Effect of Medium and Cytokinin Types on Banana Micropropagation during Multiplication Stage

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Abstract: Banana (*Musa* spp. family Musaceae) is a fourth most important fruit crop in the world. This investigation was carried out in tissue culture laboratory in Horticulture Department, Agriculture Faculty, Suez Canal University, Ismailia during the period 2013 – 2015, to study the effect of medium type (solid or liquid) and cytokinin types (BAP or Kin at 0, 1, 3, 5, 7 mg/l) during multiplication stage, in a complete randomized design with two factors. The explants were collected from suckers grown around banana fruiting mother plants, cultivar Grande Naine. In liquid medium supplemented with 3.0 mg/l Benzyl amino purine (BAP) increased number of shoots (7.20). While 5 mg/l Kinetin (Kin) improved shoot length (7.40 cm) in solid medium compared with other treatments. The liquid media is preferable within mass production and commercial advantages. The BAP promotes shoot initiation and development either with solid or liquid media more than Kin.

Keywords: Banana, *Musa* spp., Tissue culture, micropropagation, cytokinin, medium type.

INTRODUCTION

Banana is a fourth most important fruit crop in the world. It is a rich source of carbohydrates and vitamins, particularly vitamins B, potassium, phosphorus, calcium and magnesium (Sahijram *et al.*, 2003). Banana production occupies an important share in the total fruit production of Egypt. The total cultivated area of banana reached to 74622 faddans, fruited area 65510 faddans and total banana production was 1283644 tons with average of about 19.595 tonsfaddan^{-1} (Egyptian Ministry of Agriculture, 2014).

The increase in cultivated area was due to the introduction of micropropagation technique in several tissue culture (TC) labs in Egypt during the past 20 years. If all the area are to use tissue culture plants then the required amount of TC plants is about 35 million transplants there for, any system which enable banana micropropagation to be increased will be valuable to growers.

Cytokinin such as BAP and Kin are known to reduce the apical meristem dominance and induce both auxiliary and adventitious shoot formation from meristematic explants in banana (Ngomuo and Ndakidemi, 2014).

Optimal shoot proliferation from shoot tip rates were achieved due to the pulse treatment of BA and kin combination (1:1) at the concentration of 50 mg/l for 60 min (Madhulatha *et al.*, 2004). In a review by (Strosse *et al.*, 2004), adding 5 mg/l BA to the culture medium resulted in suppression of the apical dominance in shoot-tip cultures and a reduction of corm and leaf tissue between meristematic tissues.

Treatment with BAP or TDZ with IAA increased shoot length more than BAP or TDZ only. On the other hand, cytokinin types and their concentrations enhanced shoot proliferation rate. However, the application of higher BAP concentrations inhibits elongation of adventitious meristems and the conversion into complete plants according to (Gubbuk and Pekmezci, 2006 and Ngomuo and Ndakidemi, 2014)

The main objective of this investigation was to identify the effect of medium type (solid or liquid) and cytokinin types (BAP or Kin) during the multiplication stage of banana micropropagation.

MATERIALS AND METHODS

The present study was carried out in the plant tissue culture laboratory in the Department of Horticulture, Faculty of Agriculture, Suez Canal University, Ismailia during the period 2013 – 2015.

Preparation of Explants:

The suckers were collected from healthy fruiting mother plants, and at the time of separation, these were 30 cm in height. In the laboratory, outer leaves were peeled off until the explants were 3 cm in height and 1 cm at the base, were soaked on anti-oxidant solution (100 mg/l citric acid and 150 mg/l ascorbic acid) for 2 hours. These explants were surface-sterilized with 5.25% sodium hypochlorite for 10 min. A few drops of Tween-20 were added during sterilization, and explants were shaken continuously for uniform sterilization. After the explants were washed with sterile distilled water, further trimming was carried out under an aseptic environment (in a laminar air flow hood) to the required size (4–6 mm).

Culturing medium:

In the starting stage, Murashige and Skoog (1962) salts and vitamins supplemented with 30 g/l sucrose, 3 mg/l BA, and 7g/l Agar was used. The pH of the culture medium was adjusted at 5.7± 0.1 prior to addition of agar. The culture medium was distributed into culture tubes 25 x 150 mm where immediately capped with polypropulin closure and autoclaved at 121ºC at 15 lb/in^2 for 20 min and then incubated at 25 □ C ± 1º C for testing against contamination.

Explant establishment:

Sterilized shoot tip explants were cultured on the specific culture medium as mentioned before in the starting stage. Culture tubes were incubated at 27± 2 ºC

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and 16/8 hours (day/night) light using white fluorescent tubes giving intensity of about 1500 Lux. After four weeks, all survival explants were transferred into the medium of multiplication stage.

Effect of medium type and cytokinin types:

Survived and established shoot tip explants were transferred and re-cultured on medium which consisted of MS basal nutrient medium supplemented with BAP (0, 1, 3, 5, 7 mg/l) or Kin (0, 1, 3, 5, 7 mg/l) + 30g/l sucrose + 7.0 g/l agar in solid medium and without agar in liquid medium and pH was adjusted at 5.7±0.1 in solid medium and 5.5±0.1 in liquid medium prior to the addition of agar. These cultural medium were distributed into glass culture jars 350 ml where each one contained 50 ml of prepared medium. The cultural jars were immediately wraped with polypropulin closure and autoclaved at 121 □C at 15 lb/in^2 for 20 min.

At the end of multiplication stage data recorded per cluster were: shoot number (No.), shoot length (cm), fresh weight (g) and number of leaves (No.).

Statistical analysis:

Experiment in this work was designed as factorial experiment in a complete randomized design. Data were computerized and subjected to statistical analysis using SPSS "version 19" statistical software. The differences between means of treatments were tested using Duncan Tests at 0.05 level according to Snedecor and Cochran, (1980).

RESULTS AND DISCUSSION

Main effect of medium type, cytokinin types and concentrations:

Data presented in Table (1) showed that shoot tips produced approximately the same number of shoots when cultured in solid or liquid medium. The values of shoots number appeared no significant differences.

Although it was slightly higher within liquid medium.

Shoot length was significantly higher in solid medium than liquid one (5.76 and 3.77 cm, respectively).

Plantlet fresh weight significantly increased on solid medium. Leaves number was not affected by media type.

The shoot numbers were significantly affected by using cytokinin where, BAP gave higher shoot numbers than Kin. In contrast Kin increased shoot length, fresh weight and number of leaves more than BAP.

The shoot numbers were significantly increased as cytokinin concentration increased. This increment correspond to cytokinin concentrations up to 3 mg/l, while the higher concentration (5.0 and 7.0 mg/l) significantly decreased this parameter compared with control. The shoot length showed no significant differences among all the concentrations of cytokinin, although the control plantlets had significant higher shoot length than other cytokinin concentrations.

Table (1): Main effect of medium type, cytokinin types and concentrations on number of shoots, shoot length, fresh weight and number of leaves of "Grand Nain" banana micropropagated *in vitro* during multiplication stage.

Treatment		No. of shoots per clump	Shoot length (cm)	Fresh weight (g)	No. of leaves
Media	**Solid**	2.62 a	5.76 a	9.63 a	4.98 a
	Liquid	2.76 a	3.77 b	3.43 b	4.92 a
Cytokinin	**BAP**	3.28 a	3.83 b	5.20 b	4.52 b
	Kin	2.10 b	5.71 a	7.87 a	5.38 a
Cytokinin conc. mg/l	**0**	1.00 c	6.03 a	6.77 a	5.45 a
	1	3.00 b	4.59 b	5.78 b	4.95 ab
	3	4.05 a	4.76 b	7.30 a	4.70 b
	5	2.85 b	4.40 b	6.49 a	4.75 b
	7	2.55 b	4.07 b	6.31 a	4.90 b

Means of each column in each treatment have the same letter/s are not significantly different at 0.05 level of probability according to Duncan's multiple range test.

Cytokinin at 3.0 mg/l gave the highest fresh weight with no significant differences with the other concentrations except 1.0 mg/l had the lowest fresh weight than the other cytokinin concentrations.

The control and 1.0 mg/l cytokinin gave the highest leaves number (5.45 and 4.95) without significant differences in between. While the other concentrations showed very closed values.

Interaction effect of media type and cytokinin types.

In any medium type (solid or liquid), medium supplemented with BAP gave a significant higher shoot numbers than that supplemented with Kin Table (2).

However, solid medium with Kin produced the highest significant shoot length than liquid media with BAP. The highest fresh weight was significantly showed in solid media with Kin followed by the same medium with BAP. In significant reduction in fresh weight was observed in liquid medium with BAP or Kin. Kin in either solid or liquid medium produced the highest number of leaves with non-significant. While, the inverse trend showed with BAP in solid or liquid medium with insignificant differences.

Table (2): Effect of interaction between media type and cytokinin types on number of shoots, shoot length, fresh weight and number of leaves of "Grand Nain" banana micropropagated *in vitro* during multiplication stage.

Media	Cytokinin	No. of shoots per clump	Shoot length (cm)	Fresh weight (g)	No. of leaves
Solid	BAP	3.08 a	4.59 b	7.29 b	4.56 b
	Kin	2.16 b	6.93 a	11.97 a	5.40 a
Liquid	BAP	3.48 a	3.06 c	3.11 c	4.48 b
	Kin	2.04 b	4.48 b	3.76 c	5.36 a

Means of each column have the same letter/s are not significantly different at 0.05 level of probability according to Duncan's multiple range test.

Effect of interaction between media type and cytokinin concentrations.

Liquid medium supplemented with 3.0 mg/l gave significant the longest shoot number than other cytokinin concentrations Table (3). In solid medium all cytokinin concentrations showed significant differences with control and non-significant differences among them.

The control (0.0 mg/l cytokinin) in solid medium gave the longest shoot length followed by the same medium supplemented with 3.0 or 5.0 mg/l cytokinin with no significant differences in between. Also, in liquid medium the control showed the highest shoot length followed by other concentration in descending order.

Fresh weight of plantlet in solid media supplemented with different cytokinin levels was higher than those of plantlets cultured in liquid media with significant differences. On the other side non-significant differences appeared among plantlets in solid media or between those in liquid media.

Leaves number showed much closed values among solid or liquid media supplemented with different cytokinin levels although there were significant differences among few values.

The effect of interaction between cytokinin types and concentrations:

Data in Table (4) showed that number of shoots were the highest when media was supplemented with BAP at 3.0 mg/l with significant differences as compared with the other cytokinin levels. Data also revealed that BAP promoted shoot initiation and development more than kinetin. On the other hand, kin appeared more promotion for shoot length and fresh weight than BAP. This was obvious within the same level and at different concentrations of both BAP and Kin. The different concentrations of Kin significantly increased the leaves number higher than BAP. The differences among various BAP concentrations and among those of Kin were not significant.

The interaction effect of media type, cytokinin types and concentrations:

Data presented in Table (5) and Plate (1) showed that number of shoot increased gradually with increasing BAP concentration in the solid media from 1 mg/l up to 5 mg/l then significantly decreased within the highest concentration. The liquid media showed similar response to BAP like that of solid media although the increments of shoot number were observed when increasing its concentration up to 3 mg/l then decreased within the other two higher concentrations. The solid media supplemented with Kin showed that increased Kin concentration only up to 3 mg/l significantly increased shoot number while the other two higher concentrations decreased shoots number. The addition of Kin to liquid media increased the shoot number higher than control at the lowest concentration. Increasing the Kin concentration showed no increments in the shoots number.

Table (3): Effect of interaction between media type and cytokinin concentrations on number of shoots, shoot length, fresh weight and number of leaves of "Grand Nain" banana micropropagated *in vitro* during multiplication stage.

Media	Cytokinin conc. mg/l	No. of shoots per clump	Shoot length (cm)	Fresh weight (g)	No. of leaves
	0	1.00 c	7.05 a	10.18 a	5.10 ab
	1	2.90 b	5.37 bc	8.53 a	5.10 ab
Solid	**3**	3.30 b	6.21 ab	10.54 a	5.00 b
	5	3.10 b	5.60 ab	9.46 a	4.90 b
	7	2.80 b	4.58 bcde	9.44 a	4.80 b
	0	1.00 c	5.00 bcd	3.37 b	5.80 a
	1	3.10 b	3.80 cde	3.03 b	4.80 b
Liquid	**3**	4.80 a	3.30 e	4.06 b	4.40 b
	5	2.60 b	3.20 e	3.53 b	4.60 b
	7	2.30 b	3.55 de	3.19 b	5.00 b

Means of each column have the same letter/s are not significantly different at 0.05 level of probability according to Duncan's multiple range test.

Table (4): Effect of interaction between cytokinin types and concentrations on number of shoots, shoot length, fresh weight and number of leaves of "Grand Nain" banana micropropagated *in vitro* during multiplication stage.

Cytokinin	Concentration mg/l	No. of shoots per clump	Shoot length (cm)	Fresh weight (g)	No. of leaves
BAP	**0**	1.00 e	6.20 a	6.58 a-d	5.30 a
	1	3.70 b	3.07 c	3.78 d	4.50 b
	3	5.30 a	4.16 bc	6.53 a-d	4.20 b
	5	3.50 bc	2.70 c	4.20 bcd	4.20 b
	7	2.90 bcd	3.00 c	4.90 bcd	4.40 b
Kin	**0**	1.00 e	5.85 a	6.97 a-d	5.60 a
	1	2.30 d	6.10 a	7.78 abc	5.40 a
	3	2.80 cd	5.35 ab	8.07 ab	5.20 a
	5	2.20 d	6.10 a	8.79 a	5.30 a
	7	2.20 d	5.13 ab	7.73 abc	5.40 a

Means of each column have the same letter/s are not significantly different at 0.05 level of probability according to Duncan's multiple range test.

Shoot length values significantly differed according to media type, cytokinin types and concentrations. The highest value (7.4 cm / plantlet) was found in control plantlets cultured in solid medium supplemented with BAP or 5.0 mg/l Kin. The five concentrations of Kin added to the solid media showed the highest values with no significant differences among them. The different concentration of BAP with both solid or liquid media and Kin with liquid media indicated significant differences among shoot length per plantlets of all treatments.

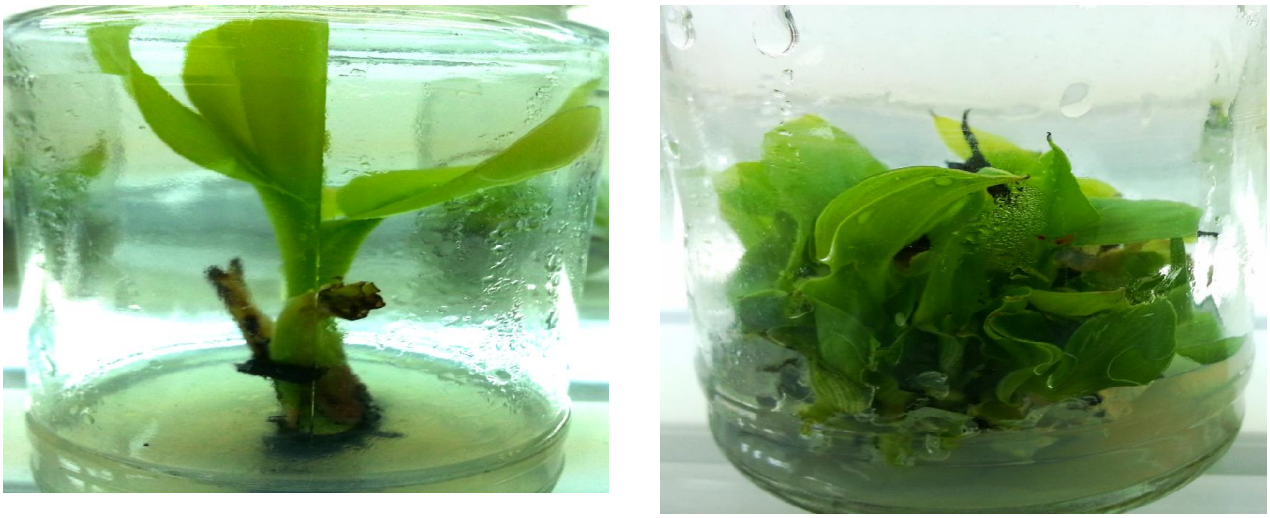
The fresh weight of plantlets in solid media supplemented with the various concentrations of Kin was the highest in comparison with the other treatments with highly significant differences. The plantlets grown in liquid medium supplemented with BAP or Kin at different concentration appeared the lowest values with significant differences among them.

The number of leaves per plantlets showed very closed values although there were significant differences between some of these values.

Table (5): Effect of interaction between media type, cytokinin types and concentrations on number of shoots, shoot length, fresh weight and number of leaves of "Grand Nain" banana micropropagated *in vitro* during multiplication stage.

Media	Cytokinin	Conc. mg/l	No. of shoots per clump	Shoot length (cm)	Fresh weight (g)	No. of leaves
Solid	BAP	0	1.00 g	7.40 a	9.79 cd	4.80 bcd
		1	3.60 bc	3.54 de	4.60 fg	4.80 bcd
		3	3.40 cd	5.72 bc	8.92 d	4.80 bcd
		5	4.20 b	3.80 de	5.83 f	4.40 cde
		7	3.20 cde	2.50 ef	7.31 e	4.00 de
	Kin	0	1.00 g	6.70 ab	10.58 bc	5.40 ab
		1	2.20 f	7.20 ab	12.46 a	5.40 ab
		3	3.20 cde	6.70 ab	12.16 a	5.20 abc
		5	2.00 f	7.40 a	13.09 a	5.40 ab
		7	2.40 ef	6.66 ab	11.57 ab	5.60 ab
Liquid	BAP	0	1.00 g	5.00 c	3.37 gh	5.80 a
		1	3.80 bc	2.60 f	2.96 gh	4.20 de
		3	7.20 a	2.60 f	4.14 gh	3.60 e
		5	2.80 def	1.60 ef	2.57 h	4.00 de
		7	2.60 ef	3.50 de	2.50 h	4.80 bcd
	Kin	0	1.00 g	5.00 cd	3.37 gh	5.80 a
		1	2.40 ef	5.00 cd	3.10 gh	5.40 ab
		3	2.40 ef	4.00 de	3.97 gh	5.20 abc
		5	2.40 ef	4.80 cd	4.48 fg	5.20 ab
		7	2.00 f	3.60 de	3.88 gh	5.20 ab

Means of each column have the same letter/s are not significantly different at 0.05 level of probability according to Duncan's multiple range test.



0.0 mg/l BAP solid medium 5.0 mg/l BAP solid medium

Plate (1): The interaction effect of solid medium and BAP concentration during multiplication stage.

The above mentioned results are in agreement with those of Gübbük and Pekmezci (2004), Gubbuk and Pekmezci (2006), Kalimuthu *et al.* (2007), Shiragi *et al.* (2008), Al-Amin *et al.* (2009), Bhosale *et al.* (2011), Mahadev *et al.* (2011), Jafari *et al.* (2013), Rahman *et al.* (2013), Anbazhagan *et al.* (2014), Mahdi *et al.* (2014), Ngomuo and Ndakidemi (2014) and Shankar *et al.* (2014). All of them found that BAP and kinetin increased the shoot number initiated from shoot tips of banana during micropropagation through tissue culture. The shoot length also increased as a result of their cytokinin treatments. They added that the increase in each cytokinin concentration till 3 or 5 mg/l increased shoots number, shoot length while the higher concentrations showed less value in this respect.

CONCLUSION

In conclusion, the results showed that the liquid media is preferable within mass production and commercial advantages. The BAP promotes shoot initiation and development either with solid or liquid media more than Kin. The optimum BAP concentration depends on medium type (5 mg/l for solid medium and 3 mg/L for liquid medium).

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تأثير نوع بيئة الزراعة والسيتوكينين على الإكثار الدقيق في الموز أثناء مرحلة التضاعف

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يعتبر نبات الموز رابع أهم نباتات الفاكهة من حيث الأهمية على مستوى العالم.أجريت هذه الدراسة في معمل زراعة الأنسجة بقسم البساتين – كلية الزراعة – جامعة قناة السويس بالإسماعيلية خلال السنوات ٢٠١٣ حتى ٢٠١٥ - تم جمع الخلفات المستخدمة في زراعة الأنسجة من نباتات موز مثمرة وخالية من الأمراض تنتمي إلى صنف جراندنان ونامية في حديقة خاصة بمحافظة الجيزة واستخدمت القمة النامية بعد تعقيمها وزراعتها بأوعية النمو وذلك بهدف معرفة تأثير نوع بيئة الزراعة (صلبة و سائلة) مضافا إليها السيتوكينين (بنزايل امينو بيورين وكينيتين بتركيز صفر، ١، ٣، ٥، ٧ ملجم/ لتر) على الإكثار الدقيق للموز أثناء مرحلة التضاعف. وقد أظهرت النتائج أن أعلى معدل للتضاعف (٧.٢٠ برعم/ كتلة متبرعمة) كان باستخدام ٣ ملجم/ لتر بنزايل امينو بيورين في البيئة السائلة وجد أطول النموات (٧.٤٠ سم) عند استخدام ٥ ملجم / لتر كينيتين في البيئة الصلبة.