Comparative Evaluation of Seed Germination, Semi-Woody Stem Cuttings, and *In Vitro* Rooting for Propagation of *Paulownia tomentosa* L. Using Growth Regulators and Natural Bio-Stimulants

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ABSTRACT

This study tests the propagation potential of *Paulownia tomentosa* through seed germination, vegetative propagation using semi-woody stem cuttings, and *in vitro* rooting, to identify efficient and practical techniques for mass-scale production. The seed germination experiment evaluated germination percentage, germination rate, and early seedling vigor under controlled greenhouse conditions. The results indicated that the seeds exhibited high germination ability when provided with suitable environmental conditions, particularly adequate temperature, light and moisture. Germination occurred rapidly and uniformly, confirming the plant's strong propagation potential through seeds. Vegetative propagation experiments focused on semi-woody cuttings treated with different concentrations of Indole-3-butyric acid and natural bio-stimulant solutions consisting of milk–honey solutions. The experiment measured rooting percentage, number of roots, root length, and survival rate after several weeks of cultivation. The results indicated variable responses depending on the treatment applied. Although some treatments enhanced survival and early growth, overall rooting success remained relatively limited, suggesting that semi-woody cuttings of *P. tomentosa* require further standardization of plant growth regulator balance and environmental conditions to achieve consistent root formation. *In vitro* rooting was also evaluated using Naphthaleneacetic acid and Indole-3-butyric acid. The results demonstrated that NAA at concentrations of 0.25–0.75 mg L^{-1} produced the best rooting performance, reaching up to 100% rooting, with the highest root number and longest root length. In general, the results show the superiority of *in vitro* rooting over both seed germination and semi-woody cuttings, providing higher rooting efficiency and more uniform plantlet development. These results highlight the importance of *in vitro* propagation as an effective approach for future large-scale propagation and mass production of this valuable tree species locally.

Keywords: Paulownia propagation, Micropropagation, Stem cuttings, Gibberellic Acid, Indole-3-butyric acid, Rooting enhancement, Milk–honey bio stimulant

INTRODUCTION

Paulownia which is known as the Empress tree or Princess tree, is a fast-growing deciduous hardwood species originally native to China and widely planted in Asia, North America, and Europe. It is mainly valued for its fast growth, rapid biomass production,

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lightweight, high-quality timber, ornamental characteristics, potential of carbon sequestration, and adaptation to diverse environmental conditions (Toma, 2019).

Paulownia can be propagated both sexually, by seeds, and vegetatively, by cuttings, root segments, or plant tissue culture. Seed propagation allows for mass production at low cost but may suffer from variability and low germination rates under suboptimal conditions. Vegetative propagation ensures clonal uniformity and preservation of elite genotypes; however, rooting success may vary depending on hormone treatments and environmental factors (Antwi-Wiredu *et al*., 2021).

While traditional propagation methods such as seed germination and root semi-woody cuttings are frequently employed, they often struggle with low germination rates, high susceptibility to soil borne diseases, and significant genetic variability. In contrast, micropropagation under *in vitro* conditions offers a superior alternative by enabling the mass production of true-to-type, pathogen-free plantlets under a controlled environment regardless of seasonal constraints. This biotechnological method ensures a much higher multiplication rate as compared to the conventional cuttings and bypasses the dormancy issues often associated with seeds, leading to providing a standardized and efficient pipeline for large-scale reforestation and commercial plantations of such important trees (Toma, 2019).

Gibberellic acid is the most commonly used growth regulator to enhance seed germination by ending dormancy and stimulating embryo growth. On the other hand, indole-3-butyric acid is widely used to stimulate adventitious root formation in cuttings (Kumatkar *et al*, 2025). Natural additives like milk and honey contain different sugars, amino acids, vitamins, and anti-microbial compounds that might enhance rooting and reduce pathogen infection, giving a low cost and environmentally friendly alternative to plant growth regulators (Altay and Gultekin, 2023).

Even with the increasing interest in Paulownia cultivation under greenhouse condition and plantation systems, many challenges remain facing the growers; low or insufficient seeds germination collected from paulownia mature trees, poorness in rooting success of stem cuttings, limited information on optimal Gibberellic acid concentrations for seed germination under greenhouse conditions and insufficient testing of natural additives such milk or honey as eco-friendly alternatives or supplements to usually applied auxins like IBA (Nasraoui *et al*., 2025).

In vitro techniques also provide a platform for producing healthy or hybrid plants, facilitating controlled deployment in agroforestry and reforestation programs (Ipekci and Gozukirmizi, 2003). Successful micropropagation of paulownia remains constrained by species-specific responses to culture media strength, plant growth regulator combinations, and the accumulation of phenolic compounds, which often affect shoot regeneration and acclimatization (Sota *et al*., 2025).

That's why there is a need to optimize both seed and vegetative propagation procedures for *Paulownia tomentosa* to enhance propagation efficiency and reduce plantation costs to ensure that *in vitro* propagation is always a better means for mass propagation of paulownia. Generally, this study aimed to enhance seed germination and vegetative propagation efficiency of *Paulownia tomentosa* under local greenhouse conditions using GA_3 , IBA, and natural additives (milk and honey) and specifically aimed to evaluate the effect of

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various GA_3 concentrations on seed germination percentage and vigor, to assess the germination ability of seeds collected from mature trees. Also to determine the effect of various IBA concentrations on the rooting of stem cuttings. Besides evaluating the potential role of milk and honey as natural rooting enhancers. As well as making a clear comparison between these two propagation methods with *in vitro* rooting of Paulownia. As a result, to compare the effectiveness of synthetic growth regulators versus natural additives on rooting performance (Bowden *et al*., 2025).

MATERIALS and METHODS

Paulownia species, *Paulownia tomentosa* L. was initially tested for its propagation under local greenhouse conditions. For the seed germination experiment seeds were collected from mature trees of *Paulownia tomentosa* L. For vegetative propagation experiment, semi-hardwood stem cuttings of 15–20 cm in length carrying 2 to 3 nodes) were collected from healthy, disease-free mother plants. For *in vitro* rooting, two cm long segment propagules were taken from initially established and multiplied experiments from single node explant and were cultured on WPM supplemented with different NAA and IBA concentrations.

1. Experiment of Sexual Propagation (Seed Germination)

The experiment was designed according to CRD (Completely Randomized Design). After soaking for 5 hours at normal room temperature in GA_3 solutions of 0.0, 100, 200 and 300 $mg.L^{-1}$, seeds were surface dried and sown uniformly on sterilized peatmoss surface and lightly covered. Three seeds were sown in each pot (15 cm diameter) and each treatment consisted of 15 pots (replicates). After two weeks, germination percentage (%), mean germination time (days) and seedlings vigor were recorded. Seed trays were usually irrigated regularly to maintain consistent moisture without water-logging. The experiment was conducted under greenhouse conditions during the spring season for a period of 4 weeks.

2. Experiment of Vegetative Propagation (Cuttings Rooting Test)

Semi-woody cuttings were collected from the Biesfki area in Duhok Governorate. A total of 120 cuttings were taken from healthy, disease-free mature trees. Each cutting consisted of five nodes**.** The cuttings were divided into two groups, each containing 60 cuttings. Distilled water was prepared using a distillation apparatus. A milk–honey solution was also prepared by mixing 1 liter of local cow milk with 250 g of local honey**,** ensuring the honey was completely dissolved in the milk.

The first Sixty cuttings were soaked in distilled water for two hours and the second sixty cuttings were soaked in the milk–honey solution for two hours. After soaking, each group of 60 cuttings was subdivided into three subgroups of 20 cuttings each. The subgroups were treated with IBA at the following concentrations: 20 cuttings: Control treatment (immersed in distilled water for 10 minutes), 20 cuttings: 500 $mg.L^{-1}$ IBA (immersed for 10 minutes), and 20 cuttings: 1000 $mg.L^{-1}$ IBA (immersed for 10 minutes).

After treatment, each set of 20 cuttings was planted in pots (approximately 15–20 cm in diameter) containing sterilized peatmoss as the growing medium. The experiment was conducted in the greenhouse of the Department of Horticulture at the College of Agricultural Engineering Sciences (UoD). The same procedure (IBA concentrations: 0, 500, and

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1000 ppm) was applied to the second group of 60 cuttings that had been soaked in the milk–honey solution for two hours. The cuttings were irrigated periodically to keep the growing medium moist and support root initiation. The experiment was carried out in a greenhouse during the spring season and continued for approximately 6 weeks.

The experiments were arranged in a Completely Randomized Design (CRD). Collected data were subjected to analysis of variance (ANOVA) using the Statistical Analysis System software (SAS, 2023). Mean comparisons among treatments were performed using Duncan's Multiple Range Test at a 5% probability level ($p \leq 0.05$) to determine significant differences among treatment means.

Data recorded included rooting percentage (%), number of days required for rooting, number of roots per cutting, mean root length (cm), fresh and dry weights of roots, fresh and dry weights of roots, time to root initiation day and survival rate of seedlings after 6 weeks.

3. Experiment of *in vitro* rooting of propagules:

Explants of 2 cm-long propagules were taken from *in vitro*-multiplied cultures on WPM treated with various concentrations of BA and kinetin. Root formation was performed using WPM supplemented with different auxin concentrations, including IBA and NAA, at 0.0, 0.25, 0.5, 0.75, and 1.0 $mg.L^{-1}$. After four weeks of culture under a temperature of 25± 2°C, which facilitates steady metabolic activity without inducing heat stress, the light regime was equally vital; a photoperiod of 16 hours of illumination followed by 8 hours of darkness, typically provided by cool white light of LEDs at an intensity of 1000 lux. Additionally, maintaining a relative humidity of 60%–70% within the growth chamber. The rooting stage performance was evaluated by recording the number of roots per explant, the mean length of roots (cm), and the rooting percentage. Each treatment consisted of five replicates, with 3 explants cultured per jar. Cultures were maintained under controlled growth room conditions (25 ± 2 °C, under a 16 h light/ 8 h dark photoperiod, with a light intensity of approximately 1000 lux provided by cool white LED lamps) and subcultured every 4 weeks to fresh rooting medium to sustain growth and rooting development.

All experiments were designed in a Completely Randomized Design. The collected data were statistically analyzed by the analysis of variance (ANOVA) using the SAS software (SAS, 2023). Means comparison among the treatments was performed using Duncan's Multiple Range Test (5%) probability level of $p \leq 0.05$ to determine the significant differences among the treatment means.

RESULTS and DISCUSSION

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1. Experiment of Sexual Propagation (Seed Germination)

Seed germination response to plant growth regulators is usually species and genotype dependent, especially in forest trees where seed dormancy and early seedling establishment play a notable role in propagation success. Gibberellic acid is widely used to break dormancy and stimulate seed germination by promoting embryo growth, enzyme activation, and reserve mobilization. However, its effectiveness depends on endogenous hormonal balance and the physiological status of a certain seed.

Contextually, these results evaluate the effect of different Gibberellic acid (GA_3) concentrations on the germination behavior and seedling vigor of *Paulownia tomentosa*, to determine the optimal concentration for promoting germination and early seedling performance under greenhouse-controlled conditions.

Table 1 shows a clear differential response of *Paulownia tomentosa* seeds to the increasing concentrations of GA_3. The highest germination percentage reached to 65%, was recorded in the control treatment (0.0 $mg.L^{-1}$ GA_3), which was significantly higher than all GA_3 treatments (Figure 1). As GA_3 concentration increased, germination percentage progressively decreased to 30%, 20%, and 5% at 100, 200, and 300 $mg.L^{-1}$, respectively. This indicates that exogenous GA_3 application was not required to promote germination in this species and, at higher concentrations, had an inhibitory effect. The reduction at raised doses may be attributed to hormonal imbalance or possible phytotoxic effects, suggesting that *P. tomentosa* seeds possess enough endogenous gibberellins for optimal seed germination (Toma, 2019).

A short mean germination time estimated at 6.75 days was not significantly affected by GA_3 concentration. This demonstrates that GA_3 treatments influenced the final germination percentage rather than the speed of germination. The uniform germination time period across treatments shows that once germination was initiated, seed metabolic processes proceeded at a similar rate regardless of PGRs treatment.

Seedling vigor aligned closely with germination performance. A strong vigor was found in the control and 100 $mg.L^{-1}$ treatments, while vigor decreased to medium and weak at higher GA_3 concentrations of 200 and 300 mg L^{-1}. This decrease supports the hypothesis that excessive GA_3 interrupts normal seedling development (Figure 1).

Generally, the results clearly demonstrate species specific responses to GA_3 application. *P. tomentosa* does not require exogenous GA_3 for germination, and higher concentrations are inhibitory. Excessive gibberellin concentrations inhibit the species, pointing out the importance of optimizing hormonal dosage to avoid inhibitory or phytotoxic effects.

While this study found rapid and uniform seed germination under the greenhouse conditions, other reports indicate that *P. tomentosa* seed germination can be inconsistent or influenced by seed-borne diseases and environmental factors, which can make seed propagation less dependable in practice (Fan *et al*., 1997 & Posta *et al*., 2022).

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Table (1): Effect of different concentrations of GA_3 on seed germination of *Paulownia tomentosa* L.

GA_3 ($mg.L^{-1}$)	Germination Percentage (%)	Mean Germination Time (Days)	Seedling Vigor
0.0	65 a	6.75 a	Strong
100	30 b	6.75 a	Strong
200	20 c	6.75 a	Medium
300	5 d	6.75 a	Weak

Figure (1): Seed Germination test of *Paulownia tomentosa*.
A. Seed soaking in different GA_3 concentrations.
B. Initially sown seeds in sterilized peatmoss.
C. Initial germination of seeds.
D. Vigorous seedling treated with 100 $mg.L^{-1}$ GA_3.

2. Experiment of Vegetative Propagation (Cuttings Rooting Test)

This experiment was applied to evaluate the response of *Paulownia tomentosa* root cuttings to various treatments aimed at promoting root initiation, root development, and overall semi-woody cuttings performance. Particular effort was given to the role of IBA treatments and natural bio-stimulant solutions in promoting adventitious root initiation and improving the general growth characteristics of the cuttings. Measurements including rooting percentage, number of roots per cutting, mean root length, and survival rate of cuttings were recorded to assess the effectiveness of the tested treatments.

The achieved results provide valuable declare into the physiological responses of *Paulownia tomentosa* root cuttings to these treatments and help clarify the most suitable conditions for improving asexual propagation. These findings are important for enhancing large-scale propagation programs and for expanding the plantation of this economically important tree species in the local forestry,

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agroforestry, and environmental rehabilitation programs.

The results shown in figure 2 declare the effect of indole-3-butyric acid and milk–honey solution on the rooting percentage of *Paulownia tomentosa* semi-woody cuttings after six weeks of culture. The control treatment (distilled water) resulted in a low rooting percentage (25%), indicating that *P. tomentosa* cuttings possess a natural but limited capacity to form roots without added exogenous auxins. Application of IBA at 500 ppm combined with distlled water reduced the rooting percentage to only 10%, suggesting that this concentration might not be sufficient for enhancing rooting under the conditions of the experiment. In contrast, IBA at 1000 ppm + DW produced the highest rooting percentage reaching to 25%, equal to the control treatment (Figure 3). This declares that increasing the concentration of IBA improved rooting as compared with the lower concentrations, identifying the positive role of auxins in enhancing adventitious root formation in semi-woody cuttings.

The treatment of using milk+ honey solution alone resulted in the lowest rooting percentage of only 5%, declaring that this natural solution alone had very limited effectiveness in improving root initiation in *P. tomentosa* cuttings.

When IBA at 500 ppm was combined with milk+ honey, rooting percentage increased to 15%, which was higher than milk+ honey alone and also higher than IBA 500 ppm with DW. This declares that the milk–honey mixture may provide some supportive nutrients or carbohydrates that promote the rooting response when used together with auxin. However, the treatment IBA (1000 ppm)+ milk+ honey produced only 10% rooting, which was lower than IBA (1000 ppm) alone, declaring that the application of milk–honey did not further promote the effect of the higher IBA concentration. Generally, these results indicate that IBA at 1000 ppm was the most effective treatment for rooting, while milk–honey solution alone gave minimal rooting promotion. The combination of IBA and milk–honey solution resulted in variable effects, showing that the concentration of IBA and the composition of the natural additives play important roles in determining the rooting success of *P. tomentosa* semi-woody cuttings.

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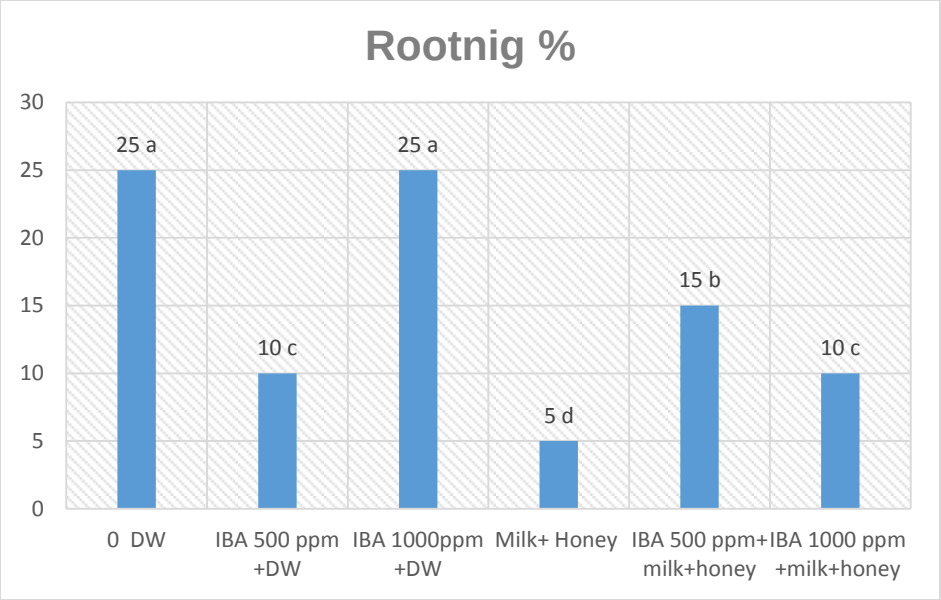

Figure 2: Effect of IBA and Milk-Honey Solution on Rooting Percentage of *Paulownia tomentosa* Semi-Woody Cuttings after 6 Weeks of Culture

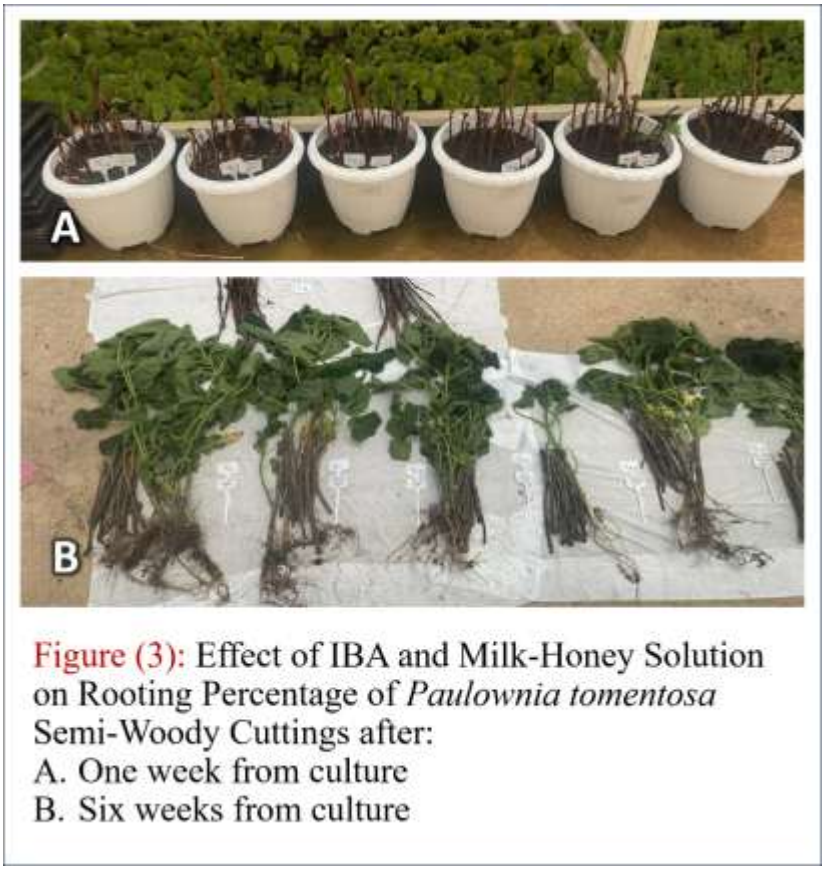

Figure (3): Effect of IBA and Milk-Honey Solution on Rooting Percentage of *Paulownia tomentosa* Semi-Woody Cuttings after:
A. One week from culture
B. Six weeks from culture

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The data presented in Figure (4) show the effect of IBA and milk–honey solution on the number of roots per cutting of *P. tomentosa* semi-woody cuttings after six weeks of culture. The DW treatment (control) produced an average of 5 roots/ cutting, indicating that the cuttings have a natural capacity to initiate roots even without auxin application. A similar result was found when IBA (1000 ppm) + DW was applied, which also produced 5 roots/ cutting, showing that this concentration of IBA can effectively enhance root formation and maintain a high rooting performance as compared to the control.

In contrast, the application of IBA at 500 ppm + DW gave only 2 roots/ cutting, indicating that this low IBA concentration was less effective in enhancing root development. The use of milk+ honey solution alone produced the lowest number of roots reaching only 1 root/ cutting), declaring that this natural solution alone had very limited ability to stimulate root formation in *P. tomentosa* cuttings. However, when IBA at 500 ppm was combined with milk+ honey solution, the number of roots increased to 3 roots/ cutting, which is an improvement as compared with either milk+ honey alone or IBA 500 ppm with DW. This may indicate that the milk–honey mixture provides certain nutrients, sugars, or bioactive compounds that enhance root formation when used together with IBA. On the other hand, the IBA 1000 ppm+ milk-honey solution produced 2 roots/ cutting, which was lower than the same concentration of IBA added with DW. This shows that the addition of milk–honey did not stimulate the effect of the higher IBA concentration and may have slightly reduced its efficiency. In general, the results suggest that IBA at 1000 ppm with DW and the control treatment produced the highest number of roots, while the milk–honey mixture alone showed the weakest effect on root formation. The combination of IBA and milk–honey mixture appears to provide moderate improvement at lower IBA concentration but not at higher levels, indicating that the response of *P. tomentosa* cuttings depends on the balance between IBA concentration and the added natural components **(Ipekei and Gozukirmizi, 2003).**

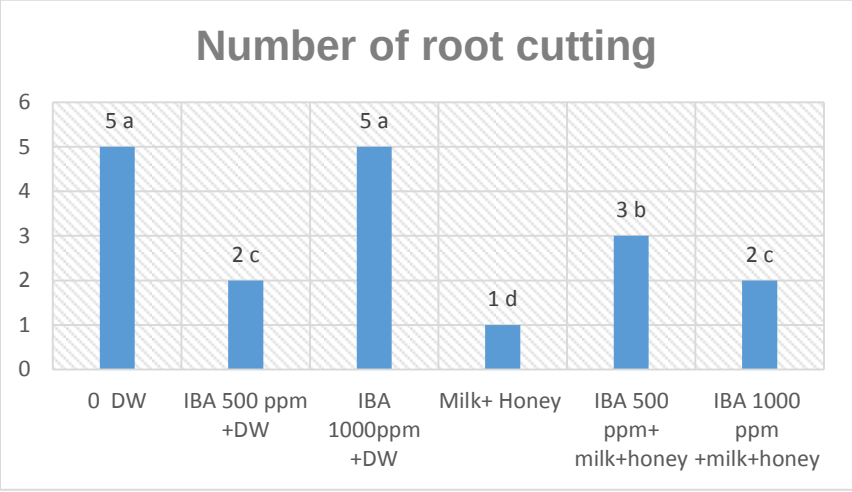

Figure 4: Effect of IBA and Milk-Honey Solution on Number of Roots per Cutting of *Paulownia tomentosa* Semi-Woody Cuttings after 6 Weeks of Culture

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Figure 5 shows the effect of IBA and milk–honey mixture on the mean root length of *P. tomentosa* semi-woody cuttings after six weeks of culture. The outcomes indicate clear differences among the tested treatments. The DW treatment (control) recorded the highest mean root length reaching to 86.2 cm, declaring that the roots produced under natural conditions were longer than those achieved with the other treatments. This may be due to the absence of external substances that could interfere with the normal elongation of roots after initiation. The addition of IBA (500 ppm)+ DW resulted in a mean root length of only 26 cm, which was significantly lower than the control but still higher than some other treatments. Increasing IBA concentration to 1000 ppm + DW further reduced the root length to only 18.88 cm, suggesting that although higher IBA concentrations may stimulate root initiation, they may also inhibit root elongation. The treatment of milk + honey solution alone gave a mean root length of 29 cm, which was higher than both IBA treatments with DW. This outcome suggests that the milk–honey mixture may provide nutrients, sugars, or organic compounds that support root elongation (**Toma, 2019).**

When IBA 500 ppm was interacted with milk + honey solution, the mean root length decreased to only 17.6 cm, while the treatment IBA 1000 ppm + milk + honey resulted in the lowest root length estimated at only 14.75 cm. These results indicate that the combined application of IBA and milk–honey solution did not stimulate root elongation and may have negatively affected root growth at higher concentrations. In general, the results suggest that while IBA treatments may influence root initiation, root elongation was greatest in the DW treatment, whereas higher concentrations of IBA and its interaction with milk–honey mixture led to reduced root length in *P. tomentosa* semi-woody cuttings (Gulteken *et al.*, 2023).

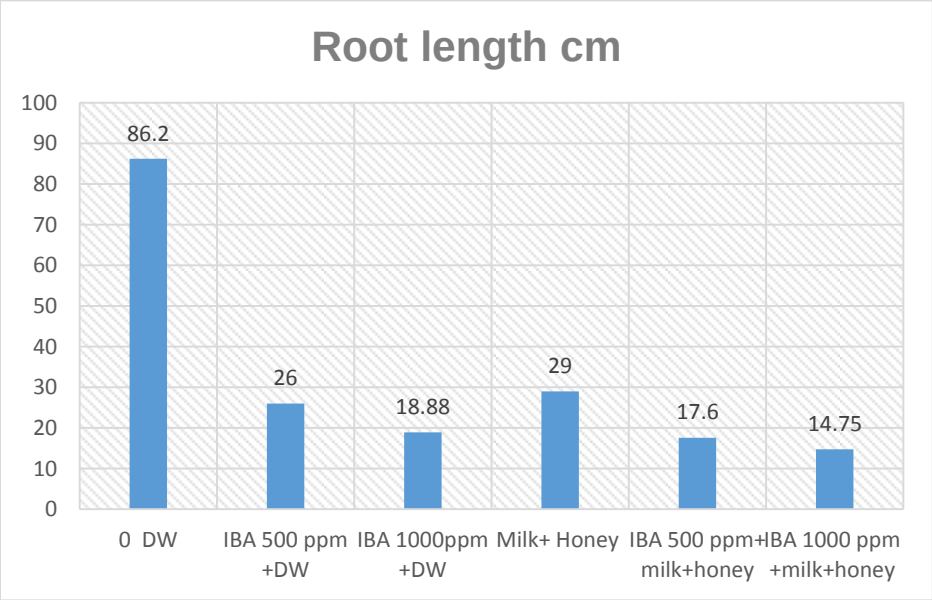

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Figure 5: Effect of IBA and Milk-Honey Solution on Mean Length of Roots of *Paulownia tomentosa* Semi-Woody Cuttings after 6 Weeks of Culture

Figure 6 declares the effect of different treatments on the root fresh weight of *P. tomentosa* semi-woody cuttings after six weeks of culture. The highest root fresh weight was recorded in the DW treatment (control), which gave 94.16 g. Among the treated semi-woody cuttings, IBA 500 ppm combined with milk and honey solution produced the second highest value, reaching 69.33 g, followed by IBA 1000 ppm + DW giving 61.3 g and IBA 500 ppm + DW giving 59.39 g. Lower root fresh weight was observed in IBA 1000 ppm + milk and honey solution (44.46 g). The lowest value was found in the milk+ honey treatment alone, which produced only 23.06 g of fresh weight of roots. The results declare that the untreated cuttings produced the greatest fresh weight, while the milk and honey treatment alone resulted in the lowest root development. Treatments containing IBA showed moderate effects as compared with the distilled water treatment.

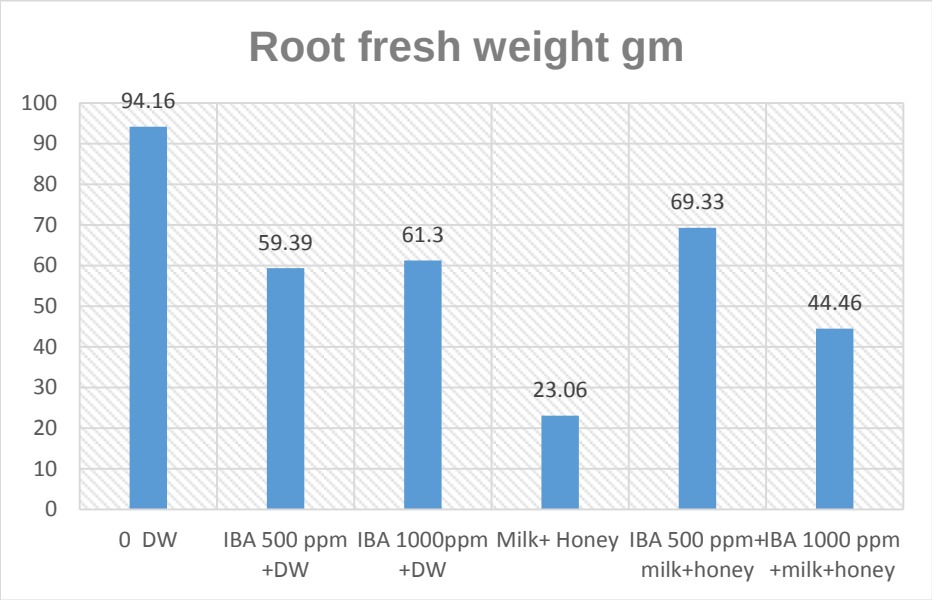

Figure 6: Effect of IBA and Milk-Honey Solution on Fresh Weight of Roots of *Paulownia tomentosa* Semi-Woody Cuttings after 6 Weeks of Culture

Figure 7 indicates the effect of different treatments on the root dry weight of *P. tomentosa* semi-woody cuttings after six weeks of culture. The highest dry weight of roots was recorded in the DW treatment (control) with a value estimated at 43.18 g. Among the treated semi-woody cuttings, IBA 500 ppm combined with milk+ honey solution produced the second highest root dry weight reaching 28.76 g. This was followed by IBA 500 ppm + DW which gave 25.32 g, and IBA 1000 ppm + DW which recorded 24.18 g. Lower dry weight of roots was observed in IBA 1000 ppm + milk and honey reaching 19.67 g. The lowest value was

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recorded in the milk and honey treatment alone, which formed only 14.11 g. These results show that the untreated cuttings produced the highest root dry weight, while the milk and honey treatment alone appeared in the lowest root development. Treatments containing IBA showed moderate effects as compared with the DW treatment.

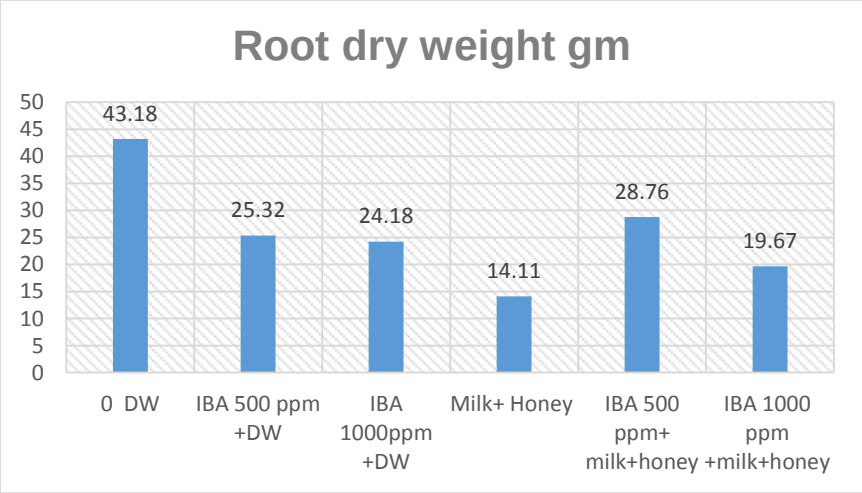

Figure 7: Effect of IBA and Milk-Honey Solution on Root Dry Weight of *Paulownia tomentosa* Semi-Woody Cuttings after 6 Weeks of Culture

Figure 8 shows the effect of different treatments on the number of days required for root induction in semi-woody cuttings of *P. tomentosa* after six weeks of culture. The results suggest that all treatments required the same time for root initiation, which was about 3 days. This value was noticed for the DW treatment (control), IBA 500 ppm + DW, IBA 1000 ppm + DW, milk + honey, IBA 500 ppm + milk + honey, and IBA 1000 ppm + milk + honey. Overall, the treatments did not result in any difference in the time required for root initiation, indicating that neither IBA nor the milk-honey mixture affected the number of days needed for roots to begin forming in the cuttings.

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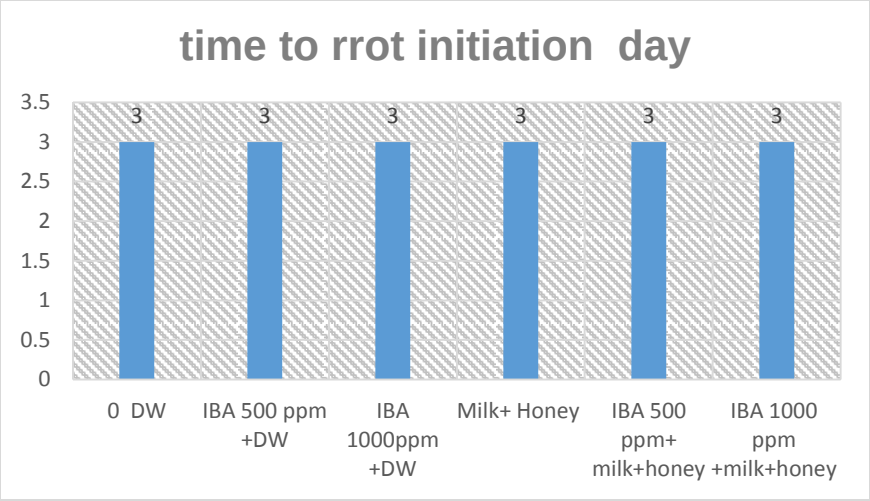

Figure 8: Effect of IBA and Milk-Honey Solution on Number of Days Required for Root Initiation of *Paulownia tomentosa* Semi-Woody Cuttings after 6 Weeks of Culture

The results in Figure (9) show the effect of IBA and a natural milk–honey mixture on the survival rate of semi-woody cuttings of *P. tomentosa* after six weeks of culture.

The highest survival rate was found in DW treatment (control), which showed 5 surviving cuttings. This indicates that under experimental conditions, the control cuttings were able to maintain relatively good viability, possibly due to their inherent physiological capacity and enough internal reserves that supported survival during the early stages of culture. The combination treatment of IBA 500 ppm with milk–honey mixture resulted in a relatively higher survival rate of 3 cuttings as compared with the other treated groups of cuttings. This improvement may be due to the synergistic effect of auxin and the natural bio-stimulant properties of the milk–honey solution, which may provide amino acids, vitamins, carbohydrates, and antimicrobial elements that help maintain tissue vitality and reduce stress during rooting.

In contrast, treating with IBA 500 ppm + DW and IBA 1000 ppm + DW resulted in moderate survival rates of only 2 cuttings each, suggesting that the use of IBA alone had a limited effect on improving survival under the tested conditions. The lowest survival rate (1 cutting) was recorded in the milk–honey mixture alone, which may indicate that although it may provide nutritional benefits, it is not enough by itself to maintain high cutting viability without the presence of rooting hormones. Generally, these results suggest that while untreated cuttings (control) maintained the highest survival in this experiment, the combined application of IBA and milk–honey solution showed a relatively better performance among the treated groups of cuttings. This indicates the potential role of integrating synthetic auxins with natural bio-stimulants to enhance the survival and establishment of Paulownia tomentosa semi-woody cuttings during the early propagation stage.(Toma, 2019).

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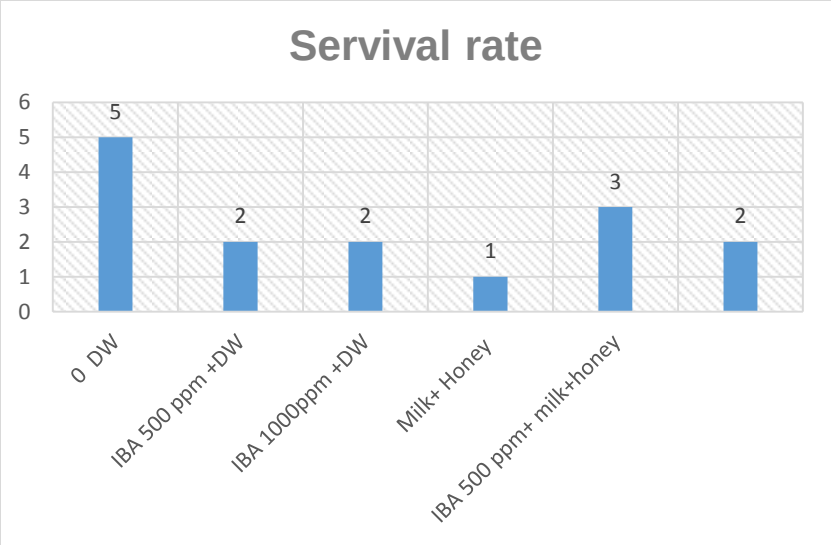

Figure 9: Effect of IBA and Milk-Honey Solution on Cuttings Survival Rate of *Paulownia tomentosa* Semi-Woody Cuttings after 6 Weeks of Culture

These results showed that semi-woody cuttings had limited rooting success, whereas some older vegetative propagation literature like root cuttings or shoot cuttings under certain conditions; reports more effective clonal propagation methods. While these traditional techniques vary widely in success, they have historically been reported as potential propagation methods for Paulownia under suitable treatments and environmental conditions (Mahmood *et al*., 2017; Ede *et al*., 1997; Nasraoui *et al*., 2025).

3. Experiment on *in vitro* rooting of propagules:

To make a comparison between the *in vitro* rooting of paulownia propagules, and to show an alternative successful means for better rooting performance to seed propagation and semi-woody cutting rooting, NAA and IBA were added to WPM media under *in vitro conditions.* It has been noticed that auxin kind and concentration had a notable effect on the rooting response of *Paulownia tomentosa* micro-shoots cultured on WPM medium after four weeks (Table 2). Generally, NAA applications were dramatically more effective than IBA in enhancing root initiation, number of roots, root elongation, and rooting percentage. Since, in terms of the number of roots/explants, NAA significantly overcomes IBA. The highest number of roots (12.30 roots/ explant) was recorded with NAA at 0.5 mg. L^{-1}, followed by 0.25 and 0.75 mg. L^{-1} NAA (11.60 roots/ explant). These applications were statistically superior to all tested IBA concentrations (Figure 10). In contrast, IBA induced a relatively low number of roots, with numbers ranging from 1.40 to 2.75 roots/ explant. The low rooting response in the control treatment (0.30 roots/ explant) confirms the clear role of exogenous auxins in adventitious root initiation in Paulownia. Similarly, the mean length of roots was significantly affected by auxin treatments. Since adding NAA at 0.25, 0.5, and 0.75 mg.L^{-1} gave the longest roots (6.62, 6.00, and 6.40 cm), respectively, which were statistically

superior to all other tested treatments. The least length of roots was found at the highest NAA concentration of 1.0 mg. L^{-1}, resulting an inhibitory effect at the optimized auxin levels. IBA treatments showed moderate root lengths of 3.00–4.70 cm, that declares that although IBA could induce root elongation, its efficiency was less than that of NAA under the same study conditions (Figure 10).

The rooting percentage shows the superiority of NAA over IBA. A very high rooting percentage (100%) was achieved with NAA at 0.25, 0.5, and 0.75 mg.L^{-1}, indicating highly uniform and reliable rooting responses. Raising NAA concentration to 1.0 mg.L^{-1} reduced rooting percentage to 83%, likely because of auxin-induced toxicity or excessive callus formation at the basal end of the shoots. On the other hand, the highest rooting percentage obtained with IBA was 75% at 0.75 mg.L^{-1}, while lower concentrations resulted in significantly lower rooting percentages (44–58%).

The best rooting abilities found with NAA may be due to its strong activity, which effectively promotes cell division and their differentiation at the basal part of shoots. However, higher auxin concentrations negatively affected root number and quality, emphasizing the necessity of optimizing auxin concentration. Generally, NAA (0.25–0.75 mg. L^{-1}) proved to be the most effective treatment for root formation in *Paulownia tomentosa* L., producing a relateively high number of long enough roots with 100% rooting efficiency. The obtained highly developed root systems are expected to promote plantlet survival during the acclimatization stage.

Table 2: Effect of NAA and IBA on rooting stage of *Paulownia tomentosa* L. after four weeks of culture on WPM medium.

Auxins	Concentrations/ (mg.L^{-1})	Number of Roots/Explant	Mean Length of Roots (cm)	Rooting Percentage (%)
NAA	0.00	0.30 g	2.30 c	22 e
	0.25	11.60 b	6.62 a	100 a
	0.50	12.30 a	6.00 a	100 a
	0.75	11.60 b	6.40 a	100 a
	1.00	1.75 e	0.87 d	83 b
IBA	0.25	1.80 e	4.70 b	58 c
	0.50	1.40 f	3.00 c	44 d
	0.75	2.75 c	4.12 b	75 a
	1.00	2.50 d	4.60 b	58 c

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Figure 10: *In vitro* rooting of *Paulownia tomentose* L. propagules after four weeks of culture on WPM.
A. Effects of NAA on rooting from left to right: 0.00, 0.25, 0.50, 0.75 and 1.00 mg.L^{-1}.
B. The best rooting performance with NAA 0.50 mg.L^{-1}.

These results demonstrated a clear superiority of *in vitro* rooting when compared with both seed germination and semi-woody cuttings rooting in the propagation of *Paulownia tomentosa*. The *in vitro* technique produced higher rooting efficiency, more uniform plantlets, and a faster establishment rate under lab and greenhouse-controlled conditions. In contrast, seed germination showed variability in growth and lower consistency, while semi-woody cuttings exhibited comparatively limited rooting success. These achievements highlight the effectiveness of *in vitro* rooting as a reliable propagation method for *P. tomentosa*. Therefore, it is recommended that this valuable technique be adopted in future propagation programs aimed at the large-scale and efficient mass production of this important tree species at the local level. These findings proved that *in vitro* propagation is effective for *Paulownia tomentosa* which can achieve high rooting and strong plantlet development which is supported by earlier work showing successful micropropagation and shoot regeneration protocols for *P. tomentosa* using tissue culture techniques (Toma, 2018).

High concentrations of GA_3 may inhibit seed germination despite its general role in enhancing germination. Excess GA_3 can disrupt the internal hormonal balance between gibberellins and inhibitors such as abscisic acid (ABA), which is essential for normal embryo growth and metabolic regulation, thereby reducing germination percentage. In case of vegetative propagation, IBA effectively promotes root initiation because it stimulates cell division and differentiation at the basal region of cuttings, leading to the formation of root primordia. However, high IBA concentrations may suppress subsequent root elongation due to hormonal imbalance and increased ethylene formation, which restricts cell elongation. In contrast, NAA is often more effective under in vitro conditions because it is chemically stable and less susceptible to degradation, allowing it to maintain a consistent concentration in the culture medium and support sustained auxin transport and cell

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differentiation. Natural additives like milk and honey may sometimes fail to enhance growth because their high sugar and nutrient content can enhance microbial contamination and create osmotic or nutritional imbalances in the medium, which may interfere with hormonal regulation, auxin transport, and normal cell differentiation processes required for successful germination and rooting.

CONCLUSIONS

In conclusion, the study declares that while seed propagation provides a reliable and rapid method for establishing *Paulownia tomentosa* seedlings, vegetative propagation through semi-woody cuttings remains inconsistent and requires further optimization of plant growth regulators and environmental conditions. In contrast, *in vitro* rooting proved to be the most efficient propagation method, producing higher rooting percentages, greater root development, and more uniform plantlets. Basically, it is recommended to prioritize micropropagation techniques for mass-scale production of Paulownia planting material, conducting further research to standardize plant growth regulator combinations and concentrations for both *in vitro* and cutting propagation, and support the establishment of local micropropagation facilities to ensure the sustainable large-scale production of high quality, disease-free Paulownia plants for forestry, agroforestry, and environmental applications.

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