival without contamination. Although 3-min application of 0.01% $HgCl_2$ gave 75% disinfected seeds and rate of contamination decreased as the exposure time increases, but 0.1% $HgCl_2$ for 5 min was the threshold, further increase in concentration and time leads blackening of seeds which finally leads mortality in seeds [Figure 1].

3.4. Effect of GA_3 Treatments

Worldwide, the requirement of GA_3 for seed germination is indicated by various studies, in *Arabidopsis* sp. [30]; tomato [31]; watermelon seeds [32], etc. Therefore, different GA_3 pre-sowing treatments were tested for the present study and results related to germination responses are depicted in Table 1. Among used treatments of GA_3, 50µM GA_3 treatment for 24 h induced maximum seed germination 86.11 ± 10.01% with 439.24 ± 307.76 SVI, followed by 100 µM GA_3 treatment for 48 h with 75.00 ± 25.00% seed germination and 287.14 ± 205.71 SVI, respectively [Table 1]. Interestingly, in the present study, seed germination took place in each treatment including control, which indicated that GA_3 is not solely responsible for seed germination in *O. indicum*. Sowing conditions have a significant influence on *ex situ* seed germination in *O. indicum* [Table 2]. The best germination response, i.e., 93.33 ± 4.83% seed germination within 10.18 ± 1.37 days of germination time, having 7.96 ± 0.94 cm seedling height and 760.33 ± 122.27 SVI was recorded in condition (i) [Plate 1c, e]. In condition (ii) except (83.33 ± 8.74%) seed germination, the germination time 18.58 ± 2.49 day; seedling height 1.94 ± 0.10 cm and SVI 162.45 ± 18.64 showed significantly different ($P < 0.5$) responses from condition (i) [Plate 1d]. Seeds under condition (iii) took more time to germinate (29.68 ± 3.65 days) and only 24.99 ± 4.56% seeds were able to germinate within 60 days sowing period. This difference in seed germination response might be due to the difference in light and temperature received by seeds in sowing conditions. In condition (i) and (ii) the light (60 µ/mol/m^{-2} s), temperature (25 ± 2°C), relative humidity (60 ± 5%), and photoperiod (16 h light/8 h darkness), however, condition (iii) received fluctuating temperature (−1°C-30°C), relative humidity (80-40%) during night and daytime and low daylight intensity (45 µ/mol/m^{-2} s). Seed germination and emergence are temperature dependent, and light intensity positively influences the seed germination [33]. The results of present study are close agreement with the results of Motsa *et al.* [33] in which seed germination response of 80 African leafy vegetables was studied.

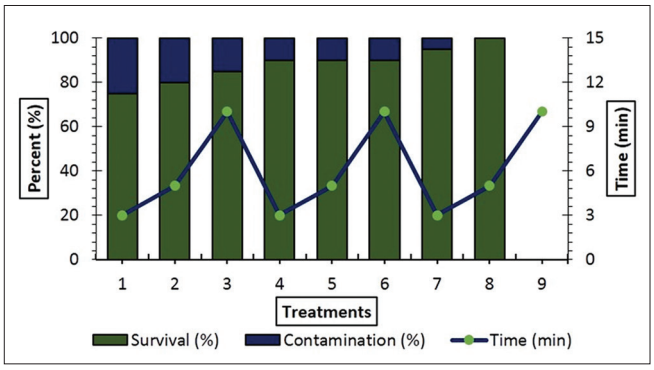

Figure 1: Standardization of mercuric chloride ($HgCl_2$) for seed disinfection treatments 1-3: $HgCl_2$ (0.01%), 4-6: $HgCl_2$ (0.05%), 7-9: $HgCl_2$ (0.1%)

3.5. Synergistic Influence of GA_3 and Sowing Conditions

Seed germination rate varied according to treatments and conditions they received for germination. The germination response was better when seeds received synergetic stimulus of GA_3 and sowing conditions. Germination percent was significantly influenced by the GA_3 treatments as well as sowing conditions and it was observed that in spite of 100 µM GA_3 and control, in which germination percent reached up to 91.67 ± 8.33% and 75.0 ± 14.43%, respectively, in remaining treatments the germination percent (100.0 ± 0.00%) was not significantly different from each other at $P < 0.5$ significance level. Further, the germination time was recorded best (7.92 ± 0.58 days) for seeds those were placed in condition (i) and treated with 50 µM GA_3 for 24 h. Seeds treated with 100 µM GA_3 for 24 h germinated slowly and took maximum time (38.33 ± 4.91 days) to germinate, while in remaining combinations there was no significant difference in germination time [Table 3]. The seedlings height was maximum (10.5 ± 0.76 cm) when seeds were placed in condition (i) and treated with 50 µM GA_3 for 24h and recorded minimum (0.63 ± 0.63 cm) when seeds were placed in condition (iii) and treated with 50 µM GA_3 for 48 h. There was no significant difference at $P < 0.5$ in seedling height when

Table 1: Effect of GA_3 treatments on *ex situ* seed germination in *O. indicum* across different sowing conditions

GA_3 treatments		Germination (%)	Germination time (days)	Seedling height (cm.)	SV
Concentration (µM)	Time (h)				
50	24	86.11±10.01[a]	4.67±2.69[a]	21.53±8.33[a]	439.24± 307.76[a]
100	24	61.11±27.35[a]	4.38±2.19[a]	20.69±8.96[a]	386.25±247.67[a]
50	48	61.11±21.69[a]	3.61±2.48[a]	13.72±2.69[a]	344.99±258.19[a]
100	48	75.00±25.00[a]	3.73±2.12[a]	18.57±5.77[a]	287.14±205.71[a]
-	-	52.78±19.45[a]	2.52±0.98[a]	22.88±3.67[a]	170.36±78.96[a]

SV: Seedling vigor, values representing the mean of three replicates ±SE. Values followed by the same letter within a column are not significantly different and determined by Duncan's multiple range test (P<0.5). Each treatment consisted of 12 explants, *O. indicum: Oroxylum indicum*

Table 2: Effect of sowing conditions on *ex situ* seed germination in *O. indicum* across tested GA_3 treatments

Sowing conditions	Germination (%)	Germination time (days)	Seedling height (cm)	SV
i	93.33±4.85[b]	10.18±1.37[a]	7.96±0.94[b]	760.33±122.27[b]
ii	83.33±8.74[b]	18.58±2.40[b]	1.94±0.10[a]	162.45±18.64[a]
iii	24.99±4.56[a]	29.68±3.65[c]	1.45±0.28[a]	54.00±11.19[a]

Values are representing the mean of three replicates ±SE. Values followed by the same letter within a column are not significantly different and determined by Duncan's multiple range test (P<0.5). Each treatment consisted of 12 explants. Condition (i): MS (Murashige and Skoog; 1962) basal medium, (ii): Placed over sterilized double layers of Whatman No. 1 filter paper containing Petri plates, wetted with MS medium, (iii) sown directly in the soil in bed where in polyhouse. *O. indicum: Oroxylum indicum*, SV: Seedling vigor

Table 3: *Ex situ* seed germination response of *O. indicum* under synergistic influence of GA_3 treatments and sowing conditions

GA_3 treatments		Sowing conditions	Germination (%)	Germination time (days)	Seedling height (cm)	SV
Concentration (µM)	Time (h)					
50	24	MS medium	100±0.00^{e}	7.92±0.58^{a}	10.5±0.76^{c}	1050±76.38^{d}
		Petri plate	91.67±8.33^{de}	20.0±2.52^{ab}	2.00±0.29^{ab}	200±28.87^{ab}
		Soil	25.00±14.43^{ab}	36.67±19.22^{ab}	1.75±0.89^{ab}	67.71±41.71^{a}
100		MS medium	100±0.00^{e}	9.08±0.58^{ab}	8.76±0.42^{c}	875.83±41.77^{cd}
	24	Petri plate	100±0.00^{e}	14.67±0.46^{ab}	2.07±0.34^{ab}	206.67±34.20^{ab}
		Soil	33.33±8.33^{abc}	38.33±4.91^{b}	2.30±0.33^{ab}	76.25±19.93^{a}
50		MS medium	100±0.00^{e}	9.00±0.58^{ab}	8.54±1.79^{c}	854.17±179.17^{cd}
	48	Petri plate	100±0.00^{e}	13.83±0.93^{ab}	1.65±0.22^{ab}	165±21.55^{ab}
		Soil	8.33±8.33^{a}	18.33±18.33^{ab}	0.633±0.633^{a}	15.83±15.83^{a}
100		MS medium	91.67±8.33^{de}	9.33±0.67^{ab}	7.96±2.09^{c}	695.83±115.55^{c}
	48	Petri plate	66.67±22.05^{cde}	17.22±0.94^{ab}	1.78±0.2^{ab}	123.92±35.20^{ab}
		Soil	25.0±14.43^{ab}	29.17±14.59^{ab}	1.27±0.75^{a}	41.67±20.88^{a}
Control		MS medium	75.0±14.43^{de}	15.58±0.36^{ab}	4.47±0.73^{b}	325.83±61.34^{b}
	-	Petri plate	58.33±16.67^{bcd}	27.16±1.64^{ab}	1.42±0.30^{ab}	116.67±50.69^{ab}
		Soil	33.33±22.05^{abc}	25.89±13.53^{ab}	1.31±0.66^{a}	68.58±47.88^{a}

Values are representing the mean of three replicates ±SE. Values followed by the same letter within a column are not significantly different and determined by Duncan's multiple range test (P<0.5). Each treatment consisted of 12 explants. SV: Seedling vigor, *O. indicum: Oroxylum indicum*

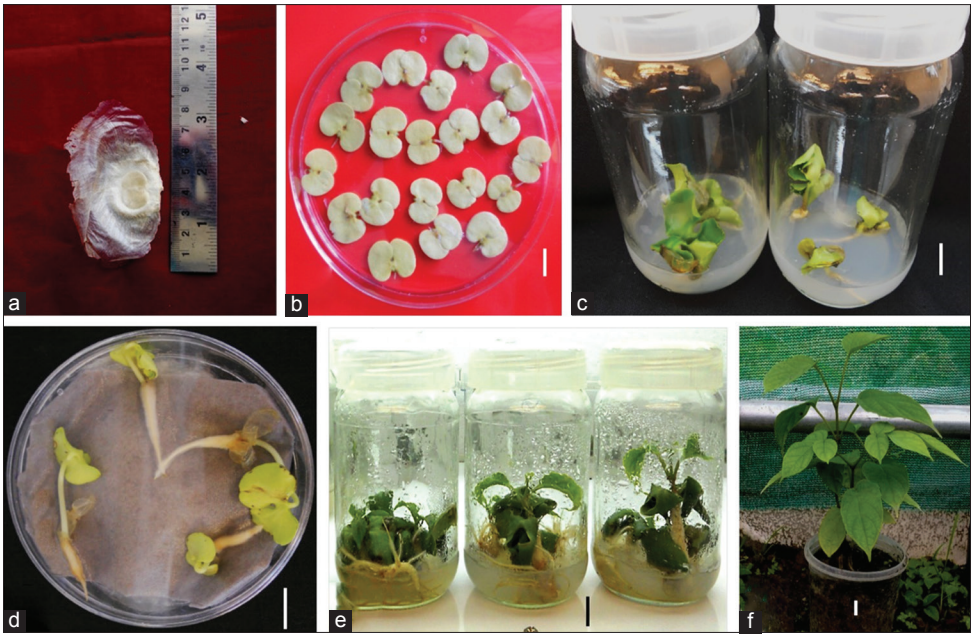

Plate 1: *Ex situ* seed germination of *Oroxylum indicum,* (a) seed of *O. indicum*, (b) disinfected seeds before sowing (c) seed germination responses in condition (i), after 20 days of inoculation (d) seed germination responses in condition (ii), after 20 days of inoculation (e)Germinated seedlings (30 days) in condition (i) (f) well-developed plant (45 week). The bar indicating 1 cm scale

seeds were treated with 100 µM GA_3 for 24 h and 48 h or 50 µM GA_3 for 48 h, respectively [Table 3]. The SVI was highest for seeds sown in condition (i) and treated with 50 µM GA_3 for 24 h and lowest for 50 µM GA_3 treated seeds for 48 h and sown under condition (iii). Properly germinated seeds of all conditions were acclimatized gradually, and for acclimatization, seedlings were initially kept under greenhouse for 6 months [Plate 1f], and finally, well-developed plantlets were planted in the departmental garden facing direct sunlight. Studies are available regarding the synergistic influence of variables such as changing light and temperature during day and night [30,33]; light, chilling and GA_3 [31]; and GA_3, light and catalase activity [32] are available which supports the preeminence of synergistic influence of GA_3 treatments

and sowing conditions in seed germination of *O. indicum* in present study.

Ex situ conservation through micropropagation of species like *O. indicum* is difficult due to a high degree of callusing at the basal end, high phenolic excretion into the medium and consequent blackening of explants. Seeds are best sources for natural regeneration of angiosperm plants [34], but seed abortion and self-incompatibility in pollination are major constraints in natural regeneration of *O. indicum* [35]. According to Singh *et al.* [29], despite being a high-value plant, so far, there is not much documentation on *O. indicum* seed germination, therefore, much more visions are needed on this aspect [36]. Thus, in the present study, seed germination response of *O. indicum* under the influence of different GA_3 treatments and sowing conditions were studied. Gibberellic acid has been known to break dormancy and increase germination in several genera [37]. Further pre-treated seed with growth regulators such as GA_3 has been found to improve the seedling growth of many species, as GA_3 induces the production of hydrolytic enzymes [38] and increases cell plasticity [39]. The artificial application of GA_3 reduces the concentration of abscisic acid, which leads acceleration in seed germination since abscisic acid is known contributing a factor of seed dormancy [40].

The best germination rate occurred in seeds inoculated in MS medium kept under condition (i), similar results were observed by Dhami *et al.* [19] and Tiwari *et al.* [23]. In condition (i) and (ii) the consistent temperature (25 ± 2°C) during the experiment may have reinforced the seed germination rate. While in condition (iii), seeds were incubated at alternating temperatures, i.e., during day and night (polyhouse) showed low germination, similar results were observed by Macchia *et al.,* [41] in *Echinacea angustifolia*. The strong reason for poor seed germination response in soil, i.e., condition (iii) may be the presence of certain mycoflora which are distracting the seeds [42]. To address this problem Pande and Gupta [43] germinated seeds of *O. indicum* in two different conditions, namely, nursery and laboratory, they found only 48% seed germination in nursery, while 96% in laboratory condition, which further supports the results of our study and the possible reason of best seed germination responses in condition (i).

4. CONCLUSION

The present study reveals that the pre-sowing treatment of seeds with GA_3 and MS medium were not solely responsible factors for germination enhancement, but there were the conditions, in which seeds were placed, also influenced seed germination. Seeds, which were kept under culture room condition and supplemented by MS medium, possess best results with all treatments, while seeds, those directly sown into the soil, has unveiled overall poor response in seed germination. Results of our study will be helpful for the conservation of this endangered and high-value medicinal tree so it can support a reforestation program and fulfill industrial demand sustainably.

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REFERENCES

1. Gokhale M, Bansal YK. Cytological studies with reference to chromocenters in a monotypic, threatened medicinal tree *Oroxylum indicum* (L.) vent. Caryologia 2012;65:171-5.
2. Theobald WL. Bignoniaceae. In: Dassanayake MD, Fosberg FR, editors. A Revised Handbook to the Flora of Ceylon. New Delhi, India: Amerind Publishing Co., Pvt., Ltd.; 1981.
3. Sasidharan N. Biodiversity Documentation for Kerala-Flowering Plants. Vol. 6. Peechi, Kerala, India: Forest Research Institute; 2004. p. 321-4.
4. Chopra RN, Nayar SL, Chopra IC. Glossary of Indian Medicinal Plants. New Delhi: CSIR; 1992. p. 182.
5. Ravikumar K, Ved DK. 100 Red listed Medicinal Plants of Conservation Concern in Southern India. Bangalore, India: FRLHT; 2000.
6. Joshi KK, Joshi SD. Genetic Heritage of Medicinal and Aromatic Plants of Nepal Himalayas. Kathmandu, Nepal: Buddha Academic Publishers and Distributors Pvt. Ltd.; 2001.
7. Biswas KP, Ghosh E. Bharater Bonoushodi. 2nd ed., Vol. 3. Calcutta: Calcutta University; 1994. p. 858.
8. Dinda B, SilSarma I, Dinda M, Rudrapaul P. *Oroxylum indicum* (L.) kurz, an important Asian traditional medicine: From traditional uses to scientific data for its commercial exploitation. J Ethnopharmacol 2015;161:255-78.
9. Karnati M, Chandra RH, Veeresham C, Kishan B. Anti-arthritic activity of root bark of *Oroxylum indicum* (L.) vent against adjuvant-induced arthritis. Pharmacognosy Res 2013;5:121-8.
10. Zaveri M, Gohil P, Jain S. Immunostimulant activity of n-butanol fraction of root bark of *Oroxylum indicum* vent. J Immunotoxicol 2006;3:83-9.
11. Knight DC, Eden JA. A review of the clinical effects of phytooestrogens. Obstetr Gynecol 1996;87:897904.
12. Subramanian SS, Nair AG. Flavonoids of stem bark of *Oroxylum indicum*. Curr Sci 1972;41:623.
13. Roy MK, Nakahara K, Na TV, Trakoontivakorn G, Takenaka M, Isobe S, *et al.* Baicalein, a flavonoid extracted from a methanolic extract of *Oroxylum indicum* inhibits proliferation of a cancer cell line *in vitro* via induction of apoptosis. Pharmazie 2007;62:149-53.
14. Singh J, Kakkar P. Oroxylin A, a constituent of *Oroxylum indicum* inhibits adipogenesis and induces apoptosis in 3T3-L1 cells. Phytomedicine 2014;21:1733-41.
15. Yan RY, Cao YY, Chen CY, Dai HQ, Yu SX, Wei JL, *et al.* Antioxidant flavonoids from the seed of *Oroxylum indicum*. Fitoterapia 2011;82:841-8.
16. Chen LJ, Games DE, Jones J. Isolation and identification of four flavonoid constituents from the seeds of *Oroxylum indicum* by high-speed counter-current chromatography. J Chromatograph 2003;988:95-105.
17. Yasodha R, Ghosh M, Santan B, Gurumurthi K. Importance of biotechnological research in tree species of dashmula. Indian Forester 2004;130:79-88.
18. Anonymous. The Wealth of India: A Dictionary of Indian Raw Materials and Industrial Products. New Delhi, India: CSIR; 1995. p. 351.
19. Dhami N, Bhatt GD, Gurung S, Gurung R, Pant B, Joshi SD. *In vitro* shoot proliferation of *Oroxylum indicum* (L.) kurz. Bot Orient 2005;5:1-2.
20. Jain SP, Singh J, Singh SC. Rare and endangered medicinal and aromatic plants of Madhya Pradesh. J Econ Tax Bot 2003;27:925-32.
21. Jayaram K, Prasad MN. Genetic diversity in *Oroxylum indicum* (L) vent. (Bignoniaceae) a vulnerable medicinal plant by random amplified polymorphic DNA marker. Afr J Biotechnol 2008;7:254-62.
22. Gokhale M, Bansal YK. Direct *in vitro* regeneration of a medicinal tree *Oroxylum indicum* (L.) vent. Through tissue culture. Afr J

Biotechnol 2009;8:3777-81.
23. Tiwari S, Singh K, Shah P. *In vitro* propagation of *Oroxylum indicum* - An endangered medicinal tree. Biotechnol 2007;6:299-301.
24. Grabe DF. Tetrazolium testing handbook for agricultural seeds. Contribution Number 29 to the Handbook on Seed Testing. North Brunswick, New Jersey, USA: Association of Official Seed Analysts; 1970.
25. Pandey A, Tamta S. *In vitro* propagation of the important Tasar Oak (*Quercus serrata* Thunb.) by casein hydrolysate promoted high frequency shoot proliferation. J Sus Forestry 2014;33:590-603.
26. Murashige T, Skoog F. A revised medium for rapid growth and bioassays with tobacco tissue culture. Plant Cell Physiol 1962;15:473-97.
27. Pandey A, Tamta S. Effect of pre-sowing treatments on seed germination in *Quercus serrata* Thunb. and *Quercus semecarpifolia* Sm. Int J Biodivers Conserv 2013;5:791-5.
28. ISTA. International rules for seed testing. Proceeding of the seed. Test Assoc 1976;31:1-52.
29. Singh M, Singh KK, Badola HK. Effect of temperature and plant growth regulators on seed germination response of *Oroxylum indicum* - A high value threatened medicinal plant of Sikkim Himalaya. J Plant Sci Res 2015;1:115.
30. Oh E, Yamaguchi S, Kamiya Y, Bae G, Chung WL, Choi G. Light activates the degradation of PIL5 protein to promote seed germination through gibberellin in *Arabidopsis*. Plant J 2006;47:124-39.
31. Groot SP, Karssen CM. Gibberellins regulate seed germination in tomato by endosperm weakening: A study with gibberellin-deficient mutants. Planta 1987;171:525-31.
32. Evensen KB, Loy JB. Effects of gibberellic acid and gold light on germination, enzyme activities, and amino acid pool size in a dwarf strain of watermelon. Plant Physiol 1978;62:6-9.
33. Motsa MM, Slabbert MM, Van Averbeke W, Morey L. Effect of light and temperature on seed germination of selected African leafy vegetables. South Afr J Botany 2015;99:29-35.
34. Pandey A, Brijwal L, Tamta S. *In vitro* propagation and phytochemical assessment of *Berberis chitria*: An important medicinal shrub of Kumaun Himalaya, India. J Med Plant Res 2013;7:930-7.
35. Bawa KS, Webb CJ. Flower, fruit and seed abortion in tropical forest trees: Implications for the evolution of paternal and maternal reproductive patterns. Am J Bot 1984;71:736-51.
36. Dalal NV, Rai VR. *In vitro* propagation of *Oroxylum indicum* vent. a medicinally important forest tree. J Forest Res 2008;9:61-5.
37. Bewley J.D, Black M. Seeds: Physiology of Development and Germination. New York, NY: Plenum Press; 1985. p. 175-236.
38. Mozer TJ. Control of protein synthesis in barley aleurone layers by the plant hormones gibberellic acid and abscisic acid. Cell 1980;20:479-85.
39. Taylor A, Cosgrove DJ. Gibberellic acid stimulation of cucumber hypocotyl elongation: Effects on growth, turgor, osmotic pressure, and cell wall properties. Plant Physiol 1989;90:1335-40.
40. Tillberg E. Levels of endogenous abscisic acid in achenes of *Rosa rugosa* during dormancy release and germination. Physiol Plant 1983;58:243-8.
41. Macchia M, Angelini LG, Ceccarini L. Methods to overcome seed dormancy in *Echinacea angustifolia* D.C. Sci Hortic 2001;89:317-24.
42. Mehrotra MD. A study on fungal deterioration of seed and damping off in *Oroxylum indicum* and their control. Indian Forester 1990;116:977-9.
43. Pande BJ, Gupta RC. Role of seed mycoflora on seed germination of *Oroxylum indicum* (L.) Vent. In Kumaun region of Indian Central Himalaya. Int J Biodvers Conserv 2011;3:715-20.

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