|  |  |  |  |
| --- | --- | --- | --- |
|  |  | **Seed germination and somatic embryogenesis studies in Ailanthus excelsa Roxb.- ‘A medicinal tree of heaven’**  **Seed germination and somatic embryogenesis in Ailanthus excelsa Roxb a medicinal tree of heaven** |  |

Original Research Article

**Abstract**

**Aim:**

This study aimed to induce *in vitro* somatic embryogenesis from germinated *Ailanthus excelsa* seedlings and evaluate the effect of various cytoknines on embryo development and shoot induction.

**Method**

Mature, dry *Ailanthus excelsa* seeds were surface sterilized using 0.1% Tween-20, 0.2% Bavistin, 70% ethanol, and 0.1% HgCl₂, followed by sterile water rinses. Seeds were cultured on MS medium with gibberellic acid (0.1–3.5 mg/L) and incubated in the dark at 25 ± 2 °C. Germination was monitored every 48 hours for 30 days (10 replicates/treatment). *In vitro* plantlets were sectioned and cultured in MS medium with 2.0 mg/L NAA, 0.1 mg/L ascorbic acid, and 0.15 mg/L citric acid. Callus was subcultured once every two weeks and transferred to liquid MS medium with NAA and/or BAP, Kn, or TDZ. Somatic embryos were cultured on solid MS medium with BAP (0.1–2.0 mg/L), alone or with Kn (0.1 mg/L) or TDZ (0.1 mg/L), and anti-browning agents. Cultures were maintained at 25 ± 2 °C under a 16/8 h photoperiod. The controls were deficient in hormones. Data were analyzed by ANOVA and Duncan’s Multiple Range Test (p ≤0.05) using SPSS v19.

**Results:**

MS medium with 1.0 mg/L gibberellic acid improved seed germination from 3.3% to 26.7%. Plantlets were cultured on MS medium with 2.0 mg/L NAA, 0.1 mg/L ascorbic acid, and 0.15 mg/L citric acid, inducing callus under dark conditions at 25 ± 2 °C. Subcultured callus developed into somatic embryos. Liquid MS medium with NAA, BAP, TDZ, and/or Kn promoted the formation of bipolar structures. The highest number of somatic embryos (81.00 ± 3.90) was achieved with 0.5 mg/L NAA, while maximum shoot induction (7.33 ± 0.58 and 7.33 ± 1.00) occurred with 0.5 and 2.0 mg/L BAP. Treatments significantly influenced somatic embryogenesis (*p* = 0.055) and shoot induction (*p* = 0.004).

**Conclusions:**Gibberellic acid at 1.0 mg/L increased seed germination nearly 8-fold. *In vitro* seedlings were effective explants for callus induction on 2.0 mg/L NAA. Low NAA levels in liquid MS medium promoted somatic embryogenesis, while BAP induced shoot elongation. However, hyperhydricity delayed full plantlet regeneration. Further studies are needed to control hyperhydricity for successful plantlet growth and artificial seed production.

**Key Words**

*Ailanthus excelsa* Roxb.; Callus; Growth regulators; Micropropagation; Plant growth regulators,repetition; Somatic embryo

1. **INTRODUCTION**

India has a rich ethnobotanical heritage, with the use of medicinal plants documented for over 3,000 years (Swami *et al*., 2022; Gogoi *et al*., 2024). Archaeobotanical and historical evidence suggest that the utilization of plant resources for disease treatment in India dates back to approximately 6000–4000 BCE, during the Buddhist period (Pan *et al*., 2014; Ganguly *et al*., 2024). An estimated 25,000 plant-based formulations are employed within various traditional and folk medicinal systems (Ghosh *et al*., 2023). From the 700 plant species predominantly used in the Indian herbal industry, nearly 90% are harvested directly from wild populations, raising concerns about sustainability and conservation (Singh and Kumar, 2021)

*Ailanthus excelsa* Roxb. is a fast-growing multipurpose tree species of the family *Simaroubaceae* (Pal *et al*., 2023) popularly known as the "Tree of Heaven," which has gained attention for its wide range of therapeutic properties, including anticancer (Vinmathi and Jacob, 2015), antiparasitic (Dell’Agli *et al*., 2008), antiallergic (Kumar *et al*., 2011), analgesic (Patel and Nataraj, 2018), antidiarrheal, and anti-inflammatory activities (Singh, 2016). In addition to its medicinal uses, the tree also serves as fodder, a source of softwood for packaging and material for making soft toys such as puppets (Patel and Nataraj, 2018), matchboxes, and sword handles (Tomar *et al*., 2004). Common names for *Ailanthus excelsa* include ‘Arduso’ (in Gujarati), ‘Maharukh’ (in Hindi), and ‘Arlu’ or ‘Araluvrksa’ (in Sanskrit). It is found in India, Australia, China, and Japan (Singh, 2016; Kumar *et al*., 2011). This deciduous tree thrives in arid and semi-arid environments and is recognized as a fast-growing, multipurpose species recommended by the National Medicinal Plants Board (NMPB), New Delhi, under the contractual farming scheme (Chavan *et al*., 2015; Pal *et al*., 2023). This deciduous tree endured with low seed viability and frequent fungal contamination, which includes delignification by *Inonotus hisidus* and branch invasion by *Bjerkandera adusta* (Koyani *et al*., 2015; Nayak *et al.,* 2019). Moreover, very few studies are available for the *in vitro* clonal propagation of the tree, ensuring contamination-free and healthy seedlings.

Seeds are the primary source for generating healthy plants with minimal microbial contamination. Seed germination is a critical physiological process in all seed-bearing plants and is influenced by various environmental and physiological factors (Ravindran and Kumar, 2019). Under controlled conditions, this process can be effectively regulated. Somatic embryogenesis has emerged as a pivotal technique for conserving the genetic integrity of woody and fruit-bearing tree species. It mirrors zygotic embryogenesis and is considered one of the most promising methods for large-scale propagation in forestry and agriculture (Aronen *et al*., 2025). Furthermore, somatic embryogenesis is important in developing stress-resistant and genetically modified plant varieties (Abate *et al*., 2019). The present study was aimed to develop somatic embryos from the sterile seedlings obtained from *in vitro* germinated seeds. The seeds of *A. excelsa* were germinated under *in vitro* conditions to obtain disease-free, genetically stable plantlets using an appropriate amount of GA3. The plantlets were used as sterile explants for the induction of somatic embryos *via* callus formation. Furthermore, the effects of different cytokinins on somatic embryo development were evaluated.

**2. METHODOLOGY**

**2.1 Collection and preparation of explants**

Dry and mature seeds of *A. excelsa* were collected in April and May (2019) from the Railway station of Vadodara Junction (India) near Platform number 7 (73.19 °E; 22.30 °N), and each seed was manually separated from wings. Such separated seeds (without wings) were inoculated after surface sterilization.

**2.2 Surface sterilization**

Wingless seeds (explants) were washed under running tap water for 5–10 minutes, followed by treatment with 0.1% (v/v) Tween-20 solution for 3–5 minutes to remove surface debris. The explants were then rinsed thoroughly with running tap water and washed twice with sterile distilled water. Later, the process of surface sterilization was carried out under aseptic conditions in a laminar airflow cabinet. Seeds were sequentially treated with 0.2% Bavistin (15 minutes), 70% ethanol (5 minutes), and 0.1% mercuric chloride (HgCl₂) (2 minutes), followed by rinsing with sterile distilled water two times after each chemical treatment. Finally, seeds were washed with sterile distilled water three times to remove traces of chemicals.

**2.3 Seed germination experiment**

Murashige and Skoog (MS) medium supplemented with varying concentrations of gibberellic acid (GA₃) was utilized used for seed germination. GA₃ was incorporated into the medium at concentrations of 0.1, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, and 3.5 mg/L. The experiment was conducted in triplicate, with each treatment comprising 10 replicates. Seeds were inoculated into borosilicate glass test tubes (16 × 250 mm) containing 15 mL of medium. The inoculated tubes were maintained in complete darkness at 25.0 ± 2.0 °C. Germination was monitored at 48-hour intervals for 30 days. Data were recorded for each treatment and expressed as mean ± standard deviation (SD).

**2.4 Callus induction and somatic embryo embryogensis**

Plantlets obtained from *in vitro* seed germination, exhibiting adequate shoot length, were aseptically excised into 1–2 cm segments and cultured on a previously standardized callus induction medium (Patel and Nataraj, 2018). This medium consisted of MS medium supplemented with 2.0 mg/L α-naphthalene acetic acid (NAA), 0.1 mg/L ascorbic acid, and 0.15 mg/L citric acid, the latter two serving as anti-browning agents. Cultures were maintained in darkness at 25.0 ± 2.0 °C for 15 days to induce callus formation. Subculturing was performed every 15 days under aseptic conditions to promote biomass accumulation and to monitor the development of granular callus. Afterwards, a scoopful of callus was transferred to 30 mL of liquid MS medium (in 150 mL Erlenmeyer flasks) supplemented with 2.0 mg/L NAA and/or 6-benzylaminopurine (BAP), alone or in combination with kinetin (Kn) or thidiazuron (TDZ), to evaluate further morphogenetic responses. Cultures were maintained at 25 ± 2 °C on a rotary shaker at 80 rpm in dark conditions until visible morphological changes were observed in the callus.

**2.5 Effect of different cytoknines on somatic embryo**

Somatic embryos developed in liquid medium were transferred to solid MS medium supplemented with various concentrations of 6-benzylaminopurine (BAP: 0.1, 0.5, 1.0, and 2.0 mg/L), either alone or in combination with kinetin (0.1 mg/L) or thidiazuron (TDZ: 0.1 mg/L). Ascorbic acid (0.1 mg/L) and citric acid (0.15 mg/L) were included in the medium as anti-browning agents combined with the growth regulators. Each treatment was evaluated in triplicate, with 10 replicates per treatment. Cultures were incubated at 25 ± 2 °C under a 16/8-hour (light/dark) photoperiod. MS medium without growth regulators served as the control.

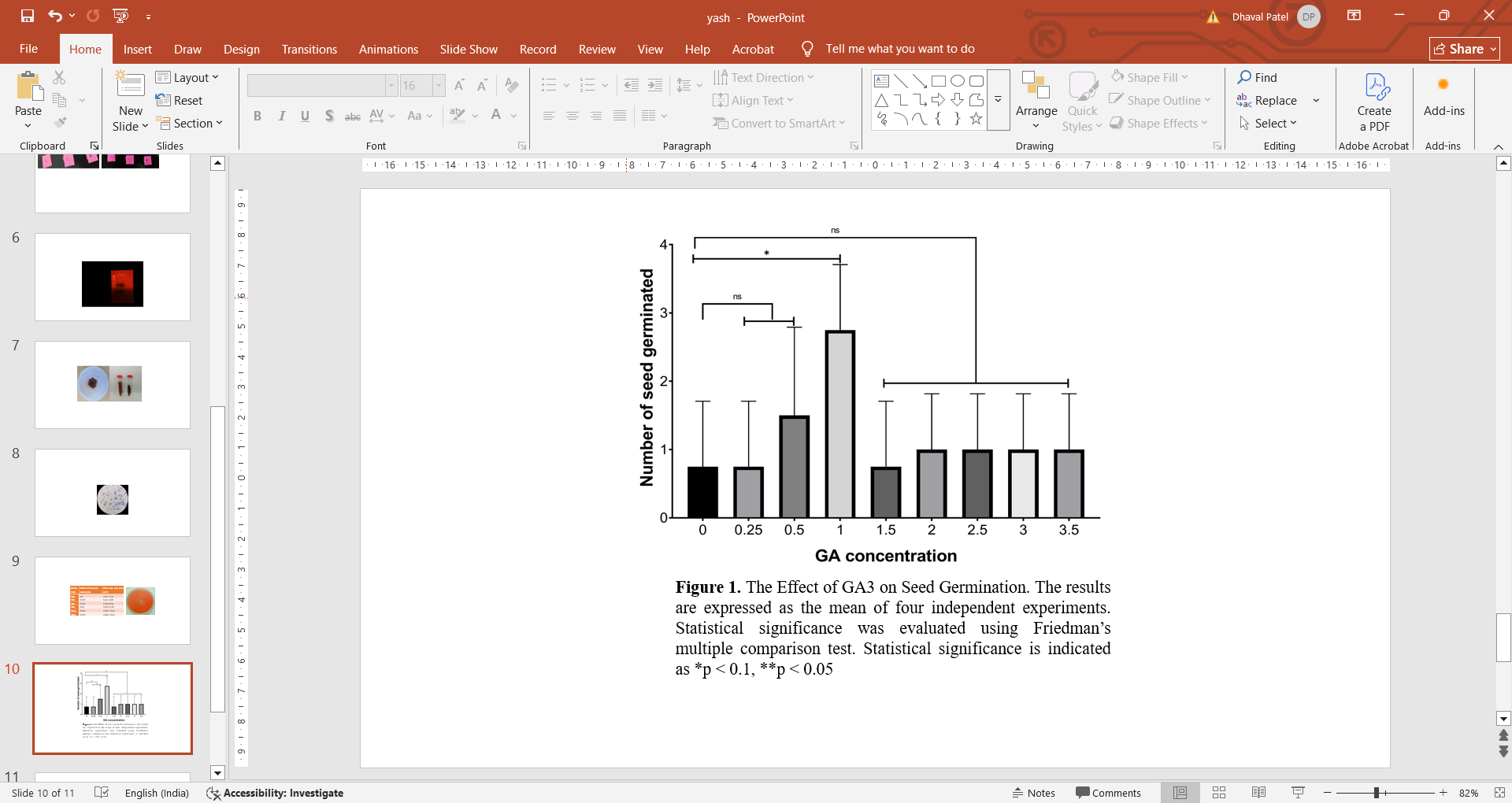
**2.6 Statistical Analysis**

All the experiments were carried out in a randomized complete block design, and each trial contained 10 replicates. Results were noted as mean ± standard deviation. Data obtained were subjected to analysis of variance (ANOVA) to assess treatment difference and interactions, and Duncan’s Multiple Range Test at *p*≤0.05 using statistical software SPSS (version 19; SPSS Inc., Chicago, IL, USA). Certain analysis was carried out using Prism (v.18).

**3. RESULTS AND DISCUSSION**

**3.1 Effect of Gibberellic Acid (GA₃) on Seed Germination of *Ailanthus excelsa***

Seed germination is a dynamic biological process that initiates the plant life cycle and has important implications in agriculture and forestry. This process is controlled by external environmental factors (light, temperature, and moisture) and internal signals, especially phytohormones (Ravindran and Kumar, 2019). Achieving the highest germination percentage in the shortest time is essential for successful propagation (Iralu and Upadhaya, 2018). GA₃ is well known for its key role in promoting seed germination by stimulating hydrolytic enzymes that break down stored food reserves within the seed (Shah *et al*., 2023). In the present study, the effect of different concentrations of GA₃ on *in vitro* seed germination of *A. excelsa* was evaluated. Results obtained are depicted in the following figure (**Fig. 1**).



The results revealed that supplementation of MS medium with 1.0 mg/L GA₃ resulted in the highest germination percentage (27.5%; *p ≤ 0.05*). Other concentrations of GA3 did not significantly affect the seed germination compared to the control. These findings confirm that GA₃ significantly improves seed germination, with a nearly 3.6 times high at 1 mg/L of concentration. The observed variability in germination is attributed more to physical dormancy than to physiological constraints. Rokas-Arechiga et al. (2011) documented that the GA3 did not improve seed germination in Cacti species. Similarly, *Carica quercifolia* seeds also exhibited a low germination rate at high GA concentrations (Gerber *et al.,* 2014).

The effect of GA₃ in enhancing seed germination has been documented across several plant species. In grapevine (*Vitis vinifera* L.), GA₃ combined with sodium nitroprusside improved seed germination by 58.33% and 56.67% after 24 h and 48 h treatments, respectively (Kara *et al.,* 2020). Seed priming with 800 mg/ L GA₃ for 48 hours significantly improved germination in *Amaranthus retroflexus*, indicating its effectiveness as a cost-efficient dormancy-breaking treatment (Nejad *et al*., 2025).In *Tilia miqueliana*, GA₃ combined with magnetically treated water accelerated dormancy release and increased germination from 29% to 75% within 75 days of cold stratification (Yao and Shen, 2018; Shi *et al*., 2024). Similarly, in *Juglans nigra* L. (Eastern black walnut), GA₃ at 400 ppm with a two-month chilling treatment achieved 69.27% germination and improved seedling growth parameters (Parvin *et al*., 2015). In *Elaeocarpus prunifolius*, the highest germination percentage (31%, T₅₀ = 56 days) was achieved with 500 mg L¹ GA₃ and 1.0% KNO₃, compared to 24% and 213 ± 5 days in control seeds (Iralu and Upadhaya, 2018). In *Santalum album*, seeds soaked in 500 mg/L GA₃ for 24 h exhibited a 74.33% germination rate (Sutheesh *et al*., 2016), while *Parkia timoriana* seeds treated with 500 ppm GA₃ for 24 h showed 64% germination (Thangjam and Sahoo, 2017). The highest germination rate (96.1%) was recorded in *Feronia limonia* (wood apple) using 100 ppm GA₃, with notable improvements in seedling growth and physiological traits (Sau *et al*., 2019). In *Argania spinosa*, GA₃ application reduced the dormancy period of argan kernels from 25.6 ± 4.5 to 20.7 ± 7 days, thus enhancing *in vitro* seed germination efficiency (Justamante *et al.,* 2017). These studies confirm GA₃ as a potent dormancy-breaker, enhancing seed germination in species like *A. excelsa* with low natural germination rates.

**3.2 Somatic embryogenesis**

Seed propagation poses significant challenges due to poor seed storability, the recalcitrant nature of many tree species, and the resultant heterogeneity among seedlings. These limitations can be effectively addressed through somatic embryogenesis (SE), an *in vitro* propagation technique that enables the large-scale production of genetically uniform plantlets. SE exploits the totipotency of somatic cells, where in differentiated cells are induced to form somatic embryos under specific stress conditions and in the presence of plant growth regulators. This multistep process provides a reliable alternative for mass clonal propagation, particularly for species with limited seed viability or propagation difficulties. (Sivasankarreddy *et al*., 2024)

A collage of a plant growth in a test tube

AI-generated content may be incorrect.In the following figure (**Fig.2**), the seeds of *A. excelsa* with and without wings are depicted. The figure also includes the germinated seedlings which used for callus formation. (see **Fig.2**)

**Figure 2 (a)** Seeds of Ailanthus excelsa Roxb. with wings and without wings **(b)** Seed germination events **(c)** Callus after two successive subculturing **(d)** Excised plantlets inoculated in MS medium, crystaline callus induced after 15 days, and granular friable callus

Seedlings obtained through *in vitro* germination were used as explants to induce callus (see **Fig. 2b**). Dark incubation in media supplemented with NAA produced watery fragile callus – ‘crystalline callus’ (**Fig. 2d**). On subsequent incubation in the same medium and under the same conditions, callus became yellowish and granular (**Fig. 2c**). Later, when granular structure or friable callus was cultured in liquid MS medium with different concentration of NAA (0.5, 1.0, 1.5 and 2.0mg/L) under shaking condition (80 rpm), it produced the bipolar structures i.e. somatic embryos.

The following table (**Table 1**) describes the treatment and results obtained through the experimentation.

**Table 1 (a)** Effect of BAP, TDZ, Kn, and/or NAA on friable callus to propagate somatic embryo. The results include the mean somatic embryo obtained from three independent experiments. The data was analysed using Duncan’s multiple range test (p=0.05). For ease of comparison, the codes are used. **(b)** shows the analysis table.

**(a)**

|  |  |  |  |
| --- | --- | --- | --- |
| **Growth Regulator (mg/L)** | | **Appearance of elongated root buds and shoot buds** | **Comparative response** |
| **NAA** | **BAP** |
| 0.0 | 0.0 | No | — |
| 0.5 | — | Yes: Root buds were longer than shoot buds | + |
| 1.0 | — | Yes: Root buds were longer than shoot buds | ++ \* |
| 1.5 | — | Yes: Root buds were longer than shoot buds | ++ |
| 2.0 | — | Yes: Root buds were longer than shoot buds | +++ \* |
| — | 0.5 | Yes: Shoot buds and root buds with variable size | + \* |
| — | 1.0 | Yes: Shoot buds and root buds with variable size | + |
| — | 1.5 | Yes: Shoot buds and root buds with variable size | + |
| — | 2.0 | Yes: Shoot buds and root buds with variable size | + |
| 0.5 | 1.5 | Yes: Shot buds were longer than root buds | ++ |
| 1.0 | 1.0 | Yes: Shot buds were longer than root buds | +++ |
| 1.5 | 0.5 | Yes: Shot buds were longer than root buds | ++ |
| + → Approximately 20 germinated somatic embryos  ++ → Approximately 40 germinated somatic embryos  +++ → More than 50 germinated somatic embryos  ++++ → More than 70 germinated embryos | | | |

**(b)**

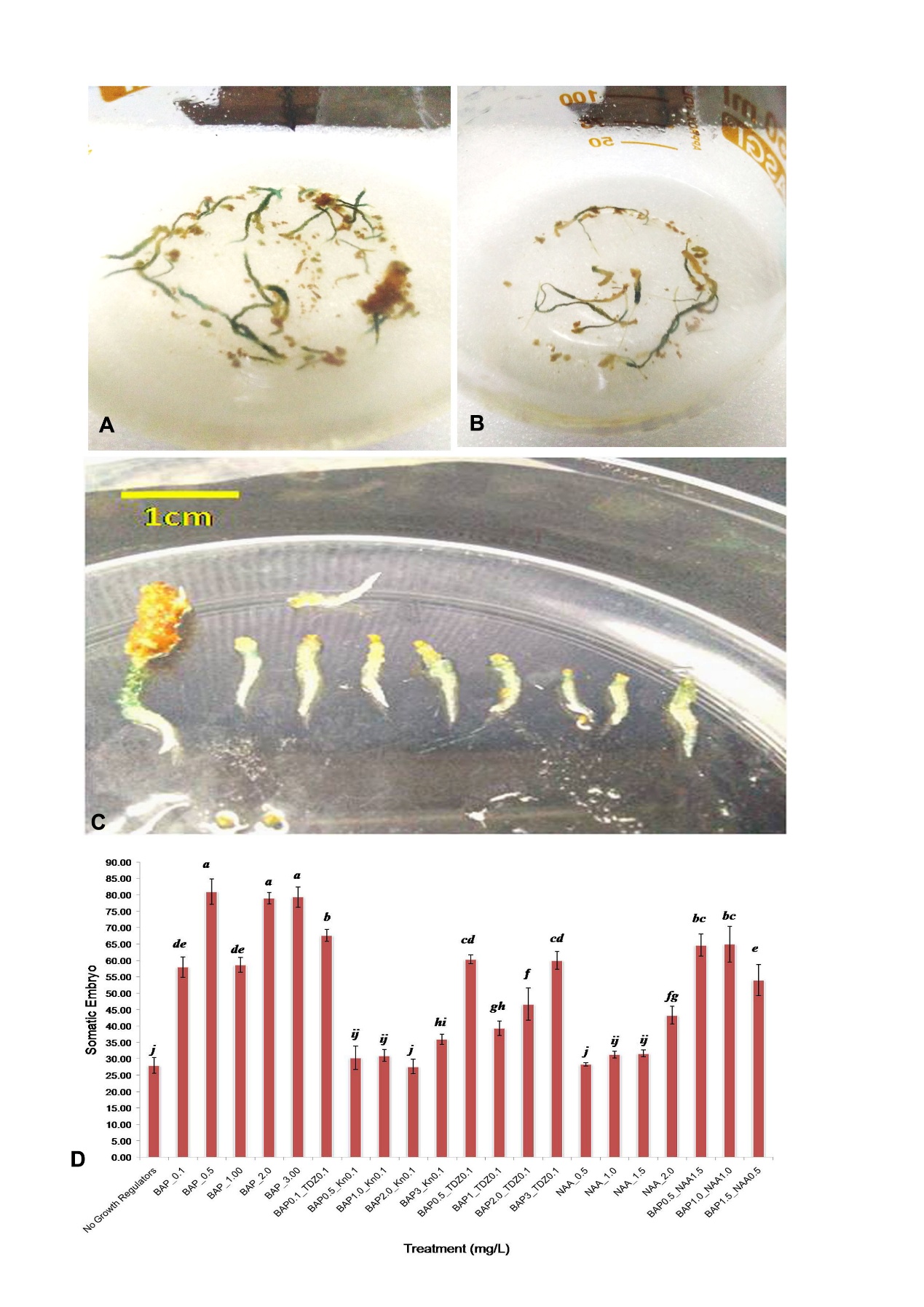
|  | Sum of Squares | df | Mean Square | F | Significance |
| --- | --- | --- | --- | --- | --- |
| Between Groups | 1907.300 | 38 | 50.192 | 1.809 | .055 |
| Within Groups | 749.200 | 27 | 27.748 |  |  |
| Total | 2656.500 | 65 |  |  |  |

As it can be seen from **Table 1a** the maximum somatic embryo was developed when granular fragile callus was cultured in NAA at a concentration of 0.5mg/L, 2mg/L, and 3mg/L compared to media without any growth regulators (served as control). Addition of other growth regulators, i.e., TDZ, Kn, and BAP, considerably supported somatic embryogenesis along with NAA. Interestingly, it was also observed that the media added with BAP and TDZ in contrast to NAA alone, supported somatic embryogenesis. Data analysis using Duncan’s multiple range test showed that the treatment significantly affected somatic embryogenesis. Comparison of mean values of obtained data from independent experimentation (described in **Table 1a**) followed by one factor analysis of variance (ANOVA) resulted in to value of ‘*p* = 0.055’ (**Table 1b**), which was similar to the considered “*p*” value. It indicated the model or treatment was significant. In addition, hyperhydricity was observed in bipolar structures that were grown in liquid medium.

The success of micropropagation mainly depends on the totipotency and disease-free, healthy stage of the explant with the least microbial contamination. The major reason for choosing such explants was their gnotobiotic and high totipotent nature. Data on somatic embryo induction are comparable with the results documented by Hazubska-Przybył *et al*. (2020). Authors reported that auxin induced different physiological responses in plant materials. NAA promotes the proliferation of embryogenic tissues in *Picea abies* by lowering oxidative stress (Hazubska-Przybył *et al*. 2020). Hypocotyls of *Paeonia ostii* gave rise to somatic embryos when grown in MS medium supplemented with thidiauron (TDZ) (0.5mg/L) and NAA (0.5mg/L) from the compact callus produced in media added with BAP (3.0mg/L) and NAA (1.0mg/L) (Ren *et al*., 2020). Moringa oleifera Lam. 's somatic embryos were proliferated in medium containing NAA (Chand *et al*., 2019). Cassava (*Manihot esculenta* Crantz) embryo germination, maturation, and plant recovery optimally happen in medium containing NAA (0.02mg/L) along with BAP (1mg/L) and GA3 (1.5mg/L) (Syombua *et al*., 2019). Somatic embryos of sugar palm (*Arenga pinnata* Wurmb Merr) matured in MS medium fortified with NAA (1.0mg/L) and BAP (1.0mg/L) (Muda and Awal 2017). In case of *Abutilon indicum* (L.) highest somatic embryos were generated from leaf-derived callus cultured when it was supplied with NAA (2.68μM), BAP (13.32μM), ascorbic acid (11.54μM), and activated charcoal (200mg/L) (Muda and Awal 2017). When 1.0 mg/L of 2,4-D was applied to *Euryodendron excelsum*, it produced light yellow, granular callus within 6 weeks. Transferring this callus to WPM medium supplemented with 1.0 mg/L of NAA resulted in yellow, friable callus, accompanied by the formation of adventitious roots from leaf explants. When WPM was supplemented with 1.0 mg/L of TDZ, BA, or KIN and 0.2 mg/L of NAA, it induced limited compact callus formation, with a few adventitious shoots emerging after 6 weeks. Upon transferring to hormone-free WPM, both adventitious shoots and somatic embryos developed on the surface of the callus. (Xiong et al., 2022)

**3.3 Effect of cytokines on somatic embryo**

We tried to propagate the somatic embryo using various plant hormones at different concentrations. The results are depicted in **Fig. 3**.



**Figure 3 (a)** and **(b)** Bipolar somatic embryos grown in liquid medium **(c)** Isolated bipolar somatic embryo **(d)** Effect of growth regulators on somatic embryo induction. Statistical significance the title ???

Among the tested concentrations of BAP, the highest shoot induction (7.33±0.58 shoots and 7.33±1.00 shoots) was observed from bipolar structure (i.e., somatic embryo) in media supplemented with 0.5mg/L or 2.0mg/L of BAP. The high concentration of BAP (2.0mg/L) supported maximum shoot induction with higher shoot length compared to other concentrations *i.e.,* 0.5mg/L. Duncan's multiplicity test for the mean of the independent experimentations showed the *p-value* was 0.004, which was less than 0.05. It indicated the treatment was significant.

Somatic embryos exhibited hyperhydricity, though a distinct bipolar structure—with chlorophyll-containing shoots and white root projections—confirmed their embryogenic nature. Despite this, they failed to develop into complete platelets, likely due to hyperhydricity. Treatment with cytokinins, particularly 6-BAP, enhanced shoot and root elongation *et al*., 2022). Further studies are needed to mitigate hyperhydricity through optimized culture conditions or media supplementation. TDZ was added to the medium for shoot multiplication in tree species (Corredoira *et al*., 2008). Addition of 0.5mg/dm-3 TDZ in MS medium induced multiple shoots in *Plutea lanceolata* (Kher *et al*., 2014)*.* BAP, in combination with low TDZ (0.1 mg/L), effectively induced shoot formation from nodal explants in *A. excelsa*. Higher TDZ concentrations led to callus formation instead of shoot induction(Patel and Nataraj, 2018)*.* Additionally, the induced shoots exhibited vitrification and failed to survive upon subsequent subculturing. Similar results were reported in grapevine axillary bud cultures, where TDZ concentrations exceeding 0.1 µM led to shoot vitrification and reduced viability(Gribaudo and Fronda, 1991). Hyperhydricity was also observed in adventitious shoots of the strawberry sepal when grown in medium supplemented with TDZ (Debnath, 2005). In *Primulina tabacum*, leaf explants initially treated with TDZ followed by BAP produced shoots, whereas the reverse treatment induced somatic embryo formation instead of shoot regeneration. This suggests that the sequence of TDZ and BAP application influences developmental outcomes. Notably, TDZ alone promoted somatic embryogenesis but not shoot induction in this species. (Yang *et al*., 2011). In the present study, TDZ was replaced by kinetin in combination with BAP to check the response. BAP, together with kinetin, showed almost equally significant shoot induction as TDZ with no hyperhydricity. Similar results were reported in the *in-vitro* propagation of ginger, i.e., BAP alone was more effective than a combination of BAP and kinetin (Nkere and Mbanaso, 2010; Sukarnih *et al*., 2021). *Withania coagulans* (Stocks) nodal explants resulted in the formation of shoots in media supplemented with 2.5mg/L TDZ, 0.1mg/L NAA and 50mg/L adenine sulphate, which showed hyperhydricity within one month (Joshi et al. 2016).

**4. CONCLUSION**

This study aimed to overcome low seed viability and the lack of clonal propagation methods in *Ailanthus excelsa* (Tree of Heaven). In vitro germination of *Ailanthus excelsa* was significantly enhanced on MS medium with 1.0 mg/L GA₃ (2.67 ± 0.6 seeds). Seed-derived plantlets cultured on MS medium with 2.0 mg/L NAA under dark conditions (25 ± 2 °C) produced crystalline callus, which upon subculturing, transitioned to yellowish, granular, and subsequently friable callus. When cultured in liquid MS medium with NAA, BAP, Kn, and TDZ under shaking (80 rpm), the friable callus developed bipolar structures indicative of somatic embryos. The highest embryo number (81.00 ± 3.90) was observed with 0.5 mg/L NAA, with significant treatment effects (p = 0.055). Somatic embryos transferred to solid MS medium with BAP, TDZ, or Kn induced shoots (max. 7.33 ± 1.00 with 0.5 and 2.0 mg/L BAP), but failed to develop into plantlets due to hyperhydricity (p = 0.004). Optimization is needed to overcome hyperhydricity and enable full regeneration. The findings of this study provide a foundation for developing a protocol for somatic embryogenesis and artificial seed production in *A. excelsa*, contributing to its mass propagation and conservation.

**DISCLAIMER (ARTIFICIAL INTELLIGENCE)**

The authors hereby state unequivocally that no generative artificial intelligence (AI) tools, such as text-to-image generators or big language models, were used in the drafting of this work. The English in this manuscript was polished with the assistance of OpenAI’s ChatGPT (GPT‑3.5/4) for grammar to improve readability. All content was reviewed, edited, and approved by the authors, who take full responsibility for its accuracy and originality. No text creation by AI is used; all content is the original creation of the human author or authors.

**List of abbreviations:**

ANOVA: analysis of variance; BAP: 6-Benzylaminopurine; °C: degree centigrade; GA3: Gibberellic acid; Kn: kinetin; L: liter; mg: milligram; MS medium: Murashige and Skoog medium; NAA: α-napthalene acetic acid; NMPB: National Medicinal Plant Board; Sec: second; TDZ: thidiazuron; WHO: World Health Organization; %: percentage

**COMPETING INTERESTS DISCLAIMER:**

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

**References**

Abate S, Mekbib F, Gebre E (2019). *In vitro* somatic embryogenesis and plantlet regeneration in anchote [ *Coccinia abyssinica* ( Lam .) Cong .]. *Plant Physioogy Rep* 24: 351-358. doi: 10.1007/s40502-019-00465-9

Aronen, T., Varis, S., & Tikkinen, M. (2025). Somatic embryogenesis: concept, principles, and applications.  *Forest Microbiology* (pp. 373-388). Academic Press. [https://doi.org/10.1016/B978-0-443-21903-0.00022-](https://doi.org/10.1016/B978-0-443-21903-0.00022-9)9

Carnelos, D., Lozano-Miglioli, J., Giardina, E., Tognetti, J., & Benedetto, A. H. D. (2022). Cytokinin action revisited: leaf anatomical changes play a key role in 6-benzylaminopurine-driven growth promotion in pot-grown lettuce. *Revista Chapingo. Serie horticultura*, *28*(2), 109-133. <https://doi.org/10.5154/r.rchsh.2021.07.015>

Chand, S., Pandey, A., & Verma, O. (2019). In vitro regeneration of Moringa oleifera Lam.: A medicinal tree of family Moringaceae. *Indian Journal of Genetics and Plant Breeding*, *79*(03), 606-613. doi: 10.31742/IJGPB.79.3.10

Chavan, S. B., Keerthika, A., Dhyani, S. K., Handa, A. K., Newaj, R., & Rajarajan, K. (2015). National Agroforestry Policy in India: a low hanging fruit. *Current Science*, 1826-1834. <https://www.jstor.org/stable/24905606>

Corredoira, E., Ballester, A., & Vieitez, A. M. (2008). Thidiazuron-induced high-frequency plant regeneration from leaf explants of Paulownia tomentosa mature trees. *Plant Cell, Tissue and Organ Culture*, *95*(2), 197-208. doi:10.1007/s11240-008-9433-6

Debnath, S. C. (2005). Strawberry sepal: another explant for thidiazuron-induced adventitious shoot regeneration. *In Vitro Cellular & Developmental Biology-Plant*, *41*(5), 671-676. doi:10.1079/IVP2005688

Dell’Agli M, Galli GV, Parapini S, Basilico N, Taramelli D, Said A, Rashed K, Bosisio E (2008). Anti-plasmodial activity of *Ailanthus excelsa*. *Fitoterapia* 79(2): 112–116. doi: 10.1016/j.fitote.2007.11.003

Ganguly, R., Ingty, P., Yadav, S. K., Ngoruh, A., Devadasan, V., & Bhattacharjee, A. (2024). Herbal Medicine: History, Contemporary Use and the Future. In *Herbs for Disease Prevention and Treatment* (pp. 148-173). Bentham Science Publishers. Doi.org/10.2174/97898152748821240101

Gerber T, Mergener R, Pinto T, Ramlov. (2014). Effect of gibberellic acid on germination potential *in vitro* seed *Carica quercifolia* (St. Hil).Hieron. (*Caricaceae*). *Ciencias Biologicas*. 5. <https://doi.org/10.36560/50201460>

Ghosh, S., Bishal, A., Ghosh, S. K., Jana, K., Gayen, B., Sahu, S., & Debnath, B. (2023). Herbal medicines: A potent approach to human diseases, their chief compounds, formulations, present status, and future aspects. *International Journal of Membrane Science and Technology*, *10*, 442-464. https://doi.org/10.15379/ijmst.v10i3.1470

Gogoi, I., Dowara, M., & Chetia, P. (2024). Traditional Medicinal Plants and Their Ethnomedicinal Values. In *Traditional Resources and Tools for Modern Drug Discovery: Ethnomedicine and Pharmacology* (pp. 377-399). Singapore: Springer Nature Singapore. https://doi.org/10.1007/978-981-97-4600-2\_14

Gribaudo I, Fronda A (1991). Effects of thidiazuron on grapevine axillary buds cultivated in vitro. *HortSci* 26(8): 1083. doi.org/10.21273/HORTSCI.26.8.1083

Hazubska-Przybył T, Ratajczak E, Obarska A, Pers-Kamczyc E (2020). Different roles of auxins in somatic embryogenesis efficiency in two picea species. *International Journal of Molecular Sciences.* 21(9): 3394. doi: 10.3390/ijms21093394

Iralu, V., & Upadhaya, K. (2018). Seed dormancy, germination and seedling characteristics of Elaeocarpus prunifolius Wall. ex Müll. Berol.: a threatened tree species of north-eastern India. *New Zealand Journal of Forestry Science*, *48*, 1-10. https://doi.org/10.1186/s40490-018-0121-y

Joshi H, Nekkala S, Sonar D, Kher M, Nataraj M (2016). *In-vitro* shoot multiplication of *Withania coagulans* (stocks) Dunal*. Plant Tissue Culture Biotechnology* 26(2): 187-195. doi: <https://doi.org/10.3329/ptcb.v26i2.30569>

Justamante MS, Ibáñez S, Villanova J, Pérez-pérez JM (2017). Vegetative propagation of argan tree ( *Argania spinosa* ( L .) Skeels ) using *in vitro* germinated seeds and stem cuttings. *Sci Hortic* 225: 81–87. doi: 10.1016/j.scienta.2017.06.066

Kara Z, Yazar K, Doğan O, Vergili E (2020). Sodium nitroprusside and gibberellin effects on seed germination and seedling development of grapevine (*Vitis vinifera* L.) Cvs. Ekşi Kara and Gök Üzüm. Erwerbs-Obstbau 62: 61-68. doi: 10.1007/s10341-020-00497-8

Kher MM, Joshi D, Nekkala S, Nataraj M (2014). Micropropagation of *Pluchea Lanceolata* (Oliver and Hiern.) using nodal explant. Journal of Horticulture Research. 22(1):35–39. doi: 10.2478/johr-2014-0004

Koyani, R. D., Pramod, S., Bhatt, I. M., Rao, K. S., & Rajput, K. S. (2015). The delignification pattern of Ailanthus excelsa wood by Inonotus hispidus (Bull.: Fr.) P. Karst. *Journal of Sustainable Forestry*, *34*(5), 502-515. doi:10.1080/10549811.2015.1033554

Kumar, D., Bhat, Z. A., Singh, P., Khatanglakar, V., & Bhujbal, S. S. (2011). Antiasthmatic and antiallergic potential of methanolic extract of leaves of Ailanthus excelsa. *Revista Brasileira de Farmacognosia*, *21*, 139-145. doi: 10.1590/S0102-695X2011005000032

Muda NA, Awal A (2017). Somatic embryogenesis in sugar Palm ( *Arenga pinnata* Wurmb Merr.) from zygotic embryo explant. *Pertanika Journal of Science and Technology* 25: 133–144.

Nayak AK, Babu BK, Srivastava AK (2019). Identification of *Erysiphe quercicola* associated with powdery mildew disease on *Ailanthus excelsa* in India. *Australasian Plant Pathology.* 48: 267–270. <https://doi.org/10.1007/s13313-019-0626-8>

Nejad, M. R., Emami Bistgani, Z., Hashemi, M., & Barker, A. V. (2025). Breaking of Seed Dormancy in Redroot Pigweed. Communications in *Soil Science and Plant Analysis*, 1-12. <https://doi.org/10.1080/00103624.2025.2507362>

Nkere C, Mbanaso E (2010). Optimizing concentrations of growth regulators for *in-vitro* ginger propagation. *Journal of Agrobiology* 27(2): 61–65. doi: 10.2478/s10146-009-0009-9. doi: 10.2478/s10146-009-0009-9

Pal, V., Sharma, V., & Gour, V. S. (2023). Ailanthus excelsa Roxb. in India: A multipurpose “tree of Heaven” for semi-arid regions. Forests, Trees and Livelihoods, 32(4), 268-283. <https://doi.org/10.1080/14728028.2023.2236122>

Pan, S. Y., Litscher, G., Gao, S. H., Zhou, S. F., Yu, Z. L., Chen, H. Q., ... & Ko, K. M. (2014). Historical perspective of traditional indigenous medical practices: the current renaissance and conservation of herbal resources. *Evidence‐Based Complementary and Alternative Medicine*, *2014*(1), 525340. <https://doi.org/10.1155/2014/525340>

Parvin P, Khezri M, Tavasolian I, Hosseini H (2015). The effect of gibberellic acid and chilling stratification on seed germination of eastern black walnut ( *Juglans nigra* L.). J Nuts 6(1): 67–76.

Patel D, Nataraj M (2018). Callus Induction in *Ailanthus Excelsa Roxb*. – A Multipurpose Tree. *International Journal of Scientific Research and Reviews* 7(1): 116–129

Ravindran P, Kumar PP (2019). Regulation of seed germination : the involvement of multiple forces exerted via gibberellic acid signaling. CellPress: *Molecul Plant* 12(1): 24–26. doi: 10.1016/j.molp.2018.12.013

Ren X, Liu Y, Jeong BR (2020). Enhanced somatic embryo induction of a tree peony, Paeonia ostii ‘fengdan’, by a combination of 6-benzylaminopurine (BA) and 1-naphthylacetic acid (NAA). *Plants* 9 (1):1–12 . doi: 10.3390/plants9010003

Roja-Arechiga M, Aguilar K, Golubov J, and Mandujano M (2011). Effect of gibberelllic acid on germination of seed of five species of Cacti from the Chihuahuan desert, North Mexico. *The Southwestern Naturalist*, 56 (3): 393-400

Sau, S., Pal, B., Sarkar, S., & Sarkar, T. (2019). Influence of seed priming on germination and seedling vigour of wood apple (Feronia limonia Swingle). *International Journal of Bio-resource and Stress Management*, *10*(2), 128-136. doi: 10.23910/IJBSM/2019.10.2.1967

Shah, S. H., Islam, S., Mohammad, F., & Siddiqui, M. H. (2023). Gibberellic acid: a versatile regulator of plant growth, development and stress responses. *Journal of Plant Growth Regulation*, *42*(12), 7352-7373.

Shi, F., Cao, Y., Gao, Y., Qiu, Y., Lu, Y., Han, B., & Shen, Y. (2024). The impact of magnetic field and gibberellin treatment on the release of dormancy and internal nutrient transformation in Tilia miqueliana Maxim. Seeds. *Forests*, *15*(2), 311**.**  <https://doi.org/10.3390/f15020311>

Singh, R. K. (2016). Acute-toxicity, anti-inflammatory and anti-diarrhoeal activity of Ailanthus excelsa in mice and rats. *International Journal of Research*, *4*(2), 7-12.

Singh, S., & Kumar, S. (2021). Medicinal plant sector in India: Status and sustainability. *International Journal of Economic Plants*, *8*(2), 81-85. [10.23910/2/2021.0414b](http://dx.doi.org/10.23910/2/2021.0414b)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| |  |  |  |  | | --- | --- | --- | --- | | |  |  |  | | --- | --- | --- | | |  |  | | --- | --- | | |  | | --- | |  | | | | |

Sivasankarreddy, K., Ashwath, M. N., Shilpa, K. S., Joseph, J., Santhoshkumar, A. V., & Shukla, G. (2024). Somatic Embryogenesis and Plant Regeneration in Forest Trees. In Biotechnological Approaches for Sustaining Forest Trees and Their Products (pp. 51-75). Singapore: *Springer Nature Singapore*. https://doi.org/10.1007/978-981-97-4363-6\_3

Sukarnih, T., Rudiyana, Y., Hanifah, N. F., & Sa’adah, N. (2021). Micropropagation of red ginger (Zingiber officinale Rosc. Var. rubrum) using several types of cytokinins. In *Journal of Physics: Conference Series* (Vol. 1751, No. 1, p. 012051). IOP Publishing. **DOI** 10.1088/1742-6596/1751/1/012051

Sutheesh VK, Jijeesh CM, Divya TP (2016) Evaluation of organic and inorganic pretreatments for better seed germination and seedling vigour in *Santalum album* L. *Plant Archives* 16(1): 143–150.

Swami, D. V., Anitha, M., Rao, M. C. S., & Sharangi, A. B. (2022). Medicinal plants: perspectives and retrospectives. In *Medicinal plants* (pp. 1-28). Apple Academic Press. ISBN9781003277408

Syombua ED, Wanyonyi CN, Adro MO, Mbinda WM, Ngugi MP, Alakonya AE, Oduor RO (2019). Explant type and hormone regime influences somatic embryogenesis and regeneration of cassava. *African Journal of Biotechnology*, *18*(25), 532-539. doi: 10.5897/AJB2019.16853

Thangjam U, Sahoo UK (2017). Effect of Different Pre-treatments and germinationmedia on seed germination and seeding growth of *Parkia timoriana* (DC.) Merr. *Journal of Experimental Biology and Agricultural Sciences* 5(1): 98–105. <http://dx.doi.org/10.18006/2017.5(1).098.105>

Tomar UK, Negi U, Sharma N, Emmanuel C (2004). Sucessful grafting in *Ailanthus excelsa* Roxb.- A Brief Rep *MyForest* 40(1): 35–37.

Vinmathi V, Jacob SJP (2015). A green and facile approach for the synthesis of silver nanoparticles using aqueous extract of *Ailanthus excelsa* leaves, evaluation of its antibacterial and anticancer efficacy*. Bulletin of Material Science*. 38: 625–628. doi: 10.1007/s12034-015-0916-x

Xiong, Y., Chen, S., Wu, T., Wu, K., Li, Y., Zhang, X., ... & Ma, G. (2022). Shoot organogenesis and somatic embryogenesis from leaf and petiole explants of endangered Euryodendron excelsum. *Scientific reports*, *12*(1), 20506. <https://doi.org/10.1038/s41598-022-24744-y>

Yang, X., Lü, J., da Silva, J. A. T., & Ma, G. (2012). Somatic embryogenesis and shoot organogenesis from leaf explants of Primulina tabacum. *Plant Cell, Tissue and Organ Culture (PCTOC)*, *109*(2), 213-221. doi: 10.1007/s11240-011-0087-4

Yao, W., & Shen, Y. (2018). Effects of gibberellic acid and magnetically treated water on physiological characteristics of Tilia miqueliana seeds. *Canadian Journal of Forest Research*, *48*(5), 554-558. doi: 10.1139/cjfr-2017-0289