

Integrative conservation approaches for the endemic seasonal ethnomedicinal plant *Iphigenia magnifica*

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Abstract

Iphigenia magnifica Ansari and R. S. Rao (Colchicaceae) is a potential seasonal medicinal herb that grows on plateaus and open grasslands with well-drained soil on gentle slopes. This plant has remarkable therapeutic potential and is used in the Indian medicinal system to treat various diseases. Currently, this species is on the verge of extinction due to the unregulated exploitation of traditional medicine and various anthropogenic activities. Seed dormancy and low seed germination rates limit natural propagation. For the first time, the effectiveness of *in vivo* pre-sowing and *in vitro* treatment with various growth regulators was evaluated to enhance seed germination and induce healthy corms. Sulfuric acid scarified seeds for 20 s significantly improved seed germination with 66.7 ± 2.7 % germination, 8.53 ± 0.32 of germination speed, 262.98 ± 15.78 of vigor index (VI), and 8.33 ± 0.33 of emergence index (EI). In this study, we also confirmed that scarified seeds treated with sulfuric acid for 20 s and cultured on half-strength Murashige and Skoog (MS) medium containing $1.5 \mu\text{M}$ GA3 proved superior for breaking residual dormancy and enhancing the germination (99 ± 0.58 %) and seedling performance; hence, it can be used for the conservation of *I. magnifica*. Corms cultured on MS medium containing $2.5 \mu\text{M}$ GA3 showed maximum sprouting (98.67 ± 0.33 %). Plantlets derived from seeds and corms were successfully acclimatized under natural conditions, confirming a stable protocol for the multiplication, conservation, and restoration of the endemic seasonal medicinal plant, *I. magnifica*. This protocol can also be used for sustainable utilization of these active metabolites.

1. Introduction

The geophytic genus *Iphigenia* belongs to the family Colchicaceae and contains 12 species worldwide (POWO, 2025). Eight of these species are endemic to India: *I. magnifica*, *I. mysorensis*, *I. pallida*, *I. sahyadrica*, *I. stellata*, *I. indica*, *I. ratnagirica*, and *I. devrukhensis* (Lekhak et al. 2016; POWO, 2025). *Iphigenia* species are known for their pharmaceutically important alkaloid, colchicine, in significant amounts. Among the different species, non-toxic colchicine is isolated from seeds of *Iphigenia* species with about 0.9% (Sapra et al. 2013). Recently, Mahajan et al. (2024) suggested that *Iphigenia* can serve as a commercial source of colchicine. According to the Indian Medicinal Plants Database, corm and seeds of the genus *Iphigenia* have been used in traditional medicine for the treatment of abdominal pain, rheumatic arthritis, skin disease, liver diseases, acne, calculous infection, muscular and stomach disorders, kidneys, snakebites, and migraine (Kiewhuo et al. 2023).

Iphigenia magnifica Ansari and R. S. Rao, commonly known as grass lily or Bhuechakra, are important wild medicinal plant. It is a small seasonal herb with a corm or globose rootstock covered with hairy scales (Mukhopadhyay et al. 2002). It has been sparsely reported in plateau regions of open grasslands with well-drained soil or shallow sandy soil on gentle slope areas with approximately 700 mm rainfall from Karnataka, Maharashtra, Goa, Tamil Nadu, Andhra Pradesh, and Kerala states of India (Rajasekar et al. 2023; More et al. 2025). In Maharashtra, it has been reported in the Ratnagiri, Sindhudurg, Kolhapur, Satara, Raigad, Nashik, and Pune districts (Sangale et al. 2025). In the Bihar state of India, *I. magnifica* bulbs are used to treat colic and headache (Chopra et al. 1969). A coconut oil-based decoction from

corms and seeds has been used to treat perforated eardrums (Jagtap et al. 2008). Among the different tribal communities, the Korku community is aware of the significance of corm oil in the treatment of pain and migraine. They used corm oil to prevent sleep after snakebites and applied it to snakebites. *I. magnifica* contains important phytoconstituents such as alkaloids, flavonoids, catechol, steroids, and saponins. In addition, some valuable amino acids, such as glutamic acid, Tyrosine, DL-Alanine, Histidine, D-Methionine, and Valine, and several ashes contain sulfur, calcium, magnesium, iron, chlorine, and phosphorus (Bhogaonkar and Devarkar 2011).

I. magnifica is typically grown from seeds and tubers, but seed viability under natural conditions is low and unpredictable. In the post-monsoon period, the red bug (*Scantius aegyptius*) is found on *I. magnifica* capsules, completing its life cycle by eating juvenile pods and seeds (Arakelian G. 2009). These seeds and tubers are extensively collected by local healers and practitioners for ethnomedicinal purposes (Gopi and Panneerselvam 2011). Furthermore, it faces several anthropogenic disturbances, including early grass cutting, mining, industrialization, overgrazing, and agricultural expansion (Sangale et al. 2025). Therefore, it is possible that this plant will become endangered in India. Immediate action is required to protect this species before its population declines (Sangale et al. 2025). *In vitro* propagation and conservation methods must be implemented to prevent the extinction of endemic medicinal herbs (Lakshmi et al. 2023). Plant tissue culture is an efficient tool for rapid and mass multiplication of medicinal plants for the production of valuable secondary metabolites in a short period of time (Sangale et al. 2023; Patil et al. 2013; Naikawadi et al. 2016). This is the most reliable approach for *ex situ* conservation of rare, endemic, and endangered medicinal plants (Shahzad et al. 2017).

Little information is available on seed germination, corm production, and mass multiplication of *I. magnifica*. have received minimal attention in *in vitro* propagation studies. Thus, in the present study, we developed *in vivo* and *in vitro* seed germination and corm production protocols and standardized nursery techniques for sustainable colchicine production and uniform biomass production from seasonally available *I. magnifica*.

2. Materials and Methods

2.1. Collection of Plant Material and Authentication

Plant material was collected with permission from the Maharashtra Biodiversity Board, Nagpur, Maharashtra, India (Ref. No: MSBB/Desk-5/Research/995/2024-25). *I. magnifica* was collected from open grasslands with drained soil or gentle slopes of plateau areas, at altitudes ranging from 600 to 800 m in Shirur (N 18° 49' 33.564", E 74° 22' 47.4132") and Parner (N 19° 10' 48.", E 74° 38' 94.") regions of the Pune and Ahilyanagar districts of the State of Maharashtra, respectively. The collected plant material was identified and authenticated by the Dr. A. Benniamin, Scientist F and Head of the Botanical Survey of India, Western Circle, Pune, India, and a voucher specimen was deposited in herbaria (Ref. No: BSI/WRC/Tech./2021/RM/04).

2.2. Collection of seeds

Fully matured and healthy capsules of naturally grown *I. magnifica* were collected from the Pune and Ahilyanagar districts of Maharashtra, India, during August and October 2021–2024 from the same locations where the plants were collected. The seeds germinated between the last week of May and June, immediately after the early pre-monsoon showers. The fruits mature between August and September each year. Mature fruits were collected during August and September.

2.3. Seed viability

The collected mature capsules were dried at room temperature and allowed to break. The number of capsules per plant, seeds per capsule, and capsule morphology were recorded visually. Seeds were stored in airtight glass vials until further use. The percent moisture content was calculated using 100 seeds using the following formula:

$$\text{Percent moisture content} = (\text{Initial weight} - \text{Final weight}) / (\text{Final weight}) \times 100.$$

Mature dry seeds were stored for different periods (0, 4, 8, 12, 16, 20, and 24 months) to evaluate seed viability. Seed viability was assessed using 2,3,5-triphenyltetrazolium chloride (TTC) assay (HiMedia, Mumbai, India). For this, randomly selected 100 seeds were soaked in a TTC solution for 24 h in the dark. The seeds were then washed thrice with sterilized distilled water and dissected into cotyledons. The dissected embryos were observed under a simple microscope for a change in color to red (Naikawadi et al. 2012).

2.4. Sterilization of seeds for in vivo germination

Freshly harvested seeds did not germinate for up to 30 days because of extensive dormancy. Mature seeds were used for germination studies after 30 days. The seeds were washed in a 4% liquid detergent solution for 10–12 min at room temperature. The seeds were then soaked in a 0.1% fungicide solution (Bavistin) for 30 min and air-dried. Further, the seeds were surface sterilized by rinsing in distilled water, followed by a quick 30 s wash in 70% ethanol and 10 min in 0.15% HgCl₂ solution (Mukhopadhyay et al. 2008). Finally, the seeds were rinsed three–four times with sterile distilled water. Surface-sterilized seeds with or without treatment were germinated on 1 mm thick germination paper (Modern Paper Ltd., Pune, India). An approximately 10 mm thick overlaying layer was made using sterilized absorbent cotton in a Petri dish (90 × 15 mm; Axygen, India). Twenty-five seeds were added to each Petri dish, 25 seeds were added. Germination paper and cotton were moistened with 25 ml sterilized distilled water, and 10 ml sterile distilled water was added at four-day intervals.

2.5. In vivo seed germination

2.5.1. Sandpaper scarification

Sterilized seeds without any pre-treatment were placed in petri dishes and used as controls. Seeds were placed between the flaps of the 0-grade sandpaper and manually scarified by applying a small frictional force for 10 s. During scarification, the damaged seeds were visually identified and discarded. Intact,

undamaged seeds (100) were selected and placed on germination paper in Petri dishes for the germination test.

2.5.2. Water treatment

Surface-sterilized seeds were treated with normal water and cold water at 4°C for 6, 12, 18, and 24 h. Seeds then treated with hot water at 70°C for 10, 20, 30, and 40 s. A total of 100 seeds were used for each treatment. After each treatment, seeds were transferred to sterile water at room temperature. Finally, the seeds were placed on germination paper in petri dishes for germination.

2.5.3. Acid scarification

Sterilized seeds were pre-soaked in 75% H₂SO₄ (Qualigens, Mumbai, India) for 10, 20, 30, or 40 s. The pre-soaked seeds were washed five times with sterile distilled water to remove traces of acid and were used for germination. For each treatment, 100 seeds germinated.

2.6 In-vitro seed germination

To verify the presence of endophytic fungi in the seeds, acid-scarified seeds were sown in compost. After one week, healthy plantlets were developed without any fungal infection on germinated seeds and one-week-old seedlings under greenhouse conditions. Therefore, similarly scarified seeds were used for the *in vitro* germination experiments. Acid-scarified seeds sterilized with 75% H₂SO₄ for 20 s were cultured on half-strength Murashige and Skoog (MS) medium (Murashige and Skoog 1962) supplemented with different concentrations of 6-Benzylaminopurine (BA), Indole-3-butyric acid (IBA), and gibberellic acid (GA3), individually and in combination. The MS medium with individual and different combinations of membrane-filtered growth regulators were used as follows: 2.5–10 µM IBA, 0.65–5 µM GA3, 5.0 µM BA + 2.5 µM IBA, 5.0 BA µM + 5.5 µM IBA, 5.0 µM BA + 7.5 µM IBA and 5.0 µM BA + 10.0 µM IBA. The seeds were used as controls without growth hormone supplementation. The treated and control seeds were placed in petri dishes containing half-strength MS medium and observed for germination.

2.7. In vitro corm induction

MS medium with 3% sucrose and 100 mg l⁻¹ myo-inositol was used for corm germination. The pH of the culture medium was adjusted to 5.5–6.2 using 1 N HCL or 1 N NaOH, before adding 0.8% (w/v) of agar. The medium was autoclaved at 121°C for 15 min at 105 kPa pressure. Membrane-filtered growth hormones, such as IBA, GA3, and BA, were added at different concentrations and in combination with 2.5–10 µM IBA, 0.65–5.0 µM GA3, 2.5 µM BA + 2.5 µM IBA, 2.5 BA µM + 5.0 µM IBA, 5.0 µM BA + 2.5 µM IBA, and 5.0 µM BA + 5.0 µM IBA. Inoculated Petri dishes and test tubes were maintained at 25 ± 2°C for 8 h photoperiod with approximately 30 µmol m⁻²s⁻¹ light intensity provided by cool white fluorescent tube lights.

2.8. Data Collection

2.8.1. Cumulative germination counts

Both *in vivo* treated and *in vitro* cultured seeds were observed regularly, and seed germination counts were recorded until the completion of germination or for a maximum of 20 days. Seeds with 0.5 mm or more radicals were considered germinated seeds. The final germination percentage was calculated based on the total number of germinated seeds on the day of completion, using the following formula (Patil et al. 2012; Naikawadi et al. 2012)

Germination percentage: No. of seed germinated on day of completion / No. of seed sowed × 100

2.8.2. Germination speed

Germination value (GV) is a composite value that combines both germination speed (GS) and total germination, providing an objective means of evaluating the results of germination values. It was calculated using the formula described by Kulkarni et al. (2007) and Alem et al. (2024) as follows:

Germination speed = Final germination percentage/Days to complete germination.

2.8.3. Seedling vigor

Normal seedlings from the standard germination tests were further classified as strong or weak based on visual observations and expressed as a percentage. Erect and sturdy seedlings with well-developed roots and shoots were considered strong (Jaleel et al. 2007). The root and shoot lengths of the seedlings were recorded. The seedling Vigor Index (SVI) was calculated according to the Pathak et al. (2025):

SVI = Germination percentage × shoot length

2.8.4. Emergence Index

The observed seedlings were categorized as strong or weak. The seedling emergence index (EI) was calculated using the following formula (Pankaj et al. 2021; Chawhan and Chintapalli 2025)

EI = $\sum$ (number of emerged seedlings at each time point / corresponding number of days)

2.9. Hardening Condition

Both *in vivo*-and *in vitro*-germinated seeds and corms were transferred into pots containing sterile sand and soil (75:25). The acclimatized seedlings were then transferred to pots containing garden soil and placed under shaded conditions (Longchar and Deb 2022). Finally, the plants grown for four weeks were transferred to the field.

2.10. Statistical analysis

A completely randomized design (CRD) was used for all experiments. The experiments were performed in triplicate. Data were analyzed using analysis of variance (ANOVA), followed by Duncan's multiple range test (DMRT) (Duncan 1955) at $P < 5\%$.

3. Results

3.1 Collection of seeds

The naturally grown mature plants had a height of 17–18 cm with 1–3 capsules per plant. After maturity, the capsule becomes 3-valved, loculicidal, dehiscent, elongated, smooth, and yellowish-brown, measuring 0.8–1 cm long and 0.4–0.8 cm in breadth. Each mature capsule contained > 25–30 seeds (Fig. 1). The mature seeds were oven-dried at 80°C for 24 h, showing a total moisture loss of 21.29%. However, seeds stored at room temperature lost 2.91% moisture after 30 days. Imbibed seeds used for germination had a higher moisture content of 29.89%.

3.2 Storage conditions and seed viability

Seed viability indicates the ability of seeds to germinate and produce normal seedlings under suitable germination conditions (Copeland and McDonald 2001). Total germination depends largely on seed viability and vigor (Harrington and Kozlowski 1972; Hay and Probert 1995). It is necessary to analyze the viability of any seed used for seedling growth of seedlings (Walt and Witkowski 2017). The TTC test provides an indication of propagation success relative to the maximum potential germination (Scianna 2001). In the present study, mature dry seeds were stored for 0, 4, 8, 12, 16, 20, and 24 months to evaluate seed viability using the TTC test. No significant decrease in viability was observed up to 8 months; however, after 8 months, viability decreased by approximately 20%. Viability gradually decreased with increasing seed storage time. The Seeds store for 20–24 months showed only 10 – 5% viability (data not shown). The increase in storage time results in a decline in viability due to moisture, temperature, and the initial health of seeds (Naikawadi et al. 2012; Pradhan and Badola 2012; Kamaei et al. 2024).

3.3. In-vivo seed germination

Different pre-treatments have been employed by many researchers to increase seed germination. The seeds of several monocotyledonous species fail to germinate because of impermeable seed coats (Nautial et al. 2023). Seeds pretreated by scarification, followed by overnight soaking in sterile distilled water, resulted in improved germination rates. Scarification artificially increases seed coat permeability. When freshly harvested seeds were sown in the soil, seed germination was observed after nine months. Compared to non-scarified seeds, scarified seeds showed significantly higher germination rates of up to 38 to 66%.

The effects of various pre-soaking treatments (normal, cold, and hot water) on the seed germination percentage, germination speed, vigor index, and emergence index are shown in Table 1 and Fig. 2. In both normal and cold water pre-treatments, germination percentage, germination speed, VI, and EI increased with increasing time up to 12 h and then decreased with increasing time. Among the different normal water pre-treatments, 12 h recorded highest germination percentage ($22.7 \pm 3.5\%$), germination speed (0.67 ± 0.09), VI (80.96 ± 9.7), and EI (1.96 ± 0.35), followed by 18 h pre-treatment with $18.7 \pm 3.5\%$ germination, 0.57 ± 0.09 germination speed, 60.87 ± 4.78 of VI and 1.67 ± 0.35 of EI. Similarly, in cold

water pre-treatment, 12 h showed $29.3 \pm 3.5\%$ seed germination, 1.27 ± 0.18 germination speed, 88.47 ± 14.43 of VI, and 2.1 ± 0.25 of EI. Moist chilling has been reported to be a very effective method for germination in *Acer pensylvanicum* and *Prunus companulata* (Bourgoin and Simpson 2004; Szymajda and Maciorowski 2025).

The seeds pre-treated with hot water for 10, 20, 30, and 40 s showed a decline in germination percentage and other parameters after 20 s, except for EI. The highest seed germination ($25.3 \pm 1.3\%$), germination speed (2.13 ± 0.07), and VI (96.4 ± 6.82) were observed in seeds pretreated with hot water for 20 s. Similar results were observed for *E. alsinoides* (Naikawadi et al. 2012).

Seeds pre-treated with 75% H_2SO_4 showed a higher germination percentage than untreated seeds. The highest germination percentage, germination speed, VI, and EI were observed in the 20 s acid pre-treatment, followed by the 30s treatment with 66.7 ± 2.7 and $56 \pm 2.3\%$ germination, 8.53 ± 0.32 and 6.63 ± 0.42 of germination speed, 262.98 ± 15.78 and 197.11 ± 18.13 of VI, and 8.33 ± 0.33 and 6.46 ± 0.13 . Pre-treatment of seeds with acid softens the seed coat and destroys the barrier in natural openings, which supports the entry of water required for germination (Baskin and Baskin 1998). Among the different pre-treatments, acid treatment for 20 s resulted in the highest germination percentage, germination speed, VI, and EI.

3.4. In vitro seed germination:

In this study, successful *in vitro* seed germination was achieved using half-strength MS medium. Embryo development, single leaf blades, and one–two root formations were observed within five days. After 21 days of inoculation, the leaf lamina up to 3–4 cm long and the lower part (corm) were swollen, which was approximately 2–3 mm in diameter. After subsequent inoculation on the parental medium, leaf size increased to approximately 9–12 cm and three to four roots were induced from the corm. The swollen part induced one to two roots, and shoot development with a single leaf blade was significant under control conditions. A significant difference was observed in acid-scarified seeds under *in vitro* conditions compared to *in vivo* conditions for healthy plantlet development (Fig. 3).

In vitro techniques have been exploited for successful germination of various types of seed dormancy (Haruna et al. 2025). In the case of small seeds, a high salt concentration in the nutrient medium causes osmotic stress because of low water potential and nitrogen toxicity (Munnes and Tester 2008, Muslihatin and Ratnadewi 2012; Reguera et al. 2020). Growth medium without nutrients and sucrose resulted in seed germination when the embryos were autotrophic (Martendal et al. 2013). Various researchers have reported that reducing the strength of MS medium is sufficient to increase the frequency of seed germination in *Citrus reticulata*, *Ludisia discolor*, *Givotia rottleriformis*, *Encyclia cordigera* and *Buchanania cochichinensis* (Hassanein and Azooz 2003; Shiau et al. 2005; Vibhute et al. 2017). Conservation through *in vitro* seed germination has been achieved in endangered and threatened plant species such as *Adansonia digitata* and *Bupleurum latissimum* (Singh et al. 2010). The application of various concentrations of individual and combined growth regulators significantly affected germination percentage, germination speed, VI, and EI. The effects of the growth regulators on seed

germination are presented in Table 2. Different concentrations of GA₃ resulted in the highest seed germination and other parameters compared to IBA and combinations of IBA and BA. (Sevik and Guney 2013) reported that high concentrations of IBA or BA-IBA combinations might lead to hormonal imbalances, negatively affecting germination rates and seedling vigor. Among the different concentrations GA₃, 1.5 µM GA₃ recorded the highest germination ($99 \pm 0.58\%$), germination speed (54.93 ± 5.19), VI (471.41 ± 12.96), and EI (38.44 ± 5.28). Followed by 2.5 and 5.0 µM GA₃ with 91.33 ± 0.88 and $77.67 \pm 1.45\%$ germination with high germination speed (25.67 ± 3.18 and 16.03 ± 2.18), VI (380.98 ± 7.3 and 310.59 ± 9.89) and EI (30.44 ± 0.29 and 16.83 ± 1.36), respectively. The present findings align with previous reports, in which GA₃ promoted enzymatic activity during germination and improved the emergence of rice seedlings (Nie et al. 2022; Luo et al. 2025). Similar results have been reported for *B. cochichinensis*, *Buchanania lanzan*, *Dendrocallamus strictus* and *Sideritis* species (Jose and Sivaprasad 2025; Su et al. 2025; Sarropoulou et al. 2025)

3.5. In vitro corm induction

The effects of the growth regulators on corm induction and root formation are shown in Table 3 and Fig. 4. Sprouting percentage, shoot length, number of roots/shoots, and root length were measured. Among the different growth regulators (IBA, GA₃, and combinations of IBA-BA), GA₃ exhibited the highest sprouting percentage, shoot length, number of roots/shoots, and root length. 2.5 µM GA₃ recorded significantly highest sprouting ($98.67 \pm 0.33\%$) with 6.67 ± 0.33 roots/ shoots, 8.87 ± 0.03 cm long shoots, and 6.33 ± 0.33 cm long roots.

The 1.5 and 0.65 µM GA₃ treatments resulted in 95 ± 0.58 and $92 \pm 0.58\%$ sprouting, 5.33 ± 0.33 and roots/ shoots in each, 8.37 ± 0.03 and 8.1 ± 0 cm long shoots and 5.33 ± 0.33 and 4.33 ± 0.33 cm long roots, respectively. The number of roots/shoots and length of roots were significantly higher in 5.0 µM IBA, with 88.67 ± 0.33 roots/shoots and 6.33 ± 0.33 cm long roots. Similar significant root lengths were observed with 7.5 µM IBA. This study found that 2.5 µM GA₃ was optimal for corm sprouting and shoot elongation, whereas 5.0 µM IBA was most suitable for root elongation and proliferation of roots and shoots. Our results are consistent with those of previous studies on *Dierama erectum*, *G. superba*, *C. sativus*, and *Gladiolus hybridus* (Koetle et al. 2010; Balamurugan et al. 2019; Mosoh 2023)

3.6. Acclimatization of plants:

Two-month-old *in vivo* grown plantlets were successfully acclimatized under natural conditions. *In vitro*-grown plantlets with single shoots and small tubers were hardened and placed in a shaded net house before planting under natural conditions. *In vivo* and *in vitro* plants grown in the natural environment showed comparable growth and development (Fig. 5).

4. Conclusion

I. magnifica is a seasonal medicinal plant. However, this is declining because of overexploitation and low seed germination potential. In the present study, we developed an efficient method to break seed

dormancy and enhance seed germination. Scarification with 75% sulfuric acid for 20 s resulted in maximum seed germination percentage, germination speed, vigor index, and emergence index. Under *in vitro* conditions, scarified seeds treated with 75% sulfuric acid for 20 s along with 1.5 μM GA₃ recorded the highest seed germination percentage. Similarly, scarified seeds treated with 75% sulfuric acid for 20 s along with 2.5 μM GA₃ showed 98% sprouting in corm. The plantlets derived from *in vitro* and *in vivo* seed germination exhibited healthy growth under natural conditions, confirming the development of a robust protocol. This method can be used for the large-scale multiplication, conservation, and restoration of endemic *I. magnifica*. Integrated biotechnological approaches, such as the manipulation of culture conditions, selection of high-yielding cell cultures, and application of elicitors and precursors, can be used to produce active metabolites.

Declarations

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Ethical Approval: This study followed the ethical guidelines for plants and plant materials for sample collection and identification (Maharashtra State Biodiversity Board, Letter no. MSBB/Desk-5/Research/995/24-25). The authors observed and reported *Iphigenia magnifica* Ansari and R. S. Rao from the Ramling and Davalmalik plateaus of Shirur Tehsil in the Pune District (Maharashtra), India.

Conflicts of interest: The authors declare no conflicts of interest.

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Tables

Table 1. Effect of seed pre-soaking treatments on *in-vivo* seed germination of *I. magnifica*

Treatments	Germination %	Germination speed	Vigor Index (VI)	Emergence Index (EI)
Normal Water (NW) Hrs.				
6	16±2.3 gh	0.4±0.06 gh	51.31±5.8 efgh	1.27±0.18 ghi
12	22.7±3.5 efg	0.67±0.09 fgh	80.96±9.7 def	1.96±0.35 efgh
18	18.7±3.5 fgh	0.57±0.09 fgh	60.87±4.78 defg	1.67±0.35 fghi
24	12±2.3 h	0.3±0.06 h	32.99±4.78 gh	1.16±0.23 hi
Cold Water (CW) Hrs.				
6	26±1.2 ef	0.93±0.03 efgh	81.72±11.49 def	1.78±0.12 fghi
12	29.3±3.5 e	1.27±0.18 defg	88.47±14.43 de	2.1±0.25 efg
18	24±2.3 ef	1.4±0.1 def	43.4±2.37 gh	2.07±0.26 efg
24	12±2.3 h	0.4±0.06 gh	21.44±5.54 h	1±0.19 i
Hot Water (HW) Sec.				
10	20±2.3 fh	1.23±0.12 efgh	48.82±4.16 fgh	2.33±0.36 def
20	25.3±1.3 ef	2.13±0.07 d	96.4±6.82 d	2.81±0.15 de
30	24±2.3 ef	1.63±0.15 de	80.18±5.4 def	3.01±0.25 d
40	12±2.3 h	0.67±0.15 fgh	32.36±8.23 gh	1.46±0.29 fghi
H2SO4 75 % Sec.				
10	48±2.3 c	4.93±0.07 c	84.6±9.54 def	5.33±0.26 c
20	66.7±2.7 a	8.53±0.32 a	262.98±15.78 a	8.33±0.33 a
30	56±2.3 b	6.63±0.42 b	197.11±18.13 b	6.46±0.13 b
40	38.7±3.5 d	4.2±0.93 c	129.6±26.94 c	4.83±0.44 c

Mean values were obtained from the observation of randomly selected 100 plants.

Tables 2. Effect of growth regulators on acid pre-treated seeds on *in vitro* seed germination of *I. magnifica*

H ₂ SO ₄ 75% (20 Sec)	Germination %	Germination speed	VI	EI
Control	16±2.3 hi	0.4±0.06 gh	51.31±5.8 efgh	1.27±0.18 ghi
2.5 µM IBA	66.67±2.67 efg	3±0.1 d	229.78±11.58 d	9.1±0.05 ef
5.0 µM IBA	72±4.62 def	2.57±0.16 d	306±18.62 c	11.09±0.62 def
7.5 µM IBA	74.67±7.06 cde	2.66±0.25 d	286.89±28.3 c	11.44±0.58 def
10.0 µM IBA	62.67±5.81 fg	2.09±0.19 d	225.27±22.11 d	9.87±0.61 def
0.65 µM GA ₃	84±2.31 bc	22±3.46 bc	378.4±4.17 b	19.53±1.01 c
1.5 µM GA ₃	99±0.58 a	54.93±5.19 a	471.41±12.96 a	38.44±5.28 a
2.5 µM GA ₃	91.33±0.88 ab	25.67±3.18 b	380.98±7.3 b	30.44±0.29 b
5.0 µM GA ₃	77.67±1.45 cde	16.03±2.18 c	310.59±9.89 c	16.83±1.36 c
BA 5.0 µM + 2.5 µM IBA	60±2.31 g	3±0.12 d	208.24±5.8 d	14.48±1.06 cde
BA 5.0 µM + 5.5 µM IBA	78.67±3.53 cd	4.33±0.18 d	302.07±23.93 c	16.93±0.58 c
BA 5.0 µM + 7.5 µM IBA	61.33±2.67 fg	2.2±0.1 d	298.11±5.47 c	15.33±0.67 cd
BA 5.0 µM + 10.0 µM IBA	38.67±3.53 h	1.93±0.18 d	122.33±10.29 e	7.29±0.76 f

Mean values were obtained from the observation of randomly selected 100 plants.

Table 3. Effect of growth regulators on *in vitro* corm induction and rooting of *I. magnifica*

Treatments	Sprouting %	Length of shoots	No. of roots/ shoots	Length of roots
Control	22±0.23 ij	3.3±0.7 jh	1.78±0.31 gh	1.45±0.56 ef
2.5 µM IBA	84.5±0.29 ef	5.1±0.06 i	4.67±0.33 de	5.33±0.33 ab
5.0 µM IBA	88.67±0.33 cde	5.53±0.03 g	8.33±0.33 a	6.33±0.33 a
7.5 µM IBA	76.33±0.33 h	5.37±0.03 h	6.33±0.33 bc	6.33±0.33 a
10.0 µM IBA	71±0.58 i	4.93±0.03 j	3.33±0.33 f	5.33±0.33 ab
0.65 µM GA ₃	92±0.58 bcd	8.1±0 c	5.33±0.33 cd	4.33±0.33 bc
1.5 µM GA ₃	95±0.58 ab	8.37±0.03 b	5.33±0.33 cd	5±0 bc
2.5 µM GA ₃	98.67±0.33 a	8.87±0.03 a	6.67±0.33 b	6.33±0.33 a
5.0 µM GA ₃	92.67±4.33 bc	8.17±0.03 c	5.33±0.33 cd	5±0 bc
2.5 µM BA +2.5 µM IBA	88±0.58 de	7.47±0.03 d	5.67±0.33 bcd	5±0.58 bc
2.5 µM BA +5.0 µM IBA	86.33±0.33 e	7.03±0.03 e	6.33±0.33 bc	5.33±0.33 ab
5.0 µM BA +2.5 µM IBA	82±0 fg	6.1±0.06 f	5.33±0.33 cd	4±0 cd
5.0 µM BA +5.0 µM IBA	80±0.58 gh	5.43±0.03 gh	4±0.58 ef	3.33±0.33 d

Mean values were obtained from the observation of randomly selected 100 plants.

Figures



Figure 1

Details of *I. magnifica* (A. fully grown plant, B. flower, C. matured capsule, D. and E. dehiscent capsule, F. seeds, G. corm)



Figure 2

Effect of pre-treatments on *in vivo* seeds germination of *I. magnifica*. (A: Seeds scarification, a: normal water for 12 h, b: cold water for 12 h, c: hot water for 20 s, d: 75 % H_2SO_4 for 20 s; B: Corm induction after 22 days of seed germination: a: normal water for 12 h; b: cold water for 12 h, c: hot water for 20 s, d: 75 % H_2SO_4 for 20 s)

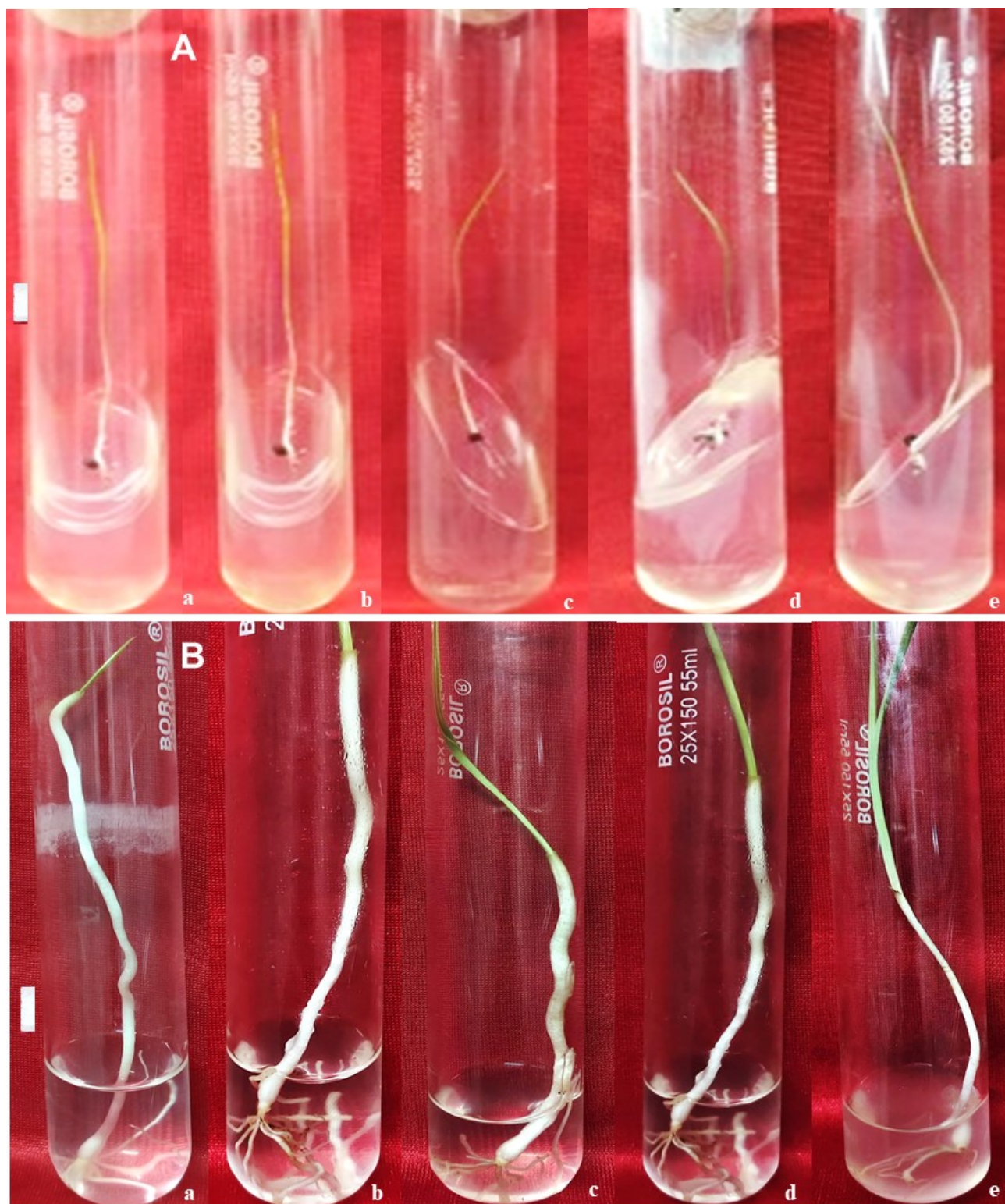


Figure 3

In-vitro* germination of seeds of *I. magnifica (A: control, a: 2.5 μ M IBA, b: 5.0 μ M IBA, c: 0.65 μ M GA_3 , d: 2.5 μ M BA + 2.5 μ M IBA, e: 2.5 μ M GA_3 ; B: After three weeks of inoculation a: 2.5 μ M IBA, b: 5.0 μ M IBA, c: 0.65 μ M GA_3 , d: 2.5 μ M BA + 2.5 μ M IBA, e: 2.5 μ M GA_3)

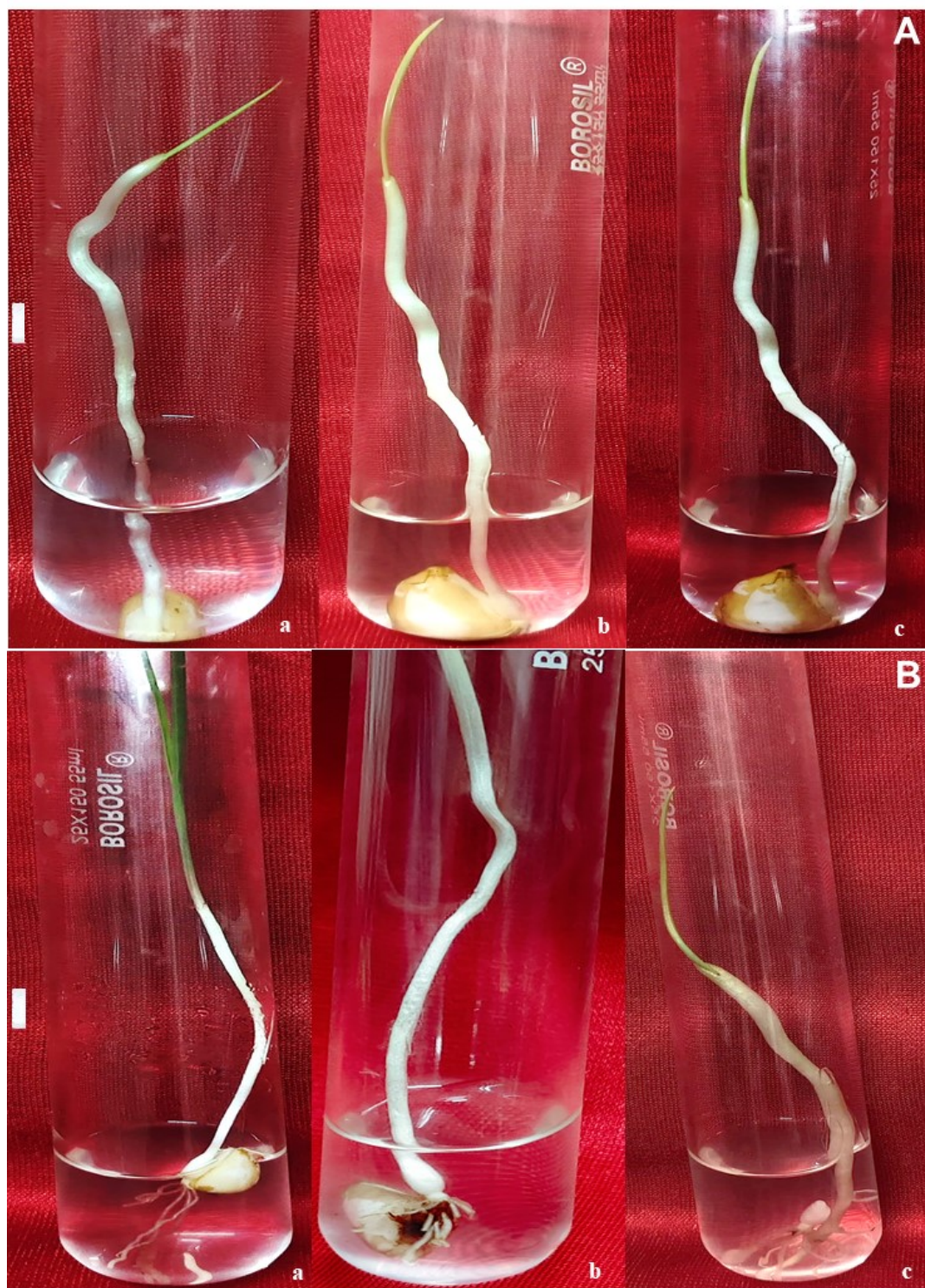


Figure 4

In-vitro* corm induction of *I. magnifica (A: control, a: 2.5 μ M IBA, b: 5.0 μ M IBA, c: 0.65 μ M GA₃; B: After three weeks of inoculación a: 2.5 μ M IBA, b: 5.0 μ M IBA, c: 0.65 μ M GA₃)



Figure 5

Hardening of corm of *I. magnifica* (A: Three-month-old corms, B: Corms germination after one month, C. Fully grown plantlets, D. Hardened plantlets under nursery conditions)

Supplementary Files

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