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In Vitro Multiplication of *Musa* (ABB group) 'Kluai Hin'

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In vitro multiplication of Musa (ABB group) 'Kluai Hin' was conducted in Murashige and Skoog (1962) medium supplemented with 1 3 5 and 7 mg/l Benzyl adenine (BA) .Planned trial Completely Randomized Design (CRD). Subculture was undertaken every 4 week was duration 12 week. The MS medium supplemented with 7 mg/l BA the number of shoot, mean width and mean lenght were 2.60 1.24 cm and 1.30 cm, respectively, That differences were statistically significant in each methods. MS medium supplemented with 1 mg/l BA produced average lenght of 3.06 cm, that differences were statistically significant in each methods. So that the MS medium supplemented with 7 mg/l BA extracts was to investigated for multiplication of Musa (ABB group) 'Kluai Hin'.

Keywords: Banana , Musa (ABB group) 'Kluai Hin'. Benzyladenine, *In vitro* Multiplication

Introduction

Banana is a major produced in temperate countries. Musa (ABB group) 'Saba', synonyms is as follows:- 'Saba' (Philippines), 'Kluai Hin' (Thailand), 'Pisang Kepok' (Indonesia); 'Pisang Abu Nipah' (Malaysia). It is the most important cooking banana cultivar in the Philippines. Fruit size are ranged from medium to large, stout and angular, skin thick, yellow, pulp creamy-white, finely textured, with a well-developed core, bunches bear 10-16 hands of tightly packed fruit. Other Filipino clones, such as the larger fruits as 'Cardaba' and 'Guboa', the waxy fruits as 'Abuhon, and the fused-fingered 'Inabaniko' and others may form a distinct Saba subgroup. Edible bananas are derived from either *Musa acuminata* (A) or *Musa balbisiana* (B), or a combination of both. Cultivars are diploid or triploid, with some new tetraploids are developed by breeding. Somatic variation has considred to a great range of cultivars. Cultivars are described by their name and genomic make up, e.g. 'Pisang Raja' AAB, the the AAB indicating that it is a hybrid with two genomes of A and one genome of B. Most dessert bananas are AA or AAA, with the triploid AAA

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which become the most important in trading. The different groups and subgroups have some distinct fruit characteristics. *M. acuminata* is a number of morphological characters that separates it from *M. balbisana*. For example, *M. acuminata* is an open petiolar canal, which in *M. balbisiana* is closed. *M. acuninata* has prominent bract scars, bracts that are lanceolate and curl, and two regular rows of ovules, compared with four irregular rows in *M. balbisiana*. Using and scoring 15 morphological characters allows the relative contribution of the two species to be determined in hybrid cultivars.Triploids and tetraploids are larger and more robust than diploids. Classification of 137 accessions in the Thai banana genebank are in the score technique according to 15 plant characters, allows for a range of total score from 15 (pure *M. acuminata*) to 75 (pure *M.balbisiana*) (Robert E.Paull and Odilo Duarte, 2011).

Tissue culture propagation of banana has received attention due to its potential to provide genetically uniform, pest and disease free planting materials. (Navarro, C., 1997, Khalil, S., 2002). Tissue culture technique for rapid clonal propagation and storage are under minimal growth conditions. Shoot-tip cultures of Musa cultivars (both banana and plantain) are induced by culturing small excised shoot apices on modified MS semi-solid medium supplemented with various concentrations and combinations of auxins and cytokinins. The effects of cytokinin concentration in the medium as well as the genotypic configuration of the cultivars on the rate of shoot-bud proliferation have been reported. The established shoot-tip cultures grown on modified MS semisolid medium supplemented with IAA (0.18 mg/l) and BA (2.30 mg/l) were successfully stored at 15 ℃ with 1000 lux light intensity up to 13–17 months depending on the cultivar. The cultivars was tested in this investigation varied in their ability to withstand the minimal growth temperature (Banerjee, N. *et al*.,1985).

Banana plants produced from tissue culture are free from diseases at the time to supply and give a high yield, they are made from selected high yield mother plants. The proper care is taken, and grown into strong healthy plants and give high yield of good quality fruits. They are produced under controlled laboratory condition using selected nutrients, and usually give yield in one or two months earlier than conventional propagated plants. (Smith et al.1998). Shoot-tip cultures of Musa cultivars (both banana and plantain) are induced by culturing small excised shoot apices on modified MS semisolid medium supplemented with various concentrations and combinations of auxins and cytokinins.

The culture medium suplememted with growth regulators becomes obligatory to induce repeated shoot multiplication from shoot buds of banana.

Exogenous supply of a single cytokinin was sufficient to induce shoot multiplication to some extent in cv. Nanjanagudu Rasabale (NR). However, a high rate of shoot multiplication needs high level as well as a precise combination of different cytokinins, because each cytokinin is known to trigger different molecular pathways (Letham and Gollnow 1985; Blackesley and Lenton 1987). The synergistic action of BA and kinetin in inducing multiple shoots has also been reported in other genotypes of Musa (Mendes et al. 1999). The multiple shoot formation occurred in all the combinations of growth regulators with significant differences in the number of shoots formed under each treatment. Although BA at higher concentrations (≤6 mg /l) was fostered an enhanced the rate of shoot multiplication. It was detrimental to shoot proliferation at very high concentrations (7–10 mg /l). This may be due to exudation of phenolics. Similar suppressive effects of BA at very high concentrations are previously reported in AAB genotype of banana (Vuylsteke et al. 1991). BA and kinetin at high concentrations (5–10 mg/ L) caused exudation of phenolics with a concomitant reduction in shoot number as well as shoot length. Exudation of phenolics is due to high levels of cytokinins is reported in earlier studies. The effect of kinetin is reported in other genotypes of banana, such as AA and AAB (cv. silk) (Mukunthakumar and Seeni 2005).

The objective was to formulate the culture medium *in vitro* propagation of edible banana, *Musa acuminata* (ABB group) 'Kluai Hin'.

Materials and methods

Plant materials

The sword suckers of edible banana *Musa acuminata* (ABB group) 'Kluai Hin' were used to excise the meristem and establish shoot cultures. Suckers of selected plants from *Musa acuminata* (ABB group) 'Kluai Hin' were collected, and the meristem was isolated in situ plants. They were washed in running tap water for 20 min. The shoot tips were surface sterilized successively in rectified spirit and in 15% sodium hypochlorite solution for 15 minutes, and followed by repeated washing with sterile distilled water. Finally, shoot meristems of 2-3 mm. size along with primordia was exised aseptically in laminar flow and immediately placed onto Murashige and Skoog (1962) medium which supplemented with different concentrations (1 3 5 and 7 ppm) of 6- benzyl adenine (BA) for multiplications.

Preparation of culture medium

This experiment was used the shoot tips from *Musa acuminata* (ABB group) 'Kluai Hin'. The explants were inoculated aseptically in MS medium (Murashige and Skoog, 1962) containing 30 g/L sucrose for culture establishment. The MS medium was variously supplemented with benzyl adenine (BA) in various combinations. After the interval of 1 month, the established shoots were sub cultured to the same media. Explant were culture in modified MS medium supplemented with BA at 4 levels of concentration as 1, 3, 5 and 7 ppm. Sub culture was undertaken for 4 weeks in the same medium. The culture medium used contains 0.8% agar and the pH was adjusted to 5.8 prior to autoclaving at 121 ^{0}C 15 pound/inch for 20 minutes. The cultures were incubated at 25^{0}C under constant light of 3000 lux intensity provided by white fluorescent lamp for 16 hours photoperiod and 70% relative humidity.

Statistical analysis

Experiment was set up as Completely Randomized Design (CRD) with 4 treatments and 10 replications. Data were recorded by number of shoots (shoots), the height of shoot (cm) and the width of explant (cm). Analysis of variance was statistically computed. Treatment means were compared using Duncan's Multiple Range Test (DMRT) at P = 0.05 and 0.01.

Results and Discussions

Result showed that the shoot-tip cultures of Musa cultivars (*Musa acuminata* (ABB group) 'Kluai Hin') are induced by culturing small excised shoot apices on modified MS semisolid medium supplemented with various concentrations of cytokinins. The effects of cytokinin concentration in the medium as well as the genotypic configuration of the cultivars on the rate of shoot-bud proliferation were tested. *In vitro* shoot-tip culture was a suitable alternative to the traditional methods of propagation of banana (*Musa* spp). In the present study, *Musa acuminata* (ABB group) 'Kluai Hin'was tested *in vitro* multiplication. Result revealed that shoot-tip culture technique can be used for mass propagation of the local cultivars of banana. A variation in multiplication rate was seen not only different genomic groups but also among cultivars of the same group. In general, from about 7-8 weeks after inoculation, shoot-tip proliferation was recorded. The meristem of the explant was multiplied producing several meristems/shoots. Each can be seen a bud due to its ability to develop into a plant. The results showed that the score of plant growth, the number of shoots and the length of shoots of *Musa acuminata* (ABB group)

'Kluai Hin'at 3 months of incubation varied among treatments. In term of the number of shoots, the treatment supplemented with 7 ppm BA was significantly higher than other treatments. The treatment at concentration of 1 and 3 ppm BA gave the lowest number of shoots. The width of explants at 3 months was found to be highest in treatment at 7 ppm BA. The treatment at 7 ppm BA was significantly higher than treatments of 1 and 5 ppm BA (Fig 1). The lenght of explants was the highest in treatment at 7 ppm BA and significantly higher than the treatment supplemented at 1 and 5 ppm BA (Fig 1). Finally, the height of shoots was found to be the highest in treatment at 1 ppm BA. There was significant difference from the treatments at 7 ppm BA and non-significantly differed from each other. However, treatments at 1,3 and 5 ppm BA were also non significantly differed.

It is suggested that a rapid banana multiplication protocol from shoot meristem was shown by using a medium with optimized concentration of cytokinins. Many reports were found *in vitro* propagation with complicated protocols, but less shoot proliferation percentage which shown less yield in number of regenerated plants per culture. It is shown a very simple, efficient, economical, rapidly multiply and highly reproducible protocol for the micro-propagation of banana for commercial scale.

MS+1 ppm BA MS+3 ppm BA MS+5 ppm BA MS+7 ppm BA

Fig.1. Different developmental stages of *Musa acuminata* (ABB group) 'Kluai Hin'. growth on MS medium supplemented with different concentrations of benzyl adenine (BA) at 4 level at 3 months

Conclusion

It is concluded that the best culture media for micro propagation of *Musa acuminata* (ABB group) 'Kluai Hin' for multiple shoot regeneration was directly expressed from shoot tips. The suitable medium was recorded as MS medium supplemented at 7 ppm BA for shoot multiplication. The MS medium supplemented with 7 mg/L of BA gave a high the number of shoot, width and length. MS medium supplemented with 1 mg/L of BA was significantly

differed in each method. The MS medium supplemented with 7 mg/L of BA was recorded for multiplication of *Musa acuminata* (ABB group) 'Kluai Hin'.

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