Study on The Response of Different Cytokinins in Micropropagation of Kolikuttu (Silk) Banana (*Musa* Spp.)

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Introduction

Kolikuttu is one of the most preferred banana varieties. But the planting materials are less available to the farmers and are usually limited to dry-zone. Therefore, the most appropriate option is to multiply Kolikuttu banana plantlets by macro propagation using tissue culture techniques. However, Kolikuttu banana shows least responses in multiplication under in-vitro conditions. In concern with the huge demand for Kolikuttu banana plantlets it is of immense importance to identify the suitable protocol to increase the multiplication rate of Kolikuttu banana under in-vitro conditions. Shoot multiplication depends on the type and level of cytokynin used in the medium, therefore this research study was focused to identify the most suitable type of cytokinin and its concentrations to enhance the multiplication rate of silk banana.

Materials and methods

Sources of Explant

Vigorous sward suckers of Silk banana were collected from a field grown cultivation situated at Anuradhapura. The apical buds were taken as the ex-plants in 2 cm x 2 cm base with a 2.5 cm height to establish in cultures.

Culture Medium and Culture Conditions

Basic Murashige and Skoog (1962) (MS) was prepared and supplemented with myo-inositol 100 mg/L, ascorbic acid 100 mg/L, sugar 30 g/L, and IAA 1 mg / L. Twelve different treatments with cytokinins were added to the basic medium as in Table 3.1. At a rate 3 g/L agar was added to solidify the media. The pH was adjusted to 5.8 $\pm$ 0.1. Aliquots of 35 mL of the culture media were dispensed into glass vessels (6 cm diameter, 10 cm height). Vessels were plugged and autoclaved at 120 kPa at 121^{0}C for 20 minutes.

Table 3.1 Different treatments used in the experiment

Treatment no.	Hormone/ Combination	Concentration (mg/L)
T_1	BAP	5
T_2	BAP	6
T_3	BAP	7
T_4	Adenine sulfate + BAP	5
T_5	Adenine sulfate + BAP	6
T_6	Adenine sulfate + BAP	7
T_7	2ip	2
T_8	2ip	4
T_9	2ip	5
T_{10}	Kinetin	4
T_{11}	Kinetin	5
T_{12}	Kinetin	6

Explants were dipped over night in Captan. All the outer layers of the corm tissue and the pseudostem were removed and the size of the banana shoot tips were reduced up to about 10" height, 5" diameter pieces and the size reduction was continued in the sterile working area. Banana shoot tips of 5 cm height, 3 cm diameter were subjected for surface sterilization. Shoot tips were sterilized using 15% Clorox solution for 15 minutes followed by 0.2% $HgCl_2$ for 3 minutes then quick dip in 70% Ethanol and finally washed thrice with sterilized distilled water. Explants of 2 cm x 2 cm base with a 2.5 cm height were inoculated as single ex-plant per one vessel. Weekly observations were made before and after sub-culturing based on the colour, swollen nature of the explants and the number of buds in the explants. Experiment was set up as Complete Randomized Design (CRD) with three replicates and the data were analyzed through Kruskal-Wallis and ANOVA using MINITAB statistical package. Turkey's test was used for the mean comparison at 5% level of significance.

Results

The used sterilization protocol was successful as it recorded the 92% of survival rate. Best colour score (Score 7; pinkish green) was resulted in the explants established in the media treated with 6 mg/L BAP (T_2) and the values were highly significant ($P = 0.001$) at 5% significant level. Media treated with 5 mg/L BAP and 2 mg/L Adenine Sulfate (T_4) were shown the least score for the colour(Score 2.3; cream). Explants established in the media treated with 6 mg/L BAP (T_2) showed the highest score (score 3; well swollen) for the increment of the size while the media treated with 4 mg/L Kinetin (T_{10}) have also resulted a better size increment (score 2.6) compared other treatments ($P = 0.013$). Explants established in the media treated with 6 mg/L BAP (T_2) showed the highest score (score 3; well swollen) for the swollen nature. Media treated with 5 mg/L Kinetin (T_{11}) was recorded the least score (score 1; no change) for the size increment after the sub culturing phase. The highest number (5) of buds were recorded in the treatment 6 mg/L BAP (T_2) where as no buds were recorded in the treatments (T_1, T_7, T_8, T_{11}) at 5% significant level. Nine replicates showed the activation of the apical bud initiation out of the 36 explants.

Discussion

Just after the establishment of the explants in the culture media all the explants have shown a vigorous growth, but after one month the growth of the explants were retarded and this might be a result of blackening. A very tough black crust like appearance could be observed around the base of the explants. Alahakoon (2003) reported that the media and explants discolouration could be successfully prevented by the addition of ascorbic acid. So the present study has not agreed with the findings of Alahakoon (2003) as 100 mg/L of ascorbic acid was not sufficient to control the blackening in the explants. This might be due to large size of explants or insufficient addition of ascorbic acid. But, Farahani (2008) has reported that ascorbic acid affects the solidity of the media. Therefore, the amount of ascorbic acid added to the media should be of great concern.

Apical bud initiation could be observed in nine treatments, which resulted in reduction of auxiliary bud formation. This might be due to apical dominancy. Longitudinal splitting of the shoots is commonly employed to reduce apical dominance, and to stimulate the production of multiple shoots (Vuyisteke, 1998).

Results of the experiment were shown that the treatment 6 mg/L BAP (T_2) was successfully established. These explants were quickly established and pinkish green colour and shoot base enlargement was observed with in one to four weeks after culture initiation. Successfully established explants resulted in quick bud formation.

The results showed that the types of cytokinines and their concentrations affect multiplication responses among Kolikuttu banana. Arinaitme *et al.* (2000) and Gubbuk and Pekmezci (2004) revealed a significant effect of both types and concentrations of Cytokinins on the multiplication rate. Arinaitme *et al.*(2000) also studied the effect of BAP on banana cultivas. They reported no significant increase in shoot proliferation in ABB types from 1.68 – 2.88 mg/L. In this research, higher number of regenerated shoots was recorded at 6 mg/L BAP (T_2). Previous reports (Kalimuthu et al., 2007) indicated that 5 mg/L of BAP is the optimum cytocinin concentration for most banana tissue culture media and this slightly supports the results of the experiment.

Conclusions

MS medium supplemented with 6 mg/L BAP was the most responsive medium for better establishment and proliferation of Kolikuttu banana as it recorded the highest number of buds (6), best colour (pinkish green) and the highest enlargement during establishment and after subculturing.

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