Effect of bean sprout on i*n vitro* multiplication of *Musa acuminata*

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Abstract. *Musa acuminata* or Pisang Berangan is popular in Kelantan, but due to a lack of knowledge on modern farming technologies, many *Musa acuminata* are affected by diseases and are of low quality. Plant tissue culture has numerous advantages, including a rapid rate of multiplication, and the prevention of disease. Plant growth regulator such as Benzyl Amino Purine (BAP) is commonly used in media to grow banana explants, however, Murashige and Skoog (MS) media production with BAP hormone is expensive for low-income farmers. Therefore, this research had been done by substituting the BAP hormone by using bean sprout extract as an organic supplement for banana culture. The additives that were used in this project were non-centrifuged and centrifuged bean sprout extracts at different concentrations, which were 5%, 10%, 15% and 20% applied in the MS media. Data were collected based on number of leaves, length of leaves and length of roots. In this study, the positive control treatment with MS + 5 mg/L BAP media recorded the highest mean value in the number of leaves, length of leaves, and length of roots with values of 12.5 ± 0.59, 5.69 ± 0.13cm, and 6.45 ± 0.36cm respectively followed by MS media with centrifuged bean sprout extract. From the observation, the bean sprout has the potential to use as an additive for *Musa acuminata* media to substitute synthetic hormones such as BAP.

1 Introduction

Bananas are a tropical fruit that is popular all over the world and are a staple food in many countries. It is believed to be originated in Southeastern Asia, specifically in India, the Philippines, and Malaysia. *Musa acuminata,* often known as Pisang Berangan, is a Malaysian banana variety that belongs to the Musaceae family[1]. Pisang Berangan is high in potassium, which helps to maintain fluid balance in the body, fibre, which helps to accelerate digestion, and Vitamin C, which helps to improve the immune system while lowering inflammation.

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Eating bananas also helps in lowering blood pressure and may reduce the risk of cancer [1].

In Kelantan, the number of consumptions was high due to the nature of staple food. Bananas are in high demand in Kelantan due to a lack of experience in the employment of modern technology, and the people of Kelantan prefer to cultivate bananas using traditional ways [2]. Traditional propagation methods could cause diseases in bananas, therefore banana production became low [2]. Production of bananas in Kelantan could be stable if used tissue culture method. Tissue culture was a technique for cultivating plants in a laboratory environment using the tissue or cells of a specific plant. Tissue culture has been suggested as an effective method of producing disease-free, high-quality planting material as well as the quick production of huge numbers of uniform seedlings [3].

Media treatments mostly used chemicals such as BAP for hormones, but to that, making MS media is quite costly. Thus, an alternative additive such as bean sprouts that are low in cost had been investigated to promote the banana tissue culture. Bean sprouts were cheap and easy to get at any type of market or store nearby. Bean sprouts also had vitamins and minerals that support growth performance and multiplication of plantlets. Vitamins and minerals found in bean sprout extract include Vitamin C, Thiamin, Riboflavin, Niacin, Vitamin B, Vitamin A, and Vitamin E. whereas, minerals found in bean sprouts include Calcium (Ca), Ferum (Fe), Magnesium (Mg), Phosphorus (P), Sodium (Na), Zinc (Zn), Copper (Cu) dan Mangan (Mn). It also contained auxin and diphenyl urea, which serves as a cytokinin [4]. The vitamins and mineral composition of bean sprout extract indicates that it could be employed in tissue culture to produce plantain explants [5].

A study from the University of Brawijaya at East Java, Indonesia, was using bean sprout extract on *Physalis angulata L.* and focused on shoot regeneration. Effect of bean sprout extract also used on *in vitro* shoot multiplication of *Colocasia esculenta L* (Japanese Taro) [6]. This study happens at the University of Hassanudin, South Sulawesi, Indonesia. Bean sprout extract is also used in types of orchids such as *Dendrobium* sp [7]. The objective of this research was to study the effect of bean sprout extract on *in vitro* multiplication of *Musa acuminata* plantlets.

2 Materials and Method

2.1 Plant Material

The *Musa acuminata* (Pisang Berangan) sucker had been collected from the banana plantation area at Ayer Lanas, Kelantan. The suitable sucker for this study was 3 – 4-month-old from the mother plant and in healthy and free disease condition [8]. The suckers were washed under tap water for 30 min and the outer layer was removed carefully. Then it was rinsed 3 times with distilled water. The explants were washed with sterilized distilled water three times and rinsed for 5 minutes, followed by soaking in Mercuric chloride (0.1%) and Ethanol (70%) for 2 minutes intervals [9]. In the final step, the suckers were again washed with sterilized distilled water three times and were trimmed, cut and cultured in MS media with different concentrations of bean sprout.

2.2 Preparation of bean sprout extract by non-centrifuged and centrifuged method

The *Vigna radiata* (mung bean) was purchased from the local market and soaked for 24 hours using tap water. The beans were drained and spread using a wet cloth on an empty tray and placed in a dark room for 2 days. 500 g of bean sprout was taken and blended with 100 ml distilled water, before being filtered using a filter cloth size five with 4000 microns. The non-centrifuged bean sprout extract had been stored in the fridge for further use. For centrifuged bean sprout extract, the extract was further centrifuged at 5000 rpm for 3 minutes at a normal temperature of 26-27ºC and the supernatant was collected for further use [10].

2.3 Media preparation

MS media containing 7.5g of sucrose, 1g of gelrite and different concentrations of bean sprout extract namely 5%, 10%, 15% and 20% were prepared. Negative control (without bean sprout extract) and positive control (addition of 5mg/L BAP) media also were prepared as in Table 1. The media were set to pH 5.7 before being sterilised in an autoclave for 15 minutes at 15 psi and temperature to 121ºC.

Table 1. Experimental design of addition of bean sprouts extract in MS media

Media	Concentration	Treatment code
Positive control	MS + 5 mg/L BAP	T1
Negative control	MS	T2
Non-centrifuged bean sprout (NCBS)	MS + 5% NCBS	T3
	MS + 10% NCBS	T4
	MS + 15% NCBS	T5
	MS + 20% NCBS	T6
Centrifuged bean sprout (CBS)	MS + 5% CBS	T7
	MS + 10% CBS	T8
	MS + 15% CBS	T9
	MS + 20% CBS	T10

2.4 Data collection and statistical analysis

The Complete Randomized Experimental Design (CRD) was used in this study. The data had been acquired over one month by examining the banana plantlet's height, number of leaves, and root length. The entire data were analysed by using Statistical Package for Social Science (SPSS) and the mean value of the treatment was analysed by using Analysis of Variance (ANOVA).

3 Results and discussion

3.1 Effect of bean sprout extract on *in vitro Musa acuminata*

The plantlets of *Musa acuminata* were cultured on MS media with the addition of bean sprout extract as a substitute for the growth regulator. The growth of the plantlets in terms of length of leaves, number of leaves and length of roots depending on the type of treatment at the different concentrations of the bean sprout extract was determined. The bean sprout extract that was used in the treatment was 5%, 10%, 15% and 20% applied in the MS media respectively. The additive supplement of bean sprout extracts shows significantly reduce

growth of the *Musa acuminata* when compared with the positive control treatment. The effect of treatments was shown and summarised in Table 2.

Table 2. The effect of difference concentration of bean sprout (%) on *in vitro* propagation of *Musa acuminata* in MS media

Treatment code	MS Media	Number of Leaves	Length of Leaves (cm)	Length of Roots (cm)
T1 (+ve control)	MS + 5 mg/L BAP	12.5 ± 0.587a	5.69 ± 0.132a	6.45 ± 0.361a
T2 (-ve control)	MS	8.00 ± 0.299bc	2.84 ± 0.156c	2.23 ± 0.068b
T3	MS + NCBS (5%)	0.18 ± 0.083cd	2.66 ± 0.111c	0.85 ± 0.045d
T4	MS + NCBS (10%)	0.17 ± 0.078d	2.48 ± 0.120c	0.79 ± 0.063d
T5	MS + NCBS (15%)	0.09 ± 0.052d	2.67 ± 0.095c	0.85 ± 0.045d
T6	MS + NCBS (20%)	0.12 ± 0.059d	3.13 ± 0.169b	0.82 ± 0.062d
T7	MS + CBS (5%)	7.70 ± 0.703bc	2.93 ± 0.211c	1.35 ± 0.089c
T8	MS + CBS (10%)	5.85 ± 0.590bc	3.71 ± 0.136b	1.20 ± 0.128cd
T9	MS + CBS (15%)	9.95 ± 0.343b	3.64 ± 0.070b	2.00 ± 0.014b
T10	MS + CBS (20%)	9.93 ± 0.252b	3.83 ± 0.207b	1.22 ± 0.033cd

Data presented as the mean ± standard error (SE). Value in columns with similar superscripts is not significantly different at ($p < 0.05$) according to Duncan's multiple range test. The experiment of *Musa acuminata* was examined after 2 months of culture. Each treatment consisted of 5 replicates of different treatments that contained 3 plantlets of *Musa acuminata.* NCBS = non-centrifuged bean sprout extract, CBS = centrifuged bean sprout extract.

Based on the result in Table 2., the highest number of leaves was obtained in positive control media which is MS media containing 5mg/L BAP (12.5 ± 0.587), followed by MS media with the addition of 15% and 20% centrifuged beans sprout extract (9.95 ± 0.343 and 9.93 ± 0.252 respectively). MS media without the addition of any additional substances (T2) have not significantly different from MS with 5% and 10% centrifuged bean sprout extract (7.70 ± 0.703 and 5.85 ± 0.590 respectively). MS media with non-centrifuged bean sprout extract showed the lowest number of leaves in *Musa acuminata* plantlets.

The highest length of *Musa acuminata* leaves was found in MS media with 5mg/L BAP with 5.69 ± 0.132cm which has a significant difference with all other treatments (Fig.1.). MS media with 10%, 15%, 20% centrifuged bean sprout extract and 20% non-centrifuged bean sprout extract (3.71 ± 0.136, 3.64 ± 0.070, 3.83 ± 0.207 and 3.13 ± 0.169 respectively) have been as a second highest for length of leaves after positive control and have a potential to use as a substitute for BAP. The result also showed that MS media without any additional substances (negative control) have not significantly different from MS media with non-centrifuged bean sprout extract (5%, 10%, and 15%) and MS media with 5% centrifuged bean sprout extract. The highest length of *Musa acuminata* root was found in MS media with 5mg/L BAP with 6.45 ± 0.361cm followed by MS without any additional substances (2.23 ±

0.068) and MS with 15% centrifuged bean sprout extract (2.00 ± 0.014). The lowest length of the root was found in all MS media with non-centrifuged bean sprout extract.

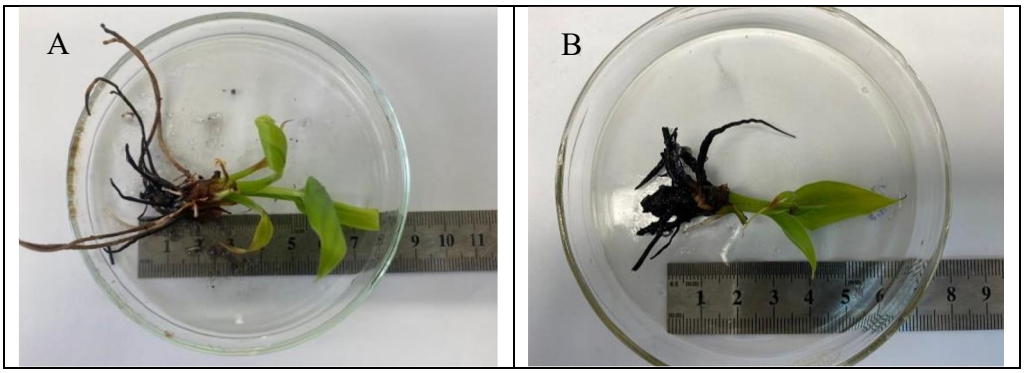

Fig. 1. *Musa acuminata* plantlet. A) MS + 5mg/L BAP, B) MS + 15% centrifuged bean sprout extract

The result showed that centrifuged bean sprout extract has the potential to use as an organic substance in *in vitro* culture of *Musa acuminata* to improve the growth of the plantlets although the effect was not as good as the addition of BAP hormone that performed very well in all the parameters tested. According to [5], many vitamins and minerals are found in bean sprout extracts such as Vitamin C, Thiamin, Riboflavin, Niacin, Vitamin B, alpha-carotene acid, Vitamin A, and Vitamin E. As opposed to this, Calcium (Ca), Ferum (Fe), Magnesium (Mg), Phosphor (P), Sodium (Na), Zinc (Zn), Copper (Cu), and Manganese (Mn) are the minerals found in bean sprouts that can help *Musa paradisiaca* grow more quickly.

According to [11], the treatment that supplements with bean sprout extracts shows positive results in the number of leaves since it contains a high number of vitamins and other substances for *Musa paradisiaca*. Natural additives like bean sprout extracts help accelerate the growth of the explant due to its high nutritional content and hormones substance in the bean sprout extract include auxin, which aids in promoting the growth of the plantlets, and diphenyl urea, which serves as a cytokinin [4].

Bean sprouts also contained an essential vitamins for all plant tissue cultures such as Thiamin (Vitamin B1), which [11] state is crucial for nearly all plant tissue cultures. Thiamin's function in the root meristem was to promote cell divisions and act as a coenzyme in reactions that generate energy. According to [11], the auxin effect may boost protein synthesis, osmosis, and cell permeability toward water, allowing water to enter the cell and increase cell volume. In low concentrations, auxin also can stimulate the growth of roots [12].

Based on the results, the addition of non-centrifuged bean sprout extract in MS media did not promote the growth performance of *Musa acuminata.* This is because bean sprout that has been germinating and put in the media without being properly filtered maybe has active bacteria after a few weeks that can contaminate the plants and the media. According to [13], bean sprouts may contain bacteria or mould if not contained properly. Bean sprouts can be stored in the refrigerator and can last for 2 to 3 weeks before turning bad.

4 Conclusion

In conclusion, the addition of 15% centrifuged bean sprout extract in MS media has the potential to use as an organic substance *in vitro* culture of *Musa acuminata* to improve the number of leaves, length of leaves and length of the root of *Musa acuminata* plantlets although the effect was not as good as the addition of BAP hormone that performed very well in all the parameters tested.

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