Micropropagation of Banana Plant (*Musa paradisiaca*) cv. Raja Bulu through Tissue Culture for Diversification of Food and Feed

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ABSTRACT

Banana cv. Raja Bulu is a perennial herbaceous monocot plant which is one of the main fruit crops in Indonesia. Banana plant residues can be used as feed for livestock. In order to produce faster and healthier plant, it is required an efficient micropropagation method. This research was aimed to optimize initiation, multiplication, and regeneration of shoot tip culture. Shoot tips from young suckers of 40-100 cm height were used as explants. From the selected sucker a cube of tissue of about 1-2 cm^3 containing the apical meristem was excised. This block of tissue was dipped in 75% ethanol for 10 s, surface sterilized in a 1% sodium hypochlorite solution for 15 min, and rinsed three times in sterile water for 5 min. Then, a shoot tip of about 3 × 5 mm, consisting of the apical dome covered with several leaf primordial was aseptically dissected. The explant was placed on a multiplication-inducing culture medium which were Murashige and Skoog (MS) based medium supplemented with phytohormone BAP and NAA were used for culture initiation and shoot multiplication. Of various treatment combinations, MS medium + 6 mg/L BAP with 0.2 mg/L NAA showed highest multiple shoots formation. For rhizogenesis, individual shoot was transferred to MS medium + 1 mg/L NAA and 50 mg/L activated charcoal.

Keywords: Micropropagation, Banana cv. raja bulu, Initiation, Multiplication, Regeneration

INTRODUCTION

Banana is an important crop in Indonesia. Bananas are perennial tropical plants which grow in a wide range of environments. They can be cultivated in integrated farming in backyard garden and agroforestry system, and also can be grown in monoculture system in commercial plantations. Banana cultivar of raja bulu are usually grown mainly for food, but in an animal farming area they can be integrated with ruminant livestock. The by-products of banana such as stems, leaves, and banana peel often used as feed for ruminant. One of the problems in banana production is susceptibility to diseases like fusarium fungi. To ensure sustainable banana cultivation it is important to supply of disease free seedlings via tissue culture. In order to

produce faster and healthier plant, it is required an efficient micropropagation method. This research was aimed to optimize initiation, multiplication, and regeneration of shoot tip culture.

MATERIALS AND METHODS

The explant materials of cv. raja bulu of banana were obtained from Yogyakarta area. Young suckers of 50-60 cm height from healthy and vigorous banana plants are used as explant. From the sucker a cube of tissue of about 1-2 cm^3 containing the apical meristem is excised. This block of tissue is dipped in 75% ethanol for 10 s, surface sterilized in a 1% sodium hypochlorite solution for 15 min, and rinsed three times for 5 min in sterile water. Then, a shoot tip of about 3 × 5 mm, consisting of the apical dome covered with several leaf primordia, is aseptically dissected.

The explant was placed on a multiplication-inducing culture medium, namely Murashige and Skoog (Murashige & Skoog, 1962) based media supplemented with 30 g/l sucrose as a carbon source, and 8 g/l agar as gelling agent. To reduce blackening, 20 mg/l ascorbic acid was added. Two growth regulators, a cytokinin and an auxin, are added to the banana growth medium. Their concentration and ratio are as follows: 2, 4, 6, & 8 mg/L benzylaminopurine (BAP), and 0.2 & 0.5 mg/L naphthalene acetic acid (NAA). The shoot-tip cultures are incubated at temperature of 25 °C in a light cycle of 12 h for 4 weeks. After culture initiation, new axillary and adventitious shoots arise from the shoot-tip explant. Clusters then be separated, trimmed and repeatedly sub-cultured at 4 week intervals in multiplication media.

Individual shoot or shoot clumps are transferred to a rooting medium to stimulate root formation. The rooting medium was MS supplemented with 1 mg/L NAA and 50 mg/L activated charcoal. After rooting, plants are hardened *in vitro* for 2-4 extra weeks on the regeneration/rooting medium prior to transplantation to soil.

RESULTS AND DISCUSSION

Table 1. Average number of shoots produced in shoots multiplication media

BAP; NAA (mg/L)	Average no. of shoots
2 ; 0	2.2
4 ; 0	4.6
6 ; 0	6.2
8 ; 0	4
2 ; 0.2	2.8
4 ; 0.2	6.2
6 ; 0.2	8.2
8 ; 0.2	4.4
2 ; 0.5	2.6
4 ; 0.5	5
6 ; 0.5	7.4
8 ; 0.5	5.6

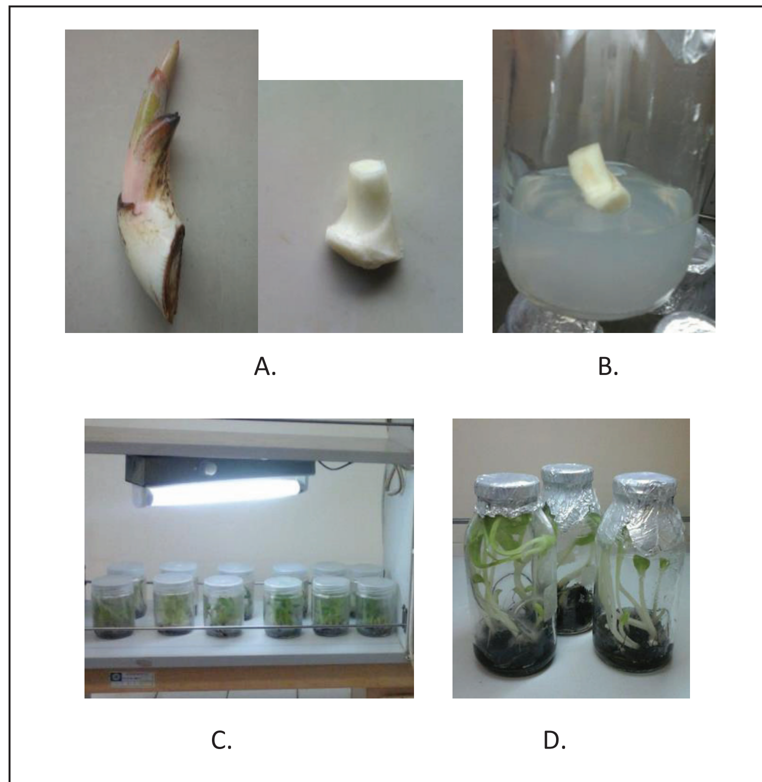

Figure 1. Micropropagation of banana cv. Raja bulu. (A. Banana sucker and shoot tip, B. Shoot initiation, C. Shoot multiplication, D. Rooting)

Previous works on banana tissue culture showed shoot multiplication using addition of cytokinin BAP on shoot induction media (Yusnita *et al*., 2015; Sipen & Davey, 2012). In this research, addition of auxine NAA showed beneficial effects on the number of shoots produced. The results revealed 0.2 and 0.5 mg/L NAA can increase the shoots compared with media with BAP only. Concentration of 6 mg/L BAP showed the highest multiple shoot formation, which supplementation of 0.2 mg/L NAA can induce up to 8.2 shoots in average.

CONCLUSIONS

This research reports shoot multiplication and plant regeneration of the banana cultivar raja bulu. The culture method can be developed as micropropagation protocol. This information may support banana research and sustainable banana cultivation and productivity.

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