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Micropropagation of Ironwood (*Xylia xylocarpa* (Roxb.) Taub.) by tissue culture

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Abstract It was found that the sterilization procedure was highly effective, resulting in a 100% survival rate of *Xylia xylocarpa* (Roxb.). After sterilization, the seeds were cultured on a half-strength Murashige and Skoog (MS) medium without adding plant growth regulators (PGRs). A maximum germination percentage of 100% was achieved after 2 weeks of culture. 1.5 cm-sized node segments from both seeds and mother trees were used for shoot induction. These node segments were treated with the same sterilization conditions as the seeds but with the addition of 0.1% $HgCl_2$. The node segments achieved a 90% survival rate despite the additional chemicals. The node segments from seeds were then cultured on a full-strength MS medium supplemented with 0.50 mg/l of BAP. The results showed a maximum number of shoots, reaching 4.10±1.20 per explant and a mean shoot length of 3.87±0.30 cm. The part of node segments derived from mother trees showed 3.50±1.27 shoots per explant, accompanied by a mean shoot length of 3.18±0.89 cm when using BAP concentration of 0.25 mg/l after 12 weeks of cultivation. The rooting medium contained half-strength MS medium supplemented with 0.75 mg/l of IBA. After 8 weeks of being cultured in the rooting medium, the shoots exhibited a maximum rooting percentage of about 80%. The technique provides a valuable method for rapidly propagating and conserving this plant species in a controlled and efficient manner.

Keywords: Fabaceae, Micropropagation, Node segments, Shoot induction, *Xylia xylocarpa* (Roxb.) Taub

Introduction

Xylia xylocarpa (Roxb.) Taub., commonly known as Ironwood, is a species of tree belonging to the family Fabaceae. It is native to South Asia and Southeast Asia and can be found in deciduous dipterocarp forests and mixed deciduous forests in Thailand. This tree is designated as the provincial tree of Tak in Thailand. It is one of the 58 tree species recognized for their substantial value as

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collateral for financial institutions in Thailand's financial sector. It holds significant economic value attributed to its desirable wood properties (Tungmitracharoen, 2018). The morphology of *X. xylocarpa*, the tree can attain a height of 25 to 30 meters. The outer bark displays shades of creamy brown or red-gray, and the inner bark takes on a reddish-brown coloration. The leaves are bipinnate, showcasing a single pair and compound leaf and these leaflets have ovate shapes. The white or yellow flowers are densely arranged in spherical heads. The fruit is boomerang-shaped and has an oblong, nearly round seed with a brown coloration inside. (Nakmee *et al.,* 2015). The wood has distinctive characteristics, including being sourced from large to giant evergreen trees. It is classified as a hardwood, exhibiting a captivating reddish-brown color and a smooth texture. Notably, this wood showcases remarkable strength properties, rendering it highly resilient against elevated temperatures, humidity, and even termite attacks (Wattanakulpakin *et al.,* 2015). Furthermore, its quality remains intact throughout the drying and processing phases. With a substantial weight and notable dimensional stability, the wood of *X. xylocarpa* exhibits a comparatively low to moderate shrinkage rate. Moreover, the ratio of tangential to radial shrinkage is balanced, suggesting minimal risk of deformation during the drying process (Josue, 2004). Given these exceptional attributes, Ironwood finds widespread applications, including flooring, construction, pillars, beams, frameworks, and furniture, among other uses. Beyond its economic significance, *X. xylocarpa* offers valuable ecological advantages. Culturing these trees contributes to elevated moisture levels, the preservation of soil fertility, environmental enhancement, and erosion mitigation (Reichel and Boonyaputthipong, 2021). The leaf litter of *X. xylocarpa* has a high decomposition rate in the soil, contributing to the increased availability of minerals. This results in improved soil fertility (Marod *et al.,* 2017). Additionally, this tree acts as a carbon sink, effectively capturing and absorbing carbon dioxide from the atmosphere. This process, known as carbon dioxide sequestration, plays a pivotal role in curbing the escalation of atmospheric carbon dioxide levels. It also aids in alleviating the repercussions of climate change resulting from human activities and natural combustion processes. A research study focusing on Biomass and carbon stocks of *X. xylocarpa* in enduring and effectively sequestering more carbon within the region's tropical dry forest. This is particularly noteworthy under various disturbances (Srinivas and Sundarapandian, 2018). Furthermore, the ethanolic extract obtained from the stem, seed, and bark of *X. xylocarpa* has demonstrated notable antiradical scavenging activity against DPPH (Ramli *et al.,* 2008). Extracts derived from the leaves of *X. xylocarpa* have been found to possess medicinal properties effective in treating various ailments, such as leprosy, wound healing, gonorrhea,

rheumatism, anemia, diarrhea, and ulcers (Chowdhury *et al.,* 2021). However, the cultivation of *X. xylocarpa* comes with its share of challenges. Its relatively slow growth rate compared to other economically valuable wood species has hindered Thailand's widespread cultivation for trade and economic purposes. Consequently, the commercial availability of *X. xylocarpa* has been relatively constrained. Typically, the propagation of trees is achieved through the utilization of seeds, wherein trees produce seeds that can be collected and subsequently planted to cultivate new trees. However, this propagation mode may entail limitations, including sluggish growth rates and the potential for mutations. A study that delved into germination rates and the implementation of high-yield seed sowing methods for various forest trees, along with an analysis of costs and returns on investment, unveiled that *X. xylocarpa* exhibited the lowest returns on investment due to its notably diminished average germination rate of approximately 63.75% (Nuchit *et al.,* 2021). The main issue encountered in propagating *X. xylocarpa* is that it can only be propagated through a single method: seed propagation. The duration of seed storage affects the germination rate and makes it prone to mutations. Tissue culture provides a potential solution for the rapid propagation of plants (Loyola-Vargas and Ochoa-Alejo, 2018). Therefore, tissue culture propagation becomes an alternative to rapidly increase the quantity of *X. xylocarpa* within a short time. It can maintain the genetic identity of the tree due to its low mutation rate and will positively impact trade in Thailand's economically valuable wood market.

The experiment aimed to study suitable methods for pathogen sterilization and appropriated nutrient formulations for inducing successful shoot and root development from seeds and node segments of *X. xylocarpa.*

Materials and methods

Cultural media and conditions

The MS (Murashige and Skoog, 1962) medium was utilized in all experiments, solidified with 0.8% (w/v) Crystal Agar Gel (Central Gel co., Ltd.), and supplemented with 3% (w/v) Sucrose as a carbon source, along with plant growth regulators. The pH of the medium was adjusted to 5.8 ± 0.2 and sterilized by autoclaving for 15 minutes at a temperature of 121°C and a pressure of 15 psi. Every experimental culture was maintained in a tissue culture room at 25±2 ºC under a 16/8 h (light/dark) cycle condition.

Sterilization and germination of seeds

The seeds were sourced from the commercial trade of economically valuable tree plants. High-quality seeds with no injuries or damage were selected. The seeds were washed with detergent and water to remove surface pathogens and then soaked in hot water at 60˚C for 60 minutes. After that, they were shaken in 70% (v/v) ethanol for 1 minute and then in fungicide 0.1% v/v (Carbendazim® fungicidal solution) for 10 minutes. The seeds were washed with distilled water twice and then shaken in different concentrations of commercial bleach containing 6% and 12% (v/v) (Sodium hypochlorite solution; NaOCl), along with 0.1% (v/v) Antibiotic Antimycotic solution (Penicillin, Streptomycin, Amphotericin; [100X]; Sigma), 0.1% (v/v) PPM (Preservative for Plant Tissue Culture Media Active, Plant Cell Technology), 0.1% (v/v) Cefotaxime (Nida Pharma Inc), and 3 drops of Tween-20 for 10 minutes. Finally, they were washed two times with sterile distilled water. Subsequently, sterilization without NaOCl was served as a control. The germinated seedlings were cultured on a half-strength MS medium without adding plant growth regulators (PGRs). Survival rates and germination percentages were recorded after 2 weeks of culture.

Sterilization of node segment

The node segments used for this procedure were sourced from commercially traded economically valuable tree plants. The trees were 1-2 years old, and node segments measure 1.5 cm in size and were without injuries or damage were selected. The surface sterilization process for these node segments using detergent and running tap water for 10 minutes. Afterward, they were shaken in 70% (v/v) ethanol for 1 minute, followed by a fungicide treatment of 0.1% v/v Carbendazim® for 10 minutes. The segments were then washed twice with distilled water and shaken in different concentrations of $HgCl_2$ (mercuric chloride solution), which included 0.1% and 0.2% (w/v), along with 6% v/v commercial bleach (NaOCl), 0.1% (v/v) Antibiotic Antimycotic solution, 0.1% (v/v) PPM, 0.1% (v/v) Cefotaxime, and 3 drops of Tween-20 for 10 minutes. Finally, they were washed two times with sterile distilled water. Subsequently, sterilization without $HgCl_2$ was served as a control. The node segments were then cultured on a full-strength MS medium without adding plant growth regulators (PGRs). Survival rates were recorded after 2 weeks of culture.

Shoot induction from seed and node segment

The node segments obtained from the germination of seeds were cultured on half-strength MS medium without the addition of plant growth regulators after

8 weeks and were cut to approximately 1.5 cm in size. Subsequently, node segments from mother trees were sterilized and cultured in vitro after 2 weeks. Node segments from both sources were then transferred to full-strength MS medium supplemented with cytokinins such as BAP (6-benzylaminopurine) or Kinetin at different concentrations 0, 0.25, 0.5, 1.0, 2.0, and 3.0 mg/l. The number of shoots per explant, the average length of shoots, and subculturing were recorded every 4 weeks.

Root induction

The completely developed multiple shoots obtained from node explants were cultured for 12 weeks and then trimmed down to single-shoot explants. These single shoot explants were 3–4 cm long and had 4–5 leaves. Before initiating the rooting process, they were pre-cultured on half-strength MS medium without adding plant growth regulators (PGRs) for 1 week. Following the pre-culture, the shoots were transferred to a half-strength MS medium supplemented with various concentrations of IBA (indole-3-butyric acid), including 0, 0.25, 0.5, 0.75, and 1.0 mg/l. The number of roots per shoot and the percentage of rooting were recorded every 4 weeks.

Statistical analysis

The experiment was conducted with 10 replicates per treatment to establish *in vitro* shoot and root induction. An analysis of variance (ANOVA) was carried out, and the normal distribution of the data was confirmed before analysis. The significance of differences in means was evaluated using the Duncan multiple comparison test ($p < 0.05$).

Results

Effect of sterilization seeds and node segments

The survival rate of seeds was assessed under various sterilization conditions. Subsequently, these seeds were cultured on a half-strength MS medium without plant growth regulators for 2 weeks. Result showed both the maximum observed survival rate, germination percentage, and reaching 100%, were achieved through sterilization using a 6% v/v NaOCl solution. The seeds started germination after 1 week of culture (Figure 1B). The subsequent condition resulted in a 100% survival rate and a 70% germination percentage

with a 12% v/v NaOCl treatment because it led to the death of plant cells (Table 1).

Table 1. Effects of different concentrations of Sodium hypochlorite solution (NaOCl) for sterilization of *X. xylocarpa* seeds and culture on half-strength MS medium without plant growth regulators for 2 weeks

Concentration NaOCl (%v/v)	Survival[/1] (%)	Contamination[/2] (%)	Germination[/3] (%)	Death[/4] (%)
Control (0)	60	40	50	10
6	100	0	100	0
12	100	0	70	30

[1] /Survival was observed after 2 weeks of culture;
[2] /Contamination was observed after 2 weeks of culture;
[3] /Germination was observed after 2 weeks of culture;
[4] /Death was observed after 2 weeks of culture.

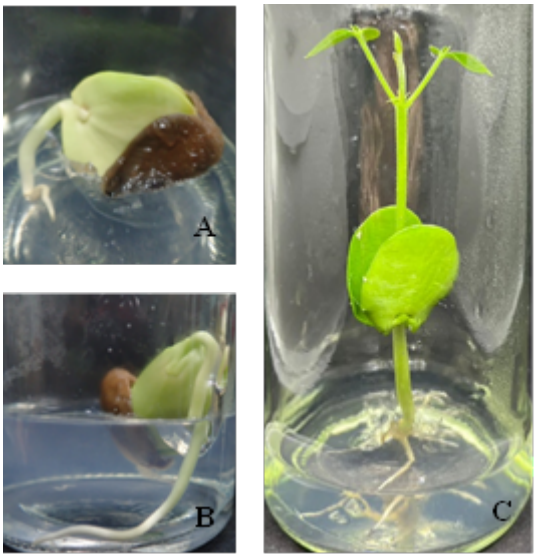

Figure 1. The seeds of *X. xylocarpa* through sterilization with 6% NaOCl and duration after 1 week of cultured (A), after 2 weeks of culture (B), and after 4 weeks of culture (C)

The node segments from mother trees were cultured under various sterilization conditions on full-strength MS medium without plant growth regulators for 2 weeks. The results showed a maximum observed survival rate of

90%, achieved through sterilization using 0.1% w/v $HgCl_2$. The characteristics of the explants sterilized and cultured after 2 weeks showed healthy explants and the initiation of shoot tip growth (Figure 2A). The following condition resulted in a 60% survival rate with a 0.2% w/v $HgCl_2$ treatment because it led to the death of plant cells (Figure 2B). The characteristics of control showed node explants indicated contamination from fungi and bacteria (Figure 2C, Table 2).

Table 2. Effects of different concentrations of Mercury Chloride ($HgCl_2$) for sterilization of *X. xylocarpa* node segments and culture on full-strength MS medium without plant growth regulators for 2 weeks

Concentration $HgCl_2$ (%w/v)	Survival[1] (%)	Contamination[2] (%)	Death[3] (%)
Control (0)	40	40	20
0.1	90	0	10
0.2	60	0	40

[1] /Survival was observed after 2 weeks of culture;
[2] /Contamination was observed after 2 weeks of culture;
[3] /Death was observed after 2 weeks of culture.

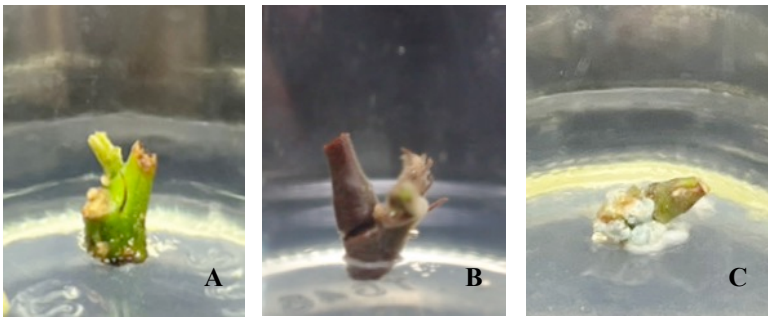

Figure 2. Node segments of *X. xylocarpa* were subjected to sterilization using different concentrations of $HgCl_2$. After 2 weeks of culture with 0.1% $HgCl_2$ (A), characteristics of node explant death were observed when 0.2% $HgCl_2$ was used (B). In the control group, without $HgCl_2$ sterilization, node explants exhibited contamination by fungi and bacteria (C)

Effect of shoot induction

The node segments from seed germination and mother trees were cultured for 8 weeks and recorded every 4 weeks. Node segments obtained from

germinated seeds were induced to develop shoots and were cultured on a full-strength MS medium supplemented with various concentrations of BAP or Kinetin for 4 weeks. The results showed an average number of shoots and an average shoot length of 2.80±1.23 shoots per explant and 1.94±0.24 cm, respectively (Figure 3A, Table 3). Similarly, the results showed that a maximum average number of shoots, as well as an average shoot length of 4.10±1.20 shoots per explant and 3.87±0.30 cm, respectively, were attained after 8 weeks of culture, using a BAP concentration of 0.5 mg/l (Figure 4A, Table 3).

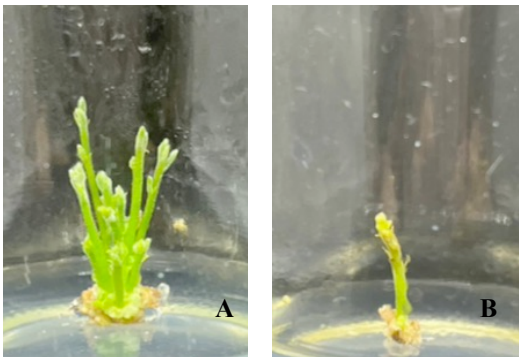

Figure 3. The shoot induction from seeds of *X. xylocarpa* cultured on full-strength MS medium supplemented with 0.5 mg/l of BAP and duration after 4 weeks culture (A), and the control cultured on full-strength MS medium without plant growth regulators and duration after 4 weeks of culture (B)

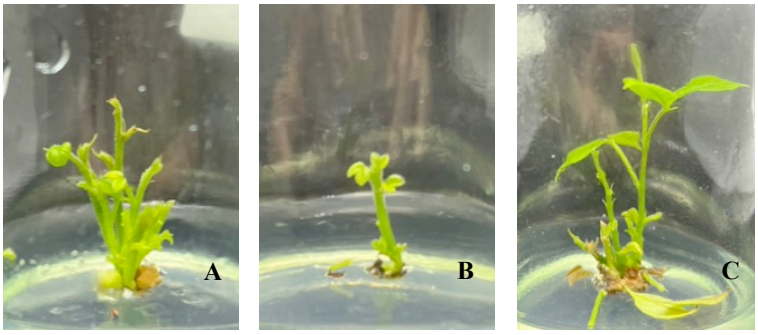

Figure 4. The shoot induction from seeds of X. xylocarpa cultured on full-strength MS medium supplemented with 0.5 mg/l of BAP and duration after 8 weeks of culture (A), and the control cultured on full-strength MS medium without plant growth regulators and duration after 8 weeks of culture (B), and after 12 weeks of culture, the shoots from 0.5 mg/L of BAP were entirely ready for rooting (C)

Table 3. Effects of shoot induction from *X. xylocarpa* seeds and culture on full-strength MS medium supplemented with different concentrations of BAP and Kinetin for a duration of 4 and 8 weeks of culture

Time (weeks)	Plant Growth Regulators (mg/l)		The average number of shoot[/1,2,3] (shoots/explant)	Average of length shoot[/1,2,3] (cm)
4	Control	0	1.00±0.00^{c}	0.60±0.04^{e}
	BAP	0.25	1.90±0.74^{b}	1.56±0.10^{b}
		0.5	2.80±1.23^{a}	1.94±0.24^{a}
		1.0	1.0±0.00^{c}	0.80±0.04^{d}
		2.0	1.0±0.00^{c}	0.59±0.03^{e}
		3.0	1.0±0.00^{c}	0.50±0.03^{f}
	Kinetin	0.25	1.0±0.00^{c}	1.28±0.04^{c}
		0.5	1.0±0.00^{c}	0.51±0.03^{f}
		1.0	1.0±0.00^{c}	0.33±0.06^{g}
		2.0	1.0±0.00^{c}	0.26±0.11^{gh}
		3.0	1.0±0.00^{c}	0.23±0.07^{h}
8	Control	0	1.00±0.00^{c}	0.80±0.05^{ef}
	BAP	0.25	2.60±0.52^{b}	2.69±0.63^{b}
		0.5	4.10±1.20^{a}	3.87±0.30^{a}
		1.0	1.0±0.00^{c}	1.20±0.05^{d}
		2.0	1.0±0.00^{c}	0.90±0.06^{e}
		3.0	1.0±0.00^{c}	0.73±0.03^{f}
	Kinetin	0.25	1.0±0.00^{c}	1.97±0.12^{c}
		0.5	1.0±0.00^{c}	0.83±0.06^{ef}
		1.0	1.0±0.00^{c}	0.50±0.06^{g}
		2.0	1.0±0.00^{c}	0.47±0.06^{g}
		3.0	1.0±0.00^{c}	0.40±0.09^{g}

[1]/Cultured on full-strength MS medium after 4 and 8 weeks,

[2] /Each value represents the mean ± SD of three repeats per treatment,

[3] /The data were statistically analyzed using Duncan's multiple range test DMRT In the same column, significant differences according to significant differences at the $p \leq 0.05$ level are indicated by different letters.

In the part where node segments obtained from mother trees were stimulated to develop shoots and were cultured on a full-strength MS medium supplemented with various concentrations of BAP or Kinetin for 4 weeks, the results showed that an average number of shoots, as well as an average shoot length of 2.40±0.70 shoots per explant and 1.63±0.09 cm, respectively (Figure 5A, Table 4). Similarly, the results showed that a maximum average number of shoots, as well as an average shoot length of 3.50±1.27 shoots per explant and

3.18±0.89 cm, respectively, were attained after 8 weeks of culture, using a BAP concentration of 0.25 mg/l (Figure 6A, Table 4).

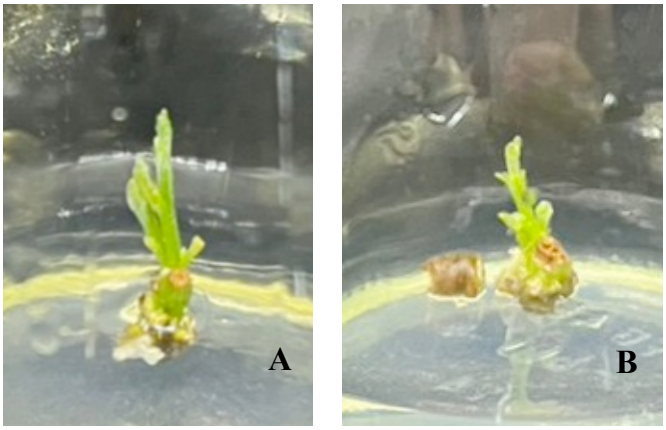

Figure 5. The shoot induction from node segments of *X. xylocarpa* culured on full-strength MS medium supplemented with 0.25 mg/l of BAP and duration after 4 weeks of culture (A), and the control cultured on full-strength MS medium without plant growth regulators and duration after 4 weeks of culture (B)

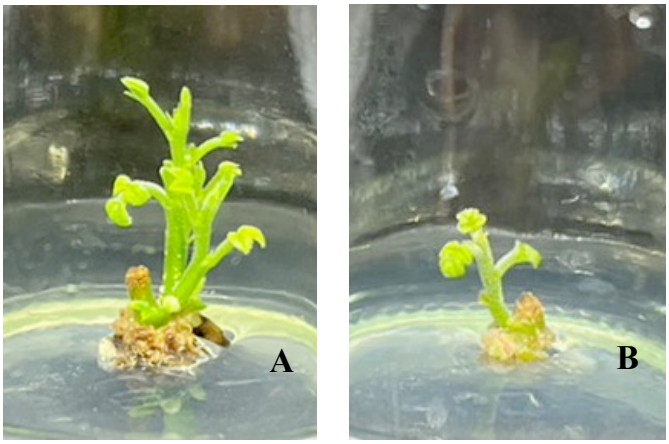

Figure 6. The shoot induction from node segments of *X. xylocarpa* culured on full-strength MS medium supplemented with 0.25 mg/l of BAP and duration after 8 weeks of culture (A), and the control cultured on full-strength MS medium without plant growth regulators and duration after 8 weeks of culture (B)

Table 4. Effects of shoot induction from *X. xylocarpa* node segments and culture on full-strength MS medium supplemented with different concentrations of BAP and Kinetin for a duration of 4 and 8 weeks of culture

Time (weeks)	Plant Growth Regulators (mg/l)		Average number of shoot[/1,2,3] (shoots/explant)	Average of length shoot[/1,2,3] (cm)
4	Control	0	1.0±0.00^{c}	0.60±0.03^{ef}
	BAP	0.25	2.40±0.70^{a}	1.63±0.09^{a}
		0.5	1.80±0.80^{b}	1.34±0.13^{b}
		1.0	1.0±0.00^{c}	0.78±0.10^{d}
		2.0	1.0±0.00^{c}	0.67±0.08^{e}
		3.0	1.0±0.00^{c}	0.55±0.08^{g}
	Kinetin	0.25	1.0±0.00^{c}	1.05±0.12^{c}
		0.5	1.0±0.00^{c}	0.51±0.07^{f}
		1.0	1.0±0.00^{c}	0.29±0.05^{g}
		2.0	1.0±0.00^{c}	0.23±0.^{07gh}
		3.0	1.0±0.00^{c}	0.18±0.09^{h}
8	Control	0	1.00±0.00^{c}	0.81±0.04^{d}
	BAP	0.25	3.50±1.27^{a}	3.18±0.89^{a}
		0.5	2.30±0.82^{b}	2.37±0.34^{b}
		1.0	1.0±0.00^{c}	0.97±0.11^{d}
		2.0	1.0±0.00^{c}	0.82±0.05^{d}
		3.0	1.0±0.00^{c}	0.73±0.05^{d}
	Kinetin	0.25	1.0±0.00^{c}	1.55±0.16^{c}
		0.5	1.0±0.00^{c}	0.82±0.06^{d}
		1.0	1.0±0.00^{c}	0.40±0.06^{e}
		2.0	1.0±0.00^{c}	0.33±0.05^{e}
		3.0	1.0±0.00^{c}	0.29±0.07^{e}

[1]/Cultured on full-strength MS medium after 4 and 8 weeks,
[2] /Each value represents the mean ± SD of three repeats per treatment,
[3] /The data were statistically analyzed using Duncan's multiple range test DMRT In the same column, significant differences according to significant differences at the $p \leq 0.05$ level are indicated by different letters.

Effect of root induction

The shoots of germinated seeds obtained from the micropropagation process were transferred entirely to a rooting medium containing half-strength MS medium supplemented with various concentrations of IBA. The results showed a rooting percentage of around 70% and an average number of roots of 0.90±0.74 per shoot when using an IBA concentration of 0.75 mg/l after 4 weeks of culture (Figure 7A). Similarly, after 8 weeks, the results showed a maximum rooting percentage of around 80% and an average number of roots of 2.90±1.73 per shoot using the same concentration of IBA (Figure 7B-C, Table 5).

Table 5. Effects of root induction from shoot germinated seeds *X. xylocarpa* seeds and culture on half-strength MS medium supplemented with different concentrations of IBA for a duration of 4 and 8 weeks of culture

Time (weeks)	Plant Growth Regulators (mg/l)		Percentage of root[/1] (%)	Average number of shoot[1,2,3] (roots/shoot)
4	Control	0	0	0.00±0.00[b]
	IBA	0.25	0	0.00±0.00[b]
		0.5	0	0.00±0.00[b]
		0.75	70	0.97±0.11[a]
		1.0	0	0.00±0.00[b]
8	Control	0	0	0.00±0.00[b]
	IBA	0.25	0	0.00±0.00[b]
		0.5	0	0.00±0.00[b]
		0.75	80	2.90±1.73[a]
		1.0	0	0.00±0.00[b]

[1]/Cultured on full-strength MS medium after 4 and 8 weeks,
[2] /Each value represents the mean ± SD of three repeats per treatment,
[3] /The data were statistically analyzed using Duncan's multiple range test DMRT In the same column, significant differences according to significant differences at the $p \leq 0.05$ level are indicated by different letters.

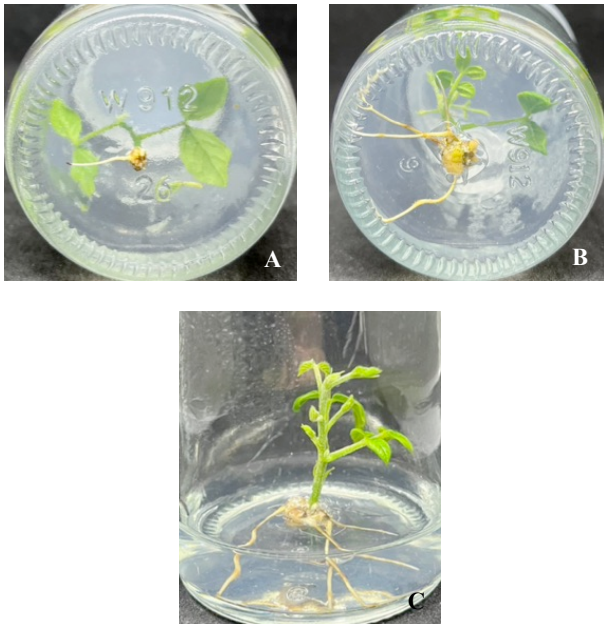

Figure 7. The root induction from shoots of germinated seeds of *X. xylocarpa* culured on half-strength MS medium supplemented with 0.75 mg/l of IBA and duration after 4 weeks culture (A), and after 8 weeks culture (B) and (C)

Discussion

In the seed sterilization process of *X. xylocarpa*, it was observed that the control experiment exhibited contamination by bacteria and fungi at relatively high percentages, resulting in low survival rates when compared to experiments involving NaOCl solutions at 6% and 12% concentrations. The NaOCl solutions experiments showed the highest survival rates with no microbial contamination. Additionally, germination rates were found to be the highest at 100% when using a 6% NaOCl solution. This finding is consistent with results of a previous reported by Kumar *et al.* (2019), who discovered that sterilizing sugarcane explants with a 6% NaOCl solution for 10 minutes resulted in a maximum survival rate of 66±1.41%. Behmand *et al.* (2022) also used a 6% NaOCl solution for sterilizing Chickpea seeds. It is like the previous research report by Ozkan and Khawar (2017), which used a 5% NaOCl solution for 20 minutes to sterilize Faba Bean seeds. Minutes to sterilize *Acacia farnesiana* and. However, germination percentages were significantly lower when a 12% NaOCl solution was employed due to seed death. A pale or light brown coloration characterized seed death, and the color of the culture medium changed. Subsequent attempts at cultivation following seed death did not yield germination. For the experiment

described above, the results are consistent with prior research findings on using NaOCl on sterilized seeds survival and germination (Hesami *et al.,* 2017). The culture medium formula plays a pivotal role in influencing seed germination. Studies and experimentation have shown that a half-strength MS medium without adding plant growth regulators leads to the optimal germination of seeds. It is consistent with the findings reported by Erisen *et al.* (2010) that the cultivation of *Astragalus nezaketae* seeds on half-strength MS medium without adding plant growth regulators resulted in a germination rate of 100%.

Additionally, cultivating seeds of *Ulex europaeus* on a half-strength MS medium resulted in better germination than the full-strength MS medium (Ramirez *et al.,* 2012). However, it is worth noting that the failure of seeds to germinate may be attributed to other factors, such as the duration of harvesting or seed storage, as well as the internal viability of embryos within the seeds. These factors can directly influence the germination rate of seeds (Dagata *et al.,* 2017).

In disinfecting node segments, it was observed that contamination by bacteria occurred in a manner like the seed disinfection experiments. The contamination percentage was relatively high, leading to the death of some of the node segments. The death of node segments was characterized by a color change from green to shades of brown and black. In contrast, surviving node segments retained their green color and exhibited the development of new shoots after two weeks of cultivation.

In experiments where 0.1% $HgCl_2$ was added to the node segments, a maximum survival rate was achieved, and there were no instances of bacterial contamination, along with minimal occurrences of node segment death. This finding is consistent with results of a previous reported by Jaiswal *et al.* (2015), found that sterilization of *Pterocarpus marsupium* Roxb. nodal explants and sterilization of Carob (*Ceratonia siliqua*) explants (Ahmad *et al.,* 2021).

On the other hand, experiments with 0.2% $HgCl_2$ showed no bacterial contamination but had survival and death rates that were quite similar. This could be attributed to the higher concentration of $HgCl_2$, which might have had an inhibitory effect on plant growth, as reported in previous research observed that surface sterilization of sugarcane explants using $HgCl_2$ of 0.1% for 5 minutes resulted in a higher survival rate (73.2±1.56%) compared to treatments with 0.2% $HgCl_2$ for the same duration (53.2±1.39%) (Kumar *et al.,* 2019).

Furthermore, apart from NaOCl and $HgCl_2$, various other substances have been utilized for seed and node segment disinfection, each impacting the survival of these plant materials. These substances include dishwashing detergent, ethanol, fungicides, antibiotics such as PPM Cefotaxime, and surfactants like tween-20,

among others (Mng'omba *et al.,* 2012) (Mahmoud and Al-Ani, 2016) (Kanjanawattanawong *et al.,* 2019) (Kumar *et al.,* 2019).

Each agent brings unique properties and effects to the disinfection process. The choice of disinfection agent must consider its efficacy in controlling pathogens and its influence on the viability and health of seeds and node segments in experimental settings. Consequently, selecting the appropriate disinfection method becomes a critical aspect of conducting successful plant-related experiments while maintaining the integrity and vitality of the plant material under investigation.

The node segments from the germination of seeds exhibited growth and were ready for shoot induction after 4 weeks of cultivation. Node segments derived from *X. xylocarpa* seeds were induced to form shoots through cultured on MS medium supplemented with 0.5 mg/l of BAP for 8 weeks. The results showed a maximum average number of shoots and average shoot length. These results are consistent with those reported in previous research, shoot induction from *Mimosa pudica* L. explants. (Bianchetti *et al.,* 2017). Similatly, the research by Bertsouklis *et al.* (2021), found that shoot induction of Senna artemisinins, showed a maximum proliferation rate of 2.4 shoots/explant. Additionally, Simoes *et al.* (2022), the results of nodes *Dalbergia nigra* in show the best concentrations for the in vitro propagation.

When it comes to inducing shoot from node segments of *X. xylocarpa* mother trees, cultivation on MS medium supplemented with 0.25 mg/l of BAP for a duration of 8 weeks showed a maximum average number of shoots and average shoot length. This finding is consistent with results of a previous report found that a maximum number of shoots with an average of 2.0 shoots/explant in 13 days of *C. spinosa* explants (Yumbla and Ortega, 2023). Additionally, showed the shoot induction an average of 8.20 ± 0.66 shoots per explant and an average shoot length of 4.14 ± 0.13 for *Cassia siamea* Lam. Moreover, it was observed that increasing the concentration of BAP had an inhibitory effect on shoot induction, found that when using a 1.0µM (0.225 mg/l) of BAP showed (8.20 ± 0.66 shoots/explant and 4.14 ± 0.13 cm.) when compared to using a 2.0µM (0.45mg/l) of BAP (6.20 ± 0.37 shoots/explant and 3.60 ± 0.13 cm.) (Parveen *et al.,* 2010).

Furthermore, a comparative study of shoot induction in *X. xylocarpa* was conducted using two growth regulators within the cytokinin group: BAP and Kinetin. It was found that, under low-concentration conditions, BAP outperformed Kinetin in promoting shoot induction, This finding is consistent with results of a previous reported by Parveen *et al.* (2010) found that the regeneration of shoots from *C. siamea* Lam. using low concentrations of BAP resulted in a maximum average number of shoots and average shoot length

compared to all concentrations of Kinetin. Similarly, the shoot induction from *Bauhinia racemosa* Lam. nodal explant using a 3.0 mg/l of BAP resulted in a maximum average number of shoots and average shoot length compared to all concentrations of Kinetin (Sharma *et al.,* 2017).

In the context of inducing root formation from healthy shoots of *X. xylocarpa*, experimental results have revealed that culturing these shoots on half-strength MS medium supplemented with 0.75 mg/l of IBA showed a maximum percentage of rooting and the average number of roots per shoot. Consistent with the previous results of Kasthurirengan *et al.* (2013), who found that in vitro-generated shoots of *Samanea saman* (rain tree) showed a maximum rooting percentage of 90%. Notably, it was observed that higher or lower concentrations of IBA had an inhibitory effect on root formation, consistent with previous research by Hakim *et al.* (2019), found that the induction of roots from Carob (*C. siliqua* L.) shoot using a 0.5 mg/l of IBA showed a maximum average number of root and average length root compare to using a 0.1 and 1.0 mg/l of IBA.

The research finding represented a significant breakthrough as it can be introduced for tissue culture cultivation in *X. xylocarpa* plants in the first time. The results obtained from this study presented with opportunities for further exploration and research into the genetic variations within *X. xylocarpa* plants.

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