https://www.waikoloadryforest.org/plants/iliahi/

Explant Sterilization of Sandalwood (*Santalum Album*) and Teak (*Tectona Grandis*) Micropropagation with Commercial Disinfectant

Contributor: Helmi Sulaiman[1+], Shabrina Yasmin Ramadhani[1+], Kresna Fajri Wicaksana[1+], Rosadi Kartiwijaya[2], Dewi Rahmawati[2], Dewi Suryani[2], Rhomi Ardiansyah[2]*

[1]Agriculture Engineering, School of Life Sciences, Bandung Institute of Technology, Indonesia
[2]SEAMEO BIOTROP, Bogor, Indonesia
[+]Equal contribution

*Corresponding author: : rhomi@biotrop.seameo.id

Abstract

Sandalwood (*Santalum album*) and Teak (*Tectona grandis*) are essential wood sources for furniture. Sustainable micropropagation methods can benefit wood availability. This study evaluates NaOCl and Povidone-iodine effects, immersion duration, and concentrations on teak and sandalwood micropropagation. Teak is treated with 10% & 15% NaOCl and 10% & 15% Povidone-iodine. No significant difference was observed between teak treatments; however, povidone-iodine shows potential as an effective teak sterilizing agent. Sandalwood's best sterilization: NaOCl at 10% & 15%.

Introduction

Sandalwood (*Santalum album*) is a prized tree known for its aromatic properties. It belongs to the Santalaceae family and is native to the Indian subcontinent, particularly India, Sri Lanka, and parts of Southeast Asia. These evergreen trees can reach up to 10 meters in height and have fragrant leathery leaves. The most valuable part is the heartwood, used in perfumes, incense, and traditional medicine. Sandalwood thrives in tropical and subtropical regions with well-drained soil and moderate rainfall (Kumar et al., 2012). Teak (*Tectona grandis*) is a valuable tropical timber native to Indonesia. The tree belongs to the family Verbenaceae. Teak dominates dry deciduous forest, with 5-7 months of dry season and 1000-1400 mm mean annual rainfall (Sankaran & Ratnam, 2013). The teak forest in Java also represents a lowland subhumid tropical forest ecosystem (Hartshorn, 2013). It has high quality wood and versatile uses. Evaluations on plant parts exhibit biological activities of antioxidant, antipyretic, analgesic, hypoglycemic, wound healing and cytotoxic (Asdaq et al., 2022). Conventional breeding is limited by seed availability and genetic variation, which can lead to differences in appearance. Woody tree plantation requires large amounts of uniform and high-quality planting materials obtained through vegetative propagation methods like shoot cutting or micropropagation. Micropropagation grows entire plants from small plant tissue sections in a controlled laboratory setting with a nutrient-rich medium (Pant & Husen, 2022). Sterilization is crucial to prevent contamination, which can hinder growth and cause mortality. Commercial disinfectants like bleach and Betadine® are commonly used for sterilizing plant cultures (Wamaedeesa et al., 2021; Pratiwi et al., 2020; Neliyati et al., 2019; Wiyastuti et al., 2018). This study examines the effects of different disinfectants and their concentrations on teak and sandalwood tissue culture to improve propagation efficiency.

Methods

The experiment was conducted at the Biotechnology Laboratory, SEAMEO BIOTROP Bogor. The Murashige-Skoog (MS) (Murashige and Skoog, 1962) media was made using the following components in Table 1. Hormone 6-benzylaminopurine (BAP; Sigma®) were added into the media. BAP stock solution was made by diluting 100 mg with HCl 1 N and 100 mL dH_2O. The optimum pH range for MS medium was adjusted to 5.8.

The basic sterilization techniques were employed to ensure the cleanliness of the explants. They were first rinsed three times with sterile water, followed by soaking in Tween-20 solution for 30 minutes, and then rinsed again with sterile water. Subsequently, explants were soaked in Fungicide and Bactericide solution for each one hour, followed by three more rinses with sterile water. After completing these sterilization steps, the explants were ready for further treatments and experimental procedures inside the Laminar Air Flow (LAF) chamber.

Table 1. Composition of Murashige and Skoog (1962) Stock Medium

Stock	Chemical name	Concentration (mg/L)	Stock Solution		Volume (mL) for one litre media
			g/L	Concentration	
A	$NH_4NO_3^-$	1650	41.25		20
B	KNO_3	1900	47.5	50	20
C	KH_2PO_4	170	17	100	10
	H_3BO_3	6.2	0.62		
	KI	0.83	0.083		
	$Na_2MoO_4.2H_2O$	0.25	0.025		
	$CoCl.6\ H_2O$	0.025	0.0025		
D	$CaCl_2.2H_2O$	440	44	100	10
E	$MgSO_4.7H_2O$	370	37	100	10
	$MnSO_4.4H_2O$	22.3	22.3		
	$ZnSO_4.7H_2O$	8.6	0.86		
	$CuSO_4.5H_2O$	0.025	0.0025		
F	$FeSO_4.7H_2O$	27.8	1.39	100	10
	Na_2.EDTA	37.3	3.73		
Myoinositol		100	Measured when making the media		
Vitamin	Niacin	0.5	0.05	100	1
	Pyridoxine.HCl	0.5	0.5		
	Thiamine-HCl	0.1	0.01		
	Glycine	2	0.2		
Sucrose/Sugar		30000	Measured when making the media		
Agar		7000			

The experiment was conducted to observe the success rate of sterilization of teak explant using several commercially available disinfectants, such as Betadine® (contains Povidone iodine 10%, Registration Number Kemenkes RI: PKD20501710078) and Bayclin® (contains 5,25% sodium hypochlorite, made in Indonesia). Fresh shoots were isolated from the axillary shoots of young Teaks in the nursery then kept in solution of three drop of tween per 100 ml sterilized water (Figure 1). First is the control group (A), following the standard procedure of double disinfection in NaOCl at 15% (v/v) and 10% (v/v) for 15 minutes and 10 minutes respectively. The second group (B) used NaOCl 15% (v/v) solution for 15 minutes, then povidone-iodine (PI) 10% (v/v) solution for 10 minutes. The last group (C) used PI solution at 15% (v/v) and 10% (v/v) for 15 minutes and 10 minutes, respectively. For each treatment with NaOCl and PI, explants were followed by three rinses in sterile water. Afterward, shoots were rinsed with alcohol 70% for one minute and then rinsed five times with sterilized water. Next, shoots were cut so each explant has one or two axillary buds.

Explant Sterilization

Figure 1. The process of teak sterilization. (Clockwise from top left: Harvesting teak shoots from the mother plant; immersion of the shoots in a povidone-iodine solution; immersion of the shoots in a NaOCl solution; condition of the explant after establishment).

To begin with the experiment for sandalwood (Santalum album). The necessary tools and materials were prepared, including a Tween-20 solution made by mixing 3 drops of tween-20 per 100 ml of sterile water. Suitable plants meeting specific criteria, such as being healthy, having usable shoots, and appropriate size and age, were selected as the source of explants. The plant shoots were carefully taken from the lateral shoots, preferably from the first or second plant branch from the bottom for easy collection. During the explant collection process, no additional treatments like pesticides or fungicides were applied, and the apical buds were left intact. The obtained explants were then placed into the prepared Tween-20 solution and brought into the lab.

Inside LAF, advanced sterilization techniques were applied. Tools and materials needed for this stage were prepared and placed inside LAF, and the UV light was turned on for 15 minutes and then turned off. The lights and blower of the LAF were turned on before use, and the cabinet shelves were opened. For the control group, explants were soaked in 70% (v/v) alcohol for one minute and then rinsed five times with sterile water. For treatment 1, explants were soaked in 15% (v/v) NaOCl solution for 15 minutes, rinsed with sterile water, then soaked in 10% (v/v) NaOCl solution for another 10 minutes, and finally rinsed again with sterile water. They were then soaked once more in 70% (v/v) alcohol for one minute and rinsed five times with sterile water. For treatment 2, the explants followed a similar procedure with soaking in 30% (v/v) NaOCl solution for 15 minutes and then in 20% (v/v) NaOCl solution for 10 minutes, followed by rinsing and alcohol treatment.

After all the treatments were completed, plant explants were ready for planting. Inside LAF, tools and materials required for the planting stage were prepared. Explants were carefully taken with sterile tweezers and placed in a petri dish containing tissue. They were then trimmed to ensure cleanliness and ease of planting in the media. This process was repeated for all explants, and each media jar was labelled with the corresponding experimental treatment code for easy observation during the experiment. Finally, the explants were placed in culture medium containing MS culture medium supplemented with 2,2 µM BAP and 30 $g.l^{-1}$ of sucrose. Culture medium pH was adjusted to 5.8. The cultured explants are then incubated in culture room at 22 ± 2 °C for seven days under continuous fluorescent light.

Results and Discussion

Sterilization technique for the Micropropagation of Sandalwood

The sterilization test for sandalwood was conducted with a total of 12 samples for each treatment. There are three treatments in total for the experiment that is control (without any treatment), treatment 1, & treatment 2. Treatment 1 is a sandalwood explant sterilization treatment by immersion in a solution of Bayclin® 15% (v/v) concentration for 15 minutes and Bayclin® 10% (v/v) for 10 minutes. While treatment 2 is the treatment of sandalwood explants sterilization by immersion in a solution of Bayclin® 30% (v/v) concentration for 15 minutes and Bayclin® 20% (v/v) for 10 minutes.

Table 2. Sandalwood Sterilization Experiment Result Data

Sample	G0	G1	G2
U1S1	X + XB	V + shoot	XJ
U1S2	X + XB	X	X + XB
U1S3	XB	X + XB	X + XB
U1S4	XJ	X + XB	X + XB
U2S1	X + XJ	X + XB	X + XJ
U2S2	X	V + shoot	V
U2S3	X + XJ	V + shoot	V
U2S4	X + XJ	V	X + XB
U3S1	V	X + XB	X
U3S2	V	V	XB + XJ
U3S3	X + XB	XB	X
U3S4	X	X + XB	X

Description: v = Sterile, x = browning, xb = bacterial contamination, xj = fungal contamination

Results of the experiment are shown in Table 2 and Figure 2, respectively. Each result can have multiple symptoms happening in a single sample; for example, sample U1S1 has bacterial infection while also inflicted with a case of browning. Based on the results shown it can be concluded that treatment 1 is the best treatment for the sterilization of the sandalwood because the treatment provides the best overall result in the experiment, while the control treatment and treatment 2 provide good enough results, but not the best one. The results showed through the experiment are with control treatment, the sample of sandalwood has a high percentage of being inflicted with browning cases while also resulting in some equal cases of bacterial and fungal contamination (Figure 2). In treatment 1, fungal contamination is absent with a high level of browning and bacterial contamination but still has high percentage for sterile cases too. While in treatment 2, the result shows not much difference with the control treatment. Some sample showed more than one condition, for example, a sample could be contaminated with bacteria and shown browning at the same time or contaminated by both bacteria and fungi. To comprehensively evaluate the explants, two evaluation types were employed: one based on the total number of explants under specific conditions and the other based on the final condition (Table 2; Figure 2). The purpose of this differentiation is to determine the predominant cause of contamination and facilitate further studies. Total explant with the condition counts samples with two or more condition more than one time. Meanwhile final condition counts based on these premises; (1) all fungi contaminated sample would also have bacterial contamination, therefore classified to fungi contamination; (2) browning samples with contamination will be classified as contaminated; (3) If successfully inducted samples show contamination, they will be classified as contaminated, even if they are still potentially salvageable.

https://pagargeserminimalis.blogspot.com/2020/04/populer-26-contoh-gambar-kayu-cendana.html

Explant Sterilization

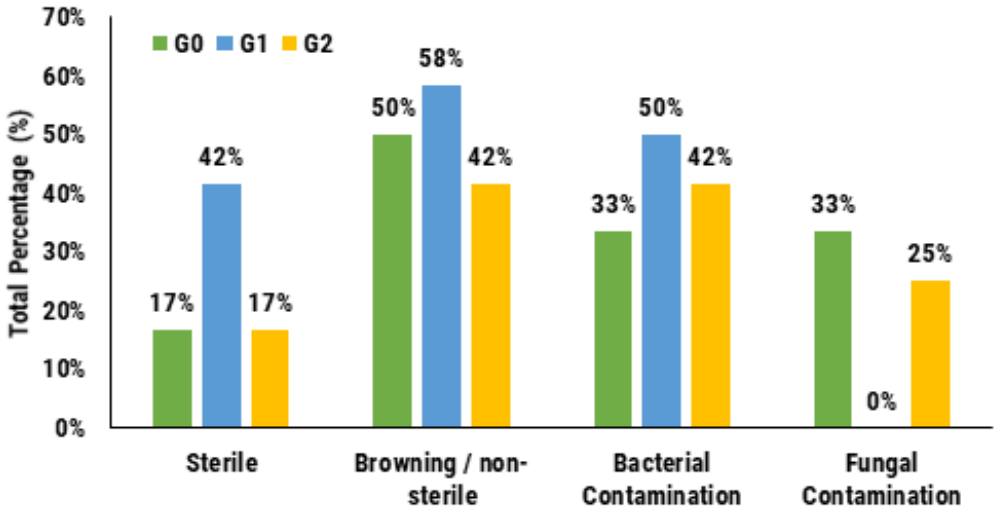

Figure 2. Bar Chart of Percentage of Sandalwood Sterilization Experiment Results

NaOCl is a strong oxidizing agent commonly found in household bleach. It is effective at killing a broad spectrum of microorganisms, including bacteria, fungi, and viruses. When used in tissue culture, NaOCl is typically diluted to a specific concentration, such as 1-10%, to ensure effective sterilization while minimizing potential damage to the explants. The treatment helps eliminate surface contaminants on plant material, reducing the risk of introducing unwanted microbes into the tissue culture environment (Teixeira et al, 2016). Some reason for its frequent usage is that NaOCl is effective against a wide range of microorganisms, including bacteria, fungi, and viruses. NaOCl is commercially available in various concentrations and forms, including household bleach, which makes it accessible in many laboratories. Dilutions of NaOCl can be easily prepared to achieve the desired concentration for effective sterilization. NaOCl is also a relatively inexpensive sterilizing agent compared to some other chemical disinfectants, making it an affordable option for tissue culture laboratories. Finally, when used at appropriate concentrations, NaOCl can effectively sterilize explants while minimizing damage to the plant tissues. It is relatively less harmful to the cells compared to certain other disinfectants, allowing for the successful initiation and growth of cultures. (Weber et al, 2015). NaOCl acts as a strong oxidizing agent and exhibits broad-spectrum antimicrobial activity. The concentration and exposure time of NaOCl is critical to achieving effective sterilization while minimizing tissue damage. Generally, a lower concentration (e.g., 1-10%) is used for tissue culture sterilization. (Teixeira et al, 2016).

The usage of NaOCl for the sterilization of sandalwood is used extensively globally. Many other research and production purpose for the culture or micropropagation of sandalwood uses NaOCl as the sterilization agent in the process. General techniques of sandalwood sterilization are advised to use NaOCl for the surface sterilization of the explant (Krishnakumar et al., 2018; Warakagoda et al., 2013). For the optimum dosage used in the sterilization, the experiment that we conducted showed that the dosage is 15% (v/v) and 10% (v/v) sourced from the bleach Bayclin® (which contains NaOCl 5,25%). Previous studies showed that the optimum dosage of NaOCl used for sterilization purposes was 0.01% of pure NaOCl (Krishnakumar et al, 2018), or if sourced from a commercial bleach (such as Bayclin® or Chlorox® which generally contain 5,25% of NaOCl) the usual dosage is 15% (v/v) (Warakagoda et al., 2013).

Sterilization technique for the Micropropagation of Teak

The result of teak sterilization showed that the treatment B, which uses both NaOCl and Povidone-iodine, showed the highest sterile explant percentage, followed by treatment A, that followed standard teak sterilization procedure (Figure 3). Treatment C that uses double sterilization with povidone-iodine showed poor sterilization by 0%. Bacterial contamination is predominantly found in treatment A, while fungi are more commonly found in treatment B. Treatment C showed a similar contamination rate for fungi and bacteria. Bacterial contaminations were found on the agar surrounding the explants, exhibiting a circular shape. Establishment phase in teak micropropagation is the most sensitive because the explants are the most vulnerable to contamination and limited in vitro response (Aguilar et al., 2019). The highest browning rate was observed in treatment C, followed by treatment A. Meanwhile, treatment B had no browning explant. The ANOVA analysis showed no significant difference between each treatment with respect to final condition at α=0.05 level of significance.

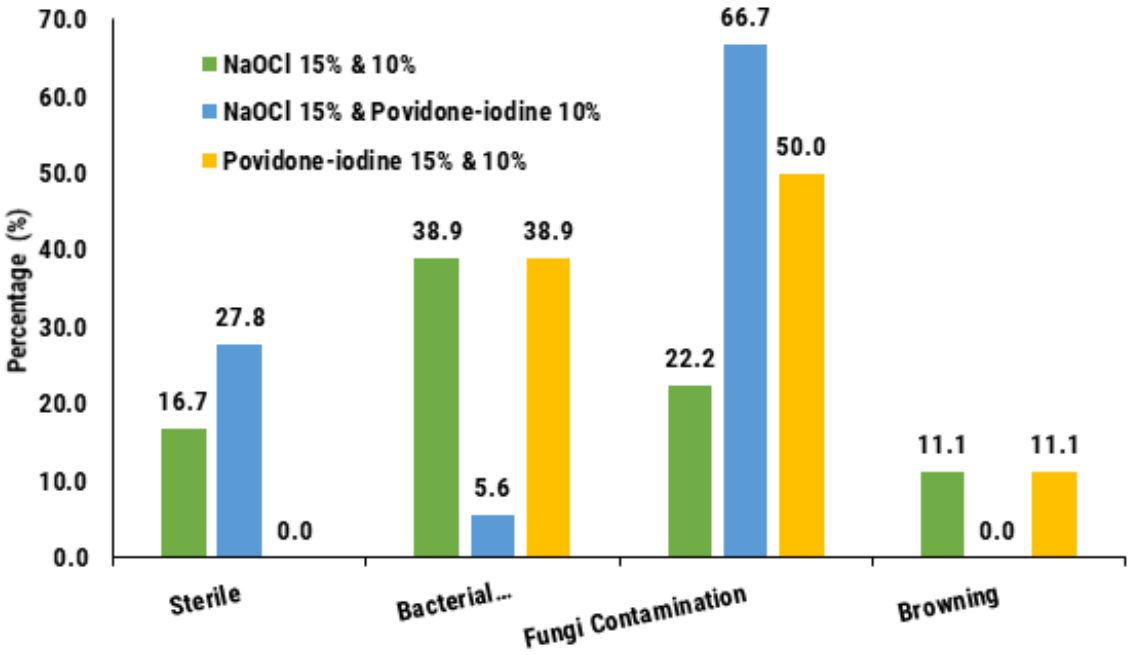

Figure 3. Bar chart of percentage of teak sterilization result based on final condition.

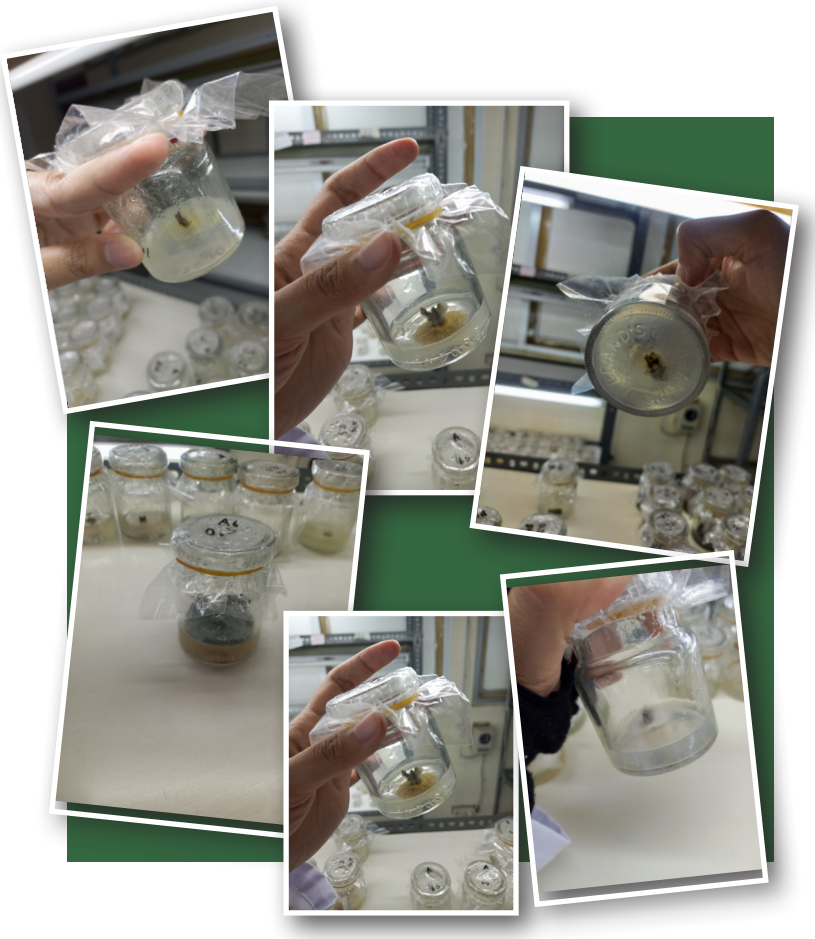

Figure 4. Examples of contaminated explant by bacteria (up) for each treatment (A, B, C); examples of contaminated explant by fungi (down) for each treatment A, B, C)

Sodium hypochlorite commonly used as broad-spectrum disinfecting agent that is effective for bacteria, viruses, fungi, and mycobacterium. It is not effective against bacterial spores and prions (Girotti, 2015). In the sterilization process, chlorine present in sodium hypochlorite operates via an oxidation mechanism. The chlorine ions stimulate inactivation of enzyme and degrade lipids and fatty acids (Pais et al., 2016). Povidone iodine is a water-soluble iodine-releasing agent, also known as iodophor. It consists of a complex between iodine and polyvinylpyrrolidone, a solubilizing carrier. Free iodine (I_2) act as the active bacterial agent, capable of rapidly penetrates microorganism and oxidizing vital proteins, nucleotides, and fatty acids. This process eventually results in cell death (Lepelletier et al., 2020).

Based on the result, treatment A (control) has the highest induction quality and number. Callus formations were observed at the bottom of the explant as a mass of white tissue (Figure 4). Some inducted explants grow one shoot from the axillar/lateral bud. Hormones that were added into the Murashige-Skoog media can affect explant growth. For instance, BAP is used to initiate bud formation and vegetative growth acceleration (Simanjuntak et al, 2015), while NAA (1-Naphtalenacetic acid) is used to initiate root growth (Arya & Husein, 2019). Hormones can also be added during the mixing process of stock solution. Although they may degrade during the heating process on stove and the sterilization process in the autoclave, leading to double degradation of hormones. Hormones that have fully degraded won't be effective to initiate growth on explants.

Explant Sterilization

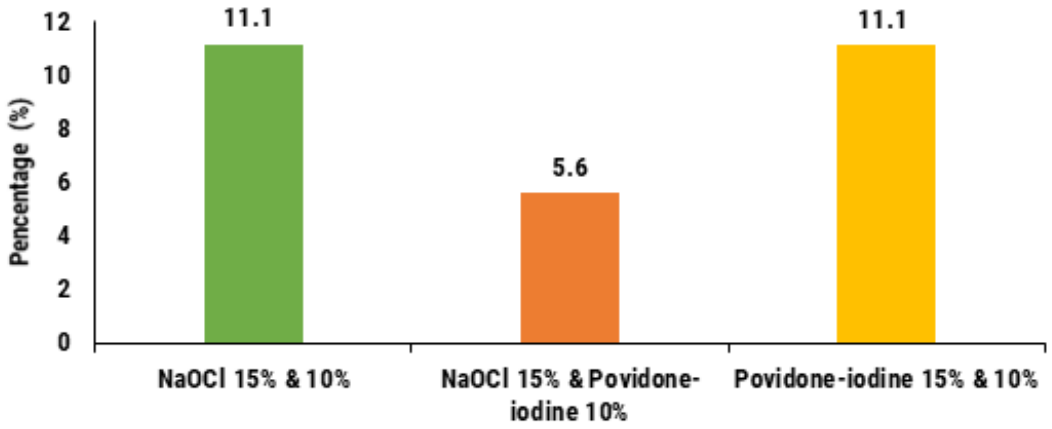

Figure 5. Bar chart of percentage of successfully inducted explant. Note the chart showing the percentage of successfully inducted explants, including the contaminated ones.

Figure 6. Examples of initiated explants. Left to right: explant with callus formation from treatment A, explant with growing shoot from treatment A, explant with callus formation from treatment B, explant with callus formation from treatment D but contaminated.

It was also observed that teak explants were still capable of growing calluses and shoots even in a contaminated state, either by bacteria or fungi (Figure 5 and Figure 6). Successful explants with bacterial contamination still had the chance to be salvaged, but the ones with fungi contamination needed to be discarded immediately. This aligns with the review by Permadi et al. (2023), which suggests that while bacterial proliferation can cause substantial damage to plant material, some explants are able to survive and thrive even when bacteria are present. Contaminants such as endophytic bacteria are difficult to eliminate because systemic steriliser could potentially harm the explants and can emerge after multiple subcultures. The explant can be salvaged after new shoots elongated and then cut into segments with one nodal. Each segment then can be used as new inoculum (Witjaksono et al., 2020). Fungi contamination has faster expansion than the explant's growth. Most species also capable of producing and releasing secondary metabolites that negatively affect the explant (Zhao et al., 2014).

The low rate of successful sterilization could be attributed to several factors. In micropropagation, NaOCl is typically used for surface disinfection, but this method has demonstrated limited effectiveness in controlling explant contamination (Widyastuti et al., 2018). Surface sterilization alone is insufficient to reach contaminants that reside dormant within the explants (Permadi et al. 2023). Another factor would be the cross contamination within the culture room. Fungi releases air borne spore, which can transfer from one explant to another. Any explant that exhibits signs of fungal contamination should be discarded immediately. During the experiment, contaminated explant wasn't immediately separated, thus increasing the risk of cross contamination. Another factor, according to Widyastuti et al., (2018), each explant may have different surface contamination levels. This is especially true since the teak mother plant was not subjected to any pretreatments, such as the application of fungicides or bactericides, and was grown in a semi-open environment.

Conclusion

Sterilization is an essential step for a successful micropropagation. In this research, for the sterilization of sandalwood explants it was evaluated using two treatments. The study confirmed the effectiveness of Sodium hypochlorite (NaOCl) as the preferred sterilizing agent for sandalwood, with the optimal dosage being 15% (v/v). Meanwhile, sterilization tests for teak were conducted using varying concentrations of NaOCl (15% (v/v) and 10% (v/v)) and povidone-iodine (15% (v/v) and 10% (v/v)). The results revealed no significant differences between the treatments. Combination of NaOCl 15% (v/v) and Povidone-iodine 10% (v/v) showed the best sterilization outcome with 27,8% sterile rate. However, control treatment with double NaOCl immersion showed higher number of successfully inducted explant of 11,1%.

Acknowledgement

The authors would like to express sincere gratitude to SEAMEO BIOTROP for supporting this research. Authors also like to express their sincerest appreciation to Dr. Rhomi Ardiansyah and Mr. Rosadi Kartiwijaya for the guidance, assistance and mentorship during the creation of this article.

References

Aguilar, M. Garita, K., Kim, Y., Kim, J. and Moon, H. (2019). Simple protocol for the micropropagation of teak (Tectona grandis Linn.) in semi-solid and liquid media in RITA® Bioreactors and ex Vitro rooting. American Journal of Plant Sciences, 10, 1121-1141. doi: 10.4236/ajps.2019.107081.

Asdaq, S. M. B., Nayeem, N., Abida, Alam, M. T., Alaqel, S. I., Imran, M., Hassan, E. E., & Rabbani, S. I. (2022). Tectona grandis L.f: A comprehensive review on its patents, chemical constituents, and biological activities. Saudi journal of biological sciences, 29(3), 1456–1464. https://doi.org/10.1016/j.sjbs.2021.11.026

Girotti, N. (2015). Guidelines for using sodium hypochlorite as a disinfectant for biological waste. Western University Canada. https://www.uwo.ca/animal-research/doc/bleach-sop.pdf

Hartshorn, G. (2013). Tropical forest ecosystem. Encyclopedia of Biodiversity (Second Edition). 269-276. https://www.sciencedirect.com/science/article/abs/pii/B9780123847195001465

Krishnakumar, N., & Parthiban, K. T. (2018). Micropropagation (in vitro) techniques for sandal wood (Santalum album L.). Journal of Pharmacognosy and Phytochemistry, 7(3), 620-627.

Kumar, A. A., Joshi, G., & Ram, H. M. (2012). Sandalwood: history, uses, present status and the future. Current Science, 1408-1416.

Lepelletier, D., Maillard, J. Y., Pozzetto, B., & Simon, A. (2020). Povidone Iodine: Properties, Mechanisms of Action, and Role in Infection Control and Staphylococcus aureus Decolonization. Antimicrobial agents and chemotherapy, 64(9), e00682-20. https://doi.org/10.1128/AAC.00682-20

Murashige, T; Skoog, F (1962). "A Revised Medium for Rapid Growth and Bio Assays with Tobacco Tissue Cultures". Physiologia Plantarum. 15 (3): 473–497. doi:10.1111/j.1399-3054.1962.tb08052.x

Neliyati, N., Lizawati, L., & Zulkarnain, Z. (2019). The evaluation of sterilization protocol for sprout explants in oil palm (Elaeis guineensis Jacq.) tissue culture. Journal of Physics: Conference Series, 1402(3).

Pais, A.K., A.P. da Silva, J.C. de Souza, S.L. Teixeira, J.M. Ribeiro, A.R. Peixotol, C.D. da Paz. (2016). Sodium hypochlorite sterilization of culture medium in micropropagation of Gerbera hybrida cv. Essandre. African Journal of Biotechnology 15(36), 1995-1998.

Pant, M., & Husen, A. (2022). In vitro micrografting to induce juvenility and improvement of rooting. In A. Husen (Eds.), Plant biology, sustainability and climate change: Environmental, physiological and chemical controls of adventitious rooting in cuttings (pp. 439-453). Academic Press. https://doi.org/10.1016/B978-0-323-90636-4.00014-3.

Permadi N, Nurzaman M, Alhasnawi AN, Doni F, Julaeha E. (2023). Managing lethal browning and microbial contamination in Musa spp. tissue culture: synthesis and perspectives. Horticulturae, 9(4), 453. https://doi.org/10.3390/horticulturae9040453

Pratiwi, D.R., Wening, S., Nazri, E., Yenni,Y. (2021). The use of alcohol and sodium hypochlorite as single sterilizing agents for oil palm explant sterilization. Jurnal Penelitian Kelapa Sawit, 29(1), 1-10. https://doi.org/10.22302/iopri.jur.jpks.v29i1.120

Sankaran M., & Ratnam, J. (2013). African and Asian savannas. Encyclopedia of Biodiversity (Second Edition). 58-74.

https://www.sciencedirect.com/science/article/abs/pii/B9780123847195003555

Teixeira da Silva, J. A., Kher, M. M., Soner, D., Page, T., Zhang, X., Nataraj, M., & Ma, G. (2016). Sandalwood: basic biology, tissue culture, and genetic transformation. Planta, 243, 847-887.

Wamaedeesa, R., Ali, B., Chedao, N., Kanjanawattanawong, S. (2021). Chemical sterilization in MS culture medium for in vitro culture of Philodendron sp. "ruaysap". Princess of Naradhiwas University Journal, 13(1), 377–387. https://li01.tci-thaijo.org/index.php/pnujr/article/view/246876

Weber, B. N., Witherell, R. A., & Charkowski, A. O. (2015). Low-cost potato tissue culture with microwave and bleach media preparation and sterilization. American journal of potato research, 92, 128-137.

Widiastuti, Y., Bariyyah, K., Istianingrum. P., Restanto, D.P., Hartatik. S. (2018). In vitro sterilization method of the Banyuwangi's local red durian leaf ex plants to several combination types of sterilization materials. International Journal of Agriculture, Environment and Bioresearch, 3(5), 262 – 273.

Witjaksono, Riastiwi, I., Nugraheni, K.U., Windarsih, G (2020). The effect of sucrose concentrations and shoot inoculum density on the growth of in vitro shoot of teak (Tectona grandis L.). IOP Conf. Series: Earth and Environmental Science, 591(012018).

Zhao, P., Quan, C., Wang, Y., Wang, J., Fan, S. (2014). Bacillus amyloliquefaciens Q-426 as a potential biocontrol agent against Fusarium oxysporum f. sp. Spinaciae. J. Basic Microbiol. 54(5), 448–456.