In Vitro Culture of Cavendish Banana (*Musa acuminata* Colla) on Media with Corn (*Zea mays* L.) Seed Extract

Adam Saepudin[1*], Yaya Sunarya[1], Rafly Anggara[1]

[1]Agrotechnology Study Program, Faculty of Agriculture, Siliwangi University Jalan Siliwangi No. 24, PO Box 164, Tasikmalaya 46115. Phone: (0265) 330634 Fax: (0265) 325812

Abstract. One effort to develop Cavendish bananas in response to climate change is the use of superior, uniform seeds with desired traits through tissue culture. In vitro banana culture is determined by various factors, including the concentration of growth regulators. One of the organic materials that are rich in growth regulators is corn (*Zea mays* L.) seeds. This study aimed to determine the effect of corn seed extract concentration on the multiplication of Cavendish banana explants. This research was carried out at the Biotechnology Laboratory, Faculty of Agriculture, Siliwangi University from May to July 2022. This experiment used a Completely Randomized Design (CRD) method, with six treatments and was repeated 5 times. Treatments J0 (without the addition of corn kernel extract), J1 (4 ppm corn seed extract), J2 (6 ppm corn seed extract), J3 (8 ppm corn seed extract), J4 (10 ppm corn seed extract) and J5 (12 ppm corn kernel extract). Observational data were analyzed using variance and further tested with Duncan's multiple-distance test with a significance level of 5%. The results showed that the addition of corn kernel extract affected the number of shoots at the age of 28, 42 and 56 days after planting (DAP), shoot length at the age of 14 DAP, and the number of leaves at the age of 56 DAP. The highest number of shoots per bottle and the highest number of leaves were produced from the medium supplemented with 10 ppm corn seed extract. It is hoped that the results of this study will reduce the use of synthetic growth regulators, making them more environmentally friendly and contributing to the supply of high-quality seeds that can adapt to environmental changes.

1 Introduction

Currently, there are over 1,000 edible banana varieties. The Cavendish variety is one of the most widely consumed and dominates the global banana market, accounting for nearly half of international banana production [1]. Cavendish bananas are extensively cultivated worldwide because of their high productivity, good fruit quality, and resistance to lodging due to their relatively short pseudostems.

Banana production in Indonesia reached approximately 8.18 million tons in 2020, with East Java, West Java, and Lampung as the major producing provinces [2]. Despite this high production, Indonesia's banana export volume remains relatively low compared with other Asian countries such as the Philippines, India, China, and Thailand [3].

Increasing banana production requires a sufficient supply of high-quality planting materials. Because conventional vegetative propagation is relatively slow, in vitro culture has become an efficient method for mass propagation of banana seedlings. Plant tissue culture involves culturing cells, tissues, or organs on artificial nutrient media under aseptic conditions and consists of initiation, multiplication, elongation, rooting, and acclimatization stages [4]. Among these stages, multiplication is particularly important because it enables rapid production of large numbers of shoots from a single explant. Shoot proliferation during this stage is largely regulated by the balance between auxins and cytokinins supplied in the culture medium [5].

Although synthetic plant growth regulators (PGRs) effectively promote shoot development, their relatively high cost has encouraged the search for natural alternatives. Young corn kernels contain natural cytokinins (zeatin) as well as auxins and gibberellins, making them a potential organic source of PGRs for tissue culture [6,7]. Therefore, this study aimed to determine the optimum concentration of young corn kernel extract (*Zea mays* L.) for in vitro shoot multiplication of Cavendish banana (*Musa acuminata* Colla).

2 Material method

2.1. Making corn seed extract

The corn used was hybrid corn harvested at 70 days after planting and characterized by soft kernels that were easily crushed. Corn kernel extract was prepared : (a) selecting corn ears with soft kernels that could be easily crushed using a fingernail; (b) separating the

*Corresponding author: adamsaepudin@unsil.ac.id

kernels from the cob; (c) blending 100 g of kernels with 100 mL of sterile distilled water to obtain the extract; and (d) filtering the extract through a sieve and storing it in a sterile container.

2.2. In vitro banana culture

The explants used were sterile explants from in vitro Cavendish banana cultures initiated from shoot-tip cultures on Murashige and Skoog (MS) medium supplemented with 2 ppm BAP and subcultured on the same medium for 8 weeks. The planting procedure followed the method described by Ratnasari et al. [9]. Planting was performed under aseptic conditions in a laminar airflow cabinet (LAF). All instruments, including scalpels, forceps, culture bottles, and Petri dishes, were sterilized by immersion in 96% ethanol followed by flaming over a Bunsen burner. Stem explants were removed from the culture bottles, transferred to sterile Petri dishes, and cut vertically into approximately 0.5 cm segments using a sterile scalpel. The explants were cultured on MS basal medium supplemented with 1 ppm BAP, 0.5 ppm ascorbic acid, and corn kernel extract according to the treatments: J0 = 0 ppm, J1 = 4 ppm, J2 = 6 ppm, J3 = 8 ppm, J4 = 10 ppm, and J5 = 12 ppm. The experiment was arranged in a Completely Randomized Design (CRD) consisting of six treatments with five replications, and two explants were cultured in each bottle. The culture bottles were sealed with plastic caps and cling film, labeled, and incubated on culture racks under continuous illumination (40-W fluorescent lamps, 24 h photoperiod) at 18–22°C. Observations were conducted on the time to shoot emergence, number of shoots, shoot height, number of leaves, and number of roots.

The experiment was arranged in a Completely Randomized Design (CRD) consisting of six treatments and five replications, with two explants cultured per experimental unit. The observed variables included the time to shoot emergence, number of shoots, shoot height, number of leaves, and number of roots. Data were tabulated using Microsoft Excel 2021 and subjected to one-way analysis of variance (ANOVA) at the 5% significance level. When the ANOVA indicated significant differences among treatments, Duncan's Multiple Range Test (DMRT) at the 5% significance level was performed to compare treatment means. Results are presented as treatment means, and values followed by the same letter are not significantly different according to DMRT at $P < 0.05$.

3 Result and Discussion

3.1. Time to shoot emergence per bottle

The analysis of variance (ANOVA) showed that the concentration of corn kernel extract had no significant effect ($P > 0.05$) on the time required for shoot emergence (Table 1).

Table 1. Effect of corn kernel extract concentration on the time to shoot emergence per bottle.

Corn kernel extract concentration	Time to shoot emergence (DAP)
J0 (control) = 0 ppm	7.8 a
J1 = 4 ppm	7.8 a
J2 = 6 ppm	7.3 a
J3 = 8 ppm	6.7 a
J4 = 10 ppm	6.3 a
J5 = 12 ppm	6.2 a

Note: Values followed by the same lowercase letter are not significantly different according to Duncan's Multiple Range Test (DMRT) at the 5% significance level.

As shown in Table 1, explants from all treatments successfully produced shoots (Figure 1). Shoot initiation occurs through cell differentiation within the explant, resulting in tissue thickening followed by the formation of shoot primordia [9]. The absence of a significant effect of corn kernel extract concentration on the time to shoot emergence may be attributed to the endogenous hormone content of the explants. Endogenous plant growth regulators are believed to be sufficient to induce initial shoot development [10]. The response of explants to exogenous growth regulators depends on their physiological condition and endogenous hormone levels [11,12].

Shoot induction is primarily regulated by cytokinins. A high cytokinin-to-auxin ratio promotes cell division and stimulates shoot initiation. Cytokinins play a central role in inducing shoot formation during in vitro culture, and an appropriate balance between endogenous and exogenous cytokinins enhances shoot development [13].

Fig. 1. Shoot emergence of Cavendish banana explants cultured on MS medium supplemented with 12 ppm corn kernel extract.

3.2. Number of shoots per bottle

The statistical analysis showed that the concentration of corn seed extract significantly affected the number of shoots at 28, 42, and 56 days after planting, but had no significant effect at 14 days after planting (Table 2).

Table 2. Effect of corn seed extract concentration on the number of shoots per bottle

Corn seed extract concentration	Number of shoots per bottle			
	14 dap	28 dap	42 dap	56 dap
J0= 0 ppm (control)	2,5 a	5,2 a	6,2 a	6,7 a
J1= 4 ppm	3,6 a	6,4 ab	9,2 ab	10,3 b
J2= 6 ppm	4,7 a	7,8 abc	9,8 ab	10,6 b
J3= 8 ppm	6,8 a	8,9 bc	10,6 b	12,1 bc
J4= 10 ppm	5,5 a	10,1 c	12 b	14,5 c
J5= 12 ppm	5,3 a	7,8 abc	9,8 ab	10,7 b

Note: Numbers followed by the same lowercase letter in a column indicate no significant difference according to Duncan's Multiple Range Test at the 5% level.

Table 2 shows that the concentration of corn seed extract had no effect on the number of shoots per bottle observed 14 days after planting. This is due to the simultaneous emergence of shoots in each treatment. Furthermore, explants require time to absorb nutrients from the media [8].

Observations 28 days after planting showed that a concentration of 10 ppm corn seed extract produced a higher number of shoots (Figure 2 and Figure 3), with an average of 10.1 shoots per bottle. However, these results were only significantly different from the control treatment without corn seed extract and with a concentration of 4 ppm corn seed extract. This is because the 10 ppm corn seed extract concentration has the correct and effective cytokinin ratio for shoot formation. Corn seed extract contains zeatin, a type of natural cytokinin that plays a role in shoot formation [6,7].

Observations 42 days after planting showed that the corn seed extract treatment affected the number of shoots per bottle. The 10 ppm corn seed extract concentration in the media produced a higher number of shoots, with an average of 12 shoots per bottle. However, this value was not significantly different from the other corn seed extract concentration treatments and was only significantly different from the control treatment. Meanwhile, corn seed extract concentrations of 4 ppm, 6 ppm, and 12 ppm were not significantly different from the control treatment without corn seed extract. This may be due to the cytokinin ratio being less suitable for explant growth. Furthermore, different endogenous hormone levels in explants can also influence shoot formation [5].

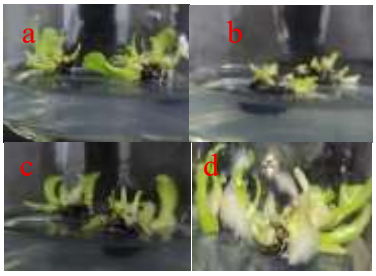

Fig. 2. Shoot growth in the treatment of adding 10 ppm corn seed extract concentration at; (a) 14 dap; (b) 28 dap; (c) 42 dap; and (d) 56 dap.

Observations 56 days after planting (DAP), with a corn seed extract concentration of 10 ppm, showed a higher average shoot count of 14.5 shoots per bottle, but this was not significantly different from the 8 ppm corn seed extract treatment. The lowest average shoot count occurred in the control treatment, with an average of 6.7 shoots per bottle. This indicates an effect of the addition of corn seed extract on the number of shoots per bottle. The addition of exogenous growth regulators to media with low auxin concentrations, higher cytokinins, and appropriate concentrations can result in good shoot formation and shoot growth [5,13]. Corn seed extract contains several important growth regulators that can stimulate shoot growth, including cytokinin hormones. Adding corn seed extract at a concentration of 10 ppm is thought to meet the need for exogenous growth regulators. A high ratio of endogenous and exogenous cytokinin hormone concentrations stimulates shoot growth by encouraging cell division [13]. Furthermore, the ratio between several types of hormones can also influence the number of shoots formed [4].

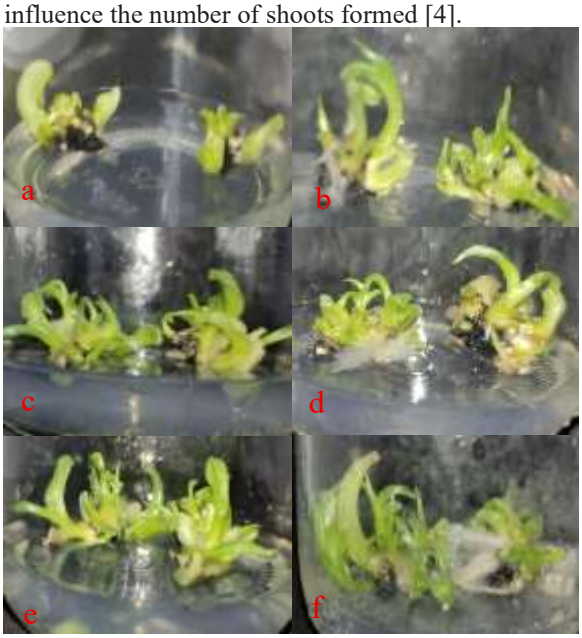

Fig. 3. Number of shoots for each treatment at 56 days after planting. (a) J0 = control; (b) J1 = 4 ppm corn kernel extract; (c) J2 = 6 ppm corn kernel extract; (d) J3 = 8 ppm corn kernel extract; (e) J4 = 10 ppm corn kernel extract; (f) J5 = 12 ppm corn kernel extract.

3.3. Shoot Height

The results of statistical analysis showed that the concentration of corn seed extract had a significant effect on shoot height at 14 days after planting, but had no significant effect at 28, 42 and 56 days after planting (Table 3).

Table 3. Effect of corn seed extract concentration on shoot height.

Corn seed extract concentration	Shoot height			
	14 dap	28 dap	42 dap	56 dap
J0 = 0 ppm (control)	3.62 a	5.69 a	6.90 a	8.71 a
J1 = 4 ppm	3.98 a	6.72 a	8.18 a	10.02 a
J2 = 6 ppm	6.20 b	7.93 a	9.53 a	11.98 a
J3 = 8 ppm	4.29 a	7.26 a	8.74 a	10.39 a
J4 = 10 ppm	4.82 ab	7.29 a	9.19 a	10.16 a
J5 = 12 ppm	5.14 ab	7.09 a	9.40 a	10.61 a

Note: Numbers followed by the same lowercase letter in a column indicate no significant difference according to Duncan's Multiple Range Test at the 5% level.

Table 3 shows that the concentration of corn seed extract affected shoot height observed 14 days after planting. A corn seed extract concentration of 6 ppm showed higher yields, with an average shoot height of 6.20 mm, but this was not significantly different from the 10 ppm and 12 ppm treatments. These results are thought to be influenced by the appropriate ratio of cytokinin and auxin hormones, which can influence shoot height. Another factor influencing shoot height is shoot number. Consistent with [8], shoot height growth tends to decrease with the greater number of shoots growing on an explant.

At 28, 42, and 56 days after planting, the corn seed extract concentration showed no effect. This shoot elongation process is stimulated by cytokinin and auxin hormones at appropriate concentrations [4,5]. Auxin plays a role in promoting cell elongation by increasing the size of the cell wall and the number of cells in the apical meristem, while cytokinin promotes cell division at the shoot tip [5]. This increase in shoot size encourages the growth of other organs, thus forming a complete plant or plantlet [4]. High cytokinin levels stimulate shoot formation in explants. Increasing the number of shoots in explants inhibits shoot elongation. This is because the use of nutrients in the media prioritizes the formation of potential shoots, which can inhibit shoot height [13].

3.4. Number of leaves

Statistical analysis showed that corn seed extract concentration significantly affected leaf number at 56 days after emergence, but had no significant effect at 28 and 42 days after emergence (Table 4). The number of leaves observed was based on leaves growing on open explants. Once the shoots are fully formed, the explants will stimulate leaf formation, stimulated by the cytokinin hormone [4].

Table 4. Effect of corn seed extract concentration on the number of leaves.

Corn seed extract concentration	Number of leaves		
	28 dap	42 dap	56 dap
J0 = 0 ppm (control)	0.75 a	1.15 a	1.70 a
J1 = 4 ppm	0.60 a	1.35 a	2.10 a
J2 = 6 ppm	1.15 a	2.10 a	3.30 ab
J3 = 8 ppm	1.05 a	1.95 a	3.15 ab
J4 = 10 ppm	1.15 a	2.55 a	4.95 b
J5 = 12 ppm	1.10 a	2.25 a	4.15 b

Note: Numbers followed by the same lowercase letter in a column indicate no significant difference according to Duncan's Multiple Range Test at the 5% level.

Table 4 shows that the concentration of corn seed extract had no effect on leaf number at 28 and 42 days after emergence. This is suspected because leaf formation in explants requires a longer time. Furthermore, the continuous formation of microshoots in explants inhibits leaf formation due to the energy being focused on shoot formation, so the more shoots that emerge, the more difficult leaf differentiation becomes [13].

At 56 days after emergence, the concentration of corn seed extract affected leaf number. Corn seed extract concentrations of 10 ppm and 12 ppm resulted in a higher average number of leaves (Figure 4). The cytokinin hormone in corn seed extract stimulates shoot formation, which then differentiates into leaves. This is in line with [4], who stated that the addition of corn seed extract containing zeatin in an appropriate ratio can increase leaf number.

Fig. 4 Leafy explants in the treatment of 10 ppm corn seed extract concentration at the age of 56 dap

3.5. Root Number

The results of statistical analysis showed that the concentration of corn seed extract had no significant effect on the number of roots at 28, 42, and 56 days after planting (Table 5, Figure 5).

Table 5. Effect of corn seed extract concentration on the number of roots.

Corn seed extract concentration	Number of roots		
	28 dap	42 dap	56 dap
J0 = 0 ppm (control)	0.20 a	0.20 a	0.30 a
J1 = 4 ppm	0.15 a	0.30 a	0.50 a
J2 = 6 ppm	0.35 a	0.60 a	0.90 a
J3 = 8 ppm	0.55 a	1.05 a	1.85 a
J4 = 10 ppm	0.60 a	0.90 a	1.50 a
J5 = 12 ppm	0.55 a	0.95 a	1.50 a

Note: Numbers followed by the same lowercase letter in a column indicate no significant difference according to Duncan's Multiple Range Test at the 5% level.

Table 5 shows that corn seed extract had no effect on root number. Root growth in explants is influenced by various factors, including the content of plant growth regulators. According to [12], root growth can be stimulated by the hormone auxin in the medium. A higher ratio of cytokinin to auxin in corn seed extract can inhibit root growth [13]. The antagonistic interaction between cytokinin and auxin affects differentiation in explants. Higher levels of cytokinin promote shoot formation and inhibit auxin biosynthesis, which plays a role in root formation. Conversely, higher levels of auxin promote root formation and inhibit cytokinin biosynthesis, which is involved in shoot formation [5].

Fig. 5 (a) Rooted explants in the treatment with 8 ppm corn seed extract concentration; (b) Rooted explants in the control treatment without the addition of corn seed extract.

Root emergence in each treatment is thought to be due to the presence of endogenous auxin in the explants. Consistent with [5], explants are able to form their own roots due to endogenous auxin. Furthermore, root growth occurs due to the explant's own tendencies. Most explants that have formed shoots will be able to form roots. Therefore, the cytokinin hormone factor can also aid in shoot formation. According to [13], cytokinins can stimulate physiological processes that support root formation under certain conditions.

Conclusion

Corn seed extract affected the number of Cavendish banana shoots at 28, 42, and 56 days after planting, shoot length at 14 days after planting, and leaf number at 56 days after planting, but had no effect on root number. Corn seed extract at a concentration of 10 ppm in MS medium produced a higher number of shoots per bottle and a higher number of leaves than other corn seed extract concentrations. Further research is needed by combining corn seed extract with the plant growth regulator auxin to promote root regeneration in the resulting shoots.

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