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Induction of Adventitious Roots from Leaf Explants of *Talinum fruticosum* (L.) Juss. through the Application of Different IBA Concentrations In Vitro

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Abstract: This study aimed to evaluate the effect of indole-3-butyric acid (IBA) concentrations on adventitious root induction from *Talinum fruticosum* leaf explants under in vitro conditions. The experiment was conducted using a completely randomized design with three IBA concentrations (2, 4, and 6 mg/L) and 20 replicates for each treatment. Leaf explants with an approximate diameter of 1.5 cm were cultured on Murashige and Skoog (MS) medium supplemented with IBA and observed for approximately 30 days. The evaluated parameters included explant survival percentage, root induction percentage, root initiation time, root number, root length, and root biomass (fresh and dry weight). Data were analyzed using analysis of variance (ANOVA). The results showed that IBA supplementation significantly affected root initiation time, with the fastest root emergence occurring after 8 days, and promoted root elongation, reaching a maximum root length of 1.23 cm. Explant survival and root induction percentages reached 100% in all treatments, indicating no significant differences among IBA concentrations. However, IBA application did not significantly affect root number or root biomass. Among the treatments, 2 mg/L IBA exhibited the best response by accelerating root initiation and increasing root length compared with the other concentrations. Therefore, the application of IBA at 2 mg/L effectively optimized selected parameters associated with adventitious root induction in *T. fruticosum* under in vitro conditions.
Keywords: Adventitious root; IBA; tissue culture; in vitro; *Talinum fruticosum*

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INTRODUCTION

The increasing demand for plant-derived therapeutic compounds has stimulated considerable interest in the exploration and sustainable utilization of medicinal plant resources. Medicinal plants serve as important sources of bioactive compounds for both traditional and modern healthcare systems. Therefore, the development of local plant species with high bioactive potential has become increasingly important, particularly for the production of herbal-based products (Widowati et al., 2020). One promising medicinal plant is Javanese ginseng (*Talinum fruticosum* (L.) Juss.), locally known as kolesom Jawa. This species belongs to the family Talinaceae and is widely distributed throughout tropical regions, including Indonesia. It has traditionally been used as both a vegetable and a medicinal plant due to its beneficial health properties (Ekawati, 2018). *T. fruticosum* contains various pharmacologically valuable secondary metabolites, including saponins, alkaloids, flavonoids, tannins, and phytosterols (Adeyemi, 2018; Airaodion et al., 2019). These compounds contribute to its diverse biological activities and support its traditional use as a natural tonic.

Conventional propagation of Javanese ginseng is commonly performed through seeds and stem cuttings; however, these approaches have several limitations, including low germination rates, limited seed availability, and relatively slow root

development (Restiani et al., 2022). Furthermore, the plant requires approximately 3–4 years to accumulate optimal levels of saponins (Khanam et al., 2022), which restricts the efficiency of conventional cultivation for continuous biomass production. This limitation is particularly relevant because root biomass represents an important source of bioactive compounds in medicinal plants. Therefore, sustainable approaches for producing root biomass under controlled conditions are required. Plant tissue culture, particularly in vitro adventitious root culture, offers a promising alternative because it enables rapid and controlled root biomass production, maintains genetic stability, and preserves the capacity to produce valuable bioactive compounds comparable to those of the mother plant (Murthy et al., 2020; Hussain et al., 2022).

The success of adventitious root induction in in vitro systems is regulated by various internal and external factors, including explant physiological conditions and the type and concentration of plant growth regulators. Among these regulators, auxins play a crucial role in stimulating root formation by promoting cell dedifferentiation, root primordium initiation, and subsequent root development. Indole-3-butyric acid (IBA) is one of the most widely used auxins for root induction due to its effectiveness in enhancing rooting responses, including rooting percentage, root number, and root elongation, although the optimal concentration varies among plant species (Isah, 2019; Parveen & Shahzad, 2021; Singh et al., 2022). Previous studies have demonstrated the effectiveness of IBA application in inducing adventitious roots in *Talinum paniculatum*, where a concentration of 2 mg/L IBA produced favorable rooting responses (Manuhara et al., 2023). However, the optimal IBA concentration for adventitious root induction in *T. fruticosum* remains unknown.

Despite the potential of *T. fruticosum* as a medicinal plant, studies focusing on adventitious root induction from its leaf explants are still limited. Differences in physiological characteristics and morphogenetic responses among plant species highlight the need for species-specific optimization of growth regulator treatments. In particular, information regarding the effects of different IBA concentrations on root initiation, root growth characteristics, and biomass accumulation in *T. fruticosum* is currently unavailable. Addressing this knowledge gap is essential for developing an efficient in vitro root culture system for this species.

Therefore, this study aimed to evaluate the effects of different IBA concentrations on adventitious root induction, development, and biomass production from *T. fruticosum* leaf explants under in vitro conditions. The findings of this study are expected to provide fundamental information regarding the response of *T. fruticosum* to IBA supplementation and serve as a basis for the development and optimization of adventitious root culture techniques for sustainable production of root biomass in this medicinal plant.

METHOD

Plant Material and Experimental Design

The study was conducted at the Plant Physiology Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Andalas, Padang, Indonesia, from December 2025 to March 2026. The equipment used included Petri dishes, culture bottles, 100 mL measuring cylinders, 250 mL Erlenmeyer flasks, spatulas, Bunsen burners, 500 and 1000 mL beakers, magnetic stirrers, pH indicator strips, a laminar air flow cabinet (LAF), autoclave, droppers, 25 cm straight forceps, scalpel (No. 4), blades (No. 23), analytical balance, oven, scissors, hand sprayer, gloves, hot plate, refrigerator, black cloth, labels, masks, culture racks, stationery, and a camera. The materials used included *Talinum fruticosum* leaves, indole-3-butyric

acid (IBA) as a plant growth regulator, Murashige and Skoog (MS) medium (Phytotech), sterile distilled water, detergent, povidone-iodine, spirit, polypropylene plastic, rubber bands, 10% Clorox, 70% ethanol, agar, sucrose, aluminum foil, tissue paper, 1 N NaOH, and 1 N HCl.

Leaf explants of *T. fruticosum* were used for adventitious root induction under in vitro conditions. Root induction was performed using Murashige and Skoog (MS) basal medium supplemented with IBA at concentrations of 2, 4, and 6 mg/L. Explants were cultured in culture bottles containing four explants per bottle and incubated at room temperature (24–25°C) for approximately 30 days until root emergence. The experiment was arranged using a completely randomized design (CRD) with a single treatment factor, namely IBA concentration, consisting of three levels (2, 4, and 6 mg/L). Each treatment was replicated 20 times, resulting in a total of 60 experimental units. The observed parameters included: (1) explant survival percentage, (2) root formation percentage, (3) root initiation time, (4) root number, (5) root length, and (6) root biomass.

Experimental Procedures

1. Sterilization of equipment

Heat-resistant culture equipment, including culture bottles, forceps, scissors, and culture media containers, were sterilized using an autoclave at 121°C and 1.2 atm pressure for 15 minutes. Heat-sensitive equipment was sterilized using 70% ethanol. The working area was sterilized prior to use by spraying with 70% ethanol and exposing it to ultraviolet (UV) irradiation.

2. Preparation of adventitious root induction medium

The culture medium was prepared using MS basal medium supplemented with 30 g/L sucrose as a carbon source and 6 g/L agar as a solidifying agent. IBA was subsequently added to the medium according to the treatment concentrations (2, 4, and 6 mg/L). All components were dissolved thoroughly using distilled water, and the medium pH was adjusted to 5.8–6.0 using 1 N NaOH or HCl before heating. The homogenized medium was dispensed into culture bottles and sterilized by autoclaving at 121°C and 1.2 atm pressure for 15 minutes.

3. Explant sterilization

Leaf explants of *T. fruticosum* were collected from healthy mother plants, specifically the third to fifth leaves from the shoot apex. Explant sterilization was performed through a series of sequential steps. Initially, explants were washed with detergent solution to remove surface contaminants. Subsequently, explants were immersed in povidone-iodine solution, followed by treatment with 10% Clorox and 70% ethanol. After each sterilization step, explants were rinsed with sterile distilled water to remove residual sterilizing agents before inoculation onto the culture medium.

4. Adventitious Root Initiation

Adventitious root induction was initiated by cutting *T. fruticosum* leaf explants into approximately 1.5 cm segments and placing them onto culture media according to the respective treatments. Explant inoculation was conducted aseptically inside a laminar air flow cabinet to maintain sterile conditions. The cultures were subsequently incubated at approximately 25°C under dark conditions for 30 days to promote adventitious root induction, following the procedure described by Manuhara et al. (2023).

Observation Parameters

1. Explant survival percentage (%)

Explant survival percentage was determined on day 30 after inoculation by

calculating the number of viable explants relative to the total number of cultured explants and expressing the value as a percentage.

2. **Root formation percentage (%)**
   Root formation percentage was assessed on day 30 by calculating the number of explants that successfully developed adventitious roots relative to the total number of cultured explants and expressed as a percentage.
3. **Root initiation time (days after planting, DAP)**
   Root initiation time was recorded based on the first appearance of adventitious roots after explant inoculation and expressed as days after planting (DAP).
4. **Number of roots per explant**
   The number of roots was determined at the end of the observation period (day 30) by counting the total number of adventitious roots produced by each explant.
5. **Root length (cm)**
   Root length was measured at the end of the observation period using a ruler. Measurements were taken from the root base to the tip of the longest root and expressed in centimeters (cm).
6. **Root biomass**
   Root biomass consisted of fresh and dry weight measurements. Fresh weight was determined immediately after harvesting by weighing the roots, whereas dry weight was obtained after drying root samples in an oven at approximately 60°C until a constant weight was achieved. Biomass measurements were performed using an analytical balance and expressed in grams (g).

Data Analysis

Data obtained from measurements of explant survival percentage, root formation percentage, root initiation time, root number, root length, and root biomass were analyzed using one-way analysis of variance (ANOVA). When significant differences were detected, the analysis was followed by Duncan's New Multiple Range Test (DNMRT) at a significance level of $p \leq 0.05$. Statistical analyses were performed using SPSS version 27.0.

RESULTS AND DISCUSSION

Explant Survival and Root Formation Percentage

The survival rate and root formation of *Talinum fruticosum* leaf explants reached 100% across all IBA treatments. These results indicate that all explants were able to survive and respond positively to root induction following IBA supplementation in the culture medium, as presented in Table 1.

Table 1. Percentage of root-forming explants and survival rate of *T. fruticosum* leaf explants

IBA concentration (mg/L)	Root formation (%)	Explant survival (%)
2	100	100
4	100	100
6	100	100

Table 1 shows that all IBA treatments (2, 4, and 6 mg/L) resulted in 100% explant survival and root formation in *Talinum fruticosum* leaf explants. These findings indicate that all tested IBA concentrations were sufficient to support explant viability and adventitious root induction, demonstrating the high regenerative capacity of *T. fruticosum* under in vitro conditions.

The high survival percentage was consistent with the successful root formation observed in the explants, as visually presented in Figure 1.

Figure 1. Condition of *T. fruticosum* explants showing successful root induction under 2 mg/L IBA treatment after 30 days

The high explant survival rate suggests that the culture medium provided adequate macro- and micronutrients, vitamins, and carbon sources required to support cellular metabolism during the culture period (Garg et al., 2024; Pasternak & Steinmacher, 2024). Furthermore, successful root formation in all treatments indicates that *T. fruticosum* leaf explants possess high totipotency and regeneration capacity under in vitro conditions. This response was also influenced by optimal culture environmental conditions, including incubation temperature, aseptic conditions, and appropriate medium composition. Exogenous IBA, as an auxin, plays an important role in activating adventitious root formation by stimulating parenchymal cell division and tissue differentiation into root primordia (Khan et al., 2022). Therefore, IBA supplementation effectively supported rhizogenesis in *T. fruticosum* leaf explants under in vitro conditions.

Root Initiation Time

The results showed that IBA treatments affected the initiation time of adventitious roots in *T. fruticosum* leaf explants under in vitro conditions. The 2 mg/L IBA treatment produced the fastest root emergence, occurring at 8 days after planting (DAP), whereas the 4 and 6 mg/L IBA treatments required 10 and 11 DAP, respectively (Figure 2). These findings indicate that increasing IBA concentrations tended to delay adventitious root initiation.

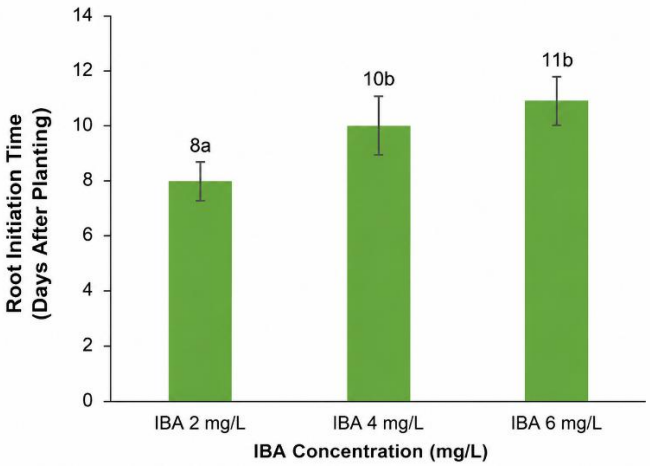

Figure 2. Root initiation time (DAP) of *T. fruticosum* leaf explants under different IBA treatments

Physiologically, IBA is a synthetic auxin that plays an essential role in stimulating root formation through cell dedifferentiation, root meristemoid formation, and vascular tissue differentiation (Sekhukhune & Maila, 2024). At an optimal concentration, IBA can enhance cell division activity and protein synthesis associated with root primordium formation. The 2 mg/L IBA concentration may have provided an appropriate hormonal balance between endogenous and exogenous auxin levels, thereby accelerating the rhizogenesis process. In contrast, increasing IBA concentration to 6 mg/L may have resulted in excessive auxin accumulation within explant tissues, which could inhibit physiological cellular processes, including cell division and early differentiation of root-forming cells. Excessive auxin levels may also stimulate ethylene production, which acts as an inhibitor of root growth and consequently delays adventitious root emergence (Bai et al., 2020).

Similar responses have been reported by Bayoudh et al. (2026), who demonstrated that the application of 1.5 mg/L IBA stimulated faster root emergence compared with other treatments, with roots appearing within 14.33 days. Likewise, Welehaweria and Sbhatu (2023) reported that 1.25 mg/L IBA resulted in the fastest root initiation, occurring within 12 days.

Root Number

IBA application did not significantly affect the number of roots produced by *T. fruticosum* explants. The highest average root number was recorded in the 2 mg/L IBA treatment (6.63 roots per explant), followed by 4 mg/L IBA (6.53 roots per explant), whereas the lowest value was observed in the 6 mg/L IBA treatment (5.57 roots per explant) (Figure 3).

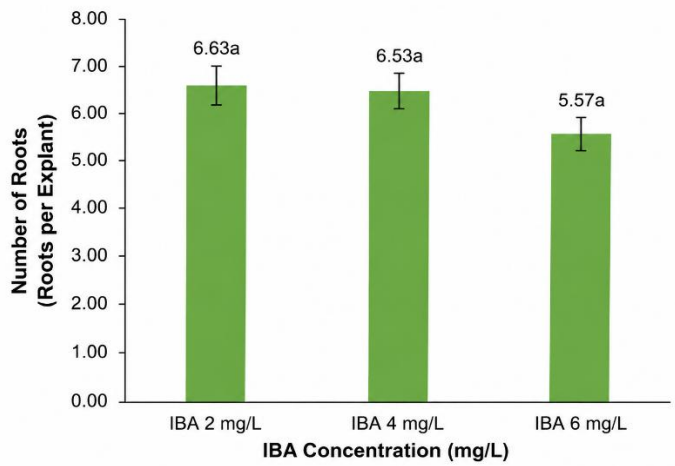

Figure 3. Number of roots produced by *T. fruticosum* leaf explants under different IBA treatments

Adventitious root formation is regulated by complex interactions among auxin concentration, explant cellular competence, and tissue metabolic activity (Devi et al., 2024). IBA is considered more stable than IAA and is therefore more effective in stimulating adventitious root formation in tissue culture systems (Tien et al., 2020). At 2 mg/L, IBA may have induced the expression of genes involved in root primordium formation, including genes associated with cell division and meristematic tissue differentiation, resulting in more optimal root development (Li, 2021).

Conversely, the reduction in root number observed under 6 mg/L IBA suggests an inhibitory effect caused by excessive auxin accumulation. High auxin concentrations may disrupt cellular hormonal balance and induce physiological stress

in explant tissues. Moreover, excessive auxin levels may enhance phenolic compound accumulation and ethylene production, which can negatively affect root organogenesis (Nazir et al., 2022). These findings indicate that adventitious root formation is determined not only by auxin availability but also by the optimal auxin concentration required to support explant cellular metabolism (Pasternak & Steinmacher, 2024). A similar response was reported by Ramadhani et al. (2025), where the application of 2 mg/L IBA combined with 1 mg/L NAA produced the highest number of roots in *Tithonia diversifolia* (Hemsl.) culture compared with other treatments.

Root Length

The longest adventitious roots were obtained from the 2 mg/L IBA treatment, with an average length of 1.23 cm, whereas the 4 and 6 mg/L IBA treatments produced roots with an average length of 0.90 cm (Figure 4). These results indicate that a lower IBA concentration was more effective in promoting root elongation than higher concentrations.

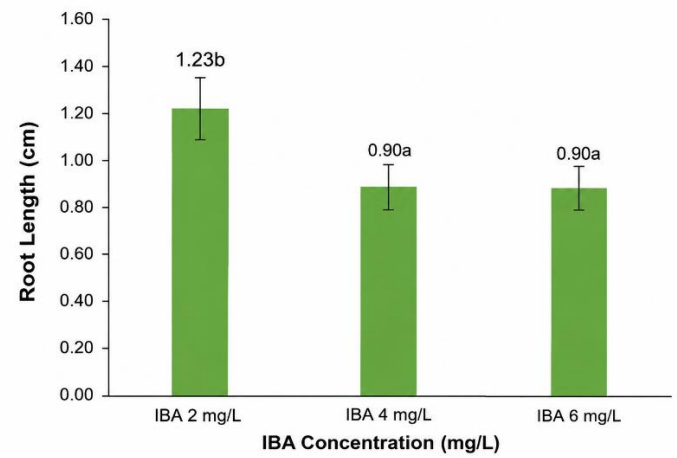

Figure 4. Root length of *T. fruticosum* leaf explants under different IBA treatments

Root elongation is closely associated with cell division and elongation activity in root meristem tissues (Zhukovskaya et al., 2020; Verslues & Longkumer, 2022). At an optimal concentration, IBA can increase cell wall permeability and stimulate enzyme activity involved in cell wall loosening, thereby promoting root cell elongation. In addition, IBA contributes to nutrient translocation and metabolite accumulation required for root growth (Xiao et al., 2020).

At higher concentrations, IBA may preferentially stimulate new tissue formation rather than cell elongation, resulting in shorter roots (Wang et al., 2022). Excessive auxin accumulation may also disrupt osmotic balance and increase oxidative stress in explant tissues, ultimately inhibiting root growth. Therefore, although roots were successfully formed in all treatments, excessive IBA concentrations were less effective in promoting adventitious root elongation (Smolko et al., 2021). Similar results were reported by Bhat et al. (2022), who found that 2 mg/L IBA produced the longest roots (34.67 mm) in *Jasminum nudiflorum* culture.

Root Biomass (Fresh and Dry Weight)

The application of IBA resulted in variations in fresh and dry root biomass of *T. fruticosum* leaf explants. The highest average fresh weight was obtained from the 2 mg/L IBA treatment (0.048 g), followed by 6 mg/L IBA (0.046 g), whereas the lowest fresh weight was observed under 4 mg/L IBA (0.042 g). A similar pattern was observed for dry weight, with the highest value recorded under 2 mg/L IBA (0.0029 g), followed

by 6 mg/L IBA (0.0026 g), while 4 mg/L IBA produced the lowest dry weight (0.0023 g) (Table 2). However, statistical analysis showed that IBA treatments did not significantly affect either fresh or dry root biomass of *T. fruticosum*.

Table 2. Fresh and dry weight of roots produced from *T. fruticosum* leaf explants

IBA concentration (mg/L)	Fresh weight (g)	Dry weight (g)
2	0.048 ± 0.033[a]	0.0029 ± 0.0019[a]
4	0.042 ± 0.026[a]	0.0023 ± 0.0014[a]
6	0.046 ± 0.028[a]	0.0026 ± 0.0010[a]

Note: Values followed by the same letter are not significantly different according to DNMRT ($p \leq 0.05$).

The higher fresh root weight observed under 2 mg/L IBA indicates that this concentration provided a more favorable condition for root tissue growth and development. Fresh root weight is closely associated with cell division, cell enlargement, tissue water content, and biomass accumulation resulting from cellular metabolism (Bhattacharya, 2021). At optimal concentrations, IBA enhances meristematic activity and accelerates root tissue formation, thereby increasing fresh biomass accumulation (Gangani et al., 2023). Furthermore, auxin contributes to increased cell membrane permeability and water uptake by tissues, which may further support higher fresh root weight (Manna et al., 2025).

The reduction in fresh weight observed at 4 mg/L IBA suggests that increasing IBA concentration does not always promote root biomass accumulation. Higher auxin concentrations may disrupt hormonal homeostasis and interfere with cellular physiological processes, resulting in suboptimal root growth (Kurepa & Smalle, 2022). Although fresh weight increased under 6 mg/L IBA compared with 4 mg/L IBA, it remained lower than that obtained with 2 mg/L IBA. This finding suggests that lower IBA concentrations are more suitable for supporting adventitious root biomass accumulation.

Dry root weight reflects the accumulation of organic compounds produced through plant metabolism, including carbohydrates, proteins, and structural compounds formed during root growth (Hao et al., 2024). The highest dry weight observed under 2 mg/L IBA indicates that this concentration supported metabolic activity and biomass synthesis more effectively than the other treatments. This response may be associated with the ability of IBA to stimulate root cell division and differentiation, leading to increased tissue formation (El-Banna et al., 2023).

Conversely, the reduction in dry weight at higher IBA concentrations suggests that excessive auxin accumulation may inhibit metabolic activity in root tissues. High auxin levels can induce physiological stress and disturb endogenous hormonal balance, resulting in reduced biomass synthesis. Moreover, increased IBA concentrations may enhance ethylene production, which can inhibit root tissue growth and development (Tien et al., 2020; Ullah et al., 2023). Similar findings were reported by Şener and Kurt (2022), who demonstrated that 2 mg/L IBA produced the highest fresh and dry root weights compared with other treatments, reaching 0.279 g and 0.0368 g, respectively.

Overall, this study provides valuable information regarding the response of *T. fruticosum* leaf explants to different IBA concentrations during adventitious root induction under in vitro conditions. All treatments resulted in 100% explant survival and root formation, indicating the strong regenerative capacity of *T. fruticosum* leaf explants under the applied culture conditions. Among the tested concentrations, 2 mg/L IBA showed the most favorable response by accelerating root initiation and increasing root

length, although it did not significantly improve root number or biomass accumulation. These findings provide preliminary information for the development of adventitious root induction protocols in *T. fruticosum*. Further studies should investigate additional culture factors, including different plant growth regulator combinations, medium composition, and culture conditions, to optimize adventitious root growth and biomass production.

CONCLUSION

Adventitious root induction from *T. fruticosum* leaf explants under in vitro conditions resulted in 100% explant survival and root formation across all IBA treatments, indicating that the culture medium used was suitable for supporting adventitious root development. Variations in IBA concentration significantly affected root initiation time and root length, whereas root number and root biomass did not show significant differences among treatments. Among the tested IBA concentrations (2, 4, and 6 mg/L), 2 mg/L IBA produced the most favorable response, particularly by accelerating root initiation and promoting root elongation. Therefore, 2 mg/L IBA can be considered a preliminary optimal concentration for adventitious root induction in *T. fruticosum*. However, further optimization is required to achieve improved root growth and biomass production.

RECOMMENDATION

Further studies should investigate a broader range of IBA concentrations or evaluate combinations of IBA with other plant growth regulators to obtain more optimal root induction responses. In addition, longer culture periods, together with physiological and biochemical analyses, are recommended to provide a more comprehensive understanding of the mechanisms underlying adventitious root formation in *T. fruticosum*.

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