

COMPARATIVE EFFICACY OF *Phaseolus aureus* EXTRACT AND SYNTHETIC GROWTH REGULATORS ON CALLUS INDUCTION IN *Curcuma xanthorrhiza*

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ABSTRAK

Kata kunci:
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Temulawak (*Curcuma xanthorrhiza* Roxb), tanaman yang berasal dari Indonesia, semakin diakui karena aktivitas farmakologinya dan menjadi dasar penting dalam pengobatan tradisional, dengan penggunaan tahunan yang menunjukkan laju pertumbuhan signifikan sekitar 5,4%. Seiring dengan meningkatnya kesadaran akan potensi terapeutiknya, permintaan terhadap sumber daya ini juga meningkat, sehingga membutuhkan upaya perbanyakan dan ekstraksi senyawa bioaktifnya. Penelitian ini bertujuan untuk menyelidiki efektivitas regulator pertumbuhan dalam induksi jaringan kalus dari *C. xanthorrhiza* melalui kultur jaringan tanaman, yang merupakan metode kunci untuk meregenerasi material tanaman yang berharga. Penelitian berfokus pada efek komparatif dari regulator pertumbuhan alami, khususnya ekstrak air dari tauge (*Phaseolus aureus*), dan regulator pertumbuhan sintetis seperti 2,4-Diklorofenoksiasetat (2,4-D) dan Asam Indol-3-butirat (IBA) dalam konsentrasi yang bervariasi. Desain eksperimen mencakup variasi konsentrasi ekstrak air *P. aureus* pada 10% dan 15%, di samping konsentrasi tetap dari agen sintetis. Temuan menunjukkan bahwa regulator pertumbuhan sintetis, khususnya kombinasi 2,4-D dan IBA pada konsentrasi masing-masing 0,4 ppm, menghasilkan proliferasi kalus yang lebih baik dibandingkan dengan perlakuan ekstrak alami. Namun, ekstrak air tauge juga menunjukkan efek positif pada kemunculan kalus dan peningkatan ukuran eksplan ketika digunakan pada konsentrasi 10% dalam media Murashige-Skoog. Oleh karena itu, penelitian ini menekankan potensi integrasi antara regulator pertumbuhan alami dan sintetis untuk meningkatkan induksi kalus dan pada akhirnya memfasilitasi penggunaan berkelanjutan *C. xanthorrhiza* sebagai sumber obat tradisional.

ABSTRACT

Keywords:
Callus
Plant
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Temulawak

Temulawak (*Curcuma xanthorrhiza* Roxb) a plant indigenous to Indonesia, is increasingly recognized for its medicinal properties, formulating a cornerstone of traditional medicine with annual usage marking a significant growth rate of approximately 5.4%. As awareness of its therapeutic potential expands, too does the demand for this valuable resource, necessitating enhanced efforts in its propagation and the extraction of its bioactive compounds. This study investigates the efficacy of different growth regulators in the induction of callus tissue from *C. xanthorrhiza* through plant tissue culture, a key method for regenerating valuable plant material. Specifically, the research focuses on the comparative effects of natural growth regulators, particularly water extracts from *Phaseolus aureus*, and synthetic growth regulators such as 2,4-Dichlorophenoxyacetic Acid (2,4-D) and Indole-3-butyric Acid (IBA) in varied concentrations. The experimental design included varying concentrations of *P. aureus* water extract at 10%, and 15%, alongside a fixed concentration of the synthetic agents. Findings indicated that synthetic growth regulators, particularly the combination of 2,4-D and IBA at a concentration of 0.4 ppm each, yielded superior callus proliferation compared to the natural extract treatments. However, *P. aureus* aqueous extract showed positive effects also on callus emergence and on increasing explant size when used at 10% concentration in Murashige-Skoog medium. Consequently, the study underscores the potential of integrating both natural and synthetic growth regulators to enhance callus induction and ultimately facilitate the sustainable use of *C. xanthorrhiza* as a medicinal resource.

INTRODUCTION

Temulawak, scientifically identified as *Curcuma xanthorrhiza* Roxb, is a prominent medicinal plant indigenous to Indonesia, gaining recognition in traditional and contemporary medicinal circles. This herb is embraced for its culinary applications and therapeutic properties, reflecting a growing inclination towards natural products in healthcare. Current statistics indicate a rising demand for *C. xanthorrhiza*, often associated with a broader trend towards plant-based remedies and the expanding global herbal market (Faramayuda *et al.*, 2022). The integration of traditional knowledge with modern scientific inquiry has fostered increasing interest in the propagation and extraction of bioactive compounds from this plant, advancing agricultural and pharmaceutical research.

The exploration of different growth regulators is central to effective *in vitro* regeneration through plant tissue culture, a technique vital for the mass propagation of valuable plants like *C. xanthorrhiza*. Recent studies highlight the importance of selecting appropriate plant growth regulators (PGRs) for inducing callus formation and subsequent shoot and root regeneration from explants. The effectiveness of PGRs is influenced by their type and concentration, as reported in the literature (Kumari *et al.*, 2015; (Rani *et al.*, 2024). Synthetic auxins such as 2,4-

Dichlorophenoxyacetic Acid (2,4-D) and Indole-3-butyric Acid (IBA) have demonstrated significant effects on callus proliferation, suggesting a critical role in enhancing the dimensions and viability of explanted tissues (Shoja & Shishavani, 2021; Gunadi *et al.*, 2022).

In addition to synthetic growth regulators, integrating natural compounds from other plants supports a holistic approach to improving propagation methods. For instance, the water extracts from *Phaseolus aureus* have been investigated for their potential in promoting callus induction alongside established synthetic methods. While studies suggest that synthetic treatments tend to outperform natural extracts in callus proliferation, these natural sources can still be beneficial, particularly in enhancing explant size and initial callus emergence at appropriate concentrations (Mutte *et al.*, 2018). Such findings advocate for a combined strategy that utilizes both natural and synthetic regulatory frameworks to optimize tissue culture protocols.

The goal is to document the comparative efficacy of these growth regulators and to contribute to the sustainable propagation of *C. xanthorrhiza*, which aligns with contemporary demands for reliability in medicinal plant cultures. The insights from this study, highlighting the synergistic potential of integrative propagation techniques, aim to enhance

the accessibility and affordability of *C. xanthorrhiza*, thus solidifying its place in the herbal medicine domain.

The application of growth regulators derived from *Phaseolus aureus* could mitigate oxidative stress induced by environmental factors (El-Okkiah *et al.*, 2022). In this context, *Phaseolus aureus* may act as a crucial adjunct to traditional tissue culture techniques, particularly when combined with specific growth regulators like auxins and cytokinins.

The convergence of traditional knowledge, modern scientific research, and biotechnological innovation is critical for the successful propagation of *Curcuma xanthorrhiza*. By understanding the comparative efficiencies of using both natural and synthetic PGRs, this research aspires to enrich the discourse on medicinal plant sustainability while optimizing methodologies related to plant tissue culture practice. The exploration of synergistic benefits from various cultural techniques represents a significant step toward enhancing the regenerative capacity of essential medicinal flora such as *C. xanthorrhiza*. This research investigates the specific effects of varied concentrations of water extracts from *P. aureus* compared to synthetic growth regulators, particularly focusing on concentrations of 2,4-D and IBA utilized in experiments conducted on Murashige-Skoog medium.

METHODS

Experimental Design

Plant Material and Preparation

The experiment commenced with the selection of healthy *C. xanthorrhiza* explants. The explants were subjected to a sterilization process, typically involving soaking in 70% ethanol followed by a rinse with 0.1% mercuric chloride, ensuring the elimination of contaminants.

Culture Media

Murashige and Skoog (MS) medium served as the basal nutrient medium. The experimental set-up included various concentrations of natural and synthetic growth regulators. Specifically, the study employed:

- Water extracts from *Phaseolus aureus* at concentrations of 10% and 15%;
- Synthetic regulators, 2,4-Dichlorophenoxyacetic Acid (2,4-D) and Indole-3-butyric Acid (IBA), each at a fixed concentration of 0.4 ppm.

This design is congruent with prior analyses highlighting the significance of selecting appropriate growth regulator concentrations in optimizing callus induction processes.

Replications and Controls

To ensure the reliability of results, the experiments included multiple replicates. Each treatment was evaluated in several iterations, thus strengthening statistical validity. Control groups that lacked growth regulators were also

maintained to benchmark the response of explants under normal growth conditions without hormonal intervention.

Incubation Conditions

The cultures were incubated under a standard photoperiod (16 hours light/8 hours dark) at a specific temperature ($25\pm 2^\circ\text{C}$), replicating optimal growth conditions reported in similar studies.

Data Analysis Methods

The collected data included the percentage of explants showing callus formation, the rate of callus proliferation, and changes in explant size. All measurements were quantitatively assessed to allow for comprehensive statistical analysis.

Instrumentation

Micropipettes (Thermo, USA), laminar Air Flow (Labolytic, Indonesia), analytical balances (Shimadzu, Japan), pH meters (Metler Toledo, German) and autoclaves (Gea, China).

Materials

The explants used were rhizome shoots of the *C. xanthorrhiza* plant aged 9 months. Murashige and Skoog (MS) (Himedia, USA), 2,4-dichlorophenoxyacetic acid (2,4-D) (Himedia, USA) and Indole-3-Butyric Acid (IBA) (Himedia USA), *Phaseolus aureus* infusion extract 10% and 15%.

Explant Preparation

This study used temulawak (*C. xanthorrhiza* Roxb) obtained from the Manoko Experimental Garden, Lembang,

West Java. Explants were taken from young shoots.

Sterilization Room and Instrument

The culture room is cleaned and sterilized for 24 hours by spraying 10% formalin, for the work surface in Laminar Air Flow is cleaned by spraying 70% alcohol. The tools used are sterilized, by washing them thoroughly using washing soap and drying them, after which they are wrapped in paper (except for the culture bottle) and put into an autoclave at a pressure of 1 atm and a temperature of 121°C for 15 minutes.

Preparation and Sterilization Media

The composition used is Murashige-Skoog (MS) basic media added with agar and sugar, then added with distilled water to a volume of 1000 mL as a basic medium. The pH of the culture medium is set at 5.8. In this study, synthetic growth regulators 2,4-D were used, and Indole butyric acid (IBA) (D0). The natural growth regulators used are *Phaseolus aureus* extract 10% (D1) and 15% (D2). The heated media solution is put into a bottle sterilized culture, added with synthetic growth regulators (D0) and natural growth regulators (D1 and D2). The bottle is covered with aluminum foil. The media is autoclaved for 15 minutes at the temperature used of 121°C .

Sterilization Explant

The explants were washed under running water for 5 minutes, soaked in detergent for 30 minutes. The explants

were soaked in 0.1% dithane solution for 30 minutes. The explants were soaked in 30% and 15% hypochlorite solutions for 5 minutes. The explants were then rinsed 3 times with sterile distilled water for 1 minute each. The explants were cut lengthwise with a size of approximately 2-3 cm² and immediately planted in the prepared media.

Induction Callus from Explant

Induction of explants in media is done in laminar air flow (LAF). Explant pieces that are ready to be planted (already sterile) are taken using sterile tweezers, Induction in a culture bottle containing MS + 2,4-D 0.4 ppm + IBA 0.4 ppm (D0) and *P. aureus* extract 10% (D1) and 15% ppm (D2).

RESULT AND DISCUSSIONS

Sterilization Optimization

The Sterilization of explants is conducted with the aim of removing soil-borne microbes. The explants are washed using running water to cleanse their surfaces. Subsequently, they are immersed in a detergent that acts as a stain remover or eliminates residual soil adhering to the surfaces of the buds that cannot be removed by water alone. A 0.1% Dithane solution serves as a fungicide, effectively eliminating fungal diseases in plants. Seventy per cent alcohol functions as a disinfectant by denaturing proteins through dehydration, thereby damaging cell membranes and inactivating enzymes.

Additionally, a sodium hypochlorite immersion is necessary to eradicate microorganisms, particularly those present in the explant tissues. The optimal Sterilization method for *C. xanthorrhiza* explants involves the use of a sodium hypochlorite solution at a concentration of 30% and 15%, each for a duration of 5 minutes, followed by immersion in a 70% alcohol solution for 1 minute.

The emergence of callus and the increase in size response of the explants serve as parameters for the success of this research. Growth is visually observed as elongation and enlargement of tissues, as well as the quantity of callus formed. Monitoring the appearance of callus is essential to ascertain the activity of growth regulators in producing callus. The analysis results indicate that the addition of *P. aureus* extract 15% (D2) concentration of boiled bean sprouts does not significantly affect callus growth and enlargement in the temulawak (*C. xanthorrhiza* Roxb.) explants, while a concentration of *P. aureus* extract 10% (D1) demonstrates callus growth along with a response of elongation and enlargement in the explants. Furthermore, the analysis results for the synthetic growth regulators 2,4-D and IBA (D0) reveal a greater quantity of callus growth and a visibly significant response in the explants (**Table 1**).

The subsequent analysis involves observing the development of explants in

terms of elongation and enlargement across each medium (D0, D1, and D2). The results of the observations regarding the size of the explants, as illustrated in the graph above, indicate that the explants subjected to a 10% concentration water

extracts from *Phaseolus aureus* exhibit a more rapid development and elongation in size. (**Figure 1; Figure 2**). However, the quantity of callus formed in this treatment is notably lower in comparison to the control group (D0).

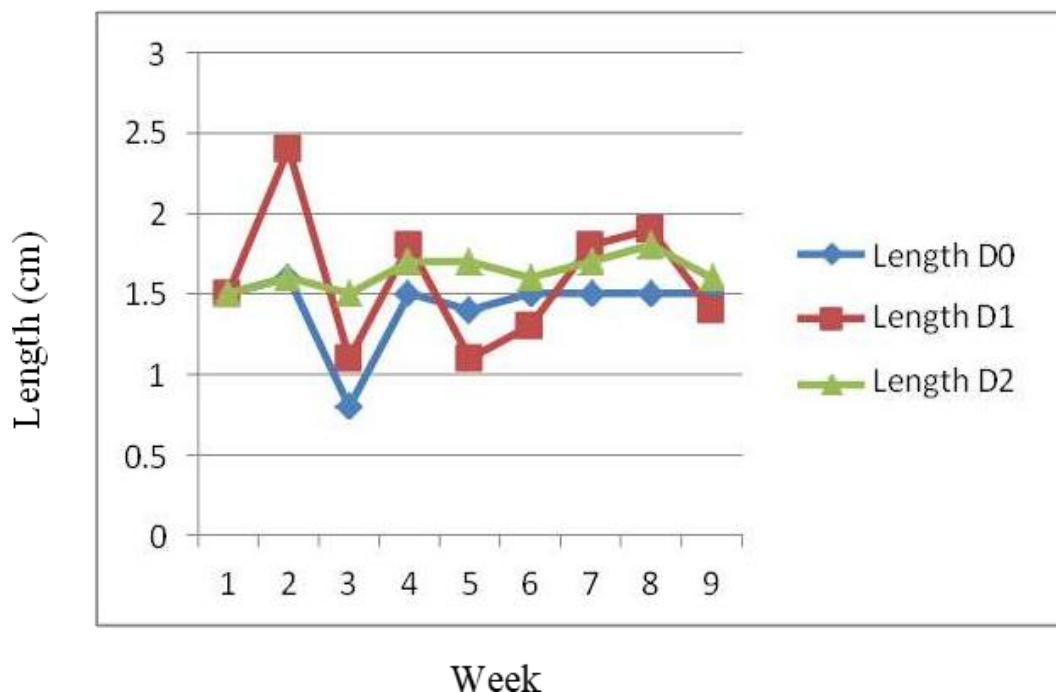


Figure 1. Graph of length development in explants during 8 weeks

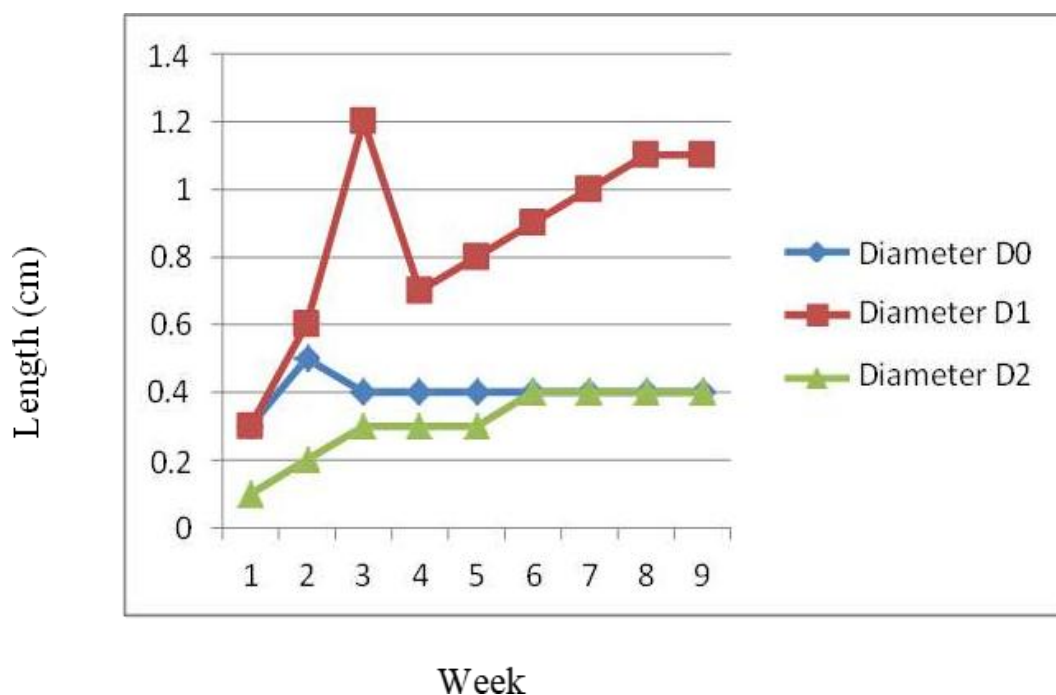
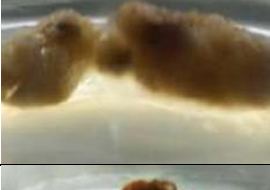


Figure 2. Graph of diameter development in explants during 8 weeks

Table 1. Development of explant expansion and callus for 8 weeks

Week	D0 (MS + 2,4D + IBA)	D1 (MS + P. aures extract 10%)	D2 (MS + P. aures extract 15%)
0			
1			
2			
3			
4			
5			
6			
7			
8			

Sterilization Optimization

In the field of plant tissue culture, the sterilization of explants is a critical procedure aimed at eliminating surface contaminants such as soil-borne microbes, which can lead to contamination in *in vitro* cultures. The process begins with thorough washing of the explants under running water to remove any adhering soil particles. Following this initial cleaning, the explants are treated with a detergent, which serves to remove any residues that might remain on the surface and is crucial for ensuring that the sterilization agents can penetrate effectively to sanitize the explant surfaces. Sodium hypochlorite has been widely reported as an effective agent for surface sterilization, with effective concentrations typically ranging from 0.1% to around 10%, depending on the specific plant species being cultured and the tissue type (Mihaljević *et al.*, 2013; Sirohi *et al.*, 2021; Agogbua & Okoli, 2022).

The application of sodium hypochlorite as a sterilizing agent is often complemented by immersion in alcohol solutions, specifically 70% ethanol, which serves to denature proteins, disrupt cell membranes, and inactivate enzymes that could interfere with successful culture establishment. This dual action of sodium hypochlorite and ethanol has been supported by various studies, indicating that such methods effectively eliminate a broad range of contaminants, thereby increasing the likelihood of obtaining

sterile cultures (Hesami *et al.*, 2018). Research has shown that using a sodium hypochlorite solution at concentrations around 10% for about 5 minutes, followed by a brief alcohol treatment, can adequately reduce microbial contamination (Zahid *et al.*, 2021). In addressing the specific sterilization protocol for *C. xanthorrhiza* explants, it is important to note that the effective sterilization regime consists of a sequential treatment: immersing explants in sodium hypochlorite for about 5 to 10 minutes, followed by immersion in 70% alcohol for an additional minute.

This combination has proven to be efficient not only in eradicating microbial life but also in preserving the viability of explant tissues, which is critical for successful growth and regeneration in a controlled *in vitro* environment (Muggia *et al.* 2015; Lamba *et al.*, 2020). Continuous research suggests that adjustments in the duration and concentration of sterilizing agents can yield varying results based on plant species and even the specific types of tissues being cultured (Hesami *et al.*, 2018).

The comparative efficacy of sodium hypochlorite against other disinfectants. Studies indicate that sodium hypochlorite often outperforms agents such as mercuric chloride and hydrogen peroxide in terms of safety and effectiveness, primarily due to its relatively lower toxicity and lesser likelihood of causing phytotoxicity (Afendi

et al., 2013; Marcelina & Ireneusz, 2014). Mercuric chloride, while historically used for explant sterilization, poses significant health risks both for humans and the plants themselves and can lead to detrimental effects on tissue viability when used at higher concentrations (Cardoso & Inthurn, 2018). In contrast, sodium hypochlorite's adaptability across diverse protocols allows for broader applications across multiple plant species and tissues.

The effectiveness of disinfectants hinges on their concentration and contact time with the explants. Research shows that increasing the immersion time can significantly decrease contamination rates without extensively sacrificing explant integrity. For example, studies have illustrated that immersion times of up to 10 minutes with sodium hypochlorite yield better disinfection with minimal adverse effects on explant growth compared to shorter immersion periods or less concentrated solutions (Mihaljević et al., 2013). Varying outcomes are influenced by factors such as the type of surface contaminants present, as noted in studies evaluating the effectiveness of sodium hypochlorite against several pathogens (Deein et al., 2013).

The use of sodium hypochlorite in plant tissue culture has garnered attention due to its antibacterial and antifungal properties, which are critical to preventing infections during the multiplication and

rooting phases of in vitro culture (Kaushik et al., 2022). It has been well-documented that such sterilization techniques enhance the likelihood of obtaining clean, contaminant-free explants capable of developing into healthy plantlets. Given the role of biotechnological applications in modern agriculture, the rigorous application of sterilization protocols remains a focal point in enhancing micropropagation techniques (Eliwa et al., 2024).

Observation of Callus Growth

Tissue culture techniques, specifically the use of natural and synthetic growth regulators, are essential methods for the regeneration of valuable plant materials. Synthetic agents like 2,4-Dichlorophenoxyacetic Acid (2,4-D) and Indole-3-butyric Acid (IBA) have garnered attention for their capacity to promote callus proliferation in various species, including *C. xanthorrhiza* (Rohman et al., 2020). The present study found that a combination of 2,4-D and IBA at a concentration of 0.4 ppm each significantly enhanced callus induction compared to treatments with natural water extracts from *Phaseolus aureus*. This finding aligns with existing evidence that highlights the superior efficacy of synthetic growth regulators over natural extracts in promoting adventitious shoot formation and overall growth in plant tissue culture systems (Atun et al., 2020).

Nevertheless, the positive results observed with the 10% concentration of *P. aureus* water extract in Murashige-Skoog medium provide a promising avenue for the integration of natural products in cultivation practices. Recent studies indicate that plant extracts can play a role in mitigating the negative effects associated with synthetic growth regulators, including their potential toxicity. The positive outcomes of *P. aureus* in callus emergence and explant size enhancement highlight the potential benefits of using a synergistic approach that combines both natural and synthetic treatments in optimizing tissue culture protocols for *C. xanthorrhiza*.

C. xanthorrhiza is recognized for its diverse range of therapeutic properties. Compounds such as curcuminoids, which include curcumin and demethoxycurcumin, have been implicated in various health benefits, including antioxidant, anti-inflammatory, antimicrobial, and even anticancer activities (Adekoya *et al.*, 2014; Suniarti *et al.*, 2022). The integration of certain growth regulators, which may enhance the biosynthesis of these beneficial compounds, could further augment the plant's medicinal value. Evidence suggests that biotic and abiotic factors, as well as growth regulators used in tissue culture, can influence the phytochemical composition of turmeric plants, potentially

increasing the yield of bioactive compounds.

Phaseolus aureus has been observed to regulate growth in other plant species through its endophytic relationships, which involve symbiotic bacteria that can facilitate nutrient uptake. Endophytes isolated from legumes have shown promise in enhancing plant growth and resistance to pathogens (Abdelaziz *et al.*, 2023). Suggesting that such organisms may also be able to leverage the physiological benefits of *Phaseolus aureus*. The intricate symbiotic relationships established within the plant's rhizosphere may significantly influence plant tissue culture outcomes, potentially increasing the efficiency of in vitro regeneration strategies.

The versatility of *Phaseolus aureus* is further emphasized by its exceptional ability to accumulate beneficial nutrients, which can induce positive responses in neighboring plants under cultivation. Recent studies have illustrated the potential of *Phaseolus aureus* to enhance the nutritional status of plants cultivated in suboptimal conditions through improved mineral uptake. However, specific references that showcase this effect in the context provided were not found (Malviya *et al.*, 2023). This dynamic of nutrient facilitation may extend to plant tissue culture environments where nutrient efficiency is vital for successful propagation.

Moreover, the implications of *Phaseolus aureus* extracts extend beyond growth promotion and stress alleviation. The plant has exhibited promising antimicrobial properties that lend themselves to improving the integrity of tissue culture environments. The discovery of antibacterial compounds in the extracts of legumes has indicated a viable pathway to reduce contamination risks during tissue culturing, offering an added layer of safety, although more research is needed to fully elucidate these effects (Shatnawi *et al.*, 2021). This capability is crucial, especially in a laboratory setting where sterility is paramount for successful plant propagation.

Synthetic growth regulators like 2,4-D have been widely utilized in tissue culture applications due to their potent effects on promoting callus induction from various explant types. For instance, a study examining the callus induction of different rice genotypes demonstrated that 2,4-D significantly boosted callus production, marking it as a robust and effective SGR in tissue culture systems (Carsono *et al.*, 2021). The versatility of SGRs, particularly 2,4-D, is attributed to their chemically stable structures, enabling reliable performance across various plant species. In contrast, the efficacy of NGRs, traditionally derived from plant sources, may exhibit variability influenced by environmental factors or specific plant

species, which can lead to inconsistent outcomes in some instances (Faramayuda *et al.*, 2022).

The integration of *Phaseolus aureus* as a natural growth regulator in plant tissue culture presents a multifaceted approach to enhancing plant propagation and resilience. From improving metabolic pathways and inducing callus formation to establishing beneficial symbiotic relationships with endophytes and providing stress mitigation, *Phaseolus aureus* represents a promising avenue for ongoing research and application in plant biotechnology. Future studies should focus on elucidating the specific mechanisms through which *Phaseolus aureus* exerts its growth-promoting effects, as well as exploring its potential in combination with other tissue culture strategies to optimize outcomes and improve the efficiency of plant cultivation practices. The regulation of auxin distribution is crucial in this process. Hormonal influences, notably auxins, play a pivotal role in determining growth patterns; they promote cell elongation on one side of a tissue, allowing for directional growth and subsequent alterations in overall tissue length

The medicinal applications of temulawak are closely tied to its biochemical composition, with curcumin being one of the major bioactive compounds responsible for its therapeutic effects (Jantan *et al.*, 2012). Previous research supports that curcumin

demonstrates efficacy against various ailments, including liver diseases and inflammation, reinforcing the need for sustainable practices in cultivating *C. xanthorrhiza* to ensure a stable supply of this essential resource (Ramadhana *et al.*, 2021). The present findings, showcasing superior callus proliferation through specific synthetic combinations, could facilitate the mass production of

C. xanthorrhiza, ensuring that more considerable quantities of curcumin and other bioactive compounds become readily available for research and therapeutic applications. It is crucial to emphasize that while synthetic growth regulators have proven effective in tissue culture, the potential side effects associated with their long-term use warrant careful consideration. Over-reliance on such chemicals can lead to unintended ecological consequences. Thus, the findings from this study, which indicate that natural extracts can also play a role in callus induction, suggest that a balanced approach is essential for optimizing tissue culture methodologies while preserving the ecological integrity of cultivation practices (Kristianto *et al.*, 2022).

Moreover, the growing interest in the biological activities of *C. xanthorrhiza*, including its usage as a natural remedy for various health conditions, calls for extensive phytochemical studies to elucidate the full scope of its therapeutic

potential (Murwani *et al.*, 2024). Exploring the interaction between different growth regulators and environmental factors may yield valuable insights into the optimal conditions required for enhancing the bioactive profile of this plant through in vitro propagation techniques.

As the demand for *C. xanthorrhiza* continues to surge, the proposed strategies in this study offer potential pathways for sustainable and efficient cultivation, vital for meeting both traditional medicine practices and modern pharmacological needs. This is especially relevant given the plant's historical significance in Indonesian culture as a staple in herbal medicine (jamu), used to treat a host of ailments (Anggayanti *et al.*, 2024). Considering the promising results observed through the integration of various growth regulators, this research lays a solid foundation for future inquiries into the optimization of callus induction and the overall enhancement of bioactive compound yields.

CONCLUSIONS

The callus induction of *C. xanthorrhiza*, employing an MS medium enriched with synthetic growth regulators such as 2,4-D and IBA at specified concentrations is recommended over the use of more generalized natural extracts. The synthetic treatments not only outperform their natural counterparts but also provide a more controlled

environment for achieving higher rates of callus proliferation, thereby enhancing the potential for propagation and bioactive compound extraction. Thus, protocols should focus on the integration of these synthetic PGRs within MS base media.

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