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Effect of BA and chitosan on *In vitro* Growth of *Musa* (ABB Group) 'Kluai Namwa Mali-Ong'

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Abstract

Fruit crops are very important in agricultural production. Bananas are the fourth most consumed food in the world, behind rice, wheat and maize. Despite its significant commercial value, banana production is faced with the problem of unreliable and unsafe planting materials, which hinder its production. Conventional planting materials (suckers) are of poor quality and also not enough to meet the increasing demand. Tissue culture techniques can however be exploited in solving this problem. Nevertheless, there are a lot of challenges hindering the micropropagation of banana. These challenges need to be addressed to enhance the effectiveness of this technique in banana production. The effect of chitosan on *in vitro* growth of micropropagated *Musa* (ABB Group) 'Kluai Namwa Mali-Ong' (ABB) was studied. Plantlets were cultured on MS medium supplemented with 0 and 5 mg/l Benzyladenine (BA) and chitosan at the concentrations of 0, 5, 10, 15, and 20 mg/l. The experiment was set up using Completely Randomized Design (CRD) with 5 replications. After 4 months, explants cultured on MS medium supplemented with 5 mg/l BA and 10 mg/l chitosan produced the maximum number of shoots (9.80±0.3 shoots/explant) and buds (2.25±0.3 buds/explant). The highest number of roots (2.67±0.4 roots/explant) were identified on plantlets cultured on MS medium supplemented with only 20 mg/l of chitosan. Supplementing MS medium with chitosan for the micropropagation of banana in this study helped to accelerate growth by increasing the number of shoots.

Keywords: *In vitro*, chitosan, Benzyladenine (BA), *Musa* (ABB Group) 'Kluai Namwa Mali-Ong'

1. Introduction

Banana is a tropical plant which originated from South East Asia. It is one of the most popular fruit crops because of the ability to use all parts of this plant. The fruits can be eaten raw or cooked into many kinds of food. Products from banana are sold both domestically and internationally. Interviews with banana processors in Phitsanulok province of Thailand revealed that they mostly use local banana varieties. Phitsanulok has many farmers who grow *Musa* (ABB Group) 'Kluai Namwa Mali-Ong'. Banana processing companies in Phitsanulok province process the fruit (ABB) into dried banana. Growing and processing of banana has led to the creation of a large banana community in Phitsanulok province. Dried banana (ABB) is a souvenir of the famous Phitsanulok city and it is exported to other countries. Sale of dried banana is a major source of income in Phitsanulok province, generating more than 100 million baht per year. Export value reached 1,370 tons, valued at 163.36 million baht [1].

ABB is used as the raw material in the production of dried banana in Phitsanulok province of Thailand. This raw material is however not enough to meet the high demand. Propagation of bananas by tissue culture can help to meet the increased demand through mass propagation within the shortest possible time. The banana shoot-tip

culture system was developed by supplementing Murashige and Skoog (MS) basal media with 5 mg/l BA [2]. Chitosan has been used as a dietary supplement in tissue culture to stimulate plant growth. Chitosan, a plant growth stimulator, stimulates the induction of plant defence mechanisms in numerous plants [3 & 4]. Chitosan also increases the production of secondary metabolites in cells and calli of several plant species [5 - 8]. The use of chitosan during micropropagation can help to enhance the quality of plantlets in vitro and subsequently help these plantlets to adapt to ex vitro conditions [9 & 10]. Kowalski et al. [11] observed an increase in seed quality of potato minitubers after applying different concentrations of chitosan on potato cv. Désirée plantlets *in vitro*. This resulted in the production of high number of tubers thereby increasing yield. However, several factors including genotypes, explant types and tissue culture methods will determine the exact concentration of chitosan to be applied in the culture media to enhance the quality and yield of minituber [11]. Chitosan can promote plant growth by stimulating the immune system through the expression of genes involved in self-protection, increase seed germination and stimulate chitinase production in seeds. The nitrogen component in the chitosan structure can also be released for plant use. To enhance the effect chitosan on growth, the optimum concentration must use. Chitosan concentration above or below the optimum may affect the amount of nitrogen released, which may intend affect plant growth. Chitosan application in tissue culture is mostly found in orchids. The use of chitosan in banana tissue culture to help accelerate growth by increasing the number of shoots has not been reported. This research was therefore carried out to determine the appropriate concentration(s) of chitosan to enhance shoot multiplication of ABB.

2. Materials and Methods

2.1 Plant material

Healthy banana (ABB) suckers (sword suckers) from disease-free mother plants were collected. The peels were removed after which the suckers were cut into sizes of 3 cm x 4 cm (basal diameter x length). The surface of sucker pieces was then sterilized by dipping in 95% ethanol for 1 minute. The sucker explants were then soaked in 30% Clorox plus 2 drops of Tween-20 for 30 minutes and 15% Clorox plus 2 drops of Tween-20 for 15 minutes. Sterilized distilled water was used to wash sucker's trice in a laminar airflow, after which they were trimmed, cut and cultured in MS medium for 4 weeks. The suckers were used as source of meristem to investigate the effect of BA and chitosan, each at different concentrations alone or in combinations, on shoot multiplication. Two levels of BA (0 and 5 mg/l) and 5 concentrations of chitosan (0, 5, 10, 15 and 20 mg/l) were tested (Table 1).

Table 1 Different treatments used for multiplication of shoot

Treatment	Solid medium
1	MS (control)
2	MS + 5 mg/l BA
3	MS + 5 mg/l BA + 5 mg/l chitosan
4	MS + 5 mg/l BA + 10 mg/l chitosan
5	MS + 5 mg/l BA + 15 mg/l chitosan
6	MS + 5 mg/l BA + 20 mg/l chitosan
7	MS + 5 mg/l chitosan
8	MS + 10 mg/l chitosan
9	MS + 15 mg/l chitosan
10	MS + 20 mg/l chitosan

All cultures were kept in the incubator at a temperature of 25±2 °C. A photoperiod (2000 lux) of 16 hours using cool white fluorescent tubes was also provided. The medium pH was adjusted to 5.6 before autoclaving. Sub culturing was carried out in a fresh medium every 30 days for up to 4 months.

2.2 Statistical analysis

The study was undertaken using the Completely Randomized Design (CRD) with 5 replications. Statistical analysis was performed using SPSS version 17.0 software package (SPSS, Germany). Statistical significance at 95% confidence level was tested using ANOVA and comparison of means done with DMRT tests. Parameters measured include number of shoots, buds and roots.

3. Results and Discussion

3.1 Effect of different concentrations of chitosan on shoot formation and multiplication

Different concentrations of chitosan application had a significant effect on shoot formation and multiplication. Using only MS medium (control) and MS medium supplemented with only 5 mg/l BA or in combination with chitosan significantly ($p \leq 0.05$) affected shoot multiplication. However, different concentration regimes of chitosan with or without BA significantly affected the number of shoots, buds and roots. Other studies by Vuylsteke and Lanhe [12], Venkatachalam et al. [13] and Bairu et al. [14] identified 5 mg/l BA as the most effective concentration for bud proliferation of many banana cultivars *in vitro*. In a review on banana cell and tissue culture, Strosses et al. [15] indicated the addition of a wide range of concentrations (0.1-20 mg/l) of BA to the culture medium. In the current study, 5 mg/l BA as according to Hui et al. [16] was used.

3.2 Effect of chitosan on the number of shoots and buds

This part of the study was carried out using the Completely Randomized Design (CRD) with 5 replications. After four months, plantlet cultured on MS media supplemented with 5 mg/l BA and 10 mg/l chitosan produced maximum shoot (9.77 ± 0.25 shoots/explant) and buds (2.25 ± 0.26 buds/explant) numbers (Figure 1, 2 and 3). Kanchanapoom et al. [17] conducted an *in vitro* study of bulb scale explants of Lily (*Lilium longiflorum* Thumb. 'Ester Lily'). Results from their study revealed that, shoot numbers decreased with an increase in chitosan concentration, and 10 mg/l chitosan yielded the highest number (3.3) of shoots. Similarly, *Phalaenopsis gigantea* J.J.Sm., cultured on VW [18] supplemented with 10 mg/l chitosan induced maximum number of protocorms and fresh weight [19].

3.3 Effect of chitosan on the number of roots

After 4 months, the highest of number of roots (2.67±0.40 roots/explant) were found on plantlets cultured on MS medium supplemented with only 20 mg/l of chitosan (Figure 4). *Cymbidium dayanum* cultured on MS medium supplemented with 1 mg/l of chitosan promoted the best shoots and roots [20]. Santiwong et al. [21] reported that stevia (*Stevia rebaudiana* Bertoni) cultivated on MS medium with 4 types of chitosan produced more roots compared to the non-chitosan control. Boonkerd et al. [22] reported that the chitosan structure contains nitrogen as a constituent that is released for the plant to use. Chitosan must be appropriately used. If it is applied in excess or less, the amount of nitrogen released will affect the growth of the plant.

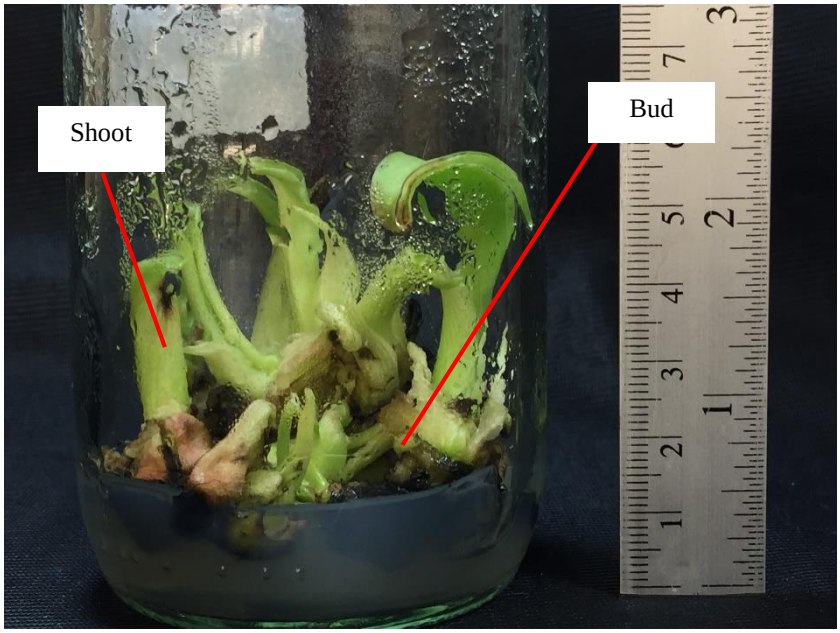

Figure 1 Banana shoot and bud development in micropropagation

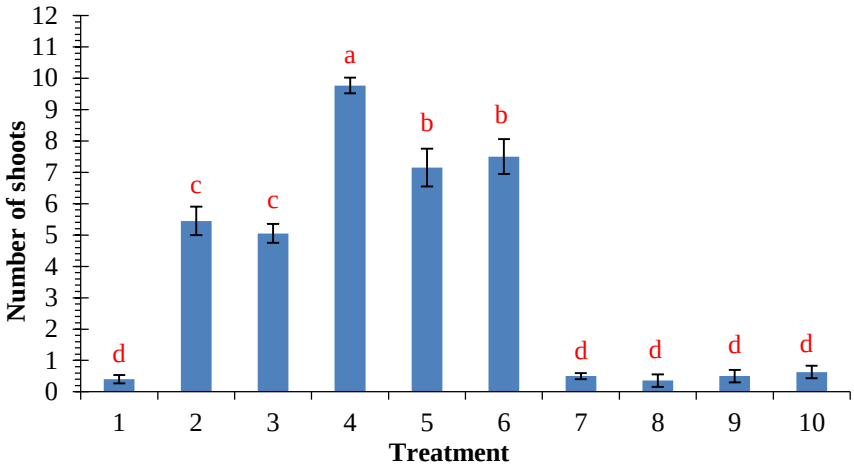

Figure 2 Effect of BA and chitosan on the number of shoots

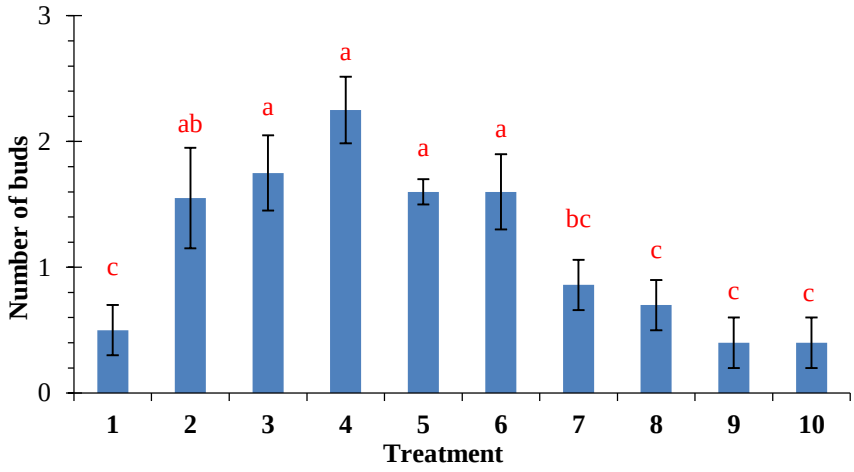

Figure 3 Effect of BA and chitosan on the number of buds

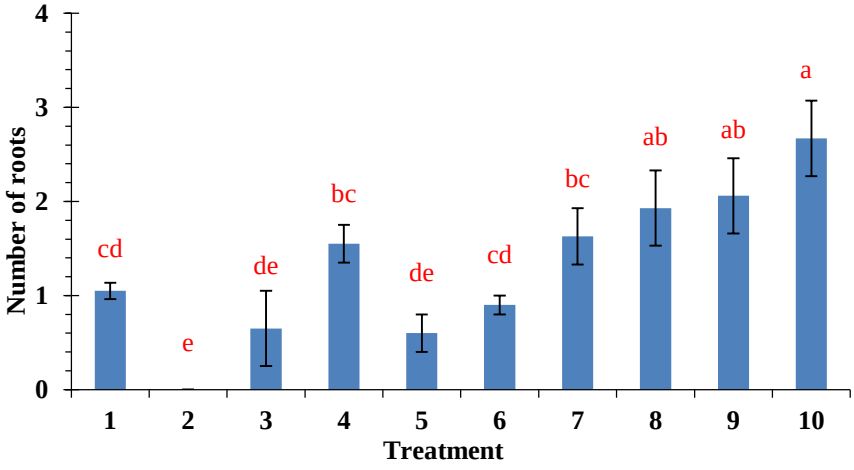

Figure 4 Effect of BA and chitosan on the number of roots

4. Conclusions

The addition of 10 mg/l chitosan in MS medium supplemented with 5 mg/l BA was the best concentration to promote growth of ABB since it resulted in the highest number of shoots, buds and roots. MS supplemented with 20 mg/l chitosan alone and MS without growth regulator resulted in the best root stimulation but did not promote the growth of ABB. The use of chitosan in banana tissue culture helped to accelerate growth by decreasing the time for shoot development as well as increasing the number of shoots. This will help reduce the cost of micropropagation of banana plantlets. Plantlets developed also stand the chance of better adaptation due to the development of shoots and roots after micropropagation.

5. Acknowledgements

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