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Research Article

EFFECTIVENESS OF ANTIOXIDANTS AS BROWNING INHIBITORS AGAINST SHOOT *Cyrtostachys renda* AS CALLUS CULTURE EXPLANT

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Abstract
Cyrtostachys renda has various pharmacological activities. *C. renda* roots contain alkaloids and flavonoids as secondary metabolites' main components responsible for anti-inflammatory and anticancer activities. To produce secondary metabolites from *C. renda*, tissue culture can be used through callus and cell suspension culture techniques. Browning is one of the main challenges in plant tissue culture, which inhibits the success of callus formation, especially in plants with a high content of phenolic compounds such as *C. renda*. This study tested the effect of soaking in polyvinylpyrrolidone (PVP) and ascorbic acid (AA) in inhibiting the browning process in *C. renda* explants. Explant colour change testing was done in vitro by soaking the explants in five concentrations of PVP and AA (50, 100, 150, 200 and 250 ppm). Changes in explant colour were then analyzed using the Royal Horticultural Society (RHS) colour chart. The results showed that the PVP antioxidant with a concentration of 200 ppm could suppress explant browning until the fourth day. In comparison, explants treated with AA antioxidants with a concentration of 200 ppm showed browning symptoms on the second day. It can be concluded that antioxidants can reduce the browning level in *C. renda* shoot explants, and the antioxidant PVP can reduce the browning level better than AA. The mechanism of PVP as an anti-browning agent through inhibition of phenolic oxidation. This study's novelty is obtaining the type of anti-browning PVP with an optimum concentration of 200 ppm to inhibit the browning of *C. renda* shoot explants.

Keywords: Antioxidant, Ascorbic Acid (AA), *Cyrtostachys renda*, Polyvinylpyrrolidone (PVP), Shoot Explants.

INTRODUCTION

Cyrtostachys renda is an ornamental and medicinal plant that has biological activities that are beneficial for health, including antioxidant, antibacterial, anticholinesterase and cytotoxic activity against cancer cells (Amir Rawa et al., 2019; Z. Liu et al., 2022; Utami et al., 2023; Syamsurizal et al., 2024; Syamsurizal & Utami, 2024). This is due to secondary metabolites, which play a role in this

activity. Research to increase the production of bioactive compounds by *C. renda* encourages researchers to research alternative methods that produce secondary metabolites in large quantities.

Plant tissue culture is a modern biotechnology method that can be applied to produce secondary metabolites (Sehgal & Khan, 2020). Aseptically, this technique can be developed in artificial media to produce callus. A callus is a mass of undifferentiated cells. Callus cells have genetic material identical to their parent to produce secondary metabolites like the parent (Mohanlall, 2020; Halder & Jha, 2021; BoangManalu, Iqbal, & Garcia, 2024). Callus culture is the most effective tissue culture technique for growing cells from plant tissue (Babich et al., 2020). Callus cells will expose the explants to a stressed environment that encourages the production and release of brownish phenolic compounds (Abdalla et al., 2022). The release of phenolic compounds into the growing media by explants has been identified as browning in plant tissue culture (Permadi et al., 2023; Xu et al., 2023). Phenolic compounds are released by plant explants as a protective strategy (Wagay et al., 2020). The brown pigment of the plant tissues and culture medium results from these chemical's oxidation (Chandran et al., 2020). Healthy plant tissues naturally contain phenolic chemicals, which can build up in specific cell types (Nurzy´nska & Nurzy´nska-Wierdak, 2023). These substances are created in vast quantities and released as a protective mechanism, particularly in response to stress or injury to plant tissues (Aguirre-Becerra et al., 2021).

Browning is a natural process, especially in plants with high phenolic content, such as *C. renda* (Vhangani & Van Wyk, 2021; Dessi & Shah, 2023; Fitriani, Triandafillidis & Thao, 2023; C. Liu, Fan, et al., 2024). Therefore, this natural defence mechanism may produce toxic compounds that damage the explant plant's cells and tissues, delaying or preventing the development of callus (Ashokhan et al., 2020). The entire process may suffer due to the browning process, represented by colour changes in the explants and culture media (Chumburidze et al., 2023; Rahmayani et al., 2023; Permadi et al., 2024). To enhance cell multiplication from plant tissues and synthesise secondary metabolites, the issue of browning in tissue culture procedures must be carefully controlled. Therefore, blocking oxidative, enzymatic, and non-enzymatic browning is required to handle this browning problem (Hamdan et al., 2022; Setiyani, Baharin, & Jesse, 2023; Yolviansyah et al., 2023; Chaudhary et al., 2024). Controlling the browning of explants is a critical challenge. It significantly impacts the success of callus and secondary metabolite production through tissue culture, both callus and cell suspension culture (C. Liu, Zhang, et al., 2024).

To be able to grow *C. renda* callus, treatment needs to be carried out to prevent browning. One treatment that can prevent browning is using antioxidants (Sari et al., 2021; Gemechu & Amante, 2021; Rini et al., 2023; C. Liu, Fan, et al., 2024; Permadi et al., 2024). Antioxidants can suppress browning by adding antioxidants to the growth medium or using antioxidants to soak the explants (Permadi et al., 2023; Xu et al., 2023). Polyvinylpyrrolidone (PVP) and ascorbic acid (AA) are antioxidants reported to suppress browning in several plants (Wen et al., 2021; Taher et al., 2022; Yusnidar et al., 2023). However, the effectiveness of PVP and AA in suppressing browning on *C. renda* callus has not been reported, so with this research it can be reported that antioxidants are effective in suppressing browning on *C. renda*.

The goal is to cultivate *C. renda* cells that can control and produce active anticancer substances in more significant amounts than the initial plant by producing callus from *C. renda* plants. By isolating explants in growth culture conditions and adding the hormones auxin and cytokinin, it is possible to increase the synthesis of active compounds from *C. renda* plants using the callus culture method (Grzegorczyk-Karolak et al., 2021; Herawati, Khairinal, & Idrus, 2023; Sultanuddin et al, 2023; Khoviriza et al., 2024). The novelty of this research is that the research report on *C. renda* is still limited, including for future development as an explant in tissue culture, so that active compounds that play a role in pharmaceutical interests can be mass synthesized and managed stably.

RESEARCH METHOD

Two types of antioxidants were used Ascorbic acid (AA) and Polyvinylpyrrolidone (PVP). Each antioxidant was dissolved into five different concentrations (50, 100, 150, 200 and 250 ppm), which were then used as a soaking solution while cutting the explants before planting them in culture media. The research was carried out from May to October 2023 at the Agrotechnology Laboratory, Faculty of Agriculture, Universitas Jambi and the Biotechnology Laboratory, Universitas Gadjah Mada, Yogyakarta.

The subject in this research (*Cyrtostachys renda*) was harvested in Muara Bungo, Jambi Province and identified in Jatinangor Herbarium, Plant Taxonomy Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Padjadjaran University, Bandung, Indonesia (No.48/HB/04/2022). The other materials needed in this research are the Murashige and Skoog (MS) Medium (Phytotechlab, US), bactericide (Agrept), fungicide (Amistartop), AA (Sigma Aldrich, US) and PVP (Sigma Aldrich, US). Chemicals in making MS medium include macronutrients, micronutrients, iron stock, vitamins, myo-inositol, sucrose, agar, 1 N HCl, 1 N KOH, and distilled water. Other materials are sterile distilled water, 70% alcohol (Brataco, Indonesia), methylated spirits, Clorox, label paper, millimetres block paper, and tissue.

Shoots from *C.renda* were utilized as the raw material. Shoots that were healthy and disease-free were rinsed under running tap water and then dipped for 15 minutes in a fungicide (1 mL L^{-1}), bactericide (1,5 g L^{-1}), and 100% Clorox. The cleaned shoot surface was rinsed three times with sterile deionized water for five minutes each after being sterilized for five minutes with 70% alcohol. The sterilized shoots are then cut into 0.5 cm thickness. During the cutting process, the shoots were soaked in a solution of PVP and AA antioxidants with different concentrations (50, 100, 150, 200, and 250 ppm). Furthermore, the shoot pieces were cultured on MS media.

Many MS medium compositions were weighed, dissolved, and swirled with a magnetic stirrer; the pH should be measured between 5.6 and 6.3. Add 30 g of sucrose and 10 g of agar powder to the medium. Heat while stirring until smooth. Immediately after boiling, the medium was put into the ready culture bottles and sterilized in an autoclave for 15 minutes at 121^{o}C and 15 Psi. Prepare an antioxidant solution of 100 mg each of PVP and AA dissolved in 100 mL of sterile deionized water (1000 ppm antioxidant stock solution). Furthermore, sterilized using 0.45 micron and 0.2 micron filtration. The antioxidants were then diluted into five concentrations: 50, 100, 150, 200, and 250 ppm.

The rate of browning inhibition was evaluated every 24 hours until day four by observing colour changes in the explants. Explant colour changes were noticed by comparing the explant's colour to the colour on fan 4 of the Royal Horticultural Society (RHS) colour chart, sixth edition (2015; 2019 reprint). The colour code was subsequently entered as data, and all matched shades were reported. To determine the degree of browning, *C. renda* shoots were cross-sectioned. The degree of browning in the outer and inner tissue of the affected shoot was measured using the following criteria: no browning = 0; slight browning (brown spots on the surface/outer part) = 1; slight to 20% browning = 2; 21–50% browning = 3; and more than 50% browning = 4. The shoot browning index was determined by the formula (Yan et al., 2013):

$$Browning\ index = \frac{\sum(browning\ level\ x\ shoot\ number\ of\ this\ level)}{higest\ browning\ level\ x\ total\ shoot\ number} \ldots (1)$$

This study used three explants in each of the three treatment repetitions. All data values were shown as means with standard deviations (SD). The Student's t-test was employed to identify differences of statistical significance, with a limit of $p<0.05$.

RESULTS AND DISCUSSION

Browning is when plant cells produce phenolic compounds in response to injury (Gemechu & Amante, 2021; Murata, 2022; Nath et al., 2022). Each plant's browning level varies according to species, age, and physiological conditions (Anwar et al., 2021; Miranda et al., 2020). *Cyrtostachys renda* is a plant species that shows a relatively high amount of browning in shoot explants, with browning occurring in less than 24 hours. This browning condition inhibits growth hence therapy is required (Ren et al., 2020; Zhang et al., 2023).

This study used different concentrations of the antioxidants PVP and AA as explant soaking components to suppress browning in *C. renda* shoot explants. The change in explant colour to brown accurately indicates the level of browning that has occurred. Explant colour changes were observed using the sixth version of the RHS colour chart (Table 1).

Table 1. Colors noticed in Fan 4 RHS of *Cyrtostachys renda* shoot explants

No	RHS Code	Colour Name	Colour Group
1	164A	Brownish Orange	Greyed-Orange Group
2	164B	Moderate Orange Yellow	Greyed-Orange Group
3	165C	Moderate Orange Yellow	Greyed-Orange Group
4	166A	Greyish Brown	Greyed-Orange Group
5	166B	Moderate Reddish Brown	Greyed-Orange Group
6	166C	Brownish Orange	Greyed-Orange Group
7	166D	Moderate Orange	Greyed-Orange Group
8	167A	Moderate Orange	Greyed-Orange Group
9	N167A	Brownish Orange	Greyed-Orange Group
10	N167B	Brownish Orange	Greyed-Orange Group
11	N167D	Moderate Orange	Greyed-Orange Group
12	N170B	Moderate Orange	Greyed-Orange Group
13	173C	Moderate Orange	Greyed-Orange Group
14	173D	Moderate Yellowish Pink	Greyed-Orange Group
15	174B	Greyish Reddish Orange	Greyed-Orange Group
16	177A	Moderate Reddish Orange	Greyed-Orange Group
17	177B	Light Reddish Brown	Greyed-Orange Group
18	177C	Greyish Reddish Orange	Greyed-Orange Group
19	183B	Dark Red	Greyed-Purple Group
20	N186B	Dark Greyish Purple	Greyed-Purple Group
21	N199B	Dark Greyish Yellowish Brown	Grey-Brown Group
22	N199C	Moderate Yellowish Brown	Grey Brown Group
23	200B	Greyish Reddish Brown	Brown Grup
24	200D	Moderate Brown	Brown Grup

Effectiveness of Polyvinylpyrrolidone (PVP) in Inhibiting Explant Browning

According to the observations, the color of the explants on the day 0 (the day the explants were planted) was brownish orange 164A for both the control and PVP treatments. During day one, the control explants transformed to Greyish Reddish Orange (177C), while the explants treated with 50 and 200 ppm PVP remained brownish orange, and concentrations of 100, 150, and 250 ppm were Moderate Orange Yellow. On day two, the control explants were Light Reddish Brown (177B), but all PVP treatment explants were Moderate Orange. On the third day, the control explants were dark red (177A), followed by the 50 ppm PVP treatment, which was greyish reddish orange (177C), the 100 ppm PVP treatment, which was mild yellowish pink (173D), and the 150, 200, and 250 ppm PVP treatments, which were still moderate orange (N170B). In control explants, the first signs of browning appeared on the first day of observation, whereas at 50 and 100 ppm they appeared on the third day. The PVP concentrations that effectively prevented browning until the third day were 150, 200, and 250 ppm. On day 4, the control explants were Dark Greyish Purple (N186B), 50 ppm were Moderate Yellowish Brown (N199C), and 100 and 150 ppm PVP were Moderate Brown (200D). A concentration of 200 ppm was successfully maintained in the color Greyish Reddish Orange (200B), whereas 250 ppm was achieved in the color Moderate Yellowish Brown (N194C) (Figure 1).

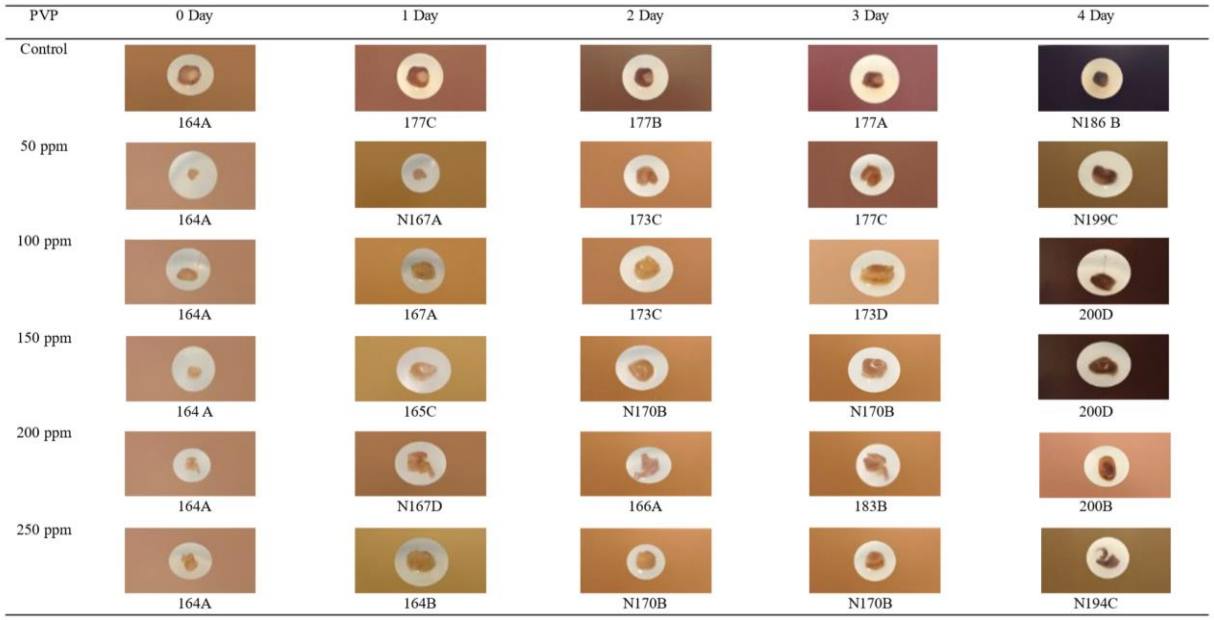

Figure 1. Browning level through *C. renda* shoots after PVP treatment. Observation of colour changes in a shoot based on Fan 4 RHS colour chart sixth edition

The findings of the observations above reveal that employing a concentration of 200 ppm PVP has the lowest impact on browning the colour. The previous evidence also suggests that the PVP soaking treatment on *C. renda* prevented browning until the third day. This finding is consistent with previous research reports, which found that soaking *Paeonia lactiflora* shoot in 0.5 and 1 ppm PVP specifically for 30 minutes can prevent the browning process (Cai et al., 2020), who found that soaking *Paeonia lactiflora* and *Cocos nucifera* shoots in 0.5 and 1 ppm PVP respectively for 30 minutes could prevent the browning process. A concentration of 1000 ppm PVP may reduce the browning of *Paeonia suffruticosa* leaf and petiole explants during culturing (Gao et al., 2020). To prevent further browning, cultures should be carried out on day three or before day four (Cai et al., 2020). The browning process begins with a reddish colour shift in the explant, and if it persists, callus production will be impeded. According to Zhao et al. (2021), browning of the explant can cause mortality and hence inhibit callus induction.

Effectiveness of Ascorbic Acid (AA) in Inhibiting Explant Browning

The antioxidant treatment on day 0 was 50 ppm AA, and the control showed a brownish-orange colour (164A and 166C). In contrast, the 100, 150, and 200 ppm AA treatments showed a moderate orange colour (166D), and the 250 ppm AA treatment displayed a moderate orange-yellow colour. (164B). On the first day of observation, the 50 ppm AA treatment was still brownish orange (166C), but the 100, 150, 200, and 250 ppm AA treatments were moderately orange. On day two, colour changes indicating browning problems were suggested at AA doses of 50 ppm Moderate Reddish Brown (166B), 100 ppm Brownish Orange (N167B), 150 ppm Moderate Reddish Brown (166B), 200 ppm Greyish Brown (166A), and 250 ppm Greyish Brown (N199B). The browning in the explants increased, evident on the third day when the concentration of 50 ppm AA was dark red (183B), 100 ppm AA was greyish reddish orange (177C), 150 ppm AA was moderate brown (200D), 200 ppm AA was dark red (183B), and 250 ppm AA was dark greyish yellowish brown (N199B). On the fourth day, the level of browning had reached a high level, with explants containing 50 ppm AA Greyish Reddish Brown (200B), 100 ppm AA Dark Greyish Yellowish Brown (N199B), 150 ppm and 200 ppm AA Greyish Reddish Brown (200B), and 250 ppm AA moderate Brown (200D) (Figure 2).

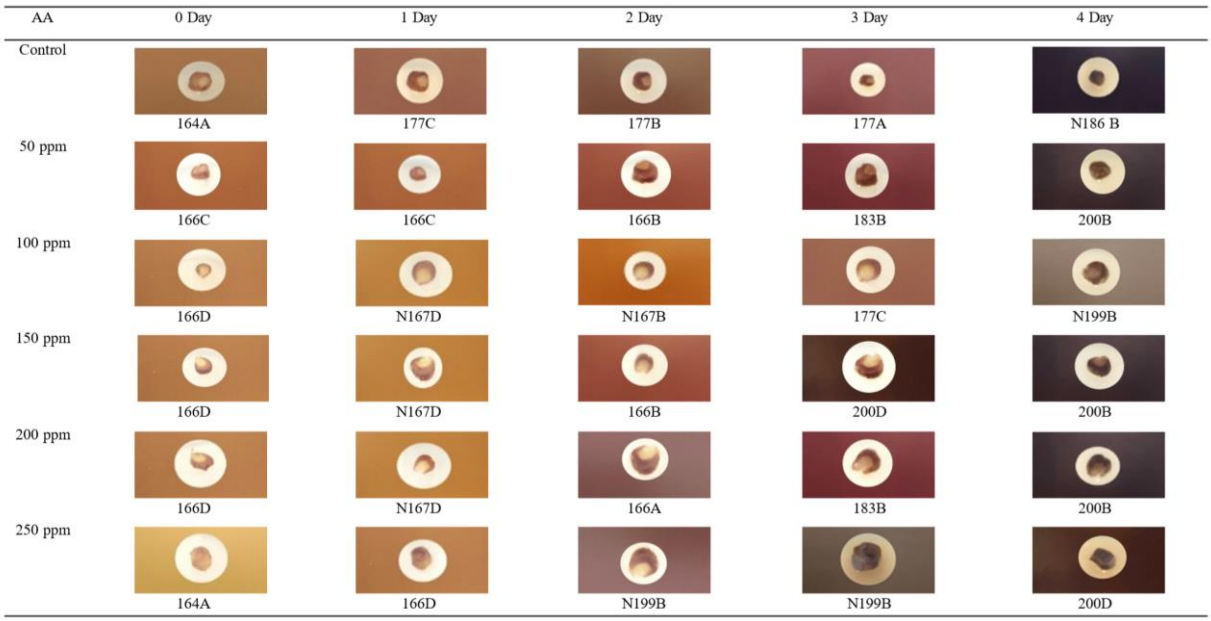

Figure 2. Browning level through *C. renda* shoots after AA treatment. Observation of color changes in shoot based on Fan 4 RHS color chart sixth edition

According to the results above, ascorbic acid can reduce browning in explants. This finding is consistent with earlier research findings that 1.2 g/l ascorbic acid effectively controlled the browning level in local *Musa* spp.cv explants (Permadi et al., 2023). Other studies found that 5-15 mg/L ascorbic acid could prevent browning in *Paeonia suffruticosa* leaf and petiole explants during culturing (Gao et al., 2020). Ascorbic acid is commonly used as an anti-browning agent. The mechanism underlying ascorbic acid's antibrowning action appears based on its reducing activity. Although ascorbic acid does not directly interact with the PPO enzyme, it suppresses enzymatic browning by decreasing the oxidized substrates. Anderson 1984 Other research revealed that the anti-browning activity of ascorbic acid can be linked to the conversion of enzymatically produced o-quinones to their precursor diphenols (Ito et al., 2020). Nonetheless, when applied to fresh-cut pears, ascorbic acid's anti-browning properties may be limited. When ascorbic acid is entirely oxidized to dehydroascorbic acid, o-quinones can be converted to diphenols, and browning probably occurs because of melanin production (Moon et al., 2020).

The Effects of Different PVP and AA Antioxidants on the Browning Index of C. renda Shoots

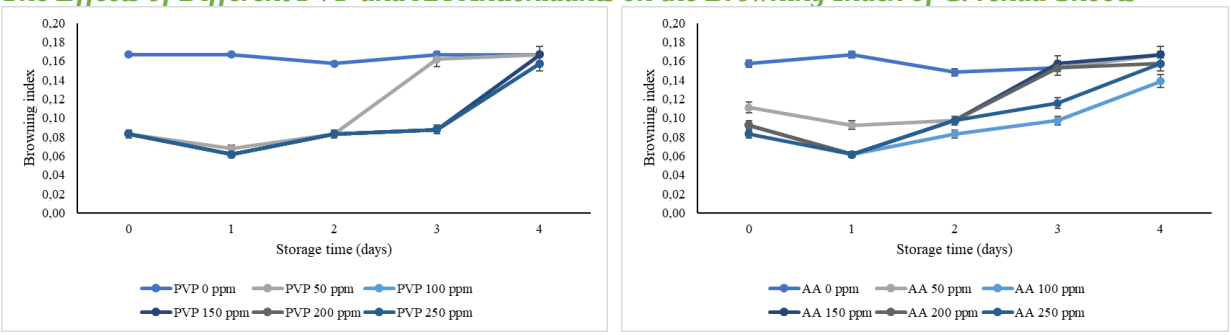

Figure 3. The browning index of *C. renda* shoots explants. (A) Treatment with PVP, and (B) treatment with AA. Browning index was reported as mean ±SDs of three independent assays

The browning index in *C. renda* shoot tissue increased during incubation (Figure 3A and B). Shoots that were untreated with PVP and AA antioxidants began to brown earlier. Meanwhile, the group of shoots treated with PVP and AA antioxidants experienced browning more slowly compared to the untreated group. *C. renda* shoots treated with PVP began to turn brown on the second day and developed slowly until the fourth day. However, treatment with AA resulted in browning changes in *C. renda* shoots occurring more quickly from the first to the fourth day than with PVP treatment. The

browning process in shoots treated with PVP and AA was significantly different on the second and third days of incubation ($p<0.05$). Shoots given PVP experienced a slower increase in browning than those given AA. This can also be seen in the browning index values of the two antioxidant compounds when applied as agents to suppress the browning process on *C. renda* shoots. These results indicate that PVP has a smaller browning index value than AA, which indicates that PVP can suppress browning more strongly than AA. At 200 and 250 ppm concentrations, PVP showed stronger browning inhibition activity than lower concentrations but did not show a significant difference in the browning index between 200 and 250 ppm concentrations. These results follow previous reports that PVP effectively inhibits browning compared to AA (de Assis et al., 2018; Permadi et al., 2024) PVP can inhibit browning with a non-browning ratio of 50%, while AA is only 5% (Ren et al., 2020). The results of other studies reported that PVP at a concentration of 300 ppm provided the best browning inhibition effect on kiwi fruit explants with a browning rate of 40%. In comparison, AA at a concentration of 200 ppm showed a browning rate of up to 60% (Chai et al., 2018). With the anti-browning effect on explants or embryos, the rate of callus formation and germination will increase (Chai et al., 2018; C. Liu, Fan, et al., 2024; J. Wang & Fang, 2023; X. Wang et al., 2022). By providing browning inhibitor treatment, phenol oxidation in explants, callus, and embryos can be inhibited so that browning and necrosis of explants or callus, which can inhibit growth and cause cell death, can be prevented (Jaiswal et al., 2021; C. Liu, Fan, et al., 2024; Sharma et al., 2025; Yang et al., 2024). Apart from browning, success in embryo germination is also influenced by the substrate used, fertilizer, and microbes that promote plant growth, such as endophytic fungi (Asniwita et al., 2024; Rikah et al., 2023; Wilyus et al., 2024) Based on these results, PVP with a concentration of 200 ppm can be applied to inhibit the browning of *C. renda* shoot explants to increase the potential for callus formation for in vitro culture purposes. Therefore, overcoming browning is very important for the in vitro propagation of *C. renda* plants. The limits of this study are the need to increase the concentration and type of anti-browning agents and the duration of observation. Combining two or more anti-browning agents is also needed to increase the browning inhibition ratio in *C. renda* shoot explants to be cultured.

CONCLUSION

The application of PEG 6000 at a 3% concentration positively influenced latex production from September to December compared to untreated plants. Additionally, the A1 treatment, consisting of 3 g of salicylic acid and 3 g of palmitic acid, demonstrated the most significant impact across all observed parameters, including latex production, rubber dryness, physiological responses, and histological characteristics. However, the combination of PEG 6000 (3%) with oleochemical treatment (A1) showed no statistically significant effect on these parameters, although a positive trend was observed in the P1A1 treatment group. These findings suggest that while PEG 6000 alone enhances latex production, its combination with oleochemicals requires further investigation to determine optimal formulations for improving rubber yield and quality. Future research should explore different concentrations and application methods of PEG 6000 and oleochemicals to maximize their synergistic effects. Additionally, long-term studies assessing environmental conditions, soil health, and sustainable latex production practices would provide valuable insights for improving rubber plantation management and productivity.

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AUTHOR CONTRIBUTIONS

Conceptualization, A.B. and B.H.; Methodology, A.B; Software, B.H.; Validation, S.Y., L.I. and E.L.; Formal Analysis, L.I.; Investigation, B.H.; Resources, D.T.; Data Curation, D.T. and E.L.; Writing – Original Draft Preparation, B.H. and D.T.; Writing – Review & Editing, S.Y. and L.I.; Visualization, D.T.; Supervision, S.Y.; Project Administration, E.L.; Funding Acquisition, S.Y.

CONFLICTS OF INTEREST

The author(s) declare no conflict of interest.

REFERENCES

Abdalla, N., El-Ramady, H., Seliem, M. K., El-Mahrouk, M. E., Taha, N., Bayoumi, Y., Shalaby, T. A., & Dobránszki, J. (2022). An Academic and Technical Overview on Plant Micropropagation Challenges. *Horticulturae 2022, Vol. 8, Page 677*, *8*(8), 677. https://doi.org/10.3390/HORTICULTURAE8080677.

Aguirre-Becerra, H., Vazquez-Hernandez, M. C., Saenz de la O, D., Alvarado-Mariana, A., Guevara-Gonzalez, R. G., Garcia-Trejo, J. F., & Feregrino-Perez, A. A. (2021). Role of Stress and Defense in Plant Secondary Metabolites Production. *Advanced Structured Materials*, *140*, 151–195. https://doi.org/10.1007/978-3-030-54027-2_5.

Amir Rawa, M. S., Hassan, Z., Murugaiyah, V., Nogawa, T., & Wahab, H. A. (2019). Anti-cholinesterase potential of diverse botanical families from Malaysia: Evaluation of crude extracts and fractions from liquid-liquid extraction and acid-base fractionation. *Journal of Ethnopharmacology*, *245*, 112160. https://doi.org/10.1016/J.JEP.2019.112160.

Anwar, S., Khan, S., Almatroudi, A., Khan, A. A., Alsahli, M. A., Almatroodi, S. A., & Rahmani, A. H. (2021). A review on mechanism of inhibition of advanced glycation end products formation by plant derived polyphenolic compounds. *Molecular Biology Reports*, *48*(1), 787–805. https://doi.org/10.1007/S11033-020-06084-0/METRICS.

Ashokhan, S., Othman, R., Rahim, M. H. A., Karsani, S. A., & Yaacob, J. S. (2020). Effect of Plant Growth Regulators on Coloured Callus Formation and Accumulation of Azadirachtin, an Essential Biopesticide in Azadirachta indica. *Plants 2020, Vol. 9, Page 352*, *9*(3), 352. https://doi.org/10.3390/PLANTS9030352.

Asniwita, A., Novalina, N., Syarif, M., Bestari, A. V., & Obura, B. O. (2024). Exploration of indigenous plant growth promoting fungi (PGPF) as biological control agents and biofertilizer. *Jurnal Ilmiah Ilmu Terapan Universitas Jambi*, *8*(1), 240-250. https://doi.org/10.22437/jiituj.v8i1.31783.

Babich, O., Sukhikh, S., Pungin, A., Ivanova, S., Asyakina, L., & Prosekov, A. (2020). Modern Trends in the In Vitro Production and Use of Callus, Suspension Cells and Root Cultures of Medicinal Plants. *Molecules 2020, Vol. 25, Page 5805*, *25*(24), 5805. https://doi.org/10.3390/MOLECULES25245805.

BoangManalu, E. N., Iqbal, M., & Garcia, C. (2024). Analysis of the relationship between interest and learning outcomes of physics in senior high school. *EduFisika: Jurnal Pendidikan Fisika*, *9*(1), 46-53. https://doi.org/10.59052/edufisika.v9i1.29641.

Cai, X., Wei, H., Liu, C., Ren, X., Thi, L. T., & Jeong, B. R. (2020). Synergistic effect of NaCl pretreatment and PVP on browning suppression and callus induction from petal explants of paeonia lactiflora pall. 'Festival Maxima.' *Plants 2020, Vol. 9, Page 346*, *9*(3), 346. https://doi.org/10.3390/PLANTS9030346.

Chai, J., Gao, Y., Dong, Y., Kong, L., & Zhang, Y. (2018). Browning treatment in tissue culture of 'hongyang' kiwifruit. *IOP Conference Series: Materials Science and Engineering*, *452*(2), 022075. https://doi.org/10.1088/1757-899X/452/2/022075.

Chandran, H., Meena, M., Barupal, T., & Sharma, K. (2020). Plant tissue culture as a perpetual source for production of industrially important bioactive compounds. *Biotechnology Reports*, *26*, e00450. https://doi.org/10.1016/J.BTRE.2020.E00450.

Chaudhary, N., Tiwari, V., Sharma, A., Kumari, A., Garg, M., Bhatnagar, A., Kansal, S. K., & Krishania, M. (2024). New Strategy for Browning Prevention in Apple Pomace Processing and Toxicity Tested in a Rodent Model. *ACS Omega*. https://doi.org/10.1021/ACSOMEGA.4C03966/ASSET/IMAGES/LARGE/AO4C03966_0007.JPEG.

Chumburidze, M., Setiabudi, E., Vassiliadou, M., Hasanov, R., & Duangpaserth, K. (2023). Unveiling the complex interplay between active learning and teacher development: Insights from TIMSS 2022 in Georgia. *Interval: Indonesian Journal of Mathematical Education*, *1*(2), 118-125. https://doi.org/10.37251/ijome.v1i2.1363.

de Assis, F. A., Rodrigues, F. A., Pasqual, M., de Assis, G. A., Luz, J. M. Q., Janoni, F., Costa, I. de J. S., Costa, B. N. S., & Soares, J. D. R. (2018). Antioxidants in the control of microorganism contamination and phenol oxidation in Eugenia pyriformis. *Biosci. j. (Online)*, *34*(1), 49–58. https://doi.org/10.14393/BJ-V34N1A2018-36311.

Dessi, L. C., & Shah, M. (2023). Application of the numbered head together type cooperative learning model to improve student learning outcomes in mathematics subjects. *Interval: Indonesian Journal of Mathematical Education*, *1*(2), 67-72. https://doi.org/10.37251/ijome.v1i2.773.

Fitriani, F., Triandafillidis, T., & Thao, L. P. (2023). Exploring the integration of computational thinking and mathematical modelling in STEM Education. *Interval: Indonesian Journal of Mathematical Education*, *1*(2), 73-82. https://doi.org/10.37251/ijome.v1i2.1341.

Gao, J., Xue, J., Xue, Y., Liu, R., Ren, X., Wang, S., & Zhang, X. (2020). Transcriptome sequencing and identification of key callus browning-related genes from petiole callus of tree peony (Paeonia suffruticosa cv. Kao) cultured on media with three browning inhibitors. *Plant Physiology and Biochemistry*, *149*, 36–49. https://doi.org/10.1016/J.PLAPHY.2020.01.029.

Gemechu, E. C., & Amante, G. (2021). Control of browning in Plant Tissue Culture: A Review. *Journal of Scientific and Innovative Research*, *10*(4), 89–93. https://doi.org/10.1371/journal.pone.0076802.

Grzegorczyk-Karolak, I., Hnatuszko-Konka, K., Krzeminska, M., Olszewska, M. A., & Owczarek, A. (2021). Cytokinin-Based tissue cultures for stable medicinal plant production: Regeneration and phytochemical profiling of salvia bulleyana shoots. *Biomolecules 2021, Vol. 11, Page 1513*, *11*(10), 1513. https://doi.org/10.3390/BIOM11101513.

Halder, M., & Jha, S. (2021). *Morphogenesis, Genetic Stability, and Secondary Metabolite Production in Untransformed and Transformed Cultures*. 663–722. https://doi.org/10.1007/978-3-030-30185-9_15.

Hamdan, N., Lee, C. H., Wong, S. L., Fauzi, C. E. N. C. A., Zamri, N. M. A., & Lee, T. H. (2022). Prevention of enzymatic browning by natural extracts and genome-editing: a review on recent progress. *Molecules 2022, Vol. 27, Page 1101*, *27*(3), 1101. https://doi.org/10.3390/MOLECULES27031101.

Herawati, H., Khairinal, K., & Idrus, A. (2023). Harmonizing nature and knowledge: Crafting engaging thematic teaching tools for expedition on environmental preservation. *Tekno - Pedagogi : Jurnal Teknologi Pendidikan*, *13*(1), 21-31. https://doi.org/10.22437/teknopedagogi.v13i1.32525.

Ito, S., Sugumaran, M., & Wakamatsu, K. (2020). Chemical reactivities of ortho-quinones produced in living organisms: Fate of quinonoid products formed by tyrosinase and phenoloxidase action on phenols and catechols. *International Journal of Molecular Sciences 2020, Vol. 21, Page 6080*, *21*(17), 6080. https://doi.org/10.3390/IJMS21176080.

Jaiswal, N., Verma, Y., & Misra, P. (2021). High frequency in vitro callogenesis and plant regeneration of Glycyrrhiza glabra L. *Vegetos*, *34*(3), 495–504. https://doi.org/10.1007/S42535-021-00219-9/FIGURES/3.

Khoviriza, Y., Azzahra, M. Z., Galadima, U., & Salsabila, W. S. (2024). Revealing the impact: Meta analysis of problem based learning models on improving communication skills in science learning. *EduFisika: Jurnal Pendidikan Fisika*, *9*(1), 38-45. https://doi.org/10.59052/edufisika.v9i1.32650.

Liu, C., Fan, H., Zhang, J., Wu, J., Zhou, M., Cao, F., Tao, G., & Zhou, X. (2024). Combating browning: mechanisms and management strategies in in vitro culture of economic woody plants. *Forestry Research*, *4*(0), e032. https://doi.org/10.48130/FORRES-0024-0026.

Liu, C., Zhang, J., Wu, J., Zhou, M., Cao, F., Tao, G., & Zhou, X. (2024). Combating browning: mechanisms and management strategies in in vitro culture of economic woody plants. *Forestry Research*, *4*(0), e032. https://doi.org/10.48130/FORRES-0024-0026.

Liu, Z., Zhao, M., Wang, X., Li, C., Wang, J., Liu, Z., Shen, X., & Zhou, D. (2022). Response surface methodology-optimized extraction of flavonoids with antioxidant and antimicrobial activities from the exocarp of three genera of coconut and characterization by HPLC-IT-TOF-MS/MS. *Food Chemistry*, *391*, 132966. https://doi.org/10.1016/J.FOODCHEM.2022.132966.

Miranda, A., Lara, A., Altamirano, A., Di Bella, C., González, M. E., & Julio Camarero, J. (2020). Forest browning trends in response to drought in a highly threatened mediterranean landscape of South America. *Ecological Indicators*, *115*, 106401. https://doi.org/10.1016/J.ECOLIND.2020.106401.

Mohanlall, V. (2020). Plant Cell Culture Systems for the Production of Secondary Metabolites-A Review. *IOSR Journal of Biotechnology and Biochemistry (IOSR-JBB*, *6*(1), 35–47. https://doi.org/10.9790/264X-0601023547.

Moon, K. M., Kwon, E. Bin, Lee, B., & Kim, C. Y. (2020). Recent Trends in Controlling the Enzymatic Browning of Fruit and Vegetable Products. *Molecules 2020, Vol. 25, Page 2754*, *25*(12), 2754. https://doi.org/10.3390/MOLECULES25122754.

Murata, M. (2022). Food chemistry and biochemistry of enzymatic browning. *Food Science and Technology Research*, *28*(1), 1–12. https://doi.org/10.3136/FSTR.FSTR-D-21-00130.

Nath, P., Pandey, N., Samota, M., Sharma, K., Kale, S., Kannaujia, P., Sethi, S., & Chauhan, O. P. (2022). Browning Reactions in Foods. *Advances in Food Chemistry: Food Components, Processing and Preservation*, 117–159. https://doi.org/10.1007/978-981-19-4796-4_4.

Nurzynska, R., & Nurzynska-Wierdak, N. (2023). Phenolic Compounds from New Natural Sources—Plant Genotype and Ontogenetic Variation. *Molecules 2023, Vol. 28, Page 1731*, *28*(4), 1731. https://doi.org/10.3390/MOLECULES28041731.

Permadi, N., Akbari, S. I., Prismantoro, D., Indriyani, N. N., Nurzaman, M., Alhasnawi, A. N., Doni, F., & Julaeha, E. (2024). Traditional and next-generation methods for browning control in plant tissue culture: Current insights and future directions. *Current Plant Biology*, *38*, 100339. https://doi.org/10.1016/J.CPB.2024.100339.

Permadi, N., Nurzaman, M., Alhasnawi, A. N., 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.

Rahmayani, F., Hambali, S. M., Moghadam, A. A., Ripeanu, D., & Nkambule, T. (2023). Unveiling college student preferences: integrating numerical and factor analysis in understanding choices for mathematics majors. *Interval: Indonesian Journal of Mathematical Education*, *1*(2), 83-98. https://doi.org/10.37251/ijome.v1i2.1346.

Ren, X., Liu, Y., & Jeong, B. R. (2020). Callus induction and browning suppression in tree peony Paeonia ostii 'Fengdan.' *Horticulture Environment and Biotechnology*, *61*(3), 591–600. https://doi.org/10.1007/S13580-020-00246-6/METRICS.

Rika, M., Oktavia, S. W., Fitriani, R., & Javed, M. A. (2023). Seed germination test technology practicum (standard development test). *Jurnal Ilmiah Ilmu Terapan Universitas Jambi*, *7*(2), 184-188. https://doi.org/10.22437/jiituj.v7i2.29761.

Rini, E. F. S., Raptis, P., Sozcu, O. F., Abdelrhman, S. A. A. M., & Lara-Valenzuela, C. (2023). Technology and ethics in social media: A study of the phenomenon of digital bullying in the young generation. *Journal of Educational Technology and Learning Creativity*, *1*(2), 106-114. https://doi.org/10.37251/jetlc.v1i2.1400.

Sari, E. P., Lestari, U., & Syamsurizal, S. (2021). Uji Sifat Fisikokimia Lotion Fraksionat Ekstrak Diklorometan Kulit Buah Artocarpus altilis. *Jurnal Ilmiah Ilmu Terapan Universitas Jambi*, *5*(2), 122-136. https://doi.org/10.22437/jiituj.v5i2.15893.

Sehgal, D., & Khan, T. (2020). Plant Tissue Culture: Beyond Being a Tool for Genetic Engineering. *Environmental Microbiology and Biotechnology: Volume 1, Biovalorization of Solid Wastes and Wastewater Treatment*, *1*, 175–200. https://doi.org/10.1007/978-981-15-6021-7_9.

Setiyani, E. N., Baharin, Z. H. Z., & Jesse, S. N. (2023). Development of POE-Based student worksheets (Predict, Observe, And Explain) for students' mathematical representation abilities. *Journal of Educational Technology and Learning Creativity*, *1*(2), 78-87. https://doi.org/10.37251/jetlc.v1i2.792.

Sharma, S., Sharma, S., Kumar, P., Verma, P., Sharma, R., & Sharma, N. C. (2025). Overcoming Challenges in Walnut (Juglans Regia L.) Micropropagation: A comprehensive approach to optimizing explant selection, sterilization, and nutrient media. *Applied Fruit Science 2024 67:1*, *67*(1), 1–13. https://doi.org/10.1007/S10341-024-01234-1.

Sultanuddin, S., Saharudin, S., Yamin, M., Waite, V., & Baral, L. N. (2023). Implementation of portfolio assessment in english lessons at man insan cendekia jambi. *Tekno - Pedagogi : Jurnal Teknologi Pendidikan*, *13*(1), 32-40. https://doi.org/10.22437/teknopedagogi.v13i1.32569

Syamsurizal, S., Elisma, E., & Pratiwi, P. D. (2024). Breadfruit peel as the most potent radical scavengers for skin protection. *Jurnal Ilmiah Ilmu Terapan Universitas Jambi*, *8*(1), 325-333. https://doi.org/10.22437/jiituj.v8i1.33966.

Syamsurizal, S., & Utami, D. T. (2024). The combination of fraction A3 of Cyrtostachys renda Blume with doxorubicin improved cytotoxicity against T47D cell line through cell cycle arrest and apoptotic induction. *Journal of Applied Pharmaceutical Science*, *14*,(9), 270–278. https://doi.org/10.7324/JAPS.2024.173607.

Taher, M. A., Lo'ay, A. A., Gouda, M., Limam, S. A., Abdelkader, M. F. M., Osman, S. O., Fikry, M., Ali, E. F., Mohamed, S. Y., Khalil, H. A., El-Ansary, D. O., El-Gioushy, S. F., Ghazzawy, H. S., Ibrahim, A. M., Maklad, M. F., Abdein, M. A., & Hikal, D. M. (2022). Impacts of Gum Arabic and Polyvinylpyrrolidone (PVP) with Salicylic Acid on Peach Fruit (Prunus persica) Shelf Life. *Molecules 2022, Vol. 27, Page 2595*, *27*(8), 2595. https://doi.org/10.3390/MOLECULES27082595.

Utami, D. T., Efrini, E., Ikhsan Jumardi, M., Farmasi, J., Kedokteran dan Ilmu Kesehatan, F., & Jambi, U. (2023). Toxicity test of red palm (Cyrtostachys renda Blume.) using the brine shrimp lethality test (BSLT) method. *Journal of Pharmaceutical and Sciences*, 407–413. https://doi.org/10.36490/JOURNAL-JPS.COM.V6I5-SI.429.

Vhangani, L. N., & Van Wyk, J. (2021). Heated plant extracts as natural inhibitors of enzymatic browning: A case of the Maillard reaction. *Journal of Food Biochemistry*, *45*(2), e13611. https://doi.org/10.1111/JFBC.13611.

Wagay, N. A., Lone, R., Rafiq, S., & Bashir, S. U. (2020). Phenolics: A game changer in the life cycle of plants. *Plant Phenolics in Sustainable Agriculture*, 241–275. https://doi.org/10.1007/978-981-15-4890-1_11.

Wang, J., & Fang, S. (2023). Effects of different anti-browning agents on enzyme activity and growth in callus of Cyclocarya paliurus. *Journal of Nanjing Forestry University*, *47*(6), 167. https://doi.org/10.12302/J.ISSN.1000-2006.202203071.

Wang, X., Li, G., Chen, X., Yu, C., Nie, J., Yang, H., Ji, W., Xu, G., Zhu, H., Jin, S., & Zhu, X. (2022). The Effect of Anti-browning Agent Activated Carbon and Polyvinyl Pyrrolidone on the Rooting of Embryo Seedlings of "FengDan" and Its Transcriptome Analysis. *Front. Plant Sci.*, *13*, 1–13. https://doi.org/10.3389/fpls.2022.832619.

Wen, P., Hu, T. G., Wen, Y., Li, K. E., Qiu, W. P., He, Z. L., Wang, H., & Wu, H. (2021). Development of nervilia fordii extract-loaded electrospun pva/pvp nanocomposite for antioxidant packaging. *Foods*, *10*(8), 1728. https://doi.org/10.3390/FOODS10081728/S1.

Wilyus, W., Nurdiansyah, F., Irianto, I., Asniwita, A., & Oktavia, S. (2024). Honey pumpkin stem borer, apomecyna saltator fabricius (coleoptera: cerambycidae) can be controlled with phosphorus and potassium fertilizer. *Jurnal Ilmiah Ilmu Terapan Universitas Jambi*, *8*(2), 634-646. https://doi.org/10.22437/jiituj.v8i2.33717.

Xu, X., Zhu, D., Huan, Z., Geng, X., & Ran, J. (2023). Mechanisms of tissue culture browning in five Magnoliaceae family species. *Plant Cell, Tissue and Organ Culture*, *155*(1), 183–195. https://doi.org/10.1007/S11240-023-02568-6/TABLES/4.

Yan, S., Li, L., He, L., Liang, L., & Li, X. (2013). Maturity and cooling rate affects browning, polyphenol oxidase activity and gene expression of 'Yali' pears during storage. *Postharvest Biology and Technology*, *85*, 39–44. https://doi.org/10.1016/J.POSTHARVBIO.2013.04.016.

Yang, Y., Chan, Y., Wang, Y., Guo, H., Song, L., Zhang, H., Sun, L., Cong, R., & Zhang, H. (2024). Preliminary establishment of genetic transformation system for embryogenic callus of Acer truncatum 'Lihong.' *Frontiers in Plant Science*, *15*, 1419313. https://doi.org/10.3389/FPLS.2024.1419313/BIBTEX.

Yolviansyah, F., Amin, N. F., Retutas, M., Tuan, N. A., & Peiling, L. (2023). Implementation of information technology algorithms based on ICT media to make teachers have 21st century skills. *Journal of Educational Technology and Learning Creativity*, *1*(2), 98-105. https://doi.org/10.37251/jetlc.v1i2.1398.

Yusnidar, Y., Kartika, E., Iqbal, M., Vitoekpon, I., Husseini, M. M. E., Hagos, H., & Khan, Y. (2023). Formulation of red bean meatballs as a local food alternative: Increasing protein and fiber using design expert D-Optimal. *Journal of Educational Technology and Learning Creativity*, *1*(2), 115-139. https://doi.org/10.37251/jetlc.v1i2.1405.

Zhang, W., Pan, Y., Jiang, Y., & Zhang, Z. (2023). Advances in control technologies and mechanisms to treat peel browning in postharvest fruit. *Scientia Horticulturae*, *311*, 111798. https://doi.org/10.1016/J.SCIENTA.2022.111798.