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Effectiveness of Antioxidants on In Vitro Regeneration of *Musa paradisiaca* var. Raja to Prevent Browning and Enhance Embryo Development

Putri Aisiyya Qutlana Munawaroh[1)], Sulistyani Pancaningtyas[2*)], and Mukhamad Su'udi[1)]

[1)]Department of Biology, Faculty of Mathematics and Natural Science, University of Jember, Jl. Kalimantan Tegalboto No. 37, Jember, Indonesia
[2)]Indonesian Coffee and Cocoa Research Institute, Jl. PB Sudirman, No. 90, Jember, Indonesia
[*)] Corresponding author: listya.1606@gmail.com
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Abstract

The cultivation of raja banana is widespread in Indonesia. The process of increasing banana propagation through in vitro culture encounters a specific issue, namely the occurrence of browning during the initiation stage, which hinders the regeneration process. The objective of this study is to determine the effectiveness of antioxidants on the in vitro regeneration of raja banana to prevent browning and enhance embryo development. The study was conducted using a completely randomized design with the treatment of the antioxidant compounds ascorbic acid (AS) and melatonin (MN). The treatments consisted of control (without antioxidants), ascorbic acid (100 mg L^{-1}, 150 mg L^{-1}, and 200 mg L^{-1}) and melatonin (10 mg L^{-1}, 12 mg L^{-1}, and 14 mg L^{-1}). The results showed that ascorbic acid and melatonin treatment had no significant effect on the percentage of viable explants and the level of browning intensity, but provided significant results on the regeneration process. The application of melatonin at 14 mg L^{-1} significantly increased callus regeneration. Furthermore, at a concentration of 12 mg L^{-1} showed the highest callus percentage value compared to other treatments. The melatonin 12 mg L^{-1} treatment showed the earliest scutellar embryo formation, whereas ascorbic acid at a concentration of 100 mg L^{-1} resulted in the most optimal regeneration of globular and scutellar embryos. The highest concentrations of ascorbic acid and melatonin inhibit the formation of coleoptilar embryos.

Keywords: Browning, explants, in vitro, raja banana, regeneration, ascorbic acid, melatonin

INTRODUCTION

Banana plants (*Musa paradisiaca*) exhibit promising potential as agricultural commodities with positive prospects for expansion. Indonesia emerged as the top global producer of bananas in 2021, achieving a remarkable output of 8,741,147 tons. In period 2021-2022, banana production experienced a growth of 9.79%, reaching a total of 9,596,972 tons (BPS, 2022). The banana producers are distributed throughout many regions in Indonesia with East Java Province being the largest one, with 2.63 million tons of bananas, followed by Lampung Province with 1.39 million tons, and West Java with 1.32 million tons (BPS, 2022).

Banana crops can be used as temporary shade crops for coffee and cocoa crops (Jezeer *et al.*, 2017). It has been used by farmers in coffee and cocoa plantations in Indonesia. Putri *et al*. (2021) stated that cocoa farmers in Pasaman Regency utilize banana crop as a temporary shade to protect young cocoa

crop from excessive sunlight and protect the soil from erosion. Coffee farmers in the Central Aceh area also used banana crop to shade Arabica coffee crop (Syadida *et al*., 2024).

Raja banana (*Musa paradisiaca*) is widely cultivated, especially in the Brebes (Arafat &Wasdiun, 2020), Cianjur, Sukabumi, Demak, and Lumajang (Probojati *et al.*, 2019). Raja banana has a triploid genome (AAB) and is the result of a hybridization between the wild bananas *Musa acuminata* and *Musa balbisiana* (Li & Ge, 2017). Raja bananas can be consumed in their fresh state or following processing. Raja bananas have a sweeter taste and strong aroma than other banana types (Ismail *et al.*, 2015). In addition, raja bananas are also widely used in traditional events such as weddings (Supriyati *et al.*, 2017). The benefits of raja bananas are the factors that contribute to their high demand among the general population. The productivity of the raja banana can be realized by propagating through tissue culture.

Tissue culture is a plant propagation technique that involves cultivating cells, tissues, and plant organs in an aseptic environment nutrientrich medium. Tissue culture generates a rapid and abundant production of new plants (planlet) (Espinosa *et al.*, 2018). Banana tissue culture is a method used to enhance and preserve local banana varieties. The process of in vitro propagation of bananas enables the production of disease-free, high-quality, and genetically uniform planting material or seedlings (Lusiyanto *et al.*, 2021). Nevertheless, a recurring difficulty in banana tissue culture is the occurrence of browning at the initiation stage, which hinders the regeneration process.

Browning occurs due to the excretion of phenolic compounds on the surface of explants that have been scratched or cut during the initiation stage. The enzyme polyphenol oxidase (PPO) catalyzes the process of phenolic oxidation, resulting in appearance on explants blackish-brown. Browning induces cellular growth inhibition and leads to cell death (Ren *et al.*, 2020). Supplementing culture media with antioxidant compounds can decrease the oxidation of phenolic compounds.

Ascorbic acid and melatonin are antioxidant substances that can be used to prevent browning. Ascorbic acid is an antioxidant that can inhibit phenol exudation hence reducing the occurrence of browning in explants. According to Ngomuo *et al.* (2014), the addition of 100 mg L^{-1} and 200 mg L^{-1} of ascorbic acid in culture medium effectively decreased the level of browning of Mzuzu variety banana with a percentage of viable explants reached 53.3% and 53.8% respectively. In addition, prior to sterilization, immersing explants in a solution containing 150 mg L^{-1} ascorbic acid and 50 mg L^{-1} citric acid for 30 minutes effectively decreased the occurrence of browning in yellow Ambon banana cultures (Yusnita *et al*., 2015).

Melatonin is a molecule belonging to the indoleamine family, which functions as an antioxidant and reduces oxidative damage. Li *et al.* (2022) found that exogenous melatonin can reduce the activity of enzymes associated with browning in sweet potatoes. This is achieved by reducing the amount of reactive oxygen species (ROS) and the level of membrane lipid peroxidation. This study aimed to assess the effectiveness of antioxidant chemicals in preventing browning and enhancing embryo development on the in vitro regeneration of raja banana (*Musa paradisiaca* var. Raja).

MATERIALS AND METHODS

The research was conducted at the Tissue Culture Laboratory of Indonesian Coffee and

Cocoa Research Institute between March and December 2023. The research material consisted of raja banana flowers explants obtained from Kaliwining Experimental Station which is part of the Indonesian Coffee and Cocoa Research Institute.

The study employed a completely randomized design, utilizing antioxidant compounds treatment of ascorbic acid (AS) and melatonin (MN) as treatments. The study comprised 6 treatments and 1 control, with each treatment being replicated 3 times. Each repetition includes one petri dish with three explants. The treatments included controls (without antioxidants), 100 mg L^{-1} ascorbic acid (AS1), 150 mg L^{-1} ascorbic acid (AS2), 200 mg L^{-1} ascorbic acid (AS3), 10 mg L^{-1} melatonin (MN1), 12 mg L^{-1} melatonin (MN2), and 14 mg L^{-1} melatonin (MN3).

The culture media used was Murashige and Skoog (1962) media supplemented with 30 g L^{-1} glucose, 4 g L^{-1} gellan gum (agar), and addition of growth regulators such as 0,2 mg L^{-1} NAA, 2 mg L^{-1} thidiazuron, and 2 mg L^{-1} BA.

Explant Sterilization

The flowers of bananas were subjected to sun drying for 24 hours to decrease the amount of sap present. The bracts and flowers are trimmed down to a size of 10 cm and thereafter washed with water. The sterilization process in a laminar air flow (LAF) was performed by repeatedly rinsing the banana flowers with 500 mL sterile distilled water five times. Subsequently immersed in solution A, consisting of 500 mL sterile distilled water and 2 mL of polysorbate, for 15 minutes. The banana flowers were rinsed with 500 mL of sterile distilled water five times. Afterward, immerse it in solution B which consists of 500 mL of sterile distilled water, 125 mL of 25% sodium hypochlorite, and 2 mL of polysorbate, for 30 minutes. The banana flowers were rinsed again with 500 mL of distilled water eight times. Subsequently, the sap should be immersed in a solution containing 96% alcohol and then subjected to combustion until it completely vanishes.

Explant Initiation and Subculture

Sterilized explants were placed on MS medium to screen any contamination. Before planting, the bracts of the banana flower were removed in 3-5 layers and then cut into small pieces with a thickness of approximately 1 cm. Thereafter, the explants were incubated in a controlled environment at a temperature of 25-27 °C.

Subculture was carried out after the explants were 3-4 weeks old in the treatment medium. Each petri dish included three explants, and this process was repeated three times for each treatment. The explants were then incubated in an incubation room at a temperature of 25-27 °C in dark conditions for 4 weeks.

Data Collection

The variables observed in this research included explant growth, percentage of viable explants, and intensity of browning. The growth of explants is observed by monitoring the development of callus and embryo formation (globular, scutellar, and coleoptilar). Globular embryos are characterized by the appearance of a white and round nodule which then develops into two nodules (scutellar) and then elongates to form a bud (coleoptilar) (Gokul *et al.*, 2023; Wehbi *et al.*, 2022; Enríquez-Valencia *et al*., 2019). Callus explants are quantified as a percentage, whereas explants that generate embryos are counted based on the number of embryos per phase.

The percentage of viable explants represents the ratio of viable explants in each treatment. Explants are considered viable if they are free from contamination and necrosis caused by browning.

The quantification of viable explants is determined by employing the subsequent formula:

$$\% \text{ viable explant} = \frac{Total\ live\ plant}{Total\ explants\ for\ treatment} \times 100\%$$

The intensity of browning was determined by quantifying the amounts of phenol exudation (browning) in both explants and the secretion on the planting medium (Figure 1). Observations were carried out once a week for 5 weeks.

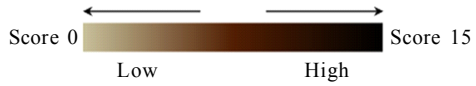


Figure 1. Level of browning intensity

Browning intensity can be quantified by the method of browning intensity scoring (Table 1).

Table 1. Browning intensity scoring

Score	Information
0	The explants do not exhibit any signs of browning.
1-5	The level of browning is low, only a few parts of the explant are browned or darkening and there is no release into the media.
6-10	The level of browning is moderate, with browning occurring in approximately half of all parts of the explant without spreading to the media.
11-15	The level of browning is high or significant, with the explant exhibiting darkening that extends to the surrounding media.

Data Analysis

The acquired data were analyzed by ANOVA (analysis of variance) test using SPSS version 29. If there are significant differences in the outcomes of these treatments, then Duncan's multiple range test (DMRT) was conducted at a 5% confidence level.

RESULTS AND DISCUSSION

Explants Initiation

The flowers of raja banana used in this research were collected from Kaliwining Experimental Station at Indonesian Coffee and Cocoa Research Institute which has been certified by the Seed Supervision and Certification Center. The efficacy of plant tissue culture is reliant upon the selection of appropriate plant parts as explants. The explants commonly used in banana tissue culture are derived from zygotic embryos, sliced rhizomes and leaf sheaths, female flowers, male flowers and bracts (Wang *et al.,* 2022). The explants used in this research were immature male flowers. The bracts and male flower of the banana flower are removed once they reach a size 5 cm (Figure 2a) in sterilized condition and then cut into small pieces measuring about 1 cm (Figure 2b) for initiation processes.

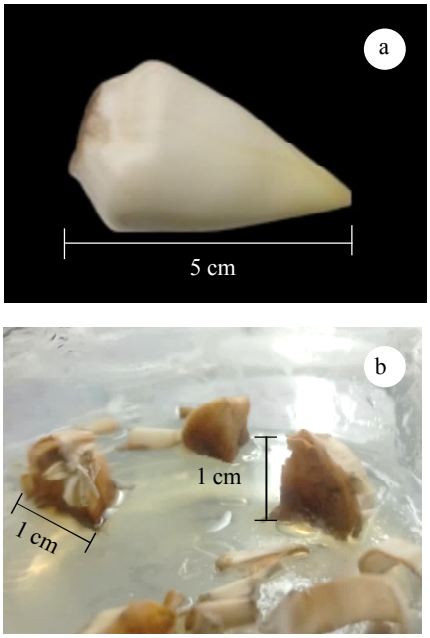


Figure 2. Banana flower used in explant preparation

Viable Explants

The viability of the explants was assessed to determine the ability of the explants to survive in the treatment media and to evaluate the level of browning intensity and regeneration during subsequent observations. The analysis indicated that the application of ascorbic acid and melatonin in the culture media had no significant effect on the percentage of viable explants of raja banana (Table 2). The application of AS3 treatment with a concentration 200 mg L^{-1} of ascorbic acid, resulted in a decrease in the percentage of viable explants in the second week due to contamination and remained stable the following week.

The addition of ascorbic acid and melatonin to culture media did not interfere with the life ability of raja banana explants. This can be seen from the high percentage value of living explants, reaching 89-100%. This result allows us for further observations, namely the measurement of browning intensity and explant regeneration. Levai *et al.* (2023) stated that ascorbic acid concentrations of 0-100 mg L^{-1} produce more than 80% of plantlets that can survive on some banana cultivars. Huang *et al.* (2022) also reported that melatonin concentrations of 0-10 ¼ M significantly increased the survival rate of *Cymbidium goeringii* explants.

Explants Browning Intensity

Browning corresponds to the secretion of phenolic compounds in response to biotic and abiotic stress (Ahmad *et al.*, 2013). Browning occurs when the explant is incised during the initiation stage (Permadi *et al.*, 2023). The presence of various levels of browning in raja banana explants is indicated by the observed browning intensity, as shown in Figure 3. The level of browning intensity impacts the ability of an explant to regenerate. The high level of browning intensity can inhibit the explant regeneration process due to the presence of phenolic compounds that are oxidized and accumulate on the surface of the explant or media. These compounds may inhibit the absorption of nutrients in the culture media (Ahmad *et al.*, 2013).

The level of browning or browning intensity of the explants was observed every week for five weeks. The results showed that all explants exhibited browning. The browning that occurred in explants generally increased in the third and fifth weeks, but the 200 mg L^{-1} ascorbic acid treatment (AS3) showed a decrease in browning intensity (Table 3). The decrease in browning intensity was caused by contamination so that the explants died and the level of browning in the explants could not be measured.

Table 2. Percentage of viable explants of raja banana during 5 weeks of observation due to ascorbic acid and melatonin treatments

Antioxidant (mg L^{-1})	Time (week)				
	1	2	3	4	5
Control	100% ± 0	100% ± 0[a]	100% ± 0[a]	100% ± 0[a]	100% ± 0[a]
Ascorbic acid (100)	100% ± 0[a]	100% ± 0[a]	100% ± 0[a]	100% ± 0[a]	100% ± 0[a]
Ascorbic acid (150)	100% ± 0[a]	100% ± 0[a]	100% ± 0[a]	100% ± 0[a]	100% ± 0[a]
Ascorbic acid (200)	100% ± 0[a]	89% ± 0.19[a]	89% ± 0[a]	89% ± 0[a]	89% ± 0[a]
Melatonin (10)	100% ± 0[a]	100% ± 00[a]	100% ± 0[a]	100% ± 0[a]	100% ± 0[a]
Melatonin (12)	100% ± 0[a]	100% ± 0[a]	100% ± 0[a]	100% ± 0[a]	100% ± 0[a]
Melatonin (14)	100% ± 0[a]	100% ± 0	100% ± 0[a]	100% ± 0[a]	100% ± 0[a]

Note: Values (Mean ± SD) followed by the same letters in a column are not significantly different based on the DMRT test at the 5% significance level

The data analysis results (Table 3) showed that ascorbic acid and melatonin treatments did not significantly affect the level of browning intensity in raja banana. The lowest browning intensity was observed in 12 mg L^{-1} melatonin treatment (MN2). MN2 treatment has the potential to suppress browning that occurs in explants compared to the control and other treatments. Huang *et al.* (2022) reported that the addition of melatonin to culture media can reduce browning and at a concentration of 1.0 µM showed the lowest level of browning in *Cymbidium goeringii* cultures.

Figure 3. Browning intensity (1x magnification), (a) No browning (score 0); (b) Low degree of browning (score 3); (c) Medium browning level (score 9); (d) High degree of browning (score 14), Bar: 5 mm.

Table 3. The average of browning intensity level on raja banana explants during 5 weeks of observation due to ascorbic acid and melatonin treatments

Antioxidant (mg L^{-1})	Week				
	1	2	3	4	5
Control	9.67 ± 0.58 ^{ab}	9.67 ± 0.58 ^{ab}	10.67 ± 0.58 ^{ab}	10.67 ± 0.58 ^{ab}	11.33 ± 1.20 ^{ab}
Ascorbic acid (100)	9.89 ± 3.02 ^{ab}	9.89 ± 3.02 ^{ab}	10.22 ± 3.36 ^{ab}	10.22 ± 3.36 ^{ab}	10.56 ± 3.02 ^{ab}
Ascorbic acid (150)	11.67 ± 1.45 ^{ab}	11.67 ± 1.45 ^{ab}	12.56 ± 1.95 ^{ab}	12.56 ± 1.95 ^{ab}	13.33 ± 2.33 ^{ab}
Ascorbic acid (200)	10.11 ± 1.95 ^{ab}	9.22 ± 0.51 ^{ab}	9.33 ± 0.58 ^{ab}	9.33 ± 0.58 ^{ab}	9.33 ± 0.58 ^{a}
Melatonin (10)	10.78 ± 3.02 ^{ab}	10.78 ± 3.02 ^{ab}	11.11 ± 3.36 ^{ab}	11.11 ± 3.36 ^{ab}	11.22 ± 3.53 ^{ab}
Melatonin (12)	7.78 ± 3.34 ^{a}	7.78 ± 3.34 ^{a}	8.56 ± 3.83 ^{a}	8.56 ± 3.83 ^{a}	9.22 ± 3.83 ^{a}
Melatonin (14)	13.22 ± 0.77 ^{b}	13.22 ± 0.77 ^{b}	14.00 ± 0.67 ^{b}	14.00 ± 0.67 ^{b}	14.89 ± 0.19 ^{b}

Note: Values (Mean ± SD) followed by the same letters in the same column are not significantly different based on the DMRT test at the 95% significance level. Browning level: o (no browning), 15 (highest level of browning).

Melatonin has antioxidant properties which are thought to inhibit the exudation of phenolic compounds that cause browning in male flower explants. Cutting explants during the initiation stage induces oxidative stress leading to the accumulation of reactive oxygen species (ROS) such as hydrogen peroxide in plant tissue (Hesami *et al*., 2020). Hydrogen peroxide acts as a stimulant for the primary enzyme responsible for explant browning, namely peroxidase (POD) (Kaewjumpol *et al*., 2021). According to Li *et al.* (2022), melatonin has the ability to decrease the level of reactive oxygen species (ROS) and inhibit the activity of enzymes associated with browning.

Callus and Embryo Regeneration

The occurrence of browning in banana tissue culture may inhibit the regeneration of the explant. The addition of antioxidant compounds to the culture media is expected to stimulate explant growth such as the formation of callus and somatic embryos. The results of this study showed that the callus formed had the character of friable texture, yellowish-white or translucent in color, and a glossy surface (Figure 4a). Debbarma *et al.* (2019) reported the same research findings, indicating that the embryogenic callus developed on several banana cultivars exhibits a translucent white appearance and contains proembryonic masses on its surface.

The formation of somatic embryos in banana tissue culture consists of three distinct phases: globular, scutellar, and coleoptilar embryos. Figure 4b showed the globular phase with the characteristic by the appearance of small white round-shaped nodules on the surface of the explant. Gokul *et al.* (2023) reported that the globular embryo in the Nendran banana cultivar initially appears as transparent and round, followed by maturation and transformation into a white, and round shape.

In the scutellar phase, the embryo exhibits two protrusions on each side, giving it a heart-shaped appearance (Figure 4c). Wehbi *et al.* (2022) reported that scutellar embryos exhibit a protrusion originating from the scutellar epithelium on the dorsal side of the explant, which is subsequently followed by the growth of the scutellum and the development of two wings that enclose the embryo. During the

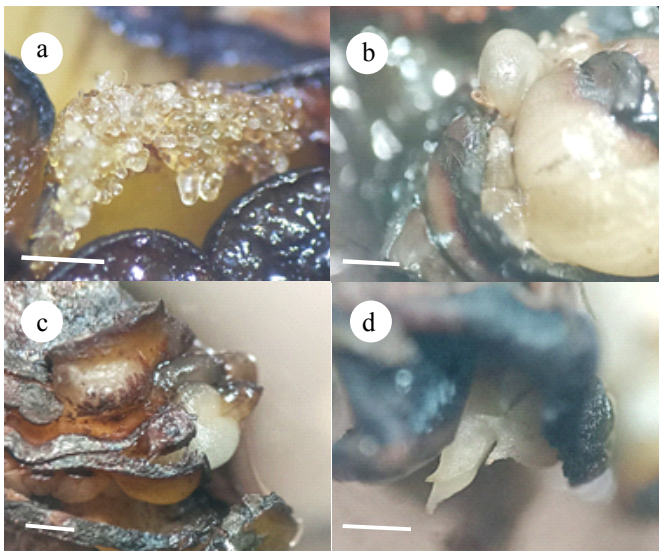
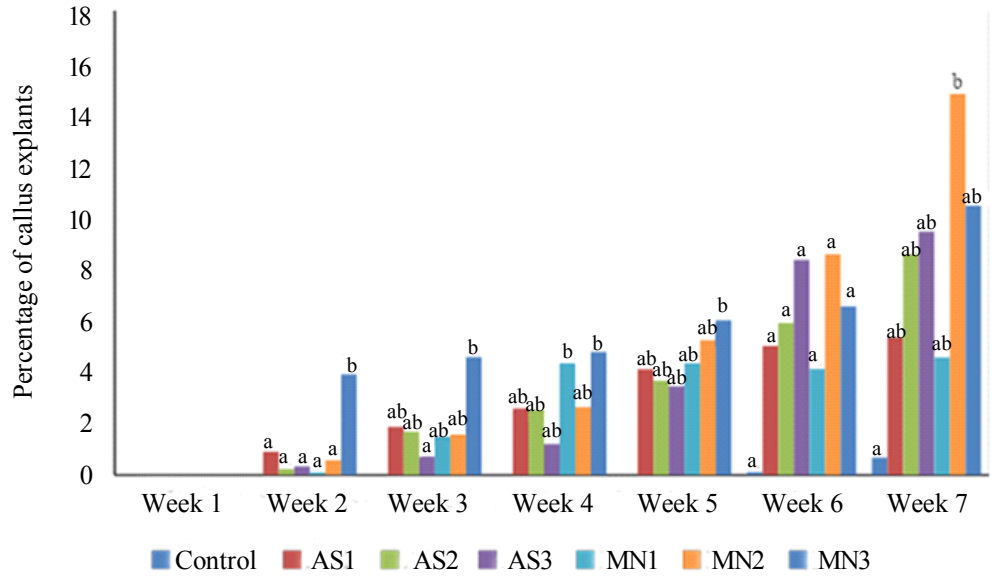


Figure 4. Callus and embryo (a) Embryogenic callus (4x); (b) Globular embryos (2x); (c) Scutellar embryo (1.5x); (d) Coleoptilar embryo (2.5x), Bar: 3 mm

coleoptilar phase, the two protrusions undergo elongation, which subsequently develops into the primordia of shoots and roots (Figure 4d). Enríquez-Valencia *et al*. (2019) additionally reported the existence of coleoptilar embryos during the somatic embryo stage of banana cv. Manzano shows the development of the coleoptilar fissure during its initial phases, followed by the emergence of plumules from the coleoptilar openings during the later stages of this phase.

The addition of ascorbic acid and melatonin in the culture media has a significant effect on the regeneration of explants (Figure 5). The formation of the callus begin to occur in the second week after initiation of the explants in the treatment media. The application of melatonin 14 mg L^{-1} significantly increased callus regeneration during the whole observation period. The application of melatonin with a concentration of 12 mg L^{-1} showed significant increase in callus regeneration. This improvement became detectable in the fifth week and reached its peak in the seventh week compared to other treatments.

Melatonin is involved in the processes of plant growth, development, and adaptability. Melatonin exhibits favorable interactions with phytohormones including auxin, gibberellins, and cytokinins (Erland & Saxena, 2018). The addition of antioxidants such as melatonin at a concentration of 50 mg L^{-1} into the culture media can prevent the accumulation of phenolic compounds. This, in turn, promotes the growth of Grand Naine banana callus throughout the regeneration process (Nandhakumar *et al.*, 2018). Bano *et al.* (2022) also reported that the combination of melatonin 1.5 mg L^{-1} with 2,4-D 1 mg L^{-1} + BAP 1 mg L^{-1} produced the highest fresh weight and dry weight in *Salvia moorcroftiana* callus.



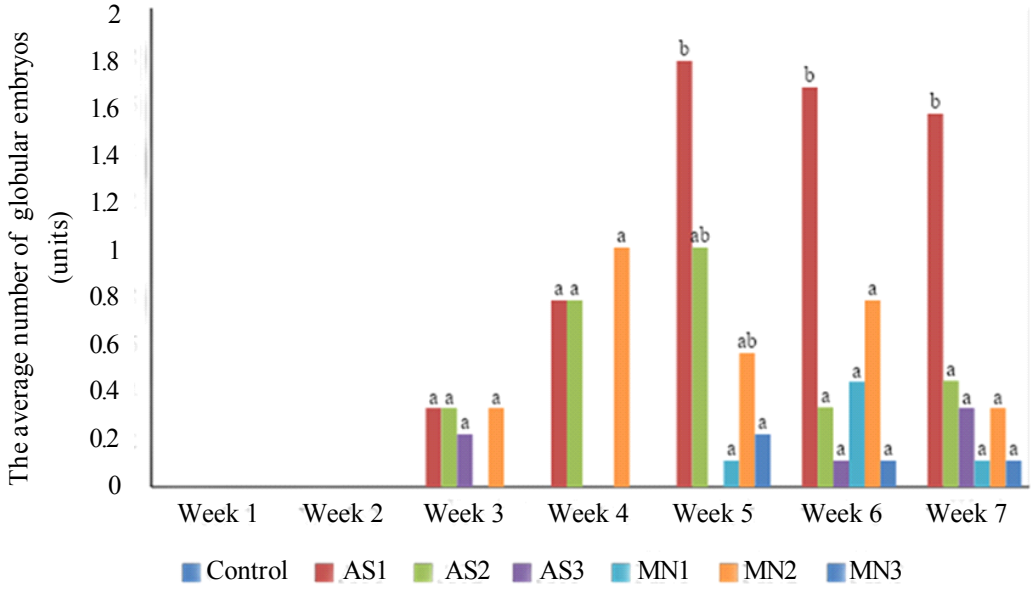
Note: Values followed by the same letters in the same week are not significantly different according to Duncan multiple range test at the 5% significance level. AS1 (ascorbic acid 100 mg L^{-1}), AS2 (ascorbic acid 150 mg L^{-1}), AS3 (ascorbic acid 200 mg L^{-1}), MN1 (melatonin 10 mg L^{-1}), MN2 (melatonin 12 mg L^{-1}), MN3 (melatonin 14 mg L^{-1})

Figure 5. Percentage of callus formation in raja banana flower explants during 7 weeks of observation due to ascorbic acid and melatonin treatments

The application of ascorbic acid resulted in a progressive rise in the proportion of explants exhibiting callus formation every week (Figure 5). The evaluation of callus regeneration in the sixth and seventh weeks revealed that a concentration of 200 mg L^{-1} of ascorbic acid resulted in the highest regeneration rate. Lekshmi *et al.* (2016) reported that the application of 20 mg L^{-1} ascorbic acid effectively decreases browning on explants, leading to an increase in callus formation on banana cv. Nendran. The statistical analysis of ascorbic acid concentration did not show statistically significant differences among the three treatments. Therefore, it was required to enhance the range of the concentration determination.

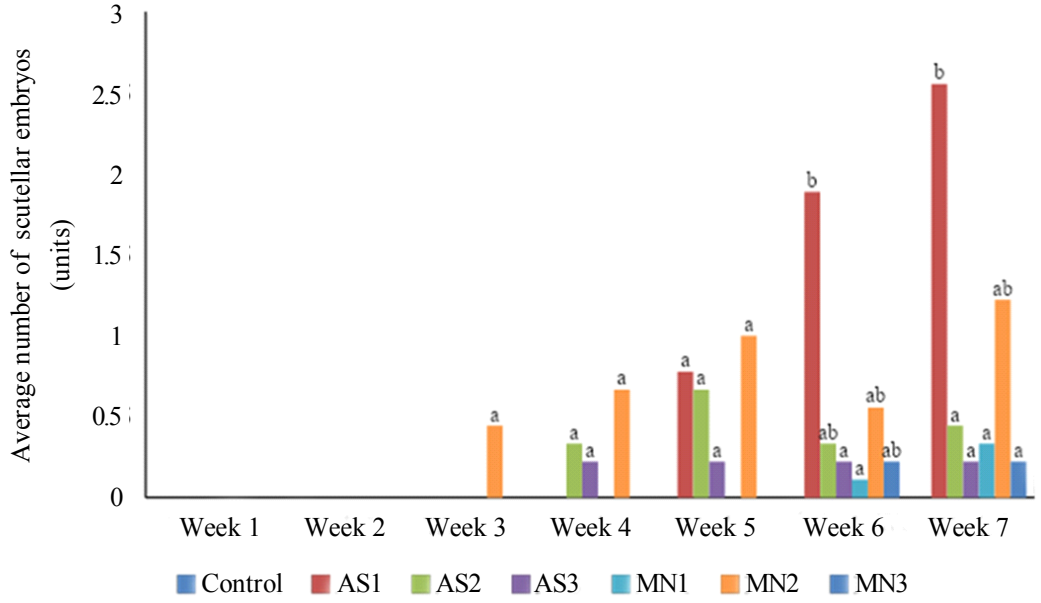
According to the analysis of the data of embryo regeneration, the ascorbic acid treatment with a concentration of 100 mg L^{-1} resulted in the highest average number of globular embryos during the fifth to seventh weeks of observation (Figure 6), as well as scutellar embryos during the sixth and seventh weeks of observation (Figure 7) compared to other treatments. The addition of 11.35 µM ascorbic acid and 100 mg L^{-1} malt extract to liquid MS media produced the highest number of somatic embryos in banana cv. Matti (Smitha & Nair, 2020). Ascorbic acid as a substrate for the enzyme ascorbate peroxidase is closely related to endogenous ascorbic acid. Ascorbic acid regulates cellular metabolism and cell division and is thought to be involved in the maturation of banana somatic embryos (Kumaravel *et al.*, 2020).

The development of the scutellar phase embryo began to appear in the third week of observation (Figure 7). The application of melatonin 12 mg L^{-1} resulted in an alteration of the scutellar phase starting from the early globular phase compared to other treatments.



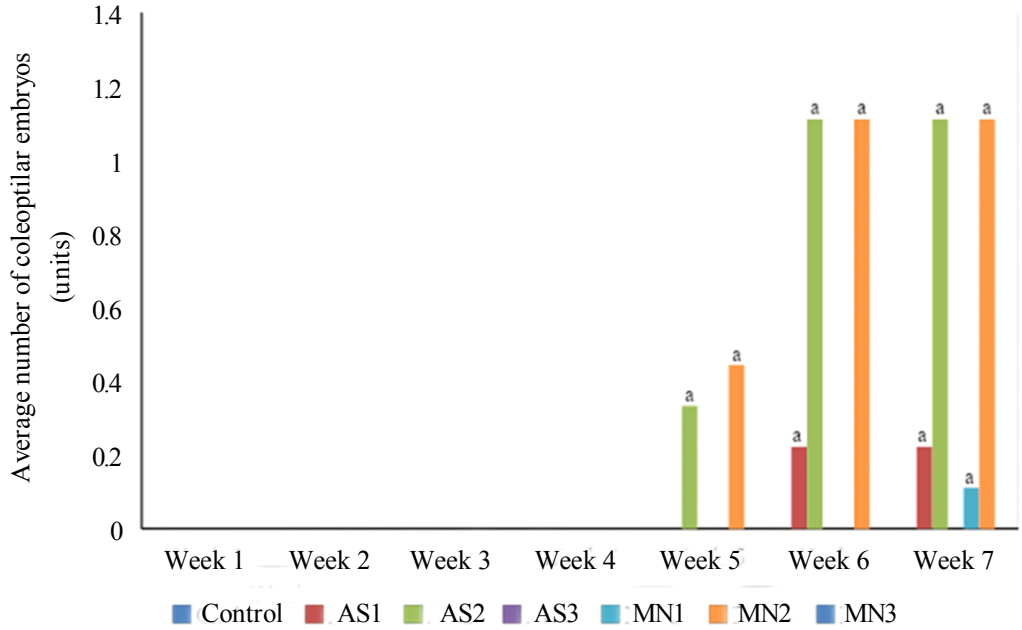
Note: Values followed by the same letters in the same week are not significantly different results by Duncan multiple range test at the 5% significance level. AS1 (ascorbic acid 100 mg L^{-1}), AS2 (ascorbic acid 150 mg L^{-1}), AS3 (ascorbic acid 200 mg L^{-1}), MN1 (melatonin 10 mg L^{-1}), MN2 (melatonin 12 mg L^{-1}), MN3 (melatonin 14 mg L^{-1}).

Figure 6. Average number of raja banana globular embryos during 7 weeks of observation due to ascorbic acid and melatonin treatments



Note: Values followed by the same letters in the same week are not different according to Duncan multiple range test at the 5% significance level . AS1 (ascorbic acid 100 mg L^{-1}), AS2 (ascorbic acid 150 mg L^{-1}), AS3 (ascorbic acid 200 mg L^{-1}), MN1 (melatonin 10 mg L^{-1}), MN2 (melatonin 12 mg L^{-1}), MN3 (melatonin 14 mg L^{-1}) .

Figure 7. Average number of raja banana scutellar embryos during 7 weeks of observation due to ascorbic acid and melatonin treatments



Note: Values followed by the sameletters in the same week are not significantly different according to Duncan multiple range test at the 5% significance level. AS1 (ascorbic acid 100 mg L^{-1}), AS2 (ascorbic acid 150 mg L^{-1}), AS3 (ascorbic acid 200 mg L^{-1}), MN1 (melatonin 10 mg L^{-1}), MN2 (melatonin 12 mg L^{-1}), MN3 (melatonin 14 mg L^{-1}).

Figure 8. Average number of raja banana coleoptilar embryos during 7 weeks of observation due to ascorbic acid and melatonin treatments

Zhang *et al.* (2022) stated that melatonin has a chemical structure and biosynthetic pathway similar to IAA. Melatonin in low concentrations can increase IAA synthesis in plants so it is thought to have a role in the formation of somatic embryos. The addition of 100 µM melatonin in ½ MS medium resulted in a significant enhancement of somatic embryo induction, leading to the production of Arabica coffee up to 62±6 embryos (Ramakrishna *et al*., 2012).

The regeneration of coleoptilar phase embryos did not show significantly different results across all treatments (Figure 8). Elevated level of ascorbic acid and melatonin inhibit the formation of coleoptilar embryo. The transition from scutellar phase to the coleoptilar phase is a lengthy process. This finding is consistent with the study conducted by Enríquez-Valencia *et al*. (2019), which revealed that the development of the initial coleoptilar phase in banana cultures cv. Manzano takes around 60 days and requires an additional 90 days to reach the final coleoptilar phase.

CONCLUSIONS

The application of antioxidants such as ascorbic acid and melatonin did not have a substantial impact on the percentage of viable explants and the level of browning intensity. The addition of ascorbic acid and melatonin to the culture media stimulates the regeneration of callus and somatic embryos. Increased callus regeneration was found in the treatment of melatonin 12 mg L^{-1} and melatonin 14 mg L^{-1}. The highest mean value of globular and scutellar embryos was found in the mg L^{-1} ascorbic acid treatment. The higher concentration of ascorbic acid and melatonin inhibited the formation of coleoptilar embryos.

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