Research Article

Effects of 6-Benzylaminopurine and Indole-3-acetic Acid on Growth and Root Development of Banana Explants in Micropropagation

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Abstract | The purpose of the experiment was to find out the impact of various combinations of growth regulators 6-Benzylaminopurine (BAP cytokinins family) and Indole-3-acetic acid (IAA auxin family) on growth and root induction of banana explants micro-propagated *in vitro*. Explants were obtained from young suckers of 8818-william, Pisang and Brazilian varieties. The explants were cultured *in vitro* containing MS media and different BAP and IAA combinations. The results showed banana varieties exhibited differences for the shoot and root development and also for the number of shoots and leaves. Wiallium-8818 hybrid gave more shoots (7.33) when the concentration of BAP and IAA was 2.5 and 1 mg L^{-1}, respectively. Alike, Wiallium-8818 hybrid produced the longest shoot (12.20 cm) with more leaves (6.23) at 1 mg L^{-1} of each BAP and IAA. But Pisang variety produced maximum fresh weight (15.45 g) with a greater number of roots at 5 + 1 mg L^{-1} of BAP + IAA, respectively.

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Introduction

There is an imperative role of plant growth regulators to develop a specific mode of growth in the cultured tissues that might be due to increase of particular biochemicals in them. Application of single or mixture of various hormones in the media causes specific maintenance and keeps balance between inorganic and organic contents in the developing tissues. This leads the tissues to produce either into roots/or shoots or even death (Ikram-ul-Haq and Dahot, 2007). Growth regulators are applied in various combinations or proportions to enhance shoots growth or formation and break dormancy (Madhulatha *et al*., 2004). The auxins and cytokinins have significant value in *in vitro* culture as the later are used to develop or initiate shoot development and buds formation while the former are used in the media for root formation (North *et al*., 2007). These growth hormones are needed in suitable combination in growth media and the difference in their concentration can effectively alter the behavior of plant growth in the culture (Dixon and Gonzales, 1994). Benzyl aminopurine (BAP) belongs to cytokinin family and is known to induce axiliary and adventitious shoot initiation from meristematic explants in banana. It also decreases the apical meristem dominance in banana (Jafari *et al*., 2011) However, higher concentration of BAP can inhibit the adventitious meristems elongation and transformation into complete plant (Buising *et al*., 1994). Likewise, indole acetic acid (IAA) is the best

auxins and plays essential functions in growth of plants and cell differentiation (Bohidar *et al*., 2008). IAA has been documented to induce and enhance rooting in plants during *in vitro* propagation (Hussein, 2012).

Hamide and Pekmezei (2004) revealed that BAP below 20 µM plus 1 µM TDZ (Thidiazuron) had no effect on shoot length while BAP + TDZ greater than 20 and 2 µM, respectively, suppressed elongation of shoot. The exogenous concentration of hormones in *in vitro* is the main factor that influences regeneration and elongation. Gubbuk and Pekmzci (2004) stated that BAP with moderate concentration improved the shoot growth rate however very high concentration reduced multiplication and reduced shoot elongation. Alike, Arinaitwe *et al*. (1999) and Buah *et al*. (2010) revealed that use of high dose of auxin in growth medium decreased the shoot growth rate in different cultivars of banana. Low auxin/cytokinin ratios (16.8/1.2 and 16.8/1.0) produced single shoot in banana due to apical dominance (Buah *et al*., 2010). Similarly, mobility, stability, rate of fusion and oxidation of hormones also affect the shoot initiation during *in vitro* propagation. Therefore for successful *in vitro* propagation of banana, suitable concentration of growth regulators is very essential. In this regard, an experiment was conducted to determine the most suitable combination of BAP and IAA for banana under micropropagation technique.

Materials and Methods

The experiment was started at plant genetic resource institute (PGRI), National Agriculture Research Center (NARC) Islamabad, Pakistan during 2015. The treatments of experiment were as; T_0 = 0 +0 (IAA + BAP mg L^{-1}), T_1= 0.5 + 1 (IAA + BAP mg L^{-1}), T_2 = 0.5 + 2.5 (IAA + BAP mg L^{-1}), T_3 = 0.5 + 5 (IAA + BAP mg L^{-1}), T_4 = 1+1 (IAA + BAP mg L^{-1}), T_5 =1 + 2.5 (IAA + BAP mg L^{-1}) and T_6 = 1+ 5.0 (IAA + BAP mg L^{-1}). The explants (3.5 cm length × 2.4 cm width) were obtained from diseased free, vigorous suckers of three banana cultivars (Brazilian, Pisang and William-8818 hybrid). The young suckers were taken from district Thatta, Sindh, a sub center of NARC. The explants were washed with autoclaved distilled water for 10 minutes. The meristem with shoot primordia were cultured as per treatment mentioned above along with MS medium. The temperature of growth room was kept at 27±2°C with 16 hr light. Data regarding shoots of explants, shoot length, number of leaves, roots and fresh weight were recorded.

Statistical analysis

Data were analyzed through Statistix 8.1 version and mean comparison was checked through LSD at 5% significance level.

Results and Discussion

Growth traits

The highest shoots per explants (6.53) was recorded for 2.5 mg L^{-1} BAP + 1 mg L^{-1} IAA followed by 5.0 mg L^{-1} BAP + 1 mg L^{-1} IAA while the minimum shoots per plant (1.5) were depicted for control treatments (Table 1). Among different varieties, Wiallium-8818 hybrid produced more shoots (4.94) followed by Brazillian (4.31) while least shoots (3.38) were noted for Pisang. Interaction effect showed that Wiallium-8818 hybrid produced maximum shoots (7.33) at 2.5 mg L^{-1} BAP + 1 mg L^{-1} IAA. The least number of shoots per plant was depicted for control treatments in all banana varieties (Table 1). Alike, BAP and IAA had significant effect on shoot length of banana varieties as shown in Table 2. Maximum shoot length (8.95 cm) was measured for BAP + IAA each at 1 mg L^{-1} while minimum shoot length (3.07 cm) was noted for control treatments. Similarly, varieties differed for shoot lengths. Wiallium-8818 hybrid had greater shoot length (8.52 cm) followed by Brazillian (5.18 cm) whereas Pisang had least shoot length (4.50 cm). Likewise, Wiallium-8818 hybrid supplemented with BAP + IAA each at 1 mg L^{-1} attained more shoot length (12.20 cm) followed by same variety (11.44 cm) at 2.5 mg L^{-1} BAP + 1 mg L^{-1} IAA (Table 2).

Table 1: *Effect of BAP and IAA on number of shoots of banana plants in in vitro condition.*

Treatments	Banana varieties			
BAP mg L^{-1} + IAA mg L^{-1}	Brazilian	Pisang	Wiallium-8818 hybrid	Means
0	1.54 jk	1.31 k	1.82 ij	1.56 F
1.0 + 0.5	2.80 h	2.03 i	3.44 g	2.76 E
2.5 + 0.5	3.35 g	2.71 h	3.91f	3.32 D
5.0 + 0.5	4.54 e	3.26 g	5.15 d	4.32 C
1.0 + 1.0	5.62 c	4.27 e	6.41 b	5.43 B
2.5 + 1.0	6.60 b	5.62 c	7.33 a	6.53 A
5.0 + 1.0	5.70 c	4.41 e	6.50 b	5.54 B
Means	4.31 B	3.38 C	4.94 A	
LSD at 5%	Hormones = 0.18, Varieties = 0.12, Interaction = 0.31			

Table 2: *Effect of BAP and IAA on shoot length (cm) of banana plants in in vitro condition.*

Treatments	Banana varieties			
BAP mg L^{-1} +IAA mg L^{-1}	Brazilian	Pisang	Wiallium-8818 hybrid	Means
0	2.57 n	3.38 lm	3.26 m	3.07 G
1.0 + 0.5	3.84 k	3.59 kl	5.31 h	4.25 F
2.5 + 0.5	4.72 i	4.21 j	6.82 e	5.25 E
5.0 + 0.5	6.99 e	5.69 g	10.39 c	7.69 B
1.0 + 1.0	8.27 d	6.37 f	12.20 a	8.95 A
2.5 + 1.0	5.36 h	4.61 i	11.44 b	7.14 C
5.0 + 1.0	4.55 i	3.65 kl	10.23 c	6.14 D
Means	5.18 B	4.50 C	8.52 A	
LSD at 5%	Hormones = 0.17, Varieties = 0.11, Interaction = 0.29			

In case of fresh weight, BAP and IAA greatly increased the fresh weight of banana plants. Maximum improvement (12.96 g) was recorded for BAP (5 mg L^{-1}) + IAA (1 mg L^{-1}) followed by 2.5 mg L^{-1} of BAP + 1 mg L^{-1} IAA (Table 3). Control treatments showed minimum fresh weight (2.95 g) during study. Banana varieties also showed significant difference regarding fresh weight. Pisang variety with BAP + IAA at 5 and 1 mg L^{-1}, respectively showed maximum fresh weight (15.45 g) followed by Wiallium-8818 hybrid (14.39 g). All the varieties without growth regulators had minimum fresh weight and there was no difference among them.

Table 3: *Effect of BAP and IAA on fresh weight (g) of banana plants in in vitro condition.*

Treatments	Banana varieties			
BAP mg L^{-1} + IAA mg L^{-1}	Brazilian	Pisang	Wiallium-8818 hybrid	Means
0	3.53 n	2.82 p	2.49 q	2.95 G
1.0 + 0.5	3.76 n	3.72 n	3.27 o	3.58 F
2.5 + 0.5	4.20 m	5.78 k	4.16 m	4.71 E
5.0 + 0.5	5.48 l	10.06 f	5.61 kl	7.05 D
1.0 + 1.0	6.40 j	11.06 e	8.77 h	8.74 C
2.5 + 1.0	8.17 i	13.74 c	13.08 d	11.66 B
5.0 + 1.0	9.05 g	15.45 a	14.39 b	12.96 A
Means	5.80 C	8.95 A	7.40 B	
LSD at 5%	Hormones =0.15, Varieties =0.09, Interaction = 0.25			

Alike, maximum leaves per explants (5.70) were noted at 1 mg L^{-1} of BAP + 1 mg L^{-1} of IAA. Among varieties, Wiallium-8818 hybrid at 1 mg L^{-1} of BAP + 1 mg L^{-1} of IAA produced more number of leaves (4.47) followed by Pisang (Table 4). Similarly, BAP and IAA significantly influenced number of roots in explants. Maximum roots per plant were noted (4.67) when BAP + IAA concentration was 5.0 and 0.5 mg L^{-1}, respectively. Pisang variety produced more roots (5.50) when 5.0 mg L^{-1} of BAP and 0.5 mg L^{-1} of IAA was used (Table 5).

Table 4: *Effect of BAP and IAA on number of leaves of banana plants in in vitro condition.*

Treatments	Banana varieties			
BAP mg L^{-1} + IAA mg L^{-1}	Brazilian	Pisang	Wiallium-8818 hybrid	Means
0	2.50 i	2.60 hi	2.57 hi	2.56 E
1.0 + 0.5	2.93 gh	3.23 fg	3.47 f	3.21 D
2.5 + 0.5	3.87 e	4.07 e	4.53 d	4.16 C
5.0 + 0.5	4.57 d	4.90 cd	5.53 b	5.00 B
1.0 + 1.0	5.27 bc	5.60 b	6.23 a	5.70 A
2.5 + 1.0	2.50 i	2.60 hi	2.57 hi	2.56 E
5.0 + 1.0	2.93 gh	3.23 fg	3.47 f	3.21 D
Means	3.83 C	4.08 B	4.47 A	
LSD at 5%	Hormones =0.22, Varieties =0.17, Interaction = 0.38			

Table 5: *Effect of BAP and IAA on number of roots of banana plants in in vitro condition.*

Treatments	Banana varieties			
BAP mg L^{-1} + IAA mg L^{-1}	Brazilian	Pisang	Wiallium-8818 hybrid	Means
0	2.40 g	2.57 fg	2.17 g	2.38 E
1.0 + 0.5	2.93 ef	3.23 e	2.37 g	2.84 D
2.5 + 0.5	3.83 d	4.60 b	3.10 e	3.84 C
5.0 + 0.5	4.57 b	5.50 a	3.93 cd	4.67 A
1.0 + 1.0	4.30 bc	4.67 b	3.70 d	4.22 B
2.5 + 1.0	2.40 g	2.57 fg	2.17 g	2.38 E
5.0 + 1.0	2.93 ef	3.23 e	2.37 g	2.84 D
Means	3.61 B	4.11 A	3.05 C	
LSD at 5%	Hormones =0.11, Varieties =0.08, Interaction = 0.19			

All the three banana varieties showed significant differences as Wiallium-8818 variety produced maximum shoots (7.33) at BAP + IAA (2.5 + 1 mg L^{-1}) and had greater shoot length (12.20 cm) at BAP + IAA (each at 1 mg L^{-1}). Alike, Wiallium-8818 variety produced more number of leaves (6.23) at BAP + IAA (1+1 mg L^{-1}). But Pisang variety showed the highest fresh weight (15.45 g) and more number of roots at

BAP (5 mg L^{-1}) + IAA (1 mg L^{-1}), 5.0 mg L^{-1} of BAP + 0.5 mg L^{-1} of IAA, respectively. Ngomuo *et al.* (2013) also depicted that various mixtures or combinations of BAP with IAA showed significant positive effects for *in vitro* banana multiplication and plantains. Improvement in shoot length leaves and fresh weight may also be presumed due to enhanced cytosolic calcium levels resulting from higher absorbance or uptake from media at suitable combination of BAP and IAA. BAP and IAA in improving banana growth during tissue cultured trials is also documented by Hussein (2012). Alike, Al-Amin *et al.* (2009) documented that MS media along with BAP and NAA exhibited an improvement in growth of banana. Similarly, Rahman *et al.* (2013) conducted tissue culture experiment and documented that that greater root length (3.69 cm) was depicted at 1.0 mg L^{-1} IBA for explants of banana. Kadota and Niimi (2003) also revealed that BAP had very important role in inducing and increasing number of shoots in banana genotypes. BAP is widely used and significantly affects the shoot induction in plants. Kalimutha *et al.* (2007) noted that 2.0 mg L^{-1} BAP with 0.1 mg L^{-1} NAA exhibited greater response in induction of buds in banana. Sreeramanan *et al.* (2008) depicted that 8 mg L^{-1} of BAP produced more shoots while less shoots were noted at 4 mg L^{-1} of BAP. Habib *et al.* (2016) noted more number of shoot when 3.0 mg L^{-1} BAP was applied in liquid medium. Rahman *et al.* (2004) observed maximum shoot (51.17) at 6 mg $L^{-1}$of BAP. Alike, Shah *et al.* (2020) depicted maximum roots per plant (9.25 ±2.08) in banana at IAA (2.0 mg L^{-1}) and BAP (0.5 mg L^{-1}) along with MS media. However, they reported that MS basal media with IAA (3.0 mg L^{-1}) and BAP (1.0 mg L^{-1}) exhibited more shoots per plant (7.81±1.03). Khatun *et al.* (2017) reported that application of sole BAP at 5.0 mg L^{-1} recorded highest number of shoots (3.4) in banana variety Sabri during in vitro condition.

Conclusions and Recommendations

The use of BAP with IAA was found to enhance number of shoots, leaves, roots and fresh weight if different banana varieties. Due to the genotype specificity, it is suggested that 1 mg L^{-1} of BAP and 1 mg L^{-1} of IAA may be used for *in vitro* propagation for these banana varieties.

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Novelty Statement

This protocol optimization would be helpful for mass scale production of high yielding and disease-free banana plants in future.

Author's Contribution

Arifa Khan, principal author, prepared the 1st draft. Jabar Zaman Khan Khatak planned and supervised the research and experiments. Shazia Erum, co-supervised the research. Aish Muhammad guide and reviewed the manuscript. Arsalan Bashir help in data recording and generation of the tables, All authors have read and agreed to this version of the manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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