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|  |  | ***In-vitro* Seed Germination and Somatic Embryogenesis in *Ailanthus excelsa* Roxby: A Medicinal Tree of Heaven** |  |

**Abstract**

**Aim:**This study aimed to induce *in vitro* somatic embryogenesis (SE) from germinated *Ailanthus excelsa* seedlings and evaluate the effect of various cytokinins on embryo development and shoot induction. **Method**:Mature, dry *Ailanthus excelsa* seeds were surface sterilized and cultured on MS medium with gibberellic acid (0.1–3.5 mg/L). *In vitro* germinated plantlets were sectioned and cultured in MS medium with 2.0 mg/L NAA, 0.1 mg/L ascorbic acid (an anti-browning agent), and 0.15 mg/L citric acid (an anti-browning agent) to induce callus. Callus was subcultured once every two weeks and transferred to liquid MS medium with NAA and/or BAP, Kn, or TDZ to promote SE. Embryos were further cultured on solid MS medium with BAP, alone or with Kn or TDZ, and ascorbic acid. Statistical analysis was performed by ANOVA and Duncan’s Multiple Range Test (p ≤0.05). **Results**: Seed germination was significantly improved from 3.3% to 26.7% with 1.0 mg/L gibberellic acid. Callus derived from *in vitro* seedlings produced bipolar somatic embryos with the highest number (81.00 ± 3.90) achieved with 0.5 mg/L NAA. The maximum shoot induction (7.33 ± 0.58 and 7.33 ± 1.00) occurred with 0.5 and 2.0 mg/L BAP, respectively. Growth regulator treatments significantly influenced SE (*p* = 0.055) and shoot induction (*p* = 0.004). **Conclusions:** Germinated seedlings served as effective explants for SE in *A excelsa.* Low concentration of NAA in liquid MS medium promoted SE, while BAP induced shoot formation. Although hyperhydricity was observed during shoot elongation. These findings lay the foundation for future work on somatic embryo conversion, artificial seed production, large-scale propagation, and conservation of *A. excelsa* Roxb.

**Key Word**

*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 which 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 medicinal uses, the *A. excelsa* 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 *A. 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 tree species recommended by the National Medicinal Plants Board (NMPB), New Delhi, under the contractual farming scheme (Chavan *et al*., 2015; Pal *et al*., 2023). *Ailanthus excelsa,* 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). Currently, limited studies have focused on the *in vitro* clonal propagation of *Ailanthus excelsa* species, which is essential for producing 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 (SE) 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). SE is important in developing stress-resistant and genetically modified plant varieties (Abate *et al*., 2019). The present study 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. The effects of different cytokinins on somatic embryo development were also 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). Each seed was prepared for the germination under *in vitro* condition by manually separating from wings. Such separated seeds (without wings) were subjected to surface sterilization.

**2.2 Surface sterilization**

The explants (here wingless seeds) 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. Later, the explants were rinsed thoroughly under running tap water and washed twice with sterile distilled water. 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. The explants were washed with sterile distilled water 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 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 the results are expressed as mean ± standard deviation (SD).

**2.4 Callus induction and SE**

Plantlets obtained from *in vitro* seed germination, exhibiting adequate shoot length (to three-fourths of the test tube), 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). The anti-browning agents (0.1 mg/L) ascorbic acid and 0.15 mg/L citric acid were also added to the medium. 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. Later, a scoopful of granular 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 cytokinins 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 ± SD. 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**).

**Figure 1** The effect of GA3 on seed germination. The results are expressed as the mean and SD of four independent experiments. Statistical significance was evaluated using Friedman’s multiple comparison test. Statistical significance is indicated as \*p< 0.1, \*\*p<0.05

 The results revealed that supplementation of 1.0 mg/L GA₃ in MS medium 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 higher. 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 GA3 concentrations (Gerber *et al.,* 2014). GA3 at 600 mg/L was reported to be optimum for *Malania oleifera* seed germination (Su *et. al.,* 2025). Similarly, 500 mg/L GA3 reduced the time-to-germination and enhanced germination probability, significantly in *Zostera marina* L. (Pieraccini *et al.,* 2025). GA3 treatment increases the transcriptional gene expression of α-amylase and its isoforms, proteases, and hydrolases, which helps in the initial period of seed germination (Damaris *et al.,* 2019).

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-hour and 48-hour 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 (**SE)

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 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)

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)** Different stages of seed germination events **(c)** Friable callus formed after two successive subculturing of excised in vitro germinated plantlets **(d)** Excised plantlets inoculated in MS medium supplemented with growth regulators, crystaline callus induced after 15 days, and granular friable callus. The arrow indicated the progressive stages from explant inoculation to the formation of 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 concentrations 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)** 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 SE along with NAA. Interestingly, it was also observed that the media added with BAP and TDZ in contrast to NAA alone, supported SE. Data analysis using Duncan’s multiple range test showed that the treatment significantly affected SE. 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. This indicates that the treatment has a statistically significant effect. In addition, hyperhydricity was observed in bipolar structures that were grown in a 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). The 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 growth regulator-free WPM, both adventitious shoots and somatic embryos developed on the surface of the callus. (Xiong et al., 2022)

**3.3 Effect of cytokinins on somatic embryo**

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



**Figure 3 (a)** and **(b)** The bipolar somatic embryos grown in the 150 mL Erlenmeyer flask containing 50 mL liquid media **(c)** Isolated bipolar somatic embryo exhibiting green and white regions similar to shoot and root regions of a plantlet**(d)** Effect of growth regulators on somatic embryo induction from friable callus. (The results shown here are the mean ± SD of three independent experiments. Statistical significance was evaluated using Duncan’s multiple range test at p ≤ 0.05. Different lowercase letters on the bar indicate statistically significant differences between treatments.)

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 i.e., 2.0mg/L supported highest shoot induction with (comparatively) maximum shoot length to other concentrations *i.e.,* 0.5mg/L. The results indicated that 2.0 mg/L BAP supplemented in MS media significantly affects the shoot induction (*p-value* = 0.004).

Somatic embryos exhibited 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. Hyperhydricity is a challenge (Abiri *et al.,* 2025) that occurs due to high water potential of MS media, or high amount of ethylene generated by *in vitro* plants, or sharp decrease in ethylene or inhibition of ethylene synthesis, leading to a decrease in lignin biosynthesis (Zeng *et al*., 2025). This reduction in lignin synthesis resulted in cell wall and cell wall pressure (Zeng *et al*., 2025). Hyperhydricity is a common morphological and anatomical disorder in plant tissue culture (Abiri *et al.,* 2025). The plantlets with low cell wall pressure and low lignin in the cell wall tend to absorb more water from the media which resulted in hyperhydricity (Zeng *et al*., 2025).

 TDZ was used as the medium for shoot multiplication in tree species (Corredoira *et al*., 2008). Addition of 0.5mg/L TDZ in MS medium induced multiple shoots in *Plutea lanceolata* (Kher *et al*., 2014)*.* BAP, in combination with low TDZ (0.1mg/L), effectively induced shoot formation from nodal explants in *A. excelsa* (Patel and Nataraj, 2018). Higher TDZ concentrations lead to callus formation instead of shoot induction(Patel and Nataraj, 2018)*.* Additionally, the induced shoots exhibited vitrification and failed to survive upon subsequent subculturing. In the current study, we used liquid media to induce SE from friable callus, which is possibly the reason for hyperhydricity in somatic embryos. Similar results were reported in grapevine axillary bud cultures, where TDZ concentrations exceeding 0.1µM 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 SE but not shoot induction in *P. tabacum* species (Yang *et al*., 2011). In the present study, TDZ was replaced by kinetin in combination with BAP to check the shoot induction. 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, in which the 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). To mitigate hyperhydricity the optimization of culture conditions or media supplementation is advisable. To reduce hyperhydricity, it was recommended to increase the sucrose concentration, incorporate activated charcoal or L-ascorbic acid in MS media (Zeng *et al*., 2025). Variation in photoperiod was used to reduce hyperhydricity traditionally (Leis *et al.,* 2025). The chemical additives, such as calcium nitrate and calcium chloride, have been used to lower the hyperhydricity to 34.30% in combination with ammonium nitrate and metatopolin in *Pistacia khinjuk* stock  *in vitro* shoots (Ersali*,* 2024)

**4. CONCLUSION**

This study aimed to overcome low seed viability and the lack of clonal propagation methods in *Ailanthus excelsa* (aka ‘Tree of Heaven’). *In vitro* germination of *Ailanthus excelsa* seeds was significantly enhanced (2.67 ± 0.6 seeds) in MS medium with 1.0 mg/L GA₃. Seed-derived plantlets were cultured on MS medium with 2.0 mg/L NAA under dark conditions (25 ± 2 °C) to produce crystalline callus, which upon subculturing, transitioned to yellowish, granular, and subsequently friable callus. The friable callus was cultured in liquid MS medium with NAA, BAP, Kn, and TDZ under shaking (80 rpm), the callus developed bipolar structures which indicated the formation of somatic embryos. NAA (0.5 mg/L) significantly (*p=* 0.055) induced the highest embryo (81.00 ± 3.90). Somatic embryos transferred to solid MS medium with BAP, TDZ, or Kn induced shoots (maximum 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. These findings lay the foundation for future work on somatic embryo conversion, artificial seed production, large-scale propagation, and conservation of *A. excelsa* Roxb.

**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

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