

Studies On In Vivo Macropropagation Of Banana

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Abstract

In vivo macropropagation is an alternative simple and cheap technique for banana multiplication. The effect of Benzyl Amino Purine (BAP) at different concentrations viz. S0=0 mg/L (Control), S1=10 mg/L, S2= 20 mg/L, S3= 30 mg /L on *in vivo* macropropagation of banana was studied. Different concentrations of Benzyl Amino Purine (BAP) performed significant variation on the growth and development of Banana sucker. Results indicated that the days of first sucker emergence, number of suckers, suckers height, sucker collar diameter (cm), number of leaves, number of roots and roots length attributed the highest responses to BAP at 30 mg/L. On the contrary, the lowest values of vegetative growth were observed in control condition (0 mg/L). In this study, 4 levels of BAP concentrations were used in cavity method. It is recommended to investigate corms soaking method in different BAP Concentrations.

Keywords: Banana, *in vivo*, Micropropagation, Benzyl Amino Purine (BAP) and sucker

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I. Introduction

Banana (*Musa spp*; Musaceae) is a fruit crop with the highest global production (127.3 million tonnes), and ranked fourth in terms of agricultural commodity value (63.6 billion US\$) after rice, wheat and milk [1]. Banana is one of the most important commercial tropical fruits traded. Banana has great socio economic significance in Asia and pacific regions. India is the top most (30.80 million tons) producer of banana in the world followed by China (13.32 million tons) [1]. Bangladesh ranks 34th largest banana producing country. Banana is one of the most important commercial tropical fruits traded. They are perennial and are a source of steady income all the year round. Bananas are one of the most popular and widely consumed fruits in Bangladesh. Banana is a valuable source of potassium, vitamin A, B6, and C. Bananas can be eaten fresh when ripe or after cooking or processing. The total per capita consumption of banana in Bangladesh is about 4.7 kg. This is very much lower than that consumed by Europe specially Belgium (26.7 kg), Sweden (16.7 kg), and Germany (14.5kg) while USA consumed 13.1 kg and UK 10.5 kg [2]. Bangladesh exports Champa kola (Apple banana, *Musa sapientum*) throughout the year (Hortex Foundation, 2013). In Bangladesh bananas were cultivated in 120,709 ha of land and total production was 8,33,309 MT in 2018-2019 (BBS,2019). In Asia the highest half of the total some 62.6 million metric tons of banana were produced. FAOSTAT estimated that in 2018 a total of 155.2 million tons of banana were produced in the world [1].

Bananas are seed sterile and parthenocarpic in nature and are normally propagated by suckers. In this method the rate of multiplication is highly limited [5]. Banana production sometimes becomes seriously affected by different fungal and viral diseases such as panama and bunchy top diseases. As a result banana productivity decreases and the yield becomes very poor and static as well. Moreover, it is very difficult to carry the bulk volume of suckers from one place to another. Another major constraint to the expansion of banana and plantain cultivation is the scarcity of healthy planting material [6]. Planting materials produced through tissue culture becomes costlier and the small and the marginal farmers cannot afford the higher cost. Under the above circumstances macro-propagation of banana becomes popular [7]. Macro-propagation techniques involve methods that employ whole suckers or relatively large pieces of corm tissue to produce planting material in a propagator. Benzyl Amino Purine (BAP) is a synthetic cytokine [8]. is used to induce multiple shoots production [9]. *In vivo* multiplication of suckers can be increased through application of BAP which induces sprouting of axillary buds and adventitious shoots [10, 11]. However, considering the above circumstances, the present study was undertaken to evaluate the effect of BAP at various concentrations on *in vivo* proliferation of banana.

II. Materials and Methods

Study area, design and period:

The present study was conducted at the experimental field of the Horticulture Centre, Department of Agricultural Extension (DAE), Kushtia, Bangladesh. The experiment was laid out in Randomized Complete Block Design (RCBD) with three replications. The experiment was conducted during April 2021 to April 2022.

Planting materials:

The banana crop cultivar **Malbhog** was used as a test crop. The planting materials (selected germplasm of banana corms) were collected from the farmers of the study area. About 05 months old suckers were used for experimentation. A careful selection of sword suckers was done from healthy mother plants grown by that farmer. Sword suckers were pared to remove roots and packed into plastic bags for transport to Horticulture centre, Kushtia.

Preparation of plantlets

The remnants of the pseudostem and roots were removed and external layer of the corm was scrapped using a sharp knife to ensure freeness from all nematodes and external root borne pathogens. The apical meristem was removed to a depth of 2cm leaving a cavity of 2cm diameter in the rhizome. The rest of the corm was given 4 cross cuts and incised upto 0.25-0.50 cm depending on the sucker size. Cross cuts were made on the exposed axillary buds and apical meristem to encourage sprouting of axillary buds and to kill apical dominance respectively. The corms were washed with fungicides Tilt-250 EC at 2ml/L solution for 30 minutes and air dried 3-4 hours before planting. The decapitated corms were planted individually in different media peat. Corms were buried 6cm deep in the substrate to protect from intensity of direct sunlight and then respective treatments were imposed. The apical meristem when scooped out to a depth of 2cm near the crown region and the corms were given 4-6 transverse incisions to a depth of 2 mm, in this cavity 4ml of each concentrated BAP was poured into left by the removal of the apical meristem. The same treatment was imposed after first time sucker collection to start second time sucker regeneration.

Treatments

Different doses of Benzyl Amino Purine (BAP) viz. S₀=0 mg/L (Control), S₁=10 mg/L, S₂= 20 mg/L, S₃= 30 mg /L were prepared by following the standard procedure. 5 mg powder of BAP was poured into 1(one) liter of distilled water. This way 5 mg/L solution was made. In the same way the concentrations of BAP solutions were made. Control corms cavity that is 0 mg/L water, No BAP with water was poured.

Data collection and analysis

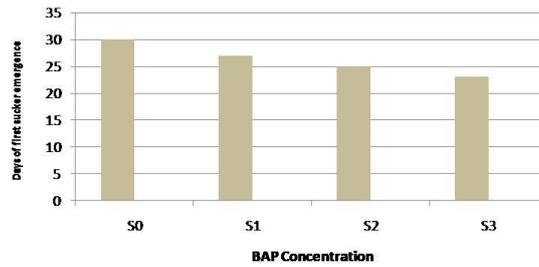
Observations were recorded for different growth parameters like i. Number of days to first sucker emergence, ii. Number of sucker emerged per corm, iii. Sucker height (cm), iv. Number of leaves per sucker, iv. Sucker collar diameter (cm), vi. Number of roots per sucker, vii. Length of root.

The recorded data on different parameters of height, length, and other contributing characters were statistically analyzed using statistix 10 software to find out the significance of variation due to applied treatments. The mean for all the calculated and the analysis of variance for each of the characters under study was done by F (variance) ratio test and mean separation was done by LSD test at 5% level of probability by Gomez and Gomez [12].

III. Results and Discussion

Days of first sucker emergence:

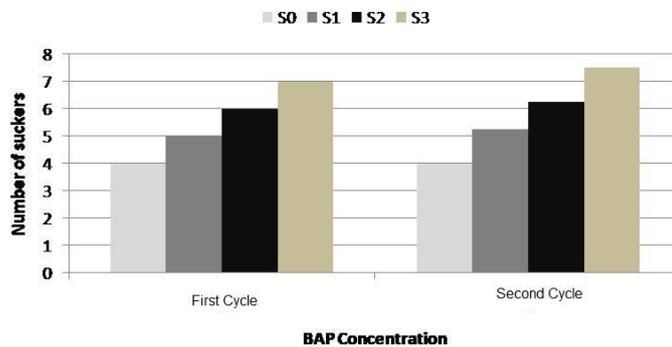
Application of different levels of BAP performed significant difference on the days of first sucker emergence. However Shortest days required for bud sprouting was in S₃ (BAP 30 mg/l) and longest time period required in control condition S₀ (No BAP application). The shortest and longest time (days) required for sprouting bud was recorded 23 days and 30 days, respectively. In case of 20mg/L and 30mg/L BAP concentration, 27 days and 25 days were required, respectively. Additionally, it is observed that more concentration of BAP up to 30mg/L showed reverse impact of sucker emergence, i.e; in this solution days required less compare to other concentration (**Figure 1**). Previous results showed that BAP concentration at 1.5 mg L⁻¹ significantly ($P < 0.05$) reduced the number of days to first shoot emergence of 15.78 days followed by BAP at 3.0, 6.0 and 0.0 mg L⁻¹ with 25.18, 28.39 and 36.43 days, respectively [12].



BAP Concentrations, S0=0 mg/L (Control), S1=10 mg/ L, S2= 20 mg/L, S3= 30 mg /L
Figure1. Effect of BAP Concentrations on days of first sucker emergence

Number of suckers produced in each corm

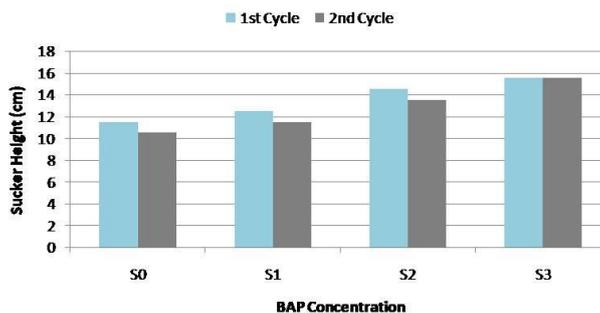
Application of different BAP concentrations showed significant variations on number of sucker production. In the 1st cycle, the highest numbers of suckers (7) were produced in S3 and the lowest (4) suckers were produced in S0 concentrations. At the same time, S1 and S2 concentration produced 5.0 and 6.0 suckers, respectively. On the flip side in 2nd cycle, the highest numbers of suckers (7.5) were produced in S3 and the lowest (4) suckers were produced in S0 concentrations But S1 and S2 concentration produced 5.25 and 6.25 suckers, respectively (**Figure 2**). Similarly, BAP concentration at 1.5 mg L⁻¹ significantly ($P < 0.05$) increased sucker productivity with 17.11 suckers per corm followed by BAP at 0.0, 3.0 and 6.0 mg L⁻¹ with 15.23, 13.08 and 12.96 suckers per corm, respectively [13].



BAP Concentrations, S0=0 mg/L (Control), S1=10 mg/ L, S2= 20 mg/L, S3= 30 mg /L
Figure2. Effect of BAP concentrations on sucker production

Sucker Height

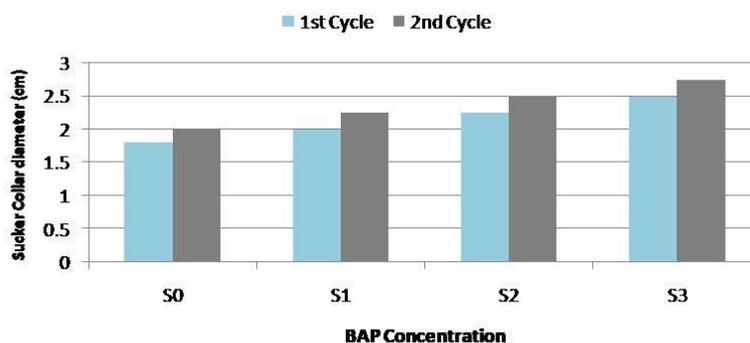
In case of BAP application, in the first cycle, the tallest sucker (15.5 cm) was observed in S3 condition and the smallest sucker (11.5) was observed in control (S0) condition. In S1 and S2 condition, 12.5 cm and 14.5 cm sucker height was recorded, respectively. On the other hand, in second cycle, the tallest (15.5 cm) and the smallest (10.5 cm) suckers were found in S3 and control (S0) condition, respectively. In S1 and S2 condition, 11.5 cm and 13.5 cm sucker height was recorded, respectively (**Figure 3**). Previous results showed that corms treated with BAP at 1.5, 3.0, 6.0 mg L⁻¹ significantly ($P \leq 0.05$) produced taller shoots with length of 27.0, 27.3 and 26.7 cm followed by corms treated with BAP at 0.0 mg L⁻¹ with shoot length of 22.7 cm [13].



BAP Concentrations, S0=0 mg/L (Control), S1=10 mg/ L, S2= 20 mg/L, S3= 30 mg /L
Figure3. Effect of BAP concentrations on sucker height

Sucker Collar diameter

Application of different BAP concentrations showed significant impact on sucker collar diameter. In the first cycle, sucker collar diameter was the highest (2.5 cm) in S3 (30 mg/L) condition whereas the lowest (1.8 cm) was found in S0 (0 mg/L) condition. In second cycle, the highest (2.75 cm) and the lowest (2 cm) sucker collar diameter was found in S3 (30 mg/L) and S0 (0 mg/L) concentrations, respectively (**Figure 4**). Conversely, corms treated with BAP at 0.0 and 6.0 mg L⁻¹ produced suckers with larger collar diameter of 3.4 and 2.4 cm followed by suckers from corms treated with BAP at 3.0 and 1.5 mg L⁻¹ with collar diameters of 2.2 and 2.0 cm, respectively [13].

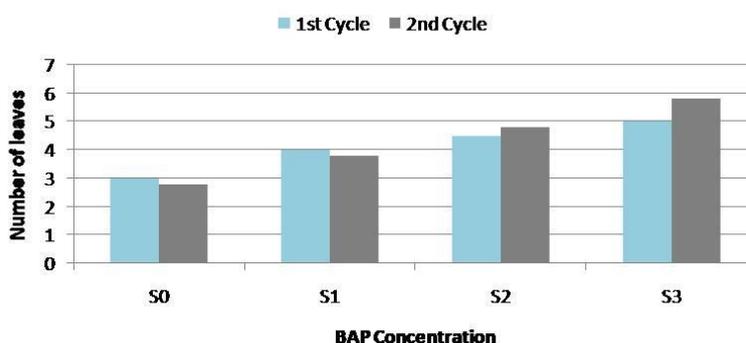


BAP Concentrations, S0=0 mg/L (Control), S1=10 mg/ L, S2= 20 mg/L, S3= 30 mg /L

Figure 4. Effect of BAP Concentration on Sucker Collar Diameter

5. Number of leaves in each sucker

The number of leaves in each produced sucker had no significant effect by BAP application. Due to use of BAP in different concentrations, the highest numbers (5) of leaves were found in S3 condition whereas the lowest numbers (3) of leaves were found in control condition (S0) in the first cycle. In S1 and S2 condition, 4 and 4.5 leaves were found, respectively. In case of second cycle, the highest numbers (5.8) of leaves were found in S3 condition whereas the lowest numbers (2.8) of leaves were found in control condition (S0). In S1 and S2 condition, 3.8 and 4.8 leaves were found, respectively (**Figure 4**). Previous results showed that suckers from corms treated with BAP at 0.0 and 3.0 mg L⁻¹ had larger number of leaves of 4.8 and 4.6 per sucker followed by suckers from corms treated with BAP at 1.5 and 6.0 mg L⁻¹ with 4.0 and 3.8 leaves per sucker, respectively [13]. Sajith et al. also stated that application of BAP with *Bacillus subtilis* produced higher number of primary, secondary and tertiary suckers leaves [14]. Kindimba also found BAP (0.04%) that reflects higher number of leaves in produced sucker [15].



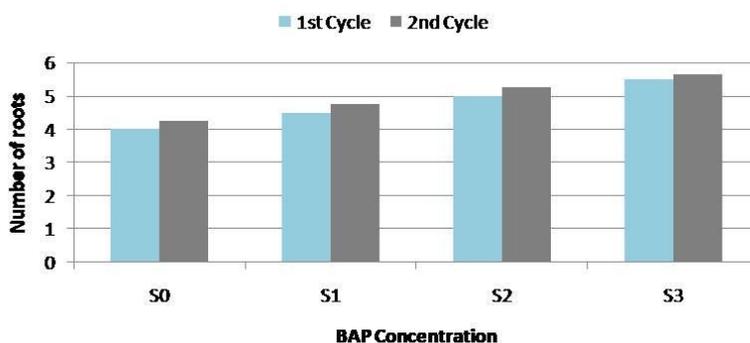
BAP Concentrations, S0=0 mg/L (Control), S1=10 mg/ L, S2= 20 mg/L, S3= 30 mg /L

Figure 5. Effect of BAP Concentrations on number of leaves of banana sucker

6. Number of roots in banana sucker

Application of different BAP concentrations showed significant variations on number of roots production. In first cycle, the highest numbers (5.5) of roots were produced in S3 (30 mg/L BAP) condition whereas the lowest numbers (4) of roots were produced in control condition (S0). Besides, in S1 and S2 concentration, 4.5 and 5 roots were produced, respectively. On the other side in second cycle, the highest numbers (5.65) of roots were produced in S3 (30 mg/L BAP) condition whereas the lowest numbers (4.25) of

roots were produced in control condition (S0). In S1 and S2 concentration, 4.75 and 5.25 roots were produced, respectively (Figure 5). Thungon also found similar result of higher roots number in BAP (0.04%) application [16].

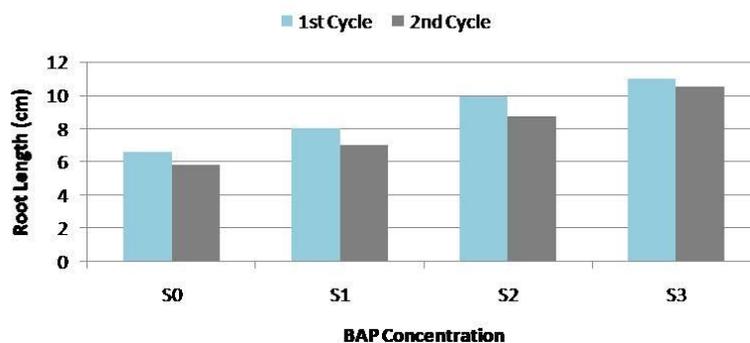


BAP Concentrations, S0=0 mg/L (Control), S1=10 mg/ L, S2= 20 mg/L, S3= 30 mg /L

Figure 6. Effect of BAP Concentrations on number of roots of banana sucker

7. Length of roots in banana sucker

Due to BAP application, the longest (11 cm) root was observed in S3 condition whereas the smallest (6.6 cm) root was observed in control (S0) condition in the first cycle. Besides, in S1 (10 mg/L) and S2 (BAP-20 mg /L) condition, the length of roots was 8 cm and 9.9 cm, respectively. On the other hand, the longest (10.5 cm) and the smallest (5.5 cm) root was found in S3 (30 mg/L) and control S0 (0 mg/L) condition in second cycle. Moreover in the same cycle, S1 (10mg/L BAP) and S2 (20mg/L BAP) concentration produced 7 cm and 8.75 cm roots (Figure 6).



BAP Concentrations, S0=0 mg/L (Control), S1=10 mg/ L, S2= 20 mg/L, S3= 30 mg /L

Figure 7. Effect of BAP concentrations on length of roots of banana sucker

IV. Conclusion

Effect of Benzyl Amino Purine (BAP) in different concentrations on *in vivo* macro-propagation of banana is investigated in this study. Application of different concentrations of Benzyl Amino Purine (BAP) performed significant variation on first shoot emergence, number of suckers produced, sucker height, sucker collar diameter, number of sucker root and sucker roots length. On the contrary, there was no significant effect was found by BAP application on the number of leaves in each produced sucker. The findings of this study provide evidence for the use of *in vivo* macropropagation with BAP as an alternative simple and cheap technology for rapid and mass production of banana suckers. Further studies are required to test the responses of other suitable cultivars to *in vivo* macropropagation in combination with different BAP concentrations. Research is also recommended to evaluate the responses of cv. **Malbhog** to *in vivo* macropropagation combined with other plant hormones.

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