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# Effect of BAP and IBA on *in vitro* Regeneration of Local Banana Variety of Sabri

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***Authors' contributions***

*This work was carried out in collaboration between all authors. Author FK designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author KAUZ managed the analysis of the study. Authors MA and MHR managed the literature searches. Authors MEH and HH assisted in designing the study. All authors read and approved the final manuscript.*

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## ABSTRACT

The experiment was carried out to study the effect of benzylaminopurine (BAP) (1.0, 2.0, 3.0, 4.0 and 5.0 mg/L) and indole-3-butyric acid (IBA) (0.5, 1.0, 1.5, 2.0 and 2.5 mg/L) for *in vitro* regeneration of sabri variety of Banana using shoot tip explants. Highest response of explants (84%) and maximum number of shoots per explant (3.4) were observed with 5.0 mg/L BAP in sabri. In contrast, due to combined effect, 5.0 mg/L BAP+2.5 mg/L IBA showed best response (90%). The highest shoot number per explant (3.4) was found with 5.0 mg/L BAP+2.0 mg/L IBA. The maximum number of roots (3.4 and 5.2) was observed in 1.5 mg/L and 3.0 mg/L BAP+1.0 mg/L of IBA. In

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controlled environment, the regenerated healthy rooted plantlets were transferred from culture media to soil in plastic pots where 90% plantlets were survived and in open atmosphere, the survival rate was 88.89%. This protocol has the applicability *in vitro* rapid propagation of Banana.

*Keywords: In vitro; regeneration; BAP; IBA; survival rate.*

## 1. INTRODUCTION

Banana (*Musa* sp.) belonging to the family Musaceae is one of the world's most important subsistence crops. In terms of gross value of production, Bananas are the world's fourth most important crop after rice, wheat and maize [1]. In international trade, Bananas account for ~22 per cent of world's fresh fruit production and are ranked second most important fruit crop after citrus [2]. Presently, Banana is grown in around 150 countries across the world on an area of 4.84 million ha [3] with an annual production of 92.38 million tons [4]. They contribute to food security by producing fruit year around and provide income to rural population [5]. As a diet, Banana is an affluent source of carbohydrate with calorific value of 67 calories per 100 g fruit and is one of the most well liked and widely traded fruits across the world [6,7]. In Bangldesh, Banana is popular for its year round availability, abundant production as well as high acceptability to the consumers. Total estimated production of Banana was 801000 metric tons and cultivated area is 131 acres [8] in the country. There are many cultivars of Banana, such as Sagar, Sabri, Champa, Mehersagar, Dudsagar, Kabri, Agniswar, Genasundari, Kanaibashi, Basrai, Binisuta etc. Sabri is also known as Malbhog, Onupam and Martaman. It is a popular dessert cultivar, widely grown in the north and western areas of Bangladesh. Sabri is a commercial variety in Bangladesh.

Banana and plantain plants are susceptible to a wide range of diseases and pests. As a result, Banana productivity decreases and the yield become very poor. Only 5 to 10 suckers can be obtained from a plant per year in conventional method whereas in traditional clonal propagation method appears unable to satisfy the increase in demand for disease free and healthy planting materials of Banana. Tissue cultured plants grow vigorously, establish more quickly and take a shorter time to bunch emergence and harvest. Tissue culture technique produce 39% higher yield than conventional sword suckers [9]. Under Bangladesh condition, tissue culture derived plantlets of Banana performed better than the conventional sword suckers [10]. With the increasing demand and vast export potential coupled with the farmers desire to grow *in vitro* propagated banana on a large area are becoming increasingly important in planting material for rapid multiplication of economically important commercial varieties [11] and plant multiplication can be continued throughout the year irrespective of seasonal variation [12].

In tissue culture, plant growth regulators (PGR) are critical media components in determining the developmental pathway of the plant cells. The effectiveness of BAP over other cytokinins in inducing multiplication of shoot tip cultures has been reported in different cultivars of Banana [13,9,14] BAP has a marked effect in stimulating the growth of axillary and adventitious buds and foliar development of shoot tip cultures [15,13]. In shoot tip culture, cytokinins are known to enhance buds growth and shoot formation, while auxins promote root induction and development [16,17]. Benzylaminopurine (BAP) combined with auxins (indole acetic acid and naphthalene acetic acid) exhibit synergistic effect and hence has also been used by number of researchers [18,19,5,20]. Meanwhile, combinations of BAP with auxins such as indole acetic acid (IAA) or indole-3-butyric acid (IBA) were also used for *in vitro* multiplication of Bananas [21].Generally, cytokinin helps in shoot proliferation and auxins helps in rooting of proliferated shoots. However, the requirement of cytokinin and auxins depends on the variety of Banana and culture conditions [22]. In our national economy sabri varieties play a vital role due to its popularity and acceptability to marginal and commercial farmers. Henceforth, the present study aimed to find out the effect of benzylaminopurine (BAP) and indole-3-butyric acid (IBA) with different concentration for *in vitro* regeneration of sabri Banana using shoot tip explants. The present study was undertaken with the following objectives:

i. To study the individual effect of BAP and IBA on *in vitro* regeneration of sabri variety of Banana.

ii. To study the combined effect of BAP and IBA on *in vitro* regeneration of sabri variety of Banana, and
iii. To develop an efficient protocol for *in vitro* rapid propagation of Banana varieties.

## 2. MATERIALS AND METHODS

### 2.1 Plant Materials

The experiment was conducted at the Biotechnology Laboratory, Department of Biotechnology, Sher-e-Bangla Agricultural University, Bangladesh for *in vitro* regeneration of sabri variety of Banana. The three months age of sabri variety was used as experimental materials in the present investigation and collected from Agargau nursery, Sher-e-bangla nagar, Dhaka.

### 2.2 Explants

The healthy, disease free shoot tips of 2 cm length were used as explants for the study for *in vitro* regeneration.

### 2.3 Culture Media

MS (Murashige and Skoog) medium supplemented with different phytohormones as per treatments were used as culture medium for shoot induction, shoot multiplication, maintenance, and regeneration of roots. Hormones (BAP and IBA) were added either in combination or separately to the media according to the treatment. Five levels of BAP (1.0, 2.0, 3.0, 4.0 and 5.0 mg/L) and 5 levels of IBA (0.5, 1.0, 1.5, 2.0 and 2.5 mg/L) were used separately and three levels of BAP (3.0, 4.0 and 5.0 mg/L) with 5 levels of IBA (0.5, 1.0, 1.5, 2.0 and 2.5 mg/L) were used as treatment. The pH was adjusted to 5.8 before placing in microwave oven which was used for melting agar (semi solidifying agent).

### 2.4 Surface Sterilization

Isolated shoot tips were collected from Banana suckers of three months having 4 cm in length were surface sterilized with 70% ethanol for 1 min and 0.2% $HgCl_2$ separately for 12 min, respectively followed by 3-4 times rinse with sterilized distilled water and then outer layer of explants were removed carefully. Further the explants were sterilized with 0.1% $HgCl_2$ for 5 min then washed for at least 3 times with sterilized distilled water. Another outer layer of the explants were removed carefully. The final size of explants were 1-2 cm which had 6/8 overlapping leaf base enclosing auxiliary bud.

### 2.5 Culture of Explants

The isolated and surface sterilized shoot tips were collected carefully through maintaining aseptic condition inside the laminar air flow cabinet and inoculated to each of the culture tube containing 50 mL of MS medium supplemented with different concentrations of hormones as per treatment. The culture vials transferred to culture racks and allowed to grow within 25±1℃ temperature by an air conditioner and 16 hour photoperiod was maintained along with light intensity of 3000 lux for proper growth and development of culture. To control blackening of the tissues on the explants which occurred within 6-7 days after inoculation, were removed and the explants were transferred to similar fresh medium. It was repeated at the interval of 10 days interval for about one month to minimize further blackening of the tissues.

### 2.6 Maintenance of Proliferating Shoots

For subculturing, the entire samples of *in vitro* shoot were cut into small pieces so that each piece would contain about one shoot and inoculated into a similar fresh medium. The subculturing was done at the interval of 20-25 days.

### 2.7 Regeneration of Plants from *in vitro* Proliferated Buds

*In vitro* proliferated micro shoots were separated and each of the micro shoot was placed on culture medium, which was supplemented with particular concentration of hormone for shoot differentiation. When the shoots grew about 3-5 cm in length with 3-6 well developed leaves they were cultured on freshly prepared medium containing different combinations of hormonal supplements for root induction.

### 2.8 Establishment of Plantlets

Regenerated plantlets were transplanted to pots (10×15 cm) containing sandy soil and cowdung in 1:1 ratio. Occasional spray of water was done to prevent sudden desiccations and maintain high humidity (98%) around the plantlets. Initially the plantlets were hardened in controlled

environment. Then after 2 weeks, exposed to lower humidity and higher light intensity (3000 lux). Finally, after 20 days plantlets were transferred to natural environment.

### 2.9 Data Collection and Statistical Analysis of Data

Data were recorded at 2, 4 and 6 weeks after inoculation (WAI) on the effect of different treatments on shoot and root proliferation. Data were analyzed using MSTAT-C statistical package where means were compared by the Duncan's Multiple Range Test (DMRT).The experiment was conducted in Completely Randomized Design (CRD) with five replications in culture room.

## 3. RESULTS AND DISCUSSION

The effect of BAP and IBA was investigated with different concentrations for *in vitro* regeneration of local sabri variety of banana using shoot tips as explants. The results are discussed based on the nature of morphogenetic response of variety, hormones (BAP & IBA) with different concentrations and their combination.

### 3.1 Effect of BAP Concentrations on Multiple Shoot Proliferation

The effect of BAP on shoot proliferation and elongation from shoot tip of sabri variety was investigated by adding different concentrations of BAP to a basal MS medium (semi solid). *In vitro* culture of shoot tip results green globular ball like structure with hard coating within 7-10 days of inoculation in media containing different concentrations of BAP and combination of BAP and IBA (Plate 1B). From these balls like structure adventitious shoots were developing (Plate 1C). This finding was supported by Azam et al. [23] who found that cultured shoot tips were visible as a swelling and greenish color after 10–15 days of inoculation in MS media supplemented with different concentrations of BAP. Al-amin et al. [18] observed meristematic ball like structure in regeneration media containing different concentrations of BAP and NAA.

Significant variations at 1% level were observed among different treatments of BAP on percent response of explants (%) and on number of shoots per explant using MS media in the laboratory condition the results are presented in the Figs. 1 and 2.

The highest percent response of explants (84%) was observed in 5.0 mg/L BAP. The second highest (72%) was observed in 4.0 mg/L BAP while the lowest (44%) was observed in control (0.0 mg/L). Ahirwar et al. [24] found the highest frequency of shoot regeneration (52.25%) at 5.0 mg/L BAP using Banana (*Musa paradisiaca* L.) that is supported by the present study. The findings of the present study is not fully supported by Darvari et al. [25] where they found that all the cultivars of Malaysian Banana and plantain (*Musa* spp.) showed their highest response to regeneration at 8.0 mg/L of BAP.

At 2, 4 and 6 weeks after inoculation (WAI), the highest number of shoots (2.00, 3.00 and 3.40 shoots per explant) was observed in medium supplemented with 5.0 mg/L BAP. The better performance was observed in 4.0 mg/L of BAP (3.00 shoots per explant) at 6 WAI. The 3.0 mg/L of BAP (1.60, 2.00 and 2.40 shoots per explant) also showed good performance. Lowest number of shoot was observed in control (1.00, 1.00 and 1.00 shoots per explant). The result of current investigation is supported by Rahman et al. [10] where they found the highest number of shoots (5.9) for each explant at 4.0 mg/L BAP using Banana (*Musa* sp.) cv. Agnishwar. The high performance of BAP over other cytokinins in the multiplication of shoot tips has also been reported in different cultivar of banana by Gilmar et al. [26]. The initial response of cytokinin may be mediated by an increase in the cytosolic calcium concentration by promoting calcium uptake from the medium. Calcium affects the cytoskeleton, which can regulate exocytosis [27].

### 3.2 Effect of IBA Concentrations on Root Regeneration

The significant variations were observed among different treatments of IBA on growth parameter of number of root at 2, 4 and 6 WAI in MS media at 1% level of significance (Fig. 3). The highest number of root (2.20, 3.40 and 3.40 per plantlet) was produced by 1.5 mg/L of IBA, which was statistically similar with 1.0 mg/L IBA (2.20, 2.60 and 2.60 per plantlet) at 2, 4 and 6 WAI, respectively (Fig. 3). Vigorous roots of *in vitro* grown plantlet of sabri variety of Banana on MS media supplemented with 1.5 mg/L IBA were shown in Plate 2. The results of present study agree with the findings of Rahman et al. [28]. They observed that IBA at a concentration of 1.0 mg/L was found most suitable for rooting of shoot in Banana cv. Agnishwar. Huq et al. [29] found that the best response of Banana (*Musa* spp.)

cv. Sabri towards root induction was achieved on half MS medium supplemented with 0.5 mg/L IBA. This variation in number of roots per plantlet might be due to the difference in genotype and culture environments.

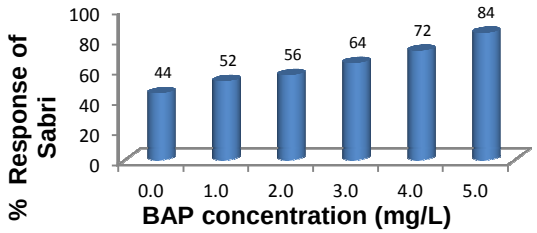



**Fig. 1. Effect of BAP on the percent response of shoot tip explants of sabri variety of banana**

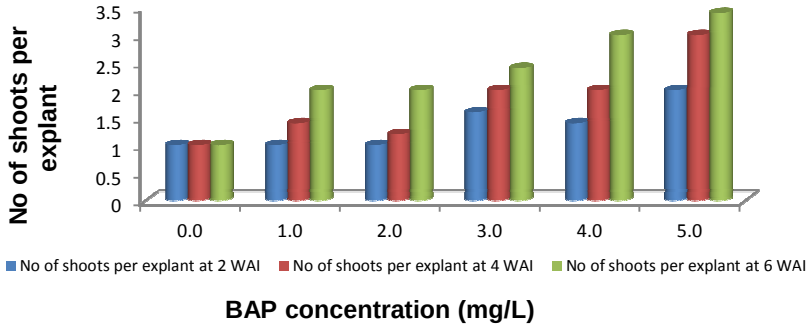
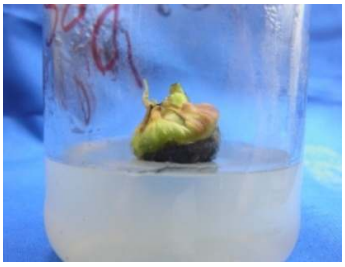
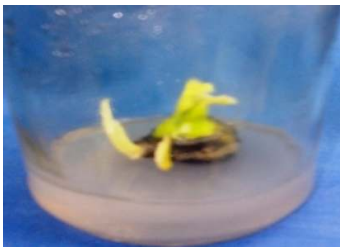



**Fig. 2. Effect of BAP on the number of shoots per explant of sabri variety**

**A. Shoot tip inoculation in MS media**



**B. Green globular ball like structure with hard coating produced from shoot tips at 8 days after inoculation**



**C. Adventitious shoot initiation from ball like structure at 2 weeks after inoculation (WAI)**

**D. Multiple shoots produced from shoot tip at 6 WAI**

**Plate 1(A-D). Shoot regeneration from shoot tip of sabri variety of banana cultured on MS medium containing 5.0 mg/L of BAP**

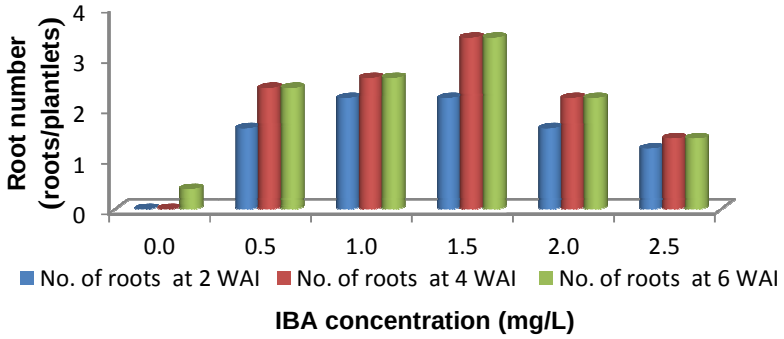



**Fig. 3. Effect of IBA on number of roots/plantlet for sabri variety of banana**

**Plate 2. Vigorous roots grown with 1.5 mg/L IBA on MS media**

### 3.3 Combined Effect of BAP and IBA on Shoot and Root Regeneration

In present study, the effect of 3.0, 4.0 and 5.0 mg/L BAP with different concentrations of IBA was investigated. Muhammad et al. [30] found highest response of shoot multiplication in 5.0 mg/L BAP and 1.0 mg/L IAA while Ahmed et al. [31] obtained maximum multiplication on treatment involving 4 mg/L BAP and 2 mg/L IAA. In the present study, the effect of BAP at higher concentrations (3.0, 4.0 and 5.0 mg/L) reveals better performance. Among them, 5.0 mg/L of BAP was found to be better performance in shoot. The differences obtained in the requirement of phytohormones as reported by different researchers and also in the present investigation may be attributed to the differences in the levels of endogenous phytohormones, nutrients, metabolites and interaction between various factors. According to Skoog and Miller [32], quantitative interaction between diverse growth factor may have decisive role in organogenesis. Ammirato et al. [33] observed that the factors involved in the control of organogenesis in culture are more complex and plant hormones, organic and inorganic nutrient and osmotic concentration exert a performed influence on organogenesis.

The different concentrations of BAP and IBA showed significant variations on percent response of explants (%), on number of shoots per explant (Table 1) and on number of roots (Table 2) at 1% level of significance. The highest percent response of explants (90%) at 5.0 mg/L BAP + 2.5 mg/L IBA and the lowest response (35%) at 3.0 mg/L BAP +2.0 mg/L IBA. The results of present study are partially supported by Huq et al. [29] where they found the highest percentage of shoot regeneration (90%) when cultured on MS + 4.0 mg/L BAP + 2.0 mg/L IAA + 13% (v/v) coconut water using Banana (*Musa* spp.) cv. Sabri. According to Cronauer and Krikorian [22], the requirement of cytokinin and auxins depends on the variety of Banana and culture conditions.

The highest number of shoots was produced by the treatment of 5.0 mg/L BAP+2.0 mg/L IBA (3.40 shoots per explant) (Plate 3A). But the treatment of 5.0 mg/L BAP + 2.5 mg/L IBA showed good number of shoots proliferations (3.00 shoots per explant). On the other hand, the lowest number of shoots (1.60 shoots per explant) was produced by the treatment concentrations of 4.0 mg/L BAP + 2.0 mg/L IBA. The treatment 3.0 mg/L BAP + 2.5 mg/L IBA produced lower shoots which was not statistically different with the treatment of 4.0 mg/L BAP +2.5 mg/L IBA. Iqbal et al. [34] found that the highest number of shoots was produced with 5.0 mg/L BAP +1.0 mg/L IAA + 10% CW (10 shoots/explant) at 40 DAI (Days After Initiation) and 6.0 mg/L BAP + 1.0 mg/L IAA +10% coconut water (8 shoots/explant) at 40 DAI. The result of current investigation is partially supported by Huq et al. [29] where they found maximum number of shoots (10) per explant

when cultured on MS + 4.0 mg/L BAP + 2.0 mg/L IAA + 13% (v/v) coconut water using Banana (*Musa* sp.) cv. sabri. This variation might be due to the differences in genotypes and explants used. Culture environment might be another reason for different results. Besides this variation might be due to interaction of both endogenous and exogenous hormones (BAP and IBA).

The highest number of root (5.20 per explant) was found in the treatment of 3.0 mg/L BAP+1.0 mg/L IBA. On the other hand, the lowest numbers of roots (2.00 per explant) were produced by the treatment concentrations of 5.0 mg/L BAP+0.5 mg/L IBA. More number of vigorous roots of sabri variety of Banana were grown on MS media supplemented with 3.0 mg/L BAP+1.0 mg/L IBA (Plate 3B). Gubbuk and Pekmezc [35] found that supplementation of 2.0 µM TDZ, and 1.0 µM IAA or 20.0 µM BAP and 1.0 µM IAA on MS medium, followed 5.0 g/L charcoal were the best combinations for the *in vitro* root regeneration of banana types.

### 3.4 *Ex vitro* Acclimatization and Establishment of Plantlets on Soil

After sufficient shoot and root development at 6 weeks of culture, the small plantlets were taken out from culture vessel carefully without damaging any roots. Excess media around the root was washed off by running tap water to prevent further microbial infection. The plantlets were then transplanted in plastic pots filled with sterilized soil: cowdung (1:1) and soil mixture were treated with a solution of 1% IBA due to proper rooting in plastic pots. Immediately after transplantation the plantlets were irrigated with a fine spray of water. Occasional spray of water was given to prevent sudden desiccations and maintain high humidity around the plantlets and the plantlets were placed under controlled environment for proper hardening (Plate 4A). The highest survival rate 90.00% found in controlled environment (Table 3). After 20 days of hardening the plantlets were transplanted to soil (Plate 4B). In the open atmosphere, the survival rate of Sabri varieties was 88.89% (Table 3).

**A. Multiple shoots produced on MS media supplemented with 5.0 mg/L BAP +2.0 mg/L IBA at 4 WAI**

**B. Vigorous roots grown with 3.0 mg/L BAP + 1.0 mg/L IBA**

**Plate 3(A-B). *In vitro* regeneration of sabri variety of Banana with combined effect of BAP & IBA**


**A. Plantlets in controlled room**

**B. Established plants in soil**

**Plate 4 (A-B). Hardening in controlled room and well established plants of sabri variety of Banana in open atmosphere**

Mortality of the plantlets occurred during transplantation of plantlets due to shifting between containers, injuries to the root system and excessive evaporation. Rahman et al. [28] found that the survival rate was 90% in Banana (*Musa* spp.) cv. Agnishwar to the main field.

**Table 1. Combined effect of BAP and IBA on shoot regeneration in sabri varieties of banana**

| BAP (mg/L) | IBA (mg/L) | Number of explant inoculated | Percent response of explants (%) | Number of shoot |
|---|---|---|---|---|
| 3.0 | 0.5 | 20 | 50 | 2.00 ±0.0b |
| | 1.0 | 20 | 45 | 1.80 ±0.45b |
| | 1.5 | 20 | 65 | 1.80 ±0.45b |
| | 2.0 | 20 | 35 | 2.00 ±0.0b |
| | 2.5 | 20 | 45 | 1.60 ±0.55b |
| 4.0 | 0.5 | 20 | 40 | 3.00 ±0.0a |
| | 1.0 | 20 | 45 | 2.00 ±0.0b |
| | 1.5 | 20 | 55 | 2.00 ±0.0b |
| | 2.0 | 20 | 65 | 1.60 ±0.55b |
| | 2.5 | 20 | 50 | 1.60 ±0.55b |
| 5.0 | 0.5 | 20 | 70 | 3.00 ±0.0a |
| | 1.0 | 20 | 75 | 2.00 ±0.0b |
| | 1.5 | 20 | 65 | 2.00 ±0.0b |
| | 2.0 | 20 | 80 | 3.40 ±0.55a |
| | 2.5 | 20 | 90 | 3.00 ±0.0a |
| $LSD_{(0.01)}$ | | | | 0.55 |
| CV (%) | | | | 14.94 |
| SE | | | | 0.146 |

*Values in the column are the means of five replicates. In a column, mean values with the same letters are not statistically different from each other at 1% probability by DMRT*

**Table 2. Combined effect of BAP and IBA on number of root in sabri variety of banana**

| BAP (mg/L) | IBA (mg/L) | Number of root |
|---|---|---|
| 3.0 | 0.5 | 3.40 ±0.90a |
| | 1.0 | 5.20 ±0.84a |
| | 1.5 | 2.80 ±0.84a |
| | 2.0 | 2.20 ±1.1a |
| | 2.5 | 2.20 ±0.45a |
| 4.0 | 0.5 | 2.60 ±0.55a |
| | 1.0 | 2.20 ±1.1a |
| | 1.5 | 2.40 ±0.55a |
| | 2.0 | 3.20 ±0.45a |
| | 2.5 | 2.40 ±0.55a |
| 5.0 | 0.5 | 2.00 ±0.0a |
| | 1.0 | 2.40 ±0.55a |
| | 1.5 | 2.20 ±0.84a |
| | 2.0 | 2.40 ±0.55a |
| | 2.5 | 2.40 ±0.55a |
| $LSD_{(0.01)}$ | | 2.92 |
| CV (%) | | 24.08 |
| SE | | 0.78 |

*Values in the column are the means of five replicates. In a column, mean values with the same letters are not statistically different from each other at 1% probability by DMRT*

**Table 3. Survival rate of *in vitro* regenerated plants of local sabri variety of Banana**

| Acclimatization | No. of plants transplanted | No. of plants survived | Survival rate (%) |
|---|---|---|---|
| Plantlet in plastic pots under controlled environment | 20 | 18 | 90.00 |
| Plantlet in open atmosphere | 18 | 16 | 88.89 |

## 4. CONCLUSION

In present study we found that the 5.0 mg/L BAP and 1.5 mg/L IBA concentration performed best in *in vitro* shoot and root regeneration. In case of combined effect, 5.0 mg/L BAP ± 2.5 mg/L IBA and 5.0 mg/L BAP ± 2.0 mg/L IBA showed the highest results for *in vitro* regeneration of sabri variety of banana. Therefore, suitable protocol was established which could be used for *in vitro* rapid propagation of Banana plantlets. In future more research works have been conducted to assess the field performance for ultimate improvement of Banana under Bangladesh condition.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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